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ORIGINAL RESEARCH PAPER

Empowerment key factors in shaping women's awareness of household waste management

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ABSTRACT

BACKGROUND AND OBJECTIVES: Empowering activities is the key in building awareness and individual capacity of household waste management, especially for women as the main actors. This study aimed to explore empowering activities as the key factor in shaping women's awareness of household waste management.

METHODS: This study was conducted using quantitative methods. The data collection technique used was survey. The sampling was carried out by purposive sampling technique. The criteria for sampling were those women who attended training in waste management in Jagakarsa Sub District, South Jakarta, Indonesia. The analytical technique used was Ordinary Least Square regression.

FINDINGS: Based on the findings of this study, it was known that women who had good adaptability to technology were likely to have a greater chance score of 0.908. Education in schools was also found to have a positive impact on the opportunity score to earn good living environment. It was found that an increase in 1 year of schooling will increase the score of chance by 0.0755 (estimation 5). This is not significantly different from estimation 4 which would increase the chance by 0.0745. In waste management training, The womens' participation are likely to increase the score chance of having a good environment by 0.944 points (estimation 5). Besides, the womens' participation were found to be statistically significant at 95% confidence level in all estimations, particularly in the waste management training. Based on the comparison of the participation coefficient parameters in waste management training, it was found that there were no significant differences or signs (+ and-) between the estimations. All coefficient parameters ranged from 0.83 to 0.94.

CONCLUSION: Empowerment activities that utilize access to education and easily adapt to a technology might have a significant correlation with women's involvement in waste management training. This is the basis for building awareness to carry out more sustainable household waste management and achieve change to get a good living environment.

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INTRODUCTION

Waste causes environmental problems for its environmental pollution (both soil, water, and air pollution) and disrupts species in the ecosystem. It is also one of the factors rise climate change that causes flood disasters. Waste problem should immediately be resolved using proper management so that it will not disrupt urban development any further and urban resilience can be reduced. The waste problem is faced by almost all countries, including Indonesia, especially due to the mismanaged waste (Jambeck *et al*., 2015). Efforts are needed to overcome this problem, one of which is by increasing community involvement in household waste management (Asteria *et al*., 2020). The main factor in waste management is to reduce the amount of waste and conduct waste sorting (according to the type of waste) so that it can take advantage of the value that is still contained in the waste through recycling activities. At the household level, women have the main role to be responsible for household waste management, women's involvement in reducing the amount of waste through non-consumptive household consumption patterns and reusing goods, and sorting waste so that waste can be recycled (Almasi *et al*., 2019; Asteria *et al*., 2020). In connection with the effectiveness of waste management, efforts are needed to overcome gender problems and gender role inequality in waste management involvement from the household level. Empowerment activities for women are needed so that women can get information that shapes women's awareness and knowledge in managing waste. In accordance with the goals of sustainable development goals (SDGs) number 11 about urban sustainability, it must be integrated with SDGs number 4 about quality education and SGDs number 5 about gender equality (OECD, 2018). The three aspects of the SDGs relate to the rights of citizens can be achieved through empowerment. The waste problem is very complex in the urban area. The existence of urbanization problems that cause population density and limited land, along with the lack of awareness about waste management, it is important to do studies in urban areas. Sustainable waste management in urban areas is determined by the contribution of the community as human resources (Asteria *et al*., 2018). Waste problems that foccur in urban areas can be managed properly using community empowerment (Pasang *et al*., 2007). Tarigan *et al.* (2020) found another important finding regarding the community participation in waste management. It is realized that improper management of waste can be a source of health and environmental problems. Community participation contributes to waste management that is effective (Ramendai, 2020). Empowerment is a process of empowering people from those who do not have ability to become capable so that they can gain control over the right to achieve goals (Kishor and Gupta, 2004). Regarding the gender aspect of waste management activities, women and men often have different needs and preferences. Women, as the primary users of waste management, have a correlation with the responsibility for managing household waste. To optimize waste management, especially household waste management, it is very important to know the needs of women in increasing their involvement in waste management. Their involvement is also essential to prevent any risk of virus transmission (Yeni *et al*., 2020), especially caused by improper waste management. According to Ocean Conservancy (2019), women in Indonesia, the Philippines, and Vietnam are eager to learn about the waste management process. They are more likely to want to understand and learn about what happens to waste after disposal. Tiwary (2015) wrote about the role of women in domestic waste management and found that the most significant challenge related to urban population growth is maintaining a sustainable environment development of infrastructure facilities and amenities that are required. Weak public awareness of waste management is partly due to low levels of education or knowledge. Individuals who have environmental concerns tend to feel proud of doing environmentally-friendly behavior. The Indonesian government's efforts in managing waste from the first source in household waste have been regulated by the policy of Presidential Regulation Number 97 of 2017 concerning Policies and National Strategies for Household Waste Management and Household-like Waste (*Jastranas*) based on the spirit of recycling (form 3R principles). Waste problems in urban areas are generally caused by insufficient availability of trash bins, inadequate availability of trash bins and low awareness, and knowledge of the community (Bahri *et al*., 2020; Desa *et al*., 2012). For the empowerment, the management strategy is carried out by improving the social aspects of the community or the surrounding community through increasing knowledge. Citizen participation is the key to success in urban waste management (Meng *et al*., 2019). Gender equality and women's empowerment associated with innovation can foster women's capabilities in terms of decision-making, strategic life choices and change processes where they can then have welfare in individual maintenance, household control, and a broader level of society and community. The empowerment of women is very important because of gender disparities. Eventhough waste management does not have a specific gender link, just like in the wider community, it is known that women and men may have different perceptions and views about waste. The empowerment indicator consists of 5 points, that is women have access and control over natural resources, freedom of action, protection from acts of violence, and make decisions (Salehi *et al*., 2020). There are 4 dimensions of women's empowerment consisting of self-esteem, control over natural resources, mobility, and participation in decision making (Hussain and Jullandhry, 2020). Empowerment of women starts when women have a desire to participate in decision making, so that they have control over resources (Hussain and Jullandhry, 2020). Regarding to SDGs number 5, gender equality gives access for women's participation since they have the ability to make environmental changes (OECD, 2018). Women have their ability to promote environmental behavior to support sustainable development (Luke and Munshi, 2011). Education is not only obtained from formal, but also from informal institutions. Informal and formal institutions both provide opportunities for women to interact with society and make them become more empowered to democratize their rights and gender equality (Mejuini and Ebrary, 2013). Educated women have higher knowledge, behavior, and practices than uneducated women (Almasi *et al.*, 2019). Optimal waste management can be carried out through a communication approach, improved education and provided infrastructure (Brown *et al*., 2010). The empowerment of women in waste management can be judged based on their education. Individual awareness will be created through education (Asteria *et al*., 2020). Even though the infrastructure cannot be found, but if individuals have awareness then they will have control over environmental management. One form of environmental behavior is managing the first source of waste, namely waste from the household level. Awareness of household waste management is closely related to the commitment of individuals to improve their abilities and skills in managing waste which can be done by implementing the reduce, reuse and recycle (3R) principle through training and similar activities. The obstacle found in this case is the limited mobility experienced by women which make them find it difficult to maintain their social networks (Hussain and Jullandhry, 2020). Participation in social networks will be difficult to maintain, so it is likely to harm women, both socially and psychologically, which can hinder the development of women's abilities(Malik and Courtney, 2011). One of the factors that support the success of empowerment is internet access. The internet in this case is able to mobilize and develop women's social networks (Hanasuma, 2019). Providing digital freedom to women can help them to increase their empowerment (Salehi *et al*., 2020). Digital freedom is like building empowerment through increasing women's social capital. Therefore, it is hoped that they can become agents of social change. Regarding access and technology adaptation for household waste management with the existence of waste composting technology using different application methods and production capacities, including aerobic composting, semi-aerobic composting, composting with worm reactors, and composting using additives. In addition, recycling technology for plastic waste is differentiated from paper, cloth, glass, and other types of non-organic waste. Access to the internet will make it easier for women to get information and knowledge about technology that can be used to manage household waste by recycling non-organic waste and making compost from organic waste. Women are both generators and recipients of waste at the household level because of their responsibility in managing domestic waste and understanding the environmental implications. Their significant contribution to resource recovery is needed. The purpose of this study is to explore empowerment activities as a key factor in shaping women's awareness in household waste management. The study was conducted in Jagakarsa District, South Jakarta, Indonesia for about two years from December 2019 until June 2020. This study has been carried out in Jagakarsa sub-district, South Jakarta, Jakarta, Indonesia during 2019 to 2020.

MATERIALS AND METHODS

This study used quantitative methods to measure the effect of activities variables empowerment measured based on three dimensions of the level of education, internet accessibility, and access of technology (as independent variables), and the level of awareness of household waste management with involvement in waste management training (as dependent variable). The data collection was carried out by the survey. Sampling was done by using the purposive sampling technique. The criteria for sampling were women who attended training in waste management in Jagakarsa sub-district, South Jakarta, Indonesia. The analytical technique used was Ordinary Least Square (OLS) regression.

Research location

The waste problem in Indonesia, especially in Jakarta, still cannot be resolved. Even in 2019, the volume of plastic waste in Jakarta was increasing up to 1,000 tons. This study was conducted in Jagakarasa sub district, South Jakarta, Indonesia (Fig. 1). The location was chosen because there have been many socialization and training in waste management provided by the South Jakarta Environmental Agency and waste care communities in South Jakarta from 2017 to 2020. One of those activities was South Jakarta Clean Up program (called "South Jakarta Clean Up"/BBJS program). There are also community service activities as a form of corporate social responsibility (CSR) from private companies, state-owned companies, and several universities in this area. The number of women in Jagakarsa sub district is the largest (reaching 206,000) compared to other sub districts in South Jakarta (BPS, 2020).

Data description and collection methodology

The sample size used in this study consisted of f 400 respondents, which was taken based of the slovin's formula with margin of error 5% and confidence level of 95% (Slovin, 1960). The survey was conducted at the individual level (not at the household level) using purposive sampling (Neuman, 2014). The criteria used was based on Ocean Conservancy (2019) which stated that majority of women in Indonesia eager to learn about waste management. Therefore it is

Fig. 1: Geographic location of the study area in South Jakarta, Indonesia

only women that will be enrolled in this sample. The survey technique conducted in this study was carried out in an offline survey (filling out the questionnaire by meeting respondents face to face) by visiting the respondent's house. The sample selection to obtain respondents who filled out the questionnaire was done based on data from the heads of the local neighborhood and hamlet (it's called RT and RW) which were recorded on 54 hamlet units located in the Jagakarsa sub district, South Jakarta. Based on the purposive sampling technique used, the samples taken and used in this study were women who had attended waste management training (both training programs from the South Jakarta Provincial Government and training activities from regional waste banks) that had been implemented in Jagakarsa Village, South Jakarta. The women in this survey came from various educational backgrounds, economic status and ages based on clasification of women profession (BPS, 2020). Regardless of their marital status, both dependent and independent women participated in this survey. Researchers noted the estimated number of independent women is likely to increase the likelihood of achieving a suitable living environment. There were more than 60 questions in the primary data collection consisting of 20 main questions with some several branch-off questions about their educational history, length of life in their current home, perspective on urban issues, economic status, and their capacity for a unique experience in segregation, equality, governance, protection, awareness and access to the labor market, environmental and financial aspects. In the questionnaire for the access variable to participate in waste management training, there are several questions with an emphasis on time and the frequency of participating in the training, the implementer of the training activities, and the sources of information regarding training activities, and posibility of obstacles to attending the training.

Analytical technique: Economic modelling and regression technique

Ordinary Least Square (OLS) regression method (Howell, 2013) was used in this study to predict the score of chance of women getting good quality of environment with certain socio-economic conditions and background. Through OLS regression methodology, the chance to earn good living environment was predicted by constant and coefficient parameters of independent variables. Each independent variable might affect in either statistically significant or insignificant way. In this study, the score chance to earn good living environment was set up as dependent variable, alongside with several independent variables. The scoring of the chances of obtaining a good living environment was carried out by using an ordinal scale with a score of 1-5 (1 = very low, 2 = low, 3 = moderate, 4 = high, and 5 = very high). The indicators of the chances to obtain a good living environment refer to environmental conditions and facilities in the area where the respondent lives. This study's limitations are that this study only shows the correlation between dependent and independent variables, for the specific case of respondent (Jagakarsa, South Jakarta). Independent variables used in this study were the dummy variables of waste-handling training, access to internet and access to technology, categorical dummy of activities and economic status, and years of schooling. Specifically, the regression model is as presented by Eq. 1.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 + \varepsilon \quad (1)$$

Where,

Y = Score of chance to earn good living environment
β_0 = Constant
$\beta_1 - \beta_6$ = Coefficient parameters of independent variables
X_1 = Dummy of waste handling training (1=trained; 0=untrained)
X_2 = Categorical dummy of economic status (base=high income; medium; low)
X_3 = Dummy of internet accessibility (1=yes; 0=no)
X_4 = Dummy of able to adapt with technology (1=yes; 0=no)
X_5 = Education, approximated by years of schooling (in years)
X_6 = Categorical dummy of activities (base=housewife)
ε = Error terms

RESULTS AND DISCUSSION

According to the stated regression model, the following Table 1 presents the regression result of the interaction between dependent variable and other exogenous variables. Meanwhile, the

Table 1: Significant test results from regression analysis

Variables	(1) est1	(2) est2	(3) est3	(4) est4	(5) est5
	Chance to Have Good Living Environment				
Waste management Training (dummy)	0.830**	0.905**	0.875**	0.861**	0.944**
	(0.368)	(0.369)	(0.369)	(0.369)	(0.377)
Categorical Dummy of Economic Status, base: High Income					
Econ. Status = Low Income	0.413	0.439	0.428	0.506	0.482
	(0.432)	(0.430)	(0.430)	(0.434)	(0.521)
Econ. Status = Middle Income	0.191	0.261	0.310	0.362	0.416
	(0.374)	(0.375)	(0.375)	(0.377)	(0.405)
Access to Internet (dummy)		0.732*	0.663*	0.564	0.495
		(0.377)	(0.380)	(0.388)	(0.397)
Able to adapt with technology (dummy)			0.805	0.829	0.908*
			(0.539)	(0.539)	(0.547)
Years of Schooling				0.0745	0.0755
				(0.0598)	(0.0659)
Categorical Dummy of Activities, base: Housewife					
Work Status = Part time worker					0.684
					(0.557)
Work Status = Retired					1.389
					(1.687)
Work Status = Self-employed					0.159
					(0.521)
Work Status = Student					0.210
					(0.555)
Work Status = Unemployed					-0.238
					(0.862)
Work Status = Employed					-0.346
					(0.581)
Constant	14.07***	13.47***	12.80***	11.92***	11.73***
	(0.256)	(0.398)	(0.602)	(0.926)	(0.997)
Observations	393	393	393	393	392
R-squared	0.015	0.025	0.030	0.034	0.044

Standard errors in parentheses
*** $p<0.01$, ** $p<0.05$, * $p<0.1$

dependent variable shows probability to attend waste management training.

In the dummy variable of waste management training, it is found to be significantly affecting the chance to earn good living environment. According to the results in Table 1, women's participation on waste management training will likely to increase the score chance to have good environment by 0.944 points (estimation 5). Based on the regression table, it is found that participation in waste management training is statistically significant at 95% confidence level on all estimations. Then, based on the comparison of the participation coefficient parameter in the waste management training, there was no significant difference or difference found in signs (+ and -) between the estimation. All coefficient parameters range from 0.83 to 0.94. The next variable is the dummy category of economic status. The comparison of economic status in this study is high-income individuals. Referring to the survey questionnaire, those included in low income are respondents who have monthly expenditure per capita below USD 70 (<IDR 1,000,000), while those included in middle income are respondents with per capita expenditure per capita between USD 70 to USD 210 (IDR1.000.000 to IDR3.000.000) per month. The high income in this study, which is the base comparator are respondents with expenditure more than USD 210 (IDR3.000.000) per month. On the regression table, the result shows that both low and middle-income women have more chance to earn good living environment (Dhokhikah *et al.,* 2015). It is shown by the coefficient parameter, where low income women will likely to have 0.482 more score of chance (estimation 5) and middle-income women will likely to have 0.416 more score of chance (estimation 5). Recall that in this study, the opportunity to obtain a good living environment does not depend on the ability to pay (or buy) housing under certain conditions (Desa *et al.,* 2012). The opportunity to earn a good living environment according to this study shows the environmental conditions and city facilities that are agglomerated in the observation area. This is in accordance with Hussain and Jullandhry (2020) about women opportunity to control of resources. Overall, the chances of low-income women having a good living environment ranged from 0.413 to 0.506 while middle-income women ranged from 0.191 to 0.416. The next independent variable is the internet access dummy variable. The regression table shows a statistically significant effect (90% confidence level) on the first and second estimation. However, due to the better R-square estimation model, estimation 5 has been used for the main interpretation. Women who have access to the internet are likely to have a good environmental score of 0.495 (estimate 5), although this is not statistically significant. In estimation 1 and 2, women with internet access are most likely to have scores of 0.732 and 0.663, respectively, than women who could not access the internet. These findings indicate that the internet provides useful references for women regarding how to make an ideal living environment (Hasunuma, 2019). Furthermore, internet can also be the place for them to learn and socialize in terms of waste-handling and environmental caring from others or communities since on the survey, most of them use for social media and entertainment access (Salehi *et al*., 2020). The next independent variable is the ability to adapt to technology. According to the regression table above, it is shown to have a detrimental effect on the likelihood of women attending enrichments for waste handling. Increasing the score chance of discrimination by 1 is likely to decrease the probability of enrichment in waste-handling by -00390 (estimation 6). Nevertheless, statistically, it is not significant, as shown by other estimates as well. This finding shows that the possibility of discrimination will halt the involvement of women in waste management enrichment. However, it may offer inconclusive results at some stage, as shown in Fig. 2. Eventhough it is not statistically significant, author tried to plot how the chance of discrimination affects the probability of attending enrichments.

The dummy variable of technology separates women who can understand, utilize, and optimize technology to facilitate daily activities. Referring to the regression table, the dummy variable shows a positive effect in all estimations, although only estimation 5 shows a statistically significant effect (90% confidence level). Women who have the ability to adapt to technology are likely to have a greater score of 0.908. This indicates that they can utilize and optimize technology to facilitate their activities, including in terms of waste management and environmental maintenance (Pasang *et al.,* 2007). The independent variable related to years of schooling also has a positive effect on the opportunity score for a good living environment. The authors found that an increase in 1 year of schooling could increase the chance value by 0.0755 (estimate 5) which is not significantly different from estimation 4, which would increase the chance by 0.0745. Yet, both estimation 4 and 5 shows statistically insignificant effect to the score of chance. The next independent variable is categorical dummy, the activities of the respondents. Recall that the base comparison of activities of the respondent is housewife. In this study, were not used the terms `working status' since in Indonesia's BPS definition (BPS, 2020) mentioned that housewife is not classified as working status, hence housewife women are not calculated as the part of labor market entities. The term of 'activity' is also used because women students also included. In this study, there are seven activities of women that were measured: housewife (base), part-time worker, retired, self-employed, student, unemployed and employed women. On the regression table, there is no category which is statistically significant affects the score of chance to earn good environment. According to the regression table, women who are part-time workers will likely to have 0.684 more score of chance to earn good environment, compared to housewife (Sekito *et al.*, 2013). Women who are retired will likely to have 1.38 more score of chance rather than a housewife, while self-employed women have 0.159 more score of chance. This result suggests that the greater the possibility of women having equal participation, rather than unequal participation, their likelihood of engaging in such enrichment would likely increase. Besides, granting women equal participation in most aspects could increase their awareness of the environment, including their participation in the enrichment for waste management (Fig. 3). This study also plotted the predicted probability of women's participation on such enrichments, given their value score of equal participation.

Women students also have positive correlation to score of chance to get good environment. They will likely to have 0.210 more score of chance. In contrast either worked or unemployed women has negative relationship with score of chance to get good environment where they will likely to have -0.238 and -0.346 len'ess score than housewife respectively. This finding indicates that women who are workers do not significantly increase their chance of getting good environment as it is shown by the negative and insignificant coefficient parameter. In other words, there is no guarantee for independent women to have better chance to get good living environment since they don't show significant increase compared to housewife. Since these relationships are expected to be shown, a U-shaped curve also expected to be shown, depicting the relationship between squared years of schooling and probability (indicating there's minimum years of schooling to takes effect). This research has plotted the predicted probability by the squared years of schooling, whether they really have quadratic relationship to the probability in Fig. 4.

In the result presented on summary statistics, women who have been trained to handle waste are mostly housewife and student, there are 42% students, 37% housewives, 19% self employed, and 2% working women who are involved in waste management training. This finding confirms that woman who have access to work (financially more independent) does not guarantee women to participate more on waste management (Salem, *et*

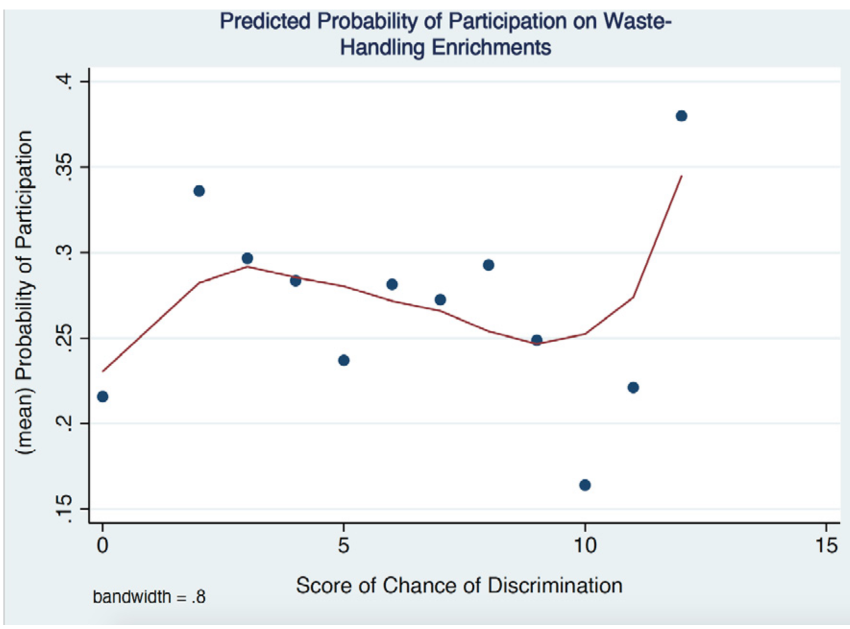

Fig. 2: Chance of Discrimination has fluctuated correlation over the probability of participation on waste-handling enrichments

al., 2020). However, this does not indicate that working women will not care about household waste management, as in Ocean Conservancy (2019). The last variable is constant. According to the estimation 5, average women will likely to have 11.73 initial score of chance, given that all independent variables are zero. This constant is statistically significant at 99% confidence level. Other estimations (1 to 4) also show the same level of significance (99% confidence level), where the initial score slightly differ from estimation 5. All constants from each estimation is vary ranges from 11.74 to 14.07. Estimation 5 has been considered as the best estimation due to its highest R-square: 0.044. It means that 4.4% variance of dependent variable can be explained by the variance of independent variables. After the dataset has been cleaned there are around 392 to 393 samples could be regressed, out of 400 samples recorded. Women who have access to information and training activity to improve their knowledge may cause social change. As in Alessa *et al*. (2003), knowledge gained from education will trigger individual curiosity and make them tend to test their knowledge or apply their curiosity/knowledge). Naturally, the education level has quadratic relationship with almost every human development indicator (in this context, is enviromental score of living). The natural quadratic relationship of education comes from basic rule, where there is always a minimum requirement for education to influcence or reshape human's behavior. In this case, author approximate the quadratic relationship between education and score, since there is minimum years of education (or certain education level) to take effect in influencing respondents behavior on environmental caring (Figs. 2 to 4). Malik and Courtney (2011) provided recommendations for developing educational strategies to further empower women. More educated women are more courageous in expressing their opinions. They can also encourage others to emulate their participation. Educated women are also more likely to have higher social capital, enabling them to connect with society, being able to increase self-confidence, courage, hope and optimism. Educated women can be effective social agents to influence other individual where they can support other women to increase their knowledge. Educated individuals play a very important role in developing the community in their area (Abu-Saad, 2016). In Dhokhikah *et al*. (2015) which stated that individuals get education are more willing to be active in environmental management. The knowledge

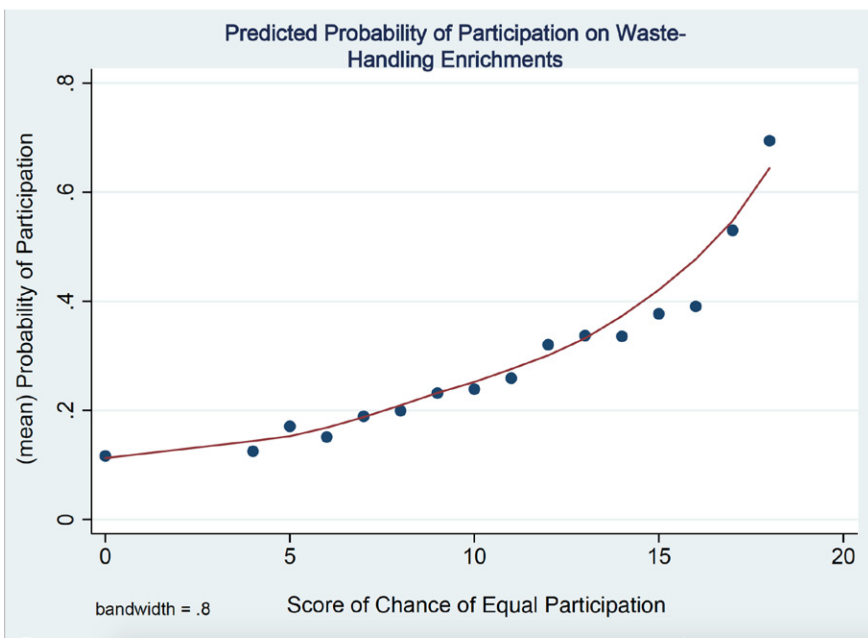

Fig. 3: Higher chance of equal participation given to women will be followed by higher participation rate in waste-handling activities

provided in formal education is able to increase environmental awareness and increase the willingness of individuals to participate in society (Meng *et al*., 2019). Sustainable waste management requires knowledge from the community where the willingness is influenced by the level of education. Access in education substantially increases women's awareness (Malik and Courtney, 2011). Awareness, knowledge, commitment, and individual responsibility will affect the consistency of individual attitudes and behavior towards the environment (Desa *et al*.,2012). Educated women will have a powerful influence on the increasing empowerment (Malik and Courtney, 2011). They have their own views that are more respected by other individuals. They are therefore more able to influence other individuals. Educated women can be characterized as women who have more knowledge, wisdom and skills (Khurshid, 2016). It makes them can provide knowledge to women who do not have access to education. Educated women can help to increase the wealth of the local community in developing management (Del Mar Alonso-Almeida, 2012). Empowerment with an educational approach in this research is obtained both formally and informally as in lifelong learning. Education is related to receiving information that creates awareness and knowledge for women so that women have ability and skills to carry out household waste processing by applying the 3R principle. The results of this study are supported by several previous studies. The residence of the household affects the behavior of the environment and the lifestyle of the place of residence (Thogersen, 2017). The variation in lifestyle in the form of housing conditions is associated with the level of openness they accept environmental management in the community. Weaver (2015) argued that a better socioeconomic level of housing has a function as a pro-social descriptive norm. A good residential environment will limit anti-environmental behavior. People living in these areas will tend to be more responsible for the environment. The level of cleanliness is considered luxury where areas with good housing conditions will better adapt to the behavior of the environment (Salem *et al*., 2019). People who live in a clean environment will participate more in environmental management activities (Permana *et al*., 2015) since they are already accustomed to living in clean conditions. By using a positive image of living conditions, people will tend to be enthusiastic about environmental responsibility activities and encourage them to actively participate in environmental protection. However, the result

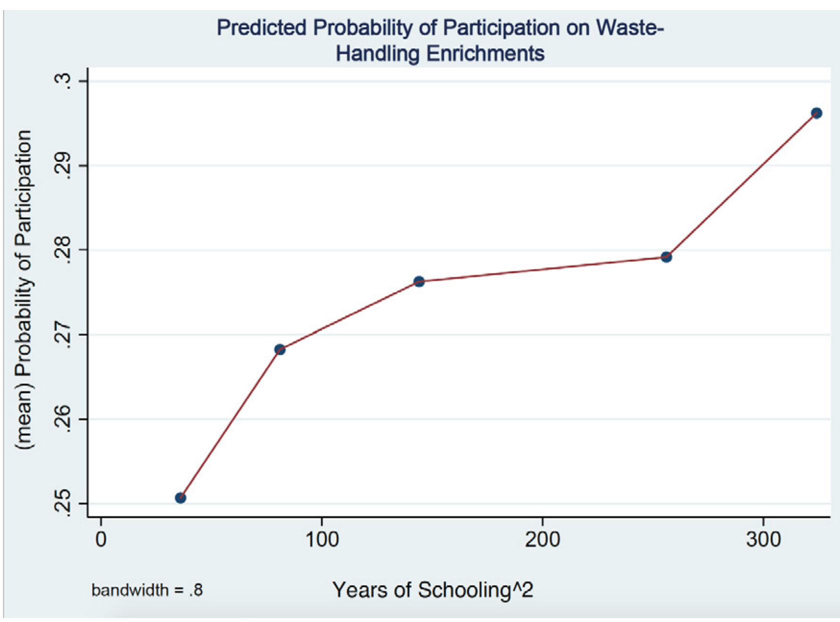

Fig. 4: The squared years of schooling in this case do not have quadratic relationship to the probability of participations.

shows that women who have middle income with similar patter (having less likelihood of participation by 0.713 times lower) with statistically insignificant results. A strong correlation was found in income level, education, and willingness to participate in environmental management (Sekito *et al*., 2013). Support for this research was carried out by study of Brotosusilo and Handayani (2020). High-income individuals have better living conditions. They tend to be willing to participate in environmental management. However, there is a concern that when the economy is high, individuals only participate through the economy not actively. Dodds and Holmes (2018) mentioned the willingness to pay for environmental management. Participation in waste management tends to spend a lot of time, and this prevents individuals from participating (Al Hassan *et al*., 2020). Individuals tend to prefer to earn a living rather than actively participate in time-consuming management. Barriers to active readiness for environmental management include time allocation, laziness, and lack of knowledge (Dhokhikah *et al*., 2015). Yet, the result is statistically insignificant. Media technology is one of the tools used as social capital (Salehi *et al*., 2020). Through social media, women will build social capital and expand it. They can disseminate information about waste management. However the existence of this social media tends to make individuals not actively participate in training or physical activities, they are more to disseminate information. Exacerbated social inequality, whether in the form of education rather than economy or work (Kuppens *et al.,* 2018) will have a more psychological impact on individuals. Education in empowerment activities is the key to giving women more control over their lives (Desa *et al.,* 2012; Malik and Courtney, 2011) and as a key in international development (Khurshid, 2016. Women's empowerment proves that women have a voice to express it; have the resources to build a country; forming, developing, and influencing society (Hanasuma, 2019). The insignificant relationship between education and the dependent variable is caused by the questionnaire. In the questionnaire, respondents' education is asked in the form of last level of education finished (not exact on what classes currently are they). Hence, to avoid autocorrelation, the dummy variable of training has been added as the proxy of respondents' environmental awareness since their awareness on environment can't be measured precisely from their level of education. Empowerment is important to increase community involvement for the sustainability of sustainable waste management. The policy of implementing an integrated waste management system is carried out by combining approaches from the 3R principle so that waste is reduced from waste sources, reuse, and recycling (by composting), then incineration and final disposal (landfilling) are carried out at a location close to the waste source. Emphasis on management at locations close to waste sources is the main issue of the importance of waste management at the household level. As the core of integrated waste management with the 3R principle is carried out by reducing waste as much as possible by processing waste in locations close to waste sources, supported by approaches through legal and regulatory aspects, institutional aspects, operational technical aspects, financing aspects, and the main thing is the aspect of community participation. In this regard, empowering women by providing access to education, access to technology, and information will greatly contribute to women's involvement in environmental protection through more independent and sustainable household waste management.

CONCLUSION

Empowering activities has a strong connection with women's involvement in waste management training as it is the basis for increasing awareness of more sustainable household waste management. The probability of access to education has a positive effect on the probability of women's involvement in waste management training. This research illustrates that the empowerment activity through training is able to develop the understanding of household waste management and broad knowledge can improve pro-environmental awareness and practical knowledge. Access to technology will create resources for women in conjunction with empowerment events since women who have ability to adapt to technology will have more involvement in waste management activities. This study shows that equality for access to get knowledge are very important in the formation of human resources as human capital in urban development. In community-based household waste management, empowerment must be carried out using a triple bottom line approach which includes 3 aspects, namely social, economic and environmental. This management approach must integrate several parties involved, including educational institutions, community organizations, private companies, and other stakeholders to support community empowerment. Support from various sectors and levels of society (multi-stakeholder) are therefore needed in increasing community empowerment through access to education, especially environmental management education. It aims to achieve more resilient and sustainable urban development. The suggestion can be given from this study is that it is necessary to carry out further testing of women's experiences in managing waste and in receiving knowledge from empowerment activities studied with a qualitative approach. Besides, it is recommended that in further research, it should also be carried out the data elaboration on the women used 3R principle for household waste management with quantitative research. Evaluation of waste management policies that are more integrated and involve multiparties is also necessary so that community empowerment, especially women's empowerment, can be further enhanced. Further research also needs to conduct studies in rural areas.

AUTHOR CONTRIBUTIONS

D. Asteria performed the elaboration of research problem, the literature review, compiled the data, interpreted the data, and prepared and edited the manuscript. J.T. Haryanto performed literature review and prepared the manuscript.

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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

$\propto$	Level of significance
A_t	Observed value
β_i *(1,2,3 ... k)*	Regression coefficients
β_0	Constant
β_1-β_6	Coefficient parameters of independent variables
ε	Error terms
Eq.	Equation
P	Probability of attending waste management trainings
e	Exponential number
X	Independent variable
X_1	Dummy of waste handling training (1=trained; 0=untrained)
X_2	Categorical dummy of economic status (base=high income; medium; low)
X_3	Dummy of internet accessibility (1=yes; 0=no)
X_4	Dummy of able to adapt with technology (1=yes; 0=no)
X_5	Education, approximated by years of schooling (in years)
X_6	Categorical dummy of activities (base=housewife)
Y	Dependent variable
3R	Reduce, reuse, and recycle
BBJS	*Bersih-Bersih Jakarta Selatan* (South Jakarta Clean Up)
BPS	*Badan Pusat Statistik* (Central Bureau of Statistics)
CSR	Corporate Social Responsibility
Jakstranas	*Kebijakan dan Strategi Nasional Pengelolaan Sampah Rumah Tangga dan Sampah Sejenis Sampah Rumah Tangga* (National Policies And Strategies For Managing Household Waste And Household-Like Waste)
RT	Rukun Tetangga (Neighbourhood)
RW	Rukun Warga (Hamlet)
SDGs	Sustainable Development Goals

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ORIGINAL RESEARCH PAPER

Bio-oil production by pyrolysis of *Azolla filiculoides* and *Ulva fasciata* macroalgae

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ABSTRACT

BACKGROUND AND OBJECTIVES: In this study, the characteristics of bio-oil samples produced through slow pyrolysis of two different macroalgae, i.e. *Azolla filiculoides* and *Ulva fasciata*, at optimized conditions were determined and compared.

METHODS: For this purpose, the effects of temperature (300-500 °C), carrier gas flow rate (0.2-0.8 L/min), and heating rate (10-20 °C/min) on the final bio-oil production were optimized using response surface methodology established by a central composite design.

FINDINGS: The highest bio-oil yield from *U. fasciata* (34.29%) was obtained at the temperature of 500 °C, nitrogen flow rate of 0.2 L/min, and heating rate of 10 °C/min. As for *A. filiculoides* feedstock, the highest bio-oil yield (30.83%) was achieved at the temperature of 461 °C, nitrogen flow rate of 0.5 L/min, and heating rate of 20 °C/min. Both bio-oil samples contained saturated and unsaturated hydrocarbons. However, the average hydrocarbon chain length was relatively shorter in *U. fasciata* bio-oil (C4-C16) than in bio-oil from *A. filiculoides* (C6-C24). Although both bio-oils had almost identical heating values, the *U. fasciata* bio-oil showed more appropriate properties, i.e. lower viscosity and density. Furthermore, the energy recovery from *U. fasciata* pyrolysis was calculated as 56.6% which was almost 1.5 times higher than the energy recovery from *A. filiculoides* pyrolysis.

CONCLUSION: The results indicated that *U. fasciata* bio-oil, with its superior characteristics, could be proposed as a promising candidate for application in diesel-based automotive industries.

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INTRODUCTION

Pollution of the environment, over-reliance on non-renewable traditional fuels, shortage in fossil fuel supply, and global warming are among the great challenges in the world (Lam *et al.,* 2019). To maintain sustainability and meet current energy demands, production of renewable energy and neutral fuels seems to be imperative (Ho *et al.,* 2014). Among various alternatives, biomass has gained considerable attention as a renewable energy resource to replace the conventional fossil fuels, due to its potential for stable energy supply and inherent nature of being environmentally friendly (Akia *et al.,* 2014; Zeng *et al.,* 2013). However, due to several issues associated with some of these resources, there is a strong motivation to develop biofuel production from non-agricultural food crops (Ahmad *et al.,* 2011; Correa *et al.,* 2017; Suganya *et al.,* 2016). In this context, algae can provide a potentially renewable source for biofuel production due to their fast growth rate, efficiency in CO_2 biofixation, ability to grow in non-arable lands and non-potable water, no competition with agricultural food/land, and higher areal productivity compared to traditional/terrestrial sources (Kositkanawuth *et al.,* 2014; Pourkarimi *et al.,* 2020; Ullah *et al.,* 2015). Algae resources can be converted to environmentally friendly biofuels such as bio-oil (biodiesel), biohydrogen, bioethanol, biomethane, and many other bio-based products (Javed *et al.,* 2019; Ortigueira *et al.,* 2015). Among the techniques developed for biomass conversion, such as fermentation, transesterification, hydrothermal liquefaction, and gasification, pyrolysis has been known as an efficient and popular technology for biofuel production because of its simplicity, product diversity, operating at relatively mild conditions, cheapness, and potential to be used in industry (Isahak *et al.,* 2012; Lin *et al.,* 2014). This technique involves thermal degradation of organic components under a non-oxidative environment by which biomass resources can be converted to the carbon-rich solid residues (bio-char), liquid products (bio-oil) and gaseous products (syngas) (Jahirul *et al.,* 2012). The yields and compositions of pyrolysis products depend considerably on the type and composition of the feedstock, pyrolysis type, and operating conditions (Azizi *et al.,* 2018). Considerable efforts have been made to study the pyrolysis behavior of algae biomass (Francavilla *et al.,* 2015; Grierson *et al.,* 2009; Pan *et al.,* 2010). Recently, macroalgae have received much attention as promising sources for biofuel production owing to their availability, high growth rate, and high photosynthesis efficiency (Choi *et al.,* 2017). Azolla is a small aquatic fern growing very fast and is widely found at the surface of Anzali wetland in north of Iran (Sadeghi *et al.,* 2014). *Ulva fasciata*, belonging to the family Ulvuceue, is a green macroalga that proliferates fast and occurs abundantly worldwide (Khan and Hussain, 2015). This macroalga is usually detrimental to local ecosystems and economy as it can change the biological and physicochemical properties of aquatic ecosystems (Sadeghi *et al.,* 2013). Therefore, its proper utilization can be considered as a good strategy to tackle with the environmental issues associated with its high distribution. More importantly, the usage of macroalgae as potential sources of renewable bioenergy would pave the way for energy security in the country, mitigate the worldwide challenges related to fossil fuels scarcity and global warming, and improve the economy by creating more job opportunities (Chang *et al.,* 2018). Some studies have focused on biofuel production from Azolla and Ulva species using the pyrolysis process (Biswas *et al.,* 2017; Ma *et al.,* 2020; Pirbazari *et al.,* 2019; Zhang, 2017). Biswas *et al.,* (2017) investigated the effect of temperature range 300-450 °C on pyrolysis of water rich *Azolla pinnata* in a glass reactor using carrier gas nitrogen flow rate of 50 ml/min and heating rate of 25 °C/min. They finally reached the maximum bio-oil yield of 38.5% at 400 °C. Moreover, Ma *et al.,* (2019) studied the capacity of bio-oil production from water rich Ulva prolifera in a fixed bed reactor and obtained the maximum bio-oil yield of 38.5% at 400 °C and carrier gas nitrogen flow rate of 30 ml/min. However, these studies primarily focused on the effects of one or more process variables on quality and yield of bio-oil using the conventional methods. Response surface methodology (RSM) is a statistical method widely applied in modelling and optimization of the processes (Bobadilla *et al.,* 2017). In contrast to conventional methods, RSM can consider the effects of independent variables and their interaction on the response while keeping the number of experiments minimized. In this way, it saves the time and experimental costs of the production process. The present study aimed to study the simultaneous effects of three experimental parameters (temperature, carrier gas flow rate, and heating rate) on slow pyrolysis of two types of natural macroalgae, *Azolla filiculoides* (*A. filiculoides*) and *Ulva fasciata* (*U. fasciata*), in order to achieve efficient biofuel production. To optimize the effects of process variables on bio-oil yield, a statistical analysis was performed using the RSM. The central composite design (CCD) of experiments was employed to develop the corresponding mathematical response surface equations. To the best of authors' knowledge, no study yet has been done on pyrolysis of Azolla or Ulva by RSM considering the optimal condition of the process. It should be noted that the authors will consider the experimental analysis of the mentioned parameters and cost analysis of bio-oil production through pyrolysis of *Azolla filiculoides* and *Ulva fasciata* in their future work. This study has been carried out in Biofuel Research Laboratory, Caspian Faculty of Engineering, College of Engineering, University of Tehran, Rezvanshahr, Iran during 2017-2019.

MATERIALS AND METHODS

Biomass preparation

Freshwater macroalga *A. filiculoides* was collected from Anzali wetland located in the northern part of Iran, and marine green alga *U. fasciata* was harvested from Makran Sea located at the southeast of Iran. Both macroalgae were harvested during the summer of 2019. The collected samples were thoroughly washed and dried under sunlight for 10 h. Afterwards, they were dried in an oven at 70 °C for 2 h. The feedstock was then pulverized by a grinding mill and sieved to an average particle size of 500-1000 µm. The ultimate analysis was performed using a Thermo Finnigan-CE Instruments Flash EA 1112 series elemental analyzer. The lipid and protein contents were determined by Soxhlet-extract method and Kjeldehl method, respectively. Moreover, the moisture and ash contents were obtained through ASTM E 1756 and ASTM E 1755, respectively. The obtained results are summarized in Table 1.

Experimental setup

The experimental system had three main parts: pyrolysis reactor (4), reformer (10), and condenser (11) (Fig. 1). The pyrolysis reactions were performed in a lab-scale fixed-bed reactor made of a stainless steel tube (23-cm long, 7.8-cm internal diameter) which was placed inside an electrical furnace (5). The stainless steel-made reformer had a length of 20 cm and diameter of 14 cm. The volumes of the pyrolysis reactor and the reformer were 1 and 0.45 L, respectively. The pyrolysis process was performed without using a catalyst in the reformer. Before pyrolysis, nitrogen (1) was fed into the system as an inert carrier gas with a controlled flow rate using a mass flow controller (3). The regulator (2) set the output gas pressure to 1 atm. An electric furnace was used to heat the reactor, and a K-type thermocouple connected with a temperature-controller system (8) was applied for measuring the temperature and controlling the heating rate. The algal feedstock (6) was loaded into the sample boat (7) inside the reactor. After completing the reaction, the pyrolysis products including biochar, bio-oil, and bio-gas were collected in the reactor, condenser (11-13), and gas bag (14), respectively.

Experimental procedure

To perform each experiment, 14 g dried biomass was placed in the reactor. Before the pyrolysis experiment, the nitrogen gas was passed through the system with a flow rate of 100 mL/min for 30 min to eliminate oxygen. The samples were heated from room temperature to different temperatures (300-500 °C) according to the designed heating rates (10 - 20 °C/min) and the nitrogen gas flow rates of 0.2-0.8 L/min. The reactor was kept at the desired temperature for 30 min. At the end of each run, the system was turned off and allowed to cool down to room temperature and then the solid fraction (bio-char) was collected from the reactor. The liquid phase (bio-oil) generated from the cooling down of condensable gases was collected in a falcon (13), weighed, and stored for further analysis. The non-condensable gases were stored

Table 1: Chemical composition and ultimate and proximate analyses of *A. filiculoides* and *U. fasciata* biomass samples

Element	Ultimate analysis					Biochemical composition			Proximate Analysis				HHV (MJ/kg)
	C	H	N	S	O[a]	Protein	Carbohydrate[b]	Lipid	Moisture	Volatiles	Fixed carbon[c]	Ash	
Azolla	42.5	7.12	3.15	0.12	47.11	18.34	56.38	6.98	10.90	75.38	6.32	7.40	16.13
Ulva	34.5	7.02	1.88	2.27	54.33	15.9	51.26	2.23	9.87	56.80	12.59	20.74	12.20

[a] Calculated by difference: % O = 100 - C - H - N - S

[b] Calculated by difference: % Carbohydrate = 100 - lipid - protein - ash – moisture.

[c] Calculated by difference: Fixed carbon = 100 - volatile - moisture – ash.

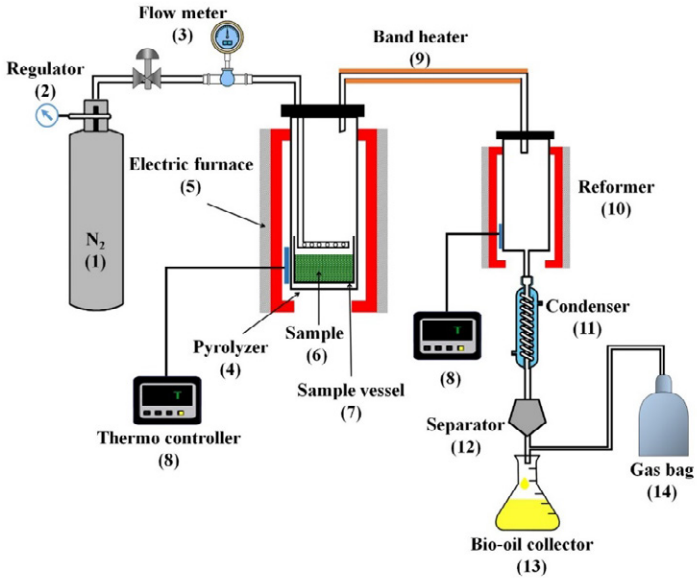

Fig. 1: Schematic representation of the pyrolysis experimental setup

Table 2: Variables and their levels used in the central composite design

Factor	Symbol	Levels				
		-α [a]	-1	0[b]	+1	+α [a]
Temperature (°C)	T	268	300	400	500	532
Carrier gas flow (L/min)	Q	0.1	0.2	0.5	0.8	0.9
Heating rate (°C/min)	H	9	10	15	20	22

[a] Axial points are equal to ±1.682 unit.
[b] Central point.

in a gas bag. All the experiments were performed in duplicates and the average values were reported. Finally, the pyrolysis performance was evaluated by measuring each fraction yield according to Eqs. 1-3 (Biswas *et al.,* 2017).

$$\text{Bio}-\text{oil yield, wt. \%} = \frac{\text{mass of bio}-\text{oil}(\text{g})}{\text{mass of feed}(\text{g})}\times 100 \quad (1)$$

$$\text{Bio}-\text{char yield, wt. \%} = \frac{\text{mass of bio}-\text{char}(\text{g})}{\text{mass of feed}(\text{g})}\times 100 \quad (2)$$

$$\text{Bio}-\text{gas yield wt. \%} = 100\% - \left(\text{Bio}-\text{oil yield, wt. \%} + \text{Bio}-\text{char yield, wt. \%}\right) \quad (3)$$

Experimental design

An experimental design combined with the RSM was used to optimize the pyrolysis process and minimize the number of experiments required. The experimental data and response surfaces were analyzed using the software package Design-Expert 11 trial version. A CCD was applied to evaluate the effects of three important factors, including temperature (T), carrier gas flow rate (Q), and heating rate (H), on bio-oil yields of *A. filiculoides* and *U. fasciata*. The variable ranges for the three parameters were determined based on previous studies and experimental setup specifications. Each independent variable was coded as high and low levels (+1 and -1, respectively) and the ranges were selected based on previous experiences. The factors and their variation levels are presented in Table 2.

The total number of experimental runs (N), suggested by CCD methodology, was calculated based on equation $N = 2^f + 2f + N_0$, where f is the number of variables, and N_0 is the replicate number of the center point. Therefore, 20 experimental runs (randomized) derived from three factors and six center points were conducted to investigate and optimize the variables effecting the bio-oil yield (Table 3). The relationship between the response (Y) and experimental variables (x_i, x_j) can be predicted by a quadratic polynomial model as presented by Eq. 4.

$$Y = \beta_0 + \sum_{1}^{3} \beta_i x_i + \sum_{i,j,j=2}^{3} \beta_{ij} x_i x_j + \sum_{1}^{3} \beta_{ii} x^2 \quad (4)$$

Where, β_0, β_i, β_{ij}, and β_{ii} represent the intercept, linear, interaction and quadratic coefficients, respectively. The significance of the predicted models was evaluated using the analysis of variance (ANOVA) with 95% confidence level.

Analysis

To evaluate the thermal decomposition characteristics of the algal samples, the thermogravimetry analysis (TGA) and the derivative thermogravimetry analysis (DTGA) were performed using the Perkin Elmer TG/DTA STA 6000 model. The samples were heated from 30°C to 900 °C at a constant heating rate of 10 °C/min under N_2 atmosphere. The chemical composition of bio-oils was analyzed by an Agilent 7890-5975c gas chromatography-mass spectrometry (GC-MS) instrument. The separation was performed on a UA-5 column (30 m × 0.25 mm ID × 0.25 µm film thickness). Helium (> 99.999%) was employed as a carrier gas at a flow rate of 1.0 mL/min. The initial oven temperature was set at 40 °C for 10 min, and later it was increasing to 300 °C at a rate of 10 °C/min, and finally held at 300 °C for 30 min. The injection volume of the samples was 0.5 µL. Some of the important qualitative parameters of the produced bio-oils, such as cetane number (CN), iodine value (IV), density, and viscosity, were specified based on the results obtained by GC-MS analysis and Biodiesel Analyzer version 2.2 software tool. The higher heating value (HHV) was obtained according to Dulong's formula, using Eq. 5 (Pourkarimi *et al.,* 2019).

$$HHV\left[\frac{MJ}{kg}\right] = 0.338 * C + 1.428 * \left(H - \frac{O}{8}\right) + 0.095 * S \quad (5)$$

Where, C, H, O, and S are the weight percentages of carbon, hydrogen, oxygen, and sulfur, respectively. The energy recovery (ER) was determined according to Eq. 6 (Babich *et al.,* 2011).

$$Energy\ Recovery\left(ER\right) = \frac{HHV_{bio-oil} \times m_{bio-oil}}{HHV_{biomass} \times m_{biomass}} \times 100 \quad (6)$$

Where, $m_{bio\text{-}oil}$ and $m_{biomass}$ are the weights of bio-oil and biomass, respectively.

Table 3: Central composite design arrangement and responses for Azolla and Ulv

Run No.	T (°C)	Q (L/min)	H (°C/min)	Bio-oil, wt. (%)		Bio-char, wt. (%)		Bio-gas, wt. (%)	
				Azolla	Ulva	Azolla	Ulva	Azolla	Ulva
1	400	0.5	22	27.86	23.93	44.29	50.07	27.86	26.00
2	400	0.5	15	29.57	25.91	41.43	50.36	29.00	23.73
3	532	0.5	15	23.79	31.36	37.21	45.71	39.00	22.93
4	300	0.2	20	15.36	21.12	48.29	53.93	36.36	24.95
5	500	0.8	20	27.86	27.00	37.71	46.57	34.43	26.43
6	268	0.5	15	18.71	18.29	56.57	58.21	24.71	23.50
7	500	0.2	10	21.71	34.43	44.00	49.00	34.29	16.57
8	400	0.5	15	30.64	26.86	42.21	50.36	27.14	22.79
9	400	0.5	15	28.07	26.43	43.29	50.79	28.64	22.79
10	400	0.5	15	29.29	26.00	40.71	50.57	30.00	23.43
11	400	0.5	9	26.43	25.90	42.86	50.00	30.71	24.10
12	300	0.8	10	13.29	21.29	51.14	52.43	35.57	26.29
13	400	0.5	15	28.14	26.21	42.07	50.43	29.79	23.36
14	400	0.9	15	18.86	23.00	45.00	49.57	36.14	27.43
15	500	0.8	10	18.36	29.50	37.36	47.71	44.29	22.79
16	300	0.2	10	21.07	22.43	51.86	59.00	27.07	18.57
17	400	0.1	15	24.64	27.71	43.00	50.29	32.36	22.00
18	300	0.8	20	15.57	19.29	60.86	52.86	23.57	27.86
19	400	0.5	15	30.00	25.29	40.00	50.29	30.00	24.43
20	500	0.2	20	26.43	31.50	44.14	48.57	29.43	19.93

RESULTS AND DISCUSSION

Biomass characterization

The results of chemical composition, ultimate and proximate analyses of the two macroalga samples have been shown in Table 1. According to the elemental analysis, the contents of C (42.5%) and H (7.12%) were higher in *A. filiculoides* than in *U. fasciata* (34.5 % C and 7.02% H). The carbon contents in both samples were higher than the carbon contents previously reported for green tide algae such as *U. fasciata* (25.64%) (Trivedi *et al.,* 2013), *Enteromorpha clathrata* (32.65%) (Wang *et al.,* 2013), and *Cladophorpha linum* (26.0%) (Bird *et al.,* 2011). The higher quantities of C and H and lower oxygen content in *A. filiculoides* led to a greater HHV, compared to *U. fasciata*. The higher HHVs obtained in this study can be comparable with the higher HHVs reported for other macroalgae, such as *Cladophora glomerata* (Norouzi *et al.,* 2016), *Laminaria japonica* (Choi *et al.,* 2015), *Enteromorpha clathrata* (Wang *et al.,* 2013), and *Porphyra tenera* (Bae *et al.,* 2011), ranging from 12 MJ/kg to 16 MJ/kg. *A. filiculoides* showed a higher nitrogen percentage (3.15%) compared to *U. fasciata* (1.88%), probably due to its higher protein content. Based on the proximate analysis, *A. filiculoides* macroalga had a low ash content of about 7.40 wt%, while *U. fasciata* had a high ash content of 20.74%. The ash content in Ulva was in the rang of the ash content previously observed in green algae *Ulva prolifera* (24.46%) (Ceylan and Goldfarb, 2015), brown algae *Saccharina japonica* (20.21%) (Kim *et al.,* 2012) and red macroalgae *Gracilaria gracilis* (19.98) (Francavilla *et al.,* 2015). Moreover, *A. filiculoides* had a higher volatile matter content (75.38%) compared to *U. fasciata* (56.80%). High volatile matter resulted in high efficiency in conversion of feedstock to products. The chemical composition analysis indicated that both macroalgae contained low amounts of lipid (<7%). However, the high contents of protein and carbohydrates as well as good volatile matters in both macroalgae made them suitable for bio-oil production through pyrolysis process.

Model analysis of CCD

Yields of pyrolysis products of *A. filiculoides* and *U. fasciata* based on the designed experiments with CCD have been presented in Table 3. It could be seen that the highest bio-oil yield for *A. filiculoides* (30.64%) was obtained at the temperature of 400 °C, heating rate of 15 °C/min, and N_2 flow rate of 0.5 L/min (experiment 8). Meanwhile, pyrolysis of *U. fasciata* at the temperature of 500 °C, heating rate of 10 °C/m, and N_2 flow rate of 0.5 L/min (experiment 7) led to the highest bio-oil yield (34.43%). Compared to the highest biochar yield for *A. filiculoides* (60.86%) (experiment 18), the highest biochar yield for *U. fasciata* (59%) (experiment 16) was observed at the same temperature (300 °C) but at lower heating rate and N_2 flow rate. The maximum syngas yields produced from the pyrolysis of *A. filiculoides* and *U. fasciata* were found to be of 44.29% (experiment 15) and 27.86% (experiment 18), respectively. The results revealed that the bio-oil yields of both biomasses enhanced with the increase of temperature to a certain point and then decreased at higher temperatures. According to Biswas *et al.* (2017), the maximum bio-oil yield of Azolla (38.5 wt.%) was achieved at the temperature of 400 °C, but the bio-oil yields decreased at the temperatures above 400 °C. Zhang (2017) and Ma *et al.* (2020) also reported that the bio-oil yield of *Ulva prolifera* improved with the increase of temperature and then gradually decreased at higher temperatures. It is generally believed that temperature is one of the significant factors affecting the bio-oil yield, since biomass depolymerization is extended with the increase of temperature. However, further increase of temperature above the specified optimum point does not usually lead to an improved bio-oil production. Two mechanisms can reduce the bio-oil production at high temperatures: 1) the secondary decomposition of pyrolysis vapors into non-condensable gases, which leads to the generation of more gaseous products; and 2) the repolymerization reaction of cracked species at high temperatures, which results in char formation (Pan *et al.,* 2010).

Analysis of variance

The results of ANOVA for bio-oil yields of *A. filiculoides* and *U. fasciata* are summarized in Table 4. According to the results, both models, with p values less than 0.0001, were significant. Besides, the lack of fit (LOF) values of 2.42 and 1.99 for *A. filiculoides* and *U. fasciata*, respectively, confirmed that the LOFs had no significant relationship with the pure errors and the models fitted well with the responses. The quality of fit of the models was also tested by determination coefficients (R^2, adjusted-R^2, and adequate precision). The R^2-values implied that the data variabilities for bio-oil yields of *A. filiculoides* and *U. fasciata* were 96.89% and 98.18%, respectively. Moreover, the good agreement observed between the predicted R^2 values (Azolla = 0.8022, Ulva = 0.9639) and the adjusted R^2 values (0.9410, 0.9734) indicated the well-fit of the experimental data with the predicted values. The adequate precision ratios greater than 4 (16.130 for Azolla and 37.540 for Ulva) represented the adequate model discrimination. Based on the data analysis rendered by multiple regression analysis, a quadratic model and an interactive model (2FI) were suggested by the software for *A. filiculoides* and *U. fasciata*, respectively, as the satisfactory response surface models to fit the experimental data. The model terms are significant at $p<0.05$. For *A. filiculoides*, all terms (A, B, C, AC, BC, A^2, and B^2), except AB and C^2 interactions, were significant model terms, while for *U. fasciata*, A, B, C, and AB variables, with p values less than 0.05, had significant effects on the bio-oil yields. The final predicted models for the bio-oil yield of *A. filiculoides* (AOY%) and *U. fasciata* (UOY%) were expressed using Eqs. 6 and 7:

$$AOY\% = +29.16 + 3.11\ A - 1.49\ B + 1.17\ C + 2.21\ AC + 1.60\ BC - 4.32\ A^2 - 3.96\ B^2 \quad (7)$$

$$UOY\% = +25.68 + 4.84\ A - 1.62\ B - 1.00\ C - 0.8064\ AB \quad (8)$$

The sign of the coefficient (+ or -) determines the direction of the relationship between the related

Table 4: Analysis of variance (ANOVA) for the bio-oils derived from Azolla and Ulva

Source	Sum of squares [a]	Df [b]	Mean square [c]	F-value [d]	p-value [e] Prob > F	Significance
Azolla:						
Model	556.63	9	61.85	34.67	< 0.0001	Significant
A [f]	111.38	1	111.38	62.43	< 0.0001	
B [g]	25.64	1	25.64	14.37	0.0035	
C [h]	15.42	1	15.42	8.64	0.0148	
AB	3.98	1	3.98	2.23	0.1661	
AC	38.91	1	38.91	21.81	0.0009	
BC	20.43	1	20.43	11.45	0.0070	
A^2	131.40	1	131.40	73.65	< 0.0001	
B^2	113.07	1	113.07	63.38	< 0.0001	
C^2	7.98	1	7.98	4.47	0.0606	
Residual [i]	17.84	10	1.78			
Lack of fit [j]	12.63	5	2.53	2.42	0.1770	Not significant
Pure error [k]	5.21	5	1.04			
Cor total [l]	574.47	19				
Ulva:						
Model	316.32	6	52.72	116.81	< 0.0001	Significant
A [f]	268.81	1	268.81	595.59	< 0.0001	
B [g]	30.23	1	30.23	66.99	< 0.0001	
C [h]	11.51	1	11.51	25.49	0.0002	
AB	5.20	1	5.20	11.53	0.0048	
AC	0.5618	1	0.5618	1.24	0.2848	
BC	0.0086	1	0.0086	0.0191	0.8921	
Residual [i]	5.87	13	0.4513			
Lack of fit [j]	4.47	8	0.5582	1.99	0.2325	Not significant
Pure error [k]	1.40	5	0.2804			
Cor total [l]	322.19	19				

[a] Sum of the squared differences between the average values and the overall mean.
[b] Degrees of freedom.
[c] Sum of squares divided by d.f.
[d] Test for comparing term variance with residual (error) variance.
[e] Probability of seeing the F-value if the null hypothesis is true.
[f] Temperature.
[g] Carrier gas flow rate.
[h] Heating rate.
[i] Consists of terms used to estimate experimental error.
[j] Variation of the data around the fitted model.
[k] Variation of the response in replicated design points.
[l] Totals of the whole information corrected for the mean.

variable and the response. The positive sign shows that the variable and the response move in the same direction. Conversely, when the variable and response move in opposite directions, the sign of the coefficient is negative. The strength of each relationship is measured by the absolute value of the related coefficient. As expressed by Eqs. 6 and 7, for both models, temperature variable (A) was the most effective factor in the bio-oil yields of both *A. filiculoides* and *U. fasciata* with absolute coefficients of 3.11 and 4.84, respectively. However, the effect of this variable on *U. fasciata* was greater. Gas flow rate (B) had almost the same negative effect on the bio-oil yields of both species. It meant that the bio-oil yields decreased with the increase of N_2 flow rate. The heating rate (C) had a positive effect on the bio-oil yield of *A. filiculoides* and a negative effect on the bio-oil yield of *U. fasciata*.

Response surface plots

The response surface plots of the models were used to display the graphical representation of the interactions between process variables and their effects on the bio-oil yield. These plots illustrated the effect of any two experimental factors while maintaining the third factor fixed at its central level. The related response surface plots of responses versus significant factors are shown for *A. filiculoides* and *U. fasciata* in Figs. 2 and 3*,* respectively. As illustrated in Fig. 2a, the bio-oil yield of *A. filiculoides* increased with the increase of temperature and carrier gas flow rate, and reached the maximum level at the temperature of 400 °C and gas flow rate of 0.5 L/min. However, a prolonged heating rate had a little positive effect on the response (Fig. 2b-c). Moreover, increase of temperature had a positive effect on the bio-oil yield of *U. fasciata*. In fact, it reached the maximum level at the temperature of 500 °C when the other two factors were kept fix at their lowest values (Fig. 3a-b). However, increase of carrier gas flow rate and heating rate led to the decrease of the bio-oil yield of *U. fasciata* (Fig. 3c).

Optimization of conditions

To achieve the optimum conditions for both *A. filiculoides* and *U. fasciata*, the maximum bio-oil yield was considered as the optimization goal. Based on the overall desirability function value of 1.0, the optimized conditions were determined as: a) temperature = 461 °C, heating rate = 20 °C/min, and N_2 flow rate = 0.5 L/min for *A. filiculoides*; and b) temperature = 500 °C, heating rate = 10 °C/min, and N_2 flow rate = 0.2 L/min for *U. fasciata*. Under these conditions, the optimum predicted bio-oil yields were found to be 30.64% and 34.43% for *A. filiculoides* and *U. fasciata*, respectively. Verification of the predicted results was performed by pyrolyzing of both biomasses under the optimized conditions. The experimental bio-oil yields of *A. filiculoides* and *U. fasciata* were obtained to be 30.83% and 34.29%, respectively. The results revealed that the experimental bio-oil yields were closely correlated with their model predictions with an error of 0.58% and 0.41% for *A. filiculoides* and *U. fasciata*, respectively. These obtained results were not in agreement with results reported by Biswas *et al.* (2017) for *Azolla pinnata* and by Ma *et al.,* (2019) for *Ulva prolifera* who reached the maximum bio-oil yield (38.5%) at the temperature of 400 °C.

Thermogravimetric analysis of Azolla and Ulva

Thermogravimetry and DTG analyses of *A. filiculoides* and *U. fasciata* seaweeds (Fig. 4a and 4b) were conducted to study the weight loss profile associated with thermal degradation, oxidation, and reaction with other gases. Fig. 4a and b showed the weight loss curves obtained from the pyrolysis of *A. filiculoides* and *U. fasciata*. The thermal degradation curves exhibited three different stages of weight loss. The first stage of weight loss occurred at temperatures lower than 180 °C, due to the removal of the adsorbed water in the macroalgae samples (Mulligan *et al.,* 2010). For *A. filiculoides* (Fig. 4a), the second and the third decomposition stages took place at 300 and 460 °C, corresponding with the decompositions of carbohydrates (cellulose, hemicellulose) and proteins, respectively (Kim *et al.,* 2013). This reflected that the pyrolysis of *A. filiculoides* should be performed at temperatures ranged 300-460 °C, since the thermal decomposition of organic matters occurred in this range. For *U. fasciata*, the main weight loss occurred in the second stage at the temperature of 220 °C, due to the volatilization of cellulose and hemicellulose materials present in the *U. fasciata* structure (Fig. 4b). Compared to the second stage, a lower DTG peak height in the third stage at the temperature of 630 °C was mainly due to the thermal degradation of lignin materials (Roslee and Munajat, 2018; Wu *et al.,* 2014). The lignin components in the biomass have a main role in biochar production. Higher lignin content produces more biochar yields and higher amounts of ash. According to Ma *et al.* (2020), temperature range of 300-500 °C was responsible for main product formation via the pyrolysis of *U. fasciata*. This was in line with the results obtained in the present study.

Properties of biomasses and produced bio-oils

Table 5 shows the elemental contents of the bio-oils derived from the pyrolysis of *A. filiculoides* and *U. fasciata.* Both algae oils had high amounts of nitrogen (3.25% and 4.21%), and sulfur contents of 0.11% and 2.15 %. Moreover, the S/N ratio for Ulva oil (0.66) was much more than the S/N ratio for Azolla oil (0.02). The large nitrogen contents and S/N ratios observed in the bio-oils might be attributed to the initial biomass compositions which were mainly composed of chlorophyll and proteins (Miao *et al.,* 2004). Therefore, further treatment seemed to be necessary before using the bio-oils for combustion to reduce the nitrogen and sulfur contents, especially for Ulva bio-oil because of its high S/N ratio. The reported results also showed that the *U. fasciata* pyrolytic bio-oil had lower carbon contents and higher

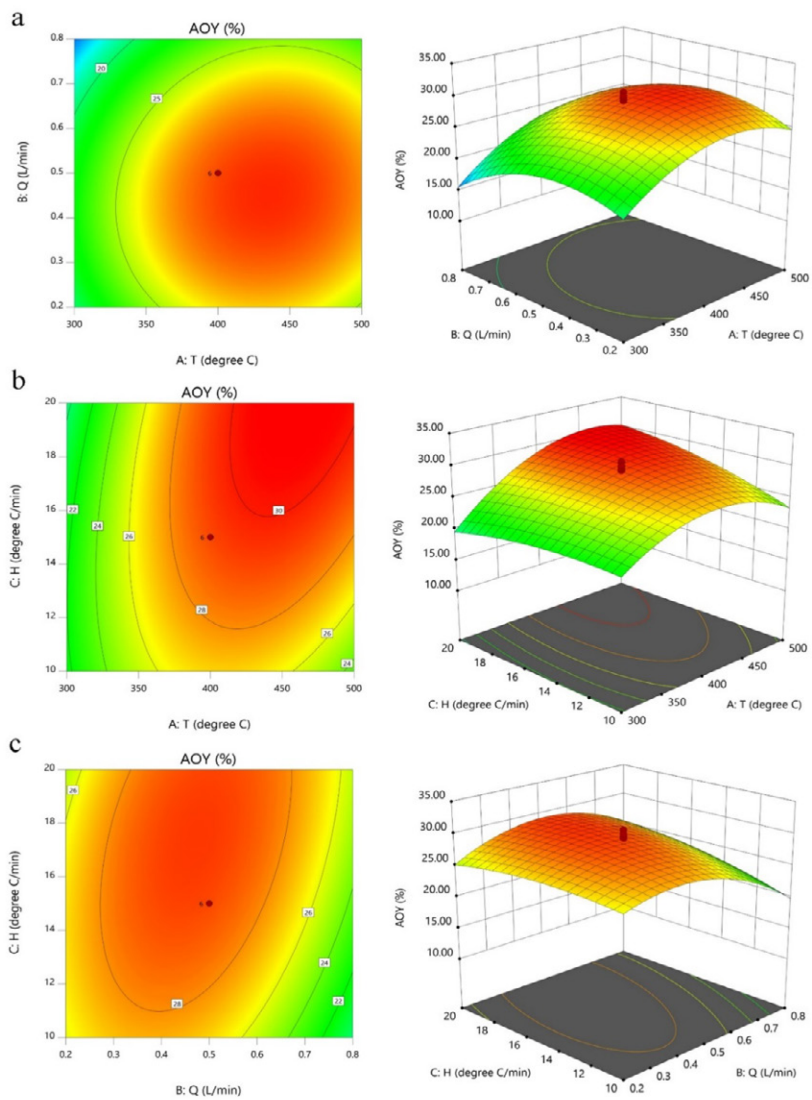

Fig. 2: The contour plots and three-dimensional response surface plots for the bio-oil yield of *A. filiculoide*: (a) temperature versus carrier gas flow rate; (b) temperature versus heating rate; (c) carrier gas flow rate versus heating rate

oxygen amounts, compared to *A. filiculoides.* This was probably due to the high concentration of cellulose in the initial *U. fasciata* biomass with a considerable amount of oxygen (Morris, 2011). The HHV, calculated by the Dulong's formula (Eq. 5), and the ER values of both bio-oil samples are also presented in Table 5. The bio-oil from *A. filiculoides* exhibited higher HHV values than the bio-oil derived *U. fasciata*, due to its relatively large carbon and hydrogen concentrations and small oxygen content. Conversely, the ER value of *U. fasciata* bio-oil (54.6%) was remarkably higher than that of *A. filiculoides* bio-oil (36.8%), because of its higher bio-oil yield and $HHV_{bio\text{-}oil}/HHV_{biomass}$ ratio. Table 6 shows the elemental contents of the biochars derived from the pyrolysis process. It can be seen the carbon amounts in both biochars are higher than the carbon amounts of both bio-oils. Moreover, the carbon amount of the Azolla-based biochar is higher than that of the Ulva-based biochar.

Effect of pyrolysis temperature on the products yield

As already discussed, temperature was the most effective parameter involved in the pyrolysis yields of *A. filiculoides* and *U. fasciata* biomasses. To compare

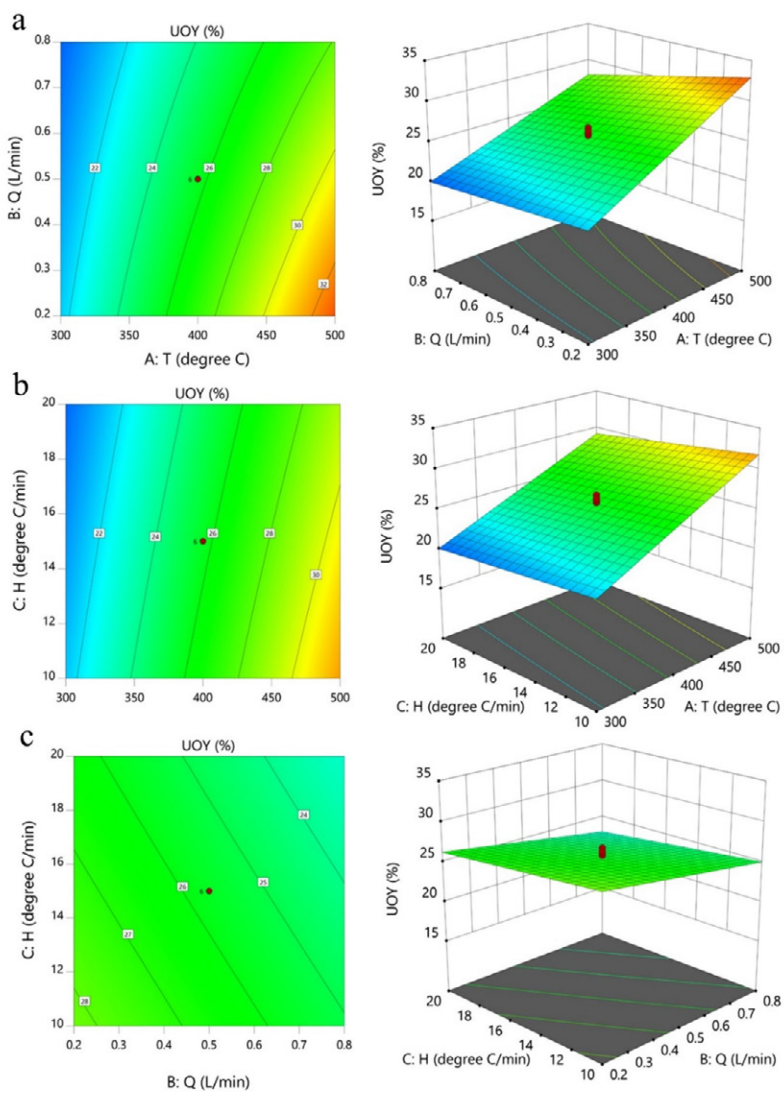

Fig. 3: The contour plots and three-dimensional response surface plots for the bio-oil yield of *U. fasciata*: (a) temperature versus carrier gas flow rate; (b) temperature versus heating rate; (c) carrier gas flow rate versus heating rate

the pyrolysis behavior of the two macroalgae, the yields of bio-oil, biochar, and biogas were investigated at several temperatures (268, 300, 400, 500, and 532 °C) (Fig. 4c and e). According to the presented plots, the bio-gas yields of *A. filiculoides* were significantly larger than those of *U. fasciata* at all temperatures. However, the yields of the bio-oil and biochar produced by *A. filiculoides* were lower than those of the bio-oil and biochar produced by *U. fasciata* at different temperatures. As shown in Fig. 4c, the maximum yields of bio-oils from *A. filiculoides* and *U. fasciata* were obtained at the temperatures of 400 °C (30.64 wt%) and 500 °C (34.43 wt%), respectively. At lower temperatures, the predominant reaction is carbonization and the bio-oil yields are small. When the temperature increases to the optimum point, more volatile components are formed during the algal pyrolysis, producing more liquid products. However, when the temperature exceeds this point, the secondary cracking of volatiles into more incondensable gases decreases the bio-oil yields. Therefore, the biochar yields of the two biomasses were significantly reduced as the pyrolysis temperature exceeded the temperature of 300 °C (Fig. 4d). However, the bio-gas yields of *A. filiculoides* and *U. fasciata* enhanced from 24.71 wt% and 23.5 wt% to 44.29 wt% and 27.43

Table 5: Ultimate analysis, high heating values and energy recovery values for the bio-oils obtained from the pyrolysis of *A. filiculoide* and *U. fasciata*

Element	Ultimate Analysis					HHV	Energy
	C (wt%)	H (wt%)	N (wt%)	S (wt%)	O (wt%)	(MJ/kg)	Recovery (%)
Azolla oil	51.23	6.87	4.21	0.11	37.58	20.43	36.8
Ulva oil	49.25	6.65	3.25	2.15	38.7	19.44	54.6

Table 6: Ultimate analysis for the biochars obtained from the pyrolysis of *A. filiculoide* and *U. fasciata*

Elements	C (wt%)	H (wt%)	N (wt%)	S (wt%)	O (wt%)
Azolla based biochar	62.43	4.19	2.71	0.08	30.59
Ulva based biochar	57.14	4.06	2.07	1.52	35.21

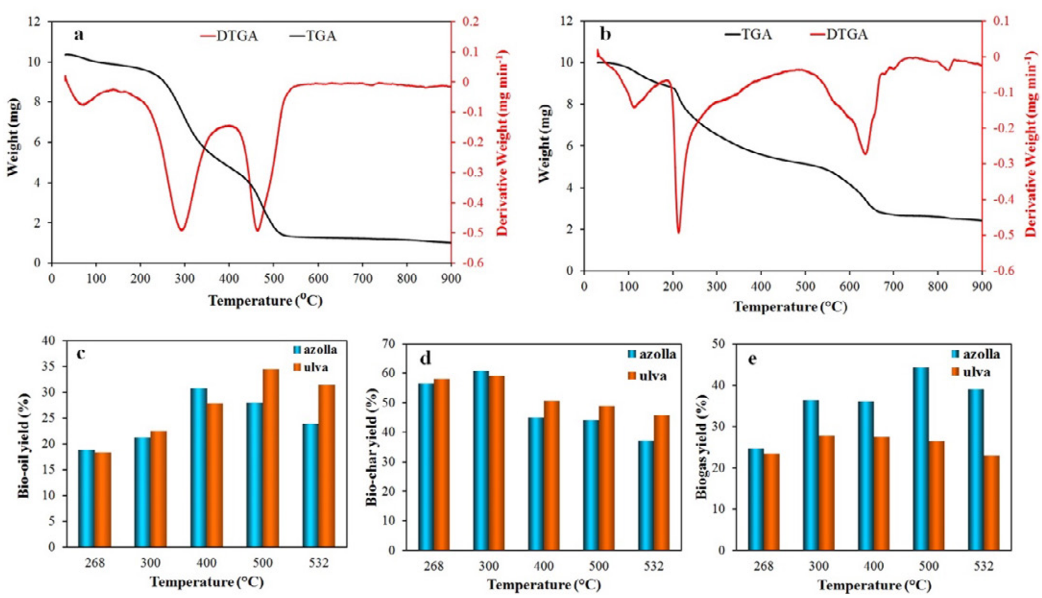

Fig. 4: Thermogravimetric curves for the pyrolysis process of (a) *A. filiculoide*; and (b) *U. fasciata* macroalgae, and yields of the products obtained from the pyrolysis of *A. filiculoide* and *U. fasciata* macroalgae at different temperatures: (c) bio-oil yield; (d) biochar yield; and (e) bio-gas yield

wt% by increasing the temperature from 260 °C to 500 °C, respectively (Fig. 4e). This could be explained by further thermal decomposition of the biochar and release of volatile components.

Qualitative analysis of the produced bio-oils

To evaluate the composition of organic compounds, the produced bio-oils were analyzed using a GC-MS instrument. As shown in Table 7, the produced bio-oils contained complex mixtures of aromatic and unsaturated hydrocarbons, alkanes, alkenes, and alcohols. Carbon distribution of the obtained bio-oil ranged from C_6-C_{24} to C_4-C_{16} for *A. filiculoides* and *U. fasciata*, respectively. Phenol (15.5%), p-Cresol (11.2%) and Catechol (9.2%) were mainly obtained from pyrolysis of *A. filiculoides*, while Ethyl acetate (11.9%), 1H-Pyrrole (9.9%) and 1-Tetradecene (7.2%) were largely obtained from pyrolysis of *U. fasciata*. This difference was probably due to the distinction of the chemical structures in the two biomasses. It should be noted that the higher contents of aromatics, phenols, and nitrogen-containing compounds in the *A. filiculoides* bio-oil rather than the *U. fasciata* bio-oil might be attributed to the higher protein content of *A. filiculoides* biomass (Table 1). According to Table 7, the aromatic hydrocarbons such as benzene, toluene, diol, and especially phenols were dominant components in the oil fraction of *A. filiculoides*. These products were associated with the degradation of the proteins containing the amino acids with aromatic rings in their structures. The majority of phenolic compounds, which are of great commercial importance, were typically present in the form of phenol, 4-methylphenol, 2-methyl phenol, 4-ethyl phenol, 2,4- dimethyl phenol, 3,5- dimethyl phenol, and 2,5- dimethyl phenol in the oil fraction of *A. filiculoides*. However, most of these compounds were absent in the pyrolytic oil of *U. fasciata* (Table 7).

Proteins were responsible for the formation of some nitrogenous compounds such as pyridines,

Table 7: Main compounds detected in the bio-oils of *A. filiculoide and U. fasciata*

No.	*A. filiculoide.*			*U. fasciata*		
	Formula	Component	Wt. %	Formula	Component	Wt. %
1	C_5H_5N	Pyridine	1.6	C_6H_6	Benzene	2.8
2	C_7H_8	Toluene	3.1	C_7H_8	Toluene	3.25
3	$C_5H_6O_2$	2-furan methanol	2.3	C_6H_6O	Phenol	2.7
4	C_6H_7N	3-methyl pyridine	1.6	C_8H_8	Styrene	3.3
5	C_8H_{10}	Ethyl benzene	2.0	$C_4H_8O_2$	Ethyl Acetate	11.9
6	C_6H_{10}	3-methyl-2- cycloPentene	0.8	C_4H_4N	1H-Pyrrole	9.9
7	C_8H_7N	Indole	7.6	$C_4H_8O_2$	2-Butene-1,4-diol	5.8
8	C_9H_{12}	1-Ethyl-3-methylbenzene	1.75	C_7H_5N	2-Ethynyl pyridine	3.7
9	C_9H_{12}	1,2,3-trimethylbenzene	1.2	C_6H_8O	2-Methylene-4-pentenal	2.3
10	$C_6H_8O_2$	3-methyl, 1,2-cyclopentanedione	1.5	C_5H_7N	1H-Pyrrole, 3-methyl	3.2
11	C_7H_{12}	2,3-Dimethyl-3-cycloPentene	0.7	C_7H_8O	Phenol,4-methylene	5.8
12	C_6H_6O	Phenol	15.5	$C_{10}H_{22}O$	1-Octanol,3,7-dimethyl	0.9
13	C_7H_8O	2-Methyl phenol	4.4	$C_6H_8O_3$	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	2.4
14	C_7H_8O	p-Cresol	11.2	$C_{10}H_8$	1H-Indene, 1-methylene	3.2
15	$C_7H_8O_2$	2-Methoxyphenol	0.7	C_8H_7N	Benzonitrile, 3-methyl	1.1
16	C_5H_5NO	3-Pyridinol	2.6	$C_{11}H_{24}$	Nonane,2,6-dimethyl	0.8
17	$C_8H_{10}O$	2-Ethyl phenol	1.00	C_5H_9O	2-methyl-3-butenyl	2.5
18	$C_8H_{10}O$	2,4-Dimethyl phenol	2.7	$C_9H_{18}O$	Nonan-4-one	2.5
19	$C_8H_{10}O$	2,5-Dimethyl phenol	1.6	C_8H_7N	Indolizine	3.2
20	$C_8H_{10}O$	4-Ethyl phenol	3.1	$C_{14}H_{28}$	1-Tetradecene	7.2
21	$C_8H_{10}O$	3,5-Dimethyl phenol	2.0	C_8H_7N	Indole	0.8
22	$C_6H_6O_2$	Catechol	9.2	$C_{14}H_{28}O$	E-2-Tetradecen-1-ol	5.6
23	$C_9H_{10}N_2$	2-Ethyl benzimidazole	1.7	$C_{13}H_{24}$	1-Tridecyne	1.3
24	$C_{11}H_{10}$	2- Methyl naphthalene	1.3	$C_{16}H_{32}O$	Hexadecanal	3.2
25	$C_7H_8O_2$	4-Methyl-1,2-benzenediol	4.42	$C_{10}H_{20}O_2$	Decanoic Acid	1.1
26	$C_{13}H_{26}$	1-Tridecene	1.1	$C_{11}H_{21}N$	Undecanenitrile	1.1
27	$C_{14}H_{28}$	2-Tetradecene	1.6	$C_{12}H_{22}O_2$	Allyl nonanoate	2.8
28	$C_{16}H_{34}$	Hexadecane	1.6	$C_{12}H_{24}$	3-Dodecene	1.00
29	$C_{24}H_{38}O_4$	Bis(2-ethylhexyl) phthalate	1.0	$C_{14}H_{29}NO$	Tetradecanamide	3.8
30	$C_{19}H_{40}$	Nanodecane	1.5	$C_{12}H_{24}$	2-Undecene, 3-methyl	1.3

Table 8: Biodiesel properties of *A. filiculoide* and *U. fasciata* and the EU standards for biodiesel

Quality parameter	Azolla oil	Ulva oil	Standard EN14214 (2012)
Cetane Number (CN)	69.8	62.4	51<
Iodine Value (IV) g I_2/100g oil	140	125	120>
Density (g/cm^3)	0.95	0.84	0.86-0.9
Kinematic viscosity (mm^2/s)	4.3	3.9	3.5-5

furans, indoles, and imidazoles. Alkenes, such as 1-Tridecene, 2-Tetradecene, 3-methyl-2- cycloPentene, and 2,3-Dimethyl-3-cyclopentene, could be produced from the conversion of unsaturated fatty acids in *A. filiculoides*. Other components identified in the *U. fasciata* bio-oil (Table 7), besides aliphatics and aromatic hydrocarbons, were alcohols, esters, ketones, and fatty acids with relatively long carbon chain amide and nitrile. Some physicochemical properties of the produced bio-oils (CN, IV, density, Kinematic viscosity, and CP) were determined using the Biodiesel Analyzer v2.2 software, and the obtained parameters were compared with the European Diesel and Biodiesel Standard (EN14214) as presented in Table 8. The CN shows the time delay in the fuel ignition. The minimum CN recommended by the European standards EN 14214 is 51 for dieselbiodiesel. In the present study, the CN values for pyrolysis of the bio-oils derived from *A. filiculoides* and *U. fasciata* were found to be 69.8 and 62.4, respectively. Although both bio-oil samples had a good quality according to the European standards, the bio-oil from *A. filiculoides* had a higher CN compared to the bio-oil produced from *U. fasciata*. This could be due to higher amount of oxygenated compounds and long-chain hydrocarbons present in the *A. filiculoides* bio-oil, which improved the combustion rate and CN (Costa *et al.,* 2018). Oxygen helps the formation of air-fuel mixture and reduces the ignition delay in order to achieve a good combustion. IV, as a factor for biodiesel tendency to react with O_2, is used to evaluate the degree of unsaturation (Saber *et al.,* 2016). The lower the iodine value, the better the combustion quality. The maximum IV recommended by the European standards EN 14214 for biodiesel is 120 g I_2/100 g of a sample. According to Table 8, the *U. fasciata* bio-oil showed a lower IV compared to the *A. filiculoides* bio-oil, probably due to the lower content of unsaturated products such as benzene, phenol, and their derivatives. The kinematic viscosity of the bio-oil produced from *A. filiculoides* was about 4.3 mm^2/s at the temperature of 40 °C, whereas the kinematic viscosity of the bio-oil derived from *U. fasciata* was about 3.9 mm^2/s at the same temperature (Table 8). The obtained values (i.e. 3.5-5 mm^2/s) were almost in the middle of the acceptable range proposed by the European standards EN 14214, indicating that the produced bio-oils could be consumed as a fuel in the current diesel engines without any modification. As shown in Table 8 (supplementary material), the density of the bio-oil produced from *A. filiculoides* was higher than that of the *U. fasciata* bio-oil. The higher density and kinematic viscosity of the *A. filiculoides* bio-oil could be explained by the presence of longer-chain hydrocarbons and higher amount of unsaturated compounds in the oil (Nascimento *et al.,* 2013). Considering the density and kinematic viscosity parameters, it was concluded that Ulva macroalgae would produce a higher quality pyrolytic bio-oil compared to *A. filiculoides* algae. The *A. filiculoides* bio-oil with higher heating value, higher viscosity and higher density can be consumed in the boilers and generators burning heavy oil, while the low viscosity and low density of the *U. fasciata* bio-oil make it more suitable to be used, either in neat form or blended, as a fuel in diesel vehicles (Kumar *et al.,* 2015).

CONCLUSION

In this study, the non-catalytic pyrolysis experiments were performed in a fixed-bed reactor on two types of biomass, *Azolla filiculoides* and *Ulva fasciata*. The effects of main pyrolysis parameters, including pyrolysis temperature, carrier gas flow rate, and heating rate, on bio-oil yields were investigated, and an experiment design method (Response Surface Methodology) was employed to determine the test conditions and analyze their results. The results obtained from the mathematical models indicated that the effect of pyrolysis temperature was more significant than the effects of nitrogen flow rate and heating rate on the bio-oil yields. Accordingly, at the optimum operating conditions, the highest bio-oil yields of 30.83 % (at the temperature of 461 °C, nitrogen flow rate of

0.5 L/min, and heating rate of 20 °C/min) and 34.29% (at the temperature of 500 °C, nitrogen flow rate of 0.2 L/min, and heating rate of 10 °C/min) were obtained from the pyrolysis of *A. filiculoides* and *U. fasciata*, respectively. The results showed that the burning quality of Azolla fuel was higher than that of Ulva fuel. Moreover, the Azolla bio-oil, showing a higher heating value and higher density and viscosity, proved to be more suitable for application in boilers and turbines. Considering the physicochemical properties, the bio-diesel obtained from *U. fasciata* macroalgae, with their superior quality, was a good candidate for application in diesel vehicles, compared to the bio-diesel derived from *A. filiculoides*. Technically, the results indicated that both biomasses could be considered as potential sources for producing the third-generation biofuel. Since further studies seem necessary to deepen the overall knowledge about the bio-oil production through pyrolysis of *A. filiculoides* and *U. fasciata*, the effect of catalytic pyrolysis on the yield and properties of the bio-oil derived from these species will be systematically investigated in our future work.

AUTHOR CONTRIBUTIONS

S. Pourkarimi has performed experiments, experimental design and data collection as well as partial analysis. A. Alizadehdakhel performed data analysis in the study. A. Hallajisani contributed to the literature, supervision and writing of the original draft. A. Nouralishahi edited and corrected the manuscript.

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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

$\propto$	Level of significance
%	Percentage
<	Less than
=	Equal
>	Greater than
° C	Degrees Celsius
°C /min	Degrees Celsius per minute (Heating rate = H)
° K	Degrees Kelvin
AOY	Bio-oil yield of Azolla
β_i (1,2,3 ... k)	Regression coefficients
C	Weight percentage of carbon
Cm	Centimeter
CN	Cetane number
DTGA	Derivative thermogravimetry analysis
et al.	"and others" in Latin
Eq.	Equation
ER	Energy recovery
Fig.	Figure
G	Gram
g/cm^3	Gram per cubic centimeter (Density)
H	Weight percentage of hydrogen
HHV	Heating value
IV	Iodine value
Kg	Kilogram
L	Litre
L/min	Liter per minute (Carrier gas flow) = Q
M	Meter
Mg	Milligram
Min.	Minutes
mL	Milliliter
mL/min	Milliliter per minute
Mm	Millimeter
mm/s	Square millimeters per second (Kinematic Viscosity)
MJ/Kg	Megajoule per kilogram
O	Weight percentage of oxygen
$P<0.05$	Probability that the null hypothesis is rejected
p-value	Probability value
RSM	Response surface methodology
R^2	Coefficient of determination
S	Weight percentage of sulfur
T	Temperature
TGA	Thermogravimetry analysis
UOY	Bio-oil yield of Ulva
X_i (1,2,3 ... k)	Explanatory variables
Y_i	Value of each individual observation

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REVIEW PAPER

Microplastics on the growth of plants and seed germination in aquatic and terrestrial ecosystems

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ABSTRACT

Growth of plants, apart from being complex and highly dynamic, is directly dependent on the environmental conditions, particularly the quality of soil for terrestrial plants and the water quality for aquatic plants. Presence of microplastics in the environment may affect the plant growth in numerous ways depending on the contents of the growing medium. However, increasing presence of microplastics at an alarming rate due to its pervasive usage and mismanagement of plastics have led to significant environmental problems. Several research studies have been conducted as well as reviewed to investigate the toxic effects of microplastics on aquatic systems, but studies that investigate the toxic effect of microplastics on the terrestrial systems are limited. Hence, in this review the individual and the combined effects of microplastics on the growth of plants and seed germination in both aquatic and terrestrial ecosystems are concisely discussed. At the beginning accumulation of microplastics on aquatic and terrestrial ecosystem is discussed and the reasonable solutions are highlighted that can mitigate the effects from the widespread increase of the plastic debris. Thereafter, the individual and combined effect of microplastics on seed germination and plant growth is reviewed separately while summarizing the important aspects and future perspectives. This review will provide an insight into the existing gap in the current research works and thus could offer possible implications on the effect of microplastics on plant growth and seed germination in aquatic and terrestrial ecosystem.

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INTRODUCTION

Widespread usage and mismanagement of plastic have been identified as a growing environmental effect in both aquatic (Wright *et al.,* 2013) and terrestrial ecosystems (Bläsing and Amelung 2018). Microplastics are considered to be contributing to plastic debris which are mainly categorized as primary and secondary microplastics depending on their formation (Nel and Froneman, 2015). The plastic debris whose initial particle size is less than 5 mm when manufactured are called "primary microplastics" and they are intentionally added to such as scrubbing agents, toiletries and cosmetics (Boucher and Friot 2017) or as ingredient for larger plastic productions (Nel and Froneman, 2015). The secondary microplastics is the most common source of pollution in the aquatic system which originates from fragmentation of larger plastic particles through photodegradation, biodegradation and other weathering processes such as thermo-oxidative degradation, mechanical degradation, and physical stress (Andrady, 2011). Different definitions exist for describing the range of size of microplastics and nanoplastics, but in general microplastics is considered as particles ranging from 100 nm to 5 mm in size and nanoplastics as particles lower than100 nm in size (Hernandez *et al.,* 2019). Fig. 1 gives an illustration of the types of sources in the terrestrial and aquatic environments, methods of degradation and fragmentation processes with the corresponding ranges in the size of plastic debris.

Several research studies regarding the effect of microplastics on plant growth have been conducted (Bhattacharya *et al.,* 2010; Jiang *et al.,* 2019; Prata *et al.,* 2018). According to the studies, the effect of microplastics on plants is directly proportional to its concentration. The presence of microplastics can delay the relative seed germination, root and shoot growth by inhibiting water uptake through short-term and transient mechanical blockage of pores in the seed capsule. Moreover, microplastics accumulate near the root hair results in a reduction of the growth rate (Bosker *et al.,* 2019). Initially, most of the investigations focused on examining the effect of microplastics on aquatic plants (Sjollema *et al.,* 2016) because of its accumulation in aquatic ecosystems

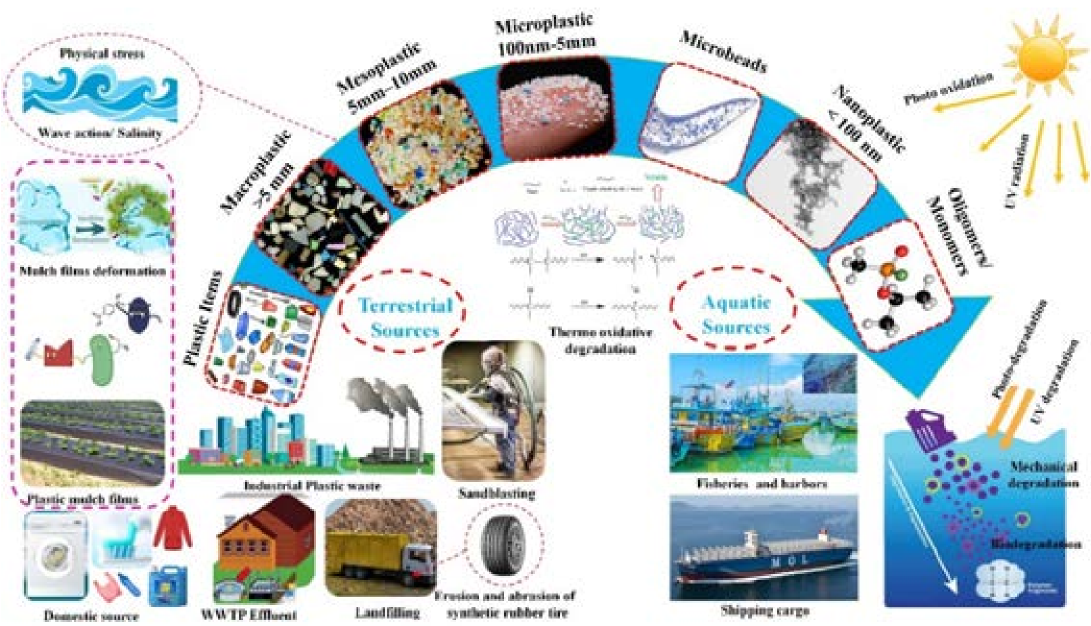

Fig. 1: Nano- and microplastic accumulation sources with the encircled (dotted lines) circle on the left showing the terrestrial sources and that on the right showing the aquatic sources. The range in the size of plastic debris resulting from the degradation and fragmentation process of plastic items to oligomers/monomers is shown across the arc in blue. Each of the squares represented in the red dotted line shows the standard size range as a result of different fragmentation cause

(Claessens *et al.,* 2011; Lechner and Ramler, 2015) in comparison to terrestrial ecosystems. Recently, attention has shifted to include effects of microplastics on terrestrial systems too. The effect of microplastics and engineered nanomaterials (Miralles *et al.,* 2012a) on plant growth varies as a function of plant species, structure and size of particles, chemical composition, and surface area. Furthermore, the effects on plant growth can be negative or positive (Rillig *et al.,* 2019). There are also reports related to the increase in the adverse effect from combination of microplastics with other chemical compounds in comparison to the effect of microplastics alone (Prata *et al.,* 2018). Consequence of expanded microplastic use becomes a threat to the human health (Cox *et al.,* 2019; Forte *et al.,* 2016; Schirinzi *et al.,* 2017) since microplastics can be transferred from prey to predators through the food chain. Furthermore, health of aquatic and terrestrial biota (Deng *et al.,* 2017; Hurley *et al.,* 2017) could be affected by nano and microplastics resulting in a number of unexplored health hazards. In highly urban areas, there exists report that a mean of 3223 and 1063 microplastic particles per year is ingested, respectively by children and adults released as road dust (Dehghani *et al.,* 2017). A few research studies have been implemented to assess the risk assessment of microplastics in food (Rainieri and Barranco, 2019) and it was found that the microplastics content of commercial salt is between 550−681 particles/kg in sea salts, 43−364 particles/kg in lake salts, and 7−204 particles/kg in rock/well salts (Yang *et al.,* 2015). It is estimated that the annual microplastic consumption of Americans' caloric intake was between 39000 to 52000 particles depending on age and sex. The estimated value could reach 74000 and 121000 from taking into consideration the effects of inhalation. Moreover, for people who drink only bottled water, they may ingest an extra 90000 microplastics annually and those who drink only tap water may be ingesting 4000 microplastics annually (Cox *et al.,* 2019). Furthermore, plastic teabags release approximately 11.6 billion microplastics and 3.1 billion nan plastics into a single cup at around 95°C temperature (Hernandez *et al.,* 2019). An abundance of microplastics was found in untreated and treated drinking water of water treatment plants (Koelmans *et al.,* 2019; Pivokonsky *et al.,* 2018). In order to mitigate the severity of the problem, a few countries have started regulating and banning the production and use of microbeads (Kramm *et al.,* 2018; Rochman *et al.,* 2015). This study focused on the individual and the combined effects of micro/nano plastics on the growth of plants and seed germination in both aquatic and terrestrial ecosystems. Here, the main topic of microplastics in different systems was categorized into three subtopics namely, the accumulation, effect on seed germination and plant growth. Firstly, the accumulation of microplastics in plants under both aquatic and terrestrial environments is discussed in detail. Next, in relation to the effect on plant growth, studies related to the individual and the combined effects of microplastics on seed germination and growth were considered. This study currently ongoing as a part of a doctoral dissertation entitled "Application of Optical Interferometric Techniques in monitoring the synergic effect of Microplastic with heavy metals on seed germination and plant growth" at Saitama University, Japan during 2019 - 2022.

Microplastic accumulation

It is a well-known fact that the abundance of microplastics exists all around the world affecting the growth of plants and can be found in oceans including deep oceans (Claessens *et al.,* 2013), coastal waters (Moore *et al.,* 2002), pelagic zones (Doyle *et al.,* 2011), coastal sediments (Claessens *et al.,* 2013) and beaches (McDermid and McMullen, 2004). Moreover, the river (Castañeda *et al.,* 2014; Driedger *et al.,* 2015; Nizzetto *et al.,* 2016a) and other terrestrial environments are also potential sites for microplastic accumulation. In 2018, world and European plastic production respectively reached almost 359 million tones and 61.1 million tons. Particularly, in Europe, plastic had been widely used for different purposes such as packaging (39.9%), building and construction (19.8%), automotive industry (9.9%), electrical and electronic appliances (6.2%), household and sport (4.1%), agriculture (3.4%) and others (16.7%). Furthermore, under the absence of control measures to cut production rate and mismanagement of plastic disposing methods, it is predicted that by 2050, approximately plastic waste in landfills or in the environment would reach 12,000 Mt (Geyer *et al.,* 2017). The plastic waste is largely accumulated in oceans due to its overwhelming usage and mismanagement (Jambeck *et al.,* 2015; Siegfried *et al.,* 2017; Van Sebille *et al.,* 2015). Consequently, the growth rate of aquatic plant and life expectancy of fish have been affected. The source of microplastics classified as primary is originating from synthetic fibers from clothes and houses and those due to the degradation of macroplastics classified as secondary microplastics arise from waste incineration and landfills (Dris *et al.,* 2016). Fibers including microplastics and small plastic debris generated through machining processes are transported by wind (Airborne microplastics) into the aquatic system (Dris *et al.,* 2016) or precipitated on plant surface (Chen *et al.,* 2020) leading to negative impact on both the aquatic and terrestrial ecosystems. The gravity of atmospheric fallout of the airborne microplastics has been expanded to human life arising in a number of health hazards, particularly in the respiratory system with 250 µm sized microfibers found in deeper lung regions of human (Enyoh *et al.,* 2019; Pauly *et al.,* 1998; Prata, 2018).

Microplastic accumulation in aquatic ecosystem

Recently, researchers reported different kinds of potential sources as the reason for accumulation of microplastics in aquatic system having an ecological impact. Among them, effluents from wastewater treatment plants (WWTP) contribute significantly as a potential source in urban rivers (McCormick *et al.,* 2014). Such effluents present in the domestic wastewater arise from microplastic particles that are intentionally added as ingredients for facial scrubs (Cheung and Fok, 2017; Lei *et al.,* 2017), cosmetics (Duis and Coors, 2016; Leslie, 2014), hand cleansers (Gregory, 1996) and fiber present in synthetic textiles (Browne *et al.,* 2011) and the removal of which being difficult due to their small sizes and low buoyancy (Fendall and Sewell, 2009). Thus, the domestic microplastics from household wastewater is directly transported to the rivers, and the rivers further transport that plastic and consequently to oceans. Hence, urban rivers are a potentially important source of the microplastic accumulation, particularly in the aquatic system. The plastic pollution in the Laurentian Great Lakes ecosystem was investigated in 2012 and an average count density of 43,157 ± 115,519 particles/km^2 was found (Eriksen *et al.,* 2013). The ocean is playing the role as the main sink for microplastics and the accumulation rate is rapidly increasing. Estimating the exact amount has been very challenging not only because of the lack of collection methods for microplastics (MPs) and nanoplastics (NPs) and further in characterizing them. Hence, different models have been developed to predict the transport of microplastic pollutions (Ballent *et al.,* 2013) and their accumulation (Pini *et al.,* 2019). As of 2014, estimated total amount in oceans across the world is more than 5 trillion floated plastic debris corresponding to a weight of around 250,000 tons (Eriksen *et al.,* 2014). The total accumulated microplastic amount on the water surface was estimated as 93,000–236,000 tons per year in the global ocean (Nizzetto *et al.,* 2016a). The accumulation of microplastics is enhanced through the commercial shipping process (Barnes and Milner, 2005). Moreover, microplastics are ingested (Cole *et al.,* 2013; Desforges *et al.,* 2015) and taken up (Besseling *et al.,* 2013; Ferreira *et al.,* 2016; Lu *et al.,* 2016) by marine biota leading to numerous health hazards. Sea urchin embryos (Della Torre *et al.,* 2014) and Mytilus (Bråte *et al.,* 2018) have been used to estimate MPs accumulation, pollution and toxicity. Furthermore, more plastics are being ingested by pelagic fish and more fibers are being ingested by benthic fish (Browne *et al.,* 2008; Lusher *et al.,* 2013; Murray and Cowie, 2011; Neves *et al.,* 2015) as a result of contamination of their natural habitat (Davison and Asch, 2011). Consequently, presence of microplastics resulted in decreased life expectancy of fish with abnormalities in their behavior possibly from the penetration of the microplastics through the blood brain barrier (BBB) into brain (Mattsson *et al.,* 2017).

Microplastic accumulation in terrestrial ecosystem

There is growing evidence that a considerable amount of microplastics is accumulating in the terrestrial environment through different potential sources such as mulch films (He *et al.,* 2018), greenhouse materials, road dust microplastics (Dehghani *et al.,* 2017), sewage sludge (Mahon *et al.,* 2017), land filling and atmospheric deposition (Klein and Fischer, 2019). Because of the anthropogenic activities, microplastics have become an emerging threat to the terrestrial system impacting the soil environment (De Souza Machado *et al.,* 2018) and eventually might become persistent organic pollutant (Lohmann, 2017) affecting the biodiversity of the soil (Rillig, 2012). The plastic mulch films (smallest size below 80 µm) widely used in farmlands reduce soil quality, thus producing microplastics on the terrestrial environment. Annual growth of the plastic mulch film is estimated to be around 5.7% in 2019 (Steinmetz *et al.,* 2016). China plays a key role in this aspect where 80% (20 million hectares) of agricultural land is enveloped by mulch film and in Europe that value is around 427,000 hectares. It was estimated that in China, the growth rate per year of mulch film was around 25% corresponding to 700,000 tones/ year (Espí *et al.,* 2006) that reached up to 1.25 million tons in 2011 (Boucher and Friot, 2017). The emerging threat of microplastic accumulation in the terrestrial environment is the road dust that contains microplastics. In such cases, automobile vehicle tire, road making paint, construction and building materials are considered as the main sources. In general, more than 50% of tires are manufactured from artificial rubber, the abrasion of which release the microplastic particles into the terrestrial environment (Sommer *et al.,* 2018). The annual emission owing to the abrasion of tires in Norwegian roads was calculated to be around 4,300 – 5,700 tonnes/year for microplastics (Vogelsang *et al.,* 2019). Moreover, the municipal wastewater treatment plants (WWTPs) sludge (biosolids) contributes highly to the accumulation of microplastics in agricultural soil leading to adverse effects of soil biota (Mason *et al.,* 2016). The sewage sludge adopted as a fertilizer to enhance the production of agricultural crop in turn increases the rate of microplastic accumulation in the soil at an alarming rate (Horton and Dixon, 2018; Saruhan *et al.,* 2010; Singh and Agrawal, 2008). In some countries, around 50% to 80% of biosolids were processed for agricultural purpose and more than 10 million tons of sewage sludges were generated through WWTPs in Europe Union (EU) in 2010 (Mahon *et al.,* 2017). Furthermore, generated microplastics from WWTP has a very high retention rate (99%) (Magnusson and Norén, 2014). A rough extrapolation of the existing data suggests that in European and North American farmlands microplastic accumulation through biosolids are in between 63,000 - 430,000 and 44,000 – 300,000 tons per year, respectively (Nizzetto *et al.,* 2016b). In Australian agroecosystem, that value is between 2800 – 19,000 tons per year (Ng *et al.,* 2018). Furthermore, tremendous amount of microplastics is accumulating in the terrestrial environment, enhancing the ecological impact on soil (Chae and An, 2018; Rillig *et al.,* 2017a; Zhu *et al.,* 2019). Consequently, soil biota, especially earthworms and gut microbiota are exposed to high microplastic concentration with significant reduction in their weight and reproduction (Huerta Lwanga *et al.,* 2016; Zhu *et al.,* 2018a; 2018b). Thus, the immune system of earthworms can be damaged by accumulated microplastics (Rodriguez-Seijo *et al.,* 2017). The effects are not restricted to the surface, as the earthworm exposed to microplastics could transport microplastics from the surface to groundwater level thus contaminating the groundwater as well exposing other soil biota (Rillig *et al.,* 2017b).

Regulating microplastic pollution

Microplastic pollution has been identified as an emerging threat for both aquatic and terrestrial environments. Recent research works have documented conversion or degradation of plastic waste through a combination of biodegradation, photo-degradation, and thermo-oxidative and thermal degradation processes over a long duration of approximately more than 100 years (Karan *et al.,* 2019). Hence, it is important to take prompt measures to prevent this long-lasting problem. One of the most reasonable solutions for this problem is to reduce, reuse and recycle plastics. Most of the countries have formulated different policies to reduce the plastic usage. For example, Scotland from banning the use of plastic bags has prevented 650 million bags entering into the waste system. Moreover, Ireland has successfully reduced the usage of plastic bags by 90% through increasing the taxes and introducing fine for plastic bag users. USA has also reduced plastic bags usage within the country in the range of 60% to 90% (Sharma and Chatterjee, 2017). Furthermore, most countries banned the use of microbeads in different domestic products such as facial scrubs, washing detergents, creams and toothpaste (Jiang, 2018). The microplastic accumulation rate could be reduced to a considerable amount through the reuse and recycle process. Plastic has been recycled within and outside of EU by 81% and EU 19% respectively in 2018. Especially in EU countries, plastic bottle collection machines installed close to the supermarket encourages the recycling and reusing process through providing monetary benefits for every returned bottle. One practical solution toward reducing the use of plastics could be to switch to the biodegradable materials such as polylactide (PLA) and polyhydroxyalkanoates (PHA)

(Wu *et al.,* 2017). Biodegradable microplastics offers the same advantages as conventional plastic in terms of usage (Shruti and Kutralam-Muniasamy, 2019) while the bioplastic degradation or fragmentation was approximately estimated to be lower than 48 days (Accinelli *et al.,* 2019), thus becoming a viable alternative. Furthermore, the particle separation efficiency of WWTP can be enhanced through new technology development that could capture even small microplastic debris avoiding pollution of terrestrial as well as aquatic environments.

Effect of microplastics on seed germination

New technologies have been incorporated in seed germination process to enhance seedling health, vegetative growth and curing plant injuries from many diseases and insect pests in which bioplastic are added as nano coating for seed coat with some active ingredients such as fertilizer or pesticide (Accinelli *et al.,* 2016). On the other hand, such techniques directly contribute to the longtime accumulation of microplastics in the terrestrial environment (Shruti and Kutralam-Muniasamy, 2019). Moreover, the presence of microplastics could delay the relative seed germination, relative root growth and relative shoot growth by inhibiting water uptake through short-term and transient mechanical blockage of pores in the seed capsule. The germination rate was significantly reduced after 8h exposure and gradually increased with passage time and the effect was dose–dependent and size-dependent (Bosker *et al.,* 2019). In this experiment three different sized plastic particles (50 nm, 500 nm, 4800 nm) were used with five different concentrations ranging from 10^3 to 10^7 particles/mL to observe the effect of MPs on seed germination. The decline of seed germination was observed from lowest to highest concentration for each MPs size as 67% - 38% for 50 nm, 50% - 30% for 500 nm and 55% to 17% for 4800 nm MPs emphasizing the dose-dependent adverse effect. Moreover, a clear size-dependent adverse effect was observed in highest MPs concentration resulting the reduction of seed germination up to 38% for 50 nm, 30% for 500 nm and 17% for 4800 nm. Interestingly, same tendency could be observed in the reduction of seed germination for lower concentrations as well. Thus, adverse effects on germination could increase with increase in the size of MP and its concentration. Moreover, microplastics accumulated near the root hair resulted in a reduction of growth rate (Bosker *et al.,* 2019). A significant difference was found in root growth after 24 h of exposure for 50 nm and 500 nm with respective rates of 16% and 21% for highest concentration in comparison to control. In contrast, 15% reduction of root growth was observed for 4800 nm for the highest concentration whereas the effect was not significant as other conditions. Furthermore, after 72h exposure to 500 nm particles, a significant difference in shoot length was observed with respective reduction rate of 19% for 10^6 particles/mL concentration compared to the control. Polystyrene nanoplastics (PSNPs) (100 nm) adsorbed and accumulated onto the spore of the plant surface (*Ceratopteris Pteridoides*) cause reduction of final spore size by 2.3% − 22.4% and inhibit water uptake reducing germination ratio by 10.4% − 88.0%. This is believed to be due to the physical blockage (Yuan *et al.,* 2019). Moreover, seed germination and seedling growth of wheat (*Triticum aestivum L.*) were examined and significant increment of root elongation (by 88.6% - 122.6%) was recorded with respect to control condition after exposure to polystyrene nanoplastics (PSNPs). Consequently, reduction of shoot to root biomass ratio (S.R ratio) was observed during the experiment after 5 days of exposure. Macronutrient such as C and N and plant biomass increased while micronutrients Fe, Mn, Cu and Zn accumulation decreased in varying degrees. Finally, it was found that PSNPs were taken up by root tips and transported to shoot across the xylem tissue (Lian *et al.,* 2019). Furthermore, synthetic fiber and biodegradable polylactic acid (PLA) can hinder seed germination of perennial ryegrass (*Lolium perenne*) (Boots *et al.,* 2019). The summary of microplastic effects for seed germination in aquatic and terrestrial ecosystem is shown in Table 1.

Individual effect of microplastics for plant growth

Microplastics accumulation and contamination have become an emerging problem since mass production of plastic begun in 1940 (Cole *et al.,* 2011). The adverse effect of microplastics has been increasing at an astonishing rate, giving negative impact to both aquatic (Capozzi *et al.,* 2018) and terrestrial biota (Jiang *et al.,* 2019) in which the microplastics debris are accumulated near the seed coat or root hair inhibiting imbibition, causing reduction of the seed germination rate and plant

Table 1: Microplastic effects on seed germination in both aquatic and terrestrial ecosystem

Seed type	Scientific name	Plastic type	Size of plastic	Tested concentration	Exposure time	Ecosystem	Effects for the germination	Reference
Vascular	*Lepidium sativum*	Nano and Microplastics	50nm, 500nm, 4800nm	10^3, 10^4, 10^5, 10^6, 10^7 particles/mL	8 h, 24 h, 48 h, 72 h	Terrestrial	Seed germination was temporally delayed through mechanical blockage of pores in seed capsule.	Bosker *et al.* (2019)
Plants	*Ceratopteris pteridoides*	Polystyrene nanoplastics (PSNPs)	100 nm	0, 0.16, 0.8, 4, 20, 100 µg/mL	28 days	Aquatic	PSNPs were adsorbed and accumulated on the spore surface while reducing spore size and germination ratio.	Yuan *et al.* (2019)
Wheat	*Triticum aestivum L.*	Polystyrene nanoplastics (PSNPs)	100 nm	0.01–10 mg/L	1, 5, 7, 14, 21 daya	Terrestrial	Reduced shoot to root biomass ratio and micronutrient adsorption. PSNPs were taken up and transported to shoot via the xylem.	Lian *et al.* (2019)

growth by considerable amounts (Kalčíková *et al.,* 2017). The literature available so far suggests that most of the time, the effect has dose-dependent and size-dependent response. Moreover, the adverse effect of MPs on the seed germination is enhanced with the increase of particle size as large plastic particles can inhibit imbibition of water and nutrient through physical blockage compared to small particles. In contrast the adverse effect of MPs on plant growth is enhanced with decrease of particle size inducing genotoxic and cytotoxic effect. Based on existing research works, nano scale plastic particles could enter the roots and probably block cell connections or cell wall pores and disrupt the nutrient uptake inducing observed toxic effect. Furthermore, the cell damage was observed from internalized plastic particles leading to reduced root growth and shoot growth. Hence, the nano scale plastic particle gives adverse effect on root growth while reducing growth of plants. However, there is a lack of a clear evidence for nano and microplastic uptake by plants and toxicity effect on plants. Nevertheless, a few studies suggest that, the uptake and translocation ability depend on different parameters such as the organic and geometric property of plastic debris (material, size, shape), root and xylem properties (surface area, volume) and plasma membrane potential (Trapp, 2000). Consequently, *Nicotiana tabacum* BY-2 cells can uptake 20 nm and 40 nm nanobeads through endocytic internalization into turgescent and plasmolyzed cells and 100 nm beads are accumulated or adhere near the cell wall. The large nano beads from 20 nm to 1000 nm excluding 2000 nm can be internalized in protoplasts (Bandmann *et al.,* 2012). The hypothesized that the mechanism of uptake and translocation of MPs can be the same as reported for nanoparticles uptake by plants. Nanoparticles are adsorbed to plant surfaces and taken up through natural nano or micrometer scale plant openings (Dietz and Herth, 2011). Moreover, newly developed roots have small cracks through which the small particle can enter. Those microplastics can then travel from the roots up to the edible parts of the crop along the xylem. There are several pathways exist or are predicted for nanoparticles association and uptake in plants as illustrated in Fig. 2. This type of nanoparticles uptake by plant is inversely proportional to the particle size and can provide adverse effect on plant growth and crop yield. More recently, presence of micro- and nano-plastics in edible fruit and vegetables were examined emphasizing the great risk for human health (Conti *et al.,* 2020). In that experiment apple

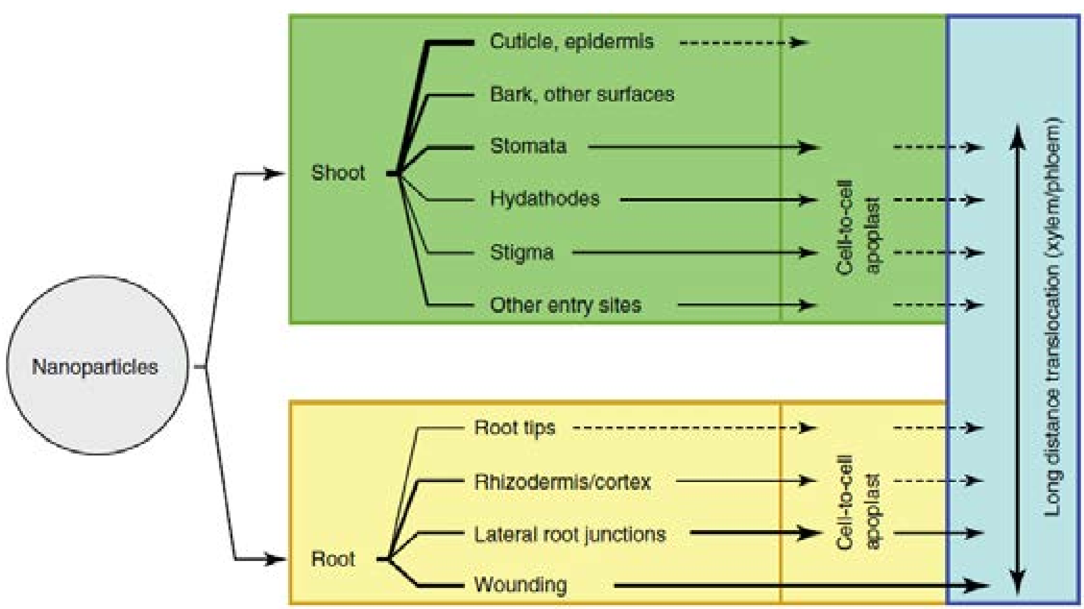

Fig. 2: Pathways of nanoparticle association, uptake, and translocation in plants. The assumed significance of the pathways is represented by the line thickness and the assumption of very low rates of transport is indicated by broken line (Dietz and Herth, 2011)

and carrot were identified as the most contaminated fruit and vegetable, respectively. The smallest size of MPs was found in the carrot samples (1.51 µm), while the biggest ones were found in the lettuce (2.52 µm).

Microplastic effects on the aquatic plant growth

It is a well-known fact that, oceans, and rivers act as the main sink for the accumulation of microplastics. Hence, the adverse effect of microplastics for aquatic plant is comparatively high. MPs effects can be categorized in to three main parts as adsorption, uptake, and toxicity. Recent research studies document that, charged nano and microplastics tend to be physically adsorbed by algae species (*Chlorella and Scenedesmus*) causing reduction of photosynthesis activity and growth rate, inhibiting air flow and light through physical blockage. Moreover, the adsorption process of MPs may enhance the production of reactive oxygen species (ROS) in algae (Bhattacharya *et al.,* 2010). The negatively or positively charged nano plastic at a high concentration (> 50 µg/mL) reduce microalgal growth. This is due to the adsorption of nano particles onto microalgal surface (Bergami *et al.,* 2017). The microalgal growth can be reduced by the uncharged polystyrene (PS) microplastics up to 45% at the highest concentration of 250 mg/ L and 72 h bioassay, compared to the control. The increasing adverse effect was observed when the particle size was decreased. The experiment was implemented by deploying three different sizes of uncharged and negatively charged microplastics (0.05, 0.5, 6 µm) to investigate small plastic particle effect for photosynthesis and growth of microalgal. However, the experimental results do not imply any obvious change in photosynthesis effect (Sjollema *et al.,* 2016). A few measurements done with atomic force microscopy (AFM) showed that adsorption capacity of neutral or positively charged microplastics onto algae cell wall (*P. subcapitata*) is comparatively higher than that for negatively charged plastic particle (Bhattacharya *et al.,* 2010; Nolte *et al.,* 2017). The root growth of the floating plant, such as duckweed species (*Lemna minor, Spirodela polyrhiza*) was negatively affected by nanoplastics and microbeads through mechanical blocking as a result of microplastic adsorption. Here again, the chlorophyll content and photosynthesis activities were not affected (Dovidat *et al.,* 2019; Kalčíková *et al.,* 2017). The nano and microplastic effects on macrophytes species (*Myriophyllum spicatum, Elodea sp*) have been studied and shoot to root ratio (S.R) reduction was examined in both macrophytes for nanoplastics whereas root length and shoot length of *M. spicatum* were reduced by microplastics in high concentration owing to the reduction of nutrient imbibition (van Weert *et al.,* 2019). The growth rate and photosynthetic activity of algae (*Chlorella pyrenoidosa*) was reduced by PS microplastics (0.1, 1 µm) under three different concentrations (10, 50, 100 mg/L), inducing dose-dependent adverse effects. In contrast, the distortion and damages of thylakoids and cell membrane were observed after 13 days exposure to confirm physical damage and oxidative stress. The acute toxicity effect was found due to biological adaptation of algae and, cell structure recovered to its normal stage after 25 days exposure while enhancing the growth rate (Mao *et al.,* 2018). Furthermore, PSNPs can reduce chlorophyll concentration and population growth in green algae (*Scenedesmus obliquus*) after 72h bioassay (Besseling *et al.,* 2014). Zhang *et al.* (2017), examined the toxic effect of microplastics (mPVC, 1 µm) and bulk plastic debris (bPVC, 1mm) on algae (*Skeletonema costatum*) at respective concentrations of 5mg/L and 50 mg/L and found that mPVC reduced growth rate of algae with the inhibition ratio reaching a maximum of 39.7% after 92 h bioassay. Besides, at high plastic concentrations, chlorophyll content and photosynthesis efficiency decreased. However, bPVC does not have any adverse effect on both the growth rate and the chlorophyll content. Moreover, moss species (*Sphagnum palustre L*) can be used as biomonitors to examine nano and microparticle pollution in the aquatic environment. Large and small plastic debris accumulated more on dead moss materials than living moss. This is because of the increased accumulation damaged cell membrane of dead moss (Capozzi *et al.,* 2018). Table 2 illustrates the individual effects of microplastic for plant growth in the aquatic environment.

Microplastic effects on the terrestrial plant growth

The knowledge about the impact of microplastics for the terrestrial environment is very limited compared to the aquatic ecosystem (Horton *et al.,* 2017). Nonetheless, the terrestrial ecosystem has been subjected to a high microplastic accumulation due to widespread usage and mismanagement of

Table 2: Individual effect of microplastics for plant growth in aquatic ecosystem

Plant species	Scientific name	Plastic type	Size of plastic	Tested concentration	Exposure time	Effect for the plant growth	Reference
Mosses	*Sphagnum palustre*	Fluorescent unmodified PSNPs	50 nm	3 x 10^{11} NPs/mL (18 mg/L)	7, 14, 21 Days	More NPs were accumulated in devitalized moss than in living moss and number of particles increase with exposure time.	Capozzi *et al.* (2018)
Algae	*Chlorella pyrenoidosa*	Polystyrene (PS)	0.1 µm, 1 µm	10, 50, 100 mg/L	30 Days	Adverse effects (physical damage and oxidative stress) were observed at the beginning and enhance the growth with passage of time.	Mao *et al.* (2018)
Algae	*Skeletonema costatum*	Microplastics and Bulk plastic	1 µm, 1 mm	5, 50 mg/L	1, 24, 48, 72, 96 h	There is a size and dose-dependent effect. At high concentration, microplastics had adverse effect on chlorophyll content and photosynthetic efficiency. The adverse effect decreased with time.	Zhang *et al.* (2017)
Algae	*Scenedesmus obliquus*	Polystyrene nanoplastic	~70 nm	44 - 1100 mg nano-PS/L	72 h	Polystyrene NPs reduce the population growth and chlorophyll concentration of algae.	Besseling *et al.* (2014)
Algae	*Chlorella and Scenedesmus*	polystyrene (PS) beads	20 nm	0.08-0.8	2, 24 h	Inhibited photosynthesis activity.	Bhattacharya *et al.* (2010)
Algae	*P. Subcapitta*	PSNPs	110 nm	0–100 µg/mL	72 h	NPs are adsorbed onto algae cell wall and inhibited algal growth	Nolte *et al.* (2017)
Algae	*D.tertiolecta,*	Polystyrene nanoparticles	40, 50 nm	0.5–50 µg/mL	2, 3 and 14 Days	NPs were adsorbed on microalgal and diminished microalgal growth at high concentration (> 50 µg/mL).	Bergami *et al.* (2017)
Microalgal	*D.tertiolecta, T. pseudonana, C. vulgaris*	Polystyrene MPs	0.05, 0.5 and 6 µm	25, 250 µg/mL	72 h	Growth was reduced up to 45% compared to the control at the highest concentration (250 mg/ L) and the adverse effects increased with decreasing particle size.	Sjollema *et al.* (2016)
Duckweed	*Spirodela polyrhiza*	Red fluorescent	50, 500 nm	10^2, 10^4, 10^6 particles/mL	120 h	MPs were adsorbed to the roots. No significant effect was observed on plant growth and chlorophyll production.	Dovidat *et al.* (2019)
Duckweed	*Lemna minor*	polyethylene microbeads	30 to 600 µm /40 to 400 µm	0, 50, 10, 100 mg/L	7 Days	MPs were adsorbed to the roots and root growth were significantly affected by mechanical blocking	Kalčíková *et al.* (2017)
Macrophytes	*Myriophyllum spicatum*	Polystyrene NPs and MPs	50 – 190 nm,	Polystyrene NPs and MPs	21 Days	The shoot to root ratio was significantly reduced by the NPs for both macrophytes due to reduction of nutrient accumulation.	van Weert *et al.* (2019)
Plant	*Nicotiana tabacum* BY-2 cells	Nano beads	20, 40, 100, 1000 nm	10, 30 µM	30 min	Small nano beads (20 and 40 nm) were internalized rapidly and accumulated partially.	Bandmann *et al.* (2012)

plastic waste causing growth reduction and toxicity effect for plants. Thus, a significant growth reduction of the higher plant (*Vicia faba*) was observed after 48 h exposure to microplastics only at the highest concentration (50,100 mg/L). Furthermore, the biomass weight and catalase (CAT) of plant root decreased by a considerable amount for 5 µm plastic debris whereas peroxidase (POD) and superoxide dismutase (SOD) enzyme activities were enhanced. The experimental results imply that the genotoxic (micronucleus test) and oxidative damage (enzymes activity) to *V. faba* is inversely proportional to particle size and thus the toxic effect of microplastics increased with decreased particle size. Same as in aquatic plants, microplastics can be accumulated near the root tip of terrestrial plants (*V. faba*) in which water and nutrient imbibition would be inhibited through the mechanical blockage of cell wall pores (Jiang *et al.,* 2019). Further, polystyrene (PS) nanoplastics induced cytotoxic, genotoxic, and oxidative damages on treated root of *allium cepa* due to external mechanical contact of nano PS with the root surface. The effect was dose-dependent and the internalization of nano PS occurred in different cellular compartments increasing the possibility of entering microplastics into the food chain (Giorgetti *et al.,* 2020). There exists limited evidence regarding microplastic uptake and translocation in terrestrial plants. However, some research studies have provided evidence about the uptake of microbeads by wheat (*Triticum aestivum*) under three different concentrations (Table 1). There was size-dependent effect where the plants ability to uptake microbeads is higher for smaller microbeads (0.2 µm) than that of the large ones. Hence, 0.2 µm microbeads were easily transported to stem and leaves across the vascular system through apoplastic pathway (Li *et al.,* 2019). Moreover, for wheat, microplastics were found to affect both the vegetative and reproductive growth. Thus, the above-ground and below-ground parts of wheat were affected by small plastic particles (Qi *et al.,* 2018). The material of plastic mulch film used for the farming process also has a significant effect on wheat growth. Moreover, the effects of microplastic in both the above and below ground soil ecosystem was observed by (Boots *et al.,* 2019), using grown grass (*Lolium perenne*) in earthworms (*Aporrectodea rosea*) under three different microplastic types (biodegradable polylactic acid (PLA), conventional high-density polyethylene (HDPE), and microplastic clothing fibers). PLA reduced both shoot height and seed germination rate in which fiber was able to decrease only seed germination rate (6% – 7%). Furthermore, HDPE reduced the soil pH value by a significant amount. The root biomass differed significantly between treatments and shoot biomass did not exhibit much difference. As a result of that, dry shoot to root ratio differed significantly under different treatments. Chlorophyll-a, chlorophyll-b content did not show a clear difference. The individual effect of microplastics on plant growth in the terrestrial environment is given in Table 3. So far, the knowledge about uptake, translocation, and toxic effect of nano and microplastics for terrestrial plant species are very limited. Some studies documented about uptake (De La Torre-Roche *et al.,* 2013), translocation (Zhang *et al.,* 2019), and bioaccumulation (Lee *et al.,* 2008) of carbon nanoparticle into the whole plant of rice (Lin *et al.,* 2009), maize and soybean (Zhao *et al.,* 2017). Plants could take up ENPs through root tips and transport it to shoot and leaves with the help of vascular system (Ma *et al.,* 2010). Consequently, cell damage may occur causing significant cell death at a high concentration ENPs (Shen *et al.,* 2010). Furthermore, ENPs accumulated on root tips of alfalfa and wheat plants while taking up some particles into other plant parts through the vascular system (Miralles *et al.,* 2012b).

Combined effects of microplastic for plants growth

Recent studies report that the combined effect of microplastics with different persistent organic pollutants (POPs) that contain pharmaceutical, chemical, and heavy metal can give high effect on plant growth compared with the effect of microplastics alone. Existing knowledge regarding the combined effect of microplastic on plant growth is very limited and more research studies are needed to estimate the risk. The combined effect of pharmaceutical (procainamide, doxycycline) and microplastics have been studied under specific growth rate and chlorophyll-a concentration as observation parameters. The significant adverse effect of microplastics alone on microalgae (*Tetraselmis chuii*) growth was observed only at a high concentration (41.5 mg/L). On the other hand, reduction of chlorophyll was observed only in low concentrations (0.9, 2.1 mg/L). Nevertheless, even in low concentrations,

Table 3: Individual effect of microplastics for plant growth in the terrestrial ecosystem

Plant species	Scientific name	Plastic type	Size of plastic	Tested concentration	Exposure time	Effect for the plant growth	Reference
Bean	*Vicia faba*	Polystyrene Fluorescent Microplastics	100 nm, 5 µm	10, 50, 100 mg/L	48 h	Microplastics induce higher genotoxic and oxidative damage. A significant decrease of growth was observed.	Jiang *et al.* (2019)
Onion	*Allium cepa*	Polystyrene nanoplastics	50 nm	0.01, 0.1, 1 g /L	72 h	The cytotoxic, genotoxic, and oxidative damages were observed. Nano PS were Internalized in cellular compartment.	Giorgetti *et al.* (2020)
Grass	*Lolium perenne*	PLA, HDPE, Microplastics clothing fibers	>2000 µm, 2000–1000 µm, 1000–250 µm, 250–63 µm, <63 µm	0.1% (w/w) (HDPE and PLA) (1 g/kg), 0.001% (w/w) synthetic fibers (10 mg/kg)	30 days	Reduced both shoot height and seed germination. The root biomass and shoot to root ratio differed significantly between treatments.	Boots *et al.* (2019)
Wheat	*Triticum aestivum*	Polyethylene and biodegradable plastic debris	50, 250, 500, 1000 µm	1% (w/w)	2, 4 months	Above-ground and below-ground parts of wheat plant were affected during vegetative and reproductive growth.	Qi *et al.* (2018)
Wheat	*Triticum aestivum*	PS microbeads	0.2, 2, 7 µm	0.5, 5.0, 50 mg/L	10 days	PS microbeads uptake by roots and transported to stem and leaves.	Li *et al.* (2019)

both pharmaceuticals were toxic to *T. chuii*, and the mixtures of microplastics-pharmaceutical were more toxic than the pharmaceuticals alone (Prata *et al.,* 2018). These results imply that the combined adverse effect is significantly high compared to the effect of microplastics alone or the pharmaceutical alone, owing to the toxicity effect of fragmented product through the degradation process. Microplastics are capable of adsorbing POPs (Bakir *et al.,* 2012) and trace metal (Holmes *et al.,* 2012) leading to a combined effect that could be either negative or positive to aquatic and terrestrial biota. The heavy metal adsorption ability of MPs is directly depending on the characteristics such as specific surface, porosity, and morphology. The adsorption isotherms were better described by Langmuir model, which indicates that the main adsorption mechanism might be chemical adsorption (Godoy *et al.,* 2019). However, the interaction between heavy metal and aged MPs are always greater than virgin MPs owing to their long-term pre-modification through photooxidation and attrition of charged material (Turner and Holmes, 2015). Moreover, different polymer type of MPs exhibits different adsorption capability, being the adsorption order of PE > PVC > PS > PP > PET. Especially on a significant adsorption of lead, chromium, and zinc on polyethylene and polyvinyl chloride a significant adsorption of lead, chromium, and zinc on microplastics was observed. The adsorption of Cd was quite rapid initially, and the equilibrium time was approximately 90 min. An increase in the pH of the Cd solution led to an increase

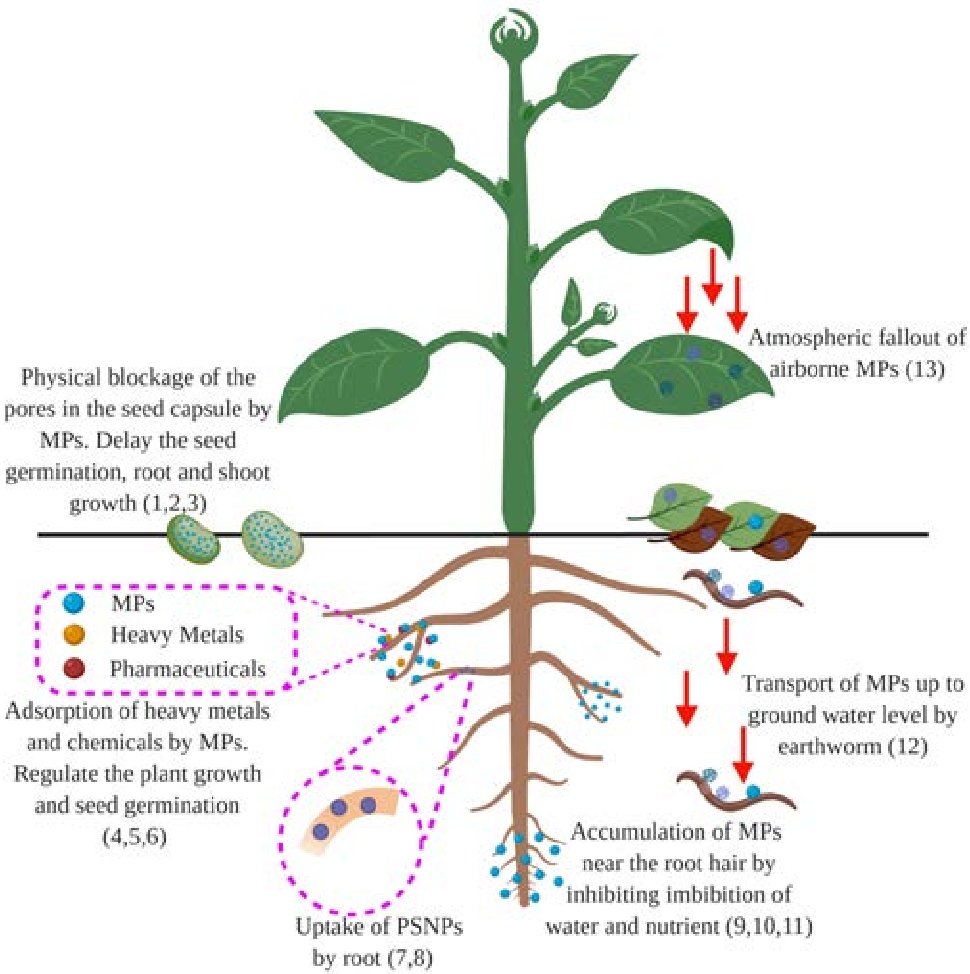

Table 3: Individual effect of microplastics for plant growth in the terrestrial ecosystem

Table 4. Combined effects of microplastic on plant growth in the aquatic ecosystem

Plant species	Scientific name	Plastic type	Size of plastic	Plastic concentrations		Chemicals concentrations			Exposure time	Effect on plant growth	Reference
				Alone	With chemical	Used chemical	Alone	With plastic			
Microalgae	*Tetraselmis chuii*	Red fluorescent polymer microspheres	1–5 µm	0.75, 1.5, 3, 6, 12, 24 and 48 mg/L)	1.5 mg/L	Procainamide	104 and 143 mg/L	125 and 31 mg/L	96 h	Toxicity of combined solutions is higher than individual solutions. Growth rate and chlorophyll content were significantly reduced.	Prata *et al.* (2018)
						Doxycycline	22 and 14 mg/L	11 and 7 mg/L			
Algae	*Microcystis aeruginosa*	Nanoplastic (nPS-NH_2)	200 nm	-	1, 5, 9, 11, and 15 mg/L	Glyphosate	-	5mg/L	96 h	Glyphosate adverse effect was significantly reduced by nPS-NH_2 owing to high adsorption ability.	Zhang *et al.* (2018)
Microalgae	*Tetraselmis chuii*	Fluorescent red	1 - 5 µm	0.046 to 1.472 mg/L	0.184 mg/L	Copper (Cu)	0.02 to 0.64 mg/L	0.02 to 0.64 mg/L	96 h	Considerable combined adverse effect was not observed for virgin MPs.	Davarpanah and Guilhermino (2015)
Fungi	*arbuscular mycorrhizal fungi*	PE and PLA	100-154 µm	-	0, 0.1%, 1% and 10% (w/w)	Cadmium (Cd)	-	5 mg/ kg Cd Soil	-	Synergetic effect of MPs and Cd can alter plant growth emphasizing the risk for agroecosystems.	Wang *et al.* (2020)

in Cd adsorption (Wang *et al.,* 2019). Furthermore, increasing pH heavy metal solution resulted in an increase in adsorption of Ag, Co, Ni, Pb and Zn, a reduction in adsorption of Cr and no clear trend for Cu or Hg. Moreover, due to the high adsorption ability microplastics facilitated the transport of toxic chemicals such as metal or POPs, particularly in the aquatic environment (Bakir *et al.,* 2014; Verla *et al.,* 2019). The dioxin-like chemical could be adsorbed on to microplastics, enhancing the risk associated with microplastics affecting plant growth causing increased health hazards (Chen *et al.,* 2019). Zhang *et al.* (2018), demonstrated such combined effect of nanoplastics (nPS-NH_2) with glyphosate on algae (*Microcystis aeruginosa*) growth. Based on the above study, nPS-NH_2 did not exhibit a significant effect for algae growth while the glyphosate alone exhibited an extremely high adverse effect on algae growth. Nonetheless, clear reduction of glyphosate adverse effects was observed when the combined effect of nPS-NH_2 and glyphosate was considered because of the high adsorption ability of NPs. In addition, the combined effects of microplastic (1-5 µm) with heavy metal (Cu) on microalgae (*Tetraselmis chuii*) growth was investigated and a significant negative effect was observed for copper (Cu) alone whereas negligible effect was observed for microplastics alone. Furthermore, no significant adverse effect was observed after considering the combined effect of virgin microplastics and Cu due to low adsorption ability of virgin microplastics (Davarpanah and Guilhermino, 2015). The experimental results indicated that no significant difference in the toxicity curves of copper in the presence and absence of virgin microplastics for the tested concentrations. Nevertheless, author emphasized that the toxic effect may be enhanced for nano-sized aged microplastics than virgin one as the aged microplastics have a high tendency to interact with metals and other chemicals. Consequently, polystyrene (PS) beads and aged polyvinyl chloride (PVC) fragments adsorbed considerable amount of heavy metals, such as copper (Cu) and zinc (Zn) in an aquatic ecosystem that was exposed to plastic debris just for 14 days (Brennecke *et al.,* 2016). Further studies are required to examine the combine effect of aged microplastics with heavy metal under long term exposure and smaller plastic debris. The effects of nano and microplastics for plant growth and seed germination is shown in Fig. 3. The combined effect of two types of microplastics (polyethylene (PE) and polylactic acid (PLA)) with cadmium (Cd) was examined by deploying arbuscular mycorrhizal fungi (AMF) community in an agricultural soil. The results of that study suggested that the combined effect of microplastics and Cd can alter the plant performance and root symbiosis while raising the risk for agroecosystems and soil biodiversity (Wang *et al.,* 2020). PLA alone showed phytotoxicity for highest concentration, reducing chlorophyll content and biomass in leaves, whereas the phytotoxic effects due to PE were considerable small. For root biomass, the combined interactive effect of PE and Cd was significant whereas such effect due to PLA and Cd combination was insignificant. Moreover, soil pH and DTPA-extractable Cd concentrations increased by both PE and PLA, but no considerable accumulation of Cd was observed. Furthermore, PLA resulted in stronger adverse effect on soil properties, plant growth and AMF community than PE due to a higher degradation ability of PLA than PE. The fragments from the PLA degradation process can interact with metals giving stronger impact.

The combined harmful health effects of microplastic and chemicals were also reported for animal and human. The effect is significant especially for aquatic biota owing to the abundance of microplastic accumulation in aquatic medium. Zebrafish (*Danio rerio*) after three weeks of exposure to the combined effects of microplastics containing chemical contaminants resulted in the combination having a significantly higher effect in comparison with either the microplastics or the chemical contaminants alone (Rainieri *et al.,* 2018). The combined effects of nickel and microplastics on Daphnia magna was investigated for two different microplastic types. A clear difference was observed for the combination than just for the individual cases (Kim *et al.,* 2017). Furthermore, the combined effects of microplastic with pyrene was able to delay the death of fish induced by pyrene and the pyrene concentration of fish bile was enhanced (Oliveira *et al.,* 2013). Table 4 shows the combined effects of microplastic for plant growth in the aquatic ecosystem.

CONCLUSION AND FUTURE PERSPECTIVES

This review discussed about up to date existing knowledge of the effects of microplastics on the growth of plants in the aquatic and terrestrial ecosystem including seed germination. Limited research studies were found for the effect on terrestrial plant compared to that for aquatic ecosystem. Therefore, more research studies need to be implemented to examine the effect of microplastics on the growth of terrestrial plants. Further studies are required to monitor the effect of microplastics for aquatic and terrestrial biota and how does it affect edible plant growth, biomass accumulation and crop yield. It is a well-known fact that microplastics serve as a vector for chemical transportation; thus microplastics can adsorb heavy metal in the environment increasing the possibility of combining microplastics with different metals or chemicals. Hence, more research studies are needed to observe the combined effect of microplastics with chemicals on plant growth. Individual effect of microplastics on seed germination has been studied. However, no research evidence has been presented so far that examines the combined effect of microplastics with chemicals and heavy metal on seed germination. Thus, it is important to observe the combined effects of microplastic for seed germination while selecting different seeds having different germination rates. Moreover, airborne microplastics are transported by wind or precipitated onto the plant surface giving a negative impact to the aquatic and terrestrial environments. It can be combined with different toxic chemical and might give rise to a high negative impact to plant growth. Therefore, more research studies are required to investigate the combined effect of airborne microplastics on plant growth. A few research studies have documented that nano and microplastic accumulation, translocation and uptake are dependent on plant species, chemical and geometrical properties of plastic debris. Thus, further studies are required to evaluate how chemical and geometric properties of plastic debris affect for the growth of plants. The toxic effect might be high for nano-sized aged microplastics than virgin ones as the aged microplastics have a high tendency to interact with metals and chemicals. Hence, further studies are required to examine the combine effect of aged microplastics with heavy metal under long term exposure and smaller plastic debris. Therefore, future works are required to investigate the effect of microplastics on the growth of plants and seed germination in the aquatic and terrestrial ecosystem to evaluate and mitigate the effects of ever increasing plastics usage of current pandemic times.

AUTHOR CONTRIBUTIONS

Y.S.K. De Silva was responsible for searching the bibliography, selecting the relevant references, revising the final version of manuscript, writing and original draft preparation. R. Uma Maheswari was responsible for conceptualization, supervision, revising the final version of manuscript, reviewing and editing. H. Kadono was responsible for conceptualization, supervision, investigation and reviewing.

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CONFLICT OF INTEREST

The authors declare that there is 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

AFM	Atomic force microscopy
AMF	Arbuscular mycorrhizal fungi
BBB	Blood brain barrier
bPVC	Bulk plastic (pure polyvinyl chlorid)
C	Carbon
CAT	Catalase
Cd	Cadmium
Cu	Copper
ENPs	Engineered nanoparticles
EU	Europe union
Fe	Iron
h	Hours
HDPE	High-density polyethylene
mg/mL	Milligram per milliliter
min	Minutes
mm	Millimeter

Mn	Manganese
MPs	Microplastics
mPVC	Microplastics (pure polyvinyl chlorid)
N	Nitrogen
nm	Nanometer
NPs	Nanoplastics
nPS-NH2	Polystyrene cationic amino-modified nanoparticles
PE	Polyethylene
PET	polyethylene terephthalate
PHA	Polyhydroxyalkanoates
PLA	Polylatic acid
POD	Peroxidase
POPs	Persistent organic pollutants
PP	polypropylene
PS	Polystyrene
PSNPs	Polystyrene nanoparticles
PVC	Polyvinyl chloride
ROS	Reactive oxygen species
SOD	Super oxide dismutase
S.R	Shoot to root ratio
WWTPs	Wastewater treatment plants
Zn	Zinc
µg/mL	Microgram per milliliter
µm	Micrometer
°C	Degrees Celsius
%	Percent
%(w/w)	Percentage weight/weight
>	Greater than
<	Less than

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ORIGINAL RESEARCH PAPER

Enterprise energy supply system design management based on renewable energy sources

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ABSTRACT

BACKGROUND AND OBJECTIVES: The problem of energy saving and the transition to technologies that allow to partially or completely move away from the use of gas and other fossil fuels are a priority in Ukraine today. By consuming traditional energy sources using outdated technologies for energy supply of industrial facilities, Ukraine consumes 3-4 times more fuel per unit of Gross Domestic Product compared to developed countries. For industrial enterprises, the energy intensity of costs is 35-40% of the total cost. At the same time, obsolete fixed assets, especially its active part (the degree of physical wear and tear of industrial equipment is 60-65%), are characterized by a large share of energy and heat loss.
METHODS: Modeling, structural analysis, and theoretical research based on current advances in the theory and practice of creating energy-efficient buildings using energy from alternative sources.
FINDINGS: Calculations and structural analysis of costs by stages of the life cycle of the design solution of the hot water supply and heating system with energy-efficient fencing and heat pump have been made. Peculiarities of calculation of assessment and design solutions in accordance with the Ukrainian legislation have been determined. The study has been conducted in seven Ukrainian industrial enterprises in the energy sector.
CONCLUSION: It is determined that the structural analysis of costs by stages of the project life cycle on the basis of standard costing should be used at the stage of designing a new power system or upgrading an existing one through marketing research. All this will contribute to the formation of a fundamentally new approach to solving technical and economic problems of the introduction of modernized energy supply systems for industrial purpose.

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INTRODUCTION

The introduction of new engineering and design solutions in energy supply systems, which provide for the integrated use of energy from renewable sources (solar and geothermal energy, environmental heat, etc) will solve an important economic and scientific problem of reducing the consumption of traditional fuel and energy resources for Ukraine. The most important direction of these measures is the construction of buildings with minimal energy consumption, which will allow to achieve a high level of energy efficiency. An integrated approach to the design of energy supply systems that use renewable energy sources (RES) includes a number of factors. Among them are; 1) cost and time distribution of renewable energy sources; 2) realized technical potential of renewable energy sources corresponds to certain climatic zones; 3) technical and institutional problems and the cost of implementing various renewable energy technologies in energy systems and markets; 4) comprehensive assessment of socio-economic and environmental aspects of the introduction of renewable energy sources and other energy efficient technologies; 5) political, institutional, and financial mechanisms to ensure the cost-effective use of renewable energy sources in a variety of conditions. The presence of a set of these indicators is essential and allows to objectively choose the composition of the energy supply system that uses renewable energy sources (Dwijaksara *et al.,* 2019).

Existing solar heating and cooling technologies, which are used in residential and commercial buildings, are an established market. It is distributed differently in most countries of the world and continues to grow at a rate of about 16% per year (Choi *et al.,* 2018). In Europe, the corresponding market volume has more than tripled recently. Despite these achievements, the use of solar energy is relatively small in Europe in hot water and heat supply systems (Cai *et al.,* 2019). For example, in Germany, the share of solar energy use in small buildings in the above systems does not exceed 15% (Wang *et al.,* 2018). This market is dominated by vacuum collectors, which today are structurally and technologically advanced and suitable for mass production; important production sites of vacuum collectors are located in Europe, Turkey, Brazil, China, and India (Guo, Qiao and Liu 2019). A significant share of the export market is made up of integrated solar water heating systems, not solar collectors. The largest exporters of these systems are Australia, Greece, USA, and France (Rafiei and Ricardez-Sandoval 2020). Competition in this area is the increase in the energy efficiency of energy supply systems through the use of a passive component. Close co-operation between the two industries is not enough but the spread of systematic design methodologies used by different countries has improved design opportunities. For example, windows are an important part of passive heating of buildings, and the presence of new generation windows with high efficiency (low emissivity, argon filling) affects the growth of solar energy in meeting the heat supply needs in the construction sector (Tezel *et al.,* 2018). Another characteristic of passive design is the addition of internal mass to the structure of the building. New materials, which are often used for more efficient heat retention, are readily available, designed to accumulate heat materials that use the heat of phase transformations (eg, paraffin), and are considered materials of the future (Zhang *et al.,* 2018). Economic indicators of renewable energy use depend on the appropriate design of the innovative energy supply system in accordance with the needs for energy services. In some regions, such as southern China, solar water heating systems are competitive in cost with traditional systems (Sun *et al.,* 2018). Solar water heating systems are usually more competitive in regions with high levels of solar radiation but this situation is changing with regard to space heating, which is usually associated with a higher total heat load (Gao *et al.,* 2019). In colder regions, capital expenditures may be spread over a longer heating season, and solar thermal energy may become more competitive in this case (Marinakis and Doukas 2018). Investment costs for solar heating systems vary greatly depending on the complexity of the technology used, as well as market conditions in the country of operation. Costs for innovative water supply systems range from a low of 83 c.u./m^2 to 1.200 c.u./m^2 (for some space heating systems) (Panetto *et al.,* 2019). The normalized cost of heat reflects a wide range of investment costs and depends on a number of variables, including the specific type of system, investment costs for the system, solar radiation available in a specific place, efficiency of conversion of this system, operating costs, system strategies, and discount rate (Li *et al.,* 2020). The normalized cost of heat for solar thermal systems, taking into account a wide range of initial parameters, was calculated in a wide range of 9 to 200 c.u./GJ (Kot 2018). The normalized cost of heat is in the range from 30 c.u./GJ to 50 c.u./GJ in the regions of some areas of Central and Southern Europe and reaches almost 90 c.u./GJ in regions with less solar radiation (Pan *et al.,* 2020). Over the last decade, for every 50% of increase in the installed capacity of solar water heaters in Europe, investment costs have fallen by 20% (Drobyazko 2020). The tendency to reduce the cost of power supply systems is achieved through the use of cheaper materials, more efficient production processes, mass production and direct inclusion in the design of buildings collectors as multifunctional building components and modular systems that are easy to install (Al-Tarazi and Chang 2019). Reducing costs is a key issue in making direct solar energy more commercially viable and able to claim a larger share of the global energy market (Mafini and Loury-Okoumba 2018). Potential use depends on actual resources and the availability of appropriate technology. At the same time, the current regulatory framework can significantly promote or deter the spread of direct solar energy (Máša *et al.,* 2018). Minimum building standards for the orientation and insulation of buildings can significantly reduce the energy needs of buildings and increase the share of renewable energy supply without increasing overall demand (Sahu 2018). Transparent and streamlined administrative procedures related to the installation and connection of a solar energy source to existing grid infrastructures can also reduce the cost of direct solar energy. In many countries, the development of energy supply systems over the decades has made it possible to ensure efficient and cost-effective distribution of electricity, gas and heat, as well as the transportation of energy to provide useful energy services to end users (Drobyazko *et al.,* 2020). Enhanced integration can lead to a full range of energy services for large and small settlements in both developed and developing countries based on renewable energy sources (Wei *et al.,* 2019). Power supply systems are constantly evolving to increase the efficiency of renewable energy conversion technologies (Xiao *et al.,* 2018). For different countries, the increase in the use of RES technologies can help reorient foreign exchange flows from energy imports to imports of goods that cannot be produced locally such as high-tech industrial goods (Ma *et al.,* 2019). The use of integrated energy supply systems in buildings, which include heat pumps, heat accumulators, energy barriers, is the result of finding ways to the most economical means of energy conservation and recovery of energy flows of objects (Li *et al.,* 2019). The introduction of such systems contributes to the spread of technical support for energy production directly at the facility. These systems are able to partially or completely replace the energy generated by traditional energy sources. The lack of modern analytical support for cost management, adapted to the economic activity of the enterprise in operational and strategic aspects, which leads to unreasonable management decisions due to misunderstanding of the target cost mechanism, and as a result the revenue of the individual enterprise, is one of the main reasons for irrational management of operating costs of Ukrainian manufacturers (Lopes *et al.,* 2019). This actualizes the study of improving the cost analysis of operating activities of industrial enterprises in terms of formulating its objectives, identifying new objects of cost analysis of operating activities, modeling the system of factors and reserves to minimize costs in a competitive environment, expanding the information base analysis, analysis of operating costs. This is especially relevant during implementation control systems for the design of the enterprise's energy supply system on the basis of renewable energy sources – the theory of life cycle is one of the fundamental factors. There are phases of the life cycle (pre-production costs, production costs, after production costs), at each stage there is a change in costs (deviation from the actual value). It is necessary to choose an adapted method of determining costs at each stage by designing the energy supply system of the enterprise. The purpose of this study is to scientifically substantiate theoretical approaches and improve methodical support for economic cost analysis in the design of energy supply systems taking into account different concepts of product costing and strategic cost control. This study was conducted for the period 2017-2019 in Central Ukraine (in three regions - Kirovograd, Dnepropetrovsk, Zaporozhye) at seven industrial enterprises.

MATERIALS AND METHODS

Description of the empirical basis of the study

When calculating the proposed design solution of the energy supply system of enterprises, the data of Ukrainian enterprises were used: Dniprenergotechnologiya LLC; PCF Velta LLC; RPE Ecoenergo-ORGCHIM LLC; Ukrtorgtara LLC; Promelectropostach LLC; Plant-firm OS LLC; RPA RAKURS LLC. It should be noted that all 7 industrial enterprises belong to the sector of equipment production in the energy industry, therefore, the energy intensity of costs is 35-40% of the total cost. At the same time, old fixed assets, especially its active part (the degree of physical wear and tear of industrial equipment is 60-65%), are characterized by a large share of energy and heat loss.

These enterprises are located in Central Ukraine in three regions: Dnepropetrovsk, Kirovograd, Zaporozhiye. They have the same climatic conditions, so natural factors favor the use of solar energy as an alternative source of energy. This study presents the selected design solution of the power supply system at these enterprises, the calculations performed and the implementation of the project. During 2017-2019, this design solution was implemented at these enterprises, and during the production stage of the life cycle, it was necessary to adjust the costs in accordance with the real conditions of the activity of the enterprises.

Formation of research hypotheses

The research proceed from certain concepts, which are based on modeling, structural analysis, and theoretical research, which rely on modern achievements in the field of theory and practice of creating energy-efficient buildings that use energy alternative sources. The underlying hypothesis (H0) is the hypothesis that the management of alternative energy sources in the activity of enterprises leads to a synergistic effect, consisting of three effects - economic (H1), environmental (H2), and social (H3). When evaluating the efficiency while selecting among alternative projects, it is necessary to take into account not only the economic effect (as the achievement of the highest results by the production enterprise at the lowest cost of living and ngible labor or the reduction of total costs per unit) but the social and environmental one. At the same time, it is necessary to assess this efficiency from the position of the appearance: internal and external. In this study, the authors focused on the economic effect at the lowest cost based on following statements:

H1.1 - depends on the life cycle of the project. When implementing energy saving projects, the business entity is faced with the need to choose from the available limited alternative resources: cash, labor, material, etc. Decisions on the implementation of energy saving projects are made by the company's management, which increases the subjectivity of this decision for many reasons, such as: mentality, professional experience, judgment, etc. To reduce the subjectivity of the calculation of profit growth through the implementation of the project, it is necessary to turn to the methods of evaluating projects in the field of social diagnostics.

H1.2 - depends on the chosen method of assessment and calculation, taking into account the calculation of the budget for the implementation of projects, the cost of deferred decisions. The cost of deferred decisions allows to identify alternative scenarios 'with a project - without a project.' The implementation of energy saving projects (programs) affects the market value of the enterprise, is a key factor, the dominant factor of competitiveness, economic and energy security, etc. It is necessary to dwell on this in more detail.

Description of the main calculation method

A set of scientific methods was used to accomplish the tasks and achieve the purpose of the work: methods of scientific generalization, comparison, grouping and system analysis (to determine the nature of costs, mechanisms of their creation and behavior, to develop a classification of operating costs, to study conceptual approaches to the management of operating costs); methods of analysis and synthesis (to develop a technology for enterprise operating costs analysis); economic-mathematical methods, tabular techniques (when conducting experimental procedures for analysis of production costs using different systems of product costing, analysis of operating costs using strategic cost control systems, and when determining their impact on making decisions regarding the efficiency of an enterprise. When using the 'standard costing' calculation, the cost of a particular product is influenced by the following factors: production volume, direct material costs, direct labor costs; other direct costs, variable production overheads and fixed allocated production overheads. In our view, a factor model for the analysis of the cost of a separate type of product (*P*) based on the use of information found out using the 'standard costing' system, using Eq. 1.

$$P = Q \times (C_1 + C_2 + C_3 + C_4 + C_5) \quad (1)$$

Where, Q – the number of products manufactured, C_1 – the direct material costs per product, C_2 – the direct labor costs per product, C_3 – the other direct costs per product, C_4 – the variable production overheads per product; C_5 – the fixed allocated production overheads per product.

Based on the research results of foreign scientists and the experience of developing the concept of intelligent energy systems, it can be noted that the process of creating an intelligent energy system in developing countries should be considered as a complex of interrelated tasks of political, economic, environmental and social nature (Wu *et al.,* 2019). As of today, society and economic sectors are interested in implementing research results related to the development of innovative design solutions for an integrated energy supply system including a heat pump, energy-active enclosures, a seasonal soil heat accumulator and using alternative energy sources such as energy of solar radiation, heat of soil and air, incl. ventilation air (Huang *et al.,* 2019). The proposed organizational-methodical stages (technology and tools) of the complex analysis of the enterprise operating costs provide the achievement of the management and strategic goals of a modern strategically oriented industrial enterprise (Table 1).

There were also singled out factors of influence on the cost of a separate type of production when using direct costing calculation, such as: direct costs and variable (general) production overheads. This

Table 1: Organizational-methodical stages (technological map and methodical tools) of the complex analysis of the enterprise operating costs

Technology of analytical research	Methodical tools for analysis of operating costs	Level of making management decisions
	Traditional approach to the analysis of operating costs	
Stage 1	Analysis of the total cost of products sold: - estimation of validity of the planned level of cost of finished goods (by norms, prices for materials); - analysis of production budget execution; - analysis of costs by economic elements - analysis of costs by items of calculation and changes in left-over stock of finished products in storage; - analysis of costs for 1 dollars USA of products sold; - cost analysis of separate types of products	Accountants, economists, cost engineers, technologists
	Operational analysis of production costs	
Stage 2	Cost analysis of a separate type of production by composition of groups: - in the standard-costing or the standard method; - in direct costing; - in absorption costing	Mid-level management – heads of subdivisions, leading specialists
Stage 3	Cost analysis of a unit of production by product functions and processes: - cost analysis using the Kaisen costing process; - analysis of costs using the target costing process; - process analysis of general expenses of production (AB costing)	Mid-level management – heads of subdivisions, leading specialists
	Strategic analysis of operating costs	
Stage 4	Analysis of costs by value chain; - analysis of costs by life cycle of operations; - competitive and comparative analysis by the method of benchmarking; - analysis of costs for quality assurance	Top level management (top management)

allows to define a new object of analysis – the cost of production at variable costs, which enables the analysis of marginal profitability for a separate type of production, and making management decisions on the issues of assortment and the development of an assortment policy (Rafiei and Ricardez-Sandoval 2020). In the process of study, it has been found out that the methods of accounting production costs and calculating the historical cost of production sold did not take into account the limitation of the price offer of competitors, which reduces the amount of planned profit, and the marginal utility (consumer value) of the product for buyers (Marinakis and Doukas 2018). The search for reserves to reduce operating costs can be carried out based on identifying sources of competitive advantages in the value chain. To identify the sources of competitive advantage, it is necessary to analyze the nine interrelated business activities that form a value chain in strategic management (Yuyin and Jinxi 2018): five main (inbound logistics, manufacturing, outbound logistics, marketing and sales, after sales) and four auxiliary (enterprise infrastructure, personnel management, technological development, logistics). Strategic analysis of operating costs by the value chain is carried out using a comparative analysis of the value chain of the enterprise with direct competitors and in the branch of operation (Variny *et al.,* 2019). Taking into account the division of operating costs for quality assurance into two large groups; 1) costs related to achieving product quality compliance, or preventative costs that characterize attempts to ensure and guarantee an adequate level of product quality (Choi *et al.,* 2018); 2) costs of product quality compliance, or costs for elimination of defects related to product quality restoration, it is proposed to carry out a structural analysis of costs for quality assurance of operating activity, which allows to allocate them across responsibility centers and provides greater control over costs for quality assurance and search for reserves for their minimization at all stages of the operating activity of the enterprise (*Wang et al.,* 2018). When using the method of absolute differences, the pattern of calculating the effect of the deviations of the listed factors on the change in production cost of products is illustrated as follows (Table 2).

The proposed factor model of cost analysis of a particular type of product on the basis of 'standard-costing' calculation provides the identification of the centers that allowed the greatest deviation of direct production costs from the set standards and gives essential information for making management decisions in the area of direct cost standards planning in preparation of production cost budgets for separate types of products. Using the 'absorption costing' system can confirm the influence of the following factors on the cost of a particular type of production: direct costs, fixed allocated (general) production overheads, and fixed unallocated (general) production overheads. Since unallocated fixed (general) production overheads are not attributed to period costs but are included in

Table 2: Pattern of calculating the effect of the deviations of the listed factors on the change in production cost of products when using the 'standard costing' system

Item No.	Effect of the deviations of the listed factors on the change in production cost of products	Equation
1	Effect of deviation (change) in scope of activity	$dP_Q = dQ \times (C_1^{s\tan} + C_2^{s\tan} + C_3^{s\tan} + C_4^{s\tan} + C_5^{s\tan})$
2	Effect of deviation in standards of direct material costs per product	$dP_{C_1} = Q^{fact} \times dC_1$
3	Effect of deviation in standards of direct labor costs per product	$dP_{C_2} = Q^{fact} \times dC_2$
4	Effect of deviation in standards of other direct costs per product	$dP_{C_3} = Q^{fact} \times dC_3$
5	Effect of deviation in standards of variable production overheads per product	$dP_{C_4} = Q^{fact} \times dC_4$
6	Effect of deviation in standards of fixed allocated production overheads per product	$dP_{C_5} = Q^{fact} \times dC_5$
Total amount of change in the cost of production must be equal to the amount of all types of deviations		$dP = dP_Q + dP_{C_1} + dP_{C_2} + dP_{C_3} + dP_{C_4} + dP_{C_5}$

the cost of production by types of production when using the concept of 'absorption costing' calculation, full cost of production is becoming the object of analysis, the relevant information on which enables the analysis of price elasticity of customer demand and the analysis of price offers of competitors in conditions of fluctuations in demand for a particular type of production and ensures making management decisions in the area of pricing and development of a pricing policy regarding the assortment positions. Using 'direct costing' calculation allows to distinguish the factors influencing the cost of production of a particular type of production, such as: direct costs and variable (general) production overheads. This allows to define a new object of analysis – the cost of production at variable costs, which enables the analysis of marginal profitability for separate products, and making management decisions on the issues of assortment and the development of an assortment policy. The peculiarity of using traditional methods of calculation in the management accounting of Ukrainian enterprises is the peculiarities of the national legislative framework. Firstly, the instructions for industrial enterprises in determining the cost of work, services, and products are very outdated, originating with the Soviet method. Outdated standards, lack of the concept of 'life cycle' of both the enterprise itself and works, services and products, taking into account market factors, inflationary processes. Secondly, accounting legislation in Ukraine, including parts of management accounting, calculation, has only declaratively harmonized standards in this area for 21 years. At the same time, the system of integration of accounting information in the management system of the enterprise has not yet been determined. Therefore, in this study, the methods for determining the value are brought to the harmonization of Ukrainian legislation (first of all, the concepts of 'liquidation value', 'residual value') in the conditions of real industrial enterprises, where the methods of valuation and calculation are in accordance with Soviet standards of the 1970s. This dissonance in the methods leads to many problems, according to the top management of the surveyed enterprises, the main one is the impossibility of entering foreign markets. At the same time, it is the asymmetry of information in the entire management system that acts as an internal factor, first of all, the discrepancy between the methods of assessment and calculation as the primary link of the entire system.

RESULTS AND DISCUSSION

Currently, the problems of energy conservation, efficient use of energy resources are very relevant for many reasons. First, it is the constant rise in prices for traditional energy and the reduction of their reserves on Earth. Secondly, it is a significant environmental burden on the environment when using traditional energy sources. In addition, when using traditional energy sources in various areas of the economy, where thermal energy is consumed, there are significant costs for the maintenance of pipeline heat distribution systems. District heating systems are branched, reversible type, with unstable modes of operation, ie those where the heat load changes significantly during the heating period and during the day. With a long service life, they are crucial in terms of assessing the effectiveness of the heating system itself. Therefore, in Ukraine and around the world, to provide consumers with electricity and heat, their combined generation when using energy from alternative sources becomes popular. The introduction of these energy generation technologies will solve the problem of inefficient use of energy by consumers because it leads to an extremely high level of specific energy consumption in the country.

The efficiency of the energy supply system in terms of economical energy use requires simultaneous resolution of issues: 1) selection of appropriate design solutions for a complex system of energy supply and air conditioning (heating, air conditioning, ventilation, and hot water supply) depending on the purpose of the building; 2) determining the impact of the components of the energy supply system on the level of energy efficiency of buildings; 3) selection of a simple and effective method of technical and economic assessment of the efficiency of operation of a complex system of energy supply and air conditioning.

When designing energy supply systems, the main goal is to minimize the total investment in their construction under conditions of high efficiency. That is, it is necessary to consider the following: 1) compliance with system efficiency and rational use of energy resources; 2) increase of energy efficiency. Such a comprehensive approach is possible provided that a systematic analysis and development of a methodology for building energy-efficient energy supply systems and air conditioning of buildings with energy-efficient fencing and the use of energy from alternative sources. To do this, it is necessary to assess the effectiveness of the selected options for the implementation of energy supply systems from the standpoint of the consumer, developer, and the state. Therefore, it is advisable to consider the design solutions and methods of calculating a comprehensive system of energy supply and air conditioning, taking into account construction and installation costs.

There are many options for power supply schemes. Examples are as:

a) traditional implementation of energy supply systems: Heating and hot water supply, centralized from the district heating network (Wu *et al.,* 2019); Heating and hot water supply from the roof boiler room, etc (Ünal *et al.*, 2019);

b) use of alternative energy sources: Scheme with heat pump with one heating circuit with mixer, "natural cooling" function, energy-active fencing for hot water preparation and buffer capacity of the heating circuit (Ashraf *et al.*, 2020); design solution with two heating circuits with mixer, "natural cooling" function, hot water supply function by means of energy-active barriers and buffer capacity of the heating circuit (Epoh and Mafini 2018); Design solution, which provides for the presence of two heating circuits with a mixer and a buffer tank of the heating circuit, energy-efficient fencing, which is used for hot water supply and "natural cooling" air conditioning system (Giama and Papadopoulos 2018); Design solution, which includes one heating circuit without mixer, air conditioning, and hot water preparation function (Hong *et al.,* 2018).

The choice of the optimal variant of the design solution of the power supply system is possible due to the system analysis. The basis of this method is to compare the efficiency of options for power supply systems from three positions: 1) consumer: When considering the options of energy supply systems from the consumer's point of view, the selected options for the implementation of energy supply systems should provide minimal operating costs, provided that sufficient comfortable conditions are created; 2) state: From the standpoint of the state, engineering systems must ensure minimum integrated consumption of all types of energy resources; 3) service organization. From this point of view, engineering systems must provide the greatest profit, which inversely depends on the capital costs of construction of these engineering systems with the necessary energy supply. In te current study, the choice was made by users - Dniprenergotechnologiya LLC; PCF Velta LLC; RPE Ecoenergo-ORGCHIM LLC; Ukrtorgtara LLC; Promelectropostach LLC; Plant-firm OS LLC; RPA RAKURS LLC. Top management chose 'minimum operating costs' as the main priority criterion. The amount of operating costs, in accordance with Ukrainian legislation and internal regulations and standards, consists of: salaries of service personnel, depreciation, maintenance costs, costs of auxiliary materials, other operating costs. The lowest operating costs are the Design solution of the hot water supply and heating system with energy-efficient fencing and heat pump. The use of one heating circuit and hot water supply when using an energy-efficient fencing is one of the options. This variant of the design solution is presented in Fig. 1.

For efficient operation of the given scheme of hot water supply, it is necessary to use the heat pump with the built-in water heater (BWH), the distributing comb with a heating circuit without the mixer. BWH is structurally a large-capacity tank, which houses the heat exchangers of the heat pump and the electric heater. The minimum flow rate of the heat pump 4 through the bypass valve is provided by the built-in secondary pump. In these heating systems, the bypass valve is connected to the distribution floor of the underfloor heating system. This allows to ensure the required minimum water flow in the heating circuit. To heat premises by means of a heat pump, the primary circuit is activated when the actual temperature (measured by the temperature sensor of the return line of the secondary circuit) is below the set value (adjustable controller), the heat pump 4 and the built-in primary pump 3 are switched on. The secondary circuit is involved when the heat pump 4 supplies heat to the heating circuit. The controller of the heat pump 4 regulates the supply temperature of the coolant, which, in turn, helps to regulate the parameters of the heating circuit. The built-in secondary pump supplies the heat carrier through the three-way switching valve to a heating circuit or to the built-in BWH. The flow in the heating circuit is regulated by opening and closing the valves on the distribution comb of the 'warm floor' system. If the actual temperature on the return line temperature sensor exceeds the set value set on the controller, the

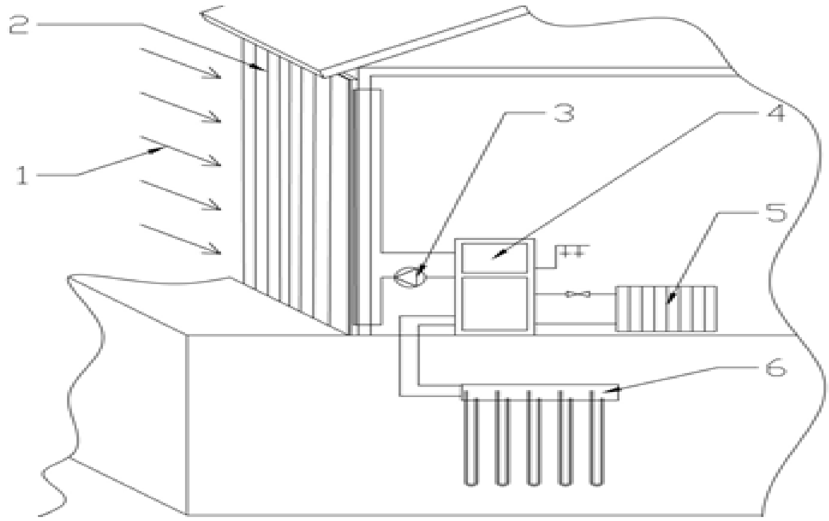

Fig. 1: Design solution of a hot water supply and heating system with an energy-efficient fencing and a heat pump
(1: Solar radiation; 2: Energy-efficient fencing; 3: pump; 4: Heat pump; 5: Heating battery; 6: Ground heat exchanger)

heat pump 4 and the secondary pump are switched off. The request for hot water preparation comes from the built-in BWH temperature sensor and the controller that controls the built-in secondary pump in conjunction with the built-in three-way switching valve or the water heater filling pump. The water temperature rises due to the presence of the controller to a value that is necessary for the consumer. If the actual value on the temperature sensor of the capacitive water heater exceeds the set one on the controller, the controller will switch the supply of coolant to the heating circuit via the 'heating/ hot water' three-way valve. The water temperature can exceed 60 ^{O}C due to the use of a built-in instantaneous heater for the coolant. The use of such a design solution is good to provide the consumer with hot water. In this mode of operation, the main elements are energy-efficient fencing, which can be located on the walls and roof of the house. BWH heating by means of energy-protective fencing is performed when the temperature difference between the temperature sensor of the energy-active fencing circuit and the built-in BWH temperature sensor exceeds the temperature difference that is set on the controller. To do this, the heat pump controller controls the pump of the energy barrier circuit. If the temperature drops below the temperature difference, the controller will switch off the energy barrier circuit again. Taking management decisions on the implementation of the project of the energy supply system of enterprises requires a structural analysis of costs by stages of the product life cycle. Based on the results of this analysis, information is formed on the comparability of costs recognized at the production stage of the product life cycle of the assortment item and other operating costs recognized at the pre-production, non-production, and post-production stages of the energy saving project life cycle. The structural analysis of expenses on stages of a life cycle of the circuit solution of hot water supply and heating system with an energy-active protection and the heat pump is carried out (Table 3).

It is necessary to determine that the planned period of implementation of the design solution of the hot water supply and heating system with energy-efficient fencing and heat pump in Table 3 is defined as 5 years. This is due to the peculiarity of Ukrainian tax legislation - a group of 5 fixed assets has a minimum service life of 5 years. In reality, with the corresponding operating costs, the term is much longer. It is 10-15 years (according to the technical documentation). In order to be able to identify changes in cost, the following valuation tools must be used. Adaptability of the proposed method of factor analysis of deviations from the standards of production costs of operating activity of the enterprise

Table 3: Structural cost analysis by stages of the life cycle of the design solution of the hot water supply and heating system with energy-efficient fencing and heat pump

No	Name of the indicator	Amount, USD	Total amount of costs, %
1	Production planned per month, sq. m	9600	X
2	Production planned per year, sq. m	75 600	X
3	PRE-PRODUCTION COSTS TOTAL pre-production costs, USD	310635	15
4	PRODUCTION COSTS Direct costs of materials and components for 1 product, USD	8.32	
5	Direct costs of piecework wages for 1 product, USD	3.57	
6	Direct production costs of the annual program, USD	898770.6	X
7	Indirect production costs of 1 item, USD	5.10	
8	Indirect production costs of the annual program, USD	385187.4	
9	Planned production period, years	5	
10	TOTAL production costs, USD [(line 6+line 8) x3 years]	1283958	62
11	NON-PRODUCTION COSTS Administrative costs in the reporting period, USD	41418	X
12	Planned period of business continuity, years	5	
13	TOTAL administrative costs, USD (line 12x3 years)	207090	10
14	Sales costs (commercial) for the annual production program, USD	33134.4	X
15	Planned implementation period, years	5	
16	TOTAL sales costs (commercial), USD (line 16x3 years)	165672	8
17	POST-PRODUCTION COSTS Utilization of technological equipment for scrap metal	103545	X
18	TOTAL post-production costs	103545	5
19	Total life cycle costs (USD)	2070900.00	100

Table 4: Input data for calculation of deviations from standards using standard costing calculation system

Designation	Indicator	Standard	Fact	Absolute deviation
P	Manufacturing cost of production, USD	2070900.00	2219950.00	149050.0
C_1	Quantity of products manufactured	30000.00	29.000.00	-1000
C_2	Direct material costs per product, USD	50.02	55.60	+5.58
C_3	Direct labor costs per product, USD	7.86	9.25	+1.39
C_4	Other direct costs per product, USD	3.00	3.16	+0.16
C_5	Variable general production costs per product, USD	4.15	4.54	+0.39
C_6	Fixed allocated general production costs per product, USD	4.0	4.0	0

on the basis of information found out using standard costing calculation system will be demonstrated with the help of Table 4, which contains the input data for calculation of deviations, as well as Table 5, which illustrates the results of factor analysis.

Absorption costing calculation confirms the influence of several other factors on the manufacturing cost of a separate type of production, namely: direct costs, fixed allocated general production costs, and fixed unallocated general production costs. Since unallocated fixed general production costs are not attributed to period costs but are included in the manufacturing cost by types of production when using the concept of 'absorption costing' calculation, the full manufacturing cost is becoming the object of analysis, the relevant information on which enables the analysis of price elasticity of customer demand and the analysis of price offers of competitors in conditions of fluctuations in demand for a particular type of production and ensures

Table 5: The results of factor analysis of changes in manufacturing cost and the effect of deviations from standards based on the use of information found out using standard costing calculation system

Designation	Indicator	Amount (USD)
dP_{C1}	effect of volume changes	-69030.00
dP_{C2}	effect of changes in the standards of direct material costs	161820.00
dP_{C3}	effect of changes in the standards of direct labor costs	40310.00
dP_{C4}	effect of changes in the standards of other direct costs	4640.00
dP_{C5}	effect of changes in the standards of variable general production costs	11310.00
dP_{C6}	effect of changes in the standards of fixed allocated general production costs	0
dP	the total amount of change in manufacturing cost	149050.00

making management decisions in the area of pricing and development of a pricing policy regarding the assortment positions. Also, there were singled out factors of influence on the manufacturing cost of a separate type of production when using direct costing calculation, such as: direct costs and variable general production costs. This allowed to define a new object of analysis - the cost of production at variable costs, which enables the analysis of marginal profitability for separate products, and making management decisions on the issues of assortment and the development of an assortment policy. In the process of research it has been found out that the methods of accounting production costs and calculating the historical cost of production sold did not take into account the limitation of the price offer of competitors, which reduces the amount of planned profit, and the marginal utility (consumer value) of the product for buyers.

CONCLUSION

The selection of projects of energy supply system of enterprises is made on the basis of renewable energy sources with the task of maximizing the economic effect, which takes into account both economic components (project life costs, project budget, cost of deferred decisions) and social and environmental effects. The method for structural cost analysis by stages of the project life cycle on the basis of standard costing, which should be used at the stage of designing a new power system or upgrading an existing one through marketing research, is stated. Product life cycle cost extends the boundaries of traditional cost management approaches by accounting for the cost of a product's lifetime to determine the target profit. At the same time, using the concept of LCC (life cycle cost) in management accounting allows to improve the methodology of internal audit of operating costs. All this allows to create a mechanism for consistent and purposeful management of the process of creating the target value of the product. According to the authors, the limitation of the proposed study is based on the principle of Ceteris paribus. This means that the economic interests of alpha stakeholders in the implementation of alternative projects for indoor climate systems are necessary. Alpha stakeholders form their requirements in accordance with the goals and motivations and influence the project based on their interests, professional competencies, and the degree of involvement in its implementation. In order to eliminate possible shortcomings and violations of the balance of interests, competencies and the degree of involvement of project agents, it is important to note some recommendations for its implementation: 1) The built alternative allows to make decisions on reducing the use of natural gas. This is possible due to: the introduction of the latest energy-saving technologies used to reduce energy intensity of production and increase the competitiveness of products; carrying out engineering and technical, scientifically implemented measures; 2) Under modern conditions of world economic development, industrial enterprises implement the optimal strategy based on energy saving and consider energy efficiency as an important component of innovative industrial development. 3) The volume and scope of renewable energy sources, which will partially replace natural gas, are increasing. Further research can be focused on: the possibility of combining these accounting cost models, as each of them focuses on solving the problem of optimal cost and performance management, development of post-production cost accounting model (waste management and process equipment).

AUTHOR CONTRIBUTIONS

S. Drobyazko developed the whole concept and led the study. M. Skrypnyk made a detailed content analysis of scientific research. N. Radionova has collected and analyzed empirical data. O. Hryhorevska formed the results of the study in graphical form. M. Matiukha focused on structuring the manuscript.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest regarding the publication of this work. In addition, 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

dP_Q	Effect of deviation (change) in the scope of activity
dP_{C_1}	Effect of deviation in standards of direct material costs per product
dP_{C_2}	Effect of deviation in standards of direct labor costs per product
dP_{C_3}	Effect of deviation in standards of other direct costs per product
dP_{C_4}	Effect of deviation in standards of variable production overheads per product
dP_{C_5}	Effect of deviation in standards of fixed allocated production overheads per product
dP	Total amount of change in the cost of production must be equal to the amount of all types of deviations
%	percent
BWH	built-in water heater
c.u./GJ	conventional units of fuel per kilojoule
c.u./m²	conventional units of fuel per square meter
C1	The direct material costs per product
C2	The direct labor costs per product
C3	The other direct costs per product
C4	The variable production overheads per product
C5	The fixed allocated production overheads per product
Eq	equation
et.al	others
etc.	Et cetera
Fact	Actual value of the indicator
Fig.	Figure
GDP	gross domestic product
H	hypothesis
i.e.,	in other words
LCC	Life-cycle costing
LCC	Limited Liability Company
P	Cost of a separate type of product
PCF	Production and commercial firm
PRA	Production and research association
PRE	Production and research enterprise
Q	The number of products manufactured
RES	Renewable energy sources
sq. m	square meters
Stand	The standard value of the indicator
USA	United States of America
USD	US dollar
°C	degrees Celsius

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ORIGINAL RESEARCH PAPER

Natural enrichment of chromium and nickel in the soil surrounds the karst watershed

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ABSTRACT

BACKGROUND AND OBJECTIVES: As a public concern, monitoring and controlling toxic metals pollution is needed worldwide. Due to the ability of poisonous metals in biomagnification and bioaccumulation, they can cause several adverse impacts on ecological and human health. The study aims to assess chromium and nickel enrichment levels and estimate the soil's ecological risk surrounds the Pangkajene watershed.

METHODS: The total concentrations of chromium and nickel were determined using the Flame Atomic Absorption Spectrophotometer. This study used contamination factor, geo-accumulation index, and pollution load index to evaluate soil enrichment status. The ecological hazard index is used to estimate the potential hazard that may occur due to contamination.

FINDINGS: The mean concentrations of chromium and nickel were 92.9 and 43.18 mg/kg, respectively. Chromium concentration exceeded the soil quality guideline for the protection of environment and human health, while Ni still below the standards. The geo-accumulation index value indicated no human-made-derived contamination in the soil. Weathering of carbonate rocks is the chromium and nickel major enrichment factor in the Pangkep regency. Contamination factor and pollution load index values showed low pollution in the studied soil. However, all study sites exceeded the ecological hazard index value (Ecological hazard index>1), which indicates a considerable ecological risk in the Pangkajene watershed area..

CONCLUSION: These findings may provide baseline information related to chromium and nickel enrichment in the soil for Pangkep regency municipality. The Pangkep regency municipality must highlight the importance of strengthening environmental standards and monitoring mechanism as the priority to maintain a healthy environment.

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INTRODUCTION

Soil heavy metals pollution becomes a public concern worldwide due to wide distribution, high latency, irreversibility, remediation distress, and contamination process complexity (Su *et al.,* 2014). This issue is also related to food security, where heavy metals can accumulate through the food chain and cause several health problems to humans. More than 10 million soil sites were polluted globally, and more than 50% of those areas were contaminated with heavy metals/metalloids (He *et al.,* 2015). This situation was impacting on the economic loss worldwide, which estimated at more than 10 billion US$ per year (He *et al*., 2015) and it can be more become serious in developing countries such as Indonesia due to the increase of financial burden. Heavy metals accumulation in the soil may come from its parent rocks (metal-enriched rocks such as serpentine and black shale) and geological activity such as volcanic activities, earthquakes, landslides, debris flows (He *et al*., 2015; Ma *et al., 2019*). Besides, anthropogenic activities such as wastewater irrigation, open dumping/landfill waste, fossil fuel combustion, incineration, coal ash, smelting, mining, industry, industrial by-products, and agriculture practices such as application of fertilizer and pesticides may enrich the soil with a high concentration of heavy metals. The soil's metal contamination can be transported via dry and wet deposition, stormwater, sewage irrigation, improper disposal of solid waste, and fertilizer application, biosolid, application of manures and pesticides. Moreover, broad land use/land cover shifts after the industrial revolution cause a faster peak of runoffs that ease contaminants' transport. Most toxic metals/metalloids such as Cadmium (Cd), Chromium (Cr), Arsenic (As), Nickel (Ni), Mercury (Hg), and Lead (Pb), are the priority substances to control by ATSDR (USDHHS., 2019). The high concentrations of heavy metals in soil can directly impact the bio-system and indirectly affect animal and human health through the food chain. The human can directly expose to soil heavy metals through inhalation and skin direct contact and indirectly through ingestion of food and groundwater contamination. Most heavy metals can be distributed in the human body through the blood to the tissue (Azeh Engwa *et al*., 2019). Long-term exposure to toxic metals was associated with a harmful effect on human health and become apparent only after several years of exposure (Khan *et al.,* 2008; Jaishankar *et al.,* 2014). The heavy metals poisoning on cellular molecules primarily caused by oxidant and antioxidant imbalance (Tchounwou *et al.,* 2012; Azeh Engwa *et al.,* 2019). Moreover, metals/metalloids such as As, Ni, Cd, Cr(VI) are classified as carcinogenic substances. Cr and Ni were known as the micronutrient for plant growth and animal in small concentrations. It becomes toxic substances at a higher concentration. Since the heavy metal accumulation in soil may pose health risks and adverse effects on the terrestrial ecosystem, the toxicity quantification of contaminated soil can be evaluated using several indices such as geo-accumulation index (I-geo), contamination factor (Cf), pollution load index (PLI), and ecological hazard quotient (EHQ). The characterization of contamination levels and ecological risk is essential to develop specific actions to reduce metal contamination hazards in the terrestrial ecosystem. This present study was conducted in Pangkajene dan kepulauan (Pangkep) regency. This regency is a part of the Maros-Pangkep karst forest area. Like other karst areas, this area is also vulnerable to environmental degradation due to anthropogenic activities such as limestone mining and cement industry and land use shifting (Duli *et al.,* 2019; UGM., 2016). A prior study from Mallongi., (2020) showed Hg contamination and low-level ecological risk in soil surrounds industrial area of Pangkep regency. Nonetheless, a research related to soil heavy metal contamination in the Pangkep Regency is very limited. Therefore, this study aims to assess other heavy metals such as Cr and Ni concentration in the surface soil samples; to characterize soil toxicity and to estimate ecological risk of Cr and Ni surrounding the Pangkajene watershed using several geochemical indices. This study has been carried out in Pangkajene dan Kepulauan (Pangkep) Regency, south of Sulawesi Province, Indonesia in 2020.

MATERIALS AND METHODS

Study area

This study was conducted in Pangkajene dan Kepulauan (Pangkep) regency, South Sulawesi, Indonesia. Pangkep regency located in the south of Barru regency, South Sulawesi Province (110^{o} longitude and 4^{o}.40' – 8^{o}.00' latitude). The total area of Pangkep regency includes 898.29 km^2 of terrestrial area, and 11.464 km^2 of sea. The climate characteristic in the Pangkep regency is tropical monsoon climate with average annual rainfall and temperature of 2500 – 3000 mm/year and 26.4°C, respectively. This area is dominated by plain and karst hills which have an elevation ranging from 100 – 1000 meters. Maros and Pangkep Regency, located in South Sulawesi Province, Indonesia, have the most prominent and beautiful Karst area worldwide. The karst area, which served to maintain the regional ecosystem's balance, was vulnerable to progressive environmental degradation due to human activities. Besides, it has thin regolith, high porosity, a low carrying capacity of heavy metals, and flexible transport of heavy metals (Zhang *et al.,* 2019). This studied area was enriched by mining materials including limestone, clay/loam, silica sand/quartz, gravel, marble, alluvial gold, chert, feldspar, kaolin, basalt, slate, coal, trachea, propylite, diorite, sandstone, and radioactive material. Marble and limestone mining are the most extensive mining sector in the studied area. In addition, land-use changes and conversion of agricultural land to non-agricultural land have been continually degraded the watershed ecosystem in the Pangkep regency. This condition is potentially enhanced the heavy metals contamination process in soil.

Soil sampling

The field sampling was done in April 2020 which is on rain season. Soil samples were obtained from an area near the Pangkajene river in Pangkajene dan Kepulauan (Pangkep) regency, Indonesia. The selected sampling area includes three sub-districts of Pangkep regency (Bungoro, Minasatene, and Pangkajene), representing watershed areas from upstream to the coast. The study site boundary was less than 5 km from the Pangkajene river. The Pangkajene river is used as irrigation water for farmland and aquaculture. The water of the Pangkajene river was used as the irrigation to the farmland. The study area includes 22 sampling sites with different land use, including; 1) agriculture soil (with 13 sampling sites) and 2) non-agriculture soil (9 sampling sites) (Fig. 1). Seven samples were taken from the upstream area (S01-S05 and S08 -S09), eight samples from the middle stream area (S06 – S07, S10, S14-S18), and eight samples were taken from the downstream region of the Pangkajene river (S11-S13, S19-S22), Pangkep regency, Indonesia. A GPS was used (Garmin 62s) to locate the sampling point during field sampling. Composite soil samples

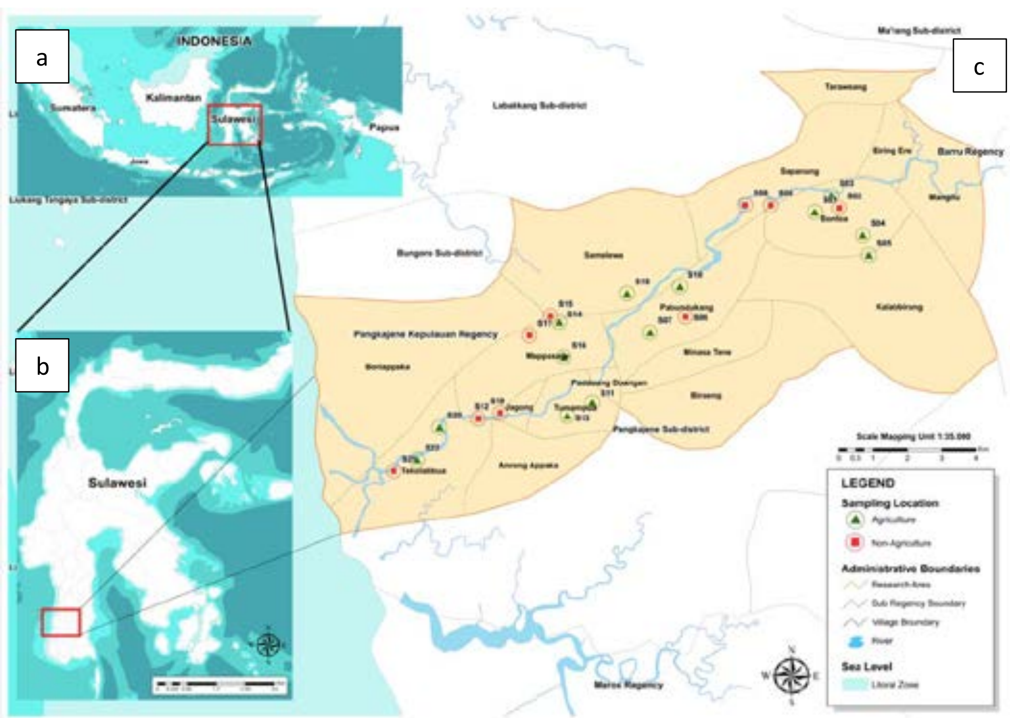


Fig. 1: Geographic location of the study area; (a) Indonesia, (b) Sulawesi Island, (c) Pangkep Regency in Indonesia

Table 1: Analysis method for total Cr and Ni concentration in the studied soil

No	Parameter	Analysis Method	Instrument
1.	Cr	EPA Method 3050b	F-AAS (PerkinElmer PinAAcle 900H)
2.	Ni	SNI 06-6992.6-2004	F-AAS (PerkinElmer PinAAcle 900H)

were collected from the depth 0 – 20 cm of surface soil using a shovel. There are ten individual soils, then they mixed. The interval between an individual sample was 200 meters in an irregular pattern. A 500 gram of soil was labeled and collected into clean zipped polyethene bags after cleaned from rubbish, gravel, grasses, and plant roots. All soil samples were placed at room temperature before brought to the laboratory. Samples were analyzed at the center for plantation-based industry laboratory in Makassar, South Sulawesi Province, Indonesia**.**

Cr and Ni analysis

The soil samples were dried at room temperature. Then, air-dried samples were crushed, sieved through a 2 mm Nylon sieve, homogenized, and placed in the polyethene bottle before acid digestion. The total metal concentration was determined following the analysis method (Table 1). Cr and Ni were analysed using the acid digestion method. To analyse Ni concentration, put 3 g of dried soil samples into Erlenmeyer glass. Added the 25 mL of distilled water and stirred the solution. Then added 5 mL of HNO_3 and three boiling chips into the Erlenmeyer glass. Then, heated the solution at 105^{0}C until the mixtures reached ±10 mL. Then, cooled at room temperature, added 5 mL of nitric acid and 1 mL of perchlorate acid to the solution, and heated until it was transparent, and filtered the mixtures using filter paper Whatman no 42. The final mixes were measured using a flame atomic absorption spectrophotometer (F-AAS, PerkinElmer PinAAcle 900H). For Cr concentration, acid digestion was done by adding 10 mL HCl to 3 grams of soil sample. Then heat the mixture at 95^{0}C for 15 minutes and filter the digested sample through Whatman no 42 and collect in a 100 mL volumetric flask. Total concentration of Cr and Ni was analysed using F-AAS with detection limit 0.2 and 0.2 mg/kg, respectively. The quality assurance and quality control of analysis included standard operating procedures and the NIST standard material (NIST 1646a estuarine sediment), all were measured attentively.

Metals Contamination assessment

Geo-accumulation index (I-geo)

The I-geo value was determined using Eq. 1 (Müller, 1986).

$$I_{geo} = log_2\left(\frac{C_x}{1.5C_b}\right) \qquad (1)$$

Where, C_x is soil-specific heavy metals in the studied area, C_b is the geochemical/background concentration of specific heavy metals. Due to lack of local background concentration, a background concentration was used from Taylor (1964). The constant of 1.5 accounted to reduce the variation of background concentration which may be influenced by lithologic variation (Laniyan and Adewumi, 2020). The I-geo value interpretation including I-geo £0 means that studied soil is unpolluted with metals; 0< I-geo £1 indicates that the studied soil is uncontaminated up to moderately polluted with metals; 1<I-geo£2 means moderately polluted; 2 < I-geo £ 3 presents that somewhat up to highly contaminated; 3 < I-geo £ 4 indicates highly polluted; 4 < I-geo £ 5 means highly up to extremely polluted; I-geo > 5 demonstrates that the studied soils are significantly/extremely contaminated with metals.

Contamination factor (C_f)

To assess heavy metals contamination in the soil effectively, several pollution indices were used. Two methods were used, including C_f, and PLI to determine the soil heavy metal pollution. Contamination factor (C_f) is widely used to determine toxic substances pollution and soil/sediment quality (Kowalska *et al.*, 2018). The pollution index can be calculated using Eq. 2 (Hakanson, 1980):

$$C_f = \frac{C_x}{C_b} \qquad (2)$$

C_f is the contamination factor for measured heavy metals. The pollution load index (PLI) is used

Table 2: The heavy metals concentration in soil at the Pangkajene watershed area (mg/kg)

Land use	Samples	HMs	Mean±SD	Min	Max	Background value*	Permissible value
Agriculture land	13	Ni	35.38±17.1	10	60	75	35
		Cr	89.9 ± 16.8	68.9	117.6	100	100
Non-agriculture land	9	Ni	54.4 ± 20.6	20	70	75	35
		Cr	97.18 ± 21.6	60.6	126.6	100	100
Overall	22	Ni	43.18 ± 20.56	10	70	75	35
		Cr	92.9 ±18.82	60.6	126.6	100	100

*Background value was obtained from continental crust background value in the study by Taylor (1964)
**Permissible value of heavy metals in soil was obtained from WHO permissible value for heavy metals (WHO, 1996)

to determine the severity level of multiple heavy metals' pollution in soils using Eq. 3 (Gati *et al.,* 2016; Kowalska *et al.,* 2018).

$$\mathrm{PLI} = \sqrt[n]{\mathrm{CF1} \times \mathrm{CF2} \times \mathrm{CF3} \times ..\mathrm{CFn}} \tag{3}$$

Where, n is the number of trace elements.

Ecological hazard quotient (EHQ)

The potential ecological risk was determined using a quantitative screening Ecological Hazard Quotient (EHQ). This index can assess the likelihood that the adverse environmental effects may exist due to toxic substance exposure. This index was used to evaluate adverse biological effects because of heavy metals contamination in the soil (Feng *et al.,* 2011; Mallongi *et al.,* 2014). The specific potential for toxicity in study area soils was estimated by calculating the hazard quotient using Eq. 4.

$$EHQ = \frac{EEC}{screening\ benchmark} \tag{4}$$

Where, EEC is the Estimated or maximum soil heavy metals concentration at sampling sites. The screening-level benchmark is the soil concentration below which toxicity is not likely to occur (Beyer and Sample, 2017). The screening value was obtained from the toxicity value for terrestrial plant by Efroymson *et al.,* (1997). The ecological hazard index is used to determine potential detrimental effects caused by multiple toxic substances (OHIO EPA, 2008). This index is calculated by summing of EHQ.

Data analysis

Cr and Ni concentrations in the soil were collected after analysis in the laboratory. Then, the statistical analysis was conducted using SPSS 24.0 version package. Descriptive analysis (mean, standard deviation, minimum and maximum value) was presented in this study. The IDW interpolation was used to map distribution of Cr and Ni concentration at whole study area.

RESULTS AND DISCUSSION

Distribution of Cr and Ni concentration in the studied area

Table 2 presented the concentration of heavy metals in the studied soil. The mean concentrations showed that Cr was higher than Ni. The Cr concentration was below the soil permissible value. Although Cr was below the standard, it can be accumulated in the soil over time. Ni concentration exceeded the allowable value. In Table 3 demonstrated that Cr concentration in present study exceeded the threshold limit value by CCME (2007). While Ni concentration was below threshold limit value by CCME (2007). Cr concentration in Pangkep regency was higher than Cr world average concentration in soil. While Ni concentration is still below the world average concentration. Compared to other study in karst area, soil in Pangkep watershed has lower concentration of Cr than soil in Huixian karst watershed, China (Huang *et al.,* 2020). Whereas, the concentration of Ni is higher in the Pangkep regency than in the Huixian karst watershed, China.

Table 3: Comparison of mean concentration in studied soils to other soil standards and studies (mg/kg)

HMs	Mean[a]	Earth crust[b]	Average shale[c]	Average surface rocks[d]	Threshold limit value[e]	World average soil[f]	Huixian, China[g]	Beke-Cave watershed, Hungary[h]
Cr	92.9	100	90	71	64	70	118.18	-
Ni	43.18	75	68	49	50	50	43.04	37.47

[a] present study, [b] Taylor (1964), [c]Turekian and Wedepohl (1961), [d,f]Martin and Whitfield (1983), [e]CCME (2007) [g]Huang *et al.,* (2020), [h]Kaszala and Barany-Kevei (2015)

Based on the Table 3, soil in present study has higher concentration of Ni than soil in the karst watershed Hungary. The soil texture can influence the total concentration of metal in soil due to the ability to absorb and mobilization of metals (Kaszala and Barany-kevei, 2015). The highest concentration of Ni is available in clay soil (Kaszala and Barany-kevei, 2015). Both Cr and Ni concentrations were higher in the non-agricultural land than in the agricultural area. Efe (2014) mentioned that soil content depends on its parent rock material and climate. Based on litho-tectonic, the Pangkep regency was classified in the west mandala structure dominating sedimentary rocks such as alluvial deposits, limestone Tonasa formation, Malawa formation, and volcanic rock (Camba volcanoes) (Sompotan, 2012). Another study by Ramli *et al.,* (2009) mentioned that Pangkep regency was developed by alluvium/ sediment material, limestone, breccias, lava, tuffs, conglomerates, ultra-basalt, basalt, trachyte, and mixed rocks. Based on Fig. 2 the highest concentration of Cr is located in S08 from Tonasa formation which has formed by limestone. Sampling site 8 is located near limestone mining and cement industry, the high concentration may be influenced by deposition of dust which is emitted from industry. While the highest concentration of Ni is located in S12, S15 and S19 formed by alluvium sediment and limestone from Tonasa formation. Carbonate rocks and alluvium deposits were dominated the soil parent material in the Pangkep regency. Heavy metal enrichment in the karst area due to the high background concentration of heavy metals in carbonate rocks and secondary enrichment was undergone when the weathering process (Tang *et al.,* 2020). Carbonate rocks may influence the high concentration of Cr and Ni in Pangkep. It has several characteristics, including poor drainage and leaching; thus, it can keep trace elements (Yolcubal and Akyol, 2007; Hasan *et al.,* 2020). Limestone is the metal stabilization agent to inhibit metal mobility by increasing pH. In contrast, when the soil has high alkaline properties, it can be enhanced metal leaching at high concentration (Yun and Yu, 2015). High rainfall and acid rainfall temporarily reduce the acid buffering capacity in karst soil. The Maros-Pangkep karst area is one of the most beautiful karst areas formed in the late Eocene to middle Miocene (40 million to 15 million years ago). The limestone forming in the Miocene era has a high concentration of $CaCO_3$ (92%) (Efe, 2014). The $CaCO_3$ content in the soil can affect heavy metals concentration where it can inhibit the absorption of heavy metals into plants (He *et al.,* 2020). A previous study by Huang *et al.,* (2016) confirmed an effect of carbonate and phosphate on the metals immobilization in the polluted soil. It is caused by the reduction of free-metal activity or the exchangeable fraction of metals in the soil. CaO content in soil karst is much higher than in non-karst soil (Li *et al.,* 2021). It can be caused by the weathering carbonate rocks and pedogenesis. Li *et al.,* (2021) showed the insoluble residues of carbonate rocks which has iron, manganese, aluminium and other trace element was remained as soil parent material, while major elements (potassium, calcium, sodium, and magnesium) were rinsed and leached by soil solution when carbonate rock dissolution in karst ecosystem.

Naturally, Cr and Ni concentrations in limestone are 10 and 20 ppm, respectively (Adriano, 1986). This study showed the enrichment of Cr and Ni in the soil because the concentration of Cr and Ni in the studied soil was higher than the natural concentration in limestone. Based on the geo-accumulation value, all the sites have I-geo < 0 (Fig. 3). It indicates that Pangkep regency's soil was uncontaminated with Cr and Ni, which derived human-made sources. This result is in line with the study by Miko *et al.,* (2003), which mentioned that soil-derived carbonate rocks

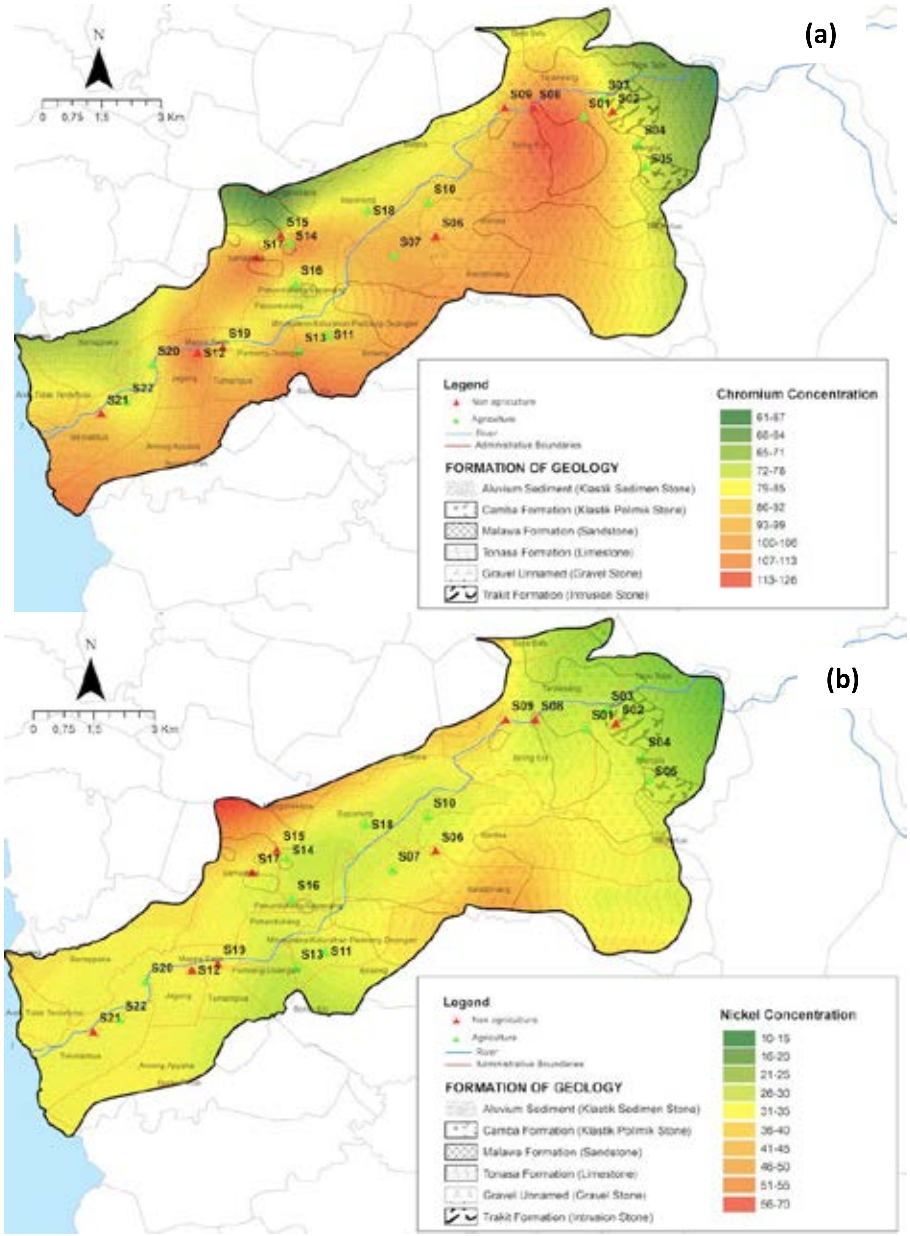
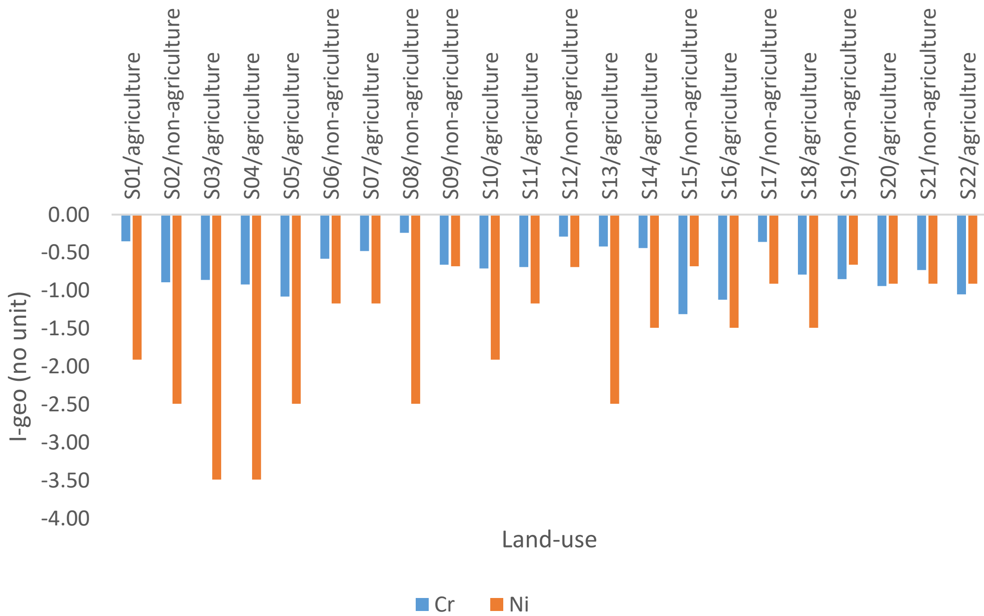
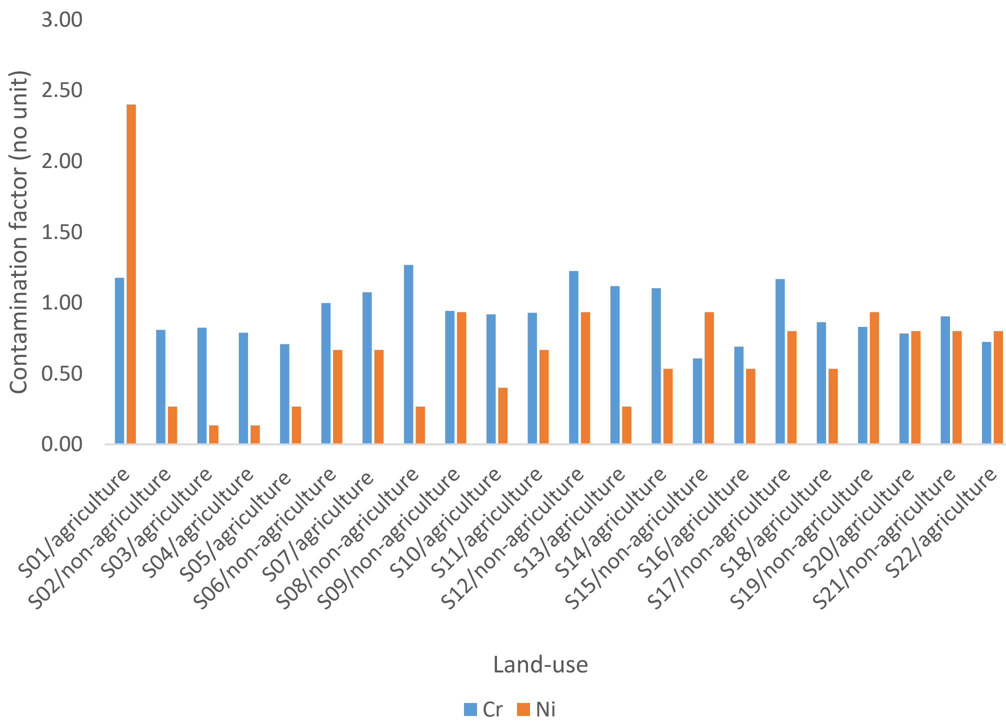


Fig. 2: The distribution of Cr (a) and Ni (b) in the studied soil (mg/kg)

have a higher concentration of Mn, Co, Al, As, Cd, Cr, Ni, Fe, La, Th, V, Cu, and Sr. The high and acid rainfall in the karst area can generate the karst soil loss and progressively enhance the rock weathering process (Kamon *et al.,* 1996; Lyu *et al.*, 2018; Wang *et al.,* 2019; Zhao and Hou, 2019). Moreover, acid rain can cause depleting the surface soil layer, degrade soil nutrients content and soil carrying capacity, and transfer soil metals to other places. Based on NCEANET (2020)'s data, acid precipitation in Maros regency has a pH ranged from 4.3 to 5.6. The main problem of acid rain is possibly caused by air pollution. Similar to the Maros regency, there are mining and industrial activities in the Pangkep regency area, including cement and



(a)



(b)

Fig. 3: Geo-accumulation index (a) and contamination factor (b) of Cr and Ni in the studied area

Table 4: Summary of metals contamination index value in the studied area

Contamination index	HMs	Mean ± SD	Range	Contamination status	
Contamination factor	Ni	0.7 ± 0.5	0.1 – 2.4	Low	Contamination factor classification: $C_f < 1$; $1 \leq C_f < 3$; $3 \leq C_f < 6$; $C_f \geq 6$, representing low, moderate, considerable, and very high pollution (Hakanson, 1980). PLI ≤1, PLI > 1 demonstrating no metals contamination and there is metal contamination, respectively.
	Cr	0.9 ± 0.2	0.6 – 1.3	Low	
Pollution load index		0.7 ± 0.3	0.3 – 1.7	No pollution	

marble. The cement industry contributed 5% of total global anthropogenic CO_2 emission (Mahasenan *et al*., 2003) and enormous quantities of acid rain precursor such as SO_2, NO_x, CO, and PM to ambient air (Lian *et al.,* 2019). On the lowlands of Pangkep regency, there are alluvial deposits, swamp, and the coast, which were Holocene aged, containing gravel, sand, loam, limestone, coral, and mud (Sompotan, 2012). Based on Fig. 2, Cr's highest concentration is located in non-agriculture land (sampling sites 8). At the same time, high Ni concentration is situated in non-agricultural land (sampling sites 9, 12, and 15). Both high concentration of Cr and Ni were located in non-agricultural land. Based on prior study by Sayadi and Rezaei (2014) high concentration of Ni and Cr may originate from residential-road soil and dairy farm land. High enrichment factor is also occur in the residential and industrial area (Sayadi and Sayyed, 2011). Another study showed that highly concentrated of toxic metals in soil located in areas where people live (Laniyan and Adewumi, 2020). The potential source of Cr are mining, pharmaceutical, metal, textile and leather industries. While, nickel is originated from batteries, power plants or incinerator, combustion of fossil fuels, rubber and plastic industry, electroplating, petroleum by-products and rocks natural weathering. Rauf *et al.,* (2020) and Mallongi *et al.,* (2020) mentioned that the area near cement industry has high concentration of Cr and Hg. The coal combustion is one of the source of metal contamination in soil. In addition to lithogenic sources, human-induced contamination such as domestic, mining, and industry sewages are potentially increased the Cr and Ni concentrations in the soil. Sampling sites 8 and 9 are the residential soil near the cement industry in the Bungoro sub-district. Thus, domestic and industrial sewage is the potential source of pollutants in these sites. Pb, Cr, Ni, and Mn are generally found in areas located near industrial sites, and they potentially pose residents at risk of detrimental health effects. Trivalent chromium is essential for human nutrient, while hexavalent chromium has carcinogenic effects (Jaishankar *et al.,* 2014; Costa and Klein, 2006). Human exposure to chromium is related to skin cancer and lung cancer. A study from McDermott *et al.,* 2014 demonstrated that a median Cr and Ni concentration of 19.13 mg/kg and 4.58 mg/kg in soil is related to low birth weight cases in pregnant women located near industrial sites in the South Carolina USA. It implicates exposure to contaminated soil metals may cause adverse impact to pregnant women living in the near of industrial contaminated sites. Contamination of Cr also effects on gastrointestinal disease (Costa and Klein, 2006). Human may expose to Cr and Ni through soil contact or through eating contaminated food. Furthermore, leaching of metals to drinking water is also possible source of Cr and Ni. Nickel is also classified as human carcinogenic substance by International Agency for Research on Cancer (Das *et al.,* 2019). Chronic exposure to nickel via soil, water or direct contact may induce allergic dermatitis (Das *et al.,* 2019).

Cr and Ni enrichment status

Based on the I-geo value (I-geo<0) in Fig. 3a, soil in our studied area was naturally enriched by Cr and Ni. Cr and Ni's concentration in the studied soil was lower than the soil's background value. It indicates that lithogenic activities in the studied area caused increased Cr and Ni concentrations. Based on Table 4, the studied soils were low contaminated with Cr and Ni (mean value of Cf<1). The result of Cf showed that the soils in Pangkep regency ranged from low to moderately contaminated with Cr and Ni. PLI mean value showed that Pangkep's soils are uncontaminated with Cr and Ni. However, ranged of pollution load index of soil indicated no pollution to moderate pollution. Based on Fig. 3b, contamination factor (Cf) at the studied area was relatively low at agriculture land for nickel contamination, except in sampling site 1 (S01) which had the highest value Cf for Ni. Geologically, sampling site 1 located in the

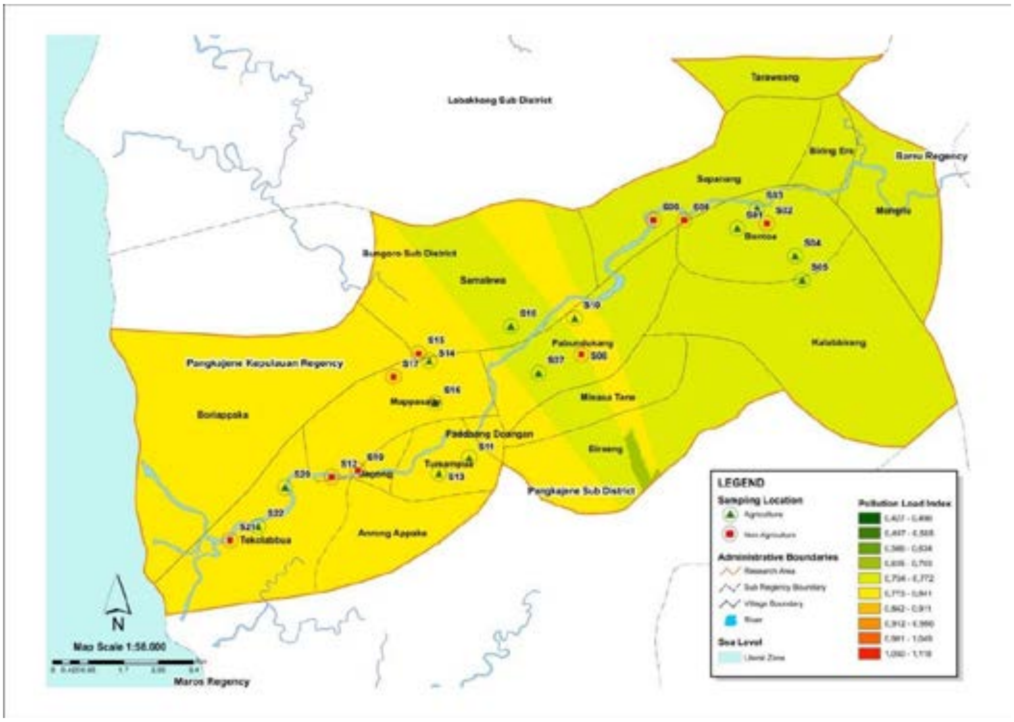


Fig. 4: Pollution load index in the studied soil

Bungoro sub-district was formed by the combination of trachyte formation, Tonasa formation, and gravel stone. Enrichment of metals and minerals little can be influenced by ultramafic and basalt rocks in Pangkep regency (Fatinaware *et al*., (2019); Suryani and Ritung, 2018; Tonggiroh, 2013; Wilson, 1996). Weathering ultramafic and basalt rocks can cause the high accumulation of Cr and Ni in the soil (Suryani and Ritung, 2018; Sayadi and Sayyed, 2011). Compared to Barru regency in study by Suryani and Ritung, 2018, the total concentration of Cr and Ni in Pangkep regency is far below the total concentration of Cr and Ni in Barru regency. The sampling sites 1 – 9 were used for the mining activities such as limestone, clay, trachyte, feldspar, sandstone, and marble. In addition, there is a cement industry that may influence the contamination of nickel and chromium. According to other study, the soil near cement industry may contaminate with high level of metals such as Pb, Cr, Cd, Ni, Hg, Zn, Cu, Co from aerial deposition (Laniyan and Adewumi, 2020; Mallongi *et al., 2020;* Ogunkunle and Fatoba, 2014; Rauf *et al.,* 2020). The distribution of metals in top soil can be influenced by the wind velocity and particle size (Ogunkunle and Fatoba, 2014). Cr and Ni mean concentration in present study was higher than concentration of Cr and Ni in study conducted by Laniyan and Adewumi, (2020). Furthermore, the high value of Cf in agriculture can be influenced by intensive tillage, and application of fertilizers and pesticides. Higher PLI values are distributed in the middle and downstream area (Fig. 4). An alluvial deposit forms the soil in the middle and downstream. Alluvial and alluvial-colluvial areas are commonly considered to have high Cr content, especially in the calcareous environment (Li *et al.,* 2009).

The middle and downstream areas of the Pangkep regency are dominated by domestic/municipal, agriculture and aquaculture activities. The studied area landscapes are dominated by karst hills in the upstream, gently sloping in the middle stream and alluvial plain in the downstream area. Bungoro and Pangkajene sub-district were classified as the critical land in Pangkep regency, and it is vulnerable to undergo worsening environmental degradation. Soil erosion can transfer metals to further sources. When

Table 5: Degree of ecological risk in soil surrounds the watershed area of Pangkajene regency, Indonesia

HMs	EHQ value	Description	Number of samples	Percentage of samples
Cr	< 0.1	No hazard	-	-
	0.1 - 1.0	Low hazard	-	-
	1.1 – 10	Moderate hazard	-	-
	>10	High hazard	22	100
Ni	< 0.1	No hazard	-	-
	0.1 - 1.0	Low hazard	8	36.4
	1.1 – 10	Moderate hazard	14	63.6
	>10	High hazard	-	-
	Total		22	100

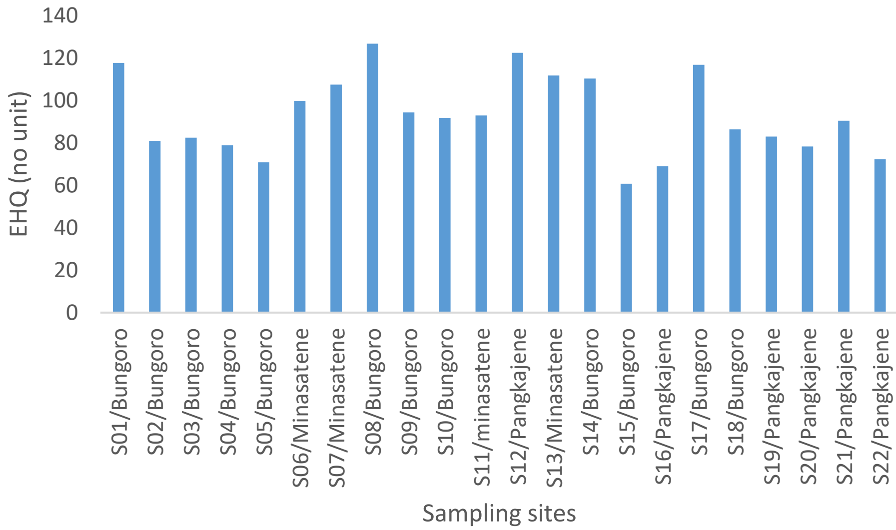
The classification of hazard based on the prior study by (Lemly, 1996)
The screening benchmark for Cr and Ni was 1 and 30 mg/kg (Efroymson *et al*., 1997)

the soil has been disturbed by human activities, including mining, industry, and agriculture, fresh deposits of heavy metals will be transported from higher elevations via the natural process of erosion. It may be remobilized together with excavated soil/sediments (Jarsjö *et al.,* 2017). Besides, soil erosion is related to the physical disturbance from mining activities and infrastructure (roads) and water use. As Mortatti and Probst, (2010) mentioned, transport of particulate heavy metals could be instantaneously discharged from the source and influence the watershed drainage area. It is expressed when the downstream area has a relatively high concentration of Zn, Cr, Ni, and Cu (Mortatti and Probst, 2010). Agricultural land can transport heavy metals to non-agricultural land by eroded soil. Mohammadi *et al.,* (2019) mentioned soil erosion in farmland could transport heavy metals to the rivers.

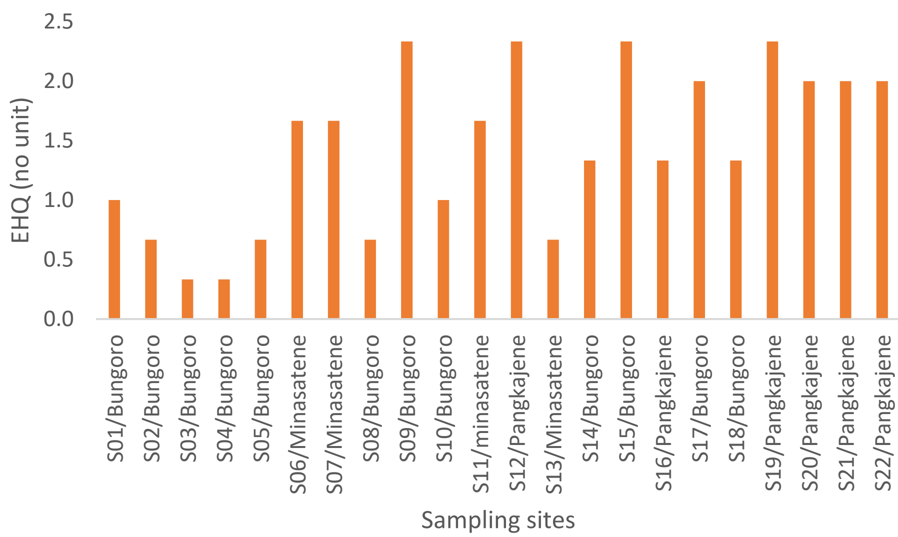
The agricultural sectors may increase heavy metals content in the soil by applying fertilizers, manures and pesticides, and wastewater irrigation. Phosphate fertilizers are the source of heavy metals (Mendes *et al*., 2006). Since Pangkep regency still used urea, SP36, and ZA as fertilizers in the farmland, it could be attributed to an accumulation of heavy metals. The soil heavy metal concentration will be influenced by many factors such as pH, soil type, land use pattern, relief of topographic and lithology aspects. Thus, it might affect the various level of content-heavy metals in this study area. The present study only analysed the total concentration of Cr and Ni in soils. Further research should be undertaken to investigate the several factors that may influence metals binding in the soil of the Pangkep regency.

The potential ecological risk

The US EPA used EHQ calculation to estimate if the risk of adverse effects to the environment is likely or not happened due to the pollutant (USEPA, 2016). Based on Table: 5, all studied soils have a high ecological risk from Cr enrichment. However, Ni enrichment has a low and moderate risk to the ecological system. The ecological risk has a critical value of 1. It means if the value of EHQ > 1, the soil contamination has a potential hazard to the environment, or there is a hazard due to pollutants at the site. Fig. 5 showed sampling site 8 in Bungoro sub-district has the highest EHQ value for Cr, while the highest value of EHQ for Ni is located in Bungoro and Pangkajene sub-district (Sampling site 9, 12, 15, and 19). These results showed that Cr's ecological risk tends to be happened in the upstream area, whereas Ni's ecological risk is likely to occur in the middle and downstream area. Soil is often contaminated with metals which are from far place. Soil erosion can attribute to metal transportation (Amorosi, 2012). Fig. 6 shows that the upstream and the middle stream areas have a high risk from Cr and Ni enrichment. Ecological hazard index (EHI) for studied soils is higher than 1, and it means there is a



(a)



(b)

Fig. 5: EHQ value related to the enrichment of Cr (a) and Ni (b) in the studied area

potential adverse impact on ecology due to Cr and Ni contamination. Cr can have beneficial and adverse effects on humans, plants, and animals. Chromium is not an essential substance for plant (Ertani *et al.,* 2017). Cr can accumulate metals on roots. The toxicity of Cr is depended on its speciation. The toxic effects of Cr and Ni on the terrestrial plant are related to the generation of reactive oxygen species (ROS), which impair the plant's metabolism and physiological process (Hassan *et al.,* 2019; Sharma *et al.,* 2020; Ertani *et al.,* 2017). Cr toxicity symptoms on plants including a decrease of germination,

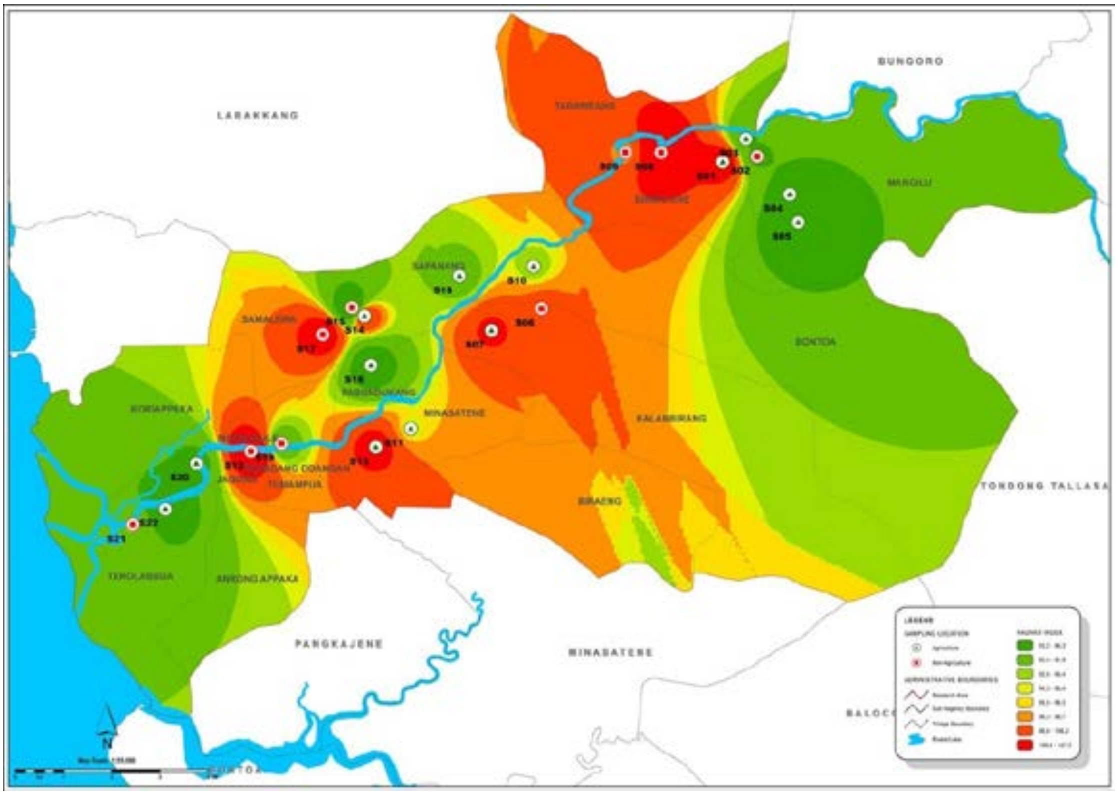


Fig. 6: EHI of Cr and Ni in the studied soil

impairment of enzymatic process, reduction of the growth process, genotoxicity, impairment of photosynthesis, and oxidative shortcoming (Ertani *et al.,* 2017). The higher EHI value is dominated in the upstream area and middle stream area of the watershed, where the mining and industry activities contribute to the accumulation of heavy metals. Besides, in the midstream area, there are municipal/ domestic and agriculture activities. Several home-based enterprises surround the middle stream area of Pangkep watersheds, such as tofu factory, automotive services, machine shops, dry cleaning, metal plating, metal mechanic and metal finishing, and furniture which may possibly discharge their pollutants into soil and water river. The upstream area (S01- S03, S08, and S09) in Bungoro subdistrict is dominated by industry and mining activities.

The cement and marble industry were established for a long time in the Bungoro sub-district. A prior study showed high Hg contamination by industrial and mining activities in the Bungoro subdistrict (Mallongi *et al.*, 2020). Another study in the karst area of Maros regency showed the contamination of Hg and Cr in the area near the cement industry of Maros regency (Rauf *et al.,* 2020). Contamination of soil may be influenced by wind direction and intensive tillage of the agriculture field (Rauf *et al.*, 2020). Study by Rauf *et al*. (2020) also mentioned that Cr tends to accumulate near the cement plant. Thus, limestone mining is one of the contributors to Cr contamination in this area. Since the Cr and Ni tend to be accumulated in the soils and have potentially exposed biota and humans, maintaining and improving the environmental quality is needed in the Pangkep regency. There are several possible actions to reduce Cr and Ni concentration at studied soil including 1) increasing the organic matter content in the soil, 2) applying agricultural lime ($CaCO_3$), and 3) planting the crops that not consumed such as agroforestry (Suryani and Ritung, 2018). The conducting comprehensive research was used on the amount of heavy metals concentration in agriculture products and heavy metals exposure on human health through the food chain. Since soil erosion has commonly happened in the watershed area, the importance of maintaining and controlling the watershed area relating to the transportation of heavy metals via the erosion process needs to be highlighted by the regional government.

CONCLUSION

The results indicated the soils in the Pangkep regency watershed were generally contain a low level of Cr and Ni. The order of mean concentrations is Cr>Ni. Cr and Ni's highest value is located in the upper and middle stream of the Pangkajene River. This location is dominated by industry, mining, and agriculture activity. I-geo and PLI values indicated no pollution of Cr and Ni in the studied area. The potential sources of Cr and Ni are mainly derived from natural sources. This study area is enriched with carbonate rocks. So, it may influence the immobilization of heavy metals in soil. Soil erosion and flood in the karst area may transport heavy metal from the upstream region to the lowland. These study results also demonstrated that Cr concentration was below WHO permissible value, while Ni concentration exceeded the WHO standard. However, Cr and Ni can accumulate in the soil over time if there is no remediation action at the site. Heavy metal controlling and monitoring activity needs to be done in the studied area due to metals bioaccumulation properties and their adverse impact that may happen in long-term exposure to biota and humans. Increasing the organic matter content in the soil, applying agricultural lime ($CaCO_3$), and developing agroforestry activities are the remediation plan for the studied area. Since lack of standards for heavy metals for soil and information on background concentration in the study area, we recommend to local authorities to develop the standards and environmental monitoring mechanism to reduce the risk of heavy metal accumulation. However, the EHI value >1 means that the metals enrichment has a potential adverse impact on ecological health from Cr and Ni contamination. The importance of landform shifting to transport heavy metals and identifying soil erosion process in the watershed area needs to be concerned in Pangkep regency to control heavy metals contamination better. For further research, there are several suggestions: 1) physical and chemical properties of soil relating to heavy metals contamination, 2) comprehensive study on the amount of heavy metals content in agriculture products, and 3) the effects of heavy metals exposure on human health.

AUTHOR CONTRIBUTIONS

R.D.P. Astuti performed research concepts and designs, assembled data, wrote the article, data analysis, and interpretation. A. Mallongi performed research concept and design and critical revision of the article. A.U. Rauf assisted in collecting data and writing the paper.

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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

Al	Aluminum
As	Arsenic
ATSDR	Agency for toxic substances and disease registry
$CaCO_3$	Calcium carbonate
C_b	The geochemical/background concentration of specific heavy metals
CCME	Canadian Council of Minister of The Environment
Cd	Cadmium
C_f	Contamination factor
Co	Cobalt
CO	Carbon monoxide
CO_2	Carbon dioxide
Cr	Chromium
Cr (VI)	Hexavalent chromium

Cu	Copper
C_x	soil-specific heavy metals in the studied area
EEC	Estimated environmental concentration
EHI	Ecological hazard index
EHQ	Ecological hazard quotient
EPA	Environmental Protection Agency
Eq.	Equation
F-AAS	Flame atomic absorption spectrophotometer
Fe	Iron
g	Gram
GPS	Global Positioning System
HCl	Hydrochloric acid
Hg	Mercury
HMs	Heavy Metals
HNO_3	Nitric acid
IDW	Inverse distance weighted
I-geo	Geo-accumulation index
La	Lanthanum
max	Maximum
mg/kg	Milligram per kilogram
mg/L	Milligram per litre
min	Minimum
mL	Millilitre
mm	Millimetre
Mn	Manganese
n	The number of trace elements.
Ni	Nickel
NIST	National Institute of Standards and Technology
NOx	Nitrogen oxide
Pangkep regency	Pangkajene dan Kepulauan regency
Pb	Lead
pH	Power of hydrogen or basicity/acidity indicator
PM	Particulate matter
PLI	Pollution load index
ppm	Part per million
ROS	Reactive Oxygen Species
SD	Standard deviation
SNI	Standar Nasional Indonesia or Indonesia's National Standard
SO_2	Sulfur dioxide
SP36	Superphosphate 36
Sr	Strontium
Th	Thorium
US$	United States dollar
V	Vanadium
WHO	World Health Organization
ZA	Diazonium sulfate (Zwavelzure ammoniac)
Zn	Zinc
%	Percent
^oC	Celsius degree

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REVIEW PAPER

Plant growth promoting rhizobacteria in promoting sustainable agriculture

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ABSTRACT

Rapid human population growth and its consequences of food shortage become a significant concern in recent decades across the world. The untold reasons behind this food shortage were industrialization, urbanization, modern civilization, etc., where the agricultural land has been deployed. With the decreasing farmland and its cultivation, food productivity declined drastically and failed to serve the world's vast human population. The present challenge is to increase productivity with the least agricultural land. Thus, excessive chemical fertilizer has been used to quickly turn out more outstanding food production, leading to more significant damages to soil ecosystem and human health. Henceforth, bio-fertilizers find the best alternatives to chemical fertilizers. This study focuses on complete nature of plant growth Promoting rhizobacteria, which is used in bio fertilizers for sustainable agricultural productivity and everlasting soil fertility. The characteristics of plant growth promoting rhizobacteria and its role in plant growth and formulation of plant growth promoting rhizobacteria biofertilizers have been revealed through intensive literature. The consortium information collected from various literatures brings the unique findings that plant growth promoting rhizobacteria is the natural boon to the global agriculturist. This study discusses plant growth promoting rhizobacteria bacterial strains' role in protecting the soil from various biotic and abiotic stresses, regulating plant growth and its role in producing biofertilizers. Besides, it is transformed into commercial products. Eventually, the future trends and research in plant growth promoting rhizobacteria bio inoculants that promote sustainable agriculture have been elucidated. The microorganism is the bio fertilizer's main ingredients, promoting the soil nutrients for efficient plant growth and increasing food productivity. Although many microorganisms efficiently contribute to the soil nutrients, this review narrows down to the plant growth promoting rhizobacteria study. Beneficial bacterium plays a vital role in nutrient mineralization and productivity among the various microorganisms. Bio fertilizers containing beneficial bacteria were economically viable and readily available in nature. This review reveals the complete essence of plant growth promoting rhizobacteria and its part in bio fertilizers.

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INTRODUCTION

One of the foremost goals of human life is the consistent and adequate food supply. Due to the increasing rate in population across the world, consistent food supply becomes a challenging phenomenon in current scenario. There are many factors in offering food supply for the rapid population growth, such as production rate, cost, climate, land utilization, adequate water supply, technology, consistency in crop productions and etc. Besides, soil fertility is the crucial element which determines the crop production quantitatively and qualitatively. Soil fertility is in the great need of continuous and consistent monitoring because of its intangible activities like nutrient cycling, structural arrangements, biotic regulation, transformation of harmful elements, holding capacity of nutrients, transferring water and nutrients to plants and etc., Shortage of soil fertility is one of the indispensable limitations in achieving expansive crop production (Franzluebbers *et al.,* 2013). In order to fulfill the world food security, excessive usage of inorganic fertilizers were practiced that in turn became one of the vital reasons for declining in soil fertility (Kumar *et al.,* 2019). The utilization of inorganic fertilizers increases the crop production; however it causes long term degradation of soil fertility over the years (Shambhavi *et al.,* 2017). Massive utilization of organic fertilizers is very essential to regain the soil fertility. The hybrid combo of inorganic with organic fertilizers retains the soil fertility that triggers off crop production rate (Emmerson *et al.,* 2016). Hybridization of fertilizers completely depends on physical, chemical and biological characteristics of soil (Walsh *et al.,* 2012).Utilization of fertilizers greatly concern with biological characteristic changes which in turn indicate the soil fertility rate (Bargaz *et al.,* 2018). Biological components of soil are more pertinent to long term crop productivity and for sustainable agricultural practices (Lima *et al.,* 2015). Improvising the soil biological composition evokes amelioration of crop productivity by means of biofertlizers. Biofertlizers is eco-friendly, which aid in sustainable agricultural practices (Santhosh *et al.,* 2018). It holds alive microorganism, which induces the soil nutrients by means of organic matter decomposition (Mazid *et al.,* 2011). Intake of mineral nutrients by plants with the help of biofertlizers brings a better outcome in terms of long term crop productivity (Malusa *et al.,* 2012). All bacterial biofertlizers has significant part in Nitrogen (N) fixing, Phosphorus (P), Potassium (K), zinc and silica solublization (Narendra *et al.,* 2017) which helps in fixing all kinds of micro and macro nutrients to the soil. In addition biofertilizers enhance the plant growth through enriching the soil fertility by release of plant growth harmones, antibiotic production and organic matter biodegradations (Sinha *et al.,* 2014). Biofertilizers involves and accumulates the nutrients in the soil and prevents the nutrient loss during intake by plants, thus helping in enhanced plant growth (Singh *et al.,* 2011). PGPR, the main ingredients of bacterial biofertlizers has hefty relationship with different species relevant to host plants. Rhizospheric and Endophytic, the two prime classes of relationship which are found in the intercellular (Imran *et al.,* 2019) and apoplastic space of the host plant respectively (Qiu *et al.,* 2016). Some of the notable Rhizospheric bacteria are Bacillus, Azospirillum, Azotobacter, Burkholderia, Enterobacter, Klebsiella, Variovorax, Comamonadaceae, Pseudomonas, Gemmatimonadetes, Streptomyces filamentosus and Bacillales. Markable endophytic bacteria are Azoarcus spp, Herbaspirillum, seropedicae and Gluconacetobacter diazotrophicus (Carvalho *et al.,* 2014). Aforementioned PGPR produces Indole-acetic acid (IAA), Cytokinins (CK), Gibberellins (GA) and inhibitors of ethylene which takes up the great responsibilities of nutrients and water uptake required for plant growth (Tsukanova *et al.,* 2017). In this study, PGPR's role in agriculture, various species and its corresponding plants, its mechanism, advantages and disadvantages, future trends were reviewed. The aim of study is to provide adequate knowledge about the characteristics and functionality of PGPR through consortium literature reviews so as to practice the same to attain sustainability in agriculture. The overall literature study and compilation were made at SRM Institute of Science and Technology, Chennai in the year of 2021.

Plant growth promoting rhizobacteria

A cluster of bacteria that colonizes the root of the plant (rizhosphere) is termed as Plant growth promoting rhizobacteria (Egamberdieva *et al.,* 2014). Rhizhosphere is considered as the maximum nutrient amalgamate zone, where active microbial activities will be carried out. Though rhizhosphere consists of various microbes such as bacteria, fungi, algae, protozoa and acticomycetes, the bacterial colonies were found abundant (Kaymak *et al.,* 2010). The sustainable plant growth has been made possible through these bacterial colonies and it has been proved under various circumstances (Saharan *et al.,* 2011; Bhattacharyya *et al.,* 2012). Apart from promoting the plant growth through its active mechanism, the bacterial colonies in the rhizosphere has a strong effect on controlling the phytopathogenic microbes (Son *et al.,* 2014).Those bacterial colonies were named as rhizobacteria and are heterogeneous in nature. The unique characteristic of rhizobacteria such as ability to produce plant growth regulators, fixation of nitrogen, siderophore production, nutrients and mineral solubilization shows the superior nature when compared to other microorganism in promoting the plant growth (Souza *et al.,* 2012). Moreover, it acts as the Biocontrol agent to the pathogenic microbes (Beneduzi *et al.,* 2012). These beneficial bacterial colonies were affected by various factors such as temperature, pH, moisture, soil mineralogy and light. Among which the composition and activities of plant associated microbes. Beneficiary microbes enhance the plant growth usually at high temperature (Stephane *et al.,* 2010). pH is the important factor in determining the sustainability of microbes in the soil. pH ranges from 6.0 to 6.5 is found advantageous for the beneficiary microbes sustainability so as to ensure healthy plant growth (Berger *et al.,* 2013). These beneficiary microbes also maintain moisture through active secretion of harmones and enzymes (Dębska *et al.,* 2016).

Role of plant growth promoting rhizobacteria
Biotic and abiotic stress tolerance

The various external factors are responsible for the unproductive growth of plants which are referred as stress in plants. These stress disturb the genetic characteristic, metabolic activities, yield of the crops etc. and it can be categorized as biotic and abiotic stresses (Verma *et al.,* 2013). The living organism especially pathogenic bacteria, virus, fungi etc are responsible for causing biotic stress in plants. This affects the host cell of the plant and modifies the genetic code of the plant which leads to mortality of the plant (Suzuki *et al.,* 2014). Abiotic factors such as soil salinity, drought, extreme high temperature, deficient or excessive water supply leads to great reduction in agricultural productivity (Nadeem *et al.,* 2010). Abiotic and biotic stress caused by pathogenic mechanism, brings pre and post-harvest troubles in crops (Nejat *et al.,* 2017). These stresses are major barriers in sustainable agricultural productivity (Srividya and Sasirekha, 2017). Stresses that cause severe damages to the crop production can be determined by the efficacious process of PGPR. The enzyme called 1-aminocyclopropane-1- carboxylate (ACC) deaminase and Hydroxyacetophenone monooxygenase produced by PGPR and Pseudomonas fluorescens respectively breaks the ethylene precursor ACC to a-ketobutyrate and ammonia, that in turn, protects plants from various destructive effects of abiotic stresses (Kumara *et al.,* 2019). The bio-inoculants, developed by acdS gene coding for enzyme ACC-deaminase against the abiotic stresses (Shaik *et al.,*2013). The salinity and drought are the most devastating stresses that lower the agricultural productivity (Hasanuzzaman *et al.,* 2013). In addition, higher levels of ethylene in the plant stimulate prematurefatuity symptoms such as leaf yellowing, abscission, or desiccation/necrosis (Elisa and Glick, 2015). PGPR plays a major role in lessening the ethylene concentrations in plants, thus deduce the stresses.Pseudomonas putida and Enterobacter cloacae improvise the plant resistance to salt stress (Zhenyu *et al.,* 2012). Azospirillum brasilense, Pseudomonas chlororaphis (Egamberdieva, 2012), Streptomyces sp. strain (Palaniyandi *et al.,*2014), Chryseobacterium (Radzki *et al.,* 2013) on tomato plants, Pseudomonas putida in soyabean plants (Sang *et al.,* 2014) tends to reduce the ethylene level in plants so as to tolerate the salinity stresses. Bacillus megaterium founds to be highest phosphate solublization under salinity stresses in the plants (Chookietwattana and Maneewan, 2012). Enterobacter sp in okra plant (Habib *et al.,* 2016), Phyllobacterium in strawberries plant (Flores *et al.,* 2015), Putida, Gigaspora rosea (Gamalero *et al.,* 2010), Promicromonospora sp and Burkholderia cepacia in cucumber plants (Sang *et al.,*2014), Bacillus licheniformis, Brevibacterium iodinum,Zhihengliuela alba in red pepper plants (Siddikee *et al.,* 2011), Bacillus in alfalfa plants (Sokolova *et al.,* 2011), Pseudomonas sp in eggplant (Fu *et al.,* 2010) were some of the PGPRs actively diminish the salinity stresses. The functionality of the plants gets affected severely by turgor pressure and water potential during drought conditions. This leads to drought stress, causing severe damages to agricultural productivity (Rahdari and Hoseini, 2012) and flow of nutrients such as sulfates, nitrates, Calcium, Silica, Magnesium (Selvakumar *et al.,* 2012) and photosynthesis process (Anjum *et al.,* 2011; Rahdari *et al.,* 2012)founds to be reducing. The bacterial colonies of rhizosphere and endorhizosphere induce the plant to overcome the drought stress in order to attain sustainable agricultural productivity (Grover *et al.,* 2011).The plant biomass enhanced through the inoculation of Pseudomonas putida under the drought stress condition (Sandhya *et al.,* 2010). It is found that lavandula dentata with bacillus thuringiensis induces the growth of plant under drought stress by increasing the concentration of proline in the shoot (Armada *et al.,* 2014). Bacillus polymyxa in tomato plant (Shintu and Jayaram, 2015), Pseudomonas jessenii, Pseudomonas synxantha, and Arthrobacter nitroguajacolicus strain in rice plants (Gusain *et al.,* 2015),lipoferum and Pseudomonas putida in maize plants (Sandhya *et al.,* 2010; Bano *et al.,* 2013), Bacillus isolates and Pseudomonas with mesorhizobium ciceri in green gram plants (Isha *et al.,* 2013), rhizobacterial strain bacillus subtilis in mustard plant (Zhang *et al.,* 2010) were the PGPRs that induce the growth of plants under drought stress. In case of biotic stresses the Bacillus subtilis found a better resistance to the cotton pathogen rhizoctonia solani (Flavio *et al.,* 2011). Therefore, PGPR actively overcome all the biotic and abiotic stresses and induce the plants growth for sustainable crop productivity as shown in the Fig. 1.

Soil Nutrients accessibility for the plant growth

The overall concentration of soil nutrients in the rhizosphere get increased with the help of PGPR. It helps in fixing the atmospheric nitrogen by preventing the leaching of soil nutrients (Choudhary *et al.,* 2011). The species that comes under azotobacter genus such asarmeniacus, vinelandii, chroococcum, paspali, beijerinckii, nigricans and salinestri has strong potential to fix nitrogen nutrients in the soil (Gothandapani *et al.,* 2017). Azospirillum belonging to the spirillaceae family has strong association with the roots of C4 crops and plays a significant role in fixing 20-40kg of nitrogen under aerobic conditions (Trabelsi and Mhamdi, 2013). For legumnus plants rhizobium finds greater potential in fixing the atmospheric nitrogen at major concern (Jehangir *et al.,* 2017). Cyanobacteria (Blue Green Alga, BGA) such as Nostoc, Anabaena, Cylindrospermum, Gloetrichia Tolypothrix, Aulosira and Aphanotheca tremendously increases the rice crop productivity up to 38%, by fixing the nitrogen nutrients(Mishra *et al.,* 2013). Azolla proves to be vital nitrogen source for sustainable agricultural productivity and it has the potential of fixing about 50kg of nitrogen (Yao *et al.,* 2018). Gluconacetobacter diazotrophicus which colonize enormously in monocotyledon sugarcane plants actively fix the atmospheric nitrogen so as to provide sufficient amount of nitrogen nutrients for the crop growth (Santhosh *et al.,* 2018). Apart from these strains Bacillus aerius, Bacillus amyloliquefaciens, Bacillus licheniformis, Bacillus mucilaginous, Bacillus subtilis helps in nitrogen fixation in soil (Singh *et al.,* 2019; Pahari and Mishra, 2017).Addition to nitrogen fixation, phosphorous solublization is also biologically important. PGPR has strong role in solubilizing phosphorous for the consistent plant growth (Rifat *et al.,* 2010). Pseudomonaserwinia and P. chlororaphiswere has strong tendency to solubilizing the phosphorous and promote the plant growth through proper uptake of phosphorous nutrients (Diriba *et al.,* 2013). From the biochemical characteristics of bacterial isolates of Pseudomonas putida and Bacillus sp., it has been proven that these colonies have viability to solubilizing the phosphate for consistent supply of nutrients to the

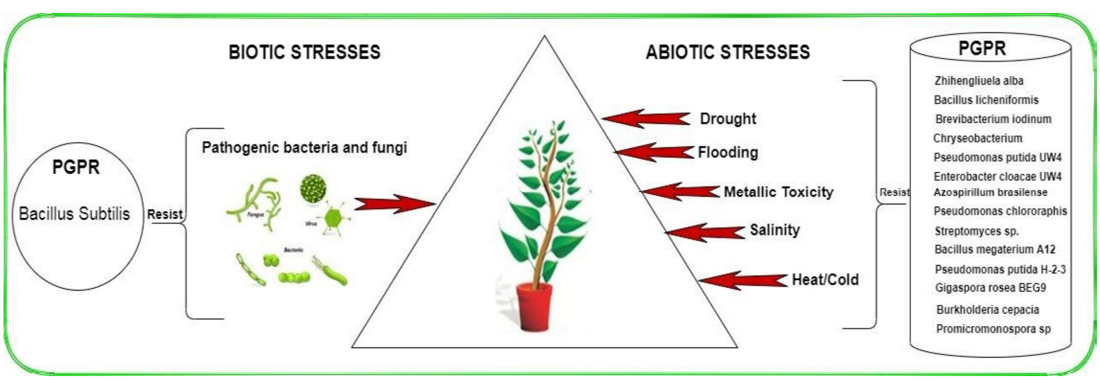

Fig. 1: PGPR in resisting biotic and abiotic stresses

plants (Grobelak *et al.,* 2015). PGPR bacterial strains such as Achromobacter xylosoxidans, Acinetobacter baumannii, Acinetobacter calcoaceticus, Aeromonas hydrophila, Arthroderma cuniculi, Aspergillus niger, Bacillus aerius, Bacillus altitudinis, Bacillus amyloliquefaciens, Bacillus licheniformis, Bacillus megaterium, Bacillus mucilaginous, Bacillus subtilis, Bacillus thuringenesis, Burkholderia cepacia, Burkholderia gladioli, Enterococcus casseliflavus, Enterococcus gallinarum, Fusarium proliferatum, Lecanicillium psalliotae, Paenibacillus favisporus, Paenibacillus taichungensis, Pseudomonas entomophila, Pseudomonas koreensis, Pseudomonas luteola, Pseudomonas simiae, Serratia marcescens, Serratia nematodiphila, Sphingomonas paucimobilis actively involves in solubilizing the phosphorous and tends to enhance the plant growth(Leite *etal.,*2018; Zhang *etal.,*2017; Martinez *et al.,* 2018; Karmakar *et al.,*2018; Gore and Navale, 2017; Kumaravel *et al.,* 2018; Vardharajula *et al.,*2011; Mussa *et al.,*2018; Kumari *et al.,* 2016; Sandhya *et al.,* 2010; Saikia *et al.,* 2018). Solublization of soil potassium is biologically an important factor and has equal importance, compared to the nitrogen and phosphorous in contributing sustainable crop production (Tri and Mutmainnah, 2016). Inorganic and organic acids, acidolysis, polysaccharides, complexolysis, chelation, polysaccharides, and exchange reactions produced by the bacteria aid to solubilizing the soil potassium (Archana *et al.,* 2013; Meena *et al.,* 2015). Bacillus licheniformis and Pseudomonas azotoformans in rice crops finds the best K solubilizing bacteria than others (Saha *et al.,* 2016). Potassium solubilizing capacity is found triggered by Enterobacter hormoecheiin cucumber crop fields (Prajapati and Modi, 2016).The species such as Burkholderia,Pseudomonas,Bacillus mucilaginosus, Bacillus edaphicus, Bacillus circulans, Acidithiobacillus ferrooxidans, and Paenibacillus spp. when inoculated with frateuria aurantiastrain shows about 39% increase in concentration of potassium in the crops (Subhashini, 2015), the aforementioned information are depicted in the Table 1.

Plant growth regulator

Plant producing organic substances in the name of harmones (also called as phytohormones) such as auxins, gibberellins (GA), abscisic acid (ABA), cytokinins (CK), salicylic acid (SA), ethylene (ET), jasmonates (JA), brassinosteroids (BR), and peptides were the key factors in developing the immune system against the pathogenic microbes and brings out tremendous agricultural outcomes (Dong *et al.,* 2013). These hormones act as the plant growth regulator for boosting up the crop productivity. Rhizobacterial traits make a major impact on the status of plant hormones by inducing the hormone secretion activities and increase the concentration of the hormones (Dodd *et al.,* 2010).Bacillus spp. producing auxin is proven as the deciding molecules which regulates the primary plant growth process (Ahmed and Hasnain, 2010).The most eminent auxin in the plant is indole-3-acetic acid. Lower the amount of IAA causes stimulation of elongation in primary roots, whereas higher concentration of IAA stimulates lateral root formation and root hair production. PGPR plays a vital role in synthesis of IAA which increases the both elongation of primary roots and lateral root formation (Vacheron *et al.,* 2013). Next to auxin, gibberellin is considered as most viable hormone responsible for seed germination, floral induction, flower, fruit, leaf and steam maturation. Sphingomonas produces gibberellins (GA) when inoculate with tomato plant, induces all the characteristics discussed above (Khan *et al.,* 2014). The plant organ size and stomata closure functionality is purely regulated by the plant hormone, namely abscisic acid (ABA) which is abundantly synthesized by the strains of Bacillus amyloliquefaciens (Raheem *et al.,* 2017). Bacillus subtilis strains synthesize the plant hormone called cytokinins which are responsible for plant cell division, inhibiting roots, stems, vascular differentiation and cambium sensitivity(Liu *et al.,* 2013).Reduction in antioxidant enzymes activity carried out by the salicylic acid (SA) were regulated majorly by the PGPR strains such as Mycobacterium spp., Pseudomonas spp., Azospirillum, lipoferum and Pseudomonas cepacia (Khan *et al.,*2020).Abscission of leaves and ripening of fruits were primarily regulated by ethylene plant hormones. 1-aminocyclopropane-1-carboxylate (ACC) is a plant, synthesized predecessor of ethylene hormone, which helps in exposing to environmental stress, pathogenic microbes and heavy metal presence (Glick *et al.,* 2012). Hence, PGPR plays a viable activity in decaying cycle of roots through degrading the ACC thus achieving the healthy root system(Glick *et al.,*2014). Jasmonates (JA) takes part in responding to the wounds in plant tissues and redirects the metabolism to repair the damages, which are mediated by PGPR. Bacillus subtilis in wheat

Table 1: Plant nutrients and its corresponding PGPR bacterial strains

Nutrients	PGPR	References
Nitrogen (N)	A. chroococcum	Gothandapani *et al.*,2017
	A. vinelandii	Gothandapani *et al.*, 2017
	A. beijerinckii	Gothandapani *et al.*, 2017
	A. paspali	Gothandapani *et al.*, 2017
	A. armeniacus	Gothandapani *et al.*, 2017
	A. nigricans	Gothandapani *et al.*, 2017
	A. salinestri	Gothandapani *et al.*, 2017
	Azospirillum	Trabelsi and Mhamdi, 2013
	Cyanobacteria	Mishra *et al.*, 2013
	Azolla	Yao *et al.*, 2018
	Gluconacetobacter diazotrophicus	Santhosh *et al.*, 2018
	Bacillus aerius	Singh *et al.*, 2019
	Bacillus amyloliquefaciens	Singh *et al.*, 2019
	Bacillus mucilaginous	Pahari and Mishra, 2017
	Bacillus subtilis	Pahari and Mishra, 2017
Phosphorous (P)	Pseudomonaserwinia	Diriba *et al.*, 2013
	P. chlororaphiswere	Diriba *et al.*, 2013
	P. putida	Grobelak *et al.*, 2015
	Bacillus sp	Grobelak *et al.*, 2015
	Achromobacter xylosoxidans	Leite *et al.*,2018
	Acinetobacter baumannii	Zhang *et al.*,2017
	Aeromonas hydrophila	Martinez *et al.*, 2018
	Arthroderma cuniculi	Karmakar *et al.*,2018
	Aspergillus niger	Gore and Navale, 2017
	Bacillus aerius	Singh *et al.*, 2019
	Bacillus altitudinis	Kumaravel *et al.*, 2018
	Bacillus thuringenesis	Vardharajula *et al.*, 2011
	Enterococcus casseliflavus	Mussa *et al.*, 2018
	Enterococcus gallinarum	Mussa *et al.*, 2018
	Lecanicillium psalliotae	Bilal *et al.*, 2018
	Paenibacillus taichungensis	Zhang *et al.*,2017
	Pseudomonas entomophila	Sandhya *et al., 2010*
	Pseudomonas koreensis	Kumari *et al.*, 2016
	Pseudomonas luteola	Martinez *et al.*, 2018
	Pseudomonas simiae	Kumari *et al.*, 2016
	Pseudomonas stutzeri	Sandhya *et al.*,2010
	Serratia nematodiphila	Saikia *et al.*, 2018
	Sphingomonas paucimobilis	Martinez *et al.*, 2018
Potassium (K)	Bacillus licheniformis	Saha *et al.*, 2016
	Pseudomonas azotoformans	Saha *et al.*, 2016
	Burkholderia	Subhashini, 2015
	Bacillus mucilaginosus	Subhashini, 2015
	B. edaphicus	Subhashini, 2015
	B. circulans	Subhashini, 2015
	Pseudomonas	Subhashini, 2015
	Acidithiobacillus ferrooxidans	Subhashini, 2015
	Paenibacillus sp	Subhashini, 2015
	Enterobacter hormoecheiin	Prajapati and Modi, 2016

seedling finds great response of synthesis of jasmonates harmones (Veselova *et al.*, 2014).PGPR has viable part in synthesizing harmones namely brassinosteroids (BR), and peptides which are responsible for various stress tolerance amelioration (Sharma *et al.*, 2017). Apart from aforementioned hormones PGPR Oozes out small, high-affinity iron-chelating compounds called Siderophores which is responsible for enhancing the plant growth through iron intensification (Flores *et al.,* 2015). Bacillus subtilis, Bacillus megaterium, Azotobacter vinelandii, Pantoeaallii and Rhizobium radiobacter were some of the PGPR strains that have strong ability to chelate iron in the form of Siderophores compounds (Ferreira *et al.,* 2019). In addition to iron magnification, PGPR produce volatile organic compounds (VOC) in order to manage plant pathogens, disease resistivity abatement and stunted plant growth (Hafiz *et al.,* 2017). Bacterial species such as Bacillus, Pseudomonas, Serratia, Arthrobacter, and Stenotrophomonas enhance the crop productivity through synthesizing the VOC (Yong *et al.,* 2015). Thus the various hormones and compounds synthesized by PGPR regulate the mechanism of plant growth and crop production as shown in the Fig. 2. Hence, PGPR is referred as plant growth regulator (Porcel et *al.,* 2014).

Need for inhibiting PGPR as biofertilizers

Inspite of various beneficial characteristics of PGPR, it has natural inducing quality without any external agents (Klett *et al.,* 2011). However, it is a need of utilizing these PGPR as biofertilizers (Salme *et al.,* 2017) to overcome tangible and intangible soil infertility problems due to excessive utilization of chemical fertilizers.

Increased utilization of chemical fertilizers

In late 1970s the utilization of chemical fertilizers has been drastically increased, since the labor cost increased day by day, the practice of blending biofertlizers with soil get decreased (Yan *et al.,* 2010). Over the past few decades huge quantity of chemical fertilizers has been applied in the cultivable ground (Sun *et al.,* 2015; Savci, 2012). This leads to serious issues such as degradation and compaction of soil, lowering of soil organic matter and soil carbon. Over the years, due to the continuous usage of chemical fertilizers leads to decline crop productivity (Sun *et al.,* 2015; Nkoa, 2014). In the modern agriculture systems, a chemical fertilizer raises the acidity nature of soil, which forces the nitrogen cycle in the soil to being complete (Guo *et al.,* 2010). It shows the path to heavy metals mobility in the soil which could be taken up by the plants (Yang *et al.,* 2010). Although some of the microbes have strong tendency to absorb the heavy metals, the entire structure and biomass of microbes get affected (Carpio *et al.,* 2014). Soil contaminants bioaccumulation take place which results in overall deflation in crop productivity due to uncontrolled utilization of phosphate and superphosphate fertilizers (Carvalho, 2017). Accumulation of chemical fertilizer such as dithiothreitol (DDT), endosulfan, heptachlor, lindane in the soil leads to perishing soil organic content (Jayaraj *et al.,* 2016). Moreover, the chemical fertilizers block the process of photosynthesis which leads to stunted growth in plants (Pesce *et al.,*

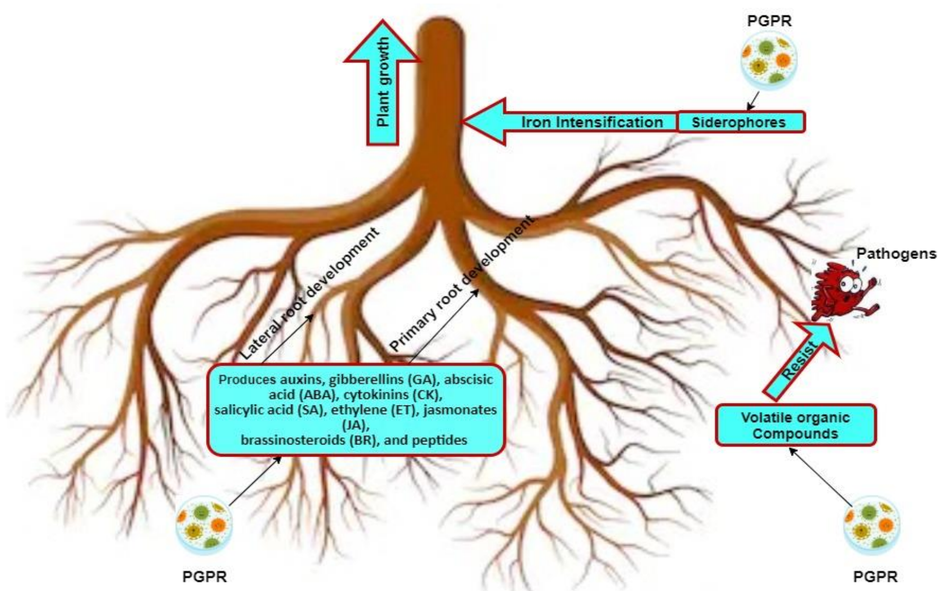

Fig. 2: Mechanism of PGPR in plant growth regulating

2011). Various evidential reports clears that chemical fertilizers volatilize and blend with the atmospheric air causes extreme atmospheric pollution (Chandrima *et al.,* 2020). The agricultural activities based on chemical fertilizers brings huge volume of crop productivity at short duration, however for the long term, it may not be suited for sustainable agricultural activities. It also have negative impacts on flora and fauna (Pimentel and Burgess, 2014; Goulson, 2014). To overcome these sort of problems and to achieve sustainable crop productivity, without any side effects, utilization the rhizosphere bacterial communities PGPR as a biofertilizers is essential (Wu *et* al.,2016).

Formulation of PGPR biofertilizers

The biofertilizers that comprises of live microbes needed to be formulated for its existence. Before formulation there is need to identify the beneficiary microbes which have to be cultivated and formulated for the further process. In identification of microbes, genotype and phenotype distinction were usually carried out. In genotype distinction of microbes rRNA functionalities were identified whereas in phenotype distinction microbial colonies, cell morphology, gram staining, metabolic and growth characteristics were identified in order to confirm the specific beneficiary bacteria. Confirmatory test is done usually for the precise identification of microbes (Lehner, 2013). After identifying the beneficiary bacterial culture it is isolated and cultured followed by formulation of biofertilizers (Rassem *et al.,* 2017). Formulation of biofertilizers includes a carrier that supports live microorganism existence, long term storage, and maintenance. It aids to supply the active live microbes to blend with the soil or plant so as to undergo aforementioned PGPR activities to improvise the crop yield and soil fertility (Sahu and Brahmaprakash, 2016).These formulations were prepared to strengthen both the crop yield and soil health (Arora *et al.,* 2010). Metabolically viable, adjustments in pH, non-toxic in nature and biodegradable are some of the basic characteristics for good formulation of bio fertilizers (Divjot *et al.,* 2020). Among the different types of commercial bio fertilizers, the liquid formulation found to be more convenient in handling compare to conventional solid base carrier inoculants(Herrmann and Lesueur, 2013; Brar *et al.,* 2012). A formulated bio fertilizer is demanding growth media for any selected bacteria. Although the cost of growth media for the microbes is high, still there are some natural media such as whey, water sludges, composts and etc., are can be used as source for the growth of microbes (Samer *et al.,* 2012). Rock phosphate along with agro based industrial residuals and bioflims can also be used as source for growth media (Allah *et al.,* 2017; Gamini *et al.,* 2010). Proper inoculation in formulation of bio fertilizers has equal importance for the plant growth (Indra *et al.,* 2014). Crop growth rate pattern increases through simultaneous inoculation with different PGPR rather than single inoculation mechanism (Martinez *et al.,* 2010). As per the literature, nitrogen and phosphorous content increases tremendously when A. brasilense is inoculated with maize(Shrivastava, 2015) and nodule-inducing rhizobia with AM fungi (Xiurong *et al.,* 2010). Mycorrhizal fungi were co-inoculated with PGPR to gain increased root colonization (Josef *et al.,* 2010). Thus co-inoculation resultant in formulation of biofertilizers is consistent for different PGPR microorganism consortium (Malusa *et al.,* 2012). Hence, effective commercial usage of biofertilizers, genuine inoculation of PGPR consortium under different species with different field condition is inevitable (Cristian *et al.,* 2017). The inoculants consist of carriers which helps the consortium PGPR microbes to be delivered at satisfactory physiological state (Jambhulkar *et al.,* 2016). Good moisture, absorptions, easy processing, sterilizing, pH buffering capacity, low cost and its availability are some of the most essential properties of carriers (Rawat *et al.,*2020).The physical form of biofertilizers is solely depends on the carriers used, such as peats, coal, clays, compost, soybean meal, wheat bran, saw dust, vermiculture, perlite and etc., (Herrmann and Lesueur, 2013). Solid type carriers are in different forms such as powders, granules and beads (John *et al.,* 2011). Alternative to carriers, freeze drying mechanism which is commonly known as lyophilization can be used where the bacterial survival rate is high (Fernanda *et al.,* 2014). In order to avoid dehydration, cryprotectant is added during the process. Henceforth, combination of growth media, inoculation and good carriers are helping to formulate strong PGPR biofertlizers. The above mentioned formulation processes of biofertlizers has been portrayed in Fig. 3.

Role of PGPR Bio fertilizers in plant growth

More than 90% of plant growth is purely depends on photosynthesis, since the plant biomass is derived from carbon dioxide assimilation. A photosynthetic process rate increases when rice gets inoculated with various rhizobia strains (Mia and Shamsuddin, 2010). Bacillus lentus, Pseudomonas sp., and Azospirillum brasilens increases the antioxidant and photosynthetic pigment that leads to rise in chlorophyll content in the plant (Heidari and Golpayegani, 2012).Bacillus sp., when inoculated with potato gives positive growth in photosynthetic performance (Gururani *et al.,* 2013). Thus PGPR biofertilizers induces photosynthesis mechanism for sustained growth of the plant even under various stress conditions. Amino acid plays an important role in plant growth by supporting the roots to intake water and nutrients from the soil (Berg *et al.,* 2014; Hildebrandt *et al.,* 2015).The active synthesis of amino acids is greatly relay on plant species and their associated microbes(Kang *et al.,* 2010). Thus PGPR biofertilizers increases the synthesis of amino acids for active performance of root system that nourishes the plant growth. Though there are certain factors that are responsible for sustainable plant growth, still few agents that causes the adverse hindrance to the plant growth. It can be perished with the help of PGPR biofertilizers. The major barrier to the plant growth is contamination in soil. The various factors contributing to the contamination in soil are accumulation of heavy metals in the soil, dumping of plastics, usage of chemical fertilizers etc. Heavy metal presence is mainly due to the rapid industrialization and population growth (Shinwari *et al.,* 2015).Heavy metals are non-biodegradable in nature and biodegradation is the only effective strategy to minimize the effects of heavy metals in the soil biosphere (Akhtar *et al.,* 2013; Lim *et al.,* 2014). In this connection, PGPR bacterial strains such as Azotobacter, Bacillus, Brevibacillus, Kluyvera, Mesorhizobium, Pseudomonas, Achromobacter, Psycrobacter, Bradyrhizobium, Rhizobium, Sinorhizobium, Ochrobactrum, Ralstonia, Variovox, and Xanthomonas were widely used for the purpose of biodegradation of heavy metals (Shinwari *et al.,* 2015). PGPR strains such as Azospirillum sp., Bacillus sp., Acinetobacter sp., Achromobacter sp., Cronobacter sakazakii, Agrobacterium sp., Alcaligenes sp., Mesorhizobium sp., Burkholderia sp., Klebsiella sp., Enterobacter, sp., Halomonas sp., Ralstonia sp., Methylobacterium fujisawaense, Pseudomonas sp., Rhizobium sp., Serratia sp., Variovorax paradoxus and Zhihengliuela alba(Chen *et al.,* 2013; Gontia *et al.,* 2017; Jha *et al.,* 2012; Siddikee *et al.,* 2011) were capable to synthesis ACC deaminase, that reduces the concentration of ethylene in plants which overcome the heavy metal stress. Thus, with the combination of these bacterial strains biofertlizers can be formulated that tends to degrade the heavy metal and reduce the contamination of the soil so as to enhance the plant growth. As it is discussed, the modern agriculture includes the practice of utilizing chemical pesticides for increasing the crop productivity which leads to adverse soil contamination and sustainability in agricultural practice (Kumar and Puri, 2012). Hence, there is a need of eco-friendly pesticides as alternatives to existing practice. The bacterial strains such as Azospirillum, Azotobacter, Bacillus, Enterobacter, Gordonia, Klebsiella, Paenibacillus, Pseudomonas and Serratia possess greater tendency to fight and degrades the harmful effects of pest, thus leading to reduce the soil contamination and provide pathway for sustained plant growth (Shaheen and Sundari, 2013). Microbial activity induce the plant growth through degradation of pesticides by synthesizing enzymes such as esterases, hydrolases and glutathione (Hernandez

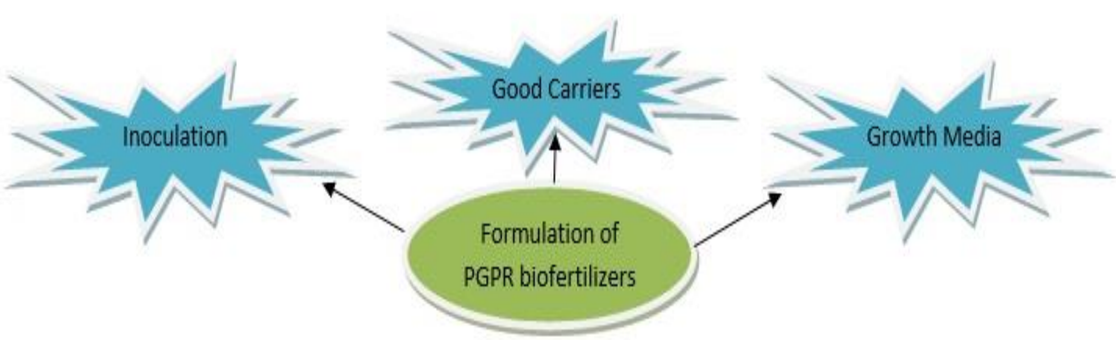

Fig. 3: Formulations of PGPR Bio-fertilizers

et al., 2013). Hence PGPR biofertilizers actively eradicate the harmful nature of pesticides that causes soil contamination so as to boost the plant growth. Moreover PGPR act as biocontrol for plant pathogens in which metabolites productions such as antibiotics and hydrogen cyanide were the primary biocontrol mechanism (Reddy, 2014). This mechanism involves wide variety of compounds having many antimicrobial activities which act as defence layer to pathogenic microbes. Phytopathogenic proliferation can be minimized or eradicated by antagonist mechanism. Antagonist mechanism includes production of siderophores, bacteriocins and antibiotics (Beneduzi *et al.,* 2012).In case of plastic waste, the bacteriological harmones such as IAA, GA and kinetins help to resist the effects causes by the plastic waste. Thus PGPR biofertilizers effectively resist the effects of plastic and reduces the soil contamination (Ikhwan and Nurcholis, 2020). It is reported that lack of micronutrients is basic problem of plant growth, specifically zinc (Zn) deficiency create a major barriers to the plant growth, especially to the cereals crops (Ashish *et al.,* 2012). PGPR bacterial strains increase the zinc and iron nutrients in the soil which is mandatory for the sustainable plant growth (Yadav *et al.,* 2017). Besides, bacterial colonies which includes Pseudomonas alcaligenes, Pseudomonas aurantiaca, Pseudomonas aureofaciens and Pseudomonas chlororaphis, Bacillus subtilis, Bacillus pumilus, Achromobacter xylosoxidans, Serratia marcescens, Pseudomonas extremorientalis, P. fluorescens, Serratia plymuthica, and Stenotrophomonas rhizophila, Phyllobacterium brassicacearum fight against biotic and abiotic stresses that are major barriers to the plant growth (Verma *et al.,* 2017; Yadav *et al.,* 2018; Liu *et al.,* 2013; Forchetti *et al.,* 2010; Lavania and Nautiyal, 2013; Egamberdieva, 2011;Timmusk *et al.,* 2014; Bresson *et al.,* 2014). In overview, it is revealed that PGPR bacterial inoculated biofertlizers has multi-disciplinary role in enhancing the sustainable plant growth. The roles of various PGPR strains utilized in biofertlizers are tabulated as shown in Table 2.

Commercialization of PGPR bio fertilizers

Around 24 countries were commercially engaged in producing PGPR biofertilizers both in large and small scales (Bharti *et al.,* 2017). Phosphorous solubilizing bacteria and atmospheric fixing nitrogen bacteria have been used for commercialization (Lesueur *et al.,* 2016). Non-rhizobial PGPR inoculants containing azospirillum were most frequently used commercial biofertilizers in global market (Herrmann *et al.,* 2013). The Non-rhizobial PGPR biofertilizers reached only 5% of global market and remaining were occupied by the chemical fertilizers because of its expensiveness (Wellesley, 2014). Later in developed countries legume and nitrogen fixing inoculants were dominated (GVR, 2020). In global biofertilizers market about 78% were occupied by rhizobial inoculants, whereas 15% and 7% has been occupied by Phosphorous solubilizers and other bioinoculants respectively (Owen *et al.,* 2015). Zinc and potassium based biofertilizers were the emerging commercial products that address the soil nutrient deficiencies (Shaikh *et al.,* 2017; Khatibi, 2011). Among this, potassium based biofertlizers has been increasing tremendously in most of the countries (Teotia *et al.,* 2016).In this regard, India stands in fourth whereas nations like USA, China and Brazil stands first in producing potassium solubilizing biofertilizers (Investing News Network, 2019). In PGPR biofertilizers commercialization, Asia-pacific nations started attaining maximum growth from 2014 and the global biofertlizers market expanded to increase the sustainable food productivity (Verma *et al.,* 2019).This shows the progress of potential nature of PGPR in the commercialization aspects.

Limitations and future trends in PGPR utilization in agriculture

In recent days the utilization of bio fertilizers became an integral part of sustainable agricultural practices and major developed countries achieved the sustainability (Weekley *et al.,* 2012). In developing countries, there is a minimum impact of PGPR bio fertilizers, due to the poor quality in inoculants and stringent regulatory legal frameworks (Berninger et *al.,* 2018). In addition, the bio fertilizer takes time to bring out the productivity in agriculture which makes the investors and scientist difficult to bring the PGPR inoculants to general farmers (Mahanty *et al.,* 2017). Large scale commercialization of PGPR inoculants requires large volume trials in understanding the bacteriological characteristics and their activities which is not an economically feasible for the farmers (Qiu *et al.,* 2019). In this connection, research in biofertilizers should be focused on cost effective, faster benefits, sustainable higher productivity under various environmental conditions (Ijaz *et al.,* 2019). Besides,

Table 2: PGPR bacterial strains utilized in bio-fertilizers and its role in plant growth

Role of PGPR Bio fertilizers in inducing:	PGPR	References
photosynthesis	Pseudomonas sp Bacillus lentus Azospirillum brasilens	Heidari and Golpayegani, 2012
	Bacillus sp	Gururani *et al*, 2013
Amino acid	B. subtilis P. putida Rhizobium sp	Luke *et al*, 2013
Biodegradation of heavy metal	Achromobacter Azotobacter Bacillus Bradyrhizobium Brevibacillus Kluyvera Mesorhizobium Ochrobactrum Pseudomonas Psycrobacter Ralstonia Rhizobium Sinorhizobium Variovox Xanthomonas	Shinwari *et al.,* 2015
Controlling pesticides	Azospirillum Azotobacter Bacillus Enterobacter Gordonia Klebsiella Paenibacillus Pseudomonas Serratia	Shaheen and Sundari, 2013

these researches promote the usage of bio inoculants and develop the confidence among the local farmers, based on their utilization and performance (Gupta *et al.,* 2015). Future world looking to bring novel biofertilizers which are very affordable, safe and best substitute to agrochemical fertilizers by the usage of consortium multi-tarit PGPR strains. It enhances the communication with the plant through quorum sensing (Ijaz *et al.,* 2019; Vassilev *et al.,* 2015; Khan *et al.,* 2017). Utilization of bioflims protects the bio inoculants from varying environments. It is expected to evolving in future that leads to increase in microbial population (Sahai *et al.,* 2017; El-Ghamry *et al.,* 2018). According to the literatures, future strategies could focus on biofertilizers which involves interactions of microbes with nano particle. It is mainly to improvise the micronutrients to bacteria and plants. It became the revolution in future agricultural practices by introducing nanofertilizers (Tarafdar *et al.,* 2013). The efficiency of nano fertilizers has been proven as best alternatives to all traditional ones because of its characteristics of reducing nitrogen loss, leeching, toxic effects, long term sustainability of microbes in the soil (Suman et al., 2010). Thus Nanoencapsulation technology will be a versatile tool to protect PGPR from various environmental changes and to extract its complete benefits so as to make sustainable crop productivity with maintained soil fertility.

A summary of present and forthcoming ideas

The present studies on PGPR in sustainable agriculture focus on formulation of biofertilizers. Moreover the PGPR bacterial strains are isolated and cultured for the formulation purpose. Followed with the formulation, the biofertilizers were commercialized so as to use in agricultural field. PGPR due to its unique characteristic such as nutrients intake, stress tolerance, wide hormonal secretion etc. finds wide range of scope in inducing the plant growth. Forthcoming challenges in agriculture can be faced with the help of genetically modified PGPR bacterial strains. Genetically modified PGPR bacterial strains can be produced without disturbing its beneficiary nature and tends to increase the efficiency. In terms of biofertilizers efficiency can be increased through these modified PGPR bacterial strains. Thus sustainable agricultural practice will tends to grow through continuous research in genome sequence analysis and characteristic of PGPR bacterial strains.

CONCLUSION

In the late 1960s, green revolution tremendously increases the crop productivity by triggering the utilization of chemical fertilizers along with other advances in agricultural practices. Thus more than billions of world population were protected from starvation and ensured the food security. But, due to the excessive population and civilization, the need of individuals were increased which leads to over utilization of chemical fertilizers in order to achieve the rapid crop productivity. Besides, the mono-cultivation strategy was widely followed because of easy handling and management resulting in complete dependency on chemical fertilizers. It leads to complete disturbance in soil ecosystem and challenge to attain sustainable crop production. Hence, it is mandatory to switch over from inorganic to organic agricultural practices for the welfare of future agricultural productivity. Organic agricultural practices needs frequent analysis of soil report for every cropping season which helps them to choosing proper organic manure and suitable crops. Although the manual soil testing gives effective information about the major soil parameters N, P and K, it is essential to identify the major minerals which supports for sustainable productivity. Though, there is a least chance to utilize the traditional agricultural practices in this modern era, the good natures of PGPR can be imported in agricultural practices. As it is discussed in this extensive literature review, PGPR plays major role in handling biotic and abiotic stress by utilizing the aforementioned bacterial strains. PGPR bacterial strains induce the plants for its effective uptake of soil macro, secondary and micro nutrients. PGPR bacterial secretion of different plants hormones such as auxins, gibberellins, abscisic acid, cytokinins, salicylic acid, ethylene, jasmonates, brassinosteroids, and peptides helps in nourishing plant growth. Adapting the PGPR in fields, makes viable impact in crop productions due to its unique features such as protecting against different environmental stresses, regulating the plant growth, influencing the crop productivity and soil ecosystem so on. In addition, PGPR utilization comes in reality and finds best alternative to various strategies in agricultural sustainability. Biofertilizers were formulated as liquid as well as solid fertilizers through proper usage of PGPR inoculation, carriers and growth medium. This formulated biofertlizers were commercialized and makes perfect alternatives to the chemical fertilizers in all means. Though the usage of these biofertlizers takes more time to show the productivity, the sustainability is rich enough. But the scientists and researchers target to bring back the organic farming and resolve the time consumption problems through major innovations such as consortium multi-trait PGPR strains, bioflims, Nanoencapsulation technology. It leads to practice multiple cropping in agricultural fields. In this modern agricultural era many farmers and policy makers aims to bring sustainable profit in agriculture through multi cropping. This makes to have more focus on PGPR biofertlizers and advanced nanotechnology intrusion in agriculture. Thus the plant growth promoting Rhizobacterial products and its corresponding technologies will be a boon to upcoming world agriculturist in ensuring the sustainable crop productivity and soil ecosystem.

AUTHOR CONTRIBUTIONS

V. Dhayalan performed the literature review, experimental design, analyzed and interpreted the data; K. Sudalaimuthu prepared the manuscript text, and manuscript edition.

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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

ABA	Abscisic Acid
ACC	1-aminocyclopropane-1-carboxylate
acdS	ACC deaminase structural gene
a-ketobutyrate	alpha-ketobutyrate
AM fungi	Arbuscular Mycorrhiza fungi
BGA	Blue Green Alga
BR	Brassinosteroids
CK	Cytokinins
DDT	Dithiothreitol
ET	Ethylene
etc	Et cetera
Fig.	Figure
GA	Gibberellins
IAA	Indole-3-acetic acid
JA	Jasmonates
K	Potassium
Kg	Kilogram
N	Nitrogen
PGPR	Plant growth promoting rhizobacters
P	Phosphorous
pH	Potential of Hydrogen
SA	Salicylic acid
Sp.	Single bacterial type
Spp.	Number of bacteria with different names belong to one genus
USA	United States of America

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REVIEW PAPER

Remediation technologies for oil contaminated soil

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ABSTRACT

Crude oil continues to impact many nations as it is among the major sources of fuel. Its role in making life in modern societies comfortable cannot be overemphasized as it is readily available and easy to use. Contamination resulting from its use in industries such as mining, transportation and petroleum especially soil contamination cannot be overlooked. Soil pollution resulting from oil contamination can be seen as being among the twenty-first-century vulnerabilities because if not well taken care of the consequences can be devastating. Soil contamination is of interest in most societies because it affects both the environment and humans. This review highlights common sources of soil pollution and their effects, oil waste disposal methods, soil remediation techniques that are well established and those still in their infancy. Such techniques include bioremediation such as phytoremediation and landfarming, where percent removal of contaminated soils was reported from 68% to 89 % in 40 days to 1 year, respectively; physical methods such as excavation and incineration (75-86% removal); chemical methods such as oxidation (48 % by Fenton process); and photocatalysis (67% using titanium dioxide). The choice of remediation in mining, transportation and petroleum industries depends on the urgency and hazardous effects of the pollutant. In Zambia, Mopani Copper Mines uses landfarming as a means to mitigate large amounts of soil contaminated with oil wastes, but the process is slow. In the proposed research, photocatalysis coupled with adsorption of oil on clay will be used to assess the effectiveness of this emerging technology to quicken the degradation of oil in soils. Clay will be incorporated with metal ions and with hydrophobic groups to enhance light absorption and oil-clay interaction, respectively. Photochemical remediation techniques for remediation of soils polluted with oil have attracted considerable interest as the processes are reported to enhance the degradation of oils in soil compared to the biological and physical methods. The extent of photo-degradation of oil waste will be evaluated using the Soxhlet technique by determining the percent residual oil. The importance of remediating contaminated soil in any nation cannot be overemphasized as consequences of not remediating this precious resource might be devastating. Since economic development through industrialization will continue, there is need to constantly improve on methods of mitigating the impact of wastes on the environment, especially in developing countries, where engineering of cheap, nontoxic materials for soil remediation is paramount.

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INTRODUCTION

In recent years, worldwide, there has been an adverse impact on the environment due to the increased dependence on anthropogenic activities for economic development. One of the components of the environment that is severely affected by various industrial activities is the soil. Soil pollution is the build-up in the soil of toxic compounds, chemicals, salts or disease-causing agents that adversely affects plant growth and animal health (Ashraf *et al.*, 2014; Karbassi et al., 2015; Yuvaraj and Mahendran, 2020). Soil contamination is of particular interest in most societies since it does not only affect the environment but humans inclusive. When soil is contaminated, in most cases it stops being ideal for purposes of agriculture, recreation and other industrial activities. In the soil, pollutants usually stay for longer periods compared with other media such as air and water. Sources of soil pollution include heavy metals (Su *et al.*, 2014), pesticides, fertilizers, herbicides, solvents, insecticides and petroleum hydrocarbons among others (Havugimana *et al*., 2015, Sethi and Gupta, 2020). Pollutants often easily go down into the soil and quickly build up but may take a long time to be depleted. Contamination by heavy metals often decrease the productivity of the soil and can make the soil sterile and makes it difficult for plant growth making the place more vulnerable to soil erosion and a possibly unsafe and unstable land (Reicosky, 2018). Fertilizers contain impurities that come from starting materials used in their production (Khan *et al.*, 2017). The agrochemical industry uses tons of pesticides in the control of different types of pests which may be harmful to microorganisms this is because most of these pesticides as well as fertilizers are not always degraded and may end up in water bodies leading to eutrophication and its effects that include foul smell, generation of significant quantities of methane (Morelli *et al.*, 2018; Zhang *et al.,* 2020a). Soil contamination by agricultural wastes leads to decreased levels of soil fertility, high erodibility, low nitrogen fixation, loss of nutrients, reduction in the crop yield and a disproportion in the soil plant and animal species (Havugimana *et al.*, 2015). Besides, soil pollution also occurs from pharmaceutical companies that carelessly dispose of drugs into water bodies that are swept in underground water that rural people easily access through drinking river water contaminated with various pharmaceutical wastes (Grenni, 2011; Li *et al*. 2019) and its effects are chronic and sub-lethal as they are deemed persistent (Kapoor, 2015). Pharmaceuticals exist as mixtures in the environment and more often than not these are composed of various chemicals which have different regimes of operations and unspecified adverse drug reactions, more so on untargeted organisms (Pomati *et al.*, 2008). Pharmaceuticals are active biological molecules hence even when the concentrations are low, they may still be toxic and harmful (Allen *et al.*, 2010). Because of this, the health of maritime creatures along with humans must be safeguarded (Pomati *et al.*, 2008, Galus *et al*., 2013) from chronic exposure (Hernando *et al.*, 2006). Mining industries require the use of oil in huge amounts in their various mining operations and the recent past has witnessed an increase in the use of oil in form of petroleum products due to the advancement of equipment as well as machinery used in the mines. The main components of oil are generally categorized into three classes namely saturated hydrocarbons (alkanes), unsaturated hydrocarbons [unsaturated paraffins and polycyclic aromatic hydrocarbons (PAHs)] and hetero-hydrocarbon compounds as shown in Table 1 (Wang *et al.,* 2017; Srivastava *et al.*, 2019**)**. The authors indicate that saturated components of low boiling points are easily vaporized and do not linger in the soil system. However, PAHs and hetero-hydrocarbons are the most difficult to remove having higher boiling points and the reluctance to dissolve in water. Some of these substances are considered mutagenic, teratogenic and carcinogenic (Ahmad *et al.,* 2020; Gao *et al.,* 2019). An estimated 45% of natural habitats in Europe are contaminated with petroleum hydrocarbons and their products (Borowik and Wyszkowska, 2018). Petroleum hydrocarbons diffuse in the soil, blocking the pores of soil and they tend to be difficult to separate from the soil because they are often firmly sorbed to the organic matter in the soil and squeezed together in the soil (Rajabi and Sharifipour, 2019). Oil discharges by-products that are toxic and persistent to the soil (Devatha, *et al.,* 2019). In addition, crude oil contamination leads to increased soil's organic carbon content which further causes a consequential reduction in the nitrogen in the soil (Kim *et al*., 2018). Further, a reduction in the soil moisture because of the hydrophobic layer of oil created around the soil particles triggers a decline in the hydraulic conductivity of the soil by about 10% and the availability of water.

Table 1: A summary of oil components, effect on the soil and the type of remediation process

Oil component	Types	Boiling point	Some effect on soil	Source of contamination	Remediation method	Reference
Saturated hydrocarbons	Alkanes Isoparaffin cycloparaffins	Low	Changes carbon to nitrogen and phosphorus ratios, pH, and conductivity	i. Petroleum industry –diesel fractions ii. Oil spillage iii. Careless disposal of oil waste iii. Solvents, and pesticides	Natural oxidation Vaporization Biodegradation	Wang *et al.,* 2017; Ali, 2019
Unsaturated hydrocarbons	Polycyclic aromatic hydrocarbons	High	i. Oil ageing: resistance of soil to any treatment. ii. Covers soil surface prevents carbon dioxide interchange and reduces soil porosity	i. Petroleum industry ii. Oil spillage iii. Careless disposal of oil waste waste iii. Solvents, and pesticides	Physical and chemical processes	Srivastava *et al.*, 2019; Ahmad *et al.,* 2020
Hetero-hydrocarbons	Resins Asphaltenes	Higher	i. Oil ageing: resistance of soil to any treatment. ii. Covers soil surface prevents carbon dioxide interchange and reduces soil porosity iv. Increases soil salinity due to trace amounts of salts in the oil	i. Petroleum industry ii. Oil spillage iii. Careless disposal of oil waste waste iv. Solvents, and pesticides	Physical and chemical process	Srivastava *et al*, 2019; Ahmad *et al.,* 2020; Krabassi and Heidari, 2015

Table 1 shows some components of oil, effect on the soil and the type of remediation process used.

The disposal of large quantities of used oil from mining activities poses a great environmental challenge especially soil contamination. This soil contamination might result from waste oil spillages during storage, transportation, human error or negligence (Jernelöv, 2010). Other industrial activities such as oil extraction and exploration, oil fields, oil drilling as well as production sites can also lead to soil contamination. The petroleum industries also produce billions in tons of natural gas, oil and its by-products. In 2017, a report by International Energy Agency (IEA) estimated daily consumption of at least 97 million barrels of oil per day and a further 100 billion barrels each day by the year 2021 (dos Santos and Maranho, 2018). The mining sector remains a big contributor to soil pollution from the use of large quantities of oil in daily operations of heavy machinery. Oil contaminated soil is among the environmental and ecological challenges resulting from mining activities. Soil pollution resulting from oil contamination can be seen as one of the twenty-first-century vulnerabilities because if not well taken care of the consequences can be devastating. Contamination of soil by waste oil adversely affects land and marine organisms (van der Heul, 2009). Also, the toxicity ensuing from these hydrocarbons causes contamination of land, surface and groundwater adversely affecting biota and prevents agricultural activities from thriving in such soils (Pashkevich, 2017). Other adverse implications of soil pollution resulting from oil contamination include; loss of soil fertility, increased soil toxicity, reduced aesthetic attraction, insufficient nutrient and water availability. According to (Dadrasnia and Agamuthu, 2013) indigenous organisms that live in the soil can also be affected and their food chains altered. As a non-renewable source, the importance of maintaining and remediating contaminated soil cannot be overstated. Soil remediation borders on its bearing on environmental protection, public health including the economy hence the need to expedite the process. In a 2017 perspective analysis, Hansen (2017) affirms that a continued occurrence of environmental contamination from oil spills for as long as there is dependence on petroleum and its products by the mines and society. Mining companies use several methods in their quest to clean and prevent further damage from oil. Skimmers and containment booms, as well as vacuum trailers, have been used. Sawdust is another material that mines have made use of in the clean-up of spilt oil though its use is deemed to be hazardous (Godoy-Faúndez *et al.*, 2008). Incineration and excavation are other commonly used methods to clean up soil contaminated with oil. Thus, to prevent on-going destruction to the ecosystem of the contaminated sites, the medium needs to undergo a thorough treatment process. The remediation process may be as simple as the introduction of certain chemicals into the system, to highly complex reactions involving several chemical and biological processes (Agamuthu *et al*., 2013; Ashraf *et al.,* 2014). The current study was aimed at identifying established commercials as well as newer methods of decontaminating soil contaminated with large quantities of oil from various industrial activities. In addition, the study also looked at the merits and demerits of each mitigation method examined. The main objectives of the review were identifying what method can complement or substitute the bioremediation technique currently used by one of the largest copper mines in Zambia. Specifically, the review sought to find a faster and cheaper way for soil remediation from the mines. The study was performed in 2020 at the Copperbelt University and Mopani copper mines spill-sorb farm in Kitwe, Zambia, respectively.

MATERIALS AND METHODS

The study consisted of a review of several published peer-reviewed articles relating to established and emerging technologies on decontamination of oil-contaminated soil. The review used keywords to search for articles discussing remediation of oil-contaminated soils in the last 20 years. To search for these articles, keywords like "biological oil remediation", "hydrocarbons", "physical oil remediation", "chemical oil remediation", "photochemical oil remediation", "and oil waste in the mining industry" were used.

Remediation process for oil-contaminated soil

Many methods have been advanced to remediate soil polluted with oil during the past thirty years (van der Heul, 2009). To our understanding, these methods focus on ensuring that the hydrocarbons are easily separated and degraded. Nevertheless, a lot of research is still being done to find better techniques to decompose the oil in the soil.

Biological remediation techniques

Bioremediation also referred to as biodegradation is defined process through which crude oil compounds are degraded by microorganisms with the aid of enzymatic reactions turning them into carbon dioxide, biomass, and water-soluble compounds (Atlas *et al.*, 2015). Some bacterial species found in the environment such as yeast, fungi, mould and archaea are capable of feeding on compounds of crude oil (Atlas *et al.*, 2015). Bioremediation is an emergent technology which is quite promising in the management of hydrocarbon contaminated soil and when compared either with physical or chemical repair, bioremediation is widely used due to its advantages of being of good effect, rapid degradation rate, and a lack of secondary pollution (Ahmad *et al.*, 2020; Xu *et al.*, 2018). Zhang *et al.* (2020b) remediated oil-contaminated soil using agricultural wastes through use of a bacterial consortium that was capable of decomposing oil components and the results showed a 68% decomposition of oil after 40 days using swine-water among other additives confirming that certain strains of bacteria can be used in bioremediation. A recent study showed that bacteria such as *Ochrobactrum* sp., *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa* are good at degrading crude oil (3%v/v) by more than 80% (Varjani *et al*., 2015), while an 89% degradation of soil contaminated with diesel was reported after 365 days using a different set of bacteria (Szulc *et al*., 2014). However, since most hydrocarbons are highly hydrophobic and lack bioavailability, bioremediation might not be a suitable remediation approach (Zhu and Aitken, 2010). While there are two main types of bioremediation (in-situ and ex-situ bioremediation), (Azubuike *et al*., 2016), there are several forms of bioremediation such as biostimulation (Abed *et al*., 2015; Emami *et al*., 2014; Ezenne *et al.*, 2014; Hamzah *et al.*, 2014; Huang *et al.*, 2019) bioventing (Amin *et al.*, 2014; Thomé *et al.,* 2014; Mosco and Zytner, 2017) and bioaugmentation (Abdulsalam *et al*., 2011; Adlane *et al.*, 2020; Roy *et al.*, 2014; Singh *et al*., 2012) Bioremediation might not be a feasible technique at sites with high concentrations of certain substances that are toxic to most microorganisms such as cadmium, lead and sodium chloride (Speight, 2018). Besides, the process of bioremediation may take several months or even years to reach completion (Valentine *et al.*, 2010).

Phytoremediation

Phytoremediation, which is also referred to as plant-assisted bioremediation is a soil remediating technique which makes use of plants and their roots. Plant species such as *Centrosema brasilianum*, *Stylosanthes capitata*, *Calopogonium mucunoides*, *Brachiaria brizantha*, *Cyperus aggregatus*, *Eleusine indica* and *Mirabilis jalapa* have been used in phytoremediation of contaminated soils (Merkl *et al.*, 2005; Peng *et al.*, 2009). Peng *et al*. (2009) utilised *Mirabilis jalapa* to remediate contaminated soil and a percent removal ranging from 41-63% was reported. The root enzymes that are present in plants help in the degradation process as they chemically modify and decompose the noxious waste. Phytoremediation, the practice of using vegetation for remedying the contaminated soil in situ is another cheap approach that is applied to clean up oil-contaminated soil. According to (Nedunuri *et al.*, 2000), it is possible to treat the PAHs and petroleum hydrocarbons among others using this method. The treatment uses the plants' property of taking up, accumulating, and/or degrading the constituents which are readily available in soil and water environments (Rostami and Azhdarpoor, 2019). All the chemical, physical and biological activities that are induced by plants to assist and support the remedy of contaminated substrata all relate to phytoremediation (Yakubu, 2007). Fig. 1 shows some mechanisms involved in phytoremediation as illustrated by (Kamath *et al.*, 2004).

The success of this procedure is attributed to the availability of organic materials as root exudates and mucilage secretion by root caps which are easily degraded and can therefore improve the degradation of oil pollutants (Merkl *et al.*, 2005). Fertilizer can be added for an additional supply of nutrients to the microorganisms and plants. This is among the factors that influence phytoremediation. However, it is not clear how much fertilizer should be added for maximum efficiency (Lim *et al.*, 2016). To that effect, some studies have been performed to ascertain the extent to which the fertilizer must be added (Dadrasnia and Agamuthu, 2014; Merkl *et al.*, 2005) Grasses and roots are reportedly the most common plants used in phytoremediation because their roots cover a bigger area providing more biomass for rhizoremediation (Cook and Hesterberg, 2013; Moreira *et al.*, 2013). Earlier studies on the effect of root coverage on phytoremediation have shown that grass which had extensive root structure and shoot biomass was more effective in soil remediation (Soleimani *et al.*, 2010). When *Festuca arundinacea* and *Festuca pratensis* were used to degrade petroleum hydrocarbons, 80-84% of polyaromatic hydrocarbons were removed with 64-72% being total petroleum hydrocarbons (Soleimani *et al.*, 2010). Though this procedure is very cheap and is used in getting rid of would-be

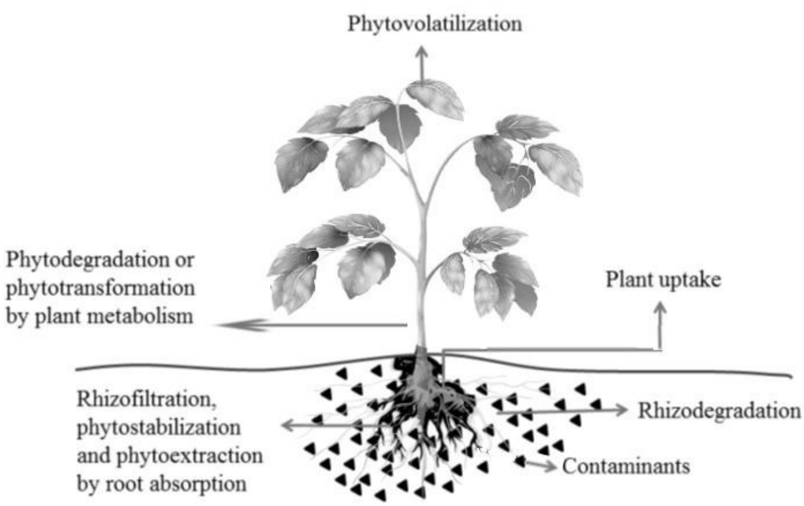

Fig. 1: Phytoremediation mechanism (Kamath *et al.,* 2004)

environmental pollution, it is time-consuming and can take approximately five to seven years (Yakubu, 2007), therefore, suitable for long term results. Other disadvantages of these phytoremediation processes are; certain oil pollutants may be harmful to the plants, some pollutants may need extended periods, the extent of the root system determines the strength of treatment and some plants may not be available throughout the year (Lombi and Hamon, 2005). Besides, nothing seems to be well-known about the bioavailability and the toxicity of the products of biodegradation (Kamath *et al.*, 2004). Despite these pitfalls, phytoremediation procedures are widely accepted and used because they are economical and ecofriendly (Guerra *et al.,* 2018). Ahmad *et al.* (2020) demonstrated that 63% of hydrocarbons can be decomposed using rye grass in combination with plant growth promoting bacteria in three years. In addition, Devatha *et al* (2019), used the topical plant *Chromolaena odorata* to evaluate its effect on oil-contaminated samples and observed that within 7 days, the total petroleum hydrocarbons (TPH) were reduced up to 50-60% for soils contaminated with 5% and 10% oil, respectively. The reduction in TPH was further evidenced by Fourier transform infrared (FTIR) spectroscopy, where certain functional groups disappeared after the 7-day interval.

Landfarming

Landfarming is a process by which the hydrocarbon polluted soils are widely dispersed in an overlay of nearly half a meter in thickness, some nutrients added, and once in a while, the soils are mixed (Paudyn *et al.*, 2008). Land farms may have different treatment routines due to variations in the locality, climate, soil type and temperature. In much warmer climatic conditions landfarming has been implemented successfully, (McCarthy *et al.*, 2004) while conflicting results have been reported in cold Antarctic and Arctic environments (Paudyn *et al.*, 2008). In this remediation technique which is normally applied to petroleum hydrocarbons, the excavated oil-contaminated soils are firstly dispersed as a shallow layer ground surface where the intervention would be done followed by stimulation activity by microbes within the soil by aeration, the addition of nutrients, water and minerals (Khan *et al.*, 2004). In contrast, when land tillage was used to remediate soil in farmland, the TPHs decreased by 50% after four months. It is important to stress that the core of bioremediation technology is the degradation of pollutants through microbial metabolic activities (Khan *et al*., 2004). Landfarming is often quite slow due to the influences of weather changes that cannot be controlled. With the landfarming technique, only percentages less than 95% can be attained and in most cases some contaminants persist in the soil (Maila and Cloete, 2004). As reported in a review by Khan and colleagues, a concentration of more than 50,000 ppm would be very difficult to remediate (Khan *et al*., 2004). Landfarming being a class of bioremediation there are high chances of generating and leaving behind (in the soil) even more toxic compounds causing further damage to the soil (Chibwe *et al*., 2015). Ultraviolet A which falls in the range of 315-387nm comprises almost 95% of the ultraviolet (UV) radiation would be better for landfarming (Yang *et al*., 2017). Also, contamination of air may result from emissions as a result of volatilization of the compounds from the contaminated soil and may pose health risks to humans (Thomé *et al*., 2014). Landfarming is often quite slow due to the influences of weather changes that cannot be controlled. Fig. 2 depicts landfarming as a soil remediation technique. The approach's effectiveness relies on the replication of the microorganisms to undertake the degradation of the pollutants; therefore, the conditions must be suitable for microbial growth (Mosbech, 2002). Being very precise and restricted to biodegradable organic compounds, the method can produce more persistent and even more toxic compounds than the parent pollutant and requires more time to generate the sought results (Sharma, 2012). Table 2 illustrates some commonly used biological remediation methods for processing soil contaminated with oil.

Physical methods

Several physical methods are also used such as soil flushing (Gitipour *et al*., 2014; Zamudio-pérez *et al*., 2013), soil washing (Achugasim *et al*., 2011; de Andrade Lima *et al*., 2018; Karbassi and Pazoki, 2015; Lim *et al*., 2016; Moon *et al*., 2016), soil vapor extraction and thermal desorption (Khan *et al*., 2004; Park and Zhan, 2003), low-temperature thermal treatment (Falciglia *et al*., 2011; Jebeli *et al*., 2019; Ren *et al*., 2019) and sonication (Effendi and Wulandari, 2019; Collings *et al.,* 2007; Collings, *et al*, 2007; Shrestha *et al*., 2009; Li *et al*., 2013).

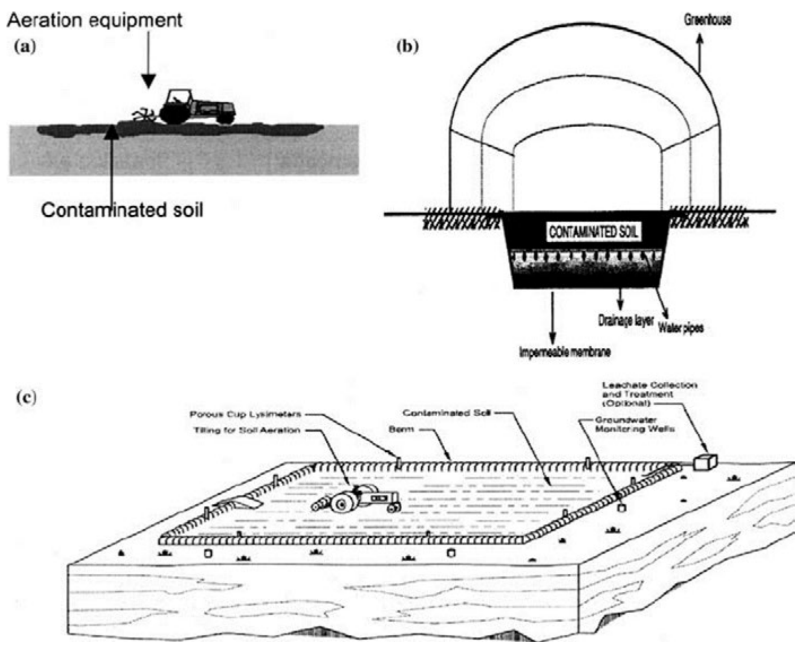

Fig. 2: Landfarming procedure (Maila and Cloete, 2004)

Using different concentrations of Triton X-100 and sodium dodecyl sulfate Gitipour *et al*. (2014) recorded removal percentages of 79.6%, 80.14%, 80.26% and 83.51% respectively. Achugasim *et al*. (2011) found that a soil washing agent can remove benzene, toluene, ethylbenzene and xylene (BTEX) from oil-contaminated soil with removal percentages being around 95%, 95% and 97% for neutral, basic and acidic pH ranges in that order. Despite the high removal percentages recorded, the procedure was found not to be effective towards polycyclic aromatic hydrocarbons present in the crude oil as was seen from the removal percentages which were less than 3%, 41% and 27%, for the PAHs in neutral, basic and acidic ranges respectively. The conclusion was that persulphate should not be used singly as a washing agent if the removal of hydrocarbons in soil contaminated with crude oil is to be effective. Different washing solutions were used by Moon *et al*. (2016) to remediate contaminated soil, and they found that tetra acetic acid performed well as a washing solution. Use of this method to treat contaminated soil has attracted debate as the detergents used are said to pollute the soil (van der Heul, 2009). The use of soil vapour extraction and thermal desorption to remove volatile organic compounds and semi-volatile organic compounds was suggested by Khan *et al*. (2004) but Park and Zhan (2003) were quick to note that contaminants from heavy oils such as diesel and kerosene are not well removed and sometimes, they are not removed at all. Falciglia *et al*. (2011) demonstrated that the efficacy of thermal desorption and adsorption as remediation techniques in diesel contaminated soil at low temperature was dependent on factors such as temperature, the texture of the soil, composition of soil and how long the treatment lasts. The researchers found that for diesel polluted sandy and silty soils; a temperature of 175 °C was enough to desorp the contamination whereas a temperature of 250 °C was needed for clay soils. The use of ultrasonic has shown a considerable effect on desorption of petroleum hydrocarbon in fine soil where the amount of TPHs removed was 61 and 49% from 27.6% silt and 55.3% clay soils, respectively (Li *et al.,* 2013) while increasing the power increased the removal rates (Shrestha *et al*., 2009). Effendi and Wulandari, 2019 reported a 61 % removal of TPHs in 15 minutes using 160 watts of power, however, it was reported that no further decrease occurred likely due to a steady-state re-adsorption of the TPHs onto the soil. Although studies on ultrasonic remediation are inadequate, Effendi and Wulandari, 2019 anticipate

Table 2: Common biological remediation methods for processing soil contaminated with oil

Method	Design and area of focus	Key Findings	Gaps/annotations	Reference
Bioremediation: Biological process	Contaminated soil treated with swine wastewater and wheat bran.	After 40 days a degradation efficiency of 68.27 ± 0.71% was obtained.	i. Use of pig waste to mitigate soil pollution is valuable. ii. Process needs external microbial agents to facilitate the biodegradation process.	Zhang *et al.*, 2020b; Sivakumar, 2015
Bioremediation: Biological process	Sawdust and the rice straw were used to degrade petroleum in the contaminated soil.	i. The removal percentages for the TPHs after 5 months were 23.9%, 45.2% and 27.5% while for the PAHs rates were 66.3%, 30.3% and 26.9%, for the sawdust, rice straw and control treatments, respectively. ii. Use of sawdust and the rice straw boost the removal of PAHs and TPHs, respectively.	Removal of petroleum was not associated with the variety of the microbes. Not all microbes play a role in petroleum elimination.	Huang *et al.*, 2019
Bioremediation: Biological process	Aged refuse from landfills was used in the treatment of soil contaminated with petroleum.	After 98 days of study, the removal of TPHs to 89.83% from 22.40% was observed.	I) Use of aged refuse increased the biomass of the microbes. ii) Safety of the use of aged refuse could not be ascertained. iii) No quantitative percent removal of oil was provided.	Liu *et al.*, 2018
Phytoremediation: Biological process	*Hyparrhenia rufa*, *Sorghum arundinaceum*, *Oryza longistaminata* and *Tithonia diversifolia* plant species were used.	i) After 120 days of phytoremediation combined with 5 gkg^{-1} soil of manure a reduction in the TPHs was observed. ii) No significant difference between the use of manure 5 gkg^{-1} soil and 10 gkg^{-1} soil in treatment of TPHs.	I) Phytoremediation on its own was not ideal for successful bioremediation of the contaminated soil ii) No quantitative percent removal of oil was provided.	Ruley *et al.*, 2020
Landfarming: Biological process	A combination of landfarming, phytoremediation and chemico-biological methods was used. Target components were with (PAHs) and (TPHs).	i) Landfarming method was the best method among the three remediation techniques employed with the least being phytoremediation ii) 90% degradation of TPHs and PAHs was observed	Landfarming produced better results because of the combination of inorganic and organic fertilizers	Okonofua *et al.*, 2020
Bioremediation (Natural Process) Landfarming; Bioagumentation (Aided landfarming)	The study used three bioremediation methods to remediate petroleum hydrocarbons polluted soil.	After a 90-days trial, about 86% of TPHs were removed by bioagumentation; 70% by landfarming and bioremediation (57%).	Best results for bioremediation are obtained with a combination of different bioremediation approaches.	Guarino *et al.*, 2017

that ultrasonic remediation can help to sought out the inability of bioremediation to degrade clayey soil when used together or as a pretreatment to bioremediation.

Excavation

The soil that is polluted is removed from the site where contamination occurred to a site or facility where the risk of exposure to potential pollutants may be controlled effectively. This approach can be used for the treatment of both inorganic as well as organic pollutants (Lombi and Hamon, 2005). Likewise, the treatment of excavated soil could also be done on the site, away from the site or the soil may not be treated but dumped in a landfill. However, the fastest and safest way to remedy crude oil-contaminated soil is excavation except that this approach is neither advanced nor cheap (Ahmad *et al*., 2020). Though this method is simple and efficient, it is very prolonged; can be damaging, time- consuming and also very costly due to the transportation of contaminated soil to the disposal site and the need to take care of the landfill mines created in the process. Because almost 75% of waste created by humanity comes from mining and manufacturing industries, the amount of waste in the mine dumps determines the dangers posed to the environment; as such air pollution from vapours of the contaminants is indiscriminately discharged into the atmosphere (Pashkevich, 2017; Iqbal *et al.,* 2015).

Incineration

During incineration, the polluted soil is burnt in an incinerator at high temperatures under regulated conditions. Once heated the vaporized pollutants are collected or eliminated by pyrolysis. This method is one of the easiest ways to get rid of oil in the soil (Rushton *et al*., 2007). Using a combustor on a pilot scale, it has been shown that almost all the oil contaminants can be removed from contaminated soil at temperatures of about 800°C (Anthony and Wang, 2006). Onsite incineration is mostly used because it is the cheaper process for remediating contaminated soil, though it must be stressed that this method is not environmentally friendly because the volatile and flammable chemical compounds that are in crude oil may cause environmental pollution (Diphare and Muzenda, 2014). Cost of transportation to access the incinerator may also render the technique expensive (Ball *et al*., 2012). As can be seen from Fig. 3, incineration produces emissions that lead to secondary pollution (Shen *et al*., 2016). Table 3 illustrates some commonly used physical remediation methods for processing soil contaminated with oil.

Chemical methods

A number of chemical methods are also used to get rid of oil in contaminated soil. These chemical methods include thermal stripping (Vidonish *et al*., 2016), chemical oxidation (Ahmad *et al*., 2020; Ershadi *et al*., 2011) and photochemical oxidation (Cheng *et al*., 2016).

Oxidation

Chemical oxidation is another technique employed in removing harmful substances from the soil. Oxidation of the organic contaminants is achieved by the use of oxidants such as hydrogen peroxide, (H_2O_2), ozone (O_3), permanganate (MnO_4^-) and persulphate ($S_2O_8^{2-}$) that are reactive and the method is dependent on the soil medium for efficiency (Ahmad *et al*., 2020; Sutton *et al*., 2014). A Fenton's reagent, a combination of hydrogen peroxide and ferric ion (Fe^{3+}) is also often used in chemical oxidation. During Fenton's reaction, the ferric ion catalyzes the reactions while hydrogen peroxide being an oxidizing agent produces hydroxyl ions (Ahmad *et al*., 2020). The efficacy of the use of Fenton's reagent on oil removal from sand at a low pH has been demonstrated in the past (Goi *et al*., 2006). This technique, when employed under the right conditions, has been found to remove 48% of TPHs in contaminated soils within two hours suggesting that the method might be used to eliminate TPHs from contaminated soils in a short period (Adipah, 2018). Other researchers have made use of ozone as another oxidative method for removing oil from soil because it is easily generated, stored and handled especially, in-situ remediations (Adipah, 2018). Further, soils treated with this technique can be reused as ozone returns back to oxygen in no time (Chen *et al*., 2019). Some researchers have reported combining ozonation with bioremediation to increase the efficiency of the degradation process (Russo *et al*., 2012; Javorská *et al*., 2009; Derudi *et al*., 2007). Despite the many benefits associated with the use of ozone, it is known to kill indigenous microorganisms in the soil and augmentation is usually required for soil restoration (Jung *et al*., 2005). The oxidation method is often used in highly contaminated soil because its

Table 3: Comparison of key findings and disadvantages associated with different physical methods of oil remediation

Method	Design and Area of Focus	Key Findings	Gaps/Annotations	Reference
Incineration	Focused on different remediation techniques including their limitations.	Incineration is a cheap remediation method compared to other techniques.	Incineration produces secondary pollutants.	Diphare and Muzenda, 2014
Excavation	Highlighted some modern remediation techniques.	Excavation is the fastest and safe way to treat contaminated soil.	Excavation is quite time-consuming and damaging at the same time.	Ahmad *et al.*, 2020
Landfill	Focused on the environmental impact of mine dumps.	Three quarters (75%) of waste originates from mining and manufacturing industries.	Amount of waste in the mine dumps determines the dangers posed to the environment.	Pashkevich, 2017
Soil washing	Use of organic solvents for hydrophobic organics Use of aqueous solvents Sodium dodecyl sulfate was used as a surfactant.	Various solvents used were able to remove the organics from 75-86% Removal of contaminants was achieved from 60-86% After seven hours an efficiency of 88.32% was actualized.	The efficiency of oil removal is dependent on oil type, soil type, weather conditions, and penetration depth. Preventive measures are necessary to prevent oil contamination of soils. No single method can completely mitigate oil-contaminated sites. Improvement in the soil quality in terms of physicochemical was reported.	Diphare and Muzenda, 2014 Akpoveta *et al.*, 2012
Sonication	Removal of persistent organic pollutants.	Rate of removal of pollutants depends on the type of soil, frequency and power of the ultrasound used.	Increasing power increased the removal rates	Shrestha *et al.*, 2009
Soil washing	Heavy metals and petroleum hydrocarbons	Tartaric acid was suitable for washing away up to 82% TPHs. Hydrochloric acid was found to be a better washing solution for heavy metals.	A suitable washing solution must be determined based on the contaminant, mineralogy of the soil, and the type of the soil.	Moon *et al.*, 2016

application is short term and contaminants can be oxidized within a few weeks. It is yet worth noting that the method might not necessarily be a good choice in low permeability soils (Ogunkeyede, 2016).

Photochemical oxidation

Photochemical oxidation involves the transformation of a chemical compound in presence of sunlight (Hadnadjev-Kostic *et al.*, 2014). In this method, a mixture of the contaminant with a catalyst is irradiated with light (UV or sunlight) which cause oxidation of the organic pollutants leading to the formation of compounds such as water and carbon dioxide. Semiconductor catalysts have a valence band with energetically stable electrons and an unoccupied conduction band of higher energy. Irradiation of these semiconductors activates photocatalytic reactions where an electron (e^-) is promoted into the conduction band and a hole (h^+) created in the valence band (Liu and Zhang, 2014; Xiang *et al.*, 2011). Consequently, through photocatalytic processes, hydroxyl (OH^-) radicals along with superoxide (O_2^-) anion radicals are formed which causes the photochemical oxidation of the organic pollutant at the semiconductor surface. The importance of a high temperature, increased exposure time or the concentration of oxygen is that

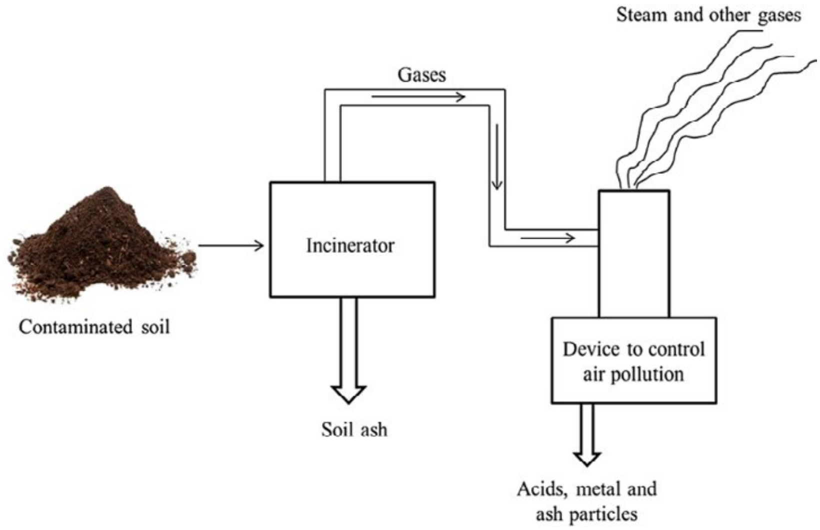

Fig. 3: Incineration of contaminated soil (Koul and Taak, 2018)

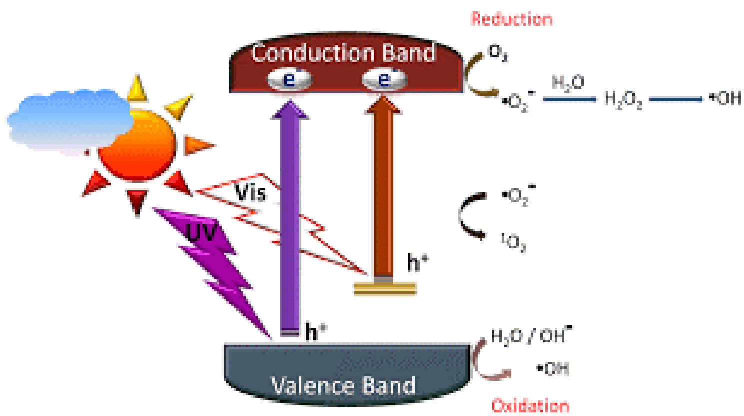

Fig. 4: A general scheme for generation of hole-electron pairs in an ideal photocatalyst (Banerjee *et al*., 2014)

it boosts photochemical oxidation in nature (Clark *et al*., 2007). Fig. 4 shows the generation of hole-electron pairs in an idealized semiconductor.

Photochemical oxidation is a powerful remediation method for decomposition of oil in the soils. Some researchers have used photooxidation with semiconductors as photocatalysts over the years to treat organic pollutants (Liu and Zhang, 2014). Photocatalytic pre-treatment enhanced degradation by improving the solubility of oil and biodegradability (Brame *et al*., 2013). Progressive combination of the photocatalytic remedy of oil polluted soil with titanium dioxide (TiO_2) and bioremediation can effectively remove TPHs in polluted soil only when sufficient infiltration of light is realized (Yang *et al*., 2017). Previous reports indicate that photooxidation removed all forms of TPHs suggesting non-selective removal of TPHs while removal increased with increasing moisture content which is in line with the assumption for hydroxyl radical generation water is required (Brame *et al*., 2013). Photochemical oxidation of oil by use of TiO_2/zeolite composite is a sustainable way of destroying oil deposits minus titanium dioxide diffusing into the environment.

Photochemical oxidation is the most appropriate method in the breaking down of oil (van der Heul, 2009) and organic pollutants (Wang *et al*., 2014).

Recent reports have concluded that the interactive influence of UV illumination and TiO_2 catalysis is responsible for the decomposition of PAHs in contaminated soil (Zhang *et al*., 2008). However, they also noted that a small number of investigative studies on the photocatalytic oxidation of polyaromatic hydrocarbons on the soil surfaces have been done using TiO_2 as the catalyst under UV irradiation. Effendi and Aminati (2019) explored the likelihood of enhancing biodegradation of the TPHs in soil contaminated with crude oil by integrating chemical oxidation using TiO_2 under sunlight irradiation as a photocatalyst and bioremediation through the landfarming system. Their study revealed that the amount of TPHs removed increased when a photocatalyst was included as compared to exclusive bioremediation. Despite this positive result, increased degradation rates were not at all times relative to the concentration of titanium dioxide. Natural microbial action and UV light derived energy had an effect on the process of degradation but the effect of UV intensity was not considerable. Addition of a concentration of 2% TiO_2 resulted in the greatest TPHs degradation rate up to 67 % removal of TPHs within 12 weeks. Because biodegradability and solubility are enhanced during chemical oxidation, Effendi and Aminati (2019) were able to investigate the proficiency of blending photocatalytic oxidation using TiO_2 and bioremediation to deal with the constraint of hydrocarbon bioavailability to indigenous microbes and to ensure that the frequency at which TPHs are removed in oil-contaminated soil increased using the landfarming technique. Photochemical oxidation is a non-selective remediation technique which can degrade a variety of contaminants.

The general problem

The use of oil in bulk quantities by mining industries leads to soil contamination (New climate economy, 2014) because of the equally large amounts of waste oil generated which often pose a disposal challenge. Spills from oil waste happen due to human negligence and leaking of oil from storage tanks and poorly maintained and serviced machinery. Indiscriminate disposal of waste oil often causes soil contamination leading to land, surface and groundwater contamination. When oil is present in water bodies, aquatic organisms are affected. Waste oil also causes degradation of the environment thereby reducing its capacity to support soil organisms as well as the growth of plants.

The problem of soil contaminated with oil in mining industries in Zambia

Mining industries require the use of oil in large amounts in their various mining operations and the recent past has witnessed an increase in the use of oil as petroleum products due to the advancement of equipment as well as machinery used in the mines (New climate economy, 2014). This high oil usage has caused a spike in environmental pollution especially soil contamination resulting from large amounts of oil waste generated from various mining activities. Challenges of waste oil disposal are also being experienced in Zambia, a country that is located in southern Africa. Zambian mining companies such as Mopani copper mines (MCM) also consume large quantities of oil in their daily operations in the process producing equally vast amounts of waste oil. This oil waste often causes soil pollution resulting from indiscriminate disposal as well as other factors related to storage and transportation. In mitigation, MCM uses bioremediation (landfarming) with spill sorb but this method is often slower and takes longer compared to other treatment methods (Maila and Cloete, 2004), thus larger oil spills might remain in the soil for some time, (Zhang *et al.,* 2020b) and may allow even more toxic compounds to linger in the soil. Since the half-lives of some hydrocarbons are as long as 28 years and some may be resistant to degradation, bioremediation is probably not the correct treatment technique (Goi *et al*., 2006). In response to this problem, the use of photochemical oxidation is an emerging technology that offers the most effective means of treating soil contaminated with oil in addition to providing a sound basis for developing a culturally, socially, economically, and ecologically sustainable remedy for contaminated soils (Yang *et al*., 2017).

Spill Sorb Technique at MCM

Mopani Copper Mines is one of the major players in Zambia's mining industry and uses vast quantities of oils in its operations. Oil spillage is therefore inevitable and the company has been spending huge sums of money on bioremediation of hydrocarbons in soils. The mining firm uses landfarming with spill sorb to treat contaminated soil. Spill sorb is a manufactured organic oil absorbent that has been utilized in adsorbing oil. It is formed from Canadian peat moss once dried after harvesting. Spill sorb has a biological catalyst inside the cell structure. The cells are also a storage for oxygen which is required in the ecosystem and for biodegradation. The catalyst in spill sorb acts together with the microorganisms present to initiate the process of bioremediation. Although the method is biological, it is time-consuming, affected by climatic conditions and usually result in incomplete degradation (Zhang *et al.*, 2020b) because some compounds in the oil are stubborn and not all microorganisms found in the soil can degrade them (Khan *et al*., 2004). This might lead to increased toxicity levels in the soil (Chibwe *et al*., 2015), hence cannot be a suitable method.

The proposed method

Photochemical oxidation emerges as a green technology for sustainable oil waste management and soil pollution resulting from hydrocarbons. The on-going study at the Copperbelt University proposes and seeks to promote this method in remedying soils contaminated with hydrocarbons present in the oils. The use of abundant and free sunlight in conjunction with readily available clay inorganic materials in the photochemical oxidation of contaminated soil is not only environmentally friendly but it is also in line with sustainable development methods. Hence, this study will focus on photochemical oxidation and assess its effectiveness in treating oil-contaminated soils with locally engineered clay using sunlight. To increase adsorption capabilities, the clay materials will be chemically modified by incorporating hydrophobic groups within the microstructure of the materials. Chemical modification typically involves the sol-gel process using room temperature reactions. Generally, the material is dispersed in an alcoholic solution followed by addition of hydrophobic groups to increase oil-material interaction and by addition of transition metal solutions in correct amounts to ensure photocatalysis effects are incorporated (Deshmukh *et al.*, 2020). X-ray fluorescence (XRF) and inductively coupled plasma mass spectrometry (ICP-MS) will be used to the percent incorporation of the transition metals. Furthermore, infra-red spectroscopy will be employed to substantiate the added hydrophobic groups onto the clay while UV-visible spectroscopy will determine whether the modified clay will absorb in the visible region of the spectrum. One of the parameters employed to ascertain the degradation process is oil reduction in the soil. This is achieved through Soxhlet extraction and later using Eq.1.

$$\%R = \frac{c_0 - c_e}{c_0} \times 100 \quad (1)$$

Where, C_o = initial amount of oil; C_e = amount of oil at time t and % Residual (R) oil = the percentage of the residual oil after photo-degradation, (Narayan,

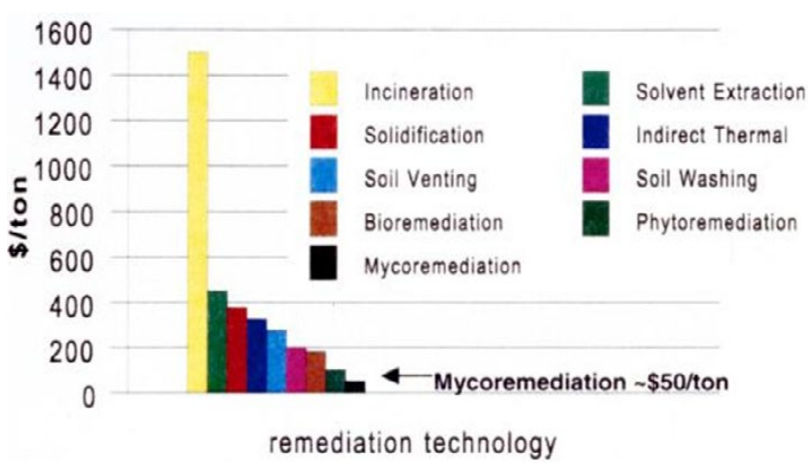

Fig. 5: Economic cost comparison of the different remediation techniques used in soil remediation (Stamets, 2013)

Table 4: Summary of chemical remediation methods for processing soil contaminated with oil

Method	Design and Area of Focus	Key Findings	Gaps/Annotations	Reference
Oxidation: Oxidants such as hydrogen peroxide, ozone was used.	Considered many remediation techniques for soil contaminated with oil.	Chemical methods offer a quick way to remediate contaminated soil.	Some techniques such as incineration must be used with caution as they are generally not acceptable in most societies.	Ahmad *et al*., 2020
Oxidation: sulfate radicals	Sulfate radicals were used to remove dissolved ammonia and organic matter from landfill leachates.	Increasing the sulfate dose reduces the efficiency of the sulfate radicals and the pH of the solution.	The method might produce nitrates from ammonia and also a reduction in the pH due to HCl production.	Chen *et al*., 2019
Oxidation: Fenton Reagent.	Oxidation using Fenton reagent was used effectively to get rid of contaminants from soil contaminated with the total petroleum hydrocarbon.	A 48%removal rate of the contaminants within 2hours of treatment was obtained.	Fenton process was hailed as a quick method to remove contaminants from the soil.	Adipah, 2018.
Conventional and microwave pyrolysis.	Conventional carbonization and microwave pyrolysis were compared.	Conventional pyrolysis produced 70% removal of total organic carbon (TOC) while microwave pyrolysis was able to extract up to 77% of TOC. Microwave pyrolysis was suitable for both in situ and ex situ remediation.	Microwave pyrolysis has little adverse impact on animals and the environment hence eco-friendly.	Ogunkeyede, 2016
Photo-oxidation: using TiO_2 under UV irradiation.	Combination of photochemical oxidation using TiO_2 as a photocatalyst and the landfarming technique in the remediation of crude oil-contaminated soil.	Addition of 0.5% and 2% of TiO_2 to bacteria soaked oil-contaminated soil removed up to 59% and 67% of TPHs in 12 weeks.	i. Photocatalysis enhanced the degradation of the organic pollutants compared to biostimulation alone. ii. No correlation between the amount of TiO_2 and rates of pollutant decomposition.	Effendi and Aminati, 2019
Photo-oxidation: using TiO_2 under UV irradiation followed by bioremediation.	Contaminated soils underwent pretreatment using TiO_2 with various concentrations of Ultraviolet radiations (UV A and UV C).	Oil-contaminated soils pretreated with UV radiation removed about 26% of TPHs compared to 15% of non-pretreated soils.	Limited penetration of light into the soil depth hinders the capability of TiO_2 to aid bioremediation	Yang *et al*., 2017
Photo-oxidation: clay-based photocatalysts.	Various advanced technologies involving the use of clay-based photocatalysts were reviewed.	Clay-based photocatalysts can be applied in environmental remediation.	Generation of H_2 and CO_2 reduction can be done using layered clay	Liu and Zhang, 2014

2010) to calculate the percentage reduction. Some common chemical methods including photochemical degradation are illustrated in Table 4.

Environmental economic evaluation of remediation strategies

According to Lombi and Hamon (2005), the economic benefits of particular soil mitigation processes largely depend on many factors such as the type of soil contamination, the hazardous effects of the pollutant, and the period of remediation of the chosen method as well as the urgency of the reclamation of the polluted soil. According to a summary provided by the authors, Fig. 5, biological-based approaches are among the most cost-effective methods. Ultimately, the real cost of each method is dependent on the length of remediation procedure involved. Depending on the economic strength of a country and the urgency of land reclamation, different strategies have to be adopted to suit the needs of that particular nation or institution.

CONCLUSION

The remediation of the contaminated soil could be viewed as a multi-dimensional crisis resulting from complex factors that influence the efforts to reverse the damage. Also, this crisis could be brought about to choose an appropriate remediation technique for a particular type of contaminants taking into account the cost associated with each particular treatment option. There are several methods of mitigating soils contaminated with oil with each method having its advantages and disadvantages. Bioremediation is particularly attractive as it is a natural process that does not introduce secondary chemicals into the soil and is capable of complete mineralization of oil into benign water and carbon dioxide. However, this process is usually slow as most micro-organisms are incapable of breaking down heavy or long chain components of the oil. Bioremediation is a cheap method, gets rid of wastes without causing disruptions to the environment. However, the method requires a lot of time to get the best results and may therefore prove to be costly in the long run. Phytoremediation as a biological technique may be inefficient as some plants may be sensitive to salinity, pH, water, soil fertility and climate. Also, it might not be appropriate in high concentrations of contaminants. Other plants may be affected by plant diseases and pests. Physical methods such as incineration may be carried out under any weather conditions and are regarded as a fast remediation method. However, this method may not be cheap to set up and operate as it requires a lot of energy. The technique may also cause air pollution. Low temperature technique can remove much as 90% of the pollutants but the method is also quite costly and not environmentally friendly. Soil washing is an easy and effective method though it is prolonged, takes too much time and is expensive. The surfactant used may be harmful because of the likelihood of sticking to the soil particles. Chemical methods such as the use of ozone are often speedy in treating contaminated soil but the use of chemicals may adversely affect the soil and living organisms as a result of leaching and other side effects. Thus, alternative methods that would enhance the breakdown of these fractions are sought to improve the remediation process. Photochemical oxidation processes as chemical remediation techniques are not only economical but also effective as compared to the biological and physical methods in the treatment of oil-contaminated environments. In response to seeking a low cost and accelerated approach to remediating soil-oil contamination, the use of abundant and naturally occurring clay is of considerable interest. In this regard, a combination of adsorption and photocatalysis using various adsorbents has been utilized. Although clay is non-conducting due to the presence of silicon (Si) and aluminium (Al) ions as the backbone of the structural integrity of clay, the materials can be modified by simple sol-gel methodology. The sol-gel process allows for incorporating eco-friendly transition metals such as iron or manganese to increase absorption to 45% of visible light but also allows for the introduction of chelating or hydrophobic groups to increase oil-clay interaction. Additionally, photocatalysis mitigation can help break down long chain hydrocarbon fractions of oil into simple forms that then microorganisms can break down naturally. Thus, reducing the time it takes to reclaim the polluted soil or land. Finally, the remediation of contaminated soil in any nation must be looked at as a crisis requiring immediate attention as consequences of not remediating this precious resource might be devastating.

AUTHOR CONTRIBUTIONS

M. Mambwe wrote the manuscript text with input from other authors. K.K. Kalebaila and T. Johnson analyzed and edited the manuscript.

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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
Al	Aluminum
C_b	Conduction band
C_e	Concentration of oil at time zero
C_o	Concentration of oil at time, t
CO_2	Carbon dioxide
Fe^{3+}	Ferric ion
FTIR	Fourier transform infrared
H_2O	Water
H_2O_2	Hydrogen peroxide
IEA	International energy agency
ICP-MS	Inductively coupled plasma mass spectrometry
IR	Infrared
MCM	Mopani copper mines
MnO_4^-	Permanganate
O_2	Oxygen
O_2^-	Superoxide
O_3	Ozone
OH^-	Hydroxyl radical
PAHs	Polyaromatic hydrocarbons
R	Residual
$S_2O_8^{2-}$	Persulphate
Si	Silicon
t	Time
TiO_2	Titanium dioxide
TPH	Total petroleum hydrocarbons
UV	Ultraviolet
V_b	Valence band
XRF	X-ray fluorescence

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REVIEW PAPER

Promising approach for composting disposable diapers enhanced by *Cyanobacteria*

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ABSTRACT

Disposable diapers have become a complicated matter due to the risk generation to the environment and human health. This study presents a description of disposable diapers characteristics and the success-proven methods used to handle this waste. In many developing countries where an inadequate waste management system occurs, the handling method selection must consider effectivity, the affordable cost, and the end product quality. Despite the diaper composting has successfully conducted in several previous studies, some issues remain for researchers to address. Thus, it requires an improvement so that the system runs effectively and sustainably. This study aimed to determine the possibility of using Cyanobacteria for enhancing the diapers composting. This study gains insights from previous studies using a literature review method, with the year of publication between 2007 to 2020. The focus of the investigation relates to disposable diapers composting and its optimation by cyanobacteria addition. And so as the future prospecting for application and implication to the environment and human life. Cyanobacteria ability to carry out nitrogen fixation, carbon sequestration, ubiquitous in natural habitat, highly adaptive in a wide range environmental condition, can live in the composting system, perform bioremediation, and its application as quality fertilizer, and potentially degrade plastic polymers, spread the expectation to cyanobacteria which associated with its advantages over other microorganisms to enhance the disposable diapers composting.This study highlights the potential utilization of cyanobacteria as an opportunity for copping disposable diapers pollution. The application of compost resulted expected to provide promising-advantages to the environmental sustainability and agriculture. This paper proposes an overarching review of the feasibility in this regard.

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INTRODUCTION

Handling solid waste is an urgent problem in almost all developing countries where infrastructure and management systems cannot deal with the waste produced. Open dumping, open burning, disposal to water bodies and drainage channel, are commonly done by the community. It leads to negative impacts on the environment, human health and infrastructure. One of municipal solid waste that brings problem is disposable diapers which can be in baby diapers, sanitary napkins, personal care wipes, and adult incontinence products. Ever since the introduction of disposable diapers in 1961, it has become an integral part of the economy that gradually expands the baby diaper industry. Further-developments occurred in the mid-1980s. Since then, the growth of the baby diaper market continue increasing (Edana, 2008). The global market for diapers exhibits a rising trend. Europe dominates the market for disposable diapers worldwide followed by the Asia Pacific, North America, and Brazil. The high birth rate in developing countries, such as Latin America, the Middle East, and the Asia Pacific predicted to positively affect the regional diaper sales due to factors such as the rising cost of national health care, the increased of affordability and awareness of the infant health issues, the rapid economic development and urbanization, also influence the diaper industry (Khoo *et al.*, 2019). In the European Union alone, disposable baby diapers occupying 36% of the market of absorbent hygiene products (Mendoza *et al.*, 2019). Disposable baby diapers are used for about 3600 to 4250 units per child, mainly in the first 18 months up to 30 months (Edana, 2008). Another study estimated that the total number of disposable diapers used during the first three years is 4600-4800 (Dey *et al.,* 2016), and can even 6,300 diapers before potty training (Khoo *et al.*, 2019). It shows a very-large market need and the diaper industry will continue to grow in the future. Although disposable diapers represent a significant problem for the environment in terms of resource consumption, the increase in waste generation, environmental pollution, huger energy use, and hazard risk for human health compared to cloth diapers, the disposable diapers is unavoidable. Many people still use disposable diapers for convenience, better hygiene, and skin protection reason. Several studies conducted related to the final handling of diaper waste, including landfill, incineration, and processing in recycling centres. All of the options require land-use changes, energy, human resources, technology, and costs that difficult to meet for many countries. It becomes a great challenge to provide appropriate, low-cost, fast, and easily applicable waste management to deal with disposable diapers pollution. With compostable cellulose content, recoverable nutrient in human waste, evidence that superabsorbent polymers increase water retention in the soil, and the plastic polymers in diapers residue are potent to decompose by microorganisms, make composting a promising method for diaper waste recovery. Some research have proven the success of composting disposable diapers where the results are satisfactory, yet, some issues remain to address. A time-consuming process (Simamora and Pandebesie, 2019), plastic polymers and cellulose that do not fully degrade (Espinosa-Valdemar *et al.*, 2014), pathogens that still exist in compost (Zulfikar *et al.*, 2019), and lack of data of compost application on plants are challenges for diapers composting and compost produced utilization. According to Rudnik (2019a), some polymers can be degraded and even composted by a suitable condition and microorganisms. The compost produced can be used in many applications. Since composting is so meaningful to reduce the waste generation, to cut off the harmful exposure to human health and the environment. Also, to produce a valuable product as waste bioconversion result, this method is worthy of continuous improvement. This study objective is to present a description of the disposable diapers characteristics of the material and waste, the hazard risk to the environment and human, the recent technologies used for their disposal or recovery, and the cyanobacteria potential as optimal biological alternative for treatment. The main focus of the review is composting treatment using cyanobacteria. Due to their ability to carry out nitrogen fixation and carbon sequestration, to survive in a wide-range habitat including stressed environmental conditions, to perform bioremediation, and reported to be used as high-quality fertilizer. This review is part of a master thesis, carried out at the Sepuluh Nopember Insititute of Technology (ITS), in Surabaya, Indonesia during 2020 – 2021.

Disposable diaper waste

The life cycle of disposable diapers divided into four such as: 1) acquisition of raw materials, 2) manufacturing processes, 3) distribution and consumption of products, and 4) disposal of the products (Edana, 2008). It requires a significant amount of natural resources and energy, also cause some environmental impacts and risk to human healths. Several studies carried out to measure the composition of disposable diaper waste. A study showed that the weight average of diaper waste is 212 grams (Colón *et al*., 2013), while another study concluded the faeces content is around 192 grams per diaper (Budyk and Fullana, 2019). Disposable diaper waste composition consist of 88% human excreta (urine and faeces), and 12% diapers material itself, that is Cellulose, Super Absorbent Polymer (SAP), Polypropylene (PP), Low-Density Polyethylene (LDPE), and others. Fig. 1. illustrates the percentage of disposable diaper waste composition in general.

Hazard risk of disposable diaper waste

Human waste is considered quite dangerous for health because it contains pathogenic microorganisms that can increase the disease transmission. If it directly discharged into open environments and water bodies, the high nutrient content in human waste can cause detrimental effects such as eutrophication, then global warming and climate change, as well as water pollution and lack of clean water (Senecal *et al.,* 2018). The ammonia oxidation process that requires large amounts of oxygen can decrease the dissolved oxygen concentrations in the waters, and it is harmful to aquatic organisms (Rochmah and Mangkoedihardjo, 2020). Dispose off diapers in the rivers led to waterborne diseases, one of the leading causes of mortality in developing countries, where diarrhoea globally accounts for more than 1.5 million deaths each year (Garg *et al.,* 2018). SAP is the second-largest component in disposable diapers material made of inert polymer material, namely polymerized acrylic acid, in granular form derived from petroleum which can absorb and hold liquids. SAP allows the surface in contact with the skin to remain dry. A study concluded that there is no systemic human safety problem for acrylic acid residues present in SAP (Dey *et al.,* 2016). However, for the environment, the sodium polyacrylate content by the extensive use of disposable diapers has caused a growing environmental impact (Wang *et al.*, 2019). Disposable diapers also contain plastic polymers such as LDPE formed by the monomer ethylene, and PP by the propylene monomer. Both types of ethylene and propylene monomers can trigger flammable gases, and to human health, ethylene can cause drowsiness or dizziness (Lithner *et al.,* 2011). Plastic debris, even microplastic in particular, could give complex impact, such as worsens aesthetic values of environment, deteriorates water resource quality, threats aquatic biota biodiversity, and human health (Lestari and Trihadiningrum, 2019). The gases emitted by the combustion of plastic wastes cause extreme risk for respiratory disorders and air pollution. The deposition of plastic debris in the soil leads to soil decay, soil corrosion and landslides, threats domestic animals by consuming plastic, decreased water permeability, while in the sea, microplastic consumed by marine organisms pose a threat to human beings. Therefore, these pollutant impact has to address carefully to identify the associated hazards (Vaikarar *et al.,* 2019). Considering the occurrence of microplastics in diaper waste, the exposure and how it occurs is still not widely known. It can be such consideration to do further investigation.

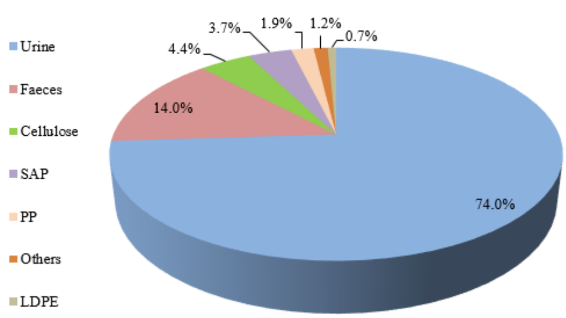

Fig. 1: Disposable diaper waste composition
(Modified from Budyk and Fullana, 2019)

Conventional disposal methods for disposable diaper waste

Due to its high volume, disposable diaper waste has become concern across the globe in recent years. In Malaysia, disposable diaper waste is 12.1% of total municipal waste (Khoo *et al.,* 2019), in Mexico is about 6.5% (Espinosa-Valdemar *et al.*, 2014). In Surabaya, one of the largest city in Indonesia, around 10.79% of the plastic waste pollutes the river, some of it is disposable diapers (Lestari and Trihadiningrum, 2019) where the diapers itself account for 6,97% of total municipal waste (Dhokhikah *et al.*, 2015). With the large volume of diaper waste, a proper handling method required at the disposal stage. The final disposal method commonly used for disposable diaper summarized in Table 1 is in landfills, incineration, and recycling facilities.

There are problems in handling diapers disposal due to the gap between developed and developing countries, viz. science and technology, the low awareness of the harmful effects of disposable diapers in developing countries, and the cost available to finance the waste management. That explains why the current diaper recycling facilities are only available in developed countries. Recycling facilities require the highest-cost compare to landfills and incinerators. It is for financing the technology, facilities investment, recycling operational, and worker health and safety.

Table 1: Summary of Conventional Disposal Methods for Disposable Diaper

Methods	Landfilling	Incineration	Recycling facilities	References
• Advantages	• The trash is immediately out of sight after being covered with a landfill cover (1) • The landfill can operate for a long-term (≥ 50 years, ≤ 100 years) (1)	• Completely burnt out wastes in a short time (1) • Potentially destroy any material containing organic carbon including pathogens (2) • Heat of combustion can be recovered and used to generate steam or hot water (2)	• Diaper waste can be separated and recycled for other purposes (1) • Reduce carbon emissions up to 71% (1)	(1) Khoo *et al*., 2019 (2) Shaaban, 2007 (3) Zhou *et al*., 2020 (4) Kim and Cho, 2017 (5) Kumar *et al.,* 2017 (6) Kerdsuwan *et al*., 2015 (7) Hoffmann *et al*., 2020 (8) Lavigne *et al*., 2014
• Limitations	• High cost in waste collection and transportation (3) • Disposable diapers would require up to 500 years to be fully degraded in landfill (1) • Pathogen contain in diaper waste can cause infectious diseases to the people nearby or landfill workers (1) • When the landfill capacity is full, it has to be shut-down, and post-decommission monitoring is needed (1) • Caused environmental problems such as land degradation, soil erosion, water and air pollution, unpleasant odours, and the greenhouse effect (4) • Require of large land space (5)	• High cost in waste transportation and collection (3) • Part of the ash produced is hazardous (1) • Constant maintenance of incinerator is needed to maintain incineration efficiency (1) • May release flue gas emissions, air pollution, and some hazardous greenhouse gases like furans and dioxins, which play a significant role in ozone layer depletion, and cause harmful problems in the human health and soil pollution (5) • The high moisture content and low heating value of the unseparated wet organic waste, causing high operating costs since the incinerator needs to run with additional fossil fuel (6)	• Additional cost for diaper collection is needed (1) • The complicated recycling processes aggravated by human excreta that attached to disposable diapers (4)	
• Example	• Brazilia (7), Malaysia (1), Indonesia (8)	• China, Japan, Sweden (1)	• Belgium, Singapore, United Kingdom (1)	
• Operating cost for one ton of waste (USD)	• $12.72 (1)	• $10.94-12.50 (1)	• $60 (1)	

Moreover, creating valuable recycled products is also a challenge because of the high level of uncertainty about the marketability and acceptance of these recycled products (Khoo *et al*., 2019; Kosemund *et al*., 2009; Mendoza *et al*., 2019). For the developing countries or low-income countries, with insufficient budget, non-sanitary and uncontrolled landfill used as the waste disposal method. At the local level, especially for areas that generate less than 5 tons per day, open dumping or open burning is the most common waste management done by the community (Kerdsuwan *et al.*, 2015). Inadequate waste collection systems of urban residents also causing waste is not transported to landfills but disposed of in the sea, rivers, and drainage (Handayani *et al.*, 2019) which end up with river sedimentation, blockages of drainage channels and watergates, result in flooding. In some extreme cases, can even lead to breaking the dam (Bott and Braun, 2019). Some researches reveal disposal methods of several countries. In Brazil, only around 40% of municipalities in the country is sent to sanitary landfill, since approximately 60% still lack proper waste management (Hoffmann *et al*., 2020). While in Vietnam, per 2014, the current practice of solid waste management in Ho Chi Minh City is landfilling about 86% of the total solid waste, and the rest 14% waste is recycled (Verma *et al.,* 2016). Out of the whole volume of waste produced in Bandung, Indonesia, in 2005, only 46% collected to landfill. Most of the uncollected waste dumped in the rivers (mainly the Citarum River), burned by the community or buried in the backyard (Lavigne *et al*., 2014). It expected that the number of untreated waste continue to decline along with the increase in waste handling. The Indonesian government targets to achieve 70% waste handling by 2025. It stated in the Indonesian National Policy and Strategy for Waste Management (Presiden Republik Indonesia, 2017). With some of the descriptions presented above, regarding the method of waste disposing, especially for diapers, it is clear that waste recycling is the best choice for environmental sustainability. However, not all countries can have these recycling facilities for the reasons previously mentioned. A waste management system can be sustainable if it meets the following essential factors (Edana, 2008).

Human health and safety; environmentally beneficial; economically affordable; and accepted by the community. If a treatment method proves to be effective in treating waste, but is financially burdensome or cannot apply by the community, then that method will stop running. Considerations for choosing a treatment method may differ based on the potential of each country. Therefore, it is necessary to carry out further studies to find sustainable processing methods, especially for countries that do not have sufficient waste management budgets. For better outlook in sustainable waste management system in the future, increasing the authority for handling waste to the Local Government will give positive-impact, since each region has its strengths, both in different resources and potential budgets. Likewise, community participation is an absolute way. Experience has shown, that in developing countries, the Central Government finds it difficult to implement a well-established waste management system without understanding and community involvement. Public participation is not a new thing in waste management. Lots of research on this subject has done, one of them is in Indonesia and India. Sorting, recycling and composting are main waste management activities done by local community using the 3R principles (Reuse, Reduce, and Recycle) (Dhokhikah *et al.*, 2015, Kerdsuwan *et al.*, 2015, Basu and Punjabi, 2020). Rather than seeing each other as an opposite, it would better the local government and the community co-operate in a harmonious relationship towards a better quality of life.

Recent technologies used for recycling disposable diaper waste

Disposable diaper waste can be treated biologically or by thermal methods. These are some processing technologies that summarized from prior research:

1. Biodegradation of by microorganisms (Moharir and Kumar, 2018),
2. Biodegradation by the cultivation of the fungus Pleurotus ostreatus (Espinosa-Valdemar *et al.,* 2011),
3. Biodegradation into compost (Mendoza *et al.,* 2019),
4. Anaerobic digestion (Torrijos *et al.*, 2014),
5. Microwave pyrolysis (Lam *et al.,* 2019),
6. Hydrothermal carbonization or wet pyrolysis (Budyk and Fullana, 2019), and
7. Steam sterilization (Maamari *et al*., 2016).

Degradation of plastic by microbes is one of the eco-friendly and innovative methods shortly. Biodegradation with the suitable microbial species is an effective-option for eco-friendly plastic waste degradation even though no protocol has developed yet to biodegrade polyethylene on a commercial scale (Kumar *et al.,* 2017, Moharir and Kumar, 2018). Among those treatment options, composting has gained attention for its benefits, such as waste hygiene, cost-effectiveness, and waste conversion into value-added products (Onwosi *et al.*, 2017). So according to the author, the improvement of this method by combining composting and microorganism addition, is worthy of continuous efforts.

Composting methods

According to Palaniveloo et al., (2020), there are some composting methods such as vermicomposting, windrow composting, aerated static pile composting, and in-vessel composting illustrated in Table 2.

From the two options for large scale composting: windrow and aerated static pile, windrow appears to be most appropriate for low-cost waste algae stabilization, while the in-vessel composting is suitable for studying algal composting at lab or pilot scale (Han *et al.,* 2014). As for diaper composting, the in-vessel and aerated static pile methods have been used in previous studies as it mentioned in Table 3.

Disposable diapers composting

Composting is a promising method to treat diaper waste for the following reasons. Composting recover nutrients from human waste (Tucho and Okoth, 2020). Cellulose is the biodegradable polymer made from renewable resources (Rudnik, 2019a), which an aerobic composting consider to be an eco-sustainable green approach for lignocellulosic biomass treatment and agricultural development (Harindintwali *et al.,* 2020; Wu *et al.,* 2020). According to previous research, SAP has the technical feasibility to improve water retention in soils and irrigation capabilities (Al-jabari *et al.*, 2019). In terms of plastic polymers LDPE and PP, require further research to obtain the optimum method for the possibility of its degradation through composting by inoculating suitable microorganism. As mentioned in Tables 1 and 2, regarding the high additional costs of transporting waste to landfills, incinerators, recycling centres, or city-scale composting, also because diaper waste contain various harmful pathogens, and the diaper can completely degraded after 500 years in the

Table 2: Characteristics of Composting Techniques

Characteristics	VermiComposting	Windrow Composting
Preferred waste input	Wastes mixed with manure	Wastes with less emission of odour such as plant-based
Land requirement	Low	High
Site selection	Anywhere equipment can be placed	Away from populated area
Cost of waste transportation	High	High
Composting period	Short	Long
Amendment	Addition of bulking agent, animal manure and microbial additives, consistent temperature	Addition of bulking agent, chemical additives and microbial additives, increase of aeration
Amendment effects on compost	Increase in production biomass by more than 20%	Reduce the total composting period by 30%
Compost quality	Good	Medium
Characteristics	**Aeratic Static Pile**	**In-Vessel**
Preferred waste input	Waste with more homogeneity, consistency and required bulking agent	Easily degraded wastes such as food waste
Land requirement	Medium	Low
Site selection	Away from populated area	Anywhere equipment can be placed
Cost of waste transportation	High	High
Composting period	Long	Short
Amendment	Addition chemical additives and microbial additives, increase of aeration	Increase the in-vessel temperature, pressure and turning rate
Amendment effects on compost	Reduce the total composting period by more than 30%	Reduce the total composting period by more than 30%
Compost quality	Medium	Good

landfill (Khoo *et al.,* 2019). Moreover, the COVID-19 pandemic era nowadays requires everyone to adapt to new life to avoid transmission of COVID-19. This new life is related to compliance with health protocol in every activity (Samudro and Mangkoedihardjo, 2020). It is critical to cut the disease transmission might be contained in domestic waste. As stated in Table 1, that pathogen in diaper waste can cause infectious diseases to the residents around the landfill area or landfill workers, so centralized waste facilities are not very efficient in handling diaper waste. The household-scale composting model provides a reliable alternative solution to address the growing waste problem. Household-scale composting saves labour costs, transport and waste sorting, compared to centralized composting systems (Zhou et al., 2020). About 87% of diaper mass reduction noted through this small-scale composting system (Espinosa-Valdemar *et al.,* 2014). Despite the difficulties in implementing household-scale composting, among others (Bernal *et al.,* 2017, Awasthi *et al.,* 2020): relatively high salt content in compost, animal disturbances, leachate and unpleasant odours due to the production of ammonia (NH_3), nitrous oxide (N_2O) and methane (CH_4), household scale composting can play a role in helping local governments achieve targets in increasing the rate of waste recovery and diverting waste from landfills (Faverial and Sierra, 2014). The innovation to handle diaper waste by composting is necessary to pursue. Table 3 shows the comparison of several studies conducted in disposable diapers composting.

Some issues remain to address include time duration, plastic polymers that do not decompose, pathogens in compost, and lack of data about compost application on plants. The initial C/N ratio of diaper waste which is high, namely 92.09, is a challenge in composting this waste (Simamora and Pandebesie, 2019). Likewise, the diaper waste is flushing (in terms of solid, liquid, semi-solid, both wet and dry condition). This problem overcome by combining composting materials or what is commonly called co-composting, which is known to have many benefits in composting. The wet material or too dry material, can be mixed with other suitable materials to obtain the initial conditions optimum

Table 3: Comparison of Disposable Diapers Composting Results

No.	1	2	3	4
Main composting feedstock	Diaper + Garden waste	Diaper + Garden waste + food waste	Diaper + Garden waste + food waste	Diaper + Vegetable waste
Regulator	-	-	-	Urea
Bulking Agent	-	Sawdust	Rice husks and bran	Rice husks
Microbial Agent	-	-	-	EM4
Optimum variation Diaper waste to organic waste	3:7	3:7	4:6	3:7
Composting method, Reactor	In-Vessel, HDPE plastic	Aerated static pile with pipes on the floor to provide aeration and to collect leachate	In-Vessel, Takakura baskets	In-Vessel, A plastic barrel with a hole at the reactor bottom to remove leachate
C/N Ratio	11	14	10-20	10-20
Pathogen in compost	Below the regulatory standard	Below the regulatory standard	above the regulatory standard at 4 of 5 reactors	not mentioned
Phytotoxicity test	On tomato plants, no phytotoxic effects detected	-	-	-
Processing time	12 weeks	106 days	90 days	60 days
References	(Espinosa-Valdemar *et al.,* 2014)	(Colón *et al.,* 2013)	(Zulfikar *et al.,* 2019)	(Simamora and Pandebesie, 2019)

for aerobic microorganisms activities in composting. Table 3 describes the materials that used in diaper co-composting research. Column number 2 is a study conducted in a city composting centre, where garbage is collected door to door. It allows the condition of the diaper waste to be inhomogeneous. However, composting for 106 days is running well. Further explanation regarding co-composting is in the next section.

The role of diverse microorganisms in the composting process

Composting is a biological conversion process where the organic material undergoes degradation by various groups of microorganisms under controlled environmental conditions in the presence of air into a stable humus-like substance that applied to the soil without affecting the environment (Bernal *et al.,* 2017; Onwosi *et al.*, 2017; Rudnik, 2019b). Co-composting is composting more than one organic waste together, for example, food waste with manure or with lignocellulosic biomass, whose benefits are not only to balance the C/N ratio, but also to increase aeration and reduce leachate (Lin *et al.,* 2019). Another example, a mixture of animal manure as a regulator, with materials from agricultural waste as a bulking agent (Said, 2020). Co-composting is a way to gain varies microorganisms in the composting system (Baharuddin *et al.,* 2009). Since the addition of compost additives determines the duration of composting, humification, and product quality, it is essential to select additive types and combinations carefully to ensure optimal effect. Various compost additives divided into bulking agents, regulators, and microbial agents. The bulking agent is a material to provide a support structure and air pores for the pile of materials. Regulators can speed up the composting rate and improve the final product quality. The addition of microbial agents expected to improve composting performance (Guo *et al.,* 2019). Although theoretically, composting does not require a special inoculum because aerobic microbes abundant in organic waste provide enough microorganisms (Bernal *et al.,* 2017), the inoculation with a suitable microbial source yielded more advantages composting performance (Harindintwali *et al.,* 2020). The diverse-combination can be applied because the mixed inoculants generate appealing results (Flores-felix *et al.,* 2019). Some of the microorganisms commonly used in the composting process are bacteria, actinomycetes, fungi (Wei *et al.,* 2007), and algae (Han *et al.,* 2014). Among the various microbial population associated with polymer degradation, only a few microbial strains capable of cellulase enzyme secretion and solid-waste degradation through cellulose hydrolysis are in light. Cellulomonas, Bacillus, Pseudomonas, Rhodococcus, Thermoactionmycetes, and Staphylococcus are the prevalent bacterial genera. Likewise, fungal species Aspergillus, Trichoderma, Sclerotium, Penicillium and white-rot fungi, are the dominant fungal genera that also produce extracellular enzymes accountable cellulose and lignin degradation during composting (Khoo *et al*., 2019, Rastogi *et al.,* 2020).

The benefits of microorganisms in the composting process are as follows:

1) Increase the mineralization of organic carbon, improve the degradation of lignocellulose, promote the humification process, accelerate the compost maturation process (Guo *et al.,* 2019; Wei *et al.,* 2007; Xu *et al.,* 2019).
2) Accelerate composting time, producing pathogen-free compost, possibly increasing the production of different enzymes resulting in a better waste degradation rate (Hashemi and Han, 2018; Rastogi *et al.,* 2020).
3) Play a role in plastic degradation (Jaiswal *et al.*, 2019; Moharir and Kumar, 2018), even microplastics (Yuan *et al.,* 2020).
4) Removal of harmful toxins or pollutants such as PCDD / F (Polychlorinated dibenzodioxin and dibenzofuran) (Huang *et al.,* 2019), dan microcystin (Han *et al.,* 2019; Han *et al.,* 2014).

The inoculation timing, whether at the initial stage, thermophilic, maturation stage, or multi-stages, affected the process to produce compost with the expected quality. Inoculation at 0, 120, and 180 days show that, lignin degradation paced maximum at the maturation phase for all the treatments (Bernal *et al.,* 2017; Rastogi *et al.,* 2020). Bacteria community play critical roles in the thermophilic and post-aeration phase while fungi take over in the maturing phase (Arab *et al.,* 2017). Nitrogen-turnover bacterial agent or nitrogen-altering bacterial inoculation at the beginning of composting, results in a high organic carbon degradation, nitrogen-loss reduction, and the compost quality improvement (Han *et al.,* 2019). Meanwhile, white-rot fungi inoculation during the thermophilic stage was effective than in the mesophilic (Lin *et al.,* 2019). During maturation, microorganisms that control plant diseases inoculated into the compost (Bernal *et al.,* 2017). An effective Microorganism (EM) inoculated compost attains better compost quality and maturity in less process time. These EM's can be isolated from various of conventional sources, such as soil, waste material or leachate and applied to the process at different stages (initial, mid or last) (Rastogi *et al.,* 2020). In terms of composting diaper waste, the commercial EM4 addition, as stated in Table 3, resulted in no effect on the composting process (Simamora and Pandebesie, 2019), the further experiments with different levels of EM4 can be conducted. It is a challenge to define a compost enhancement strategy by adjusting the relevant parameters and add the appropriate inoculants at the right point in time. Enhanced composting performance could mean better organic matter degradation, faster stability time (Arab *et al.,* 2017), and reduce nitrogen-loss (Han *et al.,* 2019). According to the reason, cyanobacteria consider as one of the most suitable microorganisms. Cyanobacteria have the exclusive capabilities called nitrogen fixation (Abatenh *et al*., 2018). Inoculation of filamentous cyanobacteria to compost, accelerate the decomposition rate and provide many nutrients for plant growth (El-Gamal, 2011). Also, the high Germination Index (GI) value (Han *et al.,* 2014). Some research has already declared success in polyethylene biodegradation by alga and cyanobacteria. Besides other microbes, the colonizing algae on the polymer surfaces found to be nontoxic and less hazardous (Sarmah and Rout, 2020), and it has concluded that among different groups of microalgae, the filamentous cyanobacteria are most effective in the biodegradation of polyethylene sheet (Kumar *et al.,* 2017).

Cyanobacteria

Cyanobacteria are a group of bacteria characterized by various structural features (El Gamal, 2010). Cyanobacteria grow autotrophically, heterotrophically, or mixotrophically (Subashchandrabose *et al.,* 2013). With the ability to adapt in diverse environmental fluctuations, making them easy to find everywhere (Yadav *et al.,* 2018). Cyanobacteria exist in almost every terrestrial and aquatic habitat, under high pressure such as UV radiation, drying, and a wide range of pH, temperature, salinity, and water potential conditions (Naik *et al.*, 2019; Pandey *et al.,* 2020). Although these microorganisms appear in most aquatic ecosystems, they can spread to the surrounding environment (Takahashi *et al.*, 2014), especially for cyanobacteria aerosols, which passively transported through the air, and released into the atmosphere from the surface of water and soil, then grow on buildings, trees or roofs (Wiśniewska *et al*., 2019). Cyanobacteria occur in soil by agricultural activities, through irrigation water and plants (Cao *et al.,* 2018). They classified as blue-green algae (Cyanophyta), have a combination of green chlorophyll and blue phycocyanin. The presence of carotenoid and phycoerythrin pigments causes cyanobacteria to be green, turquoise, brown, purple, red, and even black. Taxonomically, they included in the prokaryote group and are gram-negative bacteria (Noreña-Caro and Benton, 2018). Cyanobacteria divided into four orders, viz Nostocales, Stigonematales, Chroococcales, and Oscillatoriales. They further divided into families, subfamilies, genera, and species. Morphologically, blue-green algae identified in various forms, such as filamentous, unicellular, planktonic or benthic and colonies. They have a unique combination of plant-like photosynthesis and microbes ability (Gupta *et al.,* 2013). Cyanobacteria are capable of producing various forms of organic molecules called secondary metabolites which function as defence mechanisms under stressful conditions, facilitate reproduction, and provide the ability to survive in aquatic environments inhabited by various types of predatory macroorganisms. Overall, cyanobacteria produce three secondary metabolites, viz UV protectants, bioactive compounds, and cyanotoxins (Noreña-Caro and Benton, 2018). Several studies have conducted to increase the usefulness of cyanobacteria for human life. The unique characteristics of cyanobacteria, namely their ubiquitous habitat, short generation time, low growth requirements, and ease of genetic manipulation, make them prominent candidates in various biotechnological applications (Yadav *et al.,* 2018). These microorganisms have proven their diverse roles by offering many benefits to the environment and also their application for several valuable products, including bioenergy (such as bioethanol, biodiesel, and biohydrogen), bioremediation (ex wastewater treatment, nutrient recovery or disposal, pesticide remediation), and bioproducts made from secondary metabolites (such as pigments, vitamins, and biopolymers, and other products related to the food industry, feed additives, cosmetics, and pharmaceuticals) (Axmann *et al.,* 2014; Deviram *et al.,* 2020; Gupta *et al.,* 2013; Patel *et al.,* 2019). There are four classes cyanotoxins, including microcystin, cylindrospermopsin, anatoxin, and saxitoxin (He *et al.,* 2016). Among the diversity of peptides produced by cyanobacteria, only microcystin has studied intensively (Janssen, 2019) where microcystin-LR (MC-LR) is the most prominent because of its distribution and high toxicity (Martins *et al.,* 2017), followed by MC-YR, MC-LA, MC-YM and MC-RR (Herrera *et al.,* 2015). Some researchers declare microcystin as a poison that is harmful to plants (Cao *et al.,* 2018), animals (Al-hazmi *et al.,* 2019; Martins *et al.,* 2017), and possibly humans (Fontanillo and Köhn, 2018; Herrera *et al.,* 2018; Lone *et al.,* 2015; Preece *et al.,* 2017). Anabaena should considered as a source of high levels of microcystin-LR (Dreher *et al.,* 2019).

Potential utilization of cyanobacteria in the composting process

Nitrogen considered the prime nutrient for plant growth is the main component of air making up about 78% of the gases in the Earth's atmosphere, available in highly stable molecular form which plants and animals cannot directly use. Nitrogen fixation is converting atmospheric nitrogen into a chemical substance, namely ammonium through combination nitrogen gas with hydrogen to produce ammonia by microorganisms with the nitrogenase enzyme. Then decomposition stage called the ammonification or mineralization process occurs. It is when decomposing-microbes convert ammonia to ammonium. Furthermore, in the maturation phase, nitrification takes-place as the transformation of ammonium into nitrite then nitrate. Ammonium ion is a source of energy for microorganisms, while nitrite is poisonous, and must immediately convert into nitrate, so can be absorbed by plants (Abatenh *et al.,* 2018; El-Gamal, 2011). The nitrogen cycle occurs in the composting process illustrated in Fig. 2.

Thereby nitrogen loss is an essential aspect of the composting process from a nutrient conservation and environmental perspective. Poorly managed composting of organic waste contributes to the loss of nutrients by releasing large emissions into the atmosphere and the leachate removal (Bernal *et al.,* 2017). Total nitrogen during composting lost through ammonia and nitrous oxide emissions (Lin *et al.,* 2018; Maulini-Duran *et al.,* 2014). Cyanobacteria recover the ammonia in composting process (Koyama *et al.,* 2020). A study concluded that composting plays a significant role in blue-green algae enrichment (Pawar

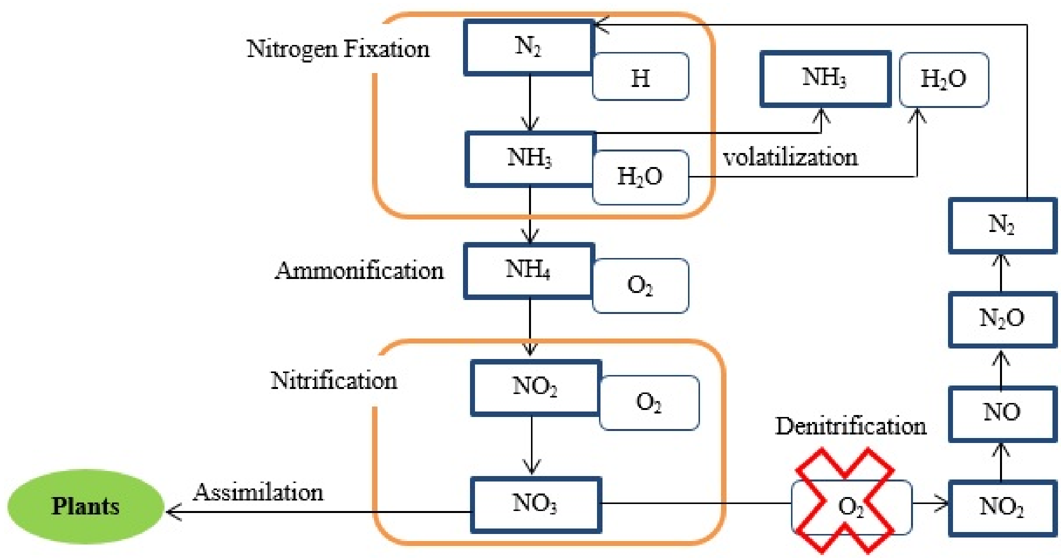

Fig. 2: The Nitrogen cycle occurs in the composting process

and Suryawanshi, 2016). Compost enrichment by blue-green algae significantly improve the nitrogen content (El-Gamal, 2011). Then, this is a mutually beneficial relationship between composting and cyanobacteria. Consideration in cyanobacteria use in composting strengthened by their resistance to high temperatures which needed to eliminate pathogenic bacteria found in organic waste. Cyanobacteria's resistance in high temperature proven by their abundance in the thermophilic phase with a temperature setting of 50-70 °C (Koyama *et al*., 2020). They reported present from early thermophilic until in mature compost (Tumuhairwe and Tenywa, 2018). Thus, the thermophilic condition can eliminate harmful pathogens without affecting cyanobacteria growth in composting system. Moisture is a dominant factor impacting growth for most cyanobacteria and microbial activity in the composting process. Water content had a significant effect on cyanobacteria growth in compost material. High moisture level resulted in nitrogen loss in compost, due to the anaerobic conditions from the waterlogged pore spaces obstructing composting processes, and also caused by denitrification process. Increasing moisture content also resulted in organic matter decreasing. The 50% moisture composition considered appropriate for cyanobacteria growth and the optimal degradation rate (Baftehchi *et al.,* 2007; El-Gamal, 2011). The carbon to nitrogen ratio (C/N ratio) of the organic material to be composted is an important factor. A higher initial C/N value may result in a long time to stabilize the compost material and shift the microbial community to include higher relative abundances of fungal biomass. A low initial C/N ratio degrades material quickly, means lots of available nutrients, but can result in nitrogen loss via ammonia emission, also the generation of unwanted odours. Generally, Blue-Green Algae has a relatively low C/N ratio, typically ranging from 4,9 to 6,06. An effective way to adjust the algal-based feedstock to have a suitable C/N value (around 30) is co-composting with a high C/N value material. Sawdust, wood chips, bark, straw, hull, bran, and animal manure commonly added as algal co-composting materials (Han *et al.,* 2014). Therefore, cyanobacteria addition to the composting of diaper waste with a high C/N ratio is proper. Cyanobacteria enrich nitrogen to the composting system that will raise the optimum C/N immediately. It also means accelerate the compost maturity. Microcystins are the obstacles for utilizing cyanobacteria since in under natural condition, cyanobacteria contain high contents of microcystins. Some microcystins residues inevitably left in cyanobacteria fertilizer. Microcystins accumulation cause the problems of food safety. However, the standard for the MC-LR in grains and vegetables has not reported yet. The composting process with microbial inoculation such as *Bacillus subtilis, Bacillus licheniformis* and *Bacillus stearothermophilus*, increased the microcystins degradation rate. A study founded the high level of MC-RR and MC-LR in the organic materials mixture without composting. However, in mature compost, it found very low, under the MC-LR limited value in drinking water which is ≤1µg/L (Han *et al.,* 2019). Another study revealed that 90% of both MC-RR and MC-LR degraded by day 48 in cyanobacteria composting. Efficient degradation of microcystins during composting may be due to the wide variety of microorganisms present in compost systems (Han *et al*., 2014).

Cyanobacteria applications for environment and sustainable agriculture

Cyanobacteria are potential oxygenic phototrophic organisms that can aid plant repair in many ways (Abatenh *et al*., 2018). The potential of new concepts of cyanobacteria-based biofilms as biological fertilizers has investigated in the last two decades. Cyanobacteria which are known as nutritional supplements (inoculants), provide high value in agriculture as biofertilizers (Renuka *et al.,* 2018), also bring some extraordinary ecological benefits (Gupta *et al.,* 2013), either individually or in combination with other biofertilizers (Hegazi *et al.,* 2015). Integration of various microorganisms with cyanobacteria produces the best results (Manjunath *et al.,* 2016). Although the use of cyanobacteria as biological fertilizers has known for a long time (Han *et al.,* 2019), recent attention has focused on the function of cyanobacteria secondary metabolites in the control of phytopathogens and their potential applications in crop protection (Yadav *et al*., 2018). Many studies have conducted to study the beneficial effects of cyanobacteria on rice, wheat, soybeans, tomatoes, oats, sugar cane, maize, radishes, muskmelons, cotton, lettuce, nuts and peppers, cantaloupe (Farrag *et al*., 2017; Naik *et al.,* 2019).

The promising functions of cyanobacteria to improve soil fertility and environmental quality noted as follows:

1) Improve soil aggregation, soil structure, soil permeability and water holding capacity of soil (Pawar and Suryawanshi, 2016), thus controlling runoff, decreasing soil loss and water conservation (Sadeghi *et al.*, 2020a; Sadeghi *et al.,* 2020b). The cyanobacteria used: Gloecapsa, Phormidium, Nostoc, and Oscillatoria.
2) Improving soil quality indicators, soil remediation, and soil stabilization (Sadeghi *et al.,* 2020b). The cyanobacteria used: Nostoc and Oscillatoria.
3) Increased soil biomass after cyanobacteria die and increased soil porosity due to fibrous structures (Renuka *et al*., 2018; Yadav *et al.,* 2018). The cyanobacteria used: Anabaena and Nostoc.
4) Increase soil fertility by releasing growth-promoting substances such as hormones, vitamins, and amino acids (Yadav *et al.,* 2018) and IAA (indole-3-acetic acid) (Chatterjee *et al*., 2019).
5) Increase in macros and micronutrients content in grains (Pandey *et al*., 2020), leaves (Rashad et al., 2019), straw (Eletr *et al.,* 2013), root (Menamo and Wolde, 2015), also in the rhizosphere of plants (Manjunath *et al.,* 2016; Prasanna *et al.,* 2016).
6) Utilized as a biocontrol agent against several pathogenic microbes, weeds, insects, fungal diseases. *Spirulina platensis* showed more allelopathic activity over other cyanobacterial strains (Dukare *et al*., 2011; Prasanna *et al*., 2015; Renuka *et al.,* 2018; Yadav *et al*., 2018).
7) Used as a bioremediation agent to remove toxic compounds such as toxic chemicals, heavy metals, and other pollutants in contaminated environments (Pandey *et al.,* 2020; Renuka *et al.,* 2018), including pesticides, lindane (Chatterjee *et al.,* 2019), salt (Li *et al.,* 2019), chemical fertilizers (Burjus *et al.,* 2020). Tolerant strains such as: Fischerella, Anabaena, Nostoc, Oscillatoria.
8) It used to detoxify industrial waste (Patel *et al.,* 2019), for example, a biological template for the synthesis of metal nanoparticles (Pandey *et al.,* 2020). The cyanobacteria used: Phormidium.
9) Reducing greenhouse gases from the environment, for example, methane mitigation (Malyan *et al.,* 2016), and carbon sequestration due to cyanobacteria showing a higher photosynthetic efficiency compared to land plants (Pandey *et al.,* 2020). *Synechocystis sp.* are one of the most beneficial for lowering methane emission from soils, presumably aiding methane oxidation via oxygenation during photosynthesis (Shankar and Strong, 2016).
10) The decomposition of polyethylene sheets by cyanobacteria in the form of filaments, where the enzymatic activity of microorganisms and breaking the polymer bonds are the main steps (Kumar *et al.,* 2017; Chia *et al.,* 2020). For example: Anabaena.
11) Reducing the chemical fertilizer utilization (Burjus *et al.,* 2020; Hegazi *et al.,* 2015).
12) Can be applied together with soil substitute planting media made of minerals such as perlite, vermiculite (Prasanna *et al.,* 2020) and biochar (Kholssi *et al.,* 2017).

According to Rudnik (2019b), compost applicable for fertilizer, soil improver/conditioner, manufactured topsoil, growing medium, mulch, and even as a landfill cover. Compost can be used in various purposes depending on the quality. High-quality compost is used in agriculture, horticulture, landscaping and home gardening, while medium-quality compost used in applications such as erosion control and roadside landscaping. Low-quality compost can be used as a landfill cover or in land reclamation projects. Moreover, with the greening trend during the COVID-19 pandemic, where people spent more home activities, the compost requirement will increase. Environment greening by the community, will lead them to participate sustainably to maintain personal and environmental health. So the diaper composting at home-scale, then used for home gardening could be one solution to the purpose: eliminating toxicants, and selected biodiversity according to local preferences and conditions (Samudro and Mangkoedihardjo, 2020). With the advantages of cyanobacteria applications previously stated, these microorganisms addition to the composting system will expand the resulting diaper compost utilization for a wide range of environmental benefits.

CONCLUSION

Nowadays, where people move dynamically and well sanitation is necessary, disposable diaper is unavoidable. The current challenges lead to the development of solutions, that guarantee both ecosystems sustainability and human life quality. Concerning the problems associated with disposable diapers pollution, there have been many technological strategies developed, such as biodegradation and thermal methods, besides disposal to landfill. Composting strategies have evolved over the years. It is a route led by biodegradation methods done by microbial activities affected by the physical and chemical condition, such as oxygen, moisture, nutrition, and particle size, which progressed towards environmentally friendly and profitable biological solutions. The mixed inoculants of different microorganisms will be the right way to solve composting challenges. Co-composting with various organic materials is required to balance the carbon sources and nutrients, also provided adequate moisture to optimize diaper waste degradation. Due to the diversity of species, widely available in nature, can grow everywhere, with highly adaptive ability, also its unique combination, to do nitrogen fixation and carbon sequestration by photosynthesize like plants but in the other hand has microbial capabilities, making cyanobacteria potentially suitable candidates for various purposes. Furthermore, their possibility to decompose plastic polymers, reinforces the reasons for cyanobacteria utilization in diapers composting. Based on the literature review carried out, the authors believed that cyanobacteria are promising candidates for enhancing disposable diaper composting in providing advantages as follow: 1) Improvement in composting performance, include: reducing composting time, increase the decomposition rate of compost, eliminating pathogens and toxic compounds, breaking down plastic polymers contained in diaper waste, and minimizing nuisance animals. 2) Production of valuable product that used for several applications. 3) Environmental protection by reducing waste disposed off into the soil and water bodies, greenhouse gasses mitigation, land remediation and restoration, biodiversity and water conservation. Nevertheless, it is clear that a more detailed investigation required to prove the viability concept of this emerging option. Further research to address challenges should include integration, optimization, modification, and social-economics aspect assessment. Those might contain several things as follows: the selection of cyanobacteria species, the planning of appropriate methods, the determination mixing variation of the material composted, the calculation of investment and operational costs, and the market share research willing to accept the compost produced. Regarding the risk of harm to humans and the environment, the quality of compost in terms of stability and maturity should be assessed respectfully, by parameters such as physics-chemistry parameters, pathogen and phytotoxin content. Planting experiments on various types of plants is a fascinating matter to researched forward. Considering the microplastic accumulation in some plants, according to the authors, is something that needs to find out whether these cyanobacteria can indeed decompose plastic polymers completely or only tear them into microscopic forms. Furthermore, it is also necessary to ensure the absence of microcystin in the compost.

AUTHOR CONTRIBUTIONS

D.I. Kusumawati performed the conceptualization, literature review, writing-original draft, visualization, administration, obtaining funding. S. Mangkoedihardjo also performed the conceptualization, methodology, supervision. critical revision of the manuscript for important intellectual content.

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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	Carbon
CH_4	Methane
C / N ratio	Carbon to nitrogen ratio
EM	Effective microorganism
H	Hydrogen
H_2O	Water
IAA	Indole-3-acetic acid
LDPE	Low-density polyethylene
MC	Microcystin
N	Nitrogen
N_2	Nitrogen
NO	Nitrogen monoxide
N_2O	Nitrous oxide
NH_3	Ammonia
NH_4	Ammonium
O_2	Oxygen
PP	Polypropylene
PCDD / F	Polychlorinated dibenzodioxin and dibenzofuran
SAP	Super absorbent polymer

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CASE STUDY

Linking the past, present and future scenarios of soil erosion modeling in a river basin

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ABSTRACT

BACKGROUND AND OBJECTIVES: Soil erosion is considered one of the major indicators of soil degradation in our environment. Extensive soil erosion process leads to erosion of nutrients in the topsoil and decreases in fertility and hence productivity. Moreover, creeping erosion leads to landslides in the hilly regions of the study area that affects the socio-economics of the inhabitants. The current study focuses on the estimation of soil erosion rate for the year 2011 to 2019 and projection for the years 2021, 2023 and 2025.

METHODS: In this study, the Revised Universal Soil Loss Equation is used for estimation of soil erosion in the study area for the year 2011 to 2019. Using Artificial Neural Network-based Cellular Automata simulation, the Land Use Land Cover is projected for the future years 2021, 2023 and 2025. Using the projected layer as one of the spatial variables and applying the same model, Soil Erosion based on Revised Universal soil loss equation is projected for a corresponding years.

FINDINGS: For both cases of projection, simulated layers of 2019 (land use land cover and soil erosion) are correlated with the estimated layer of 2019 using actual variables and validated. The agreement and accuracy of the model used in the case land use are 0.92 and 96.21% for the year 2019. The coefficient of determination of the model for both simulations is also observed to be 0.875 and 0.838. The simulated future soil erosion rate ranges from minimum of 0 t/ha/y to maximum of 524.271 t/ha/y, 1160.212 t/ha/y and 783.135 t/ha/y in the year 2021, 2023 and 2025, respectively.

CONCLUSION: The study has emphasized the use of artificial neural network-based Cellular automata model for simulation of land use and land cover and subsequently estimation of soil erosion rate. With the simulation of future soil erosion rate, the study describes the trend in the erosion rate from past to future, passing through present scenario. With the scarcity of data, the methodology is found to be accurate and reliable for the region under study.

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INTRODUCTION

Various naturally occurring resources are available around us, but soil and water resources are considered to be indicative of all. An increase in population in due course of time associates with increasing anthropogenic pressures to these resources. Checking these pressures and opting for practices that focus on the optimized use of resources with minimum stress on the environment can only lead to sustainable development, which is indeed needed in the time ahead (Renard *et al*., 1997). Land degradation is the state due to which the trait of soil is decreased both in physical composition and chemical combination, as an aftermath of a certain phenomenon due to certain factor. Soil erosion is the most significant environmental problem which leads to land degradation. It is defined as the damage of topsoil by natural agents (Gallaher and Hawf, 1997). The loose soil carried away by the agents during the soil erosion process transports anthropogenic toxic substance into drainage systems, which in turns causes other environmental pollutions (Sandra *et al*., 2015). Exposure of the topsoil during rainfall; then detachment of loose particles and deposition of the particles is the stages involved in the soil erosion phenomena. These processes are associated directly with the changing land use and land cover pattern of the region (McCool *et al*., 1978). Thus, soil erosion can be related to changing LULC patterns, changes in topography, soil composition attributed to the area. Manipur lies in the eastern part of the Himalayan range and comprises hilly terrain which covers the majority of the total geographic area, except for the valley in the central portion of the state. Due to its topography and changing LULC pattern and various anthropogenic factors, the state is prone to land degradation. According to the study regarding land degradation, in 2011-2013, 26.96% of the total geographic extent is under degradation. 25.78% of the above-mentioned percentage is due to vegetation degradation and 0.36% is due to soil erosion (SAC, ISRO, 2016). The geospatial technique is a widely used technique for studying the environmental phenomenon because of its ability to integrate the various variable parameters and gives the synoptic view of the study area. These tools and techniques can be applied in the computation of the rate of soil erosion, evaluating processes and understanding its underlying parameters about the region (Jahun *et al*., 2015). The revised universal soil loss equation (RUSLE), Geographic information system (GIS)-based model is one of the most widely used soil erosion model and is improved model of original soil erosion model developed by Wischmeier and Smith, 1978 (Renard *et al*., 1997). Models such as USLE had also been used for assessing the soil erosion rate in the river basin of the hilly terrain of North East India (Ghosh, *et al*., 2013). Physical models such as water erosion prediction project (WEPP), unit stream power-based erosion deposition (USPED) and European soil erosion model (EUROSEM) which requires complex input variables is not applied in this study due to the scarcity of data (Mitas and Mitasova, 2001; Smets, *et al*., 2011; Ahmadi, *et al*., 2020). The use of slope length factor in RUSLE enables to estimate the overland flow of the agricultural area. The model has advantages of limited data requirement, which is important for research in data-scarce regions like the study area and simplicity in computation. Hence, RUSLE is used for the assessment of soil rate in this study. NBSS & LUP, CSWCR&TI and Department of Horticulture and Soil Conservation, Government of Manipur jointly undertook the task for preparation of soil resource map and conducted soil erosion assessment for the state of Manipur, India, using USLE. The assessment detail the soil loss in the state, which also encompassed the study area for the year 1996 and 2006 (Sen *et al*., 1996; Sen *et al*., 2006). In this study, estimation of soil erosion rate in Manipur river basin for the past 9 years (2011- 2019) and predicting the soil erosion rate of 2021, 2023 and 2025 has been the main emphasis. ANN-based CA model has been successfully implemented for predicting the future changes in land use land cover pattern (El-Tantawi, *et al*., 2019; Gharaibeh, *et al*., 2020; Yang, *et al*., 2016). For predicting the future soil erosion rate, a Projected LULC is required, which is done using MOLUSCE simulation. MOLUSCE, a Quantum GIS platform-based tool enables the convenient assessment for land-use change modelling. The model employed artificial neural network (ANN) based cellular automata model for simulation (Rahman *et al*., 2017; Saputra and Lee, 2019). The neural network, which is an adaptive system, is used in the study to estimate the vegetative index of future years. Time series vegetative index data are train and test in the neural network environment (Abujayyab and Karas, 2019). Projected vegetative index named Normalized

Difference Vegetative Index is also employed as one of the inputs in the assessment. The application of the machine learning techniques for the projection of future scenarios; the use of simulated data and integration for projecting another future scenario is the main focus in this study. The primary aim of this study is a) Estimation of soil erosion rate for years (2011-2019), Spatio-temporally; b) prediction of projection of the rate of soil erosion for the year 2021, 2023 and 2025.

MATERIALS AND METHODS

Study area

The Loktak catchment and Chakpi river watershed and Manipur River watershed constitute the Manipur River Basin. Areal extent of basin is about 6872 sq.km, which is almost 31% of the expanse of Manipur state (Fig. 1). It comprises nine watersheds namely Imphal River Watershed, Upper Iril River Watershed, Lower Iril River Watershed, Thoubal River Watershed, Heirok River Watershed, Khuga River Watershed, Loktak Watershed, Chakpi River watershed and Manipur River Watershed. Soil composition of the basin differs along with the topography. Hill soil comprises of red soil due to oxidisation and a usually weathered at the foothills, and valley soil ranges from silt loam to clayey texture. The average precipitation of the basin ranges between 1200 to 1350 mm and temperature between 19°C to 21°C. The elevation of the basin is 720 to 800 m above mean sea level in the valley and rises up to 2684m in the hilly region and average slope of 15 degree. As the basin encompassed almost of all the valley region of the state, the population of the inhabitant is about 90% of the total population of the state of Manipur. Most of the agricultural practices are found in the valley region of the study area and also in some regions of hilly region. Tropical deciduous types of forest are found in the study area. (Trisal and Manihar, 2004).

Extensive percent of total built-ups and agricultural lands are situated in the valley region of the basin. Dynamic nature of built-ups and agricultural land affects the land cover, which is due to increase in inhabitant population which in turn makes land cover susceptible to soil erosion.

Data acquisition and methodology

For input as spatial variables, drainage distance raster and elevation raster is derived from DEM; and road distance raster is developed using the

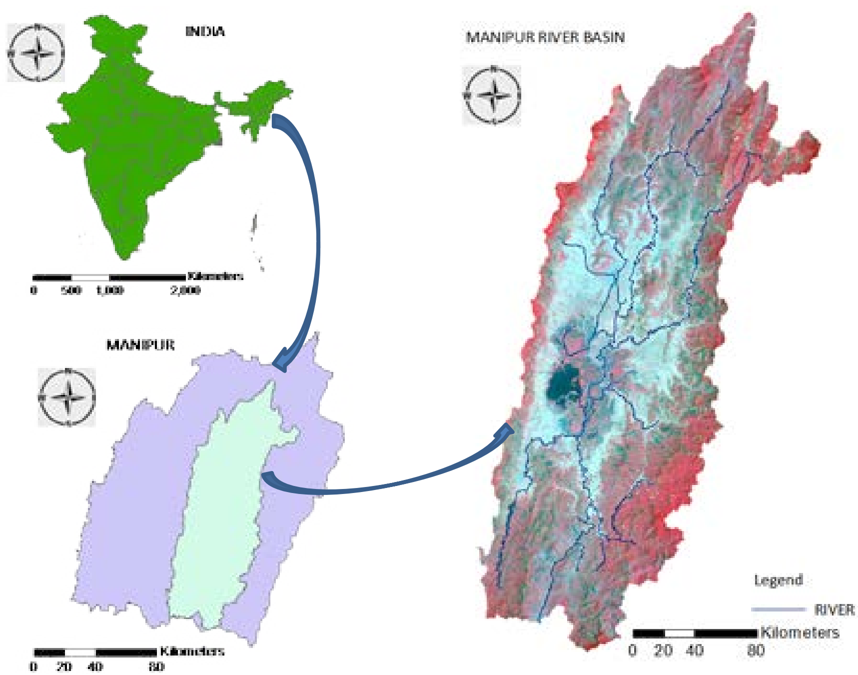

Fig. 1: Geographical location of the study area, Manipur River Basin, Manipur, India

road network layer procured from north east space application centre, India. In both cases i.e. past and future soil erosion assessment, soil layers and topographic features are considered to be constant.

RUSLE soil erosion estimation

RUSLE is based on physical variables such slope length factor; soil erodibility factor; supporting conservation practices factor; crop management factor and rainfall erosivity (Sehgal and Abrol, 1994). RUSLE was originally designed for use in plot size or field size study area but due to its applicability, it is now often used for various scales using Eq. 1 (Panos *et al*., 2014).

$$A = R \times C \times P \times K \times LS \quad (1)$$

Where, A is the rate of soil erosion in ton/ha.year; R is Rainfall Erosivity factor in MJ.mm/ha/ h/y; K is Soil Erodibility factor in ton.ha.h/ha.MJ.mm; LS is Slope Length factor; P is Practice Management Factor and C is Crop management Factor.

Variables used as input for estimation, in the model have varying spatial resolution. The entire inputs variables layer has been resampled to 30m spatial resolution to have spatial homogeneity, and thus the model is processed.

R – Factor

Intensity of the contact of rainfall drops with the ground is signified by the Rainfall Erosivity. Raindrop has compelling effect on soil erosion rate and the surface run-off and is signifies by R-Factor (Wischmeier and Smith, 1978). The gridded precipitation data are acquired from Climate hazards group infrareds precipitation with station data portal for past years and from NASA NCCS downscaled precipitation for future years. Both the acquired datasets have different spatial resolutions (Table 1) which are resample to 30m resolution for estimation of the rainfall erosivity factor. Daily precipitations data is acquire in both the case and are total monthly and eventually yearly manner for implementation in the equation. Eq. 2 describes the Rainfall erosivity equation used in this study using (Arnoldus H.M.J., 1980).

$$R = \sum_{1}^{12} 1.735 * 1010^{(1.5\log_{10}(\frac{Pi}{P}) - 0.08188)} \quad (2)$$

Where, R is Rainfall Erosivity factor in MJ.mm/ha/ h/y; P_i is Monthly Precipitation (mm); P is Annual Precipitation.

K – Factor

Impediment of loose soil against transportation and detachment based on the physical texture and carbon content is estimated as K-Factor. The factor is express in (ton/ha/h)/ (ha/MJ/mm) (Wischmeier *et al*., 1971). The soil of the study area is characterized as deep, rich in organic carbon content, slightly acidic and rich in nitrogen, potassium and low in phosphorous (Sarkar *et al.*, 2002). In this study, the soil physical properties are considered to be constant and remain unchanged, i.e. for past years and for prediction same soil layers are used for the generation of soil erodibility layer. The gridded soil layer dataset namely Sand fraction percent, Silt fraction percent, Clay fraction percent and Organic carbon content is acquired from World Soil Information data portal. The acquired data is process and resample and used for estimation of soil erodibility of past years soil erosion and predicting future soil erosion rate. K- Factor is estimated using the equation, Eq. 3 (Sharpley and

Table 1: Input data and sources

Satellite/sensor/source	Description	Resolution
Landsat-7 ETM+ C1 level1	Year: 2011 - 2013	30m
Landsat 8 OLI/TIRS C1 level 1	Year: 2014 - 2019	30m
CHIRPS*	Precipitation data: year 2011-2019	5.55km
NEX- GDDP dataset*	Precipitation data: Year 2021, 2023 and 2025	~ 25km
MODIS (MOD13Q1)*	NDVI data: year 2011-2019	250 m
World soil information data portal*	Soil physical raster data	250 m
SRTM	DEM dataset, drainage distance layer	30 m
Road network, NESAC, India	Road distance raster layer	30 m

***** Dataset spatial resolution resample to 30m

Williams, 1990)

$$K = ([0.2 + 0.3 \times \exp\{-0.0256 \times Sand \times (\frac{1-Silt}{100})\}] \times (\frac{Silt}{Clay+Silt}) \times \{1 - \frac{0.25 \times org.C}{org.C + \exp(3.75 - 2.95 \times org.C)}\} \times \{1 - \frac{0.7 \times (1 - \frac{Sand}{100})}{(1 - \frac{Sand}{100}) + \exp(-5.51 + 22.9 \times (1 - \frac{Sand}{100}))}\} \times 0.1317) \quad (3)$$

Where, K is soil erodibility factor; sand is sand fraction percent; silt is silt fraction percent; clay is *clay fraction percent* and org. c is *organic carbon content*.

LS – Factor

Topographical factor indicating the distance of the origin point of erosion to the deposition point, as a result of the slope is determined by the slope-length factor. Higher overland flows velocities are attributed to and correspondingly higher erosion (Renard *et al*., 1997; Wischmeier and Smith, 1978). In this study, the physical-based slope length factor is calculated using the satellite-based elevation model data, which is used in RUSLE (Both past years and future years), using Eq. 4 (Moore and Burch, 1986).

$$LS = (\frac{A}{22.13})^m \times (\frac{\sin\theta}{0.0896})^n \quad (4)$$

Where LS is the slope length factor; A is Catchment Area; θ is slope angle in percent (%)

m = 0.4 and n = 1.3

C - Factor

Influences of vegetation coverage in the soil erosion rate estimation are significant. C - Factor is the variable which is defined as the correlation between the soil erosions from cropped land with the defined condition and corresponding erosion till cleaned, fallow continuously (Wischmeier and Smith, 1978). Vegetation index such as NDVI has been employed for the estimation of C- factor, but the equation used for calculation differs according to the geographic region under study. The equation used for assessment in the mid latitudinal region (Van der Knijff, *et al*., 2000) is found to be unsuitable for tropical region and hence different equation is used for this region, using Eq. 5 and 6 (Durigon *et al*., 2014).

$$C = (\frac{-NDVI+1}{2}) \quad (5)$$

$$NDVI = \frac{NIR - RED}{NIR + RED} \quad (6)$$

Where, C is Crop Management Factor Management; NDVI is Normalized Difference Vegetation Index; NIR is Near Infra-Red band; RED is the Red Band of imagery used.

MODIS NDVI dataset, which is of 250 m spatial resolution, is used in this study. The composite NDVI layer for each year is generated and extracted for the study area region and is resampled to 30m for estimation of C – Factor using Eq. 5.

P – Factor

Variable used in RUSLE, which is a correlation between the soil erosion with a specific support practice and the corresponding erosion with upslope and downslope cultivation. Relation between land use variable and slope factor is developed so that the impact on runoff, drainage and velocity resulting from control practice are considered. The assigned value of P-factor to specific land use class slope is given Table 2. (Wischmeier and Smith, 1978). P – Factor layer is generated by combining the LULC layer for each year and slope layer generated from DEM. LULC layer for each years are classified into 'Agriculture' class and 'Other Land Use' class and slope layer is reclassified into six classes as shown in Table 2.

MOLUSCE

The model performs the transition potential modelling based on Markovian approach and uses four different models namely Logistic Regression (LR); Artificial Neural Network; Weight of Evidence

Table 2: Assigned P-factor value for different land use and slopes

P- factor	Land use	Slope (%)
0.100	Agriculture	0 – 5
0.120		5 – 10
0.140		10 – 20
0.190		20 – 30
0.250		30 – 50
0.330		50 – 100
1.000	Other land use	All

(WoE) and Multi-Criteria Evaluation (MCE) for training simulation model (NEXTGIS, 2017). Logistic regression analysis is a statistical approach of training the sample data to develop Relationship between dependent variable and sets of independent variable. Finally, the independent variable which has the best correlation will be selected for prediction. Artificial neural network is an adaptive system which develops the relation between variables so that it could be trained to recognize pattern, classify data and predict the data. Sigmoidal function is used to train the sample data. Weight of evidence training model was initially develop to train only binary model, but was later modified so that it could train continuous data. In this module, the continuous data are categorized into classes (maximum of 100 classes) and weights are estimated to predict the data. Multi criteria evaluation training model is based on Saaty's hierarchal analysis. It is a systematic approach to make decision for complex problems (GIS Lab, 2018). In this study, Cellular Automata - ANN approach is used for predicting future LULC and soil erosion rate for the area under study. LULC layers (Years 2011-2019) are generated using Maximum Likelihood Classification technique employing Landsat multispectral imageries as inputs. LULC of the year 2019, 2017, 2015 and 2013 is used as inputs in the MOLUSCE model to generate the Projected LULC of 2021, 2023 and 2025. Road distance raster layer, Drainage distance layer and elevation are employed as the spatial variable (Driving Factor) for the ANN Transition potential modelling. Pearson's Correlation method is used in the model tool for calculating the correlation among the spatial variables or driving factors, which are in turn used in ANN potential modelling. Projected future LULC is generated using CA Simulation. Road distance raster layer is generated using the road network file and drainage distance and Elevation are obtained from SRTM-DEM. To validate the model and to assess the degree of agreement and accuracy, LULC of the year 2019 is simulated using LULC of the 2017 and 2018. The simulated LULC of 2019 is validated using the actual LULC of 2019, which is generated using Landsat Imageries as mentioned above. MOLUSCE model tool is widely used for projection of the future LULC. However, for this study, the tool is used for simulation and projection of soil erosion class also. As the input layers should be qualitative raster, the quantitative soil erosion rate raster obtained from RUSLE is classified to qualitative form and is used as inputs. Soil erosion datasets of 2019, 2017, 2015 and 2013 are used for projecting the future soil erosion of years 2021, 2023 and 2025. In the case of Soil Erosion simulation, Drainage distance, Elevation, LULC and precipitation for the year to be simulated are used spatial variable or driving factor. Simulated LULCs of the future years are input as spatial variables for each case and future projected precipitation is acquired from NASA Earth Exchange Global Daily Downscaled Projection (NEX – GDDP) precipitation dataset (Thraser *et al.*, 2012). Similar to previous case, Pearson's Correlation method is used in the model tool for calculating the correlation among the spatial variables or driving factors, which are in turn used in ANN potential modelling and simulation is done by CA approach. As in the case of LULC future dataset simulation, validation for the agreement and accuracy of the model is done by simulating the soil erosion dataset of the year 2019 using the soil erosion dataset of 2017 and 2018 and follows by validation using the actual soil erosion dataset of the year 2019.

RESULTS AND DISCUSSION

Soil erosion rate estimation

The estimated soil erosion rate is classified into five soil erosion classes. Classification by NBSS and LUP is adapted for classifying the soil erosion class for this study. According to this adaptation, the classified soil erosion qualitative classifications are 'No Erosion' (<5 t/ha/y); 'Slight Erosion' (5 to 10 t/ha/y); 'Moderate Erosion' (10 to 20 t/ha/y); 'High Erosion' (20 to 40 t/ha/y) and 'Intense Erosion' (> 40 t/ha/y) (Table 3). According to the study adapted above, soil erosion less than 5 t/ha/y shows no effect on the effectiveness of the conservative structure. Thus the tolerable soil erosion limit of the study area is taken to be soil erosion rate less than 5 t/ha/y (Sen *et al.*, 2006). The corresponding area in square km.

Table 3: Soil erosion rate classification limit (based on national bureau of soil survey and land use planning)

Class	Range (t/ha/y)
No Erosion	< 5
Slight Erosion	5 - 10
Moderate Erosion	10 - 20
High Erosion	20 - 40
Intense Erosion	> 40

for the Soil Erosion class for the years 2011 to 2019 is shown in Fig. 2. Even though there is variation in the area under 'No Erosion' class, an increasing trend is observed overall in this class from the year 2011 to 2019. 2013 has lowest percentage with 39.36% and 2018 and 2019 have the highest with 48.69% and 48.57% respectively. 'Slight Erosion' in the study area is observed to follow the increasing trend during the period of study. With percentage 5.10% in the 2011 increases up to 14.53% in 2018 and 13.27% in 2019. Gradual increment with variation is observed in the 'Moderate Erosion' class. Except for 2013, where the percentage declines to 12.40%, remaining years shows the gradual rise in percentage from 22.81% in 2011 to 29.92% in 2017. In case of 'High Erosion' class, a positive response is observed throughout the period under study. The percentage of total area under this class has been gradually reducing since 18.16% in 2011 to 6.30% and 7.13% in 2018 and 2019, except for slight increment up to 18.64% in 2013. 'Intense Erosion' class also indicates positivity, as there is decline in the total percentage of area coverage in this class over the year. From 12.96% in 2011 to 3.32% and 3.80% in 2018 and 2019 respectively, except for an abrupt rise to 25.18% in 2013.

The range of soil erosion rate for the year 2011 to 2019 is depicted in maps in Fig. 3. Quite a variation is observed in the maximum soil erosion rate from the year 2011 to 2019. An increasing trend is followed from the year 2011 to 2015 with maximum soil erosion rate 776.63 t/ha/y to 1253.7 t/ha/y and then decreasing trend follows to 2019 estimating the maximum soil erosion rate of 266.45 t/ha/y (Table. 4).

Future LULC projection using MOLUSCE

Using MOLUSCE model tool, LULC of the future year 2021, 2023 and 2025 of the study area has been projected in this study (Fig. 4). In the projected future LULC, variations in the spatial extent of the land use class is observed as result. Area under 'Vegetation' class shows decrease in area from the year 2021 to 2023 and then grows in the year 2025. Spatial extent of classes 'Agriculture';' Water Bodies' and 'Barren' shows similar trends of change in area. Area coverage increases from 2021 to 2023 and decreases in the year 2025. 'Built Up' class shows growth in the most unique pattern. Extent of area coverage expanse over the time from 2021 to 2025 and is shown in Fig. 5.

In this study, ANN based transition potential modelling is performed and sample data are train. Then, the trained data are used for CA simulation and simulated layer is generated. For validation of the output simulated layer, accuracy assessment, kappa statistics is done and overall accuracy of the model is found to be 96.21% for 2019 simulation. Also, agreement of the model expressed in terms of Kappa Coefficient is observe as 0.92 which is almost perfect model (Landis and Koch, 1977). Regression analysis is perform between area of each classes from actual LULC 2019 and projected LULC 2019 so as

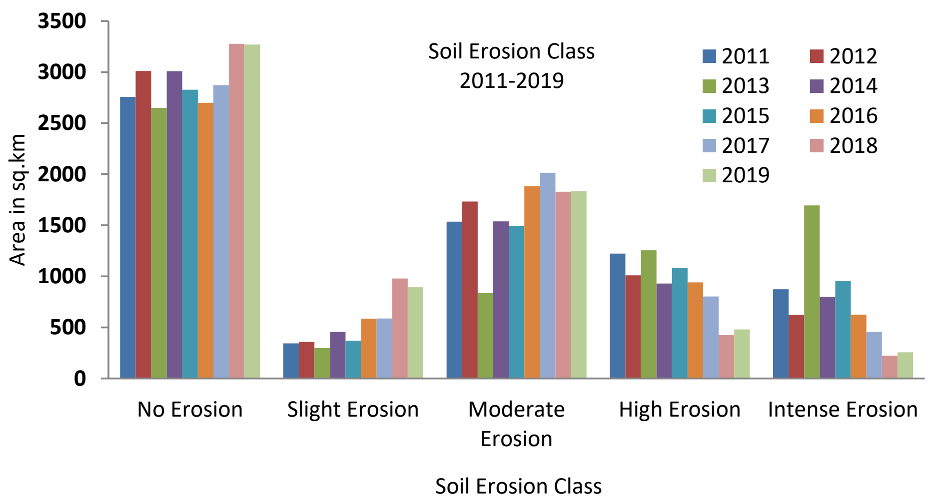

Fig. 2: Area under different soil erosion class in km^2 (2011-2019)

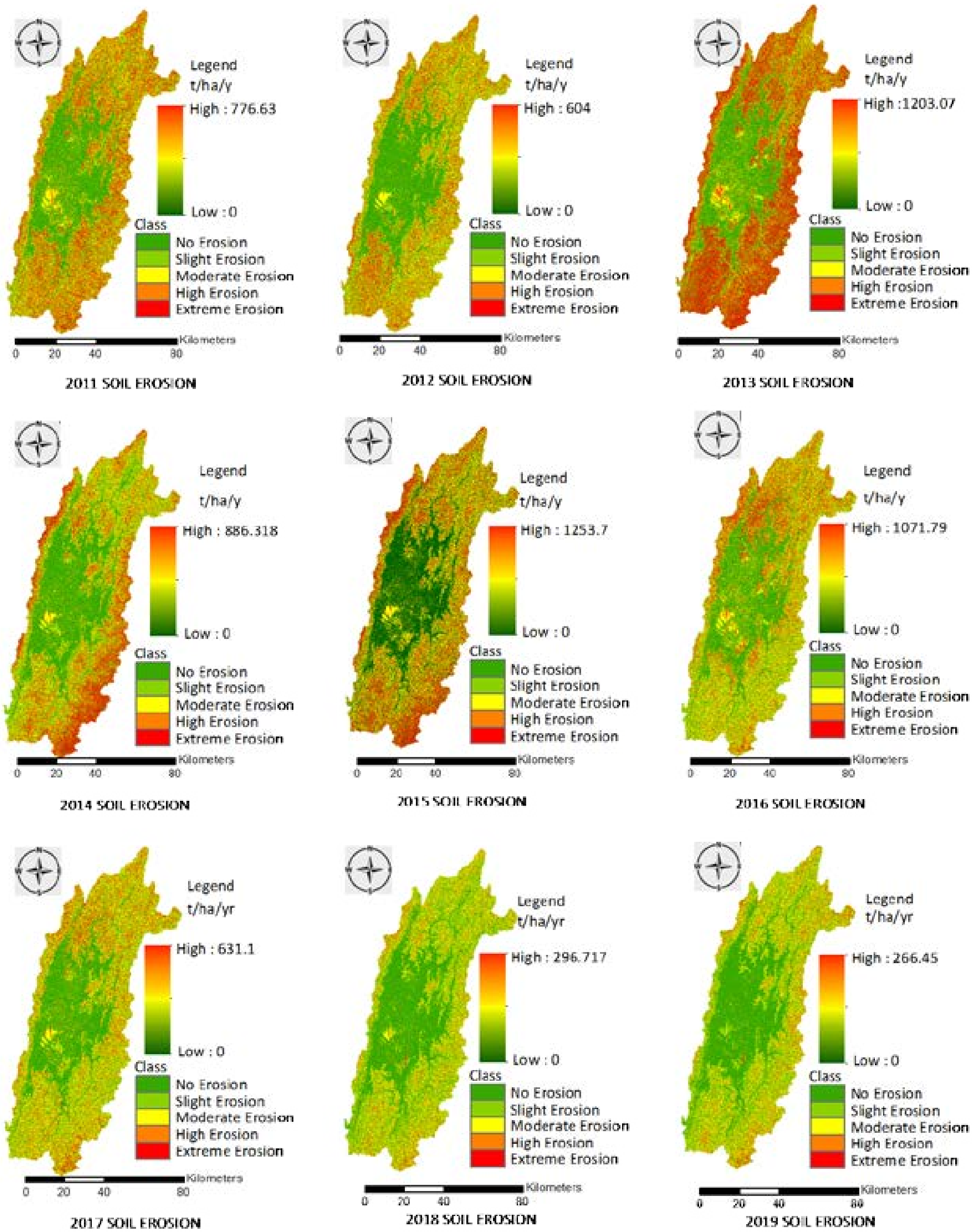

Fig. 3: Soil erosion maps (2011-2019)

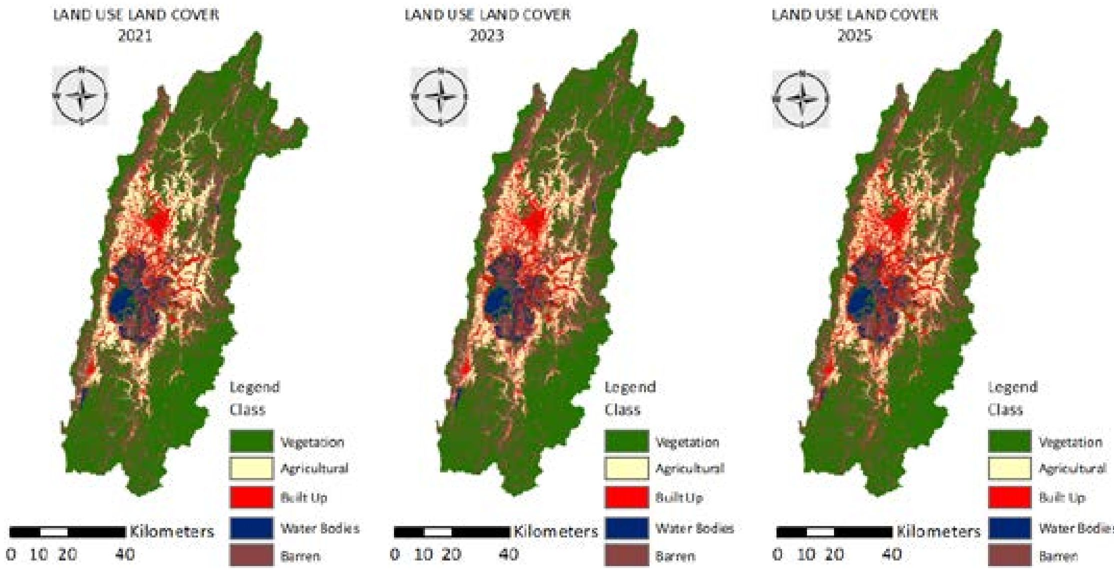

Fig. 4: Projected LULC map of 2021, 2023 and 2025

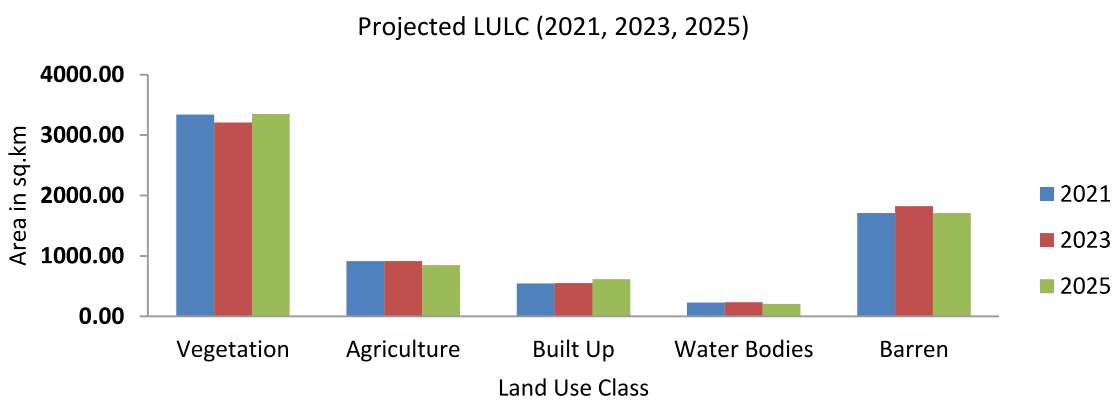

Fig. 5: Area under different LULC classes of 2021, 2023 and 2025

Table 4: Area of the land use classes (areas in km^2)

Class/Value	LULC 2019	Projected LULC 2019	Residual
Vegetation	3271.954	3394.049	96.403
Agriculture	904.691	930.366	97.240
Built Up	531.307	471.686	112.640
Water Bodies	236.993	249.148	95.122
Barren	1787.161	1686.856	105.946

to determine the proportion of variance dependent variable i.e. projected LULC 2019. The co-efficient of determination, R^2 of the regression is found to be 0.875 which shows the high accuracy of the model.

Future soil erosion projection using MOLUSCE

The projected future soil erosion depicts the future trend in quite a positive nature when comparison is done with the soil erosion layer of

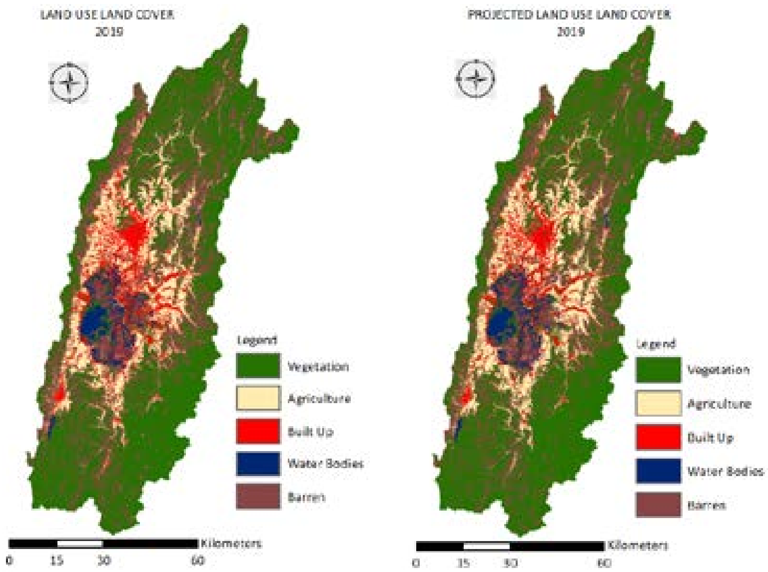

Fig. 6(a)

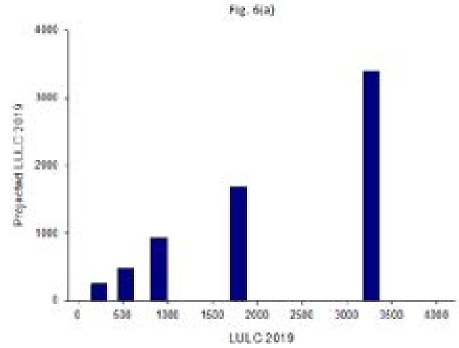

Fig. 6(b)

Fig. 6: (a) LULC 2019 vs projected LULC 2019 map; (b) LULC 2019 vs projected LULC 2019 (Areas in km^2)

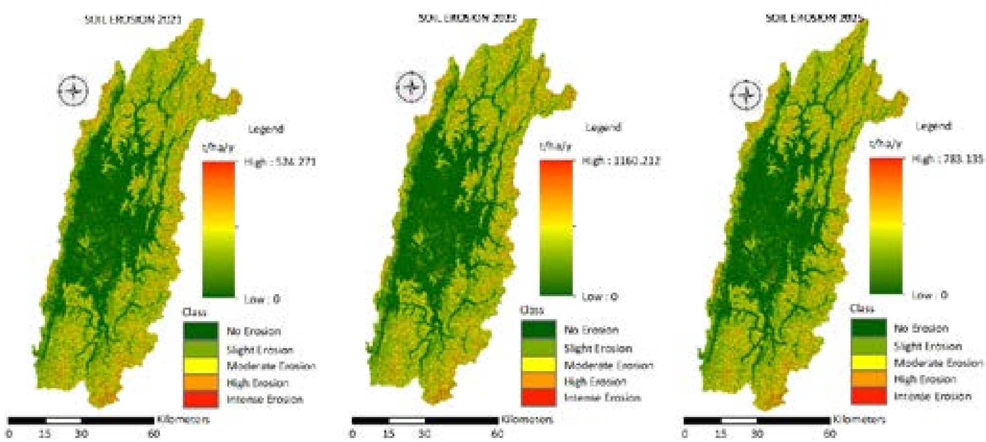

Fig. 7: Soil erosion classes map for the year 2021, 2023 and 2025

2019 (Fig. 7). Variation in the soil erosion rate is observed from the estimated layers. Maximum soil erosion rate increases abruptly from 52.4.271 t/ha/y in year 2021 to 1160.212 t/ha/y in 2023. Again, the maximum ranges down to 783.135 t/ha/y in 2025 (Fig. 7). Resultant soil erosion rate is classified in five qualitative class based on the classification shown in Table 3. As depicted in graphical format in Fig. 8, the soil erosion classes namely 'Moderate Erosion'; 'High Erosion' and 'Intense Erosion' shows the decreased in area coverage over the time and 'No Erosion' and 'Slight Erosion' class shows the increase in area as the time lapses. In case of 'No Erosion' class, there increase in area coverage is observed with 6.14%,

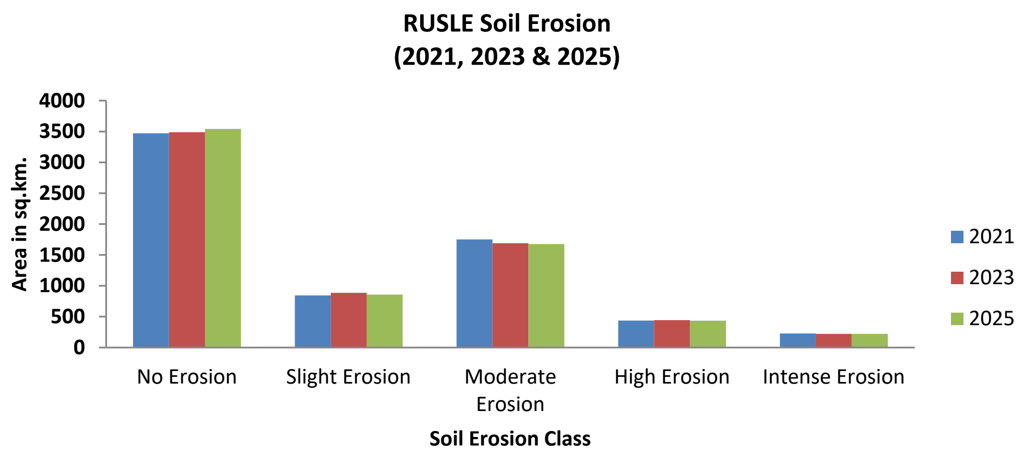

Fig. 8: Area under different soil erosion classes for the year 2021, 2023 and 2025 (Areas in km^2)

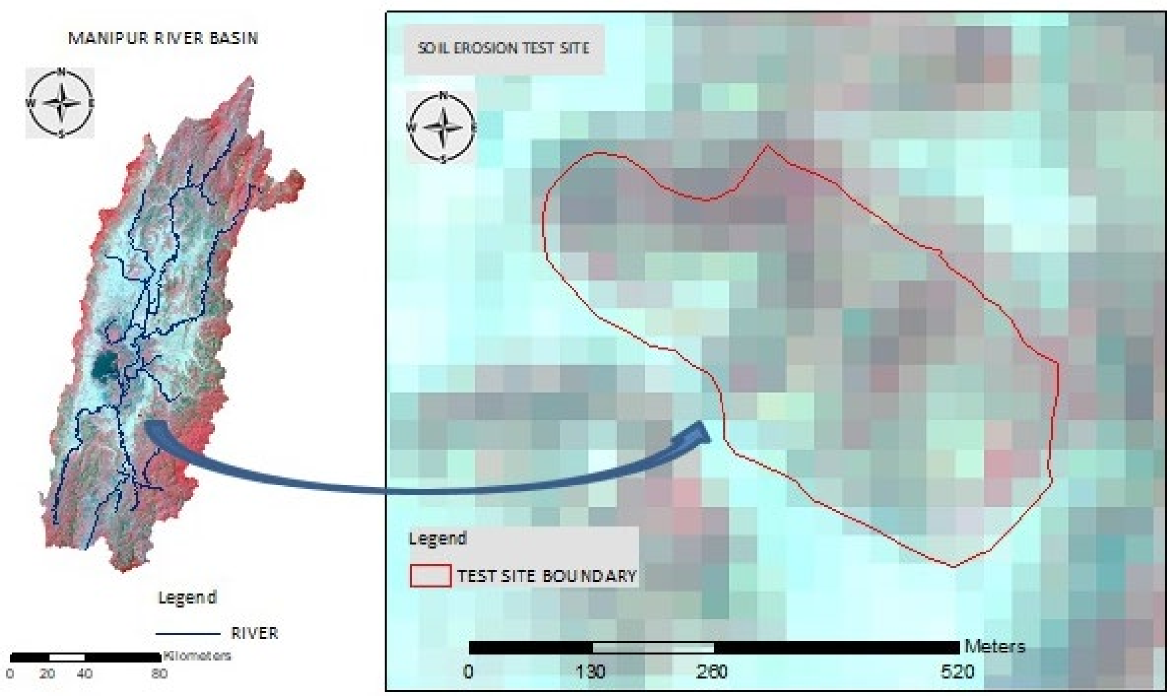

Fig. 9: Soil erosion test site within the basin

6.70% and 8.26% of total area in 2021, 2023 and 2025 respectively. Areas under 'Slight Erosion' class decreases 1.49% to 0.22% in 2021, 2023 and then, increases to 1.07% in 2025. Highest rate of decreases in the area is observed in the 'Moderate Erosion' class. The area declines by 2.49% in 2021, 4.37% in 2023 and 4.80% in 2025. The area under 'High Erosion' class reduces with 1.30% in 2021, 1.07% and 1.35% in 2023 and 2025 respectively. 'Intense Erosion' class also shows declining in the area, which is a positive indication. 0.86% is decrease in 2021, 1.05% in 2023 and 1.04% in 2025 is the percentage of area decrease under the above mention class.

Validation of future soil erosion rate model

For the validation of the model, a plot scale catchment is considered within the study area as test site. The total area of the test site is 135809.30 sq.m., which is a plantation site. For estimating the soil erosion rate of the test site, observed data such as topographic survey is conducted using total station every month for the year 2019, NDVI is measured using the NDVI meter and soil samples are taken. Elevation profile of the test site is obtained and thus slope length factor is estimated. C- Factor is estimated using the NDVI data and P-factor is estimated using the land use and slope layer which is generated using the elevation layer. K- Factor is estimated by testing the soil samples and finally R- factor is estimated using the precipitation data obtained from the automatic weather station installed nearby the test site. Thus, soil erosion rate of the test site is estimated for the year 2019.

Validation of the model is performed by using the observed soil erosion rate layer of the year 2019 (observed 2019). For model validation, using the same model-simulated soil erosion rate layer of 2019 (simulated 2019) is also generated. Random 155

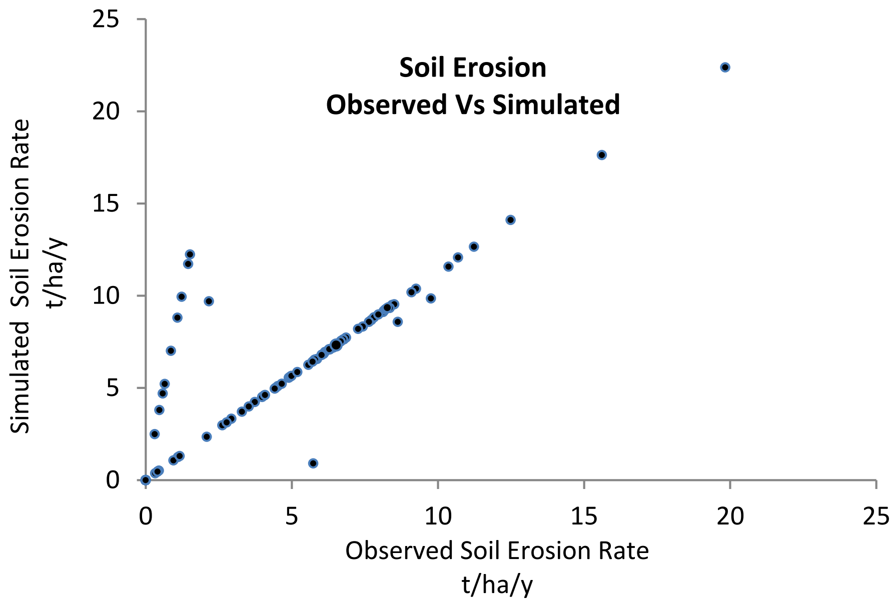

Fig. 10: Observed soil erosion rate Vs simulated soil erosion rate

Table 5: Area under each class for soil erosion 2019 and projected soil erosion 2019 (areas in km^2)

Class	Observed	Simulated	Residual
No Erosion	0.088	0.100	0.012
Slight Erosion	0.044	0.036	-0.008
Moderate Erosion	0.007	0.004	-0.004

Table 6: Range of soil erosion rate from 2011 to 2025 (t/ha/y)

	Year	2011	2012	2013	2014	2015	2016
Range t/ha/y	Minimum	0	0	0	0	0	0
	Maximum	776.63	604.00	1203.07	886.32	1253.70	1071.79
	Year	2017	2018	2019	2021	2023	2025
Range t/ha/y	Minimum	0	0	0	0	0	0
	Maximum	631.10	293.72	266.45	524.27	1160.21	783.14

sample points are selected within the test site with ground coordinates. Using geospatial techniques, the soil erosion rate value corresponding to each sample point are extracted from the observed soil erosion layer and also from the simulated soil erosion layer of 2019. As the attributes of the point have soil erosion rate value of both observed and simulated layer, the attribute is exported as the table. The soil erosion rate of the two layers ranges from 0 to 14.10 t/ha/y for the observed data and 0 to 12.49 t/ha/y for simulated data. Using the data from the table, regression analysis is performed between observed and simulated soil erosion rate, to determine the variance of simulated data (Fig. 10). The coefficient of determination value, R^2 is found to be 0.838, which shows more than 80% of the simulated data can be explained by the observed data. Apart from few outliers observed at some sampling points, the observed data and simulated data are highly correlated. With this percentage of the coefficient of determination quantitatively, the model shows a high percentage of agreement.

For qualitatively validating the model, the observed and simulated soil erosion layers are classified and 3 class is obtained as per maximum soil erosion rate (Table 5). As in case of LULC, which represents qualitative values, regression analysis is performed considering simulated data as dependent variable and observed data as independent variable. The co-efficient of determination, R^2 is found to be 0.96, which also shows high percent of agreement. The model is validated quantitatively and qualitatively with high percent of agreement.

Over estimation is observed in the 'No Erosion' class and under estimation are observed in the 'Slight Erosion' and 'Moderate Erosion' class. The soil erosion studies conducted earlier in the region were found to be of courser temporal resolution, i.e. time gap between the two studies was 10 years. Even though the output results from the earlier studies were for the whole state of Manipur, the time gap between the outputs were big and is difficult for trend analysis (Sen *et al*., 1996; Sen *et al*., 2006). In this study, the resultant outputs are provided for consecutive years and for the future also, hence trend analysis of the soil erosion in the basin can be analyse with ease. Also there is observation, such as increase in area under 'No Erosion' class and decrease rate of soil erosion as the analysis is done from past towards future (Table 6).

CONCLUSION

The present study focus on the estimation of the soil erosion rate over the period of 9 years, and also the prediction of future soil erosion rate. As the estimation of the future soil erosion rate is dependent on the projected LULC, the focus has also been over the prediction of future LULC. With the good agreement of the model is observed for prediction of LULC, the estimation of the future soil erosion rate is performed using the same model. The results of the model shows strong correlation with the observed data used for validation, hence the model can be used for estimation of further future soil erosion rate. The result of the model can be improved by using other model if the region has not face the hindrance of data scarcity.

The ranges of the soil erosion rate has been analyse in this study for 2011 to 2019 and 2021, 2023 and 2025. The trending of the maximum range decreases as the study moves forward the time and in future. Higher rate of erosion is observed the past years due to the activity like jhum cultivation, deforestation, slash and burning practice in the hilly region of the basin. As the year moves towards the recent past, there is decrease in the erosion rate due to the new land use policy, afforestation schemes and conservative measure taken up with initiation from the policy maker. Conservative structures are also encouraged in the hilly region in the recent past hence positive signs towards the environment are observed. The rate of soil erosion increases from 776.63 t/ha/y in 2011 to 1253.7 t/ha/y in 2015 and then decreases to 233.45 t/ha/y in 2019 due to conservative practices followed by the inhabitants. The projected scenario from the study is possible in the future only if we intervene in the degradation activity in the environment at present time. Intense land degradation problems may be solved and sustainable development can be achieved even in this developing region, by considering the results from this study. The result of this study is focusing on the future simulated data which can be made into better results if the policymakers and stakeholders, and none the less, the inhabitants take up conservative measure against the destruction of environments.

AUTHOR CONTRIBUTIONS

C. Loukrakpam performed the literature review, data processing, analysed and interpretation of the data, prepared the manuscript text and manuscript edition. B. Oinam helped in the conceptualization, review of manuscript and presentation of the manuscript.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest regarding the publication of this work. In addition, 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
A	Rate of soil erosion
ANN	Artificial neural network
C	Crop Management
CA	Cellular automata
CHIRPS	Climate Hazards Group InfraRed Precipitation with Station
DEM	Digital Elevation Model
Eq	Equation
EUROSEM	European soil erosion model
exp	Exponent
h	hour
ha	hectare
ISRIC	International Soil Reference and Information Centre
K	Soil Erodibilty
log	Logarithm
LR	Logistic regression
LS	Slope length
LULC	Land Use Land Cover
MCE	Multi-criteria evaluation
MJ	Mega Joule
mm	millimeter
MODIS	Moderate resolution imaging spectroradiometer
MOLUSCE	Modules for land use change simulations
NASA-NCCS	National Auronautics and Space Administration – NASA Centre for Climate Change Simulation
NBBS & LUP	National Bureau of Soil Survey and Land Use Planning
NDVI	Normalized difference vegetation index
NESAC	North Eastern Space Application Centre
NEX-GDDP	NASA Earth Exchange Global Daily Downscaled Projection
NIR	Near Infra red
P	Annual precipitation
P	Practice management factor
P_i	Monthly precipitation
R	Rainfall erosivity
R^2	Coefficient of determination
RUSLE	Revised universal soil loss equation
SRTM	Shuttle radar topographic mission

USGS	United state geological survey
USLE	Universal Soil Loss Equation
USPED	Unit stream power-based erosion deposition
WEPP	Water erosion prediction project
WoE	Weight of evidence

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CASE STUDY

Biodiversity and integration of ecological characteristics of species in spatial pattern analysis

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ABSTRACT

BACKGROUND AND OBJECTIVES: Assessment of biodiversity is a key factor in understanding of function and ecosystem management. Nevertheless, an operating procedure for assessing biodiversity and spatial pattern has not been established yet. Therefore, this empirical study was conducted to explore the role of diversity of species in the spatial patterning of tow shrub herbaceous communities.

METHODS: First, the biodiversity analysis was performed by Past3 software to compare the relationship between the two communities. Secondly, the distance and quadrat indices were employed to explore the spatial relationship of dominant species with diversity. In this regard, 64 and 84 plant species recorded in two vegetation types were investigated. Distribution patterns were extracted by distance and quadrat indices and Ecological Methodology software.

FINDINGS: The results showed that vegetation type 2 had more diversity and richness compared to vegetation type 1. Besides, the spatial distributions of dominant species (*Astragalus gossipinus* and *Bromus tomentellus*) in the two vegetation types were clumped and random with tendency to be clumped. The Scrophulariaceae, Malvaceae, Papaveraceae, and Euphorbiaceae families were not found in vegetation Type 1, and vegetation Type 2 had no species of the Boraginaceae, Rosaceae, Thumeliaceae, Capparidaceae, Oleaceae, Sistaceae, and Dispaceae families. The results showed significant differences in the number of Gaminae and Legominosea families between the two vegetation types.

CONCLUSION: It was concluded that in communities with a dominant cover of shrub, the distribution pattern was clumped, and quadrat indices were less efficient than distance indices. While, in high-diversity communities with a predominant cover of gross, spatial distribution was random and distance and quadrat indices were more convergent.

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INTRODUCTION

Spatial pattern analysis methods provide insights into where things occur, how the distribution of incidents is or how the arrangement of data aligns with other features in the landscape, and what these patterns may reveal about potential connections and correlations (Scott 2015). In general, plant's distribution pattern could be classified into three vegetation types as random, uniform and clumped (Krebs 1999; Carvalho, 1992; Buschini, 1999; Askari *et al*., 2013 ; Lohe *et al.,* 2015). In the random distribution pattern, each member is independent from the other members (Pielou 1977; Whistle Wood, 1978; Petrere, 1985). Correspondingly, this pattern is based on the environmental similarities and non-selective behavior forms. In uniform distribution pattern, the members are positioned with regular intervals. Therefore, this pattern shows the negative impacts of competition on food or niche (Elliott, 1979; Matuky and Kulma, 1982). The clumped distribution pattern occurs when all or the majority of the population prefer to concentrate mostly on the specific parts of the environment (Odum, 1986; Krebs, 1999; Carvalho, 1992; Buschini, 1999). Apparently, the occurrence of this pattern is attributed to asexual reproduction and abundance of seed production. Therefore, these patterns are affected by the environmental factors, species behaviour, and individual characteristics of the plant species. Using Pielou and Hopkins indices, the distribution pattern of plain *Artemisia* was studied in three sites located in a step zone (Jamali *et al*., 2020). Results showed that the distribution pattern of *Artemisia* in the first habitat was uniform; this distribution was clumped in the second habitat and uniform in the third habitat. Additionally, the distribution pattern of *Anadenanthera peragrina* species was determined using the proportion of variance to the mean, Morisita, standard Morista, negative binomial distribution, green indices and various measuring plots. Among the calculated indices, the standard morista index was found to be the best one, despite the plot size (Malhado and Petter, 2004). The distribution patterns of three species of *Festuca ovina*, *Prangos ferulacea,* and *Bromus tomentelus* in a pasture were investigated. Accordingly, it was proved that the distribution of *F. ovina* was as clumped vegetation type, and the distributions of *P. ferulacea* and *B. tomentellus* were random (Zare Chahooki *et al*., 2010). Furthermore, the spatial pattern of *Crategus sp*. in the Central Zagros was evaluated. All the applied indicators showed a clumped pattern for *Crataegus sp.* forests. The obtained results also demonstrated that distance distribution indices had the same pattern for one species in most of the cases, and were more accurate than quadrat indices (Askari *et al.,* 2013). Biodiversity is defined as the kinds and numbers of organisms and their patterns of distribution (Schuler, 2006). Generally, it can be said that biodiversity measurement typically focuses on the species level, and species diversity is one of the most important indices used for the evaluation and sustainable management of ecosystems. The diversity distribution can be evaluated using different spatial indices and scales such as species ecological traits, phytogeographic history, species richness, evenness and diversity (Crist *et al*., 2003; Sühs *et al*., 2019). Species diversity studied in grazed and non-grazed areas showed that herbaceous layer had the highest richness, evenness and diversity. The differences between biodiversity indices in the two areas were statistically significant in the tree, shrub and herbaceous layers (Haidari *et al*., 2013). Erfanzadeh *et al*. (2015) studied the variation of plant diversity components in arid and semi-arid regions in different scales, and reported that diversity had the highest contribution to the total diversity for all the species as well as rare species in both regions. The results of a study conducted by Luiza *et al*. (2020) on three dimensions of plant diversity change across ecological and biogeographic scales showed that two Neotropical inselbergs were taxonomically different (beta diversity); however, they had convergence in their function and diversities. The plant floristics in different parts of Kermanshah province have been studied by some scientists. For instance, Sadeghirad *et al.* (2014) showed that among 29 plant genera in Kermanshah, Poaceae (25 species), Papilionaceae (17 species) and Lamiaceae (11 species) had the highest frequencies. No study has tested the relationship between spatial pattern and assessment of diversity so far. Notably, the main objectives of this study were: i) to analyse the distribution of the different vegetation types of the existing plant species at two different altitudes in order to better understand the relationship among floristic composition, richness, and diversity to species spatial pattern; and ii) to select proper indices which can illustrate the distribution pattern of different species more accurately. This would consequently facilitate the selection of sampling methods. This study was carried out in Kermanshah, Iran in 2020.

MATERIALS AND METHODS

Study area

The study area, with an area of 11009.62 hectares, is located on Sepol and Dodgoosh mountains, at a distance of 13 km from Kermanshah city. In addition, 5725.49 hectares of the study area are covered with rangelands occupied with Poaceae and legominacea families. This area is located between latitude of 34° 10′ to 34° 19′ N and longitude of 47° 16′ to 47° 24′ E. The elevation range of the study area is 1248-1804 m above the sea level and its mean slope is in the range of 12-20%. Also, the annual rainfall is 400-450 mm. Soil in this area has a medium texture with a high percentage of gravels, and in some parts, out crops are observable. Based on the previous studies performed in this region, the area has four main vegetation types. In the present study, has only focused on two vegetation types at minimum and maximum two altitudes as follows: the first vegetation type (Type 1), with an area of 1,600 hectares, covers about 28% of the whole area and exists at an altitude of 1,248 m above the sea level on the southern hills leading to Gamasiab and Gharesoo rivers (Karimi, 2017) (Fig. 1). Accordingly, this pasture is mainly used for grazing during the growing season. The most important species of the first vegetation type are *Astragalus gossypinus* (dominant species), *Astragalus brachystachis, Bromus tomentellus*, and *Gundelia turnefortii*. The second vegetation type (Type 2), with an area of 487 hectares, has the smallest share among the vegetation types, covers 5.8% of the natural habitats, and exists at an altitude of 1,804 m. This vegetation type has a high potential for producing rangeland vegetation. Although the accumulation and density of the rangeland vegetation have been decreased due to drought, this vegetation type, due to having higher moisture, high altitude and less grazing, has the highest forage production and percentage of cover among the other vegetation types. The dominant family of the second vegetation type is Poaceae with the dominant species of *Bromus tomentellus* and other species such as *Hordeum bulbosum* and *Gundelia turnefortii* (belonging to the compositae family).

Data collection

The two vegetation types (Type 1 and Type 2) were selected for sampling. For random placement

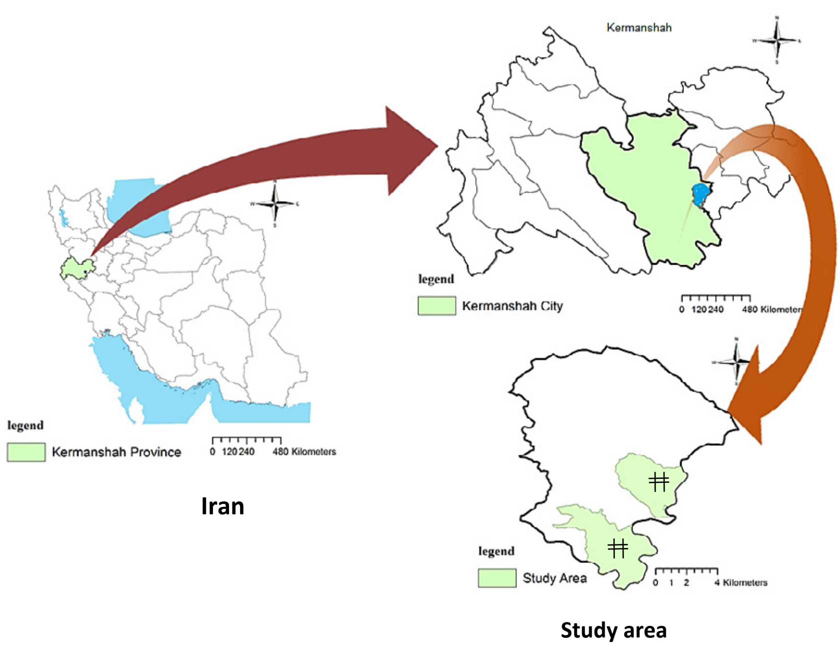

Fig. 1: Geographic location of the study area in the Northern Zagros region, Kermanshah, Iran

of transects, 10 points with a distance of 50 m were selected, so that the first 4 points were randomly selected and then transects were extended from these points. Considering the expansion of the area and the species distribution, four 300-m transects with spacing of 100 m were randomly allotted to each vegetation type. Along each transect, 25 points with intervals of 4 m were selected and 15 of them were measured. A total of 100 quadrats, with a 2-m^2 surface, were established in each vegetation type to count and identify the species. The plant species were assessed by the authors and experts in the plant phenology.

Data analysis

Information on cover, frequency, and number of species in each plot were recorded. Past3 software was then utilized to calculate the species richness and diversity indices. Menhinic and Margalef indices, presented by Eqs. 1 and 2 respectively, were utilized for calculating the species richness. Moreover, Simpson, Shannon and Brillouin indices were introduced to calculate the species diversity indices (Eqs. 3, 4 and 5, respectively).

$$R = \frac{S}{\sqrt{N}} \tag{1}$$

$$R = \frac{S-1}{Ln\left(N\right)} \tag{2}$$

$$H' = 1 - \sum_{i=1}^{s} Pi^2 \tag{3}$$

$$H' = \sum_{i=1}^{s} \left(\mathrm{Pi}\right)\left(\log 2\mathrm{Pi}\right) \tag{4}$$

$$HB = \frac{\mathrm{Ln}N! - \sum_{i=1}^{n} Lnn_i!}{N} \tag{5}$$

Where, S is the number of plant species included in the sample; N is the total frequency of all the studied plant species; and Pi is the frequency ratio of ith group to the all the studied species (Magurran, 1988; Schowalter, 2012). Compared to complete sampling methods, distance and quadrat indices required less time and costs and had higher accuracy at the same time. Therefore, these indices were selected to measure the distribution of species. For each random point, the distance to the nearest plant, the distance of the mentioned plant to the nearest neighbor, and the distance of random point from the second near plant were measured. Finally, distance and quadrat indices of the distribution were described based on the obtained information. Johnson and Zimer (1985) and Ludwig and Reynolds (1988) proposed following index for measuring the spatial pattern of distance in which if E(I) > 2 the pattern is clumped and the spatial pattern is random when E(1) = 2 (Eq. 6).

$$I = (N+1)\frac{\sum_{i=1}^{N} (d_i^{\,2})^2}{\left[\sum_{i=1}^{N} (d_i^{\,2})\right]^2} \tag{6}$$

Where, N is random points (with x and y co-ordinates), d_i is Distance from the ith point to the nearest neighbor and E(I) is the expected value of I.

Eberhart's index is suggested as the following equation (Krebs, 1989) where IE > 1.27 indicates clumped spatial pattern, IE < 1.27 represents the uniform pattern and IE = 1.27 indicates the random pattern using Eq. 7.

$$I_E = \left(\frac{S}{\overline{X}}\right)^2 + 1 \tag{7}$$

Where, I_E is the Eberhardt's index of dispersion for point-to-organism distances, S is observed standard deviation of distances and X is the mean of point-to-organism distances. Pielou (1959) presented the Pielou's index in which the P value less than 1 indicates the random spatial pattern of distance, while P=1 shows the uniform pattern and P>1 represents the clumped spatial pattern of distance (Eq. 8). Equation 8 presents the Pielou's index calculation where π equals 3.14, ΣX_i is the total distance from the nearest neighbor to the sample point, D is the Density (m^2) and N is the number of samples.

$$P = \pi D\left(\frac{\sum_{i=1}^{N} X_i}{N}\right) \tag{8}$$

Hopkines (1954) presented his spatial pattern of distance index using Eq. 9.

$$I_H = \frac{\Sigma(x_i^2)}{\Sigma(x_i^2) + \Sigma(r_i^2)} \quad (9)$$

Where, h is the Hopkins' test statistics for randomness, x_i is the distance from random point i to the nearest organism, and r_i is the distance from random organism i to its nearest neighbor. I_H = 1 shows the clumped spatial pattern of distance while I_H values equal to 0 and 0.5 indicate uniform and random spatial pattern of distance, respectively. Murcury (2000) introduced the Holgate's index as indicated in Eq. 10 in which A value greater than 0 indicates the clumped pattern, A=0 shows the random pattern while the A value less than 0 represents the uniform spatial pattern of distance using Eq. 10.

$$A = \frac{\Sigma \frac{d_i^2}{d_2^2}}{n} - 0.5 \quad (10)$$

The variance-to-mean ratio index is among the first indices for calculating the spatial pattern of quadrat using Eq. 11 (Krebs, 1989; Ludwig and Reynolds, 1988).

$$ID = \frac{S^2}{\bar{X}} \quad (11)$$

Where, $\bar{x}$ is the mean population density and S^2 is the variance of population. According to the Eq. 11, ID value equal to n and 0 indicate the clumped and uniform spatial pattern of quadrat, respectively. Ludwig and Reynolds (1988) modified the variance-to-mean ratio index and introduced the Green's index using Eq. 12; in which $\bar{x}$ is the mean population density, S^2 is the variance of population and n is the total population. If GI value equal to 1, it means that the spatial pattern of quadrat is clumped, if the value is equal to 0, indicates the random pattern, while if the GI value is less than 0, means the spatial pattern of quadrat in studied community is uniform.

$$GI = \frac{(\frac{s^2}{\bar{x}}) - 1}{n - 1} \quad (12)$$

Eq. 13 shows the Lioyd's index for spatial pattern of quadrat (Ludwig and Reynolds, 1988).

$$LI = \frac{\bar{x} + (\frac{s^2}{\bar{x}} - 1)}{\bar{x}} \quad (13)$$

Where, $\bar{x}$ is the mean population density and S^2 is the variance of population. The LI value less than 1 indicates the uniform spatial pattern of quadrat, while LI=1 shows the random pattern and LI>1 represents the clumped spatial pattern of quadrat. Morisita's index introduced for calculating the spatial pattern of quadrat using Eq. 14 (Morisita, 1962; Krebs, 1989; Ludwig and Reynolds, 1988).

$$I_d = n\left[\frac{\Sigma X_i^2 - \Sigma X_i}{(\Sigma X_i^2) - \Sigma X_i}\right] \quad (14)$$

Where, I_d is the Morisita's index of dispersion, n is the sample size, Σx_i is the sum of quadrat counts and Σx_i^2 is the sum of quadrat counts squared. The $I_d > 1$ indicates clumped spatial pattern, $I_d < 1$ represents the uniform pattern and $I_d = 1$ indicates the random pattern. Smith and Gill (1975) standardized the Morisita's index and developed two equations for uniformity index using Eq. 15 and Clumped index using Eq. 16.

$$M_u = \frac{X^2_{0.975} - n + \sum X_i}{(\sum X_i) - 1} \quad (15)$$

$$M_u = \frac{X^2_{0.025} - n + \sum X_i}{(\sum X_i) - 1} \quad (16)$$

Where, n is the sample size, Σx_i is the sum of the quadrat counts, $X^2_{0.975}$ is the Chi-squared distribution with *n-1* degrees of freedom and 0.975 quantile values and $X^2_{0.975}$ is the Chi-squared distribution with *n-1* degrees of freedom and 0.025 quantile values. According to the equations, the I_p value equal to 0, means that the spatial pattern of quadrat is random, if the value is higher than 0, indicates the clumped pattern, while if the I_p value is less than 0, means the spatial pattern of quadrat in the studied community is uniform. To reach a more accurate plants distribution, statistical distributions (poisson and negative/positive binomial distributions) were calculated using Ecological Methodology Software. Finally, the distribution curves of the species were created by the relationship between quadrats frequency and the number of individuals in each quadrat. These curves enabled us to differentiate the distribution patterns for the plant species type existing in the selected area.

RESULTS AND DISCUSSION

Diversity analysis

The floristic compositions of vegetation Type 1 and Type 2 are listed in Tables 1 and 2, respectively. Most of the species appeared in the herb layer, and the species of woody plants were very limited. The results shown in Fig. 2 indicate that the numbers of the species belonging to the different families of the two vegetation types are considerably divers. Moreover, in both vegetation types, the species belonging to Garminae had the highest abundancy. However, the dominant cover of vegetation Type 1 was found to be shrub and *Astragalus* genus. Species diversity was higher in vegetation Type 2, and the number of Graminae species was higher in vegetation Type 2 than in vegetation Type 1. The Scrophulariaceae, Malvaceae, Papaveraceae, and Euphorbiaceae families were not found in vegetation Type 1, and vegetation Type 2 had no species of the Boraginaceae, Rosaceae, Thumeliaceae, Capparidaceae, Oleaceae, Sistaceae, and Dispaceae families. The results showed significant differences in the number of Gaminae and Legominosea families between the two vegetation types. Overall, these results indicated that the plant community composition and the abundance of species were significantly different between the two vegetation types (Fig. 2). Yirga *et al.* (2019), in their study, reported the impact of altitude on plant species composition, diversity, and structure at the Wof-Washa highlands of Ethiopia. Furthermore, Al-Aklabi *et al.*, (2016) found different combinations of plant species in response to elevation and topography. The differences in species compositions among the forest sites could be mainly attributed to the dissimilarities of the sites in terms of location, altitude, human impact, rainfall, and other biotic and abiotic factors (Yirga *et al.*, 2019;

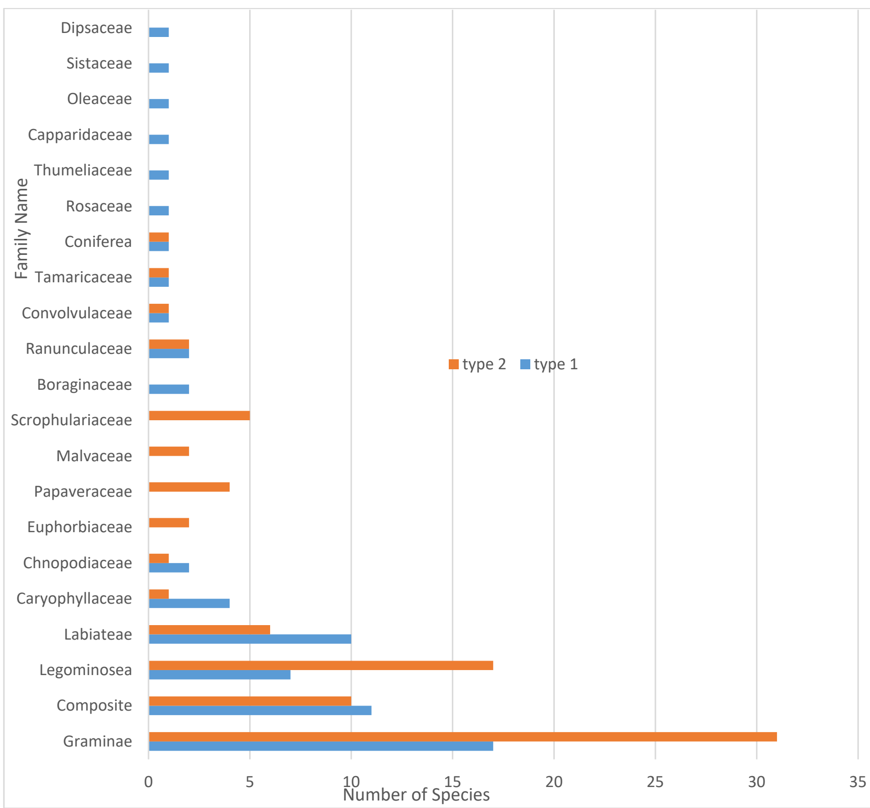

Fig. 2: Comparison of the number of species belonging to the different families in the two vegetation types (Types 1 and 2)

Table 1: The floristic list of vegetation Type 1

No.	Family	Genus	Species	No.	Family	Genus	Species
1	Graminae	*Bromus*	*tomentellus*	33	Legominosea	*Astragalus*	*gossypinus*
2	Graminae	*Bromus*	*sterilis*	34	Legominosea	*Astragalus*	*effusus*
3	Graminae	*Bromus*	*tectorom*	35	Legominosea	*Astragalus*	*raddei*
4	Graminae	*Bromus*	*inermis*	36	Labiateae	*Phlomis*	*herbaventi*
5	Graminae	*Hordeum*	*bolbosum*	37	Labiateae	*Phlomis*	*lanceolatus*
6	Graminae	*Hordeum*	*geniculatum*	38	Labiateae	*Tribolus*	*tristri*
7	Graminae	*Hordeum*	*violaceum*	39	Labiateae	*Teucrium*	*polium*
8	Graminae	*Agropyrun*	*panormitaum*	40	Labiateae	*Salvia*	*sclarea*
9	Graminae	*Agropyrun*	*inermis*	41	Labiateae	*Salvia*	*limbata*
10	Graminae	*Agropyrun*	*cristatum*	42	Labiateae	*Ziziphora*	*junior*
11	Graminae	*Stipa*	*cappensis*	43	Labiateae	*Ziziphora*	*capitata*
12	Graminae	*Stipa*	*pennata*	44	Labiateae	*Stachys*	*inflata*
13	Graminae	*Stipa*	*barbata*	45	Labiateae	*Stachys*	*kurdica*
14	Graminae	*Poa*	*annua*	46	Chnopodiaceae	*Chnopodium*	*botrys*
15	Graminae	*Aegilups*	*columnaris*	47	Chnopodiaceae	*Ferolago*	*macrocarpa*
16	Graminae	*Avena*	*clauda*	48	Rosaceae	*Amygdalus*	*scoparia*
17	Graminae	*Avena*	*ventricola*	49	Caryophyllaceae	*Acanthophyllum*	*bractetum*
18	Composite	*Onopordo*	*acanthium*	50	Caryophyllaceae	*Vaccaria*	*pyramidata*
19	Composite	*Lactuca*	*orientalis*	51	Caryophyllaceae	*Dianthus*	*szowitisianus*
20	Composite	*Tragopogon*	*collinus*	52	Caryophyllaceae	*Dianthus*	*macranthus*
21	Composite	*Tanacetum*	*policephalum*	53	Thumeliaceae	*Paliurus*	*spina*
22	Composite	*Thevenotia*	*persica*	54	Boraginaceae	*Onosma*	*latifolia*
23	Composite	*Scariola*	*orientalis*	55	Boraginaceae	*Ceccinia*	*marantera*
24	Composite	*Gundelia*	*mucronata*	56	Capparidaceae	*Noaea*	*mucronata*
25	Composite	*Artemisia*	*siberi*	57	Ranunculaceae	*Ranunculus*	*arvensis*
26	Composite	*Sirsium*	*echinus*	58	Ranunculaceae	*Anemone*	*biflora*
27	Composite	*Gundelia*	*turneforti*	59	Convolvulaceae	*Convolvulus*	*ammocharis*
28	Composite	*robostus*	*Echinops*	60	Tamaricaceae	*Reaumuria*	*stocksii*
29	Legominosea	*Aechardia*	*orientalis*	61	Oleaceae	*Fraxinus*	*excelsior*
30	Legominosea	*Astragalus*	*brachystachis*	62	Sistaceae	*Helianthemum*	*sulicifolium*
31	Legominosea	*Astragalus*	*schistusus*	63	Dipsaceae	*Ceohalaria*	*microcephala*
32	Legominosea	*Astragalus*	*macropelmatus*	64	Coniferea	*Taraxacom*	*montanum*

Girma, 2011). Table 3 shows the values of the species richness and diversity indices for the plant species existing in the study area. It was found that the values for the species richness indices, named Margalef and Menhinic, were higher in vegetation Type 2 than in vegetation Type 1. In vegetation Type 1, the species are expanded into the areas with more humidity and slope. This can be due to grazing intensity and geographical location of the region, which, in turn, have resulted in loss of the species diversity (Santos and Munhoz, 2012). Considering the values of diversity indices, it was found that all the calculated indices (Simpson-1-D, Shannon-H and Brillouin) were higher in vegetation Type 2 rather than vegetation Type 1 (Table 3).

Spatial patterns

Spatial pattern of species can indicate stand history, population dynamics, and species competition (Haas, 1995), and may explain what controls the co-existence and diversity of species in a rangeland. The spatial distributions of two dominant species in the two vegetation types are shown in Tables 4 and 5. According to the most of the indices, the distribution pattern of *Astragalus gossypinus* is random with more tendency to be clumped. Eberhart indices (the distance of random point from the nearest plant), Hopkines (based on the distance of random point from both the nearest plant and the nearest neighbor), and Holgate (based on the distance of the point from both the nearest plant and the second nearest plant) also show a clumped distribution pattern (Table 4). This finding is consistent with the results of the previous studies (Jannat Rostami *et al*., 2009; Mohebi *et al*., 2011). Since three distance indices including Eberhardt, Hopkines, and Holgate showed a clumped distribution pattern, it can be assumed that distance indices would present the distribution pattern of shrub species better than quadrat indices. Due to

Table 2: The floristic list of vegetation Type 2

No.	Family	Genus	Species	No.	Family	Genus	Species
1	Graminae	*Bromus*	*tomentellus*	43	Legominosea	*Astragalus*	*brachystachis*
2	Graminae	*Bromus*	*sterilis*	44	Legominosea	*Lathyrus*	*pratensis*
3	Graminae	*Bromus*	*tectorom*	45	Legominosea	*Lathyrus*	*aphaca*
4	Graminae	*Bromus*	*inermis*	46	Legominosea	*Visia*	*monantha*
5	Graminae	*Bromus*	*dantoniae*	47	Legominosea	*Visia*	*villosa*
6	Graminae	*Bromus*	*squarrisa*	48	Legominosea	*Robinia*	*pseudoacaci*
7	Graminae	*Hordeum*	*bolbosum*	49	Legominosea	*Glissierhisa*	*glabra*
8	Graminae	*Hordeum*	*glaucum*	50	Legominosea	*Trifolium*	*fragiferum*
9	Graminae	*Hordeum*	*murinum*	51	Legominosea	*Lotus*	*michauxianu*
10	Graminae	*Hordeum*	*geniculatum*	52	Legominosea	*Onobrychis*	*altissima*
11	Graminae	*Hordeum*	*violaceum*	53	Legominosea	*Onobrychis*	*cristugelli*
12	Graminae	*Agropyrun*	*panormitaum*	54	Legominosea	*Sophora*	*alopecuroide*
13	Graminae	*Agropyrun*	*inermis*	55	Legominosea	*Medicago*	*radiata*
14	Graminae	*Agropyrun*	*cristatum*	56	Legominosea	*Medicago*	*rigidula*
15	Graminae	*Agropyrun*	*elongatom*	57	Legominosea	*Sorghom*	*halopens*
16	Graminae	*Agropyrun*	*intermedium*	58	Legominosea	*Trigonella*	*angustifolium*
17	Graminae	*Festuca*	*ovina*	59	Labiateae	*Phlomis*	*rigida*
18	Graminae	*Festuca*	*pratensis*	60	Labiateae	*Marrobium*	*vulgar*
19	Graminae	*Festuca*	*arundinacea*	61	Labiateae	*Teucrium*	*polium*
20	Graminae	*Stipa*	*cappensis*	62	Labiateae	*Salvia*	*limbata*
21	Graminae	*Stipa*	*pennata*	63	Labiateae	*Ziziphora*	*junior*
22	Graminae	*Stipa*	*barbata*	64	Labiateae	*Ziziphora*	*capitata*
23	Graminae	*Poa*	*angustifolia*	65	Euphorbiaceae	*Euphorbia*	*strobitacea*
24	Graminae	*Poa*	*bulbosa*	66	Euphorbiaceae	*Euphorbia*	*aellenii*
25	Graminae	*Poa*	*annua*	67	Papaveraceae	*Papaver*	*tenuifolium*
26	Graminae	*Aegilups*	*columnaris*	68	Papaveraceae	*Papaver*	*lacerum*
27	Graminae	*Aegilups*	*triuncialis*	69	Papaveraceae	*Glauciun*	*elegans*
28	Graminae	*Festuca*	*pratensis*	70	Papaveraceae	*Fumaria*	*vaillantii*
29	Graminae	*Festuca*	*arundinacea*	71	Malvaceae	*Alcea*	*ficifolia*
30	Graminae	*Secale*	*montanum*	72	Malvaceae	*Malva*	*neglecte*
31	Graminae	*Boissiera*	*sqarus*	73	Scrophulariaceae	*Scrophularia*	*striata*
32	Composite	*Tragopogone*	*collinus*	74	Scrophulariaceae	*Scrophularia*	*microcarpa*
33	Composite	*Lanacetum*	*polysephalum*	75	Scrophulariaceae	*Verbascum*	*macronata*
34	Composite	*Carthamus*	*lanatus*	76	Scrophulariaceae	*Linaria*	*lineolata*
35	Composite	*Anthemis*	*brachystephan*	77	Scrophulariaceae	*Plantago*	*major*
36	Composite	*Anthemis*	*rulneraria*	78	Caryophyllaceae	*Dianthus*	*macranthus*
37	Composite	*Onopordo*	*acanthium*	79	Ranunculaceae	*Ranunculus*	*arvensis*
38	Composite	*Lactuca*	*orientalis*	80	Ranunculaceae	*Anemone*	*biflora*
39	Composite	*Tragopogon*	*collinus*	81	Convolvulaceae	*Convolvulus*	*ammocharis*
40	Composite	*Scariola*	*orientalis*	82	Tamaricaceae	*Reaumuria*	*stocksii*
41	Composite	*Gundelia*	*turneforti*	83	Chnopodiaceae	*Chnopodium*	*botrys*
42	Legominosea	*Astragalus*	*gossypinus*	84	Coniferea	*Taraxacom*	*montanum*

seeding near the mother base in *A. gossypinus* and providing appropriate moisture conditions, the spatial arrangement of these shrubs is mainly triple and quadruple or quintuple. The presence of small masses among the individual shrubs makes some changes in distribution parameters. In such a plant community, when selecting random points, more points would be observed among small clumps as compared to shrubs. In other words, the measured distances of the random points would be placed in the border of the clumps of shrubs. Therefore, the distance indices developed based on the point distance from the nearest plant, plant's distance from the nearest neighbor, and point distance from the second nearest plant would be more accurate in determining the clumped distribution pattern. However, it has been observed that individual shrubs are not appropriate

Table 3: The values of species richness and diversity indices for plant species in the studied area

Species	Index	Type 1	Type 2
Species richness	Menhinic	7.559	7.794
	Margalef	9.746	10.46
Species diversity	Simpson-1-D	0.618	0.774
	Shannon- H	4.416	4.459
	Brillouin	1.147	1.44

Table 4: The value of the distance and quadrate indices for distribution pattern of *Astragalus gossypinus*

Distance and quadrate indices	Calculated value	Distribution pattern
Johnson and Zimer	2.32	Random with tendency to clumped
Eberhart	1.80	Clumped
Pielou	1.08	Random with tendency to clumped
Hopkines	0.98	Clumped
Holgate	0.78	Clumped
Variance-to-mean ratio	1.25	Random with tendency to clumped
Green	0.43	Random with tendency to clumped
Lioyd	1.077	Random with tendency to clumped
Morisita	1.06	Random with tendency to clumped
Standardized Morisita	0.28	Random with tendency to clumped

Table 5: The value of the distance and quadrate indices for distribution pattern of *Bromus tomentellus*

Distance and quadrate indices	Calculated value	Distribution pattern
Johnson and Zimer	1.98	Random
Eberhart	1.27	Random
Pielou	0.84	Random with tendency to uniform
Hopkines	0.34	Random with tendency to uniform
Holgate	0.047	Random with tendency to clumped
Variance-to-mean ratio	0.95	Random
Green	0.0052	Random with tendency to clumped
Lioyd	1.406	Random with tendency to clumped
Morisita	1.09	Random with tendency to clumped
Standardized Morisita	0.0620	Random with tendency to clumped

options for determining the distribution pattern of these communities. This finding is consistent with Digel (1983) theory and results of the previous studies (Zare Chahooki *et al*., 2010) which considered distance indices more accurate than quadrat indices. In these communities, the quadrat indices, due to having problems of number, area, and quadrat shape, are less efficient than the distance indices. In plant population measurements, the data of distribution pattern can be used instead of field measured data for at least random and regular spatial distribution patterns (Jamali *et al.,* 2020).

Hennenberg and Steinke (2006) proved that the plant ecologists could use the distance methods in both density estimation and statistical testing when the spatial pattern of the studied population was randomly distributed (Hennenberg and Steinke 2006).

A key feature in the present study is quantification of the indices under which the distribution processes operate. Acceptance of the fact that the groups of a species with the same morphological characteristics are not randomly distributed relative to others, allows for inference about the role of exogenous and endogenous spatial diversities in determining the pattern of plant community in space. Calculation of the Johnson and Zimer, Eberhart, and variance-to-mean ratio indices demonstrated that distribution of *Bromus tomentellus* in the second vegetation type was random (Table 5). Moreover, Pielou and Hopkines indices showed a random with tendency to uniform pattern, and other indices indicated a random with tendency to clumped pattern. This means that, almost all indices, including the distance and the quadrat indices, showed a random distribution pattern; however, the accuracy of the distance indices was higher. Therefore, it was confirmed that, compared to quadrat indices, the accuracy of distance indices in determining the distribution pattern of *B.tomentellus* was higher. This could be attributed to the fact that, in the study area, *B.tomentellus* was semi-dense; therefore, the distance among the clumps was close to plants within the clumps. In such communities, the distance indices show a more accurate distribution pattern. Due to the presence of fewer plants within the quadrats and having lower variance, the quadrat indices showed a random distribution pattern. Thus, most of the indices, including the distance and quadrat, presented a random distribution pattern in this study. In addition, based on the point distance from both the nearest plant and near plant, the distance indices presented this pattern

Table 6. Distribution frequency of the species

Species	Value of df	Value of P
A. gossypinus	3	Negative binomial 0.154 Poisson 0.0016
B.tomentellus	3	Negative Binomial 0.00 Poisson 0.74

appropriately. Generally, both of the distance and quadrat indices proved to be proper for measuring the distribution of the species. The distribution patterns were calculated based on statistical distributions using Poisson and negative binomial distributions and Ecological Methodology Software (Table 6). It was shown that the distribution of *Astragalus gossypinus* was negative binominal (P ≥ 0.05) (representing the clumped distribution pattern), and the distribution of *Bromus tomentellus* was Poisson (P ≥ 0.05) (representing the random distribution pattern. It was suitable to use the counting and distance indices for determining the distribution pattern in the areas with appropriate species density, and it was better to mainly use the distance indices to determine the distribution pattern in the areas with light and small mounds. Therefore, the distance indices were of high priority in determining the distribution pattern in the two areas. The results showed that the average distances among *Astragalus gossipinus* and *Bromus tomentellus* were 130 cm and 5.8 cm respectively. Understanding of the average distance among the plants can be helpful in determining the planting distances and the number of seedlings required for the plants with forage value in similar areas.

The distribution curves of the species were created by the relationship between the quadrat's frequency and the number of individuals in each quadrat (Fig. 3). As shown in Fig. 3, the shape of the distribution frequency curve of *A. gossypinus* completely tends to the left (clumped distribution). However, the shape of the distribution curve of *B. tomentellus* is more symmetrical and tends to be slightly right (random distribution). Therefore, these curves confirm the results of the above-mentioned methods. According to the obtained results, the distribution pattern is clumped in communities with a dominant cover of shrub, and quadrat indices are less efficient compared to distance indices. However, in high-diversity communities with predominant covers of gross and forb, the spatial distribution is random and quadrat and distance indices are more convergent.

CONCLUSION

In this study, it was attempted to evaluate the biodiversity and spatial distribution patterns of two dominant types of vegetation in rangelands of Kermanshah, Iran. The most important species of the first vegetation type (Type 1) were *Astragalus gossypinus* (dominant species), *Astragalus brachystachis, Bromus tomentellus*, and *Gundelia turnefortii* and the dominant family of the second vegetation type (Type 2) was Poaceae with the dominant species of *Bromus tomentellus* and other species such as *Hordeum bulbosum* and *Gundelia turnefortii* (belonging to the compositae family). Results showed that the number of Graminae species was higher in vegetation Type 2 than in vegetation Type 1. The Scrophulariaceae, Malvaceae, Papaveraceae, and Euphorbiaceae families were not found in vegetation Type 1. Calculation of the Johnson and Zimer, Eberhart, and variance-to-mean ratio indices demonstrated that distribution of *Bromus tomentellus* in the second vegetation type was random and the distribution pattern of *Astragalus gossypinus* is random with more tendency to be clumped. It was shown that the distribution of *Astragalus gossypinus* was negative binominal (P ≥ 0.05) (representing the clumped distribution pattern), and the distribution

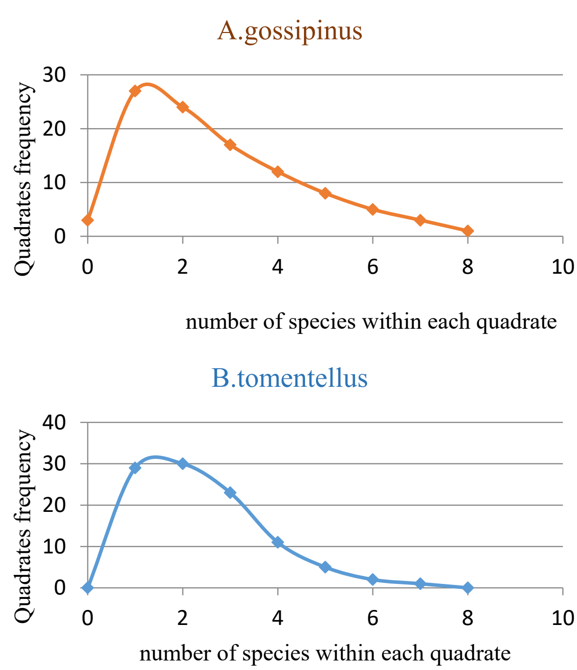

Fig. 3: Curves for distribution frequency of the species

of *Bromus tomentellus* was Poisson (P ≥ 0.05) (representing the random distribution pattern and the distribution pattern is clumped in communities with a dominant cover of shrub, and quadrat indices are less efficient compared to distance indices. All in all, results of species diversity and richness indices showed that, compared to vegetation Type 1, vegetation Type 2 had higher values for these indices. Therefore, it was recommended to protect vegetation Type 1 against adverse environmental and human factors to make it more diverse. The surveyed sites had a certain number of exclusive species, which could be due to differences in environmental factors or other aspects which were not measured in this study. The distribution patterns of two dominant species were different, which could be due to structures of species growth and reproduction. Moreover, when the spatial patterns of species were more uniform, the distance and quadrat indices were more convergent. It was found that effective public policies were required for conservation of the Northern Zagros rangelands as an important biodiversity reservoir. This study can provide a baseline for performing more detailed studies by focusing on the systems of the Zagros rangelands as well as the distribution and dynamics of the flora.

AUTHOR CONTRIBUTIONS

Z. Mohebi performed the experiments and literature review, analyzed and interpreted the data, prepared the manuscript text, and manuscript edition. H. Mirzaee performed experimental design, helped in the literature review and manuscript preparation.

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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

A.	Astragalus
B.	Bromus
Eq.	Equation
Fig.	Figure
m^2	Square meters
p	Probability level
R	Species richness
I_E	Eberhart's index
P	Pielou's index
I_H	Hopkins' test
A	Holgate's index
M_u	Morisita's index
GI	Green's index
LI	Lioyd's index
ID	The variance-to-mean ratio index

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