Global

Journal of Environmental Science and Management

CONTENTS

Volume 7, Number 1, Winter 2021

(Serial # 25)

Quarterly Publication

ISSN 3572 - 2383

Global Journal of Environmental Science and Management

Volume 7, Number 1, Winter 2021

(QUARTERLY PUBLICATION)

OPEN ACCESS

Publication authorization is certified by the Ministry of Culture and Islamic Guidance; No. 93/3629; 14 May 2014
Scientific-Research grade is accredited by the Ministry of Science, Research and Technology; No. 3/18/59975; 20 June 2015

Subscription form

Global Journal of Environmental Science and Management

Please enter my annual subscription to the Global Journal of Environmental Science and Management (GJESM), including 4 quarterly issues for the Year .... Vol. .... Nos.........

	Domestic (Rials)	International (USD)
▪ Institutional	3,000,000	150
▪ Individual	2,000,000	100
▪ Student	1,000,000	80
▪ Single Copy	500,000	50

Name: **Tel.:** **Email:**

Mailing Address:

Payment method: Check or money order must be made in order of:
Account #. 0101834143005, Account name: J. Nouri
Bank Melli Iran, IAU Branch, Code 1017, Tehran, Iran

□ Bank receipt enclosed

** Please allow 3 to 5 weeks for delivery*

Please send this filled in order form along with the Bank receipt payment to:

Global Journal of Environmental Science and Management
No. 2, Kouhestan Deadend, Janpour Street,
Darabad, Tehran 1956934461 Iran

Let's work together to publish the knowledge and science to serve better the environment

Circulation: 500

*p*ISSN 2383 - 3572

*e*ISSN 2383 - 3866

Aims and Scope

Global Journal of Environmental Science and Management (GJESM) is an international scholarly refereed research journal which aims to promote the theory and practice of environmental science and management. A broad outline of the journal's scope includes; peer reviewed original research articles, case and technical reports, reviews and analyses papers, short communications and notes to the editor, in interdisciplinary information on the practice and status of research in environmental science and management, both natural and man-made. The main aspects of research areas include, but are not exclusive to; environmental chemistry and biology, environments pollution control and monitoring, transport and fate of pollutants in the environment, concentrations and dispersion and trace of wastes in air, water, and soil, point and non-point sources pollution, heavy metals and organic compounds in the environment, atmospheric pollutants and trace gases, solid and hazardous waste management; soil biodegradation and bioremediation of contaminated sites; environmental impact assessment, industrial ecology, ecological and human risk assessment; improved energy management and auditing efficiency and environmental standards and criteria.

Vision and Mission

Global Journal of Environmental Science and Management (GJESM) publishes original. Peer-reviewed and technical environmental articles serve those environmental science and management through the on time quarterly publication with reliable information. GJESM is an integral partner with the scientific and technical communities, delivering superior information products and services that foster communication, build insights and enables individual and collective advancement in environmental research. Providing environmental science and management information to the general public with descriptions of contemporary advances in environmental issues to be used in improving environmental protection and management.

Abstracting and Indexing

Web of Science (ESCI); Scopus; Scimago Journal Rank (SJR); ProQuest (Agricultural and Environmental Science + Natural Science Collection + Ulrichsweb); Chemical Abstract (CAS); CABI Abstract; Global Health Abstract; Agricola; Committee on Publication Ethics (COPE); PubMed-NCBI; DOAJ; Open J-Gate; Google Scholar; Academia.edu; Geomar; WorldCat; Academic Resource Index; Environmental XPRT; Information Matrix for the Analysis of Journals (MIAR); Bibliothek Humburg; ScienceMedia; JournalTOCs; MSRT; ISC; RICeST; SID; Civilica; Magiran.

Serial # 25

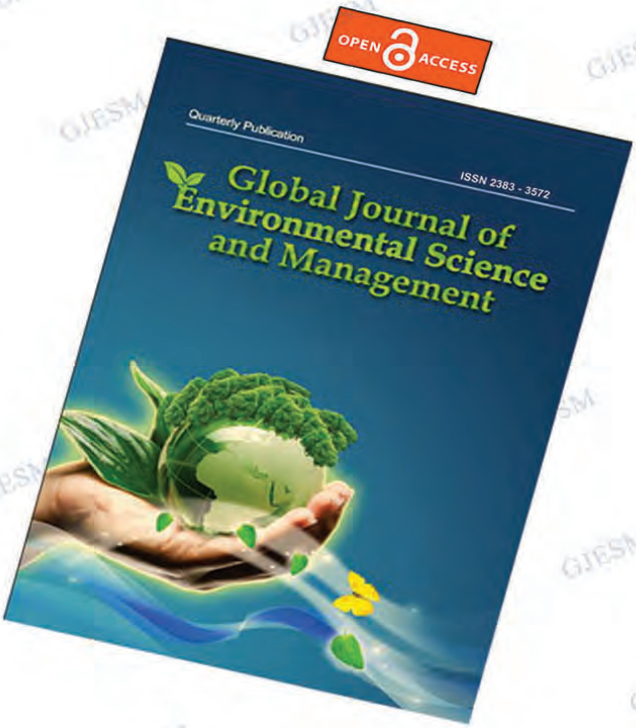
Global
Journal of Environmental Science and Management (GJESM)

Editor-in-Chief
Professor J. Nouri

pISSN 2383 - 3572
eISSN 2383 - 3866

QUARTERLY FULL OPEN ACCESS PEER REVIEWED PUBLICATION

Journal Abbreviation: Global J. Environ. Sci. Manage.



CALL FOR PAPERS

Publication benefits in
Global Journal of Environmental
Science and Management

- *Quarterly Publication journal*
- *Online submission and reviewing*
- *Online status inquiry*
- *Double blind peer reviewing*
- *Rapid evaluation and publication*
- *Immediate publication on the net*
- *Open access to all PDF full text of published articles*
- *No pay charge for publication*
- *Indexed and cited in well-known databases; particularly* Web of Science and Scopus

www.gjesm.net
www.gjesm.org

editor@gjesm.net
global.jesm@gmail.com
gjesm.publication@gmail.com

Tel.: +9821 2610 5110
Fax: +9821 2610 5111

CONTENTS

Volume 7, Number 1, Winter 2021

COVERING LETTER

Subject: **Submission of manuscript**

Dear Editor,

I would like to submit the following manuscript for possible evaluation

Manuscript Title:

Running Title (Short title):

Main Subjects:

Name and address of corresponding author:

Telephone #

Fax #

Email:

I affirm that the manuscript has been prepared in accordance with Global Journal of Environmental Science and Management guide for authors.

I have read the manuscript and I hereby affirm that the content of this manuscript or a major portion thereof has not been published in a refereed journal, and it is not being submitted fully or partially for publication elsewhere. The manuscript has been read and approved by all listed authors.

The source(s) of financial support of study (if any):

Type of Manuscript (check one):

- ☐ Original research paper
- ☐ Case report
- ☐ Research note
- ☐ Short communication
- ☐ Review paper

Name:

Corresponding Author Signature:

Date:

Global Journal of Environmental Science and Management
(GJESM)

Homepage: https://www.gjesm.net/

ORIGINAL RESEARCH PAPER

Geographic information system and process-based modeling of soil erosion and sediment yield in agricultural watershed

G.R. Puno[1,*], R.A. Marin[1], R.C.C. Puno[2], A.G. Toledo-Bruno[2]

[1]Department of Forest Resources Management, College of Forestry and Environmental Science, Central Mindanao University, Musuan, Maramag, Philippines

[2]Department of Environmental Science, College of Forestry and Environmental Science, Central Mindanao University, Musuan, Maramag, Philippines

ARTICLE INFO

Article History:
Received 17 February 2020
Revised 20 May 2020
Accepted 01 June 2020

Keywords:
Agriculture
Geospatial
Sediments
Soil conservation
Sustainability

ABSTRACT

BACKGROUND AND OBJECTIVES: The study explored the capability of the geographic information system interface for the water erosion prediction project, a process-based model, to predict and visualize the specific location of soil erosion and sediment yield from the agricultural watershed of Taganibong.
METHODS: The method involved the preparation of the four input files corresponding to climate, slope, land management, and soil properties. Climate file processing was through the use of a breakpoint climate data generator. The team had calibrated and validated the model using the observed data from the three monitoring sites.
FINDINGS: Model evaluation showed a statistically acceptable performance with coefficient of determination values of 0.64 (probability value = 0.042), 0.85 (probability value = 0.000), and 0.69 (probability value = 0.001) at 95% level, for monitoring sites 1, 2, and 3, respectively. A further test revealed a statistically satisfactory model performance with root mean square error-observations standard deviation ratio, Nash-Sutcliffe efficiency, and percent bias of 0.62, 0.61, and 44.30, respectively, for monitoring site 1; 0.65, 0.56, and 25.60, respectively, for monitoring site 2; and 0.60, 0.65, and 27.90, respectively, for monitoring site 3. At a watershed scale, the model predicted the erosion and sediment yield at 89 tons per hectare per year and 22 tons per hectare per year, respectively, which are far beyond the erosion tolerance of 10 tons per hectare per year. The sediment delivery ratio of 0.20 accounts for a total of 126,390 tons of sediments that accumulated downstream in a year.
CONCLUSION: The model generated maps that visualize a site-specific hillslope, which is the source of erosion and sedimentation. The study enables the researchers to provide information helpful in the formulation of a sound policy statement for sustainable soil management in the agricultural watershed of Taganibong.

DOI: 10.22034/gjesm.2021.01.01

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
50	12	4

*Corresponding Author:
Email: *grpuno@cmu.edu.ph*
Phone: +9166918259
Fax: +6388 356 1912

Note: Discussion period for this manuscript open until April 1, 2021 on GJESM website at the “Show Article.

INTRODUCTION

Accelerated soil erosion is the primary issue of the declining trend of soil fertility and the overall degradation of agricultural lands which in turn threatens the global socio-economic and environmental conditions (Van Leeuwen *et al*., 2019; Ghafari *et al*., 2017). Common to the other regions of the developing world, soil erosion poses a serious threat to soil sustainability and degradation of the entire agricultural systems (Zhang *et al*., 2019; Olabisi, 2012). The report shows that degraded 44% of the total land area due to soil erosion in the Philippines is affecting 33 million Filipinos (Dar, 2017). To address land degradation issues, quantification of soil erosion rate from a particular agricultural watershed is necessary. Quantification of soil erosion by water offers a variety of procedures ranging from actual field data collection to computer simulation using geographic information system (GIS) tools coupled with the standalone and process-based models (Diwediga *et al*., 2018; Brooks *et al*., 2016). The current trend of soil erosion research recognizes extensively the use of prediction models, as the measurement of soil erosion rates from large watersheds is impractical due to complex hydrologic processes under varying conditions such as climate, soils, slope, vegetation and tillage (Pijl *et al*., 2020; Zheng *et al*., 2020). Over the few decades, the experts were focusing on the development of various modeling techniques to assess soil erosion in agricultural watersheds where the Water Erosion Prediction Project (WEPP) is among of the widely applied models as identified by most works of literature (Xiong *et al*., 2019; Pandy *et al.,* 2016). In as much as the development of reasonable and scientific control measures depends on reliable data and information, the selection of an effective prediction model is critical (Han *et al*., 2016). Thus, a review of fifty erosion and sediment models in terms of worldwide applicability for best management practices implementation was conducted (Pandy *et al*. 2016). The findings revealed that only five to be the most promising, including the Soil and Water Assessment Tool (SWAT) and WEPP. Further comparison between the two models reported by Shen (2009) showed that the later had provided better results over the former for both runoff and sediment yield. WEPP is standalone software, a process-based, and a continuous soil erosion model that predicts the spatial and temporal distribution of soil loss and deposition due to surface runoff on the small agricultural watershed (Flanagan *et al*., 2001). In August 1985, the United States Department of Agriculture-Agricultural-Research Service (USDA-ARS) has initially developed the software (Flanagan *et al*., 2007). The detailed descriptions of WEPP in terms of model components, processes, and input files requirements are presented in the works of Meghdadi (2013) and Gonzàlez-Arqueros *et al*. (2016). The advancement of computer technology allows the model to enhance its capabilities through the geospatial interface known as GeoWEPP (Renschler and Zhang, 2020). The interface, therefore, is enriched with the excellent characteristics of GIS such as the processing and creation of digital data at a watershed scale (Gonzàlez-Arqueros *et al*., 2016; Flanagan *et al*., 2013). The application of GeoWEPP model is now model worldwide to assess its predictive capability under varying factors of erosion processes that are unique to a specific watershed such as climate, soil, slope, and land management (Han *et al*., 2016). The following discussions are some of the works on the application of GeoWEPP outside the USA. In Iran, the GeoWEPP model was used to identify the type of land uses and management scenarios effective to reduce runoff, soil erosion, and sediment yield (Mirakhorlo and Rahimzadegan, 2019; Narimani *et al*., 2017). The findings of several GeoWEPP modeling studies in that country enable the researchers to identify the best management practices suitable for agricultural and critical watersheds (Meghdadi, 2013). The model was also explored in Japan to assess the potential disaster caused by sediment discharge from the mountainous watershed (Amaru and Hotta, 2018). GeoWEPP was also used in Central Mexico to account for the impact of the human via land-use changes on soil erosion trends covering almost 2000 years from pre-Hispanic period to modern times (Gonzàlez-Arqueros *et al*., 2016). The model also performed satisfactorily in predicting daily runoff and sediment yield in the highlands of Northern Ethiopia. The results served as bases to assess the impact of soil and water conservation structures to prevent land degradation (Melaku *et al*., 2018). In Malaysia, GeoWEPP has accurately predicted runoff although over calculation of sediment load was observed due to steeper slopes of the study site (Ebrahimpour *et al*., 2011). Satisfactory performance of GeoWEPP in simulating streamflow and sediment yield under the prevailing environmental condition of the heterogeneous catchment in Italy was also reported (Peiri *et al*., 2014). In China, the model was used to account for the effect of slope gradients, and land uses on soil erosion intending to provide scientific evidence for a sound land use plan in the watershed (Zhang *et al*., 2015). Locally, the model was successfully applied to assess soil sustainability in the agriculturally active watershed of the Philippines (Puno, 2014). Oftentimes, model evaluation is necessary to test how results will aid as a guide to local land management in providing science-based policy implications and guidelines relative to soil conservation for sustainable agriculture. (Renschler and Zhang, 2020; Panagos and Katsoyiannis, 2019; Prasuhn *et al*., 2013). Like any other impaired watersheds in the country, Taganibong is an agricultural watershed that suffers erosional problems due to rapid land conversion and uncontrolled land tilling along steeper hillslopes, which may result in a poor soil condition when remains unabated. Site-specific information on erosion in the area to support the advocacy of sustainable soil management in the agricultural watershed of Taganibong is sought. Acquiring this kind of information needs a GIS and a process-based model like GeoWEPP, as the measurement of erosion and sediment yield at a watershed scale is almost impossible considering the complexity of the interacting environmental factors (Liu *et al*., 1997). This academic exercise aimed to explore the applicability of GeoWEPP model to predict soil erosion and sedimentation rates in the study area. This study anticipates providing preliminary information helpful in evaluating the soil condition of the watershed. The research team chose the model as it works as an extension tool of the leading GIS software, and is extensively applied worldwide. Further, only the GeoWEPP model can predict erosion distribution along hillslope on a per-event basis (Flanagan *et al*., 2001). The study was carried out for about two years from 2013 to 2015 within the watershed of Taganibong, Mindanao, Philippines.

MATERIALS AND METHODS

Study area

The study location was at the watershed of Taganibong, Mindanao, Philippines (Fig. 1). Geographically, the watershed lies between 124^{0}56’ to 125^{0}4’ east and 7^{0}48’ to 7^{0}56’ north with a total land area of 5,853 ha. The terrain is mostly undulating to rolling with 11.6% average slope and 121% as the steepest, particularly along channel and mountainside hillslopes. The area has an elevation of 284 to 1,334 meters above sea level (masl) with 595 masl on the average. The site has a heavy-textured brown clay dominated by a silt loam and a sandy clay loam type of soil texture. The area receives an average annual rainfall of 2,587 mm with a mean annual temperature of 25^{o}C. There is no very pronounced dry season, although dry periods are experienced from November to April while the rest of the year

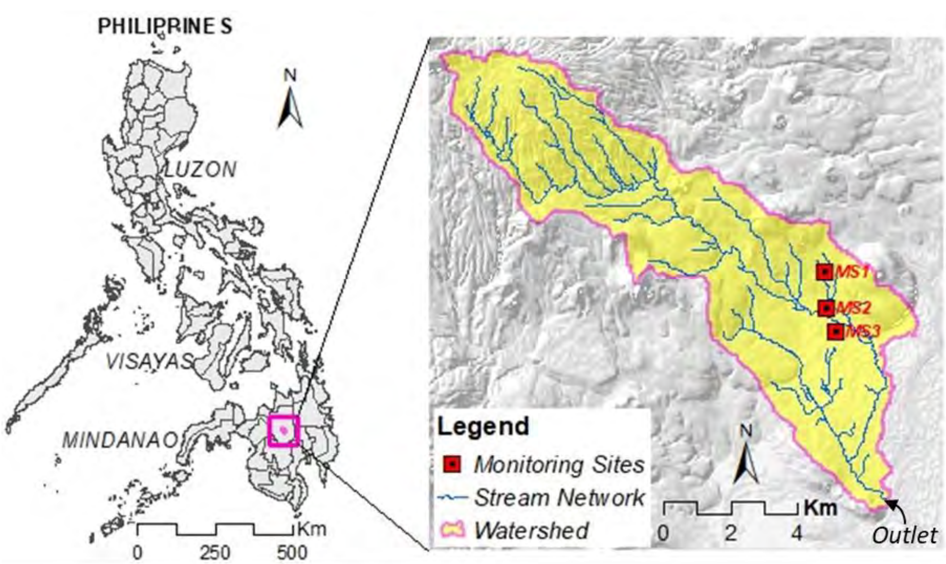


Fig. 1: Geographic location of the study area in Taganibong Watershed, Philippines

is wet. Generally, cultivation for agricultural crop production is among of the land-use practices in the watershed with the presence of some growing built-ups. The research team established three monitoring sites (M1, M2, and M3) within the watershed for the collection of soil erosion data.

The GeoWEPP Model

GeoWEPP is the interface of the WEPP model and GIS that uses the topography parameterization (TOPAZ) algorithm with ArcGIS software as a working platform. A standalone WEPP model is a daily continuous, physically, and process-based model that describes hillslope, channel, and impoundment and simulates hydrologic variables such as erosion, sediment, runoff, and deposition on a temporal and spatial base (Ebrahimpour *et al*., 2011). As a plugin in the ArcGIS software, GeoWEPP allows two simulation options, the onsite or flowpath method for the onsite assessment of erosion, and the offsite or the watershed method for the assessment of sediment yield based on a single hillslope and channel (Amaru *et al*., 2018). The TOPAZ tool allows GeoWEPP to delineate watershed boundary and generate hillslopes or subwatershed profiles using a digital elevation model. (Maalim *et al*., 2013). A detailed description of the GeoWEPP model discussing how the model runs and produces textual and spatial databases is presented in the work of Flanagan *et al*., (2013).

Data collection for model simulation

Modeling with GeoWEPP requires four significant data groups corresponding to the slope, landcover, soil, and climate (Table 1). The preparation of the slope input file needs the synthetic aperture radar-digital elevation model (SAR-DEM) availed from the University of the Philippines Diliman (UPD), Quezon City, through its Disaster Risk and Exposure Assessment for Mitigation (DREAM) Program. The slope was classified following the recommendation from the Bureau of Soil and Water Management (BSWM) that includes six categories described as flat (0-3%), undulating (3.01-8%), undulating to rolling (8.01-18), rolling (18.01-30%), step (30.01-50%), and very steep (>50%) (Fig. 2). Landcover data (Fig. 3) was collected by digitizing an image from the Google Earth tool and was

Table 1: Data for GeoWEPP simulation

Type of data	Methods of data acquisition
Slope	SAR-DEM, UPD-DREAM
Landcover	Google Earth, field survey
Soils (NPK, OM, CEC, albedo, texture, rock)	Field survey, laboratory analysis, literature
Climate (rainfall, RH, max and min temperature, solar radiation, wind speed, and direction)	Automatic Weather Station

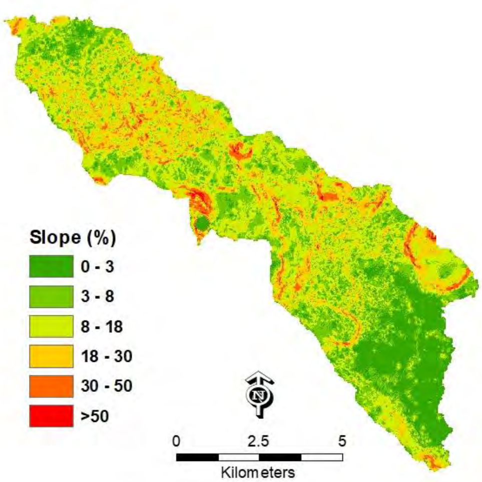


Fig. 2: Slope map of the Taganibong watershed

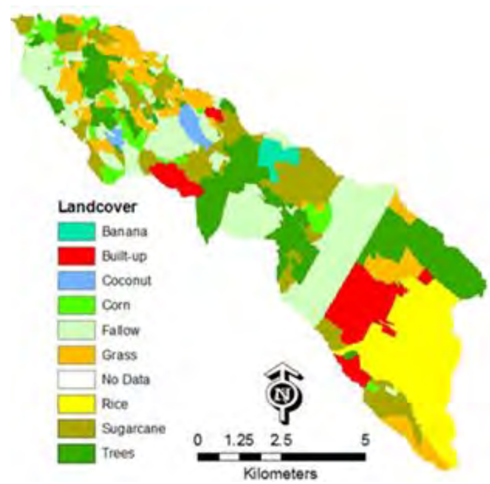


Fig. 3: Landcover map of the Taganibong watershed (2015)

Table 2. Landcover or land use of the Taganibong watershed

Landcover	Area (ha)	%
Banana	84.5	1.4
Built-up	487.0	8.3
Coconut	258.6	4.4
Corn	85.2	1.5
Fallow	1,168.8	20.0
Grass	659.0	11.3
Rice	947.5	16.2
Sugarcane	928.7	15.9
Trees	1,233.7	21.1
Total	5,853.0	100.0

validated on the ground. Crops like corn, sugarcane, banana, among others, with patches of grasslands and fallow areas were the typical landcover or land uses (Table 2). The Central Mindanao University manages both the patches of natural and mixed plantation forests at the rolling lower portion of the watershed and the rice fields at the floodplain areas. The straight line formed in the landcover reflects the real situation on the ground where the power transmission lines traverse the area, restricting the site from being grown or planted with taller perennial vegetation. Soil parameters such as nitrogen (N), phosphorus (P), potassium (K), organic matter (OM), texture (silt, loam, clay, and sand) were derived from the collected samples, which were analyzed at the laboratory. The albedo of the ground surface was availed from literature, while the cation exchange capacity (CEC) was based on the built-in database of the software. The rock information expressed in percent of the total area was obtained via an ocular survey. Fig. 4 shows the spatial distribution of the soil textural properties in the area. Climate variables comprising of the rainfall amount, relative humidity (RH), maximum and minimum temperature, solar radiation, wind speed, and direction were collected through the automatic weather station installed near the three monitoring sites within the watershed.

Map Layer and database requirements

The initial step of setting up the GeoWEPP model before the actual simulation run requires two groups of input files. The first group includes the grid-based map layers written in the American Standard Code for Information Interchange (ASCII) format comprising of dem.asc, landcov.asc, and soilsmap.asc layers. The second group includes the database files in text format corresponding to landcov.txt, soilsmap.txt, landusedb.txt, and soilsdb.txt. The first group of data was processed and prepared using the ArcGIS version 10.2.2 software of the Environmental Systems Research Institute Inc. The step by step procedure of making the above data is presented in the work of (Minkowski and Renschler, 2008).

Slope grid file preparation

The slope grid layer was derived from a 10-meter resolution SAR-DEM. As mentioned, GeoWEPP accepts the grid input file in ASCII format. Thus, the input slope map layer was saved as dem.asc.

Landcover and soil grid files preparation

The landcover in a shapefile format of the watershed was created through digitizing an image

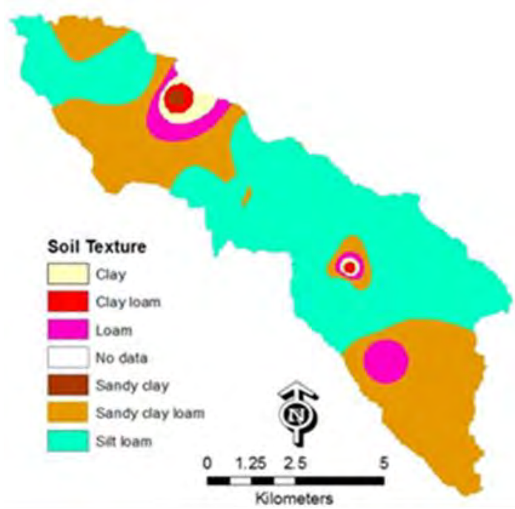


Fig. 4: Soil map of the Taganibong watershed

from the internet with the Google Earth application tool. The resulting shapefile layer was converted into a raster layer file and saved as landcov.asc. Similarly, the soil map layer in the raster format was prepared as the landcover map layer with a slight difference at the initial step. The shapefile containing all the descriptions of the soil samples was created using the coordinates of the sample pits randomly distributed in the watershed. The soil map layer in the raster format was finalized to satisfy the requirement of GeoWEPP using the ArcGIS tool capabilities.

Landcover and soil database files preparation

When using a landcover layer in ASCII format, the program will not proceed if the landcover in the text file format (landcov.txt) is missing. In the same manner, the program also fails to run if the soil in text file format (soil.txt) is missing. Finally, the created database files were saved with an extension file names of .txt corresponding to landcov.txt, landusedb.txt, soilsmap.txt, and soilsdb.txt. The landcov.txt and soilsmap.txt files were used by the GeoWEPP and WEPP/TOPAZ translator (WEPP Management and Soil Lookup) to determine the description that corresponds to the landcov.asc, and soilsmap.asc layers, respectively. Likewise, landusedb.txt and soilsdb.txt files were georeferenced in a similar manner as landcov.txt and soilsmap.txt files. The detailed procedure in making the landcover and soil database input files followed the procedure in the work of Minkowski and Renschler (2008).

Climate input file preparation

The model will run using a climate input file generated using either a climate generator (CLIGEN) or the BPCDG module. CLIGEN is the built-in capability of the WEPP model to make the required data using long historical climate data. The BPCDG, on the other hand, is a standalone software module that processes a single year observed climate datasets. This study used the module to process the climate input file for the GeoWEPP simulation. The climate parameters required by the BPCDG involve five-minute interval climate datasets comprising rainfall, minimum and maximum temperature, relative humidity, solar radiation, wind speed, and direction, which were collected using an automatic weather station installed in the site. The use of BPCDG was preferred based on its advantages over CLIGEN as it allows direct use of observed storm and other daily standard climate data sets (Zeleke, 1999).

Channel network and catchment delineation

GeoWEPP model allows automatic delineation of channel network and catchment boundary through the topographic parameterization (TOPAZ) tool following the concept of a critical source area (CSA) and minimum source channel length (MSCL) (Renschler and Zhang, 2020; Amaru *et al*., 2018). The process involved the arbitrary setting of the CSA and MSCL values to determine the desired density of the channel network and the number of hillslopes within the watershed.

Model calibration and validation

Calibration was conducted manually following the procedures from the previous study, as discussed (Ramos, 2016). The parameters adjusted in the calibration process included the soil erodibility, critical shear, and effective hydraulic conductivity factors. Other parameters like the channel width, presence or absence of rocks in the river bed, type of vegetation, mode of tillage, among others, were manually adjusted until best fit between the first set of observed data and the simulated values. Validation was carried out after a thorough calibration and series of simulation trials by comparing the simulated results of the calibrated model with the second set of observed erosion data.

Model performance evaluation

The process of performance evaluation involved the use of soil erosion data collected from the three monitoring sites (MS1, MS2, and MS3) to assess the predictive capacity of the model. The data collection activity for this purpose applied the modified erosion bar instrument to measure soil erosion values for every rainfall event (Marin and Casas, 2017). The calculated values of the coefficient of determination (R^2), root mean square error (RMSE)-observations standard deviation ratio (RSR), Nash-Sutcliffe efficiency (NSE), and percent bias ratio (PBIAS) were the basis to evaluate the performance of the model. The RSR ranges from zero to a large positive number with 0 and 0.7 indicating a perfect prediction and unsatisfactory values, respectively. NSE ranges between $-\infty$ and 1, with 1 and <0.5 being the optimal and unsatisfactory values, respectively.

Values between 0 and 1 are generally viewed as acceptable levels of performance, whereas values <0 indicates unacceptable performance (Moriasi *et al*., 2007). PBIAS assesses the average tendency of the predicted results to overestimate or underestimate the observed data (Gupta *et al*., 1999). A PBIAS of 0 indicates an accurate model performance. A positive value, on the other hand, suggests underestimation, and overestimation if negative values (Gupta *et al*., 1999). PBIAS of 55% for sediment modeling is already a satisfactory result (Moriasi *et al*., 2007). These statistical criteria are mostly applied to hydrologic modeling studies like GeoWEPP to validate model performance (Ricci *et al*., 2020; Narimani *et al*., 2017; Ramos, 2016).

GeoWEPP simulation

The calibrated and validated GeoWEPP model was then applied for the simulation of erosion and sediment yield at a watershed scale. The simulation involved two assessment methods, namely offsite or watershed method, and onsite or flow path process. The offsite determines a representative profile for the hillslope and assigned one soil and one land use to it (Amaru, 2018). This method predicts the amount of sediment, leaving each hillslope evaluated at the outlet. The onsite process helps the user identifies which hillslopes are the problem areas. This method shows which portions of a particular hillslope are the main contributors to such erosion problems considering the diversity and distribution of the soil and land use types (Minkowski and Renschler, 2008).

Soil sustainability assessment

The concept of soil erosion tolerance or threshold was used as the criteria to assess the sustainability of soil in the watershed. As defined, soil tolerance is the maximum rate of erosion to occur while permitting sustainable and high-level of crop productivity (Lenka *et al*., 2014). The soil is assessed as sustainable when the rate of erosion is not exceeding the allowable soil tolerance. For convenience, a tolerable limit of 10 t/h/y as used in the work of Melaku *et al*. (2018) was also applied in this study because the Philippines is a tropical country where the acceptable soil loss ranges from 10 to 12 t/h/y (Tacio, 2011).

RESULTS AND DISCUSSION

Predicted and observed soil erosion

The predicted soil erosion values were compared with the amount of erosion observed from the three monitoring sites. Figs. 5, 6, and 7 show the graphical representation of the compared values. The graphs show that there is a close relationship between the predicted and observed erosion rates. The result of the t-test revealed that the two sets of erosion values are not statistically different with p-values of 0.28, 0.29, and 0.29 at 0.05 level of significance for MS1, MS2, and MS3, respectively. A similar study reported comparable results where the GeoWEPP model simulated hydrologic variables closer to the measured values (Yuksel, 2008). This indicates that the model is a good predictor of soil erosion and sediment yield in the Taganibong watershed.

Model evaluation results

Table 3 summarizes the statistics of model evaluation results. Results show the linear fitting between observed and predicted erosion values in

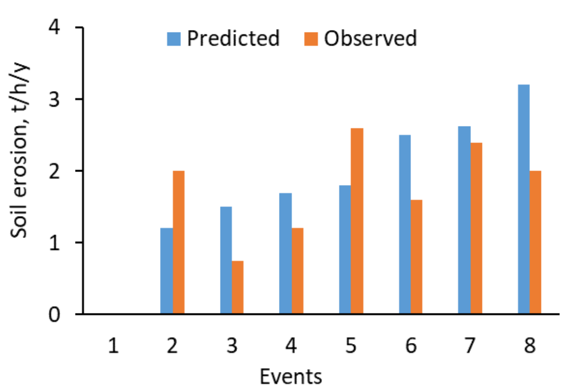


Fig. 5: Comparison of predicted and observed erosion from MS1

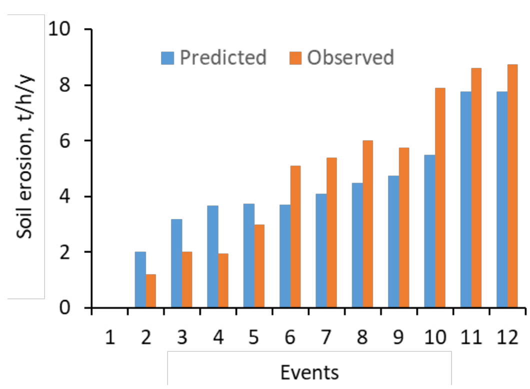


Fig. 6: Comparison of predicted and observed erosion from MS2

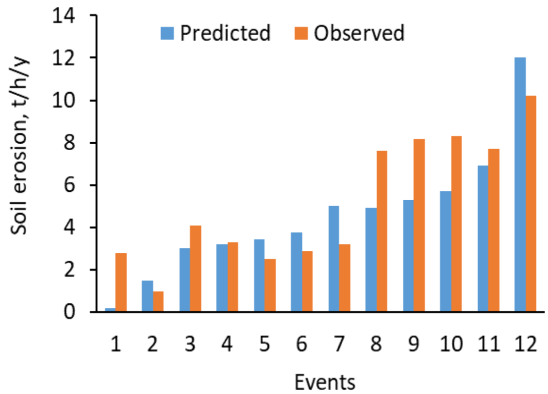


Fig. 7: Comparison of predicted and observed erosion from MS3

the three monitoring sites. Figs. 8, 9, and 10 show the direct relationship between the observed and predicted values with the coefficient of determination (R^2) of 0.64 (p=0.042), 0.85 (p=0.000), and 0.69 (p=0.001) at 95% level for MS1, MS2, and MS3, respectively. Several studies also showed R^2 of these ranges implying that the model is a good predictor of erosional processes at an acceptable parametric calibration under similar conditions (Maghdadi, 2013; Ebrahimpour *et al*., 2011; Alibuyog, 2009; Pandey, 2007). Generally, the results on model performance evaluation following the suggested statistical criteria show closer values reported in previous studies on the application of GeoWEPP and other related hydrologic models (Melaku *et al*., 2018; Fukunaga *et al*., 2015). Using RSR, NSE, and PBIAS statistical tests revealed that the model performance is satisfactory (Table 3). However, the model tends to underestimate soil loss, as shown by consistent large positive PBIAS values for the three sites. Nevertheless, Moriasi *et al*. (2007) reported that PBIAS of ±55% for sediment yield modeling is already satisfactory. Under and over prediction of the model, however, does not necessarily suggest that GeoWEPP performed poorly but rather a manifestation that erosion prediction, in general, contains large factors of error due to the interacting complex and varying environmental conditions (Liu *et al*., 1997).

Watershed scale GeoWEPP simulation

Using the main input files prepared for the model, the calibrated GeoWEPP was applied in a broader scale of Taganibong watershed with the soil tolerance or threshold set at 10 t/h/y. A total of 177 sub-catchments or hillslopes assigned with unique soil and land management type were created based

Table 3: Statistics of model evaluation

Criteria	MS1	MS2	MS3
R^2 (95%)	0.64 (*p=0.042*)	0.85 (*p=0.000*)	0.69 (*p=0.001*)
RSR	0.62	0.65	0.60
NSE	0.61	0.56	0.65
PBIAS	44.30	25.60	27.90

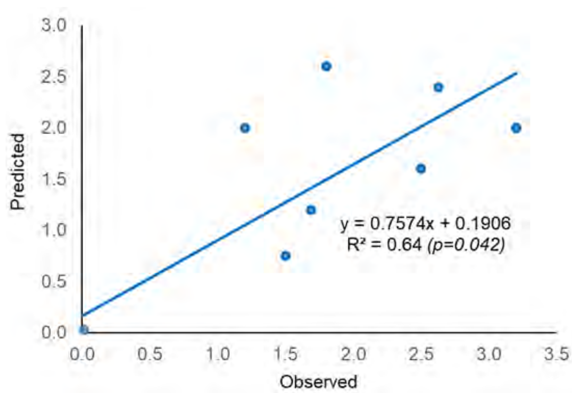


Fig. 8: Regression between predicted and observed erosion from MS1

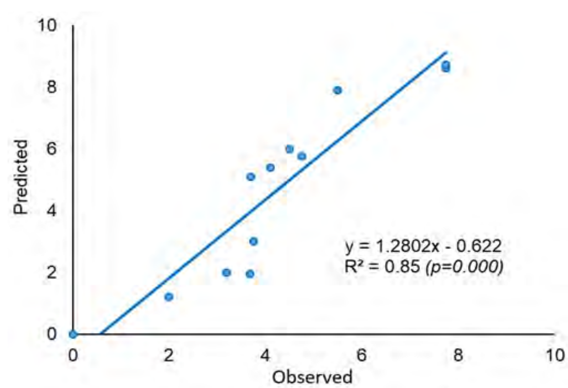


Fig. 9: Regression between predicted and observed erosion from MS2

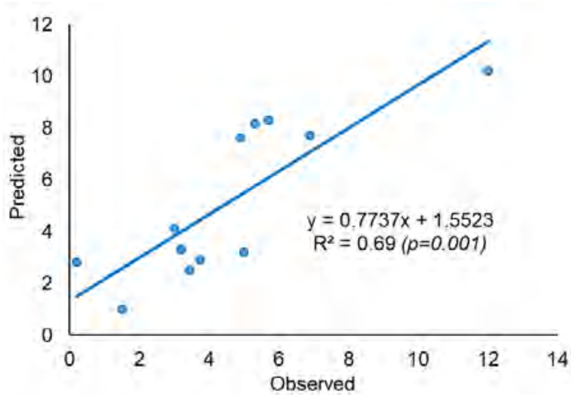


Fig. 10: Regression between predicted and observed erosion from MS3

on the CSA and MSCL values set at 50 hectares and 200 meters, respectively. The model generated two raster map layers as a result of the offsite (watershed) and onsite (flowpath) methods. The offsite method produced the sediment yield map that determines the amount of soil removed and accounted at the outlet of a particular hillslope or subcatchment with homogeneous soil and landcover assigned by the model. The runoff discharges mainly influenced it from the hillslopes and channel and determined the same at the outlet point of the modeled watershed (Maalim and Melesse, 2013).

Sediment yield assessment

The model accounted for a total of 46 out of 177 hillslopes with sediment yield beyond the threshold and 131 with sediment yield lower than the limit, depicted in Fig. 11 with shades of red and green, respectively. The hillslopes with sedimentation rate beyond the threshold are entirely shaded with red because the offsite method assumes entirely those areas as the sources of sediments and does account for the specific location where the origin

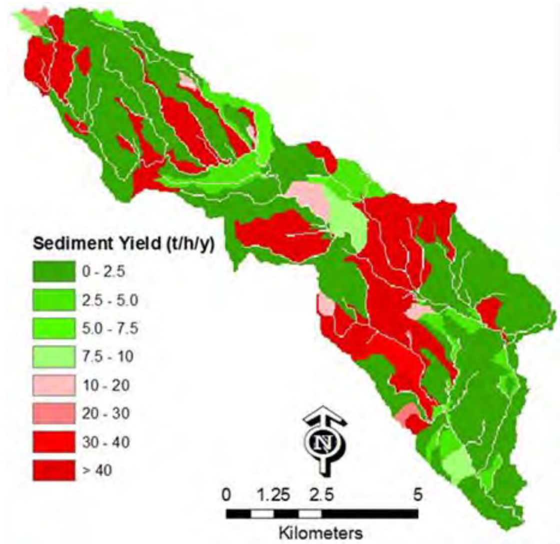


Fig. 11: Sediment yield map of the Taganibong watershed

of sedimentation has occurred. The total land area of the hillslopes with sediment yield beyond the threshold is around 26% of the watershed total area. On average, the model predicted sediment yield at the rate of 22 t/h/y for the whole watershed (Table 4).

Soil erosion assessment

The onsite method identifies the specific location of the area within the watershed where the problem of erosion has occurred (Maalim *et al*., 2013). Through this method, the model generated a more detailed soil erosion map that shows the spatial distribution of the eroded material along the hillslopes. As a result, some of the hillslopes predicted under the offsite method with sediment yields beyond threshold were further subdivided into shades of green, red,

Table 4: Predicted hydrologic values for the Taganibong watershed

Hydrologic parameters	Predicted values
Total area of watershed	5,853 ha
Precipitation volume	124,239,285 m^3/y
Water discharge	6,349,328 m^3/y
Total hillslope soil erosion	523,522 t/y
Total channel soil erosion	124,927 t/y
Sediment discharge from outlet	126,390 t/y
Soil erosion per unit area	89 t/h/y
Sediment yield per unit area	22 t/h/y
Sediment delivery ratio	0.20

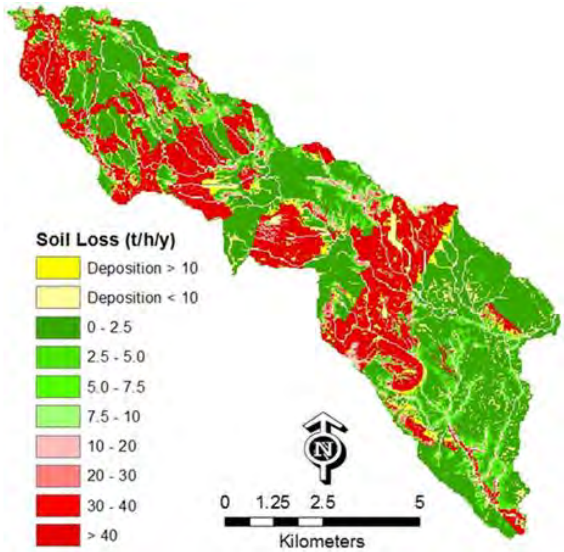


Fig. 12: Soil erosion map of the Taganibong watershed

and yellow, for areas within the threshold, beyond the threshold, and deposition respectively (Fig. 12). The red portion of the hillslope, therefore, is the area where the problem of erosion has specifically occurred. By default, the model generated the soil erosion map with a value code up to >40 for hillslopes with erosion beyond the threshold (Fig. 12). This means that the model can report the hillslopes with large erosion values. The model predicted an amount of 89 t/h/y for the whole area which is very far from the threshold of 10 t/h/y. Table 4 shows the details of the predicted hydrologic parameters for the whole watershed of Taganibong.

The predicted average erosion rate of 89 t/h/y is closed to the national average erosion rate of 80 t/h/y (Asio *et al*., 2009). The likelihood of higher values beyond erosion tolerance is explained by the steeper and tilled hillslope with a silt loam type of soil texture (Obalum *et al*., 2019). With some limitations, the model has successfully generated information on the onsite erosion and deposition variables due to the combined effect of climate, soil texture, landcover, and slope factors. Overlaying the predicted soil erosion map (Fig. 12) with the landcover input file map (Fig. 3), revealed that the erosion, depicted with shades of red in the erosion map (Fig. 12), has generally occurred in the areas with built-ups and fewer trees. On the other hand, areas with lesser erosion values, coded with shades of green in the map, are generally covered with trees. This indicates that vegetative landcover is helping to arrest soil erosion in that particular site. However, observation should take into account the combined effect of the slope. As illustrated in the erosion map, and with reference to the slope map, some areas in the watershed without trees have lesser predicted erosion values due to their flat ground surfaces. The results confirmed with the findings from the previous modeling studies, pointing out that the removal of permanent landcover, and tilling in some steeper slopes are the primary factors to accelerate soil erosion rates (Narimani *et al*., 2017). Tilling in steeper slope for short term crops and the occasional presence of some built-ups are evident in the site. Many studies reported that increased soil erosion and sediment yield is due to changes in land management from vegetated to open areas (Pieri *et al*., 2014; Alibuyog *et al*., 2009). More studies also show that tilling in steeper hillslopes resulting in excessive erosion is likely the major contributing factor to the process (Amaru and Hotta, 2018; Zhang *et al*., 2015). An increase in erosion rates attributed to the absence of forest and other permanent vegetation is further explained by less water infiltration, increased runoff velocity, and proneness to erodibility (Zheng *et al*., 2020). Steeper slope, however, with trees and perennial plant cover was predicted to have lesser erosion values below the erosion tolerance indicating sustainable soil in the area. Lower soil loss in forested areas was mainly due to the constant ground cover throughout the year, resulting in a minimal runoff and high permeability of the forest soil (Ricci *et al*., 2020; Amaru and Hotta, 2018). The predicted erosion rates in areas planted with rice located at the southeastern portion of the watershed were below the threshold as indicated with the shade of green in the soil erosion map (Fig. 12). Soil erosion in the irrigated plain and terraced paddy field for rice production was reported to have 0.77 t/h/y (Chen *et al*., 2012). The model accounted for a difference in the erosion values between the offsite and onsite method despite the same source of contributing hillslope. The concept of the sediment delivery ratio explains the expected difference between the two processes. As defined, sediment delivery ratio is a measure of sediment transport efficiency expressed as the fraction of the gross erosion and deposition from a given area with a value being inversely proportional to the size of the watershed (Dong *et al*., 2013). The lower predicted sediment delivery ratio indicates a relatively smaller volume of sediment deposited downstream. The sediment delivery ratio of 0.20 indicates about 80% of sediment were deposited or trapped within the watershed due to vegetation, size of the watershed, and slope gradient of the mainstream channel, that control the soil particles to reach the lowest point of the watershed (Nguyen and Chen, 2018). With the prevailing sediment delivery ratio, the model accounted for a total of 126,390 tons of sediments that had accumulated at the low-lying bodies of water in just one year. To some extent, the model predicted soil erosion and sediment yield with some degree of variability. As evaluated, however, the model is statistically acceptable with a satisfactory performance with the predicted hydrologic variables closed to the results of the previous studies. Nevertheless, the variability of the predicted results suggests for future modeling study based on challenging quantitative field data measurements sufficient for model calibration and validation to improve the predictive performance of the model.

Assessment of soil sustainability

The excessive predicted soil erosion and sediment yield values of 89 t/h/y and 22 t/h/y, respectively, are excessively far from the erosion tolerance of 10 t/h/y. This indicates that the soil in some hillslopes of the watershed is unsustainable. This is probably due to the uncontrolled land cultivation for crops production. Open land tilling for sugarcane, corn, banana, among others growing at steeper slopes and elevated areas, has a greater tendency to cause accelerated soil erosion. Further, silt soil being a loose-type of ground quickly releasing particles covers 55% of the total watershed land area. The presence of a dominant silt loam type of soil may have contributed to a higher rate of soil loss (Obalum *et al*., 2019). Site-specific land-use planning is encouraged for hillslopes with erosion and sedimentation rates beyond the threshold, using the type of crop suited for a certain percent of a slope category as suggested by the BSWM. Further, it is important to integrate conservation measures such as alley cropping and contour hedgerows in the sloping areas that exhibited high erosion rates to prevent accelerated erosion.

CONCLUSION

GeoWEPP was successfully applied and validated with statistically acceptable performance to predict soil erosion and sediment yield in Taganibong watershed. GeoWEPP simulation used digital files of soil, landcover, and elevation all in ASCII format prepared using the GIS tool capabilities. The use of the WEPP model interface enabled the research team to create the database files for management and soil information. The climate file was processed using the BPCDG standalone software. The modeling process involved a rigorous calibration of various parameters to fit the existing condition of the watershed and the series of model simulation trials. The model was validated by comparing the predicted with the observed soil erosion values from the three monitoring sites (MS1, MS2, and MS3), and revealed to be statistically satisfactory with R^2 values of 0.64 (p=0.042), 0.85 (p=0.000), and 0.69 (p=0.001), respectively, at 95% level. A further statistical test proved the acceptability of model performance with RSR, NSE, and PBIAS of 0.62, 0.61, and 44.30, respectively, for MS1; 0.65, 0.56, and 25.60, respectively, for MS2; and 0.60, 0.65, and 27.90, respectively, for MS3. At a watershed scale of Taganibong, the calibrated model had predicted the average soil erosion and sediment yield at 89 t/h/y and 22 t/h/y, respectively. These values are far from the erosion tolerance of 10 t/h/y indicating unsustainable soil particularly in some hillslopes of the watershed. The sediment yield and erosion maps generated by the model revealed the specific hillslopes in the watershed where the problem of erosion has occurred, coded with red color in the respective map layers. The sediment delivery ratio of 0.20 indicates that around 20% of the sediments amounting to 126,390 tons had accumulated at the downstream areas of the watershed. The remaining 80% of sediments were deposited elsewhere within the watershed. Similar to other modeling studies, GeoWEPP showed under and over prediction as indicated by consistent higher positive PBIAS values for the three monitoring sites. This observation, however, does not necessarily mean poor performance by the model but rather a manifestation that erosion modeling is subject to varying environmental factors, where the process had unintentionally missed to capture. Subject to some limitations, the overall result of this modeling exercise illustrated the applicability of GeoWEPP to predict soil erosion and sediment yield under a similar condition like the Taganibong watershed. The result offers an insight into identifying a specific location in the watershed with excessive soil erosion beyond the threshold. This study enables the research team to fill the gaps of information needed in the formulation of a site-specific policy recommendation for sustainable soil management in the agricultural watershed of Taganibong.

AUTHOR CONTRIBUTIONS

G.R. Puno performed the manuscript writing, prepared the GIS databases, thematic map layers, layout design, and graphs. R.A. Marin managed the operation of the project, collated, and analyzed the field data. R.C.C. Puno updated the landcover map using satellite images and edited the manuscript. A.G. Toledo-Bruno acted as project staff and edited the manuscript.

ACKNOWLEDGMENTS

This study is part of the project "Soil Erosion Management in Taganibong Watershed", supported by the Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development-Department of Science and Technology (PCAARRD-DOST), of the Philippines under financial account number [1 416-28]. The authors are grateful to the Central Mindanao University administration for its support; the University of the Philippines Diliman for the SAR-DEM dataset. Sincere appreciation to the editors and anonymous reviewers for their precious time and critical comments to improve the manuscript.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest regarding the publication of this work. Also, the ethical issues including plagiarism, informed consent, misconduct, data fabrication and, or falsification, double publication and, or submission, and redundancy have been completely witnessed by the authors.

ABBREVIATIONS

ArcGIS	Geographic Information System software product
ASCII	American Standard Code for Information Interchange
BPCDG	Breakpoint Climate Data Generator
BSWM	Bureau of Soil and Water Management
CEC	Cat-ion Exchange Capacity
CSA	Critical Source Area
DEM	Digital Elevation Model
dem.asc	Digital elevation map layer saved as ASCII format
DREAM	Disaster Risk and Exposure Assessment for Mitigation
GeoWEPP	Geospatial Interface for Water Erosion Prediction Project
GIS	Geographic Information System
ha	Hectare
Landcov.asc	Landcover map layer saved as ASCII format
Landcov.txt	Landcover map layer saved as text format
Landusedb.txt	Land use database saved as text format
masl	Meters above sea level
mm	Millimeters
m^3/y	Cubic meter per year
MS1	Monitoring Site 1
MS2	Monitoring Site 2
MS3	Monitoring Site 3
MSCL	Minimum Source Channel Length
NPK	Nitrogen, Phosphorus and Potassium
NSE	Nash-Sutcliffe Equation
OM	Organic Matter
PBIAS	Percent bias
R^2	Coefficient of determination
RMSE	Root mean square error
RSR	RMSE-observation Standard Deviation Ratio
SAR-DEM	Synthetic Aperture Radar-Digital Elevation Model
Soilsdb.txt	Soil database saved as text format
Soilmap.asc	Soil map layer saved as ASCII format
Soilmap.txt	Soil map layer saved as text format
SWAT	Soil and Water Assessment Tool
t/h/y	Tons per hectare per year
TOPAZ	Topographic Parameterization
UPD	University of the Philippines Diliman
USA	United State of America

USDA-ARS	United State Department of Agriculture-Agricultural-Research Service
WEPP	Water Erosion Prediction Project

REFERENCES

Alibuyog, N.R.; Ella, V.B.; Reyes, M.R.; Srinivasan, R.; Heatwole, C.; Dillaha, T., (2009). Predicting the effect of land use change on runoff and sediment yield in Manupali river subwatershed using the SWAT model. Int. Agric. Eng. J., 18(1-2):15-25 (**11 pages**).

Amaru, K.; Hotta, N., (2018). Application of GeoWEPP for evaluating sediment yield in a mountain area: Agatsuma Watershed, Japan. Int. J. Erosion Control Eng., 11(1): (**14 pages**).

Asio, V.B.; Jahn, R.; Perez, F.O.; Navarette, I.A.; Abit Jr, M., (2009). A review of soil degradation in the Philippines. Ann. Tropical Res. 31(2): 69-94 (**26 pages**).

Brooks, E.S.; Dobre, M.; Elliot, W.J.; Wu, J.Q.; Boll, J., (2016). Watershed-scale evaluation of the Water Erosion Prediction Project (WEPP) model in the Lake Tahoe basin. J. Hydrol., 533(3): 89-402 (**14 pages**).

Chen, S-K.; Liu, C-W.; Chen, Y-R., (2012). Assessing soil erosion in a terraced paddy field using experimental measurements and universal soil loss equation. Catena, 95: 131-141 (**11 pages**).

Dar, W., (2017). Sustainable soil and land management. The Manila Times.

Diwediga, B.; Le, Q.B.; Agodzo, S.K.; Tamene, L.D.; Wala, K., (2018). Modelling soil erosion response to sustainable landscape management scenarios in the Mo River Basin (Togo, West Africa). Sci. Total Environ., 625: 1309-1320 (**12 pages**).

Dong, Y.; Wu, Y.; Zhang, T.; Yang, W.; Liu, B., (2013). The sediment delivery ratio in a small catchment in the black soil region of Northeast China. Int. J. Sediment Res., 28: 111-117 (**7 pages**).

Ebrahimpour, M.; Balasundram, S.K.; Talib, J.; Anuar, A.R.; Memarian, H., (2011). Accuracy of GeoWEPP in estimating sediment load and runoff from a tropical watershed. Malaysian J. Soil Sci., 15: 25-33 (**9 pages**).

Flanagan, D.C.; Frankenberger, J.R.; Cochrane, T.A.; Renschler, C.S.; Elliot, W.J., (2013). Geospatial application of the water erosion prediction project (WEPP) model. Trans. ASABE, 56(2): 591-601 (**11 pages**).

Flanagan, D.C.; Gilley, J.E.; Franti, T.G., (2007). Water erosion prediction project (WEPP) development history, model capabilities, and future enhancements. Trans. ASABE, 50(5): 1603-1612 (**10 pages**).

Flanagan D.C.; Ascough J.C.; Nearing M.A.; Laflen J.M., (2001). The water erosion prediction project (WEPP) model. In: Harmon R.S., Doe W.W. (eds). Landscape Erosion and Evolution Modeling, 145-199 (**54 pages**).

Fukunaga, D.C.; Cecilio, R.A.; Zanetti, S.S., (2015). Application of the SWAT hydrologic model to a tropical watershed at Brazil. Elsevier. Catena, 125: 206-213 (**8 pages**).

Ghafari, H.; Gorji, M.; Arabkhedri, M.; Roshani, G.A., (2017). Identification and prioritization of critical erosion areas based on onsite and offsite effects. Catena, 156: 1-9 (**9 pages**).

Gonzàlez-Arqueros, M.L.; Mendoza, M.E.; Vazquez-Selem, L., (2016). Human impact of natural systems modeled through soil erosion in GeoWEPP: A comparison between pre-Hispanic periods and modern times in the Teotihuacan Valley (Central Mexico). Catena, 149: 505-513 (**9 pages**).

Han, F.; Ren, L.; Zhang, X.; Li, Z., (2016). The WEPP model application in a small watershed in the Loess plateau. PloS One, 11(3): 1-11 (**11 pages**).

Legates, D.R.; McCabe Jr, G.J., (1999). Evaluating the use of goodness-of-fit measures in hydrologic and hydroclimatic model validation, Water Resour. Res., 35(1): 233-241 (**9 pages**).

Lenka, N.K.; Mandal, D.; Sudhishri, S., (2014). Permissible soil loss limits for different physiographic regions of the West Bengal. Current Sci. Ass., 107(4): 665-670 (**6 pages**).

Liu, B.Y.; Nearing, M.A.; Baffaut, C.; Ascough, II J.C., (1997). The WEPP watershed model: III. Comparisons to measured data from small watersheds. Trans. ASAE, 40(4): 945-952 (**8 pages**).

Maalim, F.K.; Melesse, A.M.; Belmont, P.; Gran, K.B., (2013). Modeling the impact of land use changes on runoff and sediment yield in the Le Sueur watershed, Minnesota using GeoWEPP. Catena, 107: 35-45 (**11 pages**).Maalim, F.K.; Melesse, A.M., (2013). Modelling the impacts of subsurface drainage on surface and sediment yield in the Le Sueur Watershed, Minnesota, USA. Hydrol. Sci. J., 58(3): 570-586 (**17 pages**).

Marin, R.A.; Casas, J.V., (2017). Soil erosion assessment of the various vegetation cover in Mt. Musuan, Philippines. J. Biodivers. Environ. Sci., 11(1): 105-113 (**9 pages**).

Meghdadi, A.R., (2013). Identification of effective best management practices in sediment yield diminution using GeoWEPP: the Kasilian watershed case study. Environ. Monit. Assess., (**15 pages**).

Melaku, N.D.; Renschler, C.S.; Flagler, J.; Bayu, W.; Klik, A., (2018). Integrated impact assessment of soil and water conservation structures on runoff and sediment yield through measurements and modeling in the Northern Ethiopian highlands. Catena, 169: 140-150 (**11 pages**).

Minkowski, M., Renschler, C., (2008). GeoWEPP for ArcGIS 9.x Full Version Manual. University of Buffalo, Buffalo, New York (**129 pages**).

Mirakhorlo, M.S.; Rahimzadegan, M., (2019). Analyzing the land-use change effects on soil erosion and sediment in the North of Iran; a case study: Talar watershed. Geocarto Int., (**22 pages**).

Moriasi, D.N.; Arnold, J.G.; Van Liew, M.W.; Bingner, R.L.; Harmel, R.D.; Veith, T.L., (2007). Model Evaluation Guidelines for Systematic Quantification of Accuracy in Watershed Simulations. Trans. ASABE, 50(3): 885–900 (**16 pages**).

Narimani, R.; Erfanian, M.; Nazarnejad, H.; Mahmodzadeh, A., (2017). Evaluating the impact of management scenarios and land use changes on annual surface runoff and sediment yield using the GeoWEPP: a case study from the Lighvanchai watershed, Iran. Environ. Earth Sci., 76(353): 1-15 (**15 pages**).

Nguyen, K.A.; Chen, W., (2018). Estimating sediment delivery ratio by stream slope and relief ratio. In MATEC Web of Conferences, 192.

Olabisi, L.S., (2012). Uncovering the root causes of soil erosion in the Philippines. Soc. Nat. Resour., 25: 37-51 (**16 pages**).

Obalum, S.E.; Uteau-Puschmann, D.; Petch, S., (2019). Reduced tillage and compost effects on soil stability of a silt-loam Luvisol using different aggregate stability tests. Soil Tillage Res., 189: 217-228 (**12 pages**).

Panagos, P.; Katsoyiannis, A., (2019). Soil erosion modelling: The new challenges as the result of policy development in Europe.

Environ. Res., 172: 470-474 **(5 pages).**

Pandy, A.; Himanshu, S.K.; Mishra, S.K.; Singh, V.P., (2016). Physically based soil erosion and sediment yield models revisited. Catena, 147: 959-620 (**26 pages**).

Pieri, L.; Poggio, M.; Vignudelli, M.; Bittelli, M., (2014). Evaluation of the WEPP model and digital elevation grid size, for simulation of streamflow and sediment yield in a heterogeneous catchment. Earth Surf. Processes Landforms, 39(10): 1331-1344 (**14 pages**).

Pijl, A.; Reuter, L.E.H.; Querella, E.; Vogel, T.A.; Tarolli, P., (2020). GIS-based soil erosion modelling under various steep-slope vineyard practices. Catena, 193: 1-11 (**11 pages**).

Prasuhn, V.; Liniger, H.; Gisler, S.; Herweg, Karl.; Candinas, A.; Clement, J-P., (2013). A high-resolution soil erosion risk map of Switzerland as strategy policy support system. Land Use Policy, 32: 281-291 (**11pages**).

Puno, G. R., (2014). Runoff and Sediment Yield Modeling Using GeoWEPP in Mapawa Catchment. CMU J. Sci,, 18: 49–70 (**21 pages**).

Renschler, C.S.; Zhang, H., (2020). Long-term, process-based, continuous simulations for a cluster of six smaller, nested rangeland watersheds near Tombstone, AZ (USA): Establishing a baseline for event-based runoff and sediment yields. Sci. Total Environ., 717: 1-17 (**17 pages**).

Ricci, G.F.; Jeong, J.; De Girolamo, A.M.; Gentile, F., (2020). Effectiveness and feasibility of different management practices to reduce soil erosion in an agricultural watershed. Land Use Policy, 90: 1-15 (**15 pages**).

Shen, Z.Y.; Gong, Y.W.; Li, Y.H.; Hong, Q.; Xu, L.; Liu, R.M., (2009). A comparison of WEPP and SWAT for modeling soil erosion of the Zhangjiachong watershed in the three gorges reservoir area. Agric. Water Manage., 96: 1435-1442 (**8 pages**).

Tacio, H., (2011). Filipino hill farmers are learning how to heal their eroded land. People & Planet.

Van Leeuwen, C.C.E.; Cammeraat, E.L.H.; de Vente, J.; Boix-Fayos, C., (2019). The evolution of soil conservation policies targeting land abandonment and soil erosion in Spain: A review. Land Use Policy, 83: 174-186 (**13 pages**).

Xiong, M.; Sun, R.; Chen, L., (2019). A global comparison of soil erosion associated with land use and climate type. Geoderma, 343: 31-39 (**9 pages**).

Yüksel, A.; Akay, A.E.; Gundogan, R.; Reis, M.; Cetiner, M., (2008). Application of GeoWEPP for determining sediment yield and runoff in the Orcan Creek Watershed in Kahramanmaras, Turkey. Sensors, 8: 1222-1236 (**15 pages**).

Zeleke, G., (1999). Application and adaptation of WEPP to the tradition farming systems of the Ethiopian Highlands (with special emphasis to the new breakpoint climate data generator, BPCDG).

Zhang, Z.; Sheng, L.; Yang, J.; Chen, X-A.; Kong, L.; Wagan, B., (2015). Effects of land use and slope gradient on soil erosion in a red hill watershed of southern China. Sustainability, 7: 14309-14325 (**17 pages**).

Zhang, Y.; Zhao, Y.; Liu, B.; Wang, Z.; Zhang, S., (2019). Rill and gully erosion on unpaved roads under heavy rainfall in agricultural watersheds on China's Loess Plateau., Agric. Ecosyst. Environ., 284: 1-10 (**10 pages**).

Zheng, F.; Zhang, X-C.J.; Wang, J.; Flanagan, D.C., (2020). Assessing applicability of the WEPP hillslope model to steep landscape in the northern Loess Plateau of China. Soil Tillage Res., 197: 1-12 (**12 pages**).

AUTHOR (S) BIOSKETCHES

Puno, G.R., Ph.D., Professor, Department of Forest Resources Management, College of Forestry and Environmental Science, Central Mindanao University, Musuan, Maramag, Philippines. Email: *grpuno@cmu.edu.ph*

Marin, R.A., Ph.D., Professor, Department of Forest Resources Management, College of Forestry and Environmental Science, Central Mindanao University, Musuan, Maramag, Philippines. Email: *rm71aquarius@gmail.com*

Puno, R.C.C., M.Sc., Instructor, Department of Environmental Science, College of Forestry and Environmental Science, Central Mindanao University, Musuan, Maramag, Philippines. Email: *rccpuno@cmu.edu.ph*

Toledo-Bruno, A.G., Ph.D., Associate Professor, Department of Environmental Science, College of Forestry and Environmental Science, Central Mindanao University, Musuan, Maramag, Philippines. Email: *agtbruno@cmu.edu.ph*

HOW TO CITE THIS ARTICLE

Puno, G.R.; Marin, R.A.; Puno, R.C.C.; Toledo-Bruno, A.G., (2021). Geographic information system and process-based modeling of soil erosion and sediment yield in agricultural watershed. Global J. Environ. Sci. Manage., 7(1): 1-14.

DOI: 10.22034/gjesm.2021.01.01

url: https://www.gjesm.net/article_40280.html

Global J. Environ. Sci. Manage. 7(1): 15-32, Winter 2021

Global Journal of Environmental Science and Management
(GJESM)

Homepage: https://www.gjesm.net/

ORIGINAL RESEARCH PAPER

The effect of short-term of fine particles on daily respiratory emergency in cities contaminated with wood smoke

R. Torres[1], N. Baker[2,3], G. Bernal[3,4], F. Peres[5], A.K. Maldonado[6], D.D. Caceres[6,*]

[1]Programa de Bioestadística, Escuela de Salud Pública, Facultad de Medicina, Universidad de Chile, Santiago, CP 838046, Chile

[2]Emory University Rollins School of Public Health, Atlanta, GA 30322, USA

[3]International Exchange Program for Minority Student,Icahn School of Medicine at Mount Sinai in New York City, NY 10029, USA

[4]Departamento de Salud Ambiental, Escuela Graduada de Salud Pública, Universidad de Puerto Rico recinto de Ciencias Médicas, San Juan, CP 00921, Puerto Rico

[5]Centro de Estudio de Salud del Trabajador y Ecología Humana, Escuela Nacional de Salud Pública, Fio Cruz. Rio de Janeiro, CEP 21040-900, Brasil

[6]Programa de Salud Ambiental, Escuela de Salud Pública, Facultad de Medicina, Universidad de Chile, Santiago, CP 838046, Chile

ARTICLE INFO

Article History:
Received 25 February 2020
Revised 28 May 2020
Accepted 12 June 2020

Keywords:
Air pollution
Firewood combustion
Time-series study
Particulate matter-2.5 (PM2.5)
Respiratory emergency visits (REVs)

ABSTRACT

BACKGROUND AND OBJECTIVES: The goal of this study is to evaluate in a time-series study the short-term effects of particulate matter-2.5 exposure on respiratory emergency visits in six central-southern Chilean cities highly contaminated by wood smoke.

METHODS: Association was assessed using both distributed lag linear and non-linear Poisson models constrained to a 7-day lag period, adjusting for temporal trends and meteorological variables and stratifying seasonally into cold and warm periods.

FINDINGS: The results showed that the daily average concentrations of particulate matter-2.5 in the cold period were 3 to 6 times those recorded in the warm period, exceeding the daily norm of 50 μg/m^3 the 93.3% of the time *versus* 6.7%, respectively. The average daily number of respiratory emergency visits were between 30% and 64% higher in the cold period compared to the warm one. From linear models, cumulative relative risk ratios over 0-7 day lags per 10 μg/m^3 of fine particle increase were between 1.004 (95% confidence Interval: 0.998 - 1.010) and 1.061 (95% confidence Interval: 1.049 - 1.074); these annual effects are attributable to the cold period impact where the cumulative risk ratios were between 1.008 (95% confidence Interval: 1.004 - 1.012) and 1.036 (95% confidence Interval: 1.026- 1.047), since significant effects of fine particles on the studied risk were not found for the warm period.

CONCLUSION: With non-linear models we observed strong increasing associations with the level of particles for the overall period. High levels of fine particles from firewood are associated with respiratory effects observable several days after exposure. Health effects found in this study suggest that current policies tending to mitigate woodsmoke-related emissions should continue and reinforce.

DOI: 10.22034/gjesm.2021.01.02

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
55	6	3

*Corresponding Author:
Email: *dcaceres@med.uchile.cl*
Phone: +562 229786554
Fax: +562 27355582

Note: Discussion period for this manuscript open until April 1, 2021 on GJESM website at the “Show Article.

INTRODUCTION

Ambient air pollution is a global health crisis. More than 9 out of 10 (91%) of the world's population is exposed to fine particulate matter at levels that exceed the health-based World Health Organization (WHO) recommended limits (Osseiran and Lindmeier, 2018). Over 100 million people in Latin America and the Caribbean (LAC) are also exposed to or live in areas with high levels of air pollution (Cifuentes *et al.*, 2005; Green and Sánchez, 2013). The population in cities, particularly large cities, may generate intensive human social and economic activities that result in urban environmental pollution. The urbanization process is increasing in LAC, with increased motorization of the urban population and increased household combustion of solid fuels (Orellano *et al.*, 2018). Urban air pollution is highly attributable to fossil fuel consumption; however, energy consumption efficiency and per capita emission differ among continents and countries (Lamsal *et al.*, 2013; Mayer., 1999). The evidence shows that people with scarce resources are more exposed to environmental problems, generating problems of equity. The pollution generated in the home is also worrisome, according to the WHO, since the smoke coming from solid fuels burned in the home is one of the main risks for people living in developing countries. The use of these fuels contributes to high rates of acute and chronic respiratory diseases (Cerda and Garcia, 2010). These emissions also generate environmental impacts to surrounding populations, both for users and non-users of solid fuels, who are even willing to pay for environmental control measures or firewood certification. The combustion of biomass, especially firewood, as a source of energy for heating and cooking has occurred since records have been kept in the history of man; the book "Fire in the World" by Stephen Pyne (2014) relates "wherever human beings have lived in the vicinity of forests, the rate of combustion of firewood has increased". This occurs through forest fires, use of fire in agricultural-forest lands and the use of firewood as fuel. According to global estimates, approximately 40% of households use firewood and other biofuels for cooking and/or heating (GEA, 2012). Several studies carried out in developed countries indicate that wood smoke is the main source of exposure to particulate matter (PM) during the colder months, from the use of residential stoves (Fairley. 1999; Maykut *et al.*, 2003; Naeher *et al.*, 2007). According to FAO estimates (2010), in developing countries between 50 to 90% of the fuel used by the population for cooking or heating is firewood. In decreasing order worldwide, the continents that use firewood the most are Asia (42%), Africa (32%), America (18%) especially Latin America (FAO, 2010). It is known that, depending on the quality of the combustion, the type and characteristics of the wood used, fires can emit a series of compounds in the smoke including metals, gases (carbon monoxide, nitrogen oxides), polycyclic aromatic hydrocarbons (many carcinogenic), volatile organic compounds (aldehydes, alcohols, phenols), chlorinated compounds, free radicals, particulate matter, sulfates, endotoxins and organic constituents, many of which are very harmful to health (Naeher *et al.*, 2007). The highest percentage of particles present (> 90%) in wood smoke are less than 2.5 microns particulate matter-2.5 ($PM_{2.5}$), considered together with the ultrafine particles as the most dangerous, as they penetrate deeply into the respiratory system where they can remain for months, causing damage and chemical structural changes. These are particularly dangerous because a series of highly toxic and carcinogenic compounds can be adsorbed, which would be involved in the process of cell damage and the subsequent inflammatory response in pulmonary and cardiovascular diseases, which occur in response to the exposure to air pollutants (Bergamaschi *et al.*, 2001; Diociaiuti *et al.*, 2001; Ghio and Cohen, 2005; Hong *et al.*, 2010; Roemer *et al.*, 2000; Ward and Ayres, 2004). The air pollution by particulate matter in cities of central-southern Chile associated largely with residential use of firewood is recognized as one of the main environmental problems (Molina *et al.* 2017; MMA. 2014). Several cities in the central and southern zones of Chile have been declared saturated due to particulate matter pollution ($PM_{10/2.5}$) in the air, since national standards are repeatedly exceeded during the year (Molina *et al.*, 2017).The main source of air pollution in these cities is the use of firewood as heating and/or cooking fuel. The objective of this study was to estimate the short-term lag structure effect of exposure to $PM_{2.5}$ on the daily number of respiratory emergency visits (REVs) in six different cities in central-southern Chile. This study has been carried out in the cities of Rancagua, Talca, Temuco, Valdivia, Osorno and Coyhaique during 2014-2017.

MATERIAL AND METHODS

Study areas

The study areas comprised six urban centers located between the central and southern zones of Chile (Fig. 1) affected by air pollution mainly from burning wood for heating homes, corresponding to the cities of Rancagua, Talca, Osorno, Valdivia, Coyhaique and Temuco. Table 1 presents the geographic and climatic characteristics of these cities and percentage of use of firewood as source of primary energy for heating and cooking (Molina *et al*., 2017).

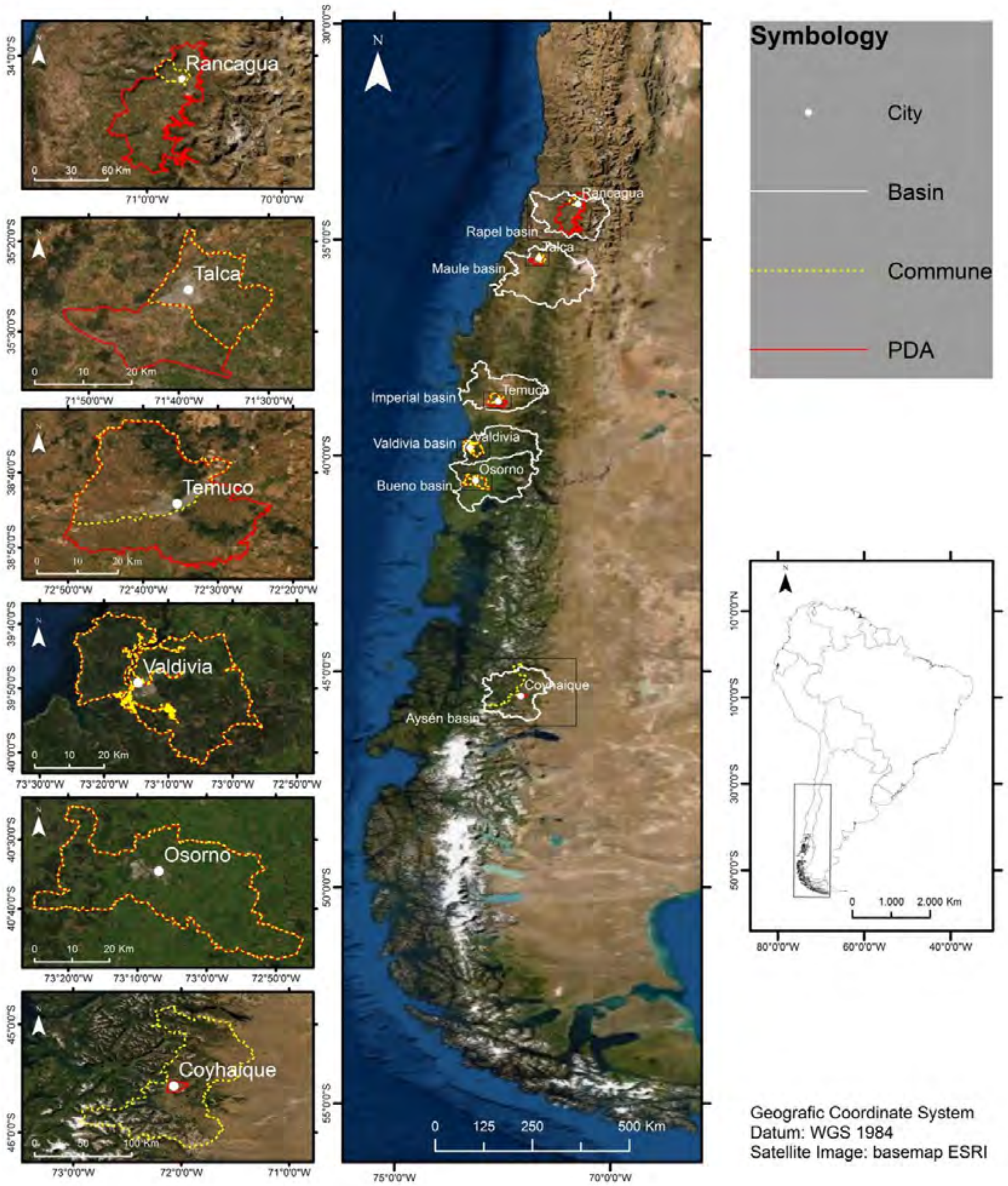


Fig. 1: Geographic location of the study areas in cities of Rancagua, Talca, Osorno, Valdivia, Coyhaique and Temuco of Chile

Respiratory emergency visit data

The daily numbers of REVs recorded between 2014 and 2017 were obtained from statistical records of the Ministry of Health of Chile (DEIS, 2019). The specific causes according to the International Classification of Diseases, ICD-10, corresponded to upper respiratory disease (J00-J06), Influenza (J09-J11), Pneumonia (J12-J18), Bronchitis/Acute bronchiolitis (J20-J21), Bronchial Obstructive Crisis (J40-J46) and other respiratory causes (J22, J30-J39, J47, J60-J98).

Air pollution and meteorological data

Air quality ($PM_{2.5}$) and meteorological (temperature and relative humidity) data were obtained with hourly frequency from automated monitoring stations located inside the study areas from the Chilean Ministry of Environment via the web site (Sistema Nacional de Calidad de Aire (SINCA, 2019). The daily average value of a variable was generated only if at least 16 hourly measurements were available. Otherwise, for the cities that have two stations (Rancagua, Temuco, and Coyhaique) data from the other station were used.

Statistical analyses

Descriptive position and dispersion statistics were used to summarize the data. Pearson correlation was used to explore the relationship between $PM_{2.5}$ concentrations and meteorological factors. Two time series approaches were used to estimate the effects of $PM_{2.5}$ on REVs, evaluating exposure-response relationships in both overall and seasonal terms, considering a "cold season" for the autumn-winter period (March 21 - September 21) and a "warm season" for the rest of the year. For each city and season both traditional distributed lag linear models (DLMs) and distributed lag non-linear models (DLNMs) were used to evaluate exposure-response association (Almon, 1965; Armstrong, 2006; Gasparrini *et al.*, 2010; Peng and Dominici, 2008; Wyzga, 1978), specifying a lag lapse of 7 days in both types of analysis. In order to model the daily counts of the REVs attentions as a function of the predictive variables considering an eventual over-dispersion of data, semi-parametric generalized additive model (GAM) Poisson regressions were used. To remove seasonal and non-seasonal cyclic effects on the health variable, functions of cubic natural splines on the calendar time variable were added to all models, in addition to a term for day of the week (Peng and Dominici, 2008). The models were initially specified using a smoother for the calendar time of 7 degrees of freedom in order to remove long-term trends and seasonal effects (Dominici *et al.*, 2004; Goldberg *et al.*, 2011) and then explore the sensitivity of the results obtained using 6, 8 and 9 degrees of

Table 1: Geographic characteristics of the cities studied

Locations	Population	Area (Km^2)	Altitude (meters)	Basin	Climate (Geiger, 1954)	Predominant wind direction	% use firewood (Molina *et al.*, 2017)
Rancagua	241,774	260	572	Middle Rapel River basin	Csb: Mediterranean warm/cool summer climate	South	57.8
Talca	220,357	232	102	Middle Maule River basin	Csb: Mediterranean warm/cool summer climate	South	64.1
Temuco	282,415	464	122	Middle basin of the Imperial River	Csb: Mediterranean warm/cool summer climate	Southwest	91.2
Valdivia	166,080	1016	14	Lower basin of the Valdivia River	Cfb (s) (i): Temperate rain with mild summer dryness and coastal influence	North - Northeast	94.6
Osorno	161,460	951	39	Middle basin of the Bueno river	Cfb (s): Temperate rain with mild summer dryness	North-northwest (NNW) and south-southeast (SSE)	96,3
Coyhaique	57,818	7,290.2	302	Middle basin of the Aysén River	Cfb: Temperate rain	WNW (West-northwest)	99.3

freedom. The potentially non-linear confounding effects of the meteorological variables were controlled in all cases using functions analogous to those used with calendar time for temperature and humidity but employing 6 and 3 degrees of freedom, respectively (Dominici *et al.,* 2000; Peng and Dominici, 2008), and then analyze the sensitivity of the effects for 5, 7 and 8 degrees of freedom in the case of temperature and 2, 4 and 5 degrees of freedom for humidity. Specifically, let Y_t^c be the total number of REVs on day *t* in the city *c*, the DLMs and DLNMs city-specific models have the general form shown in Eqs.1 and 2.

$$Y_t^c \sim Poisson\left(\mu_t^c\right) \quad (1)$$

$$\mathrm{Var}\left(Y_t^c\right) = \phi^c \mu_t^c \quad (2)$$

Where μ_t^c and ϕ^c are the expectation and overdispersion of Y_t^c, respectively. The DLMs and the DLNMs specific models are shown in Eqs. 3 and 4, respectively

$$\log\left(\mu_t^c\right) = \alpha + \sum_{l=0}^{7} \beta_l^c PM_{t-l}^c + s^c\left(t, 7x4\right) + s^c\left(temp_t, 6\right) + s^c\left(hum_t, 3\right) + \eta^c I_{dow} \quad (3)$$

$$\log\left(\mu_t^c\right) = \alpha + \mathrm{B}^{cT} PM_{t,l}^c + s^c\left(t, 7x4\right) + s^c\left(temp_t, 6\right) + s^c\left(hum_t, 3\right) + \eta^c I_{dow} \quad (4)$$

Eq.3 and Eq.4 approaches have a common structure sharing the use of natural cubic splines s^c (.) of the calendar time (*t*), temperature ($temp_t$) and humidity (hum_t) levels on day *t* and the term I_{dow} as an indicator function of the day of week, being η^c a vector of coefficients. In Eq.1 the terms $\beta_l^c\left(l = 0,1,2,\ldots,7\right)$ represent the lag *l* distributed log-relative risks of REVs for the city *c*, PM_{t-l}^c the lag *l* $PM_{2.5}$ level on day *t*. In Eq.4 the object $PM_{t,l}^c$ represent a city-specific matrix obtained by the application of DLNM methodology to the observed exposures of fine particles in order to estimate the unknown parameters represented by B^c defining the bi-dimensional shape of the relationship between the lagged and current levels of exposure and health response.

The effects of other possible confounders such as epidemics of influenza, other infectious disease epidemics, ozone pollution or other air contaminants were not controlled in this study, since this information is not available for all the areas studied and the data available is incomplete. Nevertheless, the smooth function of time is included in the models precisely to remove most of these unmeasured confounding effects (Goldberg *et al.,* 2011; Peng and Dominici, 2008). The cumulative effect was determined up to a lag of 7 days for the linear models. DLNM-based analyses represent the relationship between the exposure and its effects in a non-linear way, accounting simultaneously for the lagged effects, generalizing the linear models of distributed lags and thus increasing significantly the flexibility in the description of the exposure-response relationship. This approach forces us to adopt a two-dimensional perspective to represent non-linear associations that may change both along the level of contamination and along the temporal lags. The DLNM methodology is based on the concept of a "cross-basis" function, consisting of a two-dimensional space of available functions which allow the already mentioned specification of the potentially non-linear exposure response. Once the functions of the cross-basis are chosen, they are combined for the two dimensions chosen ($PM_{2.5}$ and its delayed effects, in our case) (Goldberg *et al.,* 2011). For the analysis the *dlnm* package written by A. Gasparrini in the R Project was used for statistical computing (version 3.5.3). Cubic natural splines were used both for the exposure and the temporal lags (Ma *et al.,* 2019), the degrees of freedom were determined for each adjustment based on the modified Akaike information criterion for models with over-dispersed response, adjusted through quasi-likelihood (Gasparrini *et al.,* 2010). We used GraphPad Prism software to make some graphs.

RESULTS AND DISCUSSION

Descriptive analysis

Table 2 shows descriptive statistics by city for REVs, $PM_{2.5}$, temperature and humidity for the entire period, and stratified by cold and warm seasons. As can be seen, the daily averages of REVs were between 30% and 64% higher in the cold period compared to the warm one. The ranges of the daily count of REVs oscillated from 19 (Coyhaique) to 688 (Talca) in the cold season, versus 0 (Coyhaique) to 533 (Talca) in the warm period. Greater variability -measured by the coefficient of variation (CV)- of the daily REVs was also observed in the warm period compared to the cold for all cities. Daily average concentrations of $PM_{2.5}$ in the cold season-warm season ratios were between 3.04 and 6.0; the four southernmost cities

Table 2: Descriptive statistics for respiratory emergency visits (REVs), daily concentrations of $PM_{2.5}$, temperature and humidity

	Overall period							Cold season (CS)							Warm season (WS)							
Variables	Mean	Min	P25	Median	P75	Max	CV(%)	Mean	Min	P25	Median	P75	Max	CV(%)	Mean	Min	P25	Median	P75	Max	CV(%)	CS/WS
REVs																						
	166,2	41	112	158	205	522	42.28	206.0	72	158.	195	236.2	522	32.31	125.8	41	90	114	155	325	37.41	1.64
Talca	276.5	72	203	268	336	688	37.19	337.7	136	274	322	385	688	25.90	214.4	72	157	205	214.	533	35.82	1.57
Temuco	135.9	31	103	135	169	329	37.32	163.1	73	133	157.5	187	329	26.29	108.2	31	70	108	138	241	39.24	1.51
Valdivia	149.8	30	101	145	189	414	41.50	179.7	56	139	172.5	212	414	31.80	119.4	30	77	110	154	286	43.08	1.50
Osorno	201.8	44	145	200	253	472	39.66	239.4	111	186	228	283	472	28.65	163.5	44	98	156	221	366	44.24	1.46
	65.49	0	43	62	83	228	49.19	74.01	19	54	68	87	221	40.02	56.90	0	30	53	76	228	57.08	1.30
$PM_{2.5}$ (µg/m^3)																						
	29.93	1.37	12.3	19.54	40.8	191.3	84.89	45.02	1.90	24.4	39.21	59.54	191.	61.26	14.66	1.37	9.69	13.00	17.4	70.15	55.46	3.07
Talca	19.09	1.00	6.00	11.60	25.7	161.2	104.1	28.64	1.42	13.2	23.63	37.83	144.	73.57	9.41	1.00	4.76	6.52	10.1	161.2	134.9	3.04
Temuco	53.21	5.00	20.9	36.00	70.3	300.3	85.46	52.02	5.00	22.6	41.52	69.52	230.	77.05	8.67	1.00	4.15	6.18	9.63	60.30	89.85	6.00
Valdivia	35.24	1.16	8.51	21.92	51.1	203.1	98.23	56.69	4.29	28.0	49.00	78.77	203.	63.70	12.97	1.64	6.06	8.71	16.0	63.67	83.19	4.37
Osorno	36.64	1.28	8.33	19.28	45.8	359	126	62.30	5.28	23.5	43.00	79.94	359.	89.28	11.93	1.28	5.23	8.23	13.8	103.7	96.98	5.22
	58.38	1.73	15.0	33.29	77.1	510.5	112.2	95.53	8.31	43.2	73.00	123.8	510.	77.10	20.66	1.73	10.1	15.11	24.0	166.6	88.04	4.62
Temperature (°C) *																						
	15.44	3.46	10.7	15.20	20.3	27.07	1.94	11.26	3.46	8.63	10.92	13.54	21.7	1.24	19.71	8.25	17.2	20.31	22.4	27.08	1.30	0,97
Talca	14.18	1.75	9.92	13.70	18.9	27.37	1.92	10.08	1.75	7.56	10.04	12.44	20.3	1.28	18.37	9.13	15.7	18.98	21.0	27.37	1.27	0,97
Temuco	11.60	3.00	9.70	11.53	13.6	22.00	1.00	9.85	3.00	8.32	9.91	11.30	16.6	0.79	13.08	7.20	11.7	13.17	14.4	18.31	0.68	0,99
Valdivia	12.04	0.45	9.17	11.85	15.1	24.75	1.45	9.46	0.45	7.26	9.53	11.52	19.8	1.12	14.70	5.33	12.4	14.83	16.9	24.75	1.13	0,98
Osorno	11.33	-1.15	8.33	11.26	14.2	23.04	1.42	8.76	-1.15	6.66	8.71	10.64	19.0	1.08	13.96	5.02	11.7	13.93	16.0	23.05	1.10	0,98
	8.81	-6.00	5.24	8.53	12.1	23.26	1.72	5.81	-6.00	3.40	5.65	7.92	18.6	1.31	11.93	2.17	9.18	11.70	14.4	23.26	1.35	0,98
Humidity (%)																						
	62.86	29.2	51.9	63.00	74.6	93.25	22.78	71.95	39.7	65.0	73.44	79.85	93.2	15.07	53.60	29.2	45.1	52.96	60.4	85.42	20.84	1.34
Talca	74.16	39.4	60.6	75,08	88.2	100.0	21.16	85.77	41.7	79.8	87.75	93.00	100.	11.40	62.32	39.5	54.5	61.04	68.2	98.33	17.78	1.38
Temuco	81.50	26.6	74.2	81.66	89.5	105.0	13.33	87.74	26.6	82.9	88.33	94.17	105.	10.53	76.33	40.3	71.3	76.04	81.1	98.67	10.31	1.15
Valdivia	79.44	44.0	70.7	81.00	89.0	104.1	15.23	85.80	50.2	81.8	86.27	90.37	104.	9.37	69.63	50.6	62.5	69.42	75.9	91.69	12.25	1.23
Osorno	72.04	45.1	64.3	72.65	79.9	93.55	13.68	78.67	45.3	75.0	79.31	83.53	93.5	8.85	65.26	45.1	59.9	64.96	70.1	88.25	11.48	1.21
Oyhaique	66.98	31.48	58.78	67.02	75.50	95.75	18.00	73.90	38.70	67.18	74.38	81.08	95.75	13.19	59.63	31.49	53.00	60.12	66.50	86.00	16.62	1.24

*CV an CS/WS ratio were calculated using the Kelvin scale

(Temuco, Valdivia, Osorno and Coyhaique) were quite remarkable in this aspect. The average daily concentrations per period varied from 1.42 to 510.57 µg/m³ in the cold season, and 1.0 to 166.64 µg/m³ in the warm season. The average concentrations in decreasing order were Coyhaique > Osorno > Valdivia > Temuco > Rancagua > Talca, and Coyhaique > Rancagua > Valdivia > Osorno > Talca > Temuco, in the cold and warm period, respectively. The daily mean concentration of $PM_{2.5}$ showed higher variability (CV) during the warm season (55.46% to 134.96%) compared to the cold season (61.26% to 89.28%) in all cities, with Osorno > Coyhaique > Temuco > Talca > Valdivia > Rancagua and Talca > Osorno > Temuco > Coyhaique > Valdivia > Rancagua, for cold and warm seasons, respectively. The cold season-warm season ratio for mean temperature (°K) varied from 0.97 (Rancagua, Talca) to 0.99 (Temuco). During the cold period, the average daily temperatures were between 5.81 °C and 11.26 °C, with a range of -6.0 °C (Coyhaique) to 21.7 °C (Rancagua). The order of the urban centers latitudinally is Coyhaique < Osorno < Valdivia < Temuco < Talca < Rancagua. The temperature variability in the cold season measured by CV was Temuco < Rancagua < Valdivia < Osorno < Talca < Coyhaique. During the warm period, daily average temperatures varied from 11.93 °C to 19.71 °C, with Coyhaique > Temuco > Osorno > Valdivia > Talca > Rancagua. The CV of temperature for this season was between 0.68% (Temuco) and 1.35% (Coyhaique). Finally, average relative humidity was in all cities higher in the cold period compared to the warm one. Valdivia, Osorno and Coyhaique recorded similar values. The average daily values in the cold period varied between 71.95% and 87.74%, in the following order Temuco > Talca > Valdivia > Osorno > Coyhaique > Rancagua. During the warm period the average daily values varied between 53.60% and 76.33%; the order was Temuco > Valdivia > Osorno > Talca > Coyhaique > Rancagua.

Fig. 2 shows the annual distribution patterns for $PM_{2.5}$, REVs and temperature, which are for all cases markedly seasonal but more homogeneous for the cities of southern Chile, that is, Temuco, Valdivia, Osorno and Coyhaique, compared to Rancagua and Talca, where a greater dispersion is observed. The daily norm of 50 µg/m³ $PM_{2.5}$ was exceeded in all cities especially during the cold periods; extreme values were recorded in Osorno and Coyhaique--the latter had days with daily averages greater than 400 µg/m³. During the whole study period the daily norm was exceeded in the studied cities a total of 2128 days,

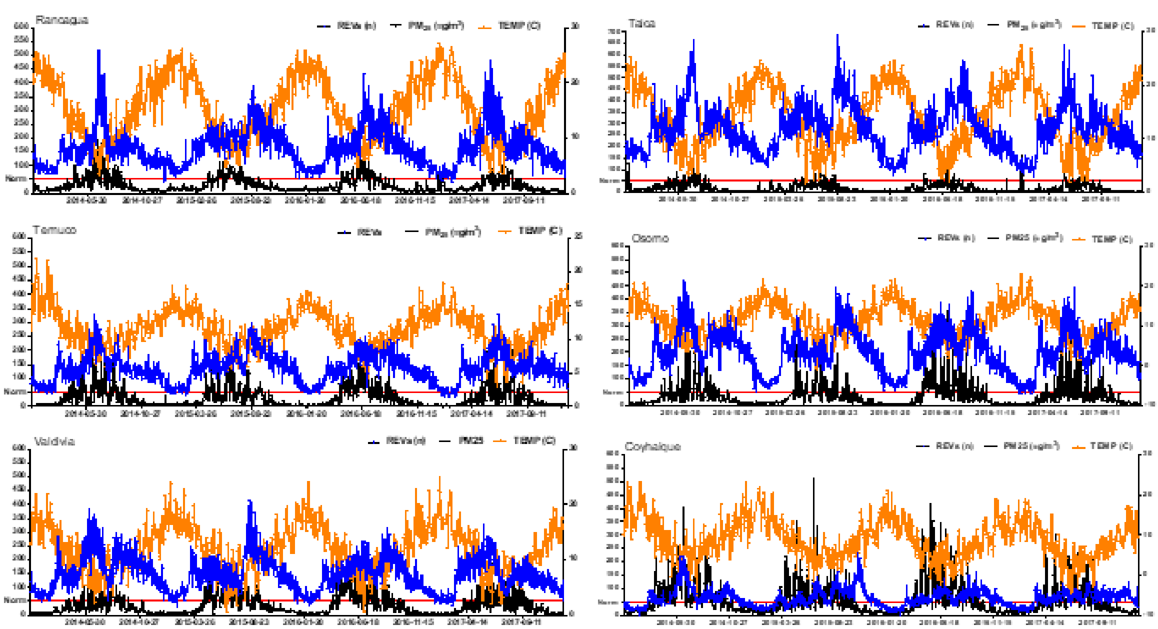


Fig. 2: Time series of $PM_{2.5}$ (Black line), REVs (Blue line) and temperature (Orange line) by cities. Red line shows the daily norm of $PM_{2.5}$ (50 µg/m³)

93.3% in the cold months and 6.7% in the warm ones, 25.7% (585 days) in Coyhaique, 24.8% (528 days) in Temuco, 17.0% (362 days) in Valdivia, 14.7% (313 days) in Osorno, 12.7% (270 days) in Rancagua and 5.1% (108 days) Talca. The daily average consultations by REVs in all cities clearly present a seasonal distribution pattern similar to the behavior of $PM_{2.5}$. The distribution of the daily average temperatures presents an inverse pattern compared to the average daily concentrations of $PM_{2.5}$ and REVs, respectively. Table 3 shows the Pearson correlation coefficients between the REVs, the meteorological variables and the concentrations of $PM_{2.5}$ for each city. In the warm season the correlations between REVs and $PM_{2.5}$ and humidity were weak to moderate (-0.08 to 0.56) and (0.01 to 0.45), respectively. REVs and temperature show a clear inverse relationship with moderate correlation values (-0.46 to -0.66). The correlation between $PM_{2.5}$ and temperature was direct but almost null for Rancagua and weak for Talca. However, this relationship was moderate and inverse for the cities of Temuco, Valdivia, Osorno and Coyhaique. $PM_{2.5}$ and humidity, generally showed weak to moderate positive correlations (-0.15 to 0.42). Temperature and humidity have a clear inverse relationship with moderate to high correlations (-0.39 to -0.72).

The correlations between REVs with $PM_{2.5}$ and humidity in the cold period were positive but weak (0.07 to 0.31) and almost null (0.04 to 0.07), respectively. The correlations of REVs with temperature were inverse and weak (-0.15 to -0.31). The temperature correlations with $PM_{2.5}$ (-0.36 to -0.60) and humidity (-0.31 to -0.58) in all cities were inverse and moderate. The correlations were positive and weak (0.04 to 0.07) for humidity versus respiratory events.

Distributed lag linear model

Based on the DLM approach, for each city is shown the current day and delayed effects of the $PM_{2.5}$ on the risk ratios (RR) of REVs for an increase of 10 μg/m³ of pollutant, for the whole year and season-stratified (the values are given in supplementary data) are presented in Fig. 3. There was a marked and positive acute effect in the entire annual cycle for each city (overall) and each lag studied in the cities of Rancagua, Talca and Valdivia; although in Osorno, Temuco and Coyhaique this positive relationship is maintained, it is less marked and the 95% confidence intervals of the cumulative lags of 0-7 day effect include the null value. Although the lag structure in the overall period has effects below 1 in some cities, most of the fitted effects are greater; cumulative effects showed a fluctuating RR between 1.004 (95% confidence Interval as CI: 0.998 -1.010) and 1.061 (95% CI: 1.049 -1.074), with Osorno being the lowest and Rancagua the highest, respectively. Stratifying the period, it can be seen that during the warm months the current and lagged effects of $PM_{2.5}$ on REVs were quite variable around the null value, with cumulative effects RR between 0.954 (95% CI: 0.910 -1.001) and 1.034 (95% CI: 1.002-1.067) with Temuco being the lowest and Osorno the highest, respectively. In contrast, for the cold period most of

Table 3: Correlations of the meteorological variables, daily average of $PM_{2.5}$ and respiratory emergency visits for six cities in central-southern Chile. 2014-2017.

Climate periods	REVs-$PM_{2.5}$	REVs- ºT	REVs- RH%	$PM_{2.5}$-ºT	$PM_{2.5}$-RH%	ºT- RH%
			Warm period			
Rancagua	0.12 *	-0.57 ‡	0.32 ‡	0.01	0.09 *	-0.72 ‡
Talca	-0.08 †	-0.65 ‡	0.45 ‡	0.19 ‡	-0.15 †	-0.68 ‡
Temuco	0.38 ‡	-0.55 ‡	0.28 ‡	-0.53 ‡	0.18 ‡	-0.44 ‡
Valdivia	0.56 ‡	-0.59 ‡	0.33 ‡	-0.69 ‡	0.42 ‡	-0.51 ‡
Osorno	0.49 ‡	-0.66 ‡	0.31 ‡	-0.59 ‡	0.27 ‡	-0.46 ‡
Coyhaique	0.38 ‡	-0.46 ‡	0.01	-0.46 ‡	0.12 *	-0.39 ‡
			Cold period			
Rancagua	0.31 ‡	-0.31 ‡	0.07 †	-0.48 ‡	0.16 ‡	-0.56 ‡
Talca	0.21 ‡	-0.24 ‡	0.06	-0.50 ‡	0.29 ‡	-0.58 ‡
Temuco	0.17 ‡	-0.27 ‡	0.07	-0.60 ‡	0.27 ‡	-0.41 ‡
Valdivia	0.07	-0.15 ‡	0.06	-0.50 ‡	0.19 ‡	-0.31 ‡
Osorno	0.16 ‡	-0.24 ‡	0.04	-0.36 ‡	0.19 ‡	-0.35 ‡
Coyhaique	0.10 †	-0.17 ‡	0.06	-0.41 ‡	0.39 ‡	-0.37 ‡

REVs: Respiratory Emergency Visits; RH: Relative Humidity; ºT: Temperature.; * : P<0.01; †: p<0.05; ‡: p <0.0001

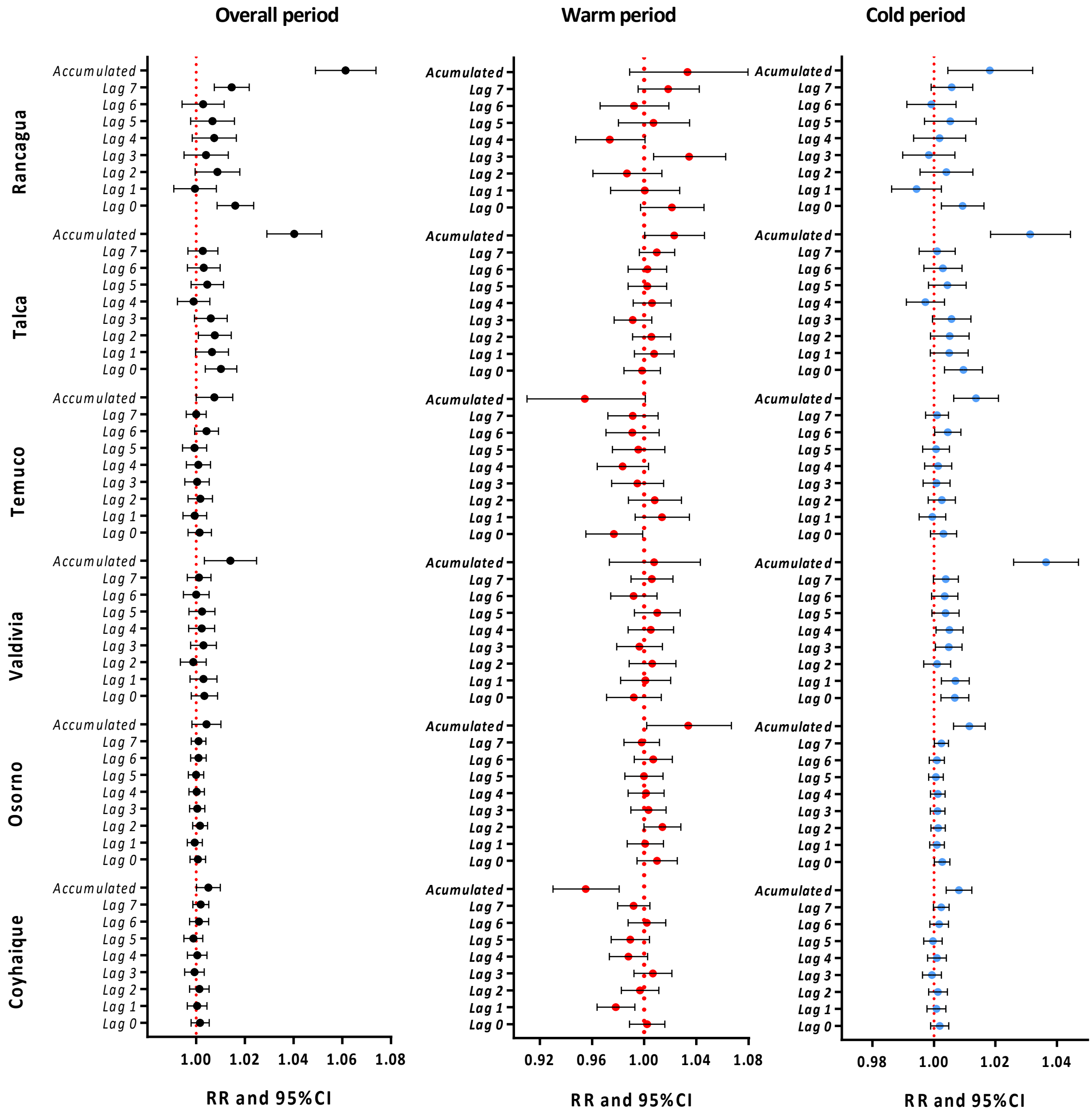


Fig. 3: Increase in the relative risk (RR) of REVs for each increase of 10 μg/m³ of $PM_{2.5}$, for the overall period and stratified by cold and warm periods in each city

the lag effect structure was positive and significant, especially for Rancagua, Talca, Valdivia, and Osorno. It was not well defined for Temuco and Coyhaique, with ranges fluctuating RR between 1.008 (95% CI: 1.004 -1.012) and 1.036 (95% CI: 1.026 -1.047) with Coyhaique being the lowest and Valdivia the highest, respectively.

Distributed lag non-linear model

The following results are based on the DLNM approach, depicting associations which may vary non-linearly with the magnitude of the pollutant in their delayed effects. Figs. 4 and 5 correspond to 3-D and contour plots, respectively, offering complementary visual summaries of the bi-dimensional association

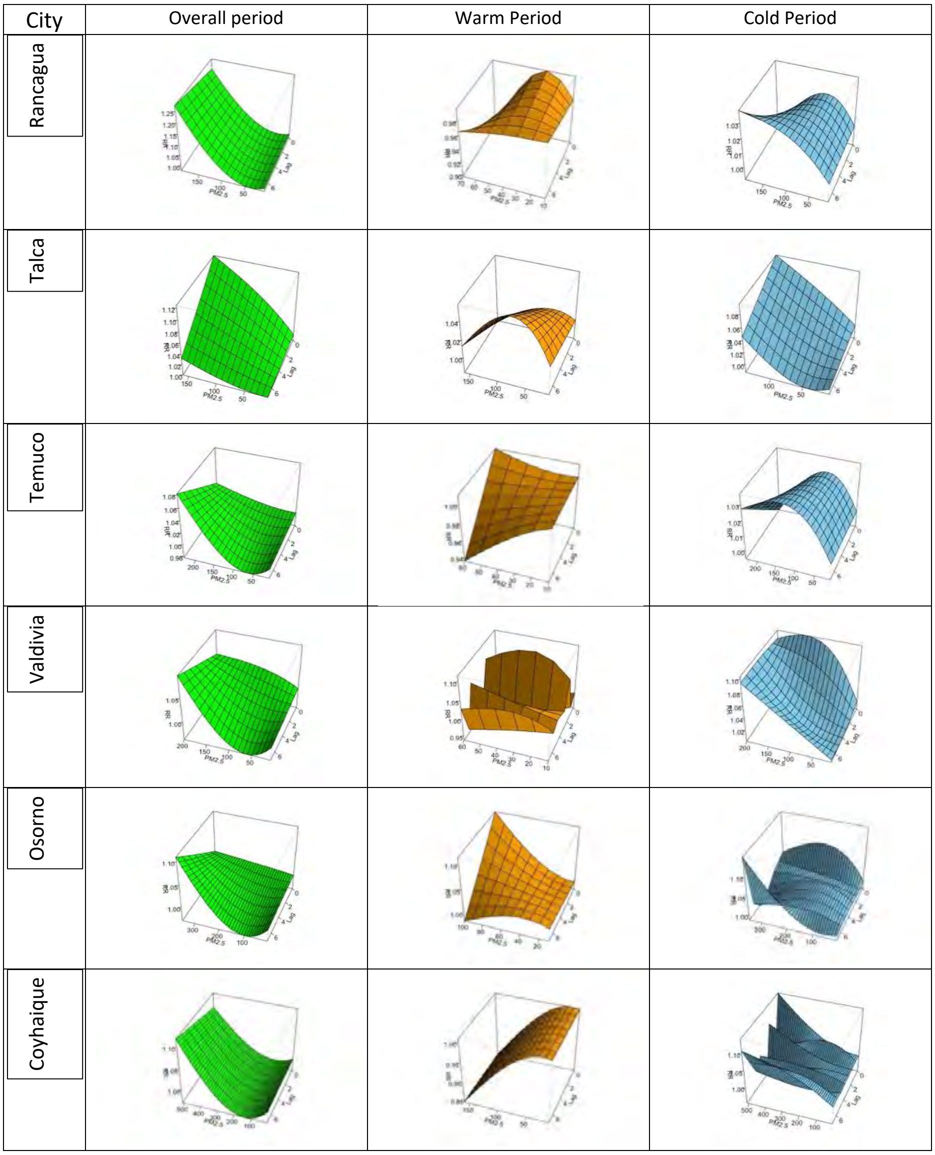


Fig. 4: Three dimensional plots of the association between $PM_{2.5}$ concentrations and RR of REVs for overall, warm and cold periods along the 7-day lag

of the contaminant with the health outcome up to a lag period of 7 days, considering 0 μg/m^3 level as reference the. It is observed that for the overall period, the southern non-coastal cities (Temuco, Osorno and Coyhaique) present a similar risk pattern, that is, the RR increases appreciably as the $PM_{2.5}$ concentrations rise over levels of 100, 150 and 300 μg/m^3, respectively, and their effects are maintained during the 7 days of lag studied. Talca and Valdivia showed something similar, but the health effects appear at lower levels of $PM_{2.5}$ concentration, increasing until day 4 and 5 of exposure to subsequently decrease, especially in Valdivia. In Rancagua there is a marked and growing effect (similar in structure to Coyhaique) above 100 μg/m^3 during the exposure period studied. The fitted RR were more dissimilar among cities during the warm period, with clear effects observed in Talca and Osorno. For Rancagua, Temuco, Valdivia and Coyhaique, the increment in concentrations was not plainly related to an increase in the risk. However, for

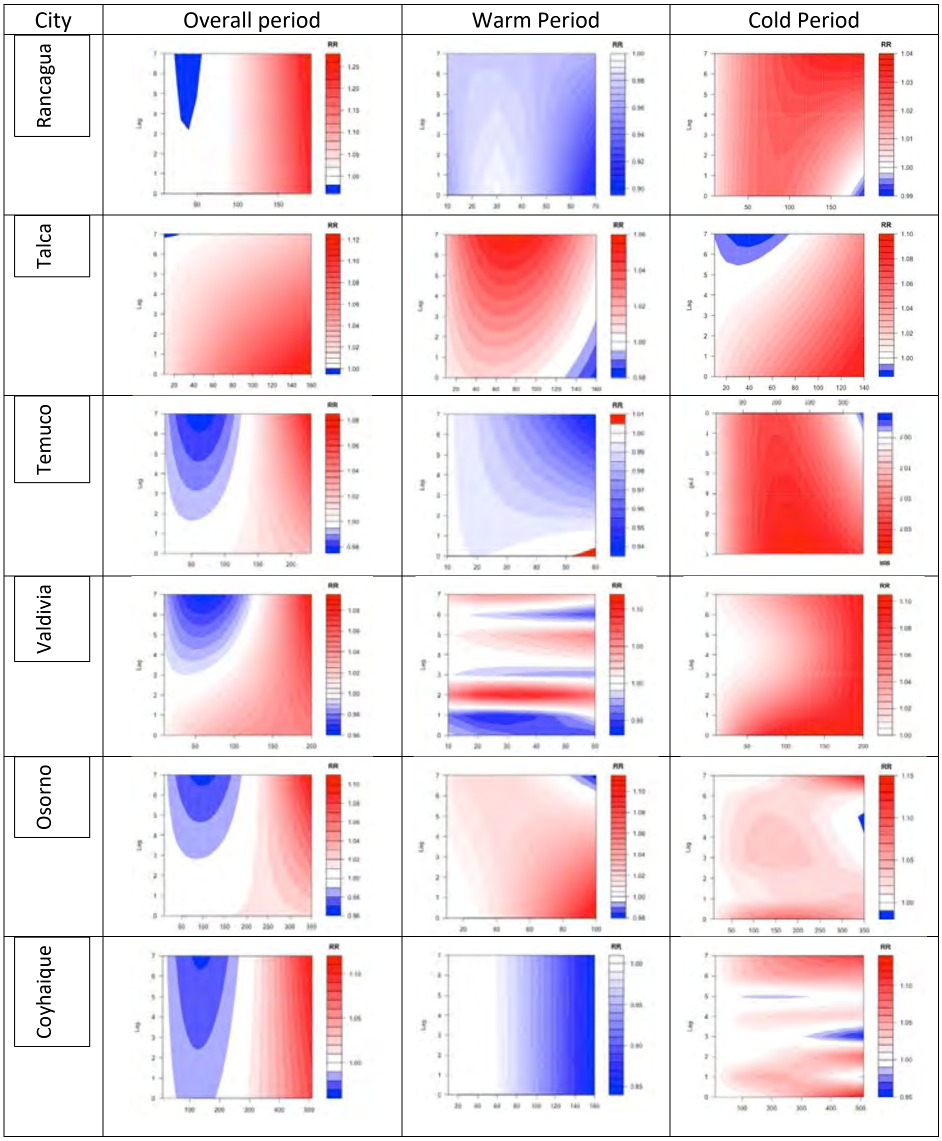


Fig. 5: Contour of cumulative effects (RR) of the association between $PM_{2.5}$ concentrations and REVs for overall, warm and cold period respectively, along 7 lags

the cold period, higher levels in $PM_{2.5}$ concentration showed a clear association with the increment in REVs in all cities. In Rancagua, Talca and Valdivia, the RR increased significantly as the concentration of $PM_{2.5}$ was higher in the first 3 to 4 days, remained constant for Rancagua and decreased for Talca and Valdivia. Temuco, Osorno and Coyhaique presented a similar pattern, increasing the RR as concentrations of pollutant increased, effects appearing at about 50 μg/m^3 in Temuco and Osorno, and over 100 μg/m^3 in Coyhaique.

Based on the same DLNM models, Fig. 6 shows "slices" of the effects surface accounting for associations along the pollutant extent at fixed lags of 1 day and 7 days, as well as through lags at 10 μg/m^3 and a high concentration level specific to the city and period, including 95% confidence intervals. The lag-fixed graphs for the overall period show that $PM_{2.5}$ level is not linearly associated with REV risk, exhibiting in some cities an apparent "protective" effect for lower pollutant levels, especially at lag 7. No evident effect in any city is observed at the 10 μg/m^3 fixed level of fine particles, except for a slight but significant effect until lag 4 days in Talca. A clear effect is observed for the city-specific high levels of pollutant, increasing along the temporal lag, except in Talca where there is a decreasing relationship. Significant effects were not observed for lag 1 and 7 in the warm season in the cities of Rancagua, Temuco, Valdivia and Osorno, but in Talca some effect is suggested at lag 7 for levels of $PM_{2.5}$ less than 100 μg/m^3 and in Coyhaique a seeming protective effect is observed, which would be reinforced along the concentration level of pollutants. The warm season graphs with low and high fixed levels of $PM_{2.5}$ do not indicate clear evidence of health effects of the pollutant with the exception of Talca, which showed a small effect from lag 3. However, for the cold season there is evidence of a significant and monotonically increasing effect along all the pollutant level range in Talca at lag 1 day, Valdivia at both lag 1 and 7 days and Osorno at lag 7 days from about the 220 μg/m^3 level. Significant effects were observed In Temuco at lag 1 as well as lag 7 days, with maximum impacts around the 100 and 120 μg/m^3 levels, respectively. Although not-significant statistically, fixed lag plots suggest the existence of effects in Rancagua at both lag 1 day and lag 7 days, Talca at lag 7 days for high levels of exposure and Coyhaique especially at lag 7 days. Weak non-significant Although not-significant statistically effects appear for the cities of Osorno and Coyhaique at lag 1 day. The 10 μg/m^3-fixed graphs do not show clear evidence of effect for the cold season, however they suggest the existence of relatively slight effects along all the studied lag dimension at this lower level of pollutant in Rancagua and Temuco, and for lags 0-2 days in Talca and Valdivia. These graphs do not show evidence of effect at 10 μg/m^3 in Osorno and Coyhaique. For the city-specific high level of fine particles analyzed, the graphs show significant effects in Talca and Valdivia through all the lag period, and near lag 7 days for Osorno. Nevertheless, non-significant effects were observed in Rancagua and Temuco from lag 2 days and in Coyhaique for most of the studied period. In general, the DLNM analysis show that the lag structure of the different cities showed a positive non-linear association between $PM_{2.5}$ daily mean concentrations and the risk of REVs, which is explained mostly by the conditions associated with the colder months. The lagging of the effects of $PM_{2.5}$ on the REVs in the different cities showed that there were excess risks in the first days, which for most cases remained during all the period evaluated. The concentration levels were relevant, observing effects from levels below 50 μg/m^3 of $PM_{2.5}$. The most intense risks for cities were observed in most of the analyses at the highest concentrations recorded of $PM_{2.5}$. However, the risks tended to decrease from north to south, being much more marked for the cities that presented less variation in the concentration levels of $PM_{2.5}$, as was the case of Rancagua and Talca during cold months.

Sensitivity analysis

We found that the effects of fine particles on REVs were mostly quite insensitive to the smoothers for time, temperature and humidity used, for all types of analyses performed. However, Osorno and Temuco showed a slight decrease in effects for the cold season when 9 degrees of freedom were used in the time smoother, which is compatible with a possible over-adjustment of data when smoothers with higher degrees of freedom are used (Goldberg *et al.,* 2011).

Results in context

There are few studies describing the impact of wood smoke on the change in the number of respiratory-related emergency visits using time series analysis in the literature reviewed. Most of these have focused on children and adults in cities with air pollution problems related to the use of firewood for residential heating. The most studied respiratory diseases have been asthmatic problems and chronic respiratory diseases, among others, with relative risks ranging between RR (1.01 to 1.12) for every 3 to 12 μg/m^3 of $PM_{2.5}$ increase (Koenig *et al*., 1993; Schreuder *et al.,* 2006; Norris *et al.,* 1999; Sheppard *et al.,* 1999; Yu *et al.,* 2000). These RR values are in accordance with those reported in the present study. In line with our results, Yañez *et al.,* (2017) studied in the cold season between 2014 and 2016 the impact of the meteorological conditions on the concentration of fine and coarse (PM_{10} -$PM_{2.5}$) particles in cities of central-southern Chile, finding great variation among cities with a marked latitudinal pattern, where the northern cities have lower levels of $PM_{2.5}$ and higher levels of coarse particles; relative humidity is one of the variables that would explain this difference. The lag period considered varies widely in studies similar to

Fig. 6: Overall and seasonally stratified effects of fine particles on daily RR of REVs along the lags (0 to 7) and $PM_{2.5}$ concentration level

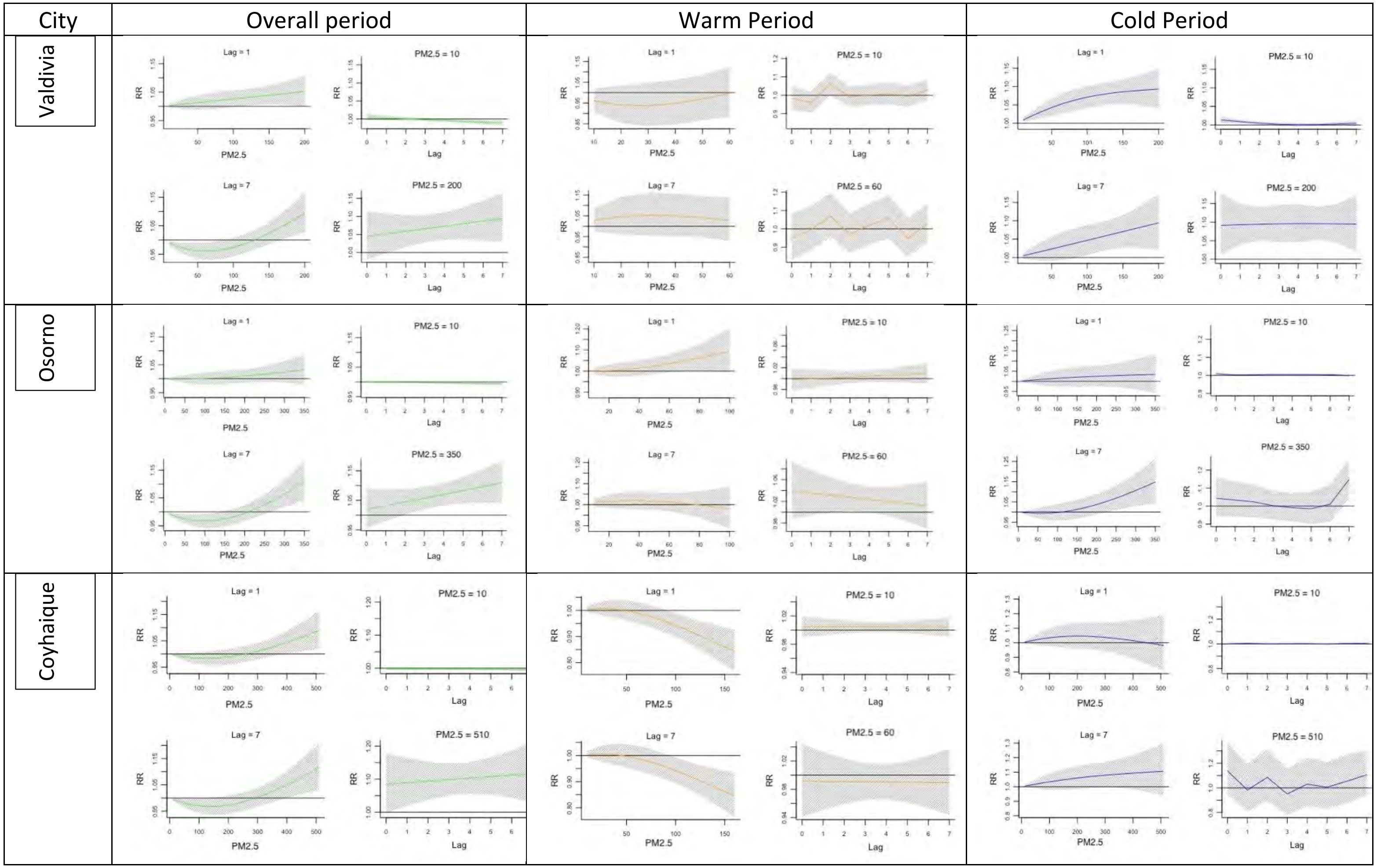


Fig. 6: Overall and seasonally stratified effects of fine particles on daily RR of REVs along the lags (0 to 7) and $PM_{2.5}$ concentration level

ours, with ranges from 0 to 30 days prior to the outcome variable. The respiratory emergency visits in our study include a number of diseases of the lower and upper tract of the respiratory system. Several studies on the Chilean population have reported greater risk in terms of morbidity and mortality related to respiratory and cardiovascular causes due to exposure to PM_{10} and $PM_{2.5}$ (Astudillo *et al.,* 2007; Cifuentes *et al.,* 2000; Ilabaca *et al.,* 1999; Ostro *et al.,* 1996; Ostro *et al.,* 1999; Pino *et al.,* 1998; Pino *et al.,* 2004; Prieto *et al.,* 2006; Roman *et al.,* 2004; Sanhueza *et al.,* 1999). Villalobos *et al.,* (2017) studied the composition of $PM_{2.5}$ in the city of Temuco, specifically organic carbon (CO), using molecular markers that identify sources of origin, reporting that 84.6% of $PM_{2.5}$ is smoke of wood burned by inefficient heating appliances. Jorquera *et al.,* (2018) measured indoor air quality in 63 houses in the urban area of the same city during the year 2014, determining that 86% of indoor $PM_{2.5}$ comes from outside by infiltration. Controlled wood smoke exposure studies in humans have not been reported. However, animal wood smoke toxicology studies have been carried out. Zelikoff *et al.*, (2002) summarized the toxicology relative to wood smoke, focusing on animal exposure but covering in part the issue from a human perspective. They concluded that the inhalation of combustion products coming from wood probably has a significant effect on pulmonary homeostasis in the exacerbation of ongoing disease processes. Wood smoke interferes with the normal development of the lungs of infants and children, increasing the risk of lower respiratory infections such as bronchitis and pneumonia (Naeher *et al.,* 2007). Exposure to smoke can depress the immune system and damage the lung epithelial tissue responsible for protecting and cleaning the airways (Zelikoff *et al.,* 2002). A higher frequency of coughs, headaches and eye and throat irritations is described in healthy people. Wood smoke is particularly harmful In vulnerable populations with asthma, chronic respiratory diseases and with cardiovascular disease; even short exposures can be very harmful (Guarnieri and Balmes, 2014). Bede-Ojimadu and Orisakwe (2020), in a systematic review in developing countries in Sub-Saharan Africa concludes that there is high level of exposure to wood smoke and this exposure is associated with a number of adverse health effects. On the other hand, an increase in the risk of cardiovascular events such as heart attacks and arrhythmias has been reported. Cardiopathic people may experience chest pain, palpitations, shortness of breath, fatigue and cardiovascular accidents. Exposure to wood smoke sharply exacerbates the respiratory symptoms of chronic diseases such as chronic obstructive pulmonary disease and bronchial asthma, leading to an increase in hospital admissions (Mott *et al.,* 2005; Xu *et al.,* 2008).

CONCLUSION

In the present study we evaluated the effect of $PM_{2.5}$ exposure on the relative risk (RR) of REVs in urban populations of six cities whose main source of air pollution is the use of firewood for heating and cooking, especially during the cold months. We found that during the cold months, the daily norm for $PM_{2.5}$ (50 μg/m^3) was exceeded over 90% of the time, more frequently in the southernmost cities (Valdivia, Temuco, Osorno and Coyhaique), which implies that at least 1,200,000 inhabitants are chronically exposed to harmful levels of $PM_{2.5}$ in these areas. In contrast, during the warm period the norm was only exceeded 7% of the time. The average 24-hour concentration of $PM_{2.5}$, during the study period, was between 3 to 6 times higher in cold months compared to warm months, reaching levels as high as 510.57 μg/m^3 in the city of Coyhaique. The number of REVs in the cold period were on average 30% to 64% higher compared to the warm period. We found a clear non-linear, short-term positive association between the $PM_{2.5}$ level and the number of REVs and this effect varied in size by city. These effects are especially strong in the colder months and at high levels of $PM_{2.5}$; they were observed during the 7-day lag period considered. On the other hand, we observed that cumulative effects of $PM_{2.5}$ distributed over seven days were significantly greater than the effect size reported for everyday lag in all cities, indicating clearly the delayed effects of air pollution on REVs. The average concentration of fine particles increases with the latitude of the studied area and with it the associated relative risks, in line with the above result found for individual cities. The results agree with previous analogous research and have a consistent biomedical explanation. The important differences found between the warm and cold seasons indicate the importance of performing this type of stratification suggesting the need for even more detailed seasonal analyzes in future research. This study shows clear acute harmful effects on the respiratory health of the population affected by pollution from wood smoke in the cities studied. Existing mitigation programs aimed at reducing exposure to PM should continue and be strengthened.

AUTHOR CONTRIBUTIONS

All authors had full access to the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. R. Torres, N. Baker and G. Bernal formulated the study goals and aims, data collection and statistical processing; A. Maldonado elaborated the maps and figures presented in this study; F. Peres, R. Torres and D. Cáceres analyzed the study data, made the interpretation and wrote the manuscript.

ACKNOWLEDGEMENTS

This study was supported by the Project of National Funding to Health Research of Chile FONIS [Nº SA17I 20207] "Air pollution by firewood combustion and its relationship with respiratory morbidity in the city of Coyhaique, Chile: a time series study". Nicole Baker and Gabriela Bernal appreciate the support of the International Exchange Program for Minority Students led by Dr. Luz Claudio at the Icahn School of Medicine at Mount Sinai in New York City. The authors also want to thank Dante Andrés Cáceres Burgos, who elaborated the graphical abstract presented in this study.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest regarding the publication of this work. In addition, the ethical issues including plagiarism, informed consent, misconduct, data fabrication and, or falsification, double publication and, or submission, and redundancy have been completely witnessed by the authors.

ABBREVIATIONS

%	Percentage
<	Less than
=	Equal
>	Greater than
>=	Greater than or equal to
3 D	Three Dimensional
° C	Degrees Celsius
° K	Degrees Kelvin
CI	Confidence Interval
CS	Cold Season
CV(%)	Coefficient of Variation
DLM	Distributed Lag Linear Models
DLNM	Distributed Lag Non-Linear Models
et al.	"and others" in latin
FAO	Food and Agriculture Organization of the United Nations
Fig.	Figure
GAM	Generalized Additive Models
GraphPad Prism	Scientific 2D graphing and statistics software
GEA	Global Energy Assessment
h	Hour
Km^2	Square kilometers
LAC	Latin America and the Caribbean
MMA	Ministry of the Environment
N	North
$P<0.05$	Probability that the null hypothesis is rejected
P25	25th percentile
P75	75th percentile
$PM_{2.5}$	Particulate Matter less than 2.5 microns in diameter
PM_{10}	Particulate Matter less than 10 microns in diameter
RR	Relative Risk
RH	Relative Humidity
REVs	Respiratory Emergency Visits
SD	Standard Deviation
SINCA	National System of Air Quality
R	The R Project for Statistical Computing
ºT	Temperature
$\mu g/m^3$	Micrograms per cubic meter
U.S.	United States
USEPA	United States Environmental Protection Agency
W	West
WHO	World Health Organization
y	Year

REFERENCES

Almon, S., (1965). The distributed lag between capital appropriations and expenditures. Econometrica: J. Econ. Soc., 178-196 **(19 pages).**

Armstrong, B., (2006). Models for the relationship between ambient temperature and daily mortality. Epidemiology. 17(6): 624-631 **(8 pages).**

Astudillo, P.; Prieto, C.; Mancilla, P., (2007). Exceso de morbilidad respiratoria en niños y adultos mayores en una comuna de Santiago con alta contaminación atmosférica por partículas. Rev. Méd. Chile. 135: 221-228 **(8 pages).**

Bede-Ojimadu, O.; Orisakwe, O.E., (2020). Exposure to Wood Smoke and Associated Health Effects in Sub-Saharan Africa: A Systematic Review. Ann. Global Health. 86: 32 **(1 page).**

Bergamaschi, E.; De Palma, G.; Mozzoni, P.; Vanni, S.; Vettori, M.V.; Broeckaert, F.; Bernard, A.; Mutti, A., (2001). Polymorphism of quinone-metabolizing enzymes and susceptibility to ozone-induced acute effects. Am. J. Respir. Crit. Care Med., 163(6): 1426-1431 **(6 pages).**

Cerda, A.A.; Garcia, L.Y., (2010). [Hypothetical health and economic benefits associated with a reduction in air pollution in a Chilean city (Talca)]. Rev. Med. Chile. 138(11): 1395-1402 **(8 pages).**

Cifuentes, L.; Krupnick, A.; O´Ryan, R.; Toman, M., (2005). Urban Air quality and Human Health in Latin America and the Caribbean **(85 pages).**

Cifuentes, L.A.; Vega, J.; Kopfer, K.; Lave, L.B., (2000). Effect of the fine fraction of particulate matter versus the coarse mass and other pollutants on daily mortality in Santiago, Chile. J. Air Waste Manage. Assoc., 50(8): 1287-1298 **(12 pages).**

DEIS., (2019) Department of Statistics and Health Information (DEIS for its acronym in Spanish). Ministry of Health. Government of Chile.

Diociaiuti, M.; Balduzzi, M.; De Berardis, B.; Cattani, G.; Stacchini, G.; Ziemacki, G.; Marconi, A.; Paoletti, L., (2001). The two PM(2.5) (fine) and PM(2.5-10) (coarse) fractions: evidence of different biological activity. Environ. Res., 86(3): 254-262 **(9 pages).**

Dominici, F.; Samet, J.M.; Zeger, S.L., (2000). Combining evidence on air pollution and daily mortality from the 20 largest US cities: a hierarchical modelling strategy. Journal of the Royal Statistical Society: Series A., 163(3): 263-302 **(40 pages).**

Dominici, F.; Zanobetti, A.; Zeger, S.L.; Schwartz, J.; Samet, J.M., (2004). Hierarchical bivariate time series models: a combined analysis of the effects of particulate matter on morbidity and mortality. Biostatistics. 5(3): 341-360 **(20 pages).**

Fairley, D., (1999). Daily mortality and air pollution in Santa Clara County, California: 1989-1996. Environ. Health Perspect., 107(8): 637-641 **(5 pages).**

Gasparrini, A.; Armstrong, B.; Kenward, M.G., (2010). Distributed lag non-linear models. Stat. Med., 29(21): 2224-2234 **(11 pages).**

GEA., (2012) Global Energy Assessment: Toward a sustainable future **(113 pages).**

Geiger., R. (1954). Klassifikation der Klimate nach W. Köppen [Classification of climates after W. Köppen]. Landolt-Börnstein Zahlenwerte und Funktionen aus Physik, Chemie, Astronomie, Geophysik und Technik, alte Serie. Berlin. 3: 603–607 **(5 pages).**

Ghio, A.J.; Cohen, M.D., (2005). Disruption of iron homeostasis as a mechanism of biologic effect by ambient air pollution particles. Inhal. Toxicol., 17(13): 709-716 **(8 pages).**

Goldberg, M.S.; Gasparrini, A.; Armstrong, B.; Valois, M.F., (2011). The short-term influence of temperature on daily mortality in the temperate climate of Montreal, Canada. Environ. Res., 111(6): 853-860 (**8 pages).**

Green, J.; Sánchez, S. (2013) Air Quality in Latin America: An overview. Washington D.C.-Updated version May 2013 **(36 pages).**

Guarnieri, M.; Balmes, J.R., (2014). Outdoor air pollution and asthma. Lancet, 383(9928): 1581-1592 **(12 pages).**

FAO., (2010) Food and Agriculture Organization of the United Nations (FAO). Criteria and indicators for sustainable wood fuels. Rome: FAO Forestry paper 160, **(103 pages).**

Hong, Y.C.; Pan, X.C.; Kim, S.Y.; Park, K.; Park, E.J.; Jin, X.; Yi, S.M.; Kim, Y.H.; Park, C.H.; Song, S.; Kim, H., (2010). Asian Dust Storm and pulmonary function of school children in Seoul. Sci. Total. Environ., 408(4): 754-759 **(6 pages).**

Ilabaca, M.; Olaeta, I.; Campos, E.; Villaire, J.; Tellez-Rojo, M.M.; Romieu, I., (1999). Association between levels of fine particulate and emergency visits for pneumonia and other respiratory illnesses among children in Santiago, Chile. J. Air Waste Manage. Assoc., 49(9 Spec No): 154-163 **(10 pages).**

Jorquera, H.; Barraza, F.; Heyer, J.; Valdivia, G.; Schiappacasse, L.N.; Montoya, L.D., (2018). Indoor PM2.5 in an urban zone with heavy wood smoke pollution: The case of Temuco, Chile. Environ. Pollut., 236: 477-487 (**11 pages).**

Koenig, J.Q,; Larson, T.V.; Hanley, Q.S.; Rebolledo, V.; Dumler, K.; Checkoway, H.; Wang, S.Z.; Lin, D.; Pierson, W.E., (1993). Pulmonary Function Changes in Children Associated With Fine Particulate Matter. Environ. Res. 63(1):26-38 **(13 pages).**

Lamsal, L.N.; Martin, R.V.; Parrish, D.D.; Krotkov, N.A., (2013). Scaling relationship for NO2 pollution and urban population size: a satellite perspective. Environ. Sci. Technol., 47(14): 7855-7861 **(7 pages).**

Ma, Y.; Zhou, J.; Yang, S.; Yu, Z.; Wang, F.; Zhou, J., (2019). Effects of extreme temperatures on hospital emergency room visits for respiratory diseases in Beijing, China. Environ. Sci. Pollut. Res. Int., 26(3): 3055-3064 (**10 pages).**

Mayer, H., (1999). Air pollution in cities. Atmos. Environ. (33): 4029-4037 **(9 pages).**

Maykut, N.N.; Lewtas, J.; Kim, E.; Larson, T.V., (2003). Source apportionment of PM2.5 at an urban IMPROVE site in Seattle, Washington. Environ. Sci. Technol., 37(22): 5135-5142 **(8 pages).**

MMA., (2014) Ministry of the Environment. Strategy of Atmospheric Decontamination Plans 2014-2018 (MMA for its acronym in Spanish), Republic of Chile **(38 pages).**

Molina, C.; Toro A, R.; Morales S, R.; Manzano, C.; Leiva-Guzmán, M., (2017). Particulate matter in urban areas of south-central Chile exceeds air quality standards. Air Qual. Atmos. Health. 10: 653-667 **(15 pages).**

Mott, J.A.; Mannino, D.M.; Alverson, C.J.; Kiyu, A.; Hashim, J.; Lee, T.; Falter, K.; Redd, S.C., (2005). Cardiorespiratory hospitalizations associated with smoke exposure during the 1997, Southeast Asian forest fires. Int. J. Hyg. Environ. Health. 208(1-2): 75-85 **(11 pages).**

Naeher, L.P.; Brauer, M.; Lipsett, M.; Zelikoff, J.T.; Simpson, C.D.; Koenig, J.Q.; Smith, K.R., (2007). Woodsmoke health effects: a review. Inhal. Toxicol., 19(1): 67-106 **(40 pages).**

Norris, G.; Young Pong, S.N.; Koenig, J.Q.; Larson, T.V.; Sheppard, L.; Stout, J.W., (1999). An association between fine particles and asthma emergency department visits for children in Seattle. Environ Health Perspect. 10: 489-493 (5 pages).

Orellano, P.; Quaranta, N.; Reynoso, J.; Balbi, B.; Vasquez, J., (2018). Association of outdoor air pollution with the prevalence of asthma in children of Latin America and the Caribbean: A systematic review and meta-analysis. J. Asthma. 55(11): 1174-1186 **(13 pages).**

Osseiran, N.; Lindmeier, C., (2018) 9 out of 10 people worldwide breathe polluted air, but more countries are taking action, World Health Organization. Geneva 2018.

Ostro, B.; Sanchez, J.M.; Aranda, C.; Eskeland, G.S., (1996). Air pollution and mortality: results from a study of Santiago, Chile. J. Expo. Anal. Environ. Epidemiol., 6(1): 97-114 **(8 pages).**

Ostro, B.D.; Eskeland, G.S.; Sanchez, J.M.; Feyzioglu, T., (1999). Air pollution and health effects: A study of medical visits among children in Santiago, Chile. Environ. Health. Perspect. 107(1): 69-73 **(5 pages).**

Peng, R.D., Dominici, F. (2008) Statistical methods for environmental epidemiology with R: a case study in air pollution and Health, 1st. Ed., **(144 pages).**

Pino, P.; Oyarzun, M.; Walter, T.; von Baer, D.; Romieu, I., (1998). [Indoor air pollution in southeast Santiago, Chile]. Rev. Méd. Chile. 126(4): 367-374 **(8 pages).**

Pino, P.; Walter, T.; Oyarzun, M.; Villegas, R.; Romieu, I., (2004). Fine particulate matter and wheezing illnesses in the first year of life. Epidemiology. 15(6): 702-708 **(7 pages).**

Prieto, M.J.; Mancilla, P.; Astudillo, P.; Román, O., (2006). Exceso de morbilidad respiratoria en niños y adultos mayores en una comuna de Santiago con alta contaminación atmosférica por partículas. Rev. Méd. Chile., 135: 221-228 **(8 pages).**

Pyne, S., (2014) World Fire: the culture of fire on Earth. (Pbk. ed.). Seattle: University of Washington Press **(399 pages).**

Roemer, W.; Hoek, G.; Brunekreef, B.; Clench-Aas, J.; Forsberg, B.; Pekkanen, J.; Schutz, A., (2000). PM10 elemental composition and acute respiratory health effects in European children (PEACE project). Pollution Effects on Asthmatic Children in Europe. Eur. Respir. J., 15(3): 553-559 **(7 pages).**

Roman, O.; Prieto, M.J.; Mancilla, P., (2004). [Atmospheric pollution and cardiovascular damage]. Rev. Méd Chile. 132(6): 761-767 **(7 pages).**

Sanhueza, P.; Vargas, C.; Jimenez, J., (1999). [Daily mortality in Santiago and its relationship with air pollution]. Rev. Méd. Chile. 127(2): 235-242 **(8 pages).**

Schreuder, A.B.; Larson, T.V.; Sheppard, L.; Claiborn, C.S., (2006). Ambient woodsmoke and associated respiratory emergency department visits in Spokane, Washington. Int. J. Occup. Environ. Health. 12: 147-153 **(7 pages).**

Sheppard, L.; Levy, D.; Norris, G.; Larson, T.V.; Koenig, J.Q., (1999). Effects of ambient air pollution on nonelderly asthma hospital admissions in Seattle, Washington, 1987-1994. Epidemiology. 10: 23-30 **(8 pages).**

SINCA, (2019) National Information System of Air Quality (SINCA for its acronym in Spanish) Ministry of the Environment, Republic of Chile.

Villalobos, A.M.; Barraza, F.; Jorquera, H.; Schauer, J.J., (2017). Wood burning pollution in southern Chile: PM2.5 source apportionment using CMB and molecular markers. Environ. Pollut., 225: 514-523 **(10 pages).**

Ward, D.J.; Ayres, J.G., (2004). Particulate air pollution and panel studies in children: a systematic review. Occup. Environ. Med., 61(4): 1-12 **(12 pages).**

Wyzga, R.E., (1978). The effect of air pollution upon mortality: a consideration of distributed lag models. J. Am. Stat. Assoc., 73(363): 463-472 **(10 pages).**

Xu, X.; Zborowski, J.V.; Arena, V.C.; Rager, J.; Talbott, E.O., (2008). Case-crossover analysis of air pollution and cardiorespiratory hospitalizations: using routinely collected health and environmental data for tracking: science and data. J. Public Health Manag. Pract., 14(6): 569-576 **(8 pages).**

Yañez, M.A.; Baettig, R.; Cornejo, J.; Zamudio, F.; Guajardo, J.; Fica, R., (2017). Urban airborne matter in central and southern Chile: Effects of meteorological conditions on fine and coarse particulate matter. Atmos. Environ., 161: 221-234 **(14 pages).**

Yu, O.; Sheppard, L.; Lumley, T.; Koenig, J.Q.; Shapiro, G.G., (2000). Effects of ambient air pollution on symptoms of asthma in Seattle-area children enrolled in the CAMP study. Environ Health Perspect 108: 1209-1214 **(6 pages).**

Zelikoff, J.T.; Chen, L.C.; Cohen, M.D.; Schlesinger, R.B., (2002). The toxicology of inhaled woodsmoke. J. Toxicol. Environ. Health. B Crit. Rev., 5(3): 269-282 **(14 pages).**

AUTHOR (S) BIOSKETCHES

Torres, R., M.Sc., Profesor Asistente, Programa de Bioestadística, Escuela de Salud Pública, Facultad de Medicina, Universidad de Chile, Santiago, Chile. Email: *rodrigotorres@uchile.cl*

Baker, N., M.P.H. Emory University Rollins School of Public Health and fellowship of International Exchange Program for Minority Student, Icahn School of Medicine at Mount Sinai in New York City, USA. Email: *nmbaker@emory.edu*

Bernal, G., M.Sc., Departamento de Salud Ambiental, Escuela Graduada de Salud Pública, Universidad de Puerto Rico recinto de Ciencias Médicas and fellowship of International Exchange Program for Minority Student, Icahn School of Medicine at Mount Sinai in New York City, USA. Email: *gabriela.bernal1@upr.edu*

Peres, F., Ph.D., Professor, Centro de Estudio de Salud del Trabajador y Ecología Humana, Escuela Nacional de Salud Pública, Fio Cruz, Brasil. Email: *fperes@fiocruz.br*

Maldonado, A.K., M.Sc. student, Programa de Salud Ambiental, Escuela de Salud Pública, Facultad de Medicina, Universidad de Chile, Santiago, Chile. Email: *vgtariana@ug.uchile.cl*

Caceres, D.D., Ph.D., Profesor Asociado, Programa de Salud Ambiental, Escuela de Salud Pública, Facultad de Medicina, Universidad de Chile, Santiago, Chile. Email: *dcaceres@med.uchile.cl*

HOW TO CITE THIS ARTICLE

Torres, R.; Baker, N.; Bernal, G.; Peres, F.; Maldonado, A.K.; Caceres, D.D., (2021). The effect of short-term of fine particles on daily respiratory emergency in cities contaminated with wood smoke. Global J. Environ. Sci. Manage., 7(1): 15-32.

DOI: 10.22034/gjesm.2021.01.02

url: https://www.gjesm.net/article_40384.html

Global J. Environ. Sci. Manage. 7(1): 33-46, Winter 2021

Global Journal of Environmental Science and Management
(GJESM)

Homepage: https://www.gjesm.net/

ORIGINAL RESEARCH PAPER

Calorific and greenhouse gas emission in municipal solid waste treatment using biodrying

B. Zaman[1], W. Oktiawan[1], M. Hadiwidodo[1], E. Sutrisno[1], P. Purwono[2,*]

[1]*Department of Environmental Engineering Faculty of Engineering Diponegoro University, Semarang, Indonesia*

[2]*Center Science and Technology, IAIN Surakarta, Pandawa, Pucangan, Kartasura, Indonesia*

ARTICLE INFO

Article History:
Received 10 April 2020
Revised 31 July 2020
Accepted 09 August 2020

Keywords:
Biodrying
Greenhouse gas
MSW
Refuse derived fuel
Temperature

ABSTRACT

BACKGROUND AND OBJECTIVES: Urban intensity and activities produce a large amount of biodegradable municipal solid waste. Therefore, biodrying processing was adopted to ensure the conversion into Refuse Derived Fuel and greenhouse gases..

METHODS: This study was performed at a greenhouse, using six biodrying reactors made from acrylic material, and equipped with digital temperature recording, blower, and flow meters. The variations in airflow (0, 2, 3, 4, 5, 6 L/min/kg) and the bulking agent (15%) were used to evaluate calorific value, degradation process and GHG emissions.

FINDINGS: The result showed significant effect of airflow variation on cellulose content and calorific value. Furthermore, the optimum value was 6 L/min/kg, producing a 10.05% decline in cellulose content, and a 38.17% increase in calorific value. Also, the water content reduced from 69% to 40%. The CH4 concentration between control and biodrying substantially varied at 2.65 ppm and 1.51 ppm respectively on day 0 and at peak temperature. Morever, the value of N2O in each control was about 534.69 ppb and 175.48 ppb, while the lowest level was recorded after biodrying with 2 L/min/kg airflow.

CONCLUSION: The calorific value of MSW after biodrying (refuse derived fuel) ranges from 4,713 – 6,265 cal/g. This is further classified in the low energy coal (brown coal) category, equivalent to <7,000 cal/g. Therefore, the process is proven to be a suitable alternative to achieve RDF production and low GHG emissions.

DOI: 10.22034/gjesm.2021.01.03

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
43	11	2

*Corresponding Author:
Email: *purwono.ga@gmail.com*
Phone: +8564 0674048
Fax: +6271 781516

Note: Discussion period for this manuscript open until April 1, 2021 on GJESM website at the “Show Article.

INTRODUCTION

Urban intensity and activities instigate the immense production of biodegradable solid waste. Therefore, proper management is required to avoid negative impacts on the environment, through odorous and pollutant emissions in soil, water, gas, and others. The current processing method involving burning or landfill is not optimal, and the availability of space for final processing (TPA) is critical. Also, identifying an alternative new location (TPA area) is difficult and expensive, especially in big cities. Moreover, waste to energy (WTE) technologies has the potential to reduce original waste volume (up to 90%) by recovering the energy, depending on the composition (Patil *et al.,* 2014). The water content is an essential factor in urban solid waste, due to the effects on the efficiency of combustion and conversion of solid waste into energy (Suksankraisorn *et al.,* 2010). However, mechanical biological treatment (MBT) is the prospective choice amongst the methods being developed, because of the environmental-friendly characteristics (Egan *et al.,* 2005). The phenomenon of natural drying, also known as biodrying, is a critical component of the MBT processes, involving the treatment of solid waste through mechanical-biological bioconversions (Rada and Ragazzi, 2015; Velis *et al.,* 2009). During practice, chopped materials with high water content are placed into the reactor. Subsequently, dry solid waste (bio-dried) are produced through a biodrying processes, before subjecting to mechanical treatment. Therefore, the heat generated from the aerobic decomposition process of organic compounds, and excess air are combined to serve as a reliable waste dryer (Velis *et al.,* 2009). Moreover, the solid waste products are also considered as Refused Derived Fuel (RDF), derived from urban, industrial, or commercial waste sources (Scheutz *et al.,* 2014). The RDF is possibly adopted as a substitute for coal (Rada and Ragazzi, 2015), and most of the biodrying process is capable of reducing the water content in solid waste to between 30% and 80% of the initial value (Li *et al.,* 2015; Zhang *et al.,* 2008; Zhao *et al.,* 2010). Furthermore, the quantity removed varies between 3.1 to 10.7 g water/g volatile solid consumed, depending on the preliminary composition and operating conditions (Frei *et al.,* 2004; Ma *et al.,* 2016). The biodrying process is performed in batch conditions, with a 20 days maximum duration, and the raw materials previously treated include manure, pulp mill sludge, food waste, MSW, and sewage sludge. . The final outcome is RDF, which is often used as co-fuel in the cement industry and boiler unit (Garg *et al.,* 2007; Wagland *et al.,* 2011). Colomer-Mendoza *et al.,* (2013) treated gardening waste with 10 reactors, characterized by an air volume of 0.88 to 6.42 L/min/kg (dry weight) and 5% bulking agents implicated in increased weight loss. However, some important aspects, including greenhouse gas emissions have not been studied, as most studies approach this phenomenon from the composting process of solid waste, e.g., sludge. González *et al.,* (2019) discussed greenhouse gases, volatile organic compounds and odor emissions in sewage sludge, without considering possible degradations during the biodrying process. In addition, composting and biodrying serve varied purposes, which require rapid and partial degradation, respectively (Goyal *et al.,* 2005). The characterization of greenhouse gas (GHG) and odorous compounds in solid sludge compost are compiled in a widely published standard scale (Maulini-duran *et al.,* 2013; Rincón *et al.,* 2019), and several related studies have been performed in full scale (González *et al.,* 2019; Shen *et al.,* 2012). In addition, emissions from the biodrying process require advanced studies because of the potential impacts on global warming (Pan *et al.,* 2018), and investigating as an alternative approach to evaluate the release of MSW, and GHG is also important. This study aims to increase the calorific value and evaluate the MSW degradation process through biodrying, and to also provide an in depth evaluation of greenhouse gas emissions. The research was conducted in 2019 at a greenhouse to avoid the disturbance of animals and to ensure optimal manipulation to the desired environmental condition.

MATERIALS AND METHODS

MSW was manually collected from the KORPRI housing complex, Tembalang, Semarang, Central Java, Indonesia, with coordinates -7.061131, 110.446709. The sample characteristics were highly similar to those produced by most people in Semarang city, which were further sorted to determine the percentage of each component (%). In addition, the percent by weight of the MSW component comprises 64% leaves, 12% paper, 16% plastic, 6% uneaten vegetables, 1,73% uneaten of meals, and 0.27% fruit peels. This material was chopped using a chopper measuring 15-20 mm, while the plastic variety was manually cut with a scissors. Subsequently, all MSW components were mixed and measured in terms of volume, before placing into a biodrying reactor. The bulking agent is mature and stable compost measuring ± 10 mm, and comprising 0.051 m^3 of MSW (85% of total volume) and 0.009 m^3 of bulking agent (15% of total volume). Therefore, the MSW volume calculation was based on the maximum reactor capacity of 60 liters (body diameter: 38 cm; total height: 65 cm; weight: 3 kg), while the biodrying reactor was constructed using polyethylene plastic, and equipped with a heat sink (Thermoshield Universal) to minimize heat loss. The reactor base is installed a stainless-steel pipe (Ø3 mm) to ensure uniform air distribution, while airflow variations (0, 2, 3, 4, 5, 6 l/ min/kg) was achieved using an aquarium pump (Resun LP-100). Furthermore, each reactor comprise of sampling holes, measuring a diameter of 7 cm, at a height of 20 cm, 40 cm, and 60 cm from the base. These orifice were tightly closed when not in use. The temperature sensor probes were placed at the top, middle, and bottom area, and the average rate was noted. Moreover, temperature measurements required stainless steel sensors, with waterproof characteristics against the nearest 0.01 °C. The degree of heat was automatically recorded every 15 minutes, and the data is saved as *xlsx* format in an SD card. The temperature probe range was -50 ° C to 200 ° C, while the leachate produced by the reactor was collected, and the volume was measured (if incurred). Fig. 1 shows the biodrying reactor scheme.

The water content parameter was measured using the gravimetric method and the analysis was performed every day, during the biodrying process. This involved measuring and mixing a total of 20 g sample obtained from three different levels of depths (top, middle, and bottom) in triplicate ways, with deviation standard set on <5%. The respective neutral detergent fiber was determined and used to calculate the cellulose content (Goering and van Soest, 1970). In addition, C-Organic was evaluated using the rapid and effective Walkey-Black method, while Nitrogen content was analyzed using the Kjeldahl method, where both assessments were performed in triplicates. Specifically, caloric/heat content was tested using Bomb Calorimeter, while Greenhouse Gas (GHG) sampling was performed at the highest temperature for CO_2, CH_4, and N_2O, using Shimadzu 14A capillary gas chromatograph, equipped with FTD at 250 °C. Limit of Detection CH_4: 0,89 ppm, N_2O: 39,22 ppb, and CO_2: 88,47 ppm. Fig. 2 shows the study flowchart.

RESULTS AND DISCUSSION

MSW degradation rate was analyzed based on the parameters of temperature, water content, cellulose, and SEM (Scanning Electron Microscopy). The GHG emissions consist of CO_2, CH_4, and N_2O.

Temperature Profile

Biodrying is an exothermic phenomenon, where aerobic processes utilize oxygen for microbial activity. In addition, temperature is a significant parameter, which serves as a crucial factor influencing

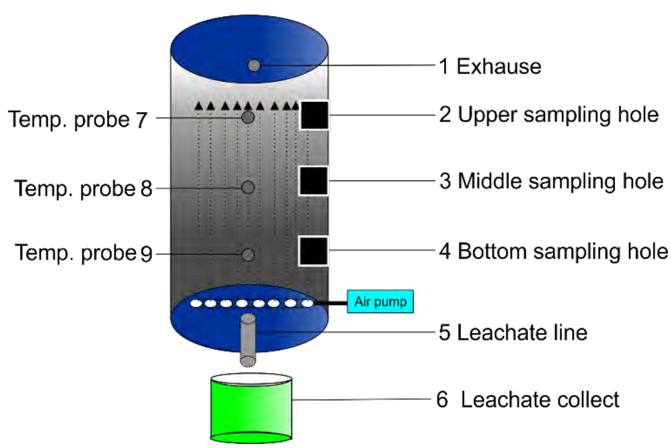


Fig. 1: The biodrying reactor scheme

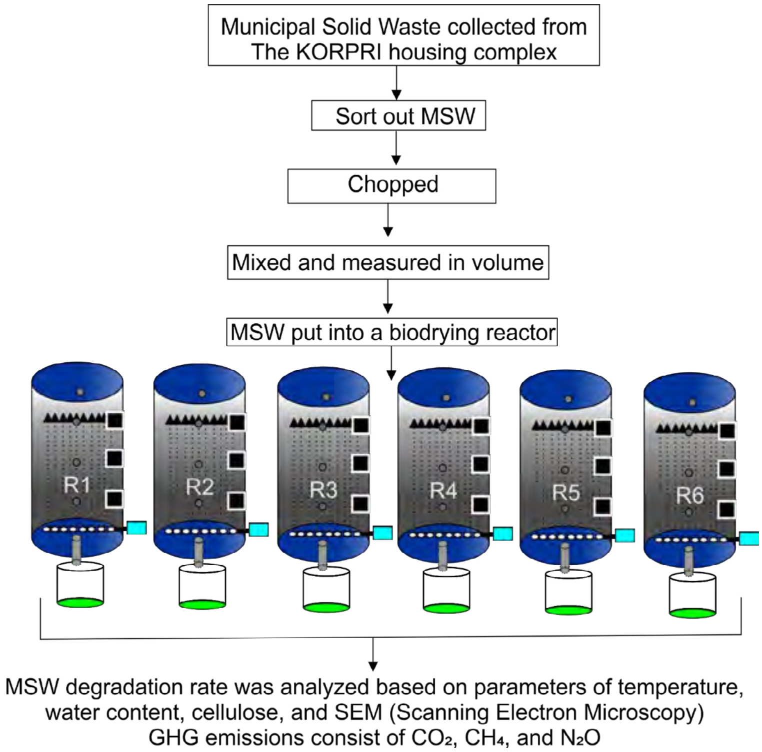


Fig. 2: Flowchart research on calorific and greenhouse gas emission in municipal solid waste treatment using biodrying

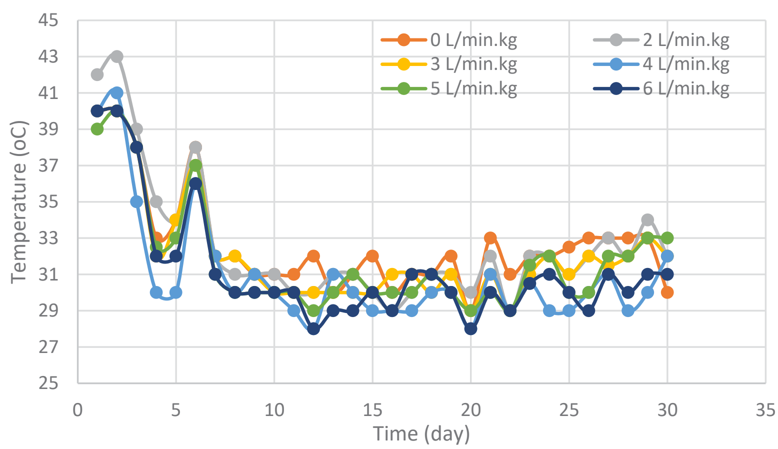


Fig. 3: Temperature profile in the biodrying process for 30 days

water evaporation and organic degradation (Fadlilah and Yudihanto, 2013; Sen and Annachhatre, 2015; Zhang *et al.*, 2008). Moreover, too high or significantly low values have the potential to slow down the drying process, due to the inactivity of decomposer microorganisms, subsequently leading to an incomplete course of action (Sudrajat, 2006). Fig. 3 shows the temperature data recorded in relation to varied air flow.

Temperature was monitored every day for 30 days to assess microorganism activities during the biodrying process (Jalil *et al.*, 2016). Fig. 3 shows the matrix temperature in each variation.

Furthermore, each reactor produces different temperatures, in relation to the distinct MSW decomposition speed, while the airflow rate influences aerobic conditions (Velis *et al.,* 2009). The amount of air in reactor 6 was more than the quantity in reactor 2, hence the variation in speed. Moreover, the highest temperature from reactor 2 (airflow 2 L/min/kg) was 43 °C on the 2nd day, followed by a decline to 39 °C on the 3rd day, and stablility was consequently achieved in the mesophilic phase up to the 8th day. Therefore, the temperature was gradually reduced to 29 °C, and the research outcome was compatible with the study of Sadaka *et al.,* (2011). This stated the presence of a temperature escalation from about 37.7 °C to 48.8 °C during biodrying on day 2 to day 3, indicating a high biodegradation process resulting from substantial microorganism metabolism (Fadlilah and Yudihanto, 2013; Jalil *et al.,* 2016). This is congruent with the report by Jalil *et al.,* (2016), based on a study performed using a reactor. Also, "mesophilic" (35 °C and 40 °C) and moderately "thermophilic" temperatures (40 °C to 45 °C) are more applicable compared to the "thermophilic" type (55˚C to 70˚C). Specifically, Jokiniemi and Ahokas, (2014) reported on the ability for a combination of high temperature and low airflow to slow down the drying process. This condition corresponds to Sadaka *et al.,* (2011). In addition, there is a rise in temperature on the second day, followed by a retraction to ambient level. Also, the moderately thermophilic as well as the mesophilic phases developed on the second and sixth day, respectively. Moreover, relatively uniform (stable) but fluctuating temperature values were recorded from day 7 to 30, ranging from 28 °C – 34 °C. This condition indicates the absence of adequately large microorganism activity required to create biological stability after the biodrying process (Adani *et al.,* 2002). Jalil *et al.,* (2016) recognized a similar condition, in the study using solid waste samples, including food scraps, papers, plastics, and woods.

Water content

Water content is an essential parameter in determining the success of a biodrying process. This constraint influences the chemical reactions associated with microbial growth and biodegradation of organic substances (Tom *et al.,* 2016; Velis *et al.,* 2009). The initial levels at the onset are generally set in the range of 50%-75%. Furthermore, extremely low values lead to reduced microbial activities, while higher amount creates anaerobic conditions. Moreover, water is more dominant in filling pores compared to air, thus limiting the oxygen availability (Colomer-Mendoza *et al.,* 2013; Fadlilah and Yudihanto, 2013; Sadaka *et al.,* 2011). Fig. 4 show the measurement results of water content in each reactor at different aeration airflows.

Water content at the inception of biodrying is not substantially low. Comparably, a significant decline was recorded on day 15, at 63,47% to 23,75% in reactor 1 (0 L/min/kg), 61,22% to 27,77% for reactor 2 (2 L/min/kg), reactor 3 (3 L/min/kg) was 66,26% to 31,84%, reactor 4 (4 L/min/kg) 63,54% to 28,87%, while 66,09% to 38,60% was observed in reactor 5 (5 L/min/kg). This reduction indicates the

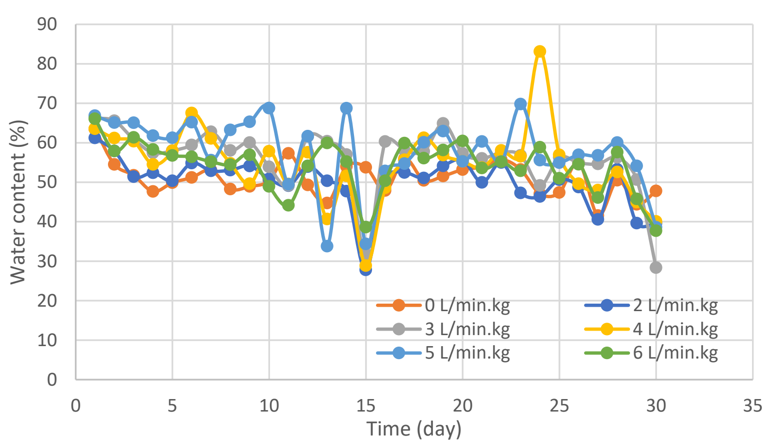


Fig. 4: Water content profile in the biodrying process for 30 days

process effectiveness, according to the literature, which ranges between days 7–15 (Velis *et al.,* 2009). The degradation characteristics of water content is compatible with the research of Jalil *et al.,* (2016), as observed on day 14 (67 ± 0,24% to 33,91 ± 2,24%). According to Adani *et al.,* (2002), it is possible for water content to reduce the decomposition level of solid waste. The level recorded in solid waste increased on day 20 for all reactors, resulting from the addition of water from the condensation process inside the reactor (Widarti *et al.,* 2015). Subsequently, evaporation is performed because of decomposition, and converted into dew on the reactor surface, due to the absence of a steam trap. This dew is further converted into saturated steam, and falls back into the pile of solid waste for another cycle of water content increase. Moreover, the solid waste in reactor 1 comprises a relatively higher water content value of 47.78%, compared to than others. This is due to the configuration without aeration, thus the absence of a biodrying process. Therefore, water content was reduced only through a biological approach (Perazzini *et al.,* 2016). Conversely, reactor 2,3,4,5 and 6 L/min/kg were equipped with aeration, which helped in physical and biological drying (Perazzini *et al.,* 2016; Sen and Annachhatre, 2015), by evaporation. In addition, a change in phase occurs and the liquid is converted to gas, as aeration accelerates the transfer of steam from the inside material to the outside air (Bilgin and Tulun, 2015; Velis *et al.,* 2009). This statement is consistent with Sen and Annachhatre (2015), where higher air flow was assumed to influence the physically dry up of solid waste, and not due to the heat generated by aerobic degradation. The final results of the research comprise the production of solid waste, with the lowest water content of 28.37% recorded in reactor 3 (3 L/min/kg). Based on this research, biodrying successfully reduced the moisture level in solid waste, compared to the control (without bio drying).

C-Organic and Total Nitrogen

C-Organic is a source of energy for the process of decomposition and cell formation, while nitrogen is an element needed by microorganisms for protein synthesis (Siswanto, M. Hamzah, Mahendra, 2012). In addition, both constituents not fully degraded in biodrying, after development with composting, hence the levels are preserved as fuel (Fadlilah and Yudihanto, 2013). Eq. 1 shows the degradation reaction of aerobic process responsible for the production of carbon and nitrogen (Sen and Annachhatre, 2015).

$$COHN + O_2 + \text{Microorganism Aerobe} \rightarrow CO_2 + NH_3 + \text{end product} + \text{Energy} \quad (1)$$

Table 1 shows the C-Organic in this study, and an insignificant decline was recorded from 50.96% - 64.82% at the beginning of the biodrying process to 47,30-60,35% after 30 days. This reduction indicates the usefulness of low carbon consumption in increasing the calorific value (Colomer-Mendoza *et al.,* 2013). However, the carbon content escalated on the 6 L/min/kg airflow, due to high level of aeration. This is assumed to inhibit microbial activity to the extent where proper organic compounds degradation is impossible (Colomer-Mendoza *et al.,* 2013; Sadaka *et al.,* 2011).

Table 1 also shows the decline in total Nitrogen (dry matter) during the 30 day period, from an initial value range of 1.07% - 1.63% to about 0.62% - 1.45%. This constituent is volatile and lower levels have been implicated in slower organic matter decomposition (Widarti *et al.,* 2015), therefore leading to the absence of any overall research sample degradation,

Table 1: C-Organic and Total Nitrogen in the biodrying process

Airflow (L/min/kg)	C-Organic (%)				Total nitrogen (%)			
	day				day			
	0	2	15	30	0	2	15	30
0	64.82	64.37	32.89	52.62	1.23	0.96	0.45	0.90
2	76.53	77.66	49.67	60.35	1.63	0.97	0.48	1.45
3	79.08	76.31	46.77	47.30	1.30	1.32	0.46	0.62
4	67.69	65..64	44.54	52.19	1.21	1.07	0.39	0.63
5	66.59	72.41	40.34	49.94	1.44	0.87	0.41	0.66
6	50.96	86.67	47.36	53.75	1.07	0.73	0.53	0.64

and for further application as fuel (Fadlilah and Yudihanto, 2013). This research corresponds with the study of Colomer-Mendoza *et al.,* (2013), where a sample garden solid waste was used in the absence of any additional bulking agents, and treated with varied airflow.

Cellulose

Under aerobic conditions, the microbes in the biodrying process have the ability to degrade semi-biodegradable organics, which is challenging as observed with cellulose (Wardhani *et al.,* 2017). This is one of the first growing cells of polysaccharides (carbohydrates), frequently attacked by microorganisms in the early stages of decomposition (Evangelou, 1998), sourced from solid waste samples, including leaf litter, paper, and food scraps. The respectivel cellulose content is about 15-20%, 85-99% (Howard *et al.,* 2003), and 13% (Astuti, 2016), although the level in the dry-weight generally varies from 15-60% (Evangelou, 1998). Furthermore, one of the potential application is as a necessary materials for fuel (Anindyawati, 2010), and Fig. 5 shows the graph of cellulose levels over a 30 day period.

Fig. 5 shows the level of cellulose produced for 30 days, and a range of about 29%-30% was reported in each reactor at the inception. Subsequently the highest degradation was recorded on day two at 26-32%, in treatments with the lowest aeration flow of 2 liters/minute, followed by those with the highest temperature, in a range of 40 °C – 43 °C, included as thermophilic. This phase facilitates the most considerable degradation (Huang, 2010), due to the optimized activity of the carboxymethyl enzyme. Hence, the subsequent period is characterized by a temperature derivation of metabolized organic matter, allowing for relatively lower degradation up to day 30, which is continuous (Huang, 2010). This research is consistent with Huang (2010), where the most significant cellulose degradation was observed from day three to 15, where the thermophilic phase occurred. However, rapid decomposition was also recorded, and Fig. 6 shows the derivation in cellulose content in each reactor over the study period.

Based on Fig. 6, a derivation of cellulose level was observed in each reactor, confirming the occurrence of degradation during the biodrying process. The statistical test shows a significance result at 0.032 (sig < 0.05), indicating the substantial effect on cellulose level, at varied air flow. In addition, cellulose is broken down to oligosaccharides and subsequently into glucose, due to the presence of extracellular microbial enzymes. This form of enzyme is produced in cells, and released into the media, with the ability to hydrolyze macromolecules. Therefore, CO_2 and water is produced. The most significant deterioration of 15.97% was observed in aeration 3 L/min/kg, while the least was recorded in aeration 6 L/min/kg, at 10.05%. This phenomenon indicates the ability for higher airflow to stop microbial activity, and inhibits proper organic compound degradation, as well as nutrient consumption (Colomer-Mendoza *et al.,* 2013; Sadaka *et al.,* 2011). Hence, airflow variation affects cellulose degradation in the biodrying process.

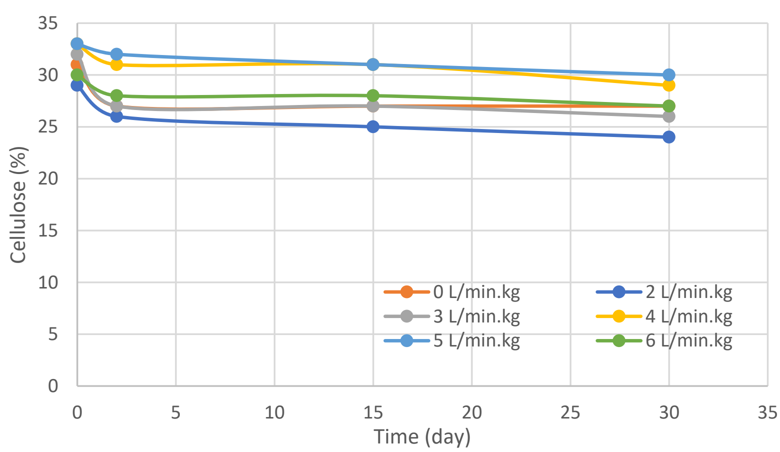


Fig. 5: The cellulose content based on variations in airflow (flow rate)

Calorific

Calor value is an indicator of energy content in a substance, including in solid waste. In addition, reliable treatment through biodrying method is expected to increase energy content by drying the solid waste, in order to produce RDF products (Fadlilah and Yudihanto, 2013). Meanwhile, each reactor produced a range 4,575.07 – 4,777.91 cal/g within the first two days. This condition was influenced by the high activities of microorganisms, shown by the moderately thermophilic temperature phase (40 °C to 50 °C). Based on the increased microorganism activity, there was significant consumption of nutrients needed by microorganisms, which influenced the calorific value. Furthermore, a significant escalation was observed on day 15, at 4,643.70 – 6,175.22 cal/g, which was stable up to day 30, in a range of 4,713.36 – 6,265.37 cal/g. This escalation results from a decline in water content. Also, there was a significant reduction on day 15 to 23.75%-38.60%, compared to 54.51%-65.56% reported on day 2. This was due to the markedly high water content and low calorific value on the second day, resulting from the use of heat during evaporation at the process inception. However, the lower value observed on day 15 was due to the relatively lower heat during evaporation, hence reduced water content is directly proportional to increased calorific value. The escalation also occurs because of the derivation of microorganisms activity, and declining temperature (Fig. 3), resulting in low nutrient consumption (Colomer-Mendoza *et al.,* 2013). This condition is congruent with the study by Fadlilah and Yudihanto (2013); Sen and Annachhatre (2015), where the most massive increase in calorific value was observed between days 12 and 16. Based on the statistical test, a significant result of 0.032 (sig<0.05) indicates the significant effect of airflow variation on calorific value, as shown in Fig. 7.

Based on Fig. 7, there was a difference between the control (without the addition of flowrate) and the biodrying reactor. This is evidenced by the insignificant increase in calorific observed in treatments without additional flow 0 L/min/kg, at only 4.58%, with an initial and final value of 4,507.46 and 4,713.36 cal/g, respectively. Conversely, the treatment reactor had an increased value by about 37.29% - 38.19%, where the minimal enhancement was recorded at the rate 3 L/min/kg, with an initial andfinal value of 4,520.98 and 6,206.78 cal/g, respectively. Meanwhile, the maximum change was recognized in the reactor of 6 L/min/kg, with corresponding initial and final value of 4,534.51 and 6,265.37 cal/g. These conditions indicate the influence of airflow rate on calorific value during the bio drying. This research is compatible with Fadlilah and Yudihanto (2013), where the biodrying process performed on solid food waste generated about 4,952 cal/g in flow rate of 6 L/min/kg and 4,064 cal/g for 4 L/min/kg. In addition, the calorific value of the biodrying process was within a range of 4,713 cal/g - 6,265 cal/g, and is further classified in the low energy (brown) coal category, according to SNI 13-6011-1999 concerning the classification of resources

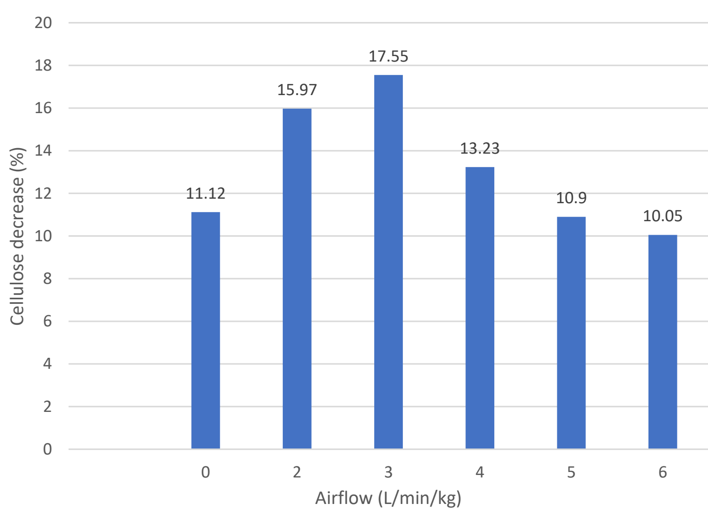


Fig. 6: Derivation cellulose levels (%) at various flow rate variations

and coal reserves, equivalent to <7,000 cal/g. The increase in value is influenced by organic substance degradation, including cellulose. This research showed the least final calorific value in treatments with maximum raw material deterioration, and vice versa. This finding is consistent with Sugni *et al.* (2005), where maximum organic matter degradation produced lower energy content.

SEM analysis (Scanning electron microscopy)

SEM analysis is used to determine the surface morphology of a sample. This shows the physical changes caused by the microbial degradation of solid waste (Sharma *et al.,* 2019). Fig. 8 demonstrates the test result from reactor 2, with an airflow of 2 L/min/kg.

Fig. 8 shows the SEM of solid waste samples on day 0L/min/kg. This features a relatively large size, with smaller cavities/pores, compared to, 15, and 30, with characteristic shrinkage of particle size and escalation of surface cavities. The findings are in line with Sharma *et al.,* (2019), where the cavity size was bigger after the degradation process. This indicates the occurrence of degradation during the 30 days of biodrying.

Greenhouse emission (GHG)

Air emissions are measured to determine the effects of biodrying on solid waste toward the gasses responsible for greenhouse effect, comprising CH_4, CO_2, and N_2O. The measurements are collected on day 0 and at the time when a peak temperature of 42.5 °C is reached. Table 2 shows the result of greenhouse gas emmitted from the decomposition of biodegradable organic matter in MSW, comprising CH_4, CO_2, and N_2O. The sources include leaves (64%), paper (12%), uneaten vegetables (6%), uneaten of meals (1.73%), and fruit peels (0.27%), while plastic waste (16%) are non biodegradable.

CH_4 emissions during the biodrying process

Fig. 9 shows the result of the CH_4 concentration test in the biodrying process, and the output on day 0 was very different between the control (without aeration) at 2.65 ppm (1.34 mg/kg) and solid waste with biodrying treatment at 1.51 ppm (0.73 mg/kg). The conversion of ppm to mg/kg for CH_4, CO_2, and N_2O was based on the calculation of fluxes used to evaluate the experimental data, through second order polynomial equation (gas concentration vs. time) (Hao *et al.,* 2002). In addition, the CH_4 emissions were very low compared to the research of Wang, *et al.,* (2018), which was performed using the combination of biochar, zeolite and wood vinegar for composting pig manure where 8.83 g/kg gas was produced. This current research shows a reduced methane yield with the presence of aeration during the biodrying process. Hellebrand (1998) reported higher values during a decomposition of grass and green waste, and also a more significant output after 30 days of urban waste decomposition. This escalation was considerably reduced by aeration. Yusuf *et al.* (2012) calculated a 28% higher methane emission during anaerobic decomposition, compared to windrow composting.

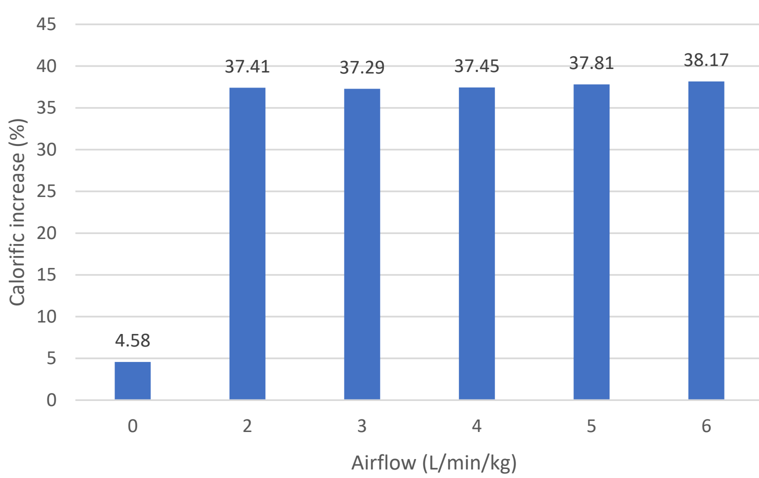
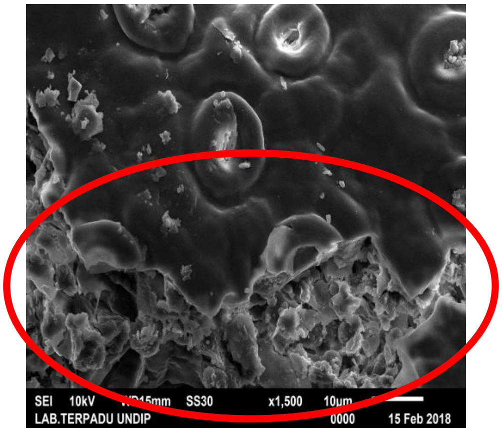
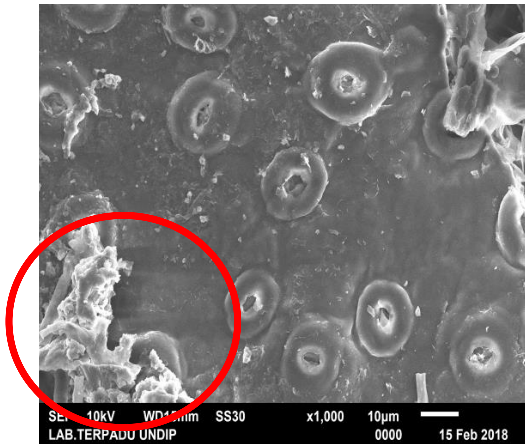


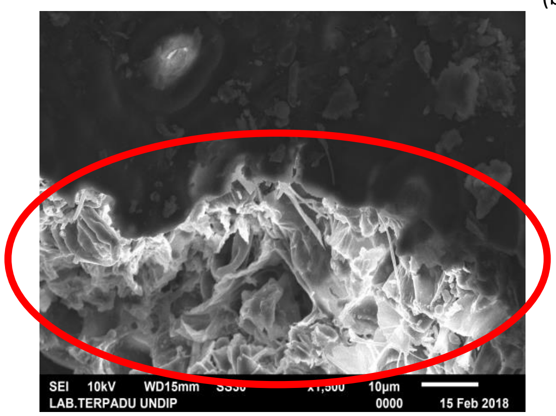
Fig. 7: Percentage increase in calorific value due to variations in flow rate (aeration)



(a)



(b)



(c)

Fig. 8: SEM test result from solid waste sample with flow rate 2 L/min/kg (a) day 0 magnification 1,000x , (b) day 15 magnification 1,500x, (c) day 30 magnification 1,500x

Table 2: Concentrations of CH_4 (ppm), CO_2 (ppm), N_2O (ppb) at day 0, and when the bio is drying reactor temperature reaches its peak

Airflow	CH_4 (ppm)		CO_2 (ppm)		N_2O (ppb)	
(L/min/kg)	1st	2nd	1st	2nd	1st	2nd
0	2.65	11.59	68,888.95	83,153.13	534.69	175.48
2	3.00	3.46	42,804.56	12,706.55	107.78	120.82
3	2.63	3.38	15,920.42	10,848.54	274.57	268.87
4	1.62	2.72	8,408.12	5,602.61	39.22	202.64
5	1.68	3.18	10,069.00	6,621.92	110.33	267.25
6	1.51	3.14	5,153.67	4,393.74	78.80	200.27

CO_2 emissions during the biodrying process

Fig. 10 shows the results of CO_2 concentration test during biodrying, and the graph describes lower levels compared to the treatments without bio-drying. Furthermore, the differences in value between control (without aeration) and solid waste with biodrying treatment was very significant on day 0, at 68.888,95 ppm (2.75 g/kg) and 5,153.67 ppm (0.27 g/kg), respectively (13:1 in comparison). Awasi *et al.,* (2016) reported a CO_2 emission of 10 g C /m^2/d on the 22nd day of sewage sludge composting. Moreover, the study conducted by Wang, *et al.,* (2018), using a combination of biochar, zeolite and wood vinegar for the composting of pig manure yielded 116.5 g/kg/d.

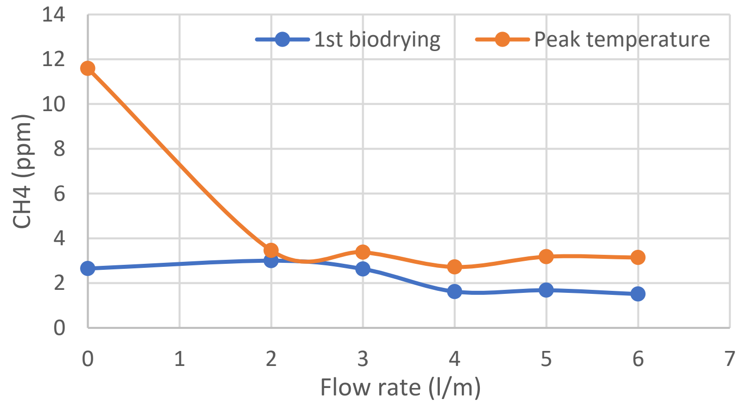


Fig. 9: The CH_4 levels (ppm) at 0 days and at the time the temperature reaches its peak.

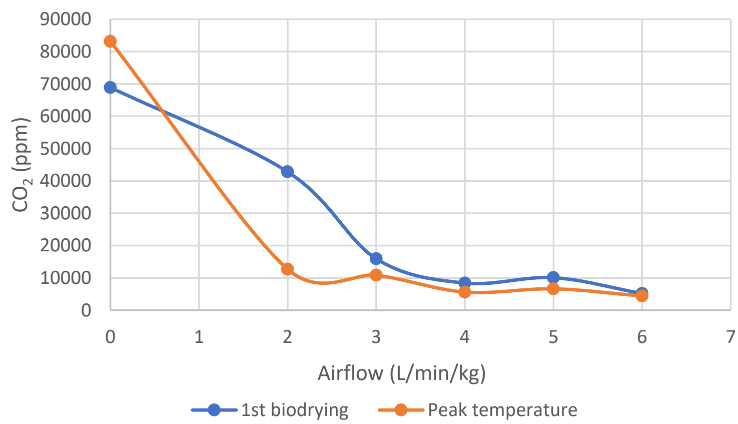


Fig. 10: Graph of CO_2 levels (ppm) at 0 days and when the temperature reaches its peak

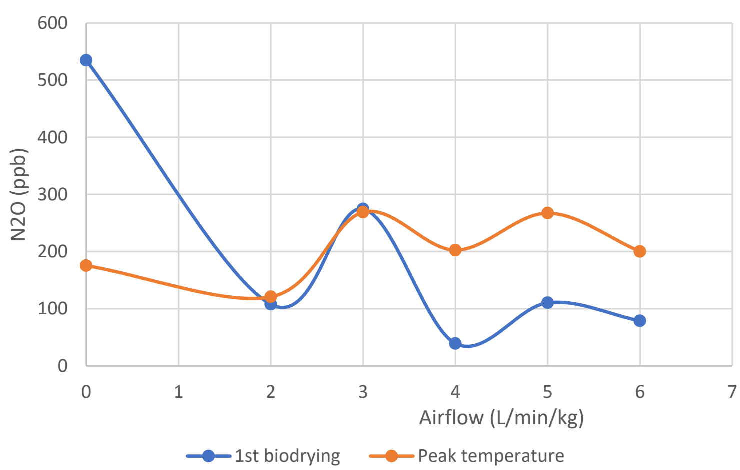


Fig. 11: Graphs of N_2O levels (ppm) at day 0 and when they reach their peak temperature

N_2O emissions during the biodrying process

Fig. 11 shows the result of N_2O concentration testing during the biodrying process, and a higher value was recorded at the peak temperature (Thermophilic). A study conducted by Wang, *et al.*, (2018), using a combination of biochar, zeolite and wood vinegar for composting pig yielded 47.29 mg/kg of N_2O emissions. According to Paul (2001), the nitric oxide released during thermophilic composting is generally higher. This often occurs as a side product of nitrification, involving the oxidation of ammonium into nitrate and denitrification. In addition, heterotrophic nitrification processes also play a major contributory role during production.

CONCLUSION

This research aims to evaluate the increase in calorific value, as well as the degradation process, and greenhouse gas emissions from MSW (refuse derived fuel), using biodrying. The results showed a higher calorific value to about 37.29% - 38.19% or 4,713 cal/g - 6,265 cal/g, which is classified in the low energy coal (brown coal) category, being <7,000 cal/g. Furthermore, the most significant temperature reached was 43 °C on second day, as observed in reactor 2 (airflow 2 L/min/kg). The lowest water content of 28.37% was produced by the solid waste in reactor 3 (airflow 3 L/min/kg). Therefore, the biodrying process ensured a successful reduction in sample moisture compared to the control. The lowest cellulose reduction of 10.05% was observed in reactor 6 (6 L/min/kg). In addition, degradation of C-Organic and Total Nitrogen was slow and not significant, hence the potential for application as fuel. Based on SEM, MSW morphology on day 0 showed larger sized molecules with smaller cavities/pores. The treatment process results in lower GHG emissions compared to the control. Furthermore, the highest CH_4 emissions, measuring 11.59 ppm was observed at the peak temperature of 43 °C, while the CO_2 concentration of control (without aeration) and solid waste exposed to biodrying was 68,888.95 ppm and 5,153.67 ppm, respectively (13: 1 ratio). Meanwhile, the N_2O concentration was 534.69 ppb and 175.48 ppb at the inception of research and during the peak temperature. The lowest level was recorded in reactor with air flow rate of 2 L/min/kg. The MSW biodrying was confirmed to increase the calorific value and reduce greenhouse gas emissions. This inhibits the possibility of sample discharging into the final processing. Appropriate Therefore, proper strategy is needed to understand other factors influencing the heat value and GHG emissions.

AUTHOR CONTRIBUTIONS

B. Zaman performed idea, developing theories, and funding. M. Hadiwidodo performed ideas, developed theories and calculations. W. Oktiawan performed ideas, verified research methods, encouraged B. Zaman and M. Hadiwidodo to investigate specific aspects, and supervised research. Purwono performed verifying research methods, analyzing data, and conducting research. E. Sutrisno performed verification methods and helped supervise the study. All authors discuss the results, and contribute to the preparation of the manuscript.

ACKNOWLEDGMENTS

Thanks to DRPM DIKTI for funding this study through PTUPT Grant No. [101-136/UN7.P4.3/PP/2018] for financing year 2018.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest regarding the publication of this work. In addition, the ethical issues including plagiarism, informed consent, misconduct, data fabrication and, or falsification, double publication and, or submission, and redundancy have been completely witnessed by the authors.

ABBREVIATIONS

°C	Derajat celcius
cal/g	Calorie/gram
cm	Centimeter
CH_4	Methane
CO_2	Carbon dioxide
FTD	Flame Thermionic Detector
gC/m²/d	Gram carbon per square meter per day
g/kg/d	Gram per kilogram per day
GHG	Greenhouse gas
m^3	Cubic metre
MBT	Mechanical biological treatment
mg/kg	Milligram per kilogram

MSW	Municipal Solid Waste
N_2O	Nitrous oxide
L/min/kg	Liters per minute per kilogram
ppb	Part per billion
ppm	Part per million
RDF	Refused Derived Fuel
SEM	Scanning electron microscopy
SD card	Secure Digital Card
SNI	Indonesian National Standard
TPA	Final processing

REFERENCES

Adani, F.; Baido, D.; Calcaterra, E.; Genevini, P.L., (2002). The influence of biomass temperature on biostabilization-biodrying of municipal solid waste. Bioresour. Technol., 83(3): 173–179 **(7 pages).**

Anindyawati, T., (2010). Potensi selulase dalam mendegradasi lignoselulosa limbah pertanian untuk pupuk organik. Berita Selulosa., 45(2): 70–77 **(8 pages).**

Astuti, F.W., (2016). Kandungan lignoselulosa hasil fermentasi limbah sayur dan jerami padi menggunakan inokulum kotoran sapi dengan variasi lama inkubasi. Universitas Muhammadiyah.

Awasthi, M. K.; Wang, Q; Huang, H; Ren, X; Lahori, A. H; Mahar, A; Ali, A; Shen, F; Li, R; Zhang, Z, (2016). Influence of zeolite and lime as additives on greenhouse gas emissions and maturity evolution during sewage sludge composting. Bioresour. Technol.,216; 172–181 **(10 pages).**

Bilgin, M.; Tulun, Ş., (2015). Biodrying for municipal solid waste: Volume and weight reduction. Environ. Technol., 36(13): 1691–1697 **(7 pages).**

Colomer-Mendoza, F. J.; Herrera-Prats, L.; Robles-Mart´ınez, F.; Gallardo-Izquierdo, A.; Pi˜na-Guzm´an, A., (2013). Effect of airflow on biodrying of gardening wastes in reactors. J. Environ. Sci., 25(5): 865–872 **(8 page).**

Egan, A.; Baddeley, A.; Joe, S.; Whiting, K., (2005). Mechanical-Biological-Treatment : A Guide for Decision Makers Processes, Policies and Market.

Evangelou, V.P., (1998). Environmental Soil and Water Chemistry : Principles and Applications. John Wiley and Sons, Inc.

Fadlilah, N.; Yudihanto, G., (2013). Pemanfaatan Sampah Makanan Menjadi Bahan Bakar Alternatif dengan Metode Biodrying. Teknik POMITS, 2(2): 289–293 **(5 page).**

Frei, K. M.; Cameron, D.; Stuart, P.R., (2004). Novel Drying Process Using Forced Aeration Through a Porous Biomass Matrix. Dry. Technol., 22(5): 1191–1215 **(25 page).**

Garg, A.; Smith, R.; Longhurst, P.J.; Pollard, S.J. .; Simms, N.; Hill, D., (2007). Comparative evaluation of SRF and RDF co-combustion with in bed combustor. Proceedings of the Eleventh International Waste Management and Landfill Symposium: 1–8 **(8 page).**

Goering, H.K.; van Soest, P.J., (1970). Forgae fibre analysis. USDA Agricultural Handbook.

González, D.; Guerra, N.; Colón, J.; Gabriel, D.; Ponsá, S.; Sánchez, A., (2019). Filling in sewage sludge biodrying gaps : Greenhouse gases , volatile organic compounds and odour emissions. Bioresour. Technol., 291: 1-8 **(8 page).**

Goyal, S.; Dhull, S.K.; Kapoor, K.K., (2005). Chemical and biological changes during composting of different organic wastes and assessment of compost maturity. Bioresour. Technol., 96: 1584–1591 **(8 page).**

Hellebrand, H.J., (1998). Emission of Nitrous Oxide and other Trace Gases during Composting of Grass and Green Waste. J. Agric. Eng. Res., 69(4): 365–375 **(11 page).**

Hao, X.; Chang, C.; Larney, F.J.; Travis, G.R., (2002). Greenhouse Gas Emissions during Cattle Feedlot Manure Composting. J. Environ. Qual.; 31: 700–700 **(10 page).**

Howard, R.L.; Abotsi, E.; Jansen van Rensburg, E.L.; Howard, S., (2003). Lignocellulose biotechnology : issues of bioconversion and enzyme production. Afr. J. Biotechnol., 602–619 **(18 page).**

Huang, D.L., (2010). Changes of microbial population structure related to lignin degradation durin lignocellulosic waste composting. Biosour. Technol., 101(1): 4062–4067 **(6 page).**

Jalil, N.A.A.; Basri, H.; Basri, N.E.A.; Abushammala, M.F.M., (2016). Biodrying of municipal solid waste under different ventilation periods. Environ. Eng. Res.; 21(2) : 145–151 **(7 page).**

Jokiniemi, H.T.; and Ahokas, J.M., (2014). Drying process optimisation in a mixed-flow batch grain dryer. Biosyst. Eng.; 121: 209–220 **(12 page).**

Li, X.; Dai, X.; Yuan, S.; Li, N.; Liu, Z.; Jin, J., (2015). Thermal analysis and 454 pyrosequencing to evaluate the performance and mechanisms for deep stabilization and reduction of high-solid anaerobically digested sludge using biodrying process. Bioresour. Technol., 175: 245–253 **(9 page).**

Ma, J.; Zhang, L.; Li, A., (2016). Energy-efficient co-biodrying of dewatered sludge and food waste : Synergistic enhancement and variables investigation. Waste Manage., 56: 411–422 **(12 page).**

Maulini-duran, C.; Artola, A.; Font, X.; Sánchez, A., (2013). A systematic study of the gaseous emissions from biosolids composting : Raw sludge versus anaerobically digested sludge. Bioresour. Technol., 147: 43–51 **(9 page).**

Pan, J.; Cai, H.; Zhang, Z.; Liu, H.; Li, R.; Mao, H.; Awasthi, M. K.; Wang, Q.; Zhai, L., (2018). Comparative evaluation of the use of acidic additives on sewage sludge composting quality improvement, nitrogen conservation, and greenhouse gas reduction. Bioresour. Technol, 270: 467–475 **(9 page).**

Patil, A.A.; Kulkarni, A.A.; Patil, B.B., (2014). Waste to energy by incineration. J. Comput. Technol., 3(6): 12–15 **(4 page).**

Perazzini, H.; Freire, F.B.; Freire, F. B.; Freire, J.T., (2016). Treatment of Solid Wastes Using Drying Technologies : A Review. Dry. Technol., 34(1): 37–41 **(5 page).**

Rada, E.C.; Ragazzi, M., (2015). Energy from waste : The role of biodrying . U.P.B. Sci. Bull.; 2: 67–72 **(6 page).**

Rincón, C.A.; De Guardia, A.; Couvert, A.; Le Roux, S.; Soutrel, I.; Daumoin, M.; Benoist, J.C., (2019). Chemical and odor characterization of gas emissions released during composting of solid wastes and digestates. J. Environ. Manage, 233: 39–53 **(15 pages).**

Sadaka, S.; Ph, D.; Eng, P.; Vandevender, K.; Costello, T.; Ph, D.; Sharara, M., (2011). Partial Composting for Biodrying Organic Materials. University of Arkansas.

Scheutz, C.; Pedersen, R. B.; Petersen, P.; Jorgensen, J.; Ucendo, I.; Monster, J., (2014). Mitigation of methane emission from an old unlined landfill in Klintholm, Denmark using a passive biocover system. Waste Manage., 34: 1179–1190 **(12 page).**

Sen, R.; Annachhatre, A.P., (2015). Effect of Airflow rate and residence time on biodrying of cassava peel waste. Int. J. Environ. Technol. Manage., 18(1): 9–29 **(21 page)**.

Sharma, A.; Ganguly, R.; Kumar, A., (2019). Spectral characterization and quality assessment of organic compost for agricultural purposes. Int. J. Recycl. Organic Waste Agric., 8: 197–213 **(17 page).**

Shen, Y.; Bin Chen, T.; Gao, D.; Zheng, G.; Liu, H.; Yang, Q., (2012). Online monitoring of volatile organic compound production and emission during sewage sludge composting. Bioresour. Technol., 123: 463–470 **(8 page).**

Siswanto, M.H.; Mahendra, F., (2012). Perekayasaan Nanosilika Berbahan Baku Silika Lokal Sebagai Filler Kompon Karet Rubber Air Bag Peluncur Kapal Dari Galangan. Prosiding InSiNas: 56–59 **(4 page).**

Sudrajat, R., (2006). Mengelola Sampah Kota. Penebar Swadaya.

Sugni, M.; Calcatera, E.; Adani, F., (2005). Biostabilization-biodrying of municipal solid waste by inverting air-flow. Bioresour. Technol., 96(12): 1331–1337 **(7 page).**

Suksankraisorn, K.; Patumsawad, S.; Fungtammasan, B., (2010). Co- firing of Thai lignite and municipal solid waste (MSW) in a fluidised bed : Effect of MSW moisture content. Appl. Thermal Eng., 30: 2693–2697 **(5 page).**

Tom, A.P.; Haridas, A.; Pawels, R., (2016). Biodrying Process Efficiency: -Significance of Reactor Matrix Height. Procedia Technol., 25: 130–137 **(8 page).**

Velis, C.A.; Longhurst, P.J.; Drew, G.H.; Smith, R.; Pollard, S.J.T., (2009). Biodrying for mechanical-biological treatment of wastes: A review of process science and engineering. Bioresour. Technol., 100(11): 2747–2761 **(15 page).**

Wagland, S.T.; Kilgallon, P.; Coveney, R.; Garg, A.; Smith, R.; Longhurst, P.J.; Pollard, S.J.T.; Simms, N., (2011). Comparison of coal / solid recovered fuel (SRF) with coal / refuse derived fuel (RDF) in a fluidised bed reactor. Waste Manage., 31: 1176–1183 **(8 page).**

Wang, Q. Wang, Q.; Awasthi, M. K.; Ren, X.; Zhao, J.; Li, R.; Wang, Z.; Wang, M.; Chen, H.; Zhang, Z., (2018). Combining biochar, zeolite and wood vinegar for composting of pig manure: The effect on greenhouse gas emission and nitrogen conservation. *Waste Manag.* 74: 221–230 **(9 page).**

Wardhani, A.; Sutrisno, E.; Purwono, P., (2017). Pengaruh Variasi Debit Aerasi Terhadap Kadar Selulosa Dan Nilai Kalor Pada Metode Biodrying Municipal Solid Waste (MSW). Universitas Diponegoro.

Widarti, B.N.; Wardhini, W.K.; Sarwono, E., (2015). Pengaruh Rasio C/N Bahan Baku Pada Pembuatan Kompos Dari Kubis dan Kulit Pisang. Integrasi Proses. 5(2) : 75–80 **(6 page).**

Yusuf, R.O.; Noor, Z.Z.; Abba, A.H., (2012). Greenhouse Gas Emissions : Quantifying Methane Emissions from Livestock. Am. J. Eng. Appl. Sci., 5(1): 1–8 **(8 page).**

Zhang, D.; He, P.; Jin, T.; Shao, L., (2008). Bioresource Technology biodrying of municipal solid waste with high water content by aeration procedures regulation and inoculation. Bioresour. Technol., 99: 8796–8802 **(7 page).**

Zhao, L.; Gu, W.; He, P.; Shao, L., (2010). Effect of air-flow rate and turning frequency on biodrying of dewatered sludge. Water Res., 44: 6144–6152 **(9 page).**

AUTHOR (S) BIOSKETCHES

Zaman, B., Ph.D., Instructor, Department of Environmental Engineering Faculty of Engineering Diponegoro University, Semarang, Indonesia. Email: badruszaman2@gmail.com

Oktiawan, W., M.Sc., Instructor, Department of Environmental Engineering Faculty of Engineering Diponegoro University, Semarang, Indonesia. Email: w_oktiawan@yahoo.com

Hadiwidodo, M., M.Sc., Instructor, Department of Environmental Engineering Faculty of Engineering Diponegoro University, Semarang, Indonesia. Email: mch323@yahoo.com

Sutrisno, E., M.Sc., Instructor, Department of Environmental Engineering Faculty of Engineering Diponegoro University, Semarang, Indonesia. Email: endrosutrisno57@gmail.com

Purwono, P., M.Sc., Instructor, Center Science and Technology, IAIN Surakarta, Pandawa, Pucangan, Kartasura, Indonesia
Email: purwono.ga@gmail.com

HOW TO CITE THIS ARTICLE

Zaman, B.; Oktiawan, W.; Hadiwidodo, M.; Sutrisno, E.; Purwono, P., (2021). Calorific and greenhouse gas emission in municipal solid waste treatment using biodrying. Global J. Environ. Sci. Manage., 7(1): 33-46.

DOI: 10.22034/gjesm.2021.01.03

url: https://www.gjesm.net/article_43616.html

Global J. Environ. Sci. Manage. 7(1): 47-58, Winter 2021

Global Journal of Environmental Science and Management
(GJESM)

Homepage: https://www.gjesm.net/

ORIGINAL RESEARCH PAPER

Willingness of end users to pay for e-waste recycling

H.T.T. Nguyen[1], C.-H. Lee[2], R.-J. Hung[3,*]

[1]*The University of Danang - University of Science and Education, Le Duan Road, Danang 550000, Vietnam*

[2]*Center for Interdisciplinary Research on Ecology and Sustainability, College of Environmental Studies, National Dong Hwa University, Hualien 97401, Taiwan*

[3]*International Bachelor Degree Program in Finance, College of Management, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan*

ARTICLE INFO

Article History:
Received 21 February 2020
Revised 28 May 2020
Accepted 29 June 2020

Keywords:
E-waste management
Logistic regression
Payment preferences
Vietnam
Willingness to pay

ABSTRACT

BACKGROUND AND OBJECTIVES: The sheer volume of electrical and electronic waste (e-waste) has presently been generated in Vietnam, posing a growing concern regarding its impact can have on the environment and human health. Therefore, the need for developing policies and regulations towards the environmentally sound management of e-waste is becoming crucial. Although the municipalities play an important role in e-waste recycling program, there does not appear to be any study involving residents' perceptions on e-waste management. This paper aims to examine the influencing factors of end users' willingness to pay and their payment preferences toward e-waste recycling.

METHODS: The logistic regression model was employed to analyze a qualified data set collected through a personal interview survey in Danang city, Vietnam. All analyses were conducted using Statistical Package for Social Sciences software (version 22.0).

FINDINGS: The results revealed that the end users' willingness to participate in recycling programs, laws and regulations, inconvenience of recycling and past experience were four key determinants significantly contributing to the willingness to pay for recycling e-waste. With regards recycling payment methods, most of the participants (36%) were in favor of deposit and refund scheme, while pre-disposal fees and advanced recycling fees came in second and third place (25.8% and 21%, respectively), making monthly payment of recycling fees the least preferred (10.2%).

CONCLUSION: These findings may provide policy-makers with crucial information for better e-waste management policy development, which helps address the conflict between development and conservation, may be applicable in Vietnam and other countries as well.

DOI: 10.22034/gjesm.2021.01.04

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
36	2	3

*Corresponding Author:
Email: *bruce@mail.npust.edu.tw*
Phone: +886-8-770-3202
Fax: +886-8-7740497

Note: Discussion period for this manuscript open until April 1, 2021 on GJESM website at the "Show Article.

INTRODUCTION

Electrical and electronic waste (e-waste) is the most emblematic by-product of the transition to a more digital world and it continues to grow at unprecedented rates around the globe (Baldé *et al.*, 2017; Balde *et al.*, 2015). Parallel with the huge number of e-waste generation, the crisis of e-waste management has quickly spread to all regions over the word (Nguyen *et al.*, 2017; Nnorom *et al.*, 2009). Similar to other developing countries, Vietnam is currently facing with an emerging concern on the excessive quantities of e-waste generated without appropriate disposal and treatment (Nguyen *et al.*, 2009). In Vietnam, there are three main sources for the generation of e-waste, those are, the disposal of electrical and electronic equipment (EEE) from households and others organizations, importation of used EEE, and waste from the manufacturing process at electronics companies (Huynh, 2014; Tran and Salhofer, 2016). According to the survey conducted by Nguyen *et al.* (2009), it is predicted that the number of e-waste generated is projected to peak at around 17.2 million pieces in 2025. Even, the quantity of e-waste is estimated to be far larger if other hidden e-waste sources (e.i. from illegal transboundary movement international trash flows under the disguise of "used goods") are detected. In addition, e-waste in Vietnam is predominantly handled by unofficial channels such as scrap dealers or unregistered units, which transfer waste to craft villages for recycling (Nguyen *et al.*, 2009; Tran and Salhofer, 2016; Truong, 2014). Therefore, one of the huge challenges when looking at the picture of e-waste in Vietnam is that the e-waste treatment activities are scattered among various craft village and illegal import-export activities that makes e-waste statistical data is very narrow (Huynh, 2014; Tran and Salhofer, 2016; Truong, 2014). Moreover, the rate of formal e-waste recycling in Vietnam is still low although recycling has been widely considered one of the waste management approaches to utilize valuable materials and reduce the burden of dumping waste (Hai *et al.*, 2017; Song *et al.*, 2012; Tran and Salhofer, 2016). The reason behind this problem is that e-waste recycling facilities and laws have not been well developed by Vietnamese government (BCRCSEA, 2016); consequently, e-waste flow is now under the control of informal sector. Under unregulated conditions, after major valuable materials of e-waste are retrieved, the remaining components are improperly treated, either dumped in the landfill, burned or illegally traded overseas (Hai *et al.*, 2017). This not only causes the enormous loss of valuable and critical raw materials from the supply chain, but also leads to serious health, environmental and societal problems through illegal transboundary treatment of waste among developing countries (Roldan and Gibby, 2018). Due to the large amount of e-waste is collected by the informal sectors, there is only a small percentage of e-waste can be collected and recovered by official recycling and disposal units (Nguyen *et al.*, 2017; Tran and Salhofer, 2016; Yoshida *et al.*, 2016). Another reason comes from the fact that the investment for establishment and operation of e-waste recycling system is extremely high; thus, it requires the cooperation among all stakeholders including the government, producers, retailers, and consumers in sharing this financial responsibility (Hai *et al.*, 2017). In fact, according to Cai *et al.* (2019), e-waste is considered not to be economical for formal recyclers to process, that is the reason why cheap manual labors can take the advantage of doing informal e-waste activity. In addition, according to Honda *et al.* (2016), the lack of Vietnamese residents' awareness and willingness to pay (WTP) a recycling fee is one of the barriers which hampers the achievement of recycling program. Many Vietnamese end users are more likely to send their e-waste to the informal mechanisms for getting the cash benefit, while the treatment activities of these sectors are causing environmental damages. Facing to this serious problem of e-waste, in 2015, the Prime Minister released Decision 16 stipulating that manufacturers and importers of electronic products must take responsibility for collecting their product waste (Nguyen *et al.*, 2017; Tran and Salhofer, 2016). Recently, Circular No. 34/2017/TT-BTNMT dated October 4th, 2017 of Ministry of Natural Resources and Environment on recall and treatment of discarded products, emphasizes the responsibility of producers on establishment of the site for collecting obsolete devices. However, e-waste still has not been put under control because there have been no other legal documents that guide the implementation of the decision (BCRCSEA, 2016). Accompanying with the governmental sector, an organization established in 2015, called "Viet Nam Tai Che" (Vietnam Recycles) made an effort to collect and recycle defective products or e-waste in a safe and environmentally friendly way. Nevertheless, the Vietnam Recycles just ran the pilot phase and took place in the Hanoi and Ho Chi Minh city regions only. As stated in several previous studies (Bhat and Patil, 2014; Nixon and Saphores, 2007; Song *et al.*, 2012), end users play an important role in the success of e-waste recycling program; hence, understanding what drives people to engage in recycling plays a fundamental role in order to design more effective environmental policies to deal with arising e-waste problems. Recent studies that have aimed to find out the relationship between individuals' WTP for e-waste recycling and both internal and external variables showed the mixed results (Saphores *et al.*, 2006). Take two studies conducted in China as example, besides education level and household income, age and region were statistically significant predictors of residents' WTP in Macau and national scale in China (Song *et al.*, 2012; Yin *et al.*, 2014). Similarly, Nixon and Saphores (2007) concluded that age, living area, income, education level, convenience, recycling habit, and environmental attitudes significantly affected the consumers' WTP. However, Vassanadumrongdee and Kittipongvises (2018) revealed that none of the socio-economic factors was significant while environmental awareness, subjective norms, and inconvenience had significant effects on households' WTP. In agreement with the latter point, it was found that environmental awareness, laws and regulations were key factors clarifying the willingness towards e-waste recycling (Wang *et al.*, 2016; Yu *et al.*, 2014). In contrast, Wang *et al.* (2011) revealed that knowledge of environmental laws were not significant factors for the explanation of the individual's willingness to recycle e-waste. It can be seen that there are mixed dimensions showed the links between demographic, socio-economic and psychological characteristics and WTP for recycling. However, little is known about end users' WTP and their preferences toward e-waste recycling in Vietnam so far. It is true that the research on the consumers' WTP for e-waste recycling is critically rare in Vietnam, compared to other countries such as America (Nixon and Saphores, 2007; Nixon *et al.*, 2009), Nigeria (Nnorom *et al.*, 2009), India (Dwivedy and Mittal, 2013), Malaysia (Afroz *et al.*, 2013), Macau (Song *et al.*, 2012), and national scale of China (Cai *et al.*, 2019; Wang *et al.*, 2011; Yin *et al.*, 2014), where various studies have placed emphasis on the WTP and preferences toward e-waste recycling. The limited published academic paper related to the WTP and preferences of Vietnamese consumers toward e-waste recycling causes difficulties for environmental managers and legislators to design effective policies to tackle e-waste recycling problems. Therefore, it is very important to explore the relationship between influencing factors and end users' decision to pay for e-waste recycling. Especially, if Vietnamese government aims to achieve appropriate future policies and develop properly e-waste recycling facilities, it is an urgent request to carry out an in-depth study on the end users' WTP for recycling programs and their preference in terms of payment method toward this waste stream. Based on the understanding of end users' preferences and their WTP for recycling e-waste, it is expected to provide the new look at Vietnamese's WTP for e-waste recycling, which is a useful reference for policy-makers to establish recycling strategies and set proper level of recycling fee. This will, in turn, encourage people to be willing to pay the fee for recycling programs, helping to improve the e-waste situation in Vietnam. Moreover, such study may contribute to a narrow current multidisciplinary literature on pro-environmental behavior (PEB) including recycling by eliciting the combined influences of both socio-economic and psychological antecedents on the willingness of end users to pay for e-waste recycling. Finally yet importantly, this kind of research will provide a useful lesson and scientific knowledge from the outlook of an emerging nation, by introducing e-waste policy schemes to the global e-waste community and sharing experience to other countries which are currently facing similar e-waste challenges. Overall, in response to the critical concern of e-waste, this study targets to examine the key socio-economic and psychological determinants motivating the willingness of end users to pay e-waste recycling fee by employing logistic regression as a data analysis tool. In addition, the end users' preferences of e-waste recycling pattern were analyzed from two pillars: recycling payment methods, and reasons for respondents' disagreement to pay for recycling. To achieve these objectives, the research survey was conducted in residential areas of Danang city, Vietnam in 2018.

MATERIALS AND METHODS

Survey design and data collection

The questionnaire used for this study included three major parts, which was developed to examine the key socio-economic and psychological factors influencing the end users' WTP for recycling e-waste and their preferences toward four recycling payment patterns. In the first section, a five-point Likert-scale was adopted to obtain the information of influencing variables namely environmental awareness, laws and regulations, inconvenience of recycling, cost of recycling, and past experience. The ranges of Likert-scale were from one to five, referring to a series from "strongly disagree" to "strongly agree". The second section uncovered the end users' willingness to participate, WTP (binary "yes or no" questions were used) and their recycling preferences (favorite payment methods, and reasons for respondents' rejection to pay for recycling). The last section gathered socio-demographic information including gender, age, education level, family size, monthly household income, and residential area. Before asking the respondents to answer all the questions in three above-described main parts, the trained interviewers informed respondents about the purposes of study and explained clearly all the specific terms used in the content of questionnaire, with the aim to make sure the respondents understood. There were six typical discarded appliances used as the targets of this study, including fridge, air conditioner, television, mobile phone, personal computer (desktop), and laptop. The questionnaire was pretested and had minor changes before conducting the actual survey. The data of this study was collected through face-to-face interviews between July and August 2018. With the aim to have better capture the variety of the city's population, the proportionate stratified random sampling was employed in this study, which involves taking random samples from stratified groups, in proportion to the population. In this approach, the sample size of each subgroup (which was classified by district) was directly proportional to the population size of the entire population of districts. That means each district sample has the same sampling fraction. After that, a systematic random sampling was taken to select the interviewees who were asked to fill the survey's questionnaires. As a result, 545 questionnaires were distributed to households living in six urban districts namely Lien Chieu, Thanh Khe, Cam Le, Ngu Hanh Son, Hai Chau, and Son Tra, shown in Fig. 1. After removing unqualified questionnaires, a set of 520 qualified ones was used for further examination. Danang, the fourth big city in Vietnam with a population of 1.08 million, is located in the zone of typical tropical monsoon. The city's average temperature is about 26.5^{0}C, the highest is 28.9 - 30.1^{0}C from May to September and the lowest is 21.5 - 24.2^{0}C from December to March. The average humidity is 79.7%, while the average annual rainfall is 2539.1 mm/ year (GSO, 2019). The reason why Danang was chosen to be a survey's area is the fact that the city government has a determination to build Danang as an environmentally-friendly city. The city's environmental plan covers the goal of achieving 70% of solid waste recycled and reused. In order to fulfill that objective and ensure the effectiveness of recycling program in the future, it is very important to have a better understanding of end users' recycling preferences, which is worthwhile for e-waste recycling policy design and implementation.

Fig. 1: Geographic location of the study area; (a) Vietnam, (b) Danang city

Analytical framework

In this study, respondents were asked to answer the question "Are you willing to pay e-waste recycling fee?" to express their WTP for recycling by making a choice from two alternatives either "Yes" (coded as "1") or "No" (coded as "0"). As the dependent variable is in 0 - 1 style, a logistic multiple regression was utilized in this work to explore the significant determinants influencing end users' WTP for recycling e-waste. The explanatory variables included socio-demographic, psychological and other external variables and were estimated in two stages. In the first step, socio-demographic predictors (gender, age, education, household size, income, and residential area) were entered into the model. Psychological and other external variables (environmental awareness, social pressure, laws and regulations, inconvenience of recycling, cost of recycling, past experience and willingness to participate) were then included in the second step. The data in this work were analyzed by using the statistical package of social sciences (IBM SPSS 22.0) software.

RESULTS AND DISCUSSION

Descriptive statistics and factor analysis for variables entering the analysis

The principal component analysis with varimax rotation was conducted to reduce a large set of Likert-scale variables (16 items of psychological and situational factors) to a small set that entered into the logistic regression model. To determine the appropriateness of the data for factor analysis, The Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy and Bartlett's test of sphericity were used to check the data. KMO test is a statistic that measures the proportion of variance among variables might be caused by underlying factors. The higher value of KMO (close to 1) is, the more suited the data is. Bartlett's test of sphericity checks the hypothesis of whether a matrix is significantly different from an identity matrix. Therefore, a significant Bartlett's test of sphericity is required ($p < 0.05$) indicates that a factor analysis may be useful with your data (Hair, 2010). The results from this run showed that the KMO value was 0.739, greater than a critical value of 0.7 (Hair, 2010), and the Bartlett's test is highly significant at p-value < 0.001, approved that the factor analysis could be applied. The result of the factor analysis performance indicates that five components were extracted with the explained variance was 72.74%. Two items whose factor loadings were smaller than 0.5 were deleted, therefore, the remainders were 14 items. To assess the reliability of the components, Cronbach's alpha test was used to check how closely related a set of items are as a factor and the results was in range of 0.672 -0.860, which was greater than the accepted value of 0.6 (Hair, 2010) (Table 1). Five factors including environmental awareness (x_8), laws and regulations (x_9), inconvenience of recycling (x_{10}), past experience (x_{11}) and cost of recycling (x_{12}) represents five out of twelve explanatory variables used in logistic model later.

It is necessary to have a brief summary of descriptive statistics of key variables entered into the logistic model, shown in Table 2. The values of explanatory variables x_1-x_6 come from section 3 of the research's questionnaire showing the socio-economic characteristics of the interviewees across the six

Table 1: Results of the principal component analysis with varimax rotation

Predictors	Number of items	Loading	Variance (%)
Environmental awareness (Cronbach's alpha = 0.860, KMO=0.769, Bartlett: p<0.001)	3	0.833 0.841 0.859	27.713
Laws and regulations (Cronbach's alpha = 0.821, KMO=0.769, Bartlett: p<0.001)	3	0.823 0.852 0.741	15.145
Inconvenience of Recycling (Cronbach's alpha = 0.777, KMO=0.769, Bartlett: p<0.001)	3	0.759 0.862 0.854	12.590
Past experience (Cronbach's alpha = 0.672, KMO=0.769, Bartlett: p<0.001)	3	0.822 0.789 0.710	9.767
Cost of recycling (Cronbach's alpha = 0.749, KMO=0.769, Bartlett: p<0.001)	2	0.889 0.868	7.525

districts. The remaining independent variables (x_7-x_{12}) come from section 1 and 2, while x_7 represents the willingness of end users to participate in the e-waste recycling program, x_8-x_{12} are five variables results from factor analysis. Before performing a logistic regression analysis, multicollinearity problem was detected by testing the tolerance and its reciprocal, called variance inflation factor (VIF). The VIF value of all variables in this study was less than 1.5, indicating that there was no multicollinearity problem among 12 explanatory variables.

Determinants of end users' WTP for e-waste recycling

A regression model was performed under two steps to measure the predictors of end users' WTP for recycling e-waste. All socio-economic and demographic variables entered into the model in the first step were categorical variables with the highest level taken as reference category except that the baselines of gender, education level, and family size were lowest levels. The remaining factors were added in step 2 including scale variables except for the willingness to participate being binary variable. The Hosmer-Lemeshow test from the SPSS output was used to measure the goodness of fit of the logistic regression model. According to the Hosmer-Lemeshow test from step 1, $p = 0.316 > 0.05$, which was not significant, indicating that the regression model is well fitted. The classification accuracy of the model was 62.3%. The set of socio-demographic variables had a mixed influence on respondent's WTP. The results of step 1 shown in Table 3 indicated that age, which was statistically significant at the 1‰ significance level, was in accordance with the results of the previous related studies (Song *et al.*, 2012; Vassanadumrongdee and Kittipongvises, 2018). This implies that the probability of paying for recycling e-waste is associated with respondents' age. Of which the age groups of (18 - 20), (21 - 30) and (41 - 50) years old were proved to be significant factors for WTP and tended to have a higher willingness than the age group of above 60 years old (the reference group) with the coefficient being 1.059, 0.941 and 0.945, respectively. Meanwhile, the results of odds ratio indicated that the odds of approval for paying recycling fee of three above age groups were 2.56 - 2.88 times higher than that of those whose age was over 60. It can be explained that the young generation who are equipped with knowledge about environmental protection and conservation seems to be more willing to pay for recycling. In terms of education level variable, only "master or above" was significant at the 10% level and its coefficient and odds ratio were 1.439 and 4.22, respectively,

Table 2: Definitions and descriptive statistics for variables entering the analysis

Variables	Scale	Sample size	Mean	S.D.
Dependent variable				
Willingness to pay (WTP)	0 = not to be WTP, 1 = to be WTP	520	0.525	0.500
Independent variables				
Gender (x_1)	0 = female, 1 = male	520	0.406	0.492
Age (x2)	1 = less than and equal 20 years, 2 = (21-30) years, 3 = (31-40) years, 4 = (41-50) years, 5 = (51-60) years, 6 = 61 years and above	520	3.073	1.276
Education level (x3)	1 = lower secondary, 2 = upper secondary, 3 = college/ vocational, 4 = university, 5 = masters or above	520	3.700	0.975
Family size (x4)	1 = one, 2 = two, 3 = three, 4 = fours, 5 = five, 6 = more than five	520	4.058	1.143
Monthly household income (x5)	1 = less than 6 million VND†, 2 = (6-10) million VND, 3 = (11-15) million VND, 4 = (16-20) million VND, 5 = more than 20 million VND	520	1.829	0.993
Residential area (x6) ‡	1 = Thanh Khe district, 2 = Hai Chau district, 3 = Lien Chieu district, 4 = Son Tra district, 5 = Ngu Hanh Son district, 6 = Cam Le district	520	2.852	1.728
Willingness to participate (x7)	0 = not participate in e-waste recycling, 1 = participate in e-waste recycling	520	0.902	0.298
Environmental awareness (x8)	1 = strongly disagree,	520	4.348	0.715
Laws and regulations (x9)	2 = disagree,	520	4.121	0.723
Inconvenience of recycling (x10)	3 = neutral,	520	3.508	0.983
Past experience (x11)	4 = agree,	520	2.181	0.861
Cost of recycling (x12)	5 = strongly agree	520	3.651	0.941

†Vietnam Dong (1USD = 23,065 VND) (data from The State Bank of Vietnam on 01/06/2019)
‡1 (31%), 2 (20.4%), 3 (13.5%), 4 (14.8%), 5 (8.5%), 6 (11.9%)

implying that the odds of WTP for respondents who get higher education were 4.22 times higher than they were for those with lower secondary education level (the reference category). It is in agreement with the results from study conducted by Song *et al.* (2012) while others previous studies reported that education had no or minor role in engaging residents' PEB and readiness to afford recycling payment (Saphores *et al.*, 2012; Vassanadumrongdee and Kittipongvises, 2018). The finding from this study may be explained that those who get higher degree have more opportunities to enrich their knowledge on e-waste and understand

Table 3: Estimated regression coefficients of the logistic regression model predicting WTP

Predictors (Independent variables)	Step 1		Step 2	
	β	Exp(β)	β	Exp(β)
x_1-Gender *(base = "female")*				
Male	-0.108	0.897	-0.176	0.839
x_2-Age *(base = age "> 60 years old")*				
<=20 years old	1.059^{*}	2.883	0.904	2.471
(21-30) years old	0.941^{*}	2.561	1.004^{*}	2.728
(31-40) years old	0.170	1.185	0.080	1.084
(41-50) years old	0.945^{*}	2.574	1.047^{*}	2.849
(51-60) years old	-0.056	0.946	-0.190	0.827
x_3-Education level *(base = "lower secondary")*				
Upper secondary	0.536	1.708	0.872	2.393
College/ Vocational education	1.193	3.296	1.126	3.083
University	1.002	2.723	0.878	2.407
Masters/ above	1.439^{+}	4.216	1.358	3.889
x_4-Family size *(base = "> 5 members")*				
1 member	-0.072	0.931	-0.058	0.944
2 members	0.000	1.000	0.274	1.316
3 members	0.543	1.721	0.648^{+}	1.912
4 members	0.085	1.089	0.198	1.219
5 members	0.073	1.076	0.120	1.127
x_5-Monthly household income *(base = "< 6 million VND")*				
(6-10) million VND	-0.108	0.898	-0.166	0.847
(11-15) million VND	-0.493	0.611	-0.512	0.599
(16-20) million VND	0.105	1.111	0.208	1.232
>20 million VND	-0.357	0.700	-0.345	0.708
x_6-Residental area *(base = "Cam Le")*				
Thanh Khe	0.423	1.526	0.411	1.509
Hai Chau	0.220	1.246	0.324	1.382
Lien Chieu	0.131	1.140	0.180	1.197
Son Tra	0.507	1.660	0.636	1.889
Ngu Hanh Son	-0.093	0.911	-0.122	0.885
Intercept	-1.719^{*}	0.179		
x_7-Willingness to participate			2.344^{***}	10.422
x_8-Environmental awareness			-0.195	0.823
x_9-Laws and regulations			0.542^{**}	1.719
x_{10}-Inconvenience of recycling			-0.219^{*}	0.803
x_{11}-Past experience			0.354^{**}	1.425
x_{12}-Cost of recycling			0.125	1.133
Intercept			-5.742^{***}	0.003
-2LL	679.122		614.746	
	χ^2=40.450, df=24, p<0.05		χ^2=104.826, df=30, p<0.001	
Nagelkerke R^2	10.00%		24.40%	
Hosmer and Lemeshow test	p = 0.316		p = 0.178	
Classification accuracy	62.30%		66.00%	

$^{+}p<0.10$, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

the importance of environmental protection; as a result, they tend to have more probability to pay for recycling fee that is used for running recycling system. Therefore, it can be concluded that education is expected to play a key factor to enhance the level of environmental awareness of households. Other variables considered include gender, monthly income, family size, and residential area but they were not statistically significant. While respondents' gender and family size were not statistically significant and supported by other studies (Cai *et al.*, 2019; Song *et al.*, 2012; Vassanadumrongdee and Kittipongvises, 2018), the residential area had insignificant influence which was different to what found by Dwivedy and Mittal (2013). Similarly, the non-significance of gender and household income was contrasted with similar findings in the study of community's willingness to join in drop-off recycling activity in California (Dwivedy and Mittal, 2013; Nixon and Saphores, 2007; Nnorom *et al.*, 2009; Saphores *et al.*, 2006).

Moving to the second step, along with demographic variables, psychological and external factors including five variables from factor analysis and one variable of willingness to participate were introduced into the model, altogether yielded a model of 66% corrected classification. The test of Hosmer-Lemeshow had $p = 0.178$, proving that the model with these factors was a perfect fit to the data. Omnibus tests of model coefficients showed a significant beyond 0.001, implying that there was an improvement over the model in step 1. Nagelkerke R^2 and the 2-Log likelihood also showed the improvement from step 1. While the values of Nagelkerke R^2 rose from 10% to 24.4%, the 2-Log likelihood fell from 679.122 to 614.746, presenting more accuracy of the predicted model. Hence, all goodness-of-fit indices indicated that the inclusion of psychological factors and other factors strengthened the accuracy of the model. In the full model, age still had a positive impact on WTP; specifically, respondents whose age at (21 - 30) and (41 - 50) years old had more WTP for recycling e-waste compared with those whose age was over 60 years old. In addition, while education level variables became insignificant which was similar to the report of previous studies (Vassanadumrongdee and Kittipongvises, 2018; Wang *et al.*, 2011), a household with three members showed the significant positive impact on the WTP of end users. The odds of WTP was 1.91 times higher for a family of three than they were for a family with over five members. Similar to the first step, it is also observed that gender, income, and residential area had no significant impact on end users' WTP in this step. Regarding one binary independent variable in this model, willingness to participate become the strongest variable which affected the willingness of end users toward recycling fees, its coefficient was 2.344 and significant at 1‰ level. From the odds ratio, it can be seen that the odds of respondents who showed their willingness to participate were 10.42 times more likely to pay for e-waste recycling fee than those who were not willing to participate. It is also found that past experience and laws and regulations could have a positive effect on end users' WTP, which is similar to the outcome of recent studies (Nduneseokwu *et al.*, 2017; Vassanadumrongdee and Kittipongvises, 2018). Their odds ratios reveal that for each one point increase on the five-point past experience and laws and regulations scale, there were 1.43 and 1.72 times of the odds that people will be willing to pay for recycling, respectively. However, it is in disagreement with the results from Wang *et al.* (2011), those authors reported that law was not an influential factor motivated Beijing residents' e-waste recycling behavior. Out of four variables from factor analysis that showed statistically significant influence on WTP, only inconvenience of recycling had negative coefficient (-0.219, sig. at 5% level) which was similar to the results from other researches (Nixon *et al.*, 2009; Nguyen *et al.*, 2018; Vassanadumrongdee and Kittipongvises, 2018). In fact, Nixon *et al.* (2009) stated that inconvenience as a major factor restricting residents from appropriate e-waste management. Alternatively, the work's finding could be interpreted that inverting the odds ratio for the inconvenience of recycling indicates that with one point increase on the five-point inconvenience of recycling scale being associated with the odds of not being willing to pay increasing by a multiplicative factor of 1.25. It indicates that the more inconvenient people feel, the less willingness they have to do e-waste recycling. The remaining variables such as environmental awareness and cost of recycling were not statistically significant, which confirms previous survey result of Nixon *et al.* (2009). From those above mentioned findings, it is obvious that the crucial importance is to encourage more and more people to engage themselves in e-waste recycling systems. Once they get used to the recycling performance, and in turn, forming their recycling habit, then the possibility of paying the recycling fee will increase. This suggestion can be proved by the study's findings demonstrated the statistically significant impacts of both the willingness of end users' participation and their past recycling experience on WTP for recycling e-waste. To fulfill the goal of encouraging the participation of end users toward recycling, it should be emphasized that the establishment of recycling services and facilities plays an extremely crucial roles, which helps to open the door to end users' WTP. With this in mind, in the context of the lack of formal recycling channels and services in Vietnam, building up e-waste recycling infrastructure should be put on the top priority as the cornerstone step for the enhancement of e-waste management system.

End users' preferences toward e-waste recycling

The end users' preferences of e-waste recycling pattern were analyzed from two aspects: payment methods, and reasons for respondents' disagreement to pay for recycling. The results from this survey showed that more than half of the respondents (52.5%) answered "Yes" for the question: "If the government develops a sustainable e-waste management infrastructure and recycling facilities, are you willing to pay e-waste recycling fee?" In other words, there were still nearly half of the respondents (47.5%) who were not willing to pay for recycling e-waste. Among 247 participants who disagreed to pay the fee, the majority of them (40.5%) stated that users did not have any responsibility to cover the e-waste recycling fee; followed by 30.8% of the respondents admitting that they preferred to pass their used EEE to informal sectors (peddlers, scrap dealers, and secondhand market) with the aim to get monetary benefits. It reflects the reality that informal e-waste recycling sectors have been predominating in Vietnam for years, while official recycling channels are extremely limited and show ineffective performance (Hai *et al.*, 2017; Tran and Salhofer, 2016). Not only in Vietnam, other previous studies also indicated that the end users were more likely to sell their old products rather than to cover the recycling fees (Islam *et al.*, 2016). While 27.1% of the households are concerned of their insufficient extra income which could not afford recycling fee, only 1.2% and 0.4% of them answered that they had other reasons or refused to answer this question, respectively. A small proportion of people gave the reasons that they did not believe that recycling service and infrastructure required for the recycling or appropriate management of e-waste could be settled in developing countries like Vietnam. In addition, they also added that the e-waste program should be launched in developed countries, but not in Vietnam. The findings exploring the reason why end users are not willing to cover the e-waste recycling cost in this study were completely similar to what Song *et al.* (2012) reported in their studies performed in Macau, a special administrative region of China. The explanation for the coincidence is that both countries are experiencing from the similar situation of e-waste with the predominance of informal sectors, not mention to the similarity between two countries' culture and lifestyles. Regarding the payment method, the end users' preferences were explored by asking all respondents to choose which payment method they preferred from four options, namely advanced recycling fee (ARF), pre-disposal fee (PDF), monthly recycling fee (MRF), and deposit and refund scheme (DRS). ARF is a system that consumers pay recycling fee at the time they buy a new product and the product price covered the fee; on the contrary, PDF refers to the method that consumers pay a fee at the point of disposal. While MRF is the style of paying the fee every month which is widely employed in municipal waste management (applied for solid waste and wastewater), DRS is a surcharge on a product when purchased and a rebate when it is returned (Dwivedy and Mittal, 2013; Walls, 2011). All the fee collected is subsequently invested in e-waste recovery and recycling projects (Nixon and Saphores, 2007). Most interviewees (36.0%) supported for the last style (DRS), while PDF and ARF were accepted by a roughly similar percentage of respondents, 25.8% and 21.0%, respectively, making MRF was the least preferred payment method, with only 10.2%. In addition, there were 37 out of 520 (7.1%) respondents reported that they could not make a decision of which payment style they prefer, shown in Fig. 2. Comparing to other studies, while Indian residents supported for PDF, Californian people considered PDF was least preferred and the majority of them chose the ARF style (Dwivedy and Mittal, 2013; Nixon *et al.*, 2009). The ARF was also accepted by the majority of Chinese residents since its convenience surpassed PDF, DRS and MRF (Song *et al.*, 2012; Wang *et al.*, 2011). Chinese people refused DRS because they assumed that the deposit system seems to be a complicated process. However, the favor of Vietnamese respondents in DRS method can be explained that end users felt at least they could receive a certain amount of money so-called refund at the end, being similar to what they sold their end

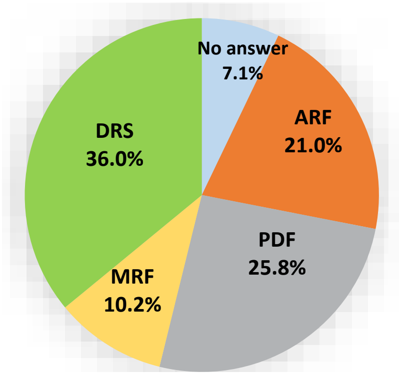


Fig. 2: End users' preferred payment modes toward e-waste collection recycling

of life devices to secondhand shops. In fact, DRS was recommended as a financial support tool for the success of e-waste collection program in Ho Chi Minh City in the study of (Le *et al.*, 2012). The study findings are also consistent with the statement of Uwasu *et al.* (2013), they concluded that DRS was relatively cheaper than the PDF payment method, especially when the consumers tended to have less concern towards product prices than recycling fees. In addition, some studies have stated that DRS are more economical than other methods of waste disposal management, among which command-and-control schemes, recycling subsidies, and advance disposal fees (Anderson and Lohof, 1997). Take a real applied DRS in South Korea as an example, the Korean consumers have responsibility for paying the e-waste recycling fee. This country is getting success in employing DRS with high rate of e-waste collection, in which deposit is set at a fixed rate on products and the refund is returned after the recycling of the items (Islam *et al.*, 2016).

CONCLUSION

This study investigated factors affecting end users' preference and WTP for recycling e-waste in Danang city, which may have contributions to establishing a proper and effective recycling system to fix the problem of e-waste in Vietnam. The empirical results indicated that end users' participation willingness toward recycling activities, laws and regulations, past experience, and inconvenience of recycling performed orderly strongest impacts on the willingness of end users to pay recycling fee. Among four above determinants, only inconvenience of recycling showed a negative effect, while three of the remaining variables were positive. With those findings, in order to launch a successful e-waste recycling program, it is very important to have high rate of end users' encouragement, especially for those who have experience of waste recycling. In addition, the establishment of recycling services and facilities also plays an extremely crucial role in easing the participation of end users in recycling e-waste. Most importantly, future legislation which targets to an e-waste recycling policy should be established which emphasizes the responsibility of end users and a need to have a cooperation mechanism amongst various stakeholders such as the government, producers, consumers, and especially informal sectors. For example, the government should provide high-level support for informal division with financial stimulus to create a partnership with the formal sector. In this way, mutual benefits can be gained: on the one hand, the government can have better control of unofficial recycling activities; on the other hand, more individuals will turn to participate in the formal recycling system. In terms of the recycling payment mode, most of the respondents in this study showed their favor in DRS method; hence, it is really a good idea to integrate DRS in extended producer responsibility implementation in order to stimulate resident's engagement in e-waste collection, and promote return and reuse, aiming to boost recycling rates. Further studies should be conducted to examine the feasibility of integration deposit refund system as a segment of the legislative initiative. On the other hand, the recycling fee, when being imposed, should be considered carefully and appropriately. If the fee is too high, a large number of poor people cannot afford recycling fee, leading to several bad consequences, even they are more likely to have illegal disposal e-waste to avoid paying the fees. In contrast, if the recycling fee is too low, it raises a concern that such fee is lower than the actual cost invested in recycling activities. Therefore, future studies should take into consideration appropriate recycling fees, which can harmonize the sake of consumers and the effective performance of recycling activities. In summary, besides the voluntary engagement of end users and the power of laws and regulations, activating end users' WTP for recycling e-waste strongly depends on not only the readiness of e-waste recycling facilities but also their e-waste recycling habits. Findings taken from this research are expected to be an academic knowledge source for getting the better understanding end users' WTP, which helps policy-makers and environmental managers to design and improve the effectiveness of recycling policies in Vietnam. Globally, this study also contributes the literature to lay the foundations for a successful e-waste management policy applying in the same social, cultural and economic regions.

AUTHOR CONTRIBUTIONS

H.T.T. Nguyen, R.-J. Hung, and C.-H. Lee were responsible for the theoretical framework formulation, research design, data analysis, and writing of the manuscript. H.T.T. Nguyen managed with data collection.

ACKNOWLEDGEMENT

The authors would like to thank a team of trained interviewers that helped us to collect data during the survey.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the publication of this manuscript. In addition, ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy were completely observed by the authors.

ABBREVIATIONS

%	Percent
‰	Per thousand
-2LL	-2 Log Likelihood
ARF	Advanced Recycling Fee
DRS	Deposit and Refund Scheme
EEE	Electrical and Electronic Equipment
E-waste	Electrical and Electronic Waste
Exp(β)	The exponentiation of the coefficient, known as Odds Ratio
KMO	Kaiser-Meyer-Olkin
mm	Millimeter
MRF	Monthly Recycling Fee
No. 34/2017/ TT-BTNMT	Circular number 34, released on October 4th, 2017 of Ministry of Natural Resources and Environment
°C	Degree Celsius
PDF	Pre-Disposal Fee
PEB	Pro-environmental Behavior
S.D.	Standard Deviation
SPSS	Statistical Package of Social Sciences
USD	United States Dollar
VIF	Variance Inflation Factor
VND	Vietnam Dong
WTP	Willingness to Pay
β	Coefficient
χ^2	Chi-Square

REFERENCES

Afroz, R.; Masud, M.M.; Akhtar, R.Duasa, J.B., (2013). Survey and analysis of public knowledge, awareness and willingness to pay in Kuala Lumpur, Malaysia–a case study on household WEEE management. J. Clean. Prod., 52: 185-193 **(9 pages).**

Anderson, R.C.; Lohof, A.Q., (1997). The United States Experience with Economic Incentives in Environmental Pollution Control Policy. Environmental Law Institute (in cooperation with United States Environmental Protection Agency, Washington, DC.

Baldé, C.P.; Forti, V.; Gray, V.; Kuehr, R.; Stegmann, P., (2017). The Global E-waste Monitor 2017: Quantities, Flows, and Resources. United Nations University (UNU), Bonn/Geneva/Vienna.

Balde, C.P.; Wang, F.; Kuehr, R.; Huisman, J., (2015). The global e-waste monitor 2014: Quantities, flows and resources. UNU-IAS institute for the Advanced study sustainability, Bonn, Germany.

BCRCSEA, (2016). Study on E-Waste Management in ASEAN Countries. Workshop 2016 of the Asian Network for Prevention of Illegal Transboundary Movement of Hazardous Wastes. Semarang, Indonesia.

Bhat, V.; Patil, Y., (2014). E-waste consciousness and disposal practices among residents of Pune city. Procedia Soc. Behav. Sci., 133: 491-498 **(8 pages).**

Cai, K.; Song, Q.; Peng, S.; Yuan, W.; Liang, Y.; Li, J., (2019). Uncovering residents' behaviors, attitudes, and WTP for recycling e-waste: a case study of Zhuhai city, China. Environ. Sci. Pollut. Res., 27(2): 2386-2399 **(14 pages).**

Dwivedy, M.; Mittal, R.K., (2013). Willingness of residents to participate in e-waste recycling in India. Environ. Dev., 6: 48-68 **(21 pages).**

GSO, (2019). Statistical yearbook of Vietnam 2018. General Statistics Office, Vietnam.

Hai, H.T.; Hung, H.V.; Quang, N.D., (2017). An overview of electronic waste recycling in Vietnam. J. Mater. Cycles Waste Manage., 19(1): 536-544 **(9 pages).**

Hair, J.F., (2010). Multivariate data analysis : a global perspective. Pearson Education, Upper Saddle River, N.J.; London.

Honda, S.; Khetriwal, D.; Kuehr, R., (2016). Regional E-waste Monitor: East and Southeast Asia. United Nations University ViE–SCYCLE, Bonn, Germany.

Huynh, T.H., (2014) Electric and Electronic Waste Recycling in Vietnam. 4th International E-waste Management Network. Hanoi, Vietnam.

Islam, M.T.; Abdullah, A.B.; Shahir, S.A.; Kalam, M.A.; Masjuki, H.H.; Shumon, R.; Rashid, M.H., (2016). A public survey on knowledge, awareness, attitude and willingness to pay for WEEE management: Case study in Bangladesh. J. Clean. Prod., 137: 728-

740 **(13 pages).**

Le, V.K.; Tran, M.C.; Pham, M.C. (2012) Assessment of E-Waste Collection Model in Ho Chi Minh City. The 5th AUN/SEED-Net Regional Conference on Global Environment. Bandung, Indonesia.

Nduneseokwu, C.K.; Qu, Y.; Appolloni, A., (2017). Factors influencing consumers' intentions to participate in a formal e-waste collection system: A case study of Onitsha, Nigeria. Sustainability, 9(6): 881 **(17 pages).**

Nguyen, D.-Q.; Ha, V.-H.; Eiji, Y.; Huynh, T.-H., (2017). Material Flows from Electronic Waste: Understanding the Shortages for Extended Producer Responsibility Implementation in Vietnam. Procedia CIRP, 61: 651-656 **(6 pages).**

Nguyen, D.-Q.; Yamasue, E.; Okumura, H.; Ishihara, K.N., (2009). Use and disposal of large home electronic appliances in Vietnam. J. Mater. Cycles Waste Manage., 11(4): 358-366 **(9 pages).**

Nguyen, T.T.H.; Hung, R.-J.; Lee, C.-H; Nguyen, T.T.H., (2018). Determinants of Residents' E-Waste Recycling Behavioral Intention: A Case Study from Vietnam. 11(1): 164 **(24 pages).**

Nixon, H.; Saphores, J.-D.M., (2007). Financing electronic waste recycling Californian households' willingness to pay advanced recycling fees. J. Environ. Manage., 84(4): 547-559 **(13 pages).**

Nixon, H.; Saphores, J.-D.M.; Ogunseitan, O.A.; Shapiro, A.A., (2009). Understanding Preferences for Recycling Electronic Waste in California: The influence of environmental attitudes and beliefs on willingness to pay. Environ. Behav., 41(1): 101-124 **(24 pages).**

Nnorom, I.; Ohakwe, J.; Osibanjo, O., (2009). Survey of Willingness of Residents to Participate in Electronic Waste Recycling in Nigeria - A case study of mobile phone recycling. J. Clean. Prod., 17(18): 1629-1637 (**9 pages**).

Roldan, J.M.; Gibby, A., (2018) Developing an e-waste national policy and regulatory framework for Malawi. Internatonal Telecommunicaton Union (ITU), Switzerland.

Saphores, J.-D.; Nixon, H.; Ogunseitan, O.; Shapiro, A., (2006). Household Willingness to Recycle Electronic Waste: An Application to California. Environ. Behav., 38(2): 183-208 **(26 pages).**

Saphores, J.-D.M.; Ogunseitan, O.A.; Shapiro, A.A., (2012). Willingness to engage in a pro-environmental behavior: An analysis of e-waste recycling based on a national survey of U.S. households. Resour. Conserv. Recycl., 60: 49-63 **(15 pages).**

Song, Q.; Wang, Z.Li, J., (2012). Residents' behaviors, attitudes, and willingness to pay for recycling e-waste in Macau. J. Environ. Manage., 106: 8-16 **(9 pages).**

Tran, C.D.; Salhofer, S.P., (2016). Analysis of recycling structures for e-waste in Vietnam. J. Mater. Cycles Waste Manage., 20: 110-126 **(17 pages).**

Truong, M.T., (2014). Current status of e-waste in Vietnam and future goals, Hanoi, Vietnam. Vietnam Environment Administration.

Uwasu, M.; Naito, T.; Yabar, H.; Hara, K., (2013). Assessment of Japanese recycling policies for home electric appliance: Cost-effectiveness analysis and socioeconomic and technological implications. Environ. Dev., 6: 21-33 **(13 pages).**

Vassanadumrongdee, S.; Kittipongvises, S., (2018). Factors influencing source separation intention and willingness to pay for improving waste management in Bangkok, Thailand. Sustain. Environ. Res., 28(2): 90-99 **(10 pages).**

Walls, M., (2011). Deposit-refund systems in practice and theory. Resources for the Future. Washington, DC.

Wang, Z.; Guo, D.; Wang, X., (2016). Determinants of residents' e-waste recycling behaviour intentions: Evidence from China. J. Clean. Prod., 137: 850-860 **(11 pages).**

Wang, Z.; Zhang, B.; Yin, J.; Zhang, X., (2011). Willingness and behavior towards e-waste recycling for residents in Beijing city, China. J. Clean. Prod., 19(9-10): 977-984 **(8 pages).**

Yin, J.; Gao, Y.; Xu, H., (2014). Survey and analysis of consumers' behaviour of waste mobile phone recycling in China. J. Clean. Prod., 65: 517-525 **(9 pages).**

Yoshida, A.; Terazono, A.; Ballesteros, F.C.; Nguyen, D.-Q.; Sukandar, S.; Kojima, M.; Sakata, S., (2016). E-waste recycling processes in Indonesia, the Philippines, and Vietnam: a case study of cathode ray tube TVs and monitors. Resour. Conserv. Recycl., 106: 48-58 **(11 pages).**

Yu, L.; He, W.; Li, G.; Huang, J.; Zhu, H., (2014). The development of WEEE management and effects of the fund policy for subsidizing WEEE treating in China. Waste Manage., 34(9): 1705-1714 **(10 pages).**

AUTHOR (S) BIOSKETCHES

Nguyen, H.T.T., M.Sc., Instructor, The University of Danang - University of Science and Education, Le Duan Road, Danang 550000, Vietnam. Email: *hongbkes@gmail.com*

Lee, C.-H., Ph.D., Professor, Centre for Interdisciplinary Research on Ecology and Sustainability, College of Environmental Studies, National Dong Hwa University, Hualien 97401, Taiwan. Email: *chlee@gms.ndhu.edu.tw*

Hung, R.-J., Ph.D., Professor, International Bachelor Degree Program in Finance, College of Management, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan. Email: *bruce@mail.npust.edu.tw*

HOW TO CITE THIS ARTICLE

Nguyen, H.T.T.; Lee, C.-H.; Hung, R.-J., (2021). Willingness of end users to pay for e-waste recycling. Global J. Environ. Sci. Manage., 7(1): 47-58.

DOI: 10.22034/gjesm.2021.01.04

url: https://www.gjesm.net/article_43154.html

Global J. Environ. Sci. Manage. 7(1): 59-78, Winter 2021

Global Journal of Environmental Science and Management
(GJESM)

Homepage: https://www.gjesm.net/

ORIGINAL RESEARCH PAPER

The ability of layered double hydroxides for nitrate absorption and desorption in crop and fallow rotation

M. Mohammadi[1], A. Mohammadi Torkashvand[1], P. Biparva[2,*], M. Esfandiari[1]

[1]Department of Soil Science, Science and Research Branch, Islamic Azad University, Tehran, Iran

[2]Department of Basic Science, Sari University of Agricultural Sciences and Natural Resources, Sari, Iran

ARTICLE INFO

Article History:
Received 14 March 2020
Revised 12 June 2020
Accepted 11 July 2020

Keywords:
Leaching
layered double hydroxides (LDHs)
Nitrate exchange
Qualitative properties

ABSTRACT

BACKGROUND AND OBJECTIVES: This study aims down to evaluate the ability of chloride magnesium- aluminium- layered double hydroxides (4:1) for nitrate adsorption from the soil solution in successive cropping periods.
METHODS: The study was conducted under long-term cropping periods, including first crop): bell pepper; second crop: mentheae; third crop: cherry tomato; and fort h crop: wheat), absorption of soil mineral nitrate in fallow periods and nitrate absorption from plants by layered double hydroxides. The effect of layered double hydroxides on qualitative and quantitative characteristics of plants was also studied.
FINDINGS: Results indicated that layered double hydroxides were able to induce long-term nitrate exchange in crop and fallow sequences. Layered double hydroxides can adsorb soil excessive nitrates in cropping periods and reduce nitrate concentration in the soil solution. Compared to control, the treatment with 16 gram layered double hydroxide/kilogram soil could reduce nitrate concentration in the soil solution by 95%. During two-week fallow periods, the amount of nitrates mineralized in the soil solution was increased, but layered double hydroxides treatments could adsorb them well and maintained the N-nitrate concentration in the soil solution at a low level. Additionally, Results indicated that application of 2, 4, 8 and 16 gram layered double hydroxides/ kilogram soil led to 34%, 44%, 58% and 69% reduction in N-nitrate concentration of soil leachates, respectively, compared to control. By increasing nitrogen availability, layered double hydroxides improved the quantitative and qualitative properties of plants. Application of 2, 4, 8 and 16 gram layered double hydroxides/ kilogram soil increased the plant height (cherry tomato) by 14%, 26%, 50% and 80%, respectively.
CONCLUSION: It is concluded that the layered double hydroxides has a potential to be used as a long-term nitrate exchanger to control the movement of nitrate in soil, and thereby reduce risks of nitrate leaching in crop production in sensible areas.

DOI: 10.22034/gjesm.2021.01.05

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
46	11	6

*Corresponding Author:
Email: *p.biparva@sanru.ac.ir*
Phone: +989114590887
Fax: +981133687367

Note: Discussion period for this manuscript open until April 1, 2021 on GJESM website at the “Show Article.

INTRODUCTION

Nitrogen is one of the plant macronutrients playing a crucial role in improving the yield of most plants. Nitrogen fertilizers are used to fulfil the plant nutritional demand (Haider *et al*., 2017). The rates of nitrogen fertilizers applied in farmlands are usually more than the real nitrogen need of crops (Canter, 2019). More than 90% of the world's urea production is used as nitrogen fertilizers. When urea fertilizer is used in the soil, urea is first converted to ammonia (NH_3) or ammonium ion (NH^{4+}) and bicarbonate ion (HCO_3^-) by urea enzyme. A group of soil microorganisms called Nitrosomonas converts ammonium to nitrite, and then nitrite is converted to nitrate by Nitrobacter. The process of converting ammonium to nitrate is called nitrification. This process is relatively quick and takes a few days (Overdahl *et al*., 1991). Nitrate leaching in crop fields depends not only on the amount of inorganic nitrogen at the harvesting time but also on the subsequent mineralization of nitrogen. To reduce inorganic nitrogen of soil at the harvesting time, the N_{min} nitrogen fertilizer method recommended by Wehrmann and Scharpf, (1986) was used during the plant growth period; in this method, nitrate leaching was observed from late fall to early spring. Nitrate leaching occurs during this period by the large amount of soil water and the mineralization of nitrogen (Köhler *et al*., 2006). To reduce the amount of inorganic nitrogen in this period, cover crops are used, which again this depends on the date of cultivation, adaptation, crop type and its root system capacity to reach deeper layers of the soil. Nevertheless, implementation of ecological farming methods may not reduce nitrate leaching (Torres-Dorante and Lammel, 2009; Köhler *et al*., 2006). However, nitrate leaching control in the soil by adsorbing it from the soil solution, in the same way that clay minerals such as vermiculites and smectites adsorb potassium and ammonium cations from the soil solution, can be an appropriate option to reduce nitrate leaching in the soil (Torres-Dorante and Lammel, 2009; Elmi *et al*., 2019). The use of a special exchanger with nitrate adsorption ability is the best way to reduce soil leaching in the soil (Torres-Dorante and Lammel, 2009).Recently, it has been suggested to apply layered double hydroxides (LDHs) or anionic clay minerals as anionic exchanger in order to increase both the efficiency of nitrogen application and the soil capacity to buffer nitrate (Halajnia *et al*., 2016; Torres-Dorante *et al*., 2008). LDHs are a group of non-silicate layered compounds with positive charges. Hydrotalcite is the main and the most dominant LDH mineral, which can be called the hydrotalcite-type compound (Berber *et al*., 2014; Bernardo *et al*., 2017). Having brucite-like layers with positive charges and relatively weak interlayer bonds, LDHs exhibit an excellent ability to adsorb inorganic anions (Halajnia *et al*., 2013; Benicio *et al*., 2018; Chubar, 2018). Koilraj *et al*. (2013); Berber *et al*. (2014) and Everaert, *et al*. (2017) mentioned LDH as a slow-release fertilizer under laboratory conditions. Halajnia *et al*. (2016) suggested LDH as a nitrate exchanger to reduce the risk of nitrate leaching when growing corn in the soil column. in preliminary experiments were conducted Mg-Al-LDH (4:1) with the highest nitrate adsorption capacity in aqueous and soil solutions of 188.67 and 107.52 mg/g (Milligram per gram) respectively, was selected for proceeding studies. Furthermore, Mg-Al-LDH (4:1) could remove nitrate preferentially in the presence of other anions. In addition, nitrate exchanged nitrate 20 times in different concentrations while its adsorption capacity was not decreased (Mohammadi *et al*., 2019). Proceeding studies were conducted to evaluate the nitrate adsorption capacity of chloride Mg-Al-LDH (4:1) under long-term cropping periods, the mineral nitrate adsorption during fallows, the plant nitrate absorption from LDH, and the effects of LDH on quantitative and qualitative characteristics of plants.

The objective of the present study was to evaluate a) the nitrate adsorption capacity of chloride Mg-Al-LDH (4:1) under long-term cropping periods, b) the mineral nitrate adsorption during fallows, c) the plant nitrate absorption by LDH, and d) the effect of LDH on quantitative and qualitative characteristics of plants. The present study was conducted during 2018 - 2019 in Tehran, Iran, under greenhouse conditions.

MATERIALS AND METHODS

Synthesis and properties of LDH

Chloride Mg-Al-LDH with the M^{2+}/M^{3+} ratio of 4:1 was synthetized by the co-precipitation method as explained in the previous study by Mohammadi *et al*. (2019). The maximum capacities of LDH for nitrate adsorption in aqueous and soil solutions were measured as 188.67 and 107.52 mg/g, respectively (Mohammadi *et al*., 2019).

Preparation of pots and treatments

This study was conducted in a 16-month period under laboratory environmental conditions at the mean daily temperature of 26±3 Centigrade (°C) and the mean might temperature of 21±3 °C. The laboratory was located in the Department of Soil Science, Science and Research Branch, Islamic Azad University, Tehran, Iran. The study was conducted in the form of completely randomized design with 5 treatments and 21 replicates. Treatments included 0 (control), 2, 4, 8 and 16 g LDH/kg soil. The soil used was collected from Maraveh Tappeh, Golestan, Iran (40° 82´ 01´´ N, 41° 94´ 77´´ E). The soils were air dried, passed through a 2 mm sieve and kept for testing. Some properties of the studied soil are presented in Table 1. Sandy soil was used for the experiment. It should be noted that the soil was poor in organic matter, and the amount of its nitrate was zero and its acidity was slightly alkaline. The soil had a sandy texture. Soil bulk density value was 1.32 g/cm^3.

First crop (bell pepper) and first fallow

To provide green bell pepper, transplant trays (55×27 centimetre (cm)) were filled with peat moss and perlite in May 2018, and Traviata variety bell pepper seeds were planted in regular distances. The seeds were covered with 0.5 cm soil on the top and irrigated uniformly. When the plants reached a height of 10 cm in June 2018, 3 of them were transplanted in each pot. Additionally, 5 kg air-dried soil, nitrogen fertilizer and LDH treatments were mixed and filled in the pots. LDH was applied as a dry powder. In preliminary trials (data not shown), this amount was found to supply 200% of the total N requirement of the vegetable. This rate was selected in order to supply “an excess” of nitrate to the soil allowing therefore to evaluate the ability of the LDH to adsorb nitrate, and its influence on plant uptake. Furthermore, 756 milligram (mg) of nitrogen from the urea fertilizer source (46.5% nitrogen) was added to each pot (with 5 kg of soil). to support the plant growth and emphasize the plant nutrient needs and soil nutrient content, Basic fertilizers rates of 150 mg Mg, 300 mg P, 500 mg K and 150 mg S from magnesium sulphate ($MgSO_4$), superphosphate (KH_2PO_4), and potassium sulphate (K_2SO_4) sources were added to the liquid form, respectively. Micronutrients were added using a solution of 5 g Hortimicro-2 in 1 L water. The fertilizer contained 7% iron chelated with EDTA, 1.7% zinc chelated with EDTA, 3% manganese chelated with EDTA, 0.25% copper chelated with EDTA, 0.25 soluble Boron and 0.35 soluble Molybdenum. There was 20-cm space between the pots. Irrigation was carried out according to the plant water requirements and retaining the soil moisture content at 65% water holding capacity. Plant height was measured when plant growth was completed (September 2018) using a ruler, and weights of plant shoots and roots were determined using a weighting scale. The plant shoots and roots were oven-dried at 65 °C for 72 hours, and then their dry weights were determined. Chlorophyll values were measured using an OS-30 chlorophyll fluorescence-meter (made in England). Number of fruits was recorded in each treatment and replicate separately. Fruit length and diameter were determined using a calliper (Extra Strong model). Measurement of vitamin C value in bell pepper fruits was performed according to the study by Ting and Rouseff, (1986) through titrating the fruit extract with indophenol. Values of total suspended solids (TSS%) were calculated using a refractometer (ATAGO Brixo-32%), and N amounts were measured in plant shoots and roots. Soil samples were collected from 3 of 21 replicates to determine N-nitrate concentration in the soil solution and N-nitrate adsorbed on LDH. After harvest, to achieve good aeration, types of soil in 18 pots remained in each treatment were mixed separately, and then the pots were filled with 4 kg of the mixed soil. During the fallow period, the pots were covered with black plastics for two weeks and placed under the conditions of 10-hours light daily, mean day temperature of 20 °C and mean night

Table 1: Soil specifications used in the study

Sand	Silt	Clay	Texture	Bulk density	Particle density	Electrical conductivity	pH	Nitrate	Total nitrogen	Lime
%	%	%	-	g/cm^3	g/cm^3	dS/m	-	mg/kg	%	%
91	2	7	Sandy	1.322	1.65	0.452	7.8	0	0.019	31

temperature of 15 °C. Soil moisture content retained at 65% of soil water holding capacity. After the fallow period, soil samples were collected from 3 of 18 replicates to determine the N-nitrate concentrations in the soil solution and LDH.

Second crop and second fallow

For the second crop, two mentheae plants (*M. spicata*) with a height of 15 cm were transplanted in each pot, and 15 replicates were rendered in the pot experiment. No nitrogen fertilizer was added. Moreover, basic and micronutrient fertilizers and irrigation were applied in the same way explained in the first crop. After completion of the plant growth period (Jan 2019), soil samples were collected from 3 of 15 replicates and used to determine the N-nitrate concentrations in the soil solution and LDH. All experiments were performed in a pot tested with the same soil and initial LDH treatments, and no soil was added. As at each stage of cultivation and fallow, 3 samples were taken from replicates to measure the concentration of N-Nitrate in the soil solution and LDH, the number of replicates of each treatment was reduced in each step. The methods explained in the first crop (bell pepper) were used to measure the number of leaves, number of side branches, plant height, and wet and dry weights of shoots and roots. Moreover, Clevenger Apparatus (Goldis Ltd., Iran) was used to measure the mentheae leaves essence by the water distillation process. Amounts of flavones and flavonols were measured using a spectrophotometer (UV/Vis T90 Company PG) at a wavelength of 420 nm as explained by Popova *et al*. (2014). Anti-oxidant activity was determined by spectrophotometry at a wavelength of 517 nm as explained by Oke *et al*. (2009). To measure the values of chlorophyll-a, chlorophyll-b and carotenoid according to the method proposed by Papadopoulos *et al*. (2000), the absorbance rates were recorded using a spectrophotometer at wavelengths of 653, 666 and 470 nm, respectively. Total phenolic compounds were evaluated according to Folin-Ciocalteu method (Folin-Ciocalteu). After harvest, to achieve good aeration, types of soil in 12 pots remained in each treatment were mixed separately, and then the pots were filled with 3 kg of the mixed soil. Additionally, 453 mg of nitrogen from the urea fertilizer source (46.5% nitrogen) was added to each pot (with 3 kg of the soil). After harvesting the mentheae, the results of experiments indicated that the amount of N-Nitrate in the soil solution and LDH treatments was low. For this reason, fertilizer was added again before the second fallow, when the soil of the repeated pots for each treatment was individually mixed to allow aeration, and no soil was added. The fallow period was repeated in the same moisture and heat regime of the first fallow. After the fallow period, soil samples were collected from 3 of 12 replicates to determine the N-nitrate concentrations in the soil solution and LDH. LDH absorbed only nitrate, and we measured the N-nitrate of the soil solution and LDH structure in all experiments.

Third crop (cherry tomato), leaching periods and third fallow

For the third crop, in March 2018, 3 plants of 15 cm high cherry tomato of Belize variety were transplanted in each pot in 9 replicates, and no nitrogen fertilizer was added. The basic and micronutrient fertilization schedules were the same as the schedules explained in the first crop. In this period, the ability of LDH to reduce nitrate leaching at plant growth period was investigated. Before irrigation and collecting the leachates, a container was placed at the bottom of each pot. In this experiment, irrigation practices were repeated four times, and the leachates from each irrigation practice were transferred to the laboratory for leachate tests. Pot weights at saturation and field capacity points were used to determine the water requirement. For this purpose, a pot filled with soil - considered the moisture control - was placed in a water basin for 24 hours to become water saturated from its bottom. Afterward, by weighting the saturated pot, the percentage of gravimetric soil water content was calculated. To prevent volatilization, the pots were covered with plastic covers and subjected to gravity force for 48 hours, and then the soil samples were collected. Since the soil gravimetric water content evaluated at field capacity for a sandy soil was 18% and there was 3 kg soil in each pot, 540 mL water was required for every irrigation practice to obtain 20% of field capacity water as leachates. During the plant growth period, soil moisture (water content) was calculated using gravimetric (mass) methods (65% of water holding capacity). After completion of the plant growth period (May 2019), soil samples were collected from 3 of 9 replicates to measure the N-nitrate concentrations in the soil solution and LDH. Methods explained in the first crop (bell pepper) were used to measure plant height, wet and dry weights of shoots and roots, number of fruits, fruit length and diameter and TSS%. To evaluate the amounts of chlorophyll-a and chlorophyll-b according to the method proposed by Papadopoulos *et al*. (2000), the absorbance rates were recorded using a spectrophotometer at wavelengths of 653 and 666 nm respectively. The value of vitamin C in cherry tomato fruits was evaluated according to Hernandez *et al*. (2006) by titration with Dichloroindophenol (DCIP). After harvest, the 6 pots remained in each treatment were mixed separately to achieve good aeration, and then the pots were filled with 2 kg of the mixed soil. The fallow period was repeated in the same moisture and heat regime of the first fallow. After the fallow period, soil samples were collected from 3 of 6 replicates to determine the N-nitrate concentrations in the soil solution and LDH.

Fourth crop (wheat)

In the fourth crop, 15-cm-high wheat plants, Chamran cultivar, were transplanted in pots in June 2019. Five plants were transplanted in each pot and no nitrogen fertilizer was added. The basic and micronutrient fertilizers and irrigation practices were applied as explained in the first crop. After the plant growth period (September 2019), plant height, dry weights of shoots and roots, 1000-seed weight, and number of seeds per spike were measured. To evaluate the amounts of chlorophyll-a and chlorophyll-b and carotenoid according to the method proposed by Papadopoulos *et al*. (2000), the absorbance rates were recorded using a spectrophotometer at the wavelengths of 653, 666 and 470 nm, respectively. Soil samples were collected from pots to evaluate the N-nitrate concentrations in the soil solution and LDH.

Nitrate measurement methods

To measure N-nitrate concentration using the soil extraction method, 140 g wet soil (at 65% water holding capacity) was centrifuged for 25 minutes at 3500 rpm (revolutions per minute). Then, N-nitrate concentration in soil extract was evaluated using a spectrophotometer (UV/Vis T90 Company PG) at a wavelength of 535 nm (Adams *et al*., 1980). To measure the total N-nitrate, (N-nitrate adsorbed on LDH and N-nitrate in soil solution), 500 ml 0.1 M $KHCO_3$ solution was mixed with 50 g wet soil and the obtained mixture was shook for 2 hours and finally filtrated. N-nitrate concentration of the obtained extract was measured using a spectrophotometer at a wavelength of 210 nm (Dorante, 2007). N-nitrate adsorbed on LDH was obtained from the difference between total soil N-nitrate and soil solution N-nitrate. According to Keeney and Nelson, (1982), nitrate concentration in leachates was evaluated using a spectrophotometer at a wavelength of 410 nm. The plant total nitrogen was measured according to the Kjeldhal method at a wavelength of 660 nm (Holz and Kremers, 1971).

Analysis of data

Statistical data analysis was performed using the SPSS and MSTAT software programs, and the Tukey test with 1% error level was used to compare the means. The Excel software was used to draw the diagrams.

RESULTS AND DISCUSSION

Ability of LDH in adsorbing soil excessive nitrate in bell pepper plantation

Fig. 1 indicates the average effects of applying LDH to the N-nitrate concentration in the soil solution, N-nitrate adsorbed on LDH, and the total N adsorbed by bell pepper plants. Results indicated that an increase in the LDH application rate increased N-nitrate adsorption on LDH, thereby decreasing the N-nitrate concentration in the soil solution. Compared to the control, treatments with 2, 4, 8 and 16 g LDH per kg soil could reduce the nitrate concentration in the soil solution by 31%, 51%, 79% and 95%, respectively. LDHs are anionic clay minerals which have anionic exchange capacity and have a layered structure with positive charge. In octahedron structure, when bivalent cations isomorphic ally substitute trivalent cations, the formed layer indicates the remaining positive charge. Thus, for electrical neutralization, presence of anions among the layers is crucial. LDH has a high selectivity for nitrate adsorption. Soil solution nitrate replaces LDH interlayer chloride and soil solution nitrate concentration is decreased after chloride adsorption by LDH. Thus, LDH controls nitrate movement in soil. LDHs have a significant anion exchange capacity, and this characteristic contributes to their good ability in nitrate adsorption (Halajnia *et al*., 2013; Torres-Dorante *et al*., 2008). Lower availability of nitrates can decrease the risk of nitrate leaching in farmlands during the winter and

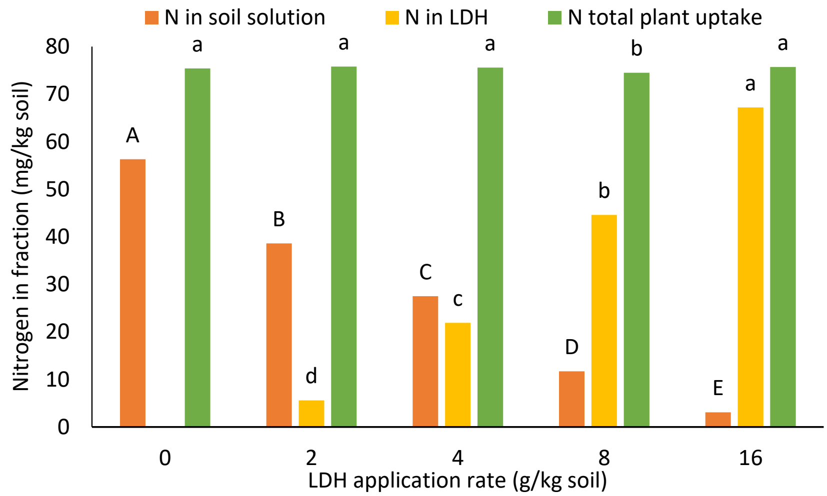


Fig. 1: Effect of LDH application rate along with urea fertilization (46.5%) on N-nitrate concentration in soil solution, N-nitrate adsorbed on LDH and total N adsorbed by bell pepper plant. Vertical bars indicate standard deviation (n=4). Vertical bars indicate standard deviation. Within fractions, treatments followed by similar letters are not statistically different at 99% confidence level of Tukey's test.

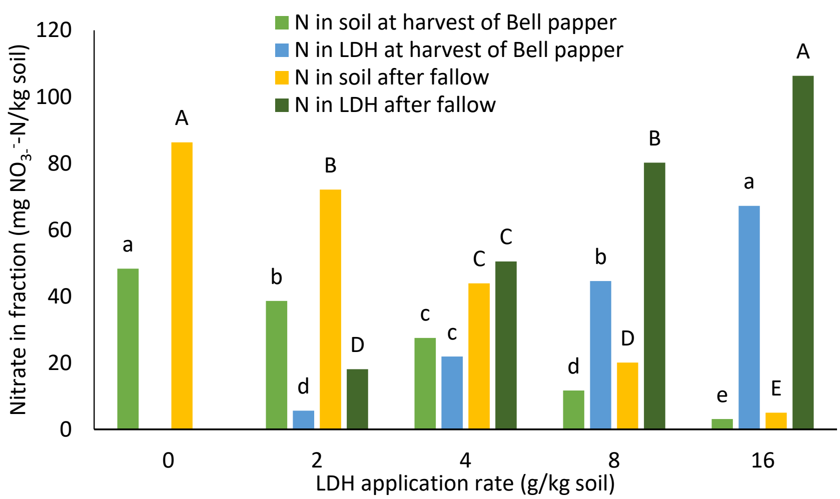


Fig. 2: Effect of LDH application rate along with urea fertilization (46.5%) on N-nitrate concentration in soil solution, N-nitrate adsorbed on LDH and total N adsorbed by bell pepper after a two-week fallow period. Vertical bars indicate standard deviation. In all treatments, the differences in soil solution-N and LDH-N between harvest and after the fallow are significantly different at 99% confidence level of Tukey's test.

in farmlands with a sandy soil texture. No significant difference was detected in the N-nitrate adsorbed by plants in treatments, LDH and control treatment (Fig. 1).

Effects of LDH on adsorbing soil mineral nitrates

Fig. 2 indicates the amounts of the N-nitrate adsorbed on LDH and N-nitrate content in the soil solution before fallow and after a two-week fallow period. During the fallow period, the immobilized (mineralized) nitrate in the soil was increased, LDH treatments adsorbed it well, and the N-nitrate concentration in the soil solution was decreased. However, in the control, the N-nitrate concentration in the soil solution was increased (86.3 mg NO_3^- N/kg soil). Treatments with 8 and 16 g/LDH kg soil adsorbed almost the whole nitrate mineralized during the fallow period. This could reduce the nitrate leaching occurred owing to nitrogen mineralization during the fall and winter (with high rainfall) in the farmlands of humid and tropical areas. Higher LDH application rates led to higher N-nitrate adsorption on LDH, and the highest N-nitrate adsorption was related to the treatment with 16 g/LDH kg soil (114.9 mg NO_3^-N/kg soil).

Fig. 3 shows the bell pepper plant with different LDH treatments and the control. Comparison of the means showed significant differences in LDH

Fig. 3: Bell pepper plants with different LDH treatments (2, 4, 8, and 16 g LDH/kg soil) and control treatment

Table 2: Effect of LDH application rate along with urea fertilization (46.5%) on the qualitative and quantitative properties of bell pepper plants

Qualitative and quantitative properties of plant	LDH application rate (g/kg soil)				
	0	2	4	8	16
Plant height(cm)	31.5 d	35.6 c	39.5 b	42.4 a	42.9 a
Plant wet weight(g)	58.1 e	64.4 d	72.8 c	86.5 b	93.3 a
Plant dry weight(g)	10.4 e	12.5 d	15.1 c	17.4 b	18.7 a
Root wet weight(g)	33.2 e	38.1 d	40.5 c	42.3 b	44.8 a
Root dry weight(g)	8.0 e	10.1 d	12.1 c	13.2 b	15.3 a
Fruit wet weight(g)	81.3 e	115.6 d	127.4 c	135.4 b	138.6 a
Fruit dry weight(g)	14.4 e	22.8 d	26.2 c	29.5 b	32.7 a
Fruit total weight(g)	829.2 e	1629.9 d	2242.2 c	3046.5 b	3534.3 a
Fruit length(mm)	65.6 c	69.3 b	69.5 b	84.1 a	85.3 a
Fruit diameter(mm)	127.3 d	142.4 c	161.7 b	173.6 a	176.2 a
Number of fruits	10.2 e	14.1 d	17.6 c	22.5 b	25.5 a
TSS (%)	6.4 d	6.5 cd	6.7 bc	7.3 ab	7.4 a
Fruit vitamin C (mg per 100 ml fruit juice)	88.4 d	119.4 c	138.1 b	156.1 a	157.6 a
relative chlorophyll content (chlorophyll meter)	33.1 e	38.1 d	40.3 c	45.7 b	46.6 a

Within columns, and soils, treatments followed by similar letters are not statistically different at 99% confidence level (Tukey test).

treatments in terms of number of fruits, wet and dry weights of shoots, roots and fruit, total weight of fruits, and relative chlorophyll. Increase of LDH application improved these traits (Table 2). Treatments with 16 and 8 g LDH/kg soil did not show significant differences in terms of plant height. The lowest plants were seen in the control (31.5 cm), and the highest plants were seen in treatments with 16 and 8 g LDH/kg soil (42.5 cm). The maximum and minimum weights of wet and dry shoots were related to the treatment with 16 g/LDH kg soil and the control, respectively. Treatments with 2, 4, 8 and 16 g/LDH kg soil led to 14.1%, 17.6%, 22.5% and 25.5% increase in the number of fruits. The least number of fruits was seen in the control (10.2). Comparison of the means demonstrated a significant difference in different treatments in terms of leaf area, but no statistically significant difference was observed between the treatments with 2 and 4 g LDH/kg soil. Maximum and minimum leaf areas were related to 16 g LDH/kg soil (54.2 cm^2) and control (40.4 cm^2) treatments, respectively. With the increase of LDH application rate, wet and dry weights of fruits increased compared to the control, so that 16 g LDH/kg soil resulted in 70% and 127% increase in wet and dry weights, respectively. Furthermore, appropriate plant nutrition is highly important. N compounds comprise 40%-50% of protein dry matter as the living material of plant cellules (Togun *et al.*, 2003). For this reason, higher N uptake plays an important role in plant growth processes, and without sufficient N supply, the plant cannot grow well (Aminifard *et al.*, 2018). LDH contributes to higher nitrogen and availability of other nutrients for plants when necessary; therefore, it plays an important role in better growth of roots and shoots (height, leaf area, number of leaves, etc.). Many researchers reported that higher plant N uptake significantly increased bush height, number of branches, number of leaves, wet and dry weights of plant shoots and roots and ripe fruit yield (number and wet and dry weights) of bell peppers (Ayodele *et al*., 2015; Aminifard *et al*., 2018). Compared to the control, application of 2, 4, 8 and 16 g LDH/kg soil increased plant chlorophyll content up to 15%, 19%, 38% and 41%, respectively, due to increases in the nitrogen absorption (uptake) by the plant. Nitrogen influence on the plant chlorophyll content might be due to nitrogen role as a chlorophyll component. Furthermore, nitrogen is the main component of all amino acids of proteins and lipids as the structural components of an active chloroplast (Ouda and Mahadeen, 2008). Aminifard *et al*. (2018) reported increases in the chlorophyll content of bell peppers due to higher plant nitrogen absorption. Higher LDH application rates resulted in longer fruits, but there was not a significant difference between treatments with 16 and 8 g LDH/kg soil and between

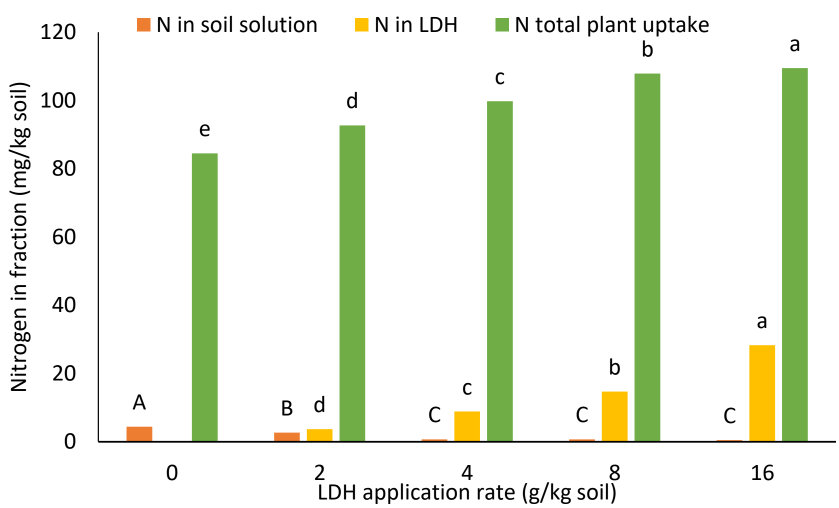


Fig. 4: The effect of LDH application rate along with urea fertilization (46.5% N) on N-nitrate concentration in soil solution, N-nitrate adsorbed on LDH and total N absorbed by mentheae plant. Vertical bars indicate standard deviation. Within fractions, treatments followed by similar letters are not statistically different at 99% confidence level of Tukey's test.

treatments 2 and 4 g LDH/kg soil. Comparison of means indicated a significant effect of LDH application rates on fruit diameter, but no significant difference was observed between applying 8 and 16 g LDH/kg soil. LDH treatments had a significant effect on vitamin C content. However, there was no significant difference between treatments with 8 and 16 g LDH/kg soil. Higher vitamin C contents can be attributed to increase of plant nitrogen absorption, being consistent with the results reported by Aminifard *et al*., (2012) suggesting that increase of plant nitrogen absorption (uptake) affects vitamin C content in bell pepper plants. Comparison of the means suggested a significant difference in terms of TSS%; however, there was no significant difference between applying 8 and 16 g LDH/kg soil, 4 and 8 g LDH/kg soil, 2 and 4 g LDH//kg soil.

Reversibility of nitrate adsorption process from LDH during mentheae growth period

In order to study the nitrate exchange capacity of LDH in the long term, successive crop and fallow periods were established. For this reason, the second crop was planted in previous pots. During the second crop growth period, plants could make use of the N-nitrate adsorbed on LDHs and absorb (uptake) the nitrates remained in the soil solution. Therefore, the N-nitrate concentration in the soil solution remained low (Fig. 4). At the end of the second crop's growth period, values of the N-nitrate adsorbed in treatments with 2, 4, 8 and 16 g LDH/kg soil reached 3.7, 8.9, 14.7 and 28.3 mg N-Nitrate/kg soil, respectively. Comparison of the means revealed the significant effect (p≤0/05) of LDH treatments on the nitrogen values of the plant biomass (Fig. 4). Application of 16 g LDH/kg soil had the largest effect on the nitrogen amount in plants, increasing the availability of nitrogen in plants to 109.5 mg N-Nitrate/kg soil and resulting in 30% increase in the amount of plant nitrogen compared to the control treatment. In fact, LDHs act as a slowly nitrogen releasing agent, which not only satisfy plant nitrogen needs, but also prevent nitrogen loss in the soil.

LDH ability to resorb soil excessive nitrate

At the end of the second crop's growth period, the N-nitrate concentration was decreased in LDH treatments and the control. In order to investigate the ability of LDHs for resorbing mineralized nitrate from the soil solution, 453 mg of nitrogen from the urea fertilizer source (46.5% nitrogen) was added to each pot (with 3 kg of the soil). Therefore, LDHs could adsorb the N-nitrate added from the urea fertilizer as well as nitrates mineralized during a two-week

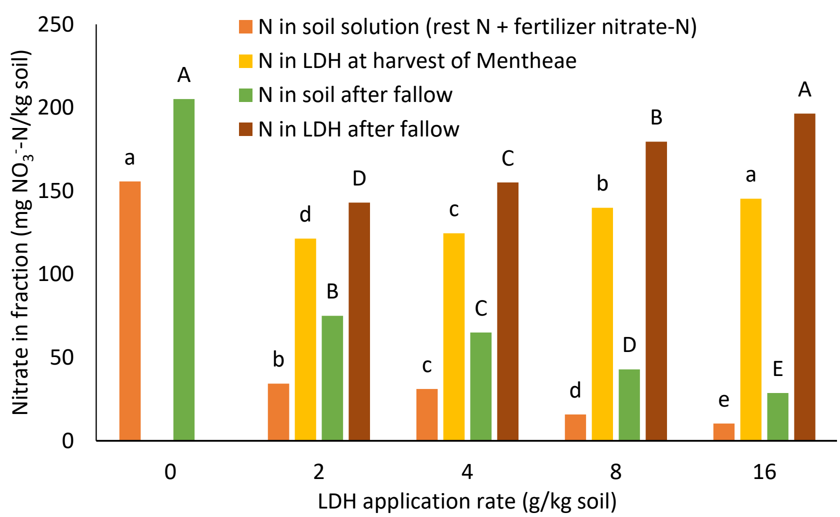


Fig. 5: The effect of LDH application rate along with urea fertilization (46.5% N) on N-nitrate concentration in soil solution, N-nitrate adsorbed on LDH and total N absorbed by mentheae plant after a two-week fallow at greenhouse. Vertical bars indicate standard deviation. In all treatments, the differences in soil solution-N and LDH-N between harvest and after the fallow are significantly different at 99% confidence level of Tukey's test.

fallow and retained the N-nitrate concentration low in the soil solution (Fig. 5). A significant difference was observed in different LDH treatments in terms of the N-nitrate adsorbed on LDH surfaces. After the fallow period, concentrations of N-nitrate adsorbed in treatments with 2, 4, 8, 16 g LDH/kg soil reached 143, 155, 179.5 and 196.38 mg N-Nitrate/kg soil, respectively. The N-nitrate concentration on the LDH surface in the treatment with 16 g LDH/kg soil was almost the same as its concentration in the soil solution of the control (205.18 mg N-Nitrate/kg soil). This suggests that LDH has a considerable ability to control nitrate movement in the soil and keep the N-nitrate concentration low. The results of Halajnia *et al*. (2016) demonstrated that application of 20 g LDH per kg soil could reduce N-Nitrate in the soil solution by 59% compared to the control. Torres-Dorante *et al*. (2009) stated that LDH could maintain N-Nitrate in its structure, and the application of 20 g LDH per kg reduced the amount of N-Nitrate in the soil solution by up to 80% compared to the control.

Fig. 6 shows mentheae pots with different LDH treatments and the control. Results of comparison of the means were significantly different in terms of plant height, but there was no significant difference between the treatments applying 2 and 4 g LDH/kg soil (Table 3). The highest plants were seen in 16 g LDH/kg soil treatment (58.2 cm), and the lowest plants were seen in the control (28.3 cm). Compared to the control, the lowest rate of LDH application (2 g LDH/kg soil) resulted in 53% increase in plant height. Apparently, LDH treatment leads to better plant growth by reducing nitrogen loss and making more nitrogen available. Considering the mean number of side shoots, comparison of the means showed a significant difference between control and LDH treatments, but treatments with 8 and 16 g LDH/kg soil were not statistically different. The most and the least numbers of side shoots were related to 16 g LDH/kg soil treatment and the control, respectively. Although, number of leaves was increased with applying higher LDH rates, comparison of the means did not show a statistically significant difference between treatments with 8 and 16 g LDH/kg soil and between the control and 2 g LDH/kg soil treatment. The least number of leaves in the control (510.4) was probably owing to lower availability of nitrogen in plants, since increase in nitrogen availability increases photosynthate content and improves plants vegetative traits, including the number of leaves (Ardalani *et al*., 2017). More leaves in LDH treatments suggests the positive role of LDH in plant nitrogen uptake. Regarding the mean wet and dry weights of roots and shoots, comparison of the means shows that LDH treatments improved these traits compared to the control. However, treatments with 8 and 16 g LDH/kg soil were not statistically different in terms of wet and dry weights of roots, and treatments with 8 and 16 g LDH/kg soil were not statistically different in terms of wet weight of root. Comparison of the means of LDH treatments showed significantly different results for wet and dry weights of plant shoots. However, the

Fig. 6: Mentheae plants in different LDH treatments (2, 4, 8, 16 g LDH/kg soil) and control

Table 3: Effect of LDH application rate along with urea fertilization (46.5% N) on the qualitative and qualitative traits of mint plants

Qualitative and quantitative properties of plant	LDH Application rate (g/kg soil)				
	0	2	4	8	16
Plant height(cm)	28.3 d	43.4 c	43.6 c	48.5 b	58.2 a
Shoots wet weight (g)	54.3 e	60.4 d	76.1 c	83.4 b	90.6 a
Shoots dry weight(g)	10.3 d	21.2 c	21.5 c	25.7 b	30.7 a
Roots wet weight (g)	9.1 c	14.5 b	14.4 b	18.2 a	18.4 a
Roots dry weight (g)	3.1 d	5.2 c	5.9 b	7.1 a	7.1 a
Number of leaves	510.4 c	513.3 c	558.2 b	662.8 a	667.3 a
Number of side branches	10.2 d	18.3 c	21.4 b	27.6 a	28.3 a
Total leaf chlorophyll (mg/g dry sample)	20.7 e	41.2 d	45.2 c	47.9 b	49.6 a
Chlorophyll-a (mg/g dry sample)	12.3 d	22.4 c	25.1 b	27.6 a	27.8 a
Chlorophyll-b (mg/g dry sample)	8.4 d	18.8 c	19.9 b	20.3 b	21.8 a
Carotenoid (mg/g dry sample)	0.68 d	4.03 c	4.4 b	4.8 a	4.8 a
Flavone and flavonol (mg/g dry sample)	10.2 c	13.3 c	18.1 bc	21.1 ab	26.5 a
Anti-oxidant (%)	77.3 e	88.1 d	93.8 c	94.5 b	98.8 a
Total phenol l (mg Galic acid/100 g dry sample)	20.4 e	29.2 d	39.4 c	40.5 b	43.4 a
Essence (%)	2.1 d	2.45 d	2.9 c	3.4 b	3.9 a

Within columns, and soils, treatments followed by similar letters are not statistically different at 99% confidence level (Tukey test).

difference between treatments with 2 and 4 g LDH/kg soil was not statistically significant. Compared to the control, application of 16 g LDH/kg soil led to 3 times heavier dry shoots. Results showed that the highest and lowest wet weights were obtained by applying 16 g LDH/kg soil (90.6 g) and the control (54.3 g), respectively. Compared to the control, application of 2 g LDH/kg soil led to 11% increase in wet weight. Lighter plant wet weight in the control was probably due lower water holding capacity and lower anion exchange capacity. Growth of shoots in hydrophilic plants such as mentheae was closely associated with water availability, so that plant beds with more available water could improve plant growth and increase the weight of plant shoots (Keshavarz *et al.*, 2018). It seems that LDH increases plant wet and dry weights by making more nitrogen available for plants and highlighting the role of nitrogen in the structure of macromolecules such as proteins, amino acids, and nucleic acids. Moreover, enough nitrogen resulted in more flowering branches and leaves, thereby increasing production per unit area (Kheirandish *et al.*, 2016). Many studies have reported the effects of increase of plant nitrogen uptake (absorption) on improving vegetative traits of mentheae (plant height, wet and dry weight of shoots, leaves and roots, number of side branches, leaves etc.) (Keshavarz *et al.*, 2018; Ardalani *et al.*, 2017). Increase in the LDH application rate improved the contents of total chlorophyll, chlorophyll-a, chlorophyll-b, carotenoids and essence in plants, and comparison of the means indicated a significant difference between LDH treatments and the control. The highest and lowest chlorophyll contents were observed in treatment with 16 g LDH/kg soil (49.6 mg/g dry sample) and the control (20.7 mg/g dry sample). Treatments with 8 and 16 g LDH/kg soil exhibited no significant difference in terms of chlorophyll-a and carotenoid contents, and treatments with 8 and 4 g LDH/kg soil did not lead to a significant difference in terms of chlorophyll-b content. LDH application increased the leaf essence content (%) as in treatments with 2, 4, 8, 16 g LDH/kg soil, it reached 2.45%, 2.9%, 3.4%, and 3.9% respectively. Nitrogen, by increasing the number of leaves and leaf area, provided an appropriate bed to obtain solar energy and was a component of chlorophyll structure. Furthermore, other enzymes involved in the photosynthetic process of carbon metabolism increased photosynthetic efficiency and played a key role in rising the essence content (Ouda and Mahadeen, 2008). Considering the considerable ability of LDH for nitrogen absorption, increase of LDH application rate improved nitrogen absorption and consequently chlorophyll and essence content. Carotenoid is one of the photosynthetic pigments, and nitrogen is a component of carotenoids. Therefore, LDH application increases the plant carotenoid content. Comparison of the results obtained for flavones and flavonols revealed that the difference between applying 4 and 8 g LDH/kg soil, 2 and 4 g LDH/kg soil, and 2 g LDH/kg soil was not significant. Increase of LDH application rate increased the total phenol content and the plant antioxidant percentage. Compared to the control, application of 16 g LDH /kg soil resulted in 112% increase in the total phenol content. The least and the most antioxidant contents were related to the control (77.3%) and the treatment with 16 g LDH/kg soil (98.8%), respectively. Phenolic compounds include flavonoids, flavonols, anthocyanin, anthraquinone, and their derivatives. Apparently, phenolic compounds, especially flavonoids and flavonols, have higher antioxidant power. Flavone and flavonole are found in flowers and leaves of green plants. Compared to anthocyanin, these compounds absorb light in lower wavelengths and act as cell protectants against harmful ultraviolet rays (Fathiazad *et al*., 2010). Phenolic content of plants depends on the genetic and environmental factors of plants. Several studies have investigated the effect of plant growth condition on second metabolites, most of which emphasize the role of habitat as an effective factor in accumulation of second metabolites. By changing the moisture condition and increasing the availability of nutrients such as nitrogen, site and crop growth, medium conditions can be effective in forming plant active ingredients (Pourmorad *et al*., 2006). LDH has a high capacity for adsorbing nitrogen and water (Berber *et al*., 2014). Bidgoli *et al*. (2018) and Keshavarz *et al*. (2018) reported that higher nitrogen content in mentheaes increased total contents of phenolic compounds, flavone content, antioxidant, and extract.

LDH ability for reducing nitrate leaching in tomato cropping condition

One of the present study objectives was to investigate the LDH ability to reduce nitrate loss in the soil and to evaluate the retention of the nitrate adsorbed on LDH under leaching conditions. For this reason, 4 phases of leaching were performed during the third crop (cherry tomato) growth period. Results indicated that application of 2, 4, 8 and 16 g LDH/kg LDH led to 34%, 44%, 58% and 69% reduction in the N-nitrate concentration of soil leachates compared to the control, respectively (Table 4). In fact, the nitrate adsorbed on LDH during crop growth was retained well against different leaching phases. This can be attributed to the structural characteristics of LDH. Some important characteristics of LDH are a layered structure for keeping interlayer anions, a significant anion exchange capacity, and a very high specific surface area (Benicio *et al*., 2018; Hu *et al*., 2017). By applying one-hour drop irrigation of 45 mL water for two days in the soil with 2500 mL N-nitrate as the initial concentration and 1.5 g Mg-Al-LDH/kg soil, Torres-Dorante and Lammel (2009)

Table 4: Effect of LDH application rate along with urea fertilization on nitrate concentration in leachates (mg/L) obtained from different leaching phases during cherry tomato growth period

LDH application rate (g/kg soil)	Leaching phases				
	First phase	Second phase	Third phase	Fourth phase	Total leachate
0	17.3 a	35.4 a	28.9 a	7.5 a	89.1 a
2	14.1 b	23.6 b	15.8 b	4.9 b	58.4 b
4	8.1 c	19.8 c	17.2 c	4.3 c	49.4 c
8	7.6 c	14.5 d	10.8 d	3.8 d	36.7 d
16	5.9 d	10.9 e	7.5 e	2.6 e	26.9 e

Within columns, and soils, treatments followed by similar letters are not statistically different at 99% confidence level (Tukey test).

found that the synthetized LDH had a capacity to leach nitrates up to 75%. The least amounts of leached nitrates were observed in the first and the fourth phases of irrigation. Reduction of N-nitrate concentration in leachate during the first phase occurred owing to transformation of urea to nitrate whose movement in the soil profile to the deep soil is a time-consuming process. The reason for reduction of the N-nitrate concentration in leachate during the fourth phase was nitrate leaching in previous phases and reduction of the soil N-nitrate concentration (Table 4). Considering the N-nitrate absorption by plants, a significant difference was observed between control and LDH treatments. The highest and the lowest plant N-nitrate absorption belonged to the treatment with 16 g LDH/kg soil (106.5 mg N-Nitrate/kg) and the control (50 mg N-Nitrate/kg), respectively (Fig. 7). In addition, LDHs could retain the N-nitrate concentration in the soil solution at low levels. LDHs not only prevented soil nitrate loss, but also led to better conditions for plant nitrogen absorption by making nitrogen available regularly and gradually. Finally, LDHs prevented the loss of this useful nutrient by keeping nitrogen inside its structure.

Mineral nitrate adsorption capacity of LDH after leaching

To investigate the capacity of LDH to adsorb soil mineral nitrate, a two-week fallow was performed after the plant growth period. During the third fallow, the nitrate content in the control was increased owing to mineralization (immobilization) (91.4 mg N-Nitrate/kg soil), but in LDH treatments, the N-nitrate concentration in the soil solution was decreased owing to LDH adsorption on LDH (Fig. 8). There was a significant difference between all LDH treatments and the control in terms of nitrate adsorption on LDH. In treatments with 2, 4, 8 and 16 g LDH/kg soil, the rates of nitrate adsorption on LDH after fallow were 79.3, 97.01, 112.1 and 126.7 mg N-Nitrate/kg soil, respectively.

Fig. 9 shows the cherry tomato plants in control and LDH treatments. Comparison of the means showed significant differences in terms of plant height, as well as wet and dry eights of shoots and roots (Table 5). Increase of LDH application rate led to increase plant height, wet and dry weights of shoots and roots. Considering the wet and dry weights of plant roots, there was no significant difference between treatments with 4 and 8 g LDH/kg soil. Application of 2, 4, 8 and 16 g LDH/kg soil increased plant height by 14%, 26%%, 50 and 80%, respectively. Nitrate had a growth-stimulating effect on plants, and LDH made more nitrate available, thereby increasing the plant height, as well as wet and dry weights of plant roots and shoots. The effect of nitrogen on increasing the vegetative growth of stems is attributed to changes of the plant hormones balance in plant vegetative

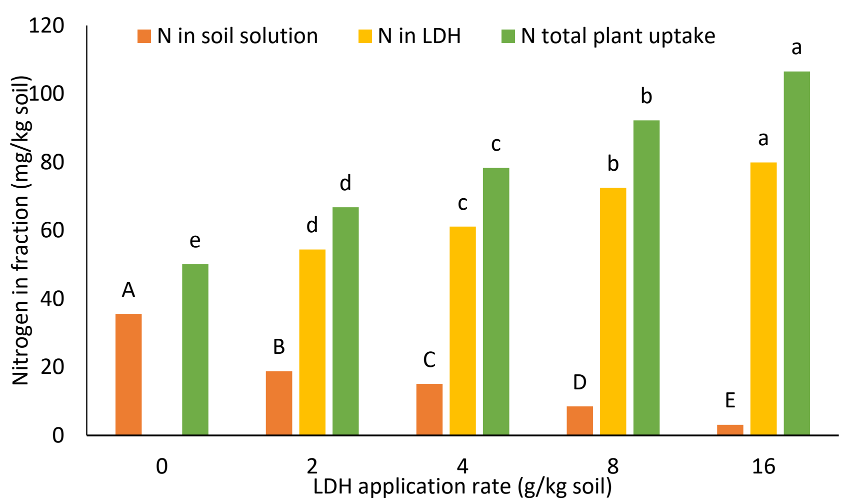


Fig. 7: Effects of LDH application along with urea fertilization (46.5% N) on N-nitrate concentration in soil solution, N-nitrate adsorbed on LDH and total plant N uptake in cherry tomato plants after leaching. Vertical bars indicate standard deviation. Within fractions, treatments followed by similar letters are not statistically different at 99% confidence level of Tukey's test.

shoots (Rahman *et al*., 2007). Many researchers have demonstrated the positive relationship between increase in nitrogen availability and vegetative growth of tomatoes (height, wet and dry weight of shoots, fruit and roots, etc.) (Rahman *et al*., 2007; Li *et al*., 2017; Chen *et al*., 2016). Comparison of the means showed significant differences in terms of number of fruits, wet and dry weight of fruit, total weight of fruits, fruit length and diameter. Higher LDH application rates increased these traits. Considering the wet and dry weights of fruit, there was no significant difference between treatments with 8 and 16 g LDH/kg soil and treatments with 4 and 8 g LDH/kg soil. Additionally, fruit length and diameter were not significantly different in treatments with 8 and 16 g LDH/kg soil. Comparison of the means revealed that the treatment with 16 g LDH/kg soil had the highest number of fruits (102.7), weight of one fruit (15.3 g), and fruit dry weight (0.76 g), while the control had the lowest number of fruits (28.4), weight of one fruit (7.3), and fruit dry weight (0.34). The mean difference of total weight of fruits in the treatment with 16 g LDH /kg soil, and the control was 1300 g. Compared to the control, application of 2 g LDH/kg soil led to 85% increase. LDH made plant nitrogen availability higher, and increased the fruit length and diameter. Many studies have reported that fruit length and diameter increases with the increase of nitrogen availability (Li *et al*., 2017). Growth improvement plays an important role in tomato yields and number of fruit per bush. Nitrogen deficiency during flowering leads to flower falling and lower number of fruits, whereas appropriate nitrogen availability leads to higher nitrogen content in the plant. This nitrogen prevents abscisic acid accumulation, thereby preventing flower falling (Streeter, 1978). In fact, LDH increases the number of fruits by making sufficient nitrogen available when it is necessary for plant growth. Comparison of the means for total chlorophyll content and chlorophyll-a content in LDH treatments showed a significant difference, but there was no statistical difference in terms of chlorophyll-b content. Higher LDH application rates led to higher chlorophyll content in plants. The highest and the lowest chlorophyll contents were related to 16 g LDH/kg soil (1.42 mg/g dry sample) and the control (0.485 mg/g dry sample), respectively. This indicates that nitrogen is part of chlorophyll molecule. Nitrogen is a component of all main amino acids in proteins and lipids as parts of an active chloroplast structure (Ouda and Mahadeen, 2008). Therefore, higher nitrogen uptake by plants leads to higher chlorophyll contents in plants. TSS had an ascending trend with the increase of LDH application rate, but there was no significant difference between treatments with 4 and 8 LDH/kg soil, treatments with 2 and 4 LDH/kg soil, and treatments with 2 and 0 LDH/kg soil (control).

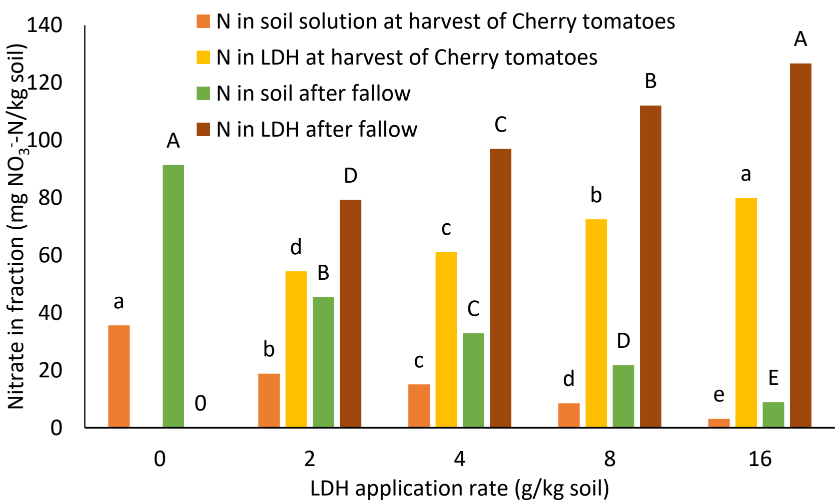


Fig. 8: Effects of LDH application along with urea fertilization (46.5% N) on N-nitrate concentration in soil solution, N-nitrate adsorbed on LDH and total plant N uptake in cherry tomato plants after a two-week fallow period in greenhouse. Vertical bars indicate standard deviation. In all treatments, the differences in soil solution-N and LDH-N between harvest and after the fallow are significantly different at 99% confidence level of Tukey's test.

Fig. 9: Cherry tomatoes with LDH treatments (2, 4, 8 and 16 g LDH/kg soil) and control treatment

Table 5: Effects of LDH application rate along with urea fertilization (46.5%) on qualitative and quantitative traits of cherry tomato plants

Qualitative and quantitative properties of plant	LDH Application rate (g/kg soil)				
	0	2	4	8	16
Plant height (cm)	21.2 e	24.3 d	26.5 c	31.5 b	38 a
Shoot weight(g)	68.4 e	120.3 d	147.7 c	152.2 b	168.2 a
Shoot dry weight(g)	8.7 e	18.4 d	23.8 c	25.1 b	27.8 a
Root weight (g)	10.5 d	14.3 c	16.1 b	17.8 ab	18.6 a
Root dry weight (g)	3.4 d	5.1 c	6.1 b	6.7 ab	7.3 a
Number of fruits	28.4 e	58.8 d	66.1 c	95.2 b	102.7 a
Weight per fruit (g)	7.3 d	12.8 c	14.5 b	15.2 ab	15.3 a
Dry weight per fruit (g)	0.34 d	0.5 c	0.63 b	0.74 a	0.76 a
Total weight of fruits (g)	204.4 e	742.4 d	957 c	1444 b	1560.6 a
Fruit length (mm)	0.7 d	1.3 c	1.7 b	2.6 a	2.8 a
Fruit diameter (mm)	0.8 d	1.5 c	2 b	2.8 a	3 a
Chlorophyll a (mg/g dry sample)	0.41 c	0.7 b	0.86 ab	0.89 a	0.96 a
Chlorophyll b (mg/g dry sample)	0.075 a	0.1 a	0.35 a	0.4 a	0.46 a
Total chlorophyll (mg/g dry sample)	0.485 e	0.8 d	1.21 c	1.29 b	1.42 a
TSS (%)	4.1 c	4.5 bc	5.7 ab	5.8 ab	6.4 a
Vitamin C (mg/100 ml fruit juice)	25.1 e	30.2 d	40.6 c	45.2 b	48.1 a

Within columns, and soils, treatments followed by similar letters are not statistically different at 99% confidence level (Tukey test).

Comparison of the means for vitamin C content in fruits showed a significant increase. Application of 16 g LDH/kg soil led to 91% increase in fruit vitamin C content. Ochoa-Velasco *et al*. (2016) have investigated the effect of nitrate absorption (uptake) on improving vitamin C amount in tomato fruits and their TSS.

LDH ability for long-term nitrate exchange during successive crop-fallow rotations in wheat plantation

To investigate the ability of LDH to desorb the nitrates adsorbed during long-term crop-fallow rotations, the nitrate released from LDH was studied by planting wheat as the fourth crop. Wheat plants were grown without any nitrogen fertilization. Results demonstrated that after one and half a year crop-fallow rotations, LDH still had a high ability to desorb the N-nitrate for the needs of plants (Fig. 10). Wheat plants consumed the N-nitrate adsorbed by LDH and N-nitrate in the soil solution (Fig. 10). At the end of growth period, the N-nitrate concentration in the soil solution of all treatments (LDH and control) was decreased considerably. However, LDHs still contained some nitrates. Amounts of N-nitrate remained in treatments with 2, 4, 8 and 16 g LDH/kg soil were equal to 8.9, 12.8, 147 and 15.6 mg N-Nitrate/kg soil. In terms of N-nitrate uptake by plants, a significant difference was observed between

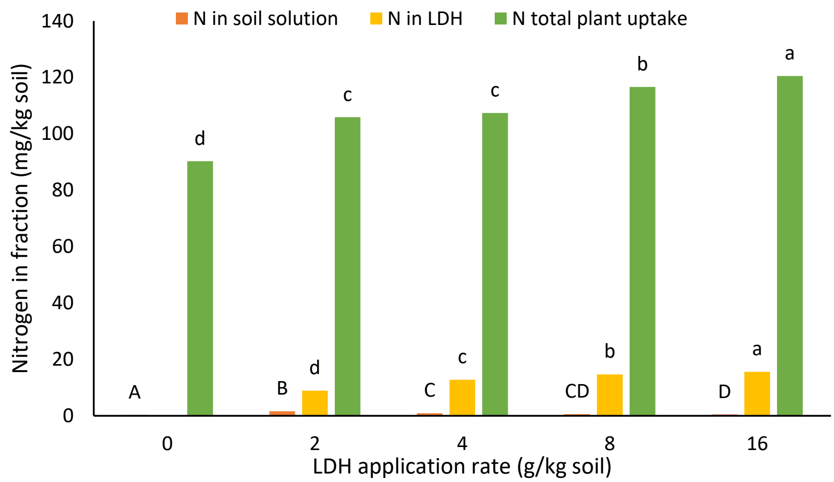


Fig. 10: Effects of LDH application along with urea fertilization (46.5%) on N-nitrate concentration in soil solution, N-nitrate adsorbed on LDH and total N uptake by wheat plants. Vertical bars indicate standard deviation. Within fractions, treatments followed by similar letters are not statistically different at 99% confidence level of Tukey's test.

Fig. 11: Wheat plants in LDH treatments (2, 4, 8, 16 g LDH/kg soil) and control

Table 6: Effects of LDH application rate along with urea fertilization (46.5%) on qualitative and quantitative traits of wheat

Qualitative and quantitative properties of plant	LDH Application rate (g/kg soil)				
	0	2	4	8	16
Bush height(cm)	39.2 e	62.3 d	64.3c	65.2 b	68.8 a
Shoots dry weight(g)	8.1 e	14.8 d	17.4 c	22.3 b	23.6 a
Roots dry weight(g)	0.6 c	1.7 b	1.7 b	1.8 b	2.2 a
Total dry weight(g)	8.7 e	16.5 d	19.2 c	24.1 b	25.8 a
Seeds per spike	16.2 d	24.1 c	27.3 b	27.3 b	29.6 a
1000-seed weight (g)	33.1 d	42.4 c	47.1 b	47.3 b	49.8 a
Total chlorophyll (mg/g dry sample)	0.3 a	0.4 a	0.5 a	0.6 a	0.7 a
Chlorophyll a(mg/g dry sample)	0.2 c	0.2 c	0.3 b	0.4 a	0.4 a
Chlorophyll b(mg/g dry sample)	0.1 c	0.1 bc	0.2 b	0.2 a	0.2 a

Within columns, and soils, treatments followed by similar letters are not statistically different at 99% confidence level (Tukey test).

LDH treatments and the control, but there was no significant difference between treatments with 2 and 4 g LDH/kg soil.

Fig. 11 indicates the wheat plants in LDH treatments and the control. Table 6 presents the effects of LDH application rate on the quantitative and qualitative traits of wheat plants. Comparison of the means showed significant differences in terms of plant height, dry weights of shoots and roots and total plant weight. However, no significant difference was seen in treatments with 2, 4 and 8 g LDH/kg soil in terms of root dry weight. The highest and the lowest plants were obtained in the treatment with 16 g LDH/kg soil (68.8 cm) and the control (39.2 cm) treatment, respectively. As explained for previous crops, LDH, by making more water and nitrogen available when necessary for plant growth, improves plants vegetative traits such as height, dry weight of roots, and plant biomass. Additionally, 1000-seed weight and seeds per spike were increased in higher LDH application rates. Comparison of the means showed no statistically significant difference between application of 4 and 8 g LDH/kg soil. The maximum number of seeds (29.6 g) and 1000-seed weight (49.8 g) were related to the treatment with 16 g LDH/kg soil, and the minimum number of seeds (16.2 g) and 1000-seed weight (33.1 g) were related to the control. Although total chlorophyll values were increased in higher LDH application rates, there was no significant difference in different LDH treatments. Chlorophyll content is nearly proportional to leaf nitrogen content (Evans, 1989). Many studies have demonstrated the effect of higher plant nitrogen uptake on wheat yield (height, dry weights of roots and shoots, 1000-seed weight, number of seeds per spike, and chlorophyll content) (Wang *et al*., 2017; Nie *et al*., 2018).

CONCLUSION

When plants require nitrate, LDH, as a useful nitrate exchanger, adsorbs nitrate if the concentration of nitrate in soil solution is high. LDH can re-adsorb nitrate If the nitrate concentration in soil solution rises again. To determine the nitrate exchange capacity of LDH in a long-term period, successive periods of crop and fallow were established. In all crop periods, LDH could adsorb soil excessive nitrate and decreased the nitrate concentration in the soil solution. Lower nitrate availability in the soil solution would reduce the risk of nitrate leaching in farmlands during the winter and farmlands with sandy-textured soil. During the two-week fallow periods, the amounts of mineralized nitrate in the soil solution were increased, and LDH treatments could adsorb them efficiently and decrease the N-nitrate concentration in the soil solution. Treatments with 8 and 16 g/LDH kg soil adsorbed almost the whole nitrate mineralized during the fallow period. This could reduce the nitrate leaching occurred due to nitrogen mineralization in farmlands of humid and tropical areas during fall and winter (with high rate of rainfall). LDHs not only prevented the loss of nitrate in the soil, but also made it available regularly and gradually, thereby improving the nitrate uptake conditions for plants. LDHs, by making more nitrogen available, improved the quantitative and qualitative traits of medical plants (mentheae), cucurbits (cherry tomato and bell pepper) and crops (wheat). LDH contributes to increase of nitrogen and availability of other nutrients for plants when necessary; therefore, it plays an important role in improving the growth of roots and shoots (height, leaf area, number of leaves, etc.). Higher plant N uptake significantly increased bush height, number of branches, number of leaves, wet and dry weights of plant shoots and roots and ripe fruit yield of plants (number and wet and dry weights). Results demonstrated that even after 18-month crop-fallow rotations, LDH had a high ability to desorb N-nitrate to fulfil the needs of wheat. Wheat consumed the N-nitrate adsorbed by LDH and the N-nitrate in the soil solution. At the end of growth period, N-nitrate concentrations in the soil solution in all treatments (LDH and control) were decreased significantly. Considering the importance of nitrate leaching risk in greenhouses, LDHs are applied as amendments for pot crops. LDH can also be used in golf courses, public greens, and sport fields. The risk of nitrate leaching losses in these areas is of high concern because of permanent irrigation and fertilizer application required to maintain the grass quality. Greens are mainly constructed on sand-based media (>90% sand) in order to increase their resistance to traffic and to ensure water percolation. As in containerized crop production, the main problems of sand-based growing media are their low cation exchange capacity and low water retention. Therefore, the nutrients applied in these media are prone to be leached. To provide cation exchange capacity, the zeolites-based products, which are silicate minerals, are recommended as amendments to sand putting greens in golf courses and turf grasses. Thus, LDH can be easily incorporated during the construction of putting greens/growing media in order to create nitrate exchange capacity.

AUTHOR CONTRIBUTIONS

M. Mohammadi analysed and interpreted the data and finalized the manuscript for publication. A. Mohammadi Torkashvand conducted the comprehensive review. M. Esfandiari helped in the sampling experiments. P. Bi-Parva performed experimental design. All authors participated in literature review and preparation of the manuscript.

CONFLICT OF INTERESTS

The author declares that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

ABBREVIATIONS

%	Percent
A°	Angstrom
C	Carbon
CO_3^{2-}	Carbonate ion
°C	Centigrade
Cl^-	Chloride ion
G	Gram
g/cm^3	Gram per cubic Centimetre
g/L	Gram per Litre
g/mg	Gram per milligram
g/mg min	Gram per gram minute
HCO_3^-	Bicarbonate ion
kg	Kilogram
L	Litre
L/g	Litre per gram
L/kg	Litre per kilogram
L/mg	Litre per milligram
LDH	Layered Double Hydroxide
M	Molar
Mg	Milligram
mg/g	Milligram per gram
mg/kg	Milligram per kilogram
mg/L	Milligram per Litre
min	Minute
mmol/g	Mill mole per gram
N	Nitrogen
NH_4^+	Ammonium ion
NO_3^-	Nitrate ion

pH	Potential of hydrogen
rpm	Revolutions per minute
S	Second
*XR*D	X-ray diffraction

REFERENCES

Adams, F.; Burmester, C.; Hue, N.; Long, F., (1980) A Comparison of Column-Displacement and Centrifuge Methods for Obtaining Soil Solutions 1. Soil Sci. Soc. Am. J., 44: 733-735 **(3 pages)**.

Aminfard, M.H.; Bayat, H., (2018). Influence of different rates of nitrogen fertilizer on growth, yield and fruit quality of sweet pepper (Capsicum annum L. var. California Wander). J. Hortic. Postharvest Res., 1**:** 105-114 **(10 pages)**.

Aminfard, M.H.; Aroiee, H.; Ameri, A.; Fatemi, H., (2012). Effect of plant density and nitrogen fertilizer on growth, yield and fruit quality of sweet pepper (*Capsicum annum* L.). Afr. J. Agric. Res., 7**:** 859-866 **(8 pages)**.

Ardalani, H.; Hadipanah, A.; Pazoki, A.; Zolfaghar, M., (2017). Phytochemical, morphological and yield responses of Mentha canadensis to organic and nitrogen fertilizers. J. Essent. Oil Bear. Plants., 20**:** 752-757 **(6 pages)**.

Ayodele, O.; Alabi, E.; Aluko, M., (2015). Nitrogen fertilizer effects on growth, yield and chemical composition of hot pepper (Rodo). Int. J. Agric. Crop Sci., 8(5)**:** 60-66 **(7 pages)**.

Benicio, L.P.F.; Eulalio, D.; Guimaraes, L.D.M.; Pinto, F.G.; Costa, L.M.D.; Tronto, J., (2018). Layered Double Hydroxides as Hosting Matrices for Storage and Slow Release of Phosphate Analyzed by Stirred-Flow Method. Mater. Res., 21(6): 1-13 **(13 pages)**.

Berber, M.R.; Hafez, I.H.; Minagawa, K.; Mori, T., (2014). A sustained controlled release formulation of soil nitrogen based on nitrate-layered double hydroxide nanoparticle material. J. soils sediments., 14**:** 60-66 **(7 pages)**.

Bernardo, M.P.; Moreira, F.K.; Ribeiro, C., (2017). Synthesis and characterization of eco-friendly Ca-Al-LDH loaded with phosphate for agricultural applications. Appl. Clay Sci., 137**:** 143-150 **(8 pages)**.

Bidgoli, R.D.; Mahdavi, M.J., (2018). Effect of Nitrogen and Two Types of Green Manure on the Changes in Percentage and Yield of Peppermentheae (Mentha piperita) Essential Oil. Notulae Sci. Biol., 10**:** 245-250 **(6 pages)**.

Canter, L.W., (2019). Nitrates in groundwater, Routledge. CRC Press.

Chen, C.; Xu, F.; Zhu, J.R.; Wang, R.F.; Xu, Z.H.; Shu, L.Z.; Xu, W.W., (2016). Nitrogen forms affect root growth, photosynthesis, and yield of tomato under alternate partial root-zone irrigation. J. Plant Nutr. Soil Sci., 179**:** 104-112 **(9 pages)**.

Chubar, N., (2018). The influence of sulfate on selenate sorption on Mg-Al-CO3 layered double hydroxides prepared by fine inorganic sol-gel synthesis studied by X-ray photoelectron spectroscopy. Appl. Surf. Sci., 459**:** 281-291 **(11 pages)**.

Dorante, L.O.T. (2007). Evaluation of a layered double hydroxide (LDH) mineral as a long-term nitrate exchanger in soil, Cuvillier Verlag., **(112 pages)**.

Elmi, C., (2019). Book Review: Infrared and Raman Spectroscopies of Clay Minerals. Am. Mineral., **(620 pages)**.

Evans, J.R., (1989). Photosynthesis and nitrogen relationships in leaves of C 3 plants. Oecologia., 78(1)**:** 9-19 **(10 pages)**.

Everaert, M.; Degryse, F.; McLaughlin, M.J.; De Vos, D.; Smolders, E., (2017). Agronomic effectiveness of granulated and powdered P-exchanged Mg–Al LDH relative to struvite and MAP. J. Agric. food Chem., 65(32): 6736-6744 **(9 pages)**.

Fathiazad, F.; Ahmadi-Ashtiani, H.; Rezazadeh, S.; Jamshidi, M.; Mazandarani, M.; Khaki, A., (2010). Study on phenolics and antioxidant activity of some selected plant of Mazandaran Province. J. Med. Plants., 9(34): 177-183 **(7 pages)**.

Haider, G.; Steffens, D.; Moser, G.; Müller, C.; Kammann, C.I., (2017). Biochar reduced nitrate leaching and improved soil moisture content without yield improvements in a four-year field study. Agric. Ecosyst. Environ., 237**:** 80-94 **(15 pages)**.

Halajnia, A.; Oustan, S.; Najafi, N.; Khataee, A.; Lakzian, A., (2013). Adsorption–desorption characteristics of nitrate, phosphate and sulfate on Mg–Al layered double hydroxide. Appl. Clay Sci., 80**:** 305-312 **(8 pages)**.

Halajnia, A.; Oustan, S.; Najafi, N.; Khataee, A.; Lakzian, A., (2016). Effects of Mg-Al layered double hydroxide on nitrate leaching and nitrogen uptake by maize in a calcareous soil. Commun. Soil Sci. Plant Anal., 47**:** 1162-1175 **(14 pages)**.

Hernández, Y.; Lobo, M.G.; González, M., (2006). Determination of vitamin C in tropical fruits: A comparative evaluation of methods. Food chem., 96(4)**:** 654-664 **(11 pages)**.

Holz, F.; Kremers, H., (1971). automatische Bestimmung des Stickstoffs als Indophenolgrun in Boden und Pflanzen. Landwirt Forsch Sonderheft., 177-191 **(15 pages)**.

Hu, Z.; Song, X.; Wei, C.; Liu, J., (2017). Behavior and mechanisms for sorptive removal of perfluorooctane sulfonate by layered double hydroxides. Chemosphere., 187**:** 196-205 **(10 pages)**.

Keeney, D.R.; Nelson, D.W., (1982). Nitrogen—Inorganic Forms 1. Methods of soil analysis. Part 2. Chem. Microbiol. prop., 2**:** 643-698 **(46 pages)**.

Keshavarz, H.; Sanavy, S.A.M.M., (2018). Yield and Oil Content of Mentheae under Different Nitrogen Fertilizer Treatments. Notulae Sci. Biol., 10(1)**:** 92-96 **(5 pages)**.

Kheirandish, E.; Roshdi, M.; Yousefzadeh, S., (2016). Effects of water stress and nitrogen fertilizer on quantitative and qualitative characteristics of Dragonhead (Dracocephalum moldavica L.). Electron. J. Crop PROD., 9(1): 109-125 **(17 pages)**.

Köhler, K.; Duijnisveld, W.; Böttcher, J., (2006). Nitrogen fertilization and nitrate leaching into groundwater on arable sandy soils. J. Plant Nutr. Soil Sci., 169: 185–195 **(11 pages)**.

Koilraj, P.; Antonyraj, C.A.; Gupta, V.; Reddy, C.; Kannan, V., (2013). Novel approach for selective phosphate removal using colloidal layered double hydroxide nanosheets and use of residue as fertilizer. Appl. Clay Sci., 86: 111-118 **(8 pages)**.

Li, S.; Juhász-Horváth, L.; Harrison, P.A.; Pinter, L.; Rounsevell, L., (2017). Effects of two slow-release nitrogen fertilizers and irrigation on yield, quality, and water-fertilizer productivity of greenhouse tomato. Agric. Water Manage., 186**:** 139-146 **(7 pages)**.

Mohammadi, M.; Mohammadi Torkashvand, A.; Biparva, P.; Esfandiari, M., (2019). Synthesis ratios of Mg-Al and Zn-Al layered double hydroxides efficiency and selectivity in nitrate removal from solution. Global J. Environ. Sci. Manage**.,** 5(4): 485-500 **(16 pages).**

Nie, Z.; Wang, J.; Rengel, Z.; Liu, H.; Gao, W; Zhao, P., (2018). Effects of nitrogen combined with zinc application on glutamate, glutamine, aspartate and asparagine accumulation in two winter wheat cultivars. Plant Physiol. Bio chem., 127**:** 485-495

(11 pages).
Ochoa-Velasco, C.E.; Valadez-Blanco, R.; Salas-Coronado, R.; Sustaita-Rivera, F.; Hernández-Carlos, B.; García-Ortega, S.; Santos-Sánchez, N.F., (2016). Effect of nitrogen fertilization and Bacillus licheniformis biofertilizer addition on the antioxidants compounds and antioxidant activity of greenhouse cultivated tomato fruits (Solanum lycopersicum L. var. Sheva). Sci. Hortic., 201: 338-345 **(8 pages)**.
Oke, F.; Aslim, B.; Ozturk, S.; Altundag, S., (2009). Essential oil composition, antimicrobial and antioxidant activities of Satureja cuneifolia Ten. Food Chem., 112: 874-879 **(6 pages)**.
Ouda, B.A.; Mahadeen, A.Y., (2008). Effect of fertilizers on growth, yield, yield components, quality and certain nutrient contents in broccoli (Brassica oleracea). Inter. J. Agric. biol., 10: 627-632 **(6 pages)**.
Overdahl, C.J.; Rehm, G.W.; Meredith, H.L., (1991). Fertilizer urea, Minnesota Extension Service, University of Minnesota., **(127 pages)**.
Papadopoulos, A.; Luo, X.; Leonhart, S.; Gosselin, A.; Pedneault, K.; Angers, P.; Gaudreau, L.; Dorais, M., (2000). Soilless greenhouse production of medicinal plants in North Eastern Canada. World Congr. Soil. Agric. Millennium., 554: 297-304 **(8 pages)**.
Popova, M.; Bankova, V.; Butovska, D.; Petkov, V.; Nikolova-Damyanova, B.; Sabatini, A.G.; Marcazzan, G.L.; Bogdanov, S., (2004). Validated methods for the quantification of biologically active constituents of poplar-type propolis. Phyto chem. Anal: Inter. J. Plant Chem. Bio chem. Tech., 15: 235-240 **(6 pages)**.
Pourmorad, F.; Hosseinimehr, S.; Shahabimajd, N., (2006). Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr. J. biotech., 5(11): 123-136 **(13 pages)**.
Rahman, M.; Mondol, A.; Rahman, M.; Begum, R.; Alam, M., (2007). Effect of irrigation and nitrogen on tomato yield in the grey terrace soil of Bangladesh. J. Soil Nat., 1: 1-4 **(4 pages)**.
Ting, S.; Rousseff, R.L., (1986). Citrus Fruits and Their Products: Analysis, Technology, Marcel Dekker New York.
Streeter, J.G., (1978). Effect of N Starvation of Soybean Plants at Various Stages of Growth on Seed Yield and N Concentration of Plant Parts at Maturity 1. Agron. J., 70: 74-76 **(3 pages)**.
Togun, A., (2003). Influences of compost and nitrogen fertilizer on growth nutrient uptake and fruit yield of tomato (Lycopersicum esculentum). Crop Res., 26: 98-105 **(8 pages)**.
Torres-Dorante, L.O.; Lammel, J.; Kuhlmann, H., (2009). Use of a layered double hydroxide (LDH) to buffer nitrate in soil: long-term nitrate exchange properties under cropping and fallow conditions. Plant soil., 315: 257-272 **(16 pages)**.
Torres-Dorante, L.O.; Lammel, J.; Kuhlmann, H.; Witzke, T.; Olfs, H.W., (2008). Capacity, selectivity, and reversibility for nitrate exchange of a layered double-hydroxide (LDH) mineral in simulated soil solutions and in soil. J. Plant Nutr. Soil Sci., 171: 777-784 **(8 pages)**.
Wang, H.; Zhang, Y.; Chen, A.; Liu, H.; Zhai, L.; Lei, B.; Ren, T., (2017). An optimal regional nitrogen application threshold for wheat in the North China Plain considering yield and environmental effects. Field Crops Res., 207: 52-61 **(10 pages)**.
Wehrmann, J.; Scharpf, H.C., (1986). The Nmin-method—an aid to integrating various objectives of nitrate fertilization. J. Plant Nutr. Soil Sci., 149: 428–440 **(13 pages)**.

AUTHOR (S) BIOSKETCHES

Mohammadi, M., Ph.D., Department of Soil Science, Science and Research Branch, Islamic Azad University, Tehran, Iran.
Email: *mohamadimaryam99@yahoo.com*

Mohammadi Torkashvand, M.A., Ph.D., Associate Professor, Department of Soil Science, Science and Research Branch, Islamic Azad University, Tehran, Iran. Email: *m.torkashvand54@yahoo.com*

Biparva, P., Ph.D., Assistant Professor, Department of Basic Science, Sari University of Agricultural Sciences and Natural Resources, Sari, Iran. Email: *p.biparva@sanru.ac.ir*

Esfandiari, M., Ph.D., Assistant Professor, Department of Soil Science, Science and Research Branch, Islamic Azad University, Tehran, Iran. Email: *mesfandiari@ut.ac.ir*

HOW TO CITE THIS ARTICLE

Mohammadi, M.; Mohammadi Torkashvand, A.; Biparva, P.; Esfandiari, M., (2021). The ability of layered double hydroxides for nitrate absorption and desorption in crop and fallow rotation. Global J. Environ. Sci. Manage., 7(1): 59-78.

DOI: 10.22034/gjesm.2021.01.05

url: https://www.gjesm.net/article_43296.html

Global Journal of Environmental Science and Management
(GJESM)

Homepage: https://www.gjesm.net/

ORIGINAL RESEARCH PAPER

Evaluation of genotoxic potential induced by marine cage culture

F. Turan*, M. Turgut

Faculty of Marine Science and Technology, Iskenderun Technical University, P.O. Box: 31200 Iskenderun, Hatay, Turkey

ARTICLE INFO

Article History:
Received 07 March 2020
Revised 24 May 2020
Accepted 26 June 2020

Keywords:
Aquaculture
Comet assay
Genotoxicity
Sparus aurata

ABSTRACT

BACKGROUND AND OBJECTIVES: The eutrophication process is increased by anthropogenic or aquaculture facilities in marine ecosystems. DNA damage biomarkers for fish species detect genotoxic parameters for ecological risk assessment. The aim of the present study was to determine genotoxic potential induced by marine cage culture in Iskenderun Bay on gilthead sea bream (*Sparus aurata*) using Comet assay.

METHODS: This study was conducted at cage and reference stations of Iskenderun Bay, Northeastern Mediterranean in January 2017. The wild and cultured samples of *S. aurata* and water samples were collected from wild and fish farm.

FINDINGS: The DNA damages at gill and liver cells of gilthead sea bream in the present study were observed with a higher level of DNA damage in gill cells compared to liver cells, and were determined at the low and minimal scale at the cage and reference stations, respectively. The present study demonstrated that the TP values were recorded at 0.020 and 0.016 mg/L in the cage and reference stations which are at border and below 0.020 mg/L. The DIN values were recorded at 0.097 and 0.075 mg/L in the cage and reference stations, which are at below 0.1 mg/L. The water bodies in the cage and reference stations exhibit Moderate/Mesotrophic water quality The correlations between physical-chemical parameters and DNA damage were shown that DIN, NH_4-N, NO_3-N and NO_2-N in water revealed significant positive correlations with DNA damage levels in gill cells.

CONCLUSION: The present study provides the first data set on genotoxic damage induced by marine cage culture in Iskenderun Bay on gilthead sea bream. The result of this research is an early warning for the marine system and further detailed research is needed to establish the source of the pollution and monitor environmental pollution.

DOI: 10.22034/gjesm.2021.01.06

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
39	3	3

*Corresponding Author:
Email: *funda.turan@iste.edu.tr*
Phone: +90 3266141866
Fax: +90 3266135613

Note: Discussion period for this manuscript open until April 1, 2021 on GJESM website at the "Show Article.

INTRODUCTION

There is increasing demand in culturing aquatic livings in coastal and inland waters. Aquaculture, likely the fastest growing food-producing sector, presently represents almost 50 percent of the world's food fish. Aquaculture production in world reached 80.1 million tons and valued at USD 237.5 billion by 2017. It is clearly accepted that world aquaculture production will remain to increase, especially in the developing countries of Asia and Africa, through the expansion of semi-intensive, small-scale pond aquaculture (FAO, 2018). Gilthead Sea bream, a species of great economic importance in Mediterranean aquaculture, thrives naturally in the coastal waters of the Mediterranean and Eastern Atlantic, often in marine lagoons, and is a fish commonly cultivated in marine cages and recirculating aquaculture systems (Basurco *et al.,* 2011). Gilthead sea bream is benthopelagic inhabiting shallow waters as well as various kinds of bottoms of coastal areas up to 100-150 m depth. Being eurythermic (2/5-32°C) and euryhaline (3‰- to full strength sea water) species. This fish is also a carnivorous species, but with a lower trophic level 3.3–3.5 (FishBase), that feeds preferentially on shellfish (mussels and oysters), crustaceous, fish and sometimes algae. Aquaculture as any aquaculture production activity, if not well managed, it can lead to ecological distraction (Gorlach-Lira *et al.,* 2013; FAO, 2014). Conventional aquaculture systems command aquaculture production in many areas, yet these are currently gradually being supplanted by intensive production approaches. Fast scale development of intensive farming systems usually causes adverse effects on surrounding environments. Intensive aquaculture has a non-stop or pulse release of nutrients that contribute to eutrophication. The main source of potentially polluting waste was discharged farm waste, uneaten feed and fish faeces (Cripps and Bergheim, 2000). Nutrients load and suspended solids in aquaculture effluent can cause eutrophication (Cho *et al.,* 1991; Ozbay *et al.,* 2014), oxygen depletion and algae blooms problems in the surrounding aquatic environments. Moreover, releasing aquaculture effluent of poor water quality may have an important effect on the marine organisms in ecosystems (Stephens and Farris, 2004). The amount of these pollutants in the effluent depends on a wide range of factors. In recent years, both worldwide and in our country, the assessment of the possible unfavourable environmental effects of aquaculture has been a salient issue. Feed-derived wastes are either dissolved, such as phosphorus (P) and nitrogen (N) based nutrients, or suspended as solids (Cripps and Bergheim, 2000). Their environmental impact can be decreased either by improved farm management, or by physical and/or biological treatment of the effluent (Moustafa *et al.,* 2020). The assessments in ecological risks, relied on molecular or biochemical markers, has been highlighted in eco-toxicological and genotoxic studies (Connon *et al.,* 2012; Baudou *et al.,* 2019) handling the reviews of marine ecologies impacted as a result of publicity to at least one or more pollution along with anthropogenic or agriculture/aquaculture services (Chapman, 2007; Kroon *et al.*, 2017). The detection procedures of DNA damage on the degree of a character eukaryotic cellular have been formerly utilized to a diffusion of research regions together with plant sciences and mammal toxicology research (Nehls and Segner, 2005; Olive and Banáth, 2006). Currently, comet assay is a standard and flexible approach, followed for eco-toxicological studies that can be applied to truly any animal and plant tissue that may be disaggregated into single cells, measuring the breaks in the DNA chain prompted via natural or inorganic pollution (De Lapuente *et al.,* 2015). The single-cell gel electrophoresis counts the DNA breaks, alkali labile sites, DNA crosslinks, damage in base or base pairs, and apoptosis in the cells of living organisms. The Comet assay was started by Ostling and Johanson (1984) and then modified via Singh *et al.* (1988). Standard dose-reaction tracking method for determining of molecular-level damages in marine animals has now commonly been used (Li *et al*., 2013; Turan and Ergenler 2019). Hallare *et al.* (2016) used the comet analysis and micronucleus test for the genotoxic effects induced by means of intensive cage aquaculture in Taal Lake (Philippines) on Nile tilapia (*Oreochromis niloticus*) that comet assay was reported as high-quality biomarkers for investigating the hazardous effects of cage-culture on freshwater quality. Arslan *et al.* (2016) stated that rainbow trout which grown in cage culture may be more pronounced with genetic damage, depending on the cage stress and concluded that these changes may be associated with nutritional conditions. Likewise, Demir *et al.,* (2015) reported that there is a correlation between water pollution that was caused by over-feeding in fish breeding farms and in vivo genotoxicity in rainbow trout (*O. mykiss*). Different anthropogenic activities (such as fish breeding farms and fertilizers) increased the genotoxicity in rainbow trout lymphocytes according to pollution rate. Despite the number of the studies, there is lack of information about evaluation of genotoxic damage in cultured and wild marine fishes, especially. The main aim of this study was to evaluate the genotoxic damage in cultured and wild gilthead sea bream (*Sparus aurata*) from Iskenderun Bay by Comet assay. This study has been carried out in Hatay, Turkey, in 2017.

MATERIAL AND METHODS

Sampling area

This study was conducted at Cage and Reference station in January 2017. As Cage station, cultured gilthead sea bream (*Sparus aurata*) and cage water samples were collected from a fish farm located in Iskenderun Bay (36°29'57.4"N 35°57'42.8"E). Wild specimens and sea water samples as Reference station were collected from the coastal zone (36°26'48.6"N 35°53'27.3"E) of Iskenderun Bay, Northeastern Mediterranean using commercial trawler (Fig. 1). Reference Station was assigned an unaffected location of the upstream area about 1 km in distance from the cage station. The Iskenderun bay receiving anthropogenic inputs and surface runoff in winter season, and indicates development of mesotrophic/eutrophic conditions locally in these semi-enclosed water bodies, due to NOx rich river in flows with modified N/P/Si ratios and direct discharges of urban wastewaters (Tugrul *et al.* 2019).

Sampling procedure and Water quality assessment

Water and fish samples were collected during the winter season in January 2017. The wild and cultured samples of *S. aurata* (10 individuals from each sampling sites) were collected from wild and fish farm, and immediately transported to the laboratory. Measurements such as total body length (cm) and wet weight (g) of sampling sea bream (±SD) were recorded at wild *S. aurata* (410.64±7.05 g, 27.50±0.70 cm) and cultured *S. aurata* (320.45±9.25 g, 24.56±0.50 cm). The sea bream samples were euthanized for gill and liver removal and immediately after dissection they were carefully washed with phosphate buffer. Water samples of sampling sites were collected at 15–30 cm below the surface, following the descriptions of DEA (2012) with Nansen hydrographical bottle. Water samples were collected in triplicate from each of the selected sites in winter season (January) 2017. The water samples were taken in 1000-mL polyethylene bottles after rinsing few times with water from the collection point and later transferred to the laboratory in cooler, containing ice to reducing the degradation of samples before analysis. The samples were filtered as soon as possible through 0.45 µm membrane filter. Immediate analysis is recommended but if it wasn't possible, samples was directly deep frozen till carrying out chemical analysis. The recommended standard methods (APHA, 2005) was used for

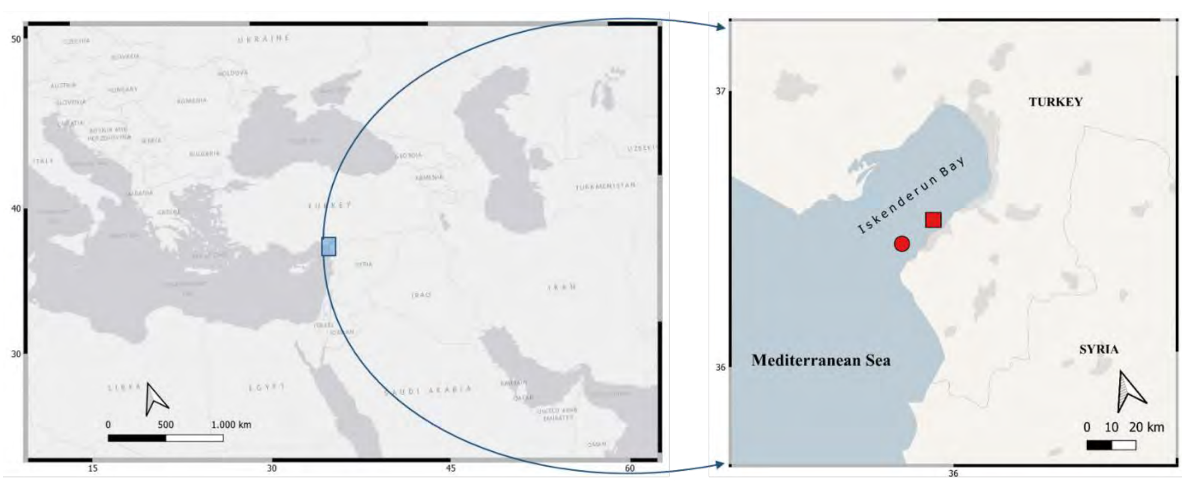


Fig. 1: Geographic location of the study area along with the sampling stations in the Iskenderun Bay, Turkey (reference station; cage station)

physicochemical analyses and preservation of water samples. These water-quality parameters tested include; temperature (ºC), pH, Alkalinity (mg/L), HCO_3 (mg/L), Total phosphate (TP) (mg/L), Dissolved Inorganic Nitrogen (DIN) (mg/L), Ammonium nitrogen (NH_4-N) (mg/L), Nitrate nitrogen (NO_3-N) (mg/L), Nitrite nitrogen (NO_2-N) (mg/L), Ammonia (NH_3-N) (mg/L), Sulphate (SO_4-S) (mg/L) were conducted in triplicates. In the sampling locations, seawater quality parameters such as temperature and pH were measured using YSI model multi probe system during the sampling.

Water quality criteria

National water quality criteria (Turkish Environmental Guideline) (TEG, 2005, 2006) were determined as proposed by Ministry of Agriculture and Forestry as the main authority responsible for regulating marine finfish aquaculture in Turkey. The TEG was determined by measuring some of the analyzed physicochemical parameters (temperature, pH, Nitrate nitrogen, Nitrite nitrogen, Ammonia etc.). In addition, National Mediterranean Coastal Waters Criteria tools were determined by Ministry of Forestry and Water Management (TEG, 2012). The Eutrophication indices obtained were classified as follows: DIN values:<0.020 mg/L; TP values:<0.010 mg/L (Good/Oligotrophic water quality); DIN values:0.020-0.1mg/L; TP values:0.010-0.020mg/L (Moderate/Mesotrophic properties); DIN values:0.1-0.2 mg/L; TP values:>0.02-0.03 mg/L (Poor/eutrophication); DIN values:>0.2 mg/L; TP values:>0.03 mg/L (Bad/ Dystrophic).

Comet assay

Cellular dissociation method modified from Cavalcante *et al.* (2008) was used in Comet assay. Gill and liver tissues of *S. aurata* were homogenized in order to get single-cell suspension and centrifuged at 3000 rpm at 4 °C for 5 min for the cell suspension, and then the cell pellet was retained. Cell viability was evaluated by the Trypan blue exclusion test (Anderson *et al.*, 1994). Singh *et al.* (1988) was followed for performing the single cell gel electrophoresis. The slides were neutralized with ice cold 0.4 M Tris buffer (pH 7.5) and stained with 80 ml ethidium bromide (20 mg/mL) and counted with an image analysis system. Images of 100 cells from each sample were scored with Comet Analysis Software, *V* 3.0. Tail density (%T-DNA), tail moment (µm) and tail migration (TM_i) were taken as the parameter of the nuclear DNA damage.

Statistical analysis

One-way analysis of variance (ANOVA) was used for statistical evaluations of data. Principal component analysis (PCA) was used to get a comprehensive view of the results and define the most important parameters involved in DNA damage (Zheng *et al.*, 2016). All data were executed using IBM SPSS Statistics 21 and R-Studio.

RESULTS AND DISCUSSION

The physical-chemical parameters of the water samples collected from the cage and reference station were given in Table 1. The data related to temperature, pH, Alkalinity, $HCO_{3,}$ SO_4-S and TP were not significantly different between the cage and reference stations (P>0.05). Dissolved Inorganic Nitrogen (DIN) in cage and sea water samples was 0.097±0.004 mg/L and 0.075±0.005 mg/L, respectively, and the data obtained for DIN was statistically different between the cage and reference stations (P<0.05). NH_4-N, NO_3-N, NO_2-N and NH_3-N parameters were also significantly different between the cage and reference stations (P<0.01). The present study demonstrated that the nutrient concentrations in the cage station were between the applicable high-quality levels of water traits according to the national marine aquaculture limits. The detected pH, temperature, NO_3-N, NO_2-N and NH_3-N parameters did not exceed the national water quality criteria (TEG 2005, 2006).

Furthermore, our findings in terms of nutrients were similar to those of previously performed research in offshore cage systems in the Aegean Sea, Turkey (Gurses *et al.*, 2019). Moreover, the TP values as Eutrophication Criteria (E.C.) tools were recorded at 0.020 mg/L and 0.016 mg/L in the cage and reference stations which are at border and below the TEG (2012) limit, 0.020 mg/L. The DIN values as Eutrophication Criteria (E.C.) tools were recorded at 0.097 mg/L and 0.075 mg/L in the cage and reference stations, respectively in this study, which are at below the TEG (2012) limit, 0.1 mg/L. The water bodies in the cage and reference stations in the Iskenderun Bay exhibit moderate/mesotrophic water quality according to the TEG (2012). Similarly, Tugrul *et al.*

Table 1: Physical-chemical parameters of the water samples collected from the cage and reference stations

Parameters (mg/L)	Reference station	Cage Station	Water quality criteria
pH	8.367±0.153	8.507±0.125	6.5–8.5
Temperature (ºC)	16.0±0.5	16.5±0.5	15–25
Alkalinity	34.333±1.155	33.333±2.887	-
HCO_3	44.000±1.732	43.333±2.887	-
Total phosphate (TP)	0.016±0.003	0.020±0.008	-
Dissolved Inorganic Nitrogen(DIN)*	0.075±0.005	0.097±0.004	-
Ammonium nitrogen (NH_4-N)**	0.039±0.001	0.047±0.001	-
Nitrate nitrogen (NO_3-N)**	0.026±0.001	0.032±0.001	< 40
Nitrite nitrogen (NO_2- N)**	0.009±0.001	0.017±0.001	< 0.5
Ammonia (NH_3- N)*	0.011±0.002	0.017±0.002	0.05–1.5
Sulphate (SO_4-S)	73.333±2.887	70.000±5.000	-

The data are shown as arithmetic mean ± standard deviation. Indicate significance level between the water samples collected from the cage and reference station (*, P<0.05; **, P<0.01). Water quality criteria: Proposed licensing requirements for marine aquaculture in Turkey (TEG, 2005, 2006); Ministry of Agriculture and Forestry as the main authority responsible for regulating marine finfish aquaculture in Turkey.

Table 2: DNA damage in the gill and liver cells of wild and cultured gilthead sea bream from the cage and reference station analyzed by Comet Assay

Gill		
Head Length (µm)**	24.845±0.419	26.668±0.401
Tail Length (µm)***	19.160±0.429	23.930±0.508
H-DNA (%)*	85.871±0.902	82.236±1.125
T-DNA(%)*	14.128±0.902	17.763±1.125
Tail.Moment (µm)**	1.846±0.157	2.958±0.244
Tail Migration (TMi)**	7.157±0.509	11.047±0.606
Liver		
Head Length (µm)**	24.168±0.263	28.423±0.835
Tail Length (µm)**	15.397±0.287	17.560±0.382
H-DNA (%)	91.749±0.530	91.004±1.118
T-DNA(%)	8.250±0.530	8.995±1.118
Tail Moment (µm)	0.793±0.075	0.885±0.005
Tail Migration (TMi)	3.592±0.327	3.349±0.264

The data are shown as arithmetic mean ± standard deviation. Indicate significance level between wild and cultured gilthead sea bream from the cage and reference station (*, P<0.05; **, P<0.01).

(2019) also reported that the Eastern Mediterranean and its offshore waters are in oligotrophic, and the inner sites of the Mersin and Iskenderun Bays are in mesotrophic conditions. DNA damage in the gill and liver cells of wild and cultured gilthead sea bream from the cage and reference stations analyzed by Comet assay are given in Table 2.

A higher level of DNA damage in gill cells compared to liver cells was observed in sea bream samples (Table 2). The highest level of DNA damage as %T-DNA, TM and TMi in gill cells were 17.763±1.125%, 2.958±0.244µm, 11.047±0.606 TMi at the cage station, respectively (Table 2). Likewise, the highest level of DNA damage as %T-DNA, tail moment and tail migration in liver cells were 8.995±1.118%, 0.885±0.005µm, 3.349±0.264TMi at the cage station, respectively. Significant differences ($P<0.01$) in DNA damage especially gill cells between the cage and reference stations from Iskenderun Bay (Table 2). The increased concerns on the genotoxicity of organic/inorganic pollutants lead to the usage of sensitive bioassays as an important instrument to monitor the genotoxicity of polluted water columns. The present study provides the first data set on genotoxic damage induced by marine cage culture in Iskenderun Bay on gilthead sea bream using Comet Assay. The DNA damages at gill and liver cells of gilthead sea bream in the present study were observed with a higher level of DNA damage in gill cells compared to liver cells in both the cage and reference stations. Gills may be more susceptible to pollutants than other tissues owing to a high respiratory blood flow and permanent contact with the water. Gill tissue are commonly used for monitoring water pollution due to their direct contact with the water. Several studies reported that gill was sensitive and target tissue for water pollution monitoring (Lenhardt *et al.,* 2015; Butrimavičienė *et al.,* 2018). On the other hand, the liver also has a high accumulation potential, and therefore, used as an important pollution indicator (Ploetz *et al.,* 2007). The DNA damages at gill and liver cells of gilthead sea bream in the present study were determined at

Table 3: Eigenvalue, proportion and cumulative contribution of physico-chemical variables to DNA damage of sea bream on first two Principal Components

Component	Eigenvalues	Variance (%)	Cumulative (%)
1	13.559	61.632	61.632
2	4.790	21.775	83.407
3	1.758	7.993	91.400
4	1.060	4.816	96.216
5	0.832	3.784	100.000

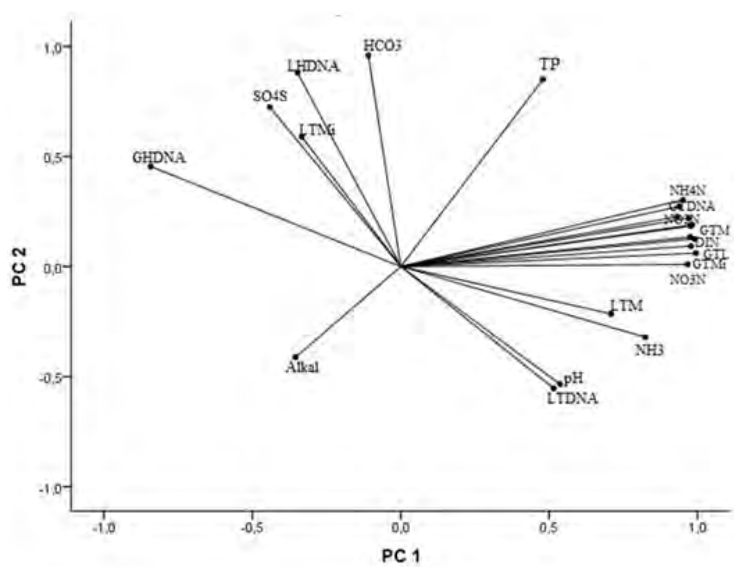


Fig. 2: Contribution and relation of analysed parameters on first two Principal Components (GTDNA: Tail density in gill cells; GTM: tail moment (TM) in gill cells; GTMi: tail migration (TM_i) in gill cells; LTDNA: Tail density in liver cells; LTM: tail moment (TM) in liver cells; LTMi: tail migration (TM_i) in liver cells).

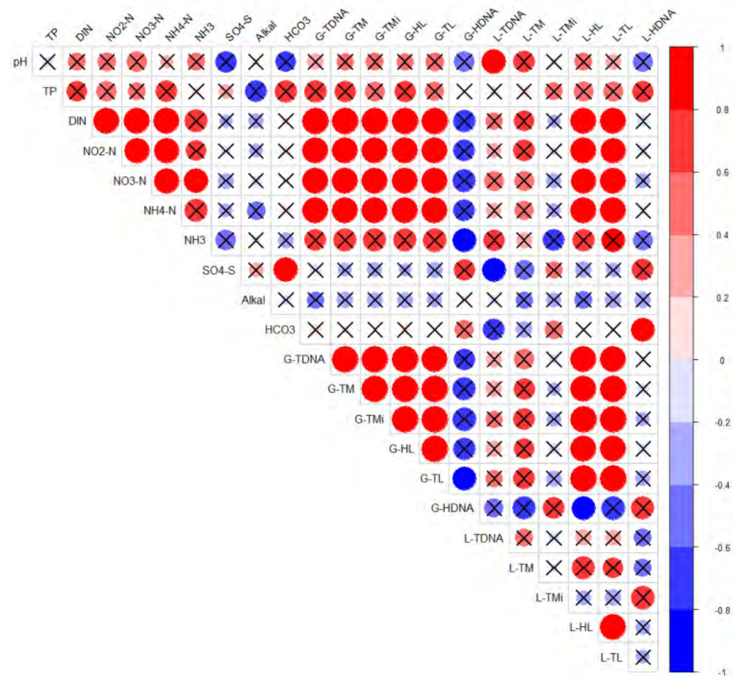


Fig. 3: Heatmap of correlations between parameters. The scale color bar indicate correlation between -1 and +1. Cross (x) indicate the insignificant correlations according to the specified significance level (P>0.05) and non-crossed circle indicate significant correlations according to the specified significance level (P<0.05)

the low and minimal scale at the cage and reference stations, respectively, based on Mitchelmore *et al.* (1998) who reported %T-DNA damage scale as <%10 T-DNA minimal damage, %10-25 T-DNA low damage, %25-50 T-DNA medium damage, %50-70 T-DNA high damage and >%70 T-DNA extreme damage. The DNA damage in fish tissue are commonly applied for detecting the genotoxic pollution of water columns (Colin *et al.,* 2016) being able to adjust exposure to low concentrations of pollutant in candidate sentinel species. Similarly, Gutiérrez *et al.* (2019) reported that mussels from mollusc farm in the Guanabara Bay revealed small amount of genomic destruction, on the other hand, mussels from aquaculture located at Rasa Beach and Forno Bay exhibited values near to zero. Furthermore, Demir *et al.* (2015) reported that the degree of DNA damage was low scale in the blood cells of rainbow trout collected from different sites from Esen stream with nutrient pollution generated from overfed fish farms. On the other hand, first two principal components revealed 61.632 % and 21.775 % of total variations, respectively in Principal component analysis (PCA) (Table 3). When the pattern of strong contribution of the DIN, NH_4-N, NO_3-N and NO_2-N (in the water column) parameters and the DNA damage in gill cells of gilthead sea bream were examined here with the PCA, the DNA damage parameters seems to be correlated with NH_3-N, DIN, NH_4-N, NO_3-N and NO_2-N which are relatively important parameters involved in DNA damage (Fig. 2).

Correlations between physical-chemical parameters of the water samples and DNA damage parameters were shown with Heatmap that DIN, NH_4-N, NO_3-N and NO_2-N in water revealed significant positive correlations (P<0.05) with DNA damage levels in gill cells (Fig. 3).

There were no significant correlations between the other physical-chemical parameters (pH, TP, NH_3-N, SO_4-S, Alkalinity and HCO_3) and DNA damage parameters both in gill and liver cells of *S. aurata*. However, a positive correlation (r^2=0.9) was observed between the gill T-DNA and other DNA damage parameters in all the examined samples (Fig. 3). The similar correlations were also reported with the previous study that Demir *et al.* (2015) showed that there is a correlation between water pollution and genotoxicity in rainbow trout (*O. mykiss*) in the Esen Stream that was caused by over-feeding in fish farms.

CONCLUSION

In conclusion, this is the first study on genotoxic damage induced by marine cage culture in Iskenderun Bay, Northeastern Mediterranean. The physical-chemical parameters and nutrient concentrations in cage station were between acceptable ranges of water quality characteristics and within the limits suitable for marine aquaculture activities. The water bodies in the cage and reference stations in coastal waters of the Iskenderun Bay, Northeastern Mediterranean exhibit moderate/mesotrophic water quality. The DNA damages at gill and liver cells of gilthead sea bream were determined at the low and minimal scale at the cage and reference stations, respectively. Furthermore, the correlations between physical-chemical parameters and DNA damage were shown that DIN, NH_4-N, NO_3-N and NO_2-N in water revealed significant positive correlations with DNA damage levels in gill cells. Correspondingly, the present study revealed the effectiveness of genotoxic markers for monitoring aqua cultural and environmental pollution by using damage DNA of the gilthead sea bream, *Sparus aurata*. Consequently, the assessment of genotoxicity by Comet Assay from cultured and wild gilthead sea bream denotes as a convenient marker to evaluate the potential pollution effect of aqua cultural activities. Aquaculture is getting an important factor in the global food supply in the future. In intensive cultural events, the main wastes are solid, chemicals and several therapeutics. Potential pollutant properties of aquaculture activities to marine habitats are progressively acknowledged, while they are a lesser quantity to land-based pollutants. Therefore, it is increasingly important to monitor genotoxic effects of any aquaculture activities in related environments. Accurately planned usage of aquaculture waste lightens marine pollution events and not only protects valuable marine assets but also profits the nutrients comprised efflux. Thus, it is greatly challenging to advance sustainable aquaculture that deliberate stocking density and pollution loadings below environmental capacity. From the management point of view, further researches are encouraged in terms of continuous monitoring of cage farm places in order to control water quality and potential culture effects for the maintainable aquaculture in the Mediterranean.

AUTHOR CONTRIBUTIONS

F. Turan performed the conception and design of the study, laboratory studies, water quality analysis acquisition of data, drafting the manuscript, reviewing and editing. M. Turgut performed laboratory studies, sampling procedure and water quality analysis.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest regarding the publication of this work. In addition, the ethical issues including plagiarism, informed consent, misconduct, data fabrication and, or falsification, double publication and, or submission, and redundancy have been completely witnessed by the authors.

ABBREVIATIONS

%H.	DNA Head Density
%T	DNA Tail Density
ANOVA	One-way Analysis of Variance
APHA	American Public Health Association
COMET	The Single-cell Gel Electrophoresis
DEA	Department of Environmental Affairs
DIN	Dissolved Inorganic Nitrogen
FAO	The Food and Agriculture Organization
N	Nitrogen
NH_3- N	Ammonia
NH_4-N	Ammonium nitrogen
NO_2-N	Nitrite nitrogen
NO_3-N	Nitrate nitrogen
P	Phosphorus
PCA	Principal Component Analysis
SO_4-S	Sulphate
TEG	Turkish Environmental Guideline
TM	Tail Moment
TM_i	Tail Migration
TP	Total Phosphate
GTDNA	Tail density in gill cells
GTM	Tail moment (TM) in gill cells
GTMi	Tail migration (TMi) in gill cells

LTDNA Tail density in liver cells
LTM Tail moment (TM) in liver cells
LTMi Tail migration (TMi) in liver cells

REFERENCES

Anderson, D.Y.T.W.; Yu, T.W.; Phillips, B.J.; Schmezer, P., (1994). The effect of various antioxidants and other modifying agents on oxygen-radical-generated DNA damage in human lymphocytes in the COMET assay. Mutat. Res-Fund. Mol. M., 307(1): 261-271 **(11 Pages).**

APHA (2005) Standard Methods for the Examination of Water and Wastewater. 21st Edition, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC.

Arslan, K.; Duman, F.; Kanbur, M.; Akyüz, B., (2016). Genotoxicological Analysis of Naturally Captured and Cultured Rainbow Trout (*Oncorhynchus mykiss*) in Pınarbasi District of Kayseri, J. Faculty. Vet. Med., Erciyes University, 13(2): 131-138 **(8 pages).**

Basurco, B.; Lovatelli, A.; García, B., (2011). Chapter 1: Current Status of Sparidae Aquaculture, in: Pavlidis, M.A., Mylonas, C.C. (Eds.), Sparidae: Biology and Aquaculture of Gilthead Sea Bream and Other Species. Blackwell Publishing 366 Ltd., West Sussex, UK. 1-50 **(50 pages).**

Baudou, F.G.; Ossana, N.A.; Castañé, P.M.; Mastrángelo, M.M.; González Núñez, A.A.; Palacio, M.J.; Ferrari, L., (2019). Use of integrated biomarker indexes for assessing the impact of receiving waters on a native neotropical teleost fish. Sci. Total Environ., 650(1): 1779-1786 **(8 pages).**

Butrimavičienė, L.; Baršienė, J.; Greiciūnaitė, J.; Stankevičiūtė, M.; Valskienė, R., (2018). Environmental genotoxicity and risk assessment in the Gulf of Riga (Baltic Sea) using fish, bivalves, and crustaceans. Environ. Sci. Pollut. Res., 25(25): 24818-24828 **(11 pages).**

Cavalcante, D.G.S.M.; Martinez, C.B.R.; Sofia, S.H., (2008). Genotoxic effects of Roundup® on the fish *Prochilodus lineatus*. Mutat. Res. Gen. Toxicol. Environ. Mutagen., 655(1-2): 41-46 **(6 pages).**

Chapman, P.M., (2007). Determining when contamination is pollution—weight of evidence determinations for sediments and effluents. Environ. Int., 33(4): 492-501 **(10 pages).**

Cho; C.Y.; Hynes, J.D.; Wood, K.R.; Yoshida, H.K., (1991). Quantification of fish culture wastes by biological (nutritional), and chemical (luminological) methods; the development of high nutrient dense (HND) diets. In: Cowey, C.B., Cho, C.Y. (Eds.), Nutritional Strategies and Aquaculture Waste. Proceedings of the First International Symposium on Nutritional Strategies in Management of Aquaculture Waste. University of Guelph, Guelph, Ont., 37–50 **(4 pages).**

Colin, N.; Porte, C.; Fernandes, D.; Barata, C.; Padrós, F.; Carrassón, M.; Maceda-Veiga, A., (2016). Ecological relevance of biomarkers in monitoring studies of macro-invertebrates and fish in Mediterranean rivers. Sci. Total Environ. 540: 307-323 **(17 Pages).**

Connon, R.E.; Geist, J.; Werner, I., (2012). Effect-based tools for monitoring and predicting the ecotoxicological effects of chemicals in the aquatic environment. Sensors, 12(9): 12741-12771 **(31 pages).**

Cripps, S.J.; Bergheim, A., (2000). Solids management and removal for intensive land-based aquaculture production systems. Aquacult. Eng., 22(1-2): 33-56 **(24 pages).**

De Lapuente, J.; Lourenco, J.; Mendo, S.A.; Borras, M.; Martıns, M.; Costa, G.P.M.; Pacheco, M., (2015). The Comet Assay and its applications in the field of ecotoxicology: a mature tool that continues to expand its perspectives. Front. Genet., 180: 1-20 **(20 pages)**.

DEA, (2012). Department of Environmental Affairs. (DEA)Chapter 9: Oceans and Coasts: Ocean and Coasts Ecosystem Services Are Important as They Directly and Indirectly Impact on Human Livelihoods, Food Security and Agriculture; Oceans and Coasts, p. 26.

Demir, E.; Turna, F.; Aksakal, S.; Emre, Y.; Emre, N.; Yağcı, A.; Kaya, B., (2015). The comet assay using rainbow trout (Oncorhynchus mykiss) for the detection of nutrient pollution generated from overfed fish farms in the esen stream. Fresenius Environ. Bull., 24(11): 3665-3671 **(7 pages).**

FAO, (2014). The state of world fisheries and aquaculture. ISBN: 978-92-5-108275-1 Rome, Italy, The Food and Agriculture Organization**,** p. 243.

FAO, (2018). The state of world fisheries and aquaculture. Rome, Italy. The Food and Agriculture Organization**, p.** 256.

Gorlach-Lira, K.; Pacheco, C.; Carvalho, L.C.T.; Melo Junior, H.N., Crispm, M.C., (2013). The influence of fish culture in floating net cages on microbial indicators of water quality. Braz. J. Biol., 73(3): 457-463 **(7 pages).**

Gürses, R.K.; Büyükateş, Y.; Yiğit, M.; Ergün, S.; Ateş, A.S.; Özdilek, H.G., (2019). Potential Environmental Impacts of Tuna Cage Farming in The Aegean Sea. Aquat. Res., 2(2): 61-72 **(12 pages).**

Gutiérrez, J. M.; Molisani, M.M.; Da Conceição, M.B.; Weber, L.I., (2019). Characterisation of coastal aquaculture sites in the state of Rio de Janeiro, Brazil, using genotoxicity biomarkers. Reg. Stud. Mar. Sci., 32: 100870-100876 **(7 pages).**

Hallare, A.; Ocampo, K.A.; Tayo, P.K., Balolong, M., (2016). Genotoxic stress induced by intensive aquaculture activities in Taal Lake (Philippines) on circulating fish erythrocytes using the comet assay and micronucleus test. Adv. Environ. Biol., 10(1): 273-283 **(11 pages).**

Kroon, F.; Streten, C.; Harries, S.A., (2017). Protocol for identifying suitable biomarkers to assess fish health: A systematic review. PLOS ONE, 12(4): 1-42 **(42 pages).**

Lenhardt, M.; Poleksić, V.; Vuković-Gačić, B.; Rašković, B.; Sunjog, K.; Kolarević, S.; Gačić, Z., (2015). Integrated use of different fish related parameters to assess the status of water bodies. Slov. Vet. Res., 52(1): 5-13 **(9 pages).**

Lı, Z.H.; Zlabek, V.; Velısek, J.; Grabıc, R., Machova, J.; Kolarova, J.; Lı, P.; Randak, T. (2013). Multiple biomarkers responses in juvenile rainbow trout, *Oncorhynchus mykis*, after acute exposure to a fungicide propiconazole. Environ.Toxicol., 28(3): 119-126 **(8 pages).**

Mitchelmore, C.L; Chipman, J.K. (1998). DNA strand breakage in aquatic organisms and the potential value of the comet assay in environmental monitoring. Mutat. Res-Fund. Mol. M., 399(2): 135-147 **(13 pages).**

Moustafa, Y. T.; Ali, A.; Gomha, S. A.; Mansour, E. M., (2020). Nitrogen and Phosphorus budget for Nile tilapia hatchery. Egypt.

J. Aquac., 10(1): 1-22 **(22 pages).**

Nehls, S.; Segner, H., (2005). Comet Assay with the Fish Cell Line Rainbow Trout Gonad-2 for Invitro Genotoxicity Testing of Xenobiotics and Surface Waters. Environ. Toxicol. Chem., 24(8): 2078-2087 **(10 pages).**

Olive, P.L., Banáth, J.P., (2006). The comet assay: a method to measure DNA damage in individual cells. Nat. Protoc., 1(1): 23-31 **(9 pages).**

Ostling, O.; Johanson, K., (1984). Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. Biochem. Bioph. Res. Co., 123(1): 291-298 **(8 pages).**

Ozbay, G.; Blank, G.; Thunjai, T., (2014). Impacts of aquaculture on habitats and best management practices (BMPs). Sustain. Aquacult. Tech., 1.

Ploetz, D.M.; Fitts, B.E.; Rice, T.M., (2007). Differential accumulation of heavy metals in muscle and liver of a marine fsh (King Mackerel *Scomberomorus cavalla* Cuvier) from the Northern Gulf of Mexico USA, Bull Environ. Con. Toxicol., 78 (2): 124–127 **(4 pages).**

Sıngh, N.P.; Mccoy, M.T.; Tıce, R.R.; Schneıder, E.L., (1988). A simple technic for quantitation of low levels of DNA damage in individual cells. Exp. Cell. Res., 175(1): 184-191 **(8 pages).**

Stephens, W.W.; Farris, J. L., (2004). A biomonitoring approach to aquaculture effluent characterization in channel catfish fingerling production. Aquacult.., 241(1): 319–330 **(12 pages).**

TEG, (2005). Regulation about the changes in the Aquaculture Regulation announced in Official Gazette No. 25967. Turkish Environmental Guidelines. Ankara. Ministry of Agriculture and Forestry, Turkey.

TEG, (2006). Circular Based on the Regulation of Aquaculture announced in Official Gazette No. 25967. Turkish Environmental Guidelines. Ankara. Ministry of Agriculture and Forestry, Turkey.

TEG , (2012). Surface water quality management regulation. Turkish Environmental Guidelines (in Turkish), Official Gazette (dated 30 November 2012) numbered 28483, Ankara. Publications of Republic of Turkey Ministry of Forestry and Water Affairs.

Tugrul, S.; Ozhan, K.; Akcay, I., (2019). Assessment of trophic status of the Northeastern Mediterranean coastal waters: eutrophication classification tools revisited. Environ. Sci. Pollut. Res., 26(15): 14742-14754 **(13 pages).**

Turan, F.; Ergenler, A., (2019). Assessment of DNA Damage by Comet Assay in Trachinotus ovatus Cells from Mersin Bay in the Northeastern Mediterranean, Nat. Eng. Sci., 4(3): 25-31 **(7 pages).**

Zheng, X.Q.; Shi, Y.J.; Lu, Y.L.; Xu, X.B., (2016). Growth inhibition and DNA damage in the earthworm (Eisenia fetida) exposed to perfluorooctane sulphonate and perfluorooctanoic acid. Chem. Ecol. 32 (2): 103-116 **(14 Pages).**

AUTHOR (S) BIOSKETCHES

Turan, F., Ph.D., Professor, Faculty of Marine Science and Technology, Iskenderun Technical University, P.O. Box: 31200 Iskenderun, Hatay, Turkey. Email: *funda.turan@iste.edu.tr*

Turgut, M., M.Sc. Student, Faculty of Marine Science and Technology, Iskenderun Technical University, P.O. Box: 31200 Iskenderun, Hatay, Turkey. Email: *mehmetturgut001@gmail.com*

HOW TO CITE THIS ARTICLE

Turan, F.; Turgut, M., (2021). Evaluation of genotoxic potential induced by marine cage culture. Global J. Environ. Sci. Manage., 7(1): 79-88.

DOI: 10.22034/gjesm.2021.01.06

url: https://www.gjesm.net/article_43089.html

Global J. Environ. Sci. Manage. 7(1): 89-102, Winter 2021

Global Journal of Environmental Science and Management
(GJESM)

Homepage: https://www.gjesm.net/

ORIGINAL RESEARCH PAPER

Palm oil plantation waste handling by smallholder and the correlation with the land fire

H. Herdiansyah*, E. Frimawaty

School of Environmental Science, Universitas Indonesia, Central Jakarta, DKI Jakarta, Indonesia

ARTICLE INFO

Article History:
Received 23 Jume 2020
Revised 12 July 2020
Accepted 13 August 2020

Keywords:
Land fire
Palm oil plantation waste
Smallholder waste
Statistical analysis
Waste handling

ABSTRACT

BACKGROUND AND OBJECTIVES: From August to October 2019, several provinces in Sumatra and Kalimantan had faced severe forest fires, causing thousands of citizens to suffer respiratory disorders. This study aims to assess waste handling in palm oil plantation manage by smallholders and the correlation palm oil plantation waste handling with the fireland in Sumatera, especially on Jambi province.
METHODS: Primary data collection was conducted in September 2019, and a purposive random sampling method was used to select respondents. Primary data collection was applied for four hundred smallholders in five districts in Jambi using a mixed method.
FINDINGS: Out of 400 correspondents that handle their waste, 50% of respondents handle the residues by stacking the waste on their field, 25% of correspondents stack the waste between trees, 17.25% of correspondents stack the waste on piles, 5% of them bury the posts, and 2.75% incinerate the waste. The average distance from home to the field for 200 correspondents is 8.825 kilometres, and they have the highest harvest quantity with a mean of 1.0940 tons. Most of them are common smallholders and self-subsistent smallholders. The 298 correspondents join a farming association. About 50% of smallholders in Jambi handle the residues by stacking the wastes on their field instead of incinerating the waste.
CONCLUSION: Out of the overall samples collected in this study, only 2.75% smallholders in Jambi incinerate their residues. Hence, the fire breakouts happened on several provinces in Sumatera and Kalimantan in late 2019 did not happen due to crude palm oil waste-handling activities.

DOI: 10.22034/gjesm.2021.01.07

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
42	1	4

*Corresponding Author:
Email: *herdis@ui.ac.id*
Phone: +628 562053791
Fax: +622131922269

Note: Discussion period for this manuscript open until April 1, 2021 on GJESM website at the “Show Article.

INTRODUCTION

Palm oil is one of the most potential commodities which have made its smallholders also be important on the past and future palm oil industry. Palm oil cultivation is related to land clearing, both from the forestry sector and from grasslands. Land clearing for palm oil plantations takes place in several regions in Southeast Asia (Gatti *et al.*, 2019; Uusitalo *et al.,* 2014). Land conversion also occurs in Brazil. Nearly 47.7% of primary forests were converted, and an increase of forest degradation by 17% in agriculture was reported. There was also an increase in land expansion in the palm oil sector by 11%. Over a 22-year period, 30% of forests in Brazil were converted into palm oil plantations (de Almeida *et al.*, 2019). Palm oil expansion is carried out in primary, secondary forests, and spread to the prairie area, which contributed to 9% of land conversion in Brazil (Benami *et al.,* 2018). The existence of palm oil plantations causes several problems, including land degradation, deforestation, and fires. Unregulated logging activities and land fire affected the degradation of land. The existence palm oil plantations certification is considered to be less than optimal. In some areas, it was found that approximately 40% of the RSPO certified area has the land cleared. Despite being certified, palm oil cultivation still leads to deforestation, hence it is considered less sustainable. There are several types of methods for land clearing, such as through burning or utilising heavy equipment. The burning method is one of the traditional methods used in clearing forests to become agricultural land. This option has a minimum economical impact, but has a negative impact to the environment. Palm oil plantations are always associated with forest fires, a phenomenon that occurs almost every year. This method contributes to the production of GHG, namely, CH4 and CO2 (Uusitalo *et al.*, 2014). Land clearing requires urgent efforts to protect degraded forests by adopting sustainable palm oil plantation strategies (de Almeida *et al.*, 2019). Identifying supporting factors is important in the opening of palm oil plantations to minimise deforestation (Benami *et al.*, 2018). In 2015, a land fire disaster happened with palm oil plantations as the main cause, burning down 16% of the concession area (Purnomo *et al.*, 2018). Incineration cause a lot of damage to the environment, human health, as well as other creatures. According to Purnomo *et al.* (2018), land burning is not necessarily free; it costs USD 15 per hectare to burn land. There are several stages in palm oil cultivation, including seedling, nursery, planting, maintenance, and harvesting. Each stage of cultivation requires different inputs and outputs. Similar to the number the utilised materials, the amount of residue also increases. Palm oil residues come from cultivation on plantations and oil processing industry (Awalludin et al., 2015). which is every year 4.5 tons (t) DM ha/year residue was produce (Bessou *et al*., 2017). The residue can be solid and liquid. A kg of palm oil contains 0.132 kg of fibre, 0.068 kg of shells, 0.227 kg of empty fruits, 0.053 kg of kernels and 0.684kg of POME (Uusitalo *et al.,* 2014). The extraction plant from FFB produces MF and PKS as waste. After FFB is cooked with pressure from the palm bunches to extract the oil in FFB, it will produce EFB as the by-product. MF is produced during the extraction process, while VFD is obtained after separation from the kernel (Awalludin *et al.*, 2015). Nevertheless, this article does not focus on residue from oil process industry. Palm oil has a vascular (Nair, 2010) and vertical stem (Hosseini and Wahid, 2014) with a crown amounting to 35-60. The height of the palm oil reaches 30 m (Mohammed *et al.*, 2011) with a branch length of about 7 m, the leaf stalk reaches 150 cm, and a rachis contains 250-350 leaflets (Nair, 2010). In 4-5 years, palm oil trees are ready to be harvested for the first time (Jaroenkietkajorn and Gheewala, 2020; Hosseini and Wahid, 2014). Mature plants are harvested annually. Harvesting is done by cutting fruit bunches and cutting the midrib. Some of the parts are not utilised for palm oil production, which then becomes biomass waste. To maintain and protect the growth of palm oil, some leaves that are not healthy and have pests need to cut down (Bessou *et al*., 2017). Palm oil plants only have 25-30 years before the plants have to be cut down and replaced with new plants (Jaroenkietkajorn and Gheewala, 2020). Cutting down of old palm oil plants will produce solid waste in the form of stems and leaves, which is often also considered as biomass waste. The waste must be treated well; therefore, it does not produce negative impacts on the environment or to humans. Solid wastes produced in the cultivation process include trunk and leaves (Hambali and Rivai, 2017; Mahidin *et al*., 2020). Leaf

Table 1: Palm oil trunk biomass production during plant rejuvenation (Hambali and Rivai, 2017)

Year	Rejuvenation area (ha)	Trunk biomass production (tons)
2002	202,682	15,302,515
2003	211,342	15,956,342
2004	211,389	15,959,863
2005	218,153	16,470,527
2006	263,797	19,916,640
2007	270,673	20,435,845
2008	294,554	22,238,818
2009	314,932	23,777,348
2010	335,416	25,323,890
2011	359,713	27,158,328
2012	382,909	28,909,599
2013	418,601	31,604,360
2014	430,192	32,479,499
2015	452,015	34,127,117

waste comes from pruning of every harvested fruit or two-year care (Chin *et al*., 2019; Mohammed *et al*., 2011). Table 1 summarises the amount of trunk biomass waste produced in Indonesia.

Table 1 describes the mass of palm oil biomass produced by trunk waste. This waste usually comes from the replanting process. Palm oil plantations have a lifespan period of 25-30 years, after that the plants need to be replanted. The process is often called the rejuvenation process. Old palm oil trees are cut down, the trunks from the harvest then become waste for farmers who cannot utilise it. Similar to palm oil leaves, the stems also have greater biomass value. Approximately 6.3 tons of leaf biomass is produced per year. The amount of stem biomass in 2015 was 34.13 million tons, which produce from the replanting or rejuvenation process (Hambali and Rivai, 2017). The amount of stem and leaf waste illustrates the abundance of biomass. That is because waste has excellent potential to retreat. The abundance of biomass from palm oil is an opportunity for oil production and economic improvement, as well as a challenge for most parties as waste (Ahmad *et al.*, 2019). The prospect of palm oil has experienced rapid development, both for increasing the area and production. The palm oil plantations in Indonesia amounted to 14,326,350 ha in 2018, with 55.09% managed by large private companies, 40.62% managed by smallholder plantations and 4.29% by large state estates (Directorate General Plantation, 2019). From that number of palm oil plantation have it by smallholder, aside a large private companies, smallholders also have important role to the development of palm oil. The increase in palm oil production affects the amount of biomass waste generated from plantations. For large private companies, waste can be treated with their own technology. However, smallholders have a limited capital that makes them handle their biomass waste in accordance with nature conservation.

Handling of palm oil waste

The issue of palm oil biomass waste presents opportunities and challenges for farmers. Farmers especially feel burdened by waste disposal, because they find it difficult to dispose of. It considers increasing their operational costs (Awalludin *et al*., 2015). Palm oil biomass waste increases proportionally with palm oil production. Therefore, rapid management is required. Minimum treatment for accumulating biomass waste can cause by a lack of technology. Smallholders prefer the utilisation of traditional methods in handling waste. Smallholder usually carries out their waste management by incinerating or piling up waste around the plantation (Zain, 2019; Nusadaily, 2020; Anyaoha *et al*., 2018). This biomass waste is organic waste, which can decompose in plantations. Stacking has been carried out by stacking on a pile, stacking up on fields, mound on the posts, and stacking up between trees. The accumulation method utilises the process of spoilage of waste, however decomposition is done naturally without the addition of chemicals, the required long period becomes the drawback. The incineration process is carried out by releasing heat that comes from burning waste into the air (Awalludin *et al.*, 2015). The mechanical method is done by chopping or cutting the waste using a small cutting machine. If there is no cutting machine, waste is usually burnt for removal (Nusadaily, 2020). The management of biomass waste has not been maximised or less effective (Ahmad *et al.*, 2019) because there are still many smallholders who utilise traditional methods. RSPO and ISPO is sustanaible certification that use in Indonesia, that applied voluntary by smallholder (Hidayat *et al.*, 2018). Waste management is include to the indicator evaluation for both certification even though RSPO has the clearest explanation in term of its environment aspect (Hidayat *et al.*, 2018; Furumo *et al.*, 2019; Nasution *et al.*, 2020). Even though sustainability certification could bring an economic, social, and environment benefits, but still small number smallholder involved in the schemes. That because the financial and knowledge barier and institutional constrain (Saadun *et al.*, 2018; Furumo *et al.*, 2019; Hutabarat *et al.*,2019). However, in this study include RSPO and ISPO as variabel to determine smallholder methods to handle their palm oil plantation. This study aims to assess 1) Agriculture process, productivity and waste-handling for palm oil plantations; 2) Social factor and waste-handling in palm oil plantation; 3) palm oil plantation waste-handling; 4) Palm oil waste handling and fireland. The study has been carried out in five districts in Jambi Province, Indonesia, in 2019.

MATERIALS AND METHODS

A survey and an in-depth interview were conducted on a field survey to palm oil smallholders in Jambi. The field survey was conducted in July 2019 until September 2019. The districts involved were Merangin, Sarolangun, Muaro Jambi, Tanjung Jabung Barat, and Tebo. The five districts were chosen as representatives from palm oil smallholders across highlands, lowlands, and coastal areas of the East Coast in Jambi. The primary data collection recorded 80 smallholders from each district, resulting in 400 observed samples as the whole sample processed in this study. All of 400 samples were smallholders who own the land and cultivate their field with palm oil. All of the smallholders in this study were chosen from a purposive random sampling method, where only palm oil smallholders who own the field will be recorded on primary data collection. In this study, smallholders were grouped based on their palm oil waste handling procedure as opposed to their farming association, or their affiliated company. As the main indicator for grouping the smallholders, methods to handle waste are divided into five types; i) incineration, ii) stacking up on field, iii) mound on the posts, iv) stacking up between trees, and v) stacking on a pile. The questionnaire was used to understand the waste handling method, where smallholders were asked about their method for palm tree waste treatment. Among smallholders, these five methods of waste handling are common methods among CPO smallholders. Inferential statistic mean and standard deviation were applied to analyse the correlation between agriculture process as well as palm oil productivity and the smallholders' waste handling methods. In this study, inferential correlation has been used to profile whether forest fires in Jambi province are caused by palm fruit smallholders' waste-handling activities. The factors of agriculture process analysed in this research were dosage of fertilisers, the number of seed planted per hectare, and distance from home to the field. The analysed factors for palm oil productivity were harvest quantity per hectare, frequency of harvest, expenditure per capita, and income per capita. Cross tabulations – tables containing methods of waste handling (columns) and observed variables (rows) were utilised to assess the correlation of social factor to waste handling methodology by smallholders. The social factors considered were a type of farmers, whether the smallholders are joining a farming association, how smallholders acquired their field, and whether smallholders use ISPO or RSPO. Based on the inferential statistic, the factor that influences the smallholders' method in handling their waste plantation was determined. A tabular form was prepared to compare the smallholders' waste handling methodology between land clearing waste and general waste on their plantation. The graph and all tables prepared for this study were carried out using a MS Excel. All survey inputs in this study were managed, cleaned and calculated using Stata MP. Calculations, tables and tabulations presented

Table 2: Agriculture process and productivity and waste handling for palm oil plantation

Quantitative variables		(i) Incineration	(ii) Stacked up on field lanes	(iii) Mound on the posts	(iv) Stacked up between trees	(v) Stacked on a pile	Total
Harvest quantity per hectare (Tons)							
	Mean	0.8000	1.0940	0.6800	0.8330	0.8000	0.9493
	S.D.	0.0000	0.1413	0.0616	0.1965	0.0000	0.2042
	Freq.	11	200	20	100	69	400
Frequency of Harvest (times/year)							
	Mean	24	24	24	24	24	24
	S.D.	0	0	0	0	0	0
	Freq.	11	200	20	100	69	400
Dosage of fertilisers (L/ha)							
	Mean	2.1364	2.2950	1.7917	1.6864	2.2500	2.1617
	S.D.	0.3233	0.3985	0.3343	0.2439	0.4287	0.4436
	Freq.	11	200	12	59	69	351
Number of seeds planted per hectare							
	Mean	129.64	130.04	137.10	137.88	130.32	132.37
	S.D.	2.94	1.92	2.71	1.31	2.20	4.00
	Freq.	11	198	20	98	69	396
Distance from home to landfields (Km)							
	Mean	5.818	8.825	6.500	6.818	5.783	7.602
	S.D.	1.471	5.804	1.850	2.771	1.962	4.608
	Freq.	11	200	20	99	69	399
Expenditure per capita (USD/person/day)							
	Mean	7.87	7.76	8.26	8.03	7.68	7.84
	S.D.	1.37	1.53	1.21	1.91	1.39	1.60
	Freq.	10	199	20	100	69	398
The income per capita (USD/person/day)							
	Mean	18.31	17.53	18.91	15.78	14.58	16.67
	S.D.	7.34	7.57	7.69	6.69	5.63	7.14
	Freq.	10	199	20	100	69	398

in this study were calculated and produced by using Stata MP, and then finished by using MS Excel.

RESULTS AND DISCUSSION

Agriculture process, productivity and waste handling for palm oil plantation

An analysis was undertaken to understand smallholder's condition based on the relationship between smallholders' agriculture process and productivity and their waste management method. That correlation is tabulated from the datasets and summarised in Table 2.

Table 2 shows that smallholders have the same number of harvests per year (24 times) since farming associations schedule palm fruit harvesting seasons. The highest dosage of fertilisers is utilised by smallholders who stacked their waste on the field. The data also indicate that the number of seeds planted by the smallholders is affected the waste handling for CPO waste. Smallholders who stack the waste between trees or mound on the posts have more space to plant seeds, where on average, they plant approximately 137 seeds per hectares, compared to other waste handling methods. The distance of their home to the field makes they tend to stack the waste instead of incinerating it. Therefore, the distance might drive their behaviour to choose the most efficient waste handling method. In regards to the economic aspect, the waste handling method is unlikely to provide a direct effect on their income or expenditure per capita. Table 2 also indicates that smallholders with the highest harvest quantity per hectares are smallholders who stacked their waste on the field. Stacking up waste on the field is still the most efficient method to harvest palm fruits. However, it is more expensive when compared to incineration. The quantity of harvest per hectares is also affected

Table 3: Social factors and palm oil plantation waste handling

Qualitative and Categorical Variables	(i) Incineration	(ii) Stacked up on field lanes	(iii) Mound on the posts	(iv) Stacked up between trees	(v) Stacked on a pile	Total
Types of Farmers						
Core Smallholders	0	0	0	75	0	75
Common Smallholders	11	120	20	25	69	245
Self-subsistent Smallholders	0	80	0	0	0	80
Total	11	200	20	100	69	400
Member of Farming Association						
No	1	86	0	6	9	102
Yes	10	114	20	94	60	298
Total	11	200	20	100	69	400
How the land field was earned						
Buy and sell	0	91	16	89	0	196
Given	1	28	0	0	10	39
Legacy	10	81	4	11	59	165
Total	11	200	20	100	69	400
ISPO Certification						
No	11	113	20	60	69	273
Yes	0	87	0	40	0	127
Total	11	200	20	100	69	400
RSPO Certification						
No	11	200	4	87	69	371
Yes	0	0	16	13	0	29
Total	11	200	20	100	69	400

by waste handling since waste handling also takes time on their post-production activities. The method of the mound on the post gives the smallest outputs since it needs longer waste-handling time when compared to the other handling method. Agroecological practices in agriculture have a more traditional view of agricultural practices or activities. Agroecological practices utilise waste or agricultural products to maintain or improve soil quality (Bessou *et al.,* 2017). Smallholders' general waste handling method is by accumulating biomass waste around the plantations. EFB or OPF biomass waste is usually left for composting at the mill or plantation (Chiew and Shimada, 2015). Male inflorescences and abscised frond bases are cut down when it becomes two years, then left on the plantation for mulching or rotting (Truckell *et al.*, 2019). Biomass decomposition includes composting, which naturally requires little human energy (Chiew and Shimada, 2015). The problem of nutrient impoverishment on plantations can be managed by palm oil residual composting (Truckell *et al.*, 2019). Composting of biomass waste reduces waste volume by 50% -75% (Chiew and Shimada, 2015). Waste is placed on the plantation will supply potassium to the soil, which improves fertility in the soil that originally depletes due to the cultivation process (Bessou *et al.*, 2017). Biomass can be utilised to replace K/Mg for nearly 10% of plantations and can increase nitrogen and phosphate in the soil, therefore maintain better soil permeability (Arvind *et al.*, 2019). Utilisation of biomass waste is less optimal because it causes the wet condition while composting and recovery of methane is considered to be more environmentally friendly compared to other technologies in terms of measured GHG (Chiew and Shimada, 2015). Palm oil cultivation requires fertiliser that contains potassium to maintain soil fertility and plant development. These chemical contents can be obtained by utilising biomass (Bessou *et al.*, 2017). Stacking has several objectives that can facilitate waste management or cultivation activities. Biomass waste placed around the plantations will decay over time. Decaying leaves are intended to control erosion in the area. However, in the long run, these decaying leaves will become additional nutrients for plantation soils. (Mohammed *et al.*, 2011). OPF and OPT waste that has been left to decompose at the plantation site is utilised for land cover and do not require additional expenditure (Chin *et al.*, 2019). Biomass waste can be utilised to increase crop yields, enrich soil nutrient content, reduce pollution, increase income, and become energy savings for farmers as well as factories (Anyaoha *et al.*, 2018).

Social factor and waste handling in palm oil plantation

Other than agriculture process and productivity, another analysis was used to determine the correlation between social factors, such as type of farmers, participation in the farming association, how the land was acquired, and whether the farmers hold ISPO and RSPO Certification, and palm oil plantation waste. The correlation is shown in Table 3

Table 3 shows that the smallholders that incinerate their waste are fewer compared to the others (core and self-subsistent smallholders) that tend to stack their waste. Mostly common and self-subsistent smallholders stack up their waste on the field, while core smallholders stack their waste between trees. According to the farming association, most of the associated smallholders stack their waste on lanes, while only a few of them incinerate the waste. The source of land ownership also does not have a direct impact on waste handling. In terms of certification aspects, all of ISPO (Indonesia Sustainable Palm Oil) certified smallholders stack the residues either on the field lanes or between trees. None of ISPO certified smallholders incinerates their waste. Uncommon findings are also found in RSPO certified smallholders since only a few of them are RSPO certified. Therefore, the likelihood of RSPO certified smallholders on waste handling could not be concluded.

Palm oil plantation waste handling

Table 4 reports the frequencies and percentage of each palm oil plantation waste handling by

Table 4: Summary of palm oil plantation waste handling method

Methods	Frequencies	Percent	Cumulative (%)
(i) Incineration	11	2.75	2.75
(ii) Stacked up on field lanes	200	50	52.75
(iii) Mound on the posts	20	5	57.75
(iv) Stacked up between trees	100	25	82.75
(v) Stacked on a pile	69	17.25	100
Total	400	100	

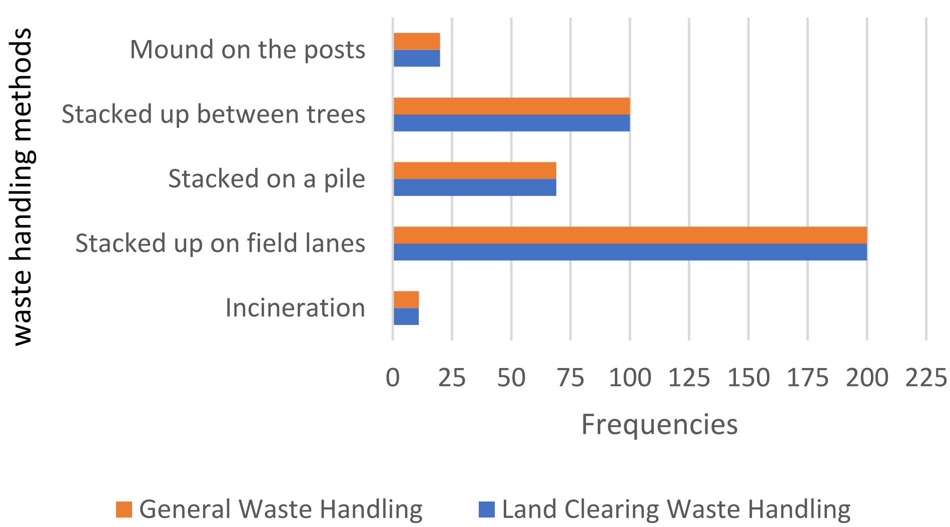


Fig. 1: Comparison of waste handling

smallholders. It was found that the waste handling method distribution are as follows: 50% stack up the waste on the field, 25% stack the waste up between trees, 17.25% stack the waste on a pile, 5% mound the waste on the posts, and 2.75% incinerate their waste for land clearing. The table shows that the method with the highest percentage is stacking up on the field and the lowest percentage incineration. Therefore, Table 4 shows general waste incineration is not preferred by most of the smallholders.

Fig. 1 shows a comparison between smallholders' general and land clearing waste handling on their land plantation. It was found that the similar waste handling process was undertaken for both general and land clearing waste. Out of 400 correspondents, 200 correspondents handle both general and land clearing waste by stacking up on the field lanes.

Based on Table 4 and Fig. 1, most palm oil smallholders in Jambi stack up their waste on-field lanes as opposed to incineration. Stacking waste, whether in field lanes, posts, piles, or between trees, are generally a common method to handle palm oil plantation waste in Jambi. At the same time, the rest (2.75%) of the respondents incinerate the waste. Management of biomass to become bioenergy and biomaterial is driven by energy supply security and pollution reduction purposes (Awalludin *et al.*, 2015). Biomass is an abundant renewable resource and has a neutral carbon cycle (Mohammed *et al.*, 2011). Biomass (plants) plays a role in preventing the release of carbon into the atmosphere because this type of plant is able to produce carbon. If biomass waste is incinerated, then carbon will be released into the atmosphere to mix with oxygen and ultimately produces CO_2 (Hosseini and Wahid, 2014). Burning of biomass will result in the release of carbon into the environment (Awalludin *et al.*, 2015). When biomass is turned into biofuel, the increase of carbon in the environment is lower. Where 60% of the biomass originates from OPT and OPF (Sulaiman *et al.*, 2010). More pollution and fog will develop if biomass waste is incinerated (Awalludin *et al.*, 2015). The incineration process can produce methane gas (CH4) as well as increasing CO2 and CH4, which are contributors to GHG, therefore inducing an increase in the earth's temperature (Cooper *et al.*, 2019; Prosperi *et al.*, 2020). The biggest GHG emissions are CO_2 and methane (Wu *et al.*, 2017). The process of cultivating plantations also produces GHG. However, palm oil plantations are more environment friendly compared to rapeseed and jatropha in terms of GHG emissions (Uusitalo *et al.,* 2014). The reason is that the nitrogen fertiliser and N2O utilised in the cultivation process are released (evaporated) in the air. Burning biomass cause a lot of pollution and low energy efficiency. Burning of palm oil biomass is carried out using a stove, which is a furnace that converts biomass into chemicals and converts into heat (Mohammed *et al.*, 2011). In electricity generation, direct combustion induces evaporation and other waste such as water and CO2 (Awalludin *et al.*, 2015). Ending the practice of incineration and replacing with making use of stems as organic fertiliser that can reduce the consumption of inorganic fertiliser. In the first four years, 20-30% of savings can be made for fertiliser consumption by relying on potassium contained in EFB waste (Nair, 2010). Incineration issues in plantation sector can be managed through coercion, giving or eliminating incentives as well as providing information. stakeholders, rules and good governance practices can be carried out with assistance from the central government, local government, and processing plants (Purnomo *et al.*, 2018). Incineration of biomass waste must be avoided in order not give an impact on the environment (Bessou *et al.,* 2017). Waste management must be done properly to avoid diversity and promote the sustainability of palm oil plantations (Awalludin *et al.*, 2015). The government does not recommend the incineration of biomass waste because it has provided higher economic value when reprocessed (Sulaiman *et al.*, 2010). Biomass waste will be more economical when processed into biofuels (Chin *et al.*, 2019). Processing biomass into other oil products can also be a solution to reduce and mitigate GHG (Wu *et al.*, 2017). Due to an increase in pollution awareness, an increase in fertilisation costs will result in a change in the waste handling method, which shifts from burning waste to processing biomass to fertilisers (Bessou *et al.*, 2017). Based on Table 5 and Fig. 1, waste handling through combustion is at a small percentage. That means the waste handling method contributes to the GHG emitting from CO2 at a low level and therefore contributes to the minimum amount of haze. In relation to the events of land fire in Jambi, the fire did not originate from incineration as waste handling for palm oil waste by smallholders. Palm oil plantations' existence will have an impact on the development of the local area. Increasing infrastructure to support plantations has started to be built, therefore causes pressure on the surrounding area even on the plantation itself. In the harvesting process, large trucks will often pass through plantation land, as such the amount of pressure applied by the trucks will have an impact on compaction of the trajectory land, which may cause land subsidence. The utilisation of OPF can help to relieve compaction pressure by placing OPF in the truck lane, in addition to reducing soil compaction (Truckell *et al.*, 2019). Biomass waste can be used as organic mulch. Mulch is used to condition soil content, maintain soil moisture, and reduce erosion (Chiew and Shimada, 2015). In addition to OPF and OPT, EFB can also be used on the plantation for mulch (Bessou *et al.*, 2017). This mulch is also able to increase chemicals and nutritional content for palm oil production. The use of biomass waste for mulch is affected by labour costs, weight, and volume ratios (Anyaoha *et al.*, 2018). The use of biomass for mulch by smallholders can increase the water content in the soil (Anyaoha *et al.*, 2018). While the use of EFB for mulch in palm oil requires additional costs because it has to be transported from the mill to the plantation (Chiew and Shimada, 2015). Mulch is able to prevent erosion (Shojaei *et al.*, 2019). Using biomass for mulch will help to retain moisture and increase soil fertility and reduce weed growth (Awalludin *et al.*, 2015). However, mulch has a negative side of attracting pests on plantations, such as beetle Oryctes rhinoceros and the bacterium *Ganoderma sp,* as well as causes diseases that attack palm oil, (Anyaoha *et al.*, 2018; Nair, 2010; Chung, 2012). Biomass waste left on plantations to decay is generally on a moist condition and rich in bacteria (Anyaoha *et al.*, 2018). These conditions attract the beetle *Oryctes rhinoceros* to breed in rotten stems or leaves (Chung, 2012; Bessou *et al.*, 2017). The fallen palm tree becomes a reservoir for stag beetle (Nusadaily, 2020). These beetles eat palm crowns and their tops and damage palm oil trees, even cause death to plants. Harvest yields can decrease by 10% due to the presence of these beetles (Arvind *et al.*, 2019). The beetle is capable of causing the death of 3-4 years old palm oil trees that have not been productive yet (Bessou *et al.*, 2017). On the other hand, *Ganoderma sp.* is the cause of basal stem root (BSR) disease. The bacterial infection in palm oil will cause stunted plant growth, pale yellow or green leaves, and rotting stems (Nair, 2010). Waste handling for palm oil biomass waste then needs to be reconsidered. Waste management through combustion and mulch use is less positive impact than the higher value of biomass waste (Sulaiman *et al.*, 2010). Burning waste creates pollution, and using waste as mulch can attract pests. Therefore, farmers need additional effort to handle pests, such as the additional use of pesticides or pheromone traps (Arvind *et al.*, 2019). That can increase the expenditure of pest eradication. However, it will affect the yield of palm oil production. Management control is needed to manage palm oil biomass waste. Waste reduces the sustainability of cultivation (Awalludin *et al.*, 2015) and a challenge if placed on a plantation. However, these challenges can be changed into opportunities for utilisation that can add their economic value (Anyaoha *et al.*, 2018). Hence, in contrast to only leaving the waste to decompose in the plantation, further processing is needed. This waste will have higher economic value if it is reprocessed into biofuels (Awalludin *et al.*, 2015). Other aspects to consider for processing biomass waste into biofuel or materials are the main characteristics of waste, treatment practices, and the available amount of waste (Mahidin *et al.*, 2020). The main characteristics of waste vary, since the form of physical or chemical characteristics of the waste is different, such as shell thickness. The climate and soil conditions are considered to be the cause of these differences (Anyaoha *et al.*, 2018). Processing biomass waste into biofuels is more profitable (Hosseini and Wahid, 2014). Biomass waste has the potential for oil production and can be a replacement for fossil oil. The shell has less water content compared to EFB, hence it is preferred for boiler fuel. Furthermore, this type of waste also has the potential to produce hydrogen (Wu *et al.*, 2017). Lignocellulose content in biomass also has a high potential to produce methane gas for biogas in high levels (Chiew and Shimada, 2015). Therefore, this material can be considered as raw materials for other materials such as cardboard, paper, road paving, briquettes (Awalludin *et al.*, 2015). Biomass waste can be processed into fertiliser or roasted kettle fuel (Wu *et al.*, 2017). The biomass waste can also be considered as raw materials for pulp and paper production. However, this process requires a large amount of energy due to the conversion of fibre into raw materials (Chiew and Shimada, 2015). High water content in biomass needs to be dried off prior to processing to reduce the produced emissions (Sulaiman *et al.*, 2010). The utilisation of biomass as bioenergy requires high costs. However, the processing costs will be replaced by the utilisation of bioenergy itself. In addition, biofuel can provide additional income to improve farmers' economy (Hosseini and Wahid, 2014). Biofuel management faces issues in terms of storage as it requires a large space due to the high mass of waste (Truckell *et al.*, 2019). Processing of biomass waste into biofuel depends on technology, knowledge, interests, and motives of farmers (Chin *et al.*, 2019). Some of the factors that become obstacles in having smallholders' participation in biomass processing include the area of the garden owned by the smallholder, the lack of information regarding biofuel processing, the type of land ownership, processing experience and labour. Other than an obstacle, there is a benefit for biofuel processing, such as the opportunity to increase farmers' income through biofuels. Smallholders prefer the utilisation of traditional methods because they put more trust in experience (Chin *et al.*, 2019). Smallholders tend to adopt practices that have been recognised and proven successful by other farmers. If the surrounding farmers have not applied the method, it is unlikely for them to use the method. Other than that, sending FFB to collectors is the reason why they do not use EFB. At the replanting stage, old plants will be replaced by new plants. This stage requires a high cost for cutting plants into small pieces and cleaning the stumps (Nusadaily, 2020). These costs can be covered by using biofuels as additional income for farmers. The utilisation of biomass to biofuel will also reduce dependence on fossil materials (Awalludin *et al.*, 2015; Idris *et al.*, 2012). According to Hosseini and Wahid (2014), the water content in biomass waste also inhibits the processing of waste into biofuels. High water content complicates the waste collection process. Moreover, this increase the transportation cost. Hence many smallholders still prefer traditional methods. Processing of biomass (stumps) is influenced by the knowledge that will affect the perception of farmers (Rahman *et al.*, 2017). That knowledge includes knowledge about the benefits of the biomass. Additional income can be used as a persuasive argument to attract smallholders. Other than gaining profit, it can also reduce the cost of preparing a new location. Reducing the ecological impact of palm oil plantations can be done by improving agricultural practices, appropriate land utilisation, and appropriate waste management.

Palm oil waste handling and fireland

Land fires can be caused by climate (Hamilton *et al.*, 2019), land-use change (Adrianto *et al.*, 2019) and waste management (Ibrahim, 2020). According to the data of fireland area in Jambi from 2015-2020, Jambi occured the highest number of fireland areas in 2015, which was around 115,634.34 ha land was burnt. The second highest happened in 2019, which was around 56,593 ha land was burnt and the smallest was happen in 2017, which is around 109.17 ha was burnt (MenLHK, 2020). That data was consistent with data from other literature, which stated that in 2015, Indonesia encountered the most significant land fires since the 1997 land fires. That land fire released some carbon (C) into the atmosphere, which affected to the neighbouring countries (Huijnen *et al.*, 2016). However, from the data in Table 4, only 2.75% of smallholders do the incineration process of their plantation waste, which can be driven to the land fire. The palm oil plantations in Jambi amounted to 1,032,145 ha in 2018, with 34.92% managed by large private companies, 63.14% managed by smallholder plantations and 1.94% by large state estates (Directorate General Plantation, 2019). Thus, how smallholders cultivated their plantation and manage their waste, its will give big impact to the environment, including to the fireland driven since smallholders is the biggest stakeholder that manage palm oil plantation in Jambi. The process of incineration waste can be one of the causes of land fires. Variable water levels, flammable surface vegetation, socio-political phenomena, and biophysical conditions allow for an increase in landscape susceptibility to annual fires that are sustained and uncontrolled (Goldstein *et al.*, 2020). Because of that, the smallholder methods of handling their waste becomes important.

Knowledge, their environments, and local wisdom may affect their decision to treat their waste. Whereas if waste incinerated done by smallholders becomes the causes of land fires due to palm oil plantations area owned by smallholders, since their plantation is spread across Indonesia and they have around 40% of palm oil plantations in Indonesia (Jelsma *et al.*, 2019). Table 4 showing the waste handling by smallholder is the least or does not of land fire-driven, especially in the study area. Since in this study show that only 2.75% palm oil plantation smallholder incinerated their plantation waste.

CONCLUSION

Waste handling on plantation process by smallholders influenced by their economic and social condition, which is affecting their decision to how they are handling their waste. The decision waste handling by smallholder influenced by their harvest productivity and the distance between their plantation and their house. That because they have low capacity to bring their waste to another area. Most of the common smallholders and self-subsistent smallholder has been handling their palm oil plantation waste through stacking the wastes on their field. The method to handle the waste also has been used by smallholder that joint a farming association. According to the survey, only 2.75% of total smallholders, handle their waste by incineration. The other 50% of smallholders stack up their waste on the field lane, including both general and land clearing waste. The smallholders might be aware that incineration will cause negative effects. Moreover, their methods of handling their palm oil plantation waste helps to minimise the probability to start land fires. If most of the smallholders still chose incineration as their preferred waste handling method, the probability of land fire to happen will be higher since smallholders are the biggest stakeholder that manage palm oil plantation in Jambi. Therefore, there is a strong argument that waste handling by smallholders does not the cause of that land fires. Moreover, there are possibilities that land-opening activities were the main cause of severe land fires since burning down the trees is the most cost-effective solution to reopen new lands. Hence, it was recommended for undertaking an analysis to understand the cause of forest fire. Aside from proper waste-handling, that most smallholders comply with the environmental preservations, regardless of their certifications. This study also found that not many palm oil smallholders are ISPO-certified. Hence, in order to exhalate sustainable operations of palm oil productions, ISPO and RSPO socializations are needed to the smallholders in order to decrease incineration activities, regardless of waste-handling or field-opening activities. ISPO and RSPO certifications can be a tool for the government and environmentalists to keep maintaining economic growth and minimize the negative impact of palm oil activities on the environment.

AUTHOR CONTRIBUTIONS

H. Herdiansyah performed the literature review, experimental design, analyzed and interpreted the data, prepared the manuscript text, and manuscript edition. E. Frimawaty performed the experiments and literature review, compiled the data and manuscript preparation.

ACKNOWLEDGEMENTS

This work was supported by International Research Collaboration Grant 2019 from Universitas Indonesia [contract number NKB-1959/UN2.R3.1/HKP.05.00/2019]. The authors extend sincere gratitude to Dr. Stefanie Steinebach from HAWK-HHG (Hochschule für angewandte Wissenschaft und Kunst-Hildesheim/Holzminden/Göttingen), who becomes an international collaborative research partner. Moreover, our sincere thank delivering to smallholders of oil palm plantation, stakeholders, Dr. Rosyanni and field assistants (M.A Aziz, V.R.A Marpaung, R.K Firdaus, and M.A Amrullah) as magister students of Environmental Science and Agribusiness of University of Jambi and A.D January for editing, as wels H.A Negoro for processing statistical data and R Sari for peer reviewing. In addition, the authors would like to thank to Khal Proof from New Zealand for English language editing.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest regarding the publication of this work. In addition, the ethical issues including plagiarism, informed consent, misconduct, data fabrication and, or falsification, double publication and, or submission, and redundancy have been completely witnessed by the authors.

ABBREVIATIONS

%	*Percentage*
BSR	*Basal stem root*
C	*Carbon*
CH_4	*Methane*
cm	*Centimeter*
CPO	*Crude palm oil*
CO_2	*Carbon dioxide*
DM	*Dry matter*
EFB	*Empty fruit bunch*
FFB	*Fresh fruit bunch*
Fig.	*Figure*
GHG	*Green house gasses*
Ha	*Hectares*
ISPO	*Indonesian Sustainable Palm Oil*
K	*Potassium*
kg	*Kilogram*
L	*Liters*
m	*Meter*
Mg	*Magnesium*
MF	*Mesocarp Fibre*
N_2O	*Nitrous Oxide*
OPF	*Oil Palm Fronds*
OPT	*Oil palm trunk*
OPL	*Oil Palm Liquid*
PKS	*Palm kernel shell*
POME	*Palm oil mill effluent*
RSPO	*Roundtable on sustainable palm oil*
Sp.	*Species*
S.D.	*Standard deviation*
(t)	*Thousand*
ton	*Tonne*
USD	*United States Dollar*
VFD	*Variable frequency drive*

REFERENCES

Adrianto, H.A.; Spracklen, D.V.; Arnold, S.R.; Sitanggang, I.S.; Syaufina, L., (2020). Forest and Land Fires Are Mainly Associated with Deforestation in Riau Province, Indonesia. Remote Sens., 12: 3 **(12 pages)**.

Ahmad, F.B.; Zhang, Z.; Doherty, W.O.S.; O'Hara, I. M., (2019). The outlook of the production of advanced fuels and chemicals from integrated oil palm biomass biorefinery. Renewable Sustainable Energy Rev., 109: 386–411 **(26 pages)**.

Anyaoha, K.E.; Sakrabani, R.; Patchigolla, K.; Mouazen, A.M., (2018). Critical evaluation of oil palm fresh fruit bunch solid wastes as soil amendments: Prospects and challenges. Resour., Conserv. Recycl., 136: 399–409 **(11 pages)**.

Arvind, K.; Rajesh, M.K.; Josephrajkumar, A.; Grace, T., (2019). Dataset of de novo assembly and functional annotation of the transcriptome of certain developmental stages of coconut rhinoceros beetle, Oryctes rhinoceros L. Data in Brief, 28: 105036 **(12 pages)**.

Awalludin, M.F.; Sulaiman, O.; Hashim, R.; Nadhari, W.N.A.W., (2015). An overview of the oil palm industry in Malaysia and its waste utilisation through thermochemical conversion, specifi cally via liquefaction. Renewable Sustainable Energy Rev., 50: 1469–1484 **(16 pages)**.

Benami, E.; Curran, L. M.; Cochrane, M.; Venturieri, A.; Franco, R.; Kneipp, J.; Swartos, A., (2018). Oil palm land conversion in Par' a, Brazil, from 2006–2014: evaluating the 2010 Brazilian Sustainable Palm Oil Production Program. Environ. Res. Lett., 13(3) **(13 pages)**.

Bessou, C.; Verwilghen, A.; Beaudoin-Ollivier, L.; Marichal, R.; Ollivier, J.; Baron, V.; Bonneau, X.; Carron, M.P.; Snoeck, D.; Naim, M.; Aryawan, A.A.K.; Raoul, F.; Giraudoux, P.; Surya, E.; Sihombing, E.; Caliman, J.P., (2017). Agroecological practices in oil palm plantations: examples from the field. OCL Journal, 24(3): D305 **(16 pages)**.

Chiew, Y.L.; Shimada, S., (2013). Current state and environmental impact assessment for utilising oil palm empty fruit bunches for fuel, fiber and fertiliser - A case study of Malaysia. Biomass Bioenergy, 51: 109-124 **(16 pages)**.

Chin, H.; Choong, W.; Alwi, S.R.W.; Mohammed, A.H., (2019). A PLS-MGA analysis of farming characteristics on the intentions of smallholder oil palm planters to collect palm residues for biofuel production. Biomass Bioenergy, 120: 404–416 **(13 pages)**.

Chung, G.F., (2012). Effcet of Pests and Diseases on Oil Palm Yield. Palm Oil Production, Processing, Characterzation, and Uses, 163-210 **(48 pages)**.

Cooper, H.V; Vane, CH; Evers, S.; Aplin, P; Girkin, N.T.; Sjogersten, S., (2019). From peat swamp forest to oil palm plantations: The stability of tropical peatland carbon. Geoderma, 342: 109-117 **(8 pages)**.

de Almeida, A.S.; Vieira, I.C.G.; Ferraz, S.F.B., (2020). Long-term assessment of oil palm expansion and landscape change in the eastern Brazilian Amazon. Land Use Policy, 90: 104321 **(8 pages)**.

Directorate General Plantation, (2019). Statistik Perkebunan Indonesia Tree Crop Estate Statistic of Indonesia 2018-2020. Jakarta: Sekretariat Direktorat Jendral Perkebunan.

Furumo, P.R.; Rueda, X.; Rodríguez, J.S.; Parés Ramos, I.K. (2020). Field evidence for positive certification outcomes on oil palm smallholder management practices in Colombia. J. Clean. Prod., 245: 118891 **(16 pages).**

Gatti, R.C.; Liang, J.; Vellichevskaya, A.; Zhou, M., (2019).

Sustainable palm oil may not be so sustainable. Sci. Total Environ., 625: 48-51 **(13 pages).**

Goldstein, J.E.; Graham, L.; Ansori, S.; Vetrita, Y.; Thomas, A.; Applegate, G.; Vayda, A.P.; Saharjo, B. H.; Cochrane, M.A., (2020). Beyond slash-and-burn: The roles of human activities, altered hydrology and fuels in peat fires in Central Kalimantan, Indonesia. Singap. J. Trop. Geogr., 41: 190-208 **(19 pages)**.

Hambali, E.; Rivai, M., (2017). The Potential of Palm Oil Waste Biomass in Indonesia in 2020 and 2030. IOP Conf. Ser.: Earth Environ. Sci., 65: 012050 **(10 pages)**.

Hamilton, R.; Stevenson, J.; Li, B.; Bijaksana, S., (2019). A 16,000-year record of climate, vegetation and fire from Wallacean lowland tropical forests. Quat. Sci. Rev., 224: 105929 **(15 pages)**.

Hidayat, N.K.; Offermans, A.; Glasbergen, P., (2018). Sustainable palm oil as a public responsibility? On the governance capacity of Indonesian Standard for Sustainable Palm Oil (ISPO). Agr. Hum. Values, 35: 223–242 **(20 pages).**

Hosseini, S.E.; Wahid, M.A., (2014). Utilisation of palm solid residue as a source of renewable and sustainable energy in Malaysia. Renewable and Sustainable Energy Rev., 40: 621–632 **(12 pages)**.

Huijnen, V.; Wooster, M. J.; Kaiser, J.W.; Gaveau, D.L.A.; Flemming, J.; Parrington, M.; Inness, A.; Murdiyarso, D.; Main, B.; van Weele, M., (2016). Fire carbon emissions over maritime Southeast Asia in 2015 largest since 1997. Sci. Rep., 6: 26886 **(8 pages)**.

Hutabarat, S.; Slingerland, M.; Dries, L., (2019). Explaining the “Certification Gap” for Different Types of Oil Palm Smallholders in Riau Province, Indonesia. J. Environ. Dev., 28: 253–281 **(29 pages).**

Ibrahim, M.A., (2020). Risk of spontaneous and anthropogenic fires in waste management chain and hazards of secondary fires. Resour., Conserv. Recycl., 159: 104852 **(11 pages)**.

Idris, S.S.; Rahman, N.A.; Ismail, K., (2012). Combustion characteristics of Malaysian oil palm biomass, sub-bituminous coal and their respective blends via thermogravimetric analysis (TGA). Bioresour. Technol., 123: 581–591 **(11 pages)**.

Jaroenkietkajorn, U.; Gheewala, S. (2020). Interlinkage between Water-Energy-Food for Oil Palm Cultivation in Thailand. Sustain. Prod. Consum., 22: 205–217 **(8 pages).**

Jelsma, I.; Woittiez, L.S.; Ollivier, J.; Dharmawan, A.H., (2019). Do wealthy farmers implement better agricultural practices? An assessment of implementation of Good Agricultural Practices among different types of independent oil palm smallholders in Riau, Indonesia. Agric. Syst., 170: 63–76 **(14 pages)**.

Mahidin; Saifullah; Erdiwansyah; Hamdani; Hisbullah; Hayati, A.P.; Zhafran, M.; Sidiq, M.A..; Rinaldi, A.; Fitria, B.; Tarisma, R.; Bindar, Y., (2020). Analysis of power from palm oil solid waste for biomass power plants: A case study in Aceh Province. Chemosphere, 253: 126714 **(23 pages)**.

MenLHK, (2020). Rekapitulasi Luas Kebakaran Hutan dan Lahan (Ha) Per Provinsi Di Indonesia Tahun 2015-2020 **(2 pages)**.

Mohammed, M.A.A.; Salmiaton, A.; Azlina, W.A.K.G.W.; Amran, M.S.M.; Fakhru’l-Razi, A.; Taufiq-Yap, Y.H., (2011). Hydrogen rich gas from oil palm biomass as a potential source of renewable energy in Malaysia. Renewable and Sustainable Energy Rev., 15: 1258–1270 **(13 pages)**.

Nasution, M.A.; Wulandari, A.; Ahamed, T.; Noguchi, R., (2020). Alternative POME treatment technology in the implementation of roundtable on sustainable palm oil, Indonesian sustainable palm oil (ISPO), and Malaysian sustainable palm oil (MSPO) standards using LCA and AHP methods. Sustainability, 12: 4101 **(16 pages).**

Nair, K.P.P., (2010). Oil Palm (Elaeis guineensis Jacquin). The Agronomy and Economy of Important Tree Crops of the Developing World, 209-236 **(27 pages).**

Purnomo, H.; Okarda, B.; Dewayani, A.A.; Ali, M.; Chdiawan, R.; Kartodihardjo, H.; Pacheco, P.; Juniwaty, K.S., (2018). Reducing forest and land fires through good palm oil value chain governance. Forest Policy and Econ., 91: 940-106 **(12 pages).**

Prosperi, P.; Bloise, M.; Tubiello, F.N.; Conchedda, G.; Rossi, S.; Boschetti, L.; Salvatore, M.; Bernoux, M., (2020). New estimates of greenhouse gas emissions from biomass burning and peat fires using MODIS Collection 6 burned areas. Clim. Change, 1-18 **(18 pages).**

Nusadaily, (2020). Pelet dari Batang Kelapa Sawit Simpan Keuntungan Menggiurkan.

Rahman, A.; Khanam, T.; Pelkonen, T., (2017). People’s knowledge, perceptions, and attitudes towards stump harvesting for bioenergy production in Finland. Renew. Sustainable Energy Rev., 70: 107–116 **(10 pages)**.

Saadun, N.; Lim, E.A.L.; Esa, S.M.; Ngu, F.; Awang, F.; Gimin, A.; Johari, I.H.; Firdaus, M.A.; Wagimin, N. I.; Azhar, B., (2018). Socio-ecological perspectives of engaging smallholders in environmental-friendly palm oil certification schemes. Land Use Policy, 72: 333–340 **(8 pages).**

Shojaei, S.; Ardakani, M.A.H.; Sodaiezadeh, H.; Jafari, M.; Afzali, S.F., (2019). Optimisation of parameters affecting organic mulch test to control erosion. J. Environ. Manage., 249: 109414 **(11 pages)**.

Sulaiman, F.; Abdullah, N.; Gerhauser, H.; Shariff, H., (2010). A Perspective of Oil Palm and Its Wastes. J. Phys. Sci., 21: 67–77 **(11 pages)**.

Truckell, I.G.; Shah, S.H.; Baillie, I.C.; Hallett, S.H.; Sakrabani, R., (2019). Soil and transport factors in potential distribution systems for biofertilisers derived from palm oil mill residues in Malaysia. Computers and Electronics in Agriculture, 166: 105005 **(9 pages)**.

Uusitalo, V.; Vaisanen, S.; Havukainen, J.; Soukka, R.; Luoranen, M., (2014). Carbon footprint of renewable diesel from palm oil, jatropha oil and rapeseed oil. Renewable Energy, 69: 103-113 **(10 pages).**

Wu, Q.; Qiang, T.C.; Zeng, G.; Zhang, H.; Huang, Y.; Wang, Y., (2017). Sustainable and renewable energy from biomass wastes in palm oil industry: A case study in Malaysia. Int. J. Hydrogen Energy, 42(37): 23871-23877 **(7 pages)**.

Zain, R., (2019). Kebun kelapa sawit dikesan terbakar.

AUTHOR (S) BIOSKETCHES

Herdiansyah, H., Ph.D., Instructor, School of Environmental Science, Universitas Indonesia, Central Jakarta, DKI Jakarta, Indonesia. Email: *herdis@ui.ac.id*

Frimawaty, E., Ph.D., Instructor, School of Environmental Science, Universitas Indonesia, Central Jakarta, DKI Jakarta, Indonesia. Email: *evi.frimawaty11@ui.ac.id*

HOW TO CITE THIS ARTICLE

Herdiansyah, H.; Frimawaty, E., (2021). Palm oil plantation waste handling by smallholder and the correlation with the land fire. Global J. Environ. Sci. Manage., 7(1): 89-102.

DOI: 10.22034/gjesm.2021.01.07

url: https://www.gjesm.net/article_44277.html

Global J. Environ. Sci. Manage. 7(1): 103-116, Winter 2021

Global Journal of Environmental Science and Management
(GJESM)

Homepage: https://www.gjesm.net/

ORIGINAL RESEARCH PAPER

The effects of glucose, nitrate, and pH on cultivation of *Chlorella sp.* Microalgae

H. Nouri[1], J. Mohammadi Roushandeh[1], A. Hallajisani[1,*], A. Golzary[2], S. Daliry[1]

[1] *Caspian Faculty of Engineering, College of Engineering, University of Tehran, Rezvanshar, Guilan, Iran*

[2] *School of Environment, College of Engineering, University of Tehran, Tehran, Iran*

ARTICLE INFO

Article History:
Received 20 March 2020
Revised 05 August 2020
Accepted 30 August 2020

Keywords:
Biomass productivity
Chlorella sp.
Environment
Growth rate

ABSTRACT

BACKGROUND AND OBJECTIVES: Bioenergy is a phenomenon that has attracted humans' attention for about a century. The desirable biological properties of *chlorella sp.* microalgae have turned it to one of the most ideal options for the production of biodiesel. However, the economic issues must be taken into account in its industrial scale production. The present study aims to investigate *chlorella sp.* biomass production and growth conditions by studying the influence of glucose concentration as a carbon source, nitrate concentration as a nitrogen source and pH, as three of the most important factors.

METHODS: For this purpose, design of experiment was done by response surface methodology and each factor was investigated simultaneously under glucose concentration in 2-20 g/L, nitrate concentration in 0-1 g/L and 6<pH<10. During the growing, pH of the culture was measured to identify the correlation between pH and growth rate change. The results were analyzed by response surface methodology as well.

FINDINGS: The results indicated that carbon concentration has maximum effect on growth and biomass production. The best results were obtained in glucose concentration of 2.6-6 g/L, nitrate concentration of 0.2-0.5 g/L and pH values 7-9. Moreover, the maximum biomass production (1.31 g/L), the highest specific growth rate (0.167 1/day), and the highest biomass productivity (0.085 g/L/Day) were obtained in the following conditions: glucose concentration of 2.6 g/L, nitrate concentration of 0.5 g/L, and pH = 8. The optimal C/N ratio was determined and significant correlation was observed between pH and growth rate change.

CONCLUSION: It was concluded that *Chlorella sp.*, if properly adjusted for both chemical and physical parameters could be a valuable source of biomass for biodiesel production in industrial scale.

DOI: 10.22034/gjesm.2021.01.08

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
34	8	5

*Corresponding Author:
Email: *hallaj@ut.ac.ir*
Phone: +9813 4460 3270
Fax: +9813 44608600

Note: Discussion period for this manuscript open until April 1, 2021 on GJESM website at the "Show Article.

INTRODUCTION

Fossil fuels have been the most important source of energy for a long time. However, the new challenges concerning the harmfulness of continuous usage of fossil fuels and imminent finishing of their production sources, have made man to look for a reliable alternative for it (Zheng *et al.,* 2017; Gouveia and Oliveira, 2009). The alternative sources must have desirable properties of fossil fuels in terms of widespread use, economical potentials and ease of use, and do not lead to environmental pollution and global warming. Among all available options, biofuel attracted the researchers' attention because of having unique capabilities (Goh *et al.,* 2019; Erazo *et al.,* 2007). Renewability, desirable environmental properties, and economical potentials are some of the characteristics of biofuel as a cheap and clean alternative for fossil fuels (Chen *et al.,* 2018; Huang *et al.,* 2010). Biofuel production sources include three different categories: first-generation fuel sources (food products such as palm oil, sunflower oil, oilseeds, etc.), Second-generation fuel sources (cellulose-containing fuels such as agricultural wastes), and the third generation fuel sources which include the fuel produced from microorganisms like microalgae (Campbell *et al.,* 2011; Schenk *et al.,* 2008). Among these, microalgae seem to be very desirable due to its high growth rate, the capability of cultivation in non-arable land, production throughout the whole year, high photosynthetic efficiency, flexibility in cultivation conditions and corrigibility by biotechnological tools (Campbell, 1988; Chisti, 2007; Chisti, 2008). However, their application in fuel production in an industrial scale is not economically affordable due to the low efficiency of lipid production (Shuba and Kifle, 2018; Benemann, 1997). In recent years, researchers have been trying to economize the fuel production from microalgae. Biomass and cellular lipid production amounts are important parameters in this process. Therefore, examination of the factors influencing biomass and lipid production in microalgae and their optimization are the most important measure (Dickinson *et al.,* 2017; Lv *et al.,* 2010). One of the most important effective factors in microalgae growth is the cultivation regime. Conducted researches in terms of cultivation regime impact show that mixotrophic regime provides the best culture conditions to achieve maximum production of microalgae biomass (Scarsella *et al.,* 2010). Gao *et al.,* (2019) and Kong *et al.,* (2011) examined the impact of triple regimes (autotroph, heterotroph, and mixotroph) on *Chlorella vulgaris* microalgae and introduced mixotroph as the best regime (Gao *et al.,* 2019; Kong *et al.,* 2011). Also, Li *et al.,* (2014) by studying *Chlorella sp.* an equivalent of *C. vulgaris*, reported that the productivity of biomass in mixotroph cultivation is 14 times greater than that of biomass in autotrophic cultivation (Daliry *et al.,* 2017; Li *et al.,* 2014). After the appropriate cultivation regime, the most important parameters influencing microalgae growth are chemical and physical conditions. The chemical conditions consist of type and amount of main nutrients. Among the chemical conditions, type and concentration of carbon and nitrogen sources are more effective. In addition to these two parameters, pH of the cultivation environment (as a physical condition) is of high importance. Chu *et al.,* (2019) and Kong *et al.,* (2011) studied *C. vulgaris* and showed that glucose was the best carbon source and increase of glucose concentration continuously increased biomass production to the extent that the maximum biomass production (2.24 g/L) was achieved at the glucose concentration of 20 g/L (Kong et al. 2011). Scarsella *et al.,* (2010) in their study on *C. vulgaris* also introduced glucose as the best source of carbon, but they measured 6 g/L glucose as the optimal concentration (Pagnanelli *et al.,* 2014). Evaluating the nitrogen source, Feng *et al.,* (2020) and Jiang *et al.,* (2010) found potassium nitrate as the best source of nitrogen for *C. vulgaris*, and reached the maximum biomass concentration (1.2 g/L) when the concentration of potassium nitrate was 0.5 g/L (Lv et al., 2010). Skorupskaite *et al.,* (2015) studied *Chlorella sp.* and reached the maximum biomass concentration and biomass productivity of 1.7 g/L and 0.103 g/L/Day, respectively, when industrial glycerol concentration was 2 g/L and ammonium nitrogen concentration of 0.09 g/L was used (Skorupskaite *et al.,* 2015). Sayadi *et al.,* (2016) studied the ability of *Chlorella vulgaris* to remove nitrate and phosphate from aqueous solutions. After cultivation of *C. vulgaris* in standard BBM medium, they examined the ability of microalgae by adding 0.25-0.45 g/L KNO3, K2HPO4 to municipal water. Finally, they reported that on day 8 the highest nitrate removal was 89.80% in the treatment with 0.25 g/L microalgae and the highest phosphate removal was 88% in the treatment with 0.45g/L microalgae. In investigate the effect of environment pH, Qiu *et al.,* (2017) and Khalil *et al.,* (2010) studied *C. vulgaris* showed that microalgae could grow under a wide range of pH values (4-10), but pH values of 9 and 10 led to the best cultivation results (Qiu *et al.,* 2017; Khalil *et al.,* 2010). Samiee *et al.,* (2017) investigated the effects of the three parameters on biomass productivity of *Chlorella sp. PTCC 6010*. They examined sodium nitrate (10-200 mg/L) as nitrogen source, dipotassium hydrosulfate (10-70 mg/L) as phosphorus source and light intensity (60-450 µmol photons/m^2/s). They, finally, reported that 200 mg/L sodium nitrate, 70 mg/L dipotassium hydrosulfate and 450 µmol photons/m^2/s^1 resulted in the highest biomass production (0.916 g/L) and biomass productivity (235.8 mg/L/d). (Samiee *et al.,* 2017). The desirable biological properties of *Chlorella sp.,* green single-cell microalgae, such as its high capability in biomass and lipid production, have made it the most ideal option for biodiesel production among other microalgae species (Daliry *et al.,* 2017; Gao *et al.,* 2019; Lv *et al.,* 2010). So far, the impact of carbon source concentration, nitrogen source concentration, and pH on growth and biomass production of *Chlorella sp*. has not been simultaneously examined in a study. Therefore, in this study different glucose and nitrate concentrations, as carbon and nitrogen sources respectively, and different pH values in a specified range were simultaneously investigated by response surface methodology and design of experiments for the first time. This study aims to evaluate the impact of each factor and binary interaction of two of them on growth rate and biomass production, in order to determine the optimal condition for maximum biomass production. It should be noted that protein and chlorophyll content of *Chlorella sp. PTCC 6010* have been also investigated in another study of ours which is under publication. This study has been carried out in Biofuel Laboratory, Caspian Faculty of Engineering, College of Engineering, University of Tehran, Rezvanshahr, Iran during 2017-2019.

MATERIALS AND METHODS

Chlorella sp. PTCC 6010 microalgae was supplied from the Persian Gulf of Iran and used after screening and purifying operations. 50 ml of the obtained microalgae was cultivated during 15-day periods in 4 levels as 0.1, 0.5, 4 and 20 L. The microalgae cultivation environment followed the standard cultivation conditions and was prepared according to Rodik medium with following chemicals in liter (Golzary *et al.,* 2015): $NaNO_3$ (0.3 g), K_2HPO_4 (0.08 g), KH_2PO_4 (0.02 g), NaCl (32.02 g), $CaCl_2$ (0.047 g), $MgSO_4.7H_2O$ (0.01 g), $ZnSO_4$ (0.1 mg), $MnSO_4$ (1.5 mg), $CuSO_4$ (.08 mg), $FeCl_3$ (17 mg), EDTA (7.5 mg) and H_3BO_4 (0.3 mg). All the chemicals were obtained from Merck Company. During the experiments, the cultivation environment was prepared without adding $NaNO_3$ because nitrate concentration was one of the intended parameters. Glucose and nitrate were added to the cultivation environment with different concentrations as carbon and nitrogen sources respectively. Also, KOH and HNO_3 (1 M) were used for initial pH adjustment.

Designing experiments with RSM method and implementation

To achieve the best results through minimum experiment runs and to perform a precise analysis, the experiments design by RSM method based on three parameters including glucose concentration, as carbon source, nitrate concentration, as nitrogen source, and pH of cultivation environment in 5 levels for each parameter. Glucose concentration, nitrate concentration, and pH range of 2-20 g/L, 0-1 g/L and 6-10 were selected according to Central Composite Design (CCD) method. Minitab 17 software was used to design 20 experiments. 250 ml of cultivation environment with the specified pH and glucose and nitrate concentrations was poured into a 500-ml Erlenmeyer and 50 ml of the microalgae was added to it. Temperature of the cultivation environment was set at 30 °C, lighting intensity of 5000 lux was provided using a white fluorescent lamp, and aeration flow rate of 0.05 L/min was provided using a RESUN ACO-004aquarium pump. The pH value of each experiment was measured every day to determine the pH range, and a 5-ml sample of each experiment was taken daily for analysis of concentration specification.

Biomass concentration specification and growth rate

The concentration specification test was conducted on the samples (15 times daily for each experiment) using a Jusco770-Japan spectrophotometer. The biomass concentration was obtained using Eq. 1 as proposed by Golzary *et al.,* (2015), and according to the desired absorption spectra of *Chlorella sp.* biomass (Golzary *et al.,* 2015).

$$W_{(g/L)} = 0.49 \times OD_{550} \tag{1}$$

Where, W is biomass concentration (on dry basis); and OD_{550} is absorption in the desired wavelength, λ=550 nm. The samples of each experiment were put under absorption analysis, and the results were illustrated using the dry biomass concentration-time graphs (growth curve). The pH values-time graphs were also provided with a better understanding of the pH change with the biomass growth.

Data analysis and mathematical model proposal

Results of initial experiments were evaluated by calculating the auxiliary values as a scale, to achieve a better analysis. Specific growth rate and biomass productivity are two vital quantities in the evaluation of growth and production of biomass. Skorupskaite *et al.,* (2015) calculated specific growth rate (μ) and biomass productivity (P) using Eqs. 2 and 3, respectively.

$$\mu = \frac{\ln X_t - \ln X_0}{t_x - t_0} \tag{2}$$

$$P = \frac{X_t - X_0}{t_x - t_0} \tag{3}$$

Where, t_0 and t_x are initial time (the first day) and final time (the 15th day) respectively; X_0 and X_t are biomass concentrations (on dry basis) in t_0 and t_x respectively. Units of μ and P are 1/Day and g/L/Day respectively. Higher specific growth rate and biomass productivity in an experiment show a better growth and higher production of biomass in experiment conditions, respectively.

A more precise analysis was performed using Minitab software, variance analysis (ANOVA) and contour plots. A mathematical model was also proposed based on the experimental results to predict the biomass concentration (W) according to C, N and pH parameters. This model is actually expansion of Eq. 4, wherein x_i values are C, N and pH parameters and β, with different indexes, is a constant factor whose values are calculated in the expanded form of the equation (using computer software). Considering β_0 as equation constant, β_{ij} as binary interaction factor, and Y as response variable or W, its experimental values are predicted by Eq. 4.

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} x_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} x_i x_j \tag{4}$$

Finally, competence and error percentage of the proposed model are examined in result prediction. Error percentage is calculated using Eq. 5.

$$Error = \frac{W_{exper} - W_{pred}}{W_{exper}} \times 100 \tag{5}$$

Where W_{exper} and W_{pred} are biomass concentration values based on experimental results and model prediction, respectively.

RESULTS AND DISCUSSION

Biomass concentration and growth rate

The final biomass concentration (on dry basis) according to absorption analysis data was calculated using Eq. 1, for all experiments. The obtained results along with the specifications of each experiment are shown in Table 1. Obviously, the best results were obtained when carbon concentration is lower than 11 g/L, nitrogen concentration is in the range of 0.2-0.5 g/L (0.2≤N≤ 0.5) and 7≤pH≤9.

Growth curves were also graphed for each experiment. Analysis of the microalgae growth rate in various experiments implied that in half of the experiments the growth rate was ascending and had a net value, while in other half, it was ascending and descending in a fluctuating behavior, leading to having no net value. Therefore, the results were examined in two groups: the experiments with constant growth rate and the experiments with ascending growth rate. Considering the main objective of this study, it was necessary for the experiments to show the significant net growth rate. Therefore, according to the results, a target group of experiments was performed to analyze the ascending growth. It should be noted that the target group had the highest biomass concentration among the experiments. The two auxiliary variables of specific growth rate (μ) and biomass productivity (P) were calculated for the target group experiments (Table 2).

As shown in Table 2, the highest specific growth rate is 0.167 1/Day, and the highest biomass productivity is 0.085 g/L/day, respectively, in carbon concentration of 2.6 g/L and nitrogen concentration of 0.5 g/L and pH value of 8. Among the target group experiments, five experiments showed the best results in terms of growth rate and amount of the produced biomass. To evaluate the growth rate of the

Table 1: Results of the final biomass concentration (dry basis) according to the experiments design

Runs	C		N		pH		Results
	Code values	Real values (g/L)	Code values	Real values (g/L)	Code values	Real values	W (g/L)
E1	0	11	0	0.5	0	8	0.613
E2	-1.68	2.6	0	0.5	0	8	1.313
E3	0	11	+1.68	1	0	8	0.268
E4	+1	16	+1	0.8	+1	9	0.246
E5	+1	16	-1	0.2	-1	7	0.221
E6	0	11	-1.68	0	0	8	0.217
E7	0	11	0	0.5	0	8	0.251
E8	-1	6	+1	0.8	+1	9	0.926
E9	0	11	0	0.5	-1.68	6.3	0.887
E10	0	11	0	0.5	0	8	0.956
E11	0	11	0	0.5	+1.68	9.7	0.447
E12	+1.68	19.4	0	0.5	0	8	0.221
E13	0	11	0	0.5	0	8	0.246
E14	-1	6	-1	0.2	+1	9	0.931
E15	0	11	0	0.5	0	8	0.300
E16	-1	6	-1	0.2	-1	7	1.289
E17	+1	16	+1	0.8	-1	7	0.314
E18	-1	6	+1	0.8	-1	7	0.59
E19	+1	16	-1	0.2	+1	9	0.419
E20	0	11	0	0.5	0	8	0.793

Table 2: specific growth rate and biomass productivity in the target group experiments

Target group	X_0 (g/L)	X_1 (g/L)	μ (1/day)	P (g/L/day)
E1: 11 , 0.5 , 8	0.128	0.613	0.112	0.035
E2: 2.6 , 0.5 , 8	0.126	1.313	0.167	0.085
E8: 6 , 0.8 , 9	0.109	0.926	0.153	0.058
E9: 11 , 0.5 , 6.3	0.100	0.887	0.156	0.056
E10: 11 , 0.5 , 8	0.157	0.956	0.129	0.057
E11: 11 , 0.5 , 9.7	0.153	0.447	0.077	0.021
E14: 6 , 0.2 , 9	0.153	0.931	0.129	0.056
E16: 6 , 0.2 , 7	0.132	1.289	0.163	0.083
E18: 6 , 0.8 , 7	0.100	0.59	0.127	0.035
E20: 11 , 0.5 , 8	0.148	0.793	0.120	0.046

microalgae under ideal conditions, the growth curves of these experiments were plotted (Fig. 1).

As can be seen in Fig. 1, E2 experiment resulted in the highest produced biomass, while E16 experiment led to the best growth rate. Among the rest of experiments having a biomass concentration of about 1 g/L, experiment E14 shows the best growth rate. The common point in E14 and E16 experiments is equal amount of the used glucose and nitrate. The pH change analysis implied that the pH value in the environment fluctuated within the alkaline range (9≤pH≤10) during the cultivation period. This contradicts with the findings of Kong *et al.,* (2011) who reported that the pH change was in the neutral range (pH=7) during mixotrophic cultivation (Kong *et al.,* 2011). The pH change is important because several studies have shown that alkaline environment can serve as a positive factor in the growth of microalgae (Qiu *et al.,* 2017; Khalil *et al.,* 2010). The range of pH fluctuation during cultivation is also important. This was investigated by plotting the pH-time curves for the five experiments (Fig. 2).

Fig. 2 shows: 1) the increasing trend of pH values in all the experiments, and 2) the tendency of pH change towards pH=10. Studies show the increasing trend of pH in all the target group experiments. The different trend of pH change in experiment E10 indicates a slower pH increase compared to other

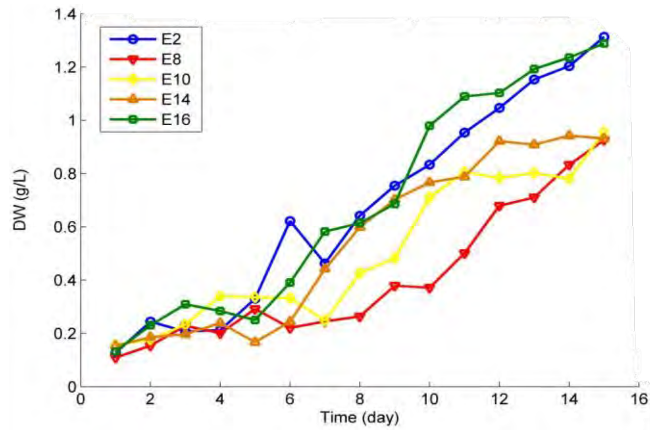


Fig. 1: Growth curves of the five experiments with the best results in terms of growth rate and amount of the produced biomass

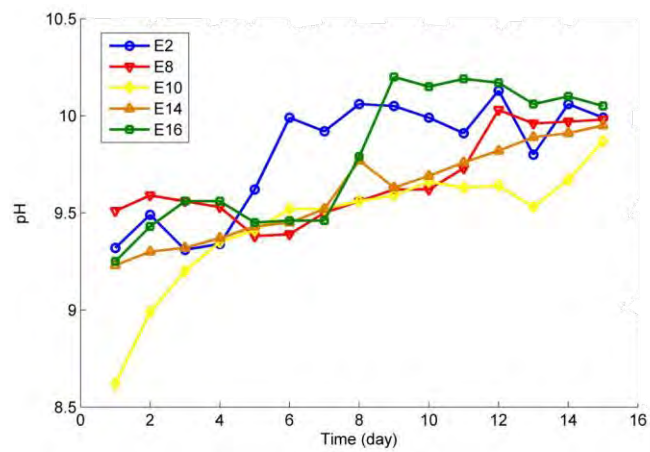


Fig. 2: pH changes for experiments with desired growth

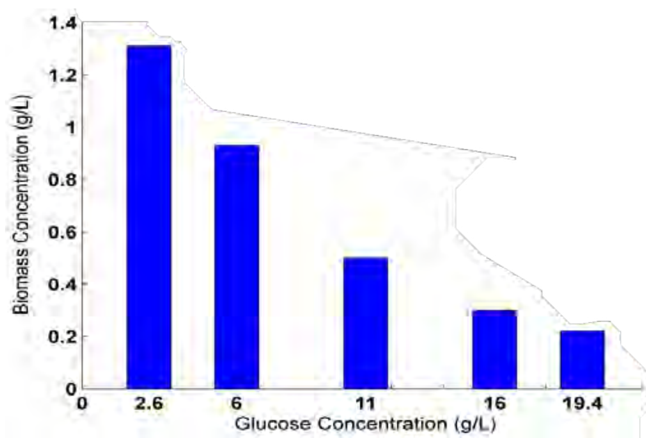


Fig. 3: The average biomass produced in each glucose concentration

experiments. This can be due to higher glucose concentration (11 g/L) in this experiment compared to other experiments.

Effect of glucose concentration on biomass production

The results of biomass production presented in Table 1 and the target group experiments reveal that glucose concentrations of over 11 g/L lead to disruption of biomass growth and thereby significant decrease of biomass production. Moreover, further increase in glucose concentration can have a more inhibitive effect on growth. Fig. 1 showed that glucose concentrations of 6 g/L and 2.6 g/L led to the best growth rate and the highest biomass production, respectively. Moreover, Fig. 2 illustrated that the highest specific growth rate of 0.16 1/Day and the highest biomass productivity of 0.08 g/L/Day were achieved in glucose concentration of 2.6 g/L and 6 g/L, respectively. The average amounts of produced biomass in different concentrations of glucose in all the experiments are illustrated in Fig. 3. It is obvious that the best range of glucose concentration is 2.6-6 g/L, leading to the best growth rate and the highest amount of produced biomass. Moreover, the glucose concentrations of over 6 g/L significantly decrease the biomass production in all the experiments. This is in agreement with the findings of Penno *et al.,* (2019), but contradicts with the results presented by Kong *et al.,* (2011) who declared that increase of glucose concentration to 20 g/L could increase biomass production, specific growth rate and biomass productivity.

Effect of nitrate concentration on biomass production

Fig. 1 shows that the highest amount of biomass (1.31 g/L) is produced in the nitrate concentration of 0.5 g/L. Growth curves illustrated in Fig. 1 indicate a better microalgae growth when nitrate concentration is 0.2 g/L. The highest values for specific growth rate and biomass productivity were also obtained in the nitrate concentration ranging 0.2-0.5 g/L. The average amounts of produced biomass in different concentrations of nitrate in all the experiments are illustrated in Fig. 4. Obviously, absence of nitrate, which means using no nitrogen source in the microalgae cultivation environment, has a significant adverse effect on biomass production. In fact, the least amount of biomass growth and production was observed in the absence of nitrate. On the other hand, the nitrate concentration of over 0.8 g/L significantly decreased the biomass production. Generally, the nitrate concentrations in the range of 0.2-0.5 g/L led to the best results, while the nitrate concentration of 0.8 g/L did not provide a suitable result in terms of biomass growth and production. These results are in agreement with the results obtained by An *et al.,* (2020) and Lv *et al.,* (2010) who reported the nitrate concentration of 0.5 g/L as the optimal concentration leading to the highest amount of chlorella biomass, and introduced the absence of nitrate as a factor leading to a significant decrease in biomass growth and production.

Effect of pH on biomass production

Effect of pH behavior on biomass production and growth rate during the growth was already discussed. However, investigation of the initial pH showed that

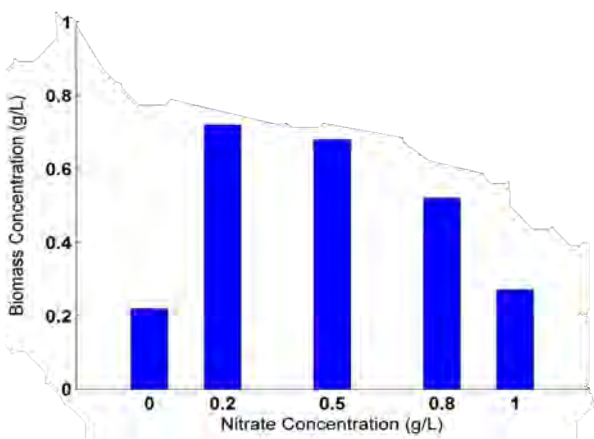


Fig. 4. The average biomass produced in each nitrate concentration

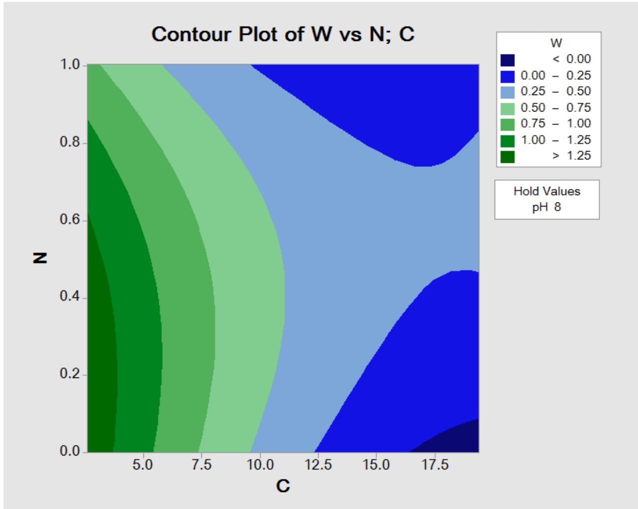


Fig. 5: Contour plot of W vs. C, N

Table 3: The average biomass production (W_{ave}), specific growth rate (μ_{ave}) and biomass productivity (P_{ave}) for different C/N ratios

C/N	5.2	7.5	11	20	22	30	38.8	80	∞
W_{avr}	1.313	0.758	0.268	0.28	0.561	1.11	0.221	0.32	0.217
μ_{avr}	0.167	0.14	0.063	0.052	0.1	0.146	0.046	0.047	0.023
P_{avr}	0.085	0.046	0.011	0.01	0.031	0.07	0.007	0.011	0.004

the microalgae maintained its biomass productivity and growth in the pH range of 6-10. This is in agreement with the findings of Qiu *et al.,* (2017) and Khalil *et al.,* (2010), in terms of growth capability of *Chlorella* in a wide pH range of 4-10. Considering the results of the target group experiments in Table 2, pH>9 decreased the growth and production of biomass and the best results were obtained at 7<pH<9. According to Table 2, the maximum biomass production of 1.31 g/L, specific growth rate of 0.167 1/day, and biomass productivity of 0.085 g/L/Day were achieved in pH=8. This result contradicts with the results obtained by Khalil *et al.,* (2010) who proposed pH of 9-10 as the optimal pH range for cultivation *Chlorella vulgaris,* and Gong *et al.,* (2014) and Gong *et al.,* (2014) who introduced an approximate optimal pH value of 10 for C. *vulgaris* cultivation.

Effects of binary interactions on biomass production

Effects of binary interactions of parameters include the effects of simultaneous change of two parameters on the target variable. The three parameters in this study would form three binary interactions: C-N, C-pH, and N-pH. Contour plots obtained from Minitab software analysis were used to study these interactions. Fig. 5 illustrates a contour plot for C-N interaction. It is obvious that, reduction of glucose concentration (C) to below 10 g/L and nitrate concentration to below 1 g/L at the same time leads to an increase in biomass production (W). Formerly, Pagnanelli *et al.,* (2014) examined the effect of C-N interaction as the effect of C/N ratio on specific growth rate. They suggested the tolerance threshold of microalgae as a specified value for the C/N ratio. According to Gao *et al.,* (2019) and Skorupskaite *et al.,* (2015), there would be a maximum concentration of glucose for each single concentration of nitrate and exceeding this maximum value would result in a significant decrease in specific growth rate and biomass production. They reported this ratio as about 17 for *Chlorella vulgaris*. The effects of different C/N ratios on average biomass production (W_{ave}), average specific growth rate (μ_{ave}) and average biomass productivity (P_{ave}) are presented in Table 3.

Table 3 confirms that there is a specific C/N ratio and exceeding it would lead to a significant decrease in W, μ and P. For *Chlorella sp*., this ratio is 30, but the mentioned decrease can be seen even in lower C/N ratios (C/N=11, 20 and 22). Study of nitrate and glucose concentrations in these ratios revealed that the mentioned decrease occurred as a result of the glucose or nitrate concentrations over the range for appropriate growth. In other words, when both nitrate and glucose concentrations are in the appropriate range, biomass production, specific growth rate and biomass productivity increase in case C/N<30. This point shows the relationship between Fig. 5 and Table 3. Table 3 also shows that the increase of C/N ratio to the values above the tolerance threshold (C/N=30) intensifies the decreasing behavior of specific growth rate and biomass productivity, and according to Fig. 5, biomass production is significantly decreased as well.

The effects of simultaneous change of C-pH and N-pH on biomass production are illustrated in Figs. 6 and 7, respectively.

As it can be seen in Fig. 6, decrease of glucose to less than 10 g/L leads to W increase in a wide range of pH values, and this increase is intensified when glucose concentration is lower than 6 g/L. According to Fig. 7, a simultaneous decrease in nitrate concentration and pH has a positive effect on increase of W. However, this applies to the nitrate concentrations below 0.8 g/L, and pH<7 or pH>9. Comparison of C-pH and N-pH interactions plots indicates that W is more increased by simultaneous decrease of glucose concentration and pH rather than simultaneous decrease of nitrate concentration and pH. This could be due to glucose concentration

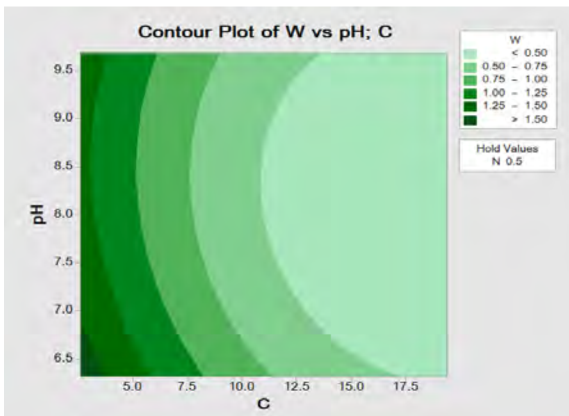


Fig. 6: Contour plot of W vs. C, pH

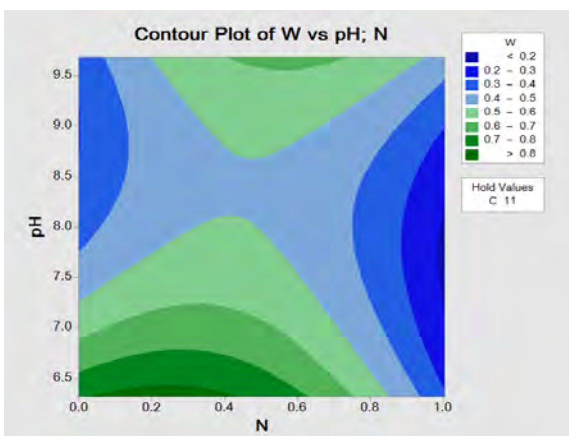


Fig. 7: Contour plot of W vs. N, pH

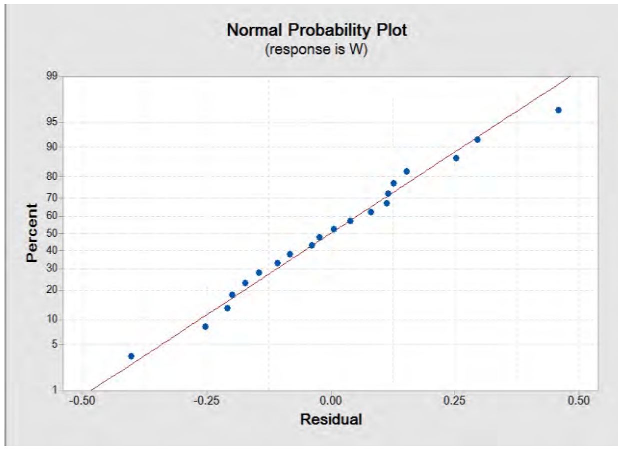


Fig. 8: The normal plot to the investigation of model competency

which has a direct effect on pH changes during the growth. Unlike nitrate, glucose is somewhat alkaline and this could also contribute to stronger interaction of C-pH to N-pH.

Prediction of biomass concentration using the mathematical model

In order to predict the amount of produced biomass (W in g/L) according to the change of glucose concentration (C in g/L), nitrate concentration (N in g/L) and pH, the software proposed a mathematical model as presented in Eq. 6 which is the expanded form of Eq. 4.

$$W=7.78 - 0.213*C - 1.28*N - 1.30*pH + 0.00423*C*C - 0.886*N*N + 0.0703*pH*pH + 0.0520*C*N + 0.0038*C*pH + 0.178*N*pH \quad (6)$$

Table 4: Analysis of variance for biomass production data

Term	P-value	Coefficient
Constant	0.002	0.499
C	0.002	-0.320
N	0.052	-0.051
pH	0.056	-0.046
C*C	0.189	0.106
N*N	0.313	-0.080
pH*pH	0.370	0.070
C*N	0.081	0.078
C*pH	0.095	0.054
N*pH	0.116	0.019

The three parameters were evaluated to investigate the model competency. The first parameter was the assumption of normality of residuals. In this approach, residual values are marked in normal plot and the fittest line crossing these points is drawn. If all the points are almost covered putting a wide pencil on this line, it is said that the assumption of normality of residuals is true and the model is competent enough. According to the proposed normal plot by Minitab 17 based on the data illustrated in Fig. 8, it can be concluded that the assumption of normality of residuals is true and the proposed model is competent enough.

The second parameter, obtained from analysis of variance, is the P-value parameter. P-value parameter is expressed based on a confidence level of the data, by two parameters of model suitability and lack of fit. Usually, the confidence level of the data is considered as 90-95%. Therefore, the P-value should be less than 0.1 (significant) for model suitability and more than 0.1 (insignificant) for lack of fit. Variance analysis of the model performed by Minitab showed that the P-value was 0.07 (<0.1) for model suitability and 0.82 (>0.1) for lack of fit, confirming the model competency. Moreover, analysis of variance was presented in Table 4 to examine the parameters consisting the P-value quantities and coefficient effects. For P-values the method is the same as above, but for coefficient effects, the sign of coefficients (positive or negative) and their values indicate the type and the amount of their effects on target quantity. As can be seen in Table 4, carbon concentration has the largest coefficient

Table 5: Performance evaluation and prediction error percentage of the model

Target group	W_{exper}	W_{pred}	*Error*
E2: 2.6 , 0.5 , 8	1.313	1.351	2.89
E8: 6 , 0.8 , 9	0.926	0.794	14.25
E9: 11 , 0.5 , 6.3	0.887	0.798	10.03
E10 : 11 , 0.5 , 8	0.787	0.519	33.97
E11: 11 , 0.5 , 9.7	0.447	0.647	44.74
E14: 6 , 0.2 , 9	0.931	0.945	1.5
E16: 6 , 0.2 , 7	1.289	1.179	8.53
E18: 6 , 0.8 , 7	0.59	0.814	37.96

effect by negative sign (-0.320). Thus, it can be stated that carbon concentration has the highest reducing effect on biomass production.

The third parameter is R^2 value, which is a measure of model validity and also the extent to which the model covers the data. The nearer is R^2 to 1, the better the model works (in here R^2=0.7). Although it was not a highly desirable value, it was acceptable according to the manner and dispersion of the data. In order to evaluate the performance of the model, the values predicted by the model were compared to the experimental results presented in Table 5. The percentage for each experiment of the target group calculated by Eq. 5 is shown in Table 5 as well.

E_{10} in Table 5 is the central point experiment, and its average value is used due to its 3-time repetition in the target group. The model performance in terms of the experiments with the maximum biomass production (E2, E8, E9, E14, and E16) was acceptable since the error percentage was 10% and proportionate to a confidence level of the data. The minimum predicting error (1.5%) was obtained for experiment E14, and the maximum error (44.74%) was related to experiment E11. In overall, the maximum biomass dry weight was obtained as 1.31 g/L in glucose concentration of 2.6 L/g, nitrate concentration of 0.5 g/L and pH=8. The validation test was done in this optimal condition and the biomass dry weight was obtained as 1.304 g/L. Thus, the optimal biomass dry weight was achieved with only 0.68% error.

CONCLUSION

Due to the desirable biological properties of *Chlorella sp.* microalgae, it is considered as one of the most ideal microalgae species for biodiesel production. Effects of three parameters of glucose concentration, nitrate concentration and pH on growth and production of *Chlorella sp.* biomass were investigated using the response surface methodology. Each factor was examined simultaneously under glucose concentration in 2-20 g/L, nitrate concentration in 0-1 g/L and 6<pH<10. During the growing, pH of the culture was measured to identify the correlation between pH and growth rate change. The results were analyzed by response surface methodology as well. Results showed that glucose concentration was the most effective parameter in biomass growth and production, so that the biomass growth was disrupted and significantly decreased in glucose concentrations of over 10 g/L. Absence of nitrate as a nitrogen source also resulted in disruption of growth and sever decrease in biomass production. It was realized that in case of eligible growth, pH of the cultivation environment increased to pH=10 and was in the range 9-10 during the growth. The best results were achieved when glucose concentration, nitrate concentration and, pH were in the range of 2.6-6 g/L, 0.2-0.5 g/L and 7-9, respectively. Effects of binary interactions of parameters on biomass production were investigated using contour plots. Comparison of C-pH and N-pH interactions plots was indicated that biomass production was more increased by simultaneous decrease of glucose concentration and pH rather than simultaneous decrease of nitrate concentration and pH. This could be due to glucose concentration which has a direct effect on pH changes during the growth. It was demonstrated a significant correlation between C/N ratio and biomass production and optimal C/N ratio of the microalgae was obtained as 30. A model was proposed to predict biomass production. The maximum biomass production, highest specific growth rate and the maximum biomass productivity were obtained as 1.31 g/L, 0.167 1/Day and 0.085 g/L/Day, respectively. It was concluded that *Chlorella sp.*, if properly adjusted for both chemical and physical parameters, could be a valuable source of biomass for biodiesel production in industrial scale.

AUTHOR CONTRIBUTIONS

H. Nouri and A. Hallajisani performed the literature review and experimental design, analyzed and interpreted the data, prepared the manuscript text, and rendered manuscript edition. S. Dalirinejad helped in the experimental design. A. Golzary and J. Mohammadi Roshande helped in the literature review.

ACKNOWLEDGEMENTS

This study was supported by the Caspian Faculty of Engineering, College of Engineering, University of Tehran. The authors also appreciate support of the Persian Gulf Microalgaes Centre for supplying the microbial species.

CONFLICT OF INTEREST

The authors declare no potential conflict of interests regarding the publication of this work. The ethical issues including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy have been completely witnessed by the authors.

ABBREVIATIONS

ANOVA	Analysis of variance
β_0	Constant factor
β_{ij}	Binary interaction factor
C	Carbon source concentration
$CaCl_2$	Calcium chloride
CCD	Central composite design
$CuSO_4$	Copper sulfate
E_i	Experiment number
EDTA	Ethylen diamine tetra acetic acid
Eq.	Equation
$FeCl_3$	Iron(III) chloride
H_3BO_4	Boric acid
HNO_3	Nitric acid
KH_2PO_4	Potassium dihydrogen phosphate
K_2HPO_4	Dipotassium hydrogen phosfate
KOH	Potassium hydroxide
$Ln\ X_0$	Natural logarithm of initial biomass
$Ln\ X_t$	Natural logarithm of final biomass
$MgSO_4.7H_2O$	Magnesium sulfate 7 hydrate
$MnSO_4$	Manganese (II) sulfate
μ	Specific growth rate
N	Nitrogen source concentration
NaCl	Sodium chloride
$NaNO_3$	Sodium nitrate
OD	Optical density
λ	Wave length
P	Biomass productivity
pH	Potential of hydrogen
p-value	Probability value
R^2	Coefficient of determination
RSM	Response surface methodology
t_0	Initial time
t_x	Final time
W	Dry biomass weight
W_{exper}	Experimental dry biomass weight
W_{pred}	Predicted dry biomass weight
X_0	Initial biomass concentration
X_t	Final biomass concentration
Y	response variable
$ZnSO_4$	Zinc sulfate

REFERENCES

An, M.; Gao, L.; Zhao, W.; Chen, W.; Li, M., (2020). Effects of nitrogen forms and supply mode on lipid production of microalga Scenedesmus obliquus. Energies, 13(3): 697 **(1 page).**

Benemann, J., (1997). CO2 mitigation with microalgae systems. Energy Convers. Manage., 38: 475-479 (**5 pages**).

Campbell, C., (1988). The coming oil crisis. Multi-science publishing co., Brentwood, England.

Campbell, P.; Beer, T.; Batten, D., (2011). Life cycle assessment of biodiesel production from microalgae in ponds. Bioresour. Technol., **102**(1): 50-56 (**7 pages**).

Chen, J.; Li, J.; Dong, W.; Zhang, X.; Tyagi, R.; Drogui, P.; Surampalli, R.Y., (2018). The potential of microalgae in biodiesel production. Renewable Sustainable Energy Rev., 90: 336-346 (**11 pages**).

Chisti, Y., (2007). Biodiesel from microalgae. Biotechnol. Adv., 25(3): 294-306 (**13 pages**).

Chisti, Y., (2008). Biodiesel from microalgae beats bioethanol. Trends Biotechnol., 26(3): 126-131 (**6 pages**).

Chu, F.; Cheng, J.; Zhang, X.; Ye, Q.; Chen, S.; Zhou, J.; Cen, K., (2019). Transcriptome and key gene expression related to carbon metabolism and fatty acid synthesis of Chlorella vulgaris under a nitrogen starvation and phosphorus repletion regime. J. Appl. Phycol., 31(5): 2881-2893 (**13 pages**).

Daliry, S.; Hallajisani, A.; Mohammadi, R.J.; Nouri, H.; Golzary, A., (2017). Investigation of optimal condition for Chlorella vulgaris microalgae growth. Global J. Environ. Sci. Manage., 3(2): 217-230 **(14 pages).**

Daliry, S.; Hallajisani, A.; Nouri, H.; Golzary, A., (2017). Effects of Environmental Factors on Chlorella sp. microalgae for Biodiesel Production Purpose: Enhanced Lipid and Biomass Productivity. Recent Innovation in Chem. Eng., 10(2): 119-126 (**8 pages**).

Dickinson, S.; Mientus, M.; Frey, D.; Amini-Hajibashi, A.; Ozturk, S.; Shaikh, F.; Sengupta, D.; El-Halwagi, M., (2017). A review of biodiesel production from microalgae. Clean Technol. Environ. Policy, 19(3): 637-668 (**32 pages**).

Erazo, J.; Sequera, A.; Parthasarathy, R.; Gollahalli, S., (2007). Comparison of spray characteristics of biodiesel and diesel fuel. Proceedings of the 5th International Energy Conversion Engineering Conference and Exhibit, St. Louis, MO, AIAA.

Feng, P.; Xu, Z.; Qin, L.; Alam, M.; Wang, Z.; Zhu, S., (2020). Effects of different nitrogen sources and light paths of flat plate photobioreactors on the growth and lipid accumulation of *Chlorella sp.* GN1 outdoors. Bioresour. Technol., 122762 (**1 pages**).

Gao, F.; Yang, H.; Li, C.; Peng, Y.; Lu, M.; Jin, W.; Bao, J.; Guo, Y., (2019). Effect of organic carbon to nitrogen ratio in wastewater on growth, nutrient uptake and lipid accumulation of a mixotrophic microalgae *Chlorella sp.*. Bioresour. Technol., 282: 118-124 (**7 pages**).

Goh, B.; Ong, H.; Cheah, M.; Chen, W.; Yu, K.; Mahlia, T., (2019). Sustainability of direct biodiesel synthesis from microalgae biomass: A critical review. Renewable Sustainable Energy Rev., 107: 59-74 (**16 pages**).

Golzary, A.; Imanian, S.; Abdoli, M.; Khodadadi, A.; Karbassi, A., (2015). A cost-effective strategy for marine microalgae separation by electro-coagulation–flotation process aimed at bio-crude oil production: Optimization and evaluation study. Sep. Purif. Technol., 147: 156-165 (**10 pages**).

Gong, Q.; Feng, Y.; Kang, L.; Luo, M.; Yang, J., (2014). Effects of Light and pH on Cell Density of *Chlorella vulgaris*. Energy Procedia, 61: 2012-2015 (**4 pages**).

Gouveia, L.; Oliveira, A., (2009). Microalgae as a raw material for biofuels production. J. Ind. Microbiol. Biotechnol., 36(2): 269-274 (5 **pages**).

Huang, G.; Chen, F.; Wei, D.; Zhang, X.; Chen, G., (2010). Biodiesel production by microalgal biotechnology. Appl. Energy, 87(1): 38-46 (**9 pages**).

Jiang, J.; Zhu, Y., (2010). Preliminary and comparative studies on the cultivations of dunaliella salina between outdoors and in the photobioreactor. J. Food Process Eng., 33(1): 104-114 (**11 pages**).

Khalil, Z.; Asker, M.; El-Sayed, S.; Kobbia, I., (2010). Effect of pH on growth and biochemical responses of Dunaliella bardawil and Chlorella ellipsoidea. World J. Microbiol. Biotechnol., 26(7): 1225-1231 (**7 pages**).

Kong, W.; Song, H.; Cao, Y.; Yang, H.; Hua, S.; Xia, C., (2011). The characteristics of biomass production, lipid accumulation and chlorophyll biosynthesis of *Chlorella vulgaris* under mixotrophic cultivation. Afr. J. Biotechnol., 10(55): 11620-11630 (**11 pages**).

Li, Y.; Tsai, W.; Hsu, Y.; Xie, M.; Chen, J., (2014). Comparison of autotrophic and mixotrophic cultivation of green microalgal for biodiesel production. Energy Procedia, 52: 371-376 (**6 pages**).

Lv, J.; Cheng, L.; Xu, X.; Zhang, L.; Chen, H., (2010). Enhanced lipid production of Chlorella vulgaris by adjustment of cultivation conditions. Bioresour. Technol., 101(17): 6797-6804 (**8 pages**).

Pagnanelli, F.; Altimari, P.; Trabucco, F.; Toro, L., (2014). Mixotrophic growth of Chlorella vulgaris and Nannochloropsis oculata: interaction between glucose and nitrate. J. Chem. Technol. Biotechnol., 89(5): 652-661 (**10 pages**).

Penno, A.; Agar, E.; Divok, J., (2019). Effects of activated carbon on the growth of *Chlorella vulgaris* in an aqueous solution. Stud. Undergraduate Res. Guelph., 11.

Qiu, R.; Gao, S.; Lopez, P.; Ogden, K., (2017). Effects of pH on cell growth, lipid production and CO2 addition of microalgae Chlorella sorokiniana. Algal Res., 28: 192-199 (**8 pages**).

Samiee-Zafarghandi, R.; Karimi-Sabet, J.; Abdoli, M.A.; Karbassi, A., (2018). Increasing microalgal carbohydrate content for hydrothermal gasification purposes. Renewable Energy, 116: 710-719 (**10 pages**).

Sayadi, M. H.; Ahmadpour, N.; Fallahi Capoorchali, M.; Rezaei, M.R., (2016). Removal of nitrate and phosphate from aqueous solutions by microalgae: An experimental study Global J. Environ. Sci. Manage., 2(3): 357-364 (**8 pages**).

Scarsella, M.; Belotti, G.; De Filippis, P.; Bravi, M., (2010). Study on the optimal growing conditions of *Chlorella vulgaris* in bubble column photobioreactors. Chem. Eng., 20: 85-90 (**6 pages**).

Schenk, P.; Thomas-Hall, S.; Stephens, E.; Marx, U.; Mussgnug, J.; Posten, C.; Kruse, O.; Hankamer, B., (2008). Second generation biofuels: high-efficiency microalgae for biodiesel production. Bioenerg. Res., 1(1): 20-43 (**24 pages**).

Skorupskaite, V.; Makareviciene, V.; Levisauskas, D.; (2015). Optimization of mixotrophic cultivation of microalgae *Chlorella sp.* for biofuel production using response surface methodology. Algal Research, 7: 45-50 (**6 pages**).

Shuba, E.; Kifle, D., (2018). Microalgae to biofuels: Promising'alternative and renewable energy: Review. Renewable Sustainable Energy Rev., 81: 743-755 (**13 pages**).

Zheng, H.; Guo, W.; Li, S.; Chen, Y.; Wu, Q.; Feng, X.; Yin, R.; Ho, S.; Ren, N.; Chang, J., (2017). Adsorption of p-nitrophenols (PNP) on microalgal biochar: analysis of high adsorption capacity and mechanism. Bioresour. Technol., 244 : 1456–1464 (**9 pages**).

AUTHOR (S) BIOSKETCHES

Nouri, H., M.Sc., Caspian Faculty of Engineering, College of Engineering, University of Tehran, Rezvanshar, Guilan, Iran. Email: *hnourip@gmail.com*

Mohammadi Roushandeh, J., Ph.D., Professor, Caspian Faculty of Engineering, College of Engineering, University of Tehran, Rezvanshar, Guilan, Iran. Email: *roshandeh@ut.ac.ir*

Hallajisani, A., Ph.D., Assistant Professor, Caspian Faculty of Engineering, College of Engineering, University of Tehran, Rezvanshar, Guilan, Iran. Email: *hallaj@ut.ac.ir*

Golzary A., Ph.D., Assistant Professor, Caspian Faculty of Engineering, College of Engineering, University of Tehran, Rezvanshar, Guilan, Iran. Email: *abooaligolzary@ut.ac.ir*

Daliry, S., M.Sc., Caspian Faculty of Engineering, College of Engineering, University of Tehran, Rezvanshar, Guilan, Iran Email: *saeeddaliry@ut.ac.ir*

HOW TO CITE THIS ARTICLE

Nouri, H.; Mohammadi Roushandeh, J.; Hallajisani, A.; Golzary, A; Daliry, S., (2021). The effects of glucose, nitrate, and pH on cultivation of Chlorella sp. Microalgae. Global J. Environ. Sci. Manage., 7(1): 103-116.

DOI: 10.22034/gjesm.2021.01.08

url: https://www.gjesm.net/article_44603.html

Global J. Environ. Sci. Manage. 7(1): 117-130, Winter 2021

Global Journal of Environmental Science and Management
(GJESM)

Homepage: https://www.gjesm.net/

CASE STUDY

Using multivariate generalized linear latent variable models to measure the difference in event count for stranded marine animals

R.E. Caraka[1,2], R.C. Chen[1,*], Y. Lee[2], T. Toharudin[3], C. Rahmadi[4], M. Tahmid[5], A.S. Achmadi[4]

[1]Department of Information Management, College of Informatics, Chaoyang University of Technology, Taiwan

[2]Department of Statistics, College of Natural Sciences Seoul National University, Shin Lim-Dong, Kwan Ak Ku, South Korea

[3]Department of Statistics, Padjadjaran University, West Java, Indonesia

[4]Museum Zoologicum Bogoriense, Research Center for Biology, Indonesian Institute of Sciences, Indonesia

[5]Indonesian Agency for Meteorological, Climatological and Geophysics, BMKG, Manado, Indonesia

ARTICLE INFO

Article History:
Received 23 April 2020
Revised 07 July 2020
Accepted 17 August 2020

Keywords:
Indonesia
Latent
Madden–Julian oscillation (MJO)
Marine species
Multivariate

ABSTRACT

BACKGROUND AND OBJECTIVES: The classification of marine animals as protected species makes data and information on them to be very important. Therefore, this led to the need to retrieve and understand the data on the event counts for stranded marine animals based on location emergence, number of individuals, behavior, and threats to their presence. Whales are generally often stranded in very shallow areas with sloping sea floors and sand. Data were collected in this study on the incidence of stranded marine animals in 20 provinces of Indonesia from 2015 to 2019 with the focus on animals such as *Balaenopteridae, Delphinidae, Lamnidae, Physeteridae*, and *Rhincodontidae*.

METHODS: Multivariate latent generalized linear model was used to compare several distributions to analyze the diversity of event counts. Two optimization models including Laplace and Variational approximations were also applied.

FINDINGS: The best theta parameter in the latent multivariate latent generalized linear latent variable model was found in the Akaike Information Criterion, Akaike Information Criterion Corrected and Bayesian Information Criterion values, and the information obtained was used to create a spatial cluster. Moreover, there was a comprehensive discussion on ocean-atmosphere interaction and the reasons the animals were stranded.

CONCLUSION: The changes in marine ecosystems due to climate change, pollution, overexploitation, changes in sea use, and the existence of invasive alien species deserve serious attention.

DOI: 10.22034/gjesm.2021.01.09

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
41	**7**	**2**

*Corresponding Author:
Email: *crching@cyut.edu.tw*
Phone: +886-4-2332-3000
Fax: +886-4-2374-2337

Note: Discussion period for this manuscript open until April 1, 2021 on GJESM website at the “Show Article.

INTRODUCTION

The Indo-West Pacific arguably offers enormous diversity in marine mammal species throughout the world, as observed in representatives of 11 of the 13 families of the Cetacean with more than 40 of the 85 species living in the sea (Rudolph *et al.,* 2009). Furthermore, there are approximately 1,250 species of sharks, rays, and ghost shark in the world. An estimate of 218 species was found in the Indonesian sea, out of which only 26 species have high economic value in the global market (Maryanto, *et al.,* 2008). The different types of sharks existing in the waters include the *Carcharhinidae, Lamnidae, Alopiidae, Sphyrnidae* families as well as the *Mobulidae* rays including the Manta and Mobula which are considered to be the most commonly used groups. The *Rhincodon typus* whale shark is the largest fish species in the world usually found in tropical to subtropical waters (Norman, 2002) as well as oceans and coastal seas including lagoons, coral atolls, and reefs. The total length of a whale shark is more than 18 meters and has been reported to be reproducing through ovoviviparity with the embryo ready to come out of the mother's stomach in sizes ranging between 55 and 64. Moreover, those categorized as adults are usually 7.05-10.26 m or more for males and 12 m or more for females (Stevens, 2007) with both gender found to have a swimming character adapted only to the aquatic environment (Colman, 1997). Whale sharks can live in deep and shallow water near the coast, and their habitat is mostly related to water quality, plankton concentration, temperature, current patterns, weather, and water location. Water quality is a critical factor for whale sharks due to its relation to the availability of nutrients for zooplankton which is their primary food source. These marine animals have a filter system feeder to feed on planktonic and nektonic biota as well as extensive migration abilities and movements thought to be related to the high productivity of zooplankton, changes in water temperature, currents, wind, and other water parameters. Zooplankton obtains food from producers that photosynthesize by phytoplankton and based on different environments considered to be significant for natural life. Meanwhile, several living space models exist with explicit attributes with some using nearness-just information while others use nearness nonattendance or tally information. Apart from the nearness-just models, which do not deal with nonappearance information such as zeros, selecting nearness nonappearance or tally-based models is a difficulty due to the reliance on contemplated species primarily when concentrating on uncommon species based on innate trouble attached to determining the models best suited to countless nonattendances. As previously referenced, uncommon species generally lead to a low number of sightings per unit exertion and this shortage of information makes it hard to determine the best appropriation models. Some studies have, however, addressed the use of models for rare species datasets (Lomba *et al.,* 2010; Mouillot *et al.,* 2013; Demos *et al.,* 2016; Kurniawan, *et al.,* 2018) but reliability and uncertainty associated with the predictions produced by these models remain pending issues (Kurniawan, *et al*., 2018). An option to address these challenges is by testing the maintenance of performance for a species distribution model when there is a decrease in the input data in order to assess its reliability in handling small datasets for rare species. Several events of animal marine species have been stranded in Indonesia in the past few decades, and Chan, *et al*. (2017) have created a database for this occurrence in addition to their mortality. Some factors causing the strandedness have been identified with the most important ones considered to be internal such as illness (Duignan, 2003), malnutrition, natural toxins, and infectious diseases (Wibowo *et al*., 2014). Meanwhile, the interactions between marine mammals and plastic debris have been the focus of studies for many years (Lusher *et al.,* 2018), the ingestion of marine litter such as plastic considered to be leading to whale shark fatality (Abreo *et al.,* 2019). The loud sound emitted during offshore industrial activities has also been reported to have an impact on marine mammals (Verfuss *et al.,* 2018). The data and information on stranded marine animals are very useful and important to understand the patterns and possible cause for the phenomenon in relation to the environment and changes in their habit due to human activities. In the policy context, the study is expected to help determine the areas with the greatest risk of stranded marine animals and the best ways to mitigate its occurrence. This study was, therefore, conducted to measure the incidence of stranded marine animals and differences in the event counts using multivariate generalized linear latent variable models on data collected in 20 Provinces of Indonesia from 2015 to 2019.

MATERIALS AND METHODS

Data collection

The data and information including species and the number of individuals on stranded marine animals were manually collected from media reports from 2015 to 2019 and verified using several sources. This data collected method is limited by the potential omission of information on some remote areas such as small islands beyond media coverage.

Multivariate latent generalized linear models

The linear regression model seeks to establish a linear relationship (Ha *et al.,* 2002) between the response variable and one or more covariates (Crawley, 2012) while the Generalized Linear Model (GLM) is a natural generalization of the Linear Regression model (Jamilatuzzahro *et al.,* 2018). This further allows linking response variables to one or more covariates via the link function (Jamilatuzzahro *et al*., 2019; Rahman *et al*., 2019) in order to explore the distribution and density of the variables. This study used $y_1,\ldots,y_n$ to represent a sequence of independent random variables (Noh *et al*., 2019; Lee *et al.,* 2012; Lee *et al.,* 2001) which were identically distributed by law to belong to the exponential family. The density was compared to the Lebesgue or a counting measure using Eq. 1.

$$f\left(y_i;\theta_i,\phi\right)=\exp\frac{y_i\theta_i-b\left(\theta_i\right)}{a\left(\phi\right)}+c\left(y_i;\phi\right) \tag{1}$$

Where, θ_i is the parameter position and ϕ is the dispersion parameter. Moreover, the expectancy and variance of *Y* are provided by $\text{Å}(Y)=b'(\theta)$ and $\text{Var}(Y)=b''(\theta)a(\theta)$, respectively. The latent variable model produces a significant instrument to investigate multivariate information, especially in ecology modeling by offering an applied structure to bring numerous divergent strategies together and serve as a base to create new techniques. Latent models are able to determine the joint dispersion of several arbitrary factors and later change to a latent variable model with a portion of these factors. Herliansyah *et al*. (2018) used these models to measure the diversity of bird species while Caraka *et al.,* (2018) used the negative binomial to determine the diversity in arthropods species counts. Moreover, Rahman *et al*., (2019) analyzed the diversity of Banteng and *Bos javanicus* while Caraka *et al.,* (2020a) used Butterfly diversity on species distribution. Anggraini *et al.,* (2020) also applied a latent factor linear mixed model to Flanders' data. It is, therefore, possible to use the dimension reduction in factor analysis to construct the model with latent variables of $x_1, x_2,\ldots,x_q$ with conditional distribution $g_i(x_i|y),(i=1,2,\ldots,p)$ (Niku *et al.,* 2019a; Niku *et al.,* 2017; Niku *et al.,* 2019b). This equation can be written as shown in Eqs. 2 and 3 (Bartholomew *et al.,* 2011).

$$g_i(x_i|y)=F_i\left(x_i\right)G_i\left(y\right)\exp\sum_{j=1}^{q}u_{ij}\left(x_i\right)\phi_j\left(y\right) \tag{2}$$

$$h\left(y|x\right)=\frac{h\left(y\right)\prod_{i=1}^{p}[F_i\left(x_i\right)G_i\left(y\right)\exp\sum_{j=1}^{q}X_j\phi_j\left(y\right).}{\int h\left(y\right)\prod_{i=1}^{p}[F_i\left(x_i\right)G_i\left(y\right)\exp\sum_{j=1}^{q}X_j\phi_j\left(y\right)dy.} \tag{3}$$

The optimization methods used in these models are Laplace (Huber *et al.,* 2004) and Variational approximations (Caraka *et al.,* 2020b). Laplace is a type of multidimensional integral approximation using Eq. 4.

$$\int_{\mathbb{R}^d} b\left(x\right)\exp-\lambda h\left(x\right)dx \tag{4}$$

Where, λ is a real parameter such that $\lambda>1$ while *h* is supposed to be an overall minimum in *x* which is regular in a neighborhood of *h* to ensure $h'\left(x\right)=0;\det[h''\left(\hat{x}\right)]>0$ and verify the following for all δ> 0. The Laplace method, while considering the integral (1) as the integral of *b,* shows the Gaussian measure for the small variance of order $\frac{1}{\lambda}$. The $h(x)$ in the integrand using the Taylor development (Herliansyah *et al*., 2018; Caraka *et al.,* 2020b) in the order is presented in Eq. 5.

$$\begin{aligned}
&\text{Inf}\left\{h\left(x\right)-h\left(\hat{x}\right):\left|x-\hat{x}\right|>\delta\right\}>0\\
&h\left(x\right)\approx h\left(\hat{x}\right)+\frac{1}{2}\left(x-\hat{x}\right)^T h''\left(\hat{x}\right)\left(x-\hat{x}\right)\\
&\int_{\mathbb{R}^d} b\left(x\right)\exp-\lambda h\left(x\right)dx\approx\int_{\mathbb{R}^d}\\
&b\left(x\right)\exp-\frac{\lambda}{2}\left(x-\hat{x}\right)^T h''\left(\hat{x}\right)\left(x-\hat{x}\right)dx
\end{aligned} \tag{5}$$

The estimation through Laplace shows each of the parameters is simultaneous (Kristensen *et al.,* 2016; Bianconcini *et al.,* 2012) as expressed in Eq. 6.

$$L=\sum_{l=1}^{n}\log f\left(y_l\right)=\sum_{l=1}^{n}\log\int_{\mathbb{R}^d} g(y_l\mid x_l)h\left(z_l\right)dz_l \tag{6}$$

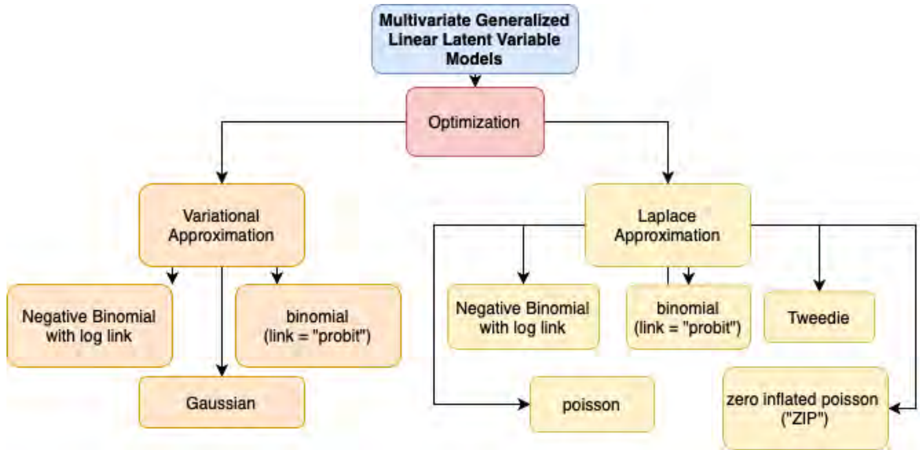


Fig. 1: Type of Optimization and Distribution Applied in Multivariate Generalized Linear Latent Variable Model

Variational approximation, however, shows that a vector $d \ll m$ underlying latent variables, u_{ij} and the parameter vector Ψ assumes responses y_{ij} are obtained from the exponential family of distributions using Eq. 7 (Hui *et al.*, 2017).

$$\ell(\Psi) = \sum_{i=1}^{n} \log\{ fy_i, \Psi\} = \sum_{i=1}^{n} \log\left(\int \prod_{j=1}^{m} f\left(y_{ij}|u_i, \Psi\right) f\left(u_i\right) du_i \right) \tag{7}$$

The distribution selection method used in this model was Kolmogorov-Smirnov (Fasano *et al.*, 1987). Meanwhile, the two optimization methods have different advantages and disadvantages based on time performance and distribution. More specifically, it is impossible to use Tweedie distribution in Laplace approximation but it is applicable in Variational approximation even though it requires extended processing time. Moreover, the distributional choice of latent variables, u_i, used in several research papers is a normal distribution with mean zero and constant variance. The types of optimization and distribution used in the multivariate generalized linear latent variable model are, however, shown in Fig. 1.

The distribution functions used for response options include Poisson (link = "log"), "negative. binomial" (with log link), binomial [(link = "probit") and (link="logit") with "LA"], zero-inflated Poisson ("ZIP"), gaussian (link = "identity"), Tweedie ("Tweedie") (with log link only with "LA"), and "ordinal" (only with "VA"). The exponential dispersion in the model was addressed mostly through the distribution of both family of two linear exponential parameters using either dispersion parameter as indicated in Eq. 8.

$$p(y|\theta,\phi) = \alpha(y,\phi)\exp\left(\frac{y\theta - z(\theta)}{\phi}\right) \tag{8}$$

The important information contained in the Tweedie model include normal (*p* = 0), Poisson (*p* = 1), gamma (*p* = 2), and gaussian inverse (*p* = 3). The Tweedie Distribution is principally an exponential family used in dispersing parameters with a variety of functions $V(y) = \mu^p$. Meanwhile, the zero-inflated Poisson is a mixed model between the distribution of Poisson and of events which is excess zero. Eq. 9 explains the random variable Y following ZIP with zero values assumed to be occurring through two scenarios. The first is the probability of π_i which produces zero observations while the second is the probability of $(1-\pi_i)$ which generates data following Poisson(λ).

$$P(y_i|\pi_i,\lambda_i) = \begin{cases} \pi_i + (1-\pi_i)\exp(-\lambda_i), y_i = 0 \\ (1-\pi_i)\dfrac{\exp(-\lambda_i)\lambda_i^{y_i}}{y_i!}, y_i = 1,2,\ldots; 0 \le \pi_i \le 1 \end{cases} \tag{9}$$

Where, *Y* is a Poisson random variable depending on parameters λ which is the value of a random variable Λ and follows Gamma to form a Poisson-Gamma mixture distribution called Negative Binomial as indicated in Eq. 10.

$$P(y|\lambda,\alpha) = \frac{\Gamma(y+\alpha^{-1})}{y!\Gamma(\alpha^{-1})}\left(\frac{\alpha}{1+\alpha\lambda}\right)^{y}\left(\frac{1}{1+\alpha\lambda}\right)^{\alpha^{-1}} \tag{10}$$

Performance Evaluation

Akaike Information Criterion (AIC) in Eq. 11 and Bayesian Information Criterion (BIC) in Eq. 12 have been used in numerous down applications to determine a model or variable (Warton, 2005; Kuha, 2004; Caraka *et al*., 2020c). The model determination criteria are factual devices with the ability to recognize an ideal measurable model from several others with the set typically called a lot of up-and-comer models. Moreover, under normality, thickness capacity strives towards keeping up the properties of $\hat{\theta}$ by assuming the density function of the exact model $g(y,\theta_0)$ belongs to $\mathcal{F}$, and where k is the element of the parameter vector ϑ. There are, however, a few expansions of AIC, such as the situation for a small dataset where the Akaike Information Criterion Corrected (AICc) ought to be progressively pertinent with the punishment term rectified to $2k\left[\frac{n}{(n-k-1)}\right]$.

$$AIC = -2\ln f(y,\hat{\theta}) + 2k \tag{11}$$

The Bayesian Information Criterion (BIC) can also be defined using Eq. 12.

$$BIC = -2\ln f(y,\hat{\theta}) + k\ln(n) \tag{12}$$

BIC is an assessment foundation for models which use the most extreme probability strategy and based on the condition that the example size n is adequately enormous.

RESULTS AND DISCUSSION

Species counts

Environmental condition is a focal component for the biological specialty of animal types and, by augmentation, its nature. This means species living space connections are one of the premises to explain the high strandedness of marine animals. Information on strandedness in natural surroundings tends to be focused on some species with several theories devoted to their territorial inclinations. It has also been discovered that different categories of animals utilize different natural surroundings for several purposes mostly due to their movement which is associated with relocating, resting, or reproducing. This, therefore, shows their living space is usually defined by both physical and natural attributes which have also been observed to be changing with time. This study determined the varying habitat preferences based on some considered time scales. For the purpose of this study, habitat preference is defined as a positive association with specific environmental conditions to produce random distributions of animals. There was, however, no attempt to relate the observed habitats with associated activities due to the inaccessibility of the information. The preference for habitats depends on species traits, therefore, this research was expected to determine different levels of variability in the ocean preferred by different species based on their life histories. The information presented in Fig. 2 showed one event was recorded in 2015, 4 in 2016, 5 in 2017, 18 in 2018, and 33 events in 2019 while the highest occurrence, 22, was with *Delphinidae*. These data are also presented in Table 1, using the number of inventory or count of individuals to represent the actual stranded population due to the fact that the data were collated manually from media reports and later pre-processed, labeled, and classified based on the type according to the expert system.

Modeling of stranding events

The difference in the diversity of event counts was practically measured using multivariate latent generalized linear models with two optimization methods which are Laplace and Variational approximations. It is also possible to use the model through different distributions such as Tweedie, Negative Binomial (NB), Poisson, Zero-inflated Poisson (ZIP), and Gaussian. The modeling, however, showed the use of Poisson with the smallest AIC value is the appropriate distribution method for Laplace approximation while Gaussian is the best for Variational approximation.

Table 2 is used to represent the performance evaluation with the likelihood being a statistical tool to summarize data evidence of unknown parameters while the log-likelihood value stands for the statistical measures for the models. The summarily means a model with relatively high value is better. It is also possible to consider the Log-Likelihood to be lying between both -Inf and +Inf while the unmitigated appearance at either value does not have the ability to provide any information. Nevertheless, the smallest AIC Laplace approximation for this model was reached at 86.1433 after which the latent value obtained from the best model was used to create the spatial difference in event counts and spatial clusters. Fig. 3,

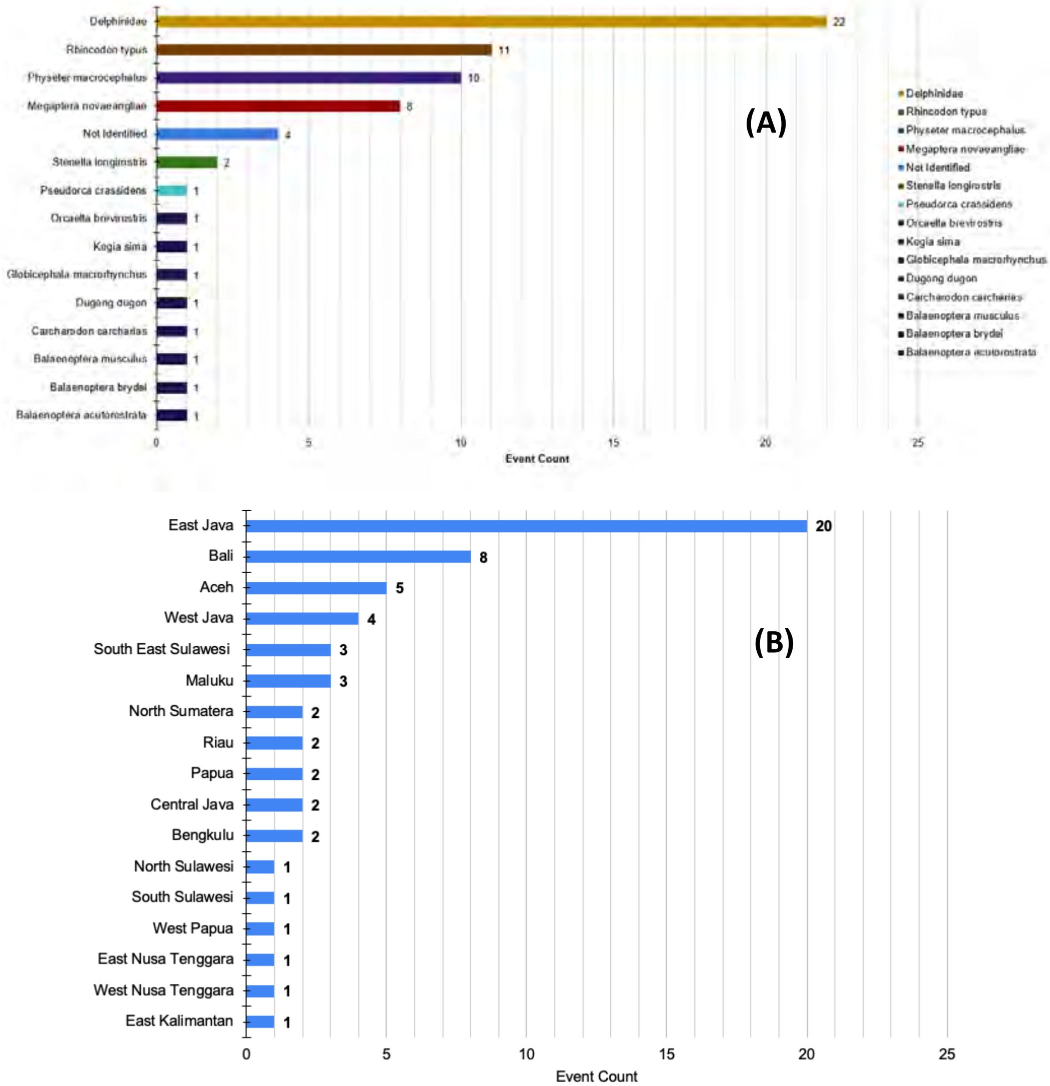


Fig. 2: count of stranded marine animals based on species (A) and Provinces (B)

however, presents the difference in event counts of stranded marine animals in Indonesia from 2015 to 2019 with the highest rate generally recorded around Java Province.

The intercept value models were, therefore, used as the information to create spatial clustering and, based on the three clusters indicated in Fig. 4, the differences between the stranded marine animals based on provinces were more apparent. Group 1 generally covers areas in Central Java, East Java, East Kalimantan, Maluku, Banten, West Kalimantan, North Sumatera, West Sulawesi, East Nusa Tenggara, West Papua, Riau, West Nusa Tenggara, and North Sulawesi. Group 2 covers Bengkulu, Papua, and South East Sulawesi while Group 3 covers the areas of Bali, West Java, and Aceh.

Reasoning

An ocean-atmosphere interaction analysis was conducted on the stranded marine phenomenon in East Java on 15 June 2016, Bali on 10 May 2019, and Bengkulu on 21 March 2018 using a dataset from BMKG Indonesia. Meanwhile, the stranded animal events are assumed to be influenced by climatological conditions and this means further study needs to

Table 1: Event counts stranded marine animals in Indonesia

Event	Year	Province	Beach	Latitude Longitude	*Type*	Size	N	Status
11/12/19	2018	Central Java	nn	-7.764416, 109.519016	*Delphinidae*	nn	1	Died
22/09/19	2019	Bali	nn	-8.634111, 115.293066	*Delphinidae*	nn	1	Died
20/09/19	2019	West Java	nn	-6.797359, 108.787466	*Rhincodon typus*	nn	1	Died
16/09/19	2019	East Java	Pantai Bambang	-8.290448, 113.105731	*Rhincodon typus*	5	1	Died
16/09/19	2019	East Kalimantan	Pantai Corong	-1.403559, 116.653588	*Pseudorca crassidens*	nn	1	Died
15/09/19	2019	East Java	Kepanjen	-8.388036, 113.382127	*Rhincodon typus*	8	1	Died
11/09/19	2019	East Java	PLTU Paiton	-7.711318, 113.583328	*Rhincodon typus*	5	1	Survived
11/09/19	2019	West Java	nn	-6.056518, 107.412216	*Delphinidae*	nn	1	Died
10/09/19	2019	East Java	Pantai Kajaran	-8.289643, 113.103304	*Rhincodon typus*	6	1	Died
02/09/19	2019	Bali	Pantai Serangan	-8.724652, 115.243014	*Kogia sima*	nn	1	Died
01/09/19	2019	East Java	Pantai Klatak	-8.269857, 111.769919	Delphinidae	nn	1	Survived
30/08/19	2019	Maluku	Liliboi	-3.741685, 128.033191	*Megaptera novaeangliae*	8	1	Died
28/08/19	2019	East Java	PLTU Paiton	-7.711318, 113.583328	*Rhincodon typus*	5	1	Survived
18/08/19	2019	West Java	nn	-6.081699, 107.425979	*Delphinidae*	nn	1	Died
16/08/19	2019	Banten	Binuangen	-6.839042, 105.900045	*Delphinidae*	nn	1	Died
04/08/19	2019	Bali	nn	-8.433401, 114.826593	*Stenella longirostris*	nn	1	Died
29/07/19	2019	Maluku	nn	-3.603043, 128.709608	*Megaptera novaeangliae*	nn	1	Died
23/07/19	2019	West Kalimantan	nn	0.421578, 108.942288	*Delphinidae*	nn	1	Died
22/07/19	2019	North Sumatera	nn	3.915655, 98.641087	*Delphinidae*	nn	1	Died
11/07/19	2019	East Java	Pantai Bambang	-8.290448, 113.105731	*Megaptera novaeangliae*	11	1	Died
11/07/19	2019	West Sulawesi	nn	-3.457637, 119.422586	*Delphinidae*	nn	1	Survived
09/07/19	2019	Bengkulu	Teluk Sepang	-3.904638, 102.280918	*Delphinidae*	nn	1	Died
26/06/19	2019	West Java	Pantai Cikadai	-7.190663, 106.443775	*Not Identified*	nn	1	Died
13/06/19	2019	East Nusa Tenggara	Pantai Doreng	-8.740595, 122.408641	*Delphinidae*	nn	1	Died
10/06/19	2019	Bali	Pantai Melaya	-8.289554, 114.498472	*Carcharodon carcharias*	1	1	Died
28/05/19	2019	West Papua	nn	-0.340917, 130.945255	*Delphinidae*	nn	1	Died
09/05/19	2019	Bali	Pantai Mengiat	-8.808089, 115.231902	Delphinidae	nn	1	Died
09/04/19	2019	Papua	nn	-4.777610, 136.542187	*Physeter macrocephalus*	nn	1	Died
09/03/19	2019	Aceh	Panga	4.549890, 95.694539	*Delphinidae*	nn	1	Died
09/03/19	2019	Aceh	nn	4.550072, 95.691889	*Stenella longirostris*	nn	1	Died
01/03/19	2019	South East Sulawesi	Pulau Bokori	-3.939953, 122.659604	*Not Identified*	nn	1	Died
05/02/19	2019	Bali	Kuta	-8.720257, 115.168991	Delphinidae	nn	1	Survived
04/02/19	2019	Papua	nn	-4.777610, 136.542187	*Balaenoptera brydei*	nn	1	Died

Continued Table 1: Event counts stranded marine animals in Indonesia

Event	Year	Province	Beach	Latitude Longitude	*Type*	Size	N	Status
27/01/19	2019	North Sumatera	Sungai Kualuh	2.611761, 100.095708	*Delphinidae*	nn	2	Survived
24/01/19	2019	East Java	Bancamara	-7.003523, 114.171978	*Rhincodon typus*	3	1	Died
14/01/19	2019	Maluku	Pulau Buru	-3.110435, 126.862872	*Balaenoptera musculus*	18	1	Died
09/01/19	2019	Aceh	nn	4.550906, 95.690956	*Delphinidae*	nn	1	Died
08/01/19	2019	East Java	nn	-8.329642, 112.221151	*Delphinidae*	nn	1	Died
19/11/18	2018	South East Sulawesi	nn	-5.326570, 123.465253	*Physeter macrocephalus*	9.5	1	Died
06/10/18	2018	East Java	Pantai Kajaran	-8.290518, 113.125963	*Megaptera novaeangliae*	15	1	Died
26/09/18	2018	Riau	nn	1.445870, 102.154649	*Orcaella brevirostris*	nn	1	Died
04/09/18	2018	Riau	nn	2.048109, 101.564324	*Dugong dugon*	nn	1	Died
15/07/18	2018	Aceh	Pantai Seragihan	2.210150, 98.067943	*Delphinidae*	nn	1	Died
17/06/18	2018	Aceh	nn	5.059513, 97.665810	*Megaptera novaeangliae*	nn	1	Died
06/06/18	2018	East Java	Randutatah	-7.700458, 113.482848	*Megaptera novaeangliae*	nn	1	Died
04/04/18	2018	East Java	Pantai Praureme k	-8.323072, 111.626680	*Rhincodon typus*	4	1	Died
29/03/18	2018	West Nusa Tenggara	Tabuan	-8.899194, 116.448733	*Physeter macrocephalus*	10	1	Died
21/03/18	2018	Bengkulu	nn	-4.702177, 103.266769	*Physeter macrocephalus*	12.5	1	Died
20/03/18	2018	Bali	nn	-8.074885, 115.138295	*Physeter macrocephalus*	15	1	Died
02/03/18	2018	East Java	nn	-7.712337, 114.182269	*Physeter macrocephalus*	17	1	Survived
01/03/18	2018	East Java	nn	-7.714664, 114.181058	*Physeter macrocephalus*	20	1	Died
01/02/18	2018	South East Sulawesi	nn	-4.801613, 121.637438	*Not Identified*	nn	1	Died
13/11/17	2017	Aceh	Ujung Kareung	5.652282, 95.423781	*Physeter macrocephalus*	nn	4	Died
13/11/17	2017	Aceh	Ujung Kareung	5.652282, 95.423781	*Physeter macrocephalus*	nn	6	Survived
30/10/17	2017	East Java	nn	-7.722134, 113.110528	*Megaptera novaeangliae*	nn	1	Died
27/09/17	2017	North Sulawesi	nn	1.117452, 124.339524	*Physeter macrocephalus*	11	1	Died
30/08/17	2017	Bali	Pantai Yeh Kuning	-8.400932, 114.659160	*Megaptera novaeangliae*	8	1	Died
15/08/17	2017	East Java	Pantai NgGenjor	-8.294141, 111.768709	*Not Identifiedi*	10	1	Died
26/12/16	2016	East Java	Pantai Sidem	-8.256870, 111.798004	*Rhincodon typus*	nn	1	Died
12/08/16	2016	Central Java	Pantai Kemiren	-7.697235, 109.057250	*Delphinidae*	nn	1	Died
15/06/16	2016	East Java	nn	-7.732271, 113.177689	*Globicephala macrorhynchus*	nn	15	Died
15/06/16	2016	East Java	nn	-7.732271, 113.177689	*Globicephala macrorhynchus*	nn	17	Survived
15/05/16	2016	East Java	Pantai Randupitu	-7.774278, 113.320638	*Physeter macrocephalus*	4	7	Died
26/12/15	2015	East Java	Pantai SIdem	-8.256870, 111.798004	*Rhincodon typus*	7.5	1	Died

Table 2: Accuracy based on the type of approximation and distribution

Approximation	Distribution	log-likelihood	Df	AIC	AICc	BIC
LA	Tweedie	-42.66616	12	109.3323	161.3323	120.6656
LA	Negative Binomial	-44.68026	12	113.3605	165.3605	124.6938
LA	Poisson	-45.06127	8	106.1225	120.5225	113.678
LA	ZIP	-45.06127	12	114.1225	166.1225	125.4558
LA	Gaussian Link Identity	-31.07117	12	86.14233	138.1423	97.4756
VA	Gaussian Link Identity	-40.57117	12	105.1423	157.1423	116.4756
VA	Negative Binomial	-44.68026	12	136.9484	188.9484	148.2817
VA	Poisson	-55.19088	8	126.3818	140.7818	133.9373

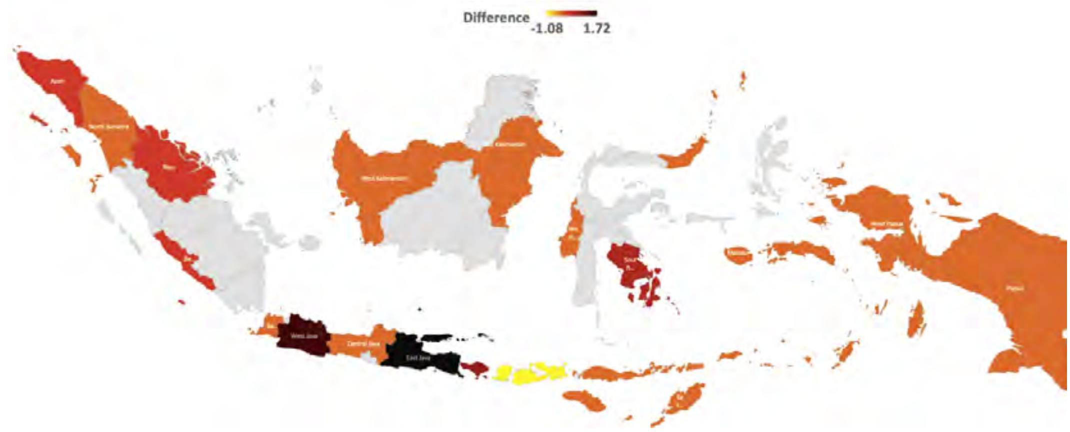


Fig. 3: Intercept models based on stranded marine animal's event counts

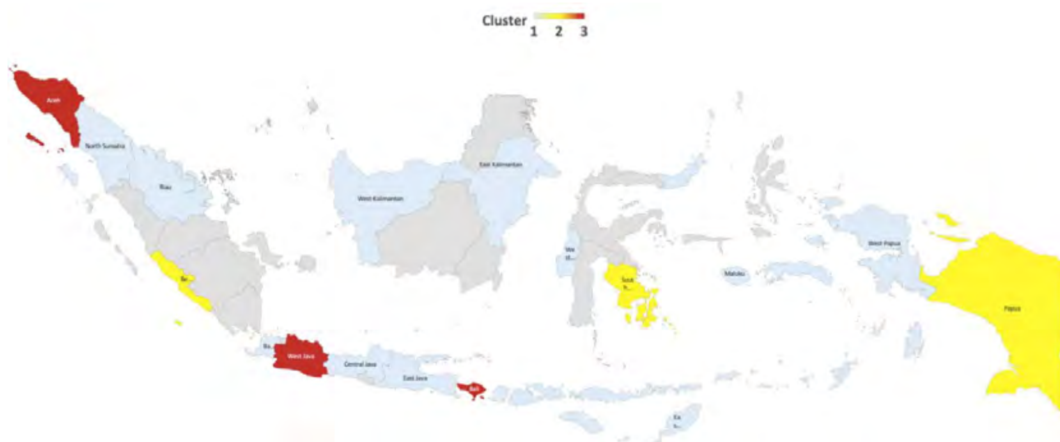


Fig. 4: Clustering based on intercept value stranded marine animal's event counts

be conducted on wind speed and direction at the location. This is important considering the fact that the changes in these factors have the ability to change the migration path of marine animals, thereby, providing opportunities for them to be stranded. Wind has been discovered to have an influence on ocean currents and waves with the wind-generated currents having varying speeds depending on their depth and, apart from its horizontal movement, wind also causes vertical water currents known as upwelling and down welling in certain areas. Upwelling is an oceanographic phenomenon which involves a solid, cold, and typically wind-driven motion which brings a nutrient-rich mass of water to the surface of the sea. Moreover, wind conditions also affect high and low waves occurring continuously at the sea level with the changes in its speed considered to be increasing sea waves and this consequently has the ability to moving water to the surface. The upwelling zone expands in the tidal sea region and this is usually followed by high wind speeds towards the land. This means an increase in the strength of ocean waves encourages mammals to search for food nutrients or fish flocks and they usually end up being drawn into coastal areas.

Bengkulu, 21 March 2018

The dominant wind recorded in Bengkulu region in March 2018 blew from South to Northwest and later in the night to Northeast with an average speed of 5 knots and a maximum speed of 24 knots and the weather was observed at this period to be dominated by rain from night to morning as well as fog and haze which were majorly experienced in the morning.

Madden–Julian oscillation (MJO) is observed to be getting stronger when it is outside the circle and in Phases 3, 4, and 5 which are its position in the Indonesian territory. The MJO in the first and second week of March 2018 is presented in Fig. 5 including phases 2 and 3 while the third week moves towards the Neutral phase and at the end of the month, it shifts from phase 6 to 7. This movement shows the MJO is quite significant and influences the growth of rain clouds in Western Indonesia especially Bengkulu in the first and second weeks after which it did not affect the increase in rainfall as indicated in its shift to neutral during the third to fourth week.

Bali, 10 May 2019

The wind in the Bali region is always not far from the western and eastern winds and this is observed from the very much influence of the winds from these areas on the region has indicated on the regional meteorological scale. It periodically blows every 6 months alternately from mainland Asia to Australia and vice versa through the concept of monsoon or western wind when it blows from the west or Asia and the east wind when blowing from the east or Australia. May is the final period of the first transition period from the rainy to the dry season which usually occurs in June, July, and August and the east wind has been observed to be very dominant at this period. Therefore, May is usually known as the end of the transition period into the dry season, especially when there are no disturbances such as El-Niño and La-Niña. MJO was, however, discovered to be non-active in this month because Bali is not in quadrants 4 and 5, therefore, a little rain is usually experienced, even for just two times, with the intensity not exceeding 1 mm per day.

East Java 15 June 2016

The MJO diagram is divided into 8 phases with phase 1 in Africa, phase 2 in the western Indian Ocean, phase 3 in the eastern Indian Ocean, phases 4 and 5 in Indonesia, phase 6 in the western Pacific region, phase 7 in the central Pacific, and phase 8 in

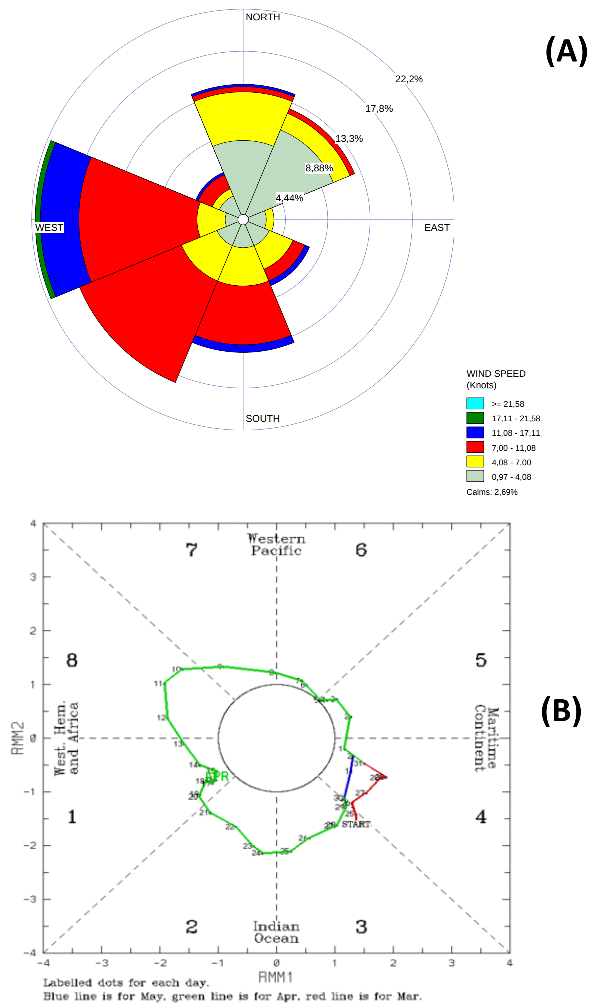


Fig. 5: Wind Speed (A) and MJO (B) in Bengkulu, Indonesia

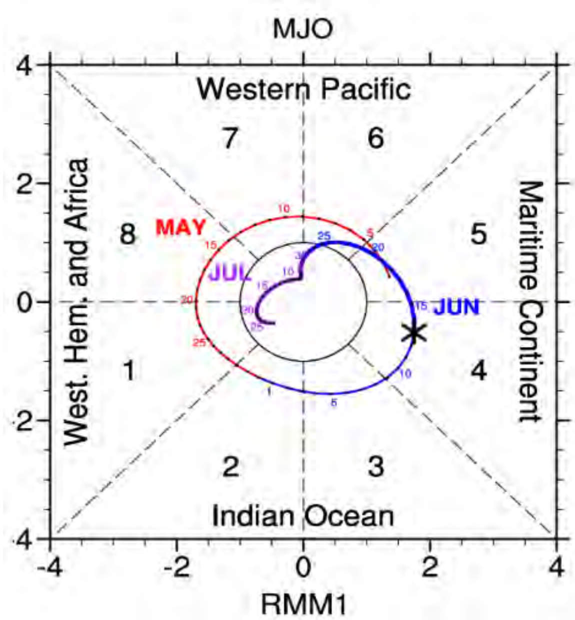


Fig. 6: Madden–Julian oscillation Bali, Indonesia

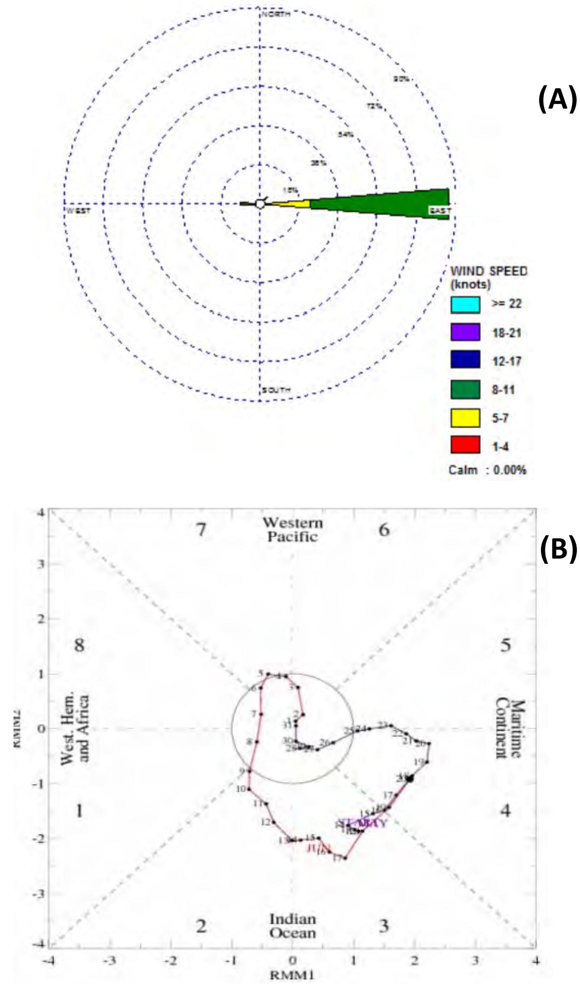


Fig. 7: Wind Speed (A) and MJO (B) in East Java, Indonesia

the convection regions of the Western Hemisphere. Fig. 6 represents the placement of the track in the small circle at the middle shows the MJO is in a weak condition while an outside placement shows it is strong or active. Fig. 7 shows MJO started to be strengthened in the West Indian Ocean of Sumatra on June 14, 2016, and observed to be entering the Sumatra and Java on June 19-20, 2016, thereby, being one of the causes of high rainfall. Another cause is the high sea surface temperature anomaly in the Madura Strait compared to other waters. In June 2016, most of the wind conditions, 87%, were from the East with an average speed of 8-11 knots while the Northeast direction has 4% with an average wind speed of 5-7 knots as shown in the Wind rose diagram.

CONCLUSION

The most frequently stranded whale mammals are those living in the deep sea and the location for the strandedness is usually very shallow areas. This is not surprising considering the fact that these animals are accustomed to swimming in the deep sea and find it difficult to return due to the less effectiveness of the echolocation capabilities they use in navigating when they are in such environments. This means it is possible the majority of whales are stranded due to navigation errors, for example, when they hunt prey to remote and dangerous areas. It was also found that there are cases the marine animals are dragged by the tide and this mostly leaves them stranded on the coast, thereby, becoming dehydrated and dead. Moreover, one or two whales were also discovered to have gotten lost and become stranded in the middle of the road while some are attached to the changes in the Earth's magnetic field caused by sunspots and high-level radio waves emitted by solar storms. There is also a large part of the radiofrequency (RF) waves range reaching the Earth and its noise has been found to be interfering with the magnetic orientation of several species. Some stranded marine mammals were also discovered after dissection to have a large lump of marine debris in their stomach containing several ropes, plastic cups, and plastic bags and this indicates the relationship between the type of food and eating behavior of whale sharks and their appearance in certain locations. This is also observed in their appearance in groups due to the abundance of planktons floating freely in waters and they are also found to be centered when they feed in the same area. Those discovered to have stranded and entangled in the South Coast and Banggai Islands were reported to be allegedly due to the existence of food sources in both waters and this was supported by the lack of differences in the appearances of both male and female whale sharks found at each of the study locations during the study period of whale shark behavior which tended to be the same between males and females. The only variation observed was the number of occurrences with the male having a higher appearance compared to the female due to the significant differences in the number of individuals. Furthermore, the wounds found on the fins, body, and mouth area are possibly due to the friction with the net of the chart or entangled fishing line of fishermen. Mass tourism activities, fishing, as well as ship collisions in Indonesian waters also have the ability to cause injuries to these marine mammals as observed in the injuries on the bodies of several whale sharks in the waters. Meanwhile, those reported to be stranded in West Sumatra have no known exact cause while the entangled fishing nets used in the Banggai Islands are observed to pose a serious threat to the population of the fishes. Therefore, it is important to apply Marine Ranching in different regions, reorganize the marine ecosystem to restore its original state, and develop the newly formed ecosystem to benefit fishermen. This is achievable by the creation of floating fish shelters and by placing blocks to protect the biota and the sea dam.

AUTHOR CONTRIBUTIONS

R.E. Caraka leads this study and has reviewed related kinds of literature, designed and developed the concept of all analysis prepared, writing, and edited the manuscript text. R.C. Chen performed the supervision and provide the study grant. Y. Lee performed the supervised the project and helped to provide the study grant. T. Toharudin performed the supervision and provides the research grant. C. Rahmadi provided and curated the dataset stranded marine animals and edited the manuscript. M. Tahmid provided and curated the climate dataset and edited the manuscript. A.S. Achmadi provided the dataset stranded marine animals and edited the manuscript.

ACKNOWLEDGMENTS

This study is fully supported by the National Research Foundation of Korea grants [NRF-2019R1A2C1002408] and the Ministry of Science and Technology (MOST) under Grant [107-2221-E-324-018-MY2] and [106-2218-E-324-002]. The research is a part of the Chaoyang University of Technology and the Higher Education Sprout Project, Ministry of Education (MOE), Taiwan, under the project name: “The R&D and the cultivation of talent for health-enhancement products”. Therefore, it was conducted in Lab Hierarchical Generalized Linear Model (H-GLM), Department of Statistics, College of Natural Sciences Seoul National University with data and information retrieved from the preliminary research conducted by Research Center for Biology, Indonesian Institute of Sciences (LIPI) on stranded marine animals in East Java and Indonesia in 2019. The CR and ASA appreciate Anang Setyo Budi and Apandi (RC Biology LIPI) for assisting during the fieldwork in Lumajang (East Java) and for visiting the stranded *Rhincodon typus* in 2019.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest regarding the publication of this work. In addition, the ethical issues including plagiarism, informed consent, misconduct, data fabrication and, or falsification, double publication and, or submission, and redundancy have been completely witnessed by the authors.

ABBREVIATIONS

AIC	Akaike information criterion
AICc	Akaike Information Criterion corrected
BIC	Bayesian Information Criterion
BMKG	Meteorology, Climatology, and Geophysical Agency is an Indonesian non-departmental government agency for meteorology, climatology, and geophysics.
cm	centimeter
df	Degree of Freedom
Eq.	Equation
Fig.	Figure

GLM	generalized linear model
Laplace approximation (LA)	Approximating Bayesian parameter estimation and Bayesian model comparison
m	meter
MGLLVM	Multivariate generalized linear latent variable models
MJO	Madden–Julian oscillation
mm	millimeter
NB	Negative Binomial
nn	Nomen nescio
Variational approximation (VA)	Techniques for making approximate inference for parameters in complex statistical models
$y_1,...y_n$	sequence of independent random variables
ZIP	Zero Inflated Poisson
Var (Y)	Variance of *Y*
E (Y)	Expectancy of *Y*
ϕ	dispersion parameter

REFERENCES

Abreo, N.A.S.; Blatchley, D.; Superio, M. D., (2019). Stranded whale shark (Rhincodon typus) reveals vulnerability of filter-feeding elasmobranchs to marine litter in the Philippines. Mar. Pollut. Bull., 141: 79–83 **(5 pages).**

Anggraini, Y.; Notodiputro,K.A.; Saefuddin,A.; Toharudin,T., (2020). Latent factor linear mixed model (LFLMM) for modelling Flanders. J. Comm. Math. Biol. Neuro., 2020: 1–14 (**14 pages**).

Bartholomew, D.; Knott, M.; Moustaki, I., (2011). Latent variable models and factor analysis: A Unified Approach: 3rd Edition, Latent Variable Models and Factor Analysis: A Unified Approach: 3rd Edition **(294 pages).**

Bianconcini, S.; Cagnone, S., (2012). Estimation of generalized linear latent variable models via fully exponential Laplace approximation. J. Multivariate Anal., 112: 183–193 **(11 pages).**

Caraka, R.E.; Shohaimi S.; Kurniawan, I.D.; Herliansyah, R.; Budiarto, A.; Sari, S.P.; Pardamean, B., (2018). Ecological Show Cave and Wild Cave: Negative Binomial Gllvm's Arthropod Community Modelling. Procedia Comput. Sci., 135: 377–384 **(8 pages).**

Caraka, R. E.; Chen, R.C.; Lee, Y.; Toharudin, T.; Kurniawan, I.D.; Asmawati.S.; Ropawandi, D.; Akbar, M.F.R.; Indra; Rustan, M.F., (2020a). Latent GLM Tweedie Distribution in Butterflies Species Counts. Ad. Int. Syst. Res., 172: 306-314 (**9 pages**).

Caraka, R. E.; Chen, R.C.; Lee, Y.; Noh, M.; Toharudin, T.; Pardamean,B.; Saputra,A., (2020b). Variational approximation multivariate generalized linear latent variable model in diversity termites. Sylwan., 164(1): 161–177 (**16 pages**).

Caraka, R.E.; Nugroho, N.T.; Tai, S.K.; Chen, R.C.; Toharudin, T.; Pardamean, B., (2020c). Feature importance of the aortic anatomy on endovascular aneurysm repair (EVAR) using Boruta and Bayesian MCMC. J. Comm. Math. Biol. Neuro., 2020:1–23 (**23 pages**).

Chan, D.K.P.; Tsui, H.C.L.; Kot, B.C.W., (2017). Database documentation of marine mammal stranding and mortality: Current status review and future prospects. Disease of AQ Organisms (**10 pages**).

Colman, J. G., (1997). A review of the biology and ecology of the whale shark. J. Fish Biol., **(15 pages**).

Crawley, M.J., (2012). Regression chapter 10. The R Book., 449–497 (**1051 pages**).

Demos, T. C.; Achmadi, A.S.; Giarla, T.C.; Handika,H.; Mahardatunkamsi .; Rowe, K.C .; Esselsstyn J.A., (2016). Local endemism and within-island diversification of shrews illustrate the importance of speciation in building Sundaland mammal diversity. Molecular Ecol., 25(20): 5158–5173 (**16 pages**).

Duignan, P.J., (2003). Disease investigations in stranded marine mammals 1999–2002., DOC Science Internal Series (**32 pages**).

Fasano, G.: Franceschini, A., (1987). A multidimensional version of the Kolmogorov-Smirnov. Month Not of the Roy. Astr. Soc., 225(1): 155-170 (**16 pages**).

Ha, I.D.; Lee, Y.; Song, J.K., (2002). Hierarchical-likelihood approach for mixed linear models with censored data. Lifetime Data Anal., 2002(8): 163–176 (**13 pages**).

Herliansyah, R.; Fitia, I., (2018). Latent variable models for multi-species counts modeling in ecology, Biodi J. Biol. Div., 19(5): 1871–1876 (**6 pages**).

Huber, P.; Ronchetti, E.; Victoria-Feser, M.P., (2004). Estimation of generalized linear latent variable models. J. Royal Stat Soc. Series B: Statistic. Method. 66(4): 893-908 (**16 pages**).

Hui, F.K.C.; Wartodm, D.I.; Ormerod, J.T.; Haapaniemi, V.; Taskinen,S., (2017). Variational approximations for generalized linear latent variable model. J. Comput. Graph. Stat., 26(1): 35-43 **(9 pages).**

Jamilatuzzahro; Caraka, R.E.; Herliansyah, R., (2018).Aplikasi generalized linear model dengan R. 1st ed., Yogyakarta: INNOSAIN GI Yogyakarta (**104 pages**).

Jamilatuzzahro; Caraka, R.E.; Aprinaldy, D.; Mahadi, A., (2019). Generalized linear model multivariate poisson with artificial marginal (GLM-MPAM): Application of vehicle insurance. AIP Conference Proceedings., 2111 (020013):1-7 (**7 pages**).

Kristensen, K.; Nielsen, A.; Berg, C.W.; Skaug, H.; Bell, B.M., (2016). TMB: Automatic differentiation and laplace approximation. J. Stat. Soft., 70: 1–21 (**21 pages**).

Kuha, J., (2004). AIC and BIC: Comparisons of assumptions and performance. Soc. Meth. Res., 33(2): 188-229 (**31 pages)**

Kurniawan, I.D.; Rahmadi, C.; Caraka, R.E.; Ardi, T.E., (2018). Short communication: Cave-dwelling arthropod community of semedi show cave in gunungsewu karst area, Pacitan, East Java, Indonesia. Biodiversitas., 19(3): 857–866 (**10 pages**).

Kurniawan, I.D.; Soesilhadi, H. R.; Rahmadi,C.; Caraka, R.E.; Pardamean,B., (2018). The difference on arthropod communities structure within show caves and wild caves in Gunungsewu karst area, Indonesia. Int. J. Ecol. Environ. Conserv., 24(1): 81–90 **(10 pages).**

Lee, Y.; Nelder, J., (2001). Modelling and analyzing correlated non-normal data. J.Statistical Modeling An Int. J., 1(1): 3–16 (**14 pages**).

Lee, Y.; Noh, M. (2012). Modelling random effect variance with double hierarchical generalized linear models. J. Statistic.

Modeling. An Int. J., 12(6): 487–502 (**16 pages**).

Lomba, A.; Pellissier, L.; Randin, C.; Vicente, J.; Moreira, F.; Honrado, J.; Guisan, A., (2010). Overcoming the rare species modelling paradox: A novel hierarchical framework applied to an Iberian endemic plant. J. Biol. Conserv., 143(11): 2647–2657 (**11 pages**).

Lusher, A. L.; Milian, G.H.; Berrow, S.; Rogan, E.; O'Connor, I., (2018). Incidence of marine debris in cetaceans stranded and bycaught in Ireland: Recent findings and a review of historical knowledge. J. Environ. Pollut., 232: 467–476 (**10 pages**).

Maryanto, I.; Achmadi, A.S.; Kartono, A.P., (2008). Mamalia dilindungi perundang-undangan Indonesia. LIPI Press (**254 pages**).

Mouillot, D.; Bellwood, D.R.; Baraloto, C.; Chave,J.; Galzin, R.; Harmelin-Vivien, M.; Kulbicki, M.; Lavergne, S.; Lavorel, S.; Mouquet, N.; Paine, N.M.C.; Renaud, J.; Thuiller, W., (2013). Rare Species Support Vulnerable Functions in High-Diversity Ecosystems. PLoS One. 11(5): 1–11 (**11 pages**).

Niku, J.; Warton, D.I.; Hui, F.K.C.; Taskinen, S., (2017). Generalized Linear Latent Variable Models for Multivariate Count and Biomass Data in Ecology. J. Agric. Biol. Environ. Stat., 22(4): 498–522 (**25 pages).**

Niku, J.; Brooks, W.; Herliansyah, R.; Hui, F.K.C.; Taskinen, S.; Warton, D.I., (2019a). Efficient estimation of generalized linear latent variable models. PLoS ONE., 14(5): 1–20 (**20 pages**).

Niku, J.; Hui, F.K.C., (2019b). Gllvm: Fast analysis of multivariate abundance data with generalized linear latent variable models. J. Meth. Ecol. Evol., 1–10 (**10 pages**).

Noh, M.; Lee, Y.; Oud, J.H.L.; Toharudin, T., (2019). Hierarchical likelihood approach to non-Gaussian factor analysis. J. Stat. Comput. Simulation. 89(3): 1555–1573 **(19 pages).**

Norman, B., (2002). CITES Identification Manual Whale Shark (Rhincodon typus Smith 1829) (**18 pages**).

Rahman, D. A.; Herliansyah, R.; Rianti, P.; Rahmat, U.M.; Firdaus, A.Y.; Syamsudin, M., (2019). Ecology and conservation of the endangered banteng (Bos javanicus) in Indonesia Tropical Lowland Forest. Hayati J. Biol.., 26(2): 68–80 (**13 pages**).

Rudolph, P.; Smeenk, C., (2009). Indo-West Pacific marine mammals. In Encyclopedia of Marine Mammals. Academic Press. 608–616 (**9 pages**).

Stevens, J.D., (2007). Whale shark (Rhincodon typus) biology and ecology: A review of the primary literature. Fish. Res., (**6 pages**).

Verfuss, U.K., (2018). Comparing methods suitable for monitoring marine mammals in low visibility conditions during seismic surveys. J. Marine Pollut. Bull., 26: 1–18 (**18 pages**).

Warton, D.I., (2005). Many zeros does not mean zero inflation: Comparing the goodness-of-fit of parametric models to multivariate abundance data. Environmetrics. 16(3): 275–289 **(15 pages).**

Wibowo, F.; Dharmadi, K., (2014). Stranding of Marine Megafauna in Indonesian Waters: Causes of the Phenomenon and Handling Methods (Seastar2000). In Design Symposium on Conservation of Ecosystem. The 13th SEASTAR2000 workshop. 19–25 **(7 pages).**

AUTHOR (S) BIOSKETCHES

Caraka, R.E., Ph.D. Candidate, Department of Information Management, College of Informatics, Chaoyang University of Technology, Taiwan and Postdoctoral Researcher, Department of Statistics, College of Natural Sciences, Seoul National University, Shin Lim-Dong, Kwan Ak Ku, South Korea. Email: *rezzyekocaraka@gmail.com*

Chen, R.C., Ph.D., Distinguished Professor, Department of Information Management, College of Informatics, Chaoyang University of Technology, Taiwan. Email: *crching@cyut.edu.tw*

Lee, Y., Ph.D., Professor, Department of Statistics, College of Natural Sciences, Seoul National University, Shin Lim-Dong, Kwan Ak Ku, South Korea. Email: youngjo@snu.ac.kr

Toharudin, T., Ph.D., Associate Professor, Department of Statistics, Padjadjaran University, West Java, Indonesia. Email: *toni.toharudin@unpad.ac.id*

Rahmadi, C., Ph.D., Researcher, Museum Zoologicum Bogoriense, Research Center for Biology, Indonesian Institute of Sciences, Indonesia. Email: *cahyo.rahmadi@lipi.go.id*

Tahmid, M., S.T., Researcher, Indonesian Agency for Meteorological, Climatological and Geophysics, BMKG, Manado, Indonesia. Email: *muhammad.tahmid@bmkg.go.id*

Achmadi, A.S., Ph.D., Researcher, Museum Zoologicum Bogoriense, Research Center for Biology, Indonesian Institute of Sciences, Indonesia. Email: *anang.setiawan.achmadi@lipi.go.id*

HOW TO CITE THIS ARTICLE

Caraka, R.E.; Chen, R.C.; Lee, Y.; Toharudin, T.; Rahmadi, C.; Tahmid, M.; Achmadi, A.S., (2021). The use of multivariate generalized linear latent variable models to measure the difference in event count for stranded marine animals. Global J. Environ. Sci. Manage., 7(1): 117-130.

DOI: 10.22034/gjesm.2021.01.09

url: https://www.gjesm.net/article_44366.html

Global Journal of Environmental Science and Management
(GJESM)

Homepage: https://www.gjesm.net/

REVIEW PAPER

Application of environmental bacteria as potential methods of azo dye degradation systems

G. Manjarrez Paba[1,2,*], R. Baldiris Ávila[1], D. Baena Baldiris[1]

[1]*Clinical and Environmental Microbiology Group, University of Cartagena, Faculty of Natural and Exact Sciences, Cartagena, Colombia*

[2]*GIA Environmental Research Group, Faculty of Engineering, Fundacion Universitaria Tecnologico Comfenalco, Cartagena, Colombia*

ARTICLE INFO

Article History:
Received 17 March 2020
Revised 20 June 2020
Accepted 08 July 2020

Keywords:
Acinetobacter
Azo dyes
Effluents
Enterococcus
Marine bacteria
Water treatment

ABSTRACT

BACKGROUND AND OBJECTIVES: The objective of this study is to present a description of the main characteristics of azo dyes and the different treatment methods used to remove them from water. There is a special emphasis given to the benefits associated with biological treatment, predominantly those related to the use of bacteria, which has to do with its competitive advantages over other microorganisms in the dye degradation processes.
METHODS: The topic to be addressed was first defined through workshops with the research group. The literature review was carried out following several inclusion/exclusion criteria: the year of publication, as the selection was limited to studies published between 2010 and 2020, the focus of the investigation, which had to be related to the efficiency of different techniques for the remediation of ecosystems contaminated with azo dyes and, lastly, that the studies also discussed the use of environmental bacteria in dye degradation processes.
FINDINGS: The efficiency of bacteria to degrade azo dyes ranges from 63-100%, the most efficient being: *Marinobacter* sp, *Sphingobacterium* sp, *Enterococcus faecalis, Enterococcus casseliflavus*. The bacteria that, reportedly, have greater efficiency for simultaneously removing the dye-metal complex are *Bacillus circulans* and *Acinetobacter junii*.
CONCLUSION: Traditional strategies for the treatment of effluents contaminated with azo dyes are limited to physical and chemical processes that have a high energy and economic cost. For these reasons, current challenges are focused on the use of environmental bacteria capable of transforming dyes into less toxic compounds.

DOI: 10.22034/gjesm.2021.01.10

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
162	1	9

*Corresponding Author:
Email: *gmanjarrezp@unicartagena.edu.co*
Phone: +573185604660
Fax: +57 (5) 6600380

Note: Discussion period for this manuscript open until April 1, 2021 on GJESM website at the "Show Article.

INTRODUCTION

Annually more than a million tons of synthetic dyes are produced around the world for use in the leather, textile, pharmaceutical, food, cosmetic, paint, plastic and paper industries (Shamraiz *et al.*, 2016), of which, at least 60% are azo dyes (Shah, 2014; Gürses *et al*., 2016). In addition to being recalcitrant towards various degradation processes (Singh *et al.*, 2014; Singh *et al*., 2015), azo dyes produce dangerous chemical substances such as aromatic amines, known for their toxic, allergenic, carcinogenic and mutagenic effect on living organisms (Das *et al*., 2015). The impact of azo dyes on the environment is proportional to the enormous amounts of hazardous waste associated with industrial processes, which is then released to water bodies, in most cases, without proper treatment. A further aggravating factor is that due to the inability of at least 35% of azo dyes to adhere to substrates, heavy metals have been incorporated during the dyeing process; these act as mordants, favoring the fixation of the dye (Vuthiganond *et al.*, 2018). Colorants associated with metals such as copper, cobalt and especially chromium, are difficult to degrade and represent an important source of environmental contamination considering their increased presence in organic load. They generate adverse and irreversible eco-toxicological effects, bioaccumulation phenomena and biomagnification in flora and aquatic fauna and alteration of biogeochemical cycles (Lončar *et al.*, 2014; Kurade *et al.,* 2016). This powerful metal-dye complex has carcinogenic and mutagenic properties for humans exposed to effluents contaminated with dyes. It can lead to: skin cancer (due to photosensitization), photodynamic damage, allergic contact dermatitis, renal, reproductive, hepatic, cerebral dysfunction, irritation of the respiratory tract and asthma (Mondal *et al*., 2017; Khan and Malik, 2018). Traditionally, physicochemical methods have been used to treat effluents contaminated with azo dyes, but their high economic and energy cost and the environmental effects associated with their use have pushed technological development towards the use of microorganisms in recent years. These are successful biological alternatives due to their survival properties, adaptability, enzymatic activity and chemical structure. Additionally, hybrid technologies have been developed, which integrate various technologies into one, taking the best of each and surpassing the limitations of current conventional treatments. (Singh *et al*., 2015; Ribera, 2019). Recent reports have indicated that molecular techniques such as metagenomics and metaproteomics are being used to explore the molecular degradation mechanism of azo dyes. These technologies can be utilized for screening and identifying crucial genes, proteins and enzymes which will be essential for achieving a deep insight into the intrinsic biodegradation mechanism of dyes. (An *et al*., 2020; Zhang *et al*., 2019; Qu *et al*., 2018). These reports indicated that the application of environmental bacteria capable of degrading azo dyes should mainly focus on bioremediation, clean technologies, genetic engineering, nanotechnology and use of metagenomics and metaproteomics analysis (Fig. 1). The objective of this review is to describe a chemical classification of dyes and their structural characteristics. It presents a description of the main characteristics of azo dyes, the treatments used for their degradation and the potential of bacteria to become an optimal biological alternative in the treatment of effluents contaminated with azo dyes. The main focus of the review are biological treatments using marine bacteria. This is due to their ability to survive in aquatic environments under adverse environmental conditions, as well as their ability to develop multi-resistance mechanisms for antibiotics and heavy metals and the enzyme systems associated with the degradation of dyes. This review also presents the mechanisms of the bacteria-heavy metals interaction and, finally, the bacterial species which are capable of degrading individual and mixed dyes, as well as remove heavy metals and dyes simultaneously and, also, metal-complex dyes. This review is part of a doctoral thesis called: Determination of the capacity of environmental bacteria for the degradation of azo dyes, a study which was carried out at the University of Cartagena, in Cartagena, Colombia during 2019 – 2020.

Overview of azoic dyes

Dyes are substances of chemical or biological origin with the ability to bind to a substrate and impart color. They can be classified according to their chemical structure, color, application and particle charge in solution. Based on their chemical structure, they are classified as: azo dyes, nitro dyes, phthalein dyes, triphenyl methane dyes, indigoid dyes and anthraquinone dyes (Ngulube *et al*., 2017; Yagub *et* al., 2014). Whereas, based on their application, they are classified as: acid dyes, basic dyes, direct dyes, ingrain dyes, disperse dyes, moderate dyes, vat dyes and reactive dyes. In general, dye molecules have a delocalized electron conjugated double bond composed of the auxochrome and the chromophore groups. The chromophores give color to the dye after the excitation of electrons, while the auxochromes intensify the color imparted by the chromophore, conferring the adhesion and solubility properties of the dye (Wardman, 2017). Table 1 describes the classification of dyes according to their chromophore group.

Azo dyes are synthetic compounds widely used due to their brilliant color, ease of handling, usage and economic feasibility in synthesis when compared to other types of dyes. They can be differentiated according to the number of azo linkages (–N=N–) present in a molecule of the dye, such as monoazo, diazo, triazo, polyazo and azoic (Pavithra *et al*., 2019). Azo linkages can bind to benzene rings, naphthalenes, aromatic heterocycles, or essential aliphatic groups, which increases the complexity of the molecule. The binding of azo linkages to these chemical groups gives the molecule special properties such as photocatalytic stability and resistance to degradation (Shah *et al*., 2014; Benkhaya *et al*., 2016). Azo dyes can form complexes with metals called metal complexes, an important feature, exploited for a long time by the textile industry. This is due to the fact that this metal-dye complex increases performance, making them resistant to fading as a result of washing or exposure to sunlight. There are two types of metal complex azo dyes: the first, those in which the azo group is coordinated to the metal (medially metallized) and the second, those in which it is not (terminally metallized). The most important metal complexes are those formed from the reaction of transition metal ions with ligands. In ligands, the ortho positions adjacent to the azo group contain a group which is capable of coordinating with the metal ion. The metals which are used commercially the most in metal complexes are copper(II), cobalt (III) and chromium (III) (Ghosh *et al*., 2016). The synthesis of these coordination complexes of transition metals with azo ligands is due to the interesting physical, chemical, photophysical, photochemical and catalytic properties. Metal complex dyes play a very important role in the textile industry. Table 2 shows a

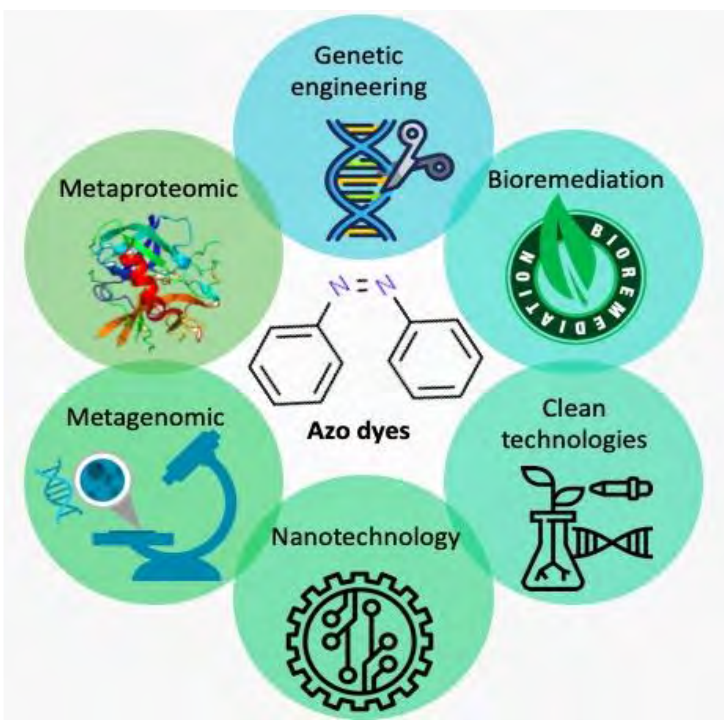


Fig. 1: Technologies for the removal of azo dyes

Table 1: Classification of dyes based on their chemical structure chromophore

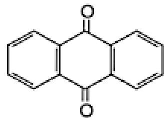
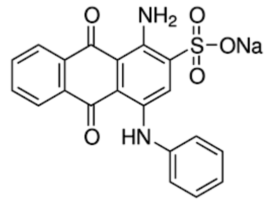
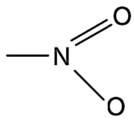
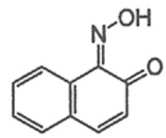
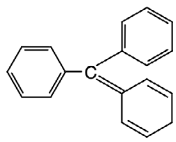
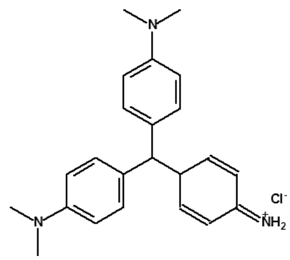
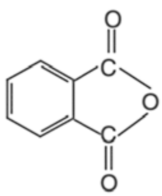
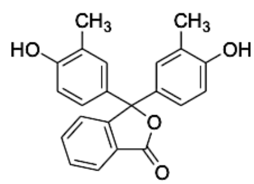
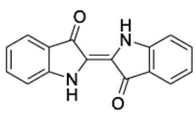
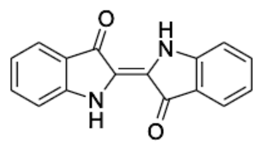
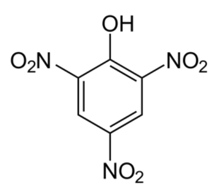
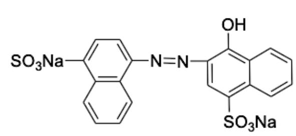
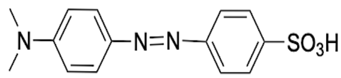
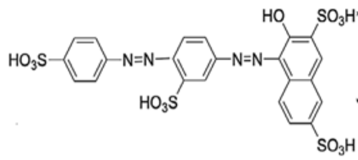
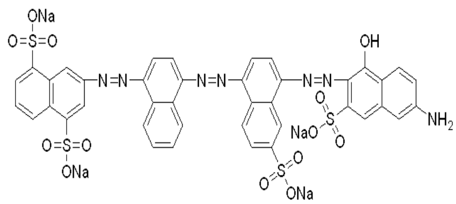
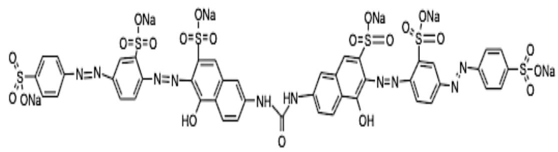
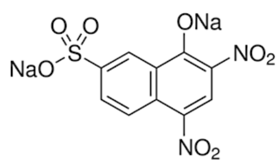
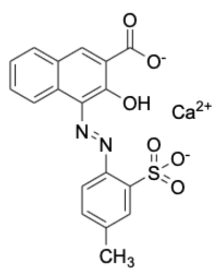
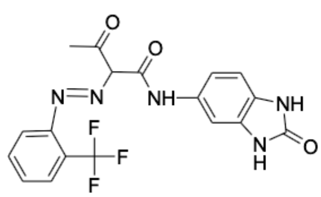
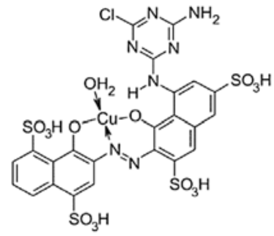
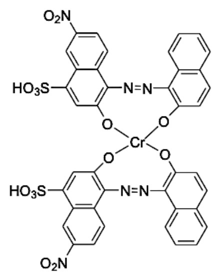
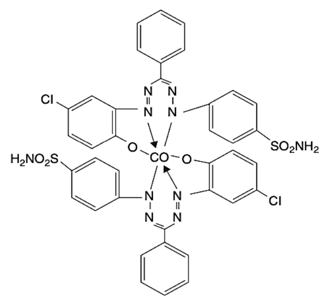
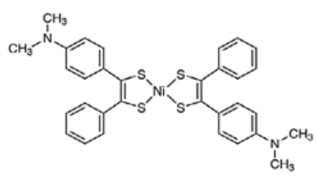
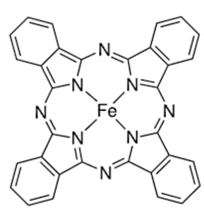
Chemical structure class	Chromophore	Dye	Chemical structure	C.I. name
Anthraquinone		Acid blue 25		62055
Nitro		Mordant green 4		10005
Triphenylmethane		Crystal violet		42555
Phthalein		o-Cresolphthalein		68995
Indigo		Indigo		10215
Nitroso	—N=O	Picric acid		10305
Azo	—N=N—	Acid red B		14720

Table 2: Classification of azo dyes according to number of azo linkages and metal complex dyes

Chromophore	Azo Dye	Chemical structure	C.I. name
Monoazo	Methyl orange		13025
Diazo	Red ponceau S		103116
Triazo	Direct blue 71		34140
Poliazo	Direct red 80		35780
Napthol	Napthol yellow S		10316
Azo lakes	Lithol rubine BK		15850
Benzimidazolone	Benzimidazolone yellow H3G		11781

Continued Table 2: Classification of azo dyes according to number of azo linkages and metal complex dyes

Chromophore	Azo Dye		Chemical structure	C.I. name
	Cu^{2+}	Reactive blue 13		181575
	Cr^{3+}	Acid black 172		23976
Metal complex	Co^{3+}	Acid black 180		13710
	B^{3+}	Boron-dibenzopyrro-methene		131818
	Ni	BDN		691182
	Fe^{2+}	Iron(II)Phthalocy-anine		23925

classification of azo dyes according to the number of azo linkages and metal complex dyes.

Azo dyes represent a significant and very versatile group of dyes used in numerous industries such as the food industry, printing, leather, pharmaceutical, cosmetic and especially in the textile industry, where they are used to dye fabrics made of protein fibers, cellulose or synthetic fibers such as polyesters and nylon (Tee *et al*., 2015; Das *et al*., 2015; Oon *et al*., 2017). Azo dyes however, are toxic, carcinogenic and mutagenic in nature. They represent a pollution hazard because they include components such as benzidine and aromatic compounds in their structure. Their breakdown products (colorless amines) are also toxic and/or mutagenic to living organisms (Xu *et al*., 2016). Once released to the environment through colored wastewater, these dyes represent a problem for the receiving waters, due to the reduction of the photosynthetic activity of aquatic plants, the decrease in the concentration of dissolved oxygen and the increase in oxygen biochemical demand (Liu *et al*., 2015; Orts *et al*., 2018; Sarkar *et al*., 2020). The bioaccumulation of these dyes in sediments and soil can generate modifications in the microbial communities and enzymatic activities, can inhibit the nitrification process, alter the activity of the urease enzyme, the ammonification rate of arginine and reduce oxidative bacteria. In plants, these dyes decrease the germination rate and produce chlorosis and anatomical changes in leaves that ultimately lead to plant death (Imran *et al*., 2015; Rehman *et al*., 2018).

Traditional methods for the treatment of contaminated effluents

Taking into account the negative environmental impact generated by the discharge of untreated or partially treated colored effluents to receiving

Table 3: Physical and chemical methods most used for the treatment of colored effluents

Method	Rationale	Limitations	References
Adsorption	Uses absorbents to remove colorants, such as activated carbon and other materials such as cobs, sawdust, vegetable and fruit peels, among others.	High cost for the preparation of activated carbon. Low cost absorbents have low dye removal efficiency. After treatment the absorbents are polluting.	Mincea *et al*., 2013 Zhao *et al*., 2014
Coagulation	Adding coagulants to water to form flocs. With the proper weight, size, and strength, sedimentation of macro-flocs occurs.	It requires the use of chemical products, and generates a large quantity of sludge that must be treated later.	Ayanda, 2018
Membrane Filtration	Uses special pore-size membranes to filter contaminants through techniques such as reverse osmosis, ultra, micro, and nanofiltration.	More efficient as a pretreatment for separation processes. It is a method of high initial and limited cost for the elimination of dyes due to their solubility in water.	Ahmad *et al*., 2012
Advanced oxidation	It involves techniques such as the oxidation of Fenton's reagent, ultraviolet photolysis, sonolysis, use of ozone and hydrogen peroxide to degrade organic pollutants at room temperature and pressure.	Inefficient for the removal of insoluble dyes and limited life. The use of the Fenton's reagent generates a large quantity of sludge	Ayanda, 2018 Gupta *et al*., 2015
Electrochemical oxidation	They use the anode of the electrolytic cell to electrochemically oxidize wastewater contaminated with dyes.	Its efficiency depends on the operating conditions and variables such as the support electrolyte, pH of the medium, temperature, concentration of the organic compound, and the type of anode material.	Ayanda, 2014 Gupta *et al*., 2015

water bodies, various treatment methods have been used. The most widely used physicochemical methods include advanced oxidation processes, adsorption, ozonation, membrane filtration, photo-catalytic degradation, coagulation and flocculation, electrocoagulation, photo-electrocatalysis, and electrochemical oxidation (Gupta *et al*., 2015; Fajardo *et al*., 2015; Solano *et al*., 2015). Unfortunately, although these methods are considered effective for removing dyes from wastewater, there are many drawbacks to these strategies: complex infrastructures, high cost, inefficient color removal, production of secondary pollutants or generation of large amounts of contaminated sludge and toxic by-products (Balapure *et al*., 2014; Liu *et al*., 2015). To minimize these drawbacks, a combination of two or more physicochemical techniques are frequently used for the treatment of waste water contaminated with dyes; however, the results remain unpromising given the recalcitrant nature of the dyes and their resistance to degradation processes (Ayanda, 2018; Kertesz *et al*., 2014). Table 3 presents the rationale and limitations of the most widely used physicochemical methods for treating colored effluents. Currently, wastewater treatment systems are not only focused on guaranteeing the ecosystem quality of the receiving water bodies and minimizing the impact to human health. Based on the principles of the circular economy, they are also focused on the need to develop treatments that allow the reuse of wastewater in response to the issue of the growing demand for water and the depletion of natural sources (Ribera *et al*., 2019).

As a consequence of the degradation processes of azo dyes, chemical compounds known as aromatic amines are generated, whose molecular structure is characterized by having one or more aromatic rings with amino substituents. The toxicity of amines depends on the metabolic activation of the amino group, which generates metabolic intermediates capable of binding to DNA molecules, producing genotoxicity and mutagenicity (Brüschweiler *et al*., 2017). Although azo dyes are considered recalcitrant, recent studies have reported that aromatic amines can be biodegraded taking into account important factors such as the type of microbial population, their conditions to adapt and the availability of oxygen (Pietruk *et al*., 2019).

Methods for removing azo dyes in different industries

The release of dyes into the environment is a consequence of industrial processes. The textile industry releases 54% of the existing dyes in the world, the dyeing industry itself releases 21%, the pulp and paper industry releases 10%, the leather tanning industry releases 8%, and 7% is released by the dye manufacturing industry. Dyes used in the textile industry are xenobiotic and recalcitrant compounds (Kurade *et al*., 2016; Ajaz *et al*., 2019). Physico-chemical alternatives for treating waste water resulting from the textile industry include: advanced oxidation processes AOP (Mondal *et al*., 2017), coagulation/flocculation (Butani *et al*., 2017), electrochemical treatments (Chellam and Sari, 2016) and physical methods (Khan *et al*., 2018). Biological alternatives are: bacterial cultures- either pure or in consortia (Kurade *et al*., 2016; Kuppusamy *et al*., 2017), algal biomass (Elgarahy *et al*., 2019), fungi cultures (Dayi *et al*., 2019) and enzymatic methods (Katheresan *et al*., 2018). The use of emerging technologies to treat waste water resulting from the dyeing industry has been reported recently. These technologies include: the combination of TiO_2 microreactor and electroflotation (Talaiekhozani *et al*., 2020), itaconic acid hydrogels (Bharathiraja *et al*., 2019) ion exchange resins (Wu *et al*., 2020), catalytic ozonation (Ghuge and Saroha, 2018) advanced photocatalytic processes (Kim and Jo, 2019; Bahadur and Bhargava, 2019) and pulsed light (Martínez-López *et al*., 2019). The pulp and paper industry treats effluents contaminated with azo dyes using advanced oxidation processes (Cesaro *et al*., 2019), ion exchange (Jaria *et al*., 2017), ozonation and biological treatment (Kumar *et al*., 2019), coagulation using polymeric ferric chloride, (Yang *et al*., 2019), aerobic granulation (Morais *et al*., 2016), bio-adsorbents (Kakkar *et al*., 2018), the microbial fuel cell, enzymatic methods with laccases, peroxidases and xylanases (Sharma *et al*., 2020; Singh *et al*., 2019) and microorganisms such as *Clostridium* (An *et al*., 2020), *Diaphorobacter nitroreducens* (Zhong *et al*., 2020) and *Saccharomyces cerevisiae* (Lin *et al*., 2012; Lin *et al*., 2017). Advanced oxidation processes such as electrochemical oxidation, electro-fenton and photoelectro-fenton are the most commonly used in the treatment of effluents contaminated with azo dyes in the leather tanning industry. The use of biosorption using solid waste from tanneries has also been reported (Gomes *et al*., 2016), as well as the use of biomass from microalgae (Da Fontoura *et al*., 2017) and fungi such as *Trametes versicolor*, *Ganoderma lucidum* and *Irpex* (Baccar *et al*., 2011). For the treatment of effluents contaminated with dyes in the dye manufacturing industry other methods are used: adsorption with magnesium oxide nanopores (Pourrahim *et al*., 2020), activated carbon (Ilnicka *et al*., 2020) and capsules with hierarchical $Mg(OH)_2$ nanostructures (Akbari, 2017).

Mechanism of microorganisms on degradation of azo dyes

Microorganisms can degrade azo dyes by means of biosorption mechanisms and/or enzymatic degradation. The biosorption capacity of a micro-organism is associated with the attraction generated between the azo dye and the components of the bacterial cell wall. This mechanism depends on pH, temperature, ionic strength, contact time, adsorbent and dye concentration, dye structure and type of microorganism. Enzymatic degradation is an anaerobic mechanism favored by the deficiency of electrons in the dye. The reduction of azo binding of the dye is mediated by azoreductase enzymes and oxidative degradation is catalyzed by peroxidases and phenoloxidases such as: manganese peroxidase, lignin peroxidase, laccase, tyrosinase, N-demethylase (Wu *et al*., 2012; Ambrosio *et al*., 2012; Solis *et al*., 2012). Fungi have ligninolytic enzymes such as manganese peroxidase enzyme, laccase and lignin peroxidase with excellent catalytic power, capable of degrading dyes using biosorption mechanisms, biotransformation or complete removal by mineralization. These mechanisms are favored by the addition of carbon and nitrogen sources, aeration, humidity and use of mixed crops (Asgher *et al*., 2014; Akdogan *et al*., 2014). For the degradation of azo dyes, bacteria have an efficient enzymatic system that allows them to carry out a series of catabolic activities, with azoreductase and laccase enzymes being responsible for the transfer of electrons to the azo bond of the dye and the production of aromatic amines. (González *et al*., 2018). The mechanism of degradation by azoreductase enzymes consists of two phases; the first, called the reducing phase, begins with the cleavage of the azo bond (-N = N-) by catalyzed reduction of the enzyme under anaerobic/anoxic or microaerophilic conditions, where NADH molecules, derived from carbohydrate metabolism are used as electron donors (Elfarash *et al*., 2017; González *et al*., 2018). In the second phase, as a result of this division, relatively simple intermediate aromatic amines are generated, which are deaminated or dehydrogenated by bacteria through aerobic processes (Garg *et al*., 2012) under aerobic conditions, which leads to complete degradation of azo dyes (Saratale *et al*., 2011; Garg *et al*., 2012; Al-Amrani *et al*., 2014). Laccases, on the other hand, are copper oxidases that degrade dyes in the presence of oxygen through mechanisms that involve direct or indirect oxidation using redox mediators to accelerate the reaction, which involves the removal of a hydrogen atom from the hydroxyl and amino groups, replacing it with phenolic substrates and aromatic amines (Tišma *et al*., 2020). Bacterial peroxidases are also involved in the degradation of dyes. These enzymes need H_2O_2 as a terminal electron acceptor rather than oxygen. Their mechanism of action is similar to that of laccases and leads to degradation of the dye without production of toxic aromatic amines (Imran *et al*., 2014). For the degradation of azo dyes, algae may involve enzymatic degradation processes, adsorption, or both. They degrade azo dyes through azoreductase enzymes or oxidative enzymes. Adsorption efficiency is influenced by dye structure, algal species and pH. Microalgae that are immobilized in alginate may remove a higher percentage of color than algae in suspension (Priya *et al*., 2011). Similar to microalgae, yeast discoloration mechanisms involve adsorption, enzymatic degradation or a combination of both. Adsorption by yeast biomass is more efficient at a pH between 2 and 4, while, degradation is associated with the presence of oxidase and reductases enzymes and the addition of carbon or glucose as an energy source. Genetically modified organisms can also degrade azo dyes through mechanisms involving genetic modification or gene transfer, that encode enzymes with different characteristics or biochemical pathway variants in a microorganism (Martorell *et al*., 2012; Solis *et al*., 2012).

The potential of bacteria for the degradation of azo dyes

The limitations associated with the use of physicochemical methods for the treatment of effluents contaminated with azo dyes have promoted the development of new treatment alternatives that are attractive, efficient, profitable, environmentally friendly and produce less sludge (Balapure *et al*., 2014; Liu *et al*., 2015; Sabaruddin *et al*., 2018; Zhuang *et al*., 2020). Table 4 compares efficiency, environmental impact and costs between biological methods and physico-chemical methods.

The effectiveness of microorganisms for the degradation of compounds depends on various factors such as survival, adaptability, the activity of the microorganism and the chemical structure (Amoozegar *et al*., 2011; Agrawal *et al*., 2014). Among the biological alternatives for dye removal is phytoremediation, which uses plants such as *Aster amellus*, which removes azo dyes mainly through their roots (Khandare *et al*., 2011). On the other hand, algae such as *Chara* sp and *Comarium* sp, are resistant to the conditions found in textile effluents and are capable of removing malachite green through degradation and sorption mechanisms. However, the long amount of time necessary to carry out these processes constitutes a disadvantage (Khandare *et al*., 2011; González *et al*., 2018). The ability of fungi to adapt their metabolism to the exploitation of various sources of carbon and nitrogen, makes them a viable option for the degradation of dyes. Such is the case of *Trametes versicolor* that degrades red dye 27 through lignins peroxidases (Rekik *et al*., 2019), or *Aspergillus niger* and *Aspergillus terreus* that degrade and absorb the red azo dye MX-5 reducing its toxicity (Almeida and Corso, 2014). Despite all of the above, bacteria are the most relevant microorganisms in bioremediation processes due to their ability to adapt to variations in chemical and biological oxygen demands at high concentrations of salinity, at variable pH levels, dissolved oxygen and heavy metals (Ajaz *et al*., 2019). To interact with heavy metals, they have specific mechanisms through which they can interact, as presented in Table 5.

In addition, they use different resistance mechanisms that include the release of metal ions by extracellular barriers such as the capsule, the cell wall and the plasma membrane, the extrusion of metal ions through efflux or diffusion pumps, intracellular sequestration of metal ions, biotransformation of toxic metal ions, and decreased sensitivity of cellular targets to metal ions (Bazzi *et al*., 2020). In the interaction between bacteria and metal, the formation of biofilm plays an important role in bacterial survival in the presence of high metal concentrations, and

Table 4: Comparison between biological and physico-chemical methods

Criteria	Biological methods	Reference	Physico-chemical methods	Reference
Efficiency	They are able to completely mineralize many azo dyes under certain environmental conditions.	Saratale *et al*., 2011 Rathod *et al*., 2017	They have low color removal efficiency. They do not completely eliminate recalcitrant azo dyes and / or their organic metabolites. Secondary waste is generated and needs additional	Guo *et al*., 2020
Impact on the environment	They are eco-friendly because they use microbial microorganisms or enzymes and the end products are not toxic. Require less water and energy consumption	Ahmadi *et al*., 2017 Dong *et al*.,2019	They generate a significant amount of sludge that can cause secondary pollution problems. Energy-intensive	Meerbergen *et al*., 2018 Guo *et al*., 2020
Costs	Low operating costs	Saratale *et al*., 2011 Dong *et al*., 2019	They are economically unviable. The large amount of sludge generated substantially increases the cost of these methods.	Saratale *et al*., 2011

efflux systems allow bacteria to interact with different amino acids as a mechanism of adaptation to the environment. These interaction mechanisms are complemented by the presence of resistance genes that encode the production of enzymes capable of reducing metals to compounds which are less toxic, and the synthesis of metalloproteins necessary for the bioaccumulation and immobilization of metals. Thermophilic and hyperthermophilic bacteria use alternative mechanisms to enzymatic production to resist metals and transfer ions to the active site (Giovanella *et al*., 2020; Artz *et al*., 2015). Bacterial action in the degradation of azo dyes is increased due to their ability to act through consortiums or synergistic associations that act as biological inducers. The union of the catabolic functions of each microorganism makes them even more useful alternatives to improve the discoloration rate of effluents contaminated with dyes, as they have greater resistance to abiotic conditions and lower rates of enzyme inactivation, especially in large-scale operations (Cervantes and Dos Santos, 2011; Khan *et al*., 2018; Balapure *et al*., 2015; Wu *et al*., 2020). Table 6 summarizes the most relevant competitive advantages that position bacteria as the most efficient microorganisms in the degradation of azo dyes.

The use of bacteria to remove azo dyes has also some disadvantages: 1) The discoloration process does not depend exclusively on these microorganisms, but also on external variables such as: agitation, oxygen, temperature, pH, dye structure, dye concentration, carbon and nitrogen sources, electron donor and redox mediator (Saratale *et al*., 2011; Al-Amrani *et al*., 2014). 2) Under anaerobic conditions, the dye penetrates with difficulty through the cell membrane, affecting the rate of degradation (Saratale *et al*., 2011; Bai *et al*., 2020). 3) As a result of the degradation process, they generate noxious and recalcitrant aromatic amines (Das *et al*., 2015; Brüschweiler *et al*., 2017). 4) Pure crops do not degrade by full azo dyes, so bacterial pools are required to make the process more productive (Saratale *et al*., 2011; Balapure *et al*., 2014). In recent years, innovative integrated processes called hybrid technologies have emerged; they provide a new treatment system, which allows eliminating the individual limitations of physical, chemical and biological methods (Shah, 2014). Among the emerging technologies associated with bacterial treatments for wastewater discoloration is biological coagulation; it consists of a prior coagulation treatment and a subsequent biological treatment dependent on variables such as the type and dose of coagulant, the amount of sludge and the degree of inhibitory and non-biodegradable substances present in wastewater. Another widely used process is the combination of advanced oxidation with activated sludge treatment. In this synergy, chemical oxidation partially degrades recalcitrant contaminants to intermediate metabolites that in subsequent processes are easily degraded by bacteria (Guan *et al*., 2018). Furthermore, the addition of adsorbents to activated sludge systems is also a viable choice for the removal of soluble organic matter. Among the most widely used adsorbents is activated carbon, which, when joined with bacteria, favors the degradation processes. Sometimes, however, carbon particles become trapped in the matrix floc and lose their adsorption properties, hindering bacterial growth

Table 5: Bacteria interaction mechanisms- heavy metals

Mechanism	Basis	Reference
Bioaccumulation	Metal enters the cytoplasm of the cell through the membrane transport system. The accumulation is favored by the action of metalloproteins or by its deposition in vacuoles.	Zhang *et al*., 2020
Biomineralization	Metal is precipitated in the cell through resistance mechanisms encoded by plasmids.	Khadim *et al*., 2019
Biotransformation	Metal is transformed inside the cell through mechanisms that favor the loss of electrons or changes in oxidation states, and the addition of methyl groups.	Johnson *et al*., 2020
Chemisorption	Metal molecules hold together with the bacteria to form a strong chemical bond, so the chemisorbed molecule does not maintain the same electronic structure.	Latif *et al*., 2020

and dye removal. Applying this process allows COD and color removal from textile wastewater in a single step without additional physicochemical treatment (Zhang *et al.,* 2019). Likewise, the new methods associated with filtration constitute a promising technology for the reuse of water, which is how the use of nanofiltration, a technique that increases the life of the membrane, has recently been reported. It provides a "closed loop" system, in which products are partially oxidized and then transferred for biological treatment by bacteria. Rinse water can be reused after membrane recovery while concentrated wastes can be degraded in anaerobic digester (Cinperi *et al.,* 2019). The membrane bioreactor also constitutes an improvement option to the conventional activated sludge treatment to treat colored water. It consists of an anaerobic reactor modified with activated carbon, that precedes the aerobic membrane bioreactor and achieves stable discoloration along with a high removal of total organic carbon, improving the dehydrability of activated sludge and reducing resistance to filtration (Bai *et al.,* 2020).

Table 6: Competitive advantages of bacteria for the degradation of azo dyes

No.	Advantages	Bacteria identified	References
1	They have short life cycles, generating faster discoloration processes.	*Proteus vulgaris.*	Britos *et al.*, 2018
2	They have a higher growth rate and adaptability.	*Bacillus* sp, *Bacillus subtilis, Aeromonas hydrophila, Bacillus cereus, Proteus mirabilis, Pseudomonas luteola, Pseudomonas* sp, *Pseudomonas aeruginosa, Escherichia coli and Klebsiella sp.*	Saratale *et al.*, 2011 Al -Amrani *et al.*, 2014
3	Their use is more viable, inexpensive and ecological.	*Bacillus subtilis, Aeromonas hydrophila, Bacillus cereus, Proteus mirabilis, Pseudomonas luteola, Pseudomonas* sp. and *Pseudomonas aeruginosa.*	Saratale *et al.*, 2011
4	Their degradation capacity is boosted when used in consortia.	*Psychrobacter alimentarius* and *Staphylococcus equorum.*	Khalid *et al.*, 2012
5	They detoxify aromatic amines produced by anaerobic discoloration.	*Aeromonas hydrophila.*	Thanavel *et al.*, 2019
6	They use complex organic compounds to carry out their metabolic activities.	*Aerococcus* sp, *Carnobacterium* sp, *Enterococcus* sp, *Lactobacillus* sp, *Lactococcus* sp, *Leuconostoc* sp, *Oenococcus* sp, *Pediococcus* sp, *Streptococcus* sp, *Tetragonococcus* sp, *Vagococcus* sp *and Weissella* sp	Sharma *et al.*, 2020
7	The effectiveness to degrade dyes does not depend on their adaptability to the environment, but has to do with the presence of enzymatic genes that can be innately expressed or over-expressed in the presence of toxic substances.	*Pseudomonas desmolyticum, Micrococcus glutamicus, Pseudomonas* sp, *Enterococcus gallinarum, Klebsiella* sp, *Lysinibacillus* sp, *Pseudomonas putida, Pseudomonas pulmonicola* and *Micrococcus* sp.	Vikrant *et al.*, 2018 Mittal *et al.*, 2018
8	They possess molecular mechanisms to acquire resistance to heavy metals similar to the antimicrobial resistance mechanisms.	*Escherichia coli, Streptomyces pilosus, Klebsiella aerogenes, Pseudomonas putida, firmicutes sp, Staphylococcus aureus, Enterococcus hirae, Ralstonia sp, Streptomyces sp, Bacillus sp and Arthrobacter viscosus.*	Nanda *et al.*, 2019

Table 7: Bacterial species reported as dye degraders

Bacteria	Degraded dye(s)	Higher percentage removal (100 mg/L)	Reference
Marinobacter sp	Direct blue 1	100%	Prasad *et al*., 2013
Galactomyces sp	Amido black	81.43%	Maqbool., 2016
Pseudomonas putida	Orange 10	70%	Mahmood *et al*., 2016
Bacillus sp	RV-5R and RBO-3R	63.33%, 96.15%	Dicle *et al*., 2014
Bacillus cohnii	Direct red-22	95%	*Prasad et al., 2013*
Brevibacterium sp	RY107, RB5, RR198 and DB71	99%	Franciscon *et al*., 2012
Providencia sp	Acid black 210	99%	Agrawal *et al*., 2014
Staphylococcus arlettae	Yellow107	99.5%	Bhardwaj *et al*., 2016
Aeromonas hydrophila	Crystal violet	99%	Bharagava *et al*., 2018
Aeromonas hydrophila	Fast yellow MR	91.25%	Thanavel *et al*., 2019
Sphingobacterium sp.	Direct red 5B	100%	Tamboli *et al*., 2010
Enterococcus faecalis	Direct red 81	100%	Sahasrabudhe *et al*., 2014
Enterococcus casseliflavus	Amaranth	100%	Chan *et al*., 2012
Enterococcus gallinarum	Reactive red 35	93.69%	Soni *et al*., 2016
Enterococcus gallinarum	Reactive red 198	91.56%	Soni *et al*., 2016
Enterococcus gallinarum	Reactive red 106	94.91%	Soni *et al*., 2016
Enterococcus gallinarum	Reactive red 120	92.69%	Soni *et al*., 2016
Enterococcus gallinarum	Reactive red 111	93.58%	Soni *et al*., 2016
Enterococcus gallinarum	Reactive black 5	91.99%	Soni *et al*., 2016
Enterococcus gallinarum	Reactive red 141	91.99%	Soni *et al*., 2016
Enterococcus gallinarum	Reactive blue 160	93.63%	Soni *et al*., 2016
Enterococcus gallinarum	Reactive blue 28	91.42%	Soni *et al*., 2016
Enterococcus gallinarum	Reactive red 152	95.95%	Soni *et al*., 2016
Acinetobacter baumannii	Reactive red 198	95.58%	Unnikrishnan *et al*., 2018
Acinetobacter baumannii	Congo red	99%	Ning *et al*., 2014
Acinetobacter baumannii	Congo red	89%	Kuppusamy *et al*., 2016
Acinetobacter baumannii	Gentian violet	90%	Kuppusamy *et al*., 2016
Acinetobacter sp	Dye disperse orange S-RL	90.2%	Cai *et al*., 2015
Acinetobacter calcoaceticus	Amaranth	91%	Ghodake *et al*., 2011
Acinetobacter iunii	RO-16. DB-19	90%	Anwar *et al*.. 2014

Table 8: Efficiency of simultaneous removal processes: dye - heavy metal

Demining process				Efficiency (%)		
Type	Methods	Dye	Metal	Colorant	Metal	Reference
Physico-chemical	Adsorption	Reactive orange 5	Pb^{2+}	97%	70%	Li *et al.*, 2019
Physico-chemical	Adsorption	Methyl orange	Pb^{2+}	90.8%	98.7%	Ge *et al.*, 2018
Physico-chemical	Adsorption	Basic red 46	Cu	99%	98%	Dolatabadi *et al.*, 2018
Biological	Bacterial: *Bacillus circulans*	Methyl orange	Cr (VI)	100%	100%	Liu *et al.*, 2017
Biological	Bacterial: *Lactobacillus paracase*	Acid Black	Cr (VI)	58.5%	51.9%	Huang *et al.*, 2015
Biological	*Pseudomonas putida*	Reactive black-5	Cr (VI)	70%	70%	Mahmood *et al.*, 2013
Biological	*Acinetobacter junii*	Reactive Red-120	Cr (VI)	83%	98%	Anwar *et al.*, 2014
Hybrid	Photocatalysis	Methyl orange	Cr (VI)	91%	91%	Xie *et al.*, 2020

Potentially degrading bacteria of the complex azoic dyes - heavy metals

Scientific reports present various bacterial species capable of degrading individual and mixed dyes, as presented in Table 7. However, few studies are associated with the use of bacteria capable of remedying effluents contaminated with dyes and heavy metals effectively and simultaneously. This is precisely because these microorganisms require exclusive properties not only to adapt to adverse environmental conditions but also for their robust enzymatic activity and chemical structure (Talaiekhozani and Rezania., 2017; Zhuang *et al.*, 2020). Table 8 compares the efficiency of physico-chemical, biological and hybrid processes for simultaneous removal of metals and azo dyes.

These properties are easily found in bacterial communities present in marine ecosystems, which have developed mechanisms that allow them to resist adverse environmental conditions such as hyper salinity, pH variations and the presence of heavy metals (Zhuang *et al.*, 2019; Zhuang *et al.*, 2020). This continuous exposure to extreme environmental conditions makes them more stable and more active, unlike other types of bacteria conserved in culture banks (Unnikrishnan *et al.*, 2018). Table 9 presents a comparison of the percentages of dye removal using bacteria isolated from water, wastewater, soil or marine environments.

One of the bacteria identified as an effective biological alternative for the removal of metal-dye synergy is *Enterococcus* sp, recognized for its ability to thrive in environments with low nutrient concentrations, persistent to temperature fluctuations, and resistant to desiccation, UV radiation, freezing, pH changes, high salinity and predation (Vignaroli *et al.*, 2018; Lee *et al.*, 2019; Thu *et al.*, 2019). Furthermore, they are considered catabolically versatile microorganisms, capable of using a wide range of unusual substrates as carbon source (Sahasrabudhe *et al.*, 2014). For a long time, the environmental importance of *Enterococcus* sp had to do with it being an excellent indicator of fecal contamination in waters (Di Dato *et al.*, 2019; Federigi *et al.*, 2019), however, recently new potential uses of this microorganism have emerged. It that can be exploited for the benefit of the environment, such as for its ability to metabolize xenobiotics, among which are azo dyes, or its affinity to bind and resist heavy metals. Furthermore, the genome of these bacteria also reveals the presence of phages, which in large-scale industrial processes could be useful elements to improve the general bioremediation capacity. They could also prove to be viable option in transferring their ability to degrade azo dyes to other *Enterococcus* through genetic engineering from hybrid strains (Chan *et al*., 2012). The ability of *Enterococcus faecalis* to metabolize azo dyes is associated with the presence of the azoA gene that encodes the production of the aerobic azoreductase enzyme, which is not secreted outside the cell, has a wide substrate specificity, requires flavin mononucleotide (FMN) as a cofactor and uses NADH as an electron donor (Rathod *et al*., 2017; Sun *et al*., 2017). The ATCC 6569 *Enterococcus faecium* strain possess the enzyme azoreductase (AzoEf1) which shares 67% identity with the azoreductase of *Enterococcus faecalis* (AzoA). However, there are differences related to coenzyme preference, residues associated with FMN binding, substrate specificity, and specific activity. The AzoEf1 sequence is found in GenBank: GQ479040.1. Chan *et al*. (2012) report a strain of *Enterococcus casseliflavus* that by the action of an enzyme with activity similar to that of azoreductase is not only able to discolor a wide range of azo dyes under microaerophilic conditions, but also catabolize by desulfonation and deamination the intermediaries generated as a consequence of the reductive cleavage. The genome of this microorganism also reveals the presence of regulatory systems possibly involved in the biodegradation of aromatic contaminants. *Enterococcus gallinarum* offers an effective ecological alternative for the remediation of environments contaminated with structurally complex and recalcitrant azo dyes such as reactive red 35. This is done through enzymatic mechanisms that involve the presence of oxidoreductases, such as laccases, tyrosinases and azoreductases under a wide range of pH, different temperature levels and with a high concentration of salinity; therefore, its use on a large scale is recommended using a suitable microaerophilic-aerobic sequential bioreactor (Soni *et al*., 2016). The binding affinity of *Enterococcus sp* to heavy metals has been attributed to the capsular polysaccharide, which contains different monomers such as glucose, galactose, mannose and fructose, capable of participating in the redox reaction of remediation processes of waters contaminated with heavy metals and dyes (Sardar *et al*., 2018). Recently, these monomers have been used for the synthesis of silver nanoparticles (AgNP) that combined with advanced oxidation processes (AOP) have shown good results in the degradation of azo dyes such as methyl orange and Congo red (Saravanan *et al*., 2017). In relation to metal removal, *Enterococcus faecalis* uses mechanisms such as copper transporting ATPases, present in the inner membrane, which not only work for the homeostasis of this metal but also to resist high concentrations of nickel, mercury, cadmium, lead and copper (Huët and Puchooa, 2017). Another bacterium present in marine ecosystems with exclusive properties to adapt to adverse environmental conditions and simultaneously degrade the metal-dye complex is *Acinetobacter sp*. This microorganism has protein coding genes capable of degrading innumerable organic compounds such as biphenyls, phenols, benzoates, acetonitrile, chlorine anilines, dichloroaniline, hydrocarbons and heavy metals, which for other microorganisms could be toxic. This makes it an important biocatalyst with high potential biotechnology to remedy various environmental pollutants (Hongsawat and Vangnai, 2011; Walter *et al*., 2020). The discoloration capacity of bacteria of the genus *Acinetobacter* is associated with the enzymatic activity of lignin peroxidases, considered enzymes with exclusive catalytic properties. The activity of these enzymes depends on hydrogen peroxide so as to transform a persistent high range of organic compounds (Bilal *et al*., 2019). There are several species of *Acinetobacter* reported with the ability to degrade dyes. Such is the case of *Acinetobacter baumanii* that degrades azo dyes using biotransformation mechanisms through peroxidase and azoreductase enzymes. The efficiency of dye degradation by this microorganism has been potentiated through microencapsulation, a technology in which the microorganism is immobilized using calcium alginate beads. This provides a higher rate of biodegradation by more easily separating the solid-liquid complex, it reduces downstream processing steps and it offers greater operational stability both by preventing leaks and by protecting the biocatalyst from environmental conditions (Unnikrishnan *et al*., 2018). *Acinetobacter junii* is capable of degrading RR-120, RO-16, RY-2, DR-28, and DB-19 in the presence of Cr(VI), a metal associated primarily with the textile and tannery industries. However, this bacterium is also capable of resisting other heavy metals such as Zn^{2+}, Cd^{2+}, Cu^{2+}, Co^{2+} and Pb^{2+}. The properties of this strain make it a multifunctional alternative and a profitable biological resource that could be exploited for the simultaneous bioremediation of more than one contaminant (Anwar *et al*., 2014). *Acinetobacter calcoaceticus* can discolor various dyes, among which is the azo dye amaranth. In this case, it is a result of the enzymatic action associated with lignin peroxidases, laccases and reductases, which, in addition to degrading the dye, are capable of decreasing phytotoxicity (Ghodake *et al*., 2011). Due to all of the above, *Enterococcus* sp and *Acinetobacter* sp constitute an important alternative solution to the problems associated with the use of azo dyes in industrial processes. The release of dyes into the environment is a global problem. Industries are now interested in using new technological alternatives to mitigate this problem. The textile, pulp and paper, as well as the leather tanning industry all use advanced oxidation, photocatalysis and adsorption methods to treat its effluents (Mondal *et al*., 2017; Cesaro *et al*., 2019). At an industrial level, all of the following have been reported as bio-treatments: the use of algal biomass (Elgarahy *et al*., 2019; Da Fontoura *et al*., 2017), fungi (Baccar *et al*.,2011; Dayi *et al*., 2019), yeasts (Lin *et al*., 2012; Lin *et al*., 2017), enzymatic methods (Katheresan *et al*., 2018; Sharma *et al*., 2020, Singh *et al*., 2019) and bacterial crops (Kurade *et al*., 2016; Kuppusamy *et al*., 2017; An *et al*., 2020; Zhong *et al*., 2020). Several authors agree that bacterial action in the degradation of azo dyes increases when they act in synergistic consortia or associations (Cervantes and Dos Santos, 2011; Saratale *et al*., 2011; Khan *et al*., 2014; Balapure *et al*., 2015; Wu *et al*., 2020) and is affected by external variables such as pH, carbon

Table 9: Comparison of dye removal using bacteria isolated from water, wastewater, soil, marine environments

Bacteria	Isolation place	Removal (%)	Dye	Reference
Rhodopseudomonas palustris	Lake Akkaya in Nigde, Turkey	100	Black azo dye K	Öztürk *et al*., 2020
Bacillus sp.	Abaya and Chamo alkaline lakes in Ethiopia	98	Reactive red 239	Guadie *et al*., 2017
Klebsiella *Buttiauxella* *Bacillus* *Escherichia* *Clostridium* sp.	Water from the textile industry in Haicheng, China	98	Methyl red	Cui *et al*., 2012
Acinetobacter baumannii	Kovalam sea shore in Tamil Nadu. India	96.2	Reactive red 198	Unnikrishnan *et al*., 2018
Oceanimonas smirnovii *Enterobacter kobei* *Citrobacter freundii*	Coastal marine sediments	95	Methyl orange	Zhuang *et al*., 2020
Aliiglaciecola lipolytica	Sea water	≥90	Congo red	Wang *et al*., 2020
Acinetobacter sp. *Klebsiella* sp.	System of activated sludge	> 80	Reactive orange 16 Reactive Green 19	Meerbergen *et al*., 2018
Pseudoarthrobacter sp. *Gordonia* sp. *Stenotrophomonas* sp. *Sphingomonas* sp.	Drainage of a textile factory in Mashhad, Iran	54	Reactive black-5	Eskandari *et al*., 2019
Lactobacillus paracase	Waste water from a tannery company in Zhengshen, Quanzhou, China	63	Acid black	Huang *et al*., 2015

and nitrogen sources, electron donor, redox mediator, dye structure and dye concentration (Saratale *et al*., 2011; Al-Amrani *et al*., 2014; Bai *et al*., 2020). The discoloration time is prolonged when the concentration of the dye increases (Chakraborty *et al*., 2013). Monoazo bonds are more easily reduced than diazo and triazo, because the activation energy required by enzymes to reduce color is lower for monoazo than for diazo and triazo (Shah, 2014). However, Oturkar *et al.* (2013) studied the degradation of azo dyes with azoreductase enzymes of *Bacillus lentus* and concluded that diazo dye showed faster discoloration than monoazo. This indicated that color degradation is not only dependent on the action of the enzyme, but also on the proximity and molecular structure of the sulfonated groups of the dye and the composition of the industrial effluent. Cofactors play an important role in the degradation of azo dyes. The azoreductase of both *Enterococcus* and *Bacillus* depends on NADH. Disturbances in the activity of this cofactor may affect bacterial physiology and growth (Rathod *et al*., 2017; Misal *et al*., 2011). The species reported in this paper as degraders show removal capacity between 63% and 100%; the most used out of them are *Enterococcus* and *Acinetobacter* (Ghodake *et al*., 2011; Chan *et al*., 2012; Anwar *et al.*, 2014; Ning *et al.*, 2014; Sahasrabudhe *et al*., 2014; Cai *et al*., 2015; Soni *et al*., 2016; Kuppusamy *et al.*, 2016; Unnikrishnan *et al*., 2018) and the most efficient are *Marinobacter sp, Sphingobacterium sp, Enterococcus faecalis and Enterococcus casseliflavus* (Tamboli *et al*., 2010; Chan *et al*., 2012; Prasad *et al*., 2013; Sahasrabudhe *et al*., 2014). Although bacteria require metals for their metabolic processes, at high concentrations they negatively affect bacterial metabolism (Zhuang *et al*., 2019). In this study, the most efficient bacteria for simultaneously removing the dye-metal complex are *Bacillus circulans* and *Acinetobacter junii* (Anwar *et al*., 2014; Liu *et al*., 2017). The metabolism of many bacteria is affected in acidic or alkaline conditions. Some studies associate a low discoloration efficiency when bacteria develop in alkaline conditions, obtaining maximum discoloration rates at acidic pH (Wang *et al*., 2017). However, this article reports the high efficiency (98% removal) of a strain of *Bacillus sp* isolated from an alkaline lake in Ethiopia (Guadie *et al*., 2017). Bacteria isolated from marine and estuarine environments were found to be highly efficient for the degradation of azo dyes (Unnikrishnan *et al*., 2018; Öztürk *et al*., 2020; Zhuang *et al*.,2020; Wang *et al.,* 2020) as opposed to waste water isolates with a low clearance rate (Eskandari *et al*., 2019; Huang *et al*., 2015). Several authors report that bacteria in marine ecosystems are more stable and more active and have mechanisms that allow them to resist adverse environmental conditions (Zhuang *et al*., 2019; Zhuang *et al*. 2020; Unnikrishnan *et al*., 2018). Furthermore, taking into account that waste waters of azo dye generally have a large quantity of salts, tolerance to high salt concentrations is a relevant indicator that these bacteria are potent bio-degradants and have great potential for industrial application (Wang *et al*., 2010). For the development of bioremediation processes it is important to prioritize the use of microbial consortia tolerant to extreme environmental conditions that simultaneously eliminate azo dyes and heavy metals, as well as to identify secondary metabolites, metabolic pathways, degradation kinetics and alternatives to minimize limiting factors. It will be relevant to advance in molecular studies of bacterial exopolysaccharide in order to use its chemical, physical and structural diversity in bioremediation processes mediated by biofilms, which can then be applied on a large scale. Transition to a circular economy boosts new bio-remediation techniques to ensure waste reduction, reuse of treated water and use of microbial fuel cells to generate renewable energy for the economic and ecological benefit of industries. The technological development associated with the degradation of dyes and metals will focus on the production of innovative biofilters, nanotubes and nanoparticles capable of immobilizing enzymes for greater efficiency. The opportunities for genetic engineering are associated with the techniques of proteomics and metagenomics for obtaining recombinant microorganisms that can over-express the genes and enzymes involved in the discoloration of azo dyes and elimination of heavy metals.

CONCLUSION

The current biotechnological challenges lead to the development of solutions that guarantee the quality of our ecosystems and the health of human beings exposed to environmental imbalances. In relation to the problems associated with the use of dyes in different industrial processes, there have been many technological strategies developed to reduce the polluting load in industrial effluents and in receiving water bodies. Dye removal strategies have evolved over the years. This has been a route led by physical and chemical methods which progressed towards the use of environmentally friendly and profitable biological solutions for the industry. These biological solutions have used plants, algae and other microbial biomasses as an alternative for dye removal. However, bacteria are the most robust microorganisms that, due to their structure and genome, become potential degraders of recalcitrant contaminants such as azo dyes. The competitive advantages of bacteria are, among others, their short life cycle, their ability to adapt and their metabolic activity, which is able to degrade and detoxify the secondary metabolites produced in the discoloration process. These properties prevail in bacterial communities present in marine ecosystems which are capable of removing, in monoculture or in consortium, individual colorants, mixtures of colorants and the metal-colorant complex. Their use, although it has been little exploited, becomes relevant with the advent of emerging technologies involving nanotechnology, alternative energy, circular economy and environmental sustainability. The mechanisms involved in the simultaneous removal of dyes and the metal-dye complex, the enzyme profile and the intermediate metabolites should be the subject of future studies based on genomics and proteomics. Likewise, due to the legal and environmental limitations for monitoring industrial discharges and for monitoring the distribution of azo dyes in the environment, it is necessary for the scientific community to provide innovative mechanisms in which monitoring discharges and bodies of water receptors are based on amine detection. Future research into the application of environmental bacteria capable of degrading azo dyes should focus on bioremediation, clean technologies, genetic engineering, nanotechnology and use of metagenomics and metaproteomics analysis.

AUTHOR CONTRIBUTIONS

R. Baldiris Ávila was responsible with preparing the work plan associated with the study, defining the bibliographic search, selection of relevant references, organizing discussion meetings, as well as revising the final version of the article. G. Manjarrez Paba and D. Baena Baldiris analyzed the documents, synthetized the information and wrote the manuscript.

ACKNOWLEDGEMENTS

The authors acknowledge that the current work would not have been possible without help of the work team of the Clinical and Environmental Microbiology Group of the University of Cartagena, in Cartagena, Colombia.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest regarding the publication of this work. In addition, the ethical issues including plagiarism, informed consent, misconduct, data fabrication and, or falsification, double publication and, or submission, and redundancy have been completely witnessed by the authors.

ABBREVIATIONS

AgNP	Silver nanoparticle
AOP	Advanced oxidation processes
ATCC	American type culture collection
ATPases	Adenylpyrophosphatase
AzoA	Azoreductase A
AzoEf1	Azoreductase from *Enterococcus faecium*
B	Boron
B^{3+}	Boron
BDN	Bis(4-dimethylaminodithiobenzyl)-nickel
C.I	Color index
Cd^{2+}	Cadmium
Co^{2+}	Cobalt
Co^{3+}	Cobaltic cation
COD	Chemical oxygen deman
Cr^{3+}	Chromium
Cr(VI)	Hexavalent chromium
Cu	Copper
Cu^{2+}	Copper
DB-19	Direct black 19 dye
DB71	Direct blue 71 dye
DNA	Deoxyribonucleic acid
DR-28	Direct red 28 dye
Fe	Iron
Fe^{2+}	Ferrous ion

FMN	Flavin mononucleotide
H_2O_2	Hydrogen peroxide
$Mg(OH)_2$	Magnesium hydroxide
MR	Methyl red
NADH	Nicotinamide adenine dinucleotide
Ni	Nickel
Pb^{2+}	Lead ion
pH	Hidrogenionic potential
RB5	Reactive black 5 dye
RBO-3R	Remazol brilliant orange 3R dye
RO-16	Reactive orange 16 dye
RR-120	Reactive red 120 dye
RR198	Reactive red 198 dye
RV-5R	Reactive violet 5R dye
RY-2	Reactive yellow 2 dye
RY107	Reactive yellow 107 dye
UV radiation	Ultraviolet radiation
Zn^{2+}	Zinc

REFERENCES

Agrawal, S.; Tipre, D.; Patel, B.; Dave, S., (2014). Optimization of triazo acid black 210 dye degradation by providencia sp. SRS82 and elucidation of degradation pathway. Process Biochem., 49(1): 110-119 **(10 pages).**

Ahmad, A.L.; Harris, W.A.; Ooi, B.S., (2012). Removal of dye from wastewater of textile industry using membrane technology. J. Teknologi., 36: 31-44 **(14 pages)**.

Ahmadi, M.; Jorfi, S.; Kujlu, R.; Ghafari, S.; Soltani, R.; Haghighifard, N., (2017). A novel salt-tolerant bacterial consortium for biodegradation of saline and recalcitrant petrochemical wastewater. J. Environ. Manage., 191: 198-208 (**11 pages**).

Ajaz, M.; Rehman, A.; Khan, Z.; Nisar, M.A.; Hussain, S., (2019). Degradation of azo dyes by alcaligenes aquatilis 3c and its potential use in the wastewater treatment. AMB Express., 9:64 **(12 pages).**

Akbari, A., (2017). Membrane capsules with hierarchical Mg (OH) 2 nanostructures as novel adsorbents for dyeing wastewater treatment in carpet industries. J. Taiwan Inst. Chem. Eng., 70: 391-400 (**10 pages**).

Akdogan, H.A.; Topuz, M.C.; Urhan, A., (2014). Studies on decolorization of reactive blue 19 textile dye by Coprinus plicatilis. J. Environ. Health Sci., 12: 49 (**7 pages**).

Al-Amrani, W.A.; Lim, P.E.; Seng, C.E.; Ngah, W.S.W., (2014). Factors affecting biodecolorization of azo dyes and cod removal in anoxic – aerobic react operated sequencing batch reactor. J. Taiwan Inst. Chem. Eng., 45(2): 609-616 **(18 pages)**.

Almeida, E.J.R.; Corso, C.R., (2014). Comparative study of toxicity of azo dye procion red mx-5b following biosorption and biodegradation treatments with the fungi aspergillus niger and aspergillus terreus. Chemosphere., 112: 317-322 **(6 pages)**.

Ambrósio, S.; Vilar Júnior, J.; Da Silva, C.; Okada, K.; Nascimento, A.; Longo, R., (2012). A biosorption isotherm model for the removal of reactive azo dyes by inactivated mycelia of Cunninghamella elegans UCP542. Molecules., 17:452–62 (**11 pages**).

Amoozegar, M.A.; Hajighasemi, M.; Hamedi, J.; Asad, S.; Ventosa, A., (2011). Azo dye decolorization by halophilic and halotolerant microorganisms. Ann. Microbiol., 61(2): 217-230 **(14 pages)**.

An, Q.; Cheng, J.; Wang, Y.; Zhu, M., (2020). Performance and energy recovery of single and two stage biogas production from paper sludge: Clostridium thermocellumaugmentation and microbial community analysis. Renew. Energy., 148: 214–222 (**9 pages**).

Anwar, F.; Hussain, S.; Ramzan, S.; Hafeez, F.; Arshad, M.; Imran, M.; Abbas, N., (2014). Characterization of reactive red-120 decolorizing bacterial strain Acinetobacter junii FA10 capable of simultaneous removal of azo dyes and hexavalent chromium. Water Air Soil Pollut., 225(8): 2017 **(16 pages)**.

Artz, J.; White, S.; Zadvornyy, O.; Fugate, C.; Hicks, D.; Gauss, G.; Peters, J., (2015). Biochemical and structural properties of a thermostable mercuric ion reductase from Metallosphaera sedula. Front Bioeng Biotech., 3: 97 (**8 pages**).

Asgher, M.; Yasmeen, Q.; Iqbal, H., (2014). Development of novel enzymatic bioremediation process for textile industry effluents through response surface methodology. Ecol. Eng., 63:1-11 (**11 pages**).

Ayanda, O.S.; Nelana, S.M.; Naidoo, E.B., (2018). Ultrasonic degradation of aqueous phenolsulfonphthalein (PSP) in the presence of nano-Fe/H2O2. Ultrason sonochem., 47: 29-35 **(7 pages).**

Baccar, R.; Blánquez, P.; Bouzid, J.; Feki, M.; Attiya, H.; Sarra, M., (2011). Decolorization of a tannery dye: from fungal screening to bioreactor application. Biochem. Eng. J., 56(3):184-189 (**6 pages**).

Bahadur, N., Bhargava, N., (2019). Novel pilot scale photocatalytic treatment of textile and dyeing industry wastewater to achieve process water quality and enabling zero liquid discharge. J. Water Process. Eng., 32:100934 (**5 pages**).

Bai, Y.N.; Wang, X.N.; Zhang, F.; Wu, J.; Zhang, W.; Lu, Y.Z.; Zeng, R.J., (2020). High-rate anaerobic decolorization of methyl orange from synthetic azo dye wastewater in a methane-based hollow fiber membrane bioreactor. J. Hazard Mater., 121753 **(10 pages)**.

Bharathiraja, B.; Selvakumari, I.; Iyyappan, J.; Varjani, S., (2019). Itaconic acid: an effective sorbent for removal of pollutants from dye industry effluents. Curr. Opin. Environ. Sci. Health., 12: 6-17 **(12 pages).**

Balapure, K.H.; Jain, K.; Chattaraj, S.; Bhatt, N.S.; Madamwar, D., (2014). Cometabolic degradation of diazo dye-Reactive blue 160 by enriched mixed cultures BDN. J. Hazard Mater., 279: 85-95 **(11 pages)**.

Bazzi, W.; Abou Fayad, A.G.; Nasser, A.; Haraoui, L.P.; Dewachi, O.; Abou-Sitta, G.; Knapp, C., (2020). Heavy metal toxicity in armed conflicts potentiates AMR in A. baumannii by selecting for antibiotic and heavy metal co-resistance mechanisms. Front. Microbiol., 11:68 **(12 pages)**.

Benkhaya, S.; Cherkaoui, O.; Assouag, M.; Mrabet, S.; Rafik, M.; Harfi, A.E.L., (2016). Synthesis of a new asymmetric composite membrane with bi-component collodion: application in the ultra filtration of baths of reagent dyes of fabric rinsing / padding. J.

Mater. Environ. Sci., 7(12): 4556-4569 **(13 pages).**

Bharagava, R.N.; Mani, S.; Mulla, S.I.; Saratale, G.D., (2018). Degradation and decolourization potential of a ligninolytic enzyme producing Aeromonas hydrophila for crystal violet dye and its phytotoxicity evaluation. Ecotoxicol. Environ. Saf., 156:166-175 **(10 pages)**.

Bharathiraja, B.; Selvakumari, I.A.; Iyyappan, J.; Varjani, S., (2019). Itaconic acid: an effective sorbent for removal of pollutants from dye industry effluents. Curr. Opin. Environ. Sci. Health., 12: 6-17 (**11 pages**).

Bhardwaj, N.; Bhardwaj, S.K.; Mehta, J.; Mohanta, G.C.; Deep, A., (2016). Bacteriophage immobilized graphene electrodes for impedimetric sensing of bacteria (Staphylococcus arlettae). Anal. Biochem., 505:18-25 (**8 pages**).

Bilal, M.; Iqbal, H.M., (2019). Lignin peroxidase immobilization on ca-alginate beads and its dye degradation performance in a packed bed reactor system. Biocatal. Agric. Biotechnol., 20: 101205 **(8 pages).**

Britos, C.N.; Gianolini, J.E.; Portillo, H.; Trelles, J.A., (2018). Biodegradation of industrial dyes by a solvent, metal and surfactant-stable extracellular bacterial laccase. Biocatal. Agric. Biotechnol., 14: 221-227 **(7 pages).**

Brüschweiler, B.J.; Merlot, C., (2017). Azo dyes in clothing textiles can be cleaved into a series of mutagenic aromatic amines which are not regulated yet. Regul. Toxicol. Pharm., 88:214-226 **(13 pages)**.

Butani, S.; Mane, S., (2017). Coagulation/flocculation process for cationic, anionic dye removal using water treatment residuals–a review. Int. J. Sci. Technol. Mange., 6:121-125 (**5 pages**).

Cai, Z.; Zhang, W.; Ma, J.; Cai, J.; Li, S.; Zhu, X.; Zhao, X., (2015). Biodegradation of azo dye disperse orange S-RL by a newly isolated strain Acinetobacter sp. Srl8. Water Environ. Res., 87(6):516-523 **(8 pages)**.

Cervantes, F.J.; Dos Santos, A., (2011). Reduction of azo dyes by anaerobic bacteria: microbiological and biochemical aspects. Rev. Environ. Sci. Biotechnol., 10: 125-137 **(13 pages)**.

Cesaro, A.; Belgiorno, V.; Siciliano, A.; Guida, M., (2019). The sustainable recovery of the organic fraction of municipal solid waste by integrated ozonation and anaerobic digestion. Resour Conserv Recy., 141:390-397 (**8 pages**).

Chakraborty, S.; Basak, B.; Dutta, S.; Bhunia, B.; Dey, A., (2013). Decolorization and biodegradation of congo red dye by a novel white rot fungus Alternaria alternata CMERI F6. Bioresour. Technol., 147: 662–666 (**5 pages**).

Chan, G.F.; Rashid, N.A.A.; Chua, L.S.; Nasiri, R.; Ikubar, M.R.M., (2012). Communal microaerophilic–aerobic biodegradation of amaranth by novel NAR-2 bacterial consortium. Bioresour. Technol., 105: 48-59 **(12 pages)**.

Chellam, S.; Sari, M., (2016). Aluminum electrocoagulation as pretreatment during microfiltration of surface water containing NOM: A review of fouling, NOM, DBP, and virus control. J. Hazard. Mater., 304: 490-501 (**12 pages**).

Cinperi, N.C.; Ozturk, E.; Yigit, N.O.; Kitis, M., (2019). Treatment of woolen textile wastewater using membrane bioreactor, nanofiltration and reverse osmosis for reuse in production processes. J. Cleaner Prod., 223: 837-848 **(12 pages)**.

Cui, D.; Li, G.; Zhao, D.; Gu, X.; Wang, C.; Zhao, M., (2012). Microbial community structures in mixed bacterial consortia for azo dye treatment under aerobic and anaerobic conditions. J. Hazard. Mater., 221: 185-192 (**8 pages**).

Da Fontoura, J.; Rolim, G.; Mella, B.; Farenzena, M.; Gutterres, M., (2017). Defatted microalgal biomass as biosorbent for the removal of Acid Blue 161 dye from tannery effluent. J. Environ. Chem. Eng., 5(5): 5076-5084 (**9 pages**).

Dayi, B.; Kyzy, A.; Akdogan, H., (2019). Characterization of recuperating talent of white-rot fungi cells to dye-contaminated soil/water. Chin. J. Chem. Eng., 27(3): 634-638 (**5 pages**).

Das, A.; Mishra, S.; Verma, V.K., (2015). Enhanced biodecolorization of textile dye remazol navy blue using an isolated bacterial strain Bacillus pumilus HKG212 under improved culture conditions. J. Biochem. Tech., 6(3): 962-969 **(8 pages)**.

Di Dato, M.; Galešić, M.; Šimundić, P.; Andričević, R., (2019). A novel screening tool for the health risk in recreational waters near estuary: the carrying capacity indicator. Sci. Total Environ., 694: 133584 (**14 pages**).

Dicle, A.R.A.R.; Doganli, G.; Sensoy, T.; Bozbeyoglu, N.; Dogan, N., (2014). Investigation of decolorization of reactive violet 5r and remazol brillant orange 3r by bacillus sp. Dt16. J. Appl. Biol. Sci., 8(1): 68-72 **(5 pages)**.

Dolatabadi, M.; Mehrabpour, M.; Esfandyari, M.; Alidadi, H.; Davoudi, M., (2018). Modeling of simultaneous adsorption of dye and metal ion by sawdust from aqueous solution using of ANN and ANFIS. Chemom. Intell. Lab. Syst., 181:72-78 (**7 pages**).

Dong, H.; Guo, T.; Zhang, W.; Ying, H.; Wang, P.; Wang, Y.; Chen, Y., (2019). Biochemical characterization of a novel azoreductase from Streptomyces sp.: Application in eco-friendly decolorization of azo dye wastewater. Int. J. Biol. Macromol., 140: 1037-1046 (**10 pages**).

Elfarash, A.; Mawad, A.M.; Yousef, N.M.; Shoreit, A.A., (2017). Azoreductase kinetics and gene expression in the synthetic dyes-degrading Pseudomonas. Egypt. J. Basic Appl. Sci., 4(4): 315-322 **(8 pages).**

Elgarahy, A.; Elwakeel, K.; Elshoubaky, G.; Mohammad, S., (2019). Microwave-accelerated sorption of cationic dyes onto green marine algal biomass. Environ. Sci. Pollut. Res., 26(22): 22704-22722 **(19 pages).**

Eskandari, F.; Shahnavaz, B.; Mashreghi, M., (2019). Optimization of complete RB-5 azo dye decolorization using novel cold-adapted and mesophilic bacterial consortia. J. Environ. Manage., 241: 91-98 (**8 pages**).

Fajardo, A.S.; Rodrigues, R.F.; Martins, R.C.; Castro, L.M.; Quinta-Ferreira, R.M., (2015). Phenolic wastewaters treatment by electrocoagulation process using Zn anode. Chem. Eng. J., 275: 331–341 **(10 pages).**

Federigi, I.; Verani, M.; Donzelli, G.; Cioni, L.; Carducci, A., (2019). The application of quantitative microbial risk assessment to natural recreational waters: A review. Mar. Pollut. Bull., 144: 334-350 **(17 pages)**.

Franciscon, E.; Grossman, M.J.; Paschoal, J.A.R.; Reyes, F.G.R.; Durrant, L.R., (2012). Decolorization and biodegradation of reactive sulfonated azo dyes by a newly isolated Brevibacterium sp. strain VN-15. SpringerPlus., 1(1): 37 **(10 pages)**.

Garg, S.K.; Tripathi, M.; Singh, S.K.; Tiwari, J.K., (2012). Biodecolorization of textile dye effluent by Pseudomonas putida SKG-1 (MTCC 10510) under the conditions optimized for monoazo dye orange II color removal in simulated minimal salt medium. Int. Biodeterior. Biodegrad., 74:24-35 **(10 pages)**.

Ge, L.; Wang, W.; Peng, Z.; Tan, F.; Wang, X.; Chen, J.; Qiao, X., (2018). Facile fabrication of Fe@ MgO magnetic nanocomposites for efficient removal of heavy metal ion and dye from water. Powder Technol., 326: 393-401 (**9 pages**).

Ghodake, G.; Jadhav, U.; Tamboli, D.; Kagalkar, A.; Govindwar, S., (2011). Decolorization of textile dyes and degradation of mono-azo dye amaranth by Acinetobacter calcoaceticus NCIM 2890. Indian J. Microbiol., 51(4): 501-508 (**8 pages**).

Ghosh, A.; Ghosh Dastidar, M.; Sreekrishnan, T.R., (2016). Recent advances in bioremediation of heavy metals and metal complex dyes. J. Environ. Eng., 142(9): C4015003-1 (**14 pages**).

Ghuge, S.; Saroha, A., (2018). Catalytic ozonation of dye industry effluent using mesoporous bimetallic Ru-Cu/SBA-15 catalyst. Process. Saf. Environ., 118:125-132 (**8 pages**).

Giovanella, P.; Vieira, G.; Otero, I.; Pellizzer, E.; De Jesus Fontes, B.; Sette, L., (2020). Metal and organic pollutants bioremediation by extremophile microorganisms. J. Hazard. Mater., 382: 121024 (**14 pages**).

Gomes, C.; Piccin, J.; Gutterres, M., (2016). Optimizing adsorption parameters in tannery-dye-containing effluent treatment with leather shaving waste. Process. Saf. Environ., 99: 98-106 (**9 pages**).

González, R.; Villagómez, R.; Madariaga, A.; Castro, J.; González, C., (2018). Biological Consortia Designed for Laccase Production and Dye Removal. Preprints., 2018040264 (**16 pages**).

Guadie, A.; Tizazu, S.; Melese, M.; Guo, W.; Ngo, H.; Xia, S., (2017). Biodecolorization of textile azo dye using Bacillus sp. strain CH12 isolated from alkaline lake. Biotechnol. Rep., 15: 92-100 (**8 pages**).

Guan, R.; Yuan, X.; Wu, Z.; Jiang, L.; Li, Y.; Zeng, G., (2018). Principle and application of hydrogen peroxide based advanced oxidation processes in activated sludge treatment: A review. Biochem. Eng. J., 339: 519-530 **(12 pages)**.

Guo, G.; Li, X.; Tian, F.; Liu, T.; Yang, F.; Ding, K.; Wang, C., (2020). Azo dye decolorization by a halotolerant consortium under microaerophilic conditions. Chemosphere., 244: 125510 (**8 pages**).

Gupta, V.K.; Khamparia, S.; Tyagi, I.; Jaspal, D.; Malviya, A., (2015). Decolorization of mixture of dyes: A critical review. Global J. Environ. Sci. Manage., 1 (1): 71-94 **(24 pages)**.

Gürses, A.; Açıkyıldız, M.; Güneş, K.; Gürses, M.S., (2016). Dyes and Pigments. Springer, Cham. **(83 pages).**

Hongsawat, P.; Vangnai, A.S., (2011). Biodegradation pathways of chloroanilines by Acinetobacter baylyi strain GFJ2.J. Hazard. Mater., 186(2-3):1300-1307 **(8 pages)**.

Huang, G.; Wang, W.; Liu, G., (2015). Simultaneous chromate reduction and azo dye decolourization by Lactobacillus paracase CL1107 isolated from deep sea sediment. J. Environ. Manage., 157: 297-302 (**5 pages**).

Huët, M.A.L.; Puchooa, D., (2017). Bioremediation of heavy metals from aquatic environment through microbial processes: A potential role for probiotics. J. Appl. Biol. Biotechnol., 5(6): 14-23 **(10 pages)**.

Ilnicka, A.; Kamedulski, P.; Aly, H.; Lukaszewicz, J., (2020). Manufacture of activated carbons using Egyptian wood resources and its application in oligothiophene dye adsorption. Arab. J. Chem., 13:5284-5291 (**8 pages**).

Imran, M.; Crowley, D.E.; Khalid, A.; Hussain, S.; Mumtaz, M.W; Arshad, M., (2015). Microbial biotechnology for decolorization of textile wastewaters. Rev. Environ. Sci. Biotechnol., 14(1): 73-92 **(20 pages)**.

Jaria, G.; Silva, C.; Ferreira, C.; Otero, M.; Calisto, V., (2017). Sludge from paper mill effluent treatment as raw material to produce carbon adsorbents: an alternative waste management strategy. J. Environ. Manage., 188: 203-211 (**9 pages**).

Johnson, N.W.; Gedalanga, P.B.; Zhao, L.; Gu, B.; Mahendra, S., (2020). Cometabolic biotransformation of 1, 4-dioxane in mixtures with hexavalent chromium using attached and planktonic bacteria. Sci. Total Environ., 706: 135734 **(10 pages)**.

Kakkar, S.; Malik, A.; Gupta, S., (2018). Treatment of pulp and paper mill effluent using low cost adsorbents: an overview. J. Appl. Nat. Sci.,10:695–704 (**10 pages**).

Katheresan, V.; Kansedo, J.; Lau, S., (2018). Efficiency of various recent wastewater dye removal methods: a review. J. Environ. Chem. Eng., 6(4): 4676-4697 (**22 pages**).

Khan, N.; Bhadra, B.; Jhung, S., (2018). Heteropoly acid-loaded ionic liquid@ metalorganic frameworks: effective and reusable adsorbents for the desulfurization of a liquid model fuel. Chem. Eng. J., 334: 2215-2221 (**7 pages**).

Kim, D.; Jo, W., (2019). Sustainable treatment of harmful dyeing industry pollutants using SrZnTiO3/g-C3N4 heterostructure with a light source-dependent charge transfer mechanism. Appl. Catal B-Environ., 242: 171-177 (**7 Pages**).

Kertesz, S.; Cakl, J.; Jirankova, H., (2014). Submerged hollow fiber microfiltration as a part of hybrid photocatalytic process for dye wastewater treatment. Desalination. 343: 106-112 **(7 pages)**.

Khadim, H.J.; Ammar, S.H.; Ebrahim, S.E., (2019). Biomineralization based remediation of cadmium and nickel contaminated wastewater by ureolytic bacteria isolated from barn horses soil. Environ. Technol. Innovation., 14: 100315 **(10 pages)**.

Khalid, A.; Kausar, F.; Arshad, M.; Mahmood, T.; Ahmed, I., (2012). Accelerated decolorization of reactive azo dyes under saline conditions by bacteria isolated from Arabian seawater sediment. Appl. Microbiol. Biotechnol., 96: 1599-1606 **(8 pages)**.

Khan, S.; Malik, A., (2018). Toxicity evaluation of textile effluents and role of native soil bacterium in biodegradation of a textile dye. Environ. Sci. Pollut. Res. Int., 25 (5): 4446-4458 **(13 pages)**.

Khan, Z.; Kunal, M.; Soni, A.; Madamwar, D., (2014). Microaerophilic degradation of sulphonated azo dye Reactive Red 195 by bacterial consortium AR1 through cometabolism. Int. Biodeterior. Biodegrad., 94: 167-175 **(9 pages)**.

Khandare, R.V.; Kabra, A.N.; Tamboli, D.P.; Govindwar, S.P., (2011). The role of Aster amellus Linn. in the degradation of a sulfonated azo dye Remazol Red: a phytoremediation strategy. Chemosphere. 82(8): 1147-1154 **(8 pages)**.

Kumar, V.; Suraj, P.; Ghosh, P., (2019). Optimization of COD Removal by Advanced Oxidation Process through Response Surface Methodology from Pulp & Paper Industry Wastewater. J. Sci. Ind. Res., 78:386-390 (**5 pages**).

Kuppusamy, S.; Sethurajan, M.; Kadarkarai, M.; Rajasekar, A., (2016). Biodecolourization of textile dyes by novel, indigenous Pseudomonas stutzeri MN1 and Acinetobacter baumannii MN3. J. Environ. Chem. Eng., 5(1): 716-724 **(9 pages)**.

Kurade, M.B.; Waghmode, T.R.; Khandare, R.V.; Jeon, B.H.; Govindwar, S.P., (2016). Biodegradation and detoxification of textile dye Disperse Red 54 by Brevibacillus laterosporus and determination of its metabolic fate. J. Biosci. Bioeng., 121(49): 442-449**(8 pages)**.

Latif, A.; Sheng, D.; Sun, K.; Si, Y.; Azeem, M.; Abbas, A.; Bilal, M., (2020). Remediation of heavy metals polluted environment using Fe-based nanoparticles: Mechanisms, influencing factors, and environmental implications. Environ. Pollut., 114728 **(15 pages)**.

Lee, T.; Pang, S.; Abraham, S.; Coombs, G.W., (2019). Antimicrobial-resistant CC17 Enterococcus faecium: the past, the present and the future. J. Glob. Antimicrob. Resist., 16: 36-47 **(12 pages)**.

Li, Z.; Li, L.; Hu, D.; Gao, C.; Xiong, J.; Jiang, H.; Li, W., (2019). Efficient removal of heavy metal ions and organic dyes with cucurbit [8] uril-functionalized chitosan. J. Colloid Interf Sci., 539: 400-413 (**14 pages**).

Lin, Y.; Wang, D.; Wang, T., (2012). Ethanol production from pulp & paper sludge and monosodium glutamate waste liquor by simultaneous saccharification and fermentation in batch condition. Chem. Eng., 191: 31–37 (**7 pages**).

Lin, Y.; Liang, J.; Zeng, C.; Wang, D.; Lin, H., (2017). Anaerobic digestion of pulp and paper mill sludge pretreated by microbial consortium OEM1 with simultaneous degradation of lignocellulose and chlorophenols. Renew. Energy., 108: 108–115 (**8 pages**).

Liu, S.; Song, H.; Wei, S.; Liu, Q.; Li, X.; & Qian, X., (2015). Effect of direct electrical stimulation on decolorization and degradation of azo dye reactive brilliant red X3B in biofilm-electrode reactors. Biochem. Eng. J., 93:294-302 **(9 pages)**.

Liu, W.; Liu, C.; Liu, L.; You, Y.; Jiang, J.; Zhou, Z.; Dong, Z., (2017). Simultaneous decolorization of sulfonated azo dyes and reduction of hexavalent chromium under high salt condition by a newly isolated salt-tolerant strain Bacillus circulans BWL1061. Ecotox. Environ. Safe., 141: 9-16 (**8 pages**).

Lončar, N.; Gligorijević, N.; Božić, N.; Vujčić, Z., (2014). Congo red degrading laccases from Bacillus amyloliquefaciens strains isolated from salt spring in Serbia. Int. Biodeterior. Biodegrad., 91:18-23 **(6 pages)**.

Mahmood, S.; Khalid, A.; Mahmood, T.; Arshad, M.; Ahmad, R., (2013). Potential of newly isolated bacterial strains for simultaneous removal of hexavalent chromium and reactive black-5 azo dye from tannery effluent. J. Chem. Technol. Biotechnol., 88(8): 1506-1513 (**8 pages**).

Mahmood, S.; Khalid, A.; Arshad, M.; Mahmood, T.; Crowley, D.E., (2016). Detoxification of azo dyes by bacterial oxidoreductase enzymes. Crit. Rev. Biotechnol., 36(4): 639-651 **(13 pages)**.

Martínez-López, S.; Lucas-Abellán, C.; Serrano-Martínez, A.; Mercader-Ros, M.; Cuartero, N.; Navarro, P.; Gómez-López, V., (2019). Pulsed light for a cleaner dyeing industry: Azo dye degradation by an advanced oxidation process driven by pulsed light. J. Clean. Prod., 217: 757-766 (**10 pages**).

Maqbool, Z., (2016). Characterization and application of dye decolorizing metal tolerant bacterial community for treatment of textile wastewater (Doctoral dissertation, Gc University, Faisalabad) (**222 pages**).

Martorell, M.; Pajot, H.; De Figueroa; L., (2012). Dye-decolourizing yeasts isolated from Las Yungas rainforest Dye assimilation and removal used as selection criteria. Int. Biodeter. Biodegr., 66:25–32 (**8 pages**).

Meerbergen, K.; Willems, K.; Dewil, R.; Van Impe, J.; Appels, L.; Lievens, B., (2018). Isolation and screening of bacterial isolates from wastewater treatment plants to decolorize azo dyes. J. Biosci. Bioeng., 125(4): 448-456 (**9 pages**).

Mincea, M.; Patrulea, V.; Negrulescu, A.; Szabo, R.; Ostafe, V., (2013). Adsorption of three commercial dyes onto chitosan beads using spectrophotometric determination and a multivariate calibration method. J. Water Resour. Prot., 5: 446-457 **(12 pages)**.

Misal, S.; Lingojwar, D.; Shinde, R.; Gawai, K., (2011). Purification and characterization of azoreductase from alkaliphilic strain Bacillus badius. Process. Biochem., 46(6): 1264-1269 (**6 pages**).

Mittal, H.; Alhassan, S.M.; Ray, S.S., (2018). Efficient organic dye removal from wastewater by magnetic carbonaceous adsorbent prepared from corn starch. J. Environ. Chem. Eng., 6(6): 7119-7131 **(13 pages)**.

Mondal, P.; Baksi, S.; Bose, D., (2017). Study of environmental issues in textile industries and recent wastewater treatment technology. World Sci. News., 61 (2): 98-109 **(12 pages)**.

Morais, I.; Silva, C.; Borges, C., (2016). Aerobic granular sludge to treat paper mill effluent: organic matter removal and sludge filterability. Desalin. Water Treat., 57: 8119–8126 (**8 pages**).

Nanda, M.; Kumar, V.; Sharma, D.K., (2019). Multimetal tolerance mechanisms in bacteria: The resistance strategies acquired by bacteria that can be exploited to 'clean-up' heavy metals contaminants from water. Aquat. Toxicol., 212: 1-10 **(10 pages).**

Ngulube, T.; Gumbo, J.R.; Masindi, V.; Maity, A., (2017). An update on synthetic dyes adsorption onto clay based minerals: a state-of-art review. J. Environ. Manage., 191:35–57 (**23 pages**).

Ning, X.; Yang, C.; Wang, Y.; Yang, Z.; Wang, J.; Li, R., (2014). Decolorization and biodegradation of the azo dye Congo red by an isolated Acinetobacter baumannii YNWH 226. Biotechnol. Bioproc. Eng., 19(4): 687-695 **(9 pages)**.

Oon, Y.S.; Ong, S.A.; Ho, L.N.; Wong, Y.S.; Oon, Y.L.; Lehl, H.K.; Nordin, N., (2017). Microbial fuel cell operation using monoazo and diazo dyes as terminal electron acceptor for simultaneous decolourization and bioelectricity generation. J. Hazard. Mater., 325: 170-177 **(8 pages)**.

Orts, F.; del Río, A.I.; Molina, J.; Bonastre, J.; Cases, F., (2018). Electrochemical treatment of real textile wastewater: Trichromy Procion HEXL®. J. Electroanal. Chem., 808: 387-394 **(8 pages)**.

Oturkar, C.; Patole, M.S.; Gawai, K.R.; Madamwar, D., (2013). Enzyme based cleavage strategy of Bacillus lentus BI377 in response to metabolism of azoic recalcitrant. Bioresour. Technol., 130: 360-365 (**6 pages**).

Öztürk, A.; Bayol, E.; Abdullah, M., (2020). Characterization of the biosorption of fast black azo dye K salt by the bacterium Rhodopseudomonas palustris 51ATA strain. Electron. J. Biotechnol., 46: 22-29 (**8 pages**).

Pavithra, K.G.; Jaikumar, V., (2019). Removal of colorants from wastewater: A review on sources and treatment strategies. J. Ind. Eng. Chem., 75:1-19 (**19 pages**).

Pietruk, K.; Piątkowska, M.; Olejnik, M., (2019). Electrochemical reduction of azo dyes mimicking their biotransformation to more toxic products. J. Vet. Res., 63(3): 433-438 **(6 pages)**.

Pourrahim, S.; Salem, A.; Salem, S.; Tavangar, R., (2020). Application of solid waste of ductile cast iron industry for treatment of wastewater contaminated by reactive blue dye via appropriate nano-porous magnesium oxide. Environ. Pollut., 256: 113454 (**10 pages**).

Prasad, A.A.; Satyanarayana, V.S.V.; Rao, K.B., (2013). Biotransformation of direct blue 1 by a moderately halophilic bacterium Marinobacter sp. strain HBRA and toxicity assessment of degraded metabolites. J. Hazard. Mater., 262: 674-684 **(11 pages)**.

Priya, B.; Uma, L.; Ahamed, A.; Subramanian, G.; Prabaharan, D., (2011). Ability to use the diazo dye C. I. Acid Black 1 as a nitrogen source by the marine cyanobacterium Oscillatoria curviceps BDU92191. Bioresour. Technol., 102:7218–7223 (**5 pages**).

Qu, W.; Liu, T.; Wang, D.; Hong, G.; Zhao, J., (2018). Metagenomics-based discovery of malachite green-degradation gene families and enzymes from mangrove sediment. Front. Microbiol., 9: 2373-2382 (**10 pages**).

Rathod, J.; Dhebar, S.; Archana, G., (2017). Efficient approach to enhance whole cell azo dye decolorization by heterologous overexpression of Enterococcus sp. L2 azoreductase (azoA) and Mycobacterium vaccae formate dehydrogenase (FDH) in different bacterial systems. Int. Biodeterior. Biodegrad., 124: 91-100 **(9 pages)**.

Rehman, K.; Shahzad, T.; Sahar, A.; Hussain, S.; Mahmood, F.; Siddique, M.H., (2018). Effect of Reactive Black 5 azo dye on soil processes related to C and N cycling. PeerJ., 6: e4802. **(14 pages).**

Rekik, H.; Jaouadi, N.Z.; Bouacem, K.; Zenati, B.; Kourdali, S.; Badis, A.; Jaouadi, B., (2019). Physical and enzymatic properties of a new manganese peroxidase from the white-rot fungus Trametes pubescens strain i8 for lignin biodegradation and textile-dyes biodecolorization. Int. J. Biol. Macromol., 125:514-525 **(22 pages)**.

Ribera Pi, J. (2019). Hybrid systems for wastewater treatment in the framework of circular economy: coupling biological and membrane technologies for a sustainable water cycle (Doctoral dissertation, Universitat Politècnica de Catalunya) **(247 pages)**.

Sabaruddin, M.F.; Nor, M.H.M.; Mubarak, M.F.M.; Rashid, N.A.A.; Far, C.G.; Youichi, S.; Ibrahim, N., (2018). Biodecolourisation of acid red 27 Dye by Citrobacter freundii A1 and Enterococcus casseliflavus C1 bacterial consortium. Mal. J. Fund., 14 (2): 202-207 **(6 pages).**

Sahasrabudhe, M.M.; Saratale, R.G.; Saratale, G.D.; Pathade, G.R., (2014). Decolorization and detoxification of sulfonated toxic diazo dye CI Direct Red 81 by Enterococcus faecalis YZ 66. J. Environ. Health Sci.,12(1):151 **(13 pages).**

Saratale, R.G.; Saratale, G.D.; Chang, J.S.; Govindwar, S.P., (2011).; Bacterial decolorization and degradation of azo dyes: a review. J. Taiwan Inst. Chem. Eng., 42(1): 138-157 **(20 pages)**.

Saravanan, C.; Rajesh, R.; Kaviarasan, T.; Muthukumar, K.; Kavitake, D.; Shetty, P.H., (2017). Synthesis of silver nanoparticles using bacterial exopolysaccharide and its application for degradation of azo-dyes. Biotechnol. Rep., 15: 33-40 **(8 pages)**.

Sardar, U.R.; Bhargavi, E.; Devi, I.; Bhunia, B.; Tiwari, O. N., (2018). Advances in exopolysaccharides based bioremediation of heavy metals in soil and water: a critical review. Carbohydr. Polym., 199: 353-364 (**12 pages**).

Sarkar, S.; Banerjee, A.; Chakraborty, N.; Soren, K.; Chakraborty, P.; Bandopadhyay, R., (2020). Structural-functional analyses of textile dye degrading azoreductase, laccase and peroxidase: A comparative in silico study. Electron. J. Biotechnol., 43: 48-54 **(7 pages)**.

Shah, M., (2014). Effective treatment systems for azo dye degradation: a joint venture between physico-chemical and microbiological process. Int. J. Environ. Bioremediat. Biodegrad., 2(5): 231-242 **(12 pages)**.

Shamraiz, U.; Hussain, R.A.; Badshah, A.; Raza, B.; Saba, S., (2016). Functional metal sulfides and selenides for the removal of hazardous dyes from Water. J. Photochem. Photobiol. B., 159: 33-41 **(9 pages)**.

Sharma, A.; Gupta, G.; Ahmad, T.; Kaur, B.; Hakeem, K.R., (2020). Tailoring cellular metabolism in lactic acid bacteria through metabolic engineering. J. Microbiol. Methods., 170: 105862. **(14 pages).**

Singh, R.P.; Singh, P.K.; Singh, R.L., (2014). Bacterial decolorization of textile azo dye acid orange by Staphylococcus hominis RMLRT03. Toxicol int., 21(2): 160-166 **(7 pages)**.

Singh, R.L.; Singh, P.K.; Singh, R.P., (2015). Enzymatic decolorization and degradation of azo dyes–A review. Int. Biodeterior. Biodegrad., 104: 21-31 **(11 pages)**.

Singh, G.; Kaur, S.; Khatri, M.; Arya, S., (2019). Biobleaching for pulp and paper industry in India: Emerging enzyme technology. Biocatal. Agric. Biotechnol., 17: 558-565 (**8 pages**).

Solano A.M.S.; Garcia-Segura S.; Martínez-Huitle C.A.; Brillas E., (2015). Degradation of acidic aqueous solutions of the diazo dye Congo Red by photo-assisted electrochemical processes based on Fenton's reaction chemistry. Appl. Catal B., 168-169: 559–571 **(13 pages).**

Solís, M.; Solís, A.; Pérez, H.; Manjarrez, N.; Flores, M., (2012). Microbial decolouration of azo dyes: a review. Process Biochem., 47(12): 1723-1748 (**26 pages**).

Soni, R.; Bhatt, N.S.; Modi, H.A.; Acharya, P.B., (2016). Decolorization, degradation and subsequent toxicity assessment of reactive red 35 by Enterococcus gallinarum. Curr. Biotechnol., 5(4): 25-336 **(12 pages)**.

Sun, J.; Kweon, O.; Jin, J.; He, G.X.; Li, X.; Cerniglia, C.E.; Chen, H., (2017). Mutation network-based understanding of pleiotropic and epistatic mutational behavior of Enterococcus faecalis FMN-dependent azoreductase. Biochem. Biophys., 12: 240-244 (**5 Pages**).

Talaiekhozani, A.; Mosayebi, M.; Fulazzaky, M.; Eskandari, Z.; Sanayee, R., (2020). Combination of TiO2 microreactor and electroflotation for organic pollutant removal from textile dyeing industry wastewater. Alex. Eng. J., 59(2): 549-563 (**15 pages**).

Talaiekhozani, A.; Rezania, S., (2017). Application of photosynthetic bacteria for removal of heavy metals, macro-pollutants and dye from wastewater: A review. J. Water Process. Eng., 19:312-321 **(10 pages).**

Tamboli, D.P.; Kagalkar, A.N.; Jadhav, M.U.; Jadhav, J.P.; Govindwar, S. P., (2010). Production of polyhydroxyhexadecanoic acid by using waste biomass of Sphingobacterium sp. ATM generated after degradation of textile dye Direct Red 5B. Bioresour. Technol., 101(7): 2421-2427 **(7 pages)**.

Tee, H.C.; Lim, P.E.; Seng, C.E.; Nawi, M.A.M.; Adnan, R., (2015). Enhancement of azo dye Acid Orange 7 removal in newly developed horizontal subsurface-flow constructed wetland. J. Environ. Manage., 147: 349-355 **(7 pages)**.

Thanavel, M.; Bankole, P.O.; Kadam, S.; Govindwar, S.P.; Sadasivam, S.K., (2019). Desulfonation of the textile azo dye acid fast yellow mr by newly isolated Aeromonas hydrophila sk16. Water Resour. Ind., 22: 100116 **(9 Pages).**

Thu, W.P.; Sinwat, N.; Bitrus, A.A.; Angkittitrakul, S.; Prathan, R.; Chuanchuen, R., (2019). Prevalence, antimicrobial resistance, virulence gene, and class 1 integrons of Enterococcus faecium and Enterococcus faecalis from pigs, pork and humans in Thai-Laos border provinces. J. Global Antimicrob. Resist., 18:130-138 **(9 pages)**.

Tišma, M.; Šalić, A.; Planinić, M.; Zelić, B.; Potočnik, M.; Šelo, G.; Bucić-Kojić, A., (2020). Production, characterisation and immobilization of laccase for an efficient aniline-based dye decolourization. J. Water Process. Eng., 36: 101327 **(9 pages).**

Unnikrishnan, S.; Khan, M.H.; Ramalingam, K., (2018). Dye-tolerant marine Acinetobacter baumannii-mediated biodegradation of reactive red. Water Sci. Eng., 11(4): 265-275 **(11 pages)**.

Vignaroli, C.; Pasquaroli, S.; Citterio, B.; Di Cesare, A.; Mangiaterra, G.; Fattorini, D.; Biavasco, F., (2018). Antibiotic and heavy metal resistance in enterococci from coastal marine sediment. Environ. Pollut., 237: 406-413 **(8 pages)**.

Vikrant, K.; Giri, B. S.; Raza, N.; Roy, K.; Kim, K.H.; Rai, B.N.; Singh, R.S., (2018). Recent advancements in bioremediation of dye: current status and challenges. Bioresour. Technol., 253: 355-367 **(13 pages)**.

Vuthiganond, N.; Nakpathom, M.; Mongkholrattanasit, R., (2018). Metal-free dyeing of cotton fabric using mangrove bark polyphenols via azoic dyeing. Fibers Polym., 19(12): 2524-2532 **(9 pages)**.

Walter, T.; Klim, J.; Jurkowski, M.; Köhling, I.; Słodownik, M.; Zielenkiewicz, U., (2020). Plasmidome of an environmental Acinetobacter lwoffii strain originating from a former gold and arsenic mine. Plasmid., 110: 102505 **(17 pages).**

Wang, N.; Chu, Y.; Wu, F.; Zhao, Z.; Xu, X., (2017). Decolorization and degradation of congo red by a newly isolated white rot fungus, Ceriporia lacerata, from decayed mulberry branches. Int. Biodeter. Biodegr. 117: 236–244 (**8 pages**).

Wang, Y.; Jiang, L.; Shang, H.; Li, Q.; Zhou, W., (2020). Treatment of azo dye wastewater by the self-flocculating marine bacterium Aliiglaciecola lipolytica. Environ. Technol. Inno., 19: 100810 (**12 pages**).

Wardman, R.H., (2017). An introduction to textile coloration: principles and practice. Hoboken, N.J: John Wiley Sons. **(376 pages).**

Wu, Y.; Li, T.; Yang, L., (2012). Mechanisms of removing pollutants from aqueous solutions by microorganisms and their aggregates: a review. Bioresour. Technol., 107: 10 -18 (**9 pages**).

Wu, J.W.; Wu, C.R.; Zhou, C. S.; Dong, L.L.; Liu, B.F.; Xing, D.F.; Yang, S.S.; Fan, J.N.; Feng, L. P.; Cao, G.L.; You, S.J., (2020). Fate and removal of antibiotic resistance genes in heavy metals and dye co-contaminated wastewater treatment system amended with β-cyclodextrin functionalized biochar. Sci. Total Environ., 723: 137991 **(8 pages)**.

Xie, W.; Pakdel, E.; Liang, Y.; Liu, D.; Sun, L.; Wang, X., (2020). Natural melanin/TiO2 hybrids for simultaneous removal of dyes and heavy metal ions under visible light. J. Photochem. Photobiol., 389: 112292 (**10 pages**).

Xu, F.; Mou, Z.; Geng, J.; Zhang, X.; Li, C., (2016). Azo dye decolorization by a halotolerant exoelectrogenic decolorizer isolated from marine sediment. Chemosphere., 158: 30-36 (**7 pages**).

Yagub, M.; Sen T.K.; Afroze, A.; Ang, H., (2014). Dye and its removal from aqueous solutions by adsorption: A review. Adv. Colloid Interface Sci., 209: 172-184 (**13 pages**).

Yang, S.; Li, W.; Zhang, H.; Wen, Y.; Ni, Y., (2019). Treatment of paper mill wastewater using a composite inorganic coagulant prepared from steel mill waste pickling liquor. Sep. Purif. Technol., 209: 238-245 (**8 pages**).

Zhang, J.; Zeng, Y.; Liu, B.; Deng, X., (2020). MerP/MerT-mediated mechanism: a different approach to mercury resistance and bioaccumulation by marine bacteria. J. Hazard. Mater., 388: 122062 **(10 pages)**.

Zhang, L.; Pan, J.; Liu, L.; Song, K.; Wang, Q., (2019). Combined physical and chemical activation of sludge-based adsorbent enhances Cr (VI) removal from wastewater. J. Cleaner Prod., 238: 117904 **(9 pages).**

Zhao, B.; Shang, Y.; Xiao, W.; Dou, C.; Han, R., (2014). Adsorption of Congo red from solution using cationic surfactant modified wheat straw in column model. J. Environ. Chem. Eng., 2: 40-45 **(6 pages)**.

Zhong, C.; Sun, S.; Zhang, D.; Liu, L.; Zhou, S.; Zhou, J., (2020). Production of a bioflocculant from ramie biodegumming wastewater using a biomass-degrading strain and its application in the treatment of pulping wastewater. Chemosphere., 253: 126727 (**9 pages**).

Zhuang, M.; Sanganyado, E.; Li, P.; Liu, W., (2019). Distribution of microbial communities in metal-contaminated nearshore sediment from Eastern Guangdong, China. Environ. Pollut., 250: 482–492 **(11 pages)**.

Zhuang, M.; Sanganyado, E.; Zhang, X.; Xu, L.; Zhu, J.; Liu, W.; Song, H., (2020). Azo dye degrading bacteria tolerant to extreme conditions inhabit nearshore ecosystems: Optimization and degradation pathways. J. Environ. Manage., 261: 110222 **(23 pages)**.

AUTHOR (S) BIOSKETCHES

Manjarrez Paba, G., PhD candidate, Clinical and Environmental Microbiology Group. University of Cartagena, Faculty of Natural and Exact Sciences, Cartagena, Colombia and researcher in GIA Environmental Research Group, Faculty of Engineering, Fundacion Universitaria Tecnologico Comfenalco, Cartagena, Colombia. Email: *gmanjarrezp@unicartagena.edu.co*

Baldiris Ávila, R., Ph.D., Professor, Clinical and Environmental Microbiology Group. University of Cartagena, Faculty of Natural and Exact Sciences, Cartagena, Colombia. Email: *rbaldirisa@unicartagena.edu.co*

Baena Baldiris, D., B.Sc. Student, Clinical and Environmental Microbiology Group. University of Cartagena, Faculty of Natural and Exact Sciences, Cartagena, Colombia. Email: *dayanabaenabaldiris@icloud.com*

HOW TO CITE THIS ARTICLE

Manjarrez Paba, G.; Baldiris Ávila, R.; Baena Baldiris, D., (2021). Application of environmental bacteria as potential methods of azo dye degradation systems. Global J. Environ. Sci. Manage., 7(1): 131-154.

DOI: 10.22034/gjesm.2021.01.10

url: https://www.gjesm.net/article_43276.html

PUBLICATION ETHICS

The ethical policy of GJESM is based on the Committee on Publication Ethics (COPE) guidelines and complies with International Committee of GJESM Editorial Board codes of conduct. Readers, authors, reviewers and editors should follow these ethical policies once working with GJESM. The ethical policy of GJESM is liable to determine which of the typical research papers or articles submitted to the journal should be published in the concerned issue. For information on this matter in publishing and ethical guidelines please visit http://publicationethics.org

Duties and Responsibilities of Publishers

1. GJESM is committing to ensure that editorial decisions on manuscript submissions are the final.
2. GJESM is promising to ensure that the decision on manuscript submissions is only made based on professional judgment and will not be affected by any commercial interests.
3. GJESM is committing to maintain the integrity of academic and research records.
4. GJESM is monitoring the ethics by Editor-in-Chief, Associate Editors, Editorial Board Members, Reviewers, Authors, and Readers.
5. GJESM is always checking the plagiarism and fraudulent data issues involving in the submitted manuscript.
6. GJESM is always willing to publish corrections, clarifications and retractions involving its publications as and when needed.

Duties and Responsibilities of Editors

1. The Editors of the journal should have the full authority to reject/accept a manuscript.
2. The Editors of the journal should maintain the confidentiality of submitted manuscripts under review or until they are published.
3. The Editor-in-Chief should take a decision on submitted manuscripts, whether to be published or not with other editors and reviewers
4. The Editors of the journal should preserve the anonymity of reviewers.
5. The Editors of the journal should disclose and try to avoid any conflict of interest.
6. The Editors of the journal should maintain academic integrity and strive to meet the needs of readers and authors.
7. The Editors of the journal should be willing to investigate plagiarism and fraudulent data issues and willing to publish corrections, clarifications, retractions, and apologies when needed.
8. The Editors of the journal should have the limit themselves only to the intellectual content.
9. The Editors of the journal must not disclose any information about submitted manuscripts to anyone other than the corresponding author, reviewers, potential reviewers, other editorial advisers, and the publisher, as appropriate.
10. Unpublished materials disclosed in a submitted paper will not be used by the editor or the members of the editorial board for their own research purposes without the author's explicit written consent.

Duties and Responsibilities of Reviewers

1. The Reviewers of the journal should assist the Editors in taking the decision for publishing the submitted manuscripts.
2. The Reviewers should maintain the confidentiality of manuscripts, which they are invited to review.
3. The Reviewers should provide comments in time that will help editors to make decision on the submitted manuscript to be published or not.
4. The Reviewers are bound to treat the manuscript received for peer reviewing as confidential, and must not use the information obtained through peer review for personal advantage.
5. The Reviewers comments against each invited manuscript should be technical, professional and objective.
6. The Reviewers should not review the manuscripts in which they have found conflicts of interest with any of the authors, companies, or institutions.
7. The Reviewers should disclose and try to avoid any conflict of interest.

Duties and Responsibilities of Authors

1. Manuscripts must be submitted only in English and should be written according to sound grammar and proper terminology.
2. Manuscripts must be submitted with the understanding that they have not been published elsewhere (except in the form of an abstract or as part of a published lecture, review, or thesis) and are not currently under consideration by another journal published by or any other publisher.
3. The submitting (corresponding) author is responsible for ensuring that the manuscript article's publication has been approved by all the other coauthors.
4. In order to sustain the peer review system, authors have an obligation to participate in peer review process to evaluate manuscripts from others.
5. It is also the authors' responsibility to ensure that the manuscripts emanating from a particular institution are submitted with the approval of the necessary institution.
6. It is a condition for submission of a manuscript that the authors permit editing of the paper for readability.
7. Authors are requested to clearly identify who provided financial support for the conduct of research and/or preparation of the manuscript and briefly describe the role of the funder/sponsor in any part of the work.
8. A copy right release and conflict of interest disclosure form must be signed by the corresponding author in case of multiple authorships, prior to the acceptation of the

manuscript, by all authors, for publication to be legally responsible towards the Journal ethics and privacy policy.

9. Under open access license, authors retain ownership of the copyright for their content, but allow anyone to download, reuse, reprint, modify, distribute, and/ or copy the content as long as the original authors and source are cited properly.
10. All authors have agreed to allow the corresponding author to serve as the primary correspondent with the editorial office, to review the edited manuscript and proof.
11. When author(s) discovers a significant error or inaccuracy in his/her own published work, it is the author's obligation to promptly notify the journal editor or publisher to retract or correct the manuscript.
12. All authors must know that that the submitted manuscripts under review or published with GJESM are subject to screening using Plagiarism Prevention Software. Plagiarism is a serious violation of publication ethics.

Violation of Publication Ethics

1. **Plagiarism:** Plagiarism is intentionally using someone else's ideas or other original material as if they are one's own. Copying even one sentence from someone else's manuscript, or even one of your own that has previously been published, without proper citation is considered by GJESM Journals as plagiarism. All manuscripts under review or published with GJESM are subject to screening using plagiarism prevention software. Thus, plagiarism is a serious violation of publication ethics. The development of CrossCheck is a service that helps editors to verify the originality of papers. CrossCheck is powered by the Ithenticate software from iParadigms, known in the academic community as providers of Turnitin. For a searchable list of all journals in the CrossCheck database, please visit: www.ithenticate.com/search
2. **Data Fabrication and Falsification:** Data fabrication and falsification means the researcher did not really carry out the study, but made up data or results and had recorded or reported the fabricated information. Data falsification means the researcher did the experiment, but manipulated, changed, or omitted data or results from the research findings.
3. **Simultaneous Submission:** Simultaneous submission occurs when a manuscript (or substantial sections from a manuscript) is submitted to a journal when it is already under consideration by another journal.
4. **Duplicate Publication:** Duplicate publication occurs when two or more papers, without full cross referencing, share essentially the same hypotheses, data, discussion points, and conclusions.
5. **Redundant Publications:** Redundant publications involve the inappropriate division of study outcomes into several articles, most often consequent to the desire to plump academic vitae.
6. **Improper Author Contribution or Attribution:** All listed authors must have made a significant scientific contribution to the research in the manuscript and approved all its claims. Don't forget to list everyone who made a significant scientific contribution, including students and laboratory technicians.
7. **Citation Manipulation:** Citation Manipulation is including excessive citations, in the submitted manuscript, that do not contribute to the scholarly content of the article and have been included solely for the purpose of increasing citations to a given author's work, or to articles published in a particular journal. This leads to misrepresenting the importance of the specific work and journal in which it appears and is thus a form of scientific misconduct.

Handling Cases of Misconduct

Once GJESM confirms a violation against GJESM's publication ethics, GJESM addresses ethical concerns diligently following an issue-specific standard practice as summarized below.

1. The first action of the journal Editor is to inform the Editorial Office of GJESM by supplying copies of the relevant material and a draft letter to the corresponding author asking for an explanation in a nonjudgmental manner.
2. If the author's explanation is unacceptable and it seems that serious unethical conduct has taken place, the matter is referred to the Publication Committee via Editorial Office. After deliberation, the Committee will decide whether the case is sufficiently serious to warrant a ban on future submissions.
3. If the infraction is less severe, the Editor, upon the advice of the Publication Committee, sends the author a letter of reprimand and reminds the author of GJESM publication policies; if the manuscript has been published, the Editor may request the author to publish an apology in the journal to correct the record.
4. Notification will be sent to corresponding author and any work by the author responsible for the violation or any work these persons coauthored that is under review by GJESM journal will be rejected immediately.
5. The authors are prohibited from serving on GJESM editorial board and serving as a reviewer for GJESM Journal. GJESM reserves the right to take more actions.
6. In extreme cases, notifications will be sent to the affiliations of the authors and the authors are prohibited from submitting their work to GJESM for 5 years.
7. In serious cases of fraud that result in retraction of the article, a retraction notice will be published in the journal and will be linked to the article in the online version. The online version will also be marked "retracted" with the retraction date.

GUIDE FOR AUTHORS

"Global Journal of Environmental Science and Management" (GJESM) is a double blind peer reviewed electronic and print quarterly publication concerned with all aspects of environmental science and management. GJESM publishes original research papers, review papers, case reports and short communications, letters to editor and authors' response about letters to editor across the broad field of environment. These include but are not limited to environmental science, environmental management, environmental engineering, environmental planning and design, urban and regional landscape design and industrial ecology. Environmentalist disciplines are invited to contribute their knowledge and experience. The publication appears at regular intervals time quarterly. The Journal database is fully open access and full text of published articles are available for everyone who can get access to the Journal website free of cost. The authors never pay any charges for submission, article processing and publication.

Guide for Authors: More details on guide for authors refer: http://gjesm.net/journal/authors.note

GENERAL

1. Authors should submit their contributions electronically through the GJESM website submission system to the Editorial Office.

2. Manuscripts must be submitted only in English and should be written according to sound grammar and proper terminology. Manuscripts should be typed in Times New Roman of 11 pt. font and in MS-Word format in one column with 2.5 cm margin at each side. Manuscript submission must be applied once in order to obtain only one submission ID number. More than one submission for a single manuscript can lose the chance of the manuscript consideration. Manuscript must be accompanied by a covering letter including title and author(s) name.

3. There are no strict formatting requirements but all manuscripts must contain the essential elements needed to convey your manuscript, for example Abstract, Keywords, Introduction, Materials and Methods, Results, Conclusions, Artwork and Tables with Captions. Please ensure the figures and the tables included in the single file are placed next to the relevant text in the manuscript, rather than at the bottom or the top of the file. There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent.

BEFORE YOU BEGIN

1. Peer-Review Process: In order to sustain the peer review system, authors have an obligation to participate in peer review process to evaluate manuscripts from others. When appropriate, authors are obliged to provide retractions and/or corrections of errors to the editors and the Publisher. All papers submitted to GJESM journal will be peer reviewed for at least one round. GJESM journal adopts a double-blinded review policy: authors are blind to reviewers, but reviewers are not blind to authors. After receiving reviewers' comments, the editorial team member makes a decision. Because reviewers sometimes do not agree with each other, the final decision sent to the author may not exactly reflect recommendations by any of the reviewers. The decision after each round of peer review may include (a) Accept without any further changes, (b) Accept with minor revision, (c) Major changes are necessary for resubmission and (d) Decline without encouraging resubmission.

2. Post-Publication Evaluation: In addition to rapid Peer Review Process, the GJESM Journal has Post-Publication Evaluation by the scientific community. Post-Publication Evaluation is concentrated to ensure that the quality of published research, review and case report meets certain standards and the conclusions that are presented are justified. The post-publication evaluation includes online comments and citations on published papers. Authors may respond to the comments of the scientific community and may revise their manuscript. The Post-Publication Evaluation is described in such a way; it is allowing authors to publish quickly about Environmental science, management, engineering and technology concepts.

3. Publication Ethics: The ethical policy of GJESM is based on the Committee on Publication Ethics (COPE) guidelines and complies with International Committee of GJESM Editorial Board codes of conduct. Readers, authors, reviewers and editors should follow these ethical policies once working with GJESM. The ethical policy of GJESM is liable to determine which of the typical research papers or articles submitted to the journal should be published in the concerned issue. The ethical policy insisted the Editor-in-Chief, may confer with other editors or reviewers in making the decision. Visit at: http://publicationethics.org

4. Conflict of Interest: Authors are requested to evident whether impending conflicts do or do not exist. A copyright transfer agreement is signed by the corresponding author, upon the acceptance of the manuscript, on behalf of all authors, for publication to be legally responsible towards the journal ethics and privacy policy. Authors will be notified as soon as possible of decisions concerning the suitability of their manuscripts for publication in the journal. The submitted materials may be considered for inclusion but cannot be returned and Editors of the journal reserve the right to accept or reject any article in any stage, if necessary. Conflict of Interest Disclosure form can be found at: www.gjesm.org/conflict_of_interest_discloure_form.docx

5. Submission Declaration and Verification: While submitting a manuscript to GJESM, all contributing author(s) must verify that the manuscript represents authentic and valid work and that neither this manuscript nor one with significantly similar content under their authorship has been published or is being considered for publication elsewhere including electronically in the same form, in English or in other language, without the written consent the copy right holder.

6. Authorship: All contributing authors should qualify for authorship and corresponding author should sign the authorship form while submitting the manuscript. It can be found at: (http://www.gjesm.net/data/gjesm/news/authorship_form.docx).

7. Changes to Authorship: After the manuscript is submitted or accepted for publication, the corresponding author is required to send a request to add or remove an author or to rearrange the author names of the submitted/accepted manuscript by sending the change of authorship form to editorial office. No authorship change is allowed after publication of manuscript. More details may be found at: (http://www.gjesm.net/data/gjesm/news/change_of_authorship_form.docx)

8. Retained Author Rights: As an author, author or authors' employer or institution retains certain rights. For more information on author rights, found at: www.gjesm.org/retained_authors_right.docx.

9. Copy Right: Journals should make clear the type of copyright under which authors' work will be published. For open access articles the publisher uses an exclusive licensing agreement in which authors retain copyright in their manuscript. More details may be found at: (www.gjesm.org/copyright_form.docx)

10. User license Agreement: GJESM provides access to archived material through GJESM archives. Manuscripts are the parts of an open archive are made freely available from GJESM website after certain period, which begins from the final publication date of the manuscript. All articles published open access will be immediately and permanently free for everyone to read and download. Permitted reuse is defined by Creative Commons user license called ***Creative Commons Attribution***. Visit at: **(**Creative Commons Attribution 4.0 International (CC BY 4.0)**)**

11. Plagiarism Prevention and Violation of Publication Ethics: All manuscripts under review or published with GJESM are subject to screening using Plagiarism Prevention Software. Plagiarism is a serious violation of publication ethics. Other violations include duplicate publication, data fabrication and falsification, and improper credit of author contribution. Thus, the Plagiarism or Fraudulent or knowingly inaccurate statements constitute unethical behavior are unacceptable and submitting the same manuscript to more than one journal concurrently constitutes unethical publishing behavior and is unacceptable. The development of CrossCheck is a service that helps editors to verify the originality of papers. CrossCheck is powered by the Ithenticate software from iParadigms, known in the academic community as providers of Turnitin. For more details visit at: www.ithenticate.com/search

12. Handling Cases of Misconduct: Once GJESM confirms a violation against GJESM's publication ethics, the following actions will be taken.

a. The work is rejected / retracted immediately. Notification will be sent to corresponding authors. In extreme cases, notifications will be sent to the affiliations of the authors.
b. The authors are prohibited from submitting their work to GJESM for 5 years.
c. Any work by the authors responsible for the violation or any work these persons coauthored that is under review by any GJESM journal will be rejected immediately.
d. The authors are prohibited from serving on GJESM editorial board. GJESM reserves the right to take more actions.

MANUSCRIPT PREPARATION

1. Title Page: The title page should include: the name(s) of the author(s), a concise and informative title, the affiliation(s) and address (es) of the author(s), and e-mail address, telephone and fax numbers of the corresponding author.

2. Manuscript Title: Title of up to 17 words should not contain the name of locations, countries or cities of the research as well as abbreviations. The title should be oriented to Environmental issues while not being obscure or meaningless.

3. Abstract: An abstract of 150 to 250 words that sketches the purpose of the study; basic procedures; main findings its novelty; discussions and the principal conclusions, should not contain any undefined abbreviations or references.

4. Keywords: Provide 5 to 7 keywords which can be used for indexing purposes. Keywords should not repeat the words of the manuscript title or contain abbreviations and shall be written in alphabetical order as separated by semicolon.

5. Introduction: The Introduction should state the purpose of the investigation and identify clearly the gap of knowledge that will be filled in the Literature review study. Date and location of the research carried out throughout the study must be mentioned at the end of this section.

6. Materials and methods: The Materials and Methods section should provide enough information to permit repetition of the experimental work. It should include clear descriptions and explanations of sampling procedures, experimental design, and essential sample characteristics and descriptive statistics, hypothesis tested, exact references to literature describing the tests used in the manuscript, number of data involved in statistical tests, etc.

7. Results and Discussion: The Results section should describe the outcome of the study. Data should be presented as concisely as possible - if appropriate in the form of tables or figures, although very large tables should be avoided. The Discussion should be an interpretation of the results and their significance with reference to work by other authors. Please note that the policy of the Journal with respect to units and symbols is that of SI symbols.

8. Tables: Do not submit tables and graphs as photograph. Place explanatory matters in footnotes, not in the heading. Do not use internal horizontal and vertical rules. Tables should be called out in the text and should have a clear and rational structure and consecutive numerical order. All tables should be numbered 1, 2, 3, etc. Give enough information in subtitles so that each table is understandable without reference to the text. Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

9. Figures: Figures/ illustrations should be in high quality art work, within 200-300 dpi and separately provided in Excel format. Ensure that figures are clear, labeled, and of a size that can be reproduced legibly in the journal. Each figure should have a concise caption describing accurately what the figure depicts. Figure captions begin with the term Fig. Figures should be with the captions placed below in limited numbers. No punctuation is to be placed at the end of the caption.

10. Conclusion: This section should highlight the major, firm discoveries, and state what the added value of the main finding is, without literature references.

11. Acknowledgements: Acknowledgments of people, grants, funds, etc. should be placed in a separate section before the reference list. The names of funding organizations should be written in full. Financial support affiliation of the study, if exists, must be mentioned in this section. Thereby, the Grant number of financial support must be included.

12. References: All the references should be cited throughout the manuscript text as well as in the Reference section organized in accordance with Harvard system. Groups of references should be listed first alphabetically, then chronologically. The number of references extracted from each journal should not exceed 3 to 5 citations, which is the average acceptable amount. The number of references should not be less than 30 for original paper, less than 100 for review paper. It is substantially recommended to the authors to refer to more recent references rather than old and out of date ones. Volume, issue and pages of the whole references must be specified according to the GJESM format.

Citing and listing of Web references: As a minimum, the full URL should be given. Any further information, if known (Author names, dates, reference to a source publication, etc.), should also be given.

Text: All citations in the text should refer to: 1. Single author: the author's name (without initials, unless there is ambiguity) and the year of publication; 2. Two authors: both authors' names and the year of publication; and 3. Three or more authors: first author's name followed by "et al." and the year of publication. Citations may be made directly (or parenthetically). Groups of references should be listed first alphabetically, then chronologically. Examples: "as demonstrated (Allan, 1996a, 1996b, 1999; Allan and Jones, 1995). Kramer *et al.,* (2000) have recently shown …".

List: References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same Author(s) in the same year must be identified by the letters "a", "b", "c", etc., placed after the year of publication.

Journal article: Nouri J.; Lorestani B.; Yousefi N.; Khorasani N.; Hassani A. H.; Seif, F.; Cheraghi M., (2011). Phytoremediation potential of native plants grown in the vicinity of Ahangaran lead-zinc mine. Environ. Earth Sci., 62(3): 639-644.

Book: Davis, M. L., (2005). Introduction to Environmental Engineering, 3rd. Ed. McGraw Hill Inc.

Book chapter: Mettam, G. R.; Adams, L. B., (1999). How to prepare an electronic version of your article, in: Jones, B. S., Smith, R. Z. (Eds.), Introduction to the electronic age. E-Publishing Inc., New York.

Conference paper: Brown, J., (2005). Evaluating surveys of transparent governance. In UNDESA, 6th. Global forum on reinventing government: towards participatory and transparent governance. Seoul, Republic of Korea 24-27 May. United Nations: New York.

Dissertation: Trent, J. W., (1975). Experimental acute renal failure. Ph.D. Dissertation, University of California. USA.

Online document: Cartwright, J., (2007). Big stars have weather too. IOP Publishing Physics Web. http://physicsworld.com/cws/article/news/2007/jun/26/big-stars-have-weather-too

AFTER ACCEPTANCE

1. Online Proof Correction: Corresponding authors will receive an e-mail with a link to our online proofing system, allowing annotation and correction of proofs online. Use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor-in-Chief. It is important to ensure that all corrections are sent back to us in one communication. Please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely the corresponding author responsibility.

2. Offprints: The offprints can be downloading from the GJESM website once the final corrected manuscripts are disseminated.

AUTHORS INQUIRIES

Authors can track their submitted article through GJESM website on author's login section at: http://gjesm.net/contacts?_action=login

Global Journal of Environmental Science and Management (GJESM)

Copyright Transfer Agreement

1. Parties of the agreement

Author (s):

Manuscript Title:

Manuscript ID:

(Herewith referred to as the "materials"),

Journal Title: Global Journal of Environmental Science and Management (GJESM)

2. Subject of the agreement

A) Copyright
1- The Author and each co-authors shall transfer and sell to the Publisher for the length of the copyright starting from the moment the present agreement comes into force the exclusive rights to the materials, including the rights to translate, reproduce, transfer, distribute or otherwise use the materials or parts (fragments) contained therein, for publication in scientific, academic, technical or professional journals or other periodicals and in derivative works thereof, worldwide, in English, in print or in electronic editions of such journals, periodicals and derivative works in all media or formats now existing or that may exist in future, as well as the right to license (or give permission to) third parties to use the materials for publication in such journals, periodicals and derivative works worldwide. The transfer under this agreement includes the right to adapt the presentation of the materials for use in conjunction with computer systems and programs, reproduction or publication in machine-readable format and incorporation into retrieval systems.
2- Reproduction, placement, transfer or any other distribution or use of the materials, or any parts of the materials contained therein, in any way permitted under this Agreement, shall be accompanied by reference to the Journal and mentioning of the Publisher, namely: the title of the article, the name of the Author (Co-authors), the name of the Journal, volume/number, copyright of the publisher.

B) Reserved Rights
The Author (Co-authors) or the employer of the Author (Co-authors) of the materials shall retain all proprietary rights (with the exception of the rights transferred to the Publisher under the present Agreement).

C) Author Guarantee
The Author (Co-authors) guarantees that the materials are an original work, submitted only to GJESM, and have not been published previously.
In case the materials were written jointly with co-authors, the Author guarantees that he/she has informed them of the terms of this Agreement and obtained their signatures or written permission to singe on their behalf.
The Author guarantees as well that:
The materials do not contain libelous statements.
The materials do not infringe on other persons' rights (including without limitation copyrights, patent rights and the trademark right).
The materials do not contain facts or instructions that can cause damage or injury to third parties and their publication does not cause the disclosure of any secret or confidential information

---

Author (Corresponding Author):

Correspondence Address:

Phone:

Fax:

Email:

Corresponding Author Name: Signature Date

On Behalf of the Publisher:

Iran Solid Waste Association,
Faculty of Environment, University of Tehran,
Postal Code: 1417854511, Tehran,
Iran

Telefax: (+9821) 2610 5110

Email: *editor@gjesm.net*

Gjesm.publication@gmail.com

Website: www.gjesm.net

Accepted for publication ☑ Signature Date

PLEASE NOTE: The accepted manuscript cannot be processed for publication until the publisher has received this signed form. The form MUST be signed by the Corresponding Author and then scanned and sent through the system or email. If the manuscript is not published in the Journal, this release will not take effect.

The sole responsibility for the whole content (s) of the article remains only with the corresponding author. However, Editor would reserve the right to adjust the style to certain standards of uniformity before publication.

CONFLICT OF INTEREST DISCLOSURE FORM

Conflict of Interest is defined as a set of conditions in which professional judgment concerning a primary interest, such as the validity of research, may be influenced by a secondary interest, such as financial gain. A Conflict of Interest Disclosure is an agreement or notification from the authors that they have not been paid for the work, or if they have, stating the source of their payment. The purpose of Conflict of Interest Disclosure form is to provide readers of authors' manuscript with information about authors' interests that could influence how the authors receive the work. The corresponding author (on behalf of all co-authors) should submit a conflict of interest disclosure form and is responsible for the accuracy and completeness of the submitted manuscript. Conflict of Interest Disclosure form can be signed by the corresponding author on behalf of all co-authors and stating that the submitted manuscript is the authors' original work, has not received prior publication and is not under consideration for publication elsewhere, permission has been received to use any material in the manuscript much as tables, figures etc. or no permissions have necessary to publish the authors' work.

1. Name of the corresponding author

2. Affiliation including e-mail and phone number

3. Manuscript Title

4. Do the authors or authors' institution at any time receive payment or services from a third party (government, commercial, private foundation, etc.) for any aspect of the submitted manuscript (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.)?

   Are there any relevant conflicts of interest? Yes / No

5. Do the authors have any patents, whether planned, pending or issued, broadly relevant to the work?

   Are there any relevant conflicts of interest? Yes / No

6. Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what the authors' information in the submitted manuscript?

   Are there any relevant conflicts of interest? Yes / No

7. Are there any aspect of the work covered in this manuscript that has involved either experimental animals or human patients has been conducted with the ethical approval of all relevant bodies or not.

   Are there any relevant conflicts of interest? Yes / No

Corresponding Author Signature	Print Name	Date

AUTHORSHIP FORM

By completing and signing the following statements, the corresponding author acknowledges and accepts the responsibility on behalf of all contributing authors, if any, concerning Authorship Responsibility.

Manuscript title:

Corresponding author:

Affiliation:

Email:

Phone No:

By signing and filling this form, the corresponding author certifies that each author has met all criteria below (A, B, C, and D) and indicates each author general and specific contributions by listing his or her name next to the relevant section.

A. I certify that

- The manuscript is authentic and valid and that neither this manuscript nor one with considerably similar content under my authorship has been published or is being considered for publication elsewhere, except as described in an attachment, nor copies of closely related manuscripts are provided.
- I will provide the data or will contribute fully in providing and obtaining the data on which the manuscript is based for examination by the editors or their assignees, if requested.
- Every author has agreed to allow the corresponding author to serve as the primary correspondent with the editorial office, to review the edited manuscript and proof.

B. Each author has given final approval of the submitted manuscript.

C. Each author has participated sufficiently in the work to take public responsibility for the whole content.

D. Each author qualifies for authorship by listing his or her name on the appropriate line of the categories of contributions listed below. List appropriate author next to each section – each author must be listed in at least 1 field. More than 1 author can be listed in each field.

- conception and design
- acquisition of data
- analysis and interpretation of data
- drafting of the manuscript
- critical revision of the manuscript for important intellectual content
- statistical analysis
- obtaining funding
- administrative, technical, or material support
- supervision
- no additional contributions
- other (specify)

Corresponding Author Signature **Print Name** **Date**

CHANGE OF AUTHORSHIP FORM

Manuscript Title:

Corresponding Author:

Please check all that apply.

☐ New author(s) have been added.

☐ There is a change in the order of authorship.

☐ An author wishes to remove his/her name. An author's name may only be removed at his/her own request in writing.

ORIGINAL AUTHORSHIP

List ALL AUTHORS in the same order as the original (first) submission.

Print Name	Print Name
Name (1)	Name (6)
Name (2)	Name (7)
Name (3)	Name (8)
Name (4)	Name (9)
Name (5)	Name (10)

NEW AUTHORSHIP

List the ALL AUTHORS in same order as the new version.

Print Name	Print Name
Name (1)	Name (6)
Name (2)	Name (7)
Name (3)	Name (8)
Name (4)	Name (9)
Name (5)	Name (10)

I attest that:

1. The manuscript is not currently under consideration, in press, or published elsewhere, and the research reported will not be submitted for publication elsewhere until a final decision has been made as to its acceptability by the journal (posting of submitted material on a web site may be considered prior publication-note this in your cover letter).
2. The manuscript is truthfully original work without fabrication, fraud, or plagiarism.
3. I have made an important scientific contribution to the study and am thoroughly familiar with the primary data.
4. I have read the complete manuscript and take responsibility for the content and completeness of the manuscript and understand that I share responsibility if the paper, or part of the paper, is found to be faulty or fraudulent.

Corresponding Author Signature Name Date