

## ASSESSING PHYTOTOXICITY OF HEAVY METALS IN REMEDIATED SOIL

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*Copper (Cu), zinc (Zn) and chromium (Cr) are pollutants that usually are accumulated in soils. Their toxicity can be decreased by applying amendments. We proposed to evaluate changes in Cu, Zn, and Cr availability, due to the application of amendments, through chemical analysis and phytotoxicity tests. The phytotoxicity test was carried out using species belonging to Sesbania genus; plant parameters were measured 48, 72, 96, and 168 hours after the start of incubation. The treatments included enriched soil, in addition to biosolid compost and triple superphosphate. Cu and Zn amounts were higher in treatments without amendments, indicating immobilization on the part of these. The amounts of Cr tended to decrease with amendments application. The amendments increased pH values and decreased EC; however, this had no impact on the results. No relationship was found among pH, EC, and plant parameters. Different behaviors were observed. S. virgata showed germination seed delay. In addition, while in S. virgata the IG increased during the assay, in S. punicea it diminished. The application of compost, fertilizer or both combined could be of interest for contaminated soils remediation. The use of chemical analysis and phytotoxicity tests allowed to estimate heavy metal availability and the effect on both Sesbania species.*

**KEYWORDS** copper, zinc, chromium, *Sesbania*, toxicity, compost, fertilizer

### INTRODUCTION

Anthropogenic activities, primarily associated with industrial processes, mining, metallurgical and energy production, are the major source of soil contamination, being able to reach also aquatic environments (Bolan *et al.*, 2003). Many pollutants, such as heavy metals are introduced into soils, accumulating mainly in the upper layers of soils (Smith, 1996). Taken in excessive amounts, these metals ions may cause toxic effects on plants and animal organisms, including humans (Antoniadis and McKinley, 2003). One of the most important problems of heavy metals contamination is that these pollutants are non-degradable (Gleyzes *et al.*, 2001). Therefore, their toxicity could be minimized by reducing their availability through immobilization using organic and inorganic amendments (Adriano, 2001; Basta *et al.*, 2001).

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Organic amendments, like mature compost, contain a high proportion of humified organic matter (humins and humic and fulvic acids). They could adsorb heavy metals temporarily through chelates formation or by the formation of stable complexes sorbing them for a longer period (Castaldi and Melis, 2004; Basta *et al.*, 2005). Furthermore, organic amendment allows the recycling of nutrients and of organic matter present in them, and the improvement in soil physical properties (Sánchez-Monedero *et al.*, 2004). On the other hand, inorganic amendments such water-soluble phosphates, provide long-term remediation through direct metal adsorption by the phosphate and precipitation of metals with solution phosphate (Adriano *et al.*, 2004). In addition, inorganic amendments can also be used as a fertilizer to provide plant nutrients (Sharpley *et al.*, 1999).

Methods for estimating metal availability include the use of chemical tests (Ali *et al.*, 2004). However, these methods do not take into consideration complex interactions between metals, soil matrix, and biota (Leitgeb *et al.*, 2007). Environmental risk assessment studies of heavy metal pollution must take into account chemical data, biological and toxicological data as well (Juvonen, 2000; Gruiz, 2005). A phytotoxicity test can be used to estimate heavy metal availability in soil extracts through morphological plant parameters (Robidoux *et al.*, 2005) such as among the germination test proposed by Zucconi *et al.* (1981). During the initial germination stage, there are many processes in which the presence of heavy metals will have a direct impact on seed viability and normal development of plants (Sobrero and Ronco, 2004). Therefore, this stage is considered a critical phase in the life cycle of an individual (Veasey *et al.*, 1999). In general, bioassays are developed to evaluate the maturity of composts used as amendments (Warman, 1999; Zubillaga and Lavado, 2006), or to evaluate the effect generated by contaminants into the soil biota (Gruiz, 2005). However, they have not been used to estimate heavy metals immobilization in soils with amendments application.

The ecosystem of Argentinean Pampas is not disparate to the contamination processes, and it has suffered transformations by human intervention, altering its biodiversity. As a result, it is important to acknowledge the behavior that each species has into the region it belongs to (Ricklefs, 1997; Brown *et al.*, 2006). One way of contributing with the native phytogenetic resources conservation, is to identify the tolerance to heavy metals of different species (Godínez-Álvarez, 1999; Carpena and Bernal, 2007). Several species from the *Sesbania* genus have a high ability to tolerate heavy metals (Ye *et al.*, 2001; Yang *et al.*, 2003). Native species from the Argentinean Pampas region, like *Sesbania punicea* and *Sesbania virgata* (Cabrera, 1994), are pioneer species with rapid growth (Vilela de Resende *et al.*, 2000); however, the tolerant behavior is not documented. In addition, it is important to compare the performance of different species, as it is common to find differential sensibility amongst them (Sverdrup *et al.*, 2003).

The objective of this work was to evaluate bioavailability changes of Cu, Zn, and Cr in contaminated soils with organic and inorganic amendments application, through chemical analysis and a phytotoxicity test, using two native species, *S. punicea*, and *S. virgata*.

## MATERIALS AND METHODS

### Sampling Methodology and Samples Contamination

Soil samples were collected from the top 0.20 m of a Typical Hapludoll (clay 19.2 g Kg<sup>-1</sup>, silt 23.2 g Kg<sup>-1</sup>, sand 57.6 g Kg<sup>-1</sup>, Sandy-loam texture), proceeding from

Buenos Aires province (35°37'S, 61°22'O). Samples were taken randomly where soil conditions were homogeneous; they were air-dried, homogenized, and passed through a 2 mm sieve. In order to simulate contamination, the samples were enriched by adding metal solutions ( $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ ,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , and  $\text{H}_2\text{CrO}_4$ ), achieving a final concentration of copper (Cu):  $450 \text{ mg kg}^{-1}$ , zinc (Zn):  $630 \text{ mg kg}^{-1}$  and chromium (Cr):  $550 \text{ mg kg}^{-1}$ . Enrichment exceeded the limits of total heavy metals in agricultural soils, established by different standards (USEPA, 1995, UECEC, 1986). In order to achieve heavy metals/colloidal fraction equilibrium, samples were wetted (field capacity) and air-dried, every five days, for a two months period, according to a modification of the methodology proposed by Martínez and Motto (2000). Two soil amendments were used. Biosolid compost (BC) like organic amendment, and superphosphate triples (PF) as inorganic amendment, being the equivalent doses  $100 \text{ Mg ha}^{-1}$  and  $100 \text{ kg ha}^{-1}$  respectively. Both amendments were left to stabilize with soil for 100 days. The resulting treatments were: i) contaminated soil (CS), ii) CS + BC, iii) CS + PF and iv) CS + BC + PF.

### Phytotoxicity Test

The phytotoxicity test was carried out based in Zucconi *et al.* (1985). Extracts were prepared by mixing 7 g of each treatment with 10 ml of distilled water. It was shaken and remained at  $60^\circ\text{C}$  for 24 hours, and after was filtered. Twenty seeds of *S. punicea* and *S. virgata*, mechanically scarified, were placed randomly on 8 cm diameter Petri dishes lined with Whatman N° 3 filter paper, impregnated with 6 ml of the obtained extracts. The control treatment was distilled water. Assays were replicated three times. The Petri dishes were incubated at  $26^\circ\text{C}$  in the dark.

Seed germination and root length in each dish were measured. In order to consider germination's delays in some treatments, measurements were done after 48, 72, 96, and 168 incubation hours. The number of germinated seeds was counted, considering germination when the primary root reached a length equal or superior than 5 mm (USEPA, 1995). Germination percentage, (%G) = (number of seeds germinated in amendment extract)/(number of seeds germinated in control)  $\times 100$ ; %RL = (Mean root length in amendment extract/Mean root length in control)  $\times 100$  and GI (%) = (G  $\times$  RL)  $\times 100$ .

### Chemical Analysis

Chemical determinations in water extracts (pH and electrical conductivity), were assessed using standard methodologies (Faithfull, 2004). In soil, heavy metals availability was extracted from each treatment using  $\text{CaCl}_2$ . In extracts, total amounts of Cu, Zn, and Cr were analyzed. Heavy metal determinations were realized by atomic absorption spectrometry in Perkin Elmer AAnalyst 300 (Cooksey and Barnett, 1979).

### Data Analysis

Results were submitted to an ANOVA and the significant differences among means were established by LSD test ( $p < 0.05$ ); previously verified the assumption of variance homogeneity (variances were stabilized when necessary using a logarithmic transformation of data). Linear correlations between analyzed variables were performed.

**Table 1** Available heavy metals amount (mg metal kg<sup>-1</sup> soil) in contaminated soil

Treatments	Cu	Zn	Cr
CS	7.227	636.800	0.790
CS + BC	5.157	520.667	1.400
CS + PF	4.820	522.000	1.190
CS + BC + PF	4.857	471.000	0.817

CS = contaminated soil; CS + BC = contaminated soil + biosolid compost; CS + PF = contaminated soil + superphosphate triples; and CS + BC + PF = contaminated soil + biosolid compost + superphosphate triples treatments.

## RESULTS AND DISCUSSIONS

### Chemical Characteristics on the Substrates and Extracts

Availability amounts of heavy metals in different treatments are presented in Table 1. It was observed that the application of amendments decreased availability of each element. These amendments had heavy metals levels below those considered as toxic by international standards (USEPA, 1995).

Heavy metals amounts in distinct extract varied significantly amongst treatments (Table 2). Amendments application to contaminated soil diminished Cu and Zn amounts, indicating their immobilization. Regarding Cu, this decrease went between 21 and 30% and for Zn between 46.8 and 55.8%. This could be due to the stable complexes formation with humic substances of compost (Madrid, 1999; Gibert *et al.*, 2003), or the precipitates formation with phosphates of the inorganic fertilizer (Basta *et al.*, 2001). The Cr amount decreased between 28 and 37.5% in treatments with amendments in relation to contaminated soil without amendment; however, this was not statistically significant, due to the high variability of the measurements. It is important to highlight that organic and inorganic amendments applied together, presented the highest decreasing percentage of metals in the extracts.

Positive relationship was found between the Cu and Zn amount in the extracts and the availability amount of those metals in the respective treatments ( $R^2 = 0.97$ ,  $p = 0.013$ ;  $R^2 = 0.92$ , and  $p = 0.039$ , respectively), not being demonstrated the same thing by the Cr. Due to the fact that at the present there is no bibliography on the use of these tests in

**Table 2** Characteristics of extracts used in phytotoxicity test

Treatments	pH	EC (ds m <sup>-1</sup> )	Cu	Zn (mg kg <sup>-1</sup> )	Cr
Control	6.01	0.02	ND	ND	ND
CS	5.06 e	0.91 a	5.27 a	138.40 a	2.08 a
CS + BC	5.37 c	0.86 b	4.16 b	73.60 b	1.50 a
CS + PF	5.26 d	0.77 c	3.81 bc	62.80 b	1.34 a
CS + BC + PF	5.42 b	0.65 d	3.69 c	61.10 b	1.30 a

CS = contaminated soil; CS + BC = contaminated soil + biosolid compost; CS + PF = contaminated soil + superphosphate triples; and CS + BC + PF = contaminated soil + biosolid compost + superphosphate triples treatments.

contaminated soils, we can hypothesize that during the obtainment of extracts, no processes of adsorption and/or precipitation or solubilization occurred, which could have had an effect on the availability of heavy metals.

The application of biosolid compost and phosphate fertilizer, significantly increased pH in contaminated soil ( $p < 0.001$ ). This result was consistent with that obtained by other authors (Bolan and Duraisamy, 2003; Castaldi *et al.*, 2005) (Table 2). On the other hand, amendment applications significantly decreased EC in relation to soil extract without amendment (Table 2). Nevertheless, no significant linear relation was found between the pH and the EC, with the plant parameters measured. Consequently, these pH and EC ranges did not influence the results obtained from the germination test.

### Phytotoxicity Test

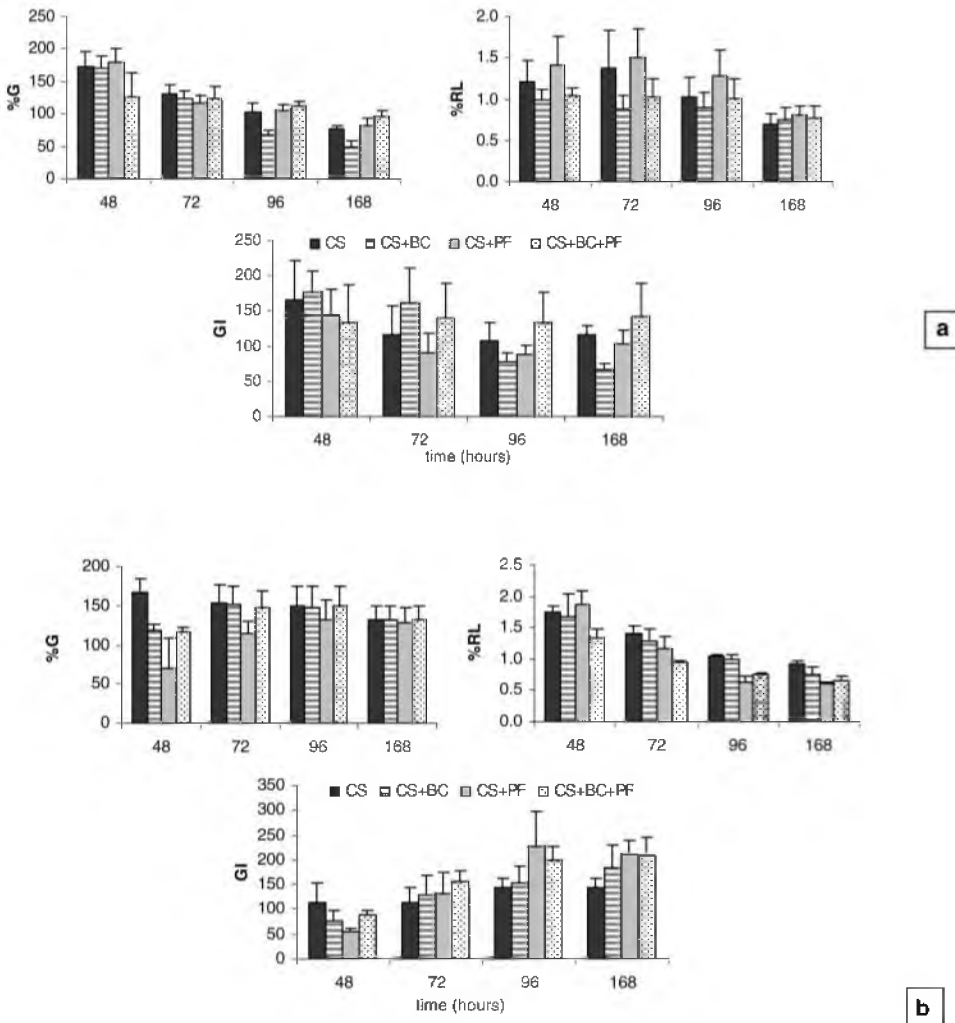
The results of the phytotoxicity test are shown in Figure 1 (a,b). In general, the germination percentage of *S. punicea* and *S. virgata* exceeded 100%. This allowed to establish that the heavy metals levels of the treatments did not negatively affect this early stage of the physiological plant development.

Different tendencies were observed between the behavior of *S. punicea* and *S. virgata*. At the beginning of the assay, the germination percentage and the germination index were superior in *S. punicea* in comparison to *S. virgata* (73 and 45.86%, respectively). These different responses from plants to amounts of heavy metals could be attributed to genetic or physiological factors, as well as to toxicological routes and to the destination of these toxins in plants (Calow, 1993). *Sesbania punicea* showed at 48 hours a generalized tendency of a higher germination percentage in all treatments in comparison to *S. virgata*. The highest percentage of germination with amendment incorporation was obtained at 72 hours. These results would be indicating a delay in seed germination with amendments application. The analyzed chemical characteristics did not allow to explain this observed delay.

Root length percentage in both species did not show significant differences. *S. virgata* showed a tendency of decrease root length with the incorporation of amendments at 72 hours. As in others studies (Wang, 1991; Zubillaga and Lavado, 2006), it was observed that root elongation was the most sensitive phytotoxicity parameter, being the variation 10% more than the germination percentage.

Both species of *Sesbania* presented a germination index superior to the 50%, indicating tolerance to toxic compounds present in the extracts (Zucconi *et al.*, 1981b). Similar to other species of *Sesbania*, these results show that *S. punicea* and *S. virgata* could be used as an appropriate pioneer species in short-term remediation projects (Chan *et al.*, 2003). The germination index in *S. punicea* did not increase with time, being considered 48 hours as the best moment for its calculation, as well as to determinate germination percentage and root length percentage. On the other hand, in *S. virgata* the germination index increased with time. There was an apparent increase in the germination index with the application of phosphorus amendments at 96 hours (Figure 1b). This increase was 38.5 at 57% compared to treatments without phosphorus amendments.

In conclusion, organic (compost) and inorganic amendments (phosphate) application decreased heavy metals availability in soils, as well as their amount in extract used on phytotoxicity test. The decrease was stronger when both amendments were used. As a result, a combination of amendments could be significant for contaminated soil remediation.



**Figure 1** Germination percentage (%G), root length percentage (%RL), and Germination Index (GI) of both *Sesbania* species. (a) *Sesbania punicea* and (b) *Sesbania virgata*. Vertical bars indicate standard error. CS, Contaminated Soil; CS + BC, Contaminated soil + Biosolid compost; CS + PF, Contaminated Soil + Superphosphate triples and CS + BC + PF, Contaminated Soil + Biosolid Compost + Superphosphate triples.

We propose testing the combined use of organic and inorganic amendments under field conditions in order to obtain a more realistic recommendation.

Compared to *S. punicea*, *S. virgata* showed a germination delay in treatments with amendments application. However, both species of *Sesbania* were able to germinate and apparently tolerate heavy metals amounts in treatments used in the present study. The short life span of *Sesbania virgata* and *Sesbania punicea* makes them ideal for quick (short-term) remediation purposes. This would allow a first indication that these species behave like others in the genus, in relation to heavy metals.

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