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BIOACCUMULATIONS OF CU AND ZN IN THE LOCAL EDIBLE ULAM *CENTELLA ASIATICA*

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Abstract: The objectives of this study were to determine the accumulations of Cu and Zn in *Centella asiatica* collected from 13 sampling sites in Peninsular Malaysia and the interactions of Cu and Zn in *C. asiatica* in different Zn/Cu ratios. The plants were collected (or bought) from 13 sampling sites in 2010. An experiment was carried out under laboratory conditions using Hoagland solution within the range of 2-6ppm for Zn and 0.1-0.5ppm for Cu. Cu and Zn accumulations were determined in the leaves and roots after 20 days. Generally, roots showed the highest accumulations of Cu and Zn compared to leaves. The treatment of Zn with Cu (Zn:Cu ratio 6:0.5) exposure significantly reduced the accumulation of Zn in leaves but significantly increased uptake in roots when higher Cu concentration was added (Zn:Cu ratio 6:0.5). Cu accumulation was increased with Zn added in leaves, but no significant difference was observed in roots. In conclusion, the accumulations of Zn and Cu in roots and leaves of *centella* were dependent on Cu and Zn availabilities in the medium.

KEYWORDS: Accumulation, Copper, Safe consumption, Zinc

Introduction

Malaysia has well-developed industrial areas with massive production of electronic products. Industries such as electronics, textiles, food processing and rubber-based industries contribute to the Zn contamination in the environment (Alkarkhi *et al.*, 2009). Besides that, agricultural practice also increases the Zn concentration in the environment. The use of fungicides and fertilisers containing organo-zinc could have caused the excess to leach into the soil (WHO, 2001). In polluted sites, Zn concentrations in the range of 150-300µg/g had been reported (Warne *et al.*, 2008). Around 30 mg/kg of Zn is adequate for plant growth and 300 to 500 mg/kg of Zn is considered toxic to plants (Miransari, 2011).

Cu naturally exists in the environment but excess Cu in the environment is contributed by anthropogenic emissions such as from smelters, iron foundries and combustion sources. WHO (2001) reported that 2% of Cu was released into the soil in agricultural sites due to excessive usage of agricultural products such as fertilisers, bactericides, fungicides and algicides. Cu is required in small amounts, namely 5-20 mg/kg in plant tissues, adequate for normal metabolism and growth (Thomas *et al.*, 1998).

Extract of *asiatica* species from the genus *Centella* is the only one from this genus to be found in commercial drugs today (Zainol *et al.*, 2003). Currently, the World Health Organisation (WHO) has acknowledged *C. asiatica* as one of the most important medicinal plant species to be conserved. There are reports on the toxicity effects of Cu or Zn alone on the growth of plants (Rout and Das, 2009) but there is lack of information about the interactions of Cu and Zn in plant-toxicity accumulation. It is a common phenomenon in nature that pollution of trace elements can occur either additively, synergistically or antagonistically (Wu *et al.*, 1995). The objective of this study was to determine the accumulations of Cu and Zn in leaves and roots of *C. asiatica* grown under hydroponic conditions.

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Methodology

Sample collection

The samples were collected from 13 sites between May and June of 2010. The samples collected were of commercial maturity (2-4 months) and the sampling sites were Permatang Pauh (PPauh), Karangan, Kluang, Butterworth, Universiti Putra Malaysia (UPM), Kapar, Seremban, Malacca, Cameron Highland (C. Highland), Bukit Mertajam (BM), Kampung Simpang Renggam (KSR), Kuala Lumpur (KL) and Pontian (Figure 1). Whole plants were collected from the sampling sites and sealed in a plastic bag for transportation to the laboratory. In total, three replicates were done at each sampling site.

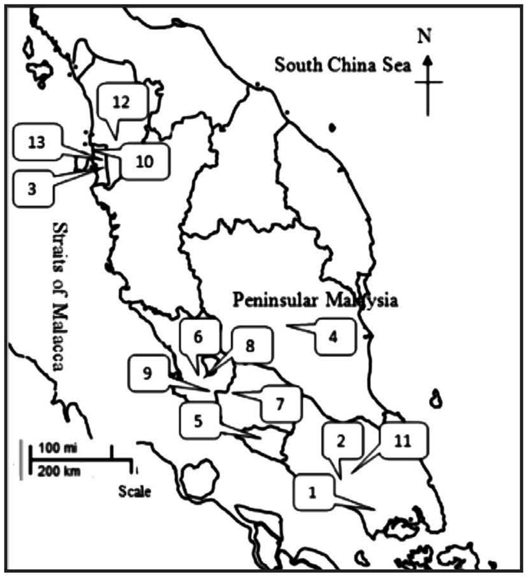
Experimental design

Young stems with 5-8 leaves of *Centella* plucked from the University Agricutural Park (Taman Pertanian Universiti, TPU), Universiti Putra Malaysia were planted in a greenhouse in hydroponic solution (Hoagland solution) in completely randomised block design (Tang *et al.*, 2009). Growth in hydroponic solution was used to easily control and prevent competition with weeds. The plants were acclimated in the eight times hydroponic solution for one week before testing with Zn and Cu (Tang *et al.*, 2009). Zn and Cu were supplemented into a medium solution based on the phytotoxicities and tolerances of plants to heavy metals as suggested by Kopittke *et al.*, (2009). Zn was applied at 2 ppm (Zn1); 4ppm, (Zn2) and 6ppm (zn3) while Cu was at 0.1ppm (Cu1); 0.3ppm (Cu2) and 0.5ppm (Cu3). The interactions between Zn and Cu were in combination of Zn1+Cu1 (ZnCu1), Zn2+ Cu2 (ZnCu2) and Zn3+Cu3 (ZnCu3). The plants were allowed to grow in the solution for 20 days under greenhouse conditions. During that period, the solutions were changed at day 10 to prevent the depletion of nutrients and metals. The nutrient solutions were adjusted to pH 5.8 with 0.1M NaOH and 1M HCl. At harvest, the roots were immersed into 20 mmoll^{-1} Na_2-EDTA for 15min to remove the metals adhering to the root surface. The shoots and roots were separated and thoroughly washed three times with de-ionised water.

Cu and Zn analysis

The plants were separated into leaves and roots and dried in an oven for 72 hours at 60 ^{0}C until constant dry weight. About 0.5g of dried plant tissues were ground into powder form and digested with 10ml of concentrated nitric acid (AnalaR grade, BDH 69%) in a digestion tube. The digestion tubes were placed in a hot block digester at 40°C for 1 hour and at 140°C for at least 3 hours (Ong *et al.*, 2011). The digested samples were left to cool down and 40ml dH_2O was added. Subsequently, the solution was filtered through Whatman No. 1 filter paper into an acid-washed pill box and stored in a safe place until analysis. Three replicates were done for each sample.

Zn and Cu were analysed using an air-acetylene Perkin-Elmer™ flame atomic absorption spectrophotometer model A Analyst 800 in the Biology Department, Universiti Putra Malaysia. Blank determination was carried out for calibration of the instrument. The recovery percentage of the certified reference material



No	*Sampling sites*
1.	Pontian, Johore
2.	Kampung Simpang Renggam (KSR), Johore
3.	Bukit Mertajam (BM) , Penang
4.	Cameron Highland (C. Highland), Pahang
5.	Malacca
6.	Kuala Lumpur (KL), Selangor
7.	Seremban, Sembilan
8.	Kapar, Selangor
9.	Universiti Putra Malaysia (UPM), Selangor
10.	Butterworth, Penang
11.	Kluang, Johore
12.	Karangan, Kedah
13.	Permatang Pauh (PPauh), Penang

Figure 1: Map of sampling sites for *Centella asiatica* in Peninsular Malaysia.

(CRM) of Soils NCS DC 7336 ranged 78.96-88.36 (Table 1).

Table 1: Recovery percentage of certified reference material (CRM) of Soils NCS DC 7336.

Metals	CRM values	Measured values	Recovery (%)
Cu	24.3 ± 1.2	19.186774 ± 0.73	78.96
Zn	68 ± 4	60.08392 ± 6.17	88.36

Statistical analysis

The STATISTICA version 8 software was used to determine the correlation coefficient and for hierarchical cluster analysis. The analysis of variance (ANOVA) and Student-Newman-Keuls (SNK) were done using the SPSS software version 17.0 for Windows to find the differences between the means of heavy-metal concentrations in the different parts of the plants from the different sites (Zar, 1996).

Results

Zn and Cu accumulations in leaves and roots

Figure 2 shows the accumulations of both Zn and Cu in roots were significantly ($P<0.05$) higher than in leaves. Comparatively, Zn was highly

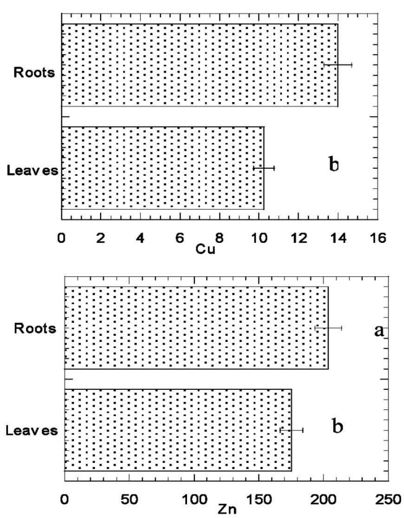


Figure 2: Mean concentrations (mean ± SD, µg/g dry weight) of Cu and Zn in leaves and roots of *Centella asiatica* collected from 13 sampling sites in Peninsular Malaysia.
Note: a,b: different alphabets in each column show the different significant means (SNK test, $P<0.05$).

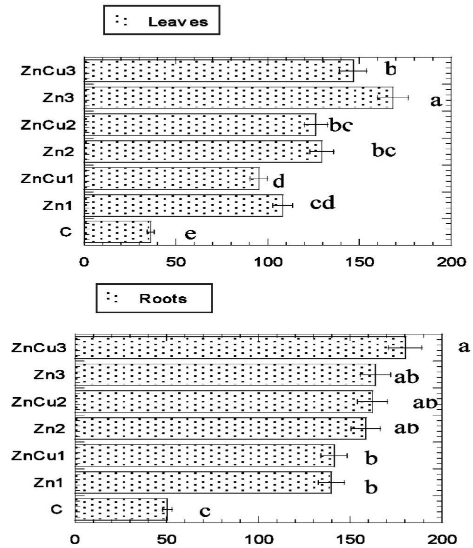


Figure 3: Mean concentrations (mean ± SD, µg/g dry weight) of Zn in leaves and roots for toxicity testing (N=3) after 20 days of being grown under hydroponic condition.
Note: a,b,c: different alphabets in each column show the different significant means (SNK test, $P<0.05$).

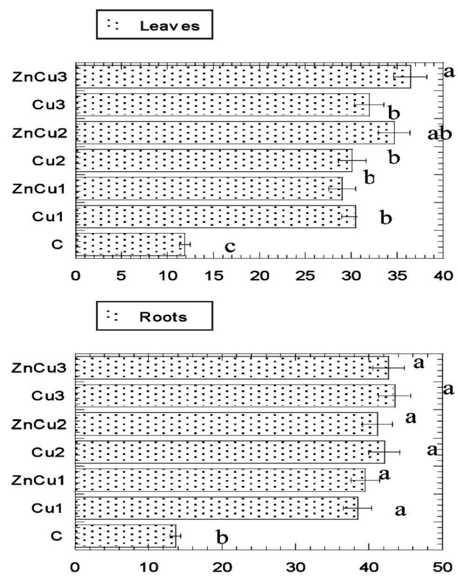


Figure 4: Mean concentrations (mean ± SD, µg/g dry weight) of Cu in leaves and roots for toxicity testing (N=3) after 20 days of being grown under hydroponic condition.
Note: a,b,c: different alphabets in each column show the different significant means (SNK test, $P<0.05$).

accumulated both in leaves (175.25 ±70.84 µg/g) and roots (203.75 ± 69.80 µg/g) compared to Cu in leaves (10.25 ± 2.78µg/g) and roots (13.99 ±3.77 µg/g).

Interaction of Cu and Zn

Figure 3 shows the Zn accumulation in leaves and roots of *Centella* when Cu was added. In leaves, overall Zn accumulation decreased when Cu was added but only ZnCu3 with the ratio of Zn:Cu of 6:0.5 showed significant ($P<0.05$) decrease. For Zn accumulation in roots, overall it showed an increased amount of accumulation when Cu was added but with no significant difference.

Figure 4 shows Cu accumulation with addition of Zn. In leaves, Cu accumulation increased when Zn was added. However, Cu accumulation showed significant ($P<0.05$) increase for ZnCu3 with the Zn:Cu ratio of 6: 0.5. For Cu accumulation in roots, although there were increase of Cu for ZnCu1 and decrease of Cu for ZnCu2 and ZnCu3, all had no significant difference.

Discussion

The accumulations in roots were higher when compared to leaves for both Cu (37%) and Zn (16%). Roots play a role as the site of water and nutrient uptake of plants by osmosis; therefore the entire metal uptake by the plant must pass through the roots before reaching the other parts (Clemens *et al.*, 2002). Hence, the excess metals that are not further transported upwards by the plant will be accumulated in the roots. The high accumulation of heavy metals in roots can also be the result of complexation of heavy metals with sulphydryl groups causing lesser translocation of metals to the shoots (Singh and Sinh, 2005). Based on the results of Arslan *et al.* (2010), the contents of the heavy metals Cd^{2+}, Cr^{3+}, Cu^{2+}, Fe^{3+}, Ni^{2+}, Pb^{2+}, and Zn^{2+} in roots were higher compared to leaves of *Verbascum bombyciferum*.

There was also metal accumulation in leaves because normally the nutrient uptake of the plant is transported to leaves and stored in them for further metabolic activities. The carbon metabolic pathways in leaves such as the photosynthetic carbon-reduction cycle and trehalose biosynthesis had provided insights into the linkages between metabolism and development (Raines and Paul, 2006). The metals were concentrated in the leaves because Zn and Cu play important roles in photosynthesis (Sawidis *et al.*, 1995). This deduction was supported by the results of Street *et al.* (2009) who found that plant roots accumulate more metals than shoots. Zn concentration in *C. asiatica* was higher when compared to Cu concentration. Zn had a higher level of accumulation because Zn plays an essential metabolic role in the plant. It is a component of several enzymes, such as dehydrogenases, proteinases, peptidases and phospohydrolases (Marschner, 1995). Owing to the relative immobility of Cu, the transfer rate of Cu was slower (Zheljazkov *et al.*, 2006) even though Cu is required as a micro-nutrient. Besides that, the lower uptake of Cu in roots might be due to competition with other essential mineral nutrients such as Mn, B, Cl, Zn and Mo. The presence of one metal influences the uptake of another metal (Peralta-Videa *et al.* (2002). Furthermore, the phytotoxicity of the trace metals was also the reason for the lower Cu accumulation where Cu was more toxic than Zn (Kopittke *et al.*, 2009).

Cu and Zn concentrations were both higher in roots than in leaves because roots are the first organ to come in contact with the metals (Singh and Sinha, 2005; Tang *et al.*, 2009). The continuous adhering of roots to soil increases their exposure to the metals. Given this, the chances of metal accumulation in roots were increased. Moreover, the large surface area of roots due to the root hairs elevates the adsorption and absorption of metals and facilitate nutrient uptake (Ong *et al.*, 2011).

The presence of Cu in the culture medium decreased the Zn accumulation in centella leaves (Figure 3). This might be due to the competition of Zn with Cu. A high concentration of Cu was reported to inhibit the rates of Zn uptake (Sunda and Huntsman, 1998). In the present study, the concentration of Zn was lower in leaves; Cu would possibly inhibit the accumulation of Zn (Ong *et al.*, 2011). Furthermore, Cu and Zn are competing for the same binding sites since both metals have similar properties (Alloway, 2008). Therefore, Cu reduced the Zn concentration in leaves when higher concentrations of Cu were added.

However, the trend of Zn accumulation in roots was different from leaves whereby the Zn accumulation increased in Cu-added medium. The significant increase of Zn concentration by addition of Cu was in the Zn:Cu ratio of 6:0.5. The effect of Cu to the uptake of Zn was to increase when high Cu was added to root. This showed that Cu had synergetic effects on Zn (Tani and Barrington, 2005) in the roots. The root-internal metals in the higher concentrated mixtures had strong synergistic effects (Sharma *et al.* (1999). Hence, the accumulation of Zn in roots of ZnCu3 was higher (180.05μg/g) compared to in Zn3 (163.85μg/g) (Figure 3). Differences of the combined toxicity of metals in plants might be attributed to the mobility of metals from roots to shoots. The heavy metals tend to be retained in root tissues. The slower transfer rate of Cu might be due to the immobility of Cu (Zheljazkov *et al.*, 2006). Zn can interact with other trace elements, especially Cu, resulting in toxicity, which usually causes the depletion of the element to a lower concentration leading to its nutritional deficiency.

Conclusion

In conclusion, roots showed higher accumulations of Cu and Zn when compared to leaves. The accumulations of Zn and Cu in roots and leaves were dependent on the levels of metal exposures. The treatment of Zn with Cu exposure reduced the accumulation of Zn in leaves but increased the Zn uptake in roots when a higher level of Cu was added. Cu accumulation increased with Zn addition in leaves but did not show significant changes in roots.

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