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Uptake of Mn and Cd by Wild Water Spinach and Their Bioaccumulation and Translocation Factors

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Abstract

Polluted ponds and lakes close to agricultural activities become the exposure route of manganese (Mn) and cadmium (Cd) to aquatic plants in near vicinity. Therefore, a study of the uptake, bioaccumulation, and translocation of Mn and Cd by the water spinach (*Ipomoea aquatica*) is presented in this paper. Different concentrations of Mn and Cd were added to the hydroponic nutrient solution that was used to grow the plants for the heavy metal uptake experiment under greenhouse conditions. The plant samples exposed to heavy metals were collected to determine the metal concentrations using atomic absorption spectroscopy (AAS) and the metal concentrations were found for Mn was between 1.589 to 9.696 μg/g and Cd from 5.309 to 10.947 μg/g. The correlation and regression results showed that the water-to-shoot bioaccumulation factor (BAF) decreased for Mn, while root-to-shoot translocation factor (TF) values increased in the order Cd > Mn to the increasing levels of metals in the water. Furthermore, it was revealed from the two-way analysis of variance (ANOVA) that the different metal types influenced the BAF and TF values at different metal concentration treatments.

Keywords: manganese; cadmium; water spinach; bioaccumulation factor; translocation factor

1. Introduction

Heavy metal contamination in surface water is a common phenomenon nowadays due to the increasing pollution sources caused by human activities (Nasir *et al*., 2012). Ponds and lakes are surface waters which are near stagnant and slow-moving when compared to rivers. The metals ending up in the ponds and lakes are dependent on the activities that are being carried out at the nearby areas. Khairiah *et al*. (2009) and Bolan *et al*. (2013) have reported that the pesticide and chemical fertilizer used in agricultural activities contribute huge amounts of Mn and Cd into the natural environment. These heavy metals leach out from the soil into the ponds and lakes and literally elevate the metal concentrations (Römkens *et al*., 2002; Bonten *et al*., 2008). The metals are then taken up by aquatic plants in the polluted ponds and lakes.

Some aquatic plants are found to have the tendency to uptake heavy metals from surface waters, for example water mint (*Mentha aquatica*) (Zurayk *et al*., 2002), water plantain (*Alisma plantago aquatica*) (Fritioff and Greger, 2003), hornwort (*Ceratophyllum demersum*) (Keskinkan *et al*., 2004), parrot feather (*Myriophylhum aquaticum*) (Kamal *et al*., 2004), and duckweed (*Landoltia punctata*) (Miranda *et al*., 2014).

Water spinach (WS) or Kangkong as called by the locals, is one of the popular consumed vegetables in Malaysia. Wild water spinach (WWS) can be found along the edges of ponds and lakes. It is a type of fast-growing aquatic plant due to its ability to reproduce vegetatively and by seeds. Nutrients including microelements are acquired from the water source and they are transferred from the root to the shoot (stems and leaves) for plant growth (Dhir, 2013). However, only little research has been done for WWS compared to duckweeds (*Lemna gibba* and *Lemna minor*) (Chaudhary and Sharma, 2014) and water hyacinth (*Eichhornia crassipes*) (Kabeer *et al*., 2014) which are widely studied for their heavy metals uptake.

To extract the metal from the plant samples, acids are commonly utilized to digest them. The digestion is usually started at a low temperature and then increased to a higher temperature. The process may take a couple of hours for the samples to be fully digested. A selection of acids had been experimented on by previous studies, for example combinations of nitric acid (HNO_3) with perchloric acid ($HClO_4$) (Yap *et al*., 2009), HNO_3-hydrofluoric acid (HF) (Khillare *et al*., 2012), HNO_3-$HClO_4$-sulfuric acid (H_2SO_4) (Opaluwa *et al*., 2012), HNO_3-H2O_2-hydrochloric acid (HCl) (Sapci and Ustun, 2012), HNO_3-$HClO_4$-hydrogen peroxide (H_2O_2) (Miranda *et al*., 2014), and HNO_3 (Nazir *et al*., 2015).

The lack of information regarding the ability of WWS to uptake Mn and Cd from the water prompted the present study to grow WWS using the hydroponic method to mimic the standing water in a pond. The hydroponic method was used because of the simplicity of the process, space-saving, and the ability to produce crops in a short amount of time in a controlled environment. The water-to-shoot bioaccumulation (BAF) and the root-to-shoot translocation factors (TF) were determined to assess the mobilities of Mn and Cd from the water to the plant.

2. Materials and Methods

2.1 Apparatus and reagents

Atomic absorption spectroscopy (AAS Model AA-6800 Shimadzu) (Shimadzu Corp., Kyoto, Japan) was employed to analyze the water and plant samples. For the preparation of hydroponic solutions, distilled water was obtained from the laboratory, the LushGro Hydro concentrated nutrient solutions A and B that consisted of nitrogen (N) 250 mg/L, nitrate (N-NO) 225 mg/L, ammonium (N-NH) 25 mg/L, phosphorus (P) 62.5 mg/L, potassium (K) 325 mg/L, calcium (Ca) 200 mg/L, magnesium (Mg) 62.5 mg/L, sulfur (S) 110 mg/L, iron (Fe) 3 mg/L, manganese (Mn) 2 mg/L, copper (Cu) 0.1 mg/L, zinc (Zn) 0.3 mg/L, boron (B) 0.7 mg/L, and molybdenum (Mo) 0.05 mg/L was purchased from Malaysia Hydroponics (Selangor, Malaysia). Standard Mn and Cd solutions (1000 mg/L) were obtained from Nacalai Tesque Inc. (Kyoto, Japan).

For the preservation and acid digestion of the water and plant samples, 65% concentrated nitric acid (HNO_3) (Bendosen Laboratory Chemicals, Bendosen, Norway) was used. Peach leaves, SRM 1547 were used as the standard reference material in the plant analysis and it was purchased from the National Institute of Standards & Technology (Maryland, USA).

2.2 Preparation of metal-contaminated water

Metal-contaminated waters which were called as treatment 1 (T1) and treatment 2 (T2) and control water (C) were used. C was water without metal treatment. Before being treated, hydroponic nutrient solutions were prepared by mixing distilled water and concentrated nutrient solutions A and B. For Mn, T1 and T2 had 10 and 50 times of the concentration found in C. Mn solutions were added into the nutrient solutions to adjust the concentrations to approximately 0.3 and 1.5 mg/L for T1 and T2, respectively. As for Cd, T1 and T2 had 100 and 500 times of the concentration found in C. Cd solutions were added to adjust the concentrations to approximately 0.1 and 0.5 mg/L for T1 and T2, respectively. To ensure the homogeneity of the nutrient and metal solutions, they were thoroughly mixed. The treatment concentrations were used because the work focuses on the uptake ability by WWS in extreme heavy metal polluted conditions that happened in Carrot River, Canada (Health Canada, 1979) and LongJiang River, China (Li, 2012) for Mn (1.7 mg/L) and Cd (0.4 mg/L) respectively. Besides that, water spinach has proven in the past to be able to accumulate high Mn which were 57.3 mg/kg (fresh weight) and 251.7 mg/kg (dry weight) as reported by Marcussen *et al*. (2008) and Kanakaraju *et al*. (2016), respectively; Göthberg *et al*. (2002) reported the dry weight of Cd accumulation was 123 μg/kg and Wang *et al*. (2008) found the bioconcentration factors of Cd were high in water spinach with results of 2227 and 144 l/kg for the roots and shoots, respectively.

2.3 Plant growth and heavy metal uptake experiment

Three greenhouses neighboring with each other were constructed with respective numbers greenhouse 1 (G2), greenhouse (G2), and greenhouse (G3) to grow the WWS. Each greenhouse had three compartments and was equipped with lighting system with time intervals of 12 h daylight and 12 h darkness. The temperature and humidity were within the range of 30.08 - 31.52°C and 53 - 56%. G2 and G3 served as the replicates for the experiment that was carried out in G1. Matured stems of the WWS were collected from the natural ponds (3°00’ 23.34”N, 101°42’ 34.97”E; 2°59’ 1.94”N, 101°42’ 41.57”E). The stems were segmented and immersed into hydroponic containers containing nutrient solutions under greenhouse conditions. The initial weights of the 3 weeks-old WWS of each individual treatment in this experiment are recorded in Table 1. The plants were transplanted and grown in C, T1, and T2 treatments for each metal with 8 plants per container. All treated plants were harvested after 7 days of metal exposures.

Table 1. Initial weights of 3-weeks old plants used (mean ± SD, n = 3)

Treatment	Fresh weight (g)
Mn	
C	11.428 ± 0.191
T1	11.549 ± 0.222
T2	11.250 ± 0.183
Cd	
C	11.859 ± 0.153
T1	11.774 ± 0.180
T2	11.824 ± 0.173

2.4 Acid digestion

The roots and shoots were separated and then they were washed and rinsed using distilled water. They were dried at 70°C for 48 h in an oven until a constant weight was achieved. The oven-dried roots and shoots were ground using a mortar and pestle into powder. The acid digestion was conducted by following the procedures used by Yap *et al*. (2003) with slight modifications. About 1 g of the powdered plant sample was used. HNO_3 was added and the sample was predigested for 24 h. The predigested sample was heated to 40°C and then 140°C on the heating block for approximately 1 h and 3 h, respectively. The clear digest was cooled, filtered, and diluted.

2.5 Metal analysis

Water samples (before and after treatments) were taken from C, T1, and T2 and preserved with HNO_3 to pH < 2 and stored at 4°C. The water samples and the plant extracts were analyzed by using AAS.

2.6 Statistical analysis

Analysis of variance (ANOVA) and regression found in the Microsoft Office Excel 2007 software were used. Moreover, correlation matrix found in StatPlus (Excel add-in) was also used.

3. Results and Discussion

3.1 Metal contents in water

The mean metal concentrations obtained for Cm (without heavy metal treatment and plants), C, T1, and T2 are presented in Table 2. The values from Table 2 are used to calculate the removal efficiency by applying the formula as follows:

$$R = [C_0 - C_1] \times 100 / C_0$$

Where R = removal efficiency (%), C_0 = initial metal concentration (mg/L), C_1 =final metal concentration (mg/L).

The histogram (Fig. 1) gives a visual picture that as the metal concentrations increased from C to T2, a lower removal of metals by WWS was observed. Correlation and regression analysis (p-values < *0.05*) showed that there was a linear relationship between the two variables for Mn. However, the analysis (p-values > *0.05*) showed that there was no linear relationship between the two variables for Cd. The results also indicated that the overall Cd removal by WWS was higher than that for Mn. The previous statement was supported by the curve slope values from the regression study. Cd had curve slope of 25.468, while Mn had curve slope of -28.713. The highest removals of Mn (48.65%) and Cd (56.03%) by WWS were in T1.

The removal efficiency of Cd by WWS was comparatively lower than that by water hyacinth (*Eichornia crassepes*), i.e. 71.28% at low treatment concentration (Narain *et al*., 2011). The water lettuce (*Pistia stratiotes*) and water hyacinth (*Eichornia crassepes*) which were studied by Nur Zaida *et al*. (2012) had a higher (86%) removal efficiency of Cd than WWS at 0.5 mg/L. Two-way ANOVA indicated that there was sufficient evidence (F statistical = *250.478* > F critical = *3.885*, and p-value = *0.0000000002* < *0.05*) of a interaction effect between type of metal and treatment.

3.2 Metal contents in plant

Table 3 shows the mean metal concentrations (μg/g) found in the dried sample of roots and shoots of WWS in natural pond, C, T1, and T2.

Table 2. Metal concentrations detected in the water samples (mean ± SD, n = 3)

Sample	Concentration (mg/L)			
	Cm[1]	C[2]	T1	T2
Before exposure				
Mn	0.035 ± 0.002	0.031 ± 0.002	0.345 ± 0.018	1.534 ± 0.018
Cd	ND*	ND*	0.105 ± 0.001	0.512 ± 0.003
After exposure				
Mn	0.037 ± 0.001	0.015 ± 0.002	0.177 ± 0.003	1.372 ± 0.021
Cd	ND*	ND*	0.046 ± 0.002	0.340 ± 0.008

Cm[1]: Control without heavy metal treatment and plants
C[2]: Control without heavy metal treatment but with plants
ND*: Cd concentration was below the detection limit of AAS

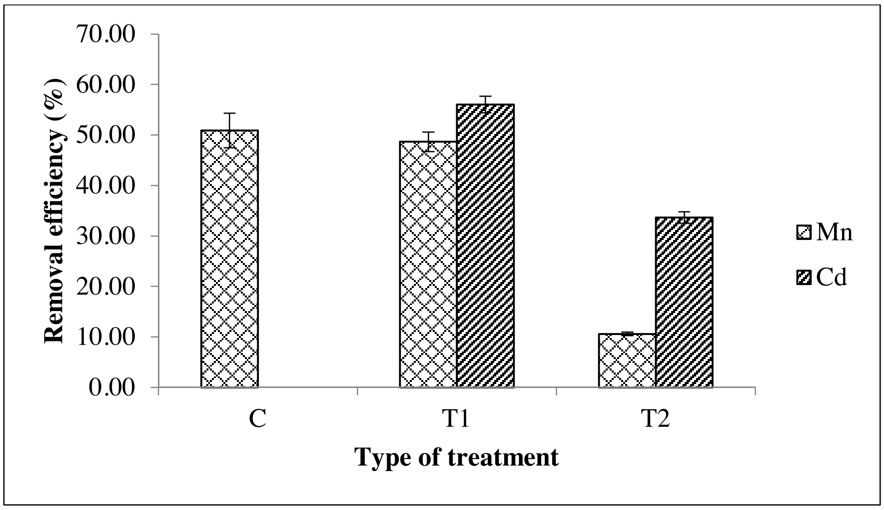

Figure 1. Removal efficiencies against types of treatments for Mn and Cd (mean ± SD, n = 3)

The results showed that both metals were more concentrated in WWS roots than shoots which agreed with the findings of Fritioff and Greger (2006) and Kumar *et al.* (2008). The metal concentrations varied in different parts of the plant as also shown by Li *et al*. (2015) and their findings revealed that the metal concentration was found to be highest in roots followed by stems and leaves. The highest Mn content was found in T2 for WWS roots and shoots with the concentrations of 9.696 and 8.787 μg/g, respectively; while the highest content of Cd was also found in T2 for WWS roots and shoots with the concentrations of 10.947 and 9.687 μg/g, respectively. The Cd content in roots was lower in WWS than in aquatic fern (17.5 μg/g) at 0.5 mg/L which was studied by Phetsombat *et al*. (2006). The precision and quality of the metal analysis procedure in plant samples were assessed by using peach leaves (SRM 1547) as the standard reference for plant material. Table 4 shows the Mn and Cd content detected in the peach leaves by AAS. The mean recoveries for Mn and Cd were 95.06 and 93.59%, respectively.

3.3 Water-to-shoot bioaccumulation factor (BAF) and root-to-shoot translocation factor (TF)

Some of the metal concentration values in water and plant samples recorded in Tables 2 and 3 were obtained to calculate the BAF and TF values by applying the formula as follow:

BAF = Cs / Cw (Yabanli *et al*., 2014)

Where BAF = bioaccumulation factor, Cs = metal concentration in plant shoot (μg/g), Cw = metal concentration in water, (mg/L).

TF = Cs / Cr (Hammad, 2011)

Where TF = translocation factor, Cs = metal concentration in plant shoot (μg/g), Cr = metal concentration in plant root (μg/g).

Table 3. Metal concentrations detected in plant samples (mean ± SD, n = 3)

Sample	Concentration (μg/g)			
	Natural pond	C	T1	T2
Mn				
Root	0.172 ± 0.010	2.028 ± 0.065	9.280 ± 0.061	9.696 ± 0.077
Shoot	0.076 ± 0.005	1.588 ± 0.093	7.413 ± 0.144	8.780 ± 0.133
Cd				
Root	0.043 ± 0.005	ND*	5.816 ± 0.069	10.947 ± 0.215
Shoot	ND*	ND*	5.309 ± 0.045	9.687 ± 0.235

ND*: Cd concentration was below the detection limit of AAS

Table 4. Metal content detected in SRM (mean ± SD, n = 3)

Metal	Certified concentration (μg/g)	Detected concentration (μg/g)	Recovery (%)
Mn	98.000 ± 3.000	93.160 ± 1.490	95.06 ± 1.520
Cd	0.026 ± 0.003	0.024 ± 0.002	93.59 ± 7.798

The BAF and TF together with high specific growth rate, large specific surface area of the portion in contact with water, and high translocation potential are important assets for a plant species to be used for phytoremediation (Jasrotia *et al*., 2015). Table 5 shows the BAF and TF values of Mn and Cd for WWS.

Bioaccumulation factor. Correlation analysis (p-value < *0.05*) showed that there was a strong negative linear relationship between the two variables. In addition, the regression statistics confirmed with 95% confidence that for each degree increase of Mn treatment concentration, the BAF values decreased between 26.397 to 85.257. On the other hand, both the correlation and the regression analyses (p-values > *0.05*) showed that there was no linear relationship between the two variables for Cd.

Nevertheless, the regression statistics showed that the overall BAF value of Cd (curve slope = -21.118) was higher than that of Mn (curve slope = -55.827). The highest BAF value for Cd was in T1 (115.035), whereas for Mn, it was in C (105.268). The two-way ANOVA indicated that the differences between the mean BAF values of the two metals were not the same for the three treatment concentrations (C, T1, and T2).

Since the BAF values were more than 1, WWS is categorised as a good accumulator for Mn and Cd. The result of the high accumulation of Mn in WWS from this study was supported by that of Dummee *et al*. (2012) who found that the bioconcentration factor of Mn was 95.07. However, WWS was less metal accumulative when compared to water lettuce (*Eichhornia crassipes*) (Lu *et al*., 2011) and hornwort (*Ceratophyllum demersum*) (Matache *et al*., 2013) which had BAF > 1000. The BAF of Cd was recorded as high as 622.3 and 653.0 in water hyacinth (*Eichhornia crassipes*) (Lu *et al*., 2004; Swain *et al*., 2014) and 184.5 in water mimosa (*Neptunia oleracea*) (Wahab *et al*., 2014).

Translocation factor. Both the correlation and the regression analyses (p-values < *0.05*) indicated that there was a positive linear relationship between the two variables for Mn. The TF of metals varied with reference to the type of metal and the metal concentrations which was in agreement with Duman and Obali (2008) and Ndimele *et al*. (2014). Regression statistics also showed with 95% confidence that for each degree of increase in the treatment concentration, the TF values increased between 0.039 to 0.131.

The TF values for Cd were slightly higher than those for Mn as confirmed by the curve values of Cd (1.244) and Mn (0.085) obtained from the regression tables. The TF value for Mn was highest in T2 (0.906), while for Cd, it was in T1 (0.913). The TF of Mn was lower for WWS than water hyacinth (*Eichhornia crassipes*) (Hua *et al*., 2012) at high treatment concentration; but the TF of Cd was higher than that of 0.60 for shichito matgrass (*Cyperus malaccensis*) (Zhang *et al*., 2011).

Table 5. BAF and TF values for C, T1, and T2 (mean ± SD, n = 3)

Treatment	BAF	TF
Mn		
C	105.268 ± 8.016	0.782 ± 0.032
T1	41.963 ± 0.507	0.799 ± 0.021
T2	6.402 ± 0.058	0.906 ± 0.013
Cd		
C	NIL*	NIL*
T1	115.035 ± 4.828	0.913 ± 0.004
T2	28.490 ± 0.153	0.885 ± 0.015

NIL*: Zero value

Two-way ANOVA showed that there was a significant interaction between the two variables because the F statistical value = $378.631 > F$ critical value = 3.885, and p-value = $0.00000000001 < 0.05$. The mobility of the metals in the plant was directly proportional to the TF value. It was observed that the TF values were less than 1 or smaller which was similar with Uka *et al*. (2013), thus it was concluded that the movement of metals in WWS from roots to shoots was not effective which mostly characterised aquatic plants (Ansari *et al*., 2015). The translocation of metals from root to shoot could be governed by biotic and abiotic factors, plant species, and environmental conditions (e.g. pH, temperature, redox, salinity, and water ion content) (Kaewtubtim *et al*., 2016).

Here, WWS functions as an excluder like other macrophytes such as reed (*Phragmites australis*) and cattail (*Typha latifolia*), which accumulated higher amounts of heavy metals in roots than shoots (Grisey *et al*., 2012). It is also known that WWS is not a hyperaccumulator for Mn and Cd because of the BAF was not more than 1000 (Xing *et al*., 2013). Although the BAF was > 1 and the TF was < 1, still it is a promising alternative for phytoremediation of heavy metal-contaminated water sources.

3.4 Uptake mechanisms in water spinach

It is normal for Mn uptake to take place because Mn is a type of micronutrient required by the plant. On the other hand, Cd being taken might be due to a few possible reasons. According to Tran and Popova (2013), one of them could be that the non-essential Cd was carried by essential-metal transporters. In other words, it was being actively transported by a carrier that was able to bind with the metal and the process was driven by metabolic energy. It was also possible that Cd was being transported by the Zn and Ca pathways (Islam *et al*., 2015). The transport of Cd within the plant could also happen through passive transport where Cd followed the water transport system in the xylem. In addition, Cd in plant might be due to its adsorption onto the membrane surface and diffusion via the cell wall (Regier *et al*., 2013). Chekroun and Baghour (2013) reported that Cd uptake could occur via processes used by plants in detoxification or defense mechanisms, for example exclusion, compartmentalization, and binding with proteins (e.g. metallothioneins) and ligands (e.g. citrate, malate, oxalate, phytate, histidine, and nicotianamine). The high amounts of Cd in the roots might be caused by vacuolar sequestration, insoluble salt precipitation, and phytochelatin complexation (Fontes *et al*., 2014). Large amounts of Cd being retained in the roots could be a type of defence mechanism to prevent the toxic element from being transported to the other parts of the plant.

4. Conclusions

The uptake of Mn and Cd from water to the plant as indicated by BAF values decreased in the order C > T1 > T2 and T1 > T2, respectively. All the BAF values were found to be more than 1 which showed that WWS is a good accumulator for Mn and Cd. As for the movement of Mn and Cd in the plant, to be precise, from root to shoot, indicated by TF values increased in the order C < T1 < T2 and T2 < T1, respectively. Higher metal content were accumulated in roots than shoots. However, it was observed that both metals moved less freely (TF < 1). Since large amounts of Mn and Cd were accumulated in WWS as shown by this study, it will be interesting to investigate the bioavailability of the metals for absorption by the human body if this edible aquatic plant were to be consumed by humans.

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