Chemical composition and Zn bioavailability of the soil solution extracted from Zn amended variable charge soils

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Abstract

A study on variable charge soils (volcanic Italian and podzolic Scottish soils) was performed to investigate the influence of soil properties on the chemical composition of soil solution. Zinc speciation, bioavailability and toxicity in the soil solution were examined. The soils were spiked with increasing amounts of Zn (0, 100, 200, 400 and 1000 mg/kg) and the soil solutions were extracted using rhizon soil moisture samplers. The pH, total organic carbon (TOC), base cations, anions, total Zn and free Zn^{2+} in soil solution were analysed. A rapid bioassay with the luminescent bacterium *Escherichia coli* HB101 pUCD607 was performed to assess Zn toxicity. The influence of soil type and Zn treatments on the chemical composition of soil solution and on Zn toxicity was considered and discussed. Different trends of total and free Zn concentrations, base cations desorption and luminescence of *E. coli* HB101 pUCD607 were observed. The soil solution extracted from the volcanic soils had very low total and free Zn concentrations and showed specific Zn^{2+}/Ca^{2+} exchange. The soil solution from the podzolic soil had much higher total and free Zn concentrations and showed no evidence of specific Zn^{2+}/Ca^{2+} exchange. In comparison with the subalkaline volcanic soils, the acidic podzol showed enhanced levels of toxic free Zn^{2+} and consequently stronger effects on *E. coli* viability.

Key words: rhizosamplers; chemical speciation; fractionation; ecotoxicological test

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Introduction

The soil liquid phase, generally referred to as soil solution, is an important soil component and contains dissolved chemicals in free and complexed forms, as well as chemicals associated with colloidal particles (Denaix et al., 2001; Holm et al., 1995). It is the medium through which not only nutrients, but also potentially toxic elements can move among biotic, solid and gas interacting soil compartments (Knight et al., 1998). Therefore, the analysis of soil solution can provide valuable information concerning bioavailability, mobility and geochemical cycling of chemicals in soil.

Zinc is a ubiquitous contaminant, which frequently occurs in agricultural, sludge amended and industrially polluted soils imposing a hazard to many plants and animals (Bostick et al., 2001; Holm et al., 1995). It is generally distributed among: (1) the soil solution, as free ions (Zn^{2+}) and organo-zinc complexes; (2) the soil colloidal fraction, as adsorbed and exchangeable Zn; (3) the soil solid phase, as insoluble complexes and secondary minerals (Kiekens, 1995). The soil type can have a strong influence on the progressive retention of potentially toxic elements (Martínez et al., 2001) and thus on the accumulation of Zn

(Alloway, 1995). Widely recognized is the importance of the variable-charge minerals of soils in sorbing nutrients and pollutants (Violante et al., 2008). The term "variable charge" is used to describe constituents whose charge varies with the pH of soil solution. Because of their large surface area, disordered network, and high charge density, poorly crystalline metal oxides and aluminosilicates react readily with anions, cations, and organic molecules. Sorption of ions onto variable-charge minerals and soils varies with pH due to a change of minerals charge. In volcanic soils, neoformed amorphous aluminosilicates (allophanes and imogolite) and organo-mineral compounds have been shown to play a key role in the high soil binding capacity for heavy metals (Nalovic et al., 1975; Tanneberg et al., 2001; Wada, 1989).

Potentially toxic elements can lead to negative effects on soil microbial populations (McGrath et al., 1999), such as reduction of biomass (Brookes and McGrath, 1984), decline of numbers or loss of specific beneficial microorganisms (Chaudri et al., 1993) and decrease of enzyme activity and substrate utilisation ability (Baath, 1989; Knight et al., 1997). The range of Zn concentrations leading to the onset of adverse effects on microbial processes is characterised by a high variability, mainly depending on the ability of soils to modify bioavailable

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Zn concentrations, the Zn sensitivity of soil microbial processes and the methodological differences among the bioassays (Giller et al., 1998; McLaughlin and Smolders, 2001). Zinc toxicity to soil microbial processes has rarely been related to Zn concentrations or Zn^{2+} activities in soil pore water (McLaughlin and Smolders, 2001). Bioluminescence-based biosensors represent a sensitive method in microbial ecotoxicity assessment (Steinberg et al., 1995). In bacteria, the expression of genes related to bioluminescence is controlled by a set of genes (*lux* genes), labelled the *lux* operon, encoding the bacterial luciferin-luciferase system. The insertion of *lux* genes, responsible for bioluminescence, into soil bacteria has enabled the development of rapid biosensors that combine the benefits attributed to Microtox® (an internationally adopted microbial biosensor, based on the use of the naturally bioluminescent marine bacterium *Vibrio fisicheri*) with ecological relevance to soils (Chaudri et al., 1999; McGrath et al., 1999; Paton et al., 1995, 1997).

The main purpose of this work was to study the speciation and bioavailability of Zn added to variable charge (volcanic and podzolic) soils during a short-term laboratory incubation and to examine the resulting soil solution properties. A bioassay with the bioluminescent bacterium *Escherichia coli* HB101 pUCD607 was used to quantify the toxicological effects of Zn. Sequential chemical extractions were performed to identify Zn geochemical forms in soil.

1 Materials and methods

1.1 Studied soils

Variable charge soils of different origin were studied: three volcanic soils from the Solofrana River valley (southern Italy) and a podzolic soil from the Grampian Region (eastern Scotland). Surface soil samples (0–30 cm depth) from two areas irrigated with the contaminated water of Solofrana River (V1 and V2), from an area of the Solofrana valley not influenced by either irrigation or overflowing with contaminated river water (V3) and from the Insch area of the Grampian region (G1) were collected and studied.

The Solofrana River valley constitutes the inner portion of the Sarno plain lands located in Campania Region (south-western Italy) between the volcanic complex of Somma-Vesuvio (NW), the Sarno Mountains (NE), the limestone Lattari Mountains (S) and the Tyrrhenian cost (W). Soil formation in the valley is strongly influenced by colluvial and alluvial processes. Volcanic material present on the mountains, sliding and eroded from the slope, concur to form very thick and fertile soils which show moderate to high andic properties (Terribile and di Gennaro, 1996). The Solofrana valley is characterised by very intensive agricultural (open field horticulture, fruit tree production and greenhouse horticulture and floriculture) and industrial activities (tanning). The widespread use of chemicals for agricultural practices and the uncontrolled dumping of polluted waste effluents from tanning plants

have produced Cr and Cu contamination of the valley's soils (Adamo et al., 2003, 2006).

The Grampian region is located in the eastern part of Scotland. The Insch valley is located about 5 km to the North of Bennachie and is described on the soil map unit 316 (Soil Survey of Scotland, 1982). This unit consists of brown forest soils, both freely and imperfectly drained, with some humus-iron podzols and noncalcareous gleys. Plant communities include arable and permanent pastures (*Lolio-Cynosuretum*) with some bent-fescue grasslands (*Achilleo-Festucetum tenuifoliae*) and herb-rich and dry boreal heather moors (parts of *Vaccinio-Ericetum cinereae*) (Soil Survey of Scotland, 1982).

1.2 Soil treatments and characterization

Soil samples were air-dried and sieved at 2 mm. Five subsamples of 1000 g each were put in plant pots, spiked with increasing amounts of Zn (0, 100, 200, 400 and 1000 mg/kg) as $ZnSO_4$ solution, and kept at 80% of the water holding capacity. All the subsamples were covered with parafilm and left to equilibrate undisturbed for 48 hr. Finally soil solution was extracted using inert rhizon soil moisture samplers. The bulk density of the soil in the pot was measured as the ratio between soil weight and occupied volume.

Soil samples were characterised for: $pH-H_2O$ (1:2.5, soil:water ratio), $pH-CaCl_2$ (1:1, soil:$CaCl_2$ 0.01 mol/L ratio), particle-size analysis (Andreasen's pipette method, pH 10, ultrasonic treatment 20 kHz, 75 W, 15 min), total carbon and total nitrogen (dry combustion, NA 1500 NCS equipment, Carlo Erba Instruments, Italy), and cation exchange capacity (CEC) ($BaCl_2$ at pH 8.1). Short-range-order aluminosilicates and amorphous iron oxides and hydrous oxides were determined in volcanic soil samples by acid-NH_4-oxalate (0.2 mol/L at pH 3.0) at a sample:extractant ratio of 1:100 after shaking for 4 hr in the dark at 25°C (Schwertmann, 1964).

The total Cr, Cu and Zn content of the volcanic soil samples was determined by acid digestion in a Milestone 900 microwave oven (Milestone S.r.l., Italy) in HF:HNO_3 1:5 solution at 600 W for 24 min. The three-step chemical extraction procedure (0.11 mol/L HOAc, 0.5 mol/L $NH_2OH·HCl$, 30% H_2O_2/1 mol/L NH_4OAc), developed by the Measurement and Testing Programme of the European Commission (Ure and Davidson, 2002), completed with an HF/HNO_3 digestion on the residual fraction, was used to fractionate Cr, Cu and Zn chemical forms in V1, V2 and V3 soils. In accordance with the applied sequential extraction procedure, the following metal fractions were extracted from the soils: (1) soluble, exchangeable and carbonate bound (HOAc-extractable), (2) occluded in easily reducible manganese and iron oxides (reducible), (3) organically bound (oxidizable), (4) metals mainly present in the mineral lattice structures (residual). The concentrations of the trace elements in the extracts were determined by a Perkin Elmer 3030 B atomic absorption spectrometer (Germany), equipped with deuterium-arc background correction in acidic samples (pH below 2.0).

1.3 Soil solution extraction and analyses

The soil solution was extracted by Rhizon Soil Moisture Samplers (Rhizosphere Research Products, Eijkelkamp, The Netherlands): two samplers were put in each pot for 24 hr. The samples of soil solution were collected in plastic bottles and stored at 4°C.

The following parameters of the soil solution were measured: total organic carbon (Labtoc TC/TOC-System by UV Digestion, Pollution & Process Monitoring, UK); pH; Na^+ and K^+ (Flame Photometer PFP 1, Petracourt Ltd., UK); Ca^{2+} and Mg^{2+} (BAIRD Atomic Absorption Spectrometer ALPHA 4, Talbot Scientific Ltd., UK); F^-, Cl^-, PO_4^{3-} and SO_4^{2-} (Dionex 45000, Dionex Corporation, USA); NO_3^- (Flow Ingestion Analyser FIAstar 5010, Tecator, Sweden).

The free Zn (Zn^{2+}) concentration in the soil solution was determined by the speciation method developed by Holm et al. (1995, 1998). The Zn concentration in the soil solution was measured before and after equilibrium between the soil solution and a Ca-saturated cation exchange resin (Amberlite CG 120, Lenntech BV, The Netherlands) was reached. The amount of free Zn was calculated by comparing the measurement values of the samples with those of a reference solution of known Ca and Zn concentration.

The total and free Zn concentrations in the soil solution were determined, after acidification with 2% HNO_3, by atomic absorption spectrometry.

1.4 *Escherichia coli* HB101 pUCD607 bioassay

A rapid bioassay based on the use of the OGM *E. coli* HB101 pUCD607 was performed. Freeze-dried cells of *E. coli* were resuscitated in 10 mL of 0.1 mol/L KCl and incubated at 25°C with shaking for 1 hr. Aliquots of 0.1 mL of the obtained cell suspension were added to cuvettes containing 0.9 mL of soil solution. After 15 min of *E. coli* exposure to the sample, light output was measured by a Jade luminometer (Labtech International Ltd., UK). The results were expressed as bioluminescence percentage of the control samples.

1.5 Statistical analyses

A correlation matrix (Pearson correlation coefficient) was calculated to find relationships between soil solution data. The evaluation of the association degree among variables, assumed as normally distributed, was based on calculating the value of the correlation coefficient (r) and testing it for 95% significance level. Scatter diagrams of all associated variables were drawn to check the uniform dispersion of plots and to help in the interpretation of significant and non-significant correlation coefficients. Only variables which resulted in clear correlation are reported and discussed in the text.

ANOVA analysis was performed, using the SPSS program, to determine differences among the studied soils. Statistical differences among means were tested using the Least Significant Difference (LSD) test at 95% confidence level.

2 Results and discussion

2.1 Soil properties

The soils from the Solofrana valley (V1, V2, V3) and from the Grampian region (G1) differed in their main physical and chemical properties (Table 1). In accordance with their volcanic nature, V1, V2 and V3 had a silty-sand texture, a very low bulk density ((0.7–0.8) × 10³ kg/m³), a WHC varying between 47.5 mL/kg (V3) and 60.4 mL/kg (V2), a neutral-subalkaline pH, a C/N ratio ranging between 9 (V3) and 15 (V2), depending on the variation of the total carbon content, and a CEC higher than 30 cmol$_{(+)}$/kg. The G1 soil presented a sandy-loam texture, a bulk density of 1.2 kg/m³, a WHC of 67.7 mL/kg, an acid pH, a C/N ratio of 13 (with C and N concentrations higher than in volcanic soils) and a CEC of 9.31 cmol$_{(+)}$/kg. As a consequence of long term irrigation with polluted river waters (Adamo et al., 2003), V1 and V2 soils showed Cu, Cr and (in the case of V1) Zn concentrations higher than the limits defined for green areas by current Italian law (Official Gazette of the Italian Republic, 2006: Cu, 120 mg/kg; Cr, 150 mg/kg; Zn, 150 mg/kg). In contrast, the unirrigated V3 soil, had Cu and Cr total contents under these limits (Table 1).

On the basis of the Fe, Al and Si oxalate extractable amounts (ox), V1 and V2 did not satisfy the Soil Survey Staff (1998) requirements for andic soils (V1: $Al_{ox} + 0.5Fe_{ox} = 1.5$; V2: $Al_{ox} + 0.5Fe_{ox} = 0.8$). Requirements were matched by V3 ($Al_{ox} + 0.5Fe_{ox} = 2.3$). Allophane (estimated according to Parfitt (1990): V1, 4.5%; V2, 2.8%; V3, 7.7%) and amorphous iron oxihydroxides (ferrihydrite

Table 1 Main physical and chemical properties of the studied soils

Soil (0–30 cm)	Particle-size fractions (g/kg)				Bulk density (× 10³ kg/m³)	WHC (mL/kg)	pH	
	2–0.2 mm	0.2–0.02 mm	0.02–0.002 mm	< 0.002 mm			H_2O	$CaCl_2$
V1	329	186	313	173	0.8	51.6	7.2	6.7
V2	266	254	319	161	0.8	60.4	7.6	7.3
V3	196	327	326	151	0.7	47.5	7.6	7.1
G1	214	363	308	115	1.2	67.7	4.4	4.1

Soil (0–30 cm)	Total C (g/kg)	Total N (g/kg)	C/N ratio	CEC (cmol$_{(+)}$/kg)	Cu (mg/kg)	Cr (mg/kg)	Zn (mg/kg)
V1	36	3	11	36.4	315	177	191
V2	49	3	15	32.3	214	151	121
V3	21	2	9	34.2	120	62	126
G1	67	5	13	9.31	n.m.	n.m.	n.m.

n.m.: not measured.

content according to Childs (1985): V1, 0.85%; V2, 0.68%; V3, 1.7%) were present in all soils although in low amounts. X-ray diffraction and FT-IR spectroscopy showed the clay fraction (< 2.0 μm) of all volcanic soils was made of 1:1 and 2:1 phyllosilicates (kaolinite, halloysite, illite, smectites, vermiculite) along with fine-grained plagioclases and quartz (Zampella et al., 2010).

2.2 Chemical composition of soil solution before and after Zn addition

An overwhelming difference in the pH of the soil solution was observed between the volcanic and podzol soils ($p < 0.001$) (Table 2). The pH of the soil solution from V1, V2 and V3 was subalkaline (7.3–8.0) and tended to decrease with the addition of Zn to the soils ($r = -0.68$, $p < 0.01$). In contrast, the very acid pH (4.3) of G1 soil solution did not appear to change with Zn addition. This might not necessarily indicate that the addition of Zn to G1 did not have any effects on the concentration of H_3O^+ (here indicated as C_H). Indeed the nature of pH (pH = $-\log C_H$) implies that a change of C_H in solution is more evident at high pH values where relatively small C_H variations produce one unit pH modification than at low pH where relatively higher C_H variations are required to produce an equivalent change.

Differences between the two soil types were also observed on TOC trends in soil solution ($p < 0.001$). The higher total carbon content of G1 compared with V1, V2 and V3 soils (Table 1) gives rise to the higher TOC amounts of the podzol soil solution (Table 2).

The pH and TOC of V1, V2 and V3 soil solutions were significantly correlated ($r = 0.75$, $p < 0.01$).

In V1, V2 and V3 soil solutions, the concentrations of base cations increased with Zn additions (Table 2 and Fig. 1) indicating the occurrence of a progressive solubilisation, due to exchange processes. Ca^{2+} appeared to be the main exchange cation for Zn because it showed the highest con-

centration and magnitude of increase. Mg^{2+}, K^+ and Na^+, in decreasing order of relevance, showed a trend similar to Ca^{2+}. In the G1 soil solution, only small and irregular variations of base cation concentration with regard to Zn addition were observed, possibly as a consequence of the low pH and CEC of the soil (Table 1).

Among the anions only Cl^- was significantly influenced by soil type ($p < 0.001$). With the exception of SO_4^{2-}, where concentration was strongly increased by the addition of Zn sulphate, anions did not show any significant and regular change in concentration with Zn addition to the soils (Table 2). The content of PO_4^{3-} was below detection limit in all soil solution samples.

G1 was characterised by higher SO_4^{2-} adsorption than V1, V2 and V3 soils, as shown by the lower concentrations of SO_4^{2-} anions in soil solution after $ZnSO_4$ additions. Soil pH is an important factor influencing the adsorption of SO_4^{2-} and the low pH may promote the adsorption of SO_4^{2-} in G1 (Curtin and Syers, 1990; Nodvin et al., 1986). No evidence of specific adsorption by soil and soil components (oxides and allophanes), with OH^- and OH_2 displacement by SO_4^{2-} (Rajan, 1978), was found. Indeed the pH of the soil solution did not increase. In the meantime, organic matter and in particular dissolved organic carbon would inhibit SO_4^{2-} adsorption, competing for adsorption sites (Nodvin et al., 1986). Therefore, the higher organic carbon of G1 soil and soil solution, compared with volcanic soils, should correspond to a lower SO_4^{2-} adsorption. Information available from this study was not sufficient to draw an unequivocal conclusion about the importance of organic matter factoring influencing SO_4^{2-} adsorption in these soils.

2.3 Zn bioavailability in the soil solution

The soil solution of G1 was always characterised by higher total and free Zn levels compared with the soil solution of V1, V2 and V3 soils (Fig. 2). In G1, a very

Table 2   Chemical properties of the soil solution before and after Zn addition

Soil (0–30 cm)	Zn treatment (mg/kg)	pH	TOC (mg/L)	Na$^+$ (mg/L)	K$^+$ (mg/L)	Ca^{2+} (mg/L)	Mg^{2+} (mg/L)	F$^-$ (mg/L)	Cl$^-$ (mg/L)	NO$_3^-$ (mg/L)	PO$_4^{3-}$ (mg/L)	SO$_4^{2-}$ (mg/L)
V1	0	7.8 abc	5.77 ab	9.4 a	15.3 ab	99.3 ab	31.3 ab	0.5	22.2 bcd	13.7	<	84.5
	100	7.6 cde	5.15 a	10.3 ab	19.0 ab	146.0 ab	45.4 abc	0.5	21.3 cd	13.7	<	379.8
	200	7.5 def	4.96 a	12.1 abc	22.5 abc	199.1 ab	65.2 abc	0.5	20.4 de	13.7	<	>500
	400	7.6 cde	5.48 a	12.6 abc	26.5 abc	372.8 bcd	94.3 bcd	0.4	21.7 cd	14.6	<	>500
	1000	7.3 f	5.45 a	17.9 bcde	37.4 bc	771.8 e	192.8 e	<	20.6 de	14.6	<	>500
V2	0	8.0 a	7.22 ab	17.9 bcde	17.5 ab	154.6 ab	34.3 ab	0.4	27.5 ad	1.3	<	114.5
	100	7.9 ab	6.77 ab	19.9 cde	20.5 ab	187.3 ab	43.0 ab	0.5	25.0 abcd	7.8	<	363.8
	200	7.9 ab	6.38 ab	20.3 cde	22.7 abc	216.4 ab	51.2 abc	0.5	26.2 abd	9.4	<	>500
	400	7.7 bd	6.30 ab	25.3 ef	25.1 abc	370.6 abcd	72.3 abc	0.5	23.4 abcd	7.8	<	>500
	1000	7.6 cde	6.69 ab	31.0 f	31.7 abc	681.9 de	127.0 cde	0.3	24.5 abcd	7.8	<	>500
V3	0	7.8 abc	6.86 ab	10.8 ab	24.0 abc	47.6 ab	20.1 ab	0.5	21.9 bcd	15.3	<	8.2
	100	7.6 cde	5.70 ab	12.1 abc	31.3 abc	93.6 ab	32.0 ab	0.4	21.8 cd	16.0	<	293.9
	200	7.6 cde	5.37 a	13.8 abc	37.1 bc	135.9 ab	45.8 abc	0.4	22.2 bcd	15.3	<	>500
	400	7.4 ef	5.09 a	17.9 bcde	46.5 c	311.4 abc	82.3 abcd	0.4	20.9 cde	15.0	<	>500
	1000	7.4 ef	5.80 ab	25.1 ef	76.6 d	578.9 cde	158.3 de	<	21.4 cd	15.3	<	>500
G1	0	4.3 g	10.67 ab	15.7 abcd	8.8 a	24.8 a	7.4 a	0.5	15.1 f	7.8	<	18.8
	100	4.3 g	16.70 bc	25.2 ef	8.2 a	29.7 ab	7.9 a	0.4	16.7 ef	7.8	<	57.6
	200	4.3 g	21.90 cd	16.0 acd	8.5 a	34.7 ab	5.0 a	0.3	16.8 ef	7.8	<	120.6
	400	4.3 g	18.28 c	19.8 cd	7.9 a	29.7 ab	6.9 a	0.2	15.2 f	7.8	<	>500
	1000	4.3 g	30.20 d	22.2 de	11.5 a	52.0 ab	9.2 a	0.6	10.2 g	94.4	<	>500
LSD		0.3	11.00	8.3	24.6	345.9	83.5	n.s.d.	4.3	n.s.d.	n.s.d.	n.s.d.

LSD: least significant difference; <: below detection limit; n.s.d.: no significant difference.
* The same letters indicated there are no significant difference at 0.05 level among soils and treatments.
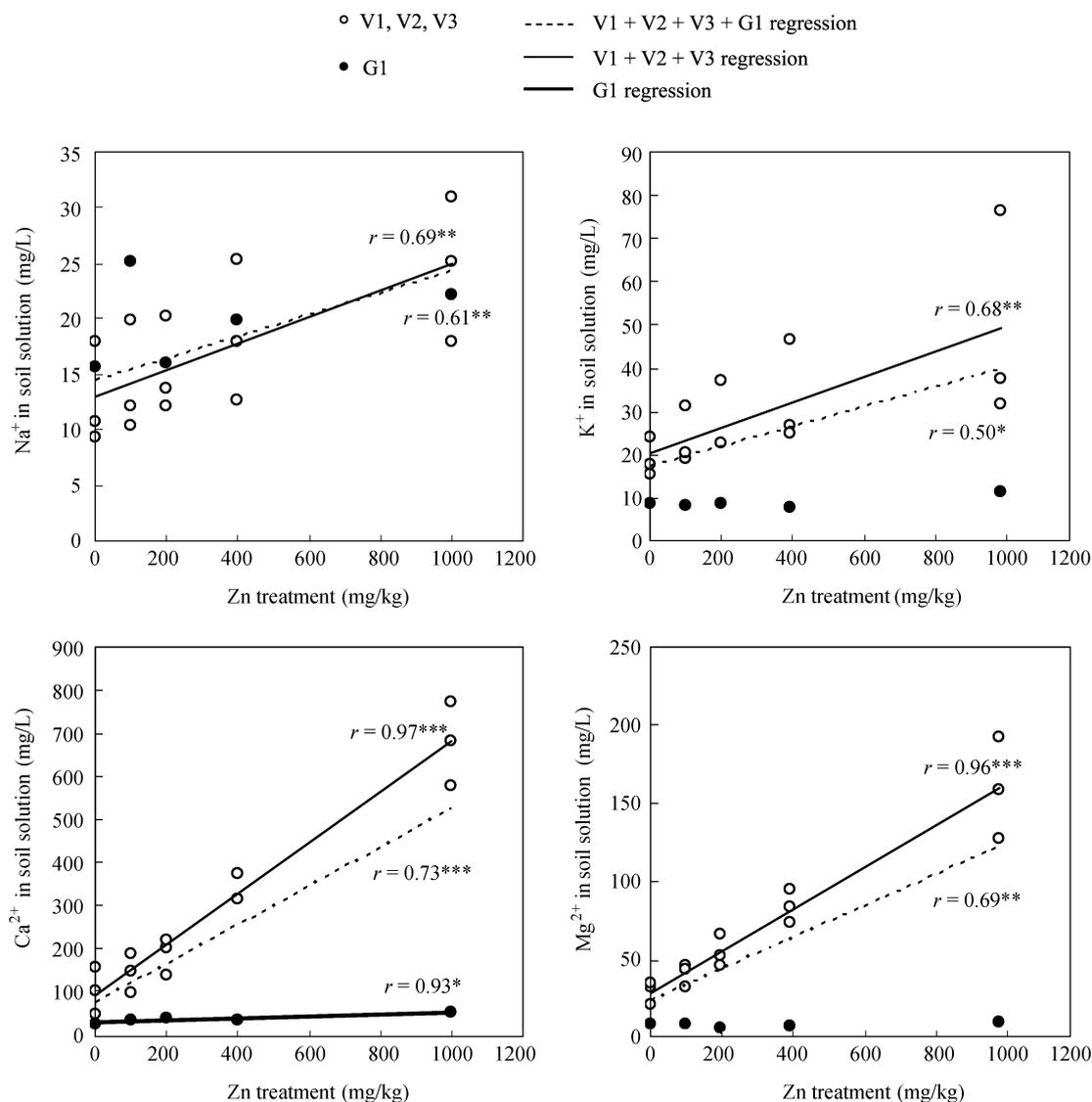
Fig. 1 Correlations among concentrations of Na^+, K^+, Ca^{2+} and Mg^{2+} in the soil solution and Zn additions. Significant correlations are marked with * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

small concentration of total Zn (0.05 mg/L) occurred in the soil solution before Zn addition. Both the total and free Zn concentrations in solution increased after the treatments and were positively correlated with the amounts of Zn added ($r \geqslant 0.90$, $p < 0.05$). In V1, V2 and V3 soil solutions, total Zn became detectable only after the addition of 200 mg/kg of Zn to the soils. Moreover, V1, V2 and V3, when compared with G1, were characterised by much lower increments corresponding to equal amounts of Zn added, showing a higher binding capacity for Zn. The subalkaline pH of V1, V2 and V3 soils, along with their high CEC, likely played a key role in reducing the Zn levels in soil solution. A decrease of Zn solubility with increasing pH has been reported by many authors (Harter, 1983; Msaky and Calvet, 1990; Pardo and Guadalix, 1996; Stahl and James, 1991). The pH of the soil solution was inversely correlated with the concentration of total and free Zn ($r \leqslant -0.60$, $p < 0.01$), suggesting an influence of pH on Zn solid:liquid partitioning. These results concur with

those found by other authors (Anderson and Christensen, 1988; Buchter et al., 1989; Janssen et al., 1997), who examined the effect of soil pH on the solid:liquid partitioning coefficient K_d and observed an inverse correlation between Zn in soil solution and pH. Sorption of Zn by Fe and Mn hydrous oxides, clays, allophanes and organic matter has been suggested (Abd-Elfattah and Wada, 1981; Cavallaro and McBride, 1984; Harter, 1983; Hickey and Kittrick, 1984). Specific adsorption reactions (Kiekens, 1995), pH-dependent adsorption sites and broken bonds may contribute to selective or preferential Zn adsorption (Maes, 1973; Van Der Weijden, 1975). Kiekens (1980) studied the reversibility of the exchange reaction between Ca and Zn and found that an important fraction of Zn was irreversibly fixed by the soil following adsorption of Zn^{2+} and desorption of Ca^{2+} during Zn^{2+}/Ca^{2+} exchange. Our results, in agreement with these studies, indicated that the volcanic soils from the Solofrana valley (V1, V2 and V3) had a high binding capacity for Zn, due to their high pH
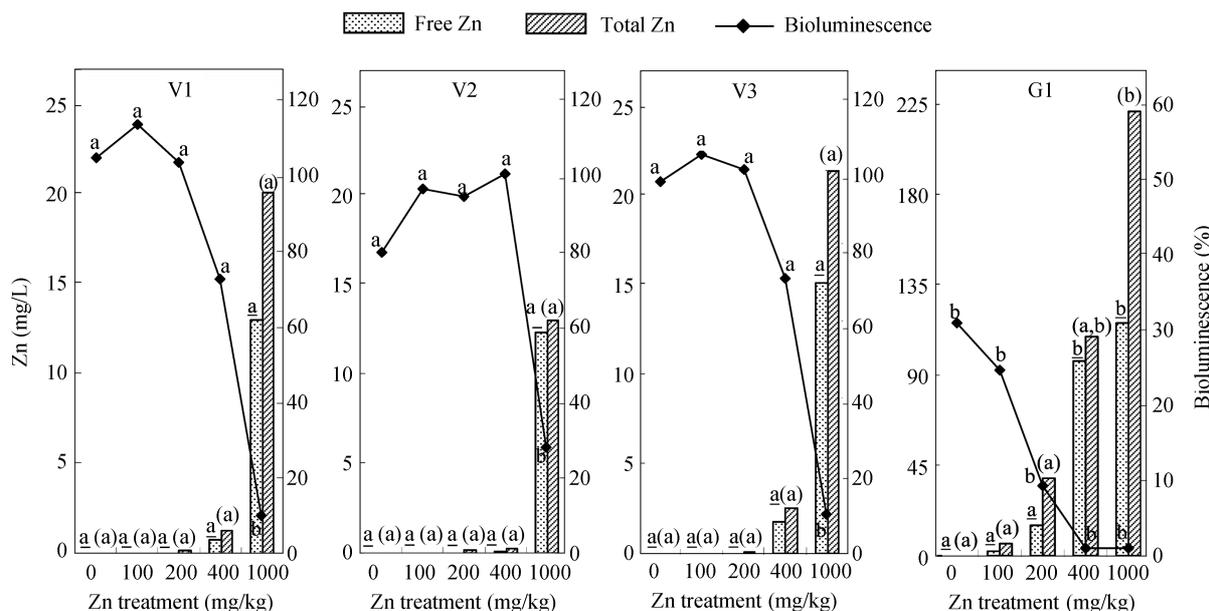
Fig. 2 Total and free Zn concentrations in the soil solution of studied soils amended with $ZnSO_4$ and bioluminescence of *E. coli* HB101 pUCD607. Significant differences among soils and treatments are marked with letters (bioluminescence), letters in brackets (total Zn) or letters underlined (free Zn); means sharing the same letter are not significantly different from each other at $p < 0.05$.

values, presence of variable charge adsorption sites and high CEC. The Zn^{2+}/Ca^{2+} exchanges may play key roles in the selective adsorption of Zn in these soils. Part of the added Zn may be irreversibly fixed by Solofrana soils, as suggested by the very low Zn concentration in the liquid phase compared with the amount of Zn added to the soils.

The studied soils showed different trends also for the bioluminescence of *Escherichia coli* HB101 pUCD607, after Zn treatments (Fig. 2). In accordance with what was previously observed by Chaudri et al. (2000), in V1, V2 and V3 soils, luminescence of *E. coli* HB101 pUCD607 was inversely correlated with Zn additions ($r < -0.92$, $p < 0.001$), and with total ($r < -0.96$, $p < 0.001$) and free ($r < -0.96$, $p < 0.001$) Zn concentration in solution. In G1 no significant correlation was found. Soil types can have a strong influence on the relationship between total and free Zn concentrations in soil solution (Knight et al., 1998; McGrath et al., 1999). The bioluminescence of *E. coli* HB101 pUCD607 in volcanic soils untreated with Zn was very high (Fig. 2). After 100 and 200 mg/kg Zn additions, when no free Zn was detected in soil solution, the bioluminescence of *E. coli* HB101 pUCD607 was even higher, presumably as a consequence of the higher availability of major nutrients due to the competition between Zn and basic cations for exchange sites. Only with further Zn additions to soil and corresponding increase of free Zn in the soil solution did a decrease of bioluminescence occur. Strong evidence of an effect of Zn on *E. coli* viability was found only after the addition of 1000 mg/kg of Zn ($p < 0.001$). The free ion is indicated by many authors as the toxic species and as a reliable chemical measure of Zn toxicity (Chaudri et al., 2000; Knight et al., 1998; McGrath et al., 1999; Ritchie et al., 2001).

In the podzol (G1), *E. coli* HB101 pUCD607 bioluminescence was already low (30%) before Zn treatments, when total Zn in soil solution was equal to 0.05 mg/L and

no free Zn was detected (Fig. 2). The bioluminescence decreased with Zn additions in the podzol but no significant difference of an effect of Zn on *E. coli* viability was found. The acid pH of the podzol is likely to have promoted the toxic free Zn^{2+} forms in soil solution, but might also have had a direct effect on *E. coli* viability.

E. coli is neutrophil, with an optimum pH between 6.0 and 7.0, and with lowest and highest pH limits of 4.4 and 9.0, respectively (Prescott et al., 1999). The pH of the podzol was far from optimal and at the limit of the acidity tolerated by *E. coli*. The pH of the volcanic soils, on the contrary, was close to the optimum pH range.

2.4 Zinc fractionation

The sequential extraction used in this study is useful to indirectly assess the potential mobility and bioavailability of Zn in the soil, assuming that the mobility/availability is inversely related to the order of extraction. Results obtained showed an increase of Zn mobility/availability in V1, V2 and V3 soils with the addition of increasing amounts of the element (Fig. 3). Zn was mainly found in the first two fractions (HOAc-extractable and reducible), where the element accumulated preferentially after additions. A small increment characterized also the organic bound forms. The absolute amounts of Zn in the first three fractions were well correlated with the amounts of Zn added to soil ($r \geqslant 0.89$, $p < 0.05$). According to the literature, it is commonly found that anthropogenic additions of heavy metals to soil cause increases in available and/or potentially available fractions that follow the same trend as the total heavy metal content (Chlopecka et al., 1996). The Zn fractions, expressed as percentages, showed different trends. The HOAc-extractable percentage increased progressively in correlation with Zn additions ($r \leqslant 0.96$, $p < 0.05$), while reducible percentage initially increased (100 and 200 mg/kg Zn treatments), and then showed no
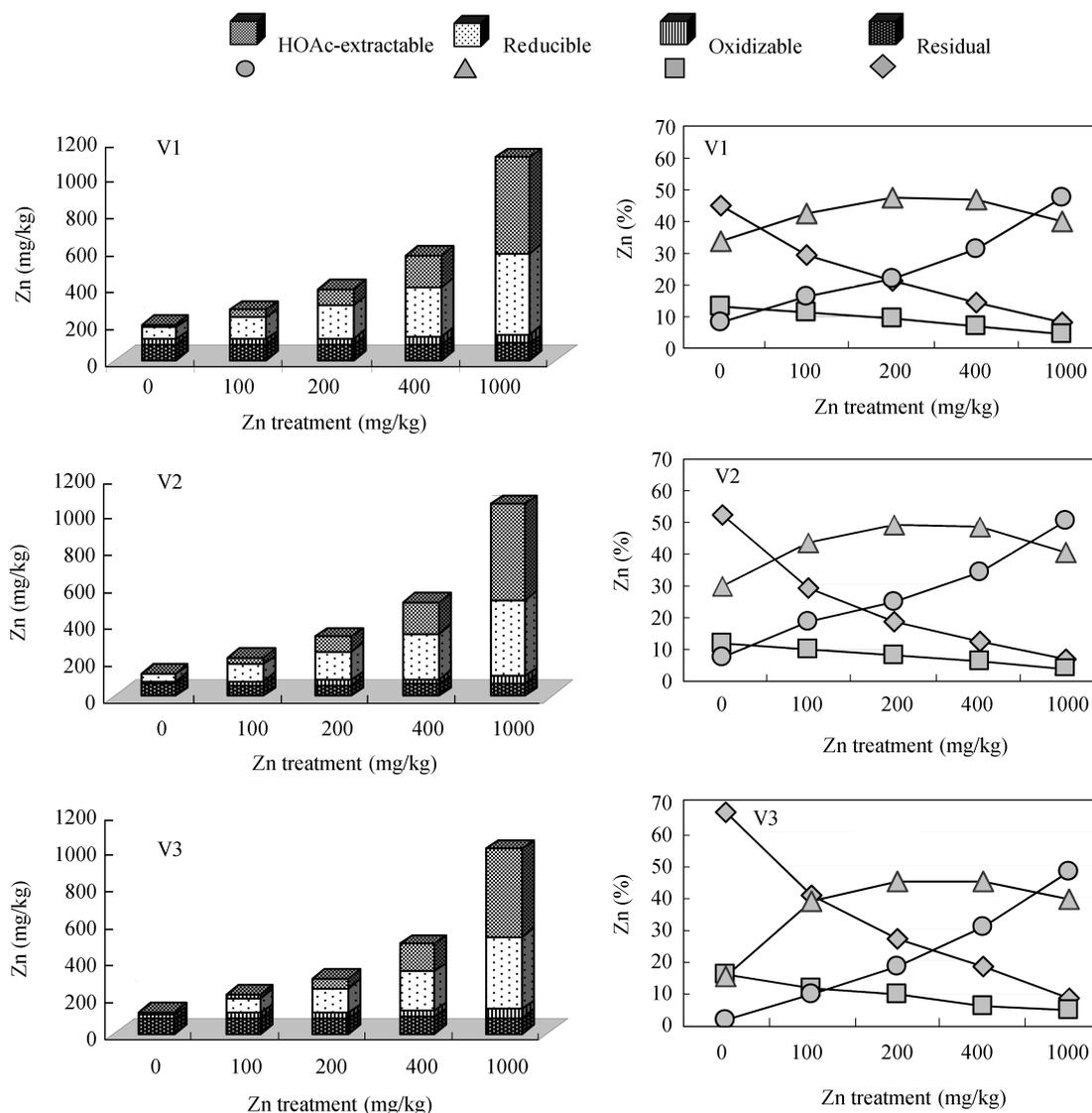
Fig. 3 Absolute (mg/kg of soil dry weight) and relative (% of the total) amounts of Zn extracted sequentially from the volcanic soils at increasing amounts of Zn added to the soil.

change or even a reduction after further additions. The oxidizable and residual percentages negatively correlated with the amounts of Zn added to soils ($r \leqslant -0.78$, $p < 0.05$). The order of Zn association with various chemical fractions was the same for the three soils collected from the Solofrana valley, but varied with Zn additions. In general, the association of Zn in the soils before Zn addition was in the decreasing order of: residual > reducible > oxidizable > HOAc-extractable. At the maximum Zn addition (1000 mg/kg) the percent of total Zn associated with different fractions in the three soils was changed in the following order: HOAc-extractable > reducible > residual $\geqslant$ oxidizable.

On the other hand, Zn additions did not affect the fractionation of the main soil metal pollutants Cu and Cr, which are on average as shown in Fig. 4. The results obtained for V1, V2 and V3 soil solution (Fig. 2) were compared with those obtained for the soils' solid phase (Fig. 3). Total and free Zn concentrations in the soil solution were much lower than concentrations of Zn extracted

from the soil in the first step of the BCR procedure. Accordingly, the Zn extracted in this first step was mainly in exchangeable and carbonate-bound forms. In addition, good correlation between total and free Zn in the soil solution and HOAc-extractable Zn was observed ($r \leqslant 0.84$, $p < 0.05$).

3 Conclusions

Considerable differences between variable charge volcanic and podzol soils were observed in total and free Zn concentrations in soil solution, basic cations desorption, and luminescence of *E. coli* HB101 pUCD607, following progressive Zn addition.

Very low total and free Zn concentrations relative to added Zn were found in the volcanic soil solutions. Evidence of specific Zn^{2+}/Ca^{2+} exchange, with consequent Zn^{2+} adsorption and Ca^{2+} desorption, suggests that part of the Zn may be irreversibly fixed. In the podzolic soil
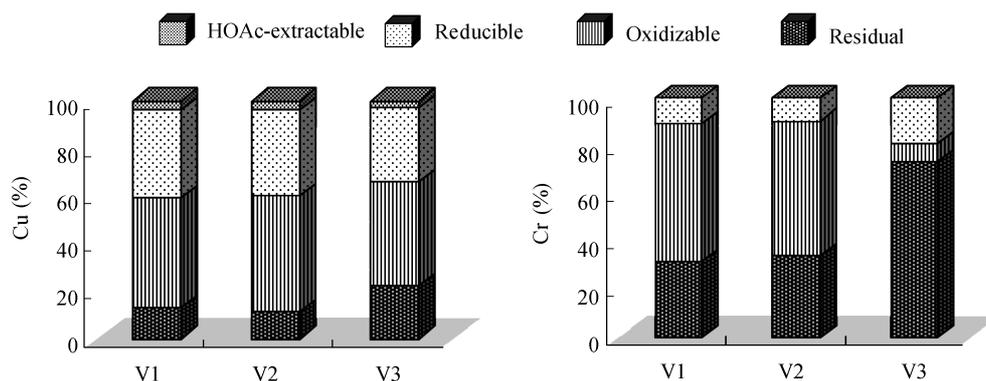
Fig. 4   Average of relative (% of the total) amounts of Cu and Cr extracted sequentially from the volcanic soils.

solution, total and free Zn concentrations were higher and no evidence of specific Zn^{2+}/Ca^{2+} exchange was observed. Differences between soil solution pH and TOC also occurred. The pH, subalkaline in the volcanic and acidic in the podzolic soils, is likely to have promoted free Zn^{2+} forms in the podzol and to have played a key role in the differences observed for the two variable charge soil types with respect to chemical forms and toxicity of Zn.

Acknowledgments

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