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Effects of Cu and Zn Supplementation on Metal Uptake by *Hibiscus sabdariffa*

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Abstract

Pot experiment was carried out in order to assess the addition effects of copper (Cu) and zinc (Zn) on their distribution and those of other metals in plant tissues. The experiment consisted of growth Hibiscus sabdariffa, a leafy vegetable, for 30 days in a soil to which copper and zinc were added alone and in combination. At the end of the experiment roots, stems and leaves of plants were analyzed for metal uptake in tissues. The results show that the Cu and Zn contents in the plant tissues varied with their single and combined additions in soil. The Cu and Zn accumulation in plant tissues was as follows roots > stems > Leaves. An antagonist or synergic effect observed in root and stem tissues according to Cu or Zn was added in soil showed that Zn and Cu uptake in plant seemed to be controlled by the concentration of both metals in soil. Generally the Cu and Zn addition in soil had antagonistic effects with Cd, Cr, Fe, Mn, Ni and Pb in root and stem tissues. In opposite, the synergic effect observed with Cr, Fe and Mn uptake in leave tissues led to the conclusion that Cu and Zn can help to have metal deficiency decrease in chain food.

Keywords: Supplementation, copper, zinc, *Hibiscus sabdariffa*, interactions.

Introduction

Micronutrient malnutrition is widespread in the industrialized nations, but even more in the developing regions of the world. It can affect all age groups, but young children and women of reproductive age tend to be among those most at risk of developing micronutrient deficiencies[1]. The growth of urban agriculture in developing countries is one of main solutions to these deficiencies. Soil fertility in urban vegetable gardens has been investigated throughout the world. Urban soil surfaces receive deposits issued from different sources such as vehicle emissions, factory activities, anthropogenic wastes, industrial discharges, and other anthropogenic activities through atmospheric transport as well as from local human activities[2-7]. Many researchers noted the increase of metal elements in soils from these sources. The metals such as copper (Cu), zinc (Zn), iron (Fe) have essential functions in plants, animals and human[2-3,8]. Others such as cadmium (Cd), lead (Pb) perform no known essential function in living[2,9].

Copper (Cu) is an essential trace element found in small amounts in a variety of cells and tissues with the highest concentrations in the liver[10-11]. The Cu deficiency is now recognized to be a common problem in many domesticated and wild animals, and marginal Cu deficiency is a problem in some human populations[12]. Cu deprivation in animals contributes to instability of heart rhythm, hyperlipidemia, increased thrombosis, breakdown of vascular tissue, cardiac lesions, cardiac hypertrophy, and altered arterial function. Much of the pathology due to Cu deficiency is thought to be associated with increased oxidative stress, which, in turn, may increase low density lipoproteins susceptibility to oxidation[13]. Conversely, Cu toxicity, typically due to genetic disorders, can also be a significant health concern[12].

Zinc (Zn) is well known to be essential for somatic growth of children[14]. Zinc constitutes about 33 µg/g of an adult body mass and it is essential as a constituent of many enzymes involved in several physiological functions, such as protein synthesis and energy metabolism[15-16]. Zinc has a close relationship with the endocrine system; it sustains normal growth, secondary sex characteristics, reproductive function and thyroid function. Therefore, Zn deficiency causes not only growth retardation, but also delays sexual maturation, hypogonadism, and thyroid dysfunction[14]. A pilot study indicated that Zn supplementation is a practical possibility comparable to that of other metal supplementation such as Fe in order to prevent marginal Zn deficiency in vulnerable groups[17].

In practice, where two nutrients like Cu and Zn are deficient, like in South Australia, supply of both or of the one, relatively shorter supply will increase yield, often greatly relative to cost. However, to add only the one less deficient will give no benefit and may decrease yield below what it would have been if nothing were done. Similarly, when only one micronutrient cation is deficient, the effect of supply of a second is mainly one of antagonism if the abundance in the soil of the second element is much better. It follows from these two cases that for maximal growth rate, all nutrients must be supplied at near optimal levels or yield, will be lost, that loss will be accelerate if another nutrient approaches high concentrations[18]. If a deficiency of Cu or Zn constitutes a hazard for human health, these two elements should be provided to human beings as part of their normal nutritional intake. The mineral elements uptake by plants has been accepted as safe and effective means for metal intake by human. A supplement of Cu and Zn in vegetables could be therefore a convenient and easy method in agriculture to improve trace elements nutrition for human.

The aim of this study was to assess effects of addition of copper and/or zinc in soil metal uptake in different parts of common leafy vegetables. A pot experiment was conducted on the Roselle or *Hibiscus sabdariffa* with addition of different concentrations of Cu and Zn in soils.

Material and Methods

Pot experiment on *Hibiscus sabdariffa:* The soil used was a sandy loam from the A horizon (0-15 cm depth) from soil near an urban garden area of Libreville city. Its pH (water, 1 : 2.5 soil : water ratio) was 7.6, and it had a carbon content of 39.6 mg/kg and cation exchange capacity of 13.6 meq/100 g. The total metals concentrations were 0.62; 167; 31; 108,301; 367; 13; 108 and 163 mg/kg of Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn, respectively.

The experiment was carried out with 36 pots containing each 3.2 kg of dry soil. The metal additions were made by adding appropriate amounts of Zn sulfate and Cu sulfate as solid powdered. The concentrations added in soil were: 10 and 110 mg/kg for Zn and 6 and 60 mg/kg for Cu. For each element pots did not receive any metal and were used as controls. There was 9 metal treatments in a complete factorial design as treatments Cu/Zn: T0 (0/0), T1 (0/10), T2 (0/110), T3 (6/0), T4 (6/10), T5 (6/110), T6 (60/0), T7 (60/10) and T8 (60/110). A basal application of fertilizer (22.4 g KH_2PO_4 and 8.6 g NH_4Cl) was also made at the same time. Deionised water was added to bring the samples to field capacity. The quantities of metal added, in the treatments described above, were intended to be weak enough to have a possibility of causing phytotoxicity, as their concentrations were always below the limits set as the maximum permissible concentrations in agricultural soils.

Hibiscus sabdariffa was chosen as the potted vegetable because, it is one of staple leafy crops in Africa diet and available all year. It was sown in a rate of 12 seeds per pot. These were allowed to germinate and establish for 10 days in an ambient environment, in an open shade structure. After this time, young plants were removed and transferred into the pots, each containing four plants. There were four replicates of each treatment and the pots were placed in four blocks into an open shade structure. Each block contained one pot of each treatment arranged randomly within the block. The pots were watered regularly with deionised water to keep them close to field capacity. The experiment was finished 30 days after sowing and the plants were uprooted. The sample vegetables were firstly washed three times with distilled water, and secondly with deionized water. Roots, stems and leaves were separated. They were dried at 70°C in a drying oven until their weight was constant, their roots, leaves and stems were subsequently separated and kept in polyethylene bags.

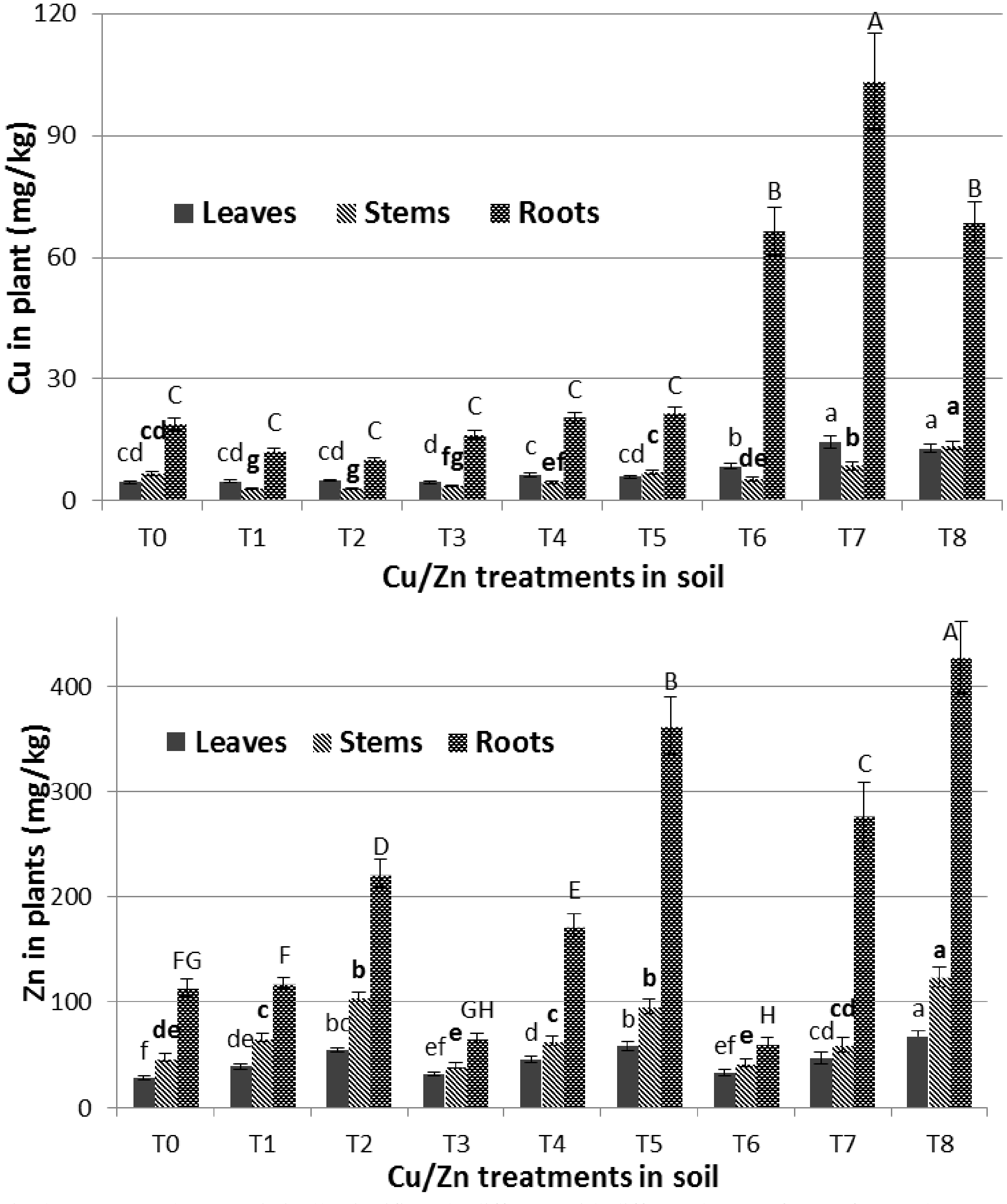
Metal concentrations in tissues of *Hibiscus sabdariffa:* Plant samples were digested at 150°C for 1 hour in a microwave mineralizer, using a mixture of nitric acid, hydrogen peroxide and ultra-pure water with a volume proportion ratio of 2:1:1 as described elsewhere[19]. The resulting solution was filtered at 0.45μm and stored at 4°C before the ICP-AES analysis in order to determine concentrations of Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn.

Quality control: Appropriate quality assurance procedures and precautions were carried out to ensure reliability of the results. Double distilled deionized water was used throughout the study. Reagents blank determinations were used to correct the instrument of readings. The detection limits (DL) were determined for the standard plant reference materials (DC 73349) from China National Analysis Center for Iron and Steel (NSC). Blank and drift standards were run after ten determinations to maintain instrument calibration. The coefficient of variation of replicate analyses was determined for the measurements to calculate analytical precision.

Statistical methods: The mean, standard error and mean comparison test were carried out. Two-way analysis of variance (ANOVA) was used to evaluate the effects of Cu and/or Zn supplementation in soil and uptake metals by the tissues of plant. XLSTAT 2010, 6.04 version software was used to perform statistical analyses.

Results and Discussion

Cu and Zn concentrations in *Hibiscus sabdariffa* tissues: The soil doping effect by Cu and Zn in the experiment could be examined by the content of these two elements in the tissues of cultivated *Hibiscus sabdariffa* samples. The concentrations of Cu and Zn in the leaves, stems and roots of *Hibiscus sabdariffa* grown at different treatments are presented in figure 1. The results show that the Cu and Zn contents in the plant tissues varied with their single and combined supplementations in soil. Thus, the single Cu supplementation in soil had a significant effect on Cu accumulation in leaves and stems ($p < 0.0001$, respectively) and Cu and Zn in roots ($p < 0.0001$, respectively); the single Zn supplementation in soil had a significant effect on Zn accumulation in leaves ($p < 0.0001$) and Cu and Zn in stems and roots ($p < 0.0001$, respectively) the combined Cu/Zn supplementation in soil had a significant effect on Cu accumulation in leaves ($p < 0.0001$), Cu and Zn accumulation in stems ($p < 0.0001$ and $p < 0.001$, respectively) and Cu and Zn accumulation in roots ($p < 0.0001$, respectively). The antagonistic effect of Cu and Zn on plant growth has been well documented[20-21]. These metals seemed absorbed by the same mechanism and therefore, each may competitively inhibit root absorption of the other[20]. The Cu additions decreased significantly Zn uptake in roots. Similar results were obtained by others while studied metal uptake in roots of *Commelina communis* treated with Cu nutrient solutions[22]. Zn additions increased significantly Cu uptake in stems and roots. A Cu/Zn synergy effect is therefore possible for metal uptake in plants. Synergism between Cu and Zn has also been shown by others in the literature[23]. Thus Zn and Cu uptake in plant appeared to be controlled by the concentration of both metals in soil.



Columns in the same graph are statistically significantly different with different letters of same font at the $p < 0.05$ level.

Figure-1

Cu and Zn content in tissues of *Hibiscus sabdariffa* for nine treatments of the pot experiment

Accumulation of Cu and Zn was significantly higher in roots than in leaves and stems. From the data in figure 1 it could be noticed that addition of Cu and Zn enhanced the Cu content until 208%, 106% and 446% in leaves, stems and roots, respectively, and enhanced the Zn content until 145%, 161% and 275% in leaves, stems and roots, respectively. These results suggest that supplementation of Cu and Zn in soil had an obvious effect on the enrichment of Cu and Zn in *Hibiscus sabdariffa*, and that the roots had better enrichment effect than leaves and stems. There is a continuing interest in supplementation of nutrients deficiency. The present study showed that *Hibiscus sabdariffa* plant can accumulate higher concentrations of Cu and Zn if the cultivated soil contains moderately higher Cu and Zn levels. The results also suggest that the uptake of Cu and Zn by *Hibiscus sabdariffa* was impacted not only by the individual Cu or Zn applications, but also by their combinations. The highest Cu enrichment effects were observed in leaves and roots for T6, T7 and T8 additions, and in stems for T7 and T8 additions, whilst the highest Zn enrichment effects were observed in all plant tissues for T8.

Cu and Zn addition effects on Cd, Cr, Fe, Mn, Ni and Pb uptake in *Hibiscus sabdariffa:* The results showed that Cu and Zn additions in soil improved the uptake of both metals in plants. The content of other metals in *Hibiscus sabdariffa* was examined. The levels of Cd, Cr, Fe, Mn, Ni and Pb in plant tissues and their dependence by Cu and Zn addition in soil (ANOVA) are presented in table 1.

The accumulation of all metals in the roots tissues decreased for all treatments compared to control, exception of Ni in T7 and Pb in T5 with an increase of 41.1% and 39.5%, respectively over control (T0). Pb contents in root tissues remained statistically unchanged up to the T5 treatment. The accumulation of Cd, Cr, Fe, Mn, Ni and Pb in root tissues decreased until a maximum of 59.8%, 66.6%, 58.8%, 82.3%, 62.5% and 50.0%, respectively at T4 compared to T0. Therefore, the results in table 1 shown that supplementation of Cu or Zn had antagonistic effects against accumulation of Cd, Cr, Fe and Mn, combined effect (synergy/antagonism) with Ni and Pb accumulation in the roots.

In the stems, Cd, Cr, Fe, Mn and Ni concentrations decreased for all treatments compared to control, exception of the treatments with the highest supplementation of Zn, there was no significant difference. Pb concentration was not detectable in these tissues. The accumulation of Cd, Cr, Fe, Mn and Ni in stem tissues decreased until a maximum of 65.0%, 62.7% and 70.2%, respectively for Cd, Mn and Ni at T6, and until a maximum of 83.4% and 85.7%, respectively for Cr and Fe at T4. Generally the concentrations of each metal at T4 and T6 were not significant in stem tissues. To note that supplementation of Cu or Zn had generally antagonistic effects against accumulation of studied metals in stems as shown table 1.

Table-1
Cd, Cr, Fe, Mn, Ni and Pb content in tissues of *Hibiscus sabdariffa* for nine treatments of the pot experiment (mg/kg; dry weight)

		T0	T1	T2	T3	T4	T5	T6	T7	T8
Leaves	Cd	0,41c	0,43c	0,65ab	0,50abc	0,66a	0,45c	0,49bc	0,49bc	0,53abc
	Cr	0,60c	1,22a	0,89b	0,62c	0,48c	0,49c	0,91b	1,07a	0,49c
	Fe	244cd	315b	321b	220d	258bcd	296bc	291bc	435a	395a
	Mn	80c	42e	56de	91bc	104b	49de	134a	87bc	63d
	Ni	0,55a	0,61a	0,54ab	0,45bc	0,45bc	0,35cd	0,59a	0,62a	0,29d
	Pb	< 2.36	< 2.36	< 2.36	< 2.36	< 2.36	< 2.36	< 2.36	< 2.36	< 2.36
Stems	Cd	0,55a	0,39cd	0,49ab	0,35d	0,33d	0,55a	< 0.30	0,45bc	0,56a
	Cr	2,29a	0,48de	1,11c	1,26c	0,38de	1,05c	0,53de	0,69d	1,97b
	Fe	337±25a	137c	63de	87d	48e	134c	54e	76de	171b
	Mn	50a	25c	22c	21c	20c	19c	36b	41b	41b
	Ni	1,26a	0,46de	0,68c	0,50d	0,50de	0,43de	0,38e	0,44de	0,89b
	Pb	< 2.36	< 2.36	< 2.36	< 2.36	< 2.36	< 2.36	< 2.36	< 2.36	< 2.36
Roots	Cd	1,76a	1,15cd	0,63g	0,97de	0,71fg	1,52b	0,86ef	1,21c	1,13cd
	Cr	9,79a	6,09bc	3,75de	4,74cd	3,27e	6,99b	4,22de	10,00a	7,08b
	Fe	4604a	3403bc	1651g	2374ef	1896fg	2567de	2826cde	3936b	3134cd
	Mn	164a	46de	25f	34ef	28f	64c	54cd	104b	55cd
	Ni	3,20b	1,78c	1,50cd	1,37cd	1,20d	2,69b	1,45cd	4,52a	2,78b
	Pb	3,80bc	3,72bc	2,73d	2,41d	< 2.36	6,21a	3,62c	4,45b	3,09cd

Values with different letters in same line are significantly different ($p < 0.05$).

The changes in the leaves tissues were more contrasted over control (T0). The accumulation of Cd, Cr, Fe and Mn increased up to 38.0%, 50.5%, 44.1% and 40.4%, respectively. The accumulation of Mn and Ni decreased with a maximum decrease of 48.1% and 47.5%, respectively. Generally, the changes occurred for the highest Zn supplementation, combined (in the case of Cd, Fe and Ni) or not (Cr, Mn decrease and Ni) with the highest Cu supplementation. The highest Mn accumulation in the leaves tissues occurred for the highest alone Cu supplementation. Supplementation of Cu or Zn had synergic effects with accumulation of Cd, Cr and Fe, antagonism effects against Ni accumulation, and synergic/antagonism effects with Mn accumulation in leaves of *Hibiscus sabdariffa*. But these effects were no significant in many cases as shown table 1.

The only Cu or Zn addition showed an antagonist effect between the metal and Cd, Cr, Fe, Mn, Ni and Pb uptake in all parts of plants, exception to these metals in leaves and Cu addition in soil which generally presented a synergic effect. Furthermore, the combined addition of Cu and Zn in soil led in some cases to the increase of Cr (treatment T7), Mn (treatments T4 and T7) and Fe (treatments T4, T6, T7 and T8). These results are particularly important because leaves are the edible part of *Hibiscus sabdariffa*. Indeed, the addition of Cu, Zn or Cu/Zn combination could not only increase Cu and Zn intake but also of other nutrients such as Cr, Mn and Fe. Chromium is slightly available to plants and not easily translocated within plants, thus it is bonded strongly to soil solids and concentrated mainly in roots, apparently because of the propensity of Cr^{3+} to bind to cell walls[20, 24]. Iron deficiency is the most common and widespread nutritional disorder in the world. As well as affecting a large number of children and women in developing countries, it is the main nutrient deficiency which is also significantly prevalent in industrialized countries. The numbers are staggering: 2 billion people – over 30% of the world's population – are anemic, many due to a deficiency of iron supply. An iron-rich diet is an inexpensive and effective solution to reduce the consequences of iron deficiency and anemia such as death rates, maternal hemorrhage, reduced school performance and lowered productivity[25]. Mn deficiencies are common on the calcareous soils, sandy soils and during episodes of over-liming in a range of soils[26-27]. Manganese deficiencies have been studied in animals, and the symptoms vary, including skeletal abnormalities, postural defects, impaired growth, impaired reproductive function, and disturbances in lipid and carbohydrate metabolism[28].

Conclusion

In this study, the concentration of Cu and Zn in the tissues of *Hibiscus sabdariffa* increased significantly with supplementation of these metals. The concentrations of other elements, such as Cd, Cr, Fe, Mn, Ni and Pb mostly decreased in roots, stems with Cu and Zn being supplemented, but showed concentration increase in leaves, edible part of *Hibiscus sabdariffa*, for many treatments. The combined addition of Cu and Zn in soils resulted in a significant increasing concentration in *Hibiscus sabdariffa* leaves of Cr, Fe and Mn, particularly when Cu supplementation is more important than Zn supplementation. The supplementation of Cu and Zn in agricultural soils could be an easy and efficient way to improve trace elements nutrition in vegetables. This result will be confirmed in the future with a farmland experiment.

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