

Availability of heavy metals in contaminated soil evidenced by chemical extractants

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ABSTRACT

Heavy metals have been accumulating in Brazilian soils, due to natural processes, such as atmospheric deposition, or human industrial activities. For certain heavy metals, when in high concentrations in the soil, there is no specific extractant to determine the availability of these elements in the soil. The objective of the present study was to evaluate the availability of Cd, Cu, Fe, Mn, Pb and Zn for rice and soybeans, using different chemical extractants. In this study we used seven soil samples with different levels of contamination, in completely randomized experimental design with four replications. We determined the available concentrations of Cd, Cu, Fe, Mn, Pb and Zn extracted by Mehlich-1, HCl 0.1 mol L⁻¹, DTPA, and organic acid extractants and the contents in rice and soybeans, which extracts were analyzed by ICP-OES. It was observed that Mehlich-1, HCl 0.1 mol L⁻¹ and DTPA extractants were effective to assess the availability of Cd, Cu, Pb and Zn for rice and soybeans. However, the same was not observed for the organic acid extractant.

Key words: plant uptake, cadmium, lead, soil pollution.

RESUMO

Disponibilidade de metais pesados a extractores químicos em solos contaminados

Metais pesados vêm se acumulando em solos brasileiros em razão de processos naturais, como deposições atmosféricas, ou atividades antropogênicas. Para alguns metais pesados, em condição de altas concentrações no solo, não existe um extrator específico que avalie sua biodisponibilidade. Objetivou-se no presente trabalho, avaliar a biodisponibilidade dos elementos Cd, Cu, Fe, Mn, Pb e Zn para plantas de arroz e soja, utilizando diferentes extractores químicos. Neste estudo foram utilizadas sete amostras de solos com diferentes graus de contaminação, em delineamento experimental inteiramente casualizado, com quatro repetições. Foram determinados os teores disponíveis de Cd, Cu, Fe, Mn, Pb e Zn extraídos pelas soluções de Mehlich-1, HCl 0,1 mol L⁻¹, DTPA e ácidos orgânicos, e os teores desses elementos em plantas de arroz e soja, cujos extratos foram analisado por ICP-OES. Observou-se que os extractores Mehlich-1, HCl 0,1 mol L⁻¹ e DTPA foram eficientes para avaliar a disponibilidade de Cd, Cu, Pb e Zn para plantas de arroz e soja. O mesmo não foi observado para os extractores derivados de ácidos orgânicos.

Palavras-chave: absorção por plantas, cádmio, chumbo, poluição de solo.

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INTRODUCTION

Heavy metals may be accumulating in Brazilian soils due to natural processes such as atmospheric deposition, or due to human interference, such as with industrial activities, disposal of sewage sludge, disposal of waste or domestic and industrial by-products, as well as with the use of fertilizers and pesticides (Amaral Sobrinho *et al.*, 2009).

The success of monitoring heavy metals in the soil depends partly on an efficient chemical method that can measure the fraction of these elements available to plants. Heavy metal availability can be assessed using a suitable extractant, in which the amount extracted is associated with the accumulation or with the concentration of the elements in plants. Among the most used extractants in the assessment of the availability of elements in the soil, there are the DTPA and the Mehlich 1 (Raij, 1994). According to Abreu *et al.* (2002) the acidic solutions that were most tested for the extraction of available forms of heavy metals in plants are HCl 0.1 mol L⁻¹ and Mehlich-1.

The DTPA extracting solution at pH 7.3, as proposed by Lindsay and Norvell (1978), was initially developed to assess the bioavailability of copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) in soils with pH near neutrality or above, and Mehlich-1, to check the availability of P and exchangeable cations. These methods, however, had their usability expanded and have now been used in the extraction of lead (Pb), cadmium (Cd) and nickel (Ni) (Mattiazzo *et al.*, 2001).

In Brazil, results found for different extractants of heavy metals in soil are quite contradictory, especially those concerning the availability of Cd, Pb, chromium (Cr) and Ni, which are elements often found in steel industry and organic waste (Abreu *et al.*, 1995; Abreu *et al.*, 1998; Mattiazzo *et al.*, 2001; Pires *et al.*, 2004).

One of the major criticisms made to chemical extractants is that they do not simulate the reactions that occur near the roots, especially those at the rhizosphere. Mench & Martin (1991) found that the exudates of roots collected from three plant species (*Nicotiana tabacum* L., *Nicotiana rústica* L. and *Zea mays* L.) have increased the solubility of Mn and Cu. They also found that Cd amounts extracted by the exudates were proportional to the amounts of the element accumulated by these three plant species when cultured in the soil. Krishnamurti *et al.* (1997) when evaluating the kinetics of Cd release in soil, stressed the importance of low molecular weight organic acids excreted by the roots to the understanding of element availability. Low molecular weight organic acids, such as acetic, citric, oxalic, fumaric and succinic, usually found in root exudates, are the most effective in solubilizing heavy metals in the soil.

The acetic, citric, lactic and oxalic acids were the main organic acids found in the rhizosphere of the different plants studied. In studies using a solution consisting of organic acids (acetic acid, 1.0 mol L⁻¹, citric acid, 0.72 mol L⁻¹, lactic acid, 0.49 mol L⁻¹ and oxalic acid 0.12 mol L⁻¹), to assess the availability of Cd, Cu, Ni, Pb and Zn in soils treated with sewage sludge, researchers have found that the extractor solution was effective in assessing the availability of heavy metals, especially Pb (Pires *et al.*, 2004).

This work aimed to evaluate the available concentrations of heavy metals in contaminated soils at the end of rice and soybean cultivation, using different extractants.

MATERIAL AND METHODS

The study was conducted in a greenhouse using soil from the surface layer (0-20 cm) of a clayey Oxisol, according to the classification of Embrapa (1999), which had different levels of contamination associated with environmental accidents. Soil sampling was conducted in an area near a factory in the city of Paulinia-SP.

Since contamination was associated with the wind, the soil was collected following the predominant direction of the wind, at equidistant points 100 m, starting from the pollution source, and organized and named as follows: Point 1 - P1 (100 m) Point 2 - P2 (200 m) Point 3 - P3 (300 m) Point 4 - P4 (400 m) Point 5 - P5 (500 m) Point 6 - P6 (600 m) Point 7 - P7 (700 m). In order to determine the level of contamination in every sampled point location, samples of approximately 1 kg were taken from soil, oven-dried, sieved in a 2 mm sieve and analyzed for pH (CaCl₂), organic matter (OM), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and active acidity (H + Al) (Raij *et al.*, 2001). The total contents of Cd, Cu, Fe, Mn, Pb and Zn were determined employing aqua regia (3:1 mixture of HCl/HNO₃) according to the methodology described in Nieuwenhuize *et al.* (1991) (Table 1).

The available concentration of Cd, Cu, Fe, Mn, Pb and Zn in the samples were extracted using four extractants: a) Mehlich-1 (Silva, 2009), b) HCl 0.1 mol L⁻¹ (Wear & Sommer, 1947), c) DTPA (Lindsay & Norvell, 1978) and d) solution of organic acids (acetic 1 mol L⁻¹, citric 0.72 mol L⁻¹; lactic 0.49 mol L⁻¹; oxalic 0.12 mol L⁻¹) whose concentrations were determined by Pires *et al.* (2004).

The experiment followed a completely randomized design with four replications, in pots of 5 dm³. The species used in the experiment were *Oriza sativa* L. cultivar IAC 202 and *Glycine max* L. cultivar BRS 133. The NPK fertilization was performed according to the needs of the crop, following the recommendations of Raij *et al.* (1997).

Ten seeds were sown per pot and after 15 days of germination thinning was carried out, leaving only four plants per pot. The watering of the pots occurred so as not to exceed a maximum of 70% and minimum of 40% of

holding capacity of water of the soil, with daily weighting of pots.

Leaves for diagnosis of nutritional status were collected following the procedure described by Malavolta *et al.* (1997). The samples were washed in tap water and then rinsed in distilled water, placed in paper bags and submitted to a drying process using forced aeration, with temperature maintained from 60 to 70°C until constant weight. After drying, they were ground in a Willey type mill and packed in plastic bags. The wet digestion of plant samples was performed in open system, using adaptation of the method described by Oliva *et al.* (2003). The concentrations of Cd, Cu, Fe, Mn, Pb and Zn in extracts of plant and soil were determined by inductively coupled plasma atomic emission spectroscopy (ICP-OES) (Miyazawa *et al.*, 2009).

The available concentrations of heavy metals in soil were subjected to analysis of variance and F test. Linear correlation analyses were also performed among the available concentrations of the elements in the soil, and the amounts accumulated by rice and soybeans. The correlation coefficient values (*r*) were compared to evaluate the effectiveness of the extractants. Statistical analyses were performed according to Pimentel Gomes & Garcia, 2002 using 5% as the significance level ($p < 0,05$).

RESULTS AND DISCUSSION

The chemical analysis of soil samples used in this study showed that they had no limitations on fertility (Table 1), showing weak acidity, proper levels of macronutrients and base saturation above 50%, except for P7 (farther point from the pollution source). However,

the limitations of this soil can be attributed to the high concentrations of heavy metals found, because they interfered with the development of rice plants, causing plant death at the beginning of the development in P1, thus demonstrating that rice may be sensitive to high concentrations of heavy metals in the soil.

The soils used in this study had high total concentrations of Cd (20 – 27 mg kg⁻¹), Cu (120 - 272 mg kg⁻¹), Fe (97 – 936 mg kg⁻¹); Mn (535 – 719 mg kg⁻¹), Pb (173 – 332 mg kg⁻¹) and Zn (101 – 553 mg kg⁻¹). For the state of São Paulo, CETESB, in 2005, published the guiding values of concentrations of heavy metals in soil for prevention, contamination control and management of contaminated sites under investigation. According to these, the concentrations of Cd, determined for this soil, are in all sampled points, above the levels of intervention for the agricultural area (3 mg kg⁻¹). The same is true for Pb, because all points had levels above the level of intervention for the agricultural area (180 mg kg⁻¹), except P3. However, the levels of P3 are above those set by CETESB as preventive level (72 mg kg⁻¹). The value of prevention is the concentration of a particular substance, above which adverse changes may occur to the quality of soil and groundwater (CETESB, 2005).

The elements Cu and Zn, only in P1, showed levels above 200 mg kg⁻¹ for Cu and 450 mg kg⁻¹ for Zn, these values are established as values of intervention for agricultural area, according to CETESB (2005). Since Fe and Mn concentrations do not have reference value established by CETESB.

Table 2 shows the concentrations of Cd, Cu, Fe, Mn, Pb and Zn in soil, extracted by the different extractants. Note

Table 1 - Soil chemical attributes and total concentrations of heavy metals in the sampled points

Determination	Unit	Soil sampled points						
		1	2	3	4	5	6	7
pH CaCl ₂		5.8	5.8	5.2	5.5	5.5	5.3	5.2
O.M.	g kg ⁻¹	29.8	36.3	34.4	37.2	35.4	36.3	33.5
P	mg dm ⁻³	59.1	36	56.1	22.1	21.5	20.8	17.5
K ⁺	mmol _c dm ⁻³	12	14.1	5.4	11.8	11.3	7.4	8.7
Ca ²⁺	mmol _c dm ⁻³	44	33	38	41	36	36	17
Mg ²⁺	mmol _c dm ⁻³	8	19	20	24	20	16	5
H + Al	mmol _c dm ⁻³	24	26	36	29	29	31	61
Al ³⁺	mmol _c dm ⁻³	0	0	0	0	0	0	0
SB	mmol _c dm ⁻³	64	66.1	63.4	76.8	67.3	59.4	30.7
T	mmol _c dm ⁻³	88	92	99	106	96	90	92
V	%	73	72	64	73	70	66	33
Cd	mg kg ⁻¹	25	23	20	23	26	28	27
Cu	mg kg ⁻¹	272	141	115	121	144	166	153
Fe	mg kg ⁻¹	537	936	861	510	99	100	97
Mn	mg kg ⁻¹	540	563	536	583	664	719	570
Pb	mg kg ⁻¹	333	208	174	198	226	244	229
Zn	mg kg ⁻¹	554	189	113	106	106	108	102

that the organic acid extractant, in general, has extracted higher concentrations of Cd, Cu, Fe and Mn in soil, when compared to other extractants, and for Fe and Mn, these values were much higher than the observed in the other soils. Low molecular weight organic acids in the rhizosphere, such as those used to make the extraction solution, are effective in the solubilization of elements linked to the solid fraction of the soil (Krishnamurti *et al.*, 1997).

For Pb and Zn, in almost all soils, Mehlich-1 extracted the most. It can also be noted that in P1, which was closest to the source of pollution, and, consequently, the most contaminated point, the heavy metals were most extracted first by the extractant Mehlich-1, followed by HCl, except for Fe and Mn. Acid extractants extract elements mainly by the dissolution of clay minerals, the amount being

dependent on the acid concentration, extraction time and on the soil/solution relationship. The acid extractant Mehlich-1, according to Nascimento *et al.* (2002) may have negative aspects for the ability to extract forms linked to specific adsorption sites due to its acidity. Extractor HCl 0,01 mol L⁻¹, which is used to extract exchangeable cations or weakly adsorbed, has demonstrated its ability to displace Cd and Zn adsorbed on Fe and Mn oxides, carbonates or decaying organic matter (Amaral Sobrinho *et al.*, 2009). Even this behavior presenting the extracted quantities correlated with the absorbed by the plants (Paula *et al.*, 1998) as can be seen in Figures 1, 2, 3 and 4.

It is interesting to observe the differential behavior of DTPA and Mehlich-1, used for assessing Zn availability to plants. Mehlich-1 was able to extract more than DTPA (Table

Table 2 - Concentrations of Cd, Cu, Fe, Mn, Pb and Zn in the soil, in mg dm⁻³, extracted by Mehlich-1, DTPA, Organic acids (OA) and HCl 0.1 mol L⁻¹ in different sampled points, before the cultivations

Extractants	Soil sampled points						
	1	2	3	4	5	6	7
	mg dm ⁻³						
	Cd						
Mehlich-1	0.85 ¹	0.22	0.16	0.09	0.06	0.06	0.04
DTPA	0.61	0.15	0.08	0.04	0.04	0.03	0.03
OA	0.39	0.16	0.25	0.10	0.13	0.08	0.13
HCl	0.76	0.16	0.09	0.05	0.02	0.01	0.01
	Cu						
Mehlich-1	101	19	11	9	12	15	13
DTPA	73	16	9	8	11	12	11
OA	87	33	23	18	22	26	21
HCl	86	15	9	7	9	12	10
	Fe						
Mehlich-1	34	31	35	34	37	39	91
DTPA	11	12	13	12	13	13	32
OA	983	869	867	938	971	957	1043
HCl	8	6	8	5	5	6	19
	Mn						
Mehlich-1	88	103	93	93	103	111	93
DTPA	23	41	36	35	40	49	62
OA	167	156	315	180	205	246	184
HCl	36	48	48	38	41	55	55
	Pb						
Mehlich-1	93	17	10	8	12	14	12
DTPA	64	15	9	8	10	11	11
OA	49	16	18	9	12	13	11
HCl	74	12	7	5	8	11	9
	Zn						
Mehlich-1	361	83	36	24	17	12	7
DTPA	250	55	21	12	8	5	3
OA	159	55	48	16	10	8	5
HCl	259	67	30	18	11	9	5

¹ Mean of four replicates

2). The greater extraction ability of Mehlich-1 is due to its relatively high acidity, which has probably solubilized part of Zn adsorbed to oxides, while DTPA, since it is an alkali extractor, showed no ability to extract the zinc of the oxides. Similar results can be found in Ribeiro Filho *et al.* (2001) with the extraction of Cd, Cu, Fe, Mn, Pb and Zn from soil taken from area of Zn processing waste, and Korcak & Fanning (1978), who found great extraction ability of Mehlich-1 in relation to DTPA for Cd, Cu, Ni, and Zn in soils with excessive concentrations of these elements.

Paula *et al.* (1998), both in field conditions and in greenhouse conditions, using dilute acid extractants such as Mehlich-1 and HCl 0.1 mol L⁻¹, have extracted more soil elements than with chelating agents, in this case DTPA. Possibly due to reduced pH of the extract at around 2.5, acidic extractants solubilize compounds that are not extracted by chelating agents.

Extractor effectiveness to assess the available concentrations at the studied points was determined by the significance of correlations. Figures 1 and 2 show the correlations for Cd, Cu, Fe, Mn, Pb and Zn among the extractants examined and the contents of rice leaves for diagnosis, and Figures 3 and 4 show the correlations among the extractants examined and the content of soybean leaves for diagnosis.

Rice

The contents extracted by DTPA, HCl and Mehlich-1 had positive correlations with the concentrations of Cd, Cu and Fe in the plant, except for the organic acid extractant (Figure 1). For Cd and Fe correlations were significant at 1% and for Cu at 5%. The organic acid extractant was positively correlated to Fe ($p < 0.05$).

Mn showed non-significant negative correlation for Mehlich-1 and organic acid extractants (Figure 2). Such negative correlations obtained with the Mn may be due to chelating capabilities (O'Connor, 1988), or to the exhaustion of the extracting ability of the chemical solution due to the high concentration of soluble ions in different elements, limiting the extraction of this element from the soil. Positive correlation was shown to DTPA and HCl extractants, and was significant only for DTPA.

For Zn, in all extractants examined correlations were significant at 1% concentration in the plant. A similar result was found in the literature for an experiment with flue dust in corn and lettuce carried out by Accioly (1996). Cunha *et al.* (2008) have found highly significant correlations ($p < 0.01$) between the concentrations of Cd and Zn in plants and those obtained by different extractants, including DTPA and Mehlich-1. Results indicate that chemical methods have similar effectiveness when assessing the availability, in the case of Cd and Zn, for plants in contaminated soil.

The analysis of the concentrations of Pb showed correlation with the DTPA extractant alone. Andrade *et al.* (2010) find significant and high correlations of the amounts extracted with DTPA with the accumulated amounts of Pb in rice plants and the concentrations in roots and shoots.

When assessing the effects of flue dust application to the soil, researchers found that Mehlich-1 and DTPA were not efficient at extracting Pb and Cd, only Zn (Accioly, 1996). In another study, using the same extractants, Santos *et al.* (2002) found that none of them was effective in assessing the availability of Pb, Cd, Cr and Ni for corn plant. The authors have described that the lack of

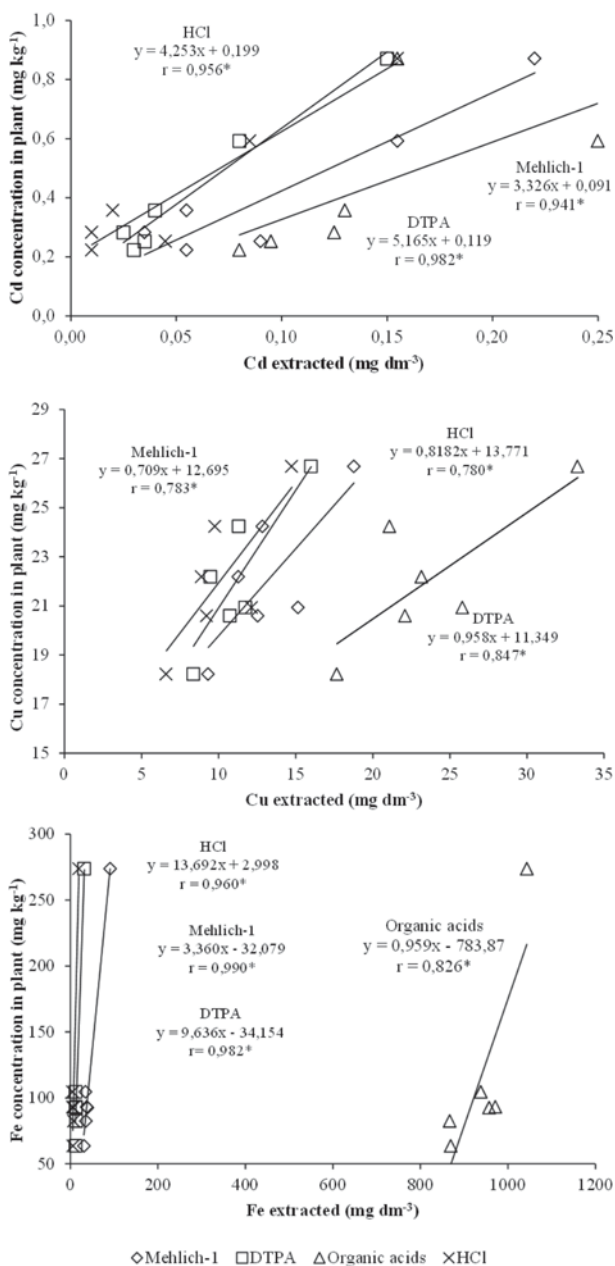


Figure 1 - Linear correlations of concentration of Cd, Cu and Fe in the leaf used for diagnostic in rice and concentrations extracted from the soil by Mehlich-1, DTPA, organic acids, and HCl (*-significant at 5%).

correlation of Cd, Cr and Ni may be due, among other factors, to low concentrations in the soil samples studied, while for Pb the lack of significant correlation can be due to the small variation of element concentrations in soil and plant. When compared to the results of this study, we may note that very high levels in the soil can also affect the extraction efficiency of chemical extractants, thereby influencing the correlation with foliar concentrations.

Unlike the results found by Accioly (1996) and Santos *et al.* (2002) levels of Cd and Pb found in this study showed positive correlation with DTPA, in agreement with Andrade *et al.* (2010). High and significant correlations among

concentrations of zinc, cadmium and lead extracted with DTPA, and concentrations of these elements in plants, have been found in several studies (Qian *et al.*, 1996; Paula *et al.*, 1998; Amaral Sobrinho *et al.*, 2009).

For the organic acid extractant, although it has extracted greater amounts of Cd, Cu, Fe and Mn in all soils, there was not correlation between the concentrations of such elements in the soil and in leaves used for diagnosis. However, a significant correlation between concentration extracted from soil and present in the leaves was found for Zn. Researchers found a significant correlation between content in soil and leaf using this

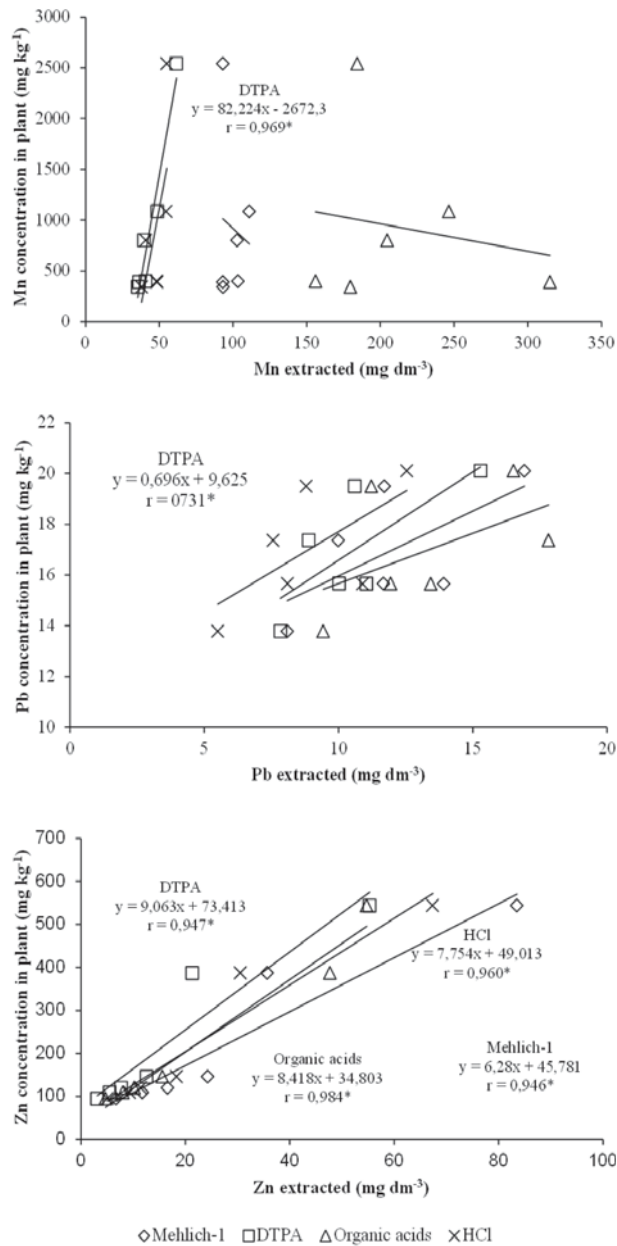


Figure 2 - Linear correlations of concentration of Mn, Pb and Zn in the leaf used for diagnostic in rice and concentrations extracted from the soil by Mehlich-1, DTPA, organic acids, and HCl (*- significant at 5%).

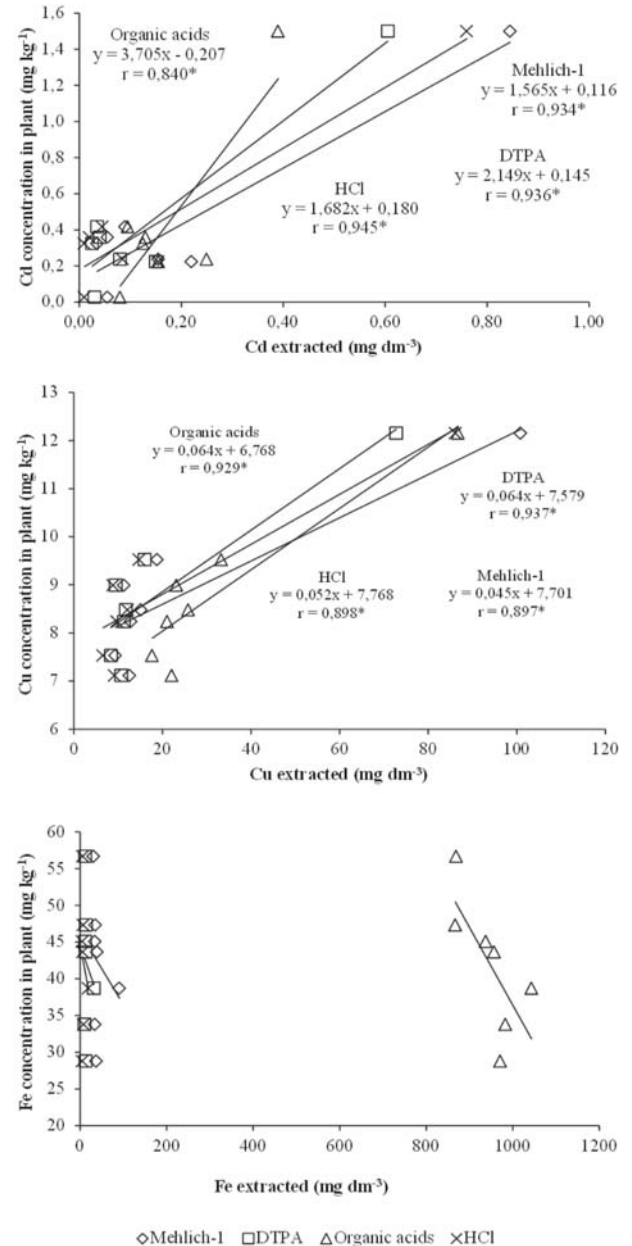


Figure 3 - Linear correlations of concentration of Cd, Cu and Fe in the leaf used for diagnostic in soybean and concentrations extracted from the soil by Mehlich-1, DTPA, organic acids, and HCl (*- significant at 5%).

method to assess Cu, Ni, Pb and Zn in leaves of banana, peach and coffee (Pires *et al.*, 2004). However, it may be noted that the heavy metals evaluated by Pires *et al.* (2004) were added through sewage sludge, rich in organic material, with 300 g kg⁻¹ organic carbon on average. This probably explains why organic acid extractant was not effective, since the organic matter content of soils in the study was around 35 g kg⁻¹. This extractor probably has ability to extract elements linked to the organic fraction and not those associated with the mineral fraction. Another possibility is that the organic acids used in this solution were not effective to solubilize the heavy metals

available for rice. To determine the types of organic acids and their concentrations, and thus draw up the extractor, Pires (2004) used crops like sugar cane, signalgrass and corn which belong to the family *Poaceae* just as rice.

Soybean

Figures 3 and 4 show the correlations in soil concentrations of Cd, Cu, Fe, Mn, Pb and Zn obtained with the extractants examined and the contents in the leaves used for diagnosis of soybean. For Cd, Cu, Fe, Mn, Pb and Zn, all extractants resulted in positive correlation between soil concentrations and the contents of leaves.

Similar results were found by Silva (1999) and Ribeiro Filho *et al.* (2001) when working with DTPA and Mehlich-1 solutions, where both proved to be appropriate to assess the availability of Cd, Ni, Zn and Pb, as well as for Pb extracted with DTPA and Mehlich-3.

For Mn only DTPA had significant correlation ($p < 0.05$), whereas for Fe no extractor was effective. One possible explanation for Fe could be its competition with Cd for absorption sites on the plasma membrane (Siedlecka & Krupa, 1999), as well as due to the similarity between the ionic radius of Fe and Zn (Woolhouse, 1983); the excess of Zn could inhibit the absorption of Fe, leading to plant deficiency, even though there is Fe in the soil solution.

The organic acid extractant, unlike what has occurred with rice, had a positive correlation with the concentrations of Cd, Cu, Pb and Zn in the leaves used for diagnosis, in agreement with previously published results (Pires *et al.*, 2004). Such results remove the assumption that this extractant would be effective only for soils with high organic matter content, since we have used the same soil for both crops. But it emphasizes the possibility that this mixture of organic acids might not be able to be used for any crop, which would require more studies on the subject.

CONCLUSION

The extractants Mehlich-1, HCl 0.1 mol L⁻¹ and DTPA were effective to assess the available concentrations of Cd, Cu, Fe, Pb and Zn in contaminated soils for rice culture.

The extractants Mehlich-1, HCl 0.1 mol L⁻¹, DTPA and organic acid were effective in assessing the availability of Cd, Cu, Pb and Zn in soils with high concentrations of these elements, when soybean was used as extractor plant.

Only DTPA was efficient in assessing the availability of Mn for plants.

The extractants Mehlich-1, HCl 0.1 mol L⁻¹, DTPA and organic acid were not effective in assessing the availability of Fe, in soils with high concentrations of this element.

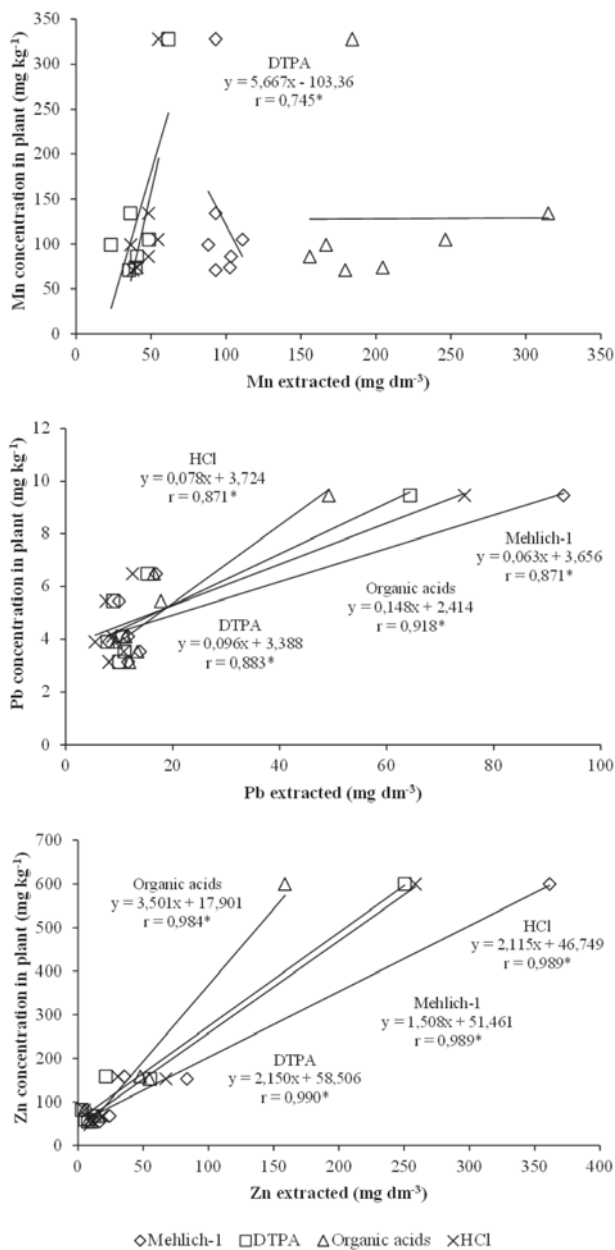


Figure 4 - Linear correlations of concentration of Mn, Pb and Zn in the leaf used for diagnostic in soybean and concentrations extracted from the soil by Mehlich-1, DTPA, organic acids, and HCl (*- significant at 5%).

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