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The Comparison of Phytoremediation Abilities of Water Mimosa and Water Hyacinth

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ABSTRACT

Water mimosa and water hyacinth are the two plants with phytoremediation ability. However, the phytoremediation abilities of these plants in terms of the heavy metals uptake, the bio-concentration factor and relative growth is rarely being assessed and reported. Present study aims to compare the phytoremediation ability of water mimosa and water hyacinth. Both plants were treated with heavy metals i.e. lead, copper and cadmium in the laboratory in a nutrient solution at concentrations of 0.5 to 20 mg/L. Treated plants were harvested and tested for heavy metals uptake, bio-concentration factor, the tolerance index and the relative growth rate. The heavy metals concentration in the root, stems and leaves was also measured in this study. The overall findings have indicated that water mimosa has lower heavy metals uptake and bio-concentration factor value compared to water hyacinth. Both plants accumulate high heavy metals in the roots compared to stems and leaves. Both plants use rhizofiltration (accumulate heavy metal in roots) process to remove heavy metals. This study concludes that both plants have the potential to be commercialized as phytoremediation agent to clean polluted water.

Keywords: *Heavy metals, phytoremediation, water mimosa, water hyacinth, polluted water*

1. INTRODUCTION

Phytoremediation is a promising and economical technology that makes use of plants to eliminate, transform, or stabilize pollutants sited in sediments, water, or soils [1-3]. Phytoremediation mechanisms can recommend appropriate methods for decontaminating polluted soil, water, and air by trace metals as well as organic substances. Normally, macrophytes such as water hyacinth, water mimosa, water spinach, smartweed and lesser duckweed has been used in water treatment as these plant growth rapidly, easy to harvest and can accumulate pollutant [4-6]. According to Gisbert et al., (2003), the use of biological materials to cleanup heavy metal has been focused on as a useful and affordable form of bioremediation [1]. Plants that take up heavy metals usually propose an alternative and less costly method to absorb heavy metals directly from the environment [1, 7, and 8].

Heavy metals such as cadmium (Cd), copper (Cu) and lead (Pb) are the examples of the toxic heavy metals which have been recognized for its negative effects on the environment where they can accumulates throughout the food chain posing serious threat to human health. Pb and Cd also can affect plant growth [9-11]. Unlike Cd and Pb, Cu is an essential and beneficial element for human bodies and plants. However, excessive concentration of Cu can become a toxic to plant and a potential hazard to human [12].

Neptunia oleracea (water mimosa) and Eicchornia crassipes (water hyacinth) are macrophytes that can be used for phytoremediation of wastewater. This biological treatment is a natural process which unlikely to leave toxic substances. This method is widely employed to clean water in Thailand and is more preferable method compared to physical and chemical treatments [13]. Previous studies have shown that water mimosa has the ability to accumulate heavy metals such as lead (Pb), copper (Cu), cadmium (Cd) and zinc (Zn) in a manner similar to other generic plants such as water hyacinth [14,15]. However, limited studies found to elaborate the efficiency of water mimosa in accumulating heavy metals as compared to other plant such as water hyacinth. Water hyacinth has been proven to be able to accumulate high concentration of heavy metals and has been extensively used in wastewater treatment [16-21].

A review on relevant literature reveals that studies on identifying new species of plants, particularly local species, is ongoing due to their promising potential in phytoremediation technology. Application of water mimosa in treating effluent has gained much attention, and a focused in this study as this plant can grow naturally in lakes and wastewater wetlands throughout the year which is suitable to be used in phytoremediation [22-24].

To date, there are lacks of studies which specifically examine the phytoremediation abilities of water mimosa in comparison to water hyacinth. Therefore, the main aim of this study is to compare the level of heavy metals uptake, bio-concentration factor, tolerance index and relative growth of water mimosa and water hyacinth. The accumulation rate of heavy metals in difference parts of these plants was also assessed in this study.

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2. MATERIAL AND METHOD

2.1 Preparation of Plants

Water mimosa and water hyacinth were cultivated for 30 days in Hoagland nutrient solution in the lab. 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 [25].

2.2 Plant Treatment with Heavy Metals

Plants in similar size and shape with a fresh weight 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, 15.0 and 20.0 mg/L concentrations. As a control, water mimosa and water hyacinth was exposed to 0 mg/L heavy metals concentration in Hoagland's nutrient solution. Experiments were carried out in Magenta boxes (Sigma). Triplicates groups of water mimosa and water hyacinth 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) [26,37]. After 10 days of treatment, plants were collected and rinsed thoroughly with distilled water. They were blotted dry, and weighed for fresh weight. All samples were oven dried at 70˚C for two days. The dried tissues were weighed, cut into small pieces and were sieved through 2 mm sieve and remaining nutrient solution were filtered for metal concentration analysis.

2.3 Sample Preparation for Heavy Metals Analyses

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 [25].

All samples (powdered form) from each experiment were accurately weighed at 0.5 g in a silica crucible and kept in a muffle furnace for ashing at 550ºC for 8 hours. Then, the sample was removed from the furnace and cooled down and 5 mL of 10% HCl was added to the crucible. Care was taken to ensure that all the ash came into contact with acid. Further, the crucible containing acid solution was kept on a hot plate and digested to obtain a clean solution. The final residue was dissolved in 10 mL of 20% HNO_3 solution and were boiled at 100˚C for one hour. The solution were cooled and then transferred quantitatively to a 50 mL volumetric flask by adding distilled water. Samples were finally filtered by using Whatman's 42 filter paper before metal concentrations in samples were determined by ICP-OES Optima 8300 Perkin Elmer [29].

2.4 Data Analysis

In study, the bio-concentration factor (BCF) was measured for both plants to determine the ability of heavy metals accumulation. 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.

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

The relative growth rate (RGR) measured the dry matter or amount of biomass production over time. RGR was calculated as;

$$RGR = \frac{\ln W2 - \ln W1}{T2 - T1}$$

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).

The tolerance index (Ti) determine the ability of plants to grow in the presence of a given concentration of metal. Ti was calculated as; [30,31].

$$Ti = \frac{\text{dry weight treated plant (g)}}{\text{dry weight control plant (g)}} \times 100\%$$

3. RESULTS

3.1 Heavy Metals Uptake and Bioconcentration Factor (BCF)

This experiment was aimed to determine the level of heavy metals uptake and BCF value of water mimosa relative to water hyacinth. **Figure 1** indicates the heavy metals uptake and the BCF values of these plants under different heavy metals concentration. Both plant species exhibited differences in accumulated concentrations and BCF values of the different heavy metals.

Among the three heavy metals tested, both plants accumulated high concentrations of Cd. Water hyacinth accumulated Cd, 2 times higher than water mimosa (3529 mg/kg for water hyacinth and 2020 mg/kg for water mimosa) at 15 mg/L Cd supply. Cd concentration in the plant tissue for both plants decreased to 3085 mg/kg (water hyacinth) and 1178 mg/kg (water mimosa) with the increased of Cd supply. Both plants attained high BCF for Cd compared to Pb and Cu. Water hyacinth attained higher BCF value compared to water mimosa. For instance, the highest BCF value for Cd for water hyacinth was 347 at 10 mg/L Cd supply while water mimosa attained the highest BCF value of 185 at 5 mg/L Cd supply.

The highest concentration of Cu uptake in water mimosa and water hyacinth was 1362 mg/kg and 2959 mg/kg respectively at 15 mg/L Cu supply. The Cu uptake in water hyacinth was twofold the value in water mimosa. The concentration of Cu in the water mimosa tissue was increased as the Cu concentration supplied increased. The accumulation of Cu were 55.4 mg/kg, 422.9 mg/kg, 810 mg/kg, 1296.3 mg/kg, 1362.4 mg/kg at Cu supply of 0.5 mg/L, 3 mg/L, 5 mg/L, 10 mg/L and 15 mg/L respectively. Similar trend was observed for water hyacinth where the Cu concentrations in plant tissue were 52.3 mg/kg, 384.9 mg/kg, 1020.2 mg/kg, 2348.8 mg/kg and 2958.6 mg/kg at similar Cu concentration supplied. The accumulation of Cu in water mimosa and water hyacinth was reduced to 1004.8 mg/kg and 2501.9 mg/kg respectively at 20 mg/L Cu supply. The peak BCF value for Cu in water mimosa, was 162 at the concentration of 5 mg/L before it reduced to 130, 91, and 50 at the concentration of 10, 15, and 20 mg/L respectively. The peak of BCF value in water hyacinth was 235 at the concentration of 10 mg/L Cu before it decreased. This result indicates that the Cu accumulation rate in water mimosa was slightly lower than water hyacinth.

Pb was the least accumulated element in water mimosa and water hyacinth. The highest Pb accumulation in water mimosa was 984 mg/kg at 15mg/L Pb supply, slightly lower than water hyacinth (1004 mg/kg). Both plant showed an increased in Pb accumulation from 0.5 mg/L to 10 mg/L Pb supply and decreased after 20 mg/L Pb supply. Both plants have similar rate of Pb accumulation. The trend of BCF value of Pb in water mimosa and water hyacinth was also in similar rate. The BCFs value for Pb in water mimosa was the highest (176.1) at 0.5 mg/L Pb supplied while in water hyacinth the BCF value was also the highest (213.9) at 0.5 mg/L Pb supplied. As the Pb supply increased, the BCF value was decreased.

3.2 Tolerance Index

Figure 2 shows the percentage of tolerance index (Ti) of water mimosa and water hyacinth, after 10 days of treatment with Pb, Cu, and Cd. The Ti values for water mimosa and water hyacinth decreased as the heavy metals concentration increased. Both plants showed a decreasing trend of tolerance as the concentration of heavy metals increased. For instance, the Ti value to Cd for water hyacinth has reduced from 91% (in 0.5 mg/l Cd) to 37% (in 20 mg/L Cd). Similar to water mimosa as the value decreased from 96% to 41%. Similar result was obtained for Cu and Pb. The Ti value for water hyacinth treated with Cu has reduced from 99% (0.5 mg/L) to 49% (10 mg/L Cu) while for water mimosa has reduced from 96% to 26%.

3.3 Relative Growth Rate (RGR)

The effects of Cd, Cu and Pb on relative growth rate (RGR) of water mimosa and water hyacinth at different concentrations were shown in **Figure 3**. The RGR in both plants showed a decrease trend by heavy metals concentration supplied. For example, the RGR for water hyacinth treated with 0.5 mg/L Cd was 0.055 g $g^{-1}day^{-1}$, a twofold decreased to 0.033g $g^{-1}day^{-1}$ (in 3 mg/L Cd supply). The RGR for water mimosa also decreased from 0.05 g $g^{-1}day^{-1}$ (0.5 mg/L of Cd) to 0.009 g $g^{-1}day^{-1}$ (3 mg/L Cd).

Similar result was obtained for Cu where the RGR in water hyacinth has reduced from 0.031g $g^{-1}day^{-1}$ (0.5 mg/L) to 0.014 g $g^{-1}day^{-1}$ (5 mg/L) and in water mimosa from 0.034 g $g^{-1}day^{-1}$ (0.5 mg/L of Cu) to 0.023 g $g^{-1}day^{-1}$ (3 mg/L Cu). The greatest reduction of biomass was recorded for water hyacinth treatment with Pb, as the RGR reduced from 0.006g $g^{-1}day^{-1}$ (0.5 mg/L Pb) to 0.005 g $g^{-1}day^{-1}$ (3 mg/L Pb). While for water mimosa the RGR reduced from 0.025 g $g^{-1}day^{-1}$ (0.5 mg/L Pb) to 0.005 g $g^{-1}day^{-1}$ (3 mg/L Pb).

3.4 Heavy Metal Concentration in Roots, Stems and Leaves

Figures 4 show heavy metals (Pb, Cu and Cd) concentration in the roots, stems and leaves of water mimosa (dotted line) and water hyacinth (solid line). All heavy metal tested in this study showed high accumulation rate in the roots compared to the stems or leaves.

Water mimosa accumulated lower heavy metals compared to water hyacinth in the roots. For instance, water mimosa accumulated 5.50 mg/L Pb slightly lower than in water hyacinth (6.45 mg/L) at 10 mg/L of Pb treatment. Water mimosa accumulated the highest Cu (6.22 mg/L) at 10 mg/L Cu supplied, while water hyacinth accumulated the highest Cu (10.92 mg/L) at 20 mg/L Cu supplied. At the highest concentration of Cd (20 mg/L) treatment, the accumulation of Cd in the roots of water mimosa and water hyacinth were 4.89 mg/L and 9.78 mg/L respectively.

Similar to the stems of these plants, water mimosa accumulated lower concentration of heavy metals compared to water hyacinth. Water mimosa accumulated the highest Cd (2.09 mg/L) in the stem compared to Pb (1.09 mg/L) and Cu (1.06 mg/L). Cd also was found to be the most accumulated in stems of water hyacinth (3.03 mg/L) compared to Pb (2.98 mg/L) and Cu (2.82 mg/L). At 20 mg/L heavy metal supplied, Cu was the least found in the stems of water mimosa and water hyacinth at 0.64 mg/L and 2.02 mg/L respectively.

Only little heavy metal was translocated to the leaves of these plants. Cd was accumulated at higher concentration in water mimosa leaves compared to Pb and Cu. For example, at 15 mg/L heavy metals supplied, the concentration of Cd, Pb, and Cu in water mimosa leaves were 0.92 mg/L, 0.11 mg/L, and 0.06 mg/L respectively. As for water hyacinth, Cu was the highest accumulated in leaves (2.0 mg/L) compared to Pb (1.95 mg/L) and Cd (1.03 mg/L).

4. DISCUSSIONS

The metals uptake was decreased in both plants as the supplied concentration increased indicates that the saturation state for the plants. When the saturation state was achieved, it is difficult for plants to further absorb heavy metals [21]. Cd was found to be the highest uptake metals in water mimosa and water hyacinth in this study

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compared to Pb and Cu. This possibly because Cd^{2+} is a mobile element by nature, easily absorbed by roots thus caused high accumulation in plant [31-34]. According to Bermond and Varrault (2004), Cd was more mobile than Cu and Pb [35]. However, less Cd accumulated in water mimosa as compared to water hyacinth was probably due to its small roots surface as compared to water hyacinth which tend to accumulate more heavy metals [16, 36].

Copper (Cu) is essential for plant growth, however it will cause toxic effects when the concentration exceeded 20 mg/kg in shoots or leaves [37]. The level of Cu uptake in water mimosa in this study was higher than was reported in Veschasit et al., (2012) study [14]. Veschasit et al., has determined 78.7 mg/kg Cu in water mimosa in 7 mg/L treatment of Cu, while water mimosa in this study accumulated 810 mg/kg of Cu in 5 mg/L Cu treatment [14].

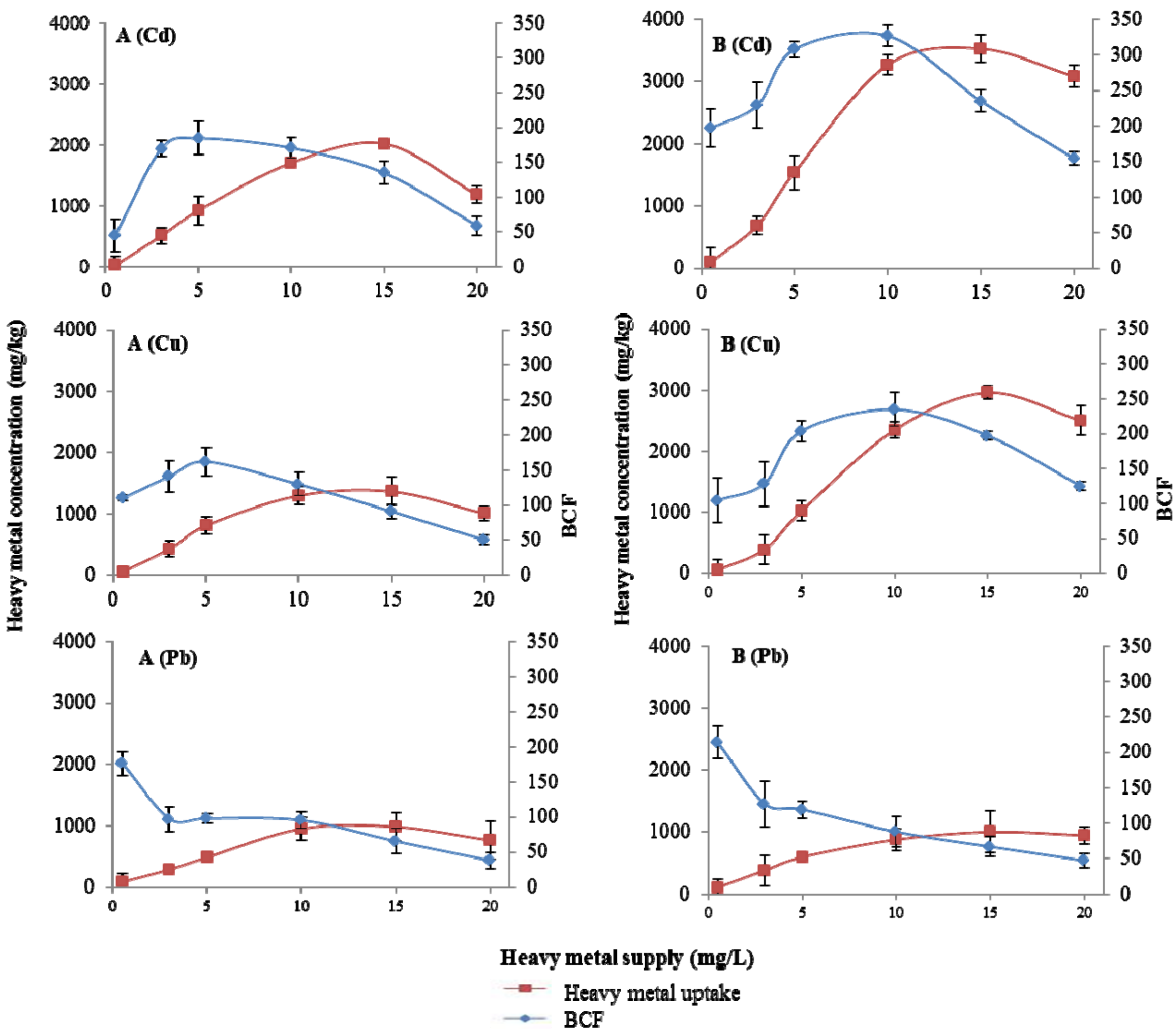

Fig 1: Cadmium (Cd) uptake in plant tissue and bioconcentration factor (BCF) of Cd in (A) water mimosa and (B) water hyacinth

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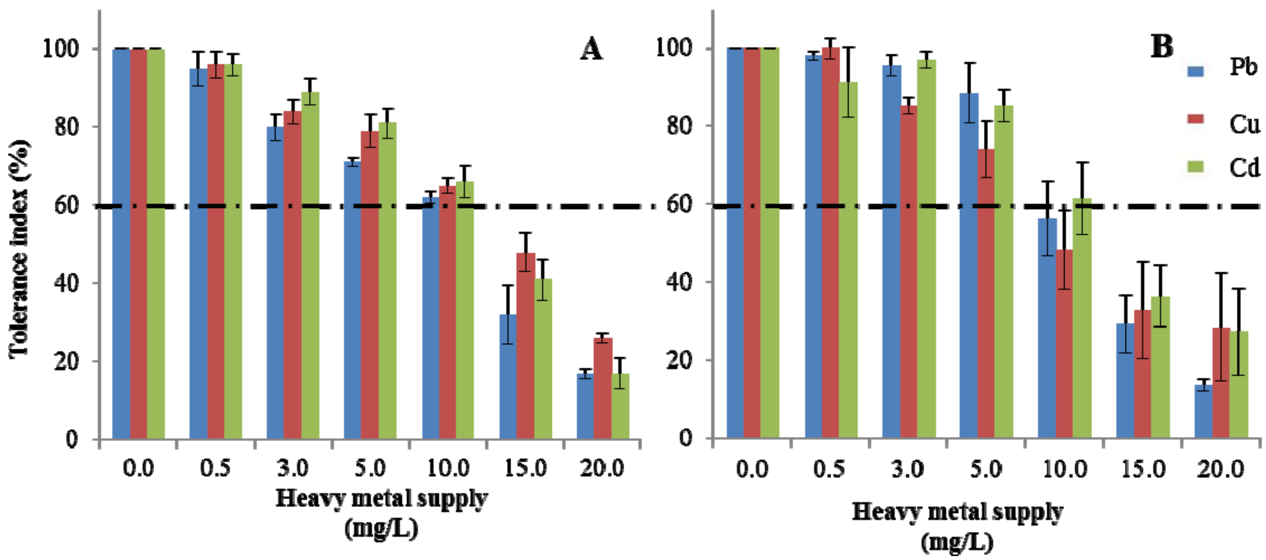

Fig 2: Tolerance index (Ti) of (A) water mimosa and (B) water hyacinth. Percentage tolerance index upper than dotted line indicates plant considered as good tolerance to heavy metals

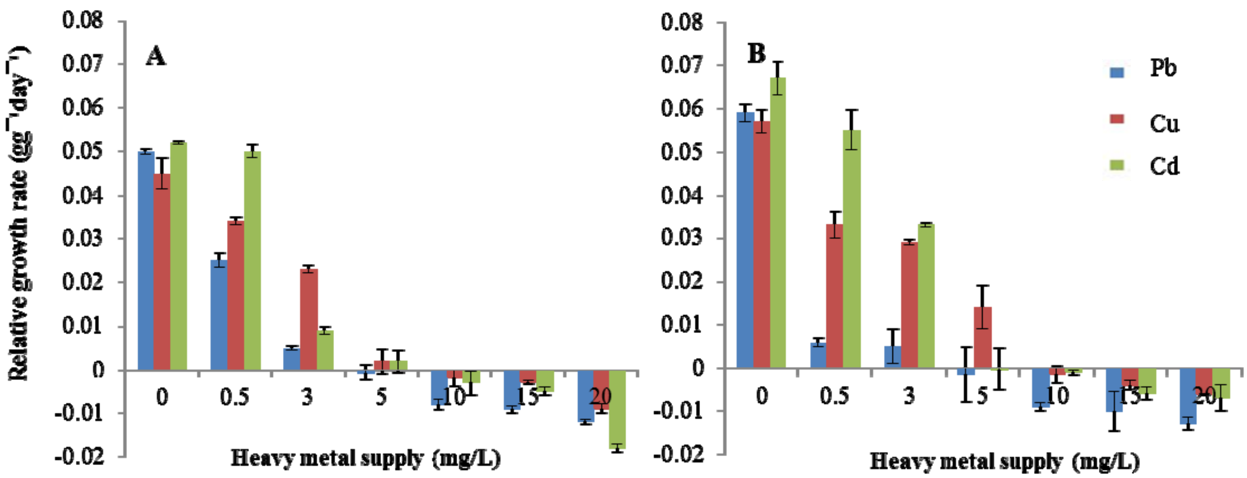

Fig 3: Relative Growth Rate (RGR) of (A) water mimosa and (B) water hyacinth.

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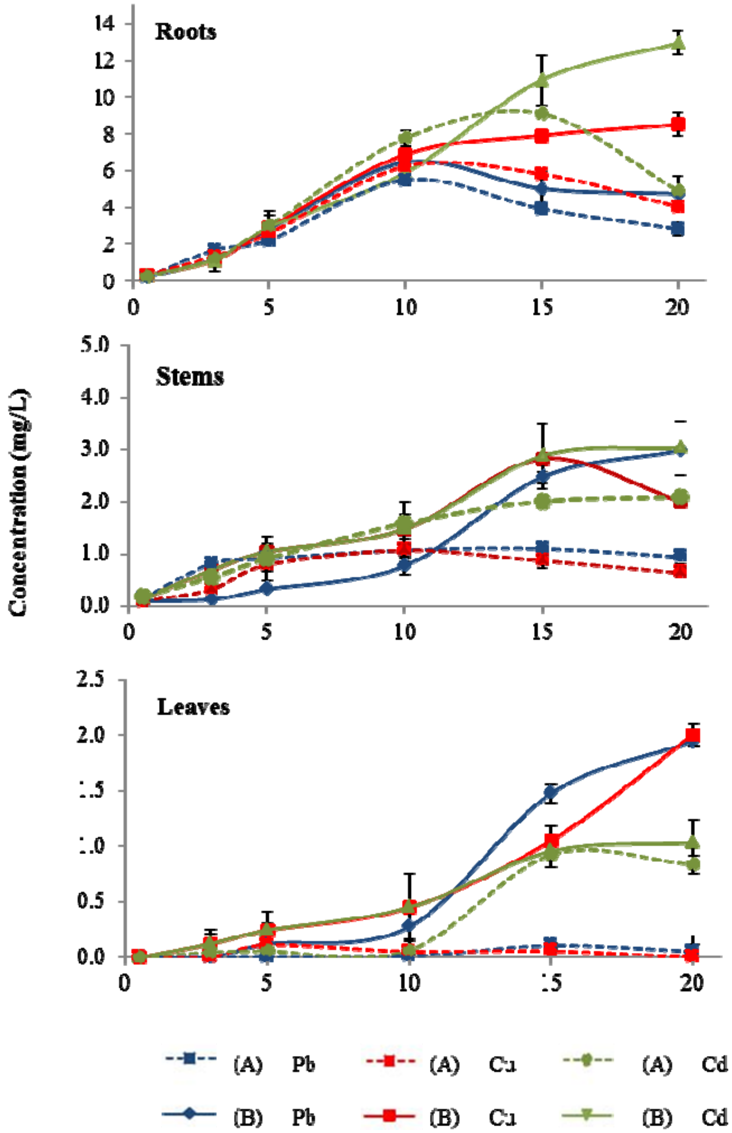

Fig 4: Heavy metals concentration in roots, stems and leaves of: (A)-water mimosa and (B)-water hyacinth. Dotted line indicates water mimosa and solid line indicates water hyacinth.

However, level of Cu uptake in water hyacinth in this study (1020.2 mg/kg) was only slightly below than a study reported by Liao and Chang, (2004) which is 1110 mg/kg at 5 mg/L initial concentration supply [36]. As the concentration of Cu supplied increased the level of Cu uptake in plant tissue was decreased. This is because when Cu concentration reached the toxic level, the root and shoot of the plant will damage and caused self-limiting to Cu uptake [38-40]. This has affected the Cu uptake in plant. Among the three heavy metals tested in this study, Pb was the least uptake by water hyacinth and water mimosa. This is because Pb solubility in plant is controlled by phosphate and carbonate precipitation and very little Pb are available to plant [41]. Phosphate or carbonate may precipitate in plant roots which came from the nutrient solutions or the originate trench where the plants were taken [25]. Phosphate and carbonate precipitation help the plant to reduce the uptake of Pb to prevent damage to plant tissue [40,41].

A plant species is considered as hyper accumulator if the BCF value is more than or equal to 1000 [42]. Hyperaccumulator describes the ability of a plant to grow in very high concentration of metal and accumulates high levels of heavy metals in their tissues [43]. Plants such as Lemna minor (duckweed), Pistia stratiotes (water lettuce) and Eicchornia crassipes (water hyacinth) are among the species reported with high BCF value [16, 44, 45].

In general, the BCF value for water mimosa and water hyacinth in this study was ranged from 38 to 327, less than 1000. This suggested that water mimosa and water hyacinth in this study are not a hyperaccumulator plant. Results in this study were consistent with Lu et al., (2004) where the BCF was ranged from 320 to 600 [21]. In contrast, Mokhtar et al., (2011) have determined high BCF value of water hyacinth for Cu in aqueous solution containing 5.5 mg/L Cu (1147.5) which lead to a conclusion that water hyacinth is a hyper-accumulator plant [46]. Result in this study also was inconsistent with Soltan and Rashed, (2003) which indicated water hyacinth as hyper accumulator type [47]. Inconsistent result was possibly due to length of treatment time where Mokhtar et al., (2011) performed the experiment for lengthier treatment time (21 days) compared to this study (10 days) [46]. Other factors that possibly contribute to the difference were due to high biomass of plants used, which cause high accumulation rate [36].

Result in this study has indicated that water mimosa has more tolerance to heavy metals compared to water hyacinth. For example, in 10 mg/L heavy metal concentration supplied, water mimosa was in good tolerance with Ti value of more than 60%. In contrast, water hyacinth only has a good tolerance to heavy metals concentration up to 5 mg/L only. The order of tolerance for water hyacinth was Zn > Cr > Ag >Pb> Cd > Cu > Ni > Hg [17]. Soltan and Rashed treated water hyacinth with several heavy metals (Cd, Co, Cr, Cu, Mn, Ni, P, Zn) and concluded that water hyacinth has high tolerance to Cd and Cu [47]. Through our literature work, there was no reported on tolerance level of heavy metal for water mimosa.

Mechanism of plants tolerance to heavy metal is associated with the capacity of plants to restrict heavy metals to the cell walls and activation of antioxidant defense system [48]. Different species may have developed different mechanisms to tolerate excess levels of metals. There are more than one mechanism to tolerate excess levels of metals within one plant species. Plants could tolerate high concentration of heavy metal through limiting the metals uptake or metal transport in plants or internal tolerance mechanism. Plant will undergo several defense mechanisms to protect themselves from metal stress. For example, phytochelatins (PCs) production in plants, the oligomers of glutathione, produced by phytochelatin synthase enzyme is important for Cd detoxification in plants [40]. Superoxide dismutase is another enzyme which will increase when plants sense an increase of heavy metals uptake in plant tissue such as Pb [40].

As the top leaves of a plant shaded the lower leaves and limiting the nutrients as well as the increase of plant and non-photosynthetic biomass (roots and stems) cause the RGR decreases over time [49]. According to Wang et al., (2002) and Lu et al., (2004), reduction in the plant growth was also related to heavy metal stress on plant [16, 21]. Since Cd and Pb are not essential for the plant growth, they may cause toxicity and cause reduction in the plant growth. There was no growth obtained as the concentration of Cd and Pb supplied increased [48, 50]. Slightly difference with Cu, even though it is essential in plant growth, at concentration above those required for optimal growth, it may inhibit plant growth and interfere with important cellular processes such as photosynthesis and respiration [38, 51]. There was very little information regarding recommended concentration of Cu for optimal growth, but according to Sandmann and Boger (1980) Cu for optimum growth of plant is between 0.1 to 1 μM [52]. However, according to Reilly, (1969) the amount of the Cu normally required by plants is very small and as little as 2 mg/L is already a toxic [53].

Results from this experiment suggested that heavy metals were retained mostly in the root. Surface-floating plants such as water mimosa and water hyacinth accumulated higher concentrations of heavy metals in the roots than in stems and leaves [47]. The root is considered crucial for the absorption of elements in free-floating plants [54]. Most of the metal ion metabolically and passively taken up by roots and transported to the shoots [14]. 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 - a binding site for metals [34].

Lu et al., (2004) also highlighted that stems and leaves had low heavy metals accumulation rate compared to roots [21]. This is due to some physical barriers in roots against metal transport to the aerial part (stems and leaves). High concentration of metals in the roots also indicated that this plant might remove heavy metals through rhizofiltration process which accumulate contaminants in the roots [55, 56]. Consideration of the amount of pollutant accumulated by plant roots is an important factor for phytoremediation of wastewater [16]. Other plants that have similar phytoprocess to water mimosa and water hyacinth (removed pollutants through rhizofiltration) are duckweed, water spinach, and calamus [14, 16, and 55].

5. CONCLUSION

The highest BCF value of water mimosa was determined for Cd. The BCF value of water mimosa was lesser than water hyacinth. This result suggests that water mimosa can accumulate heavy metals but not as high in water hyacinth. Thus, this plant has good potential in water phytoremediation. After 10 days of treatment, the tolerance of water mimosa and water hyacinth to heavy metals (Ti values) were decreased with an increased of heavy metals concentration. Result shows water mimosa has more tolerance compared to water hyacinth. This suggests that water mimosa can tolerate to high concentration of heavy metals compared to water hyacinth.

RGR of water mimosa and water hyacinth decreased with heavy metals concentration after 10 days of treatment. Both plants have high RGR values for Cu compared to Cd and Pb. This is because Cu is an essential element in plant growth. However, it can become a toxic to plant at very high concentration. Both plants accumulate high heavy metal in the roots compared to stems and leaves. Results in this study suggested that water mimosa and water hyacinth use rhizofiltration (accumulate heavy metal in roots) process to remove heavy metals.

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