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Original Article

Analysis of Heavy Metal Contents of *Marsilea crenata* Presl. Leaves and Soils from East Java Province, Indonesia

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ABSTRACT

Introduction: *Marsilea crenata* Presl. grows in East Java Province, Indonesia, and showed several pharmacological activities due to its phytoestrogen content. Research indicated its phytoremediation property, and therefore has an impact on heavy metal accumulation in the plants. Lead, cadmium, and mercury are amongst heavy metals that are toxic to the human body. This study aimed to determine and compare Pb, Cd, and Hg mineral contents of soils and leaves of the plants grew in Batu, Pasuruan, Surabaya, and Kediri cities in the province. **Method:** 10 g of dried powdered leaves and soils were put in separate crucible porcelains, and add 2ml of nitric acid. All were burnt in low heat until white smoke were not formed, and then stood to cool. Ashes obtained were examined using AA 6200 Atomic Absorption Spectrometer (AAS). **Result:** Showed that leaves samples from the areas showed no Pb and Hg mineral contents that exceeded the permissible limit. Batu City leaf sample had Cd heavy metal content of 2.79 ± 0.01 ppm, which exceeded the permissible limit. **Conclusion:** Analysis of heavy metal contents of soils from all of the areas showed no exceed limit of Pb and Hg mineral concentrations. Cd soil contents of Surabaya and Kediri cities, were 0.73 ± 0.00 and 2.61 ± 0.08 ppm respectively.

Key words: AAS, Clover, Phytoremediation.

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INTRODUCTION

Marsilea crenata Presl. is an aquatic plant and has been used through generations by people of Surabaya City and East Java Province of Indonesia as vegetables. Through unknown resources, it was known that *Marsilea crenata Presl.* leaves had antiosteoporosis property among post-menopausal women, and therefore scientific research were being conducted to prove it. A research showed that *Marsilea crenata* Presl. leaves had activity in bone formation in an in vitro study using MC3T3-E1 cell line and in vivo in female mice[1,2]. Further research was conducted to study the neuroprotective activity of the leaves by inhibition of neuroinflammation in prolonged activation of microglia HMC3 cell line classical pathway[3,4].

Research revealed, that such activities are caused by the phytoestrogen content of leaves[1,3,5,6], through its binding affinity to estrogen receptors (ERs) of cells[7,8]. Phytoestrogen is a group of compounds of plants with either estrogen-like structure or estrogenic function, and can bind to the receptor through ER dependent or ER-independent pathways[9-11].

On the other hand, the plant has heavy metal phytoremediation property[12]. An unpublished research revealed high lead and mercury concentrations of powdered leaves of plants cultivated in water in its original habitat in East Java. It proved the phytoremediation property of the plant. The phytoremediation capability has an impact on heavy metal accumulation in the plants, and is critical for human consumption[13].

Lead (Pb), cadmium (Cd), and mercury (Hg) are examples of heavy metal that are toxic to human. The average Pb rate of absorption for adults is 10 to 15% of the total consumption, and this amount can increase to 50% among infants, young children and pregnant women[14]. Animal study showed the absorption range of Cd from the intestine between 0.5% to 3%[15], but so far no information on the correlation of the blood or urine Cd content with its clinical toxicity[16]. Lead is mostly accumulated in blood, and symptoms of poisoning occurred when it reached a level of blood concentration of 0.2–0.6 ppm. Such concentration could be reached through daily intake of 0.3–1.0 mg of Hg among healthy men[17].

Soil heavy metal pollution has become a worldwide environmental issue that has attracted considerable public attention, largely from the increasing concern for the security of agricultural products. Heavy metals enter the soil agro-ecosystem through natural processes derived from parent materials, and through anthropogenic activities, and it can accumulate in the soil[18]. Therefore, further research is needed to study more on the areas of cultivation of the plant to find a better leaf quality free of toxic heavy metal pollution.

This study aims to determine and compare Pb, Cd, and Hg contents of *Marsilea crenata* Presl. leaves and soils of selected areas from Kediri, Batu, Pasuruan, and Surabaya Cities as some of the areas where the plant grows abundantly in East Java Province, Indonesia. Determination is based on the Indonesian National Food and Drug Agency Regulatory No. 12, 2014[19], and World Health Organization[20] heavy metal permissible limits. Selection of areas is also based on the altitude of each place that cause differences

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in temperature and rainfall. Those are among external factors that influence varieties of secondary metabolites and affect the plant phytoremediation property[21].

MATERIALS AND METHOD

Materials

Plant and soil materials

Marsilea crenata Presl. leaves were collected from Batu District of Batu City, Purwodadi District of Pasuruan City, Benowo District of Surabaya City, and Wates District of Kediri City. All districts had different temperatures, altitudes and rain falls (Table 1). All leaves were identified in Center for Plant Determination, Unit Pelaksana Teknis, Materia Medica, Batu, Indonesia, under specimen number 1a-17b-18a-1. The leaves were harvested at 14 days of age, as can be seen from the color, that was dark green, and the size, that was 2 cm in diameter (Figure 1).

The leaves were dried and powdered, while the soils from each selected area where plants grew were taken and analyzed for heavy metal contents.

Chemical materials

Standard solutions of Pb, Cd, and Hg (Merck), HNO_3 (Merck), and aquadestilata (Merck) were purchased from Chemical Analysis and Measurement Unit, Faculty of Mathematics and Natural Science, Brawijaya University, Indonesia.

Method

Heavy metal analysis of Marsilea crenata Presl. leaves and the soil

Each standard of heavy metal was diluted with various solution. Cd and Pb were diluted with HNO_3 1% and Hg with aquadestilata as solvents. Each solution was prepared in concentrations of 0.05; 0.1; 0.25; 0.5; and 1 ppm. Samples of 10 grams of dried powdered leaves and soils were put in separate crucible porcelains, and burnt carefully at low heat to decomposed. During this process all crucible porcelain should be covered tightly. 2 ml of nitric acid then were added into each of the crucible porcelain, and continued heating at 500 °C up to 600° C until white smoke was no longer formed. All samples were remove from heat and stood to cool, and then remove to 25-ml measuring flasks. Samples for Pb and Cd identifications were dissolved in 1% HNO_3, while for Hg identification in aquadestilata. All were then filtered with ash-free filter papers, and examined with AA 6200 Atomic Absorption Spectrometer (AAS) (Shimadzu, Japan) to calculate the concentration of Pb, Cd, and Hg[23].

RESULTS

Heavy metal analysis of Marsilea crenata Presl. leaves

Results of AAS analysis on the heavy metal contents of *Marsilea crenata* Presl. leaves from Batu, Purwodadi, Benowo, Wates Districts, East Java Province, Indonesia are as follows (Table 2).

Based on the WHO guidelines and Indonesian National Food and Drug Agency Regulatory, Batu District showed Pb and Hg concentrations within the permissible limits, while Cd concentration was exceeded the limit. Purwodadi, Benowo, and Wates Districts showed Pb, Cd, and Hg concentrations within the permissible limits. It was shown that, Batu District was the only area in the study to have heavy metal content exceeded the permissible limit, that is Cd, with concentration of 2.79 ppm.

Heavy metal analysis of soils

Results of AAS analysis on the heavy metal contents of soils from Batu, Purwodadi, Benowo, Wates Districts, East Java Province, Indonesia were as follows (Table 3).

Soil samples from all areas were within the permissible limits of Pb and Hg concentrations. Batu and Purwodadi Districts showed Pb, Cd, and Hg concentrations within the permissible limits. Benowo and Wates Districts showed exceeding limit of Cd concentrations.

A comparison between Pb, Cd and Hg mineral contents and concentrations of leaves and soils of Batu, Purwodadi, Benowo, Wates Districts, East Java Province, Indonesia was described as follows (Table 4).

Figure 1: *Marsilea crenata* Presl. Leaves.

Table 1: Demography of areas of cultivation of *Marsilea crenata* Presl.

No.	Area	Soil Type	Elevation (masl)	Rainfall/Year (mm)	Temperature (°C)
1.	Batu District, Batu City	Andosol	900-1000	2001-2500	24-26
2.	Purwodadi District, Pasuruan City	Alluvial	350-700	2001-2500	25-32
3.	Benowo District, Surabaya City	Alluvial	20-30	1001-1500	24-32
4.	Wates District, Kediri City	Regosol	50-60	15001-2000	23-30

(Indonesian National Meteorology, Climatology, Geophysics Agency, 2019)[22]

Table 2: Heavy metal contents of *Marsilea crenata* Presl. leaves from Batu, Purwodadi, Benowo, Wates Districts, East Java Province, Indonesia.

No.	Area	Heavy Metal Concentration (ppm)		
		Pb (limit < 10 ppm)	Cd (limit < 0,3 ppm)	Hg (limit < 0,5 ppm)
1.	Batu District, Batu City	0.00	2.79 ± 0.01*	0.00
2.	Purwodadi District, Pasuruan City	0.00	0.00	0.00
3.	Benowo District, Surabaya City	1.06 ± 0.01	0.00	0.00
4.	Wates District, Kediri City	0.00	0.00	0.00

*Exceed the safe limit from Indonesian National Food and Drug Agency Regulatory and WHO Guideline
Permissible limits of Indonesian National Food and Drug Agency Regulatory No. 12/2014: Pb 10 ppm, Cd 0.3 ppm, and Hg 0.5 ppm,
Permissible limits of WHO Guideline: Pb 10 ppm, Cd 0.3 ppm, and Hg 0.5 ppm.

Table 3: Heavy metals contents in soil samples from Batu, Purwodadi, Benowo, Waters Districts, East Java Province, Indonesia.

No.	Area	Heavy Metal Concentration (ppm)		
		Pb (limit < 10 ppm)	Cd (limit < 0,3 ppm)	Hg (limit < 0,5 ppm)
1.	Batu District, Batu City	6.60 ± 0.21	0.00	0.00
2.	Purwodadi District, Pasuruan City	2.77 ± 0.10	0.00	0.00
3.	Benowo District, Surabaya City	3.30 ± 0.10	0.73 ± 0.00*	0.00
4.	Wates District, Kediri City	0.00	2.61 ± 0.08*	0.00

*Exceed the safe limit from Indonesian National Food and Drug Agency Regulatory and WHO Guideline
Permissible limits of Indonesian National Food and Drug Agency Regulatory No. 12/2014: Pb 10 ppm, Cd 0.3 ppm, and Hg 0.5 ppm,
Permissible limits of WHO Guideline: Pb 10 ppm, Cd 0.3 ppm, and Hg 0.5 ppm.

Table 4: Pb, Cd and Hg mineral contents of leaves and soils of Batu, Purwodadi, Benowo, Wates Districts, East Java Province, Indonesia.

No.	Area	Heavy Metal Concentration (ppm)					
		Leaf			Soil		
		Pb (limit < 10 ppm)	Cd (limit < 0,3 ppm)	Hg (limit < 0,5 ppm)	Pb (limit < 10 ppm)	Cd (limit < 0,3 ppm)	Hg (limit < 0,5 ppm)
1.	Batu District, Batu City	0.00	2.79 ± 0.01*	0.00	6.60 ± 0.21	0.00	0.00
2.	Purwodadi District, Pasuruan City	0.00	0.00	0.00	2.77 ± 0.10	0.00	0.00
3.	Benowo District, Surabaya City	1.06 ± 0.01	0.00	0.00	3.30 ± 0.10	0.73 ± 0.00*	0.00
4.	Wates District, Kediri City	0.00	0.00	0.00	0.00	2.61 ± 0.08*	0.00

*Exceed the safe limit from Indonesian National Food and Drug Agency Regulatory and WHO Guideline
Permissible limits of Indonesian National Food and Drug Agency Regulatory No. 12/2014: Pb 10 ppm, Cd 0.3 ppm, and Hg 0.5 ppm,
Permissible limits of WHO Guideline: Pb 10 ppm, Cd 0.3 ppm, and Hg 0.5 ppm.

DISCUSSION

Phytoremediation is an ecological strategies of plants, in situ, to promote the breakdown, immobilization, and removal of pollutants from the environment. Some plants showed their ability in conducting phytoextractions, thus enable to control heavy metal contents of soil. The effectiveness of the extraction process could determine the concentration of accumulated heavy metals in various plant tissues, such as leaves, roots and other plant cells[21].

This study was aimed to compare the contents of heavy metals found in plant leaves and in the soil where the plants grew. Through Cd analysis, only leaves from Batu District of Batu city, was 2.79 ppm, which exceeded the limit. But, analyses of soil showed that Cd concentration of Batu District was within the permissible limit. This indicated a possibility of the occurrence of phytoremediation through the air, that is via the absorption of Cd particles from the air, and accumulated in the leaves. Therefore, there were no absorption of the metal from the root[24-25].

Benowo and Wates Districts showed exceed limit of Cd content of the soils, while in leaves were within the permissible limits. One of the probabilities of such occurrence is the self-defense mechanism of the plants against heavy metal absorption from the root through the secretion of exudates into the soil matrix. Such secretion is considered the first line of self-defense mechanism against heavy metal, in which root exudates play a major role in the chelation of the metal, so as to prevent their uptake by the cells[26-27].

This mechanism might caused low Pb content of leaves from all areas in the study, even though Pb occurred in the soils where the plants grew. In this case, there was a probability that Pb was converted into chelate forms by root exudates as a self-defense mechanism. Through analysis, Hg content was not found in the leaves and soil of the areas where the plants grew.

Result of the study indicated, that Purwodadi District of Pasuruan city could be considered as the most suitable area for the cultivation of *Marsilea crenata* Presl. This is because of the free heavy metal contamination of Pb and Cd contents of the leaves and soils, while Pb content of soil was within permissible limit. Purwodadi District also is located in an area with heavy rainfall per year compared to other areas. There is a possibility that heavy rainfall could help to clean the soil from heavy metal contamination, while high temperature of the area helped

to enhance the self-defense mechanism of the plant. Enhancement of self defense mechanism helped in reducing the amount of heavy metal absorption by the roots[26,28].

CONCLUSION

Based on the analysis of heavy metal contents of Cd, Hg, and Pb of *Marsilea crenata* Presl. leaves and soils in Batu, Purwodadi, Benowo, Wates Districts, East Java Province, Indonesia, it can be concluded that Purwodadi District, Pasuruan City, is recommended for the cultivation of *Marsilea crenata* Presl.

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CONFLICTS OF INTEREST

The author states that there is no conflicts of interest with the parties involved in this study.

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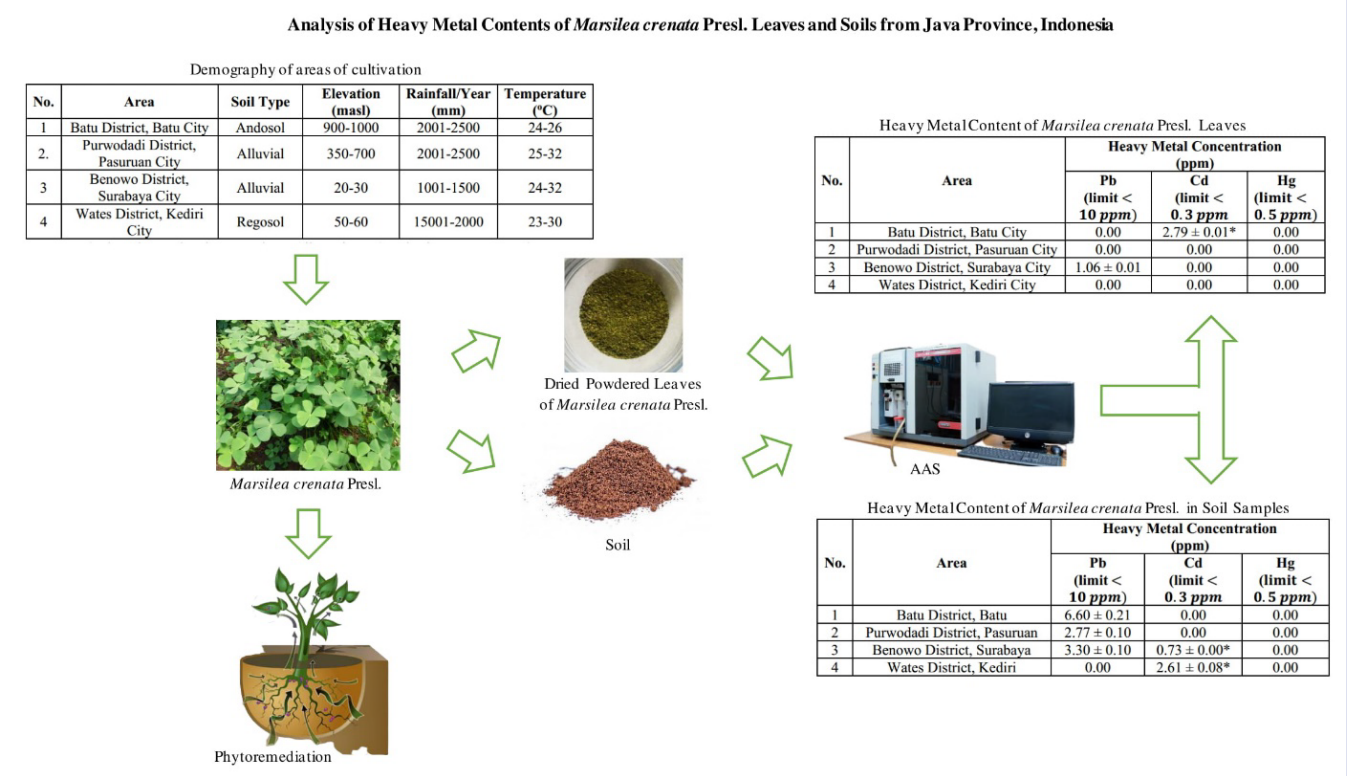
GRAPHICAL ABSTRACT



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