Journal of Environmental Protection, 2015, 6, 700-709
Published Online July 2015 in SciRes. http://www.scirp.org/journal/jep
http://dx.doi.org/10.4236/jep.2015.67063

Changes of Essential Mineral Elements Contents in Response to Cu^{2+} Treatment in *Sagittaria sagittifolia*

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Received 1 June 2015; accepted 17 July 2015; published 23 July 2015

Abstract

Changes of various mineral elements (P, K, Ca, Mg, Fe, Mn, Zn and Na) contents in roots and leaves of *S. sagittifolia* were studied with treatment of different Cu^{2+} concentrations (5 μM, 10 μM, 20 μM and 40 μM) after 15 days. The results showed that: 1) Cu accumulated in roots of *S. sagittifolia* in large quantities, while Cu content in leaves showed no significant change; 2) It can be seen from the changes of macroelements that Cu^{2+} treatments had inhibited the absorption of P, K, Ca, Mg in roots of *S. sagittifolia*, but the contents of P, K and Mg in leaves were higher than those in the roots in all Cu^{2+} treatment groups; 3) It can be seen from the changes of microelements that Cu^{2+} treatment promoted the absorption of Fe, inhibited absorption of Mn, Zn and Na in roots of *S. sagittifolia*, and hindered the transport of various microelements from roots to leaves. In all the Cu^{2+} treatment groups, contents of Fe, Mn, Zn and Na in leaves were lower than those in the roots; 4) The critical concentration of Cu^{2+} to *S. sagittifolia* was 5 μM. It could be seen from the above results that exogenous added Cu^{2+} of different concentrations broke the balance of various mineral elements in *S. sagittifolia*, which would exert a significant impact on numerous metabolic pathways and physiological processes.

Keywords

***Sagittaria sagittifolia*, Cu^{2+}, Macroelements, Microelements**

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How to cite this paper: Xu, X.Y., Xu, Y.K., Chi, Y., Wang, P.H. and Shi, G.X. (2015) Changes of Essential Mineral Elements Contents in Response to Cu^{2+} Treatment in *Sagittaria sagittifolia*. *Journal of Environmental Protection*, **6**, 700-709.
http://dx.doi.org/10.4236/jep.2015.67063

1. Introduction

Cu, as a trace mineral element which is necessary for normal growth and development of plants, is widely involved in various life activities of plants. However, when its concentration exceeds a certain threshold value, it will inhibit growth of plants, and even lead to death of organisms [1]. Cu^{2+} is a metal ion, which is widely present in water environment. The free Cu^{2+} is generally considered as the major ion form of Cu which is toxic to aquatic organisms [2].

Studies find that the Cu^{2+} stress exerts a significant effect on absorption of mineral elements in plants, which breaks the balance of various mineral elements, and results in the lack of essential mineral elements in plants, thus affecting the normal growth of plants [3]. On one hand, Cu^{2+} stress inhibited absorption of macroelements K, Ca [4] [5], N, P and S [6] in plants. On the other hand, Cu^{2+} stress also interfered with absorption and transport of microelements Mn, Zn, B [7], Na, Fe, Mn and Zn [8] [9] in plants. However, studies on the effect of Cu^{2+} stress on mineral elements in plants mostly focused on terrestrial plants, and studies on the effect of Cu^{2+} pollution in water body on mineral elements in higher aquatic plants are still very scarce.

Sagittaria sagittifolia is a perennial emerging plant and is widely distributed in China. Its underground bulbs can be used as a low-fat, high-carbohydrate health food, which can regulate and promote human functions, and has good medicinal value. Because of its important edible and medicinal value, its cultivation and planting are attracting more and more attention. *S. sagittifolia* is considered as a good material for experiment because of its wide distribution and large biomass.

In order to reveal tolerance to Cu^{2+} and the effect of Cu^{2+} treatment of different concentrations on essential mineral elements in *S. sagittifolia*, as well as provide a theoretical basis for utilization of *S. sagittifolia*, we adopted the method of nutrient solution simulated cultivation to study: 1) accumulation of Cu; 2) changes of contents of various macroelements (P, K, Ca and Mg); 3) changes of contents of various microelements (Fe, Mn, Na and Zn) in the roots and leaves of *S. sagittifolia* which had been processed by exogenous added Cu^{2+} of different concentrations (5 $\mu mol \cdot L^{-1}$, 10 $\mu mol \cdot L^{-1}$, 20 $\mu mol \cdot L^{-1}$ and 40 $\mu mol \cdot L^{-1}$) for 15 days.

2. Materials and Methods

2.1. Plants and Growth Conditions

Corms with terminal buds of *S. sagittifolia* (**Picture 1**) were collected from an unpolluted field in Nanjing, China. Similar size of corms with terminal buds were washed with distilled water and grown in nutritional soil which was composed of import peat, coco coir, perlite, clean gravel and controlled release fertilizer granules.

Picture 1. Corms with terminal buds of *S. sagittifolia*.

Plant materials were cultured in a controlled environmental growth chamber (Forma 3744, England) with a photoperiod of 12 h light (70 $\mu M \cdot m^{-2} \cdot s^{-1}$) and 12 h dark cycle and the day/night temperature of 24˚C /18˚C. After 2 weeks, plants of 15 cm with two euphylla and roots of similar density and length were selected for experiment.

2.2. Treatments

Plant materials were treated as follows: 1) control: 1/10 Hoagland solution; 2) Cu^{2+} treatment: 2 L of 1/10 Hoagland solution containing 5, 10, 20 and 40 $\mu mol \cdot L^{-1}$ Cu^{2+} (Cu^{2+} was supplied by $CuSO_4 \cdot 5H_2O$), respectively. The selected Cu^{2+} concentrations were based on preliminary experiments. All solutions were refreshed every 2 days. After 15 days, roots and leaves were cut and sampled. All experiments were performed in triplicate.

2.3. Determination of Cu and Different Mineral Element Contents

The roots and leaves were washed thoroughly with 10 mM EDTA at 4˚C for 30 min and then with double distilled water in order to remove the metals adsorbed to the surface. After roots and leaves were dried, they were digested with HNO_3-$HClO_4$ (10:1, v/v) at 160˚C until the digest solution became clear. The digested residue was dissolved in 0.7 ml HCl and diluted with pure water to 10 ml. The concentrations of P, K, Ca, Mg, Fe, Mn, Zn and Na in the extracts were determined using inductively coupled plasma atomic emission spectroscopy (Leeman Labs, Prodigy, USA).

2.4. Statistics

Each value was repeated three times with three replicates in each. The data reported in the table and figures were means of the values with standard deviation (SD). Results were statistically analyzed using analysis of variance (ANOVA). Levels of significance were indicated by Duncan's multiple range test at $P < 0.05$. The coefficients of correlation were expressed using r-values.

3. Results

3.1. Accumulation of Cu in *S. Sagittifolia*

From **Table 1**, we can know that as the concentration of Cu^{2+} in nutrient solution increased, Cu content in roots of *S. sagittifolia* sharply rose. In the 40 $\mu mol \cdot L^{-1}$ Cu^{2+} treatment group, the accumulation of Cu in roots was 97 times that of the control group. There was an extremely significant positive correlation ($r = 0.9832$, $P < 0.01$) between the Cu content in roots and the concentration of exogenous Cu^{2+} concentration; however, the Cu content in leaves did not change significantly as the concentration of exogenous Cu^{2+} increased. So in the experimental concentrations, Cu mainly accumulated in the roots of *S. sagittifolia*.

3.2. The Impact of Cu^{2+} Treatments on Macroelement Contents in *S. Sagittifolia* Roots

In roots of *S. sagittifolia*, all the Cu^{2+} treatments had made the contents of P, K, Ca and Mg significantly lower than those in the control group ($P < 0.05$). In the 40 $\mu mol \cdot L^{-1}$ Cu^{2+} treatment, contents of P, K and Mg reached their minimum values, respectively were only 35.5%, 18.9% and 42.8% of those in the control group (**Figure 1(a)**, **Figure 1(b)** and **Figure 1(d)**). However, the Ca content reached its minimum value in the 20 $\mu mol \cdot L^{-1}$ Cu^{2+} treatment groups, and the Ca content was only 85.3% of that in the control group (**Figure 1(c)**). Through statistical analysis, we found that there was a significant negative correlation between the P content in roots and

Table 1. Cu accumulation in roots and leaves of *S. sagittifolia* with application of different Cu^{2+} concentrations ($\mu g \cdot g^{-1}$ DW).

	Exogenous Cu^{2+} Concentrations ($\mu mol \cdot L^{-1}$)				
	0	5	10	20	40
Roots	1.4 ± 0.1d	20.1 ± 2.0c	24.1 ± 2.0c	55.2 ± 5.0b	135.7 ± 10.1a
Leaves	1.8 ± 0.2a	2.1± 0.2a	2.0 ± 0.2a	2.2 ± 0.2a	1.9 ± 0.2a

Note: Each value is the mean ± SD (n = 3). Different letters indicate significant differences between treatments according to Duncan's multiple range test at P < 0.05.

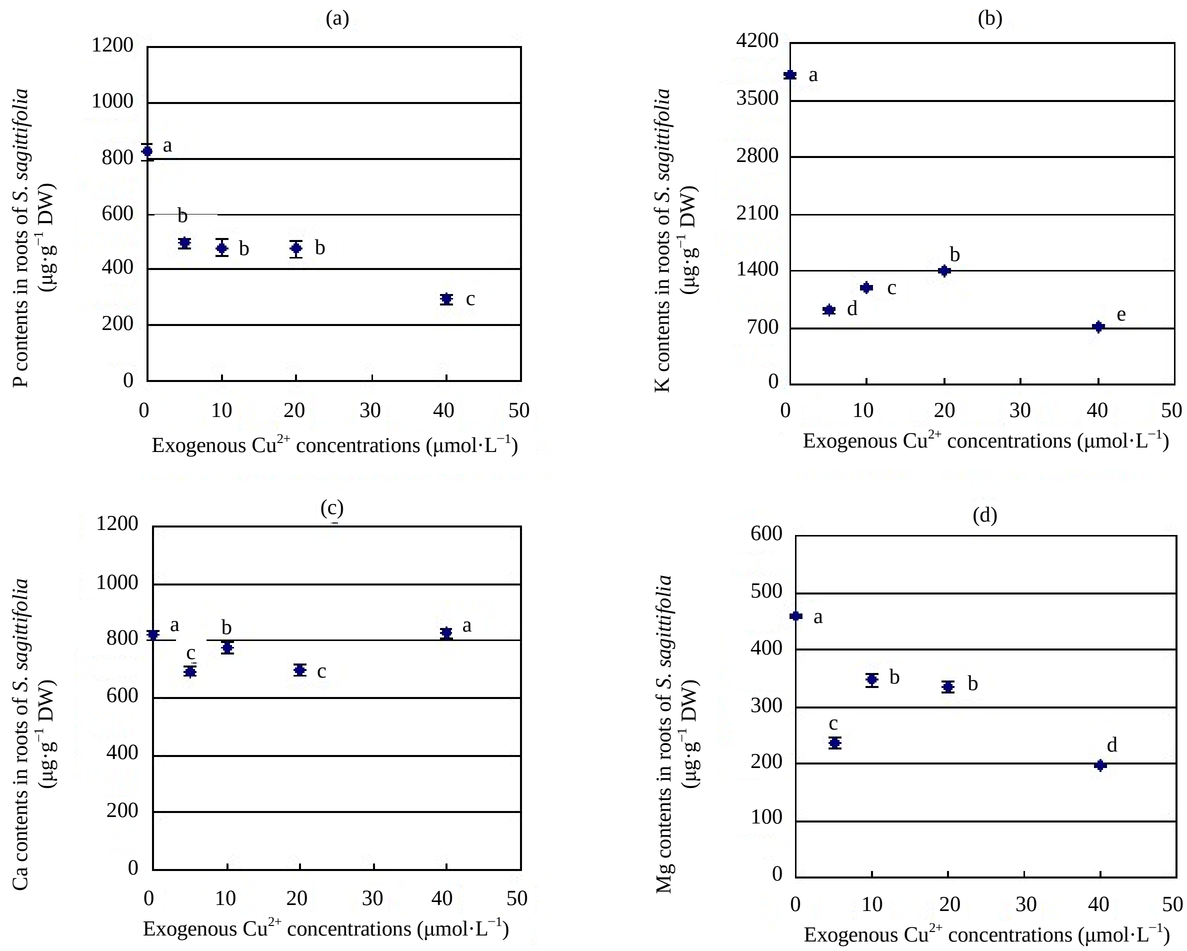

Figure 1. Changes of P (a), K (b), Ca (c) and Mg (d) contents with exogenous application of different Cu^{2+} concentrations in roots of *S. sagittifolia*. Each value is the mean ± SD of triplicates. Different letters indicate significant differences between treatments according to Duncan's multiple range test at $P < 0.05$.

the concentration of exogenous Cu^{2+} concentration ($r = -0.821$, $P < 0.05$), and K contents in Cu^{2+} treatment groups of different concentrations were significantly different from each other ($P < 0.05$).

3.3. The Impact of Cu^{2+} Treatments on Macroelement Contents in *S. Sagittifolia* Leaves

In leaves of *S. sagittifolia*, Cu^{2+} treatments of different concentrations exerted no significant effect on the P content (**Figure 2(a)**). In the 5 $\mu mol \cdot L^{-1}$ Cu^{2+} treatment group, contents of K, Ca and Mg were respectively 1.33 times, 1.16 times and 1.19 times that of the control group, so they were significantly higher than those in the control group ($P < 0.05$). With the increase of the concentration of exogenous Cu^{2+}, contents of K, Ca and Mg decreased. When the concentration of exogenous Cu^{2+} was 40 $\mu mol \cdot L^{-1}$, contents of Ca and Mg were respectively reduced to 42.2% and 83.5% of those in the control group (**Figure 2(c)** and **Figure 2(d)**). Though K content had dropped somewhat, it was still higher than that in the control group (**Figure 2(b)**). Statistical analysis showed that contents of P, K, Ca and Mg in leaves had no significant correlation with the concentration of exogenous Cu^{2+} concentration ($P > 0.05$); K contents in Cu^{2+} treatment groups of different concentrations were significantly different from each other ($P < 0.05$).

3.4. The Impact of Cu^{2+} Treatments on Microelements in *S. Sagittifolia* Roots

Fe, Mn, Zn and Na are essential microelements for growth of plants. From **Figure 3**, we can see that contents of Fe, Zn and Na in roots of *S. sagittifolia* had a brief rise in the 5 $\mu mol \cdot L^{-1}$ Cu^{2+} treatment, and then gradually

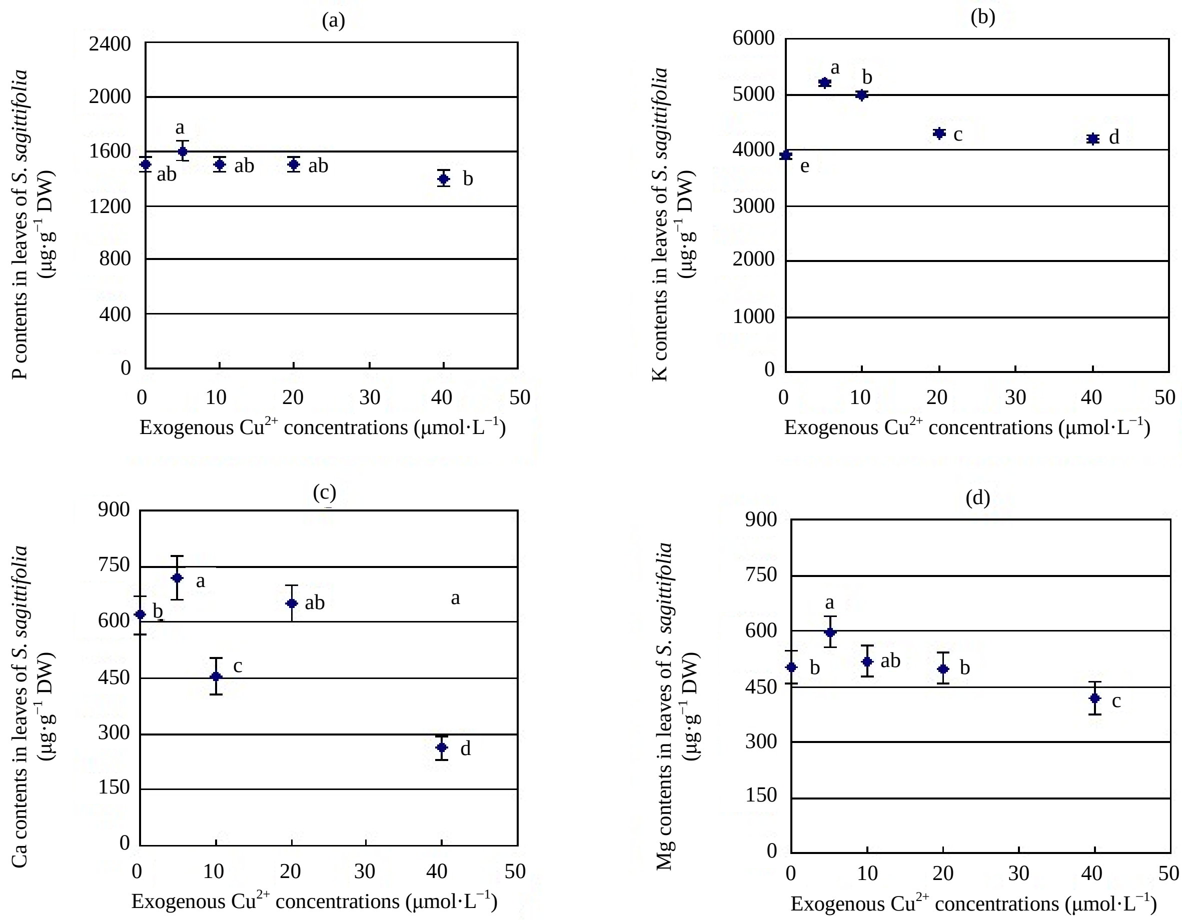

Figure 2. Changes of P (a), K (b), Ca (c) and Mg (d) contents with exogenous application of different Cu^{2+} concentrations in leaves of *S. sagittifolia*. Each value is the mean ± SD of triplicates. Different letters indicate significant differences between treatments according to Duncan's multiple range test at $P < 0.05$.

decreased with the increase in exogenous Cu^{2+} concentration. However, the Mn content in each Cu^{2+} treatment group continued to decrease. Although the Fe content was decreased by Cu^{2+} treatments of high concentrations, its value was always higher than that in the control group (**Figure 3(a)**). When the concentration of Cu^{2+} treatment was 40 μmol·L^{-1}, contents of Zn, Na and Mn were reduced to 79.7%, 39.4% and 15.1% of those in the control group, respectively (**Figures 3(b)-3(d)**). Statistical analysis showed that there was a significant negative correlation between the Zn content in roots and the concentration of Cu^{2+} treatment ($r_{Zn} = -0.8241$, $P < 0.05$), while there was an highly significant negative correlation between the Na content and the concentration of Cu^{2+} treatment ($r_{Na} = -0.968$, $P < 0.01$). What's more, Na contents in all Cu^{2+} treatment groups were significantly different from each other ($P < 0.05$).

3.5. The Impact of Cu^{2+} Treatments on Microelements in *S. Sagittifolia* Leaves

In the leaves of *S. sagittifolia*, contents of Fe, Mn, Zn and Na were all lower than those in the roots. In Cu^{2+} treatments of different concentrations, changes of contents of Fe, Mn, Zn and Na in leaves were toward the same trend, which was manifested as that contents of Fe, Mn, Zn and Na were significantly higher than those in the control group ($P < 0.05$) in Cu^{2+} treatments of low concentrations (5 μmol·L^{-1} or 10 μmol·L^{-1}). As the concentration of exogenous Cu^{2+} treatment increased, their contents decreased gradually. When the concentration of Cu^{2+} treatment was 40 μmol·L^{-1}, contents of Fe, Mn and Zn were reduced to 53.9%, 69.9% and 73.8% of those in the control group, respectively (**Figures 4(a)-4(c)**). Although the Na content decreased slightly in Cu^{2+} treatments of high concentrations, it was always higher than that in the control group (**Figure 4(d)**). Statistical analysis

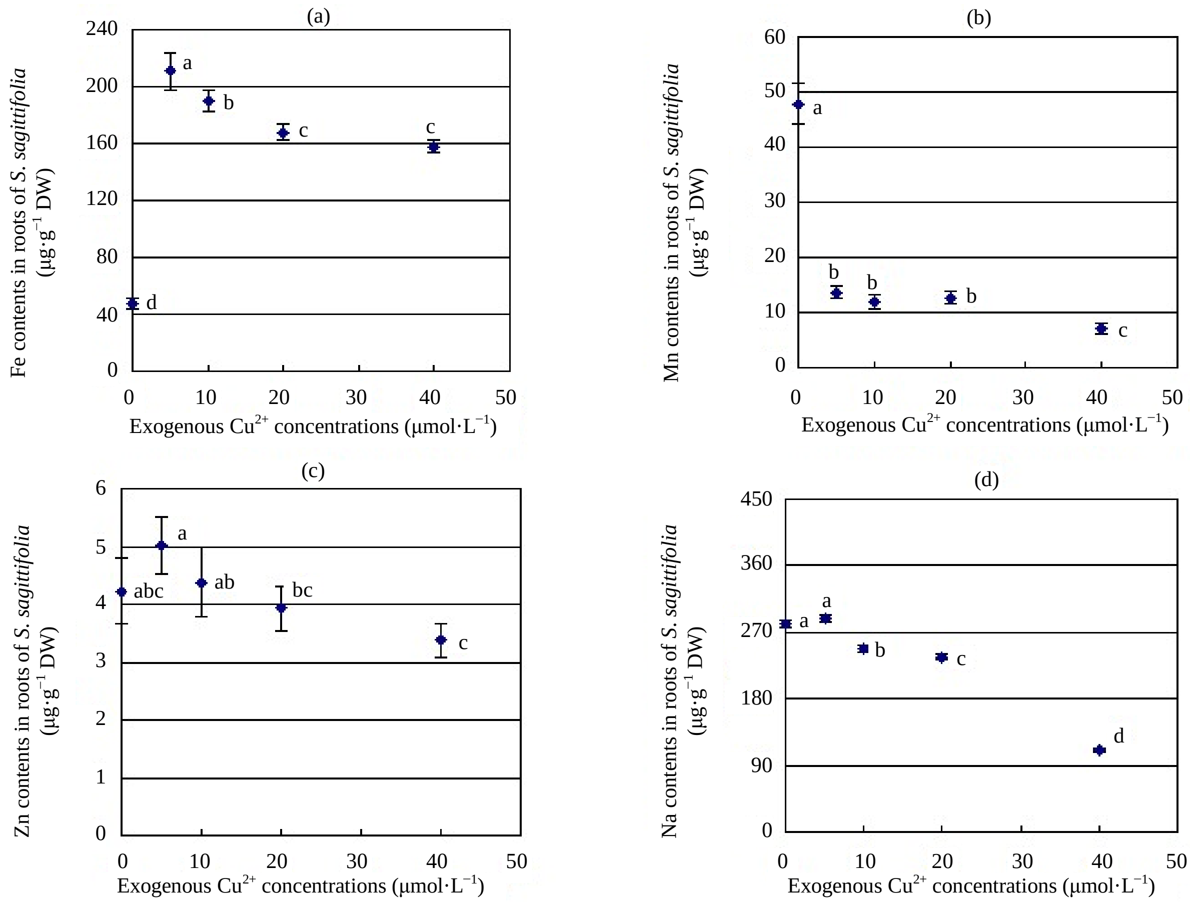

Figure 3. Changes of Fe (a), Mn (b), Zn (c) and Na (d) contents with exogenous application of different Cu^{2+} concentrations in roots of *S. sagittifolia*. Each value is the mean ± SD of triplicates. Different letters indicate significant differences between treatments according to Duncan's multiple range test at $P < 0.05$.

showed that, there was a significant negative correlation between the Mn content in leaves and the concentration of Cu^{2+} treatment ($r = -0.8811$, $P < 0.05$).

4. Discussion

When plants are subjected to heavy metal stress, contents of heavy metals in different vegetative organs generally follow the law that the accumulation levels in roots are higher than those in leaves, but sometimes this law may vary in different plants [10]. In this experiment, after Cu^{2+} treatments, the Cu content in the roots significantly accumulated. However, Cu accumulation of great quantity was not found in leaves (**Table 1**). This was consistent with the findings of predecessors' studies on *Kosteletzkya virginica* and *Halimione portulacoides* under Cu^{2+} stress [5] [6]. Thus, the distribution of Cu in plants presented the features that a relatively large amount of Cu accumulated in the organ of high metabolism, namely the roots, and a relatively small amount of Cu accumulated in nutrient storage organs such as leaves. Therefore, we inferred that roots were the main part for *S. sagittifolia* to store Cu, and root blocking could serve as an important way for *S. sagittifolia* to resist excessive Cu^{2+} stress in environment and protect the organism from harm.

In addition to causing accumulation of toxic metals, heavy metal stress can also affect the absorption of essential mineral elements in plants, and different kinds of heavy metals exert different effects on absorption of mineral elements in different plants [11]. This study found that the Cu^{2+} treatment not only inhibited the absorption of P, K, Ca, Mg by *S. sagittifolia* roots to different degrees (**Figure 1**), but also made P, K and Mg transport to leaves excessively, which eventually caused contents of P, K and Mg in leaves higher than those in the roots, while Ca content in leaves was lower than that in roots (**Figure 2**).

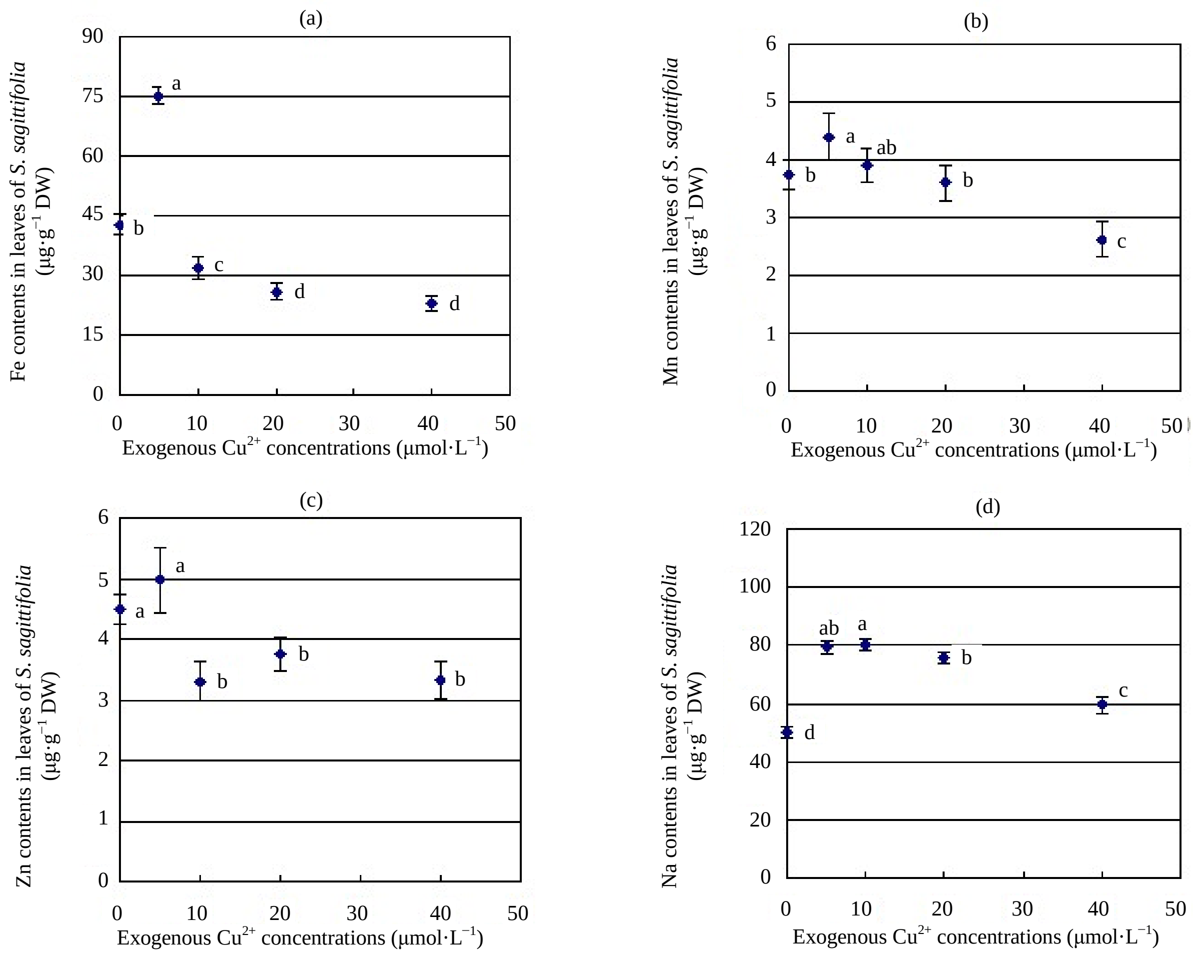

Figure 4. Changes of Fe (a), Mn (b), Zn (c) and Na (d) contents with exogenous application of different Cu^{2+} concentrations in leaves of *S. Sagittifolia*. Each value is the mean ± SD of triplicates. Different letters indicate significant differences between treatments according to Duncan's multiple range test at $P < 0.05$.

In this experiment, P content in roots of *S. sagittifolia* decreased gradually with the increase of Cu^{2+} concentrations. Mateos-Naranjo *et al*. [12] got the same results that P content in *Spartina. Densiflora* roots which were treated with Cu^{2+} decreased as the concentration increased. On one hand, Cu^{2+} had inhibited the activity of phosphatase in roots. The higher the concentration of Cu^{2+} was, the more obvious the inhibitory action was, which inhibited the P metabolism, and it could be regarded as the result of P-Cu interaction [12]-[14]. On the other hand, P usually formed unstable compounds in plants, which could decompose unceasingly, and the released ions could be transferred to other parts to be reused [15]. The Cu^{2+} treatment promoted the shift of P from roots to leaves in *S. sagittifolia*, which made the P content in roots decrease significantly, and P content in the leaves was higher than that in the roots (**Figure 2(a)**).

In the roots, the change of K content was similar to the change of P content. Cu^{2+} treatments made K content significantly lower than that in the control group (**Figure 1(b)**). We inferred that the exogenous application of Cu^{2+} significantly inhibited the absorption of K in roots, which resulted from a competition between the absorption of K and other mineral elements [8]. In the leaves, K content of each Cu^{2+} treatment group was significantly higher than that in the control group (**Figure 2(b)**). In *S. sagittifolia*, changes of K contents in roots and leaves were consistent with *Kosteletzkya virginica* (L.) Presl [5]. However, studies on *Cucumis sativus* [16], *Arabidopsis thaliana* [17] and *Hydrilla verticillata* [18] all found that K content in leaves decreased with the increase of Cu^{2+} concentration. It suggested that the Cu^{2+} treatment exerted different effects on K contents in leaves of different plants, which might be due to the different regulatory mechanisms used by different plants to respond to Cu^{2+} stress.

Compared to the contents of P and K, Cu^{2+} treatment exerted more complicated effect on Ca content in *S. sagittifolia*. Most existent studies showed that Cu^{2+} treatment would lead to the decrease in the Ca content in plants [5] [8] [19] [20]. Because Cu^{2+} could replace Ca^{2+} at some exchange sites of cells, and it could be tightly integrated in some free space of the roots and leaves, so exogenous added Cu^{2+} of different concentrations resulted in the decrease in the Ca content in roots and leaves of *S. sagittifolia* (**Figure 1(c)** and **Figure 2(c)**). However, in the leaves of the 5 $\mu mol \cdot L^{-1}$ and the 20 $\mu mol \cdot L^{-1}$ Cu^{2+} treatment groups and in the roots of the 40 $\mu mol \cdot L^{-1}$ Cu^{2+} treatment group, the Ca contents were increased. Probably it was due to that Cu^{2+} of relative high concentrations caused damages to cell membranes, and Ca flowed inward through the Ca^{2+} channels in cell membranes [21] [22]. The mechanism of the impact of Cu^{2+} treatments of different concentrations on the Ca contents in *S. sagittifolia* might also involve interference of Cu^{2+} in Ca-dependent signaling proteins and Ca receptor gene [23].

Studies on *Vigna radiata* [8], *Arabidopsis thaliana* [17] and *Alyssum montanum* [19] found that Cu^{2+} treatment would cause a decline in Mg contents, which was consistent with our findings. Mg, as a key component of chlorophyll, played an important role in photosynthesis of plants. In order to resist Cu^{2+} stress, the leaves of *S. sagittifolia* needed to be supplied with a lot of Mg to enhance photosynthesis which certified sources of material and energy for normal life activities of *S. sagittifolia*. Therefore, Mg content in leaves had been maintained at a high level (**Figure 2(d)**), which was one of the reasons why Mg content in roots continued to decrease (**Figure 1(d)**).

Fe, Mn, Zn and Na are essential microelements for the growth of plants. Although demand of plants for them is very small, they still play an important role in the process of physiology and biochemistry of plants [15]. It is noteworthy that, although Cu^{2+} treatments of relative high concentrations (>5 $\mu mol \cdot L^{-1}$) decrease Fe contents in the roots of *S. sagittifolia*, Fe contents in the roots are always higher than that in the control group ($P < 0.05$) (**Figure 3(a)**). Studies on *Triticum aestivum* and *Hydrilla verticillata* [18] [24] also found that the Cu^{2+} treatment could improve the absorption of Fe in plants. It was reported that Fe in nutrient solution was present in the form of EDTA complex, and the exogenous added Cu^{2+} would carry out the activity of complexation with EDTA, which would displace Fe from Fe-EDTA complex and make Fe easier to be absorbed by plants [17]. However, when the concentration of Cu^{2+} treatment was higher than 5 $\mu mol \cdot L^{-1}$, the transport of Fe from roots to leaves was significantly impeded, which was demonstrated by the continuous decrease of Fe content in leaves (**Figure 4(a)**). The enzyme, which catalyzed chlorophyll synthesis, needed to be activated by Fe^{2+}, so the decrease in Fe content in leaves would hinder the chlorophyll synthesis. Cu-Fe antagonism under Cu^{2+} stress had been reported by many scholars [8] [9].

It was reported that there was a competition between transfer sites of Cu and Mn in cell membranes [25]-[28]. The higher the concentration of Cu^{2+} was, the fiercer the competition of transfer sites between Cu and Mn in root cell membranes was, the less Mn was absorbed into the roots (**Figure 3(b)**). It could be seen from **Figure 4(b)**) that Mn content in the leaves of each Cu^{2+} treatment group was significantly lower than that in the roots, so Cu^{2+} treatment significantly impeded the transport of Mn in *S. sagittifolia*.

Zn can participate in the synthesis of indole acetic acid (IAA). The lack of Zn in plants will impede the synthesis of IAA, which will ultimately hinder the growth of young leaves of the plants [15]. In this experiment, the Cu^{2+} treatment exerted a more evident effect on Zn content in the leaves of *S. sagittifolia* than that in the roots. It indicated that the Zn content in leaves was very sensitive to the Cu^{2+} treatment. Even a slightly high concentration of Cu^{2+} (>5 $\mu mol \cdot L^{-1}$) would cause the lack of Zn in leaves, which affected the normal growth of leaves finally (**Figure 4(d)**).

Na can increase turgor pressure of plant cells and promote plants growth. It can also partially play the role of K in enhancing osmotic potential of cell sap [15]. The decrease in Na content reduced the cell turgor pressure and the osmotic potential of cell sap, which inhibited the transport of Na from the roots to the leaves. So in each Cu^{2+} treatment group, the Na content in the leaves of *S. sagittifolia* was lower than that in the roots (**Figure 3(d)** and **Figure 4(d)**). And the Cu^{2+} treatments of high concentrations (20 $\mu mol \cdot L^{-1}$ and 40 $\mu mol \cdot L^{-1}$) made Na contents in the leaves significantly lower than that in the control group (**Figure 4(d)**), which was attributed to the disorder of physiological metabolism induced by exorbitant Cu^{2+} concentration in *S. sagittifolia* [8].

In summary, exogenous application Cu^{2+} broke the balance of various mineral elements in roots and leaves of *S. sagittifolia*, which would exert great impact on a number of metabolic pathways and physiological processes. From the experimental results, we could draw the following conclusions:

1) *S. sagittifolia* primarily stored excess Cu in roots, which was an important mechanism for *S. sagittifolia* to tolerate Cu^{2+} stress. The specific mechanism of action of *S. sagittifolia* to Cu^{2+} treatments remained to be further

studied;

2) In roots, Cu^{2+} treatments inhibited the absorption of various mineral elements to different degrees except Fe. And the contents of P, Zn and Na had a significant negative correlation with the concentration of Cu^{2+} treatment;

3) Cu^{2+} treatment impeded the transport of Ca, Fe, Mn, Zn and Na from roots to leaves. In leaves, only the Mn content and the concentration of Cu^{2+} treatment showed a significant negative correlation;

4) The critical concentration of Cu^{2+} to *S. sagittifolia* was 5 $\mu mol \cdot L^{-1}$.

Our experiment indicated that Cu, which was an essential microelement for plant growth, was strongly phytotoxic at high concentration to *S. sagittifolia*. The research provided theoretical basis for changes of essential mineral elements contents in response to Cu^{2+} treatment in *Sagittaria sagittifolia*. Further studies considering specific effects of Cu^{2+} on uptake and transport of essential mineral elements are required.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (NO. 31300324) and the Natural Sciences Research Project of Higher Learning Institution in Jiangsu Province (NO. 13KJB180028).

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