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Research Article

ZINC AND COPPER PLANT UPTAKE IN SOILS AMENDED WITH FEEDLOT MANURE OR SOLUBLE SALTS

Laura María Ramos[1,2], Carlos Hernán Moscuzza[2], Alicia Fernández-Cirelli[1, 2*].

[1]*Instituto de Investigaciones en Producción Animal, (INPA-UBA-CONICET). Facultad de Ciencias Veterinarias – Universidad de Buenos Aires. Av. Chorroarín 280 (C1427CWO), Buenos Aires, Argentina*
[2]*Centro de Estudios Transdisciplinarios del Agua (CETA-UBA), Argentina*

** Corresponding author*
E-mail: mramos@fvet.uba.ar.

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ABSTRACT. In agriculture, inorganic and organic amendments were used to improve soil properties but those can highly modify de bioavailability of metals in the soil environment and plant uptake. In a field micro-plot trial, we compared the effect of organic fertilization (feedlot cattle manure) versus conventional inorganic fertilizers ($CuSO_4$-$ZnSO_4$) on copper (Cu) and zinc (Zn) plant composition. At first harvest (30 d), the Cu concentration in aerial biomass was higher ($P < 0.05$) in plots receiving feedlot manure compared to the control tests. Fertilization with soluble salts did not increase Cu concentration in forages samples. For Zn, the results showed a positive response for all fertilization treatments ($P = 0.0148$), but better outcomes were obtained for organic amendments. At the second harvest (90 d), Cu and Zn concentrations in leaf biomass showed minimum differences after different type of fertilization compared to the control ($P > 0.05$). These results indicate a more positive response of feedlot manure in comparison with inorganic fertilizers at first harvest. At 90 d, independently of the fertilization treatment, the Cu and Zn concentrations in aerial plant biomass decreased compared to 30 d. The study shows that at long harvest time, a second fertilization should be considered to prevent a depletion of metal concentration bellows beef cattle requirements established by National Research Council (NRC).

Keywords: *Feedlot manure, inorganic fertilizer, forage, enrichment*

INTRODUCTION

Intensive livestock production systems generate a large quantity of manure by products which may be potentially contaminant making it urgent to find environmentally friendly alternative uses. Land filling or land application of these wastes are commonly suggested disposal techniques [1] to primarily benefit from their organic carbon, nitrogen (N) and/or phosphorous (P) content. However, depending on manure composition, continuous application of this amendment may lead to soil pollution due to accumulation of different chemical elements. Use of manure or compost biosolid as organic fertilizer has been reported to increase total amount of certain metals like Cu and Zn, since they are not biodegradable with the potential of eventually becoming phytotoxic [2,3].

Trace mineral content in feed and mineral assimilation by animals are main factors determining the mineral composition of manure [4–10]. Intensive feeding systems usually use high levels of nutritional mineral and vitamin additives that in turn significantly raise trace mineral excretion to the environment and manure application appears as an important source of certain metals input in soils [11,12].

On the other hand, Cu and Zn are trace elements that frequently present primary or secondary deficiencies for plants and animals in different regions of the world. The Pampean plain of Argentina has different kinds of soils with different aptitude for agriculture and micronutrient content; deficiencies of those minerals have been reported in grassing cattle [13,14]. The adoption of high-yield genotype crops improved agricultural mechanization and use of fertilizers with a higher degree of purity [15,16] has resulted in an increased response to the accumulation of these minor elements in crops [17]. Therefore, soil amendment with feed-lot manure could assist in supplying the minerals for plant and animal nutrition.

However, minerals plants uptake is highly variable [18] and dependent on physico-chemical properties of the soil (such as pH; [19]) and mechanisms by which nutrients are captured and metabolized by plants [20]. In general terms, the intrinsic capacity of minerals to be assimilated by plants is referred to as bioavailability, which is highly dependent on chemical form of the element, and interactions among nutrients [21]. Bioavailability of micronutrients should be considered important from two point of view: from a nutrient perspective, for its association with crop growth and health, and from an environmental view, for the increased accumulation in the environment.

Availability of trace minerals to plant and soil has been largely studied. Ahumada et al. [22] evaluated Cu and Zn concentration in plant tissue growing in soils amended with stabilized sewage sludge, or biosolid, finding higher concentrations of both metals in plants roots that grow on biosolids amended soils. In addition, a satisfactory correlation has been observed between the labile fraction determined by sequential extraction and Cu and Zn contained in plants of *Lolium perenne* L. and S*ubterranean Clover*. Mantovi et al. [7] determined Cu and Zn bioavailability in maize, sugar beet and lucerne by measured their concentrations in edible plants parts cultivated in agricultural soils fertilized with different levels of liquid manure. They concluded that periodic application of manure as organic fertilizer could increase soil metal contents, but this fact did not impact in Cu and Zn concentrations in plants tissues probably due to the low bioavailability of those metals. Mendoza et al. [23] analyzed the effect in heavy metal (Cu, Zn, Ni, Pb, Mn and Fe) soil availability by sequential BCR extraction, and trace element plant uptake by *Sorghum bicolor* L. *Moench* grown in soils treated with two kinds of sludge obtained by two different processes. Unlike other studies, lower concentrations were determined in leaves for sorghum fertilized plants in comparison with control pots despite higher percentage of labile fraction of said metal in amended soils determined through sequential extraction. In turn, there was no correlation between the extractable Cu and the concentration in the plants.

In our country, Moscuzza et al. [24] assessed the capacity of forage enrichment through fertilization with composted manure from feedlots mixed with increasing concentrations of copper and zinc sulfate solutions resulting in elevated concentrations of these metals in forages. Torri and Lavado [25] investigated the association of Cd, Cu, Pb, and Zn soil availability determined by a sequential extraction procedure with plant uptake capacity of *Lolium perenne* L. grown in soils amendment with sewage sludge or sewage sludge mixed with 30% (dry matter basis) of its own incinerated ash. For Cu and Zn, the concentration observed in aerial biomass at a short term of fertilization treatment was significantly higher for all treatments respect control soils. However, they concluded that the most available fractions obtained by sequential soil extraction did not provide the best indicator of Cu and Zn availability for *Lolium perenne* L.

For Cu and Zn, sulphate, oxides, oxysulfates (partially acidulated with H_2S0_4) and synthetic or natural organic chelated compounds were fertilizer sources commonly used in agricultural practices [26]. Application of inorganic and organic amendments (like feedlot cattle manure) improves soil properties, such as microbial and enzyme activities, and promotes changes in soil organic matter content that can affect the mobility and trace element plant uptake [27-29].

The objective of this study was to assess the effect of soil amendment with feedlot effluents on Cu and Zn plant composition.

MATERIALS AND METHOD

Field preparation and raising plant

The experiment was conducted in the campus of the Veterinary School Sciences (Univ. of Buenos Aires, Argentina; latitude 34° 35' 55" S longitude 58° 28' 50" W) on a Typical Argiudol soil (clay loam textured, with medium contents of organic carbon (OC) (20 g kg^{-1}), low-pH values (6.1 in KCl) and cation exchange capacity (CEC, 19 cmolc kg^{-1}).

A field micro-plot trial was set in a 2 m × 2.5 m plot, where the experimental treatments were allocated in strings of 0.5 m wide (with 10 cm depth buffer around each one) and 2 m long (split in 4 pseudo-replicates of 0.5 m each). The experimental treatments were allocated to each field string, and consisted of:

- *T-I*, feed-lot cattle manure at rate of 1.3 kg on wet basis (WB) m^2
- *T-II*, feed-lot cattle manure at rate of 2.6 kg WB m^2
- *T-III*, Cu and Zn solution at 30 and 150 mg l^{-1} respectively
- *T-IV*, and Cu and Zn solution at (Total Zn: 840 mg l^{-1}; Total Cu: 340 mg l^{-1}), based on nominal doses to correct Cu and Zn crop deficiencies according to Voss [30] suggestion.
- *Control*, soil without any fertilization.

Cattle manure was collected from a commercial feedlot (before arriving to the laboratory it was kept refrigerated +4°C) and mixed by stirring with distilled water (1:1 m/v) before being applied on-top of the soil. The physicochemical characteristics and total and exchangeable or soluble content of Cu and Zn in organic amendment are reported in Table 1. The inorganic treatments were prepared by using a $CuSO_4$-$ZnSO_4$ chemical fertilizer and *T-III / T-IV* were poured directly on the top of the soil at the beginning of the experiment (day 0, August 30th 2014) avoiding the application on the plant foliage ("Diffusion Application").

The whole experimental plot was prepared by cut-flushing a previous *Bromus unioloides* K. pasture and re-seeded with *Lolium Perenne* L. Measurements were carried out on the regrowth of this mixed pasture between September and November 2014 (average temperature 19°C). The reported rainfall for this period was 231 mm (National Meteorological Service), and plants were watered with distilled water regularly throughout the experimental period.

Table 1. *Physical–chemical characteristics and content of heavy metals in organic amendment (feedlot manure)*

Properties	Feedlot Manure
pH[a]	7.60
CEC [b](cmol Kg^{-1})	2.20
P (mg kg^{-1}) extractable[c]	143
OC [d](%)	22.5
TKN[e] (%)	1.60
Total Cu (mg kg^{-1})	28.7
Total Zn (mg kg^{-1})	150
Exchangeable Cu (mg kg^{-1})	0.90
Exchangeable Zn (mg kg^{-1})	3.80

a pH measured potenciometrically in a 1:2.5 soil water ratio; b Electrical conductivity measured; c Extractable phosphorus by Bray-Kurtz method; d Organic carbon measured by Walkley-Black method; e Total Kjeldahl nitrogen measured by Microkjeldahl method.

Detailed description of these methods can be found in *Sparks* (1996; [73]).

Soil and plant sampling

Plant aerial biomass samples were harvested at days 30 and 90 after treatments were applied with a rectangular quadrat (0.0625 m^2) from the center of each treatment subplot. At the second harvest, a part of the new grass regrowth on the same soil section sampled at day 30, whole plant (aerial and root parts) samples were taken. Plant samples were thoroughly washed by placing them on a sieve (2 mm mesh) under running bidistilled water (MilliQ) to remove soil particles from the roots. Afterwards, plant parts (root, shoot, leaf and inflorescence) were dried at 65°C until a constant weight was achieved. Dried samples were milled and passed through a sieve of 2 mm mesh and then kept aluminum foil envelopes at room temperature until analysis were performed.

Soil samples (at depths of 0–10 cm) were taken at the end of the experiment (day 90 after fertilization) from two sub-plots and pooled to a single sample per treatment. Before being analyzed, samples were air dried and sieved to pass 2 mm mesh.

Analysis of Cu and Zn

Plant material extracts were prepared by digesting 0.5 g of milled material with HNO_3 and $HClO_4$ (3:1 v/v) following Zhao et al. [31] procedure. Cu and Zn soils extracts were prepared by digesting with *aqua regia* (HNO_3: HCl 3:1) [32]. Phytoavailable Cu and Zn soil samples were extracted by the sequential extraction [33]. All the extracts were analyzed for Cu and Zn by ICP-OES (ICP-OES Optima 3000 DV, Perkin Elmer) equipped with a cross-flow nebulizer, scot chamber and quartz torch, using an external calibration with Quality Control Standard 21 (Atomic spectroscopy standard Nº 9300281, Perkin Elmer Pure 100 mg L^{-1}). The water use for preparation of working solutions was obtained from a Milli-Q Academic water purification system (Millipore, Bedford, MA, USA) with a resistivity of 18.2 MOhm*cm. Analytical determinations were performed in triplicate with a relative standard error less than 1%. Wavelength used for quantification of the analyzed elements was chosen based on the EPA recommendations [38], Cu: (327.393) and Zn: (213.857). Water blank were run to correct the all measurements and reagent blanks were used to verify possible interferences. Certified reference materials for soil samples (WQB CRM-3) and forage

(NIST-1570a) were used for verification of calibration procedure and validation of analytical method. Accuracy test was performed and recovery percentage (trueness) was calculated, ranging from 91% - 83% of Cu and 91% - 69% of Zn in WQB-3 soil material and NIST-1570a forage material respectively.

Statistical analysis

All values were expressed as mean ± standard deviation. The means values of Cu and Zn concentrations did not fit the normal distribution and homogeneity of variance (Shapiro–Wilk, $P < 0.05$; Levene, $P < 0.05$). Kruskal–Wallis non-parametric test was carried out to compare the means of all variables analyzed for different fertilization treatments. The statistical analyses were performed using the InfoStat® software.

RESULT AND DISCUSSION

Concentration of Cu and Zn in the aerial biomass at 30 days

Different manure doses and soluble salts solution significantly increased the Cu and Zn concentrations in aerial biomass of fertilized sub-plots (Table 2).

Table 2. *Total Cu y Zn concentrations (mg kg^{-1}) in aerial biomass obtained at first and second harvest (C1- C2) of forage grown under different treatments applied.*

Metals	**Cu concentration (mg kg^{-1})**		**Zn concentration (mg kg^{-1})**	
Days of harvest	**30**	**90**	**30**	**90**
Unamendment soils	16.1 ± 6.30a	6.70 ±1.70	37.9 ± 4.10a	34.1 ± 6.30
Organic amendment treatments				
T I	32.9 ± 5.30b	7.90 ± 2.20	121 ±11.1c	35.9 ± 6.50
TII	22.3 ±1.10ab	8.80 ± 0.90	97.2 ± 6.80bc	38.0 ± 3.70
Salt treatments				
T III	15.6 ± 4.20a	6.70 ± 1.70	62.5 ±17.9abc	35.2 ± 4.70
T IV	14.5 ± 2.00a	6.10± 2.00	53.0 ± 9.00ab	30.7 ± 12.6

*All the values are means of four replicates (N = 4) ± SD. BDL – Bellow detection limit. a, b, c: Different letters indicate statistical significant differences ($P \leq 0.05$).

Although the levels of Cu and Zn determined in control samples were within the normal range values published in international literature reports for grasses (Cu: 1- 20 mg kg^{-1}; Zn: 25 to 150 mg kg^{-1}; [34,35]) and national reports for natural grassland and cultivated pastures (3.8 to 8.5 mg kg^{-1} Cu; 27.7 – 42.5 mg kg^{-1} Zn; [14]); aerial biomass Cu concentrations at first harvest (30 d) were higher (p< 0.05) in plots receiving feedlot manure compared to the control tests (Control: 16.1 mg kg^{-1}, TI: 32.9 mg kg^{-1}, TII: 20.3 mg kg^{-1}), but higher concentration were recorded for *T-I* (1.3 kg m^{2}) than for *T-II* (2.6 kg m^{-2}; Figure 1). The fertilization with soluble salts ($CuSO_4$-$ZnSO_4$) did not increase

Cu concentration in forage samples indicating an advantage of organic amendments with respect to sulfate solutions. For Zn, the results showed a positive response for all fertilization treatments (P = 0.0148), but better outcomes were obtained for organic amendments (*TI*: 109.3 mg kg^{-1}; *TII*: 89.05 mg kg^{-1}; Control without fertilization: 37.87 mg kg^{-1}).

Feedlot cattle manure increased Cu and Zn concentration in aerial biomass crop pasture at first harvest. In addition, soluble and exchangeable forms of Cu an Zn were determined in beef cattle manure coming from intensive production systems (IS) (0.9 – 3.8 mg kg^{-1} respectively) inferring an increase in labile forms and other available fractions like bound to organic matter and carbonates of trace minerals in soil fertilized with feedlot cattle manure. These results are in agreement with previous reports of organic amendments applied to soil [22, 25, 36, 37]. In our work, in *TI* fertilization dose of feedlot manure aimed to supply 30 and 150 mg kg^{-1} in soil of Cu and Zn respectively (hence each plot received 1.3 kg m^{-2} of amendment), that consequently it meant 0.018 kg m^{-2} of N (which did not exceed the 250 kg N ha^{-1} suggested by EPA [38].

The availability, leaching and runoff losses of metals added to soil are determined by the several reactions that these metals undergo including adsorption, complexation, precipitation and reduction [39]. When adding manure, we can find evidence of such reactions in different parameters such as high amount of organic carbon (dissolved organic carbon [DOC]), soluble salt concentration (salinity), and acidification caused by the mineralization of organic nitrogen. Soil chemical partitioning and availability of metals added may be largely modified by changes in soils properties such us pH, redox potential and organic matter content generated by application of cattle manure as an organic amendment [40]. In addition to this, changes in organic matter and soil pH in amendment treatments could account for the lower response in the *TII* (in comparison to *TI* and control treatments) for both metals. This outcome was in agreement with previous results where Cu and Zn availability was reduced by high doses of amendments due to chelating properties of organic matter and low availability due to soil alcalinization [7,41-43].

Cu and Zn are mostly incorporated by plants as Cu^{+2} and Zn^{+2} through diffusion. Zinc sulfate and copper sulfate are commonly used, as powder or granule form, because they are completely soluble in water and readily available to the plant after soil application. Likewise, high application rates are always suggested due to the interaction of metals with soil components, for example, Cu complexation with organic matter [44,45]. The different responses to inorganic fertilization obtained in our work, could be affected by many other factors such as nutrient concentration, physical and chemical soil characteristics, metals runoff, plants root system, water regime, and plant transpiration [46–49].

Studies on the effectiveness of inorganic fertilization compared to organic amendments as a source of trace elements, are scare and long-standing. Gupta [50] determine the effect of various sources and methods of application of Cu on the Cu concentration and on the yield of cereals and forages. They found an increase of Cu concentration in the boot stage tissue of wheat, and oats with foliar applications of copper sulfate and chelated form of Cu. Conversely, non-significant differences were found in soil applied Cu showing a small increase in plant Cu concentration. At second harvest, Cu content in forages with foliar applied treatment was considerably lower than in the first cut and showed similar values to control treatments. Cu in soils were founded mostly as immobile geochemical forms being related with the low availability of Cu in these matrix [51]. The use of water-soluble compounds of Cu as fertilizers did not provide high availability to plant roots because particles remains very close to original fertilizer sources. Wang et al. [52] evaluated the effectiveness of Zn foliar and soil application methods on Zn and Fe concentrations of maize and wheat grains. They found a significantly increased in Zn concentration in ear leaves and shoot of maize and winter wheat before a first foliar and soil Zn application during the first (spring maize: 15 %; winter wheat: 18 % respect control) and second growing season (spring maize: 21 %; winter wheat : 44 % respect control). The effect of soil Zn application on Zn concentration in shoots and leaves is related to DTPA-Zn in the soil and related to the Zn fertilizer rate. During the second growing season of maize and wheat, soil Zn application was still not as effective as foliar Zn application. The effect of soil Zn application is further related to the soil characteristics that affect Zn availability, including pH and Ca concentration [53]. Yassen et al. [54] determined the effects of organic materials (farmyard manure (FMY), humic acid) and foliar application of zinc on yield and nutrient uptake by wheat plants. Application of high rate of manure, and humic acid in combination with foliar zinc sulphate increased Fe, Mn and Zn uptake in 79,73 and 119 % respectively in wheat plants as compared with the control treatment. On the other hand, studies that analyze changes in DTPA-extractable zinc, iron, manganese and copper concentration in soils after fertilization, showed that use of inorganic fertilizers directly to soil modified the percentage of trace metal extractables forms and should be considered jointly residual power after application. In general, soluble and extractable forms of Cu and Zn decrease rapidly after the first week of application [55].

Cu and Zn Concentration in plant fractions at 90 days

At the second harvest, Cu and Zn concentrations in leaf biomass for control treatment without fertilization were within the normal range reported for these forage species (Zn: 25 to 150 mg kg^{-1}, Cu: 1 to 20 mg kg^{-1} ;[34,35]), but no differences were found after any type of fertilization ($P > 0.05$; Table 2).

Cu and Zn concentration in plants foliage was lower in the second harvest than in the first one for all treatments (Fig. 1, a-b) probably as consequence of plant growth dynamics [56], which leads to an increase in total dry matter mass and lower protein and dry matter digestibility as result of lower leaf:stem ratio and metabolic activity [57–60]. Mineral concentration reduction in the second cut could have been associated too with a dilution effect as in late maturation stages, when large and active photosynthetic areas are being formed, dry-matter production may out-strip absorption of mineral elements, leading to a reduction in the mineral content on a dry-matter basis [61].

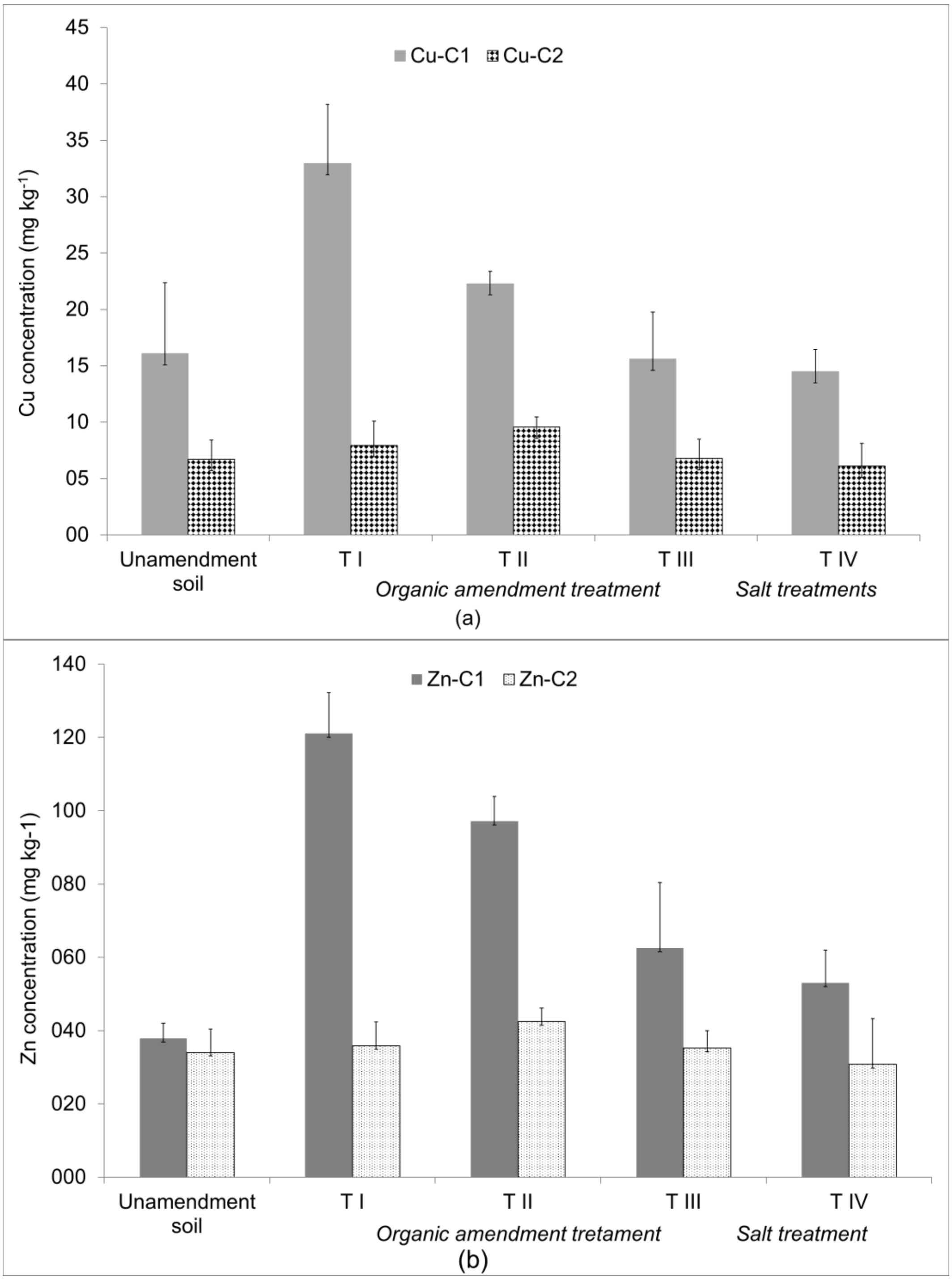

Fig. 1. *Cu (a) and Zn (b) content in aerial biomass of crops (mg kg^{-1}) after organic and mineral fertilization at first and second harvest (C1: 30 days; C2: 90 days). Data are presented as means± SD of four replicates.*

Concomitantly, there exists a redistribution of elements within the plant as well as variation among and even within different organs [62]. Table 3 shows metal contents in different parts of the plant at 90 days after fertilization treatments applied.

Table 3. *Total heavy metal concentrations (mg kg^{-1}) in leaf, shoot, root and inflorescence part of forages grown under different treatments applied.*

	Unamendment soil	**Organic amendment treatment**		**Salts treatments**	
		T I	*T II*	*TIII*	*T IV*
Leaf					
Cu (mg kg^{-1})	10.3 ± 1.70	12.1 ± 1.90	9.90 ± 3.30	11.3 ± 3.60	9.10 ± 6.00
Zn (mg kg^{-1})	40.6 ± 6.00	43.0 ± 2.90	43.2 ± 5.00	42.9 ± 7.20	73.5 ± 1.40
Shoot					
Cu (mg kg^{-1})	7.90 ± 1.70	12.3 ± 2.00	5.10 ± 1.00	13.1 ± 6.50	7.10 ± 1.80
Zn (mg kg^{-1})	45.9 ± 9.20	62.8 ± 3.30	31.6 ± 11.6	79.8 ± 6.20	31.9 ± 1.30
Root					
Cu (mg kg^{-1})	17.6 ± 1.70	19.1 ± 6.20	12.7 ± 2.20	16.7 ± 3.20	21.7 ± 2.70
Zn (mg kg^{-1})	94.4 ± 16.8	122 ± 4.80	248 ± 11.5	61.6 ± 2.80	97.5 ± 9.30
Inflorescence					
Cu (mg kg^{-1})	8.60 ± 1.90	11.9 ± 4.60	6.70 ± 5.20	18.0 ± 2.90	10.8 ± 1.30
Zn (mg kg^{-1})	41.9 ± 4.60	32.7 ± 13.0	28.2 ± 6.90	54.5 ± 2.90	43.4 ± 4.40

*All the values are means of four replicates (N = 4) ± SD.

It may be observed that Zn and Cu accumulate and are retained predominantly in plant species roots [1,63,64]. Also, they were heterogeneously distributed in different plants parts in control and in organic amended and inorganic soil treatments. Despite this, minor variations were observed in Cu and Zn concentration at second harvest among above ground plant tissues (or edible plant parts), especially leaf tissue (Fig. 2 a-b).

These results were in agreement with previous reports [7,23,25,46]. Ahumada et al. [22] found a differential Cu and Zn uptake by *Lolium Perenne* L. and *Trifolium subterraneum* L. reaching Zn higher concentrations in plant tissues than Cu in soils fertilized with stabilized sewage sludge and biosolids. However, an important amount of copper occurs in labile forms. Metals were mostly distributed and accumulated in plants roots of both plant species with about a threefold accumulation compared to the shoot. Ryegrass plants showed a better response to organic fertilization than subterranean clover. Similar results were obtained by Qian et al. [65] in wheat, where Cu accumulated mostly in roots. The root cell walls had mechanism of exchange that can limited the transmission of toxic elements to other tissues being a plant strategy to prevent heavy metal stress [66].

Low levels of exchangeable (EXCH) Cu and Zn determined from sequential extraction on soils samples at 90 days post fertilization could also have contributed to the diminished concentrations found in plant aerial parts (Cu- EXCH: <ld for all treatments; Zn- EXCH: 0.4 – 0.8%). According to our results, the application of organic amendments and inorganics forms of Cu and Zn increased the residual fraction (RES), which is related with the unavailable forms of trace elements, because they are incorporated inside crystalline lattice and clays structures of soil (Cu- RES= Unamendment soil: 18.1%, *TI:* 41.9%, *TII:* 34.2%, *TIII*: 36.0%, *TIV*: 37.1%; Zn- RES= Unamendment soil: 77.2%, *TI:* 78.6%, *TII*: 77.4%, *TIII*: 80.9%, *TIV*: 83.7%). None of

the applied fertilization treatments promote increases in the total metal content compared to the control without fertilization, after the end of the experience (90 days).

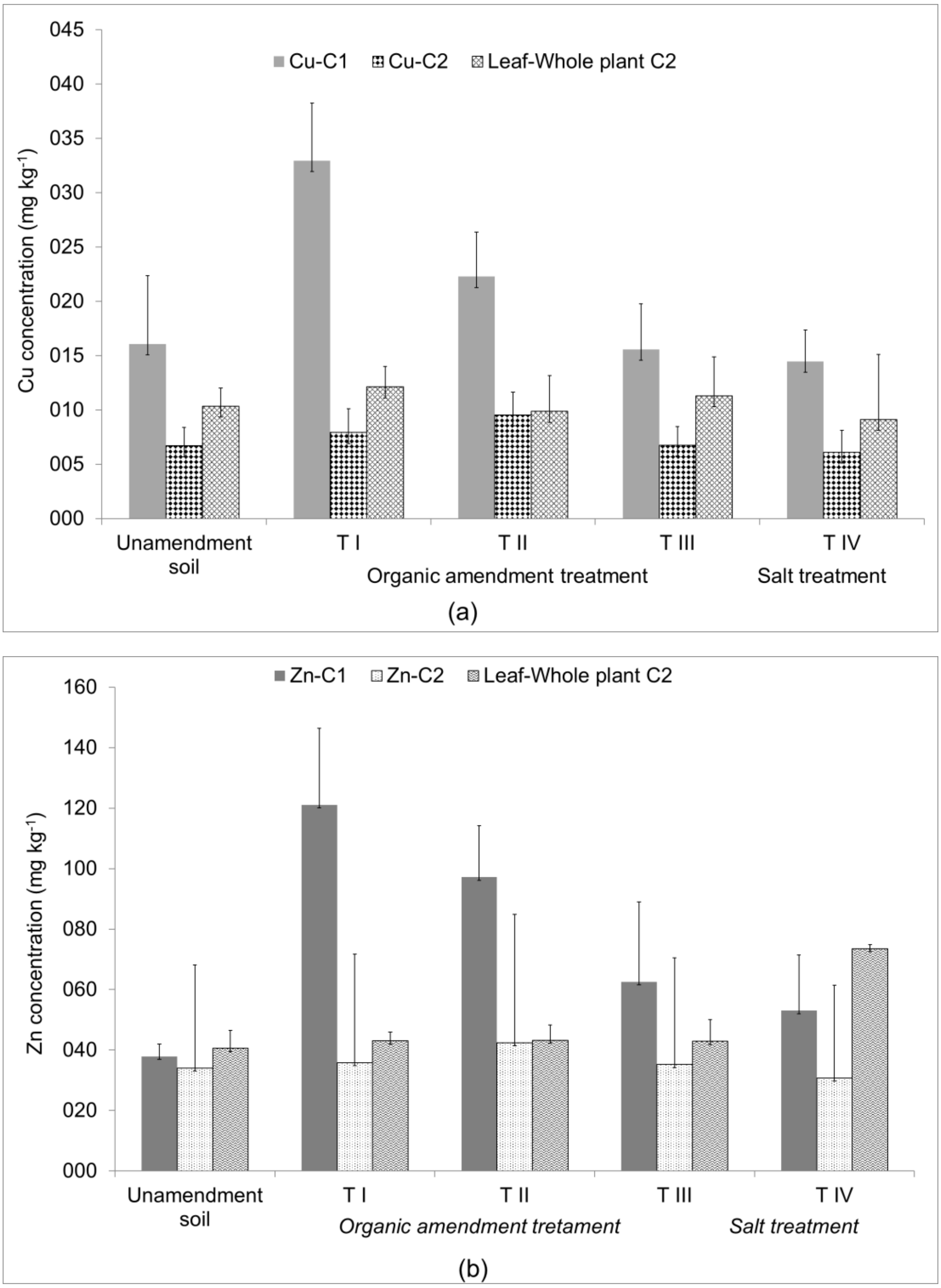

Fig. 2. *Cu (a) and Zn (b) concentrations (in mg kg^{-1}) obtained for aerial plant biomass obtained at first and second harvest (C1: 30 days; C2: 90 days) harvest and leaf of whole plant samples harvest at 90 days (C2).*

Organic amendments or mineral fertilizer and different application technologies (foliar, soils, superficial and homogenized) could modify the distribution of metal in different soil geochemical fractions. The distribution of the metal in organic amendments was different from the distribution obtained in the amended soils. Considering that copper in organic fertilizers was mainly found in the residual and oxidizable fraction and Zn in the inorganic and residual fraction, these fractions would be expected to increase more in amended soils [23]. It has been hypothesized that manure properties would predominate in trace metal chemistry at short term once organic amendments are applied to the soil, but these properties would have a smaller influence over the long time, becoming stronger control of the soil characteristics [67]. Likewise, these facts can be supported by the low percentage or absence of metals available forms in soils after long-term application of fertilizers. Less solubility and phytoavailability of trace elements have been observed as a result of a significant content of sorbents in the biosolids applied to the soil. On the other hand, the observed decrease agrees with the higher pH values found in amended soils, just as stated by Qiao et al. [68].

Though feedstuff ability to supply enough amount Cu and Zn depends on their concentration in plant organs alongside with their bioavailability and interactions with other minerals and organic compounds [69–71], their forage concentration in our experiment showed that except for the Cu in the second cut, harvested forage was always able to supply enough amounts of Cu and Zn to match all beef cattle categories requirements (NRC, [72]; Table 4).

Table 4. *Mineral requirements based on stage of production, maximum tolerable levels and concentration range determined in aerial biomass at 30 and 90 days.*

		Cows			Our work	
Mineral (mg kg^{-1} MS) *	**Growing and finishing**	**Gesta tion**	**Early lactation**	**MT L***	**1st Harvest**	**2nd harvest**
Copper	10.0	10.0	10.0	100	14,5 - 32,9	6,11 - 8,79
Zinc	30.0	30.0	30.0	500	37,9 – 121	30,7 - 37,9

Requirements based on values provided by NRC (2000)

CONCLUSION

These results show an increase of Cu and Zn concentration in aerial biomass of forage at the first harvest (30 days) after being fertilized with amendment or sulfate salts. Moreover, feedlot manure evidenced a more positive response with respect to inorganic fertilizers. At second harvest (90 d), independently of fertilization treatment Cu and Zn concentrations in aerial plant biomass decreased with respect to 30 d harvest. This can be mainly attributed to the higher metal content in the intensive excreta and the contribution of bioavailable trace element forms by the excreta, as well as to the effect that results from the application of said amendments on mobility and gradual release from fractions. less available as organic and inorganic fractions.

Likewise, the lower responses to fertilization with Cu sulfate and Zn sulfate can be related to the application form, the dose and the persistence of the product in the soil after its incorporation.

On the other hand, lower Cu and Zn concentrations were obtained in the forage samples at later harvest times (90 days after application of the treatments), regardless of the type of fertilization applied. This was related to pasture growth dynamics, plant tissue aging, and defoliation, as well as an increase in residual Cu and Zn forms in the amended soils.

Additionally, it is important to emphasize that Cu and Zn concentrations in forages tested 30 days after application were high enough to satisfy the beef cattle requirements according to NRC standard [72]. However, after 90 days of pasture growth, the Cu concentration felt slightly below the value stipulated by the nutritional tables mentioned above.

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