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MICROBIAL ACTIVITY, ARBUSCULAR MYCORRHIZAL FUNGI AND INOCULATION OF WOODY PLANTS IN LEAD CONTAMINATED SOIL

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ABSTRACT

The goals of this study were to evaluate the microbial activity, arbuscular mycorrhizal fungi and inoculation of woody plants (*Caesalpinia ferrea*, *Mimosa tenuiflora* and *Erythrina velutina*) in lead contaminated soil from the semi-arid region of northeastern of Brazil (Belo Jardim, Pernambuco). Dilutions were prepared by adding lead contaminated soil (270 mg Kg^{-1}) to uncontaminated soil (37 mg Pb Kg $soil^{-1}$) in the proportions of 7.5%, 15%, and 30% (v:v). The increase of lead contamination in the soil negatively influenced the amount of carbon in the microbial biomass of the samples from both the dry and rainy seasons and the metabolic quotient only differed between the collection seasons in the 30% contaminated soil. The average value of the acid phosphatase activity in the dry season was 2.3 times higher than observed during the rainy season. There was no significant difference in the number of glomerospores observed between soils and periods studied. The most probable number of infective propagules was reduced for both seasons due to the excess lead in soil. The mycorrhizal colonization rate was reduced for the three plant species assayed. The inoculation with arbuscular mycorrhizal fungi benefited the growth of *Erythrina velutina* in lead contaminated soil.

Key words: AMF, contaminated soil, heavy metals, mycorrhizae, semi-arid.

INTRODUCTION

Over the last few decades industrial activities have increased the level of pollution in the biosphere, including soils, with a negative impact on the environment (31). Among these pollutants, heavy metals are considered the most relevant causes of soil contamination (11).

The effect of soil contamination by metals is reflected in the nutrient cycle, which can be determined by estimating the level of microbial biomass, CO_2 emission, enzymatic activity and organic matter decomposition (2,9). A plant's response to contamination by metals varies depending to the contamination level, soil type, and the diversity of microorganisms and plants that influence the soil processes (6).

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Among the microorganisms common to many soils, arbuscular mycorrhizal fungi (AMF), which are obligate symbionts, can increase the survival rate of certain plants by helping them take up nutrients and water and promoting healthy roots (30). They can also potentially help in the recovery of heavy metals from polluted environments (18).

Even though the toxicity caused by metals can be attenuated by AMF (19,25), healthy development of plants in contaminated soil depends on other factors, such as the intensity of the contamination, bioavailability of a particular metal to the roots, and the plants ability to absorb and accumulate this metal in their leaves (7).

Considering the importance of AMF and the need to establish strategies to rehabilitate contaminated areas, the goals of this study were to evaluate the microbial activity, arbuscular mycorrhizal fungi and inoculation of woody plants in lead-contaminated soil found in the semi-arid region of northeastern Brazil.

MATERIALS AND METHODS

The soil samples were collected (0–20 cm deep) in the dry season and rainy season at two sites in the municipality of Belo Jardim, Pernambuco (08°20’08” S, 36°25’27” W). One site contained lead-contaminated soil (270 mg Kg^{-1}), derived from solid waste from a mechanical metal industry, and the other site had no contamination (37 mg Pb Kg^{-1}). From each site, eight subsamples were collected to make two samples, one called non-contaminated soil (NCS) and the other called contaminated soil (CS). Dilutions of the soil samples were prepared by adding the contaminated soil to the natural soil to obtain the following contamination proportions: 0%, used as control, 7.5%, 15%, and 30% (v:v).

The experiments were conducted in the greenhouse of the Departamento de Micologia at the Universidade Federal de Pernambuco. Physicochemical analyses of the samples were made in the Laboratório de Biotecnologia Ambiental at the Instituto de Tecnologia de Pernambuco (ITEP) and the Instituto Agronômico de Pernambuco (IPA). The soil (a sandy clay loam), from both collection periods and at each contamination proportion, was chemically analyzed. The method of acid leaching was in accordance with the standards issued by the Associação Brasileira de Normas Técnicas (3). This method was used to analyze the lead in the soil with a coupled plasma-atomic emission spectrometer.

Microbial activity in the soil

The microbial activity was evaluated by the following analyses: (a) carbon of the microbial biomass, by the fumigation-extraction method (15,28); (b) basal respiration (13); (c) metabolic quotient (qCO_2); and (d) acid phosphatase activity (26). These analyses were replicated five times for each soil treatment (0%, 7.5%, 15%, and 30%), and samples collected during the dry and rainy seasons.

Most probable number (MPN) of infective propagules and arbuscular mycorrhizal fungi (AMF) spores in the soil

Samples of NCS and CS, collected in the dry and rainy seasons, were used to estimate the MPN of infective propagules and spores of AMF (glomerospores). Maize (*Zea mays* L.) seedlings were used as hosts and autoclaved sand was used to dilute the original inocula (8). The presence of typical AMF forming structures was investigated after 30 days in roots cleared and stained with Trypan blue (24). Glomerospores were extracted from NCS and CS samples using the wet sieving method (10) and then centrifuged in water and saccharose (14), with three replicates per treatment.

AMF inoculation of wood plants in contaminated soil

Seeds from the following plants were used in this study: *Caesalpinia ferrea* C. Martius and *Mimosa tenuiflora* (Wild.) Poir., obtained from IPA; and *Erythrina velutina* Wild., obtained from the Empresa Brasileira de Pesquisa Agropecuária - Embrapa Semi-Árido. The seeds were disinfected with sodium hypochloride (0.05%) and seeded in trays with NCS soil. After growth of the first true leaves, the plantlets were transferred to plastic bags containing 2 kg of substrate each.

Plantlets of *M. tenuiflora*, *C. ferrea* and *E. velutina* were inoculated at the root region with 100 glomerospores extracted from NCS and from the 15% contaminated soil. After 90 days the plantlets were evaluated by measuring the height, stem diameter and dry weight of the aerial parts. Mycorrhizal colonization was observed on roots stained with Trypan Blue in lactoglycerol (24) and quantified by the grid-line intersect method (12). The experimental design was randomly outlined in 3 × 2 × 2 factorial, with 3 plant species, 2 inoculation treatments (with and without AMF) and 2 substrates (NCS and 15% contaminated soil), in five replicates.

The data were analyzed using analysis of variance and the means were compared by Tukey's test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Starting at 7.5% contamination, the lead levels were above the acceptable level (300 mg kg^{-1} and 50 mg kg^{-1}) for alkali and acidic soils, respectively (5). With a concentration above 58.6 mg kg Pb^{-1}, the soil's pH (±4.9) might have helped make the metals available and to reduce the carbon and nitrogen levels of the microbial biomass (16). In general, the nutrients (P, Ca, Mg and Al) were present in higher concentrations in the rainy seasons, regardless of the level of contamination, which resulted in increased microbial activity during this period (Table 1).

Table 1. Chemical characteristics of the substrate with increasing proportions of lead contaminated soil (CS) for both collection seasons: dry (D) and rainy (R).

CS (%)	Season	Pb ($mg.kg^{-1}$)	pH (H_2O)	O.M.[1] (%)	P ($mg.dm^{-3}$)	Ca ($cmol_c.dm^{-3}$)	Mg ($cmol_c.dm^{-3}$)	Al ($cmol_c.dm^{-3}$)
0	D	8.6	5.34	1.34	3.0	1.90	0.90	0.10
	R	37.0	5.07	2.73	6.0	1.70	1.85	0.15
7.5	D	58.6	4.92	1.27	2.0	2.00	1.25	0.15
	R	80.3	4.88	2.33	5.0	2.70	2.80	0.25
15	D	97.2	4.85	1.26	2.0	2.00	1.60	0.15
	R	154.5	4.82	2.48	6.0	3.25	4.05	0.30
30	D	93.8	4.76	1.02	2.0	1.85	2.85	0.20
	R	164.7	4.70	2.51	11.0	5.00	5.65	0.45

[1]O.M. = organic matter.

The increase of lead contamination in the soil negatively influenced the amount of carbon of the microbial biomass in the samples from both the dry and rainy seasons (Figure 1). It has been found that the microbial biomass activity of soil is negatively related to heavy metal concentration and positively related to the amount of organic matter (29). This reduction suggests that excess lead in soil is harmful to microorganisms, regardless of the amount of water in the soil. Physical limitations, such as lack of water, low aeration and porosity of soils, influence the biological communities that live in them, and when there is low water potential, most soil microorganisms become inactive. On the other hand, under the same stressful conditions, certain microorganisms can increase their sporulation rate in an attempt to increase the chances of survival during a dry period (22). In fact, during the rainy season, the amount of carbon of the microbial biomass was significantly higher (232 µg C g $soil^{-1}$) compared to the samples from the dry season (70 µg C g $soil^{-1}$), which could be due to the higher activity of the microorganisms and consequently the higher accumulation of organic matter in the substrates (Table 1).

The basal respiration was significantly inhibited by the increase of the soil contaminant in the samples collected during the rainy season, and was different when compared to the natural soil samples (Figure 2). Liao *et al.* (18) found that increasing heavy metal concentrations of Cu and Cd reduced basal respiration, an activity that is related to the increase of organic matter, which is responsible for complexation of available metals.

The metabolic quotient (qCO_2) estimated in the soil only differed between the collection seasons in the 30% contaminated soil. During the dry season, the highest qCO_2 rates were recorded with increasing proportions of lead-contaminated soil (Figure 3), which indicates that the ecosystem was under stress. These results show that microbial populations invest most of their energy for maintenance, a typical condition of disturbed environments (21).

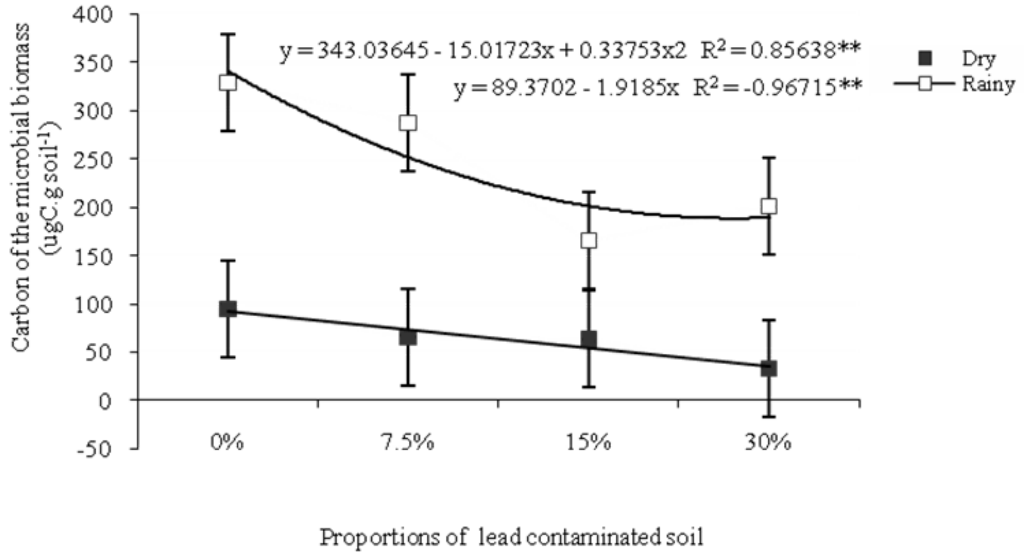

Figure 1. Carbon of the microbial biomass in non-contaminated soil (0%) and with increasing proportions of lead-contaminated soil (7.5%, 15% and 30%), from the dry and rainy seasons.

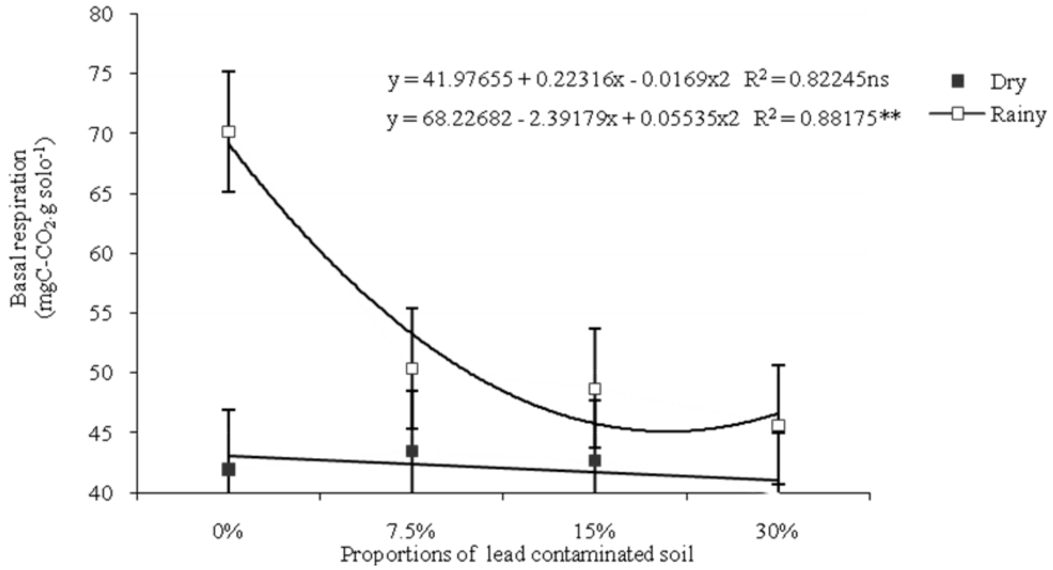

Figure 2. Basal respiration in non-contaminated soil (0%) and with increasing proportions of lead-contaminated soil (7.5%, 15% and 30%), from the dry and rainy seasons.

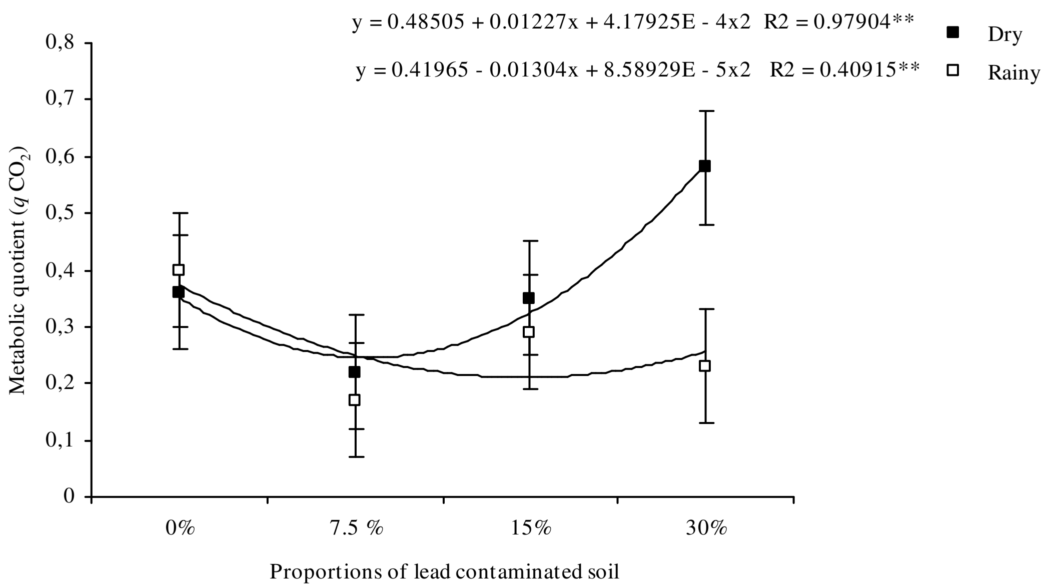

Figure 3. Metabolic quotient in non-contaminated soil (0%) and with increasing proportions of lead-contaminated soil (7.5%, 15% and 30%), from the dry and rainy seasons.

In general, the average value of the acid phosphatase activity in the dry season (3.14 µg *p*-np g soil^{-1}) was 2.3 times higher than observed during the rainy season (1.33 µg *p*-np g soil^{-1}) (Figure 4a). The NCS (2 µg *p*-np g soil^{-1}) differed only from the 7.5% contamination sample (2.4 µg *p*-np g soil^{-1}), though there was a tendency towards reduced activity when the proportion of contamination in the soil was increased (15% and 30%) (Figure 4b). These results suggest that if there is up to 80.3 mg kg^{-1} of lead in the soil (7.5%), the activity of acid phosphatase can be induced. However, above this level the activity was reduced, indicating the deleterious effect of lead on the soil microbiota. The activity of acid phosphatase was positively related to the pH and negatively related to the phosphorous available in the soil. The enzymatic activity is directly related to the physical and chemical properties of the soil, especially to the phosphatase activity, which plays an important role in the mineralization of phosphorous, related in this case to the total amounts of P found in the soil (11).

The number of glomerospores did not differ significantly between NCS and CS in the rainy and dry seasons. However, in the CS the MPN of infective propagules was drastically reduced in both the rainy and dry seasons due to the excess lead (270 mg Kg^{-1}) (Table 2). These data corroborate studies showing that the number of glomerospores, the spore germination of AMF and mycorrhizal infectivity are directly affected by the concentration and kind of metals in the soil (18). In copper-contaminated soil (484 mg dm^{-3}), the spore production of AMF was negatively influenced by higher contamination levels (75% and 100%). However, a lower contamination level (25%) favored sporulation (20). Trap cultures are being kept in a greenhouse in an attempt to recover as many glomerospores and identify species of AMF in the region (data not shown). Studies in the Amazon region have demonstrated that the spore production of AMF was more abundant in trap cultures from land uses under interference than in the pristine forest ecosystem (17).

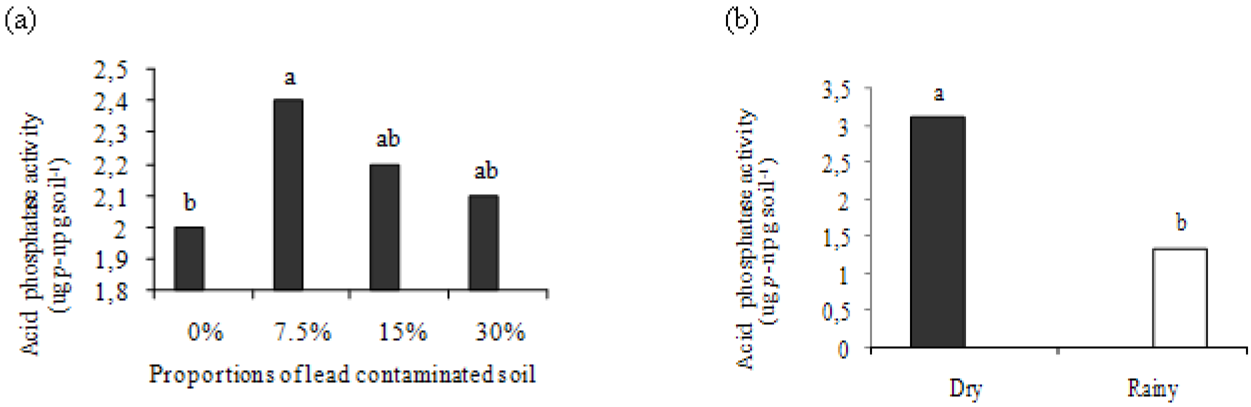

Figure 4. Acid phosphatase activity in (a) non-contaminated soil (0%) and with increasing proportions of lead-contaminated soil (7.5%, 15% and 30%) and (b) from the dry and rainy seasons. Means followed by the same letter do not differ significantly by the Tukey test at 5% probability.

Table 2. Most probable number (MPN) of infective propagules and arbuscular mycorrhizal fungi (AMF) spores (glomerospores) in non-contaminated soil (NCS) and in lead-contaminated soil (CS), from the dry (D) and rainy (R) seasons.

	NCS		**CS**	
	R	**D**	**R**	**D**
Glomerospores	144a	132a	136a	124a
MPN	350	140	40	12

Means followed by the same letter in the row do not differ significantly by the Tukey test at 5% probability.

The colonization rate of AMF was below 10% for the three plant species assayed in the NCS and 15% contaminated soil. *E. veluntina* had the lowest colonization rate (1.5%) in both soils and the rate declined in the roots of both *M. tenuiflora* (6.5% to 2.7%) and *C. ferrea* (8.7 % to 6.0%) when the proportion of contaminated soil was increased. However, this reduction was not significant. This shows that in general a high concentration of metals interferes in mycorrhizal association. The Cd toxicity to *Trema micrantha* (L.) Blum was evaluated by Soares *et al.* (25). They found that the Cd inhibited the colonization of AMF even at the lowest tested concentration (5 µmol L^{-1}). Low levels of root colonization by AMF can be a result of lead contamination of the soil. The metal can be toxic to fungi, reducing glomerospore germination, growth of mycelium and mycorrhizal colonization (23).

The growth of *M. tenuiflora* and *E. velutina* was not influenced by the addition of lead in the substrate, while *C. ferrea* appeared to be sensitive height to 15% contamination (Table 3). This can be explained by the excess lead in the soil solution, indicating an inhibitory effect. In spite of the low colonization rates for the three plant species, only *C. ferrea* and *E. velutina* responded to the inoculation in some of the growth parameters. In *E. velutina*, the stem diameter of the inoculated plants differed from the non-inoculated plants for both soil treatments (0% and 15% contamination). In addition, the amount of dry matter was higher in the colonized plants, especially in the 15% contamination treatment. Plantlets of *C.*

ferrea also showed a significant difference in height when grown in non-contaminated soil.

In a study by Trannin *et al.* (27), the cultivation of three woody plant species (*Acacia mangium*, *Enterolobium contortisiliquum* and *Sesbania virgata*) in heavy metal contaminated soil had a negative effect on the growth, dry weight of the aerial parts, height, and stem diameter of these plants. These authors observed a phytotoxic effect of the metals on the three species for soils with 15% contamination. Plantlets of *Leucaena leucocephala* (Lam.) de Wit cultivated in three soils in the caatinga (which was preserved, without the superficial layer, and contaminated with copper effluent from mining) showed lower growth in the copper contaminated treatment, due to the higher absorption of this metal by the roots (20). In another study using soybean plants, the addition of Pb to the soil reduced the growth of the plants with mycorrhizae, directly influencing the production of dry matter (1).

Recent studies in contaminated environments have shown there is a strong reduction in growth in colonized and non-colonized plants as the contamination level increases (1,20). It has also been found that AMF vary from tolerant to very sensitive when they are growing in soil with heavy metals (4) and that plant development depends on the contamination level and the type of heavy metal present. The results of this work confirm these observations and show that in lead-contaminated soil only *E. velutina* responded to inoculation.

Table 3. Growth parameters of *Mimosa tenuiflora*, *Erythrina velutina* and *Caesalpinia ferrea* inoculated (I) or not inoculated (NI) with arbuscular mycorrhizal fungi, cultivated in non-contaminated (0%) and in 15% contaminated soil, after 90 days in the greenhouse.

Plant	Treatment	Height (cm)		Stem diameter (cm)		DWAP (g)	
		0%	15%	0%	15%	0%	15%
M. tenuiflora	I	3.77 aA	4.72 aA	0.07 aA	0.08 aA	0.02 aA	0.02 aA
	NI	5.22 aA	3.95 aA	0.07 aA	0.06 aA	0.02 aA	0.02 aA
E. velutina	I	13.82 aA	14.17 aA	0.57 aA	0.53 aA	0.95 aA	0.97 aA
	NI	12.50 aA	7.77 aA	0.27 aB	0.32 aB	0.54 aA	0.36 aB
C. ferrea	I	20.55 aA	15.37 bA	0.27 aA	0.20 aA	0.76 aA	0.39 aA
	NI	10.42 aB	10.10 aA	0.19 aA	0.24 aA	0.23 aA	0.19 aA

DWAP - Dry weight of aerial parts; data are the mean of 5 replicates. Means followed by the same small letter in the rows and same capital letter in the columns do not differ significantly by the Tukey test at 5% probability.

CONCLUSIONS

The microbial activity in the semi-arid region of northeastern Brazil (Belo Jardim, Pernambuco) is negatively affected by lead contamination in the soil.

The activity of acid phosphatase in the soil from the semi-arid region can be induced when there is up to 80.3 mg kg^{-1} of lead in the soil.

Mycorrhizal infectivity in soil from the semi-arid region is directly affected by the concentration of lead in the soil.

Inoculation with arbuscular mycorrhizal fungi benefits the growth of *Erythrina velutina* in soil with moderate lead contamination levels (up to 80.3 mg Pb Kg $soil^{-1}$).

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