

# **EFFECT OF ACETATE, CITRATE, AND LACTATE INCORPORATION ON DISTRIBUTION OF CADMIUM AND COPPER CHEMICAL FORMS IN SOIL**

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## **ABSTRACT**

Some organic acids of low molecular weight, generated through breakdown of plant wastes or root exudation, increase the solubilization of heavy metals, such as cadmium (Cd) and copper (Cu) through complexation reactions and may affect the distribution of their chemical forms in soil. By using natural soil and soil spiked with Cd or Cu, the effect of acetate, citrate, and lactate incorporation on the solubilization of these metals and the distribution of their chemical forms was assessed. To this end, four concentration levels of organic acids were incorporated to soil: 0, 0.4, 0.8, and 1.6 mM at constant ionic strength in 0.02 M potassium chloride (KCl) at pH 5.5 at a soil to solution ratio of 1:20. After 24 h equilibrium, the supernatant was separated and used to determine the concentration of organic acid not retained by soil through the

technique of Zone Capillary Electrophoresis and the metal concentration through inductively coupled plasma-mass spectrometry (ICP-MS). The residue was fractionated to determine Cd and Cu chemical forms through a sequential extraction method of five steps. Results showed that acetate and lactate retention is higher in non-sterilized soils which would indicate that anion retention would partly correspond to consumption by microorganisms rather than to anion adsorption by soil particles. It was also found that a higher metal content has a negative effect on acetate retention. The presence of lactate and citrate increases the solubility of both metals, and treatment with these acids in some cases affect the distribution of their chemical forms. Acetate incorporation increased the amount of Cu associated to organic matter, and the presence of citrate affected the fractions of exchangeable Cu, carbonate, and associated to manganese oxides. The fraction of both metals associated to iron oxides was the least affected by the incorporation of organic acid anions.

## INTRODUCTION

The fate of heavy metals added to soil will be controlled by a complex set of chemical reactions and by a number of physical and biological processes acting within the soil (1). Metals in the soil system may be part of the liquid and solid phases. In the latter, they are distributed among the various soil components and their association with them gives rise to chemical forms that determine their mobility. Thus, these metals may be found in soil in the liquid phase as free cations or complexed with inorganic and organic ligands, and in the solid phase retained at exchange sites or at specific sites of oxides, organic matter and in structures of primary or secondary minerals.

In order to estimate the mobility of heavy metals and their behavior in soil, it is necessary to learn about these chemical forms through the application of sequential extraction methods which have been widely used by several authors (2, 3, 4). It has been found that Cd and Cu retention in soil is different, Cd being more mobile than Cu. The latter is firmly retained and constitutes one of the least mobile elements (5). Some authors have observed a predominance of exchangeable Cd and Cu associated to oxides or to organic matter (6, 7).

Organic acids are involved in soil formation processes directly participating in mineral solubilization through complexation and through their ability to release protons (8). Most of these acids result from microbial metabolism, plant waste decay, and root exudation. Most aliphatic organic acids are soluble in water, but

their concentration depends on the rate of production, exudation, plant uptake, microbial consumption, and immobilizing mechanisms, such as adsorption of soluble complexes or chelates on mineral constituents (9).

Some authors have detected the presence of formic, acetic, lactic, citric, and oxalic acids in forest and contaminated soils (10, 11). The presence of organic acids in the liquid phase of soil means a series of equilibrium reactions that could modify heavy metal retention and mobility in soil.

The purpose of this study was to determine the effect of the organic anions, acetate, lactate, and citrate, on the extractability and distribution of Cd and Cu chemical forms in natural soil and soils spiked with these elements to simulate contaminated soil.

## MATERIALS AND METHODS

In this study, an alluvial soil belonging to the Inceptisol order and the clayed, mixed, thermic, Vertic Xerochrepts family that had previously been used in a study involving pot plant breeding. Before cultivation, this soil was treated with Cu or Cd as 0 and 10 mM cadmium nitrate [ $\text{Cd}(\text{NO}_3)_2$ ] and copper nitrate [ $\text{Cu}(\text{NO}_3)_2$ ], respectively, in a 1:2 soil:solution ratio. Once the metal was incorporated into the samples, they were dried at room temperature, ground, and passed through a 2-mm mesh sieve. After cultivation, the samples were again conditioned so as to homogenize them, and they were characterized as to their pH, organic carbon percentage, cation exchange capacity, and total metal content. The pH was determined in a 1:1 soil to water suspension, organic carbon content was determined by the Walkley-Black procedure (12), and cation exchange capacity (CEC) was determined by the sodium acetate procedure (13). Total metal determination was done by flame atomic absorption spectrophotometry (AAS) after acid digestion [by a mixture of nitric acid ( $\text{HNO}_3$ ), hydrofluoric acid (HF), and hydrogen peroxide ( $\text{H}_2\text{O}_2$ )] of the soil in a Milestone microwave oven.

Part of the samples were sterilized with chloroform fumes. The sterilized and non-sterilized samples were equilibrated for 24 hours with acetate, citrate, and lactate solutions at four concentration levels: 0, 0.4, 0.8, and 1.6 mM with constant ionic strength in 0.02 M KCl at pH 5.5 and at a soil to solution ratio of 1:20. After equilibrium, the samples were centrifuged and the supernatant was separated to determine organic acid anion concentration by Zone Capillary Electrophoresis, and Cd or Cu concentration was determined by flame AAS or by ICP-MS, depending on the concentration. The residue was sequentially extracted using a 5-step procedure based on the methods by Chao (14), Tessier (2), and Miller et al. (15). Cadmium and Cu fractionations were done by the following model:

Sequential Extraction of Heavy Metals in Soils

Steps	Forms	Extractants*	Equilibrium
1	Exchangeable	1 M MgCl <sub>2</sub>	1 hour
2	Carbonate-associated	1 M NaOAc	5 hours
3	Mn oxide-associated	0.1 M NH <sub>2</sub> OH-HCl + 0.01 M HNO <sub>3</sub>	30 minutes
4	Organically-bound	0.1 M K <sub>2</sub> P <sub>2</sub> O <sub>7</sub>	24 hours
5	Fe oxide-associated	1 M NH <sub>2</sub> OH-HCl + 25%(v/v) HOAc	4 hours to 96°C

\* 20 mL of each extractants solutions was used.

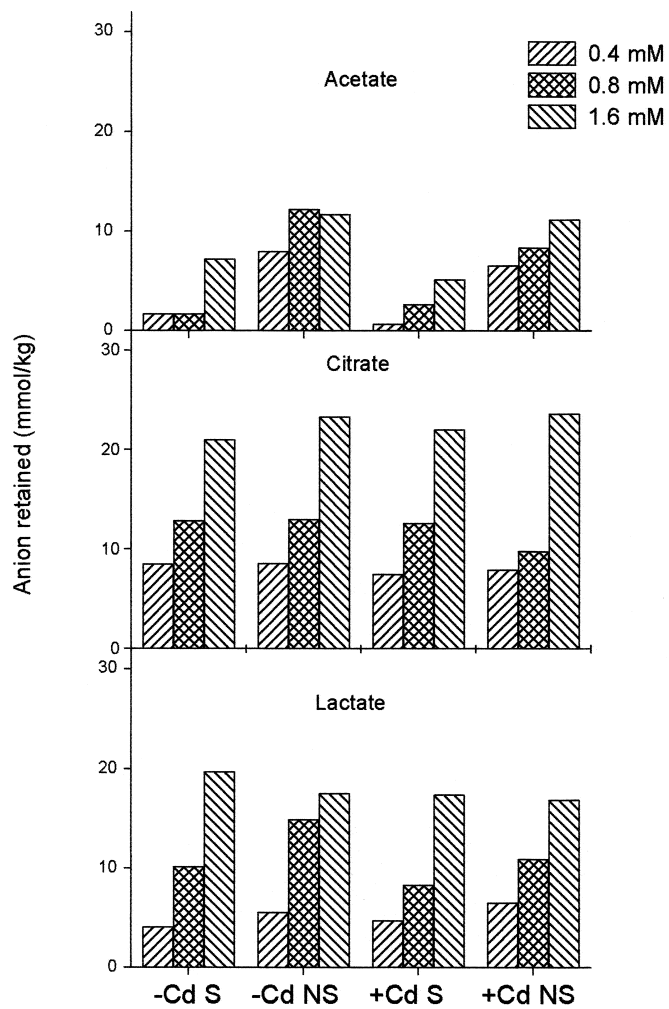
The extracting solutions were prepared equivalent to 200 mg nitriloacetic acid (NTA) L<sup>-1</sup> as described in a study by Howard and Shu (16) to prevent the effect of heavy metal resorption.

In order to determine organic anions, a Capillary Ion Analyzer (Waters) was utilized using a 60 cm × 75 μm ID capillary by means of indirect photometric detection at 254 nm and at a voltage of -20 kV at 25°C. A separation buffer consisting of 10 mM p-hydrobenzoic acid and 0.5 mM tetradecyltrimethylammonium bromide (TTAB) was used as an electrosmotic flow modifier at pH 4.5.

## RESULTS AND DISCUSSION

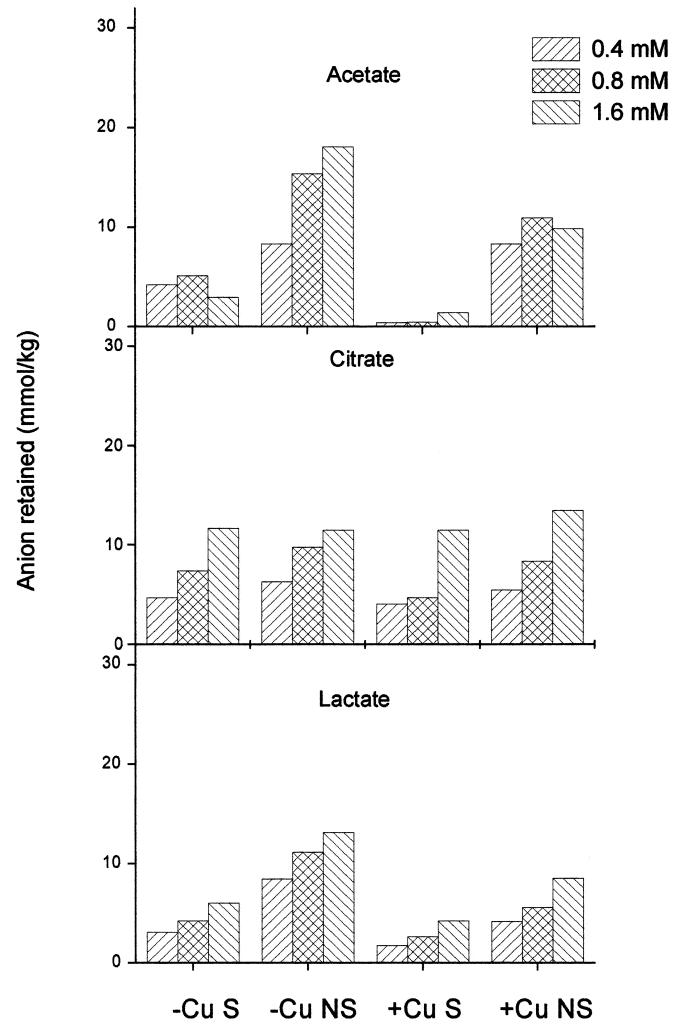
The retention of organic anions in the soil samples with a baseline level of Cd or Cu and in soil spiked with these metals are shown in Figures 1 and 2. A negative influence was observed in the retention of these anions at the highest Cu level which was evident in the case of acetate and lactate retention. Similar results were found in the sample spiked with Cd, but only when acetate was added. A larger retention of acetate and lactate was found in non-sterilized soil. The differences concerning the effect of sterilization were statistically analyzed by the Student's t-test, significant differences being found to P<0.01, only in the cases where acetate (t=5.14) and lactate (t=3.18) were added to the soil spiked with Cu. Instead for soil spiked with Cd, a similar result was found in the case of acetate incorporation (t=4.68). This would indicate that part of the incorporated organic anion disappearing from solution would be consumed by microorganisms and not being adsorbed on the soil particles (17). It should be pointed that in the samples with a baseline level of Cd or Cu, different results were observed concerning the retention of the different acids in spite of their similarity in physicochemical characteristics (Table 1).

The results concerning solubilization of the metal with acid incorporation



**Figure 1.** Acetate, citrate and lactate retention in non sterilized (NS) and sterilized (S) soil. -Cd = soil with a baseline Cd level; +Cd = soil spiked with Cd.

are shown in Table 2. Soil with the lowest Cd level exhibited a higher percentage of soluble Cd in the samples treated with lactate and citrate. In the latter case, solubility increases with increasing citrate addition both in sterilized and non-sterilized soil. A similar behavior was observed in soil with a higher Cd level when citrate was added, the non-sterilized samples showing an increase of 2.0% to



**Figure 2.** Acetate, citrate and lactate retention in non sterilized (NS) and sterilized (S) soil. -Cu = soil with a baseline Cu level; +Cu = soil spiked with Cu.

9.5%. When acetate or lactate was added, the quantity of soluble Cd was similar to that observed in soil not treated with organic acid. Krishnamurti et al. (18) found that Cd solubility was higher when citrate was present than in the presence of acetate. Similar results were obtained for Cu, but the quantities solubilized by acids are smaller compared with those observed in the case of Cd. Only an in-

**Table 1.** Some Properties of Soils Under Study

Suelos	pH	C org (%)	CIC (cmol/kg)
Level 0 Cd	7.2	1.9	45.0
Level 3 Cd	6.8	2.0	48.1
Level 0 Cu	7.2	1.9	46.9
Level 3 Cu	6.8	1.8	49.0

Level 0 = sample with a baseline metal level.

Level 3 = sample spiked with Cd or Cu.

**Table 2.** Soluble Fraction of Cd and Cu in Soil with and without Incorporated Metal Treated with 0, 0.42, 0.85, and 1.69 mM Acetate, Citrate, or Lactate. Values in % of Total Metal

Added Anion mM	S		NS		S		NS	
	0 Cd %	0 Cd %	3 Cd %	3 Cd %	0 Cu %	0 Cu %	3 Cu %	3 Cu %
Control	0.57	0.28	1.6	1.6	0.13	0.09	0.12	0.095
Acetate								
0.42	0.57	0.38	1.6	1.6	0.11	0.10	0.13	0.11
0.85	0.38	0.67	1.6	1.7	0.12	0.14	0.11	0.11
1.69	0.47	0.38	1.8	1.7	0.12	0.14	0.13	0.088
Citrate								
0.42	1.4	3.1	3.4	2.0	0.16	0.12	0.74	0.47
0.85	1.9	6.6	8.3	7.8	0.29	0.21	2.1	1.7
1.69	5.3	4.1	7.4	9.5	0.54	0.48	5.1	4.7
Lactate								
0.42	2.2	0.76	1.7	1.7	0.14	0.14	0.13	0.11
0.85	1.1	1.3	1.7	1.7	0.14	0.17	0.14	0.11
1.69	3.4	1.3	1.8	1.8	0.14	0.13	0.15	0.13
	Total metal (mmol/kg)							
	0 Cd	0 Cd	3 Cd	3 Cd	0 Cu	0 Cu	3 Cu	3 Cu
	S	NS	S	NS	S	NS	S	NS
	0.010	0.009	5.7	5.7	6.9	6.9	15.9	15.8

Level 0 = sample with a baseline metal level.

Level 3 = sample spiked with Cd or Cu.

S = sterilized soil.

NS = nonsterilized soil.

crease in the soluble metal outstands in the case of citrate incorporation, higher values being found in sterilized soil.

Similar results were reported by Mench et al. (19) in a study where they proved the effectiveness of an exudate containing citric acid for Cu solubilization.

### Sequential Extraction

The results obtained with the different treatments with organic acids in the samples of sterilized and non-sterilized soil were statistically analyzed in order to determine the effect of sterilization and the influence of organic acid incorporation on the metals under study. No significant differences were found between sterilized and non-sterilized soils, so these values were taken as repetitions in order to assess the effect of acid incorporation through the application of the Duncan's test with a probability level of 0.05.

### Cadmium

The results are shown in Table 3. Cadmium is found predominantly bound to iron oxides and is distributed according to the following order: Cd-OxFe > Cd-Exchangeable > Cd-OxMn > Cd-Organic > Cd-Carbonate. In the soil samples spiked with this metal, distribution varies, and the results are similar to those in soil spiked with Cu, exchangeable Cd and Cd bound to carbonate being the most important Cd forms. This fact, that is, finding that the exchangeable and carbonate fractions of the metals were predominant when they were incorporated agrees with Kabata-Pendias and Pendias (1993) who suggest that all the metal anthropogenically incorporated into soil would be in bioavailable forms.

Incorporation of the acids under study showed no significant changes in the distribution of Cd fractions which kept the same order as that found in the samples not treated with organic acids, except for those samples with a baseline Cd level treated with citrate or lactate where the fraction associated to carbonate was found to be third in importance.

The sample spiked with Cd showed significant differences in the exchangeable fraction of the metal among the treatments with the different acids. However, in the sample treated with acetate, the values were not significantly different from those obtained in the sample with no acid incorporated.

In soil with a baseline level of Cd, this metal was only found associated to carbonates in the samples treated with citrate and lactate, and no significant differences were found with increasing concentrations of them. The sample spiked



with Cd showed statistically different values in the case of citrate incorporation, and a decrease in Cd associated to carbonate was observed with increasing concentration of this anion. On the other hand, incorporation of citrate and lactate decreased the amount of Cd occluded in manganese oxides in soil with a baseline Cd level, but in the sample spiked with Cd this form of the metal was not detected in the sample with no acid incorporated and in the sample treated with acetate. However, this form was detected when citrate and lactate were incorporated and a decrease in this value was observed with increasing citrate. As concerns the metal bound to organic matter, no significant differences were found with the different treatments, except for lactate incorporation to soil with a baseline Cd level. In soil spiked with Cd, instead, values of this fraction differed significantly between the samples treated with citrate and lactate. As to Cd occluded in iron oxides, significant differences were observed only in the case of the sample spiked with Cd and acetate with increasing concentration of this anion.

### Copper

The results are shown in Table 4. It was found that in soil with a baseline level of Cu, this is predominantly associated to iron oxides. In the samples spiked with Cu, instead, exchangeable Cu and Cu associated to carbonates are prevailing. If acetate is incorporated into soil with a baseline Cu level, the predominant form is Cu bound to organic matter, and in soil spiked with Cu, the exchangeable form is the most important, followed by both Cu bound to organic matter and Cu associated to carbonates. In the presence of citrate and lactate, the distribution order is the same order as in soil without incorporated acid: Cu-OxFe > Cu-Carbonate > Cu-organic > Cu-exchangeable > Cu-OxMn for soil with a baseline Cu level, and Cu-exchangeable > Cu-Carbonate > Cu-OxFe > Cu-organic > Cu-OxMn for soil spiked with this metal. Citrate addition increased the exchangeable Cu form in soil with a baseline Cu level. In soil spiked with Cu, a decrease of this fraction was found with increasing acetate or citrate, but in the latter case the variation was insignificant. Likewise, citrate incorporation affected Cu associated to carbonates in the samples spiked with Cu. In soil treated with acetate, the amount of Cu associated to organic matter is significantly different from the values found for this fraction in the samples treated with citrate and lactate, and a greater amount of the metal in this form is found in soil treated with acetate, both in soil with a baseline Cu level and in soil spiked with Cu. As to Cu associated to iron oxides, no significant differences were found among the treatments with the different acids or with increasing their concentration, except for the samples spiked with Cu and with acetate, where a decrease is observed for this metal fraction in the samples treated with a higher concentration of acetate.

**Table 3.** Cd Fractionation in Soil with a Baseline Cd Level and Spiked with Cd, Treated with 0, 25, 50, and 100 mg/L Organic Anions. Values Expressed as % of Total Metal Content

Added Anion mM	% Cd Exch.	RSD %	% Cd Carbo-nate	RSD %	% Cd MnOx	RSD %	% Cd Organic	RSD %	% Cd FeOx	RSD %	Rec Total Metal %
Control	19.7 cd	15	nd		19.1 a	7	7.3 a	34	29.2 c	10	75
Acetate					Level 0 Cd						
0.4	31.4 a	4	7.5 b	8	12.4 b	10	7.8 a	36	31.0 bc	9	90
0.8	17.5 d	7	nd		19.9 a	5	nd		32.8 abc	5	70
1.6	21.3 bcd	3	nd		18.8 a	8	4.3 a	20	33.3 abc	6	78
Citrate											
0.4	19.4 bcd	4	18.3 a	7	10.8 bc	24	4.6 a	30	34.2 ab	5	87
0.8	22.4 bc	19	18.8 a	6	10.2 bc	27	5.0 a	31	35.5 a	8	91
1.6	22.0 bc	7	17.2 a	10	11.6 bc	4	7.0 a	23	35.7 ab	6	94
Lactate											
0.4	22.9 b	14	18.6 a	2	9.9 bc	17	7.5 a	30	34.4 ab	5	93
0.8	20.6 bcd	8	17.5 a	7	10.5 bc	11	6.4 ab	10	34.2 ab	5	89
1.6	18.3 cd	4	17.7 a	5	8.8 c	10	6.4 b	9	34.2 ab	5	86

	Control	70.5 a	2	10.5 b	2	Level 3 Cd	5.8 a	6	3.0 bcd	5	90
Acetato						nd					
0.4	2	69.6 a	2	10.5 b	2	nd	5.9 a	1	3.1 bcd	5	89
0.8	1	71.3 a	1	10.0 c	2	nd	6.0 a	2	2.7 e	5	90
1.6	3	71.8 a	3	9.8 c	6	nd	5.3 b	2	2.8 de	4	90
Citrato											
0.4	2	60.4 b	2	11.5 a	2	2.8 a	5.1 b	3	3.4 a	10	83
0.8	4	54.3 c	4	10.8 b	3	2.6 b	4.6 c	4	3.3 ab	4	76
1.6	1	52.1 c	1	9.8 d	3	2.1 c	4.5 c	4	3.2 abc	8	71
Lactato											
0.4	2	62.6 b	2	9.8 c	3	2.7 b	5.2 b	0.8	3.0 bcd	4	83
0.8	2	63.0 b	2	9.9 c	2	2.7 b	5.2 b	2	3.1 bcd	3	84
1.6	2	63.0 b	2	10.0 c	1	2.6 b	5.1 b	0.8	3.0 bcd	2	84

Level 0 = sample with a baseline metal level.

Level 3 = sample spiked with Cd or Cu.

RSD = relative standard deviation.

Values followed by the same letter in one column show no significant difference at a level of 5% according to the Duncan test.

**Table 4.** Cu Fractionation in Soil with a Baseline Cu Level and Spiked with Cu, Treated with 0, 25, 50, and 100 mg/L Organic Acids. Values Expressed as % of Total Metal Content

Added Anion mM	% Cu Exch.	RSD %	% Cu Carbo-nate	Level 0 Cu			RSD %	% Cu Organic	RSD %	% Cu FeOx	RSD %	Rec Total Metal %
				RSD %	% Cu MnOx	RSD %						
Control	8.0 bc	1	13.4 abc	2	2.5 c	8	14.2 b	32	21.0 a	9	59	
Acetate												
0.4	7.8 cd	1	13.7 ab	2	2.4 c	1	23.5 a	3	22.1 a	3	69	
0.8	7.6 d	3	13.5 abc	4	2.4 c	4	22.4 a	7	21.7 a	6	68	
1.6	7.7 cd	2	13.7 ab	2	2.5 c	2	24.6 a	6	21.0 a	2	70	
Citrate												
0.4	7.8 cd	1	13.8 a	0.7	2.4 c	4	8.6 c	0.7	22.1 a	2	55	
0.8	8.2 b	3	13.6 abc	2	2.3 c	2	8.6 c	2	21.7 a	2	54	
1.6	8.7 a	4	13.5 abc	1	2.1 d	2	8.9 c	2	21.0 a	2	54	
Lactate												
0.4	7.8 cd	0.4	13.5 abc	0.9	2.8 a	2	11.4 bc	0.6	22.8 a	1	58	
0.8	7.8 cd	0.2	13.1 c	0.2	2.7 ab	1	11.4 bc	0.9	22.4 a	1	58	
1.6	7.8 cd	0.6	13.2 bc	1	2.7 ab	1	11.4 bc	1.7	20.3 b	2	56	

	Control	21.0 ab	2	15.2 cd	3	Level 3 Cu	20	10.3 b	25	14.0 b	13	65
Acetate						4.6 bc						
0.4	20.4 bc	2	15.3 bcd	3	2.5 d	3	15.9 a	7	13.6 b	11	68	
0.8	21.8 a	9	15.5 bcd	2	2.7 d	3	15.8 a	2	17.0 a	9	73	
1.6	19.6 c	6	17.0 a	3	4.1 c	2	13.7 a	2	10.4 c	3	65	
Citrate												
0.4	21.0 ab	0.9	15.0 d	2	5.0 ab	2	9.5 b	2	12.6 b	2	63	
0.8	20.2 bc	1	14.2 e	3	4.6 bc	2	7.7 b	2	12.5 b	1	59	
1.6	19.7 bc	2	12.9 f	4	3.8 c	3	7.3 b	2	12.0 bc	0.9	56	
Lactate												
0.4	20.8 abc	0.1	15.6 bcd	2	5.7 a	0.5	8.2 b	0.7	12.8 b	1.3	63	
0.8	20.7 abc	0.4	15.9 b	2	5.4 ab	0.6	8.5 b	0.9	12.2 bc	0.5	63	
1.6	20.8 abc	0.2	15.8 bc	2	5.2 ab	0.7	8.5 b	0.7	12.0 bc	1.2	62	

Level 0 = sample with a baseline metal level.

Level 3 = sample spiked with Cd or Cu.

RSD = relative standard deviation.

Values followed by the same letter in one column show no significant difference at a level of 5% according to the Duncan test.

## CONCLUSIONS

Acetate retention was higher in sterilized soils, and a greater Cu content had a negative effect on acetate retention. Lactate and citrate incorporation increased the solubility of both metals both in soil with a baseline metal level and in the spiked soil sample. The exchangeable and carbonate forms of Cd and Cu predominate in the soil samples spiked with these metals, while in soil with a baseline level of the metals, these are mainly associated to iron oxides. Treatment with the different acids under study had a different effect on the distribution of chemical forms both of Cd and Cu. Thus, acetate incorporation was found to increase the amount of Cu associated to organic matter and citrate incorporation decreased the amount of this metal as carbonate and occluded in manganese oxides. This latter anion also decreased the exchangeable form of Cd which is more evident with increasing addition of citrate. In soil treated with a baseline Cd level, both citrate and lactate incorporation increased the fraction of Cd bound to carbonate, but decreased the form of Cd occluded in manganese oxides. Both for Cd and Cu, the metal fraction associated to iron oxides was the least affected by the incorporation of organic acid anions.

## ACKNOWLEDGMENTS

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