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## Copper and zinc forms in soil fertilized with pig slurry in the bean crop

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### ABSTRACT

The application of pig slurry may have different influence on copper (Cu) and zinc (Zn) dynamics in the soil compared with mineral fertilization. The aim of this research was to determine the different forms of Cu and Zn in soil and their uptake by bean plants in response to the application of mineral fertilizer and pig slurry (PS). The treatments were: mineral fertilizer (Cu and Zn oxides) and liquid pig slurry, at increasing rates (0/0, 1.7/6.0, 3.4/12.0 and 6.8/24.0 kg ha<sup>-1</sup> Cu/Zn, respectively) applied in a Rhodic Kandudox. PS increased the Cu content in soil in the exchangeable form, Fe oxides and residual, while the mineral fertilizer increased Cu contents in the fraction associated with soil organic matter. Soil Zn contents in the fractions available, exchangeable and SOM were highest under mineral fertilization, while in the soluble fraction the contents were highest under PS. The fertilizers had not impact on Cu and Zn contents associated with Al oxides, and these elements were mostly associated with Fe oxides in the soil. PS promoted the highest biomass production in shoots and roots of the bean plants, reflecting in the highest accumulation of Cu and Zn.

### Palavras-chave:

esterco de suíno  
absorção  
fracionamento de metais

## Formas de cobre e zinco no solo adubado com dejetos suíno na cultura do feijão

### RESUMO

A aplicação de dejetos suíno pode ter influência diferente na dinâmica do cobre (Cu) e zinco (Zn) no solo em relação à adubação mineral. Objetivou-se, neste trabalho, determinar as formas de Cu e Zn no solo e sua absorção pelas plantas de feijão em resposta à aplicação de fertilizante mineral e dejetos suíno, cujos tratamentos foram: fertilizante mineral (óxido de Cu/Zn) e dejetos suíno (DS) em doses crescentes (0/0, 1,7/6,0, 3,4/12,0 e 6,8/24,0 kg ha<sup>-1</sup> de Cu/Zn, respectivamente) aplicados em Nitossolo Vermelho. O DS aumentou o teor de Cu no solo nas frações disponível, trocável, óxidos de Fe e residual enquanto o fertilizante mineral aumentou na fração associada a matéria orgânica do solo (MOS). O teor de Zn no solo nas frações disponível, trocável e MOS foi maior na aplicação de fertilizante mineral enquanto na fração solúvel foi maior com o DS. Os fertilizantes não interferiram no teor de Cu nem no Zn ligados aos óxidos de Al sendo a maior parte associada aos óxidos de Fe no solo. O DS proporcionou maior produção de biomassa de parte aérea e raízes nas plantas de feijão refletindo no maior acúmulo de Cu e Zn.



## INTRODUCTION

Monitoring elements with pollutant potential in the agricultural production system is based on the premise that the factors plant, soil, water and agricultural practices are interdependent. The application of pig slurry (PS) in the soil without the proper technical criteria may favor the excess of metals in the soil, which include copper (Cu) and zinc (Zn) (Ceretta et al., 2010; Girotto et al., 2010).

The high concentration of Cu and Zn in the PS is due to the surplus used in the feed to compensate the low use by the animal, since approximately 85% of the Cu and 98% of the Zn present in the feed are excreted (Marcato, 1997). Plants absorb small amounts of these metals, while the application of PS for many years or at high doses may lead to accumulation of Cu and Zn in the soil (Mattias et al., 2010).

In order to increase the efficiency of use of this fertilizer, it is necessary to know the dynamics of these metals in the soil, because they are results of interactions, such as: pH, organic matter (OM), composition of clays, redox potential, competition in the adsorption sites, complexation with OM, temperature, microbial activity and the tendency to form precipitates and coprecipitates (Kumpiene et al., 2007; Sipos et al., 2008).

The use of PS in the soil may act differently with respect to its solubility and bioavailability of Cu and Zn in the soil, compared with mineral fertilizers, because of the complexation of these metals with soil OM (Paradelo et al., 2011). The sequential extraction in the soil provides detailed information on origin, form of occurrence, availability, mobilization and transport of metals in natural environments (Mallmann et al., 2012; Souza et al., 2012).

Thus, this study aimed to determine the different forms of Cu and Zn in the soil and their absorption by bean plants in response to PS application compared with the mineral in the form of Cu and Zn oxide in Rhodic Kandiodox.

## MATERIAL AND METHODS

The experiment was carried out in a greenhouse, at the Embrapa Swine & Poultry, in 2012, using a Rhodic Kandiodox (Nitossolo Vermelho Distroférico) (EMBRAPA, 2013) collected in the surface layer of 0-20 cm, in area of native vegetation that did not receive fertilizers or correctives, with the following chemical characteristics: 16 g kg<sup>-1</sup> of SOM; pH in water of 4.6; 1.9 mg dm<sup>-3</sup> of P; 0.8, 4.0, 1.1, 19.0 and 24.9 mmol<sub>c</sub> dm<sup>-3</sup> of K, Ca, Mg (exchangeable), H+Al and CEC pH 7.0, respectively, and V of 24%, determined as described by Tedesco et al. (1995). The soil was maintained with natural pH because the highest absorption of Cu and Zn occurs in the pH range of 4.5 to 5.0, while base saturation was increased to 60% using agricultural gypsum.

The experiment was conducted in randomized block design in a 2 x 3 + 1 factorial scheme, with eight replicates. The treatments consisted of two types of fertilizers: mineral, composed of Cu and Zn oxide, and liquid pig slurry (PS), at three increasing doses equivalent to 1.7/6.0, 3.4/12.0 and 6.8/24.0 kg ha<sup>-1</sup> of Cu/Zn in interaction between the nutrients, besides the control, without Cu and Zn only.

The chemical composition of the PS was determined according to Rice et al. (2012), with the following chemical characteristics: 5.1% (m/v) of dry matter, 4.5, 0.99 and 0.74 kg m<sup>-3</sup> of N, P and K; and 34 and 120 g m<sup>-3</sup> of Cu and Zn. N, P and K in the mineral treatment are present in the form of urea, triple superphosphate and potassium chloride, supplied to the soil in amounts equivalent to those added by the PS for each treatment.

The experimental units consisted of pots filled with 10.0 kg of soil, planted with eight seeds of 'Carioca' bean, cultivar 'BRS Estilo'. Sowing occurred on the same day of the application of the fertilizers in the soil; after germination, thinning was performed leaving only five seedlings per pot. During the experiment, the pots were maintained under intermittent irrigation in order to maintain soil moisture close to 80% of field capacity.

Bean plants were harvested after 55 days of cultivation. The plants were cut at the soil level for shoot biomass (S) and, for roots (R), the intact whole block of soil was removed from the pots and later separated and washed. Then, the materials were dried in forced-air oven at 65 °C until constant mass. After drying, shoot and root dry biomass were determined on a precision scale, with two decimal places, and the contents of Cu and Zn were determined according to the methodology described by Tedesco et al. (1995).

Total and residual Cu and Zn contents in the soil were determined according to the methodology described by Ure (1990) through the aqua regia method. Cu and Zn fractionation followed the methodology described by Gomes et al. (1997), analyzing the fractions that are soluble, exchangeable, bound to organic matter and to the oxides of Fe and Al. The elements of the soluble fraction were extracted using KCl (0.005 mol L<sup>-1</sup>); the elements of the exchangeable fraction were extracted using BaCl<sub>2</sub> (0.1 mol L<sup>-1</sup>), followed by agitation for 1 h; Cu and Zn bound to organic matter were extracted using NaClO (0.7 mol L<sup>-1</sup>) and placed in water bath at 90 °C for 15 min, and the process was repeated twice and the extracts were combined; Cu and Zn bound to Al oxides were extracted using NaOH (1.25 mol L<sup>-1</sup>) and only then placed in water bath at 75 °C for 1 h. Cu and Zn bound to Fe oxides were extracted using solution of ammonium oxalate (0.2 mol L<sup>-1</sup>) + oxalic acid (0.2 mol L<sup>-1</sup>) + ascorbic acid (0.1 mol L<sup>-1</sup>) followed by agitation in the dark, for 4 h.

Between each fractionation step, the soil was washed with distilled water, centrifuged and the supernatant was discarded. Then, the soil was dried and the samples were weighed before each extraction step to calculate the contents of Cu and Zn, since there may be removal of part of the mineral and organic phases by the extractors in the successive extractions. The samples of each fraction were centrifuged at 2000 rpm at 670 × g for 20 min, the extracts were filtered and the volume was replenished with 3 mol L<sup>-1</sup> HNO<sub>3</sub> to 50 mL, being later analyzed in atomic absorption spectrophotometer.

The data were subjected to analysis of variance homogeneity and normality. Data transformation was not necessary; after the parametric assumptions were met, the results were subjected to analysis of variance by F test and, when there was difference, the means were compared by Tukey test (p < 0.05). Additionally,

regression analysis was performed to verify the behavior of the variable as a function of the increasing doses applied in the soil.

## RESULTS AND DISCUSSION

There was interaction for the variables total Cu and Cu in exchangeable, organic matter, Fe oxide and residual fractions (Table 1). For total Cu, fertilization with PS and as Cu oxide showed increasing quadratic behavior, with maximum responses at the doses of 5.8 and 6.8 kg ha<sup>-1</sup> of Cu for oxide and PS, respectively; the contents for PS were superior to those for Cu oxide at the applied doses. It should be pointed out that, for the form of Cu oxide, the highest contents were observed at the doses of 3.4 and 6.8 kg ha<sup>-1</sup>, while for PS the highest content occurred at the highest dose.

Cu application in the form of oxide or PS until the highest dose (equivalent to 6.8 kg ha<sup>-1</sup>) did not interfere with the content of soluble Cu, and only traces of the element were found (Table 1). The low contents in this fraction may have occurred because the applied doses were not sufficient to occupy all bonding sites in the soil and allow the increase in the soluble fraction. The content of Cu in the free form is extremely low due to its affinity with organic and mineral compounds of the soil (Sipos et al., 2008; Muñoz et al., 2008).

The content of exchangeable Cu in the soil showed increasing quadratic behavior, with maximum response at the dose of 6.8 kg ha<sup>-1</sup> in both treatments; PS was superior to the mineral in the form of Cu oxide with respect to the availability of this micronutrient with emphasis for doses equal to or higher

than 3.4 kg ha<sup>-1</sup> (Table 1). The slight increase in the content of exchangeable Cu when soluble mineral sources are applied is related to its adsorption to soil colloids, covalently, leaving it little available, which does not occur with the organic form, which preserves this micronutrient in structures with organic C, avoiding its fixation in the soil (Ceretta et al., 2010; Bertol et al., 2010).

With the increase in Cu doses in the soil, there was an increasing quadratic response in Cu bound to soil organic matter (SOM) only for PS, with maximum response at the dose of 3.6 kg ha<sup>-1</sup> (Table 1). The lowest contents of Cu bound to SOM for the application of PS, in comparison to the mineral of Cu oxide, may be related to the fact that this element is bound to carbonic structures present in the PS.

OM has large specific surface, negative net charge dependent on pH, ease of imbibition of water and soil solution containing metals and capacity to form organic chelates (Plaza et al., 2013). Cu can be complexed to OM in insoluble organic forms, being little available. The main bond of Cu in the OM is in the organic acids of higher molecular weight forming stable complexes of high energy (Pires & Mattiazzo, 2007; Ceretta et al., 2010). In Cu-deficient soils, the bonding force decreases with the increase of the applied amount, but increases with the higher degree of humification and with pH (Kumpiene et al., 2007; Muñoz et al., 2008).

Only the application of increasing doses of Cu in the form of PS increased the content of Cu bound to Fe oxides in the soil, with increasing quadratic behavior and highest content at the highest dose (Table 1). The highest Cu contents in this

Table 1. Copper (Cu) content in different soil fractions in response to the application of different doses of this element in the forms of mineral oxide or pig slurry

Fertilizer	Dose (kg ha <sup>-1</sup> )				Regression equation
	0	1.7	3.4	6.8	
Total (mg kg <sup>-1</sup> )					
Cu oxide	169.4 c	172.9 Bb	178.4 Ba	179.8 Ba	$\hat{y} = 169 + 3.5x - 0.3x^2$ R <sup>2</sup> = 0.90
Pig slurry	169.4 d	181.8 Ac	185.4 Ab	194.8 Aa	$\hat{y} = 170.2 + 6.3x - 0.4x^2$ R <sup>2</sup> = 0.93
Mean	169.4 d	177.4 c	181.9 b	187.3 a	
Soluble (mg kg <sup>-1</sup> )					
Cu oxide	nd	nd	nd	nd	nd
Pig slurry	nd	nd	nd	nd	nd
Mean	nd	nd	nd	nd	
Exchangeable (mg kg <sup>-1</sup> )					
Cu oxide	0.27 c	0.30 bc	0.32 Bab	0.36 Ba	$\hat{y} = 0.3 + 0.02x - 0.0009x^2$ R <sup>2</sup> = 0.67
Pig slurry	0.27 d	0.33 c	0.51 Ab	0.77 Aa	$\hat{y} = 0.3 + 0.06x + 0.002x^2$ R <sup>2</sup> = 0.97
Mean	0.27 d	0.30 c	0.42 b	0.56 a	
Organic matter (mg kg <sup>-1</sup> )					
Cu oxide	6.41 b	5.60 Ab	6.99 Aa	6.37 Aa	$\bar{y} = 6.34$
Pig slurry	6.41 b	4.18 Bc	5.62 Ba	5.64 Ba	$\hat{y} = 6.0 - 0.65x + 0.09x^2$ R <sup>2</sup> = 0.31
Mean	6.41 b	4.89 c	6.31 a	6.00 a	
Aluminum oxide (mg kg <sup>-1</sup> )					
Cu oxide	nd	nd	nd	nd	nd
Pig slurry	nd	nd	nd	nd	nd
Mean	nd	nd	nd	nd	
Iron oxide (mg kg <sup>-1</sup> )					
Cu oxide	51.2 b	47.1 Bc	53.1 Ba	55.6 Ba	$\bar{y} = 52.3$
Pig slurry	51.2 c	52.8 Ac	57.6 Ab	62.3 Aa	$\hat{y} = 50.9 + 1.7x - 0.007x^2$ R <sup>2</sup> = 0.84
Mean	51.2 b	50.5 b	55.9 a	58.5 a	
Residual (mg kg <sup>-1</sup> )					
Cu oxide	111.2 b	118.9 Ba	116.7 Ba	117.5 Ba	$\bar{y} = 116.2$
Pig slurry	111.2 b	124.5 Aa	121.8 Aa	126.1 Aa	$\bar{y} = 118.5$
Mean	111.2 a	121.7 a	119.3 a	121.8 a	

Means followed by different letters (lowercase in rows and uppercase in columns) differ ( $p \leq 0.05$ ) by Tukey test; nd - Value below the detection limit of the analytical method

mineral fraction of the soil occurred with the application of Cu in the form of PS, in relation to the form of oxide, at both doses.

The contents of Cu bound to Al oxides were below the detection limit of the analytical method, and only traces of this element were found (Table 1). Similar response in this fraction occurred for Zn in the soil (Table 4). The lack of interference of the application of Cu and Zn in the form of oxide or PS in the soil in this mineral fraction may be due to the predominance, at higher proportions in the clay fraction, of crystalline forms of Fe in relation to those of Al.

There was an increase in Cu content in the residual fraction with the Cu doses only with the application of PS (Table 1). This fraction is considered as inert and is not extracted in the other steps of the fractionation, but is considered as a chemically stable and biologically inactive fraction (Sipos et al., 2008; Souza et al., 2012).

Most of the Cu in the soil was found in the residual fraction, followed by Fe oxides, organic and exchangeable, indicating a strong Cu adsorption to the functional groups of the soil (Table 1), with predominance of the fraction of Fe oxides. Cu is adsorbed in the soil through covalent bonds, being strongly retained and forming complexes of high bonding energy, especially on surfaces containing hydroxyl groups, such as in hydroxides and oxyhydroxides of Fe, Al and Mn (Mallmann et al., 2012).

There was no difference in the content of Cu in the shoots of bean plants in response to the application of Cu in the soil in the form of oxide or PS (Table 2); however, for the root, there was increasing quadratic behavior in the Cu content, with maximum response at the Cu doses of 3.3 and 6.8 kg ha<sup>-1</sup> for the fertilizer in the form of oxide and PS, respectively.

The application of PS promoted greater accumulation of Cu in shoots and roots, with increasing quadratic behavior and

maximum accumulation at the doses of 5.5 and 5.0 kg ha<sup>-1</sup> for bean shoots and roots, respectively (Table 3). Cu accumulation in bean plants is related to the availability of this micronutrient in the soil, mainly represented by the exchangeable fraction (Table 1).

The total Zn content in the soil showed an increasing quadratic behavior, with maximum response at the doses of 20 and 24 kg ha<sup>-1</sup> of Zn in the form of oxide and PS, respectively; the treatment with Zn oxide was superior to that with PS in the availability of this micronutrients at the applied doses (Table 4). The highest Zn availability in the soil, demonstrated by the total contents in the mineral fertilizer in relation to PS, is related to the complexation of this micronutrient by carbon chains present in the PS, unlike the form of Zn oxide.

The content of soluble Zn in the soil increased in a similar way in both treatments, with slight superiority for PS at the doses of 6 and 24 kg ha<sup>-1</sup> and for the mineral in the form of Zn oxide at the dose of 12 kg ha<sup>-1</sup> (Table 4). For this fraction, there was an increasing quadratic response, with highest Zn content at the highest dose in both treatments. Unlike Cu in the soil, Zn is more weakly bound to clay minerals and adsorbents in the form of oxides, hydroxides and oxyhydroxides in the soil, making this metal more labile and bioavailable (Sipos et al., 2008; Paradelo et al., 2011; Mallmann et al., 2012), which explains the higher presence of this element in the soluble and exchangeable fractions of the soil.

Total Zn, exchangeable Zn and Zn bound to Fe oxides in the soil showed the highest contents with the application of Zn in the form of oxide (Table 4). The lower intensity of bonding force of Zn to soil colloids, compared with Cu (Sipos et al., 2008; Uprety et al., 2009), allows the mineral fertilizer to promote greater availability, a condition that occurred in the three forms of Zn in the soil

Table 2. Copper (Cu) contents in shoots and roots of bean plants in response to the application of different doses of this element in the soil in the forms of mineral oxide or pig slurry

Fertilizer	Dose (kg ha <sup>-1</sup> )				Regression equation
	0	1.7	3.4	6.8	
Shoots (mg kg <sup>-1</sup> )					
Cu oxide	10.9	10.5	10.0	10.7	$\hat{y} = 10.6$
Pig slurry	11.2	10.5	10.6	11.1	$\hat{y} = 10.9$
Mean	11.0	10.5	10.3	10.9	
Roots (mg kg <sup>-1</sup> )					
Cu oxide	67.5 c	110.7 Aa	89.0 b	72.1 Bc	$\hat{y} = 73.5 + 16.7x - 2.5x^2$ R <sup>2</sup> = 0.57
Pig slurry	67.5 c	74.1 Bc	88.1 b	101.2 Aa	$y = 66.5 + 6.4x - 0.2x^2$ R <sup>2</sup> = 0.93
Mean	67.5 c	92.4 a	88.6 ab	86.7 b	

Means followed by different letters (lowercase in rows and uppercase in columns) differ ( $p \leq 0.05$ ) by Tukey test

Table 3. Copper (Cu) accumulation in shoots and roots of bean plants in response to the application of doses of this element in the forms of mineral oxide or pig slurry

Fertilizer	Dose (kg ha <sup>-1</sup> )				Regression equation
	0	1.7	3.4	6.8	
Shoots (mg pot <sup>-1</sup> )					
Cu oxide	0.17 b	0.20 Ba	0.20 Ba	0.20 Ba	$\hat{y} = 0.19$
Pig Slurry	0.17 d	0.25 Ac	0.42 Aa	0.33 Ab	$\hat{y} = 0.2 + 0.11x - 0.01x^2$ R <sup>2</sup> = 0.84
Mean	0.17 d	0.22 c	0.31 a	0.26 b	
Roots (mg pot <sup>-1</sup> )					
Cu oxide	0.22 d	0.39 Aa	0.31 Bb	0.25 Bc	$\hat{y} = 0.2 + 0.06x - 0.01x^2$ R <sup>2</sup> = 0.54
Pig Slurry	0.22 c	0.33 Bb	0.42 Aa	0.41 Aa	$\hat{y} = 0.2 + 0.08x - 0.008x^2$ R <sup>2</sup> = 0.97
Mean	0.22 c	0.36 a	0.36 a	0.33 b	

Means followed by different letters (lowercase in rows and uppercase in columns) differ ( $p \leq 0.05$ ) by Tukey test

Table 4. Zinc (Zn) contents in different soil fractions in response to the application of different doses of this element in the forms of mineral oxide or pig slurry

Fertilizer	Dose (kg ha <sup>-1</sup> )				Regression equation
	0	6	12	24	
Total (mg kg <sup>-1</sup> )					
Zn oxide	101.3 d	109.7 Ac	114.3 Ab	116.6 Aa	$\hat{y} = 101.4 + 1.6x - 0.04x^2$ R <sup>2</sup> = 0.96
Pig slurry	101.3 d	106.7 Bc	108.9 Bb	114.4 Ba	$\hat{y} = 101.7 + 0.8x - 0.01x^2$ R <sup>2</sup> = 0.92
Mean	101.3 d	108.2 c	111.6 b	115.5 a	
Soluble (mg kg <sup>-1</sup> )					
Zn oxide	0.34 d	0.50 Bc	0.98 Ab	1.22 Ba	$\hat{y} = 0.3 + 0.06x - 0.0008x^2$ R <sup>2</sup> = 0.92
Pig slurry	0.34 d	0.60 Ac	0.70 Bb	1.42 Aa	$\hat{y} = 0.4 + 0.02x - 0.001x^2$ R <sup>2</sup> = 0.98
Mean	0.34 d	0.55 c	0.84 b	1.32 a	
Exchangeable (mg kg <sup>-1</sup> )					
Zn oxide	1.59 c	2.11 c	5.52 Ab	7.53 Aa	$\hat{y} = 1.2 + 0.3x - 0.002x^2$ R <sup>2</sup> = 0.92
Pig slurry	1.59 d	2.50 c	4.26 Bb	7.02 Ba	$\hat{y} = 1.5 + 0.2x - 0.001x^2$ R <sup>2</sup> = 0.97
Mean	1.59 d	2.30 c	4.89 b	7.28 a	
Organic matter (mg kg <sup>-1</sup> )					
Zn oxide	0.99 a	0.77 Ab	0.67 Ab	0.36 Ac	$\hat{y} = 0.98 - 0.03x - 0.0002x^2$ R <sup>2</sup> = 0.91
Pig slurry	0.99 a	0.27 Bb	0.04 Bc	0.04 Bc	$\hat{y} = 0.96 - 0.12x - 0.004x^2$ R <sup>2</sup> = 0.97
Mean	0.99 a	0.52 b	0.35 c	0.20 d	
Aluminum oxide (mg kg <sup>-1</sup> )					
Zn oxide	Nd	nd	nd	nd	nd
Pig slurry	Nd	nd	nd	nd	nd
Mean	Nd	nd	nd	nd	
Iron oxide (mg kg <sup>-1</sup> )					
Zn oxide	13.9 d	18.6 Ac	20.2 Ab	22.8 Aa	$\hat{y} = 14.1 - 0.7x - 0.02x^2$ R <sup>2</sup> = 0.93
Pig slurry	13.9 c	14.4 Bc	18.1 Bb	19.8 Ba	$\hat{y} = 13.4 + 0.4x - 0.004x^2$ R <sup>2</sup> = 0.82
Mean	13.9 d	16.5 c	19.2 b	21.3 a	
Residual (mg kg <sup>-1</sup> )					
Zn oxide	84.5 b	87.8 a	86.9 ab	84.7 b	$\hat{y} = 84.8 + 0.05x - 0.002x^2$ R <sup>2</sup> = 0.41
Pig slurry	84.5 b	89.0 a	85.7 b	86.0 b	$\bar{y} = 86.3$
Mean	84.5 c	88.4 a	86.3 b	85.4 bc	

Means followed by different letters (lowercase in rows and uppercase in columns) differ ( $p \leq 0.05$ ) by Tukey test; nd - Value below the detection limit of the analytical method

The application of increasing doses of Zn in the soil in the form of oxide or PS resulted in decrease of the Zn bound to SOM, and the doses of 24 and 15 kg ha<sup>-1</sup> showed lower reduction (Table 4). The reduction in the content of Zn bound to SOM, in both cases, can be related to the displacement of this organic form to the exchangeable and soluble forms, resulting in greater availability to the plant.

For the fraction of residual Zn in the soil, there was an increasing quadratic response for Zn added in the form of oxide, with maximum content obtained at the dose of 12.5 kg ha<sup>-1</sup>, and there was no response for PS (Table 4). In the condition of residual Zn, there was small interference in relation to the increase of the doses of this micronutrient in the soil, in both organic and mineral forms.

For Zn contents in the shoots of bean plants, there was a decreasing quadratic behavior in both treatments as a function of the applied Zn doses, and the highest contents

were observed in the absence of application of this element in the soil (Table 5), while in the roots there was an increasing quadratic behavior, with the highest Zn contents at the highest dose applied in both treatments.

PS application resulted in greater Zn accumulation in both shoots and roots of bean plants, with increasing quadratic behavior, and the most effective dose was 24 kg ha<sup>-1</sup> in both treatments (Table 6), although the mineral treatment in the form of oxide showed higher Zn content in the forms of total, exchangeable and bound to Fe oxides (Table 4).

The application of increasing doses of PS in the soil resulted in greater production of shoot and root dry biomass in bean plants, in relation to the form of oxide, with increasing quadratic behavior (Table 7), and the dose of 3.4/12 kg ha<sup>-1</sup> of Cu/Zn showed the highest response. At the doses of 6.8/24 kg ha<sup>-1</sup> of Cu/Zn in the form of oxide and PS, there was a decrease in the production of shoot dry biomass, demonstrating probable effect of toxicity.

Table 5. Zinc (Zn) contents in shoots and roots of bean plants in response to the application of different doses of this element in the soil in the forms of mineral oxide or pig slurry

Fertilizer	Dose (kg ha <sup>-1</sup> )				Regression equation
	0	6	12	24	
Shoots (mg kg <sup>-1</sup> )					
Zn oxide	434.1 a	227.5 Bd	276.0 Ac	355.9 Ab	$\hat{y} = 412.7 - 27.8x + 1.1x^2$ R <sup>2</sup> = 0.72
Pig slurry	434.1 a	281.7 Ab	163.2 Bc	292.1 Bb	$\hat{y} = 441.3 - 37.6x + 1.3x^2$ R <sup>2</sup> = 0.94
Mean	434.1 a	254.6 c	219.6 d	324.0 b	
Roots (mg kg <sup>-1</sup> )					
Zn oxide	72.2 d	158.3 Ac	171.1 b	261.6 a	$\hat{y} = 80 + 10.3x - 0.1x^2$ R <sup>2</sup> = 0.94
Pig slurry	72.2 d	106.3 Bc	164.4 b	264.2 a	$\hat{y} = 69.8 + 6.8x - 0.05x^2$ R <sup>2</sup> = 0.99
Mean	72.2 d	132.3 c	167.7 b	262.9 a	

Means followed by different letters (lowercase in rows and uppercase in columns) differ ( $p \leq 0.05$ ) by Tukey test

Table 6. Zinc (Zn) accumulation in shoots and roots of bean plants in response to the application of different doses of this element in the soil in the forms of mineral oxide or pig slurry

Fertilizer	Dose (kg ha <sup>-1</sup> )				Regression equation
	0	6	12	24	
Shoots (mg pot <sup>-1</sup> )					
Zn oxide	6.4 a	4.3 Bc	5.6 Bb	6.7 Ba	$\hat{y} = 6.1 - 0.2x + 0.01x^2$ R <sup>2</sup> = 0.54
Pig slurry	6.4 b	6.6 Ab	6.5 Ab	8.6 Aa	$\hat{y} = 6.5 - 0.06x + 0.006x^2$ R <sup>2</sup> = 0.81
Mean	6.4 b	5.5 c	6.1 b	7.6 a	
Roots (mg pot <sup>-1</sup> )					
Zn oxide	0.24 c	0.56 Ab	0.59 Bb	0.92 Ba	$\hat{y} = 0.3 + 0.04x - 0.0005x^2$ R <sup>2</sup> = 0.94
Pig slurry	0.24 d	0.47 Bc	0.78 Ab	1.08 Aa	$\hat{y} = 0.2 + 0.05x - 0.0007x^2$ R <sup>2</sup> = 0.99
Mean	0.24 d	0.52 c	0.69 b	1.00 a	

Means followed by different letters (lowercase in rows and uppercase in columns) differ ( $p \leq 0.05$ ) by Tukey test

Table 7. Shoot and root dry biomass of bean plants in response to the application of different doses of copper (Cu) and zinc (Zn) in the forms of mineral oxide or pig slurry

Fertilizer	Cu and Zn dose (kg ha <sup>-1</sup> )				Regression equation
	0	1.7/6	3.4/12	6.8/24	
Shoots (g pot <sup>-1</sup> )					
Cu/Zn oxide	14.85	18.74	20.48 B	18.85 B	$\hat{y} = 13.1 + 3.2x - 0.1x^2$ R <sup>2</sup> = 0.96
Pig slurry	14.85 c	23.60 bc	39.79 Aa	29.43 Ab	$\hat{y} = 14.9 + 0.8x - 0.03x^2$ R <sup>2</sup> = 0.96
Mean	14.85 b	21.17 b	30.14 a	24.14 ab	
Roots (g pot <sup>-1</sup> )					
Cu/Zn oxide	3.33	3.56 B	3.46 B	3.50	$\hat{y} = 3.4 + 0.02x - 0.0007x^2$ R <sup>2</sup> = 0.52
Pig slurry	3.33 b	5.33 Aa	6.03 Aa	4.66 a	$\hat{y} = 3.4 + 0.4x - 0.01x^2$ R <sup>2</sup> = 0.99
Mean	3.33	4.45	4.75	4.08	

Means followed by different letters (lowercase in rows and uppercase in columns) differ ( $p \leq 0.05$ ) by Tukey test

## CONCLUSIONS

1. The application of Cu in the form of pig slurry (PS) increased Cu contents in the soil in the fractions available, exchangeable, Fe oxides and residual, while its application in the form of oxide increased Cu contents in the fraction associated with soil organic matter (SOM).

2. The Zn contents in the fractions available, exchangeable and SOM were higher when Zn was applied in the form of oxide, and higher in the soluble fraction when Zn was applied in the form of PS.

3. Among the evaluated fractions, the main bond of Cu and Zn in the soils occurred with Fe oxides, regardless of the source, for the condition of a Rhodic Kandiodox.

4. The highest content of Cu available in the soil occurred when it was applied in the form of PS and, for Zn, in the form of oxide, reflecting in the higher absorption of these nutrients by the bean plants.

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