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Original Article

Heavy Metals Uptake of Water Mimosa (*Neptunia oleracea*) and Its Safety for Human Consumption

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Abstract

Background: *Neptunia oleracea* or 'water mimosa' has a phytoremediation ability which is rarely being assessed. This plant also can be eaten as raw or cooked and but brought such concern on its safety for human consumption. The objective of this study was to assess the phytoremediation ability of water mimosa and its safety for human consumption.

Methods: Water mimosa was treated with Pb, Cu and Cd at concentrations of 0.5 to 20 mg/L and the level of heavy metals uptake was measured. Treated plants were also harvested and soaked in boiled water (100˚C) for 2 to 10 minutes to determine the level of heavy metals reduction. Heavy metals were detected by Inductively-Coupled Plasma-Optical Emission Spectrometry (ICP-OES). Experiment was conducted in the Environmental Health laboratory, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia from June to December 2013.

Results: Water mimosa accumulates up to 93% of Cd (5 mg/L) after 10 days of treatment, the highest as compared to Cu (80%) and Pb (50%). It also has the highest BCF when treated with 10 mg/L of Cd. The heavy metals concentration in plant tissue decreased as the boiling time increased.

Conclusion: The overall results demonstrated that water mimosa could be used to remediate wastewater polluted with Cd, Cu and Pb. The plant is not recommended for human consumption as its ability to retain heavy metals in edible parts.

Keywords: Phytoremediation, Water mimosa, Heavy metals, Food safety, Green technology

Introduction

Heavy metals pollution has been studied and reported all over the world (1). Its bioaccumulation and the toxicity allow us to classify them as pollutants. Heavy metals are non-biodegradable element and persist in the ecosystem (2). The accumulation of these metals and indestructible elements were related to various human activities such as burning fossil fuels, rapid industrialization, mining and smelting, sewage sludge contents, usage of chemical fertilizers as well as existence in pesticide and herbicide residues. Different heavy metals may exert different harmful effects that can cause a potential risk to human health. Diseases caused by heavy metals are almost untreatable due to irreversible effect towards biological systems as they can accumulate in body tissues. The pollution of heavy metals to our ecosystems is a warning to humankind about the importance to prevent the ongoing metals pollution (3-6). The use of appropriate treatment technology in industry is needed to solve the problem of water pollution. At present, water pollution treatment technology such as chemical precipitation, ion exchange, reverse osmosis and evaporation method require sophisticated instrumentation, skilled personnel, and high cost. Hence, the use of biological system such as phytoremediation has been an emerging method to remove heavy metals because they are simple, cheap and safe for environment. The use of plants to degrade, assimilate, metabolize, or detoxify contaminants is cost-effective and ecologically sound (7, 8).

The application of water mimosa (*Neptuniaoleracea*) as a biological treatment in phytoremediation of wastewater has developed rapidly. For example in Thailand, this biological treatment is widely employed rather than chemical and physical treatments as it uses natural processes and is unlikely to leave toxic substances (9). In addition, some kinds of aquatic plants such as water mimosa, java weed, morning glory and reeds, grow naturally in reservoirs or in the wastewater wetland. As a consequence, the utilization of these aquatic plants especially water mimosa to treat the effluent is of particular interest. The characteristic of water mimosa that can be grown all year round become one of the benefits in phytoremediation. This technique is found to be simple, cheap and usually high efficiency is obtained (10).

Instead of being used as phytoremediation to treat wastewater, the leaves and young shoots of water mimosa can be eaten raw or cooked (added to soups). This has made a cultivation of this plant as aquatic vegetables became an important activity that sustains the livelihoods of community around the wastewater wetland in some countries. Water mimosa has been consumed by people from Malaysia, Thailand and Cambodia due to its nutritional values. This plant has high calcium content and plays a crucial role in metabolic processes of living cells, DNA repair and in production of natural steroid hormone (11). For instance, in Phnom Penh (Cambodia), large water surface areas are overgrown with water morning glory and to a lesser extent with water hyacinth and water mimosa. There were extensive human contacts with the water during the various production activities where women and children living nearby often harvest the plants and make bundles, which are collected by middleman with a truck on a daily basis and being sold at the market (12).A study by the Ministry of the Environment of Cambodia estimated that 20% of the total daily vegetable consumption of Phnom Penh comes from these aquatic vegetables in the waste water lake within the city (13). A previous study found high metal concentrations in wastewater sludge especially lead and mercury, which is not surprising as the untreated effluent of more than 3000 industries drains into this wastewater lake. Therefore, this wastewater fed aquatic vegetables despite their potential health risks, they are very important in supplying the city's vegetable markets and thus meeting the demands of the growing population of Phnom Penh (12, 13).

Using water mimosa wastewater phytoremediationis quite new and not extensively applied. Limited knowledge was found on the level of nutrient uptake by this plant and was not a common plant in phytoremediation process. Despite of their potential as a treatment of polluted water, the potential health risk of these aquatic vegetables as daily food consumption is tremendously worrisome (12). Although heavy metals in the vegetables could possibly be reduced from the cooking process especially through boiling (13), however the safety of this vegetable remains a question.

This study was aimed to determine the heavy metals uptake by this plant and to determine the safety of this plant to be consumed by human.

Materials and Methods

Preparation of live plants and culture solution

Water mimosa with similar size and weight was freshly collected from Sabak Bernam, Selangor and was cultivated for 30 days in Hoagland nutrient solution in Environmental Health laboratory, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. The nutrient solution contained N (NH_4NO_3), 38 mg/L; P (KH_2PO_4), 3.5 mg/L; K (KCl), 30 mg/L; Ca ($CaCl_2.2H_2O$), 9 mg/L; Mg ($MgSO_4.7H_2O$), 7 mg/L (13).This is to ensure water mimosa could adapt well in the nutrient solution. Nutrient solution was replaced once a week to replace water loss.

Plant treatment with heavy metals and sample preparation for analyses

Determination of Bio-concentration factor (BCF), Relative Growth Rate (RGR) and Tolerance Index (Ti).

Whole plants with similar size and shape (fresh weight of each plant between 13 to 15 g) were established in 250 mL quarter-strength Hoagland's solution and were exposed to individual heavy metal (Pb, Cd, Cu) at 0.5, 3.0, 5.0, 10.0 mg/L concentrations. As a control, water mimosa was exposed to 0 mg/L concentration. Experiments were carried out in Magenta boxes (Sigma). Triplicates groups of water mimosa were exposed to heavy metals for 10 days (normal time used in research to know the effects of heavy metals and its accumulation in plant) (14,15).

Determination of heavy metal concentration in stems, roots and leaves

Treated plants were cut into stems, roots and leaves and weighed for fresh weight after 10 days of treatment. All samples were oven dried at 70˚C for two days. The dried tissues were cut into small pieces and sieved through 2 mm sieve. Samples were applied dry ashing methods for heavy metals analysis (8).

Heavy metal determination in water mimosa after boiling process

Treated plants were harvested and were soaked in boiled water (100˚C) for 2, 5, and 10 minutes. This was to determine the difference of heavy metals concentration in plant tissue after boiling. Normally, people will cook vegetables for 2 to 5 minutes. The plants were cooled and oven dried at 70˚C for two days. The dried tissues were weighed, cut into small pieces and sieved through 2 mm sieve and remaining nutrient solution was filtered for metal concentration analysis (16).

All plant samples from each experiment were carried out by dry ashing method and followed by measurement of total metal concentrations using Inductively-Coupled Plasma-Atomic Emission Spectrometry (ICP-AES). All samples (powdered form) were weighed at 0.5g in crucible and burned at 550˚C in furnace. Samples were digested with 5 mL of 10% HCl and 10 mL of 20% HNO_3 and were digested at 100˚C for one hour. All samples were cooled and were marked up to 50mL with distilled water. Samples were finally filtered by using Whatman's 42 filter paper before metal concentrations in samples were determined by ICP-AES Optima 8300 Perkin Elmer (17).

Data analysis

The bio-concentration factors (BCF), Relative Growth Rate (RGR) and Tolerance index (T*i*) were calculated as described in previous studies (2013). BCF measure the ability of the plant to accumulate heavy metals with respect to the heavy metals concentration in the external solution. The RGR quantify the speed of plant growth under different concentration of heavy metals treatment and Ti measure the ability of plants to grow in the presence of a given concentration of metal (18).

Bioconcentration factor (BCF)

BCF was calculated by dividing the trace element concentration in plant tissue in mg/kg at harvest with initial concentration of the element in the external nutrient solution in mg/l (Equation 1). High BCF value indicates plant tend to extract more heavy metals from water and concentrate it in plant tissue which is good for phytoremediation. However, this possibly brings serious risk to human health if the plant is being eaten.

$$BCF = \frac{Trace element concentration in plant tissue\ [mg/kg]\ at harvest}{Initial concentration of the element in solution\ [mg/l]}$$ (Equation 1)

Relative Growth Rate (RGR)

RGR was calculated as in Equation 2;

$$RGR = \frac{lnW2 - lnW1}{T2 - T1}$$ (Equation 2)

where RGR is measured as the mass increase per aboveground biomass per day (g/ g day), W1 is initial dry weight of plant at time (g), and W2 indicates final dry weight of plant (g), T1 and T2 are the initial and final times for treatment respectively (day). RGR is estimating the plant growth (19).

Tolerance index (Ti)

T*i* was calculated as in Equation 3, where plants with T*i* higher than 60% are considered as in good tolerant (18,20).

$$Ti = \frac{dryweighttreatedplant\,(g)}{dryweightcontrolplant\,(g)} \times 100\%$$ (Equation 3)

Results

Bioconcentration factor (BCF)

Figure 1indicates the heavy metals concentration in the whole plant tissue of water mimosa and the bioconcentration factor of this plant under different heavy metals concentrated treatment. As the supply of heavy metals increased, heavy metal concentration in the plant tissue also increased. The greatest Cd concentration was measured (1706.3± 35.5 mg/kg) in the whole plant tissue of water mimosa after being treated with 10 mg/L of Cd. By comparison, with similar concentration of Pb and Cu supplied (10 mg/L), the concentration of metals attained was lesser than Cd with 1296.3 ±56.9 mg/kg and 1177.8 ±81.7 mg/kg, respectively. As for the BCFs values, Cd and Cu showed a similar trend where the BCF rate increased as the supply of metals increased from 0.5 to 10 mg/L. The highest BCF value was determined for Cd (184.5 ±24.8) at the concentration of 5 mg/L before it remained fairly constant after 5 mg/L Cd was supplied. As for Cu, the peak BCF value was 162.1 ±16.1 at the concentration of 5 mg/L before it reduced to 129.6 ±25.7 at the concentration of 10 mg/L. The BCFs value for Pb was the highest at the lowest Pb supplied (0.5 mg/L). As the Pb supply increased, the BCF value decreased then remains constant at 10 mg/L Pb.

Figure 2shows the percentage of heavy metals uptake in water mimosa with the initial heavy metals supplied (Pb, Cu, Cd). The highest percentage of metal uptake was determined for 5 mg/L Cd supplied where 93% of metals were absorbed by the plant. At 0.5 mg/L supplied of metals, the highest ratio of heavy metals uptake was determined for Pb (88%) followed by Cu (56%) and Cd (22%). At the highest concentration supplied (10 mg/L), the highest ratio of uptake was determined for Cd (85%) followed by Cu (65%) and Pb (59%).

Tolerance index (Ti) and Relative Growth Rate (RGR)

Figure 3indicates the tolerance index (T*i*) of water mimosa after 10 days of treatment with Pb, Cu, and Cd. As the heavy metals concentration supply increased, the T*i* of water mimosa decreased. Water mimosa considered to be tolerance to all three heavy metals tested. This can be seen at 10 mg/L heavy metal concentration supplied; water mimosa still has good tolerance towards heavy metals, as the T*i* value was more than 60%. Figure 4 indicates the Relative Growth Rate (RGR) of water mimosa after being exposed to Pb, Cu and Cd. The RGR values for water mimosa decreased as the concentration of heavy metals supply increased.

Heavy metal concentration in roots, stems, and leaves

Table 1 indicates the concentration of heavy metals (Pb, Cu, and Cd) in roots, stems and shoots of water mimosa. The trends of the heavy metals accumulation in the roots, stems and shoots of water mimosa increased as the concentration of heavy metals supply increased. The highest concentrations of Cd supplied, 10 mg/L was determined in roots (7.76 mg/L), stems (1.59 mg/L), and shoots (0.22 mg/L).

Heavy metal concentration in water mimosa after boiling process

Figure 5 indicates the concentration of heavy metals in water mimosa after the boiling process. The heavy metals concentration in the plant tissue decreased as the time of boiling increased. This is in contrast to the heavy metals concentration in the boil water, as they increased with the boiling time (Pb.w, Cu.w, Cd.w).

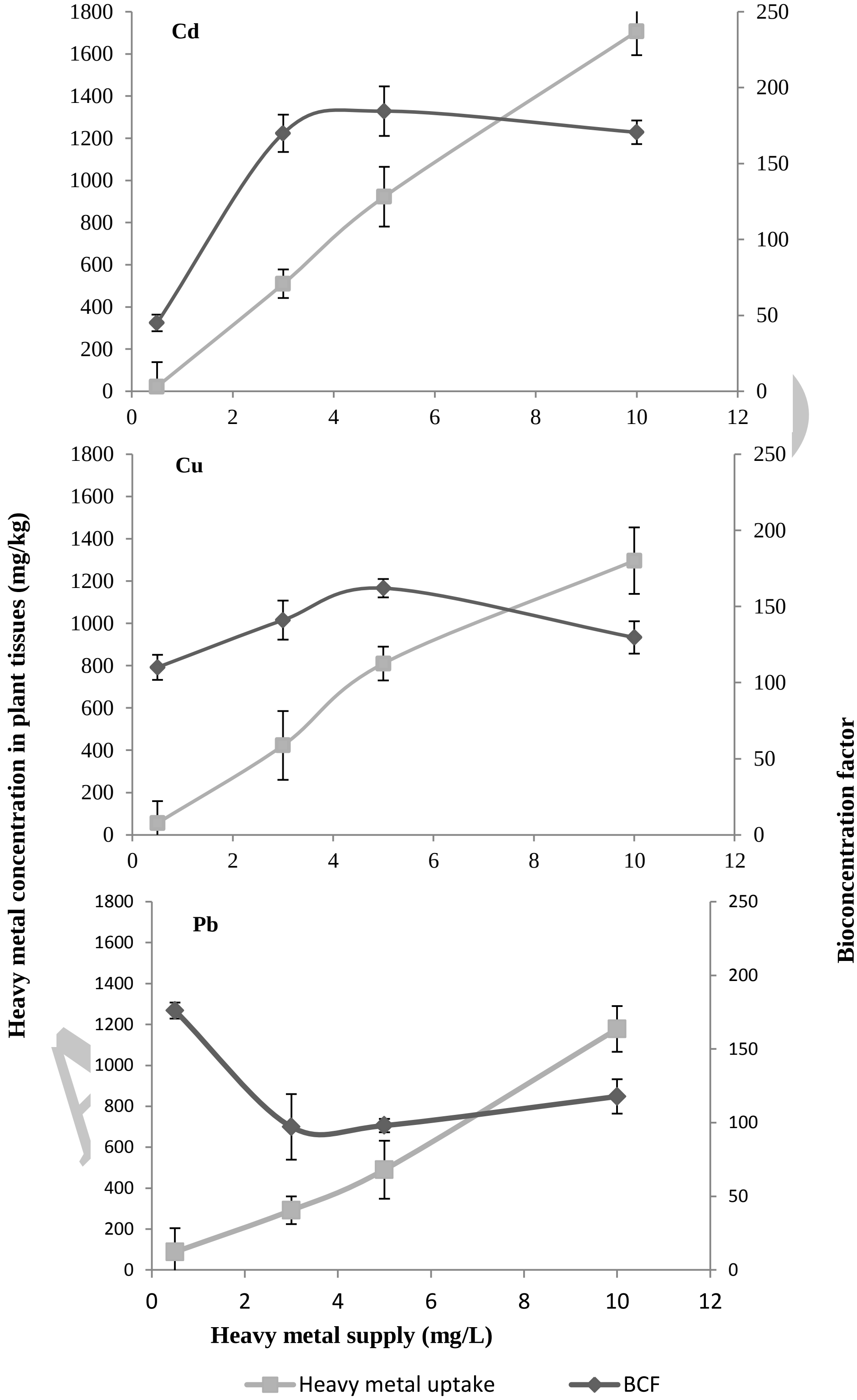

Fig. 1: Heavy metals concentration in plant tissue and bio-concentration factor of heavy metals (Pb, Cu and Cd) in water mimosa. Vertical bars indicate means of three replicates ± standard error (SE)

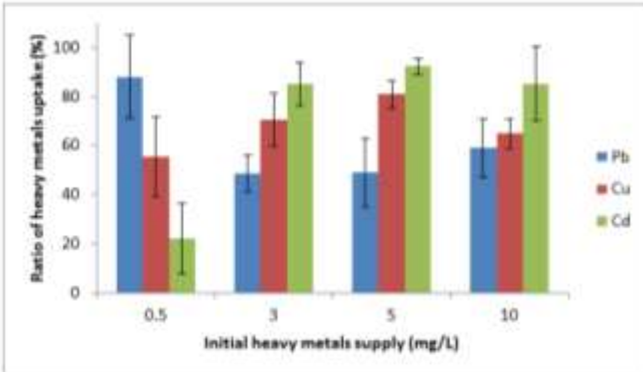

Fig. 2: Percentage of heavy metals (Pb, Cu and Cd) uptake in water mimosa dry plant tissues. Vertical bars indicate standard error of mean

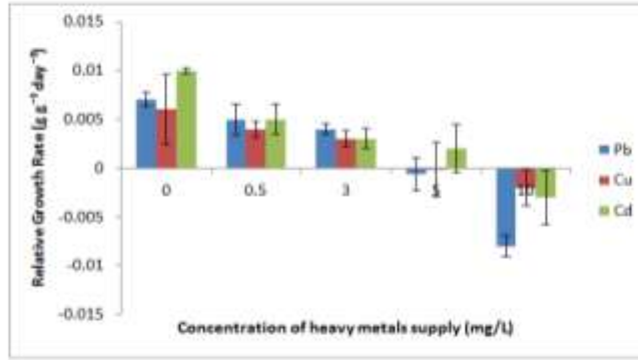

Fig. 4: Relative Growth Rate (RGR) of water mimosa treated with 0 (control), 0.5, 3, 5, and 10 mg/L of Pb, Cu and Cd after 10 days of treatment. Data are given as means of three replicates ± standard error (SE)

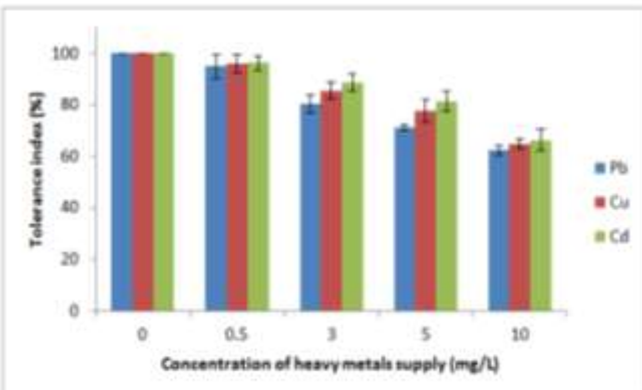

Fig. 3: The tolerance index (Ti) of water mimosa treated with 0.5, 3, 5, and 10 mg/L of Pb, Cu and Cd after 10 days of treatment. Data are given as means of three replicates ± standard error (SE)

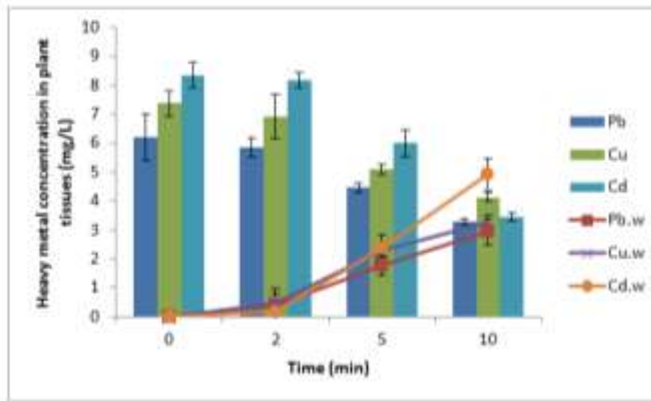

Fig. 5: Concentration of heavy metals in water mimosa after the boiling process at 0 minute (control), 2, 5 and 10 minutes. Vertical bars indicated standard error means

Table 1: Concentration (mean±SE) of heavy metals in root, stem and shoot of water mimosa in mg/L

Treatment (mg/L)	Pb (mg/L)			Cu (mg/L)			Cd (mg/L)		
	Roots	Stems	Shoots	Roots	Stems	Shoots	Roots	Stems	Shoots
0.5	0.21 ±0.03	0.15 ±0.06	*ND	0.30 ±0.11	0.09 ±0.05	*ND	0.12 ±0.08	0.08 ±0.02	*ND
3.0	1.65 ±0.01	0.81 ±0.04	*ND	1.23 ±0.08	0.32 ±0.02	*ND	1.21 ±0.22	0.55 ±0.06	0.05 ±0.01
5.0	2.21 ±0.12	0.91 ±0.42	*ND	2.57 ±0.09	0.79 ±0.13	0.09 ±0.09	2.98 ±0.61	0.90 ±0.10	0.12 ±0.07
10.0	5.10 ±0.26	1.07 ±0.92	0.01 ±0.002	6.22 ±0.02	1.07 ± 0.11	0.13 ± 0.02	7.76 ±0.13	1.59 ±0.42	0.22 ±0.11

*ND=not detected

Discussion

Bioconcentration factor (BCF)

Cd was found to be the highest concentrated metals in water mimosa as compared to Pb and Cu. This was possibly related to phytochelatins (PCs) production (21). PCs are the oligomers of glutathione, produced by the enzyme phytochelatins synthase that are important for heavy metals detoxification in plant. High concentration of Cd in plant increase the PC peptide response thus caused high Cd accumulation in plant (22-24).Water mimosa accumulated low Cd at the lowest Cd supplied. This is because of PCs formation is not very sensitive at low concentration of Cd (23,24).Large ratio of BCF indicated better phytoremediation ability. A plant species is considered as a hyper accumulator if the BCF value is more than or equal to 1000 (25). Hyperaccumulator describes the abilities of plant to grow in very high concentration of metal and concentrating high heavy metals in their tissues (19) such as in *Lemna minor* (duckweed), *Pistiastratiotes* (water lettuce), *Eicchornia crassipes* (water hyacinth). However, the BCF for water mimosa in this study was not more than 1000 and this suggested that water mimosa is a non-hyperaccumulator type of plant.

Tolerance index (Ti) and Relative Growth Rate (RGR)

Different species may have developed different mechanisms to tolerate excess metals. It is quite likely; even within one plant species, there are more than one mechanism involved. There are possibilities that plants could possibly tolerate high metal concentrations by limiting the metals uptake or transport and through internal tolerance mechanisms (PCs production) (26).Even though the plant shows a decreasing trend of tolerance as the concentration of heavy metals increased, but it can considered as in good tolerance, since the T*i* value was not below than 60% (18,20).The RGR decrease for several reasons, for instance it is stated that, RGR decreases over time as the biomass of a plant increases. The RGR also decreases as the non-photosynthetic biomass (roots and stems) increases, since the top leaves of a plant begin to shade lower leaves and nutrients can become limiting (27).

Heavy metal concentration in roots, stems and shoots

Concentrations of all three heavy metals were higher in the roots compared to the shoots and leaves of this plant. This suggests that the tested heavy metals in this study were highly retained in the root. The greatest uptake of heavy metals occurred in roots rather than in shoots because roots are very sensitive in producing glutathione (GSH), cysteine, and PCs (24).These elements function as a binding site for metals. High concentration of metals in the roots also indicates that this plant removed heavy metals through rhizofiltration process, which accumulate contaminants in roots (28, 29). Consideration of the amount of pollutant accumulated by plant roots is an important factor for phytoremediation of wastewater (30). Other plants that have similar phytoprocess to water mimosa (removed pollutants through rhizofitration) are water hyacinth, duckweed, water spinach, and calamus (30-32).

Concentration of heavy metals after boiling process

As the boiling time increase, the heavy metal concentration in plant tissues decrease while heavy metal concentration in boiling water decrease. This is because the membrane cell tissue of this plant was ruptured during the boiling process and heavy metals were leached into the water (14). During preparation of water mimosa as dishes, the water or soup as well as plant itself might be contaminated with heavy metals and might possessed harm to human health. If there is any extra boiled water contaminated with heavy metals, the water should be put into screw cap containers to take to the hazardous waste disposal (33). Even though the boiling process could reduce the heavy metals concentration, but in certain method of cooking for example if the dish is served for soup or fried, the metal could possibly remained in the food. So, water mimosa is not good to be consumed as raw food and one way to ensure that heavy metals being reduced from the plant are through boiling.

Conclusions

Water mimosa can be used to remove heavy metals pollution in water as this plant found to be a good accumulator of Cd and Cu as compared to Pb. However, the effectiveness of water mimosa in real world application need to be further studied. The level of heavy metals in water mimosa has reduced from the process of boiling, thus indicates that the risk of human poisoning is somehow manageable. However, further research is needed especially under different type of food preparation for example for soup or fried, so that the risk can be countered.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or sub-mission, redundancy, etc) have been com-pletely observed by the authors.

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