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Research Article

Soil-applied Zn effect on soil fractions

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ABSTRACT: The interaction of Zn with soil compartments influences its bioavailability and uptake by plants. In this study, rice and soybean were cultivated under greenhouse conditions with the aim of evaluating Zn bioavailability and fractionation in a clayey-textured Typic Hapludox as a function of Zn rates (4 or 8 mg kg^{-1} Zn). The experiment was conducted until grain filling. Two soil subsamples (t_1 and t_2) that referred to the seeding and flowering stages, were collected and compared with two single extraction schemes, DTPA (Zn_{DTPAt1} and Zn_{DTPAt2}) and Mehlich-1 (Zn_{Mt1} and Zn_{Mt2}) for Zn available contents. Zn fractionation was carried out with t_2 soil subsamples for the testing of the following fractions: exchangeable Zn (Zn_{Exc}), Zn bound to carbonates (Zn_{Carb}), Zn bound to organic matter (Zn_{OM}), Zn bound to oxides (Zn_{Oxi}) and residual Zn (Zn_{res}). Zn applied to soil increased the Zn concentration in labile fractions in decreasing order as follows: $Zn_{Exc} > Zn_{OM} > Zn_{Carb}$. There was no difference between the lesser or unavailable fractions, Zn_{Oxi} and $Zn_{res,}$ when there was no correlation between the rates either with total accumulated Zn in plants (Zna_{total}), or the contents extracted by DTPA or Mehlich-1. Total cumulative Zn content in rice and soybean affected by the $ZnCl_2$ rates applied were positively correlated with Zn content extracted by both solutions. Both extractant solutions presented positive correlation between available contents of Zn with Zn bound to labile fractions.

Keywords: DTPA, Mehlich-1, soil phases, soil chemistry, micronutrient

Introduction

Zinc (Zn) participates in biogeochemical processes in soil, sediments, and aquatic settings (Cloquet et al., 2008), but is the most widespread in terms of deficiency in micronutrients for a number of crops all over the world (Dobermann and Fairhust, 2000). The dynamics of Zn in soil-plant systems have important economic and environmental implications, as Zn is a bioessential element for the growth and reproduction of living organisms (Frassinetti et al., 2006). Its behavior in the soil is complex because it depends on the dynamics of the soil compounds (Alexakis, 2011), whose heterogeneity enables its presence in several forms, whether soluble or weak or as Zn bound to soil particles (Abreu et al., 2007).

Zn bioavailability and remobilization are readily modified by environmental conditions. Physical and chemical variations can significantly change the balance reactions between the soil solution and organic or inorganic fractions (Gismera et al., 2004; Tessier et al., 1979). A deep understanding of Zn chemical reactions within soil components and its geochemical behavior are fundamental to the characterization of nutrient availability to plants. Zn is sorbed mainly to organic matter, oxides and carbonates, which can be conceptually determined by sequential extractions to obtain the sorbed Zn distributed in acid soluble, reducible, oxidisable and residual fractions present in the soil (Rauret et al., 1999). Its distribution in each fraction depends on the soil organic matter content, pH, temperature (Tiller et al., 1984), texture, structure, amount and type of clay minerals (Spark and Wells, 1995), cation exchange properties, metal oxide fractions (Stahl and James, 1991; Guadalix and Pardo, 1995) and transformations in the source material (Fontes and Alleoni, 2006). The total Zn in the soil does not represent the Zn bioavailability properly, since the available Zn easily interacts in chemical reactions, which varies according to the soil physical or chemical characteristics and water parameters resulting in a temporary increasing in available Zn followed by a decreasing in available Zn for plants (Impa et al., 2012). Therefore, the Zn distribution must be distinguished between the fractions of soil.

This study addresses an evaluation of Zn bioavailability to rice (*Oryza sativa* L.) and soybean (*Glycine max* L. Merrill) and its fractionation in a clayey-textured soil as a function of soil-applied Zn rates.

Materials and Methods

Soil samples

The Zn fractionation in soil was evaluated in a typical low Zn-content humid tropical soil. Surface (0-20 cm) samples of a clayey-textured Typic Hapludox (Soil Survey Staff, 1999) were collected from a field near Piracicaba (22°42′30″ S, 47°38′00″ W; altitude: 546 m) in the state of São Paulo (SP), Brazil. Soil samples were air-dried, crushed and sieved through a 2 mm screen.

Chemical and physical characterizations were conducted according to van Raij et al. (2001) and Camargo et al. (1986), respectively (Table 1). The determination of potentially available Zn content was conducted using 20 g of dry soil sample, which was extracted with 40 mL of a DTPA solution and the concentration determined by flame atomic absorption spectrophotometry (FAAS) as described in the Chemical Analysis Manual for Tropical Soils (van Raij et al., 2001).

Table 1 – Chemical and physical properties of soil studied. Data reported as a mean of triplicate determinations, except for unreplicated textural determinations.

Parameters (unit)	Value
pH (0.01 mol L^{-1} $CaCl_2$)	3.8
P (mg dm^{-3})	7
K ($mmol_c$ dm^{-3})	1.5
Ca ($mmol_c$ dm^{-3})	4
Mg ($mmol_c$ dm^{-3})	2
H+Al ($mmol_c$ dm^{-3})	82
BS[a] ($mmol_c$ dm^{-3})	7.5
CEC[b] ($mmol_c$ dm^{-3})	90.2
V_c (%)	9
Na (mg dm^{-3})	4.5
Si (mg dm^{-3})	6.3
Zn (mg dm^{-3})	0.7
Cu (mg dm^{-3})	0.9
Fe (mg dm^{-3})	60.3
Mn (mg dm^{-3})	7.7
WHC[d] (g dm^{-3})	168.3
OM[e] (g dm^{-3})	26
Sand (g dm^{-3})	546
Silt (g dm^{-3})	83
Clay (g dm^{-3})	371

Notes: [a]BS = sum of basis; [b]CEC = cation exchange capacity; [c]V = percentage of soil base saturation; [d]WHC = water-holding capacity; [e]OM = organic matter.

Zn uptake experiment

The effects of Zn on the soil and its bioavailability were evaluated in a greenhouse experiment. Three kg of air-dried and sieved soil samples were placed in 4 dm^3 plastic pots. The soil samples' base saturation was raised to 50 % for rice and 60 % for soybean crops by liming (Cantarella and Furlani, 1997; Trani and van Raij, 1997). The samples were homogenized and incubated for 30 days and 70 % of the water-holding capacity of the soil was maintained. A $ZnCl_2$ solution was added at the rates of 4 and 8 mg kg^{-1}, in four replicates and two crops (rice and soybean), which gave a total of 24 experimental units; including 4 control samples (0 mg kg^{-1} of Zn added). These rates were selected according to Novais et al. (1991) for basic fertilization in controlled condition experiments (Zn recommendation – 4 mg kg^{-1}). The samples were watered with deionized water (70 % water-holding capacity – WHC) for two days to achieve Zn equilibrium in the soil.

Rice seeds from the IAC-203/Mococa-13 cultivar or soybean seeds of UFV-16/Capinópolis cultivar were used at a seven seed per pot rate from the growth period to grain filling. Both crops carry a certain social importance since they are the usual sources of carbohydrate and protein for the human diet; mainly in developing countries. In addition, rice and soybean are fundamental to the national economy in terms of agricultural activities.

All treatments were fertilized in two steps; the first one on the same day of sowing and the second, one month after the first germination. The nutritive solution was composed of N (250 mg dm^{-3}: 59.48 mg dm^{-3}, as urea; 155.52 mg dm^{-3}, as monopotassium phosphate – MAP, and 35.00 mg dm^{-3}, as ammonium sulphate – AS), P (297.00 mg dm^{-3} as MAP), K (95.26 mg dm^{-3} as KCl), S (40.00 mg dm^{-3} as AS), B (0.60 mg dm^{-3} as boric acid), Cu (0.60 mg dm^{-3} as copper sulphate) and Mo (0.15 mg dm^{-3} as molybdic acid), according to recommendations by Malavolta (1980) and Novais et al. (1991). The soil moisture content was watered down to 70 % WHC applied in the surface soil and two soil subsamples (t_1 and t_2) were collected in the seeding and flowering stages, respectively.

The plants were cultivated up to the physiological maturity of the grains, i.e. 98 days after germination for soybean and 108 days for rice. The stems were cut approximately 0.5 cm above the soil surface and the vegetable samples dried in a forced circulation oven at 65 °C for 72 h. After drying, the samples were weighed to obtain the dry matter. Total Zn content in the plant subsamples (Zna_{total}) for both crops was determined after nitro-perchloric digestion (Miyazawa et al., 2009) in the plant subsamples by multiplying the total Zn content by the weight of the dry matter.

Zn extraction procedures

Single extractions

Representative soil samples of each vessel in t_1 and t_2 sampling periods were extracted by two chemical reagents (DTPA and Mehlich-1) for evaluating the available fraction. Both extractants are widely used; DTPA is a stable chelating agent, while Mehlich-1 has an acid character. Although micronutrient extraction by DTPA is a widespread procedure, here we compared it with Mehlich-1.

Dry soil (20 g) comprised extracted solution mixed with 40 mL of 0.005 M diethylenetriaminepentaacetic acid (DTPA) in 0.01 mol L^{-1} $CaCl_2$ buffered at pH 7.3 with 0.1 mol L^{-1} triethanolamine (TEA), at room temperature, and shaken for 2 h (Lindsay and Norvell, 1978). Another three grams (3 g) were mixed with 15 mL of a Mehlich-1 extractant solution (HCl 0.05 mol L^{-1} + H_2SO_4 0.0125 mol L^{-1}), at room temperature, and shaken for 5 min (Embrapa, 1997). The resulting mixture was filtered at 0.45 µm and the filtrate was determined by FAAS so as to obtain the following extracted Zn parameters: Zn_{DTPAt1}, Zn_{DTPAt2}, Zn_{Mt1} and Zn_{Mt2}.

Zn fractionation in soil

Zn was sequentially extracted from soil compartments for the purpose of assessing Zn fractions (phases). The method developed by Silveira et al. (2006) calibrated for tropical soil conditions was selected from among the several fractionation procedures for estimating the extractable metal pools as regards metal-bearing soil phases (Alloway, 1995; Asami et al., 1995; Cabral and Lefebvre, 1998; Rauret et al., 1999; Tessier et al., 1979). A five-stage sequential scheme was implemented with

1 g of t_2 soil subsampling from each experimental unit (Table 2). Such a subsample is justified by the flowering stage, which enables correlation of the highest absorbed concentration of nutrients by plants.

Exchangeable Zn (Zn_{Exc}) and Zn bound to carbonate (Zn_{Carb}), organic matter (Zn_{OM}), oxides (Zn_{Oxi}) and residual phases (Zn_{res}) were determined. The same soil subsamples were used in the development of the fractions by distinct processes and under different conditions. Additionally, pseudo-total Zn (Zn_{PST}), was determined by the same method used for Zn_{res} by microwave-assisted acid digestion with HNO_3 + HCl, as described by the USEPA 3051A methodology. All concentration was determined by FAAS and the sum of extracted Zn (Zn_{SUM}) and Recovery (Rec) was calculated by equations 1 and 2.

$$Zn_{SUM} = Zn_{Exc} + Zn_{Carb} + Zn_{OM} + Zn_{Oxi} + Zn_{res} \qquad (1)$$

$$\text{Rec } (\%) = \frac{Zn_{SUM}}{Zn_{PST}} \times 100 \qquad (2)$$

Statistical analysis

The experiment was conducted in a factorial design between three $ZnCl_2$ rates (0, 4 and 8 mg kg^{-1}) and two crops, with four replications distributed in a completely randomized design for both crops. Averages and standard deviations of the replicate data were calculated and the significance of treatment factors was assessed by analysis of variance (ANOVA) using statistical software (SAS - Statistical Analysis System, v. 9.4). Treatment averages were compared by Tukey's significant difference procedure ($p < 0.05$) for detecting differences in the Zn fractions. Data behaviors were represented by fitting regression models using SigmaPlot software. After the descriptive analysis of the response variables, a Pearson's correlation analysis was performed to assess the contribution among Zn fractions in the soil (Zn_{Exc}, Zn_{Carb}, Zn_{OM}, Zn_{Oxi} and Zn_{res}) and other variables (Zna_{total}, Zn_{PST}, Zn_{DTPAt1}, Zn_{DTPAt2}, Zn_{Mt1} and Zn_{Mt2}).

Results and Discussion

Zinc fractionation in soil

Zn phases were influenced by the $ZnCl_2$ concentration and by the interaction between particle-soil solution interfaces. The Zn concentration varied according to the $ZnCl_2$ rates applied and was different for the crops, which were evaluated in a proportional distribution (Figures 1 and 2). In general, Zn_{res} was the most abundant phase for the highest rate, i.e. 8 mg kg^{-1}, followed by Zn_{Oxi}, Zn_{Exc}, Zn_{OM} and Zn_{Carb} in soil cultivated with rice. Zn_{res} was also the most expressive phase for soybean cultivation, followed by Zn_{Exc}, $Zn_{Oxi} = Zn_{OM}$ and Zn_{Carb}, with no difference between the Zn_{Oxi} and Zn_{OM} proportions. Soil samples treated with 4 mg kg^{-1} had the following designs: $Zn_{res} > Zn_{Oxi} > Zn_{Exc} > Zn_{OM} = Zn_{Carb}$ for soil cultivated with rice and $Zn_{res} > Zn_{Oxi} > Zn_{Exc} = Zn_{OM} > Zn_{Carb}$ for soil cultivated with soybean. Finally, the control samples, without any Zn addition, were described by $Zn_{res} > Zn_{Oxi} > Zn_{OM} > Zn_{Exc} > Zn_{Carb}$ for soil cultivated with rice and $Zn_{res} > Zn_{Oxi} > Zn_{OM} > Zn_{Exc} = Zn_{Carb}$ for soybean.

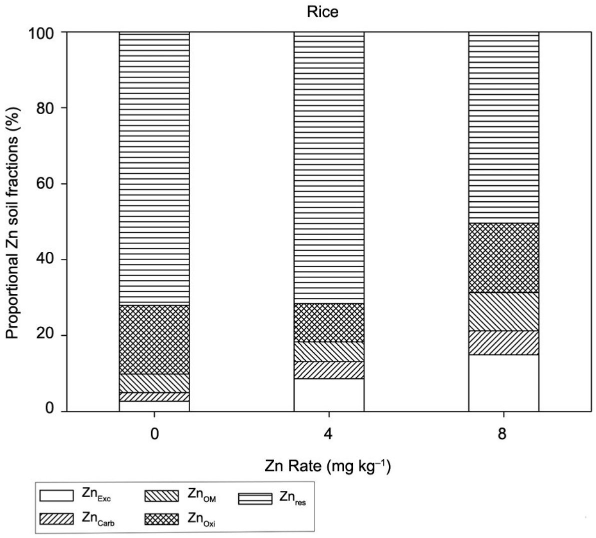

Figure 1 – Zinc fractions distribution affected by $ZnCl_2$ addition in soil cultivated with rice (exchangeable Zn, Zn_{Exc}; Zn bound to carbonates, Zn_{Carb}; Zn bound to organic matter, Zn_{OM}; Zn bound to oxides, Zn_{Oxi} and residual Zn, Zn_{res}). Data are represented by means of quadruplicate.

Table 2 – Sequential extraction procedure: extractants solution, specific condition and extraction phase of soil at each extraction step.

Steps	Extractant solution	Shaking and centrifuge condition	Other specification	Fraction
1	0.1 mol L^{-1} Calcium Chloride	180 rpm for 2 h 3000 rpm for 10 min	room temperature	Zn_{Exc} (exchangeable, soluble)
2	Concentrated Sodium Acetate	180 rpm for 5 h 3000 rpm for 10 min	pH adjusted to 5	Zn_{Carb} (bound to carbonates)
3	5 % Sodium Hypochlorite	3000 rpm 10 min	pH adjusted to 8.5 water bath (90 °C) for 30 min	Zn_{OM} (oxidisable, bound to organic matter)
4	0.2 mol L^{-1} Ammonium Oxalate 0.2 mol L^{-1} Oxalic Acid 0.1 mol L^{-1} Ascorbic Acid	3000 rpm 10 min	pH adjusted to 3 water bath (90 °C) for 30 min	Zn_{Oxi} (reducible, bound to oxi/hydroxides)
5	Concentrated Nitric Acid and Hydrochloric acid	-	microwave digestion	Zn_{res} (residual, bound to silicate minerals)

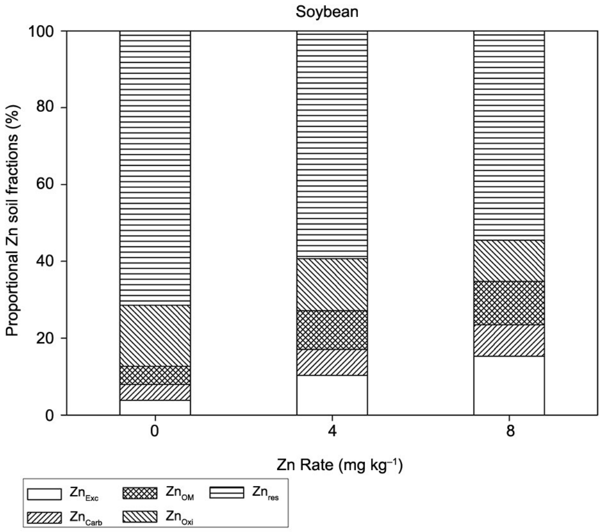

Figure 2 – Zinc fractions distribution affected by $ZnCl_2$ addition in soil cultivated with soybean (exchangeable Zn, Zn_{Exc}; Zn bound to carbonates, Zn_{Carb}; Zn bound to organic matter, Zn_{OM}; Zn bound to oxides, Zn_{Oxi} and residual Zn, Zn_{res}). Data are represented by means of quadruplicate.

Zn_{res} was proportionally the highest phase for all Zn rates in the soil samples cultivated with rice and soybean. Yang et al. (2013) also observed Zn in the residual phase representing more than 50 % of total Zn in soil associated with Zn retention in crystalline structures which characterizes a stable phase (Fuentes et al., 2008). Under an expressive weathering soil condition, Zn^{2+} metallic ions bound favorably to the broken edges of 1:1 clay minerals, which hinder the Zn bioavailability in soil solution since ions are unavailable due to the adsorption to mineral soil components (Garcia et al., 2008).

Specific binds with Fe or Al hydroxides characterize the Zn_{Oxi} phase, as they involve high energy adsorption and prevent Zn availability in the soil solution (Kalbasi et al., 1978; Cavallaro and McBride, 1984). Leleyter et al. (2012) reported Zn binds expressively to oxides for two types of soil samples: from a privative garden (100 mg kg^{-1} Zn) and from an agricultural soil (50 mg kg^{-1} Zn). A comparison between Zn behavior in soil fractions and $ZnCl_2$ rates applied to soil (Figures 3A, B and 4A, B) revealed no differences Zn_{res} and Zn_{Oxi} for the crops due to the addition of the saline source. Soil particles expressively bonds Zn_{res} and Zn_{Oxi} phases, which are considered "non-available Zn for plants". Usually some forms of Zn in soil are not available to rice: Zn tightly bound to insoluble soil organic matter, Zn tightly adhering to soil particle surfaces (e.g. iron oxides, carbonates, or cation exchange sites), precipitated Zn (e.g. with sulfides or carbonates), or Zn in structural minerals (Impa, 2012).

Zn_{Exc}, Zn_{Carb} and Zn_{OM} phases, which may interact by solubilization and-or availability, promote greater mobility in the soil solution for plants. Only a small fraction of $ZnCl_2$ became available to plants in both samples, even at higher rates (Figures 3A and 4A). Zn_{Exc}, which is weakly bound in soil particles, but became available easily in the soil solution through exchanges of chemical bonds. In general, the concentration of the most labile fractions (Zn_{Exc}, Zn_{Carb} and Zn_{OM}) increased as a function of the Zn rates applied to the soil cultivated with rice or soybean. The average concentration of extracted Zn_{Exc} (Figure 3A) ranged from 0.40 (control) to 2.72 mg kg^{-1} (8 mg kg^{-1} rate) in the soil cultivated with rice, which demonstrates how the applied rates increases Zn in the exchangeable fraction. Similarly, in the soil cultivated with soybean, the concentration ranged from 0.50 (control) to 2.78 mg kg^{-1} (8 mg kg^{-1} rate), which shows slightly higher values (Figure 4A). Yang et al. (2013) found similar results for non-agricultural (82.7 mg kg^{-1} Zn; pH 7.85) and agricultural soil samples (157 mg kg^{-1}; pH 7.81), i.e. 5 % of Zn present in the exchangeable fraction, called "weak acid soluble fraction", and they also considered it the most available phase and of higher mobility. In alkaline soils, a low Zn bound to soluble phase was verified (Wu et al., 2010) attributable to the basic conditions (high pH) that promote favorable bounds formed between the charges of metals and mineral clays, i.e., oxides and residual phases (Dai, 2006). In another context, Fernández-Calviño et al. (2017) studied fractionation of heavy metals (BCR methodology) in pine bark amended in a soil from mine activities. They found that, in untreated soil, 96 % of Zn on residual fraction and in soil samples treated with a heavy metals mixture (1.50 mmol kg^{-1} of Zn) presented 88-92 % as soluble fraction and it rose higher with a period of incubation.

The soil-applied Zn rates modified Zn_{Exc} concentrations in soil from rice and soybean cultivation, except for samples from the 4 mg kg^{-1} rate in the soil cultivated with soybean, which presented similar concentrations to the highest rate samples (Figures 3A and 4A). Soil management in the form of fertilization, contributed to the Zn bioavailability in the exchangeable fraction, since it promotes changes in the chemistry equilibrium between soil solution and particles, although this fraction was not representative in Zn_{SUM} (eq. 1, Table 3). According to Oliveira et al. (1999), a 20 mg kg^{-1} Zn fertilization rate promoted the highest exchangeable fraction attributable to the higher homogeneous distribution in comparison to the other phases evaluated (mainly Mn oxides and amorphous and crystalline Fe oxides).

The Zn_{Carb} phase, which is less expressive in acid soils, ranged from 2 to 6 % (of the Zn_{SUM}) in soil cultivated with rice (Figure 1), which varies according to the rate applied (Figure 3A). However, higher concentrations were observed in the soil from soybean cultivation (Figure 2), probably due to the higher pH of the soil, required for the crop growth (pH 6.2 after liming). The extractant solution of sodium acetate used in fractionation reacts with the soil solution and solubilizes the functional groups from colloids, which releases Zn for the soil solution previously associated with acid groups.

The carbonate chemical balance indicates a prevalence of undissociated carbonic acid ($H_2CO_3^0$) in the soil

Table 3 – Pseudo total zinc extracted from soil (Zn_{PST}) cultivated with rice and soybean, according to USEPA 3051A method; sum of Zn extracted by fractionation (Zn_{SUM}), followed mean values and mean standard error and recovery Zn (Rec) as a function of the zinc rates added into the soil.

	Soil from rice cultivation			Soil from soybean cultivation		
Rate	Zn_{PST}	Zn_{SUM}	Rec	Zn_{PST}	Zn_{SUM}	Rec
	mg kg^{-1}		%	mg kg^{-1}		%
0	13.2 ± 0.7	16.2 ± 0.6	124 ± 7	9.5 ± 0.7	14.6 ± 0.7	154 ± 9
4	13.4 ± 1.0	18.1 ± 0.5	137 ± 8	12.4 ± 1.2	15.8 ± 0.9	132 ± 17
8	12.1 ± 0.9	19.3 ± 2.2	143 ± 3	12.4 ± 0.8	17.0 ± 0.9	127 ± 15

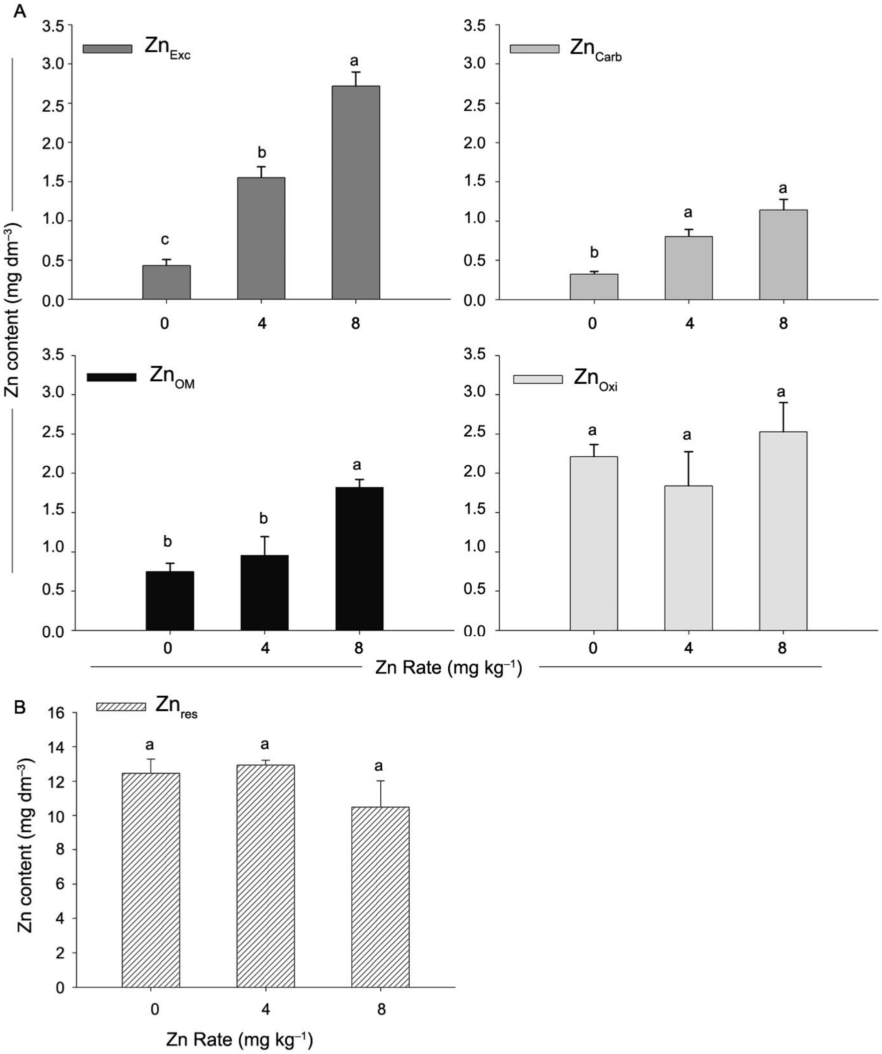

Figure 3 – A) Zinc fractions content extracted from soil cultivated with rice (exchangeable Zn, Zn_{Exc}; Zn bound to carbonates, Zn_{Carb}; Zn bound to organic matter, Zn_{OM}; Zn bound to oxides, Zn_{Oxi}) and B) residual Zn, Zn_{res}. The bars with lower case letters are significant (Tukey's multiple range test, $p < 0.05$). Data are represented by means ± standard errors.

pH, which explains the low proportion of Zn bound to carbonates (CO_3^{-2}) (see the diagram proposed by Lindsay, 1979). Under higher pH the bicarbonate (HCO_3^-) species precipitated as $Zn(HCO_3)_2$ prevails which results in a higher contribution of the Zn_{Carb} fraction. The Zn_{Carb} fraction is also associated with carbonate minerals (e.g. calcite) when an isomorphous substitution occurs between Ca^{2+} and other divalent metals, as Zn^{2+} (Auernheimer and Chinchon, 1997). The pH of the soil cultivated with soybean was 6.1, which characterizes the predominance of HCO_3^- activity in the soil solution, whereas the pH of the soil cultivated with rice was 5.9, which favors the formation of $Zn(HCO_3)_2$, as shown in the diagram proposed by Lindsay (1979).

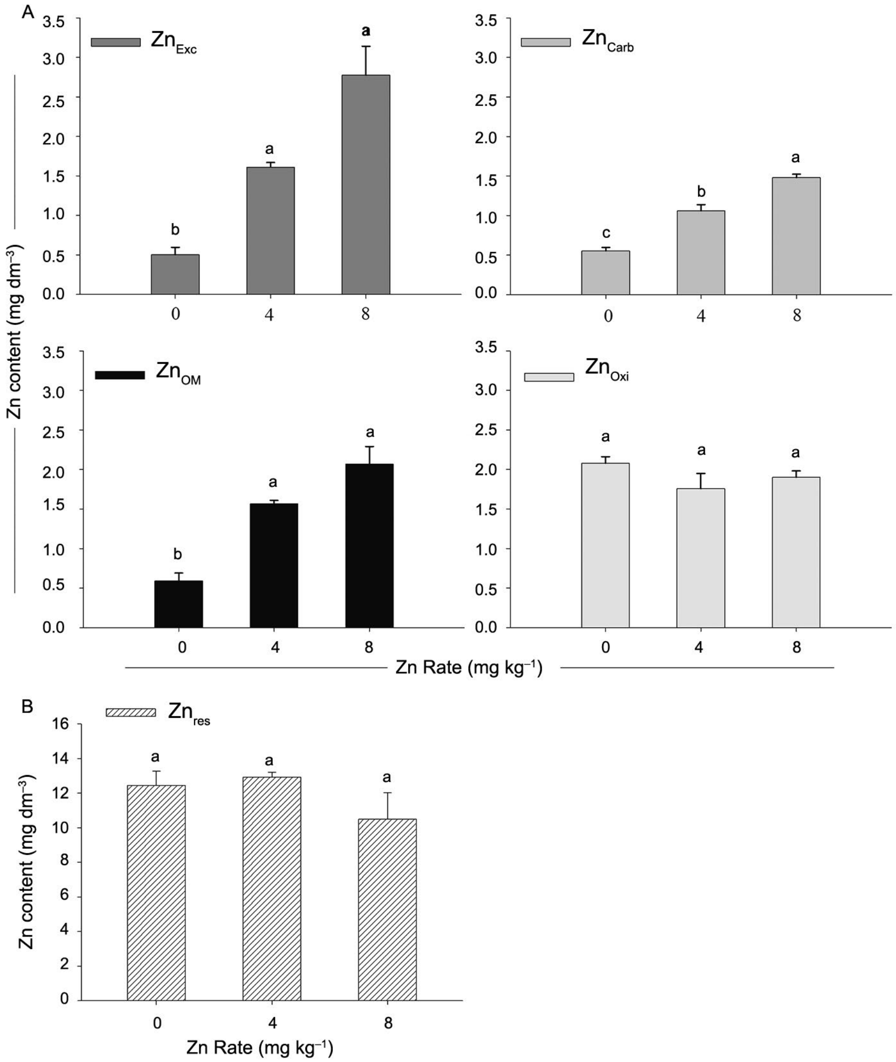

Figure 4 – A) Zinc fractions content extracted from soil cultivated with soybean (exchangeable Zn, Zn_{Exc}; Zn bound to carbonates, Zn_{Carb}; Zn bound to organic matter, Zn_{OM}; Zn bound to oxides, Zn_{Oxi}) and B) residual Zn, Zn_{res}. The bars with lower case letters are significant (Tukey's multiple range test, $p < 0.05$). Data are represented by means ± standard errors.

The concentrations of the Zn_{OM} fraction ranged from 5 to 10 % for soil cultivated with rice and from 5 to 11 % for soybean (Figures 1 and 2). The lower abundance of the Zn_{OM} fraction is due to the clayey-textured Typic Hapludox used in the experiment, which has a low OM content (Table 1). However, both crops cultivated varied as a function of the applied rates (Figures 3A and 4A). The 4 mg kg^{-1} rate for the soil cultivated with soybean exhibited the same behavior as the 8 mg kg^{-1}; however, the soil cultivated with rice presented no difference between the intermediary rate and the control, which presented a difference only with the highest rate applied. The low concentration of Zn bound to OM can be attributed to the application of the sodium hypochlorite extractant solution under alkaline conditions, which can result in sub-estimation promoted by the metal precipitation and redistribution of the element in soil phases (Miller et al., 1986; Tu et al., 1994).

The differences in soil phases can be associated with distinct rhizospheres behavior between the crops with the addition of a soluble Zn source in the soil, which changes the bioavailability condition and results in labile phases in plants. Due to the different Zn demands between these crops and the root system architecture of Poaceae (rice), and to Fabaceae (soybean), the plants' rhizospheres are different in terms of the root exudates concentrations "as organic acids" that change the rhizosphere pH and thus the Zn availability. Zn is absorbed in the rice and soybean crop by free Zn^{2+} (aq) and Zn complexes with organic compound forms (Suzuki et al., 2008). Moreover, Zn bioavailability is related to soluble inorganic compounds and the weak adsorption of organic and inorganic soil particles which characterize the cation exchange pool.

A Zn_{PST} analysis (Table 3) was conducted with a methodology that does not utilize hydrofluoric acid as extractant in order to evaluate the pseudo-total Zn and to compare to Zn_{SUM}. The method was selected because we considered that, in the environmental conditions; there is no solution capable of extracting the pools accessed by hydrofluoric acid.

The recovery was calculated for each support electrolyte rate and ranged from 124 to 143 % for the soil cultivated with rice, and from 127 to 154 % for soybean (Table 3). Zn_{SUM} was higher than Zn_{PST}, due to the inclusion of the Zn_{res} phase in the Zn_{SUM} calculation (eq. 1). Owing to the previously extracted fractions (Zn_{Exc}, Zn_{Carb}, Zn_{OM} and Zn_{Oxi}), the same soil sample used for the obtaining of Zn_{res} resulted in more efficient extraction, whereas Zn_{PST} was conducted with an unchanged soil sample. Therefore, because of sequential analysis and specificities in the determinations of trace elements such as Zn close ranges in the recovery above or below 100 % are tolerated. Additionally, we considered that the recovery values are sufficient for the main aims of this study as other similar studies have demonstrated (Colzato et al., 2018).

Interaction of Zn at the soil-plant system

The total zinc uptake by plants (Zna_{total}) exhibited positive behavior as a function of the soluble Zn rates applied to the soil and the interaction between the soil solution and soil compartments as reported by the sequential extraction analysis. The total Zn accumulated had increasing correlation for both extractant solutions (Zn_{DTPA} and Zn_{M}) with the rates applied and both soil subsamples, t1 and t2 (Figures 5 and 6). In general, Zn linked with DTPA extraction showed higher values when compared with Mehlich-1 for soil cultivated with both crops especially on t2 subsamples (flowering). The samples from soil cultivated with rice presented better responses to the extractant solutions than from soybean cultivation.

The application of $ZnCl_2$ rates increases Zn availability because of the stronger presence of Zn in the labile fractions, i.e., exchangeable fraction and fractions bound to carbonates and organic matter which could be obtained in the extraction with DTPA and Mehlich-1 solutions. Zn available to rice plants determined by a DTPA extractant solution showed positive correlation for both subsamples (Zn_{DTPAt1} and Zn_{DTPAt2}) in the soil fractions as presented in Table 4, i.e., Zn_{Exc} phase (r: 0.91 and 0.99), Zn_{Carb} (r: 0.81 and 0.94) and Zn_{OM} (r: 0.55 and 0.59).

Zn available (DTPA) to soybean plants also correlated with the same fractions, i.e., Zn_{Exc} (r: 0.93 and 0.96), Zn_{Carb} (r: 0.83 and 0.82) and Zn_{OM} (r: 0.76 for both), (Table 5). Correlations between Zn_{DTPAt1} and Zn_{DTPAt2} subsamples in Zn_{Oxi}, Zn_{res} fractions were not significant. As regards Zn_{PST}, no significance (to rice) or low correlation (r: 0.49 in t2, to soybean) was observed (Table 4 and 5)

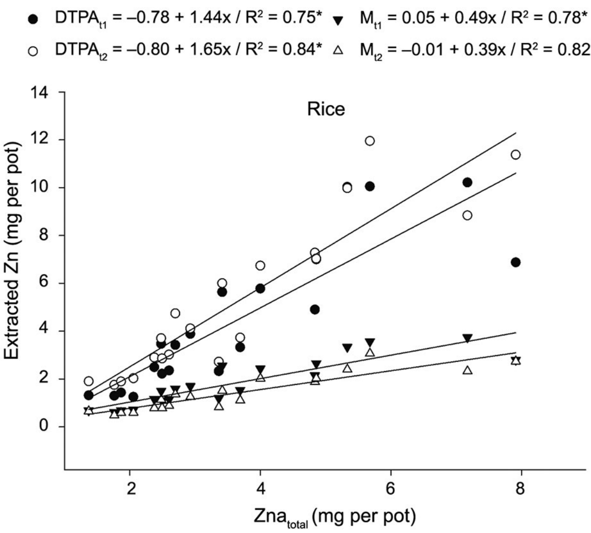

Figure 5 – Correlation coefficients between Zn extracted from soil cultivated with rice by DTPA (Zn_{DTPAt1} and Zn_{DTPAt2}) and Mehlich-1 (Zn_{Mt1} and Zn_{Mt2}) in two subsamples and the total accumulated Zn (Zna_{total}) affected by the $ZnCl_2$ rates applied (4 and 8 mg kg^{-1}). Notes: *Significant $p < 0.01$. Extracted Zn is expressed in mg per pot as regards the total amount of soil used in the experimental units (3 kg per pot) and Zna_{total} was expressed in mg per pot representing the total Zn absorbed. The following data present the rice dry matter for each treatment (Zn rates): 45.94 ± 2.42 (0 mg kg^{-1}); 49.57 ± 3.51 (4 mg kg^{-1}) and 41.57 ± 5.28 (8 mg kg^{-1}) – data are represented by means ± standard errors.

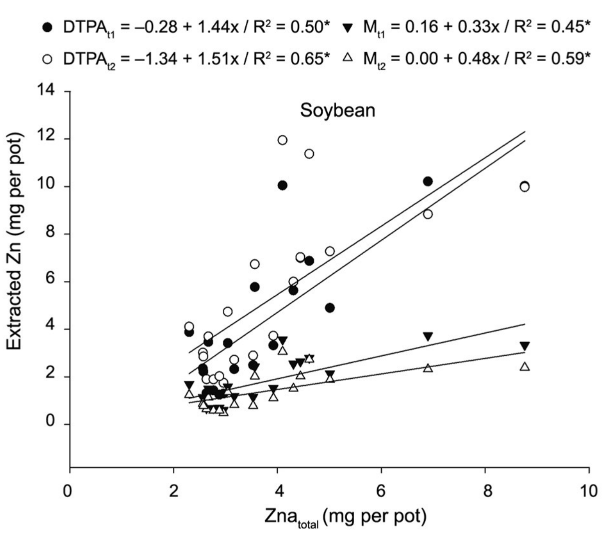

Figure 6 – Correlation coefficients between Zn extracted from soil cultivated with soybean by DTPA (Zn_{DTPAt1} and Zn_{DTPAt2}) and Mehlich-1 (Zn_{Mt1} and Zn_{Mt2}) in two sub-samples and the total accumulated Zn (Zna_{total}) affected by the $ZnCl_2$ rates applied (4 and 8 mg kg^{-1}). Notes: *Significant $p < 0.01$. Extracted Zn is expressed in mg per pot regarding the total amount of soil used in the experimental units (3 kg per pot) and Zna_{total} was expressed in mg per pot representing the total Zn absorbed. The following data present the rice dry matter for each treatment (Zn rates): 66.17 ± 5.15 (0 mg kg^{-1}); 62.55 ± 3.90 (4 mg kg^{-1}) and 59.80 ± 2.91 (8 mg kg^{-1}) – data are represented by means ± standard errors.

Table 4 – Pearson's correlation coefficients (r) for the variable response for soil cultivated with rice.

	$\text{Zna}_{\text{total}}$	$\text{Zn}_{\text{DTPAt1}}$	Zn_{Mt1}	$\text{Zn}_{\text{DTPAt2}}$	Zn_{Mt2}	Zn_{Exc}	Zn_{Carb}	Zn_{OM}	Zn_{Oxi}	Zn_{res}	Zn_{PST}
$\text{Zna}_{\text{total}}$	1	0.93	0.94	0.90	0.91	0.89	0.82	0.56	0.19 ns	-0.05 ns	-0.03 ns
$\text{Zn}_{\text{DTPAt1}}$		1	0.99	0.94	0.94	0.91	0.81	0.55^1	0.20 ns	0.13 ns	0.05 ns
Zn_{Mt1}			1	0.94	0.95	0.91	0.82	0.52^1	0.19 ns	0.12 ns	0.05 ns
$\text{Zn}_{\text{DTPAt2}}$				1	0.99	0.99	0.94	0.59	0.12 ns	0.03 ns	0.13 ns
Zn_{Mt2}					1	0.96	0.92	0.57	0.06 ns	0.08 ns	0.18 ns
Zn_{Exc}						1	0.93	0.60	0.15 ns	-0.02 ns	0.09 ns
Zn_{Carb}							1	0.53^1	-0.08 ns	-0.07 ns	0.08 ns
Zn_{OM}								1	0.29 ns	0.07 ns	0.20 ns
Zn_{Oxi}									1	-0.08 ns	-0.25 ns
Zn_{res}										1	0.55 ns
Zn_{PST}											1

Notes: r = significant correlations ($p < 0.01$), values followed by ^{1}significant correlations ($p < 0.05$) and values followed by ns = there is no significance ($p > 0.05$).

Table 5 – Pearson's correlation coefficients (r) for the variable response for soil cultivated with soybean.

	$\text{Zna}_{\text{total}}$	$\text{Zn}_{\text{DTPAt1}}$	Zn_{Mt1}	$\text{Zn}_{\text{DTPAt2}}$	Zn_{Mt2}	Zn_{Exc}	Zn_{Carb}	Zn_{OM}	Zn_{Oxi}	Zn_{res}	Zn_{PST}
$\text{Zna}_{\text{total}}$	1	0.82	0.79	0.80	0.78	0.78	0.62	0.45^1	-0.06 ns	-0.20 ns	0.55^1
$\text{Zn}_{\text{DTPAt1}}$		1	0.98	0.98	0.98	0.93	0.83	0.76	-0.11 ns	-0.19 ns	0.45 ns
Zn_{Mt1}			1	0.96	0.95	0.90	0.79	0.73	-0.12 ns	-0.09 ns	0.47^1
$\text{Zn}_{\text{DTPAt2}}$				1	1.00	0.96	0.82	0.76	-0.13 ns	-0.20 ns	0.49^1
Zn_{Mt2}					1	0.95	0.83	0.77	-0.14 ns	-0.23 ns	0.48^1
Zn_{Exc}						1	0.80	0.66	-0.09 ns	-0.30 ns	0.46^1
Zn_{Carb}							1	0.62	0.26 ns	-0.43 ns	0.23 ns
Zn_{OM}								1	-0.35 ns	-0.04 ns	0.20 ns
Zn_{Oxi}									1	-0.48 ns	-0.50
Zn_{res}										1	0.06 ns
Zn_{PST}											1

Notes: r = significant correlations ($p < 0.01$), values followed by 1significant correlations ($p < 0.05$) and values followed by ns = there is no significance ($p > 0.05$).

which suggests although the three fractions (Zn_{Oxi}, Zn_{res}, Zn_{PST}) are proportionally higher, they do not represent the Zn absorption by the plant system because they express lower lability. Zn_{Oxi} and Zn_{res} fractions are hardly bioavailable, despite the environmental condition variations simulated by different extractants and conditions used in the fractionation methodology.

Mehlich-1 extractant was efficient in the assay of Zn availability to plants. High significant correlations were observed for rice in both subsamples in the following fractions: Zn_{Exc} (r: 0.91 and 0.96), Zn_{Carb} (r: 0.82 and 0.92) and Zn_{OM} (r: 0.52 and 0.57) phases, whereas for soybean, the correlations were Zn_{Exc} (r: 0.90 and 0.95), Zn_{Carb} (r: 0.79 and 0.83) and Zn_{OM} (r: 0.73 and 0.77) in t1 and t2, respectively (Tables 4 and 5). The presence of Zn in the oxides and residual phases did not explain the Zn availability, as was observed in the DTPA solution.

Oliveira et al. (1999) found exchangeable Zn, Zn bound to organic matter and Zn bound to manganese oxide fractions in soils treated with 20 mg Zn kg^{-1} highly correlated with DTPA extraction. The same phases and Zn linked crystalline iron oxides correlated with M1 ($p < 0.01$). The Zn_{OM} fraction exhibited an expressed distinct behavior between the crops in correlation with the extractants. Soybean plants have higher access to available Zn bound to organic matter than rice plants, especially at the 4 and 8 mg kg^{-1} rates (Figures 3A and 4A). This result can be attributed to the protonation and deprotonation in functional groups of organic matter, which may favor binds with Zn^{2+} initially available in the soil solution. Since the management of soil pH is necessarily different for rice and soybean, the responses may be different. Cheng et al. (2005) and Shi et al. (2008) also addressed the difficulty in assigning the Zn absorption from organic matter Zn complexes, which may or may not become available.

This study may assemble important information about the soil-applied Zn management especially in tropical soils such as a Typic Hapludox. The incorporation of Zn in soil increases Zn interaction with the colloid particles which decreases its bioavailability in soil. Therefore, it is required to assay other fertilization techniques, e.g. fertilizer granules with coating or foliar Zn application, in order to increase the agronomic efficiency of available Zn for plants.

Conclusions

The addition of a soluble source ($ZnCl_2$) to clayey-textured Typic Hapludox samples cultivated with rice or soybean increased the Zn concentration in the labile fractions in the following order: exchangeable Zn, Zn bound to organic matter and Zn bound to carbonates. Such fractions had high positive correlation with total Zn accumulated in rice and soybean plants. Zn extracted by DTPA (Zn_{DTPAt1} and Zn_{DTPAt2}) and Mehlich-1 (Zn_{Mt1} and Zn_{Mt2}) correlated positively with Zn in labile fractions in the soil, which indicates its bioavailability. In contrast, Zn bound to oxides and residual Zn present in a high proportion in the soil did not change with increasing Zn rates. Such fractions correlated neither to the total Zn accumulated in plants, nor to DTPA and Mehlich-1 extractants, which shows the unavailability of Zn associated with the Zn bound to the recalcitrant fractions. The interaction of Zn with the different soil phases is a relevant key to the assessment of its bioavailability and uptake by plants which varies according to the $ZnCl_2$ concentration applied to soil as a supporting electrolyte.

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Conceptualization: Leite, C.M.C.; Muraoka, T.; Alleoni, L.R.F. Data acquisition: Leite, C.M.C.; Muraoka, T.; Colzato, M. Data analysis: Leite, C.M.C.; Muraoka, T.; Alleoni, L.R.F. Design of methodology: Leite, C.M.C.; Muraoka, T.; Alleoni, L.R.F. Software development: Leite, C.M.C. Writing and editing: Leite, C.M.C.; Muraoka, T.; Colzato, M.; Alleoni, L.R.F.

References

Abreu, C.A.; Lopes, A.S.; Santos, G.C.G. 2007. Micronutrients = Micronutrientes. p. 645-736. In: Novais, R.F.; Alvarez, V.H.; Barros, N.F.; Fontes, R.L.F.; Cantarutti, R.B.; Barros, N.F.; Fontes, R.L.F.; Neves, J.C.L., eds. Soil fertility = Fertilidade do solo. Sociedade Brasileira de Ciência do Solo, Viçosa, MG, Brazil (in Portuguese).

Alexakis, D. 2011. Diagnosis of stream sediment quality and assessment of toxic element contamination sources in East Attica, Greece. Environmental Earth Science 63: 1369-1383.

Alloway, B.J. 1995. Heavy Metals in Soils. 2ed. Blackie Academic & Professional, London, UK.

Asami, T.; Kubota, M.; Orikasa, K. 1995. Distribution of different fractions of cadmium, zinc, lead and copper in unpolluted and polluted soils. Water, Air, & Soil Pollution 83: 187-194.

Auernheimer, C.; Chinchon, S. 1997. Calcareous skeletons of sea urchins as indicators of heavy metals pollution. Environmental Geology 29: 78-83.

Cabral, A.R.; Lefebvre, G. 1998. Use of sequential extraction in the study of heavy metal retention by silty soils. Water, Air, & Soil Pollution 102: 329-344.

Camargo, O.A.; Moniz, A.C.; Jorge, J.A.; Valadares, J.M. 1986. Methods of chemical, mineralogical and physical analysis of soils used in the Instituto Agronômico, Campinas, State of São Paulo, Brazil. Instituto Agronômico, Campinas, SP, Brazil (in Portuguese, with abstract in English).

Cantarella, H.; Furlani, P.R. 1997. Upland rice = Arroz de sequeiro. p. 48-49. In: van Raij, B.; Cantarella, H.; Quaggio, J.A.; Furlani, A.M.C., eds. Fertilization and liming recommendations for São Paulo State = Recomendações de adubação e calagem para o Estado de São Paulo. 2ed. Instituto Agronômico, Campinas, SP, Brazil (Boletim Técnico, 100) (in Portuguese).

Cavallaro, N.; McBride, M.B. 1984. Zinc and copper sorption and fixation by an acid soil clay: effect of selective dissolutions. Soil Science Society of America Journal 48: 1050-1055.

Cheng, T.; Champhelaere, K.; Lofts, S.; Janssen, C.; Allen, H.E. 2005. Measurement and computation of zinc binding to natural dissolved organic matter in European surface waters. Analytica Chimica Acta 542: 230-239.

Cloquet, C.; Carignan, J.; Lehmann, M.F.; Vanhaecke, F. 2008. Variations in the isotopic composition of zinc in the natural environment and the use of zinc isotopes in biogeosciences: a review. Analytical and Bioanalytical Chemistry 390: 451-463.

Colzato, M.; Alleoni, L.R.F.; Kamogawa, M.Y. 2018. Cadmium sorption and extractability in tropical soils with variable charge. Environmental Monitoring and Assessment 190: 345.

Dai, S.G. 2006. Environmental Chemistry. 2ed. Higher Education Press, Beijing, China.

Dobermann, A.; Fairhurst, T.H. 2000. Nutrient Disorders and Nutrient Management. International Plant Nutrition Institute, Peachtree Corners, GA, USA.

Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA]. 1997. Manual for Methods of Soil Analysis = Manual de métodos de análise de solo. Embrapa-Centro Nacional de Pesquisa de Solos, Rio de Janeiro, RJ, Brazil (in Portuguese).

Fernández-Calviño, D.; Cutillas-Barreiro, L.; Paradelo-Núñez, R.; Nóvoa-Muñoz, J.C.; Fernández-Sanjurjo, M.J.; Álvarez-Rodríguez, E.; Núñez-Delgado, A.; Arias-Estévez, M. 2017. Heavy metals fractionation and desorption in pine bark amended mine soils. Journal of Environmental Management 192: 79-88.

Fontes, M.P.F.; Alleoni, L.R.F. 2006. Electrochemical attributes and availability of nutrients, toxic elements, and heavy metals in tropical soils. Scientia Agricola 63: 589-608.

Fuentes, A.; Lioréns, M.; Sáez, J.; Isabel, A.M.A.; Ortuño, J.F.; Meseguer, V.F. 2008. Comparative study of six different sludges by sequential speciation of heavy metals. Bioresource Technology 99: 517-525.

Frassinetti, S.; Bronzetti, G.; Caltavuturo, L.; Cini, M.; Croce, C.D. 2006. The role of zinc in life: a review. Journal of Environmenal Pathology, Toxicology, and Oncology 25: 579-610.

Garcia, G.; Penas, J.M.; Manteca, J.I. 2008. Zn mobility and geochemistry in surface sulfide mining soils from SE Spain. Environmental Research 106: 333-339.

Guadalix, M.E.; Pardo, M.T. 1995. Zinc sorption by acidic tropical soils as affected by cultivation. Journal of Soil Science 46: 317-322.

Gismera, M.J.; Lacal, J.; Silva, P.; Garcia, R.; Sevilla, M.T.; Procópio, J.R. 2004. Study of metal fractionation in river sediments: a comparison between kinetic and sequential extraction procedures. Environmental Pollution 227: 175-182.

Impa, S.M.; Sarah, E.; Johnson, B. 2012. Mitigating zinc deficiency and achieving high grain Zn in rice through integration of soil chemistry and plant physiology research. Plant Soil 361: 3-41.

Kalbasi, M.G.; Racz, J.; Loewen-Rudgers, L.A. 1978. Mechanism of Zn adsorption by iron and aluminum oxides. Soil Science 125: 146-150.

Leleyter, L.; Rousseau, C.; Biree, A.L.; Baraud, F. 2012. Comparison of EDTA, HCl and sequential extraction procedures, for selected metals (Cu, Mn, Pb, Zn), in soils, riverine and marine sediments. Journal of Geochemical Exploration 116-117: 51-59.

Lindsay, W.L.; Norvell, W.A. 1978. Development of a DTPA soil test for zinc, iron, manganese and cooper. Soil Science Society of America Journal 42: 421-428.

Lindsay, W.L. 1979. Chemical Equilibria in Soils. John Wiley, New York, NY, USA.

Malavolta, E. 1980. Elements of Plant Nutrition = Elementos de Nutrição de Plantas. Agronômica Ceres, São Paulo, SP, Brazil (in Portuguese).

Miller, W.P.; Martens, D.C.; Zelazny, L.W. 1986. Effect of sequence in extraction of trace metals from soils. Soil Science Society of America Journal 50: 598-601.

Miyazawa, M.; Pavan, M.A.; Muraoka, T.; Carmo, C.A.F.S.; Melo, W.J. 2009. Chemical analysis of plant tissue = Análise química de tecido vegetal. p. 191-233. In: Silva, F.C., ed. Manual of chemical analyses of soils, plants and fertilizers = Manual de análises químicas de solos, plantas e fertilizantes. Embrapa, Brasília, DF, Brazil (in Portuguese).

Novais, R.F.; Neves, J.C.L.; Barros, N.F. 1991. Experiment under controlled conditions = Ensaio em ambiente controlado. p. 189-254. In: Oliveira, A.J.; Garrido, W.E.; Araújo, J.D.; Lourenço, S., eds. Research methods in soil fertility = Métodos de pesquisa em fertilidade do solo. Embrapa, Brasília, DF, Brazil (in Portuguese).

Oliveira, M.F.G.; Novais, R.F.; Neves, J.C.L.; Vasconcelos, C.A.; Alves, J.C.L. 1999. Relationship between the Zinc available by different extracting solutions and Zinc fractions in soil samples. Revista Brasileira de Ciência do Solo 23: 827-836 (in Portuguese, with abstract in English).

Rauret, G.; López-Sánchez, J.F.; Sahuquillo, A. 1999. Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials. Journal of Environmental Monitoring 1: 57-61.

Shi, Z.Q.; Di Toro, D.; Allen, H.E.; Sparks, D.L. 2008. A WHAM: based kinetics model for Zn adsorption and desorption to soils. Environmental Science & Technology 42: 5630-5636.

Silveira, M.L.; Alleoni, L.R.F.; O'Connor, G.A.; Chang, A.C. 2006. Heavy metal sequential extraction methods: a modification for tropical soils. Chemosphere 64: 1929-1938.

Spark, K.M.; Wells, J.D. 1995. Characterizing trace metal adsorption on kaolinite. European Journal of Soil Science 46: 633-640.

Stahl, R.S.; James, B.R. 1991. Zinc sorption by B horizon as a function of pH. Soil Science Society of America Journal 55: 1592-1597.

Soil Survey Staff. 1999. Soil Taxonomy: A Basic System of Soil Classification for Making and Interpreting Soil Surveys. 2ed. NRC-USDA, Washington, DC, USA.

Suzuki, M.; Tsukamoto, T.; Inoue, H.; Watanabe, S.; Matsushashi, S.; Tahashi, M.; Nakanishi, H.; Mori, S.; Nishizawa, N.K. 2008. Deoxymugineic acid increases Zn translocation in Zn deficient rice plants. Plant Molecular Biology 66: 609-617.

Tessier, A.; Campbell, P.G.C.; Bisson, N.M. 1979. Sequential extraction procedure for the speciation of particulate trace metals. Analytical Chemistry 51: 844-851.

Tiller, K.G.; Gerth, J.; Brümmer, G. 1984. The sorption of Cd, Zn and Ni by soil clay fractions: procedures for partition of bound forms and their interpretation. Geoderma 34: 1-16.

Trani, P.E.; van Raij, B. 1997. Vegetables = Hortaliças. p. 157-164. In: van Raij, B.; Cantarella, H.; Quaggio, J.A.; Furlani, A.M.C., eds. Fertilization and liming recommendations for São Paulo State = Recomendações de adubação e calagem para o Estado de São Paulo. 2ed. Instituto Agronômico, Campinas, SP, Brazil (Boletim Técnico, 100) (in Portuguese).

Tu, Q.; Shan, X.Q.; Qian, J.; Ni, Z.M. 1994. Trace metal redistribution during extraction of model soils by acetic acid/sodium acetate. Analytical Chemistry 66: 3562-3598.

United States Environmental Protection Agency [USEPA]. 2007. Method 3051A: microwave assisted acid digestion of sediments, sludges, soils, and oil. Available at: https://www.epa.gov/sites/production/files/2015-12/documents/3051a.pdf [Accessed Apr 6, 2018]

van Raij, B.; Andrade, J.C.; Cantarella, H.; Quaggio, J.A. 2001. Chemical analysis to evaluate the fertility of tropical soils = Análise química para avaliação da fertilidade de solos tropicais. Instituto Agronômico, Campinas, SP, Brazil (in Portuguese).

Wu, C.; Lu, L.; Yang, X.; Feng, Y.; Wei, Y.; Hao, H.; Stoffella, P.J.; He, Z. 2010. Uptake, translocation, and remobilization of zinc absorbed at different growth stages by rice genotypes of different Zn densities. Journal of Agricultural and Food Chemistry 58: 6767-6773.

Yang, S.; Zhou, D.; Yu, H.; Wei, R.; Pan, B. 2013. Distribution and speciation of metals (Cu, Zn, Cd, and Pb) in agricultural and non-agricultural soils near a stream upriver from the Pearl River, China. Environmental Pollution 177: 64-70.