Heavy metal toxicity in rice and soybean plants cultivated in contaminated soil

Maria Lígia de Souza Silva[1], *Godofredo Cesar Vitti*[2], *Anderson Ricardo Trevizam*[3]

ABSTRACT

Heavy metals can accumulate in soil and cause phytotoxicity in plants with some specific symptoms. The present study evaluated the specific symptoms on rice and soybeans plants caused by excess of heavy metals in soil. Rice and soybean were grown in pots containing soil with different levels of heavy metals. A completely randomized design was used, with four replications, using two crop species and seven sample soils with different contamination levels. Rice and soybean exhibited different responses to the high concentrations of heavy metals in the soil. Rice plants accumulated higher Cu, Mn, Pb and Zn concentrations and were more sensitive to high concentrations of these elements in the soil, absorbing them more easily compared to the soybean plants. However, high available Zn concentrations in the soil caused phytotoxicity symptoms in rice and soybean, mainly chlorosis and inhibited plant growth. Further, high Zn concentrations in the soil reduced the Fe concentration in the shoots of soybean and rice plants to levels considered deficient.

Key words: contamination, plant nutrition, symptomatology, heavy metals, *Oriza sativa* L., *Glycine max* L.

RESUMO

Toxicidade por metais pesados em plantas de arroz e soja cultivados em solo contaminado

Os metais pesados podem-se acumular no solo, causando fitotoxicidade em plantas, apresentando alguns sintomas específicos. Por esta razão, este estudo avaliou os sintomas específicos, em plantas de arroz e de soja, causados por excesso de metais pesados no solo. As plantas de arroz e de soja foram cultivadas em vasos, contendo solos com diferentes quantidades de metais pesados. O delineamento experimental foi inteiramente casualizado, com quatro repetições, utilizando-se duas culturas e sete amostras de solos com diferentes níveis de contaminação. As plantas de arroz e de soja exibiram diferentes respostas às elevadas concentrações desses metais no solo. Por exemplo, altas concentrações de Zn disponível no solo causaram fitotoxicidade no arroz e na soja, principalmente cloroses e inibição do crescimento das plantas. As plantas de arroz acumularam maiores teores de Cu, Mn, Pb e Zn e foram mais sensíveis às concentrações elevadas desses elementos no solo, absorvendo-os mais facilmente do que as plantas de soja. Além disso, altas concentrações de Zn no solo reduziram a concentração de Fe na parte aérea das plantas de soja e de arroz para níveis considerados deficientes.

Palavras-chave: contaminação, nutrição de plantas, sintomatologia, metal pesado, *Oriza sativa* L., *Glycine max* L.

[1] Engenheira-Agrônoma. Departamento de Agronomia, Universidade Estadual do Centro-Oeste, Rua Simeão Camargo Varela de Sá, 3, Vila Carli, 85040-080, Guarapuava, Paraná, Brasil. mlsousi@hotmail.com (autora para correspondência).

[2] Engenheiro-Agrônomo. Departamento de Ciência do Solo, Escola Superior de Agricultura “Luiz de Queiroz”, Avenida Pádua Dias, 11, Caixa Postal 09, 13418-900, Piracicaba, São Paulo, Brasil. gcvitti@usp.br

[3] Químico. Departamento de Agronomia, Universidade Estadual do Centro-Oeste, Rua Simeão Camargo Varela de Sá, 3, Vila Carli, 85040-080, Guarapuava, Paraná, Brasil. aanrt@hotmail.com

INTRODUCTION

Increasingly economic growth and technological development is evident in modern times, but has brought about numerous environmental problems caused by residues derived from industrial processes. Heavy metals when applied to the soil can accumulate and persist for long periods of time. These elements can be phytotoxic and harmful to vital microbial processes in nutrient cycling (Oliveira *et al.*, 2005).

Plants have mechanisms of tolerance to high concentrations of heavy metals. These include restricting transport from the root to the leaf, accumulation in trichomes, exudates that can complex the heavy metals, type of link between the element and the cell wall component, intracellular production of compounds with chelating properties and active pumping to the vacuoles. These mechanisms can occur individually or simultaneously, conferring increased tolerance to stress caused by the presence of heavy metals (Yadav, 2010; Hossain *et al*., 2012).

The processes of heavy metal absorption by plants are basically the same as those observed for macro and micronutrients. According to Carver & Ownby (1995) this absorption generally occurs actively or passively. However, heavy metals in the soil should be primarily in exchangeable form for absorption to occur (Kabala & Singh, 2001).

Symptoms in plants caused by shortage or excess of these elements and the associated levels in tissues make up a useful guide to establish deficiency or toxicity. It is therefore very important to know the heavy metal distribution in different parts of the plant. Some heavy metals in excess may result in toxicity to animals and crops (Gupta & Gupta, 1998). In the study of plant nutrition, it is necessary to consider the nutrients as a whole because in the absorption process elements can influence one another. However, interactions may occur altering the mineral composition of the leaves (Raij, 1991).

According to Adriano *et al.* (1971) toxicity symptoms are a result of alterations in metabolic processes, but are not common in the field. However may occur in areas with high availability of heavy metals and in plants that are not tolerant to these elements. In the case of zinc (Zn), chlorosis may occur in younger leaves due to interference in the iron (Fe) function in the chloroplasts and chlorophyll synthesis or decrease in root and leaf size and, when at high levels, there is decrease in phosphorus (P) and Fe absorption.

Cadmium (Cd) can reduce growth, photosynthesis rate and cause both enzymatic and metabolic alterations (Parmar *et al*., 2013). Another symptom identified for this metal is the chlorosis caused by the Cd and Fe competition for absorption sites in the plasma membrane (Siedlecka & Krupa, 1999; Tran & Popova, 2013).

The present study evaluated the specific symptoms in rice and soybeans plants caused by excess of heavy metals in the soil.

MATERIALS AND METHODS

The soil used in the study had been accidently contaminated and presented high levels of trace elements according to the intervention values described by CETESB (2005) (Table 1). Soil samples were collected in the district of Paulínia, SP, Brazil (22°45’S, 47°09’W) and classified as Typic Hapludox, containing 590 g kg^{-1} clay, 100 g kg^{-1} silt and 310 g kg^{-1} sand. Because contamination was associated with the wind, the soil was collected following the predominant direction of the wind, at equidistant points 100 m apart, starting from the pollution source and arranged and named as follows: Soil 1 (100 m), Soil 2 (200 m), Soil 3 (300 m), Soil 4 (400 m), Soil 5 (500 m), Soil 6 (600 m) and Soil 7 (700 m), at a depth of 0 - 20 cm. Soils were air-dried, sieved (5 mm mesh) and stored. For the experiment, soil samples were placed in 5 dm^3 pots. Irrigation was applied so as not to exceed the maximum of 70% and minimum of 40% of the water retention capacity, by weighing the pots daily.

The experiments were conducted in a greenhouse, in Piracicaba, SP, Brazil, from November 2004 to April 2005. The plant species used in the experiment were rice (*Oriza sativa* L. cv. IAC 202) and soybean (*Glycine max* L. cv. BRS 133). Fertilization with NPK was applied according to the need of each crop (Raij *et al.*, 1997). A randomized complete design was used, in a 7 x 2 factorial scheme (7 soil samples and 2 plants species), with four replications, in a total of 56 experimental units.

Ten seeds were sown in each pot on November 25, 2004, for both rice and soybean. Three days after germination the plants were thinned to four per pot. The diagnostic leaves were collected following methodology described by Malavolta *et al.* (1997). For rice, the leaf Y (immediately below the newest uncoiled leaf) was collected in the middle of the tilling period, on the 50th day after sowing. For soybean, the first mature leaf starting from the tip of the branch was collected, at the end of the flowering period (on the 69th day after sowing). The samples were stored in paper bags and dried. Thereafter, the plants were grown until maturation and cut close to the soil surface. The collected material was washed in running water, and then rinsed in distilled water. Afterwards, they were dried in forced-air oven (60 - 70ºC), weighed and crushed in a Wiley mill.

Digestion was carried out in an open system, using a slight modification of the method described by Oliva *et* *al.* (2003). The concentrations of Cd, Cu, Fe, Mn, Pb and Zn in the extracts were analyzed by an inductively coupled plasma optical emission spectrometer (ICP-OES) (Miyazawa *et al.*, 2009).

The data on the concentrations of Cd, Cu, Fe, Mn, Pb and Zn were analyzed and compared statistically by the Tukey test at 5% probability according to the methodology described by Pimentel-Gomes (1990).

RESULTS AND DISCUSSION

During cultivation, the crops had different reactions and development in response to high concentrations of heavy metals in the soil. Progressive symptoms of heavy metal toxicity appeared in the rice shoots, in young leaves. The leaves had dark green ribs in contrast to the rest of the yellow leaf. With toxicity severity, the leaves became totally chlorotic and became whitish with time, progressing to the drying of the leaf (Figure 1). In the Soil 1, with higher heavy metal concentrations (Table 1), especially Zn, rice plants died 10 days after germination (Figure 2).

The toxicity symptoms in soybean were characterized by generalized internerval chlorosis in young leaves with the appearance of dark brown spots, progressing to the drying of the leaves (Figure 3). A reduction in plant growth was also observed (Figure 4), but the symptoms of toxicity in soybean was less intense in comparison with rice, since they were slower to be expressed. The rice plants presented toxicity as early as the first 10 days at higher concentrations of metals in the soil. The soybean plants completed the cycle in all soils.

Soares *et al.* (2001) observed that excess Zn reduced Fe translocation to shoots. The presence of Zn inhibits the Fe metabolism promoting the appearance of symptoms of induced Fe deficiency. In extreme cases of deficiency, there was a decrease in the chlorophyll concentrations resulting in leaves totally chlorotic, which later become white, causing accentuated delay in plant growth. Fe has

Table 1. Chemical analysis of the soils used in the cultivation of soybean and rice

Characteristic	Unit	Soil samples						
		1	2	3	4	5	6	7
(1,2) pH $CaCl_2$		5.8	5.8	5.2	5.5	5.5	5.3	5.2
(1) Organic matter	g dm^{-3}	29.8	36.3	34.4	37.2	35.4	36.3	33.5
(3) Cadmium	mg kg^{-1}	25	23	20	23	26	28	27
(3) Copper	mg kg^{-1}	272	141	115	121	144	166	153
(3) Iron	mg kg^{-1}	537	936	861	510	99	100	97
(3) Manganese	mg kg^{-1}	540	563	536	583	664	719	570
(3) Lead	mg kg^{-1}	333	208	174	198	226	244	229
(3) Zinc	mg kg^{-1}	544	189	113	106	106	108	102
(4) Cadmium	mg dm^{-3}	0.61	0.20	0.11	0.01	0.01	0.10	0.01
(4) Copper	mg dm^{-3}	77	19	12	9	11	13	12
(4) Iron	mg dm^{-3}	10	10	13	10	11	11	17
(4) Manganese	mg dm^{-3}	38	64	77	59	67	84	51
(4) Lead	mg dm^{-3}	73	17	12	8	11	13	11
(4) Zinc	mg dm^{-3}	256	62	28	14	9	7	3

(1) Raij *et al.* (2001); (2) pH in calcium chloride 0,01 mol L^{-1}; (3) Total concentration in soil, according Nieuwenhuize *et al.* (1991); (4) Extraction by DTPA pH 7.3, according Abreu & Andrade (2001).

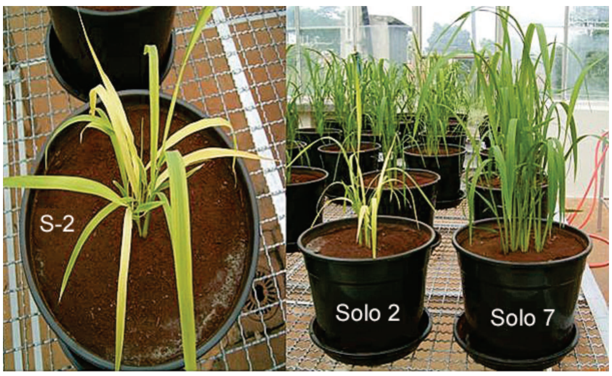

Figure 1. Characteristics of chlorotic leaves as a function of phytotoxicity by heavy metals in rice plants.

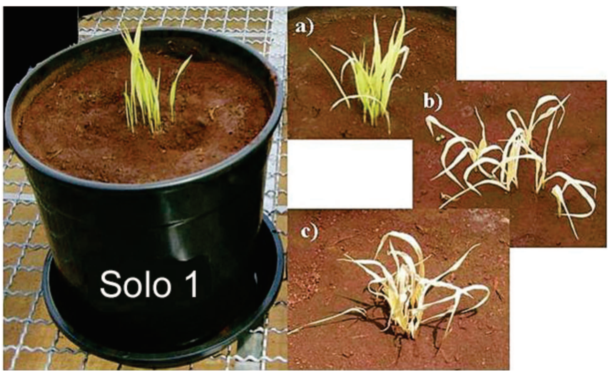

Figure 2. Phytotoxicity symptoms from a to c, evolving to death of the plants 10 days after planting.

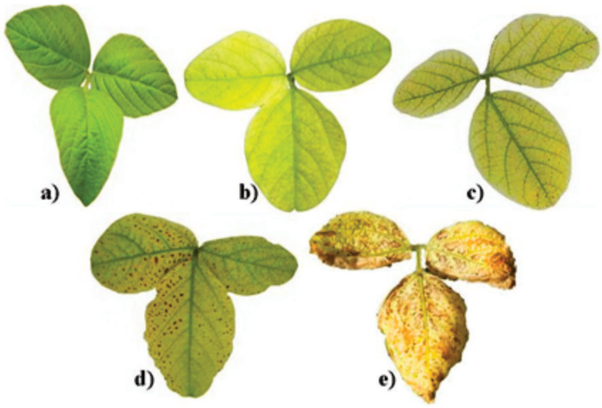

Figure 3. Evolution (25 days) of increased symptoms on soybean leaves (a, b, c, d and e).

Figure 4. Difference in growth between the plants in the most contaminated soil (Soil 1) and less contaminated (Soil 7), sixty days after the seeding.

numerous physiological functions in plants, but, for the expression of visual symptoms, its involvement in chloroplast formation and porphyrin synthesis are the most important processes (Romheld, 2001). One explanation for excess Zn causing Fe deficiency is that the ionic radii of zinc and iron happen to be nearly identical (Woolhouse, 1983; Romheld, 2001). In this work, the visual symptoms observed (Figure 1 and 3) demonstrated the interference of the Zn in the Fe metabolism, which is confirmed by the higher Zn concentration in relation to the Fe concentration (Table 2 and 3).

According to Mengel & Kirkby (2001), Alloway (2008) and Ai-Qing *et al*. (2011) high Cu, Mn and Zn concentrations can induce Fe deficiency in plants. In this study, it was observed that Zn, Cu and Cd, to a lesser extent, (Table 1) may have induced Fe loss in the plants. Cd competes with Fe for absorption sites in the plasma membrane (Siedlecka & Krupa, 1999; Tran & Popova, 2013). Excess Zn (Table 1) can cause the induced Fe loss. The presence of Zn inhibits the metabolism of Fe, causing the appearance of symptoms of induced Fe deficiency, which in severe cases is characterized by decreased chlorophyll levels, resulting in totally chlorotic leaves (Romheld, 2001; Izaguirre-Mayoral & Sinclair, 2005; Ghasemi-Fasaei & Ronaghi, 2008).

Cadmium is also responsible for absorption sites in the plasma membrane with Fe, causing Fe deficiency symptoms (Gallego *et al.*, 1996; Siedlecka & Krupa, 1999;

Table 2. Concentration of cadmium, copper, iron, manganese, lead and zinc in rice leaves

Soil	Cadmium	Copper	Iron	Manganese	Lead	Zinc
			mg kg^{-1}			
1	4.86a*	316.6a	840.9a	294.1c	322.5a	2562.1a
2	0.87b	26.7b	63.7d	397.8c	20.1b	543.1b
3	0.59c	22.2cd	82.5cd	388.9c	17.4bc	386.5c
4	0.25de	18.2e	104.6c	342.5c	13.8d	145.3d
5	0.36d	20.6de	93.2cd	800.5bc	15.6cd	119.4de
6	0.22e	20.9cde	92.6cd	1085.8b	15.6cd	108.4de
7	0.28de	24.2bc	273.7b	2540.7a	19.5b	94.2e

*Means followed by the same letters in the columns are not significantly different (Tukey test, $p \leq 0.05$).

Table 3. Concentrations of cadmium, copper, iron, manganese, lead and zinc in soybean leaves

Soil	Cadmium	Copper	Iron	Manganese	Lead	Zinc
			mg kg^{-1}			
1	1.50a*	12.1a	33.8cd	99.2cd	9.4a	599.6a
2	0.22c	9.5ab	56.7a	86.2cde	6.5b	152.4b
3	0.24c	8.9b	47.3ab	133.9b	5.4c	157.9b
4	0.42b	7.5b	45.1abc	70.8e	3.9d	68.2c
5	0.36b	7.1b	28.8d	73.7de	3.1d	55.2c
6	0.03d	8.5b	43.6abc	105.1c	3.5d	59.9c
7	0.32bc	8.2b	38.7bcd	327.7a	4.1d	81.8c

*Means followed by the same letters in the columns are not significantly different (Tukey test, $p \leq 0.05$).

Astolfi *et al.*, 2012). High Mn concentrations may reduce Fe absorption and induce deficiency. The Fe: Mn ratio must be maintained between 1.5 and 2.5 (Adriano, 1986), which did not occur in this case, where the ratio was below 0.7, except for the leaves of rice in soil 1. Increasing Zn rates in the soil (Table 1) produced an increase in the Mn concentration and Fe reduction in the leaves.

With this information, it is assumed that the observed symptoms are indeed induced deficiency of Fe, since the soil in question has available high concentrations of Cd, Mn and Zn.

The contents found in standard leaves of rice according to Malavolta *et al.* (1997) are between the ranges of 10-20, 200-300, 100-150 and 25-35 mg kg^{-1} for Cu, Fe, Mn and Zn, respectively. For soybean the standard leaves are between the ranges of 10-30, 51-350, 20-100 and 21-50 mg kg^{-1} for Cu, Fe, Mn and Zn, respectively (Malavolta *et al.*, 1997). As for Cd and Pb, the concentrations considered as normal in plants are between 0.1 and 2.4 (Bowen, 1979) and 2 and 3 mg kg^{-1} (Mengel & Kirkby, 2001), respectively.

The critical toxicity concentration in plant leaves can range from 100 to 400 mg kg^{-1} for Zn (Kabata-Pendias & Pendias, 2001) and above 600 mg kg^{-1} for Mn (Borkert *et al.*, 2001). In soybean leaves concentrations considered toxic are above 140 mg kg^{-1} for Zn (Borkert *et al.*, 1998) and 150 mg kg^{-1} for Mn (Borkert *et al.*, 2001). Without specifying the crop, the critical toxicity concentrations in leaves can range from 3 to 8 mg kg^{-1} for Cd (Kabata-Pendias & Pendias, 2001), up to 30 mg kg^{-1} for Cu (Malavolta *et al.*, 1997; Romheld & Marschner, 1991) and above 10 mg kg^{-1} for Pb (Sauerbeck & Tietz, 1983).

In relation to the ranges previously mentioned, the Cu concentration in rice was at toxic level in Soil 1, above 30 mg kg^{-1}. The concentration of Mn was above 150 mg kg^{-1} in all soils, but both rice and soybean presented Mn in this concentration only in Soil 7. The soils 1, 2, 3 and 4 for rice and 1, 2 and 3 for soybean showed Zn concentrations above 140 mg kg^{-1}, considered as toxic. Cd concentration was found only within the range 3-8 mg kg^{-1} in Soil 1 for rice. Pb concentrations were up to 10 m kg^{-1} in all soils with rice.

The differences in absorption of the elements may be due to soil characteristics, such as interaction with organic matter, interaction between the elements, chemical immobilization of elements, among other factors that were not in the scope of this study.

According to Malavolta *et al.* (1997) and Ai-Qing *et al*. (2011), in chlorotic leaves, the total Fe content may be higher or at least equal to that found in normal leaves. Data such as these suggest that in the deficient plants, part of Fe is inactivated. In this case, it is suggested to determine the “active Fe” and not the “total Fe”, which was not possible to do in this experiment, given the small amount of material produced. However, for the soybean leaves (Table 3), it is noted that, except in the treatment 2, all Fe concentrations are under the adequate concentration, but only treatment 1 presented visible signs of deficiency.

Nevertheless, the results found in rice leaves (Table 2) showed that the Fe concentration (841 mg kg^{-1}) was far above the maximum adequate concentration (300 mg kg^{-1}), which is because the leaf analysis determines the total of the elements.

In Soil 1 (Table 2), it was observed that for rice, the elements were evaluated in the range of toxic concentrations, except Mn, which was in agreement with the observed symptoms, since the plant died 10 days after germination (Figure 2). The concentrations of Mn in the Soils 5, 6 and 7 could have caused the appearance of toxicity symptoms, but this has not occurred in the plants.

With respect to Mn in Soils 5, 6 and 7 (800, 1086 and 2541 mg kg^{-1}, respectively), for rice, several factors may have inhibited the development of Mn toxicity symptoms, such as high concentrations of Fe, Cu and Zn (Pearson *et al.*, 2008) and the temperature. According to Gonzalez *et al*. (1998), temperatures above 30°C reduce the toxicity intensity and effect of manganese.

The high Zn concentration in the soil possibly interfered with the Cu absorption by soybean , since according to Alloway (1993) Zn^{+2} high activities in the soil solution have an antagonistic effect on Cu^{+2} absorption.

It was observed that the Mn content in Soil 1 was toxic. Phytotoxicity symptoms have been associated with Mn in the leaves above 600 mg kg^{-1} for rice and above 150 mg kg^{-1} for soybean (Borkert *et al.*, 2001). However, according to Rosolem *et al.* (2001), this is not a good indication of toxicity, because good soybean yields have been obtained with concentration above 200 mg kg^{-1}. This occurs because the symptom manifestation depends on other factors, as already mentioned for rice.

For soybean, Pb and Zn were above the concentrations considered adequate, however, only Zn in the treatments 1, 2 and 3 showed toxic concentrations. In the case of Fe, all soils had concentrations below adequate, indicating a possible deficiency of this element. The other elements, Cu and Mn, generally showed adequate concentrations (Table 3).

Zn was the most absorbed element, which most influenced the appearance of symptoms of toxicity in both Soils 1 and 2 and in both crops evaluated (Figure 1 to 4). Several authors found similar symptoms in guava plants (Salvador *et al.*, 1999) and tropical tree species (Soares *et al.*, 2001) under conditions of excess elements in the soil.

CONCLUSIONS

The soybean and rice crops were shown to be sensitive to high levels of heavy metals available in the soil, exhibiting phytotoxicity symptoms manifested principally as chlorosis and inhibited plant growth.

Cu, Mn, Pb and Zn were higher in rice than in soybean. Rice proved to be more sensitive to high heavy metal concentrations in soil, absorbing them more easily than soybean.

High concentration of Zn reduced the Fe concentrations in the soybean and rice shoots to levels considered deficient, interfering with the crop development.

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