Comissão 3.4 - Poluição, remediação do solo e recuperação de áreas degradadas

CADMIUM AVAILABILITY AND ACCUMULATION BY LETTUCE AND RICE(1)

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SUMMARY

Among the toxic elements, Cd has received considerable attention in view of its association with a number of human health problems. The objectives of this study were to evaluate the Cd availability and accumulation in soil, transfer rate and toxicity in lettuce and rice plants grown in a Cd-contaminated Typic Hapludox. Two simultaneous greenhouse experiments with lettuce and rice test plants were conducted in a randomized complete block design with four replications. The treatments consisted of four Cd rates ($CdCl_2$), 0.0; 1.3; 3.0 and 6.0 mg kg^{-1}, based on the guidelines recommended by the Environmental Agency of the State of São Paulo, Brazil (Cetesb). Higher Cd rates increased extractable Cd (using Mehlich-3, Mehlich-1 and DTPA chemical extractants) and decreased lettuce and rice dry matter yields. However, no visual toxicity symptoms were observed in plants. Mehlich-1, Mehlich-3 and DTPA extractants were effective in predicting soil Cd availability as well as the Cd concentration and accumulation in plant parts. Cadmium concentration in rice remained below the threshold for human consumption established by Brazilian legislation. On the other hand, lettuce Cd concentration in edible parts exceeded the acceptable limit.

Index terms: *Lactuca sativa* L., *Oryza sativa* L., soil pollution, chemical extractants, heavy metals, human health.

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RESUMO: *DISPONIBILIDADE E ACÚMULO DE CÁDMIO POR PLANTAS DE ARROZ E ALFACE*

Entre os elementos tóxicos, o Cd tem recebido atenção considerável por sua associação com diversos problemas de saúde humana. Objetivou-se com este estudo avaliar a disponibilidade de Cd no solo e seu acúmulo, taxa de transferência e toxidez em plantas de arroz e alface cultivadas em Latossolo Vermelho-Amarelo contaminado com esse elemento. Foram realizados dois experimentos simultâneos com a cultura da alface e do arroz. O delineamento adotado foi em blocos ao acaso; em cada experimento, foram avaliadas quatro doses de Cd ($CdCl_2$), com quatro repetições: 0,0, 1,3, 3,0 e 6,0 mg kg^{-1}, definidas com base nos valores orientadores de Cd no solo estabelecidos pela Companhia Ambiental do Estado de São Paulo (Cetesb). As doses de Cd aplicadas no solo aumentaram a concentração desse nutriente nele disponível (Mehlich-3, Mehlich-1 e DTPA) e diminuíram a produção de massa seca das plantas de alface e de arroz. Contudo, não foram observados sintomas de toxidez nas plantas. Os extratores Mehlich-1, Mehlich-3 e DTPA foram eficientes na predição das concentrações de Cd disponível no solo, bem como do teor e acúmulo de Cd nas partes vegetais. Os teores de Cd nos grãos de arroz permaneceram abaixo dos limites máximos estabelecidos para o consumo humano, conforme a legislação brasileira. Entretanto, para a alface, as concentrações de Cd verificadas na parte aérea excederam o limite tolerado.

Termos de indexação: Lactuca sativa *L.,* Oryza sativa *L., poluição do solo, extratores químicos, metais pesados, saúde humana.*

INTRODUCTION

In the last decades, Cd concentration in the environment have increased more than other heavy metals (Alloway, 1995). Phosphate fertilizers and atmospheric deposition are considered to be the greatest Cd pollution sources (Kabata-Pendias & Mukherjee, 2007). Due to the high soil mobility and availability of this metal, there is a general concern about Cd phytotoxicity and consequent potential risks to all living beings and human health, because Cd could rapidly diffuse into several steps of the food chain (Pierangeli et al., 2007).

Cadmium bioavailability depends on the concentration in the soil solution, which in turn depends on Cd release from soil colloids. Cadmium release from or adsorption to soil colloids is mainly affected by (in decreasing order of importance): (a) soil solution pH; (b) soil cation exchange capacity (CEC); (c) organic matter (OM) content; (d) clay content; (e) redox potential; and (f) presence of other elements in the soil system (Alleoni et al., 2005; Kabata-Pendias & Mukherjee, 2007; Sposito, 2008). The major part of soil Cd (55–90 %) occurs freely in solution as cationic species (Cd^{2+}) (Kabata-Pendias & Mukherjee, 2007). Therefore, irrespectively of soil type or contamination source, the major part of soil Cd is found in a highly available form (Sterckeman et al., 2009).

There is evidence that plants grown on heavy metal-contaminated soils are not able to prevent but restrict the metal uptake and accumulation in tissues (Peterson & Girling, 1981). However, there is a great variation not only among plant species but also among cultivars as to their ability to take up and accumulate heavy metals, as reported by Ishikawa et al. (2005) for rice and soybean and Liu et al. (2005) for rice plants.

Plant breeding for low heavy metal accumulation in edible parts is one of the measures towards reducing the entry of heavy metals into the food chain (Arao & Ae, 2003; Yu et al., 2006; Grant et al., 2008). Despite this scientific strategy, the establishment of specific legislation to ensure soil quality, prevent pollution and identify land areas threatened by contamination is an indispensable and essential action to avoid heavy metal accumulation in crops. Worldwide the establishment of guidelines for heavy metal concentrations in soils has been suggested, informing quality, alarm levels and intervention reference values, as first steps towards soil quality monitoring. However, general international guidelines could lead to misinterpretations, because of the different climatic, technological and pedological conditions of each country. These reasons justify the development of specific guidelines, appropriate for the characteristics of each country (Cetesb, 2001). Besides, a precise evaluation of the contamination risk requires the development of specific laboratory tests for each heavy metal (McLaughlin et al., 2000).

There is a great concern about Cd contamination of the food chain, because this heavy metal reaches considerably higher concentrations in crops than the others (As, Cu, Cr, Hg, Ni, Pb, and Zn). However, the Cd levels are well below the toxic concentrations at which phytotoxicity could be expressed and identified based on visual symptoms (McLaughlin et al., 2000). According to these authors, Cd evaluations in soils must be able to predict the transfer of this metal to edible plant parts. This prediction can be mainly correlated to: (i) metal concentration recovery from soil extracts obtained with Mehlich-1, Mehlich-3 and DTPA solutions, saturation extract, and ionic speciation; (ii) metal accumulation in plants; (iii) metal accumulation in plant shoots; and (iv) the transfer factor, defined by Wang et al. (2006) as the ratio between metal concentration in edible plant parts and the total metal concentration in the soil (food metal/soil metal ratio).

The Environmental Agency of the State of São Paulo, Brazil (Cetesb) has adopted guidelines for heavy metals in soils (Cetesb, 2005). When the critical value is exceeded, the area may be considered inadequate for agriculture and subject to intervention. In this sense, several studies have investigated the recommendation values proposed by Cetesb (Pereira et al., 2007; Borges Júnior et al., 2008; Mellis & Rodella, 2008; Nogueira et al., 2009; Pereira et al., 2010).

Despite the relevance of the high potential risks of Cd entry into the food chain, little information is found in Brazilian literature about detailed evaluations of Cd guidelines in soils related with its availability and transport into the soil-plant-food system.

In this context, the objectives of this study were to evaluate the Cd availability and accumulation in soil, the Cd transfer rate and toxicity to lettuce and rice plants grown in a Cd-contaminated Typic Hapludox.

MATERIALS AND METHODS

Two experiments were simultaneously carried out in a greenhouse, in Piracicaba, State of São Paulo, Brazil, from January to May 2007, one with rice (*Oryza sativa* L.) cv. 'IAC 202', and the other with lettuce (*Lactuca sativa* L.) cv. 'Vera', used as test plants.

The experiments were carried out in a randomized complete block design with four replicates and four treatments. The treatments consisted of four Cd rates, supplied as $CdCl_2$ (0.0; 1.3; 3.0, and 6.0 mg dm^{-3} Cd), based on the guidelines reported by Cetesb (2005), that is, alarm value = 1.3 mg dm^{-3}, intervention value for crop land = 3.0 mg dm^{-3}; and twice the intervention value = 6.0 mg dm^{-3}.

Each experimental unit consisted of a 3 dm^3 pot filled with soil from the 0–20 cm layer of a sandy-texture Typic Hapludox.

The soil was analyzed according to methods described by Raij et al. (2001) and Camargo et al. (1986) for chemical and physical characteristics, respectively, with the following results: pH = 4.3; organic matter (OM) = 20 g dm^{-3}, P = 2 mg dm^{-3}, S-SO_4^{2-} = 5 mg dm^{-3}; K^+ = 0.8 mmol$_c$ dm^{-3}; Ca^{2+} = 10 mmol$_c$ dm^{-3}; Mg^{2+} = 4 mmol$_c$ dm^{-3}; Al^{3+} = 5 mmol$_c$ dm^{-3}; H + Al = 38 mmol$_c$ dm^{-3}; sum of bases (SB) = 14.8 mmol$_c$ dm^{-3}; cation exchange capacity (CEC) at pH 7.0 = 52.8 mmol$_c$ dm^{-3}; base saturation (V %) = 28 %; B = 0.24 mg dm^{-3}; Cu (Mehlich-1) = 0.6 mg dm^{-3}; Fe = 208 mg dm^{-3}; Mn = 19.6 mg dm^{-3}; Zn = 1.9 mg dm^{-3}; Cd < 0.01 mg dm^{-3}; sand = 640 g kg^{-1}; silt = 160 g kg^{-1}; and clay = 200 g kg^{-1}. The procedures for soil analysis were: pH in 0.01 mol L^{-1} $CaCl_2$ solution (soil: solution 1: 2.5); Ca^{2+}, Mg^{2+}, K^+ extracted by ion exchange resin; Al^{3+} extracted by 1 mol L^{-1} KCl solution; potential acidity (H + Al) estimated by SMP-pH method (Raij et al., 2001); CEC at pH 7.0 and base saturation (V %) were calculated; OM was determined after oxidation with $K_2Cr_2O_7$ plus H_2SO_4 and the excess dichromate was titrated with $(NH_4)_2Fe(SO_4)_2.6H_2O$ (Raij et al., 2001); P, Cu, Fe, Mn, Zn, and Cd were extracted with Mehlich-1 solution (Mehlich, 1978) and determined by atomic absorption spectrophotometry (AAS) using a Perkin-Elmer AAS-700, Norwalk, CT, USA; sulfur (S) was extracted with 0.01 mol L^{-1} $Ca(H_2PO_4)_2.H_2O$ solution and determined by spectrophotometry (λ = 420 nm), using a Klett-Summerson colorimeter, 900–3, NY, USA (Raij et al., 2001); B was extracted with hot water in a microwave oven (Raij et al., 2001) and determined by spectrophotometry (λ = 420 nm); soil granulometry (particle size) was analyzed using the pipette method (Camargo et al., 1986).

The soil pots (water-holding capacity 60 %) were incubated in plastic bags during 30 days with the respective $CdCl_2$ rates plus proportional parts of $CaCO_3$ (p.a.) and $MgCO_3$ (p.a.) to reach a base saturation (V %) of 50 % (for rice) and 80 % (for lettuce) (Cantarella & Furlani, 1997; Trani et al., 1997). After liming, the soil pH$CaCl_2$ was 4.9 ± 0.02 (V = 50 %) and 5.5 ± 0.07 (V = 80 %). Afterwards, the soil was dried and incubated again for 30 days with the pre-planting fertilization (Malavolta, 1980).

The nutrients were added in solution (in mg dm^{-3}): 200 of P ($NH_4H_2PO_4$); 50 of K (K_2SO_4); 5 of Cl (KCl); 1 of B (H_3BO_3); 0.1 of Co ($CoSO_4.7H_2O$); 2 of Cu ($CuSO_4.5H_2O$); 20 of Fe (Fe-EDTA); 10 of Mn ($MnSO_4.H_2O$); 0.2 of Mo (MoO_3); 0.1 of Ni ($NiSO_4.6H_2O$); and 2 of Zn ($ZnSO_4.7H_2O$).

Rice fertilization was top-dressed at tillering and panicle initiation stages as follows (per dm^3 and per plant stage): 50 mg of N (NH_4NO_3) and 50 mg of K (KCl) in each plant stage. Lettuce top-dressing fertilization consisted of 40 mg of N (NH_4NO_3) and 40 mg of K (KCl) per dm^3: 20 % at seedling transplant and 30, 20 and 30 % at 10, 20 and 30 days after transplanting, respectively.

Rice seeds were sown (10 seeds/pot) at 1 cm depth and after emergence three seedlings (height 10 cm) per pot were left. Shoots and roots of lettuce and rice were harvested 40 and 120 days after planting, respectively. The plant parts were carefully separated and rinsed in tap and deionized water. Rice grains were separated from husks. All plant parts were dried to constant weight in a forced air oven at 60–65 °C to determine dry matter yields. Thereafter, plant part samples were ground, subjected to nitric-perchloric digestion and analyzed for Cd concentrations by atomic absorption spectrophotometry (Malavolta et al., 1997). Based on the plant element concentration and dry matter yields, the total amount of accumulated Cd in the plant parts were calculated (root, shoot and grain) by:

$$A = C \times DM$$

where A = Cd accumulated (in micrograms per plant); C = Cd concentration in plant part (mg kg^{-1}); and DM = dry matter of plant part (in gram).

The Cd transfer from soil to rice and lettuce plants was calculated using the transfer coefficient (*tc*) by means of the formula (1) (USEPA, 1992):

$$tc = TC/SC \qquad (1)$$

where *tc:* Cd transfer coefficient from soil to plants; TC: Shoot Cd concentration on dry matter basis (mg kg^{-1}); and SC: total Cd concentration in the soil (mg kg^{-1}) in this case as extractable Cd.

After plant harvest, soil samples were collected to quantify the available Cd concentration, using three extracting solutions: DTPA (Abreu et al., 2001), Mehlich-1 (Mehlich, 1978) and Mehlich-3 (Mehlich, 1984).

Experimental data were subjected to analysis of variance and polynomial regression analysis between applied Cd rates and Cd concentrations in soils or plants (Pimentel-Gomes & Garcia, 2002), by means of "SAS system of analysis 9.1.4" computer program (SAS, 2004). Correlation studies were made to evaluate the relationships among Cd concentrations in different soil extracts (different chemical extractants) and the Cd concentrations and accumulation in rice and lettuce plant parts. The models were chosen according to the parameter significance used and coefficients of determination.

RESULTS AND DISCUSSION

Plant dry matter yield and Cd concentration and accumulation

Rice dry matter and grain yields decreased with increasing Cd rates applied to the soil (Figure 1a), as well as the lettuce shoot and root dry matter yields (Figure 1d). Higher dry matter yields were found for plants from the control treatments (without Cd addition). The decreasing dry matter yields were attributed to Cd phytotoxicity (Gussarson et al., 1996; Yang et al., 1996) as reported in other experiments with lettuce (Malgorzata & Asp, 2001; Corrêa et al., 2006; Kukier et al., 2010) and rice cultivars (Liu et al., 2007). However, the plant genotypes respond with different biomass due to varied degrees of susceptibility or tolerance to the toxic element. Besides, Cd may cause alteration in the plant metabolism (Lagriffoul et al., 1998) and its effects evidenced by higher Cd concentration in soil solution and plant tissue. In this regard, Pereira (2001) observed lower dry matter yield of lettuce (varieties ´Mimosa` and ´Regina de Verão`) grown with 9.6 mg dm^{-3} Cd than of control plants. On the other hand, Pereira (2006) found no reduction in lettuce dry matter yield, despite the high Cd concentrations detected in shoot parts, evidencing greater cultivar tolerance to the metal. Likewise, for rice plants, Li et al. (2009) found no decrease in shoot and root dry matter and grain yields of rice plants grown in soil treated with soluble Cd (in the form of $CdCl_2$), where the highest Cd rate did not exceed 1.0 mg kg^{-1}.

In this study, Cd concentrations and accumulation in rice shoots, roots and grain increased proportionally to the Cd rates applied to the soil (Figure 1b,c), in the following decreasing order: Cd root > Cd shoot > Cd grain. Similar results were obtained with lettuce (Figure 1e,f), where plant parts showed Cd concentrations and contents in the following decreasing order: Cd root > Cd shoot. These results corroborated the findings of Alloway (1995) and Kukier et al. (2010) for lettuce and of Jurado (1989), Silva et al. (2007) and Li et al. (2009) for rice.

Rice plants grown with 1.3 mg dm^{-3} Cd (soil prevention value) showed grain Cd concentration of 0.6 mg kg^{-1} (Figure 1b), which is below the Cd threshold value for edible plant parts reported by the National Agency of Sanitary Vigilance (ANVISA) (ANVISA, 1965). However, this rice grain would be considered inadequate for human consumption anyway, since the Cd concentration exceeded the limit value (0.2–0.4 mg kg^{-1}) defined for rice by the "Codex Alimentarius" Committee of the Food and Agriculture Organization of the United Nations (FAO) and by the World Health Organization (WHO, 2004).

Lettuce plants grown with 1.3 and 3.0 mg dm^{-3} Cd (Prevention and Intervention Values) (Figure 1e), showed leaf Cd concentrations between 55 and 100 mg kg^{-1}, respectively, or 1.65 and 3.0 mg kg^{-1} on a fresh matter basis, considering a lettuce water content of 95 %, according to Mohsenin (1980). These values are well above the maximum value allowed for fresh biomass (1.0 mg kg^{-1}), according to ANVISA (1965), posing risks to human health. It is well known that lettuce plants accumulate high metal concentrations in the shoot (Sampaio et al., 2009).

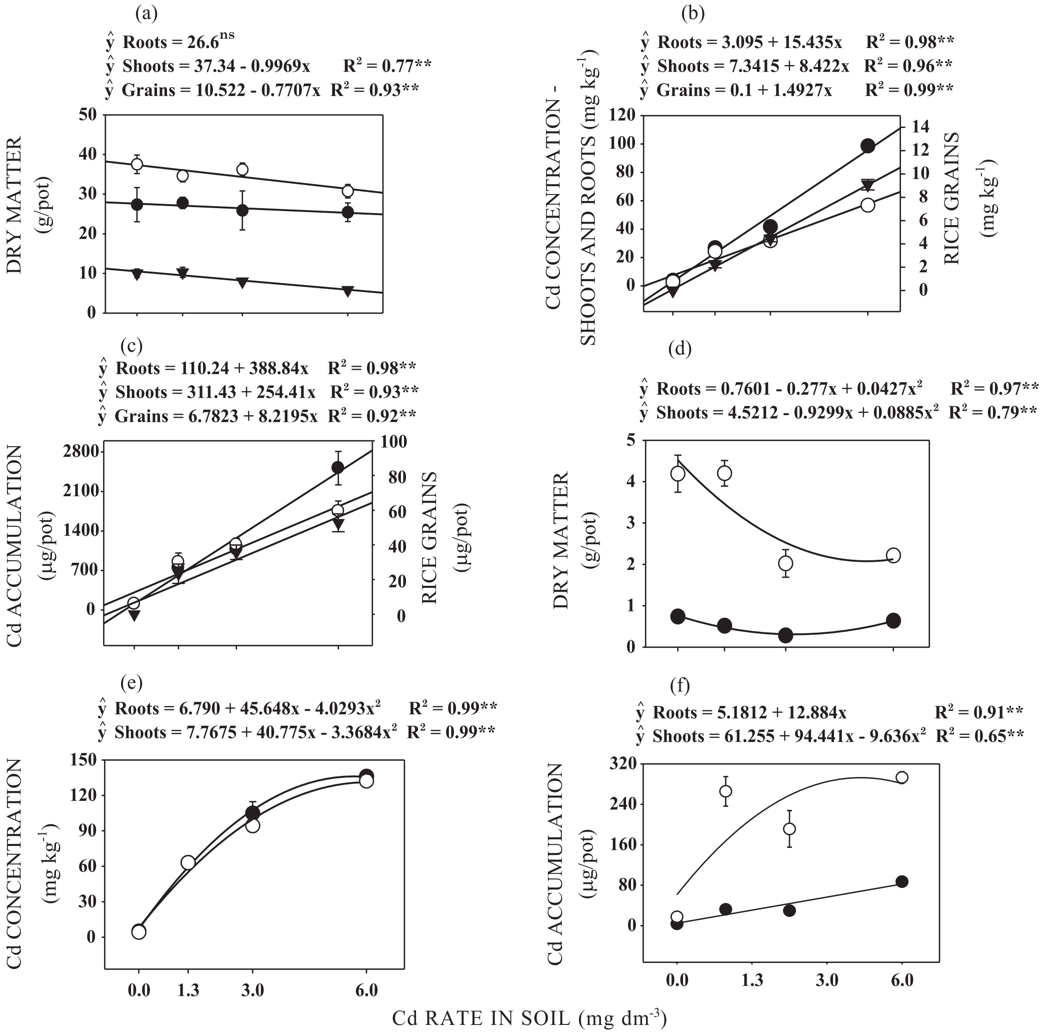

Figure 1. Dry matter yields of rice (a) and lettuce (d); Cd concentration in rice (b) and in lettuce (e) and Cd accumulation in rice (c) and lettuce (f) grown with Cd rates applied to the soil. (●) roots; (○) shoots; (▼) rice grain. (ns) and (**) non-significant and significant at p < 0.01, respectively.

According to Kabata-Pendias & Pendias (2001), the natural leaf Cd concentrations in lettuce usually vary between 0.66 and 3.0 mg kg^{-1} (on a dry matter basis). Leaf Cd concentrations in the range between 10 and 95 mg kg^{-1} are considered toxic to lettuce plants (Malavolta, 2006).

The visual Cd toxicity symptoms are characterized by darkened leaf margins, chlorosis, reddish petioles and vascular bundle, leaf curling and dark, short roots (Kabata-Pendias & Mukherjee, 2007). Nevertheless, in this study, no such visual symptoms were observed. Similar results were reported by Peterson & Girling (1981) who found no visual Cd toxicity symptoms in plants, in spite of growing in contaminated environments, which may be explained by the plant ability to accumulate inactive forms of metals in their tissues, and therefore, not expressing visual symptoms of phytotoxicity. There is a great concern not only about the harmful phytotoxic effect of Cd, but also because plants may accumulate high Cd concentrations without expressing any toxic visual symptoms, making the evaluation of risks of Cd entry into the food chain and for human health more dificult (Moustakas et al., 2001).

Soil available cadmium

There was a positive linear correlation between soil available Cd (Mehlich-1 and DTPA extractants) with Cd concentrations and accumulation in rice (root, shoot and grain) and lettuce plant parts (root and shoot) and the Cd applied to the soil (Table 1). The use of highly soluble cadmium chloride ($CdCl_2$) salt may have contributed to the high positive linear correlations (between 0.65 and 0.99). This procedure may arouse criticism of the obtained results. However, the study of Cd effects on plants would be difficult without isolated soil Cd contamination. Nevertheless, less soluble Cd sources might also be considered in

Table 1. Correlations between Cd concentrations and accumulation in rice and lettuce plant parts and soil available Cd concentrations obtained with DTPA, Mehlich-1 and Mehlich-3 extracting solutions

Extractant	Equation	r	*t* test	R^2	F test
mg dm^{-3}	Rice root Cd concentration, mg kg^{-1}				
DTPA	$\hat{y} = 4.030 + 20.6719 x$	0.99	26.25**	0.98	644.84**
Mehlich-1	$\hat{y} = 2.997 + 17.8523 x$	0.99	26.00**	0.98	646.89**
Mehlich-3	$\hat{y} = 6.449 + 15.9180 x$	0.99	26.66**	0.98	710.10**
	Rice shoot Cd concentration, mg kg^{-1}				
DTPA	$\hat{y} = 8.040 + 11.1789 x$	0.94	13.54**	0.93	256.18**
Mehlich-1	$\hat{y} = 7.627 + 9.5890 x$	0.96	12.37**	0.92	239.66**
Mehlich-3	$\hat{y} = 9.7341 + 8.4391 x$	0.95	10.83**	0.89	171.98**
	Rice grain Cd concentration, mg kg^{-1}				
DTPA	$\hat{y} = 0.212 + 1.9876 x$	0.99	26.19**	0.98	820.36**
Mehlich-1	$\hat{y} = 0.138 + 1.7050 x$	0.98	20.11**	0.97	488.08**
Mehlich-3	$\hat{y} = 0.469 + 1.5197 x$	0.98	20.18**	0.97	810.06**
	Lettuce root Cd concentration, mg kg^{-1}				
DTPA	$\hat{y} = 26.529 + 27.0428 x$	0.92	8.77**	0.85	750.76**
Mehlich-1	$\hat{y} = 24.755 + 23.5437 x$	0.93	9.25**	0.86	1126.31**
Mehlich-3	$\hat{y} = 31.695 + 19.9482 x$	0.88	6.98**	0.78	224.04**
	Lettuce shoot Cd concentration, mg kg^{-1}				
DTPA	$\hat{y} = 24.482 + 26.0793 x$	0.93	9.55**	0.87	517.59**
Mehlich-1	$\hat{y} = 22.914 + 22.6408 x$	0.94	9.93**	0.88	629.31**
Mehlich-3	$\hat{y} = 29.081 + 19.4049 x$	0.90	7.72**	0.81	220.91**
	Rice grain Cd accumulation, µg/pot				
DTPA	$\hat{y} = 7.615 + 10.8300 x$	0.93	9.32**	0.86	187.13**
Mehlich-1	$\hat{y} = 6.717 + 9.5127 x$	0.94	10.68**	0.89	261.19**
Mehlich-3	$\hat{y} = 9.5178 + 8.0614 x$	0.90	7.61**	0.81	140.72**
	Lettuce Cd accumulation, µg/pot				
DTPA	$\hat{y} = 106.033 + 45.7686 x$	0.70	3.69**	0.49	16.82**
Mehlich-1	$\hat{y} = 103.320 + 39.7174 x$	0.71	3.73**	0.50	17.81**
Mehlich-3	$\hat{y} = 117.008 + 32.7856 x$	0.65	3.23**	0.43	11.60**
	Cd applied to the soil, mg dm^{-3}				
DTPA	$\hat{y} = -0.033 + 0.7420 x$	0.99	34.84**	0.99	1629.61**
Mehlich-1	$\hat{y} = 0.027 + 0.8564 x$	0.99	28.06**	0.98	755.54**
Mehlich-3	$\hat{y} = -0.178 + 0.9571 x$	0.99	23.18**	0.97	3532.88**

**: significant at $p < 0.01$.

similar research work. According to Li et al. (2009) better correlations can be found when Cd is applied to soil as $CdCl_2$ compared to other Cd sources, such as swine effluent.

Research data on Cd adsorption in contaminated soils by anthropogenic activities significantly reinforced that a major Cd fraction is found in the cationic form (Cd^{2+}) in soil solution. This possibly explains the results obtained in this study. In most soils, 99 % of Cd is associated to the soil colloids and found in the soil solution (Kabata-Pendias & Mukherjee, 2007). By the technique of isotope dilution, Kukier et al. (2010) evidenced that soil Cd availability was the same in both treatments, Cd applied as sewage sludge or as soluble salt.

Studies on soil Cd adsorption demonstrated at least two reasons for the high Cd availability in soils: (a) Cd has low adsorption to goethite and Fe oxides; goethite is one of the main minerals found in Oxisols (Camargo et al., 2008), in which the relative decreasing order of adsorption is Cu > Pb > Zn > Co > Cd; and for Fe oxides, also abundant in Oxisols, metal adsorption follows the decreasing order: Pb > Cu > Zn > Cd (McLeand & Bledsoe, 1992); and (b) at low soil pH (pH < 6) a small Cd fraction remains adsorbed to the oxides, hydroxides and organic matter (McLeand & Bledsoe, 1992), and most Cd is found in solution as Cd^{2+}. The lowest amounts of Cd adsorbed to Oxisols and Nitosols were obtained at $pH_{CaCl_2} < 5.5$ (Dias et al., 2001). Brazilian soils are known to be predominantly acid (about 70 %) (Quaggio, 2000).

Therefore, the high soil availability of some metal elements has led researchers to believe that the metal bioavailable concentration or quantity is a better indicator than the total concentration to predict soil contamination by metals and the risks to human health via food chain transfer (McLaughlin et al., 2000; Wang et al., 2006; Baear, 2009). Nevertheless, to ensure environmental protection and safety, some authors have suggested that the eco-toxicological guidelines must be associated to the metal available fraction, determined using chemical extractants such as DTPA (Sauvé et al., 1996; Wang et al., 2006).

The chemical extractant efficiency is evaluated mainly by the correlation degree between the metal concentrations recovered from soil and leaf tissue. In this study, the soil Cd concentrations obtained with three extracting solutions (DTPA, Mehlich-1 and Mehlich-3) were positively correlated with the plant Cd concentrations. Besides, the correlation coefficients between Cd rates applied to soil and the recovered Cd concentrations of these three chemical extractants used to evaluate soil Cd availability were high. According to the angular coefficients obtained, Mehlich-3 (0.96) and Mehlich-1 (0.86) extractants were more effective to extract Cd from soil, followed by the DTPA (0.74). The similarity between Mehlich-1 and Mehlich-3 is probably due to their acid characteristics, different from the DTPA chelating agent. These acid solutions act by dissolving soil clay minerals and chelates such as DTPA can extract greater quantities of metal labile forms, without interfering with non-labile forms. In the State of São Paulo, the use of DTPA solution is recommended as official extractant of metal micronutrients (Fe, Mn, Cu and Zn) from soils, due to the positive and high correlations between soil and plant metal concentrations in several field studies (Abreu et al., 2007).

A similar efficacy of the chemical extractants was also observed by Araújo & Nascimento (2005) when evaluating Zn availability in soils treated with sewage sludge (SS) and growing maize. Teixeira et al. (2005) also studied the SS effects on heavy metal concentrations of a mine-degraded Red Yellow Oxisol and observed better results when acid extractants were used to predict soil Cd availability. On the other hand, Anjos & Mattiazo (2001) found no differences among extracting solutions when evaluating Cd availability in Oxisol treated with SS and cultivated with maize.

Rice grain yields decreased with higher soil available Cd extracted by Mehlich-1, Mehlich-3 and DTPA solutions. The negative correlations obtained between soil Cd concentrations (in three chemical extracts) and rice grain yields allowed the conclusion that plants were susceptible to Cd toxicity (Figure 2). Besides, all three extracting solutions were effective to predict the rice grain yield variation, since high and significant coefficients of determination were obtained (Figure 2). According to Cantarutti et al. (2007) the extractant should be chosen based on the best proportional variation between the plant yield or nutrient content and the soil nutrient determined in the extracting solution.

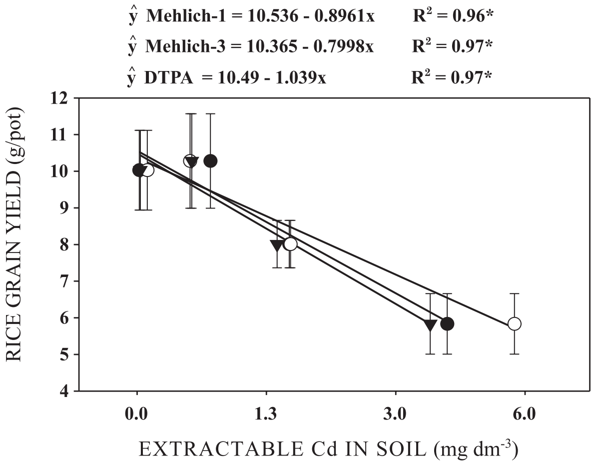

Figure 2. Correlation between rice grain yield and soil available Cd extracted with Mehlich-1(●), Mehlich-3(○) and DTPA (▼). *Significant at $p < 0.05$.

Cd transfer coefficient

The transfer coefficient (*ct*) evaluates the capacity of a plant species to take up the metal from soil metal and transport it to the edible part (shoot). It is commonly used in phytoremediation studies and, in this case, high *ct* values are desirable. The *ct* values for Cd transfer from soil to plant shoots usually vary from 1 to 10 (Pavlíková et al., 2004). In ths study, the objective is food safety; therefore, low *ct* values are desirable, because they indicate low metal transport from roots to edible plants. The *ct* coefficient is effective to evaluate the risks to human health caused by contact with metals from SS-treated soils (USEPA, 1992). Recently, studies reported the use of this coefficient to evaluate food safety (Wang et al., 2006).

The relationships between Cd rates applied to the soil and the Cd transfer coefficients (*ct*) of the two plant species fit polynomial equations (Figure 3). The estimates of points of maximum $ct\left(\frac{\partial \hat{y}}{\partial X}=0\right)$ for the lettuce and rice shoot parts were found for soil Cd rates of 3.3 and 3.4 mg kg^{-1}, respectively (Figures 1a,d). This allowed the conclusion that, in this study, maximum Cd transfer from soil to shoot parts occurred at values close to the soil intervention values for agricultural areas (3.0 mg kg^{-1}) established by Cetesb (2005).

The tendency to decreasing *ct* values at higher Cd rates (Figure 3) can most likely be explained by: (i) the negative effect of high Cd concentrations on plant metabolic activities, reducing the biomass gain; and (ii) to the higher Cd accumulation in plant roots (Figures 1c and 1d) with consequent decreasing Cd transport to shoot.

According to the polynomial equations obtained, the maximum *ct* value was 43.8 for lettuce and 8.9 for rice (Figure 3), evidencing the greater capacity of lettuce to transport Cd from the soil solution to the shoot. In general, *ct* values for lettuce vary between 2 and 5 (Crews & Davies, 1985). However, the threshold *ct* value allowed for Cd in leafy greens is 0.64. Considering the Cd reference value (1.3 mg kg^{-1}, Cetesb, 2005), the *ct* estimate by the polynomial equation was 30.6 for lettuce, confirming the high Cd translocation ability of lettuce from soil to shoots.

The determination of the Cd transfer to edible products is relevant to investigate the potential Cd intake by the population. In Brazil, a *per capita* consumption of lettuce and rice of 1.2 and 39–52 kg year^{-1} is estimated, respectively (Pereira, 2002; Mello et al., 2003). According to Zazouli et al. (2008), rice is the source of major Cd human intake in countries with high annual rice consumption.

Based on the total soil Cd concentration of 1.3 mg kg^{-1}, or 0.93 mg kg^{-1} extracted with DTPA ($y = -0.033 + 0.744x$), a lettuce shoot Cd concentration of ~55 mg kg^{-1} was estimated (Figure 1e). Consequently, considering the yearly average human lettuce *per capita* consumption of 1.2 kg year^{-1}, the calculated Cd intake is ~66 mg year^{-1} (55 x 1.2 = 66) or ~180 µg day^{-1} per capita^{-1} (66/365 x 1000 = 180). For rice consumption, based on the values of Figure 1b and the yearly rice consumption of 39–54 mg kg^{-1}, a daily Cd intake between 218 and 290 µg day^{-1} was estimated. These values exceed the maximum Cd daily intake limit for humans established by FAO/OMS, which is 70 µg day^{-1} (WHO, 2004). Thus, it was inferred that a total soil Cd concentration of 1.3 mg kg^{-1}, which is equivalent to 0.93 mg kg^{-1} of soil available Cd, can be considered risky for human health, because significant Cd amounts may be transferred to lettuce and rice products.

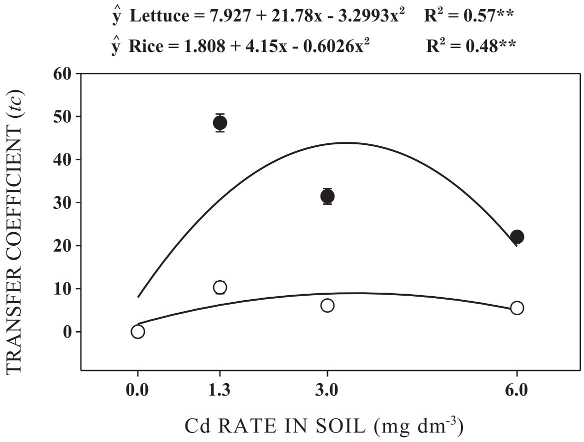

Figure 3. Effect of Cd rates applied to the soil on the Cd transfer coefficients (ratio between plant shoot Cd mg kg^{-1} / soil total Cd mg kg^{-1}) to plants of rice (○) and lettuce (●).

CONCLUSIONS

1. No visual symptoms of Cd toxicity were observed in rice or lettuce plants. However, the increasing Cd rates applied to soil reduced the dry matter yield of both species.

2. Cadmium concentrations and accumulation in the shoot and root of rice and lettuce plants, as well as of rice grain, increased proportionally to the increasing Cd rates and Cd availability in soil.

3. Rice grain Cd concentrations were below the maximum limit established for human consumption, according to Brazilian legislation. However, the Cd concentrations in lettuce exceeded these limits.

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