Global J. Environ. Sci. Manage. 9(4): 995-1004, Autumn 2023, Serial #36

Global Journal of Environmental Science and Management
(GJESM)

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

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CASE STUDY

Heavy metals contamination in geothermal medicinal plant extract (*Chromolaena odorata* Linn)

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ARTICLE INFO

Article History:
Received 06 December 2022
Revised 12 March 2023
Accepted 22 April 2023

Keywords:
Accumulator
Arsenic (As)
Cadmium (Cd)
Hot spring
Lead (Pb)
Mercury (Hg)

DOI: 10.22035/gjesm.2023.04.22

ABSTRACT

BACKGROUND AND OBJECTIVES: Medicinal plants growing in geothermal areas have been reported to possess relatively high abundance of bioactive secondary metabolites concomittant to the adaptive heat stress response. Nonetheless, the exploitation of their medicinal benefits is limited by the possible health-threatening concentrations of heavy metal contamination. *Chromolaena odorata* Linn, or also called as seurapoh, is a well-known medicinal plant but could absorb and accumulate heavy metal from the soil. Herein, this present study aimed to investigate the contents of Hg, Pb, Cd, and As in ethanolic extract of *Chromolaena odorata* Linn leaves collected from a geothermal area in Aceh of Indonesia.
METHODS: Three hot springs (Ie-Suum, Ie-Jue, and Ie-Brouk) located in the same geothermal area Seulawah Agam was selected as the sampling points, where three samples of *Chromolaena odorata* were collected in each sampling point. Extraction was carried out by means of maceration employing ethanol solvent. The contents of heavy metals in each extract were determined by priorly validated atomic absoption spectrometry and graphite furnace atomic absorption.
FINDINGS: The results revealed that arsenic (0.0482 ± 0.004 – 0.0639 ± 0.007 miligram per kilogram) and lead (0.0219 ± 0.004 – 0.0672 ± 0.006 miligram per kilogram) were found in trace levels and did not exceed Indonesian maximum safety thresholds (≤5 and ≤10 miligram per kilogram, respectively). The presence of mercury in all samples was not observable (limit of detection= 0.018 miligram per liter). Cadmium was observed in almost all samples with a concentration range of 0.0219 ± 0.005 – 1.1472 ± 0.006 miligram per kilogram exceeding the maximum threshold (0.3 miligram per liter).
CONCLUSION: Contamination of heavy metals in the ethanolic extract of geothermal *Chromolaena odorata* leaves is thought to be originated from volcanic activities. Among the heavy metals of concern, cadmium was the only one with concentration exceeding the safety limit. The presence of cadmium in the extract with high concentration could act cause its translocation to human body which eventually lead to multiple organ damages. Therefore, the extract of geothermal C. odorata leaves collected from a geothermal area should be consumed with caution for possible cadmium intoxication.

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
47	0	4

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Note: Discussion period for this manuscript open until January 1, 2024 on GJESM website at the “Show Article”.

INTRODUCTION

Geothermal has provided unique ecological conditions for living organisms such as microbes and plants to live on and adapt with. Thus, there are high chance for organisms inhabiting geothermal areas to possess different characteristics from their counterparts living in other regions (Boothroyd, 2009; Rachmilevitch *et al.*, 2006).In response to geothermal heat stress, several plants have been witnessed to experience the shifting of metabolomes with increased production of phenolic acids and terpenes (Gargallo-Garriga *et al*., 2017). This phenomenon is thought to be beneficial to enhance the therapeutic effect of medicinal plants growing within geothermal areas. The study group has reported more efficacious effects of extracts of several medicinal plants collected from Mount Seulawah Agam – a volcano located in Aceh, Indonesia. Of which, the research group has investigated the antibacterial and antioxidant activities of *Calotropis gigantea* (Kemala *et al*., 2022; Ningsih *et al*., 2022) and *Vitex pinnata* (Nuraskin *et al*., 2019a; Nuraskin *et al.*, 2019b). Other than the foregoing plants, *Chromolaena odorata* Linn (also known as *seurapoh* by the locals) is commonly used as a traditional medicine to treat various diseases, including fever, diarrhea, malaria, and skin diseases (Phumthum *et al*., 2020). Scientifically, *C. odorata* Linn has been reported effective as antiulcer, anti-inflammatory, antipyretic, antimicrobial, antioxidant, analgesic, and diuretic agents (Olawale *et al*., 2022; Sabri and Yusof, 2021). In a qualitative screening, *C. odorata* from a geothermal area in Aceh was found positive containing tannins, steroids, flavonoids, terpenoids, phenols, and saponins. Previously, a preliminary study has revealed that secondary metabolites were thought to be different between those produced in geothermal and non-geothermal *C. odorata* based on chemometric classification (Abubakar *et al*., 2021). These all are indicative that *C. odorata* from geothermal area has relatively high secondary bioactive metabolites, hence higher therapeutic efficacy. Unfortunately, based on a recent investigation, hot springs manifested in Mount Seulawah Agam were contaminated with arsenic (As) in high concentration, where bioaccumulation of this heavy metal in people exposed daily to the water was observed (Irnawati *et al*., 2021). Volcanic activities are responsible to the release of heavy metals to the surrounding environment (Nagajyoti *et al*., 2010). Since *C. odorata* has a strong bioaccumulation ability (Tanhan *et al.*, 2007), its oral uptake as herbal medicine might act as the translocation route of heavy metals from environment to human body (Behera and Bhattacharya, 2016; Yap *et al*., 2010). These findings stress the importance of performing thorough assessment of heavy metal contents in the extract of *C. odorata* before used as herbal medicine. Previously, researchers have reported the heavy metal contaminations in herbal medicine commercially available and commonly consumed in China (Meng *et al*., 2022; Wang *et al*., 2019) and Pakistan (Soomro *et al.*, 2021). However, none of the published studies has investigated the geothermal medicinal plant, particularly for *C. odorata.* Herein, the contents of mercury (Hg), lead (Pb), cadmium (Cd), and As in the ethanolic extract from geothermal *C. odorata* leaves were investigated. The objective of this study was to determine the presence of heavy metal contents in the ethanolic extract of *Chromolaena odorata* Linn leaves collected from geothermal area in Aceh, Indonesia. This study was performed in Aceh Province, Indonesia in 2022.

MATERIALS AND METHODS

Study design

This study further aimed to draw a conclusion about the safety of consuming the extract based on the heavy metal content profiles. The heavy metals analyzed herein were Hg, Pb, Cd, and As, quantitatively determined using Atomic Absorption Spectrometry (AAS) or Graphite Furnace—AAS (GF-AAS). The heavy metal concentrations were then compared with the maximum thresholds observed by the Indonesian Agency for Drug and Food Control (BPOM) (Ahmad *et al*., 2022).

Plant specimens and chemicals

Plants specimen (*Chromolaena odorata* Linn) was collected from three separated hot springs namely Ie-Suum, Ie-Jue, and Ie-Brouk, located within the same geothermal area Mount Seulawah Agam in Aceh Besar Regency, Aceh Province, Indonesia. In each location, the plant specimen was collected from three different sampling points as presented in detail in Table 1. The sampling locations are not commonly exposed to anthropogenic activities, except for Ie-Suum receiving intense tourist visits. Taxonomical identification of the collected specimen was carried

Table 1: Sampling locations and sample weights of *C. odorata* Linn leaves

Location	Label	Coordinate		Leaf sample weight (kg)	
		N	E	Fresh	Dry
Ie-Suum	IS-1	5°32'51"	95°32'53"	3.00	0.61
	IS-2	5°32'52"	95°32'49"	3.45	0.64
	IS-3	5°32'54"	95°32'32"	3.95	0.68
Ie-Jue	IJ-1	5°30'23"	95°37'45"	3.35	0.64
	IJ-2	5°30'25"	95°37'42"	3.45	0.64
	IJ-3	5°30'24"	95°37'38"	3.15	0.60
Ie-Brouk	IB-1	5°51'68"	95°62'06"	3.00	0.60
	IB-2	5°51'59"	95°62'00"	3.45	0.64
	IB-3	5°51'82"	95°61'69"	3.00	0.65

out in Department of Biology, Universitas Syiah Kuala, Indonesia, with voucher number: 179/UN11.1.8/TA.00.03/2023. To determine the concentrations of heavy metals in the plant specimen, the following chemicals or materials were required: Argon gass, nitric acid (HNO_3), hydrogen peroxide (H_2O_2) 30%, perchloric acid ($HClO_4$), sulfuric acid (H_2SO_4), deionized water, and standard solutions of Hg, Pb, Cd, and As. As for the extraction, ethanol 96% solvent was used. All chemicals were analytical grade and used without pre-treatment after being procured from Merck (Selangor, Malaysia).

Preparation of the ethanolic extract from C. odorata Linn leaves

In general, the extract was prepared following the previously published study with a few modifications (Abubakar *et al*., 2021). Briefly, the leaves of *C. odorata* Linn; 200 gram (g) were air-dried for a week prior to maceration using ethanol 96% for 2x24 hour (h) at room temperature. The filtrate was separated by filtration and proceeded to rotary evaporation to obtain the concentrated extract as much as 40 g.

Pre-treatment for heavy metal determination

Before analyzed, the leaf samples were pre-treated by acidic destruction. Leaves were weighed for 1 g and placed on a glass container following with the addition of concentrated 7 mililiter (mL) HNO_3 and 1 mL H_2O_2 30%. Afterward, the mixture was heated at 80 degree Celcius (°C) on hot plate for 30 minutes (min), consequently left-cold and filtered. The 5 mL filtrate was diluted in 50 mL volumetric flask.

Calibration curve construction and validation

Calibration curves for AS and Hg were constructed based on the signal intensities recieved at 193.7 and 253.7 nanometer (nm) generated by the solution standards, respectively. Meanwhile in the case of Pb and Cd, wavelengths of 283.3 and 228.8 nm were used, respectively. Limit of detection (LoD) and limit of quantification (LoQ) were determine for each heavy metal to measure the sensitivity based on LINEST function on Microsoft Excel (Redmond, Washington, USA) (Iqhrammullah *et al*., 2021; Nisah *et al*., 2022). Quality of the calibration curve was judged based on the linearity (R^2). Precision and recovery were also calculated to validate the analytical methods (Nisah *et al*., 2022; Safitri *et al*., 2022). The relative standar deviation (RSD) was used to determine the precision, which was obtained from 6 repetitions of a particular concentration of the heavy metal. As for the recovery (%), the value was calculated based on the signal intensity generated by the spiked sample. Validities of the used methods have been presented in Table 2.

Determination of As and Hg using GF-AAS

Determinations of AS and Hg were carried out on GF-AAS (Perkin Elmer PinAAcle 900 Series AA Spectrometers, Waltham, Massachusetts, United States). Pre-treated solution (20 µL) was injected through autosampler into the furnace tube for diatomization. Concentrations of As and Hg were determined at 193.7 and 253.7 nm, respectively. Each sample was analyzed in triplicate and the data were presented as mean±standard deviation (SD).

Plant specimens and chemicals

As for Pb and Cd, their concentrations were determined using AAS Flame (Thermo Fisher Scientific iCE 3500, Waltham, Massachusetts, United States). Wavelengths of 283.3 and 228.8 nm were

Table 2: Sensitivity, precision, and accuracy of the methods used to determine the heavy metal contents

Heavy metals	LoD (mg/L)	LoQ (mg/L)	R^2	RSD (%)	Recovery (%)
Pb	0.016	0.048	0.9995	1.01	99.86
Cd	0.024	0.072	0.9990	0.99	100.11
As	0.020	0.062	0.9993	1.05	100.28
Hg	0.018	0.055	0.9994	1.07	101.77

Table 3: Geothermal soil characteristics of the sampling locations (Idroes *et al*., 2019)

Hot spring	Temperature (°C)	Elevation (m)	pH
Ie-Suum	83.63±0.075 – 86.09±0.019	70-72	6.66±0.000 –6.68±0.004
Ie-Jue	93.49±0.172 – 98.62±0.151	264-269	3.95±0.048 –5.93±0.005
Ie-Brouk	40.04±0.013 – 47.49±0.133	197-210	7.24±0.004 –7.40±0.058

employed for the determincations of atomized Pb and Cd, respectively. The analysis was carried out in triplicate for each sample, where the values obtained were presented as mean±SD.

RESULTS AND DISCUSSION

Hot spring characteristics

It is of importance to firstly understand the factors that contributing to the distribution of heavy metal. Obviously, plant species and variety are the determining factors of heavy metal uptake and accumulation. Similarly, the age of the plant is positively correlated to the heavy metal accumulation. Environmental factors are also known to affect the uptake and accumulation of heavy metals by plants including metal concentration in the soil, cation exchange capacity, soil pH, and contents of organic matters. Regardless, the most contributing factors are those related to the environment and soil characteristics (Annan *et al*., 2013). Hence, the soil characteristics surrounding the sampling locations have been presented (Table 3), which have been published in the previous work (Idroes *et al*., 2019). As for the presence of heavy metal in the geothermal area, a previous study has suggested the presence of As in geothermal soil and water of Mount Seulawah Agam (Irnawati *et al*., 2021). Other heavy metals, including Pb, Hg, and Cd, have been reported in various locations of geothermal areas (Pulungan *et al*., 2019; Zimik *et al*., 2021).

Heavy metal contaminations and their bioaccumulation

Heavy metal concentrations contained in ethanolic extract of *C. odorata* Linn leaves collected from the geothermal hot spring manifestations have been presented in Table 3. Based on the AAS method used with LoD of 0.018 mg/L for Hg, the heavy metal was not observable in all samples. In the case of As, its presence was only found in two samples collected from Ie-Jue with concentrations of 0.0482 ± 0.004 – 0.0639 ± 0.007 mg/kg. Cd was detected in all samples collected from hot springs Ie-Jue (0.8511 ± 0.005 – 1.0517 ± 0.007 mg/kg) and Ie-Brouk (0.5738 ± 0.006 – 1.1472 ± 0.006 mg/kg), while in the case of Ie-Suum, only a single sample was detected with Cd (0.0219 ± 0.005 mg/kg). Pb was quantitatively observable in all samples, where the highest concentration range was found in Ie Brouk (0.0544 ± 0.006 – 0.0672 ± 0.006 mg/kg). The concentration ranges for Pb content were 0.0219 ± 0.004 – 0.0316 ± 0.004 and 0.0329 ± 0.005 – 0.0514 ± 0.005 mg/kg for Ie-Suum and Ie-Jue, respectively.

In this present study, the presence of heavy metal contents was found in the ethanolic extract of *C. odorata* Linn leaves predominated in relatively high concentration by Cd. Hg was not observable in all samples, while only two samples were detected to contain As. Pb was found in all samples in a variety of concentrations ranging from 0.0219 ± 0.004 to 0.0672 ± 0.006 mg/kg. Of the four heavy metals assessed herein, only Cd found exceeding the maximum threshold by the BPOM. The differences in the observed heavy metal concentrations might be attributed to their solubility in ethanol, as not all species of Pb, Cd, As, or Hg are soluble in ethanol. Therefore, their concentrations are not reflective to the real concentration accumulated in the *C. odorata* Linn leaves. Regardless, the presence of heavy metals in the samples of this present study was

Table 4: Heavy metal concentrations contained in the ethanolic extract from *C. odorata* Linn leaves

Sample	Heavy metal concentration (mg/kg)			
	As	Cd	Hg	Pb
IS-1	ND	ND	ND	0.0316 ± 0.004
IS-2	ND	N	ND	0.0304 ± 0.005
IS-3	ND	0.0219 ± 0.005	ND	0.0219 ± 0.004
IJ-1	ND	0.8511 ± 0.005	ND	0.0514 ± 0.005
IJ-2	0.0639 ± 0.007	1.0360 ± 0.004	ND	0.0366 ± 0.005
IJ-3	0.0482 ± 0.004	1.0517 ± 0.007	ND	0.0329 ± 0.005
IB-1	ND	0.9482 ± 0.007	ND	0.0544 ± 0.006
IB-2	ND	0.5738 ± 0.006	ND	0.0672 ± 0.006
IB-3	ND	1.1472 ± 0.006	ND	0.0599 ± 0.004
Max. threshold	≤5	≤0.3	≤0.5	≤10

ND = Not detected

more likely occurring naturally, rather than caused by anthropogenic activities. Pb concentrations in samples collected from Ie-Suum, a place with relatively intense anthropogenic activities, were found less as compared to that of other hot springs (Ie-Jue and Ie-Brouk). As is an example of heavy metals that are commonly released through natural weathering, geochemical reactions, and volcanic eruptions (Maity *et al*., 2019). Similarly, the contamination of Cd has been associated with natural events such as volcanic eruptions and forest fires (Briffa *et al*., 2020). As weeds, *C. odorata* Linn is known to accumulate heavy metal with high survival rate against the environmental distress and has been utilized as phytoremediator for contaminated soil (Aiyesanmi *et al*., 2012; Tanhan *et al*., 2007). The uptake of heavy metals from the soil by *C. odorata* Linn have been reported in multiple studies. According to a published literature, Pb and Cd contents in the root of *C. odorata* exposed with intense vehicle traffic were found reaching as high as 3.79±0.19 and 0.01±0.00 mg/kg, respectively (Sulaiman and Hamzah, 2018). The distribution of heavy metal in *C. odorata* Linn was varied throughout the plant parts, where its shoots and roots could accumulated Cd as high as 102.3 and 1440.9 mg/kg, respectively (Tanhan *et al*., 2007). Moreover, the study also revealed that the accumulation was concentration-dependent, reaching accumulated concentrations as high as 1772.3 and 60655.7 mg/kg for Pb and Cd, respectively, confirming high heavy metal uptake capacity of this plant (Tanhan *et al*., 2007).

Health risk related to heavy metal exposure

In a previous study investigating the urinary and blood samples, accumulation of heavy metals in human body is less likely to be associated with the geothermal-sourced contamination (Nuvolone *et al*., 2022). However, accumulation through plants or animals might give a different insight on how naturally occuring heavy metal contamination could contribute to the exposure risk to humans (Ahmad *et al*., 2022). This present study is the first in reporting the heavy metal contaminations derived from geothermal sources in medicinal plant extract. Presence of heavy metal in the extract of *C. odorata* leaves found herein alarms the users of this medicinal plant regarding the possible heavy metal intoxication. Bioaccumulated heavy metal in the plant could be translocated to human and cause a series of health problems (Behera and Bhattacharya, 2016; Yap *et al*., 2010). Herein, it is found that Cd contaminated the ethanolic extract from geothermal *C. odorata* leaves at the concerning level since its concentration exceeded the maximum safety threshold. As the easiest heavy metal to be absorbed and accumulated in plants, translocation of Cd from plants to human is common (Yang *et al*., 2022). When present in human body, even at its lowest concentration, Cd could cause serious illness and even mortality. A study suggested that Cd is accumulated in immune cells, thereby modulating immune system function which triggers the systemic inflammation cascade (Hocaoğlu-Özyİğİt and Genç, 2020). Furthermore, Cd-associated immune response causes cell apoptosis and adversely alters secretion of cytokine, subset frequency of T lymphocyte, and production of selective antibodies (Wang *et al*., 2021). Organs such as lungs, kidneys, liver, gastrointestinal tract, and bones are susceptible to Cd toxicity. As the organ with the highest likelihood of being exposed to Cd after its oral intake, gastrointestinal tract has been suggested as the target of Cd toxicity (Zhao *et al*., 2006). Other than causing inflammation in the gut, Cd intoxication further progress to the dysbiosis of gut microbiome (Ba *et al*., 2017; Ninkov *et al*., 2015). Moreover, Cd exposure through inhalation could harm the upper respiratory tract, where it induced overproduction of mucus and disrupted the cilia function and squamous cells differentiation (Xiong *et al.,* 2019). When this heavy metal is absorbed and translocated to the blood stream, it could be accumulated and triggers the damages in multiple organs. In the blood stream, Cd was shown to reduce the activity of superoxide dismutase and catalase of erythrocyte which cause the imbalance of oxidative stress (Jemai *et al*., 2007). Several research has shown the acute toxicity of this heavy metal in artificial biological systems suggesting its ability to exert lethal nephortoxicity and hepatoxicity (Ibraheem *et al*., 2016; Wang *et al*., 2016). In addition, Cd accumulation has been reported to occur in bones (Tai *et al*., 2022), and consequently contributes to reduced bone mineral density, altered the expression of bone formation genes, disrupted osteoclast activities, reduced calcium absorption, and increased the risk of ostheoporosis (Ou *et al.*, 2021; Reyes-Hinojosa *et al*., 2019).

CONCLUSION

Geothermal activity provides a unique activities for plants to produce different metabolite profiles as compared to those growing in area without geothermal activity. This encourage researchers and medical practitioners to utilize geothermal plants since they exert higher levels of bioactive secondary metabolites. However, geothermal activity promotes the release of toxic heavy metals from the earth's crust. Therefore, there is high possibility that the heavy metal would contaminate the plants inhabiting the geothermal areas. This is more pronounced in plants with an ability of high heavy metal uptake, including the *C. odorata*. In this present study, the contents of heavy metals in the extract of *C. odorata* collected from geothermal area were determined. The presence of As, Cd, and Pb contaminations in the ethanolic extract of geothermal *C. odorata* Linn leaves was found within the observable concentration range. It should be noted that the As, Cd, Pb, and Hg have different solubility in ethanol, so their concentrations in the extract are not reflective to their accumulated contents in the plant. The presence of these heavy metals are found to be non-dependent to the intensity of anthropogenic activities indicating their origins are from the natural occurrence. As for Hg, its concentration is thought to be below the LoD of the used analytical tool. In this case, the Hg could be present in lower concentration than the LoD so it could not be detected. Though the concentrations of the foregoing heavy metals are below the maximum safety level, their presence still deserve some concerns because of their capability to be accumulated in biological system. Cd concentrations in the extract exceeded the allowable limit, suggesting the potential harms of its consumption as herbal medicine. Cd exposure could lead to several serious pathologic conditions such as nephorpathy, pulmopathy, hepatopathy, and so on. Moreover, individuals intoxicated with Cd could experience an increased risk of ostheoporosis, reduced mineral density, and other ostheo-related problems. The presence of these heavy metals in the extract might counter the therpeutic benefits of *C. odorata* Linn. These findings also imply the possibility of heavy metal uptakes by other medicinal plants in geothermal area. A stringent quality control is therefore recommended in monitoring the content of toxic impurities in herbal products collected from geothermal area.

AUTHOR CONTRIBUTIONS

A. Abubakar contributed in the conceptualization, experiment, and original-draft writing. H. Yusuf acted as a supervisor and contributed in reviewing the final version of the manuscript. M. Syukri acted as a supervisor and contributed in reviewing the final version of the manuscript. R. Nasution acted as a supervisor and contributed in reviewing the final version of the manuscript. M. Yusuf assisted the experiment and performed formal análisis. R. Idroes performed the scientific, results validation, and reviewing the final version of the manuscript.

ACKNOWLEDGEMENT

Authors appreciate the assistance from Universitas Syiah Kuala for the research facilities.

CONFLICT OF INTEREST

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

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ABBREVIATIONS

%	Percent
µL	Microliter
AAS	Atomic Absorption Spectrometry (AAS)
As	Arsenic
BPOM	Indonesian Agency for Drug and Food Control
°C	Degree celcius
Cd	Cadmium
g	Gram
GF-AAS	Graphite Furnace— Atomic Absorption Spectrometry
h	Hour
Hg	Mercury
LoD	Limit of detection
LoQ	Limit of quantification
min	Minute
mg/kg	Milligram per kilogram
mg/L	Miligram per liter
mL	Milimeter
nm	Nanometer
ND	Not detected
RSD	Relative standard deviation
SD	Standard deviation

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HOW TO CITE THIS ARTICLE

Abubakar, A.; Yusuf, H.; Syukri, M.; Nasution, R.; Yusuf, M.; Idroes, R., (2023). Heavy metals contamination in geothermal medicinal plant. Global J. Environ. Sci, Manage., 9(4): 995-1004.

DOI: 10.22035/gjesm.2023.04.22

URL: https://www.gjesm.net/article_704441.html