Pot experiment of the uptake of metals by Amaranthus cruentus grown in artificially doped soils by copper and zinc

Jean Aubin ONDO*, Richard MENYE BIYOGO, Aimé-Jhustelin ABOGO MEBALE, François EBA

Laboratoire pluridisciplinaire des sciences de l'Ecole Normale Supérieure, B.P 17009 Libreville Gabon

* E-mail of the corresponding author: laplus_ens@yahoo.fr

Abstract

The supplementation of metals is a mean used to ameliorate the nutritional quality of vegetables. Pot experiment was carried to evaluate the addition effects of copper (Cu) and zinc (Zn) in soils on their distribution and those of other metals in plant tissues. The experiment consisted of growth *Amaranthus cruentus*, a leafy vegetable, for 30 days in a soil to which copper and zinc were added singly 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 accumulation of Cu and Zn was significantly higher in roots than in leaves and stems ($p < 0.0001$). There were no significant difference between Cu and Zn uptake in leaves and roots. The supplementation of Cu and Zn in soil had an obvious effect on the enrichment of Cu and Zn in *Amaranthus cruentus*, the leaves had better enrichment effect of Cu and the roots had better enrichment effect of Zn. Furthermore, the enrichment effect of Zn in leaves was also important (until 237%). The present study showed that *Amaranthus cruentus* plant can accumulate higher concentrations of Cu and Zn if the cultivated soil contains moderately higher Cu and Zn levels. Antagonist effects were observed between Cu and Zn supplementation and other metals in roots. In stems, it was synergic effects which were observed. Many synergic and antagonist effects between Cu and Zn added in soil and other metal transfer in leaves were often observed.

Keywords: pot experiment; copper; zinc; Amaranthus cruentus; metal uptake

1. Introduction

Evaluation of micronutrients and essential trace elements concentrations in vegetables is a important topic in the world. Trace elements play an important role in the metabolic regulations of the human body.

Copper (Cu) is an essential element widely distributed and always present in food. It is necessary for normal biological activities of amino-oxides and tyrosinase enzymes (Hashmi et al., 2007), it is required as cofactor in different oxidative and reductive enzymes (Ismail et al., 2011). A low uptake of Cu in human consumption can cause a number of symptoms e.g. growth retardation, skin ailments, gastrointestinal disorders etc. 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 (Turley et al., 2000). Conversely, Cu toxicity, typically due to genetic disorders, can also be a significant health concern (Uriu-Adams, 2005).

Zinc (Zn) is well known to be essential for somatic growth of children (Kaji and Nishi, 2006). It is essential as a constituent of many enzymes involved in several physiological functions, such as protein synthesis and energy metabolism (Jalbani et al., 2010; Onianwa et al., 2001). For example, Zinc is required for the proper function of 1,5′-deiodinase, the enzyme required for the conversion of thyroxine to the more active form, triiodothyronine (Kandhro et al., 2009). Zinc deficiency is a major overlapping public health concerns in developing countries. It causes not only growth retardation, but also delays sexual maturation, hypogonadism, and thyroid dysfunction (Kaji and Nishi, 2006). A pilot study of Abdulla and Suck (1998) 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 of general population in developing countries.

Because of their essentiality, vegetables contain some concentration of elements such as Cu and Zn that they acquire from cultivated soils. Unfortunately, much of the soil in which food is grown has been depleted of these nutritive minerals; therefore the mineral content in food is reduced. 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 a common leafy vegetable. A pot experiment was conducted on the Amaranth (Amaranthus cruentus) with addition of different concentrations of Cu and Zn in soils.

2. Materials and methods

2.1. Pot experiment on Amaranthus cruentus

The soils (0–15 cm) used in this pot experiment were collected from the eastern part of Libreville city. It was a sandy loam; 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. Each pot (22 cm x 50 cm) contained 3.2 kg (dry weight) of soils. The metals Cu and Zn were artificially added with solutions of copper sulfate and/or zinc sulfate to make the total concentration 6 and 60 mg Cu kg^{-1} or 10 and 110 mg Zn kg^{-1}, respectively. There was 9 metal treatments in a complete factorial design as treatments Cu-Zn: (0-0), (0-10), (0-110), (6-0), (6-10), (6-110), (63-0), (63-10) and (63-110). 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.

The leafy vegetable *Amaranthus cruentus* was chosen as the potted plant 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 there were four plants per pot. 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 stove until their weight was constant, their roots, leaves and stems were subsequently separated and kept in polyethylene bags.

2.2. Analysis of metals concentrations in tissues of Amaranthus cruentus, and quality control

2.2.1. Metal concentrations in tissues of Amaranthus cruentus

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 (Nardi et al., 2009). 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.

2.2.2. 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.

2.3. Statistical methods

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

3. Results and discussion

3.1. Cu and Zn concentrations in Amaranthus cruentus tissues

The supplementation of Cu and Zn in soil was examined for the content of both metals and other metals in the tissues of cultivated *Amaranthus cruentus* samples. The concentrations of Cu and Zn in the leaves, stems and roots of *Amaranthus cruentus* 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 soils. Thus, when the concentration of Zn (Cu respectively) not varied in soils, the Cu (Zn respectively) supplementation in soil led generally to a significant increasing of Cu (Zn respectively) in all tissues of plant ($p < 0.0001$, respectively). The antagonistic effect of Cu and Zn on plant growth has been well documented (Kabata-Pendias, 2011; Graham, 1981). These metals seemed to be absorbed by the same mechanism and therefore, each may competitively inhibit root absorption of the other (Kabata-Pendias, 2011). In the case of *Amaranthus cruentus*, the Zn additions in soils decreased significantly Cu uptake in roots, and the Cu additions in soils decreased significantly Zn uptake in roots, exception for a Zn concentration of 110 mg/kg.

Accumulation of Cu and Zn was significantly higher in roots than in leaves and stems ($p < 0.0001$). There were no significant difference between leaves and roots for Cu and Zn uptake ($p = 0.515$ and $p = 0.336$, respectively). The supplementation of Cu and Zn led to decrease the Cu uptake until 76% in steams and 24% roots, but mainly to increase Cu and Zn in leaves (422% and 237%, respectively), steams (376% and 159%, respectively) and roots (125% and 475%, respectively). Thus, it could be concluded that supplementation of Cu and Zn in soil had an obvious effect on the enrichment of Cu and Zn in *Amaranthus cruentus*, and that the leaves had better enrichment effect of Cu and the roots had better enrichment effect of Zn. Furthermore, the enrichment effect of Zn in leaves was also important (until 237%). There is a continuing interest in supplementation of nutrients deficiency. The present study showed that *Amaranthus cruentus* 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 *Amaranthus cruentus* was affected not only by the individual Cu or Zn applications, but also by their combinations. The highest Cu and Zn enrichment effects were generally observed for the supplementation of Cu to 63 mg/kg, with or without the supplementation of Zn. Some cases of high Zn uptake were also observed with the supplementations Cu/Zn of 0/110 mg/kg and 6/110 mg/kg.

3.2. Cu and Zn addition effects on Cd, Cr, Fe, Mn, Ni and Pb uptake in Amaranthus cruentus

The results showed that Cu and Zn additions in soil were helpful to the uptake of these both metals in plants. The concentration of other metals in *Amaranthus cruentus* 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. The accumulation of Cd, Cr, Fe, Mn, Ni and Pb in root tissues decreased until a maximum of 17%, 9%, 12%, 26%, 26% and 15%, respectively. It was more important for addition of 6 mg/kg of Cu and 110 mg/kg of Zn. Therefore, supplementation of Cu and Zn had antagonistic effects to accumulation of Cd, Cr, Fe, Mn Ni and Pb in the roots (Table 1).

Cd and Pb concentrations in stems and leaves were below detectable concentrations. In the stems, a decreasing was observed only for Fe and Mn uptake in the supplementation of 63 mg/kg of Cu. The metal uptake in stems often increased, particularly for Cr, Mn (all treatments with 110 mg/kg of Zn) and Ni (all treatments with Cu). The treatments with 63 mg/kg of Cu combined with Zn contributed to increase the uptake of all detected metals. The accumulation of Cr, Fe, Mn and Ni in stem tissues decreased until a maximum of 92%, 51% and 47% and 286%, respectively. To note that supplementation of Cu and Zn had presented synergic effects with accumulation of studied metals in stems (Table 1).

The changes in the leaves tissues were more contrasted over control. The accumulation of metals in leaves was observed for supplementation of 110 mg/kg of Zn, with or without 60 mg/kg of Cu supplementation. Cr, Fe, Mn and Ni uptake increased until 26%, 31%, 49% and 20%, respectively. Generally, metal accumulation in leaves decreased for the others treatments. The decrease maximum was 23%, 42%, 86% and 26% for Cr, Fe, Mn and Ni, respectively. Supplementation of Cu and Zn had antagonist effects with accumulation of Cr, Fe, Mn and Ni in leaves of *Amaranthus cruentus*, exception of high Zn supplementation where synergic effects were observed.

The Cu and Zn addition showed mainly between the metals and Cd, Cr, Fe, Mn, Ni and Pb uptake an antagonist effect in roots and leaves of *Amaranthus cruentus*, and often a synergic effect in stems. However, the treatments of soils by Zn with or without Cu showed that metals were more easily transferred into leaves. This result is particularly important because leaves are the edible part of *Amaranthus cruentus*. Indeed, the addition of Zn or Cu/Zn combination could not only increase Cu and Zn intake but also of other nutrients such as Cr, Mn and Fe. Cr 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 (Kabata-Pendias, 2011; Zayed et al, 1998). 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 (WHO, 2011). Mn deficiencies are common on the calcareous soils, sandy soils and during episodes of over-liming in a range of soils (Van der Walls and Laker, 2008; Holloway et al., 2008). 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 (Watts, 1990).

4. Conclusion

In this study, the concentration of Cu and Zn in the tissues of *Amaranthus cruentus* increased significantly with supplementation of these metals. Generally, concentrations of other elements, such as Cd, Cr, Fe, Mn, Ni and Pb mostly decreased in roots and leaves, and often increased in stems. The supplementation of Zn, combined or not with Cu in agricultural soils could be an easy and efficient way to improve the concentration of nutrients in leafy vegetables such as *Amaranthus cruentus*. Farmland experiments will be carried out to confirm or not these results but also study the cases of supplementation of toxic concentrations of Cu and Zn in agricultural soils.

5. Acknowledgements

The authors acknowledge Dimitra ONDO for her corrections and critics, and Jean Félix NDZIME for his technical assistance in laboratory analysis.

References

Abdulla, M., Suck, C. (1998). Blood levels of copper, iron, zinc, and lead in adults in India and Pakistan and the effect of oral zinc supplementation for six weeks. *Biological Trace Element Research* 61, 323-331.

Graham, R. D. (1981). *Absorption of copper by plant roots*. New York: Academic Press.

Hashmi, D. R., Ismail, S., Shaikh, G. H. (2007). Assessment of the level of trace metals in commonly edible vegetables locally available in the markets of Karachi city. *Pakistan Journal of Botany,* 39, 747-751.

Holloway, R. E., Graham, R. D., Stacey, S. P. (2008). Micronutrient deficiencies in Australian field crops. Dordrecht: Springer.

Ismail, F., Anjum, M. R., Mamon, A. N., Kaz, T. G. (2011). Trace Metal Contents of Vegetables and Fruits of Hyderabad Retail Market. *Pakistan Journal of Nutrition*, 10, 365-372.

Jalbani, N., Ahmed, F., Kazi, T. G., Rashid, U., Munshi, A. B., Kandhro, A. (2010). Determination of essential elements (Cu, Fe and Zn) in juices of commercially available in Pakistan. *Food and Chemical Toxicology* 48, 2737-2740.

Kabata-Pendias, A. (2011). Trace elements in soils and plants, 4th ed. London, New York: Taylor & Francis.

Kaji, M., Nishi, Y. (2006). Growth and minerals: Zinc. *Growth, Genetics & Hormones* 22, 1-7.

Kandhro, G. A., Kazi, T. G., Afridi, H. I., Kazi, N., Baig, J. A., Arain, M. B., Sirajuddin, Shah, A. Q., Sarfraz, R. A., Jamali, M. K., Syed N. (2009). Effect of zinc supplementation on the zinc level in serum and urine and their relation to thyroid hormone profile in male and female goitrous patients. *Clinical Nutrition*, 28, 162-168.

Nardi, E. P., Evangelist, E. S., Tormen, L., Saint´Pierre, T. D., Curtius, A. J., de Souza, S. S., Barbosa Jr, F. (2009). The use of inductively coupled plasma mass spectrometry (ICP-MS) for the determination of toxic and essential elements in different types of food samples. *Food Chemistry* 112, 727-732.

Onianwa, P. C., Adetola, I. G., Iwegbue, C. M. A., Ojo, M. F., Tella, O. O. (1999). Trace heavy metals composition of some Nigerian beverages and food drinks. *Food Chemistry* 66, 275–279.

Turley, E., McKeown, A., Bonham, M. P., O'Connor, J. M., Chopra, M., Harvey, L. J., Majsak-Newman, G., Fairweather-Tait, S. J., Bügel, S., Sandström, B., Rock, E., Mazur, A., Rayssiguier, Y., Strain, J. J. (2000). Copper supplementation in humans does not affect the susceptibility of low density lipoprotein to in vitro induced oxidation (FOODCUE project). *Free Radical Biology and Medicine* 29, 1129-1134.

Uriu-Adams, J. Y., Keen, C. L. (2005). Copper, oxidative stress, and human health. *Molecular Aspects of Medicine* 26, 268-298.

van der Waals, J. H., Laker, M. C. (2008). Micronutrient deficiencies in crops in Africa with emphasis on Southern Africa. Dordrecht: Springer.

Watts, D. L. (1990). The nutritional relationships of manganese. Journal of Orthomolecular Medicine, 5, 219-222.

WHO (2011). Micronutrient deficiencies-Iron deficiency anaemia. http://www.who.int/nutrition/topics/ida/en/ index.html

Zayed, A., Lytle, C. M., Qian, J. H., Terry, N. (1998). Chromium accumulation, translocation and chemical speciation in vegetable crops. Planta, 206, 293-299.

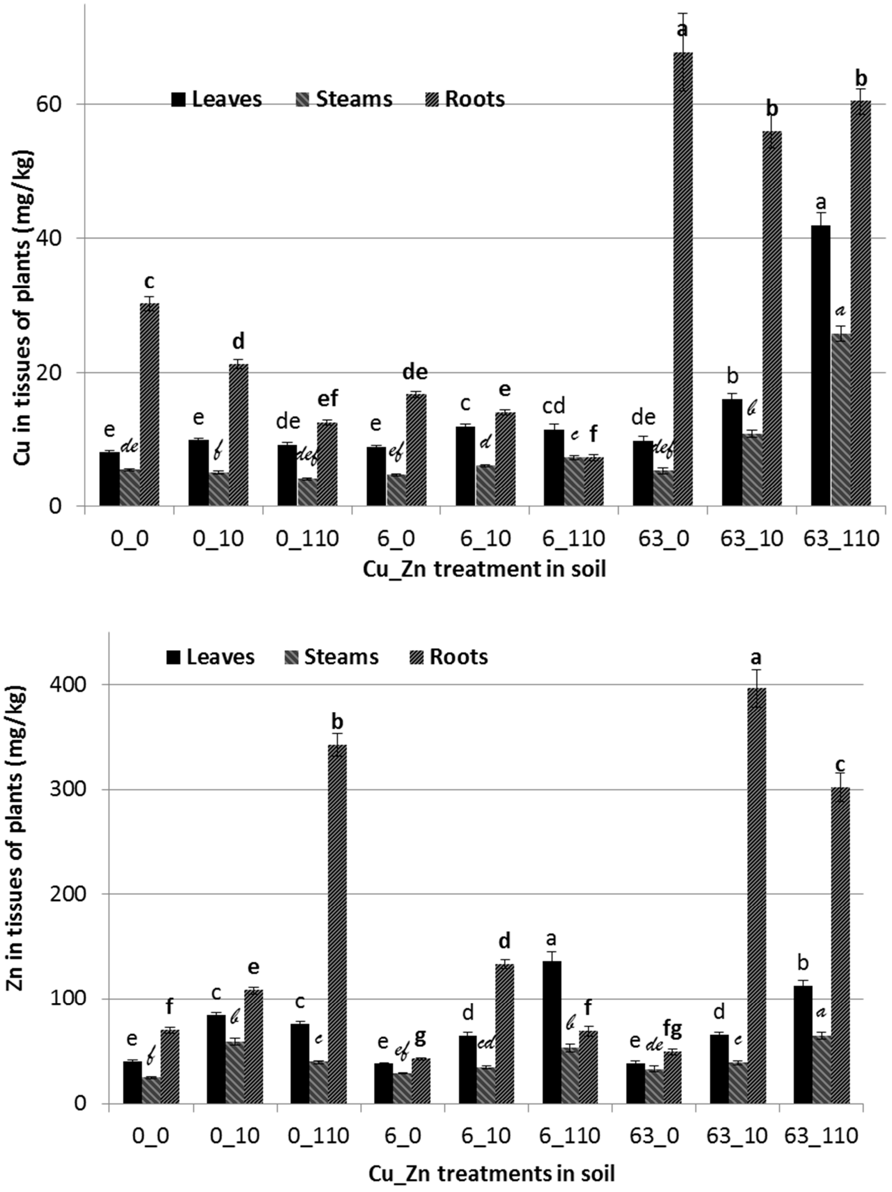

Figure1: Cu and Zn content in tissues of *Amaranthus cruentus* for nine treatments of the pot experiment

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

	0 – 0	0 – 11	0 – 111	6 – 0	6 – 11	6 – 111	63 – 0	63 – 11	63 - 111	ANOVA		
										Cu	Zn	Cu * Zn
Cd	-	-	-	-	-	-	-	-	-	-	-	-
Cr	3,54±0,13b	1,62±0,05de	4,46±0,14a	2,58±0,07c	1,65±0,05de	1,43±0,09e	0,80±0,07f	1,77±0,08d	4,41±0,20a	***	***	***
Fe	657±24b	412±12d	445±14d	413±11d	446±14d	396±26d	274±23e	567±25c	860±39a	***	***	***
Mn	30±1cd	29±1de	37±1b	28±1de	26±1de	33±2bc	26±2e	37±2b	45±2a	***	***	***
Ni	1,52±0,06c	0,76±0,02e	1,82±0,06a	1,15±0,03d	0,49±0,02g	0,62±0,04f	0,40±0,03g	0,85±0,04e	1,71±0,08b	***	***	***
Pb	-	-	-	-	-	-	-	-	-	-	-	-
Cd	-	-	-	-	-	-	-	-	-	-	-	-
Cr	0,60±0,02d	0,41±0,01d	0,72±0,02c	0,71±0,02c	0,56±0,02d	0,62±0,04d	0,62±0,0d	0,90±0,04b	1,15±0,05a	***	***	***
Fe	97±4cd	93±3cd	96±3cd	104±3c	93±3d	124±8b	78±7e	135±6ab	147±7a	***	***	***
Mn	14±0c	13±0c	17±1b	13±0c	14±0c	17±1b	11±1d	16±1b	20±1a	***	***	***
Ni	0,32±0,01ef	0,25±0,01f	0,31±0,01ef	0,60±0,02c	0,43±0,01d	1,09±0,07b	0,53±0,05c	0,37±0,02de	1,23±0,06a	***	***	***
Pb	-	-	-	-	-	-	-	-	-	-	-	-
Cd	2,45±0,09a	1,30±0,04c	1,61±0,05b	0,91±0,02d	0,42±0,01f	-	1,59±0,14b	0,60±0,03d	0,80±0,04e	***	***	***
Cr	21,29±0,78a	9,03±0,27c	10,85±0,34b	5,44±0,14d	2,30±0,07fg	1,47±0,10g	8,51±0,73c	3,00±0,13f	4,20±0,19e	***	***	***
Fe	9409±343a	4770±143c	5751±349b	2719±71d	1142±36f	160±10g	4740±404c	2130±95f	2997±135d	***	***	***
Mn	84±3a	42±2cd	62±1b	32±1f	22±1g	23±1g	37±3de	33±1ef	42±2c	***	***	***
Ni	5,41±0,20a	2,47±0,14cd	4,57±0,15b	1,82±0,05e	2,71±c	0,64±g	2,27±0,19d	1,38±0,06f	1,75±0,08e	***	***	***
Pb	8,81±0,32a	4,68±0,14c	8,48±0,42a	3,23±0,08d	-	-	5,09±0,43bc	2,76±0,12d	5,46±0,30b	***	***	***

***: $p < 0.0001$; values with different letters in same line are significantly different ($p < 0.05$).