University of São Paulo
College of Agriculture “Luiz de Queiroz”

Phytotoxicity of cadmium and barium and derivation of critical limits in soils

Leônidas Carrijo Azevedo Melo

**Thesis presented to obtain the degree of Doctor in Science.
Area: Soils and Plant Nutrition**

**Piracicaba
2010**

Leônidas Carrijo Azevedo Melo
Agronomist

Phytotoxicity of cadmium and barium and derivation of critical limits in soils

Advisor:
Prof. Dr. **LUÍS REYNALDO FERRACCIÚ ALLEONI**

Thesis presented to obtain the degree of Doctor in Science.
Area: Soils and Plant Nutrition

Piracicaba
2010

Dados Internacionais de Catalogação na Publicação
DIVISÃO DE BIBLIOTECA E DOCUMENTAÇÃO - ESALQ/USP

Melo, Leônidas Carrijo Azevedo
Phytotoxicity of cadmium and barium and derivation of critical limits in soils / Leônidas Carrijo Azevedo Melo. - - Piracicaba, 2010.
85 p. : il.

Tese (Doutorado) - - Escola Superior de Agricultura “Luiz de Queiroz”, 2010.

1. Estresse oxidativo 2. Fitotoxicidade 3. Metal Pesado 4. Poluição do solo 5. Saúde pública 6. Solos - qualidade I. Título

CDD 628.55
M528p

Aos meus avós paternos, Jaime e Ana (em memória),
e maternos, Joaquim e Maria (em memória)
pelo exemplo de vida e dignidade,
OFEREÇO

À minha esposa Patrícia pela paciência, carinho, amor
e dedicação, imprescindíveis nesta etapa.
Aos meus pais Antônio e Zilda e às minhas irmãs
Mariana e Zildinha pela confiança e apoio incondicional,
DEDICO

AGRADECIMENTOS

A Deus pela vida e saúde para chegar a esta conquista.

A toda minha família por sempre me apoiar em todos os momentos.

Ao Prof. Luís Reynaldo Ferracciú Alleoni pela orientação, apoio, convívio e amizade nesta etapa importante de minha vida.

Ao Programa de Pós Graduação em Solos e Nutrição de Plantas e ao Departamento de Ciência de Solo da ESALQ (LSO) pela oportunidade de realização do curso e pela disponibilização da estrutura, recurso e materiais para desenvolvimento do trabalho de pesquisa.

À Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) pela concessão da bolsa e pelo apoio financeiro ao projeto.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela concessão da bolsa de doutorado sanduíche.

Ao Dr. Frank Swartjes pela supervisão, amizade e sugestões no trabalho durante o período de doutorado sanduíche no Instituto Nacional de Saúde Pública e do Ambiente (RIVM), da Holanda.

Aos técnicos do laboratório de Química do Solo do LSO Luís Silva e Marina Colzato pelo auxílio nas análises, e aos pesquisadores e funcionários do Laboratório de Avaliação de Risco Ecológico (LER) do RIVM pela recepção e agradável convívio.

Ao Prof. Ricardo Antunes de Azevedo pela parceria e pela disponibilização do laboratório de Genética Bioquímica de Plantas para realização de parte das análises e à Gisele de Carvalho pelo auxílio nas análises.

Ao meu professor de inglês e amigo, Antonio Bianchi, pelo incentivo em sempre melhorar meu inglês.

Aos amigos do curso de pós-graduação Benedito Correia, Fernando Garbuio, Antonio Fernandes, Mariana Gabos, Suzana e Julius Blum, Evandro Silva, Cintia Lopes, Alexys Boim, Roberta Nogueirol, Sabrina Novaes, Anderson Braz, Rodrigo Coqui, Adriana Guirado, Suzana Araújo e muitos outros que não foi possível citar, com os quais tive o privilégio de conviver e aprender ao longo deste período.

Enfim, a todos que, diretamente ou indiretamente, contribuíram para a realização deste trabalho.

Acknowledgments

First of all, I thank God for the life and health to reach this achievement. I would like to thank all my family for always supporting me. My sincere gratitude to Prof. Luís Reynaldo Ferracciú Alleoni for his guidance, support and friendship in this important step of my life. I would like to thank the Graduate Program in Soils and Plant Nutrition and the Soil Science Department from ESALQ/USP for the opportunity of taking the course and for the availability of the structure, and resource materials for the development of the research work. I thank the São Paulo Research Foundation (FAPESP) for the scholarship and financial support to the project, as well as to the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES) for granting the scholarship for the doctorate 'sandwich' program. I am very grateful also to Dr. Frank Swartjes for his supervision, friendship and valuable suggestions to the work during the doctorate 'sandwich' at the National Institute of Public Health and the Environment (RIVM) in the Netherlands. I thank the technicians of the Soil Chemistry Laboratory (LSO) from ESALQ Luís Silva and Marina Colzato for their help with the analysis, and the researchers and staff of the Laboratory for Ecological Risk Assessment (LER) of the RIVM for the welcome and pleasant stay. I would like to thank Prof. Ricardo Antunes de Azevedo for the partnership and for the availability of the Laboratory of Plant Biochemical Genetics to carry out part of the analysis and Giselle de Carvalho for her help with the analysis. I thank also my English teacher and friend Antonio Bianchi for always encouraging me to improve my English skills. Thanks to my friends from the graduate program Benedito Correia, Fernando Garbuio, Antonio Fernandez, Mariana Gabos, Susana and Julius Blum, Evandro Silva, Cintia Lopes, Alexys Boim, Roberta Nogueirol, Sabrina Novaes, Anderson Braz, Rodrigo Coqui, Adriana Guirado, Suzana Araújo and many others that I could not mention here, with whom I had the privilege to live and learn in this period. Finally, I would like to thank all people that, although not mentioned, contributed to this work.

CONTENT

ABSTRACT

Phytotoxicity of cadmium and barium and derivation of critical limits in soils

Soil pollution by heavy metals caused by anthropogenic activities is a problem in many countries. Cadmium (Cd) is one of the most hazardous elements due to its relatively high mobility in soils and transfer to plants. The main human exposure pathway to Cd is through the vegetable consumption and depends on the exposure scenario. Thus, defining a critical limit of Cd in soil is necessary. Barium is an earth alkaline element that can be toxic to humans and plants when it is present in the free form (Ba^{2+}). However, only few studies have focused on Ba in soil. Soil characteristics influence Cd and Ba availability for plant uptake. Both metals may affect plant growth and metabolism and cause oxidative stress. The aims of this study were: (i) to compare tropical and temperate datasets to establish critical soil Cd concentrations for the State of São Paulo, Brazil; (ii) to evaluate the influence of liming on Cd availability and accumulation in lettuce in order to calculate site specific critical soil Cd concentrations for two typical tropical soils from the State of São Paulo; (iii) to evaluate the effects of Cd and Ba concentrations on plant growth, lipid peroxidation and activities of antioxidant enzymes in leaves of soybean, grown in tropical soils with contrasting properties. The Cd uptake from the tropical dataset was higher as compared to the temperate dataset. Critical soil Cd concentrations were from 1.7 to 3.2-fold lower when tropical soil data were used. It is suggested that only literature data, regarding Cd in soils and vegetables, from tropical regions should be used for the State of São Paulo to derive critical soil Cd concentrations. Cadmium accumulated linearly in lettuce at soil concentrations up to 12 mg kg^{-1}. In both soils, a slight reduction on Cd uptake was observed as a response to liming. The calculated critical soil Cd concentrations were lower in the Ultisol because of the differences in soil characteristics. Besides, there was a positive correlation with the vegetable consumption rates. Cadmium strongly reduced soybean growth at concentrations from 5.2 mg kg^{-1}, while Ba only slightly reduced at 600 mg kg^{-1} in the sandy Entisol. The activities of superoxide dismutase, catalase and glutathione reductase were dependent mainly on the soil type: soybean plants grown in the Entisol suffered higher oxidative stress than those grown in the clayey Oxisol and, consequently, responded less to the increase of metal concentrations.

Keywords: Phytotoxicity; Oxidative stress; Soil quality standard; Human health; Soil properties.

RESUMO

Fitotoxicidade de cádmio e bário e derivação de limites críticos em solos

A poluição do solo por metais pesados causada principalmente por atividades antrópicas é um problema em muitos países. Cádmio (Cd) é um dos elementos mais perigosos devido à sua mobilidade relativamente alta em solos e transferência para as plantas. A principal via de exposição humana ao Cd é por meio do consumo de vegetais e depende do cenário de exposição. Assim, a definição de um limite crítico de Cd no solo é necessária. O bário (Ba) é um elemento alcalino-terroso que pode ser tóxico aos seres humanos e às plantas, quando absorvido na sua forma livre (Ba^{2+}). No entanto, há poucos estudos sobre Ba em solos. As características do solo influenciam na disponibilidade de Cd e Ba para absorção pelas plantas. Ambos os metais podem afetar o crescimento e metabolismo das plantas e causar estresse oxidativo. Os objetivos deste estudo foram: (i) comparar resultados oriundos de regiões tropicais e temperadas para determinar concentrações críticas de Cd em solos para o Estado de São Paulo; (ii) avaliar a influência da calagem na disponibilidade e no acúmulo de Cd em plantas de alface, a fim de calcular as concentrações críticas de Cd específicas para solos do Estado de São Paulo; e (iii) avaliar os efeitos das concentrações de Cd e Ba no crescimento das plantas, na peroxidação lipídica e na atividade de enzimas antioxidantes em folhas de soja, cultivada em solos tropicais com propriedades contrastantes. A absorção de Cd a partir do conjunto de dados de solos tropicais foi maior em relação ao de dados de clima temperado. As concentrações críticas de Cd no solo foram de 1,7 a 3,2 vezes menores quando os dados de solos tropicais foram utilizados. Sugere-se que apenas resultados de literatura de regiões tropicais, em relação a Cd em solos e vegetais, sejam utilizados para o Estado de São Paulo para derivação de concentrações críticas de Cd em solo. O Cd acumulou linearmente em alface em concentrações de até 12 mg kg^{-1} no solo. Apenas uma leve redução na absorção de Cd em resposta à calagem foi observada tanto no Latossolo quanto no Argissolo. As concentrações críticas de Cd foram menores no Argissolo do que no Latossolo, devido às diferenças nas características dos mesmos. Tais concentrações críticas tiveram correlação positiva com o consumo de hortaliças. O Cd reduziu fortemente o crescimento da soja em concentrações a partir de 5,2 mg kg^{-1}, enquanto Ba causou apenas ligeira redução na dose de 600 mg kg^{-1} no Neossolo Quartzarênico. As atividades das enzimas superóxido dismutase, catalase e glutationa redutase dependeram, principalmente, do tipo de solo. Assim, as plantas de soja cultivadas no Neossolo sofreram mais estresse oxidativo do que as cultivadas no Latossolo. Conseqüentemente, a resposta das enzimas ao aumento das concentrações de metais foi pequena.

Palavras-chave: Fitotoxicidade; Estresse oxidativo; Padrão de qualidade do solo; Saúde humana; Propriedades do solo

1 INTRODUCTION

Soil pollution is a serious problem in many countries around the world. Heavy metals, also called trace elements, are of special concern because they accumulate in the environment and can reach concentrations that may pose risks to the human health and to the environment. Their availability in soils depends on lithogenic and pedogenic processes, but also on anthropogenic activities such as mining, combustion of fossil fuels, urban waste disposal, metal working industries, phosphate fertilizer and sewage sludge applications, and municipal waste disposal (HASAN et al., 2009).

Soil may filter the contaminants through adsorption process. Differences in soil properties, however, may have a huge influence on the availability and transfer of the metals that enter in the food chain. The fraction of total soil metal concentration available for plant uptake is called bioavailable fraction. The most important soil characteristics that influence metal availability in the environment are pH, clay content and type, soil organic matter and oxides contents, and the presence of other ions. The effect of pH is recognized as the most important to influence metal availability because it is related to the competition for negative charges in soil colloids. Then, the availability of cationic metals decreases with increase in pH (ALLOWAY; JACKSON, 1991). Other soil properties are related to the binding capacity and retention of the contaminants.

Among the metals, cadmium (Cd) is considered one of the most hazardous to the environment, because it can affect humans and living organisms at relatively low concentrations and it has a relatively high mobility in soils (DAS; SAMANTARAY; ROUT, 1997). Therefore, cadmium can be leached to groundwater or absorbed by food and fodder plants. As a consequence, agricultural soils must be carefully managed, since they are often subjected to the increase of Cd content mainly through the fertilization with phosphate containing Cd and the disposal of sludge sewage or municipal solid waste. Due to these aforementioned characteristics, Cd is one of the most studied metals in the world.

Conversely to Cd, only few studies have focused on barium (Ba). Barium is an alkaline earth element which occurs as a trace metal in igneous and sedimentary rocks. In nature it occurs mainly as low soluble minerals such as barite ($BaSO_4$) and witherite ($BaCO_3$). Some authors observed in nutrient solution studies that Ba may be toxic to bean (LLUGANY; POSCHENRIEDER; BARCELÓ, 2000) and soybean (SUWA et al., 2008). Though Ba is toxic to plants, its interaction with soil colloids and its precipitation with sulphate or with carbonate in soil reduces considerably its toxic effects and plant uptake. Nevertheless, Ba is regulated by the Brazilian legislation that has defined limits for different land use scenarios (CONAMA, 2009).

Metal concentrations in soils may cause plant toxicity by competing with essential mineral nutrients for uptake thereby disturbing the mineral nutrition of plants. Once absorbed by the plant, it accumulates in plant tissue and cell compartments and hampers the general metabolism of the plant (HASAN et al., 2009). The effects include an increase in the production of reactive oxygen species (ROS), which are extremely reactive and cytotoxic and their production must be minimized (ARRUDA; AZEVEDO, 2009). To deal with such a stress plants possess a number of antioxidant system, which includes enzymatic and non-enzimatic mechanisms. Enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), among others, react when the plant is exposed to metals (GRATÃO et al., 2005). The activities of these enzymes are, however, also dependent on the plant developmental stage and the growth conditions of the media.

Yet regarding potential toxicity of metals, Cd is recognized as the only one that may cause risks to human health even at non-phytotoxic concentrations in soil (PEIJNENBURG et al., 2000). Then, no visual symptoms of toxicity or reduction in the production is expected in plants grown in soils with Cd concentration below the established critical limit based on human health risks (de VRIES; RÖMKENS, SCHÜTZE, 2007), but the Cd concentration in edible parts of plants may be higher than the permissible limit for human consumption. Because of this, the consumption of vegetables containing Cd is the main human exposure pathway (SWARTJES et al., 2007).

To define a generic limit of Cd in soil, also called soil quality standard, it is necessary to take into account the soil-plant relationships, especially because of the influence of soil properties on Cd availability for plant uptake. However, it is necessary to use literature data to derive such relationships because of the lack of data for the Brazilian conditions. But, bibliographic references should be differentiated according to regions they were obtained. Otherwise, different patterns of Cd transfer from soil to plants may lead to derive inappropriate critical values for a specific region. For instance, data of Cd from temperate regions may not be comparable to those obtained in humid tropical regions, because of the differences among soils properties from such regions (McLAUGHLIN et al., 2000; RIEUWERTS, 2007). Most of the humid tropical soils are highly weathered and are supposed to have a lower Cd adsorption capacity as compared to temperate soils (NAIDU et al., 1997). Under tropical conditions, soils are normally more acidic than in temperate regions; therefore, this may contribute to increase the availability and consequently the uptake of Cd by plants. Another source of variation that might influence is the difference among vegetable species adapted to distinct climate conditions, which can result a completely different pattern of Cd uptake and accumulation by vegetables. All variables together make the direct application of a dataset from one region to another region more difficult.

Most of the Brazilian territory lies on the tropical humid region. So, most soils are highly weathered and present a mineralogy composed mainly by kaolinite and iron and aluminum oxides (FONTES; ALLEONI, 2006). Moreover, the content of soil organic matter is usually low. However, site specific differences can be high and, Cd retention capacity may differ markedly. Agricultural management practices that increase the pH, such as liming, may also reduce Cd availability and its uptake by plants. Tlustoš et al. (2006) showed that liming reduced Cd accumulation in wheat grains in a pot experiment. But, the liming effect is not always consistent in reducing Cd availability and uptake as reviewed by Smolders (2001).

The objectives of this study were:

- To select appropriate data from literature references by comparing tropical and temperate datasets, deriving soil-plant relationships and then calculating critical soil Cd concentrations valid for the State of São Paulo, Brazil.

- To evaluate the influence of liming on Cd availability and accumulation in lettuce grown in two tropical soils with contrasting characteristics, and to derive relevant soil-plant relationships, in order to calculate critical soil Cd concentrations for the State of São Paulo based on human health risks.

- To evaluate the effects of Ba and Cd concentrations (based on the Brazilian legislation) and time length of exposure, on plant growth, lipid peroxidation and activities of three antioxidant enzymes (CAT, GR, and specific isoenzymes of SOD) in leaves of soybean plants grown in tropical soils with contrasting properties.

References

ALLOWAY, B.J.; JACKSON, A.P. The behaviour of heavy metals in sewage sludge-amended soils. **Science of the Total Environment**, Amsterdam, v. 100, p. 151-176, 1991.

ARRUDA, M.A.Z.; AZEVEDO, R.A. Metallomics and chemical speciation: towards a better understanding of metal-induced stress in plants. **Annals of Applied Biology**, Warwick, v. 155, p. 301-307, 2009.

CONSELHO NACIONAL DO MEIO AMBIENTE. **Resolução n° 420 de 28 de Dezembro de 2009. Dispõe sobre critérios e valores orientadores de qualidade do solo quanto à presença de substâncias químicas e estabelece diretrizes para o gerenciamento ambiental de áreas contaminadas por essas substâncias em decorrência de atividades antrópicas.** Brasília, 2009, publicada no Diário Oficial da União de 30 de Dezembro de 2009. Disponível em: http://www.mma.gov.br/port/conama/legiabre.cfm?codlegi=620. Acesso em: 05 nov. 2010.

DAS, P.; SAMANTARAY, S.; ROUT, G.R. Studies on cadmium toxicity in plants: a review. **Environmental Pollution**, Barking, v. 98, p. 29-36, 1997.

de VRIES, W.; RÖMKENS, P.F.A.M.; SCHÜTZE, G. Critical soil concentrations of cadmium, lead, and mercury in view of health effects on humans and animals. **Reviews of Environmental Contamination and Toxicology**, New York, v. 191, p. 91–130, 2007.

FONTES, M.P.F.; ALLEONI, L.R.F. Electrochemical attributes and availability of nutrients, toxic elements, and heavy metal in tropical soils. **Scientia Agrícola**, Piracicaba, v. 63, p. 589-608, 2006.

GRATÃO, P.L.; POLLE, A.; LEA, P.J.; AZEVEDO, R.A. Making the life of heavy metal-stressed plants a little easier. **Functional Plant Biology**, Victoria, v. 32, p. 481-494, 2005.

HASAN, S.A.; FARIDUDDIN, Q.; ALI, B.; HAYAT, S.; AHMAD, A. Cadmium: Toxicity and tolerance in plants. **Journal of Environmental Biology,** Lucknow, v. 30, p. 165-174, 2009.

LlUGANY, M.; POSCHENRIEDER, C.; BARCELÓ, J. Assessment of barium toxicity in bush beans. **Archives of Environmental Contamination and Toxicology**, New York, v. 39, p. 440-444, 2000.

McLAUGHLIN, M.J.; HAMON, R.E.; McLAREN, R.G.; SPEIR, T.W.; ROGERS, S. L.A bioavailability-based rationale for controlling metal and metalloid contamination of agricultural land in Australia and New Zealand. **Australian Journal of Soil Research**, Melbourne, v. 38, p. 1037-1086, 2000.

NAIDU, R.; KOOKANA, R.S.; SUMNER, M.E.; HARTER, R.D.; TILLER, K.G. Cadmium sorption and transport in soils dominated by variable charge surfaces: a review. **Journal of Environmental Quality**, Madison, v. 26, p. 602-617, 1997.

PEIJNENBURG, W.; BAERSELMAN, R.; de GROOT, A.; JAGER, T.; LEENDERS, D.; POSTHUMA, L.; van VEEN, R. Quantification of metal bioavailability for lettuce (Lactuca sativa L.) in field soils. **Archives of Environmental Contamination and Toxicology**, New York, v. 39, p. 420-430, 2000.

RIEUWERTS, J.S. The mobility and bioavailability of trace metals in tropical soils: a review. **Chemical Speciation and Bioavailability**, Surrey, v. 19, p. 75–85, 2007.

SMOLDERS, E. Cadmium uptake by plants. **International Journal of Occupational Medicine and Environmental Health**, Attleboro, v. 14, p. 177-183, 2001.

SUWA, R.; JAYACHANDRAN, K.; NGUYEN, N.T.; BOULENOUAR, A.; FUJITA, K.; SANEOKA, H. Barium toxicity effects in soybean plants. **Archives of Environmental Contamination and Toxicology**, New York, v. 55, p. 397-403, 2008.

SWARTJES, F.A.; van BREEMEN, E.M.D.; OTTE, P.F.; van BEELEN, P.; RIKKEN, M.G.J.; TUINSTRA, J.; SPIJKER, J.; LIJZEN, J.P.A. Human health risks due to consumption of vegetables from contaminated sites. RIVM, Bilthoven, the Netherlands, 2007. (RIVM report 711701040).

TLUSTOŠ, P.; SZÁKOVÁ, J.; KOŘÍNEK, K.; PAVLÍKOVÁ, D.; HANČ, A.; BALÍK, J. The effect of liming on cadmium, lead and zinc uptake reduction by spring wheat grown in contaminated soil. **Plant, Soil and Environment**, Praha, v. 52, p. 16–24, 2006.

2 DERIVATION OF CRITICAL SOIL CADMIUM CONCENTRATIONS FOR THE STATE OF SÃO PAULO, BRAZIL, BASED ON HUMAN HEALTH RISKS

Abstract

The main cadmium (Cd) exposure pathway for humans is through diet. A database on Cd concentration in soils and accumulation in edible vegetables from tropical and temperate regions was organized, soil-plant relationships were derived, and then critical soil Cd concentrations were calculated based on human exposure parameters for the State of São Paulo, Brazil. Cadmium accumulation in leafy and root vegetables could be predicted by multiple regression analysis and most of the variance was explained when total Cd concentration and pH in soil were included as predictors. The calculated Cd bioconcentration factors for the tropical dataset were higher as compared to the temperate dataset. Consequently, critical soil Cd concentrations were from 1.7 to 3.2-fold lower for tropical conditions. Higher humidity and temperature at the tropics, as well as more weathered soils with lower retention capacity of Cd, may explain the higher Cd uptake and accumulation in tropical than in temperate regions. To protect human health, exclusive data regarding Cd in soils and edible vegetables from tropical regions should be used for the State of São Paulo to derive critical soil Cd concentrations, instead of (additional) data from temperate regions.

Keywords: Vegetable consumption; Tropical soils; Bioconcentration factor; Soil pollution; Heavy metals

2.1 Introduction

Soil contamination by metals is a problem in several countries around the world. Defining limits of these metals in soils, such as critical soil concentrations, also called soil quality standards (SQS), is necessary to protect human health and the environment. For derivation of SQS, however, first an exposure scenario must be defined to describe the conditions that are suitable for a specific type of critical soil concentration (SWARTJES et al., 2007). Such an exposure scenario is a combination of both scientific knowledge and policy decisions.

Cadmium (Cd) is one of the most dangerous metals due to its high mobility in the environment and high toxicity to humans and organisms, even at low concentrations (DAS; SAMANTARAY; ROUT, 1997; YANG et al.,, 2009). Cadmium enters the human body via many exposure pathways, i.e. soil ingestion, drinking water, air inhalation and vegetables and animals consumption. However, the main source of Cd exposure in non-occupational people except smokers is the consumption of Cd-contaminated food (GRANT; BAILEY, 1997). According to the World Health Organization (WHO), rice, wheat, all kind of vegetables (including root, tuber, leafy and other vegetables) and molluscs account for 40–85% of the total human intake of Cd (WHO, 2006). Regarding vegetables grown for private consumption, however, this pathway can represent up to more than 90% of the total human Cd intake, depending on the exposure scenario (SWARTJES et al., 2007).

The most common index used for estimating the metal accumulation in vegetables and subsequent human exposure through vegetable consumption is the bioconcentration factor (BCF), i.e. the ratio between the metal concentration in edible parts of vegetables and the total metal concentration in soil (SWARTJES et al., 2007). Because of its simple application BCF is widely used (ALONSO et al., 2003; SIPTER et al., 2008; MURRAY; THOMPSON; MACFIE, 2009), including the derivation of soil screening levels for plant uptake (USEPA, 1996). Although BCF may vary highly depending on the vegetable type (ALEXANDER; ALLOWAY; DOURADO, 2006) or even among cultivars or genotypes of the same vegetable (McLAUGHLIN et al., 1994; ALEXANDER; ALLOWAY; DOURADO, 2006; ZHENG et al., 2008), linear relationships (in a logarithmic scale) were found between total soil Cd and plant uptake, taking into account soil properties (McBRIDE, 2002; RÖMKENS et al., 2009).

Among the soil properties, pH is the most important in controlling Cd availability and uptake by vegetables (ANDERSON; CHRISTENSEN, 1988; PEIJNENBURG et al., 2000; McBRIDE, 2002; GOLIA; DIMIRKOU; MITSIOS, 2008). Thus, a soil-plant relationship can be improved by considering the main soil properties that influence Cd availability (de VRIES; RÖMKENS; SCHÜTZE, 2007). Soil-plant relationships were successful used for the derivation of SQS for Cd in several studies (OTTE et al., 2001; BRUS; de GRUIJTER; RÖMKENS, 2005; de VRIES; RÖMKENS; SCHÜTZE, 2007; FRANZ et al., 2008; RÖMKENS et al., 2009). Most of these relationships, however, were performed for the derivation of SQS based on literature data for vegetables and soils from temperate regions.

There are few studies regarding Cd and other metal concentrations in soils and vegetables from tropical regions. As a consequence, data from temperate regions have been often used to derive SQS for tropical regions, e.g. for the state of São Paulo, Brazil (COMPANHIA DE TECNOLOGIA DE SANEAMENTO AMBIENTAL – CETESB, 2001). Data from temperate regions, however, may not be appropriate, because of the differences among soils from tropical and temperate regions (McLAUGHLIN et al., 2000; RIEUWERTS, 2007). Most of the humid tropical soils are highly weathered and are supposed to have a lower Cd adsorption capacity as compared to Cd adsorption capacity of temperate soils (NAIDU et al., 1997).

Differences among vegetable species and climate conditions between both types of regions may also play a significant role, resulting in a completely different pattern of Cd uptake by vegetables. Our hypothesis is that vegetable Cd uptake is higher in tropical as compared to temperate regions. As a consequence, critical soil Cd concentrations with regard to vegetable uptake must be lower than critical concentrations under temperate conditions. We aimed to select appropriate data from literature studies, deriving soil-plant relationships and then calculating critical soil Cd concentrations valid for the State of São Paulo, Brazil. The State of São Paulo (20-25^{o} S; 44-53^{o} W) is the most populated Brazilian State, with more than 40 million inhabitants in 250,000 km^2 and 645 municipalities, representing 34% of Brazilian gross domestic product.

2.2 Material and Methods

2.2.1 Selection of Literature Data

A literature search was performed from several sources (Web of Science; Scopus, etc.) to compose a database for the derivation of soil-plant relationships for Cd in soils and vegetables. The minimum requirements to select data from a study were providing total soil Cd concentration, the paired Cd concentration in the edible parts of vegetables and pH. The data were considered reliable when information about quality analysis control of Cd in soil and vegetables was also provided. Only vegetables were selected, because they are supposed to accumulate higher Cd concentrations than other groups of plants (JINADASA et al., 1997) and they are relevant for human health risk assessment, because they are consumed directly by humans. Only field or pot experiments data with non-spiked soils were selected, since experiments with Cd-spiked soils by salts are supposed to overestimate uptake as compared to field conditions (EFFROYSOM; SAMPLE; SUTER, 2004), and such an overestimation can vary from a factor of 2.5 (McLAUGHLIN et al., 2006) up to a factor of 20 (YANG et al., 2009). Only Cd concentrations from the topsoil were considered. Cadmium concentrations in vegetables reported on a fresh weight basis were converted to dry weight considering the water content. When this information was not available, conversion factors from the literature (USEPA, 1996; SWARTJES et al., 2007) were applied.

All selected soil and vegetable Cd concentrations represent averages from composite samples. A procedure adopted to compare samples from different studies is to apply a weighting factor, based on the number of individual samples to make a composite sample (HOEFKENS et al., 2009). However, not all studies reported such information, and then no weighting factor was applied. Also, no differentiation was made between pH measured in water or in solutions such as 0.01 M $CaCl_2$, because such information was missing in some studies.

The database comprised studies from different parts of the world, in which vegetables were grown under some potential source of pollution, such as in industrial areas, soils amended with sewage sludge, wastewater irrigation, sites near highways, etc. (Table 1). None of the selected studies, however, was carried out in Brazil. To enhance the applicability for Brazilian conditions, only the vegetable species grown and consumed in the State of São Paulo, Brazil (CAMARGO; CAMARGO; CAMARGO FILHO, 2008) were selected. They were grouped into three categories of vegetables: leafy, roots (including tubers) and fruits (referred in this study as a vegetable category). Swartjes et al., (2007) distinguished eight vegetable groups, but they were not able to derive many significant plant-soil relations due to lack of data for most of these specific groups. Additionally, the dataset was separated into temperate and tropical (humid tropical).

Table 1 – Overview on the selected references for the database composition

Region	Country	Source of potential contamination	Reference
	Nigeria	Long-term wastewater irrigation	Agbenin; Danko; Welp, 2009
		Farmlands around industrial areas	Yusuf et al., 2003
		Vegetable cropping along major highways	Atayese et al., 2009
		Vicinity of open municipal waste dump	Oyedele; Gasu; Awotoye, 2008
	India	Air-borne heavy metals in an organic farm	Pandey; Pandey, 2009
		Wastewater irrigation	Gupta et al., 2009
Tropical		Peri-urban soils	Singh; Kumar, 2006
		Wastewater irrigation with tannery effluents	Sahu et al., 2007
		Wastewater irrigation	Singh et al., 2009
	Vietnam	Wastewater irrigation	Marcussen et al., 2008
	Camboja	Irrigation with industrial and domestic wastewater	Marcussen; Dalsgaard; Holm, 2009
	New Zealand*	Long-term use of agrichemicals and soil amendments	Gaw et al., 2008
	Phillipines	Irrigation with water taken from urban sites	Hardiyanto et al., 2008
	Uganda	Farming sites along major highways	Nabulo; Origa; Diamond, 2006
	Zimbabwe	Wastewater irrigation	Mapanda et al., 2007
	Bangladesh	Vegetables grown in and around an industrial area	Ahmad; Goni, 2009
	Australia	Survey in Agricultural soils	Jinadasa et al., 1997
		Biosolids application	Watmuff, 2002
	China	Long-term use of manure and fertilizer containing Cd	Yang et al., 2009
		Area around mining and smelting	Zhuang et al., 2009
		Area adjacent to a Pb/Zn mine	Li et al., 2006
		Area around mining and smelting	Li et al., 2006b
		Long-term irrigation of metal-contaminated water	Zheng et al., 2008
	UK	Long-term application of sewage sludge	Datta; Young, 2005
		Gardens contaminated by heavy metals from a lead mining	Davies, 1992
Temperate		Urban garden soils	Moir; Thornton, 1989
	Spain	Industrial and urban pollution	Alegria et al., 1991
	USA	Application of municipal sewage sludge	Page et al., 1987
		Soils mixed with mine wastes	Cobb et al., 2000
		Soil contamination with batteries	Dorris; Bassan, 2002
	Canada	Agricultural contaminated soils	Murray; Thompson; Macfie, 2009
	Romania	Areas influenced by emissions of heavy metals	Lăcătuşu; Lăcătuşu, 2008
	Slovenia	Contaminated vegetable gardens	Karo, 2007
	Greece	Vegetables Grown in an Industrial Area	Fytianos et al., 2001
	Iran	Wastewater irrigation	Behbahaninia et al., 2009

* Sub-tropical region

2.2.2 Development of the Soil-Plant Relationships

To develop the soil-plant relationships the soil Cd concentration range was selected between 0.1 and 12 mg kg^{-1}. At total soil Cd concentrations lower than 0.1 mg kg^{-1}, plants uptake rates are assumed be too low to represent unacceptable human health risks (based on PETERSEN et al., 2002: there is no unacceptable risk at concentrations lower then 0.5 mg kg^{-1}; SWARTJES et al., 2007: below 0.5 mg kg^{-1} every crop can be cultivated and consumed without unacceptable risks to human health; and using an assessment factor of 5). Moreover, such a value would be impractical, since it is lower than the background concentration normally found in soils from many countries, including that one from the State of São Paulo (0.5 mg kg^{-1}). The concentration range up to 12 mg kg^{-1} includes the current Cd Intervention Values for the State of São Paulo related to two scenarios where vegetables are supposed to be grown: agricultural (Rural) and residential (Urban), which are 3.0 and 8.0 mg kg^{-1}, respectively (CETESB, 2005). The rural scenario was defined as agricultural areas traditionally used for growing vegetables, including growth for own production. On the other hand, the urban scenario was defined as areas predominantly for residence, but also includes vegetable consumption from own gardens.

Dissolved Cd in soil solution is strongly dependent on total soil Cd concentration and soil pH (McBRIDE; SAUVÉ; HENDERSHOT, 1997; SAUVÉ et al., 2000) and can be written as the following equation:

$$\log(\text{dissolved Cd}) = a + b \log \text{Cd soil} + c \text{ pH soil} \qquad (1)$$

where log [Cd soil] = logarithm (base 10) of the total Cd concentration in soil (mg kg^{-1}).

Although this is an empirical equation, it has some theoretical basis from metal complexation theory, as described in McBride, Sauvé and Hendershot (1997). Assuming that plant uptake is described by a linear function of the dissolved Cd in soils, in a logarithmic scale, the following equation was used:

$$\log[\text{Cd veg}] = a + b \log[\text{Cd soil}] + c \text{ pH soil} \qquad (2)$$

where: log [Cd veg] = logarithm (base 10) of the Cd concentration in the edible part of the vegetable (mg kg^{-1}); log [Cd soil] = logarithm (base 10) of the total Cd concentration in soil (mg kg^{-1}); a, b and c are empirical coefficients (that can have either positive or negative sign) obtained by multiple linear regression using SPSS 16.0 software. All concentrations used were on dry weight basis, unless stated otherwise.

Other factors like soil organic matter, clay content and (hydr)oxide contents may also influence the bioavailability of Cd, however, only a limited number of studies reported this information and then they could not be included as predictors. A soil-plant relationship was considered significant when the $R^2 \geq 0.5$, the standard error (Y-*est*) ≤ 0.5 and the coefficients were significant at least at $p < 0.05$. From the significant soil-plant relationships the BCF (Cd veg/Cd soil) values were calculated for each group of vegetables within the selected soil Cd concentration range (0.1-12 mg kg^{-1}) and a particular soil pH (5.0 and 6.0), to show the impact of both pH and Cd in soil on this index. These pH values were chosen because they are compatible to most agricultural or residential garden soils at the State of São Paulo, Brazil.

2.2.3 Derivation of Critical Soil Cd Concentrations

Critical soil Cd concentrations were derived on the basis of exposure, considering all the pathways, and the toxicological reference dose (0.5 µg per kg body weight per day) (BAARS et al., 2001), by using the CSOIL 2000 exposure model (BRAND; OTTE; LIJZEN, 2007). A summary of the CSOIL model parameters is presented in Table 2.

Specific BCF values for a particular soil pH and exposure scenario were obtained through an iterative process, in which the starting soil Cd concentration and the resulting critical soil Cd concentration (Cdcrit) converge to values closer than one percent, as described in Ministry for the Environment (NEW ZEALAND, 2010). Total vegetable consumption rates (100%) and three fractions (50%; 25% and 10% of the total) are assumed to be taken from the contaminated site were evaluated. The consumption rates of vegetables were taken from the Brazilian Institute of Geography and Statistics for the State of São Paulo, Brazil (IBGE, 2004). The soil type for each standard scenario was normalized to 25% of clay content and 2% of soil organic matter content to make it compatible with many soils of the State of São Paulo.

Table 2 – Parameters used in C-SOIL model

Specific Soil Data	**value**		**unit**
soil temperature	283		K
Volume fraction air in soil	0.20		-
Volume fraction water in soil	0.30		-
Volume fraction solids in soil	0.50		-
Organic carbon fraction	0.0116		-
clay content	25		%
pH	5.0 and 6.0		-
Model Parameter Values			
water solubility	3.00×10^{3}		mg dm^{-3}
Partition coefficient metals	2.56×10^{3}		dm^{3} kg^{-1}
Bioconcentration factor (Cd vegetable/Cd soil)	dependent on the calculation as described in Material and Methods		
fraction contaminated root vegetables	0.10; 0.25; 0.50 and 1.00		-
fraction contaminated leafy vegetables	0.10; 0.25; 0.50 and 1.00		-
	value for children	**value for adults**	
body weight	15	70	kg
daily intake soil (year average)	1.00×10^{-4}	5.00×10^{-5}	kg/d
consumption of root/tuber vegetables (rural scenario)	0.028	0.056	kg/d
consumption of root/tuber vegetables (urban scenario)	0.019	0.038	kg/d
consumption of leafy vegetables (rural scenario)	0.027	0.093	kg/d
consumption of leafy vegetables (urban scenario)	0.048	0.110	kg/d
consumption of drinking water	1.00	2.00	dm^{3}/d
inhalated soil particles	3.13×10^{-7}	8.33×10^{-7}	kg/d
time spend indoors (year average)	0.211	0.229	h
time spend outdoors (year average)	2.86	1.14	h
breathing volume	0.317	0.833	m^{3}/h
body surface	0.95	1.80	m^{2}
Exposed surface indoor	0.05	0.09	m^{2}
Exposed surface outdoor	0.28	0.17	m^{2}
degree of covert skin indoor	5.60×10^{-4}	5.60×10^{-4}	kg/m^{2}
degree of covert skin outdoor	5.10×10^{-3}	0.0375	kg/m^{2}
Dermal absorption velocity	0.01	5.00×10^{-3}	L/h
Duration exposure contact soil indoor	9.14	14.90	h/d
Duration exposure contact soil outdoor	2.86	1.14	h/d

- Dimensionless

2.3 Results

Tropical and temperate data had different patterns, as related to vegetable Cd uptake (Table 3). The same pH range (5.5-8.6), and similar Cd concentrations ranges in leafy (0.05-7.4 mg kg^{-1}) and root (0.03-7.0 mg kg^{-1}) vegetables were found in the selected dataset from tropical regions. However, fruit vegetables exhibit a narrower range of Cd concentration (0.01-4.9 mg kg^{-1}) and a lower pH range (4.4-7.8). On the other hand, leafy vegetables from temperate regions had a wider range of Cd concentration (0.02-19.0 mg kg^{-1}), but root (0.03-7.1 mg kg^{-1}) and fruit

(0.30-7.1 mg kg^{-1}) vegetables presented a similar and lower range. The range of temperate soil pH was different of the tropical soil pH dataset, being lower for leafy (4.2-7.9) and root (4.1-7.8) vegetables and higher for fruit (6.9-8.2) vegetables.

Table 3 – Coefficients of the soil-plant relationships determined by multiple linear regressions from the selected dataset

Dataset	Vegetable type	Range of Cd in vegetable (mg kg^{-1} DW)	pH	soil-plant relationship	R^2	*se* (Y-*est*)	n
Tropical	Leafy	0.05–7.4	5.5–8.6	Log [Cd veg] = 1.52 + 0.71** log [Cd soil] – 0.17*pH soil	0.65	0.26	35
	Root	0.03–7.0	5.5–8.6	Log [Cd veg] = 1.49 + 0.82** log [Cd soil] – 0.27** pH soil	0.82	0.23	16
	Fruit	0.01–4.9	4.4–7.8	Log [Cd veg] = - 2.63 + 0.53 log [Cd soil] + 0.35 pH soil	0.14	0.43	19
Temperate	Leafy	0.02–19.0	4.2–7.9	Log [Cd veg] = 1.43 + 0.86** log [Cd soil] – 0.23** pH soil	0.52	0.50	56
	Root	0.03–7.1	4.1–7.8	Log [Cd veg] = 0.43 + 0.99** log [Cd soil] – 0.15* pH soil	0.70	0.40	27
	Fruit	0.30–7.1	6.9–8.2	Log [Cd veg] = 0.79 + 0.49* log [Cd soil] – 0.24* pH soil	0.24	0.66	37

** and * Significant coefficients at $p < 0.01$ and at $p < 0.05$, respectively ; *se* (Y-*est*) = standard error of the estimate; n = number of observations

Soil-plant relationships for leafy vegetables, roots and fruits are presented in Table 3. For leafy and root vegetables the relationships were significant for both tropical and temperate data. No significant soil-plant correlations were found for fruit for either tropical or the temperate datasets. Therefore, they can not be used for the calculation of BCFs and, hence, for the derivation of Cdcrit. Such absence of correlation may be explained by the pathway that Cd must follow from soil to reach the fruit, in which the membranes cell form a barrier. Consequently, it is generally accepted that accumulation of Cd in fruits is relatively low compared to leafy and root vegetables (JINADASA et al., 1997; YANG et al., 2009).

Based on the coefficients of pH (Table 3), its impact in reducing Cd availability for vegetable uptake was higher for leafy vegetables from temperate than tropical regions, whilst for root vegetables such effect was higher for tropical than for temperate data. Nevertheless, the calculated BCFs from such soil-plant relationships were higher for tropical than for temperate dataset, for both leafy and root vegetable groups (Fig. 1).

The BCFs for leafy vegetables (Fig. 1A and B) were higher than for root vegetables (Fig. 1C and D). Such results are probably related to the efficiency of leafy vegetables in uptake and accumulation of Cd in its edible parts and clearly show that different groups of vegetables have different patterns of Cd accumulation. At soil Cd concentrations below 1.0 mg kg^{-1} the calculated BCF was relatively high as compared to concentrations of Cd in soil higher than 1.0 mg kg^{-1}. The impact of pH on the BCF was higher for tropical root vegetables (Fig. 1C) as compared to other groups.

The vegetable consumption rates for the State of São Paulo, in the studied scenarios, were only available for leafy vegetables and fruits combined. Therefore, the calculations based on soil-plant relationships for leafy vegetables may be considered conservative, which is a reasonable assumption for the derivation of generic soil quality standards. The tropical dataset yielded critical soil Cd concentrations much lower as compared to the temperate dataset, for all exposure scenarios. This is a result of the higher calculated BCFs for the tropical dataset.

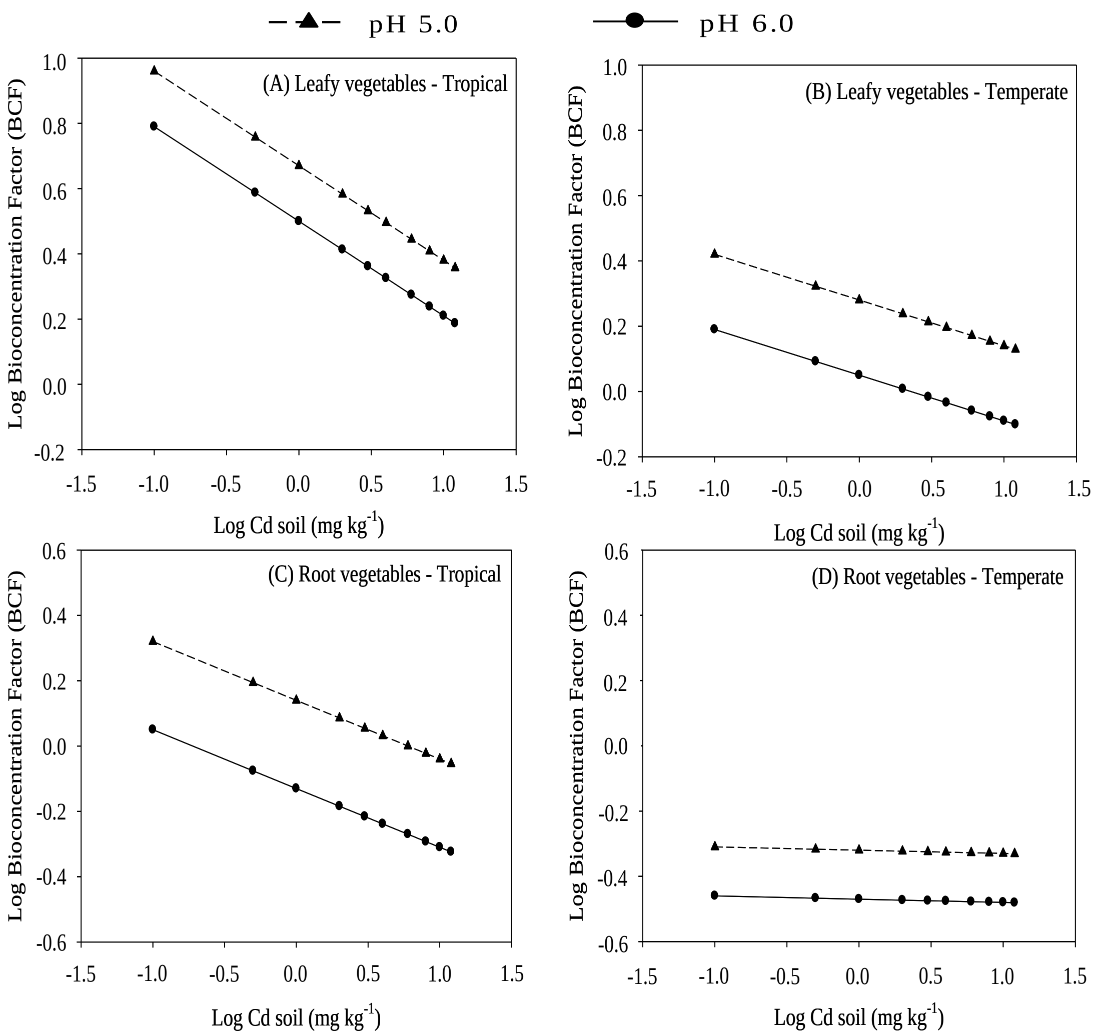

Figure 1 – Influence of soil Cd concentrations and pH on the bioconcentration factor (BCF), as calculated by multiple linear regressions

The contribution of soil ingestion to total lifelong exposure of Cd varied from 0.1 to 8.0%, whereas the contribution of vegetable consumption was from 92.0 to 99.9% (data not shown). Other exposure pathways were negligible. Based on this, one can say that the consumption rates of contaminated vegetables (taken from the contaminated site) had an almost linear impact on the derived critical soil Cd concentration, which means that the higher the consumption the lower is Cdcrit.

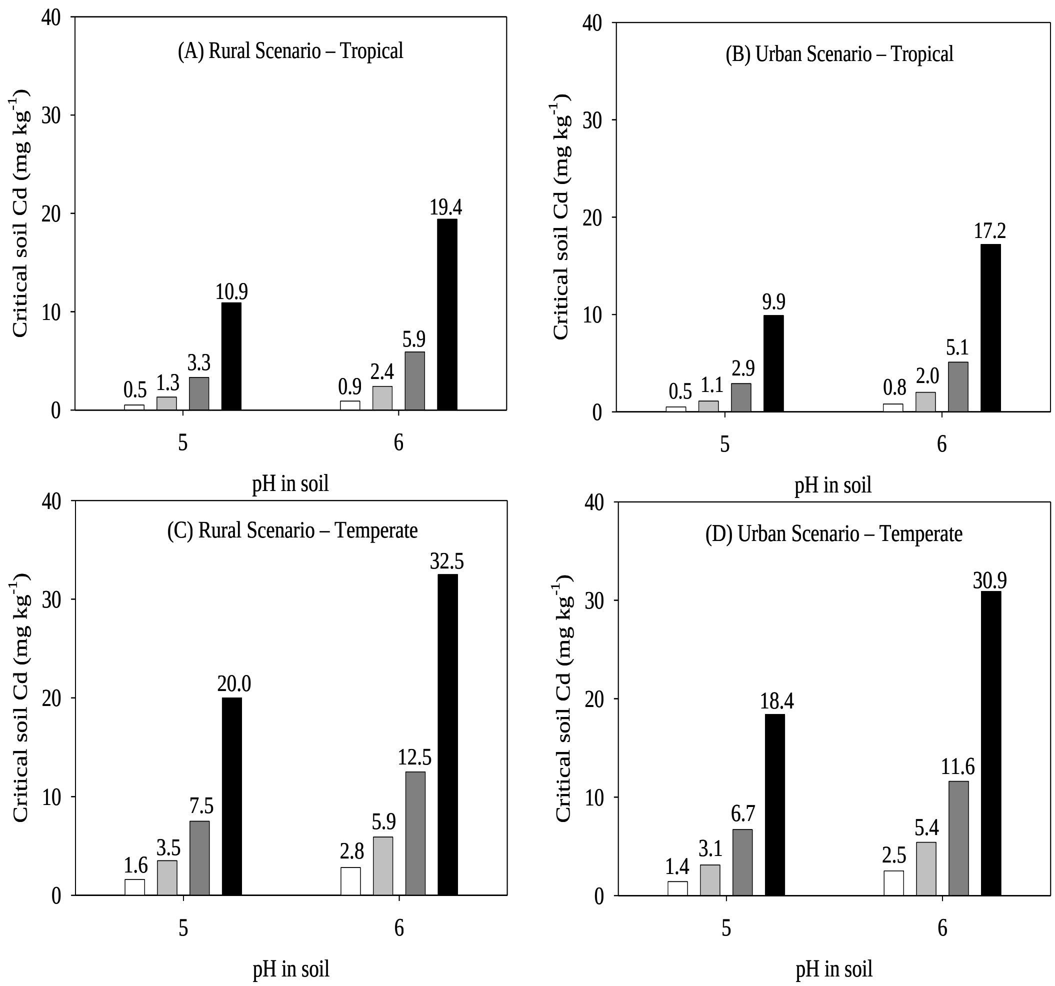

Figure 2 – Critical soil Cd concentrations in different exposure scenarios and settings of data. (□) Total vegetable consumption rate (100%); (▒) 50% of vegetable consumption; (▓) 25 % of vegetable consumption; (■) 10 % of vegetable consumption taken from a contaminated area.

Regarding the tropical dataset, values were very low, in both rural and urban scenarios when 100% and 50% of vegetable consumption were assumed as being taken from the contaminated site (Figs. 2A and B). On the other hand, Cdcrit reached relatively high values when only 10% of vegetable consumption was regarded. However, values higher than 12 mg kg^{-1} should be interpreted carefully, because they are outside the range of dataset used for the calculations.

In average, the Cdcrit was 1.7-fold more restrictive at pH 5.0 than at pH 6.0 for all exposure scenarios (Fig. 2). Also, at the same conditions, Cdcrit from temperate regions (Figs. 2C and D) were from 1.7 up to 3.2-fold higher than from the tropical dataset.

2.4 Discussion

The availability of Cd in soil for vegetable uptake depends on the soil Cd concentration and on several soil properties such as pH, the organic matter content, the clay content, the (hydr(oxide) content and the concentration of other metals in soil. The soil pH affects the metal solubility and/or precipitation, carboxyl and phenolic groups from the organic matter, clay and the (hydr)oxides act as sorbent or complexing agents to reduce the availability of dissolved Cd, and other metals competes with Cd for uptake and transport inside the plant (BRUS; de GRUIJTER; RÖMKENS, 2005). All these parameters influence the variability of Cd uptake. Additionally, the heterogenic distribution of Cd in soil increases the variability in vegetable uptake (MILLIS; RAMSEY; JOHN, 2004). Nevertheless, Effroymson, Sample and Suter (2001), McBride (2002), Li, Okazaki and Zhou (2003), Adams et al. (2004), Römkens et al. (2009) have shown that Cd uptake by different vegetable species can be predicted taking into account soil properties, and all of them included pH as a predictor.

In this study, in agreement with former findings, pH has been demonstrated to be a significant soil property to predict Cd uptake by vegetables (leafy and root). At high soil pH, Cd compounds such as Cd-phosphate, Cd-carbonate and Cd-hydroxide form in soil (DORRIS; BASSAM, 2002) and reduces its availability. However, contrary to other studies, we have grouped several vegetable species to compose the leafy and root vegetable groups, which share similarities regarding Cd uptake, but increase the variability due to the individual uptake characteristics of each species within the group. Nevertheless, the soil-plant relationships were significant.

Generally, not much attention has been paid to the differences among vegetables grown in tropical and temperate regions, regarding Cd uptake. As mentioned before, the differences among vegetable species or even genotypes of the same specie (McLAUGHLIN et al., 1994;

ALEXANDER; ALLOWAY; DOURADO, 2006; ZHENG et al., 2008) adapted to each region may play a significant difference on Cd uptake. Moreover, humid tropical regions usually have a hot, rainy season, which result in a completely different environment for growing vegetables, regarding the evapotranspiration rate and, consequently, the amount of contaminants (e.g. Cd) uptake. Our results clearly show higher bioconcentration factors (BCFs) for the same groups of vegetables grown in tropical than in temperate regions.

Besides of the aforementioned differences between both regions, the differences in soil characteristics are also important to take into consideration. Many tropical soils are highly weathered with low nutrients and organic matter contents (McLAUGHLIN et al., 2000; CRASWELL; LEFROY, 2001). Normally, their clay fraction is dominated by 1:1 layer silicates (mainly kaolinite) and iron and aluminium oxyhydroxides, which yields a low cation exchange capacity (FONTES; ALLEONI, 2006). In contrast with these variable charge soils, many soils from temperate regions are composed predominantly of permanent charged minerals which have high affinities for toxic metals (NAIDU et al., 1997), including Cd. In variable charge soils (mainly Oxisols and Ultisols) the dominant retention mechanism of Cd is outer sphere adsorption, whereas, in soils with predominance of permanent charge sites Cd is strongly bound by inner sphere adsorption (RIEUWERTS, 2007). Although, 2:1 layer-type minerals have been detected even in highly weathered Brazilian tropical soils (ALLEONI et al., 2009), they do not seem to be significant to adsorb Cd as compared to soils from temperate regions. Therefore, the relationship between Cd sorption and soil properties for one geographical region may not be applicable to another region (NAIDU; SUMNER; HARTER, 1998). This explains a higher availability of Cd in tropical soils and, consequently, higher Cd vegetable uptake as found in this study for the tropical as compared to the temperate dataset.

When the purpose is to evaluate soil quality with regard human health protection, the tiered approach (SWARTJES; TROMP, 2008) should be considered. According to this approach a human health risk assessment should be simple when possible (screening evaluation – more conservative – tier 1) and more complex when necessary (more realistic – higher tiers). Since most of the State of São Paulo is located on the humid tropical region and the exposure parameters in this study were based on São Paulo conditions, the derived Cdcrit from the tropical dataset is supposed to best meet the proposal of assessing human health regarding Cd exposure. However, even at the “tier 1”, considering 100% of vegetable consumption coming from own garden (own production) would be unrealistic, because of the limitation of space to supply such amount of vegetables, especially when an urban scenario is regarded. Thus, 50% and 25% of vegetable consumption at a rural and urban scenario, respectively, would still be conservative, but represent a more realistic situation. In this case a “trigger” value (when an investigation should be initiated) could be 1.3 and 2.9 mg kg^{-1} at pH 5.0 or 2.4 and 5.1 mg kg^{-1} at pH 6.0 for the rural and urban scenario, respectively. But, the decision of protection level is, indeed, a combination of technical (scientific) considerations and policy decisions.

When these Cd concentrations in soils are not exceeded it is assumed that vegetables can be grown and consumed, at the aforementioned proportion, without unacceptable risks for humans. These values, especially considering the pH in soil as 5.0, are actually lower than the current Cd Intervention Values for the State of São Paulo, which are 3.0 and 8.0 mg kg^{-1} at the same aforementioned scenarios (CETESB, 2005). But, they are compatible to other legislations, such as in Taiwan (tropical country) in which the current SQS for Cd is 5.0 mg kg^{-1} for arable land (RÖMKENS et al., 2009) or New Zealand which derived a SQS for Cd as 0.5 and 1.4 mg kg^{-1} for pH 5.0 and 6.0, respectively, for a rural or residential scenario when 50% of vegetable consumption is regarded (NEW ZEALAND, 2010).

Some countries have derived SQS for Cd as a function of pH in their legislations (UNITED KINGDOM, 2002; NEW ZEALAND, 2010). However, pH can change in soil in a relatively short time frame, which means a guideline value should not be dependent on an unstable parameter. Considering such situation for São Paulo conditions, in which several soils are acidic or can be easily acidified, Cdcrit derived for pH 5.0 seems to be more appropriate for a screening evaluation purpose.

2.5 Conclusions

Cadmium uptake and accumulation in leafy and root vegetables could be predicted, with most of the variance being explained by total Cd concentration in soil and pH, regardless the fact that each group was comprised by several vegetable species with similar uptake characteristics. The dataset from humid tropical regions yielded higher Cd bioconcentration factors (higher uptake) than the dataset from temperate regions. Such difference was assigned mainly due to the different climate conditions, which is warmer and wetter in the tropics and, due to the differences in soil characteristics. Tropical soils are more weathered and, in most circumstances, exhibit a lower Cd retention capacity than the temperate ones, allowing higher Cd uptake by vegetables. Critical soil Cd concentrations derived from tropical dataset were lower as compared to temperate dataset and such results confirmed our hypothesis. Tropical regions like São Paulo have a higher Cd availability in soils and accumulation in vegetables. Therefore, exhibit a higher exposure to the population. In order to protect human health exclusive data, regarding Cd in soils and edible vegetables, from tropical regions should be used for the State of São Paulo to derive critical soil Cd concentrations, instead of (additional) data from temperate regions.

Future research is recommended in the State of São Paulo to measure the accumulation of Cd and other metals in areas subjected to different sources of pollution. Local measurements would result in better predictions of human exposure considering the vegetable consumption exposure pathway.

References

ADAMS, M.L.; ZHAO, F.J.; MCGRATH, S.P.; NICHOLSON, F.A.; CHAMBERS, B.J.; Predicting cadmium concentrations in wheat and barley grain using soil properties. **Journal of Environmental Quality**, Madison, v. 33, p. 532-541, 2004.

AGBENIN, J.O.; DANKO, M.; WELP, G. Soil and vegetable compositional relationships of eight potentially toxic metals in urban garden fields from northern Nigeria. **Journal of the Science of Food and Agriculture**, London, v. 89, p. 49-54, 2008.

AHMAD J.U.; GONI M.A. Heavy metal contamination in water, soil, and vegetables of the industrial areas in Dhaka, Bangladesh. **Environmental Monitoring and Assessment**, Dordrecht, v. 166, p. 347-357, 2009.

ALEGRIA, A.; BARBERÁ, R.; BOLUDA, R.; ERRECALDE, F.; FARRÉ, R.; LAGARDA, M. J. Environmental cadmium, lead and nickel contamination - possible relationship between soil and vegetable content. **Fresenius Journal of Analytical Chemistry**, Berlin, v. 339, p .654-657, 1991.

ALLEONI, L.R.F.; PEIXOTO, R.T.G.; AZEVEDO, A.C.; MELO, L.C.A. Components of surface charge in tropical soils with contrasting mineralogies. **Soil Science**, Baltimore, v. 174, p. 629-638, 2009.

ALEXANDER, P.D.; ALLOWAY, B.J.; DOURADO, A.M. Genotypic variations in the accumulation of Cd, Cu, Pb and Zn exhibited by six commonly grown vegetables. **Environmental Pollution**, Barking, v. 144, p. 736-745, 2006.

ALONSO, J.; GARCÍA, M.A.; PÉREZ-LÓPEZ M.; MELGAR, M.J.The concentrations and bioconcentration factors of copper and zinc in edible mushrooms. **Archives of Environmental Contamination and Toxicology**, New York, v. 44, p. 180-188, 2003.

ANDERSON, P.R.; CHRISTENSEN, T.H. Distribution coefficients of Cd, Co, Ni and Zn in soils. **Journal of Soil Science**, Oxford, v. 39, p. 15-22, 1988.

ATAYESE, M.O.; EIGBADON, A.I.; OLUWA K.A.; ADESODUN, J.K. heavy metal contamination of amaranthus grown along major highways in Lagos, Nigeria. **African Crop Science Journal**, Kampala, v. 16, p. 225-235, 2009.

BAARS, A.J.; THEELEN, R.M.C.; JANSSEN, P.J.C.M.; HESSE, J.M.; van APELDOORN, M.E.; MEIJERINK, C.M.; VERDAM, L.; ZEILMAKER, M.J. **Re-evaluation of the human-toxicological maximum permissible risk levels.** RIVM, Bilthoven, the Netherlands, 2001.(RIVM report 711701025**)**

BEHBAHANINIA, A.; MIRBAGHERI, S.A.; KHORASANI, N.; NOURI, J.; JAVID, A.H. Heavy metal contamination of municipal effluent in soil and plants. **Journal of Food Agriculture & Environment**, Helsinki, v. 7, p. 851-856, 2009.

BRAND, E.; OTTE, P.F.; LIJZEN, J.P.A. **CSOIL 2000**: Exposure model for human risk assessment of soil contamination. A model description. RIVM, Bilthoven, the Netherlands, 2007. (RIVM Report 711701054).

BRUS, D.J.; de GRUIJTER, J.J.; RÖMKENS, P.F.A.M. Probabilistic quality standards for heavy metals in soil derived from quality standards in crops. **Geoderma**, Amsterdam, v. 128, p. 301–311, 2005.

CAMARGO, A.M.M.P.; CAMARGO, F.P.; CAMARGO FILHO, W.P. Distribuição geográfica da produção de hortaliças no Estado de São Paulo: participação no País, concentração regional e evolução no período 1996-2006. **Informações Econômicas**, São Paulo, v. 38, p. 28-35, 2008.

COBB, G.P.; SANDS, K.; WATERS, M.; WIXSON, B.G.; DORWARD-KING, E. Accumulation of heavy metals by vegetables grown in mine wastes. **Environmental Toxicology and Chemistry**, New York, v. 19, p. 600-607, 2000.

COMPANHIA DE TECNOLOGIA DE SANEAMENTO AMBIENTAL. **Estabelecimento de valores de referência de qualidade e valores de intervenção para solos e águas subterrâneas no Estado de São Paulo**. São Paulo, 2001. 92 p. (Relatório Final. Série Documentos Ambientais).

_______. Decisão de Diretoria nº 195-2005-E, de 23 de Novembro de 2005. Dispõe sobre a aprovação dos Valores Orientadores para Solos e Águas Subterrâneas no Estado de São Paulo – 2005, em substituição aos Valores Orientadores de 2001, e dá outras providências. São Paulo, 2005, publicada no Diário Oficial do Estado de São Paulo de 03 de Dezembro de 2005, retificada em 13 de Dezembro de 2005. Disponível em: http://www.cetesb.sp.gov.br/Solo/relatorios/tabela_valores_2005.pdf. Acesso em: 05 nov. 2010.

CRASWELL, E.T.; LEFROY, R.D.B. The role and function of organic matter in tropical soils. **Nutrient Cycling in Agroecosystems**, Dordrecht, v. 61, p. 7-18, 2001.

DAS, P.; SAMANTARAY, S.; ROUT, G.R. Studies on cadmium toxicity in plants: a review. **Environmental Pollution**, Barking, v. 98, p. 29-36, 1997.

DATTA, S.P.; YOUNG, S.D. Predicting metal uptake and risk to the human food chain from leaf vegetables grown on soils amended by long-term application of sewage sludge. **Water Air and Soil Pollution**, Dordrecht, v. 163, p. 119-136, 2005.

DAVIES, B.E. Inter-relationships between soil properties and the uptake of cadmium, copper, lead and zinc from contaminated soils by radish (*Raphanus sativus* L.). **Water, Air and Soil Pollution**, Dordrecht, v. 63, p. 331-342, 1992.

De VRIES, W.; RÖMKENS, P.F.A.M.; SCHÜTZE, G. Critical soil concentrations of cadmium, lead, and mercury in view of health effects on humans and animals. **Reviews of Environmental Contamination and Toxicology**, New York, v. 191, p. 91–130, 2007.

DORRIS, J.; BASSAM, H. Cadmium uptake by radishes from soil contaminated with nickel-cadmium batteries: toxicity and safety considerations. **Toxicology Mechanisms and Methods**, Gaithersburg, v. 12, p. 265-276, 2002.

EFROYMSON, R.A.; SAMPLE, B.E.; SUTER, G.W. II. Bioaccumulation of inorganic chemicals from soil by plants: Regressions of field data. **Environmental Toxicology and Chemistry**, New York, v. 20, p. 2561-2571, 2001.

EFFROYSOM, R.A.; SAMPLE, B.E.; SUTER, G.W. Bioaccumulation of inorganic chemicals from soil by plants: spiked soils vs field contamination or background. **Human and Ecological Risk Assessment**, Atlanta, v. 10, p. 1117-1127, 2004.

FONTES, M.P.F.; ALLEONI, L.R.F. Electrochemical attributes and availability of nutrients, toxic elements, and heavy metal in tropical soils. **Scientia Agrícola**, Piracicaba, v. 63, p. 589-608, 2006.

FRANZ, E.; RÖMKENS, P.F.M.A.; van RAAMSDONK, L.; van der FELS-KLERX, I. A chain modeling approach to estimate the impact of soil cadmium pollution on human dietary exposure. **Journal of Food Protection**, Des Moines, v. 71, p. 2504-2513, 2008.

FYTIANOS, K.; KATSIANIS, G.; TRIANTAFYLLOU P, ZACHARIADIS, G. Accumulation of heavy metals in vegetables grown in an industrial area in relation to soil. **Bulletin of Environmental Contamination and Toxicology**, New York, v. 67, p. 423-430, 2001.

GAW, S.K.; KIM, N.D.; NORTHCOTT, G.L.; WILKINS, A.L.; ROBINSON, G. Uptake of Sigma DDT, arsenic, cadmium, copper, and lead by lettuce and radish grown in contaminated horticultural soils. **Journal of Agricultural and Food Chemistry**, Easton, v. 56, p. 6584-6593, 2008.

GOLIA, E.E.; DIMIRKOU, A.; MITSIOS, I.K. Influence of some soil parameters on heavy metals accumulation by vegetables grown in agricultural soils of different soil orders. **Bulletin of Environmental Contamination and Toxicology**, New York, v. 81, p. 80-84, 2008.

GRANT, C.A.; BAILEY, L.D. Effects of phosphorus and zinc fertiliser management on cadmium accumulation in flaxseed. **Journal of the Science of Food and Agriculture**, London, v. 73, p. 307-314, 1997.

GUPTA, S.; SATPATI, S.; NAYEK, S.; GARAI, D. Effect of wastewater irrigation on vegetables in relation to bioaccumulation of heavy metals and biochemical changes. **Environmental Monitoring and Assessment**, Dordrecht, v. 165, p. 169-177, 2009.

HARDIYANTO; de GUZMAN, C.C. Identification of lead and cadmium levels in white cabbage (*brassica rapa* L.), soil, and irrigation water of urban agricultural sites in the Philippines. **Indonesian Journal of Agricultural Science,** Bogor, v. 9, p. 1-6, 2009.

HOEFKENS, C.; SIOEN, I.; de HENAUW, S.; VANDEKINDEREN, I.; BAERT, K.; de MEULENAER, B.; DEVLIEGHERE, F.; van CAMP, J. Development of vegetable composition databases based on available data for probabilistic nutrient and contaminant intake assessments. **Food Chemistry**, London, v. 113, p. 799-803, 2009.

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA. **Pesquisa de orçamentos familiares 2002-2003**. Aquisição alimentar domiciliar *per capita* no Brasil. IBGE, Rio de Janeiro, Brasil, 2004. 252 p.

JINADASA, K.B.P.N.; MILHAM, P.J.; HAWKINS, C.A.; CORNISH, P.; WILLIAMS, P.; KALDOR, C.; CONROY, J. Survey of cadmium levels in vegetables and soils of greater Sydney, Australia. **Journal of Environmental Quality**, Madison, v. 26, p. 924-933, 1997.

KARO, P. **Risk assessment of cadmium intake from home-grown vegetables on the local population of the municipality of Celje**. 2007. 132 p. Dissertation (Master in Environmental Diagnostics) Cranfield University, Cranfield, 2007.

LĂCĂTUŞU, R.; LĂCĂTUŞU, A.R. Vegetable and fruits quality within heavy metals polluted areas in Romania. **Carpathian Journal of Earth and Environmental Sciences**, Baia More, v. 3, p. 115-129, 2008.

LI, F.Y.; OKAZAKI, M.; ZHOU, Q.X. Evaluation of Cd uptake by plants estimated from total soil Cd, pH, and organic matter. **Bulletin of Environmental Contamination and Toxicology**, New York, v. 71, p. 714-721, 2003.

LI, J.; XIE, Z.M.; XU, J.M.; SUN, Y.F. Risk assessment for safety of soils and vegetables around a lead/zinc mine. **Environmental Geochemistry and Health**, Kew, v. 28, p. 37-44, 2006.

LI, Y.; WANG, Y.B.; GOU, X.; SU, Y.B.; WANG, G. Risk assessment of heavy metals in soils and vegetables around non-ferrous metals mining and smelting sites, Baiyin, China. **Journal of Environmental Sciences**, Beijing, v. 18, p. 1124-134, 2006b.

MAPANDA, F.; MANGWAYANA, E.N.; NYAMANGARA, J.; GILLER, K.E. Uptake of heavy metals by vegetables irrigated using wastewater and the subsequent risks in Harare, Zimbabwe. **Physics and Chemistry of the Earth**, Oxford, v. 32, p. 1399-13405, 2007.

MARCUSSEN, H.; DALSGAARD, A.; HOLM, P.E. Element concentrations in water spinach (*Ipomoea aquatica* Forssk.), fish and sediment from a wetland production system that receives wastewater from Phnom Penh, Cambodia**. Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering**, Philadelphia, v. 44, p. 67-77, 2009.

MARCUSSEN, H.; JOERGENSEN, K.; HOLM, P.E.; BROCCA, D.; SIMMONS, R.W.; DALSGARD, A. Element contents and food safety of water spinach (*Ipomoea aquatica* Forssk.) cultivated with wastewater in Hanoi, Vietnam. **Environmental Monitoring and Assessment**, Dordrecht, v. 139, p. 77-91, 2008.

McBRIDE, M.B. Cadmium uptake by crops estimated from soil total Cd and pH. **Soil Science**, Baltimore, v. 167, p. 62-67, 2002.

McBRIDE, M.; SAUVÉ, S.; HENDERSHOT, W. Solubility control of Cu, Zn, Cd and Pb in contaminated soils. **European Journal of Soil Science**, Oxford, v. 48, p. 337-346, 1997.

McLAUGHLIN, M.J.; WILLIAMS, C.M.J.; McKAY, A.; KIRKHAM, R.; GUNTON, J.; JACKSON, K.J.; THOMPSON, R.; DOWLING, B.; PARTINGTON, D.; SMART, M.K.; TILLER, K.G. Effect of cultivar on uptake of cadmium by potato tubers. **Australian Journal of Agricultural Research**, Melbourne, v. 45, p. 1483-1495, 1994.

McLAUGHLIN, M.J.; HAMON, R.E.; McLAREN, R.G.; SPEIR, T.W.; ROGERS, S.L.A bioavailability-based rationale for controlling metal and metalloid contamination of agricultural land in Australia and New Zealand. **Australian Journal of Soil Research**, Melbourne, v. 38, p. 1037-1086, 2000.

McLAUGHLIN, M.J.; WHATMUFF, M.; WARNE, M.S.T.J.; HEEMSBERGEN, D.; BARRY, G.; BELL, M.; NASCH, D.; PRITCHARD, D. A field investigation of solubility and food chain accumulation of biosolid-cadmium across diverse soil types. **Environmental Chemistry**, London, v. 3, p. 428-432, 2006.

MILLIS, P.R.; RAMSEY, M.H.; JOHN, E.A. Heterogeneity of cadmium concentration in soil as a source of uncertainty in plant uptake and its implications for human health risk assessment. **Science of the Total Environment**, Amsterdam, v. 326, p. 49-53, 2004.

MOIR, A.M.; THORNTON, I. Lead and cadmium in urban allotment and garden soils and vegetables in the United-Kingdom. **Environmental Geochemistry and Health**, Kew, v. 11, p. 113-119, 1989.

MURRAY, H.; THOMPSON, K.; MACFIE, S.M. Site- and species-specific patterns of metal bioavailability in edible plants. **Botany**, Ottawa, v. 87, p. 702-711, 2009.

NABULO, G.; ORIGA, H.O.; DIAMOND, M. Assessment of lead, cadmium, and zinc contamination of roadside soils, surface films, and vegetables in Kampala City, Uganda. **Environmental Research**, San Diego, v. 101, p. 42-52, 2006.

NAIDU, R.; KOOKANA, R.S.; SUMNER, M.E.; HARTER, R.D.; TILLER, K.G. Cadmium sorption and transport in soils dominated by variable charge surfaces: a review. **Journal of Environmental Quality**, Madison, v. 26, p. 602-617, 1997.

NAIDU, R.; SUMNER, M.E.; HARTER, R.D. Sorption of heavy metals in strongly weathered soils: an overview. **Environmental Geochemistry and Health**, Kew, v. 20, p. 5-9, 1998.

NEW ZEALAND. Ministry for the Environment. **Draft methodology for deriving soil guideline values protective of human health.** Wellington, 2010.

OTTE, P.F.; LIJZEN, J.P.A.; OTTE, J.G.; SWARTJES, F.A.; VERSLUIJS, C.W. **Evaluation and revision of the CSOIL parameter set.** RIVM, Bilthoven, the Netherlands, 2001. (RIVM report 711701021).

OYEDELE, D.J., GASU, M.B; AWOTOYE, O.O. Changes in soil properties and plant uptake of heavy metals on selected municipal solid waste dump sites in Ile-Ife, Nigeria. **African Journal of Environmental Science and Technology**, Victoria Island, v. 3, p. 107-115, 2008.

PAGE, A.L.; CHANG, A.C.; EL-AMAMY, M. Cadmium levels in soils and crops in the United States. In: HUTCHINSON, T.C. AND MEEMA, K.M. (Ed.). **Lead, Mercury, Cadmium and Arsenic in the Environment**. New York:John Wiley, 1987. p. 119-146.

PANDEY, J.; PANDEY, U. Accumulation of heavy metals in dietary vegetables and cultivated soil horizon in organic farming system in relation to atmospheric deposition in a seasonally dry tropical region of India. **Environmental Monitoring and Assessment**, Dordrecht, v. 148, p. 61-74, 2009.

PEIJNENBURG, W.; BAERSELMAN, R.; de GROOT, A.; JAGER, T.; LEENDERS, D.; POSTHUMA, L.; van VEEN, R. Quantification of metal bioavailability for lettuce (Lactuca ativa L.) in field soils. **Archives of Environmental Contamination and Toxicology**, New York, v. 39, p. 420-430, 2000.

PETERSEN, L.S.; LARSEN, E.H.; LARSEN, P.B.; BRUUN, P. Uptake of trace elements and PAHs by fruit and vegetables from contaminated soils. **Environmental Science & Technology**, Iowa City, v. 36, p. 3057-3063, 2002.

RIEUWERTS, J.S. The mobility and bioavailability of trace metals in tropical soils: a review. **Chemical Speciation and Bioavailability**, Surrey, v. 19, p. 75–85, 2007.

ROMKENS, P.F.A.M.; GUO, H.Y.; CHU, C.L.; LIU, T.S.; CHIANG, C.F.; KOOPMANS, G.F. Prediction of cadmium uptake by brown rice and derivation of soil–plant transfer models to improve soil protection guidelines. **Environmental Pollution**, Barking, v. 157, p. 2435-2444, 2009.

SAHU, R.K.; KATIYAR, S.; TIWARI, J.; KISKU, G.C. Assessment of drain water receiving effluent from tanneries and its impact on soil and plants with particular emphasis on bioaccumulation of heavy metals **Journal of Environmental Biology**, Lucknow, v. 28, p. 685-690, 2007.

SAUVÉ, S.; NORVELL, W.A.; McBRIDE, M.; HENDERSHOT, W. Speciation and complexation of cadmium in extracted soil solutions. **Environmental Science & Technology**, Iowa City, v. 34, p. 291–296, 2000.

SINGH, S.; KUMAR, M. Heavy metal load of soil, water and vegetables in peri-urban Delhi. **Environmental Monitoring and Assessment**, Dordrecht, v. 20, p. 79-91, 2006.

SINGH, A.; SHARMA, R.K.; AGRAWAL, M.; MARSHALL, F. Effects of wastewater irrigation on physicochemical properties of soil and availability of heavy metals in soil and vegetables. **Communications in Soil Science and Plant Analysis**, New York, v. 40, p. 3469–3490, 2009.

SIPTER, E.; RÓZSA, E.; GRUIZ, K.; TÁTRAI, E.; MORVAI, V. Site-specific risk assessment in contaminated vegetable gardens. **Chemosphere**, Oxford, v. 71, p. 1301-1307, 2008.

SWARTJES, F.A.; DIRVEN-VAN BREEMEN, E.M.; OTTE, P.F.; van BEELEN, P.; RIKKEN, M.G.J.; TUINSTRA, J.; SPIJKER, J.; LIJZEN, J.P.A. **Human health risks due to consumption of vegetables from contaminated sites**. RIVM, Bilthoven, the Netherlands, 2007. (RIVM report 711701040).

SWARTJES, F.A.; TROMP, P.C. A tiered approach for the assessment of the human health risks of asbestos in soils. **Soil & Sediment Contamination**, Boca Raton, v. 17, p. 137-149, 2008.

UNITED KINGDOM. Department for Environment, Food and Rural Affairs and the Environment Agency. **Soil guideline values for cadmium contamination**. Bristol, 2002.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY). **Soil screening guidance, office of emergency and remedial response**. Washington, 1996. (Technical Background Document. EPA/540/R95/128).

WHATMUFF, M.S. Applying biosolids to acid soils in New South Wales: are guideline soil metal limits from other countries appropriate? **Australian Journal of Soil Research**, Melbourne, v. 40, p. 1041-1056, 2002.

WORLD HEALTH ORGANIZATION. **Evaluation of certain food contaminants**. Geneva, 2006.(WHO Technical Report Series, 930).

YANG, Y.; ZHANG, F.S.; LI, H.F.; JIANG, R.F. Accumulation of cadmium in the edible parts of six vegetable species grown in Cd-contaminated soils. **Journal of Environmental Management**, London, v. 90, p. 1117–1122, 2009.

YUSUF, A.A.; AROWOLO, T.A.; BAMGBOSE, O. Cadmium, copper and nickel levels in vegetables from industrial and residential areas of Lagos City, Nigeria. **Food and Chemical Toxicology**, Oxford, v. 41, p. 375-78, 2003.

ZHENG, R.L.; LI, H.F.; JIANG, R.F.; ZHANG, F.S. Cadmium accumulation in the edible parts of different cultivars of radish, *Raphanus sativus L*., and carrot, *Daucus carota* var. sativa, grown in a Cd-contaminated soil. **Bulletin of Environmental Contamination and Toxicology**, New York, v. 81, p. 75-79, 2008.

ZHUANG, P.; McBRIDE, M.B.; XIA, H.; LI, N.; LI, Z. Health risk from heavy metals via consumption of food crops in the vicinity of Dabaoshan mine, South China. **Science of the Total Environment**, Amsterdam, v. 407, p. 1551-561, 2009.

3 CADMIUM UPTAKE BY LETTUCE AS BASIS FOR DERIVATION OF CRITICAL LIMITS IN SOILS

Abstract

Cadmium (Cd) can accumulate above the permissible limit for human consumption in edible parts of vegetables. Therefore, it constitutes the main human exposure pathway. The availability and uptake of Cd by lettuce in two common tropical soils with and without lime addition was studied in order to derive critical soil concentrations, based on human health risks. Cadmium concentrations ranging from 1 up to 12 mg kg^{-1} were added to samples from an Oxisol and an Ultisol under glasshouse conditions. After incubation, lettuce was grown during 36 days, and the edible parts were harvested and analyzed for Cd. Total (*aqua-regia*) and available Cd (extracted either by DTPA or by 0.01 mol L^{-1} $CaCl_2$ solution) were also determined. A positive linear correlation was observed between total soil Cd and the Cd concentration in lettuce. On the other hand, an exponential trend was found between Cd content in lettuce and available Cd in soil. The amount of Cd absorbed by lettuce grown in the sandy Ultisol was about twice the amount absorbed in the clayey Oxisol. Consequently, the critical soil Cd concentrations were lower in the Ultisol. Liming increased the pH and slightly reduced Cd availability and uptake. DTPA and $CaCl_2$ were good predictors of the Cd bioavailability and uptake. $CaCl_2$ was sensitive to differentiation between limed and unlimed soils. Critical Cd concentrations in the Ultisol were lower than in the Oxisol and were below the Intervention Value adopted for São Paulo State, Brazil, regardless of the exposure scenario.

Keywords: Tropical soils; Vegetable uptake; Risk assessment; Guideline values

3.1 Introduction

Cadmium (Cd) is highly toxic to humans and all other living organisms, since it has no known biological functions to aquatic or terrestrial organisms (CHEN et al., 2007). Industrial and agricultural activities have led to an increased level of Cd in many agricultural soils (SARWAR et al., 2010). Cadmium enters the soil through various anthropogenic sources and management practices such as application of phosphate fertilizers, irrigation with waste water, disposal of sewage sludge rich in Cd, and anthropogenic emissions from power stations, metal industries, urban traffic and cement industries (di-TOPPI; GABRIELLI, 1999; YANG et al., 2004; KIRKHAM, 2006). Although Cd enters the human body via soil, water, air, plants and animal products, the main source of Cd exposure to the general population, except smokers, is the consumption of Cd-contaminated food (GRANT; BAILEY, 1997). Vegetable consumption can significantly contribute to Cd exposure; contributing from 70% (SARWAR et al., 2010) up to more than 90% (SWARTJES et al., 2007) of total Cd intake by humans, depending on the land use.

Among the vegetable species, lettuce (*Lactuca sativa* L.) is of primary concern because it accumulates metals at relatively high internal contents, because of the efficient root uptake and subsequent translocation to the shoots (PEIJNENBURG et al., 2000). This crop is particularly important for the State of São Paulo, Brazil, since is one of the most produced and consumed leafy vegetables (CAMARGO; CAMARGO; CAMARGO-FILHO, 2008). Lettuce is also considered a good indicator specie for derivation of critical soil Cd concentrations, which generally are used in a first tier risk assessment. Because, such derivation should be relatively conservative, and lettuce is a relatively high Cd uptake crop (BROWN et al., 1996; de VRIES; RÖMKENS, SCHÜTZE, 2007).

Soil pH, organic matter (OM) and clay contents, presence of other ions, root exudates, types and cultivars of crop plants affect Cd bioavailability in soil (CIÉSLÍNSKI et al., 1996; BRUS; de GRUIJTER; RÖMKENS, 2005), and, hence, crop uptake. Soil pH, however, is generally recognized as the most important (McBRIDE; SAUVÉ; HENDERSHOT, 1997; McBRIDE, 2002; GOLIA; DIMIRKOU; MITSIOS, 2008). The bioavailability in Cd-contaminated acidic soils is substantially higher as compared to neutral and alkaline soils (SAWAR et al., 2010). Cadmium availability in acidic soils can be manipulated through the use of various amendments, including lime (TLUSTOŠ et al., 2006). To calculate human health-based critical soil Cd concentrations for São Paulo State, we used lettuce as reference vegetable and evaluated the influence of liming on Cd availability and accumulation in this crop grown in two tropical soils with contrasting properties.

3.2 Material and Methods

3.2.1 Experimental procedures and soil analyses

A pot experiment was carried out under glasshouse conditions in Piracicaba, State of São Paulo, Brazil. An Oxisol and an Ultisol were selected due to their wide occurrence in the State of São Paulo and because of the differences in soil properties (Table 1). Soil samples were collected from the topsoil (0-0.2m) of agricultural areas cultivated with sugar cane and then air-dried and sieved (< 4 mm). After homogenization, a subsample of each soil was sieved (< 2 mm) for characterization. The pH was determined in water (soil:solution ratio 1:2.5); soil organic carbon contents were determined by titration with ferrous ammonium sulfate after reaction with potassium dichromate and sulphuric acid (RAIJ et al., 2001). Soil physic fractionation was performed according to the densimeter method (GEE; OR, 2002). Iron oxides were determined after extraction with 9 mol L^{-1} H_2SO_4, and silicon oxides were extracted with 30% NaOH solutions. The cation exchange capacity (CEC) was determined after extraction with 1 mol L^{-1} NH_4OAc solution.

Table 1 – Soil characterization after field collection

Soil	pH in water	SOC	Sand	Loam	Clay	Fe_2O_3*	CEC	V	Total Cd
		------------------------g kg^{-1}------------------------					$mmol_c$ kg^{-1}	%	mg kg^{-1}
Oxisol	5.7±0.1	16.1±0.3	215±7	105±7	680±0	98±9	100±5	45±2	1.20±0.15
Ultisol	6.1±0.1	9.1±0.6	785±21	25±7	190±14	7.1±0.4	37±2	49±4	0.79±0.01

Mean (n = 3) ± Standard Deviation. Except marked with * (n = 2). SOC = soil organic carbon; Fe_2O_3 = iron oxide extracted by 9 mol L^{-1} H_2SO_4;; CEC = cation exchange capacity; V = basis saturation.

Soils were spiked with Cd to reach the following concentrations: 1.0; 2.0; 3.0; 5.0; 8.0 and 12.0 mg kg^{-1} by using Cd $(NO_3)_2$ solution. The same Cd concentrations were applied to the limed soil samples. Lime requirement was calculated to raise basis saturation of each soil to 80% according to the recommendation for lettuce in the State of São Paulo, Brazil. Amounts of dolomitic lime (34 % of CaO and 16% of MgO) added to the Oxisol and Ultisol were 3.5 and 1.2 g/pot, respectively. For both treatments (with and without liming), control treatments (no metal addition) were included.

The chosen Cd concentrations represent a range of concentrations around the current soil Cd Intervention Values for the State of São Paulo (CETESB, 2005). Total Cd contents below 3 and 8 mg kg^{-1} of soil are not supposed to be contaminated, for agricultural and residential sites, respectively. After mixing, soils were placed in 2 kg-pots and left to equilibrate for seven weeks. The water content was maintained to ±60% of the maximum water retention capacity. Water was replaced weekly by weight. After equilibrium, soil material of each pot was homogenized again and a subsample of each pot was collected, air-dried and sieved (< 2 mm). Three fractions of Cd were extracted: (i) total concentration (in powered samples < 0.15 mm) by extraction with aqua-regia (HCl-HNO_3, 3:1, v/v) in a microwave oven (Mars Xpress, CEM Corporation); (ii) potentially available Cd extracted with diethylenetriaminepentaacetic acid (DTPA), buffered at pH 7.3 (LINDSAY; NOVELL, 1978) and (iii) readily available Cd extracted with 0.01 mol L^{-1} $CaCl_2$ solution (HOUBA et al., 2000).

Cadmium concentrations in soils extracts were determined by Flame Atomic Absorption Spectrometry (FAAS). A Graphite Furnace Atomic Absorption Spectrometer (GF-AAS) was used when the concentrations were below the detection limit. Blank reagents were analyzed to each 20 samples to control the quality of Cd determination. The digestions recovery with aqua-regia in soil samples were verified by including a certified soil sample (Montana soil, NIST, SRM 2711) to each 20 samples.

3.2.2 Cultivation, harvest and plant analyses

Two seedlings, 30-days old, of a commercial variety of lettuce (cultivar Amanda) were placed in pots previously fertilized with nutrient solutions containing macro and micronutrients (N, P, K, Cu, Fe, Zn, Mn, B and Mo) and water to 60% of the maximum retention capacity. Nitrogen was supplied weekly at 50 mg kg^{-1} to each pot in the first three weeks as NH_4NO_3 p.a. Five days after the planting, plants were thinned to one plant per pot and left to grow for 36 further days (when plants were ready for human consumption). The experiment was carried out in a randomized blocks design, with three replicates.

The edible parts of the plants were harvested and washed with deionized water, to remove possible soil material and contaminants deposited on the leaves. The material was oven-dried at 60ºC for 72 h, and the dry weight was recorded. The plant material was ground (to pass 1 mm sieve), and 250 mg were weighted into Teflon vessels where 1.5 mL of HNO_3 + 2 mL of H_2O_2 were added and left to react for 60 min. After, 5 mL of deionized water were added to the vessels, which were sealed and placed to digest in a microwave oven (CEM, corporation), according to Araújo et al. (2002).

Cadmium concentrations in plant extracts were determined by Flame Atomic Absorption Spectrometry (FAAS). A Graphite Furnace Atomic Absorption Spectrometer (GF-AAS) was used when the concentrations were below the detection limit. Blank reagents were analyzed to each 20 samples to control the quality of Cd determination. The digestions recovery for plant samples were verified by including a certified plant sample (tomato leaves, NIST, SRM 1573a) to each 20 samples.

Linear regression analyses were performed to establish the relationships between Cd concentrations in lettuce and total Cd concentrations in soils (soil – plant relations). Data were converted to a logarithmic (base 10) scale to study the relationship between available Cd extracted either by DTPA or $CaCl_2$ and Cd in lettuce. Also, the correlation between total and available Cd in soils was studied. The bioconcentration factor ($Cd_{lettuce}/Cd_{soil}$) was calculated from the soil – plant relations (total Cd in soil and Cd in lettuce), and critical soil Cd concentrations were derived for each soil (limed and unlimed) by using the CSOIL 2000 exposure model (BRAND; OTTE; LIJZEN, 2007).

Two standard scenarios (rural and urban) were evaluated, and levels of vegetable consumption (10; 25; 50 and 100% of total vegetable consumption) were assumed as being taken from the contaminated site. The whole vegetable consumption was based on the consumption of lettuce, only. The rural scenario was defined as 'agricultural areas traditionally used for growing vegetables, including growth for own production'. The urban scenario was defined as 'areas predominantly for residence, but including vegetable consumption from own gardens' (PERES, 2007). The consumption rates of vegetables for children and adults for the State of São Paulo, Brazil were obtained from the Brazilian Institute of Geography and Statistics (IBGE, 2004).

3.3 Results

Cadmium did not reduce the lettuce yield, and liming also did not increase the dry matter yield (Figure 1). However, the dry matter production in the Oxisol was higher than in the Ultisol, given the better soil chemical conditions, e.g. SOM and CEC (Table 1).

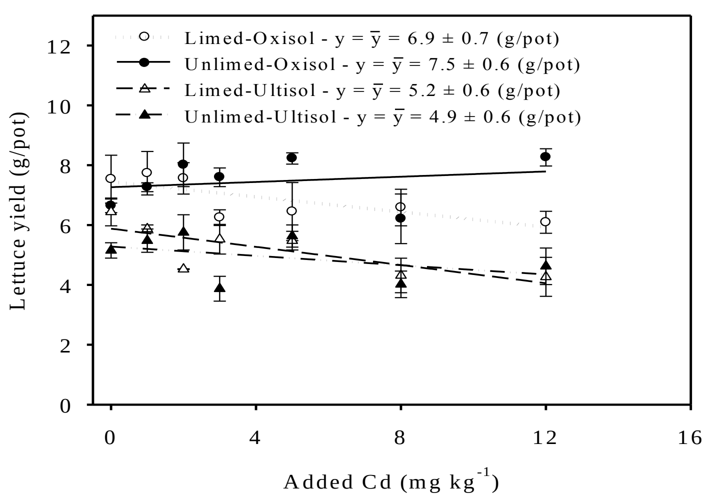

Figure 1 – Yield of Lettuce (dry weight) grown in an Oxisol and an Ultisol spiked with rates of cadmium. Error bars are ± SE

The correlation between total soil Cd and the Cd uptake by lettuce was linear in the Cd concentration up to 12 mg kg^{-1} (Figure 2A). The lettuce accumulated about twice more Cd in the Ultisol than in the Oxisol, and the pH was increased by 1.1 unit in the Ultisol and by 0.9 unit in the Oxisol after liming. Despite such increase, Cd uptake was only slightly reduced as compared to the unlimed treatments.

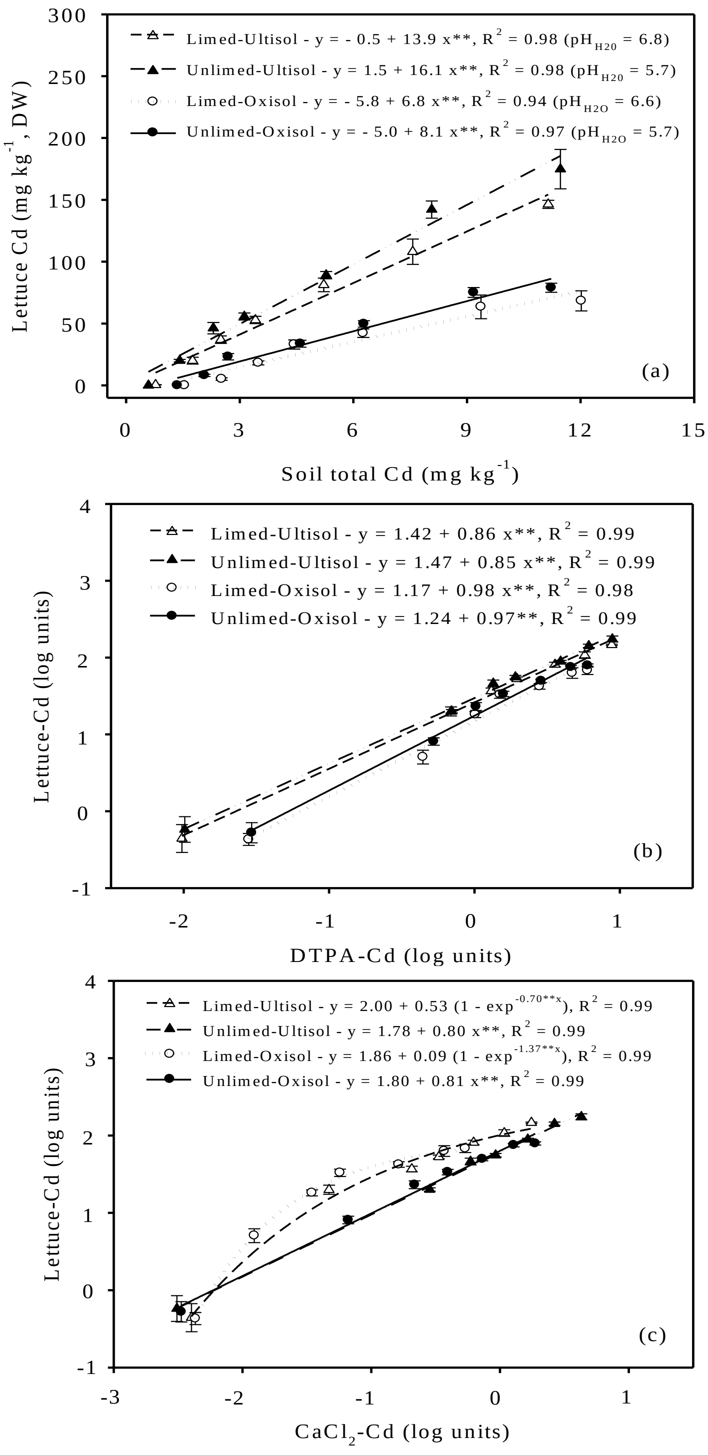

Figure 2 – (a) Soil–plant relationship between total soil Cd concentration (*aqua-regia* extraction) (b) available Cd concentration (DTPA extraction) and (c) readily available Cd concentration ($CaCl_2$ 0.01 mol L^{-1} extraction) and lettuce Cd concentration. Error bars are ± SD. Soil pH was measured in water (1:2.5 soil:solution ratio)

The soil–plant relationships between available Cd as extracted either by DTPA or $CaCl_2$ and the concentration of Cd in lettuce was linear in a log scale (Figure 2B and 2C). When soils were limed, however, the readily available Cd concentration (Cd extracted by $CaCl_2$) and the Cd concentration in lettuce followed an exponential model in a log scale, tending to reach a maximum level of Cd in lettuce (Figure 2C).

The potentially available cadmium (extracted by DTPA) was about 80% of the total Cd in the Ultisol and it was higher than in the Oxisol, where available Cd accounted for 60% of the total Cd (Figure 3A).

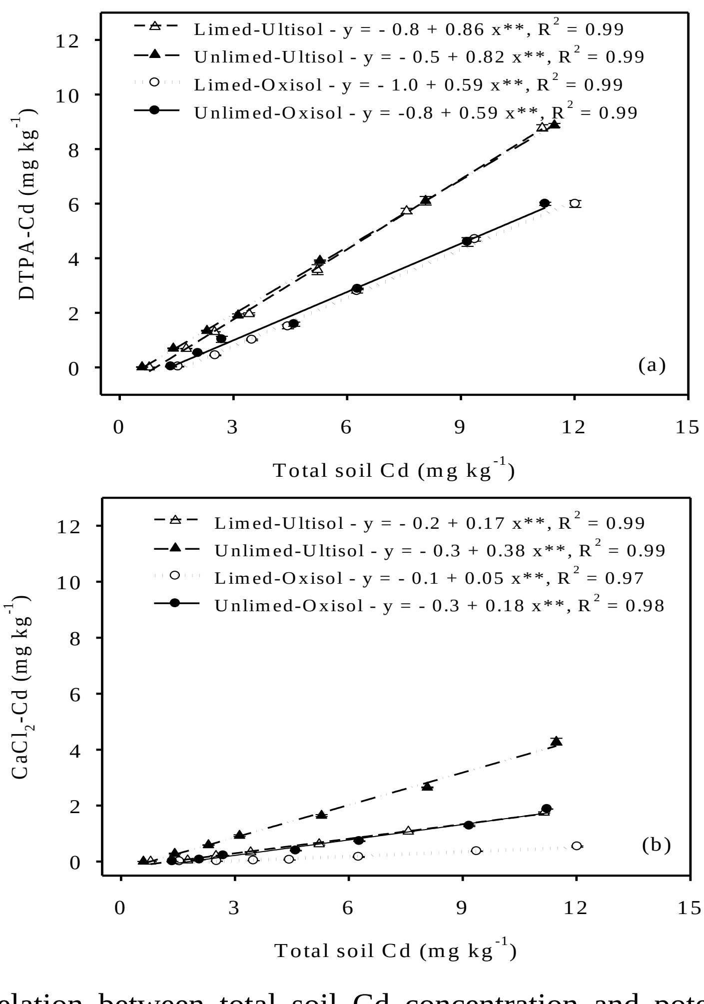

Figure 3 – Correlation between total soil Cd concentration and potentially available Cd (by DTPA extraction) (a) and readily available Cd (by $CaCl_2$ 0.01 mol L^{-1} extraction) (b). Error bars are ± SD

There was no difference on the potentially available Cd (extracted by DTPA) between the limed and unlimed soil samples. On the other hand, the readily available Cd (extracted by

0.01 mol L^{-1} $CaCl_2$) was affected by liming (Figure 3B). The readily available Cd concentration in the limed Ultisol was three times higher than in the limed Oxisol, and the difference between these soils was about twice when they did not receive lime. Based on the slope of the regressions, the readily available Cd concentration varied from 17 to 38% of the total Cd in the Ultisol and from 5 to 18% of the total Cd concentration in the Oxisol.

The derived critical soil concentrations for Cd were mainly dependent on the human exposure through vegetable consumption (Table 2). When 100% of the vegetable consumption was assumed as being taken from the contaminated site, the critical values were lower than 1 mg kg^{-1} in all exposure scenarios. However, as the exposure was reduced by lower fractions of 50%, 25% or 10% of contaminated vegetable consumption the critical soil Cd concentrations increased almost linearly.

Table 2 – Critical soil Cd concentrations in samples from limed and unlimed Oxisol and Ultisol samples under two scenarios of exposure and four fractions of vegetable consumption for own consumption from a contaminated area

	Rural Scenario				Urban Scenario			
	10%	25%	50%	100%	10%	25%	50%	100%
	(mg kg^{-1}, dry weight)							
IV*	3.0				8.0			
	Oxisol							
Limed	7.0	2.9	1.4	0.7	5.7	2.3	1.1	0.6
Unlimed	5.7	2.3	1.1	0.6	4.5	1.8	0.9	0.5
	Ultisol							
Limed	2.7	1.1	0.5	0.3	2.2	0.9	0.4	0.2
Unlimed	2.2	0.9	0.4	0.2	1.8	0.7	0.4	0.2

* Soil Intervention Value for Brazil (Conama, 2009).

The derived critical soil Cd concentrations were dependent on soil type and, to a lesser extent, on lime addition. Higher critical values were found in the Oxisol than in the Ultisol, and such a difference was a factor of 2.6 in both limed and unlimed treatments. On the other hand, critical values obtained from the treatments with lime addition, within each soil, were higher than the unlimed treatments; such a difference was about 20%. The urban scenario yielded slightly lower critical soil Cd concentrations than the rural scenario, because the vegetable consumption rates in such a scenario are higher as compared to the latter one.

3.4 Discussion

In our study there was neither a reduction in biomass production of lettuce nor any visible symptoms related to soil Cd concentration up to 12 mg kg^{-1} in the two tropical soils. The experimental conditions with spiked soils in pots certainly enhance the uptake of Cd compared to actual contaminated sites. Lettuce is considered a Cd accumulator crop and it is not sensitive to toxic effects at low or medium soil Cd concentrations (ALEXANDER; ALLOWAY; DOURADO, 2006).

Controversial results regarding levels of total Cd-spiked soils and effects on lettuce growth are found in literature. Chen et al. (2009) did not observe any reduction in biomass production of romaine lettuce grown, in a field experiment, amended with P fertilizer spiked with $CdCl_2$ at total soil Cd contents up to 20 mg kg^{-1}. The studied soil by Chen et al. (2009) was a fine, smectitic, calcareous, hyperthermic Vertic Torrifluvent with pH in water of 8.2. Conversely, Kukier et al (2010) observed a reduction in lettuce yield grown in a fine-silty, mixed, mesic Typic Hapludolls after the addition of 2.5 mg kg^{-1} of $Cd(NO_3)_2$. However, in the latter one, when 120 and 180 t ha^{-1} of biosolids were applied, there was no reduction in lettuce yield until the Cd soil addition of 10 mg kg^{-1}, showing that these biosolids rates eliminated the toxic effects of Cd. Such results show that Cd toxic effects on lettuce vary according to soil properties, growth conditions and cultivar or genotype.

The increase of pH is expected to reduce Cd availability, but Cd uptake by lettuce in this study was only slightly reduced in both soils as a response of lime addition, though the soil pH has increased by ≈ 1 unit. This limited reduction in Cd uptake after liming is a result of two factors: (i) a release of negative charges, blocked by H and Al, on the colloids surface because of the increase in pH. Consequently, Cd adsorption is increased in variable-charge sites, thus reducing its uptake; and (ii) an increase on the level of Ca in soils, which compete with Cd for the Ca channels in plants, reducing Cd uptake (SARWAR et al., 2010).

Differences in the slope of the curves relating Cd uptake as a response of the applied Cd to soils are mainly related to the differences in clay, soil OM and iron and aluminum oxides contents, which act as Cd sorbents, thus reducing its availability in soils. This explains the observed differences in Cd uptake by lettuce in the Oxisol and in the Ultisol in this study. The linear positive correlations between total soil Cd and lettuce Cd contents found in this study are in agreement with Kukier et al. (2010) that also found a linear relationship between total soil Cd in a Typic Hapludoll and lettuce Cd at concentrations up to 10 mg kg^{-1}.

When the tiered approach for soil risk assessment is considered (SWARTJES; TROMP, 2008) the first tier is supposed to be fast, easy and conservative. Therefore, a generic evaluation might be performed by measuring the total soil metal concentration. In this study, the critical soil Cd concentrations derived can be considered conservative; since the experiment was performed in pots with Cd-spiked soils. In such situation, the uptake is normally higher than under field conditions, as demonstrated by McLaughlin et al. (2006) and Yang et al. (2009). Moreover, the total vegetable consumption was related to lettuce, only, which also is a conservative assumption, since uptake in lettuce is relatively high.

When a site-specific risk assessment is necessary, the bioavailable fraction should be evaluated in higher tiers of risk assessment. We found a linear relationship between DTPA extracted-Cd and Cd absorbed by lettuce. Such linearity, however, was achieved in a logarithmic scale, corresponding to a curvilinear relationship tending to maximum in a non-logarithmic scale. Datta and Young (2005) also demonstrated that there was an approach to an asymptote as free metal ion activity increases for uptake of Cd, Cu and Zn by perpetual spinach (*Beta vulgaris*, Cicla). DTPA combined with triethylamine and $CaCl_2$ as used in this study is a strong chelating agent, which form highly stable complexes with Cd and mobilizes not readily available forms, and, hence, extracted a fraction of the total Cd concentration that can considered as potentially available. On the other hand, 0.01 mol L^{-1} $CaCl_2$ is a diluted salt that extract only the readily available Cd fraction. Then, it was more sensitive than DTPA to differentiate between limed and unlimed treatments. An exponential relationship was fitted between readily available Cd (extracted by $CaCl_2$) and lettuce Cd when lime was applied in both soils. The strength of Cd adsorption increased after pH increases due to liming. Therefore, the degree of metal extracted with this diluted $CaCl_2$ solution reflects the differences in binding strength in different soils (PEIJNENBURG, ZABLOTSKAJA, VIJVER, 2007).

The critical soil values derived for Cd had been directed correlated with the human exposure through vegetable consumption. When the higher exposure is regarded (the exposure scenario with a consumption of 100% of contaminated vegetables from the contaminated site) the critical concentration was extremely low. Anyway, it would not be realistic assuming such a scenario, because there is a limitation of the area needed to grow such amount of vegetables.

For the State of São Paulo it was adopted 25% and 12.5% of vegetable consumption as taken from private areas or own gardens, in a rural and residential scenario, respectively, for the derivation of Intervention Values of metals (including Cd) (CETESB, 2001). But, even when only 10% of vegetable consumption was assumed in our study, the critical values in both soils were below the current Intervention Value adopted for an urban scenario (8 mg kg^{-1}) and for a rural scenario (3 mg kg^{-1}), in the case of the Ultisol. This soil is more vulnerable to contamination, due to its inherent low cation retention capacity. One reasonable explanation to this result is that Cd was added to the soils, which implies that no aging has taken place and, hence, Cd was readily available for plant uptake in the pots. Such data (when Cd was added to the soils) was described by Swartjes et al. (2007) as those that might represent a worst case situation.

All critical values derived for Cd in soils were below the toxic concentrations for lettuce (de VRIES, RÖMKENS, SCHÜTZE, 2007). Cadmium might pose human health risks at plant tissue concentrations that are not phytotoxic, and this corroborates former findings of Peijnenburg et al. (2000), who also studied other metals and concluded that Cd is the only metal with such characteristic, becoming the one of most concern due to bioaccumulation through the soil-plant-animal food chain.

3.5 Conclusions

Cadmium accumulates linearly in lettuce at total soil Cd concentrations up to 12 mg kg^{-1}. By increasing the soil pH through liming, Cd vegetable uptake is only slightly reduced. In the Oxisol with high clay and oxides contents, Cd availability and uptake is much lower than in the Ultisol, whose Cd retention capacity is low. The readily and the potentially bioavailable Cd (extracted either by 0.01 mol L^{-1} $CaCl_2$ or DTPA, respectively) were positively correlated with Cd uptake by lettuce and are important indices to differentiate the soils. Such values should be used for site-specific risk assessment. The critical soil Cd concentrations were conservative, since they were derived from spiked soils and lettuce consumption, and were lower than the Intervention Values for São Paulo, regardless the exposure scenario.

References

ALEXANDER, P.D.; ALLOWAY, B.J.; DOURADO, A.M. Genotypic variations in the accumulation of Cd, Cu, Pb and Zn exhibited by six commonly grown vegetables. **Environmental Pollution**, Barking, v. 144, p. 736-745, 2006.

ARAÚJO, G.C.L.; GONZALEZ, M. H.; FERREIRA, A. G.; NOGUEIRA, A. R. A.; NÓBREGA, J. A. Effect of acid concentration on closed-vessel microwave-assisted digestion of plant materials. **Spectrochimica Acta part B: Atomic Spectroscopy**, Oxford, v. 57, p. 2121–2132, 2002.

BRAND, E.; OTTE, P.F.; LIJZEN, J.P.A. **CSOIL 2000**: Exposure model for human risk assessment of soil contamination. A model description. RIVM, Bilthoven, the Netherlands, 2007.(RIVM Report 711701054).

BROWN, S.L.; CHANEY, R.L.; LLOYD, C.A.; ANGLE, J.S.; RYAN, J.A. Relative uptake of cadmium by garden vegetables and fruits grown on long-term biosolid-amended soils. **Environmental Science & Technology**, Iowa City, v. 30, p. 3508–3511, 1996.

BRUS, D.J.; de GRUIJTER, J.J.; RÖMKENS, P.F.A.M. Probabilistic quality standards for heavy metals in soil derived from quality standards in crops. **Geoderma**, Amsterdam, v. 128, p. 301–311, 2005.

CAMARGO, A.M.M.P.; CAMARGO, F.P.; CAMARGO FILHO, W.P. Distribuição geográfica da produção de hortaliças no Estado de São Paulo: participação no País, concentração regional e evolução no período 1996-2006**. Informações Econômicas**, São Paulo, v. 38, p. 28-35, 2008.

CIÉSLÍNSKI, G.; van REES, K.C.J.; HUANG, P.M.; KOZAK, L.M.; ROSTAD, H.P.W.; KNOTT, D.R. Cadmium uptake and bioaccumulation in selected cultivars of durum wheat and flax as affected by soil type. **Plant and Soil**, The Huague, v. 182, p. 115–124, 1996.

CHEN, F.; DONG, J.; WANG, F.; WU, F.; ZHANG, G.; LI, G.; CHEN, Z.; CHEN, J.; WEI, K. Identification of barley genotypes with low grain Cd accumulation and its interaction with four microelements. **Chemosphere**, Oxford, v. 67, p. 2082–2088, 2007.

CHEN, W.; LI, L.; CHANG, A.C.; WU, L.; KWON, S.I.; BOTTOMS. R. Cadmium uptake by lettuce in fields treated with cadmium-spiked phosphorus fertilizers. **Communications in Soil Science and Plant Analysis**, New York, v. 40, p. 1124-1137, 2009.

COMPANHIA DE TECNOLOGIA DE SANEAMENTO AMBIENTAL. **Estabelecimento de valores de referência de qualidade e valores de intervenção para solos e águas subterrâneas no Estado de São Paulo**. São Paulo, 2001. 92 p. (Relatório Final. Série Documentos Ambientais).

_______. **Decisão de Diretoria nº 195-2005-E, de 23 de Novembro de 2005. Dispõe sobre a aprovação dos Valores Orientadores para Solos e Águas Subterrâneas no Estado de São Paulo – 2005, em substituição aos Valores Orientadores de 2001, e dá outras providências.** São Paulo, 2005, publicada no Diário Oficial do Estado de São Paulo de 03 de Dezembro de 2005, retificada em 13 de Dezembro de 2005. Disponível em: http://www.cetesb.sp.gov.br/Solo/relatorios/tabela_valores_2005.pdf. Acesso em: 05 nov. 2010.

DATTA, S.P.; YOUNG, S.D. Predicting metal uptake and risk to the human food chain from leaf vegetables grown on soils amended by long-term application of sewage sludge. **Water Air and Soil Pollution**, Dordrecht, v. 163, p. 119-136, 2005.

de VRIES, W.; RÖMKENS, P.F.A.M.; SCHÜTZE, G. Critical soil concentrations of cadmium, lead, and mercury in view of health effects on humans and animals. **Reviews of Environmental Contamination and Toxicology**, New York, v. 191, p. 91–130, 2007.

di-TOPPI, L.S.; GABRIELLI, R. Response to cadmium in higher plants. **Environmental and Experimental Botany**, Elmsford, v. 41, p. 105–130, 1999.

GEE G.W.; OR, D. Particle-size analysis. In: DANE, J.H., TOOP, G.C. (Ed.). **Methods of soil analysis.** Physical methods. Madison, Wisconsin, 2002. p 255-293.

GOLIA, E.E.; DIMIRKOU, A.; MITSIOS, I.K. Influence of some soil parameters on heavy metals accumulation by vegetables grown in agricultural soils of different soil orders. **Bulletin of Environmental Contamination and Toxicology**, New York, v. 81, p. 80–84, 2008.

GRANT, C.A.; BAILEY, L.D. Effect of phosphorus and zinc fertilizer management on cadmium accumulation in flaxseed. **Journal of the Science of Food and Agriculture**, London, v. 73, p. 307–314, 1997.

HOUBA, V.J.G.; TEMMINGHOFF, E.J.M.; GAIKHORST, G.A.; van VARK, W. Soil analysis procedures Using 0.01 M calcium chloride as extraction reagent. **Communications in Soil Science and Plant Analysis**, New York, v. 31, p. 1299–1396, 2000.

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA. **Pesquisa de orçamentos familiares 2002-2003**. Aquisição alimentar domiciliar *per capita* no Brasil. IBGE, Rio de Janeiro, Brasil, 2004. 252 p.

KIRKHAM, M.B. Cadmium in plants on polluted soils: Effect of soil factors, hyperaccumulation and amendments. **Geoderma**, Amsterdam, v. 137, p. 19–32, 2006.

KUKIER, U.; CHANEY, R.L.; RYAN, J.A.; DANIELS, W.L.; DOWDY, R.H.; GRANATO, T.C. Phytoavailability of cadmium in long-term biosolids-amended soils. **Journal of Environmental Quality**, Madison, v. 39, p. 519–530, 2010.

LINDSAY, W.L.; NORVELL, W. Development of DTPA soil for zinc, iron, manganese and copper. **Soil Science Society of America Journal**, Madison, v. 42, p. 421–428, 1978.

McBRIDE, M.B.; SAUVÉ, S.; HENDERSHOT, W.H. Solubility control of Cu, Zn, Cd and Pb in contaminated soil. **European Journal of Soil Science**, Oxford, v. 48, p. 337–346, 1997.

McBRIDE, M.B. Cadmium uptake by crops estimated from soil total Cd and pH. **Soil Science**, Baltimore, v. 167, p. 62–67, 2002.

McLAUGHLIN, M.J.; WHATMUFF, M.; WARNE, M.S.T.J.; HEEMSBERGEN, D.; BARRY, G.; BELL, M.; NASCH, D.; PRITCHARD, D. A field investigation of solubility and food chain accumulation of biosolid-cadmium across diverse soil types. **Environmental Chemistry**, London, v. 3, p. 428–432, 2006.

PEIJNENBURG, W.; BAERSELMAN, R.; de GROOT, A.; JAGER, T.; LEENDERS, D.; POSTHUMA, L.; VAN VEEN, R. Quantification of metal bioavailability for lettuce (Lactuca sativa L.) in field soils. **Archives of Environmental Contamination and Toxicology**, New York, v. 39, p. 420-430, 2000.

PEIJNENBURG, J.G.M.; ZABLOTSKAJA, M.; VIJVER, M.G. Monitoring metals in terrestrial environments within a bioavailability framework and a focus on soil extraction. **Ecotoxicology and Environmental Safety**, New York, v. 67, p. 163–179, 2007.

PERES, A.C. **Modelo para o estabelecimento de valores orientadores para elementos radioativos no solo**. 2007. 125 p. Tese (Doutorado em Tecnologia Nuclear – aplicações) - Instituto de Pesquisas Energética e Nucleares, Universidade de São Paulo, São Paulo, 2007.

RAIJ, B. van; ANDRADE, J.C. de; CANTARELLA, H.; QUAGGIO, J.A. **Análise química para avaliação da fertilidade de solos tropicais.** Campinas: Instituto Agronômico, 2001. 285 p.

SARWAR, N.; SAIFULLAH; MALHI, S.S.; ZIA, M.H.; NAEEM, A.; BIBI, S.; FARID, G. Role of mineral nutrition in minimizing cadmium accumulation by plants. **Journal of the Science of Food and Agriculture**, London, v. 90, p. 925–937, 2010.

SWARTJES, F.A.; van BREEMEN, E.M. D.; OTTE, P.F.; van BEELEN, P.; RIKKEN, M.G.J.; TUINSTRA, J.; SPIJKER, J.; LIJZEN, J.P.A. **Human health risks due to consumption of vegetables from contaminated sites**. Bilthoven : RIVM, 2007. (RIVM report 711701040).

SWARTJES, F.A.; TROMP, P.C. A tiered approach for the assessment of the human health risks of asbestos in soils. **Soil & Sediment Contamination**, Boca Raton, v. 17, p. 137-149, 2008.

TLUSTOŠ, P.; SZÁKOVÁ, J.; KOŘÍNEK, K.; PAVLÍKOVÁ, D.; HANČ, A.; BALÍK, J. The effect of liming on cadmium, lead and zinc uptake reduction by spring wheat grown in contaminated soil. **Plant, Soil and Environment**, Praha, v. 52, p. 16–24, 2006.

YANG, X.E.; LONG, X.X.;YE, H.B.; HE, Z.L.; CALVERT, D.; VandStoffella P.J. Cadmium tolerance and hyperaccumulation in a newZn-hyperaccumulating plant species (Sedum alfredii H.). **Plant and Soil**, The Hague, v. 259, p. 181–189, 2004.

YANG, Y.; ZHANG, F.S.; LI, H.F.; JIANG, R.F. Accumulation of cadmium in the edible parts of six vegetable species grown in Cd-contaminated soils. **Journal of Environmental Management**, London, v. 90, p. 1117-1122, 2009.

4 BARIUM AND CADMIUM EFFECTS ON GROWTH AND OXIDATIVE STRESS IN SOYBEAN GROWN IN SOILS WITH CONTRASTING PROPERTIES

Abstract

Barium (Ba) and cadmium (Cd) accumulation effects on growth and antioxidant response in soybean leaves were investigated. Plants were grown in pots using two artificially contaminated soils (Oxisol and Entisol) under glasshouse conditions. Cadmium reduced plant growth at concentrations higher than 5.2 mg kg^{-1}, while Ba only affected plant growth at 600 mg kg^{-1}. Such levels are higher than the limits imposed by the Brazilian environmental legislation. Lipid peroxidation was increased only at Cd concentration of 10.4 mg kg^{-1} in the Oxisol, after 30 d of exposure. Twelve superoxide dismutase (SOD; EC 1.15.1.1) isoenzymes were evaluated, most of them classified as Cu/Zn-SOD isoenzymes. SOD activity reduced over time in the Oxisol and remained high in the Entisol, given the more stressful conditions in this soil. Catalase (CAT; EC 1.11.1.6) exhibited few responses to Cd or Ba concentrations, but increased over time. Glutathione reductase (GR; EC 1.6.4.2) activity was reduced over time when exposed to the higher Cd concentration, but increased under Ba exposure in the Oxisol. Contrary to the normal effects observed when oxidative stress normally results in responses based on metal concentration, time length of exposure and plant tissue, the enzymes activity changes were mainly dependent on soil type, time of exposure and, in a lesser extent, metal concentration in soil. Soybean plants grown in a sandy soil with low buffer capacity, as the Entisol, suffer higher oxidative stress than those grown in a clayey soil, as the Oxisol.

Keywords: Heavy metals; Soil pollution; *Glycine max*. L.; Superoxide dismutase; Catalase; Glutathione reductase

4.1 Introduction

The increase of metal pollution in soils is a problem in many parts of the world, especially when agricultural soils are concerned. Heavy metals enter the soil through addition of sludge, composts, or fertilizers (KIRKHAM, 2006). Cadmium (Cd) is one of the most hazardous metal pollutant to the environment and human health because plants growing on contaminated soils can absorb and accumulate Cd in edible tissues in large quantities without any visible symptoms, entering the food chain (MONTEIRO et al., 2008). In different parts of the world, Miller et al. (2004), Yang et al. (2009) and Marković et al. (2010) reported contents of Cd in edible parts of vegetables above established permissible limits. Due to these problems, Cd is one of the most studied soil metal pollutant around the world, but there is a lack of data regarding Cd in Brazilian soils and plants. Barium (Ba), on the other hand, is a metal that has not received a great deal of attention and little has been reported about its effect on plants, although Llugany, Poschenrieder and Barceló (2000) and Suwa et al. (2008) have showed its potential toxicity in plants, but such studies were short-term and performed in nutrient solutions. Consequently, Ba effects on plant grown in soils containing Ba still needs to be further investigated. Though Ba is not bioconcentrated by many plants species, some are able to accumulate it. Bioconcentration factors (Ba plant/ Ba soil) from 2 to 20 have been reported for tomatoes and soybean (CHOUDHURY; CAREY, 2009). Recently, Nogueira et al. (2010) observed that successive sewage sludge applications increased soil Ba concentration and Ba accumulation in parts of maize plants grown in the State of São Paulo, Brazil.

The Brazilian National Environment Council recently established guideline values of metals in soils, including Cd and Ba (CONSELHO NACIONAL DO MEIO AMBIENTE - CONAMA, 2009). However, defining soil quality standards for metals is a difficult task, especially in tropical soils, where little information is available. Although the derivation of the aforementioned values is based on human health risk, studies focused on the relationship between heavy metals and oxidative stress in plants could be useful as a tool to identify critical soil Cd or Ba concentration for plants, since it is a more sensitive endpoint as compared to classical endpoints like biomass production (CORRÊA et al., 2006). Many studies on oxidative stress have been available in recent years (see GRATÃO et al., 2005, for a review), yet, it is still difficult to draw a general conclusion about critical toxic metal concentrations in soils (GOMES-JUNIOR et al., 2006, 2007).

When present as free ionic metals, Ba^{2+}, Cd^{2+}, and other metallic elements can affect the performance of plants from the subcellular up to the ecosystem level (ERNST; VERKLEIJ; SCHAT, 1992). The interaction of free ionic metals with cellular components can initiate a variety of metabolic responses in seconds, sometimes with direct or indirect generation of reactive oxygen species (ROS) (BABU et al., 2001). ROS are extremely reactive and cytotoxic to all organisms (ARRUDA; AZEVEDO, 2009), and their production must be minimized. The major ROS scavenging mechanisms of plants include the enzymes superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) (GRATÃO et al., 2005). These enzymes are affected by exposure of soybean to Cd (FERREIRA et al., 2002; NORIEGA et al., 2007; MUÑOZ et al., 2008).

Most of the studies focusing on oxidative stress in plants induced by metals have been performed in nutrient solution, and frequently it is evaluated less than ten days of exposure (SCHÜTZENDÜBEL; POLLE, 2002; GRATÃO et al., 2008a), but soil type plays a key role in controlling metal availability for plant uptake (PEIJNENBURG et al., 2000). On the other hand, oxidative stress responses in plants grown in tropical soils for longer periods of exposure to Cd and, especially to Ba, have not been studied yet. In this study we aimed to evaluate the effects of Ba and Cd concentrations (based on the Brazilian legislation) and time length of exposure, on plant growth, lipid peroxidation and activities of three antioxidant enzymes (CAT, GR, and specific isoenzymes of SOD) in leaves of soybean plants grown in tropical soils with contrasting properties.

4.2 Material and Methods

4.2.1 Soil characterization and experimental design

An experiment was carried out under glasshouse conditions in Piracicaba, State of São Paulo, Brazil. A Typic Quartzipsamment (Entisol) and a Typic Hapludox (Oxisol), with contrasting attributes, were collected from native forests, to ensure the absence of metal contamination. The pH was determined in a 0.01 mol L^{-1} $CaCl_2$ solution (soil:solution ratio 1:2.5); contents of C were determined in a CNS automatic analyzer (NELSON; SOMMERS, 1982); contents of oxides were determined after extraction with 9 mol L^{-1} of sulphuric acid and 30% sodium hydroxide solutions (CAMARGO et al., 1986); background concentration of Cd and Ba were extracted by aqua-regia method (McGRATH; CUNLLIFE, 1985) in sieved samples (< 0.15 mm) and determined by inductively coupled plasma optical emission spectrometry (ICP-OES). Soil physical fractionation was performed by using densimeter method (GEE; OR, 2002). The cationic exchange capacity (CEC), was calculated by cation summation (Ca^{2+}, Mg^{2+}, K^+- extracted by a ionic resin) plus Al^{3+} and H^+, estimated at pH 7.5 with 1.78 mol L^{-1} SMP solution (SHOEMAKER; MCLEAN; PRATT, 1961). The water retention capacity was determined in a tension table after 24 h of saturation with water at 0 and 0.01 MPa of pressure.

Batch-type analysis was performed by adding, in 2g of soil, 20 mL of solutions containing 5; 10; 25; 50; 100 and 200 mg L^{-1} of Cd or 25; 50; 100; 200 and 400 mg L^{-1} of Ba. After shaking for 24 h and centrifugation the metal concentration in the supernatant was determined. Then, Langmuir isotherms adsorption was adjusted for both soils and the maximum adsorption capacity (MAC) was calculated to check the capacity of each in retains the studied metals. Soil acidity was corrected to raise the base saturation to 50%. Although, for soybean it is recommended 70% at the State of São Paulo, Brazil, we chose 50% to avoid a high increase in pH. Lime requirement (*LR*) was calculated as *LR* (t ha^{-1}) = ($V_2 - V_1$) *CEC* / 10 *ECCE*, where V_2 is the established base saturation (50%), V_1 is the current base saturation (soil analysis), V_1 (%) = ($Ca_{ex} + Mg_{ex} + K_{ex}$) 100 / *CEC*. *ECCE* is the effective calcium carbonate equivalent of the lime material; Ca_{ex}, $Mg_{ex,}$ and K_{ex} are exchangeable basic cations; and *CEC* ($mmol_c$ dm^{-3}) = Ca + Mg + K+ H + Al.

After one month of incubation, the mean value for pH in a 0.01 mol L^{-1} $CaCl_2$ solution stabilized in 5.2 for the Oxisol and in 5.0 for the Entisol. To evaluate the toxic effects in plants, soils were spiked with 0.65; 1.3; 2.6; 5.2 and 10.4 mg kg^{-1} of Cd and 75; 150; 300; 600 mg kg^{-1} of Ba by using nitrate salts plus a fertilization with macro and micronutrients. These levels of contamination represent 0.5; 1; 2; 4 and 8 times the warning value for Cd and 0.5; 1; 2 and 4 times the warning value for Ba in soils for the State of São Paulo (CETESB, 2005). A control treatment (no metal addition) was also included. The experimental design consisted of randomized blocks, with three replicates. Firstly, sorghum (*Sorghum bicolor* L.) was grown in pots of 4.0 dm^3 until the flowering stage, and roots and shoots were harvested. Later, the soil of each treatment was again placed in the pots (to crop succession) and a sample was collected for posterior analysis. Each pot was watered to reach 70% of the maximum capacity of water retention, and 150 mg kg^{-1} of N, 200 mg kg^{-1} of P, 200 mg kg^{-1} of K and 20 mg kg^{-1} of S were added. Five soybean (*Glycine max* L.) seeds per pot were sown, and seedlings were thinned to two plants per pot. After, 50 mg kg^{-1} of N as NH_4NO_3 p.a. were added weekly during the first three weeks. All concentrations were expressed on a dry weight basis, unless stated otherwise.

4.2.2 Sampling and analytical determinations

Soybean leaves were collected at 10, 30 and 45 d after sowing and immediately immersed in liquid nitrogen and stored at -80ºC, for further analyses. The selected treatments used in this study were: i) for Cd – Oxisol: control; 1.3 mg kg^{-1} and 10.4 mg kg^{-1}; Entisol – control; 1.3 mg kg^{-1} and 2.6 mg kg^{-1}; ii) for Ba – Oxisol: control; 150 mg kg^{-1} and 600 mg kg^{-1}; Entisol – control; 150 mg kg^{-1} and 300 mg kg^{-1}. Due to the low plant yield, especially at the first sampling (10 d) in the Entisol, it was not possible to collect plants exposed to the highest concentration for both metals. Leaves were also collected at 10, 30 and 45 d (flowering stage) after sowing to determine metal concentration. The plant material was then rinsed in deionized water, oven-dried (60ºC) until constant weight, weighted, grounded and digested using a microwave oven (Mars Xpress, CEM Corporation) as described by Araújo et al. (2002). Cadmium concentration in the plant extracts was determined by using flame atomic absorption spectrometry (FAAS), and Ba by ICP-OES. Blank reagents and a certified sample of tomato leaves (NIST, SRM 1573a) were used to have a quality analysis control.

Soil samples were taken from each pot one day before sowing, air-dried and sieved (< 2 mm). The available contents of Cd and Ba in soil samples were determined after extraction with diethylenetriaminepentaacetic acid - DTPA (LINDSAY; NOVELL, 1978). Although only few authors (eg. IPPOLITO; BARBARICK, 2006) have evaluated DTPA to measure Ba availability in soils, we have used it because it is the official method of the State of São Paulo, Brazil. Cadmium concentration in the soil extracts was determined by FAAS and Ba by ICP-OES. Blank reagents and a certified sample of Montana soil (NIST, SRM 2711) were used to have a quality analysis control.

4.2.2.1 Lipid peroxidation

Lipid peroxidation was determined by estimating the content of thiobarbituric acid reactive substance (TBARS) as proposed by Heath and Packer (1968) and optimized and described by (GOMES-JUNIOR et al., 2006). The concentration of equivalent malondialdehyde (MDA) was calculated using an extinction coefficient of 155 mM cm^{-1}.

4.2.2.2 Enzyme extraction and assays

The following steps were carried out at $4^{o}C$ unless stated otherwise: Leaves were macerated with liquid nitrogen in a mortar with a pestle. Extracts were homogenized (3:1 buffer volume, FW) in 100 mM potassium phosphate buffer (pH 7.5) containing 1 mM ethylene-diaminetetra-acetic acid (EDTA), 3 mM DL-dithiothreitol (DTT) and 5% (w/v) insoluble polyvinylpolypyrrolidone (PVPP) (GRATÃO et al., 2008a). The homogenates were centrifuged at 10,000 x *g* for 30 min and the supernatants were kept stored in separate aliquots and stored at -$80^{o}C$ prior to SOD, GR and CAT determinations. Protein concentration was determined by the method of Bradford (BRADFORD, 1976) using bovine serum albumin as a standard.

4.2.2.3 Polyacrylamide gel electrophoresis

SOD and GR activities were determined in non-denaturing polyacrylamide gel electrophoresis (PAGE). Electrophoresis was carried out as described by Gratão et al. (2008a). Briefly, 12% polyacrylamide with a 4% stacking gels were subjected to a 20 mA constant current per gel for 3 h (gels stained for SOD activity) or for 4 h (gels stained for GR activity), and the temperature set to 4^oC. Equal amounts of leaf extracted protein (40 µg for Oxisol treatments and 20 µg for Entisol treatments) were loaded on to each gel lane and bovine liver SOD and GR used as activity standards.

After electrophoretic separation, for SOD activity staining, the PAGE gel was rinsed in distilled-deionized water and incubated in the dark for 30 min in 50 mM potassium phosphate buffer (pH 7.8) containing 1 mM of EDTA, 0.005 mM de riboflavin, 0.1 mM nitroblue tetrazolium, and 0.3% N,N,N´,N´-tetramethylethylenediamine. At the end of this period, the reaction mixture was poured off, the gels were rinsed with distilled–deionized water and then illuminated in water until the development of achromatic bands of SOD activity in a purple-stained gel were visible. In order to classify SOD isoenzymes, samples were subjected to non-denaturing PAGE and the SOD bands classified as described by Guelfi et al. (2003). Pre-treatment of the gel in H_2O_2 and KCN before SOD staining allowed the classification of the SOD into Cu/Zn–SOD, Fe–SOD or Mn–SOD. Cu/Zn–SOD is inhibited by both inhibitors, Fe–SOD isoenzyme is resistant to KCN and inhibited by H_2O_2, and Mn–SOD is resistant to both inhibitors.

For GR activity staining, the PAGE gel was rinsed in distilled-deionized water and incubated in the dark for 30 min at room temperature in a reaction mixture containing 0.25 mM Tris, 0.5 mM 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*- tetrazolium bromide (MTT), 0.7 mM 2,6-dichloro-*N*-(4-hydroxylphenyl)-1,4-benzoquinoneimine sodium salt (DPIP), 3.4 mM GSSG and 0.5 mM NADPH as described by Gomes-Junior et al. (2007).

4.2.2.4 CAT specific activity determination

CAT activity was assayed spectrophotometrically as described by Gratão et al. (2008a) at 25^oC in a reaction mixture containing 1 mL 100 mM potassium phosphate buffer (pH 7.5) added of 2.5 µL H_2O_2 (30% solution). The reaction was initiated by the addition of 25 µL of protein extract and the activity determined by following the decomposition of H_2O_2 by change in absorbance at 240 nm for 1 min. CAT activity is expressed as µmol min^{-1} mg^{-1} protein.

4.2.2.5 GR specific activity determination

GR activity was assayed spectrophotometrically as described by Gratão et al. (2008a) at 30°C in a mixture consisting of 1.7 mL 100 mM potassium phosphate buffer (pH 7.5) containing 1 mM 5,5´-dithiobis (2-nitrobenzoic acid), 1 mM oxidized glutathione and 0.1 mM NADPH. The reaction was started by the addition of 50 µL of protein extract. The rate of reduction of oxidized glutathione was followed by monitoring the change in absorbance at 412 nm for 1 min.

4.2.3 Statistical analysis

A one-way analysis of variance was performed by F test ($p < 0.05$) to determine the influence of metal (Cd or Ba) concentration in soils, within each time of sampling. When a significant difference was detected the means of the measured parameters were compared by Tukey test ($p < 0.05$). All values represent the mean of three independent replicates.

4.3 Results

4.3.1 Soil characteristics, metal uptake and effects on plant growth

The studied soils exhibited highly contrasting properties (Table 1).

Table 1 - Soil characterization under natural conditions

Property	Oxisol	Entisol
pH $_{0.01\ mol\ L^{-1}\ CaCl_2}$	3.5±0.1[(1)]	3.6±0.1
Total C (g kg^{-1})	32±1.0	8.0±0.4
Total N (g kg^{-1})	2.9±0.07	0.7±0.01
Sand (g kg^{-1})	260	811
Loam (g kg^{-1})	108	129
Clay (g kg^{-1})	632	60
Fe_2O_3 (g kg^{-1})	76	5.0
Al_2O_3 (g kg^{-1})	211	26
[(2)]WRC (%) at 0 MPa	60	46
WRC (%) at 0.01 MPa	31	9
CEC ($mmol_c$ kg^{-1})	188±11	41±1.0
Ca ($mmol_c$ kg^{-1})	9.0±0.4	5.3±0.6
Mg ($mmol_c$ kg^{-1})	4.9±0.1	1.0±0.1
K ($mmol_c$ kg^{-1})	1.8±0.03	0.4±0.0
Total Cd (mg kg^{-1})	0.30±0.02	0.01±0.01
Total Ba (mg kg^{-1})	32±3.6	3.5±0.4
[(3)]MAC of Cd (mg kg^{-1})	2,000	833
MAC of Ba (mg kg^{-1})	3,330	1,110

[(1)] Mean (n = 3) ± Standard Deviation; [(2)] WRC – Water Retention Capacity; [(3)] MAC – Maximum Adsorption Capacity.

The Oxisol had 4-fold higher total C and total N contents than the Entisol. Moreover, Oxisol also exhibited much higher clay, Fe and Al contents, yielding a higher CEC than the Entisol. Both soils exhibited naturally low levels of Cd and Ba. The water retention capacity in the Oxisol was higher than the Entisol, especially at 0.01 MPa pressure in which the water retention dropped to a very low level (9%) in the Entisol.

Despite of the different chemical properties between the soils, the available content of Cd increased linearly with the applied rates in both Oxisol and Entisol (Fig. 1 A). On average, almost 70% of the added Cd was available. Conversely, the availability of Ba in the Oxisol was low (± 2% of the added Ba) as compared to the Entisol, in which about 10% of the added Ba was available. Ba availability, especially in the Entisol, followed a more exponential model (Fig. 1 B), and there was a larger increase in the available fraction in this soil at the treatment of 600 mg kg^{-1}.

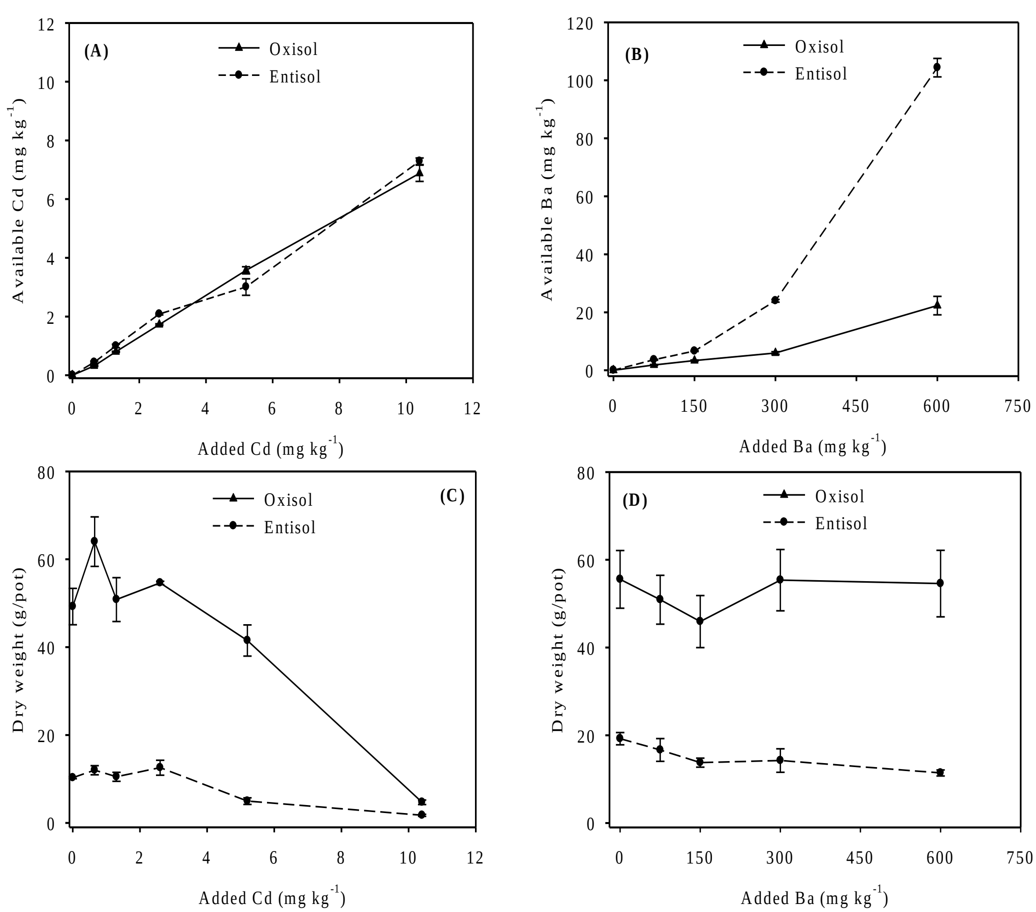

Figure 1 - Availability of cadmium – Cd (A) and barium – Ba (B) as extracted by Diethylenetriamine pentaacetate (DTPA), and total dry weight of soybean (shoot + roots) grown in an Oxisol and in an Entisol with increasing concentrations of Cd (C) and Ba (D). Points represent the means (n = 3) and bars represent standard deviation

The availability is related, among other factors, to the affinity of the metal for the soil colloids, as measured by the maximum adsorption capacity (MAC), which were about 2.5 and 3.0-fold higher in the Oxisol than in the Entisol for Cd and Ba, respectively (Table 1).

Cadmium caused a dramatic inhibition on the growth of soybean plants in both soils at the rate of 10.4 mg kg^{-1} (Fig. 1 C), although there was a reduction from the rate 5.2 mg kg^{-1} in the Entisol ($p < 0.05$). The dry matter yield in the Oxisol was much higher than in the Entisol due to better soil chemical and physical properties. The Ba treatments did not cause any reduction in the dry matter yield in the Oxisol (Fig. 1 D) ($p < 0.05$). A small reduction, however, was observed in the Entisol when 600 mg kg^{-1} of Ba was added, but no visual symptom of phytotoxicity was observed in soybean plants.

4.3.2 Metal concentration in leaves and induction of oxidative stress

Cadmium concentration in leaves increased according to the increasing concentration in both Oxisol (Fig. 2 A) and Entisol (Fig. 2 B). In the Oxisol, the highest Cd concentration (10.4 mg kg^{-1}) added caused an increase in the level of lipid peroxidation products in soybean leaves, measured as TBARS, at 30 d of exposure, as compared to the control (Fig. 2 C). In the Entisol there was a difference ($p < 0.05$) in this parameter only at 45 d with the Cd concentration of 1.3 mg kg^{-1}, as compared to the control (Fig. 2 D).

Barium concentration in leaves increased with the increasing of added Ba concentrations until 30 d of exposure in the Oxisol, when the same concentrations are compared over time (Fig. 3 A). In the Entisol, however, Ba concentration increased either with the added concentrations or over time until the last time of sampling (Fig. 3 B). Although there was an increase in Ba concentration in leaves, the level of lipid peroxidation was not changed in the Oxisol (Fig. 3 C), whereas in the Entisol was observed a small increase at 30 d of exposure at the concentration of 300 mg kg^{-1}, as compared to the control (Fig. 3 D).

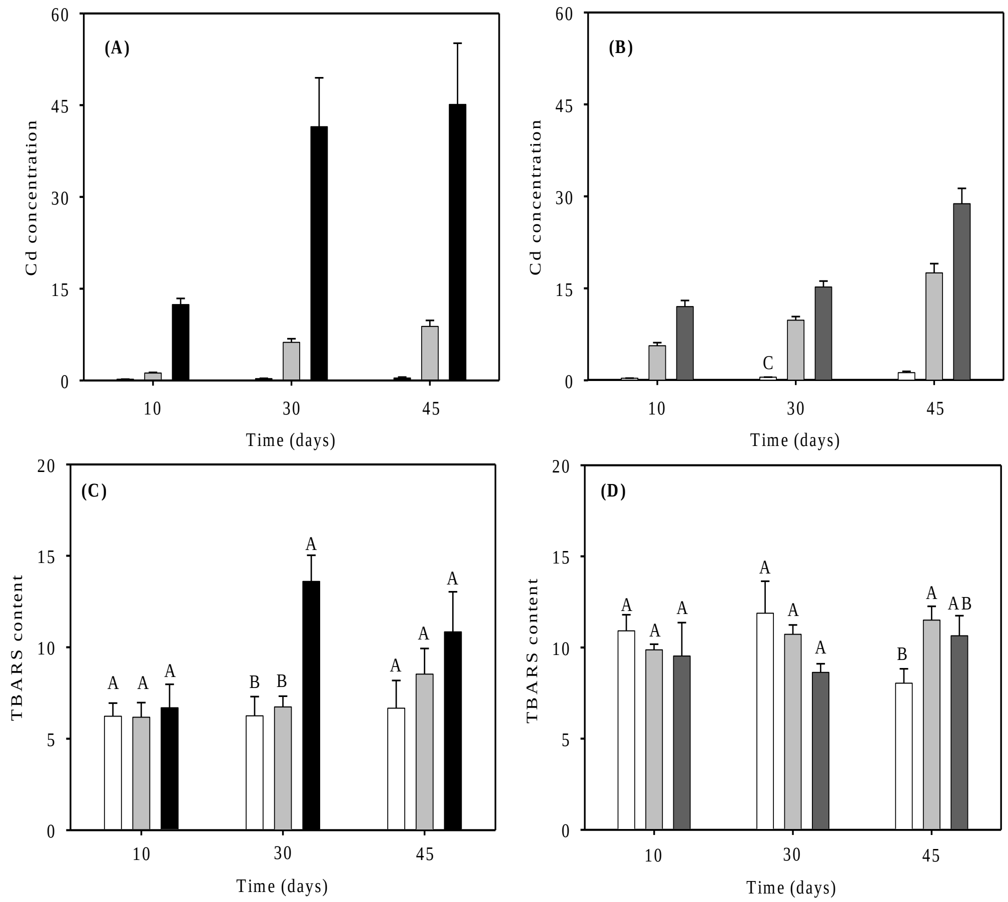

Figure 2 - Cd concentration (µg g^{-1} dry wt.) and TBARS content (nmol g^{-1} fr. wt.) in leaves of soybean plants grown in a clayey Oxisol (A and C) and in a sandy Entisol (B and D) with increasing Cd concentrations at different times. (□) Control (no metal), (▒) 1.3 mg kg^{-1}, (▓) 2.6 mg kg^{-1}, (■) 10.4 mg kg^{-1}. Means (n = 3) for Cd concentration at each time with the same letter are not different (Tukey, $p < 0.05$)

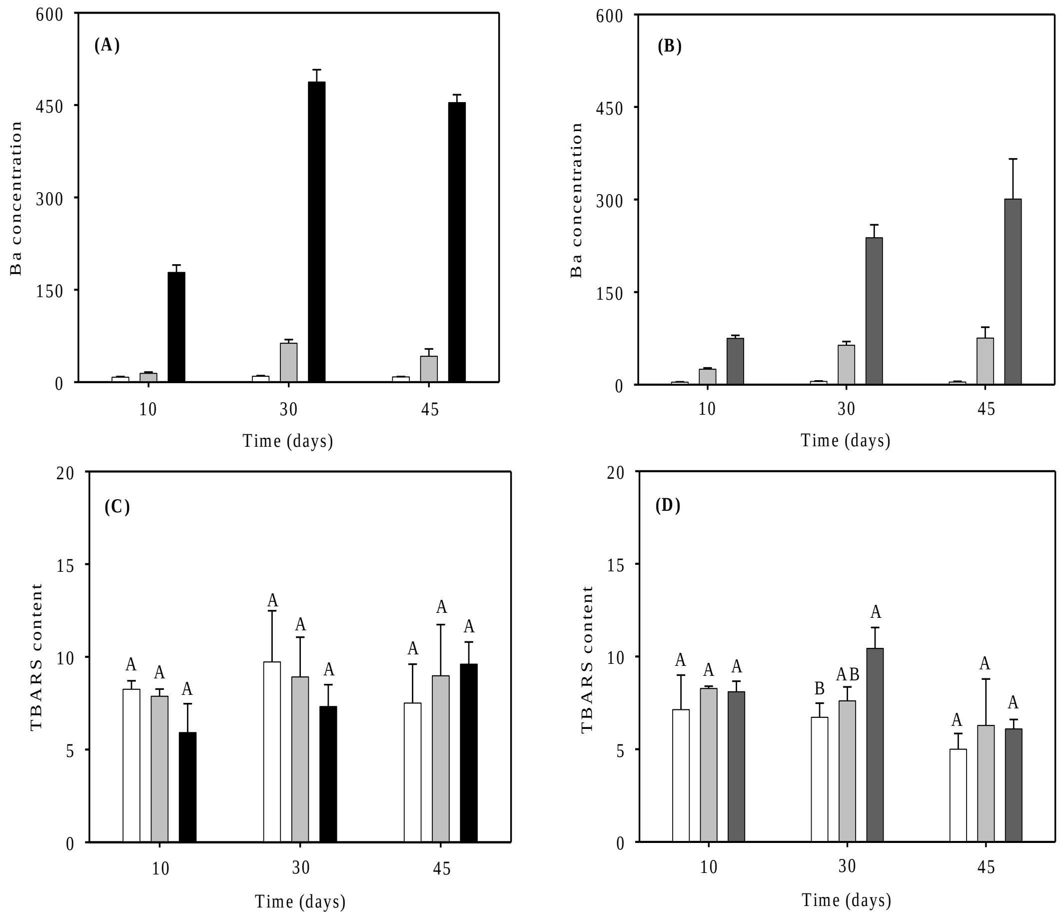

Figure 3 - Ba concentration (µg g^{-1} dry wt.) and TBARS content (nmol g^{-1} fr. wt.) in leaves of soybean plants grown in a clayey Oxisol (A and C) and in a sandy Entisol (B and D) with increasing Ba concentrations at different times. (▭) Control (no metal), (▭) 150 mg kg^{-1}, (▭) 300 mg kg^{-1}, (■) 600 mg kg^{-1}. Means (n = 3) for Ba concentration at each time with the same letter are not different (Tukey, $p < 0.05$)

4.3.3 Response of antioxidant enzymes to metal exposure

4.3.3.1 Cadmium

There was a clear difference in SOD activity between Oxisol and Entisol (Fig. 4). SOD activity staining revealed the existence of twelve isoenzymes in leaves of soybean plants grown in the Entisol (Fig. 4 B), including 1 Mn-SOD (band I), 3 Fe-SODs (bands II–IV), and 8 Cu/Zn-SOD (bands V-XII), according to the isoenzyme characterization (Fig. 4 E). Bands IX and XI did not appear in the Oxisol (Fig. 4 A). The Mn-SOD isoenzyme appeared at all times of sampling for both soils but without any major variations between soils or metal concentration. The Fe-SOD isoenzymes (bands II–IV) only clearly appeared from the second time of sampling (30 d) and were independent of soil or metal concentration. It appears, however, that their activities were slightly increased in the Entisol containing Cd at higher concentrations (Fig. 4 B) when compared to Oxisol (Fig. 4 A).

CAT activity exhibited differences only in a limited number of treatments. However, a clear trend of activity increase during the time length of the experiment was observed, regardless of the soil type. Cadmium concentrations only increased CAT activity in the Oxisol at 45 d (Fig. 5 A). GR activity, however, exhibited different trends when the Oxisol is concerned (Fig. 5 C). For instance, GR activity exhibited a tendency of reduction over time in the presence of Cd (Fig. 5 C) and only at 10 d that the highest Cd concentration increased GR activity, as compared to the control. On the other hand, Cd concentrations did not increase GR activity in the Entisol (Fig. 5 D).

GR activity was also measured based on isoenzyme activity staining (Fig. 7). Such as for SOD, and in agreement with GR activity determined by the spectrophotometer assay (Fig. 5 C, D), up to 7 clearly defined GR isoenzymes (marked as I-VII) were identified in soybean leaves. Although the activity patterns were not changed for the majority of the isoenzymes (GR IV-VII), GR isoenzymes I and II appeared to have been induced during the time length of the treatments in both soil types. The activity of these two GR isoenzymes appeared to increase over time, based on GR activity as measured by the spectrophotometer assay (Fig. 5 C, D), but such increase did not alter the GR activity observed in the treatments which might have been compensated by a decrease in GR V activity.

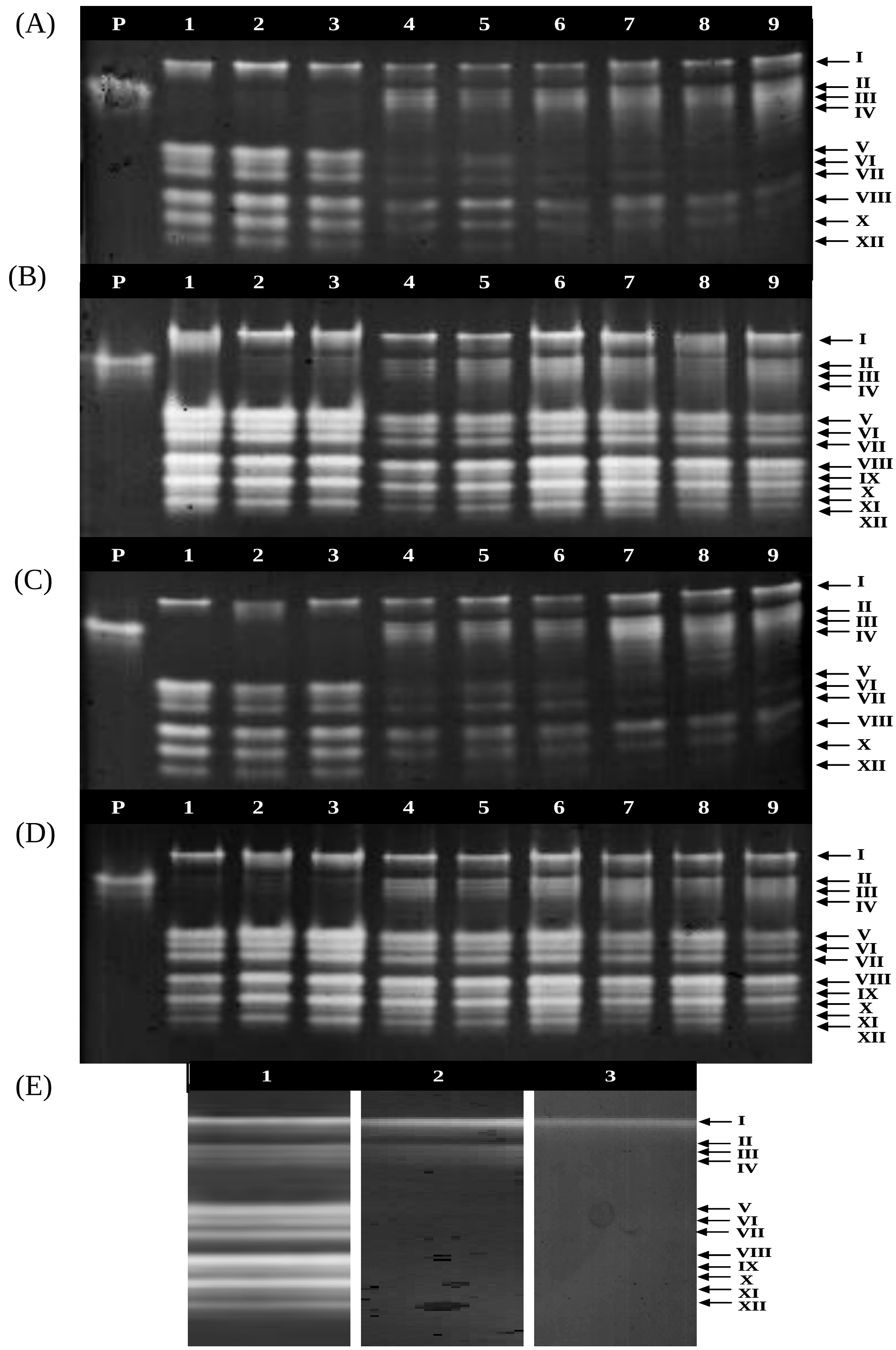

Figure 4 – Activity staining for SOD from soybean leaves grown in an Oxisol (A and C) and in an Entisol (B and D). (A) and (B) are the treatments with Cd; (C) and (D) are the treatments with Ba. Lane P (bovine SOD standard); lanes 1, 2, 3 (10 days after sowing); lanes 4, 5, 6 (30 days after sowing); lanes 7, 8, 9 (45 days after sowing). To check the concentrations of Cd and Ba in each soil and time of sampling see figures 2 and 3. (E) Represent superoxide dismutase (SOD) isoenzyme classification. Activity staining following native PAGE for soybean leaves. Lane 1 (control), lane 2 (plus 2 mM potassium cyanide) and lane 3 (plus 5 mM hydrogen peroxide). Arrows indicate SOD bands sequentially numbered

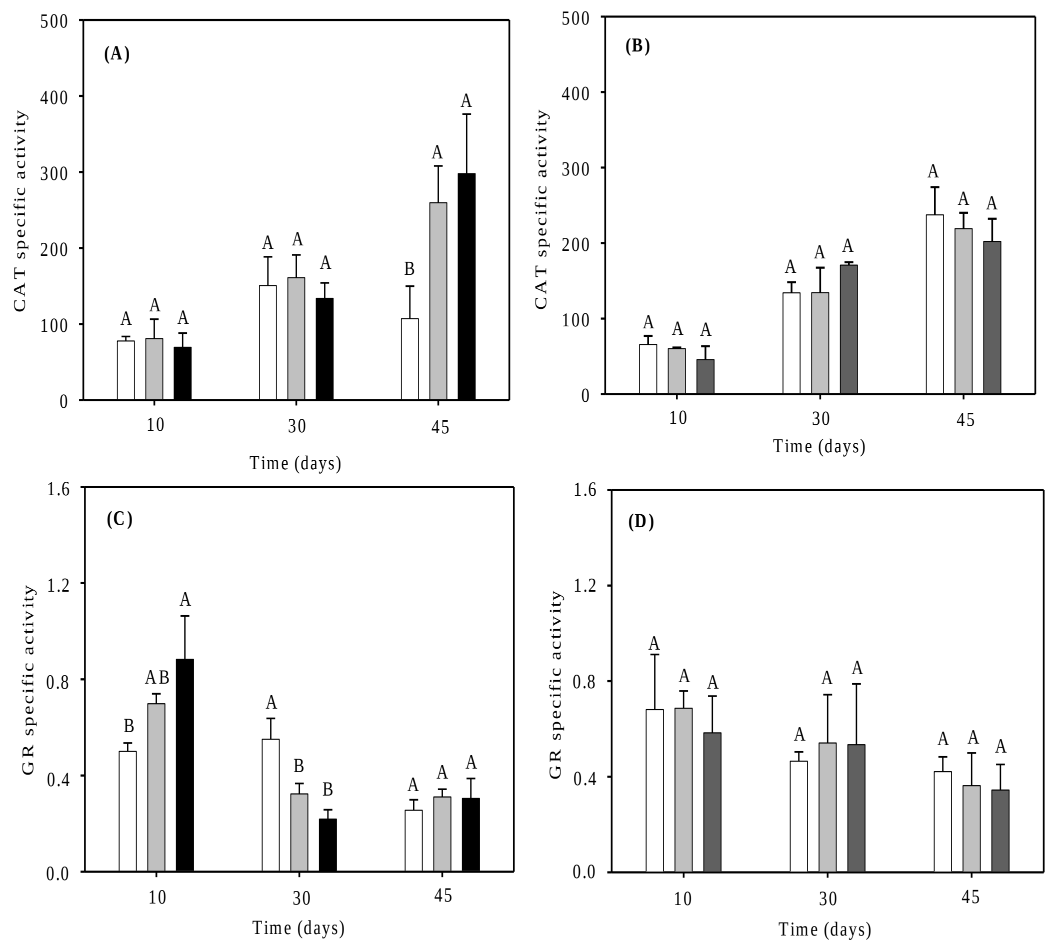

Figure 5 – Specific activity of CAT and GR in soybean leaves grown in a clayey Oxisol (A and C) and in a sandy Entisol (B and D) with increasing Cd concentrations at different times. ( ) Control (no metal), ( ) 1.3 mg kg^{-1}, ( ) 2.6 mg kg^{-1}, ( ) 10.4 mg kg^{-1}. Means (n = 3) for Cd concentration at each time with the same letter are not different (Tukey, $p < 0.05$)

4.3.3.2 Barium

When Ba is concerned (Fig. 4 C, D), the overall SOD isoenzyme pattern was not different from what was observed for Cd (Fig. 4 A, B). Thus, in a similar way, total SOD activity (sum of all band intensities) appeared to exhibit an increased SOD activity in Entisol. Cu/Zn-SOD isoenzymes exhibited the largest differences between the soils. The activity of this class of isoenzymes decreased considerably over time in Oxisol (Fig. 4 C) treated with Ba, while it remained high at all times studied in the Entisol (Fig. 4 D).

Barium concentrations only increased CAT activity in the Oxisol at 10 d (Fig. 6 A) and at 30 d in the Entisol (Fig. 6 B), as compared to the control. However, CAT activity exhibited a trend of activity increase during the time length of the experiment, regardless of the soil type.

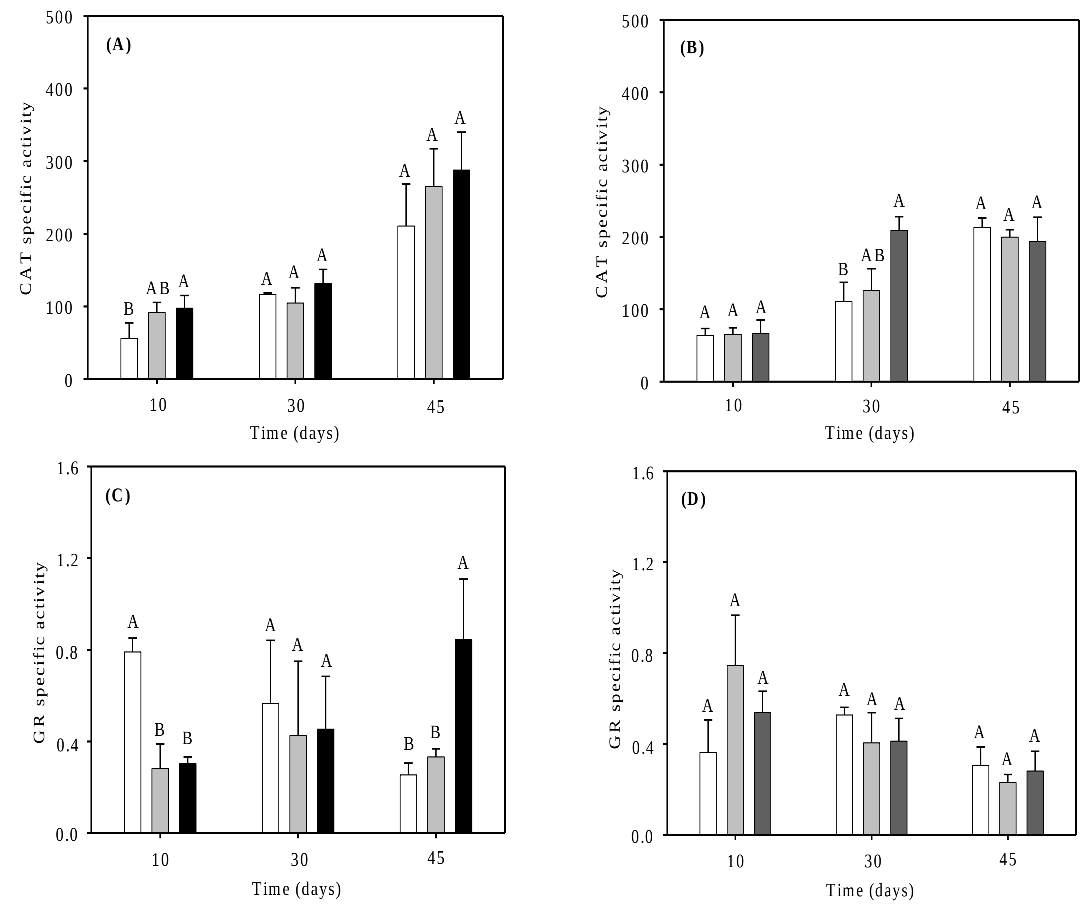

Figure 6 – Specific activity of CAT and GR in soybean leaves grown in a clayey Oxisol (A and C) and in a sandy Entisol (B and D) with increasing Ba concentrations at different times. ( ) Control (no metal), ( ) 150 mg kg^{-1}, ( ) 300 mg kg^{-1}, ( ) 600 mg kg^{-1}. Means (n = 3) for Ba concentration at each time with the same letter are not different (Tukey, $p < 0.05$)

The highest concentration of Ba exhibited a clear trend of increased GR activity over time in the Oxisol (Fig. 6 C). On the other hand, in the Entisol, GR activity exhibited a tendency of reduction over time with the Ba treatments (Fig. 6 D). For Ba treatments, GR activity measured based on isoenzyme activity staining (Fig. 7) followed the same pattern as discussed for Cd treatments.

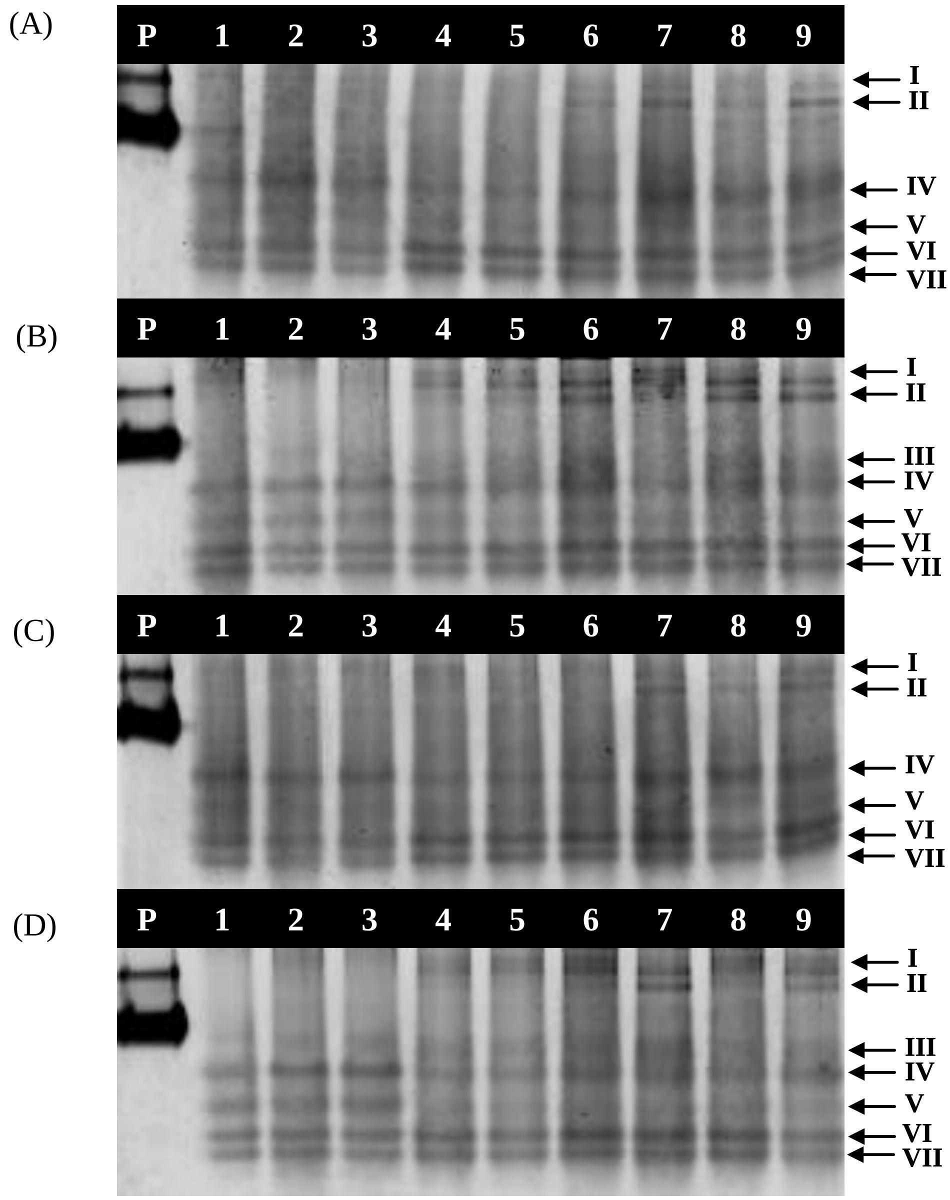

Figure 7 – Activity staining for GR from soybean leaves grown in an Oxisol (A and C) and in an Entisol (B and D). (A) and (B) are the treatments with Cd; (C) and (D) are the treatments with Ba. Lane P (bovine GR standard); lanes 1, 2, 3 (10 days after sowing); lanes 4, 5, 6 (30 days after sowing); lanes 7, 8, 9 (45 days after sowing). To check the concentrations of Cd and Ba in each soil and time of sampling see figures 2 and 3. Arrows indicate GR bands sequentially numbered

4.4 Discussion

The main differences between the studied soils that influenced the Cd and Ba availabilities and uptake by soybean were the contents of C, N, clay and oxides (iron and aluminum). Such differences in soil characteristics yielded a much higher CEC for the Oxisol as compared to the Entisol. Furthermore, the metal affinity by the soil colloids is extremely important in determining metal availability for plant uptake. In this case, Cd had a lower affinity with the soils measured by the adsorption capacity, as compared to Ba. Consequently, Cd was more available for plant uptake and caused a strong reduction in biomass production, while Ba almost did not affect biomass production. For both metals the biomass production was only affected in concentrations higher than the Intervention Values established by the State Environmental Agency of the State of São Paulo, Brazil, for agricultural soils (CETESB, 2005).Such Intervention Values are based on human health risks. So, concentrations of these metals which are unacceptable for agricultural soils were not toxic to soybean plants, even in a pot experiment like in this study. Metal availability is normally higher in spiked soils than in non-spiked soils (EFFROYSOM; SAMPLE; SUTER, 2004). Under natural conditions the concentrations of Cd and Ba were low and the soils can be considered uncontaminated (KABATA-PENDIAS; MUKHERJEE, 2007).

Although the literature is well served with reports for several plant species indicating the damaging effects of Cd toxicity on plant growth (VITÓRIA; LEA; AZEVEDO, 2001; PEREIRA et al., 2002; WÓJCIK; TUKIENDORF, 2005), some authors have also demonstrated a stimulatory effect on plant growth or even cell growth (FORNAZIER et al., 2002; GOMES-JUNIOR et al., 2006; GRATÃO et al., 2008b) and an increase in biomass production, in the presence of low concentrations of Cd (COSTA; MOREL, 1994; PINTO et al., 2004; WANI; KHAN; ZAIDI, 2008). An increase in soybean biomass production was observed in the Oxisol at the lowest Cd concentration studied (0.65 mg kg^{-1}). Such a result can be discussed as a biostimulation phenomenon, in which various processes are stimulated by low Cd concentrations, namely stomatal opening, which enhances gas exchanges, increase in amino acid metabolism and formation of enhanced concentrations of lignin or derived compounds, increasing root biomass production (PINTO et al., 2004). This phenomenon, however, still needs to be further investigated to draw a more general conclusion. For Ba, on the other hand, just a few studies have focused on plant toxicity. For instance, Ba was shown to cause a reduction in biomass production for bean (CHAUDHRY; WALLACE; MUELLER, 1977; LLUGANY; POSCHENRIEDER; BARCELÓ, 2000) and soybean (SUWA et al., 2008) grown in nutrient solution. Barium reduced soybean growth due to reduced CO_2 assimilation caused by limited photosynthetic activity. In this case, Ba acts as an efficient K^+-channel blocker (SUWA et al., 2008). In our study, Ba also caused a reduction in protein content in soybean leaves in the Entisol at the highest concentration investigated at 30 and 45 d of exposure (data not shown), which might also be a result of enhanced proteolysis.

Malondialdehyde (MDA) is one of several low molecular weight products formed via the decomposition of primary and secondary lipid peroxidation products, being the most frequently used indicator of lipid peroxidation, a consequence of oxidative damage (DEWIR et al., 2006). Increases of TBARS at higher Cd or Ba concentrations were only observed in some treatments subjected to longer periods of exposure (30 or 45 d), which does not directly indicate that the plants were under severe oxidative stress. Such a result may also explain some changes observed in this study for the antioxidant enzymes, suggesting that the experimental conditions have probably induced a certain level of oxidative stress, but the cell metabolism was able to deal with such a stress, resulting in very little changes in lipid peroxidation rates. Moreover, there was clear evidence that the metals were entering the cell system reaching the leaves, so, being able to cause a certain degree of oxidative stress which, as already commented, may likely have been dealt with the action of some of the enzymes tested. Similar results were found in rice cultivars exposed to Cd to 7, 14 and 21 days (HASSAN et al., 2005) and have been also observed in *Arabidopsis thaliana* after 21 days of exposure (CHO; SEO, 2005). However, an increase in TBARS was demonstrated to happen only after 48 h of $CdCl_2$ solution exposure in coffee cells (GOMES-JUNIOR et al., 2006), although the effect of enhancing the TBARS levels is likely to be higher in nutrient solution than in soil, as demonstrated by Teklić et al. (2008a,b) in radish and lettuce tissues.

Heavy metals can generate ROS by direct electron transfer (in case of redox active metals, such as Cu), by binding to functional groups of (membrane) proteins, by substitution of essential metals in enzymes, which can cause electron leakages, and by inactivation of (certain) antioxidant enzymes or depletion of low molecular weight antioxidants (MEERS et al., 2005). Cd and Ba are not redox metals and, therefore, can not catalyze Fenton-type reactions yielding ROS. Despite of this, Cd has been shown to increase the generation and accumulation of $O\bullet_2^-$ and/or H_2O_2 in pea leaves (SANDALIO et al., 2001; ROMERO-PUERTAS et al., 2004), pea roots (RODRÍGUEZ-SERRANO et al., 2006), wheat (RANIERI et al., 2005) and rice leaves (HSU; KAO, 2007), in a similar way to other metals (GOMES-JUNIOR et al., 2007; POMPEU et al., 2008). We have not measured the production of ROS; however, taking into account the accumulation levels of the metals and the responses obtained by the antioxidant enzymes, the production of ROS may have happened but the cell system was able to deal with any enhanced production that might have occurred.

SOD is responsible for the dismutation of $O\bullet_2^-$ into H_2O_2 and O_2 and is considered to be the first line of defense against ROS (GRATÃO et al., 2005). Although the dismutation of superoxide radicals produces H_2O_2 in the initial process of ROS removal, the enhanced production of H_2O_2 upon metal exposure might not be entirely induced by the increased production $O\bullet_2^-$ and activity of SOD (CHO; SEO, 2005). Cu/Zn-SODs were the most abundant group of isoenzymes in soybean leaves. Their activities were higher in the beginning of exposure (10 d) and declined dramatically after that with the increase of metal exposure in the Oxisol, but remained constant for the whole period in the Entisol, mainly because of the inherent low buffer capacity, related to the set of properties of this soil which caused a more stressful growth condition and induced the response of SOD. This was a significant result and such a change in isoenzyme distribution pattern was clearly not related to metal exposure, but to the soil type and during plant growth since the controls without Cd and Ba also exhibited the same changes or identical patterns. These results also indicate a lack of a specific response by SOD to the metals for soybean and under the condition tested in this research. Conversely, Fe-SOD isoenzymes were shown to be induced in soybean leaves during plant development since they only appeared after 10 d of the experiment and could not be related at all to the Cd or Ba additions to the soil or even soil type, indicating a clear metabolic natural change. Three minor bands of Fe-SOD isoenzymes were also observed in coffee cells (GOMES-JUNIOR et al., 2006), but such as observed in this study, they did not respond to the Cd treatment.

CAT activity showed a clear and consistent increase trend with time of exposure under control condition and for Cd and Ba treatments in both soils, suggesting that the observed changes were almost entirely due to the plant developmental stage, but not the metals. Yet, for Cd in Oxisol at 45 d CAT exhibited higher activity with Cd concentrations when compared to the control, whilst in the Entisol no difference was observed between control and treatments. In the Entisol other variables, such as water and nutrient supply or even a small, but higher variation in soil temperature compared to the Entisol, may have caused some stress in the plants and masked the effects of Cd concentrations. For Ba treatments, it was noticed that only the higher concentrations studied induced a small CAT increase at 10 d in the Oxisol and at 30 d in the Entisol, when compared to the control. Balestrasse et al. (2001) observed an increase in CAT activity that could be attributed to an effect of Cd toxicity in soybean, but Ferreira et al. (2002) did not observe any major changes in CAT activity in both leaves and roots of soybean plants exposed to Cd, indicating contradictory results for this plant species, even though the cultivar and the concentrations used were not the same.

GR is responsible for the reduction of oxidized glutathione (GSSG) into reduced glutathione (GSH) in the glutathione-ascorbate pathway to remove H_2O_2, which is required for the activity of dehydroascorbate peroxidase (CHO; SEO, 2005). Yet, the data for GR activity was consistent with previous reports since GR activity did not exhibit any major significant changes when metal concentration is concerned, especially in the Entisol. For Cd, only at 10 d of exposure that the highest concentration of this metal (10.4 mg kg^{-1}), in the Oxisol, differed from the control, whereas for Ba in the same soil GR activity only responded at 45 d of exposure at the highest concentration tested. On the other hand, in the Entisol, as also observed for SOD and CAT, the activity of GR did not respond to the increase in metal concentration. It seems that plants grown in this soil were clearly more subjected to stress caused by the edafic conditions and do not differentiate of those exposed to metals. Still, GR activity was also not affected in soybean leaves as reported by Ferreira et al. (2002), who only observed a GR activity increase in soybean roots when exposed to a very high Cd concentration. The lack of any major GR activity changes suggests that responses via the activation of the ascorbate-glutathione cycle for the removal of the extra H_2O_2 produced is probably not taking place and that there is no indication that extra reduced glutathione is being produced even for the synthesis of Cd-binding proteins. Not even the observed induction of GR isoenzymes I and II appears to be related to a response to oxidative stress since they were both induced during the time length of the treatments in both metals and soil types. Although other groups of antioxidant enzymes were not analyzed in this study, it appears that CAT is capable of dealing with the excess H_2O_2 produced.

It is important that further studies are conducted in order to check other biochemical and physiological parameters and also roots, apart from other metals, since the huge majority of the studies on the antioxidant stress responses by plants to metals have been performed in hydroponic conditions, which is a completely different system than the one used in this study. Soil type is critical and was the most important element influencing the plant response to the metal. Soils with contrasting properties as those of the present study deserve an especial attention regarding their influence on oxidative stress caused by heavy metals. Ongoing studies are being carried out to investigate the same effect on the root system which is particularly important considering that the large majority of plant species appears to accumulate Cd and other metals preferably in the roots system, also using phytochelatins as a defense mechanism (PEREIRA et al., 2002), which, for instance, might perhaps result in a different pattern of reduced glutathione production for the synthesis of phytochelatins. Arruda and Azevedo (2009) discussed other approaches involving not only a more analytical study and the use of metallomics to investigate oxidative stress responses induced by metals, but also distinct experimental strategies and designs, indicating particularly the benefit of using chronic and acute treatments. Finally, it is important to comment about the importance of future studies on soil type and the mutual associations between arbuscular mycorrhizal fungi and roots, which may influence the way by which metal ions are taken up and metabolized by plants (ANDRADE et al., 2009; LI et al., 2009).

4.5 Conclusions

Cadmium was phytotoxic to soybean in concentrations (5.2 mg kg^{-1} in the Entisol and 10.4 mg kg^{-1} in the Oxisol) higher than the Intervention Value (3.0 mg kg^{-1}) for an agricultural scenario, established by the legislation in the State of São Paulo, Brazil, while Ba slightly reduced the production only in the concentration of 600 mg kg^{-1} in the Entisol, which is above the Intervention agricultural Value (300 mg kg^{-1}). The antioxidant defenses, measured through three key antioxidant enzyme activities, were dependent on the soil type in which the plant was grown, time of exposure and physiological/developmental stage. Soil type was critical for the plant response to oxidative stress: SOD activity reduced over time in the Oxisol and remained high in the Entisol, whilst CAT consistently increased with the time length of exposure, but just few differences were observed with metal concentrations. GR activity did not respond to Cd and Ba concentrations in the Entisol, but distinct isoenzymes were shown to be altered based on the soil in which the soybean plants were grown, but not the metal. Soybean plants grown in a sandy soil, with low cation retention capacity, as the Entisol, suffer higher oxidative stress than those grown in a clayey soil, as the Oxisol, and consequently respond less to the increase of metal concentrations.

References

ANDRADE, S.A.L.; GRATÃO, P.L.; SCHIAVINATO, M.A.; SILVEIRA, A.P.D.; AZEVEDO, R.A.; MAZZAFERA, P. Zn uptake, physiological response and stress attenuation in mycorrhizal jack bean growing in soil with increasing Zn concentrations. **Chemosphere**, Oxford, v. 75, p. 1363-1370, 2009.

ARAÚJO, G.C.L.; GONZALEZ, M.H.; FERREIRA, A.G.; NOGUEIRA, A.R.A.; NÓBREGA, J.A. Effect of acid concentration on closed-vessel microwave-assisted digestion of plant materials. **Spectrochimica Acta part B: Atomic Spectroscopy**, Oxford, v. 57, p. 2121-2132, 2002.

ARRUDA, M.A.Z., AZEVEDO, R.A. Metallomics and chemical speciation: towards a better understanding of metal-induced stress in plants. **Annals of Applied Biology**, Warwick, v. 155, p. 301-307, 2009.

BABU, T.S.; MARDER, J.B.; TRIPURANTHAKAM, S.; DIXON, D.G.; GREENBERG, B.M. Synergistic effects of a photooxidized polycyclic aromatic hydrocarbon and copper on photosynthesis and plant growth: evidence that in vivo formation of reactive oxygen species is a mechanism of copper toxicity. **Environmental Toxicology and Chemistry**, New York, v. 20, p. 1351-1358, 2001.

BALESTRASSE, K.B.; GARDEY, L.; GALLEGO, S.M.; TOMARO, M.L. Response of antioxidant defense system in soybean nodules and roots subjected to cadmium stress. **Australian Journal of Plant Physiology**, Melbourne, v. 28, p. 497-504, 2001.

BRADFORD, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. **Analytical Biochemistry**, New York, v. 72, p. 248–254, 1976.

CAMARGO, O.A.; MONIZ, A.C.; JORGE, J.A.; VALADARES, J.M.A.S. **Métodos de Análise Química, Mineralógica e Física de Solos do Instituto Agronômico de Campinas.** Campinas: Instituto Agronômico, 1986. 94 p. (Boletim Técnico, 106).

CHAUDHRY, F.M.; WALLACE, A.; MUELLER, R.T. Barium toxicity in plants. **Communications in Soil Science and Plant Analysis**, New York, v. 8, p. 795-797, 1977.

CHOUDHURY, H., CAREY, R. Barium and barium compounds in: CONCISE INT. CHEM. ASSESSMENT DOC, 33.2009. Geneva. **Int. Progr.** Chem. Safety, World Health Organ., Geneva. Available at: http://www.inchem.org/documents/cicads/cicads/cicad33.htm. 2009.

CHO, U.H.; SEO, N.H. Oxidative stress in Arabidopsis thaliana exposed to cadmium is due to hydrogen peroxide accumulation. **Plant Science**, Limerick, v. 168, p. 113-120, 2005.

COMPANHIA DE TECNOLOGIA DE SANEAMENTO AMBIENTAL. **Decisão de Diretoria nº 195-2005-E, de 23 de Novembro de 2005. Dispõe sobre a aprovação dos Valores Orientadores para Solos e Águas Subterrâneas no Estado de São Paulo – 2005, em substituição aos Valores Orientadores de 2001, e dá outras providências.** São Paulo, 2005, publicada no Diário Oficial do Estado de São Paulo de 03 de Dezembro de 2005, retificada em 13 de Dezembro de 2005. Disponível em: http://www.cetesb.sp.gov.br/Solo/relatorios/tabela_valores_2005.pdf. Acesso em : 05 nov. 2010.

CONSELHO NACIONAL DO MEIO AMBIENTE. **Resolução n° 420 de 28 de Dezembro de 2009. Dispõe sobre critérios e valores orientadores de qualidade do solo quanto à presença de substâncias químicas e estabelece diretrizes para o gerenciamento ambiental de áreas contaminadas por essas substâncias em decorrência de atividades antrópicas.** Brasília, 2009, publicada no Diário Oficial da União de 30 de Dezembro de 2009. Disponível em: http://www.mma.gov.br/port/conama/legiabre.cfm?codlegi=620. Acesso em: 05 nov. 2010.

CORRÊA, A.X.R.; RORIG, L.R.; VERDINELLI, M.A.; COTELLE, S.; FERARD, J.F.; RADETSKI, C.M. Cadmium phytotoxicity: Quantitative sensitivity relationships between classical endpoints and antioxidative enzyme biomarkers. **Science of the Total Environment**, Amsterdam, v. 357, p. 120-127, 2006.

COSTA, G.; MOREL, J.L. Water relations, gas-exchange and amino-acid content in Cd-treated lettuce. **Plant Physiology and Biochemistry**, Paris, v. 32, p. 561-570, 1994.

DEWIR, Y.H.; CHAKRABARTY, D.; ALI, M.B.; HAHN, E.J.; PAEK, K.Y. Lipid peroxidation and antioxidant enzyme activities of Euphorbia milii hyperhydric shoots. **Environmental and Experimental Botany**, Elmsford, v. 58, p. 93-99, 2006.

EFFROYSOM, R.A.; SAMPLE, B.E.; SUTER, G.W. Bioaccumulation of inorganic chemicals from soil by plants: spiked soils vs field contamination or background. **Human and Ecological Risk Assessment**, Atlanta, v. 10, p. 1117–1127, 2004.

ERNST, W.H.O.; VERKLEIJ, J.A.C.; SCHAT, H. Metal tolerance in plants. **Acta Botanica Neerlandica,** Amsterdam, v. 41, p. 229-248, 1992.

FERREIRA, R.R.; FORNAZIER, R.F.; VITÓRIA, A.P.; LEA, P.J.; AZEVEDO, R.A. Changes in antioxidant enzyme activities in soybean under cadmium stress. **Journal of Plant Nutrition**, New York, v. 25, p. 327-342, 2002.

FORNAZIER, R.F.; FERREIRA, R.R.; PEREIRA, G.J.G.; MOLINA, S.M.G.; SMITH, R.J.; LEA, P.J.; AZEVEDO, R.A. Cadmium stress in sugar cane callus cultures: effect on antioxidant enzymes. **Plant Cell, Tissue and Organ Culture**, Dordrecht, v. 71, p. 125-131, 2002.

GEE G.W.; OR, D. Particle-size analysis. In: DANE, J.H., TOOP, G.C. (Ed.).**Methods of soil analysis.** Physical methods**.** Madison, 2002. p 255-293.

GOMES-JUNIOR, R.A.; MOLDES, C.A.; DELITE, F.S.; GRATÃO, P.L.; MAZZAFERA, P.; LEA, P.J.; AZEVEDO, R.A. Nickel elicits a fast antioxidant response in Coffea arabica cells. **Plant Physiology and Biochemistry**, Paris, v. 44, p. 420-429, 2006.

GOMES-JUNIOR, R.A.; GRATÃO, P.L.; GAZIOLA, S.A.; MAZZAFERA, P.; LEA, P.J.; AZEVEDO, R.A. Selenium-induced oxidative stress in coffee cell suspension cultures. **Functional Plant Biology**, Victoria, v. 34, p. 449-456, 2007.

GRATÃO, P.L.; POLLE, A.; LEA, P.J.; AZEVEDO, R.A. Making the life of heavy metal-stressed plants a little easier. **Functional Plant Biology**, Victoria, v. 32, p. 481-494, 2005.

GRATÃO, P.L.; MONTEIRO, C.C.; ANTUNES, A.M.; PERES, L.E.P.; AZEVEDO, R.A. Acquired tolerance of tomato (Lycopersicon esculentum cv. Micro-Tom) plants to cadmium-induced stress. **Annals of Applied Biology**, Warwick, v. 153, p. 321-333, 2008 a

GRATÃO, P.L.; POMPEU, G.B.; CAPALDI, F.R.; VITORELLO, V.A.; LEA, P.J.; AZEVEDO, R.A. Antioxidant response of Nicotiana tabacum cv. Bright Yellow 2 cells to cadmium and nickel stress. **Plant Cell, Tissue and Organ Culture**, Dordrecht, v. 94, p. 73-83, 2008 b.

GUELFI, A.; AZEVEDO, R.A.; LEA, P.J.; MOLINA, S.M.G. Growth inhibition of the filamentous fungus Aspergillus nidulans by cadmium: an antioxidant enzyme approach. **Journal of General and Applied Microbiology**, Tokyo, v. 49, p. 63-73, 2003.

HASSAN, M.J.; ZHANG, G.P.; WU, F.B.; WEI, K.; CHEN, Z.H. Zinc alleviates growth inhibition and oxidative stress caused by cadmium in rice. **Journal of Plant Nutrition and Soil Science**, Weinheim, v. 168, p. 255-261, 2005.

HEATH, R.L.; PACKER, L. Photoperoxidation in isolated chloroplasts .I. kinetics and stoichiometry of fatty acid peroxidation. **Archives of Biochemistry and Biophysics**, New York, v. 125, p. 189-198, 1968.

HSU, Y.T.; KAO, C.H. Toxicity in leaves of rice exposed to cadmium is due to hydrogen peroxide accumulation. **Plant and Soil**, The Hague, v. 298, p. 231-241, 2007.

IPPOLITO, J.A.; BARBARICK, K.A. Biosolids affect soil barium in a dryland wheat agroecosystem. **Journal of Environmental Quality**, Madison, v. 35, p. 2333–2341, 2006.

KABATA-PENDIAS, A.; MUKHERJEE, A.B. **Trace elements from soil to human**. Berlin: New York, Springer-Verlag. 2007. 550 p.

KIRKHAM, M.B. Cadmium in plants on polluted soils: Effects of soil factors, hyperaccumulation, and amendments. **Geoderma**, Amsterdam, v. 137, p. 19-32, 2006.

LI, H.F.; GRAY, C.; MICO, C.; ZHAO, F.J.; McGRATH, S.P. Phytotoxicity and bioavailability of cobalt to plants in a range of soils. **Chemosphere**, Oxford, v. 75, p. 979-986, 2009.

LINDSAY, W.L.; NORVELL, W.A. Development of a DTPA soil test for zinc, iron, manganese, and copper. **Soil Science Society of America Journal**, Madison, v. 42, p. 421-428, 1978.

LlUGANY, M.; POSCHENRIEDER, C.; BARCELÓ, J. Assessment of barium toxicity in bush beans. **Archives of Environmental Contamination and Toxicology**, New York, v. 39, p. 440-444, 2000.

MARKOVIĆ, M.; CUPAĆ, S.; ĐUROVIĆ, R.; MILINOVIĆ, J.; KLJAJIĆ, P. Assessment of Heavy Metal and Pesticide Levels in Soil and Plant Products from Agricultural Area of Belgrade, Serbia. **Archives of Environmental Contamination and Toxicology**, New York, v. 58, p. 341-351, 2010.

McGRATH, S.P.; CUNLIFFE, C.H. A simplified method for the extraction of the metals Fe, Zn, Cu, Ni, Cd, Pb, Cr, Co and Mn from soils and sewage sludges. **Journal of the Science Food and Agriculture**, Malden, v. 36, p. 794-798, 1985.

MEERS, E.; RUTTENS, A.; GEEBELEN, W.; VANGRONSVELD, J.; SAMSON, R.; VANBROEKHOVEN, K.; VANDEGEHUCHTE, M.; DIELS, L.; TACK, F.M.G. Potential use of the plant antioxidant network for environmental exposure assessment of heavy metals in soils. **Environmental Monitoring and Assessment**, Dordrecht, v. 120, p. 243-267, 2005.

MILLER, J.R.; HUDSON-EDWARDS, K.A.; LECHLER, P.J.; PRESTON, D.; MACKLIN, M.G. Heavy metal contamination of water, soil and produce within riverine communities of the Rio Pilcomayo basin, Bolivia. **Science of the Total Environment**, Amsterdam, v. 320, p. 189-209, 2004.

MONTEIRO, M.S.; SANTOS, C.; SOARES, A.M.V.M.; MANN, R.M. Does subcellular distribution in plants dictate the trophic bioavailability of cadmium to porcellio dilatatus (crustacea, isopoda)? **Environmental Toxicology and Chemistry**, New York, v. 27, p. 2548-2556, 2008.

MUÑOZ, N.; GONZALEZ, C.; MOLINA, A.; ZIRULNIK, F.; LUNA, C.M. Cadmium-induced early changes in $O•_2^-$, H_2O_2 and antioxidative enzymes in soybean (Glycine max L.) leaves. **Plant Growth Regulation**, Dordrecht, v. 56, p. 159-166, 2008.

NELSON, D.W.; SOMMERS, L.E. Total carbon, organic carbon, and organic matter. In: PAGE, A.L. et al (Ed.). **Methods of soil analysis** 2nd ed. Madison: ASA and SSSA, 1982.pt 2. p. 539-580.(Agronomy Monogr, 9).

NOGUEIRA, T.A.R.; MELO, W.J.; FONSECA, I.M.; MARQUES, M.O. Barium uptake by maize plants as affected by sewage sludge in a long-term field study. **Journal of Hazardous Materials**, Amsterdam, v. 181, p. 1148-1157, 2010.

NORIEGA, G.O.; BALESTRASSE, K.B.; BATLLE, A.; TOMARO, M.L. Cadmium induced oxidative stress in soybean plants also by the accumulation of delta-aminolevulinic acid. **Biometals**, London, v. 20, p. 841-851, 2007.

PEIJNENBURG, W.; BAERSELMAN, R.; de GROOT, A.; JAGER, T.; LEENDERS, D.; POSTHUMA, L.; van VEEN, R. Quantification of metal bioavailability for lettuce (Lactuca sativa L.) in field soils. **Archives of Environmental Contamination and Toxicology**, New York, v. 39, p. 420-430, 2000.

PEREIRA, G.J.G.; MOLINA, S.M.G.; LEA, P.J.; AZEVEDO, R.A. Activity of antioxidant enzymes in response to cadmium in Crotalaria juncea. **Plant and Soil**, The Hague, v. 239, p. 123-132, 2002.

PINTO, A.P.; MOTA, A.M.; de VARENNES, A.; PINTO, F.C. Influence of organic matter on the uptake of cadmium, zinc, copper and iron by sorghum plants. **Science of the Total Environment**, Amsterdam, v. 326, p. 239-247, 2004.

POMPEU, G.B.; GRATÃO, P.L.; VITORELLO, V.A.; AZEVEDO, R.A. Antioxidant isoenzyme responses to nickel-induced stress in tobacco cell suspension culture. **Scientia Agricola**, Piracicaba, v. 65, p. 548-552, 2008.

RANIERI, A.; CASTAGNA, A.; SCEBBA, F.; CARERI, M.; ZAGNONI, I.; PREDIERI, G.; PAGLIARI, M.; di TOPPI, L.S. Oxidative stress and phytochelatin characterisation in bread wheat exposed to cadmium excess. **Plant Physiology and Biochemistry**, Paris, v. 43, p. 45-54, 2005.

RODRÍGUEZ-SERRANO, M.; ROMERO-PUERTAS, M.C.; ZABALZA, A.; CORPAS, F.J.; GÓMEZ, M.; DEL RÍO, L.A.; SANDALIO, L.M. Cadmium effect on oxidative metabolism of pea (Pisum sativum L.) roots. Imaging of reactive oxygen species and nitric oxide accumulation in vivo. **Plant, Cell & Environment**, Oxford, v. 29, p. 1532-1544, 2006.

ROMERO-PUERTAS, M.C.; RODRÍGUEZ-SERRANO, M.; CORPAS, F.J.; GÓMEZ, M.; DEL RÍO, L.A.; SANDALIO, L.M. Cadmium-induced subcellular accumulation of $O\bullet_2^-$ and H_2O_2 in pea leaves. **Plant, Cell & Environment**, Oxford, v. 27, p. 1122-1134, 2004.

SANDALIO, L.M.; DALURZO, H.C.; GOMEZ, M.; ROMERO-PUERTAS, M.C.; DEL RIO, L.A. Cadmium-induced changes in the growth and oxidative metabolism of pea plants. **Journal of Experimental Botany**, Oxford, v. 52, p. 2115-2126, 2001.

SCHÜTZENDÜBEL, A.; POLLE, A. Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. **Journal of Experimental Botany**, Oxford, v. 53, p. 1351-1365, 2002.

SHOEMAKER H.E.; McLEAN, E.O.; PRATT, P.F. Buffer methods for determining lime requirement of soils with appreciable amounts of extractable aluminum. **Soil Science Society of America Proceedings**, Madison, v. 25, p. 274–277, 1961.

SUWA, R.; JAYACHANDRAN, K.; NGUYEN, N.T.; BOULENOUAR, A.; FUJITA, K.; SANEOKA, H. Barium toxicity effects in soybean plants. **Archives of Environmental Contamination and Toxicology**, New York, v. 55, p. 397-403, 2008.

TEKLIĆ, T.; HANCOCK, J.T.; ENGLER, M.; PARANIKOVIĆ, N.; CESAR, V.; LEPEDUŠ, H.; ŠTOLFA, I.; BEŠLO, D. Antioxidative responses in radish (Raphanus sativus L.) plants stressed by copper and lead in nutrient solution and soil. **Acta Biologica Cracoviensa Series Botanica**, Cracow, v. 50, p. 79-86. 2008 b.

TEKLIĆ, T.; ENGLER, M.; CESAR, V.; LEPEDUŠ, H.; PARANIKOVIĆ, N.; LONČARIĆ, Z.; ŠTOLFA, I.; MAROTTI, T.; MIKAC, N.; ŽARKOVIĆ, N. Influence of excess copper on lettuce (Lactuca sativa L.) grown in soil and nutrient solution. **Journal of Food, Agriculture and Environment**, Helsinki, v. 6, p. 439-444, 2008 a.

VITORIA, A.P.; LEA, P.J.; AZEVEDO, R.A. Antioxidant enzymes responses to cadmium in radish tissues. **Phytochemistry**, New York, v. 57, p. 701-710, 2001.

WANI, P.A.; KHAN, M.S.; ZAIDI, A. Effects of heavy metal toxicity on growth, symbiosis, seed yield and metal uptake in pea grown in metal amended soil. **Bulletin of Environmental Contamination and Toxicology**, New York, v. 81, p. 152-158, 2008.

WOJCIK, M.; TUKIENDORF, A. Cadmium uptake, localization and detoxification in Zea mays. **Biologia Plantarum**, Praha, v. 49, p. 237-245, 2005.

YANG, Y.; ZHANG, F.S.; LI, H.F.; JIANG, R.F. Accumulation of cadmium in the edible parts of six vegetable species grown in Cd-contaminated soils. **Journal of Environmental Management**, London, v. 90, p. 1117-1122, 2009.