



Degradation of low molecular weight organic acids complexed with heavy metals in soil

Giancarlo Renella*, Loretta Landi, Paolo Nannipieri

Department of Soil Science and Plant Nutrition, University of Florence, Piazzale delle Cascine 28, 50144, Florence, Italy

Available online 5 February 2004

Abstract

Soils contaminated with heavy metals also contain a number of organic ligands, particularly in the rhizosphere and thus, a fraction of the bioavailable metals in soils likely exists in a complexed form. The presence of soluble, metal-complexing organic ligands can influence the fate and transport of metals as well as mineralisation of organic compounds. Oxalate and citrate complexed with Cd, Cu, Mg, Pb and Zn were used as model metal–organic complexes possibly occurring in heavy metal contaminated soils. Soil respiration was used to determine the biodegradation of these compounds in a clay alkaline soil kept under different management regimes. Overall, the results indicated that metal complexes with citrate generally were more degradable than oxalate–metal complexes. For each organic acid, biodegradability of the metal–organic complexes varied for different metals, following the ranking order: $Mg > Zn > Cu \cong Pb > Cd$. Addition of Cd complexes decreased the soil respiration.

These results indicate that the formation of the complexes between heavy metals and low molecular weight organic ligands might affect the soil functionality, especially in the rhizosphere of contaminated soils, as determined by soil respiration.

© 2004 Elsevier B.V. All rights reserved.

Keywords: Biodegradation; Heavy metals; Organic complexes; Soil pollution

1. Introduction

The continuous enrichment of the pedosphere with heavy metals due to deposition from the atmosphere, the use of chemical fertilisers, and the incorporation of sewage sludge into agricultural soils pose major risks for terrestrial ecosystem health.

Soils with high heavy metal contents usually have a reduced microbial biomass, increased metabolic quotient (qCO_2) and altered biodegradation activities towards organic compounds (Brookes, 1995).

The toxicity of heavy metals to soil microorganisms has been generally attributed to the activity of the free metal ions, which can be predicted using the free-ion activity model (FIAM).

Low molecular organic acids represent a stable pool of the soil organic matter (SOM; Stevenson, 1994) resulting mainly from rhizodeposition and as microbial metabolites during soil organic matter (SOM) decomposition (Robert and Berthelin, 1994). Laboratory studies have shown that low molecular weight organic acids (LMWOAs) can solubilize heavy metals through complexation reactions (Krishnamurti et al., 1997; Naidu and Harter, 1998; Poulsen and Hansen, 2000; Taniguchi et al., 2000). Ahumada et al. (2001) reported that the addition of LMWOAs

* Corresponding author. Tel.: +39-55-3288-219; fax: +39-55-333-273.

E-mail address: giancarlo.renella@unifi.it (G. Renella).

influenced heavy metal speciation and, for example, acetate increased the amount of Cu associated to the SOM. It is therefore of interest to understand the interactions between organic acids and metal that may influence the bioavailability of heavy metals to plants and microorganisms.

In vitro studies have shown that low molecular weight organic ligands regulate the uptake of essential metals by microorganisms, and in some cases, the production of organic acids may be involved in detoxification mechanisms (Hantke, 2000). Investigations on the interactions between microorganisms and organic molecules in the presence of heavy metals and on the ability of microorganisms to degrade metal–organic complexes have given unclear results. Brynhildsen and Allard (1994) reported that citrate complexes with Cd, Cu and Zn were taken up at a similar rates that were similar to that of free citric acid, but that these complexes were not metabolized within the cell cytoplasm. Hassen et al. (1998) reported that the presence of citrate increased the biosorption of Cr, by *Pseudomonas aeruginosa* and Cu, by *Bacillus thuringiensis*. Puranik and Paknikar (1999) reported that citrate reduced Pb, Cd and Zn sorption by *Citrobacter* cells. Heavy metals like Cu have been found to interfere with citric acid production by *Aspergillus niger* (Tsekova et al., 2001).

Despite the large number of studies on metal solubilization by organic ligands and the biodegradability of metal–organic complexes in pure cultures, to our knowledge, no detailed studies have been conducted to assess the biodegradation of metal–organic complexes in soil. Most of the research on the effects of heavy metals on microbial-driven processes like respiration (Babich and Stotzky, 1985), soil enzyme activity (Moreno et al., 2001) and biodegradation of organic com-

pounds (Burkardt et al., 1993) have been carried out by adding heavy metals and organic compounds separately. This is surprising because all experimental evidences to date shows that in the rhizosphere, LMWOAs exudated by the plant roots form soluble complexes with heavy metals (Krishnamurti et al., 1997; Romer et al., 2000). Moreover, the use of citrate as a chelating agent coupled with metal citrate utilizing strains of *P. aeruginosa* and *Pseudomonas putida* has been proposed as a feasible soil remediation technique (Thomas et al., 2000). Metal complexes of low molecular weight organic ligands might contribute to the heavy metal bioavailability to freshwater algae and vertebrates by direct uptake of metal complexes (van Ginneken et al., 1999; Errecalde and Campbell, 2000).

The aim of this study was to monitor the degradation rates of LMWOAs complexed with different heavy metals in soils under different management regimes.

2. Materials and methods

The soil studied was a Vertic Xerochrept (USDA 1992) kept under wheat, lucerne, grassland and woodland, from the Vicarello experimental fields located in Tuscany (central Italy). The two arable soils were sampled from the 0–23 cm depth whereas the grassland and forest soils were sampled from the 0–10 cm depth. The main soil characteristics are listed in Table 1. Soils were sieved (<2 mm), moistened to 40% WHC, and preincubated for 7 days at 25 °C prior to the treatments with metal–LMWOAs complexes.

Two LMWOAs, acetate and citrate complexed with Mg, Cd, Cu, Pb and Zn were added (2 ml of solution or suspension) dropwise to soils; The soil

Table 1
Main physical and chemical characteristics of soils

Soil	pH [H ₂ O]	Sand (%)	Silt (%)	Clay (%)	C org (%)	N tot (%)	CEC (meq × 100 g ⁻¹)	Carbonates (%)	ATP (µg kg ⁻¹)	Basal respiration (mg CO ₂ -C kg ⁻¹ × d)
Woodland	8.1	20.5	41.0	38.5	3.8	0.35	12.5	10.8	3885.7	55.7
Grassland	8.1	20.5	41.0	38.5	2.2	0.21	11.4	11.2	2357.2	51.4
Lucerne	8.1	20.5	41.0	38.5	1.2	0.13	10.7	12.6	1425.2	43.1
Wheat	8.1	20.5	41.0	38.5	0.9	0.09	9.6	14.2	1023.5	24.7

moisture was brought to 55% WHC to give a carbon rate typical of rhizosphere and equivalent to 300 mg kg⁻¹ soil. The chemicals used were: Mg-acetate and Pb-acetate from Carlo Erba, Mg-dicitrate trihydrate from Fluka, Cd-acetate dihydrate from Merck, Cu-acetate hydrate, Cu-citrate, Pb-acetate, Pb-citrate, Zn-acetate dihydrate and Zn-citrate dihydrate from Aldrich. Cd-citrate was prepared by using equimolar amounts of citric acid and Cd (0.52 mM). All chemicals were of analytical grade.

Soils (25 g oven dry equivalent) were placed in 250 ml Quickfit flasks and incubated at 25 °C in the dark. Flasks containing soils treated with distilled water only served as control soils while empty flasks were used as blanks. The LMWOA decomposition rate was monitored for 96 h by sampling the headspace every 24 h and flushing the flask afterward. The CO₂ evolution was measured by a gas chromatograph (HP 6890) equipped with a gas sampling valve, a packed column (Poropack Q) and a thermal conductivity detector, according to Blackmer and Bremner (1977). All treatments and measurements were replicated three times.

3. Results

The results reported in Fig. 1 show the net mineralisation rates of the different metal–LMWOAs complexes, which were calculated by subtracting the basal respiration values from those of the metal complex induced respiration during the 96-h incubations.

In the woodland soil, Mg- and Zn-acetate were mineralised at similar rates. Copper- and Pb-acetate were mineralised at significantly lower rates ($P < 0.01$). Cadmium acetate addition repressed the soil respiration such that negative values were obtained after 48 h of incubation (Fig. 1). The same trends were observed with citrate complexes. Both Mg and Zn complexes were mineralised at approximately the same extent, whereas Cu and Pb complexes were mineralised at significantly lower rates (Fig. 1). As with acetate, the lowest net cumulative respiration was observed with Cd-citrate addition.

In the grassland soil, Mg-acetate, Cu-acetate and Zn-acetate were mineralised to similar extents, whereas Pb-acetate was mineralised at a significantly lower rate ($P < 0.01$). Cadmium acetate inhibited soil basal

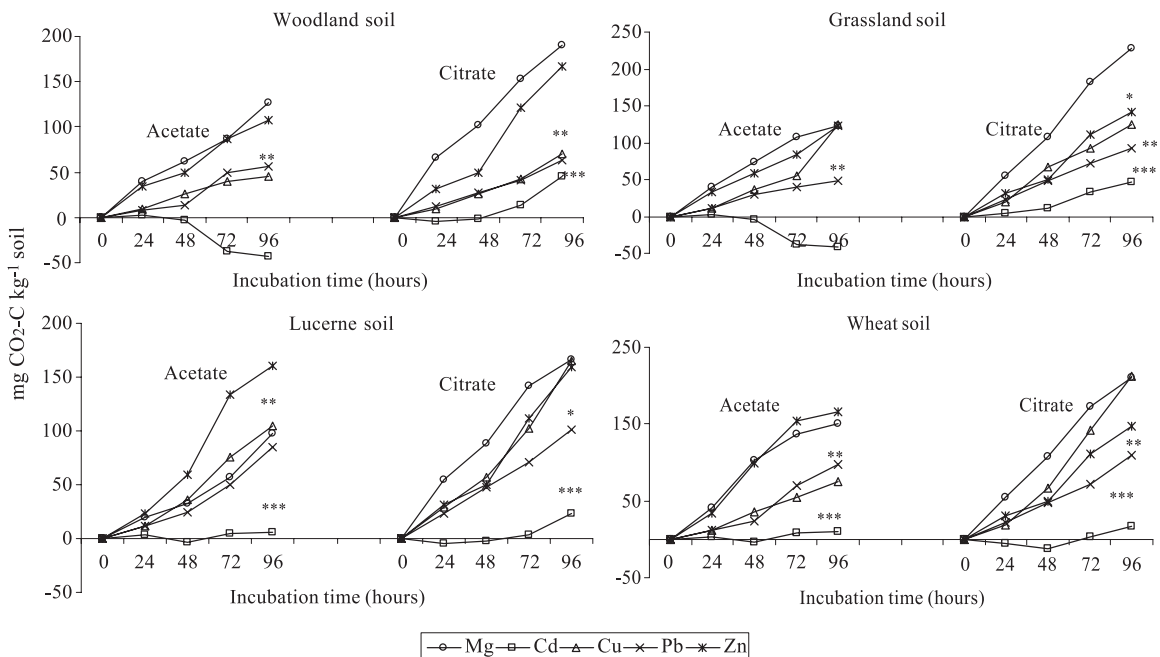


Fig. 1. Net decomposition of LMWOAs complexed with different heavy metals in the Vicarello soils. Symbols *, ** and *** indicate significant difference at P levels < 0.05 , 0.01 and 0.001 , respectively.

respiration, resulting in negative net respiration values (Fig. 1). In the case of metal–citrate complexes, the Mg-citrate gave the highest net cumulative respiration whereas Cu-, Pb- and Cd-citrate complexes were mineralised to the same extent after 96 h (Fig. 1).

In the lucerne soil, the highest net cumulative mineralisation rates were observed for Zn-acetate, whereas Mg-, Cu- and Pb-complexes were mineralised to similar extents (Fig. 1). Among the citrate complexes, those containing Mg, Cu and Zn gave similar mineralisation rates after 96 h incubation, whereas the mineralisation of Cd-citrate was very low for the duration of the experiment (Fig. 1).

In wheat soil, Mg- and Zn-acetate were mineralised to a similar extent. Copper- and Pb-acetate complexes were significantly less mineralised ($P < 0.01$). Among the citrate complexes, those containing Mg, Cu and Zn resulted in similar net cumulative respiration after 96 h of incubation whereas Pb-citrate and Cd-citrate were significantly less mineralised (Fig. 1).

4. Discussion and conclusions

The results indicated that in each soil, the mineralisation of different metal–LMWOAs complexes depended on the complexing metal. Considering the Mg complexes as a reference compound, only the LMWOAs complexed with Zn gave similar cumulative net respiration, whereas all of the other heavy metal complexes were generally degraded to a lesser extent, following the ranking order of degradability: Cu>Pb>>Cd. Cadmium acetate in particular, appeared to be toxic to the soil microflora as its addition resulted in negative values as compared to the control soils (Fig. 1).

The net percentage of mineralisation of the C added as metal–organic complex is shown in Table 2. Such values were calculated by subtracting those from the basal respiration. The percentages of mineralisation were higher with citrate than with acetate complexes. Probably, different metabolic pathways were involved in the biodegradation of the metal–organic complexes as a result of their utilization by different groups of microorganisms. Possibly, the greater complexing capacity of citrate than acetate would have led to a greater metal sequestration resulting in a lower inhibition on microbial metabolism.

Table 2

Percentage of C added as LMWOAs mineralised to CO₂ during the 96-h incubation

Me-acetate complexes decomposition (%)					
Soils	Mg-acetate	Cd-acetate	Cu-acetate	Pb-acetate	Zn-acetate
Woodland	44.1	–	15.2	18.9	35.7
Grassland	41.1	–	41.7	16.0	40.7
Lucerne	32.7	2.1	35.0	28.2	53.3
Wheat	50.4	3.2	24.8	32.3	55.1
Me-citrate complexes decomposition (%)					
Soils	Mg-citrate	Cd-citrate	Cu-citrate	Pb-citrate	Zn-citrate
Woodland	63.5	15.2	23.5	21.1	55.7
Grassland	76.3	15.3	41.8	30.7	47.7
Lucerne	55.4	7.6	55.0	33.6	53.2
Wheat	70.4	5.5	70.7	36.5	49.0

The values were calculated after subtracting the values of CO₂–C evolution of the respective control soils from those of the amended soils. For statistical analysis, see Fig. 1.

Degradation of LMWOAs is an intracellular process and the reduction of the basal respiration after the addition of Cd-acetate to woodland and grassland soils is an indirect proof that the soil microbial activity is affected by some heavy metal complexes. Francis et al. (1992) reported that the inability of microorganisms to degrade heavy metal–organic complexes could be due to their molecular configuration which might be unsuitable for enzymes involved in their metabolism, rather than by the toxicity of the heavy metals. Russell et al. (1998) reported that a mixed microbial population was able to degrade different metal–EDTA complexes according to the following ranking order: Fe>Cu>Co>Ni>Cd. It was suggested that different transporters having lower affinities for heavy metal-complexed EDTA could be used for uptake of metal complexes. However, it should be not excluded that other soil factors like nutrient availability might also be important in affecting the rates of biodegradation of metal complexes. Alaoukaty et al. (1991) reported that *Pseudomonas fluorescens* strains were able to grow on Pb-citrate as sole source of carbon if sufficient phosphorus was supplied to the growth medium.

To our knowledge, nothing is known about the indigenous microbial species involved in the degradation of heavy metal–organic complexes in situ. Results of this study suggest that it may be important to monitor the changes in microbial diversity and identify the

microbial groups that are responsible for the biodegradation of these molecules. The use of ^{14}C -labeled heavy metal complexes may also allow better discrimination of the fate of the metal complexes that are not mineralised, but may be incorporated into the soil organic matter.

References

- Ahumada, I., Mendoza, J., Escudero, P., Ascar, L., 2001. Effect of acetate, citrate, and lactate incorporation on distribution of cadmium and copper chemical forms in soil. *Commun. Soil Sci. Plant Anal.* 32, 771–785.
- Alaoukaty, A., Appanna, V.D., Huang, J., 1991. Exocellular and intracellular accumulation of lead in *Pseudomonas fluorescens* ATCC-13525 is mediated by the phosphate content of the growth medium. *FEMS Microbiol. Lett.* 83, 283–290.
- Babich, H., Stotzky, G., 1985. Heavy metal toxicity to microbe-mediated ecological processes. *Environ. Res.* 36, 591–594.
- Blackmer, A.M., Bremner, J.M., 1977. Gas chromatographic analysis of soil atmosphere. *Soil Sci. Soc. Am. J.* 41, 908–912.
- Brookes, P.C., 1995. Use of microbial parameters in monitoring soil pollution by heavy metals. *Biol. Fertil. Soils* 19, 269–279.
- Brynildsen, L., Allard, B., 1994. Influence of metal complexation on the metabolism of citrate by *Klebsiella oxytoca*. *BioMetals* 7, 163–169.
- Burkardt, C., Insam, H., Hutchinson, T.C., Reber, H.H., 1993. Impact of heavy metals on the degradative capabilities of soil bacterial communities. *Biol. Fertil. Soils* 16, 154–156.
- Errecalde, O., Campbell, P.G.C., 2000. Cadmium and zinc bioavailability to *Selenastrum capricornutum* (Chlorophyceae): accidental metal uptake and toxicity in the presence of citrate. *J. Phycol.* 36, 473–483.
- Francis, A.J., Dodge, C.J., Gillow, J.B., 1992. Biodegradation of metal citrate complexes and implications for toxic-metals mobility. *Nature* 356, 140–142.
- Hantke, K., 2000. Bacterial zinc transporters and regulators. *BioMetals* 14, 239–249.
- Hassen, A., Saidi, N., Cherif, M., Boudabous, A., 1998. Effects of heavy metals on *Pseudomonas aeruginosa* and *Bacillus thuringiensis*. *Bioresour. Technol.* 65, 73–82.
- Krishnamurti, G.S.R., Huang, P.M., van Rees, K.C.J., 1997. Kinetics of cadmium release from soils as influenced by organic acids: implication in cadmium availability. *J. Environ. Qual.* 26, 271–277.
- Moreno, J.L., Garcia, C., Landi, L., Falchini, L., Pietramellara, G., Nannipieri, P., 2001. The ecological dose value (Ed_{50}) for assessing Cd toxicity on ATP content and dehydrogenase and urease activities of soil. *Soil Biol. Biochem.* 33, 483–489.
- Naidu, R., Harter, R.D., 1998. Effect of different organic ligands on cadmium sorption by and extractability from soils. *Soil Sci. Soc. Am. J.* 62, 644–650.
- Poulsen, I.F., Hansen, H.C.B., 2000. Soil sorption of nickel in presence of citrate or arginine. *Water air soil pollut.* 120, 249–259.
- Puranik, P.R., Paknikar, K.M., 1999. Biosorption of lead, cadmium, and zinc by *Citrobacter* strain MCM B-181: characterization studies. *Biotechnol. Prog.* 15, 228–237.
- Robert, M., Berthelin, J., 1994. Role of biological and biochemical factors in soil mineral weathering. In: Huang, P.M., Schnitzer, M. (Eds.), *Interaction of Soil Minerals with Natural Organics and Microbes*. SSSA Special Publication, vol. 17. Soil Science Society of America, Madison, WI, pp. 453–495.
- Romer, W., Kang, D.K., Egle, K., Gerke, J., Keller, H., 2000. The acquisition of cadmium by *Lupinus albus* L., *Lupinus angustifolius* L., and *Lolium multiflorum* Lam. *J. Plant Nutr. Soil Sci.* 163, 623–628.
- Russell, A.P.T., Lawlor, K., Bailey, M., Mackasie, L.E., 1998. Biodegradation of metal–EDTA complexes by an enriched microbial population. *Appl. Environ. Microbiol.* 64, 1319–1322 (Humic substances from volcanic soils, *Geoderma* 80, 327–338).
- Stevenson, F.J., 1994. *Humus chemistry: Genesis, Composition, Reactions*, 2nd ed. Wiley, New York.
- Taniguchi, S., Yamagata, N., Sakurai, K., 2000. Cadmium adsorption on hydroxyaluminosilicate–montmorillonite complex as influenced by oxalate and citrate. *Soil Sci. Plant Nutr.* 46, 315–324.
- Thomas, R.A.P., Beswick, A.J., Basnakova, G., Moller, R., Macaskie, L.E., 2000. Growth of naturally occurring microbial isolates in metal-citrate medium and bioremediation of metal-citrate wastes. *J. Chem. Technol. Biotechnol.* 75, 187–195.
- Tsekova, K., Dentchev, D., Todorova, D., 2001. Effect of cadmium and copper on the production of citric acid by *Aspergillus niger*. *Folia Microbiol.* 39, 331–334.
- van Ginneken, L., Chowdhury, M.J., Blust, R., 1999. Bioavailability of cadmium and zinc to the common carp, *Cyprinus carpio*, in complexing environments: a test for the validity of the free ion activity model. *Environ. Toxicol. Chem.* 18, 2295–2304.