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Solubility of Heavy Metals/Metalloid on Multi-Metal Contaminated Soil Samples from a Gold Ore Processing Area: Effects of Humic Substances

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ABSTRACT: Bioavailability of heavy metals at contaminated sites is largely controlled by the physicochemical properties of the environmental media such as dissolved organic matter, hydroxides and clay colloids, pH, soil cation exchange capacity and oxidation-reduction potential. The aim of this study was to investigate soil pH and heavy metal solubility effect by levels of humic and fulvic acids applied in soil samples with different levels of contamination by heavy metals. The soil samples used in this study were collected in a known metal-contaminated site. Humic acid (HA) and fulvic acid (FA) were purchased as a commercially available liquid material extracted from Leonardite. The experiment was carried out in a factorial scheme of 4 × (4 + 1), with four contaminated soil samples and four treatments, comprised of two levels of HA, two levels of FA and a control. The HA treatments increased the solubility of Cu, Zn, Ni, Cr, Cd, Pb, As and Ba from soils, while FA treatments decreased, thus raising or not their availability and mobility in soil. Humic acid concentration did not influence soil pH and FA decreased soil pH until 0.7 units. The initial heavy metal concentration in soil affects the magnitude of the processes involving humic substances. The lower releases of heavy metals by FA verified the importance of the complexation properties of organic compounds. These results appear to encourage the use of HA for increased plant-availability of heavy metals in remediation projects and the use of FA for decreased plant-availability of heavy metals at contaminated sites with a risk of introducing metals into the food chain.

Keywords: soil pH, bioavailable metal, humic acid, fulvic acids, metal leaching.

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INTRODUCTION

Heavy metals are particularly problematic because, unlike most organic contaminants, they are non-biodegradable and can accumulate in living tissues, posing a great threat to both human health and for ecological environment (Lesmana et al., 2009). The most common heavy metals are Hg, Cd, Pb, Cr, As, Zn, Cu, Ni, Co, etc. These metal ions can cause toxicity and have serious side effects on human health.

Metals present in soils can be associated with several reactive components such as clay minerals, insoluble soil organic matter, oxides, or be taken up by plants and soil organisms mainly through the soil solution. The bioavailability of heavy metals at contaminated sites is largely controlled by the physicochemical properties of the environmental media such as dissolved organic matter, hydroxides and clay colloids, pH, soil cation exchange capacity and oxidation-reduction potential (Sparks, 2003; Violante et al., 2010; An et al., 2015). In addition, humic substances influence metal transport and control metal uptake into plants through carboxyl, phenolic and alcoholic hydroxyl, methoxyl, carbonyl and quinone groups present in HA acid composition. However, the functional groups identified in humic acids (HA) are also identified in fulvic acids (FA), but FA contain considerably more groups of an acidic nature, particularly -COOH (Jackson et al., 1978; Pandey et al., 2000).

Humic substances are naturally ubiquitous and have a high capacity to form complexes with heavy metals by means of the functional groups, being an obvious cleaning agent. However, although humic substances have been investigated as a washing agent for soils contaminated by polyaromatic hydrocarbons and other organic pollutants (Borggaard et al., 2009; Soleimani et al., 2010) and testing on heavy metal polluted soils is limited, in recent years some studies have been performed (Fonseca et al., 2013; Tang et al., 2014; An et al., 2015; Hwang et al., 2015).

We hypothesized that soil samples with different levels of contamination by heavy metals could have metal solubility reduced by the presence of humic substances. Second, the solubility of heavy metals could be changed by levels of humic substances incorporated and the levels of contamination in the soil samples. Finally, humic and fulvic acids could immobilize or solubilize heavy metals because these have high electronegative charges which means a higher affinity for heavy metals, reducing or not the available heavy metals in soil solution.

The aim of this study was to investigate soil pH and heavy metal solubility effect by levels of humic and fulvic acids applied in soil samples with different heavy metal contamination levels.

MATERIALS AND METHODS

Sample preparation and analysis

The soil samples were collected at a known metal-contaminated site described previously in Boechat et al. (2016). The soil of the studied field was classified as Entisol Orthent (Soil Survey Staff, 2010), a Neossolo Litólico (Santos et al., 2013). Soil samples were collected at previously determined points in order to form a systematic distribution in “square mesh” to characterize initial heavy metal distribution at the site. Soil sample collections are based on four points according to heavy metal concentrations at a depth of 0.00-0.20 m depth. Soil samples were air dried, crushed and sieved (2 mm screen) for chemical and physical characterization by methods described in Tedesco et al. (1995). The soil samples were digested using the hot-block digestion procedure according to EPA 3050 (USEPA, 1996) for total metal concentration, and were analyzed by an optically-induced plasma spectrophotometer (Perkin Elmer Optima 7000 DV, Perkin – Elmer Corporation, Norwalk, CT). Selected characteristics of the soil samples collected for this study are shown in table 1.

Humic acid (HA) and fulvic acid (FA) were purchased as a commercially available liquid material, being a humic acid product (HAP) with 16.02 % of humic acid and a fulvic acid product (FAP) with 36.04 % of fulvic acid (JDMV, Brasil). Humic and fulvic acids were extracted from Leonardite. Leonardite is a low-rank coal with significant amounts of humic materials, mainly HA. The chemical characterization of FAP and HAP was according to Rosa et al. (2000) (Table 2).

Soluble-exchangeable heavy metals

The experiment was carried out in 50 mL autoclaved conical tubes in the laboratory using a 4 × (4 + 1) factorial scheme with four contaminated soil samples, four organic acids and a control: two levels of humic acid: 0.04 µL g^{-1} (H1), 0.08 µL g^{-1} (H2), two levels of fulvic acid: 0.04 µL g^{-1} (F1), 0.08 µL g^{-1} (F2) and a control treatment with ultrapure water (0.04 µL g^{-1}).

In the first-step, 10 g of each autoclaved soil sample were transferred to autoclaved conical tubes. Soil samples were autoclaved for 40 min at a temperature of 121 °C under a pressure of 1 atm, in order to eliminate all microorganisms. Soil samples were irrigated with ultrapure water at 80 % of the field capacity, with estimated value approximately of 0.35 kg kg^{-1}. Treatments were added in each tube and then all conical tubes were incubated at 30 °C in a dark-room for 21 days.

In the second-step, soil pH was recorded prior to and after the experiments in water at a 1:2.5 soil/water ratio according to Tedesco et al. (1995) and heavy metal extraction was carried out in triplicate, using 1 g of air-dried soils. Soil samples were placed in 50 mL conical centrifuge tubes and 15 mL of $CaCl_2$ 0.1 mol L^{-1} was added into tubes. All tubes were agitated in a rotary shaker (120 rpm) at room temperature for 2 h. Following equilibration, the solution and solid phases were separated by centrifugation at 1,225 × g for 10 min.

Table 1. Chemical and physical characterization of soil samples (S1, S2, S3 and S4) from a multi-metal contaminated site

Property	S1	S2	S3	S4
P (mg dm^{-3})	30.0	7.9	7.0	3.4
K^+ ($cmol_c$ dm^{-3})	0.2	0.3	0.3	0.2
Ca+Mg ($cmol_c$ dm^{-3})	7.7	5.0	9.3	4.3
H+Al ($cmol_c$ dm^{-3})	2.8	2.2	1.0	0.7
SB ($cmol_c$ dm^{-3})	7.9	5.3	9.6	4.5
CEC ($cmol_c$ dm^{-3})	10.7	7.5	12.9	10.7
V (%)	74	71	73	42
SOM (%)	2.7	1.4	3.4	2.3
Zn (mg kg^{-1})	167.0	42.0	170.0	9.4
Cu (mg kg^{-1})	61.0	4.7	9.5	0.8
Mn (mg kg^{-1})	13.0	4.0	30.0	37.0
Cd (mg kg^{-1})	2.0	0.4	2.2	<0.2
Ni (mg kg^{-1})	8.0	5.0	9.0	3.0
Cr (mg kg^{-1})	12.0	9.0	15.0	5.0
Pb (mg kg^{-1})	599.0	46.0	227.0	72.0
Ba (mg kg^{-1})	109.0	84.0	164.0	149.0
As (mg kg^{-1})	45.0	<0.2	7.0	<0.2
Clay (g kg^{-1})	140	120	150	150

Clay was determined by the hydrometer method, using NaOH 0.1 mol L^{-1}; exchangeable Ca and Mg: extracted with 1 mol L^{-1} KCl; P and K: estimated by Mehlich-1; H+Al: extracted with calcium acetate solution 0.5 mol L^{-1} pH 7.0; organic carbon was determined by the Walkley-Black combustion method (Tedesco et al., 1995). For total metal concentration: EPA 3050 (USEPA, 1996). SB: sum of bases; CEC: cation-exchange capacity; V: bases saturation; SOM: soil organic matter.

The supernatants were filtered through a 0.45 µm membrane (Silveira et al., 2006; He et al., 2013), and then analyzed for Cu, Zn, Ni, Cr, Cd, Pb, As and Ba by inductively coupled plasma-optical emission spectrometer (ICP-OES, Perkin Elmer Optima 7000 DV, Perkin – Elmer Corporation, Norwalk, CT).

Experimental setup and statistical analyses

The experiment design used was a factorial scheme with a completely randomized design, with three replicates. The data obtained from the experiment were submitted to an analysis of variance and means to Tukey's test at 1 %. The Sisvar statistical program package (Ferreira, 2011) was used for statistical analyses of data.

RESULTS AND DISCUSSION

Soil attribute

The use of the HA did not alter the initial soil pH at either level. However, in the treatments with FA, the soil pH decreased to 0.7 units, so this substance had an acidifying effect on the soil (Table 3). Humic substances can be differentiated based on their solubility behavior into three fractions: HA, which is soluble in dilute alkali but precipitates in acid solution; FA, which is soluble in both alkali and acid solutions; and humin, which is insoluble in both alkali and acid solutions. Humic acid and FA are the two major components of

Table 2. Chemical characterization of the humic and fulvic acid products applied on soil samples from a multi-metal contaminated site

Chemical characteristic(1)	Humic acid product	Fulvic acid product
pH(H_2O)	6.6	6.6
Humic acid (g kg^{-1})	-	160.15
Fulvic acid (g kg^{-1})	360.40	-
Organic matter (g kg^{-1})	565.20	503.50
Total-N (g kg^{-1})	9.70	9.70
P (g kg^{-1})	52.10	54.50
K (g kg^{-1})	1.30	5.90
Ca (g kg^{-1})	0.30	0.30
Mg (g kg^{-1})	6.40	7.10
Cu (mg kg^{-1})	0.01	0.01
Mn (mg kg^{-1})	0.01	0.01
Fe (mg kg^{-1})	246.40	47.38
Zn (mg kg^{-1})	23.78	26.99

(1) Chemical characterization according to Rosa et al. (2000)

Table 3. Initial and final soil pH in water of soil samples from a multi-metal contaminated site after application of doses of humic acid and fulvic acid

Soil sample	pH(H_2O)				
	Initial	Final			
		H1(1)	H2	F1(2)	F2
S1	6.0	5.7	5.8	5.5	5.3
S2	6.0	5.9	5.9	5.7	5.4
S3	5.3	5.1	5.1	5.0	4.9
S4	5.1	5.1	5.0	4.8	4.8

(1) Humic acid (H1: 0.04 µL g^{-1}; H2: 0.08 µL g^{-1}); (2) Fulvic acid (F1: 0.04 µL g^{-1}; F2: 0.08 µL g^{-1}).

humic substances and, generally, differ in molecular weight, elemental and functional group contents (Weng et al., 2006; Güngör and Bekbölet, 2010).

In soils with higher clay and organic matter content (Table 1), metals are more tightly bound, which limits the efficiency of their removal (Kulikowska et al., 2015). Similarly, Hong et al. (2002) found that a high content of organic matter in soils (7.2 and 11.3 %) makes the removal of heavy metals quite difficult due to their high adsorptive capacity. However, in the present study was observed from all soils, heavy metals were solubilized or immobilized according to humic substances and initial heavy metal concentrations.

Soluble-exchangeable heavy metals/metalloid

Soluble metal contents (Table 4) were quite different and depend on the type and levels of the humic substances and heavy metal concentrations in the soil samples (Table 4).

For the Cu in soil sample S1, larger amounts of the element in solution were observed in H1 and H2 treatments that are based on humic acids, with an increase in soluble Cu concentration of 81.0 and 523.8 %, respectively, compared to the control (water). The

Table 4. Soluble heavy metals and metalloid contents in soil samples (S1, S2, S3 and S4) from a multi-metal contaminated site after application of doses of humic acid and fulvic acid

Treatment	Cu	Zn	Ni	Cr	Cd	Pb	As	Ba
	mg kg^{-1}							
				S1				
F1	0.18 dA	0.48 cB	0.007 dA	0.007 dA	0.002 dA	0.18 dA	0.100 dA	0.20 cA
F2	0.23 dA	0.37 cBC	0.009 cdA	0.007 dA	0.004 cdA	0.12 dA	0.195 bA	0.11 cA
H1	0.76 bA	1.65 bA	0.027 bB	0.039 bB	0.008 bA	1.67 bA	0.156 cA	0.48 bBC
H2	2.62 aA	4.82 aA	0.065 aB	0.087 aB	0.032 aA	3.90 aA	0.346 aA	1.53 aB
Control	0.42 cA	1.14 bA	0.020 bcB	0.030 bAB	0.005 cA	1.21 cA	0.127 cdA	0.28 bcBC
				S2				
F1	0.02 cB	0.05 dB	0.005 dA	0.007 dA	0.000 cA	0.027 cA	0.016 dB	0.15 dA
F2	0.02 cB	0.02 dC	0.007 dA	0.006 dA	0.000 cB	0.014 cA	0.034 bcBC	0.04 dA
H1	0.25 bB	1.21 bAB	0.047 bA	0.065 bA	0.007 bA	0.31 bB	0.063 bB	1.30 bA
H2	0.71 aB	3.03 aB	0.100 aA	0.140 aA	0.024 aB	0.74 aB	0.156 aB	3.56 aA
Control	0.07 bcB	0.44 cB	0.023 cA	0.031 cAB	0.001 cB	0.12 bcB	0.018 dB	0.43 cB
				S3				
F1	0.04 aB	1.18 bA	0.012 bA	0.011 bA	0.002 bA	0.039 bA	0.030 aB	0.19 bA
F2	0.05 aB	1.07 bcA	0.015 bA	0.012 bA	0.003 bAB	0.035 bA	0.047 aB	0.12 bA
H1	0.06 aC	1.02 bcB	0.016 bBC	0.030 abBC	0.003 bB	0.15 abBC	0.026 aC	0.33 abC
H2	0.15 aC	2.07 aC	0.033 aC	0.048 aC	0.009 aC	0.34 aC	0.049 aC	0.56 aC
Control	0.02 aB	0.60 cB	0.007 bC	0.014 bB	0.000 bB	0.069 bB	0.017 aB	0.14 bC
				S4				
F1	0.00 aC	0.40 aB	0.001 cA	0.003 bA	0.000 bA	0.006 aA	0.008 aB	0.17 dA
F2	0.00 aB	0.68 aAB	0.003 cA	0.006 bA	0.000 bB	0.010 aA	0.011 aC	0.15 dA
H1	0.00 aC	0.26 aC	0.009 bcC	0.013 bC	0.001 bB	0.038 aC	0.021 aC	0.66 cB
H2	0.02 aC	0.34 aD	0.017 bD	0.021 abD	0.001 bD	0.069 aD	0.025 aC	1.66 bB
Control	0.02 aB	0.51 aB	0.030 aD	0.038 aA	0.004 aA	0.093 aB	0.044 aB	2.09 aA
CV (%)	22.42	17.21	19.93	22.01	18.92	14.93	17.30	12.63

Means followed by the same lowercase letter do not differ between treatments in each soil samples and same capital letter does not differ between soil samples in each treatment by Tukey's test ($p<0.01$); CV: coefficient of variation; H1: humic acid 0.04 μL g^{-1}; H2: humic acid 0.08 μL g^{-1}; F1: fulvic acid 0.04 μL g^{-1}; F2: fulvic acid 0.08 μL g^{-1}; Control: ultrapure water 0.04 μL g^{-1}.

lowest amounts of soluble Cu were observed in F1 and F2 treatments, which are based on FA. The HA increased soluble Cu concentration, and is capable of solubilizing Cu, unlike FA which immobilized the element reducing the soluble contents in 57.1 and 45.2 %, respectively, compared to the control (Table 4).

In soil sample S2, the behavior of the treatments was similar to the previous one. In the H1 and H2 treatments, Cu concentration in solution was 914.3 and 257.2 %, respectively, which was higher than in the control. However, in the F1 and F2 treatments, Cu concentrations decreased with an immobilization of 71.4 % in both treatments compared to the control. The amounts of soluble Cu in the treatments H1, H2, F1 and F2 in soil samples S3 and S4, showed no significant differences (Table 4). Fulvic acid is the dominant fraction in humic substances for links with ions and metals in the soil solution, which explains the reduction of soluble Cu concentration compared to control (Ren et al., 2015).

Soluble content of Cu in the soil samples was different (Table 4), and the intensity of solubilizing by both the H1 and H2 treatments, as for immobilization by F1 and F2 treatments, depended on the heavy metal concentration in the soil samples (Table 1). Soluble contents of Zn were different between treatments and soil samples (Table 4). In S1, the highest concentration of Zn was observed in treatment H2 with amounts of 322.8 % higher than in the control, showing the solubilizing effect for this metal ion. However, in F1 and F2 treatments, a decrease of 57.9 and 67.5 %, respectively, was observed, when compared to the control, indicating that there was Zn mobilization. The concentration of Zn in H1 treatment is not different from the control. In S2, the behaviors between treatments were similar, except for the H1 treatment, which presented Zn contents higher than the control. The available percentages of Zn in H1 and H2 treatments were 175.0 and 588.6 %, respectively, and immobilizations in treatments F1 and F2 were 88.6 and 95.5 %, respectively, when compared to the control (Table 4).

In S3, the highest and lowest concentrations of soluble Zn were observed in the H2 and control treatment, respectively. No significant differences were observed between H1, F1 and F2 treatments in S3. Fulvic acid treatments solubilized the metallic ion Zn, with a different behavior in soil samples S1 and S2. However, in S4 there was no significant difference in the concentration of soluble Zn in treatments, suggesting that Zn concentration in the soil may change its immobilization or have no solubilization effects from humic substances. The behavior of Zn solubility depends on the ion concentration in the soil sample, with higher concentrations of soluble Zn in S1 and S3, which have the highest levels for this element (Tables 1 and 4).

Soluble concentrations of Ni in S1 decreased by 65.0 and 55.0 % in F1 and F2 treatments, respectively, and increased by 69.2 % in H2 compared to the control. However, in H1, there was no significant difference compared to the control (Table 3). These results indicate that FA was capable of immobilizing soluble Ni, while treatment with HA solubilizes this element making it available in the soil solution. Among the soil samples, no significant difference was observed in the F1 and F2 treatments. Although soil sample S2 has an intermediate total Ni concentration compared to S1 and S3, higher soluble concentrations of Ni were observed in the H1 and H2 treatments in these soil samples (Tables 1 and 4).

Soluble content of Cr in sample S1 for both treatments with FA decreased the Cr concentration in the soil solution by 76.7 %, while the treatment H2 increased Cr availability by 65.5 % and the other treatments did not differ. In S2, the Cr behavior was equal to that found for Ni, where the concentrations of Cr available in the treatments have increased with HA levels and decreased with FA levels when compared to the control. In S3, the highest levels were observed in the H1 and H2 treatments and for others there was no significant difference (Table 4). The concentrations of Cr in the soil samples within each treatment demonstrate that the treatments with FH (F1 and F2) could make this element unavailable to the soil solution. However, S1 and S3 had higher concentrations of Cr when compared to S2 and S4 (Table 1), but there was no significant difference between the Cr contents available with these treatments (Table 4), while in treatments H1 and H2 the highest soluble Cr concentration was observed in S2, followed by S1, S3 and S4, respectively.

In soil samples S1, S2 and S3, the highest available content of heavy metal Cd was observed in the H2 treatment, followed by the H1 treatment. In S1 the lower concentration singular was observed in F1, and did not differ between other treatments. In soil sample S4, the highest Cd content in soil solution was observed in the control, followed by other treatments that did not differ (Table 4). The concentrations of Cd from the soil samples show that FA compost was effective in rendering this element unavailable in the soil solution. No significant difference to concentrations of soluble Cd was observed between the soil samples.

Among the soil samples, S1 had the highest Pb concentration (Table 1). However, F1 and F2 treatments were able to decrease the availability of Pb by 90.1 % compared to the control in soil sample S1 (Table 4). The H1 and H2 treatments in soil sample S1 increased the Pb availability in soil solution by 38 and 222.3 %, respectively, compared to Cd and Cr. In soil samples S1 and S3, the available Pb concentrations increased in H1 and H2 treatments, but for others there was no significant difference and in soil sample S4 no effects of treatment were observed on the solubility of Pb (Table 4). For the Pb content available from soil samples, there was no significant difference in F1 and F2, which means that these treatments were able to immobilize Pb from soil solution. In the other treatments, it was observed that the Pb content in the solution was higher in soils with the highest total concentrations (Tables 1 and 4).

The subsequent changes in bioavailability and toxicity in the presence of organic matter may play a critical role in ecological risk assessment (An et al., 2015). The toxic effects of Pb in soil has a strong relationship with Pb in soil solution, indicating that the soil toxicity can be represented by the soil solution toxicity. Therefore, the use of higher doses of HA in soil with higher Pb concentration can be a risk to environmental quality.

For soluble As in soil sample S1, an increase of 55.5 % was observed in the As soluble concentration in F2 treatment compared to the control treatment. However, in F1 treatment, a decrease of 21.3 % in soluble As was observed compared to the control and increases of 22.9 and 172.4 % in the As concentration were observed in H1 and H2 treatments, respectively (Table 4). In soil sample S2, there was an increase in the As concentrations in soil solution in the F2, H1 and H2 treatments when compared to the control and the F1 treatment. In soil samples S3 and S4, there was no significant effect of the treatments on available As (Table 4). Among the soil samples studied, soil sample 1 showed the highest levels of As available in all treatments, probably because of the initial concentration of this element in the soil sample (Tables 1 and 3).

The soluble Ba content in S1 behaved in the same way as other elements, with a decrease of 28.6 and 60.7 % in the soluble content in F1 and F2 treatments, respectively, that do not differ, and an increase of 81.7 % in the H2 treatment. However, in the H1 treatment there was no significant difference compared to the control, but in S2, the higher concentration of soluble Ba was observed in treatment H2, followed by H1 and the lower concentrations were observed in F1 and F2 treatments, demonstrating the FA sorption capacity for Ba in soil solution, while HA increases the Ba solubility, i.e., Ba desorption (Table 4). However, in the soil sample S4, the higher Ba content in solution was observed in the control treatment, followed by treatments H2 and H1 and lower levels were observed in F1 and F2 treatments (Table 4).

In general, soil samples with higher heavy metal concentrations (S1, S2 and S3) applying HA levels resulted in the solubilization of metals, while the FA levels decreased the concentration of soluble metals in soil samples or immobilized these metals, when compared to the control (Table 4). The capacity for heavy metal solubilization by HA arises from the reaction of quinones (Rashid, 1972). In infrared analysis, the author observed that quinone groups participate in organometallic reactions; therefore, the intensity of the absorption of quinone of 1650 cm^{-1} was reduced and shifted to a lower frequency (1635 cm^{-1}).

Benzoquinone, which is believed to be present in HA along with other quinone compounds, solubilized from 8.3 to 11.8 mg g^{-1} of Cu, Zn, Co, Mn, and Ni. These are presumed to be chelation-type reactions. Major changes are also believed to occur in the ligand environment of metals when they react with quinone-containing compounds. One such change is valency change. These reactions and changes may enhance metal solubility and mobility (Rashid, 1985). The enhanced solubility of metals as a consequence of chelation reactions by organic compounds is widely accepted and frequently reported (Güngör and Bekbölet, 2010; Borggaard et al., 2011; Ren et al., 2015; An et al., 2015). However, the same behavior was not observed in FA doses. This occurred because the quinone content of HA is generally higher than for FA and a higher percentage of the total C=O in HA occurs in quinones than in FA. Essentially all of the oxygen in most soil FA can be accounted for in O-containing functional groups, whereas a smaller fraction, somewhat less than 75 %, has been recovered in these groups in most HA (Sparks, 1998).

There was a higher binding affinity between some metals and FA compared to humic acids, due to more functional groups (e.g., OH and/or COOH) in FA that could complex with metal as well as higher polarities (Tang et al., 2014). Therefore, authors observed that the presence of FA led to a strong increase in Cu(II) sorption at low pH and a decrease at high pH. This is attributed to dissociation of functional groups such as carboxylic and phenolic. Carboxyl groups dissociate at acidic or neutral pH, whereas phenolic groups dissociate at alkaline pH (Kulikowska et al., 2015). Therefore, for this study, the responses suggest that in higher FA concentration, carboxyl groups were more responsible for metal complexation than phenolic groups, because soil pH was at 4.8 to 6.0 (Table 3).

Determinations of total acidity, carboxyl groups, hydroxyl groups and carbonyl groups are important (Borggaard et al., 2011). Both carboxyl and phenolic hydroxyl groups will contribute to total acidity. Fulvic acids contain more functional groups, particularly carboxyl, per molecule than HA that only contribute 1.8-2.5 mmol COOH at a concentration of 25 mmol L^{-1} of dissolved organic C, which could explain results found in this study.

The absence of readily oxidizable quinone and amine groups in FA is compatible with a chemical degradation model for the formation of FA from humic materials. This is a significant result, as the carboxyl and hydroxyl groups of humic substances are the most likely to react with cations. Therefore, FA should have a higher capacity for interactions with metals (Tang et al., 2014; Kulikowska et al., 2015; ElBishlawi and Jaffe, 2015); the corroborating results are shown in table 4.

CONCLUSIONS

Humic acid from leonardite do not influence soil pH and increase the solubility of Cu, Zn, Ni, Cr, Cd, Pb, As and Ba from soils.

Fulvic acid decreased soil pH and metal solubility, except for As at the lower dose (0.04 µL g^{-1}).

The initial soil heavy metal concentration affects the processes involving humic substances.

The use of humic or fulvic acids as a natural and environmentally friendly substance for increased or decrease, respectively, plant-availability of heavy metals in remediation projects.

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