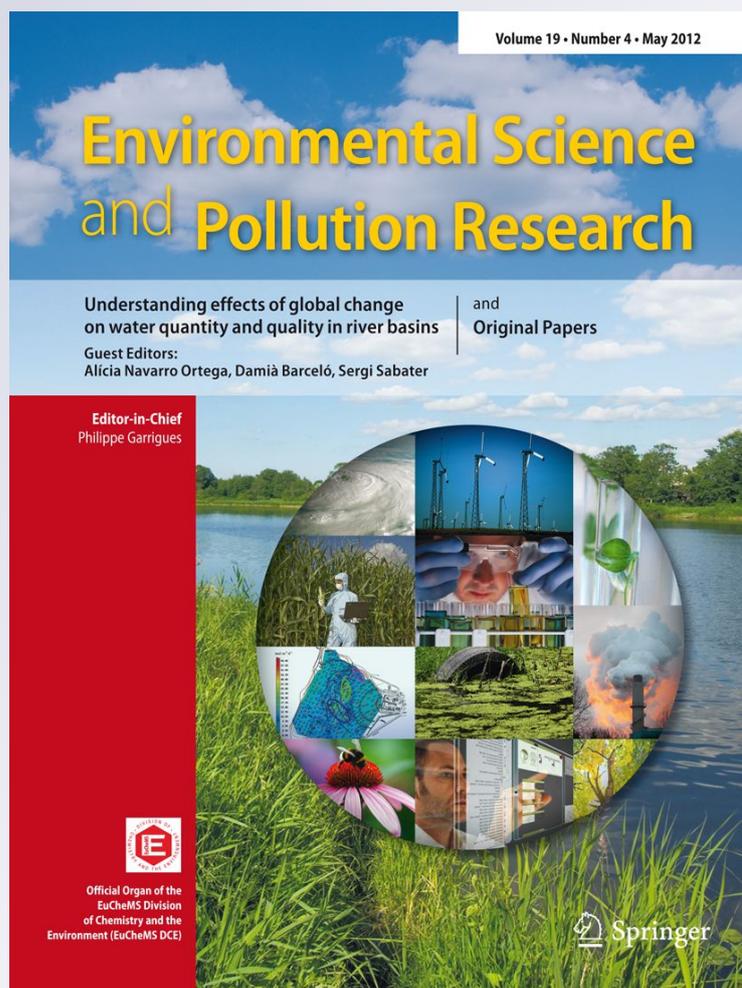# Simultaneous immobilization of metals and arsenic in acidic polluted soils near a copper smelter in central Chile

Valeska Cárcamo, Elena Bustamante, Elizabeth Trangolao, Luz María de la Fuente, Michel Mench, Alexander Neaman & Rosanna Ginocchio

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RESEARCH ARTICLE

Simultaneous immobilization of metals and arsenic in acidic polluted soils near a copper smelter in central Chile

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Luz María de la Fuente · Michel Mench · Alexander Neaman · Rosanna Ginocchio

Abstract

Introduction Acidic and metal(oid)-rich topsoils resulted after 34 years of continuous operations of a copper smelter in the Puchuncaví valley, central Chile. Currently, large-scale remediation actions for simultaneous in situ immobilization of metals and As are needed to reduce environmental risks of polluted soils. Aided phytostabilization is a cost-effective alternative, but adequate local available soil amendments have to be identified and management options have to be defined.

Materials and methods Efficacy of seashell grit (SG), biosolids (B), natural zeolite (Z), and iron-activated zeolite (AZ), either alone or in mixtures, was evaluated for reducing metal (Cu and Zn) and As solubilization in polluted soils under laboratory conditions. Perennial ryegrass was used to test phytotoxicity of experimental substrates.

Results Soil neutralization to a pH of 6.5 with SG, with or without incorporation of AZ, significantly reduces metal (Cu and Zn) solubilization without affecting As solubilization in soil pore water; furthermore, it eliminates phytotoxicity and excessive metal(oid) accumulation in aerial plant tissues. Addition of B or Z to SG-amended soil does not further reduce metal solubilization into soil pore water, but increase As solubilization due to excessive soil neutralization (pH>6.5); however, no significant As increase occurs in aerial plant tissues.

Conclusion Simultaneous in situ immobilization of metal(oid) in acidic topsoils is possible through aided phytostabilization.

Keywords Natural zeolite · Biosolids · Seashell grit · Metal phytotoxicity · Ryegrass

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1 Introduction

Soils surrounding the Ventanas copper smelter in the Puchuncaví valley, central Chile, were heavily degraded due to continuous operation of the Cu concentrate processing facility in the absence of any environmental regulations from 1964 to 1992 (Environmental Resources Management 1993). Elevated emissions of sulfur dioxide (mean of 399 t day^{-1}) to the atmosphere resulted in the occurrence of strong acid rain deposition with acute damage to local vegetation and soil acidification to a pH of 3.5–4.7 (ECUS 1996; Ginocchio 2000). Simultaneously, atmospheric fall-out of metal(oid)-rich particulate matter (mean of 12 t day^{-1}) resulted on enrichment of soils with metals (i.e., Cu, Zn, Pb, Cd) and As (ECUS 1996; Ginocchio

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2000; Ginocchio et al. 2004). For example, total Cu (the main metal pollutant) and As contents measured in topsoils (0–30 cm depth) reached values up to 1,214 and 120 mg kg^{-1}, respectively (ECUS 1996; González et al. 2008). As a result of the acid rain and metal/As pollution, plant richness and abundance were dramatically reduced, leaving soils exposed to wind and rain erosion. As a result, soils became deficient particularly in terms of both organic matter and macronutrient contents (Ginocchio 2000; Ginocchio et al. 2004).

Even though atmospheric emissions of pollutants from the processing facility were effectively controlled by 1999, several studies have demonstrated that metals/As remains in acidic topsoils (Ginocchio et al. 2004; Neaman et al. 2009). Therefore, in rainy months of the year (winter period), when plant development also occurs, metal solubility and toxicity to plants are expected to occur (Ginocchio 2000; Ginocchio et al. 2006). This may be favored by acidic soil conditions (Sauvé et al. 2000; Ginocchio et al. 2002) and the high percentage (56–67% *w/w*) of water-soluble salts (i.e., $CuSO_4$, $ZnSO_4$, $PbSO_4$, As_2O_3) contained in dusts generated by base metal smelters (Skeaff et al. 2011). Indeed, it was demonstrated that soil acidification and the increase in soluble Cu concentration in topsoils explained 10% and 7%, respectively, of the reduction in plant abundance detected toward the Ventanas Cu smelter (Ginocchio 2000). Moreover, a laboratory assay demonstrated that Cu contained in smelter dust is more likely to be bioavailable and, thus, toxic to lettuce (*Lactuca sativa* L.) plants compared to other massive mine wastes (i.e., smelter slag, tailings) at equivalent total soil Cu concentrations when artificially spiked into agricultural soils (Ginocchio et al. 2006).

Currently, large-scale remediation is needed in the Puchuncaví valley to hold and reverse strong degradation of soils and to reduce environmental risks posed by metals/As accumulated in topsoils. However, this is not an easy task as feasible treatment options for As-polluted soils are limited (Lombi et al. 2004). In situ immobilization of metals in topsoils through aided phytostabilization has been recommended as a cost-effective method of soil remediation (Berti and Cunningham 2000; Mench et al. 2000, 2010). However, the remediation of soils co-polluted by metals and As through in situ aided phytostabilization has been less evaluated (i.e., Kumpiene et al. 2006; Mench et al. 2006; Lee et al. 2011).

In the case of multielemental polluted soils, particularly those co-polluted with metals and As, successful in situ stabilization depends on the combination of critical elements in the soil and the choice of proper amendments (U.S. EPA 2007; Kumpiene et al. 2008). For example, lime and other alkaline materials are known to reduce metal solubility in polluted soils (Lombi et al. 2003; Ruttens et al. 2006; Lopareva-Pohu et al. 2011) while organic matter is known to

strongly adsorb Cu cations (i.e., Ruttens et al. 2006; Bes and Mench 2008). However, amelioration of soluble soil metals by increasing soil pH to neutral values may result in increased solubilization of As and potential bioaccumulation of this element through food webs (Sauvé et al. 1997; Adriano 2001). Therefore, appropriate soil amendments and rates of application have to be carefully defined for simultaneous in situ immobilization of metals and As in polluted soils. The U.S. EPA (2007) has suggested to adjust soil pH between 5.5 and 6.5 to decrease metal bioavailability/phytotoxicity in metal-polluted soils when As is also present. Addition of organic matter, medium rates of phosphorous (P) fertilizer, and/or sorbents such as zeolites, charcoal, and high-surface area iron oxides have been also suggested as effective sorbents for both anions and cations (U.S. EPA 2007; Kumpiene et al. 2006, 2008). Therefore, the main objective of the present study was to evaluate, under laboratory controlled conditions, the efficacy of some local available organic (i.e., municipal biosolids) and inorganic (i.e., zeolites and seashell grit) amendments to reduce the solubility of both metals and As, and thus phytoxicity and accumulation into aerial plant tissues, when added into polluted topsoils surrounding the Ventanas copper smelter. Results of the present study will allow the development of a much more focused rehabilitation program at the field scale.

2 Materials and methods

2.1 Study soils

An 80-kg batch of polluted topsoil (0–30 cm depth) was collected at Los Maitenes (34°45′00″ LS, 71°29′00″ LW), a site located 2 km to the southeast of the Ventanas Cu smelter, Puchuncaví valley, central Chile; this soil was used as negative control (C−). The same amount of topsoil was collected from a reference site (32°47′26″ LS, 71°31′20″ LW) located 5 km to the south of the smelter facility, where plant communities are considered representative of the site before contamination (Ginocchio 2000); this soil was used as a positive control soil (C+). Composite samples of topsoils were collected at each site with a stainless steel shovel and transported to the laboratory in plastic containers. Soils were homogenized, steam-sterilized (70°C) to eliminate the seed bank, dried at 30°C, and sieved to pass a <2-mm nylon mesh. Elimination of viable seeds from selected soils was required to avoid any type of interference in the plant cultivation assay described below. A summary of selected properties of study soils is given in Table 1. Polluted and reference site soils were alike with the exception of pH (one unit lower at Los Maitenes), and total Cu, Zn, Cd, Pb, and As contents (from 1.4 to 4.5 times higher at Los Maitenes; Table 1).

Table 1 General physicochemical characteristics of study soils (polluted and reference sites) collected at the Puchuncaví valley, central Chile

Parameter	Los Maitenes (polluted soil)	Reference site (reference soil)
Sand (%)	78	83
Clay (%)	3	3
Silt (%)	19	14
Density (g cm^{-3})	1.4	1.4
pH (water)	5.58	6.30
EC (dS L^{-1})	0.14	0.17
CEC (cmol+ kg^{-1})	9.07	8.55
Organic matter (%)	2.10	1.25
Total N (%)	0.09	0.06
Available N (mg kg^{-1})	29.4	15.3
Available P (mg kg^{-1})	42.5	33.1
Available K (mg kg^{-1})	216	267
Total Cu (mg kg^{-1})	685	209
Total Zn (mg kg^{-1})	159	115
Total Cd (mg kg^{-1})	0.9	0.2
Total Pb (mg kg^{-1})	85	19
Total Fe (%)	5	5
Total As (mg kg^{-1})	51	21

EC electric conductivity, *CEC* cation exchange capacity

2.2 Amendments

To reduce metals and As solubilization in polluted soils of Los Maitenes, a range of local available amendments were selected. Seashell grit (SG) from Sociedad Contractual Minera Aceituno, a natural calcium carbonate-rich material, was used for acidity neutralization as several mines exist in coastal areas of north-central Chile. Laboratory protocols for calculating the acid–base account from polluted study soil were used to determine the appropriate application rate to increase soil pH from 5.6 to 6.5, as suggested by the U.S. EPA (2007) when excess As is also present in a metal-polluted soil. Application rate was 0.5% (*w/w*), dry weight basis (d.w.).

Class B fresh biosolids (B; 76% of water content) from El Trebal plant of Aguas Andinas S.A. (central Chile), the primary organic solid product yielded by municipal wastewater treatment processes (Haering et al. 2000), were selected as organic amendments as they are readily available at free cost. Fresh biosolids came directly from the centrifuge at the end of the anaerobic stage of the municipal water treatment process. Biopolymers are used for dewatering of biosolids during centrifugation process; no alkaline materials are added into biosolids. Metals having elevated concentrations in selected biosolids were Cu (664 mg kg^{-1}, d.w.) and Zn (1,366 mg kg^{-1}, d.w.) while As levels were 18 mg kg^{-1} d.w. Application rates were

defined considering the maximum rate (50 t ha^{-1}, d.w.) allowed for degraded soils according to the Chilean guidelines and twofold this rate (100 t ha^{-1}, d.w.), as metal-contaminated sites (primarily hard rock mining sites) in the USA have been restored with biosolids added at rates of about 25 to 100 t ha^{-1} and higher (U.S. EPA 2007).

Natural zeolite (Z), ZORMET® of Andayen Ltda., was used as potential sorbent of excess free cations (i.e., Cu, Zn, Cd, and Pb). It corresponds to a mixture of clinoptilolite, huelandite, and mordenite, with particle size less than 75 μm, and cation exchange capacity of 80–100 cmol+kg^{-1}. Application rate (10% *w/w*) of natural zeolite was determined based on previous studies at similar sites in the literature (i.e., Mench et al. 1998; Moirou et al. 2001). Iron-activated zeolite (AZ) has an increased capability of As adsorption when compared to natural zeolite (Onyango et al. 2003); therefore, a batch of the natural zeolite was activated with a solution of 0.2 M of iron sulfate according to the method described by Bonnin (2000). As a result, total Fe content of activated zeolite was five times higher than in natural zeolite (42,061 and 8,248 mg kg^{-1}, respectively). Application rate of Fe-activated zeolite (4.2% *w/w*) was defined considering the total As concentration (51 mg kg^{-1}) measured in the polluted study soil (Los Maitenes).

2.3 Experimental assay

To test the efficacy of selected amendments for in situ immobilization of metals and As in polluted topsoils of Los Maitenes, 14 experimental treatments, replicated four times, were defined as described in Table 2. Batches of polluted soil were amended with selected materials at the rates indicated in Table 2. Four kilograms of each experimental substrate was prepared in polyethylene containers, homogenized with an automatic roller for 20 min, and distributed in four 1-L plastic pots with drainage at their base. Remaining substrates were used to determine the field capacity [FC; method of Klute (1986), the permanent wilting point (PWP; method of Richards 1985)] and general physicochemical characteristics as described below.

Experimental pots were kept in a plant growth room under controlled conditions (23±2°C; light intensity, 103± 14.3 μm s^{-1} m^{-2}; 12:12-h light to dark photoperiod) during 15 weeks and randomly relocated once a week. The soil moisture of every pot was daily checked, and deionized water was added when necessary (no leaching) to maintain each substrate at 70% field capacity. At the beginning of the fifth week, all pots were sown with 0.6 g of seeds of *Lolium perenne* L. var. *nui* (perennial ryegrass), as this species has been frequently used to test metal(oid) toxicity in polluted soils (i.e., Arienzo et al. 2004). Irrigation was increased up to 90% field capacity in those treatments with elevated plant yield by the end of the assay.

Table 2 Experimental treatments to evaluate the efficacy of inorganic (seashell grit, natural zeolite, Fe-activated zeolite) and organic (biosolids) amendments for in situ immobilization of metals and arsenic in polluted topsoils of the Puchuncaví valley, central Chile

Treatment code	Seashell grit (SG) (%)[a]	Biosolids (B) (%)[a]	Natural zeolite (Z) (%)[a]	Fe-activated zeolite (AZ)(%)[a]
C+	0	0	0	0
C−	0	0	0	0
SG	0.5	0	0	0
SGAZ	0.5	0	0	1
SGZ	0.5	0	10	0
SGAZZ	0.5	0	10	1
SGB50	0.5	3	0	0
SGB100	0.5	6	0	0
SGB50AZ	0.5	3	0	1
SGB50Z	0.5	3	10	0
SGB50AZZ	0.5	3	10	1
SGB100AZ	0.5	6	0	1
SGB100Z	0.5	6	10	0
SGB100AZZ	0.5	6	10	1

C− polluted soil at Los Maitenes; *C+* reference soil; *SG* seashell grit; *AZ* iron-activated zeolite; *Z* zeolite; *B50* biosolids at 50 t ha^{-1} (3%), d. b.; *B100* biosolids at 100 t ha^{-1} (6%), d.w. basis

[a] Dry weight basis

During the 1st, 3rd, and 14th week of the assay, aliquots of soil pore water (5–7 mL) were taken from three experimental pots per treatment with Rhizon® Soil Pore Water Samplers (5-cm length; Rhizosphere Research Products, Wageningen, The Netherlands) following the methodology described by Vulkan et al. (2000). A composite sample per experimental treatment and week was produced in order to have enough volume for the analysis. Soil pore water samples were kept in acid-washed polyethylene plastic vials (15 mL) and characterized as described below.

By week 15, aerial plant biomass was harvested per pot. Shoots were washed with deionized water, air-dried at 45°C to a constant weight, and weighed. Shoot Cu and As contents were determined as described below. Pots were then longitudinally cut in halves for root cover determination. A 1×1-cm grid was overimposed on the exposed soil surface of one pot half, and the number of intersection points effectively contacted by roots from total intersection points was registered. Root cover (in percentage) was then calculated from the frequency of contacts.

2.4 Analytical determinations of experimental substrates, pore water samples, and plant tissues

For determining general properties of experimental substrates, 1-kg samples were dried in a forced air-drying

cabinet at 30°C and then sieved through a 2-mm nylon sieve. Texture, pH, electric conductivity (EC), cation exchange capacity (CEC), organic matter (OM) and available nitrogen (N), P, and potassium (K) were determined after the procedures of the USDA methods manual (USDA 2004).

Samples of experimental substrates and dried ryegrass shoot samples were subjected to microwave acid digestion (1,200 Mega Microwave System, Milestone) and extraction (U.S. EPA method 3051 (U.S. EPA 1995) and USDA method 3050 (USDA 2004)) prior to trace element determinations. Every digestion batch had one blank, one standard reference material sample, one duplicate sample, and one quality control sample. Digested samples were analyzed for total Cu, Zn, As, and Ca contents by flame atomic absorption spectrometry (FAAS, AAnalyst 300; Perkin-Elmer) under U.S. EPA methods (U.S. EPA 1995). The quality assurance and quality control criteria were satisfied when the measured parameter of the standard reference material (Loam-B, catalog no. CRM-LO-B, High-Purity Standard, USA; SRM 1573a tomato leaves or SRM 1570a spinach leaves, National Institute of Standards and Technology, USA) and the quality control sample (a previously characterized soil sample with a known concentration of metals) differed by no more than 5%.

Determinations of pH, EC and free ionic copper (Cu^{2+}) were done in collected pore water samples up to 10 min after sample collection with a combination pH electrode (Sensorex 120C), a conductivimeter (Schott Gerate CG858), and a Cu-ion selective electrode (Orion, model 9629 BN); the Cu-ion specific electrode was calibrated using a diaminoacetic acid solution (Rachou et al. 2007). From Cu^{2+} values the activity of the free Cu^{2+} ions or pCu^{2+} values were calculated according to $pCu^{2+} = -\log [Cu^{2+}]$. Every pore water sample was then divided into two aliquots and fixed with either a 65% solution of HNO_3 Suprapur (Merck) for elemental (Cu, Zn, As, Na, Ca, and Mg) determinations or with concentrated H_3PO_4 Suprapur (Merck) for dissolved organic carbon (DOC) determinations. DOC was determined by combustion with the U.S. EPA method 415.1 (Keith 1996) in a carbon analyzer (Tekmar Dohrmann Carbon Analyzer, model Apollo 9000). The calibration standard solution used was 1 mg L^{-1} as carbon of dipotassium phthalate for organic carbon. As reference material ION-20 was used (National Waters Research Institute). Total Cu, Zn, As, Na, Ca, and Mg were determined by FAAS (AAnalyst 300; Perkin-Elmer) under U.S. EPA methods (U.S. EPA 1995), as described above. The sodium adsorption ratio (SAR) was calculated from pore water concentrations of Na, Ca, and Mg (in miliequivalents/liter) according to the equation $SAR = Na/\sqrt{[(Ca+Mg)/2]}$ (USDA 2004). The SAR was calculated to characterize salt-affected soils.

2.5 Statistical analyses

Factorial analysis of variance (ANOVA) was used for testing the effects of amendments and time since amendment addition on pore water chemistry (pH, EC, and pCu^{2+}). Fisher's least significant difference (LSD) was used as a posteriori test ($\alpha=0.05$). One-way ANOVA and Fisher's LSD ($\alpha=0.05$) were used to contrast plant variables (i.e., shoot biomass, root cover, Cu and As content in shoots) under experimental treatments (Zar 1984). Linear simple correlations (Pearson's r) were used to evaluate statistical relationships among soil chemical variables and plant variables (Draper and Smith 1988).

To elucidate the experimental treatments that allowed proper transformation of pore water chemical parameters of the polluted soil (C−) to those of the reference soil (C+), a principal components and classification analysis (PCCA) was performed (Jongman et al. 1995). Pore water chemical parameters (pH, EC, SAR, DOC, pCu^{2+}, total dissolved Cu, total dissolved Zn, and total dissolved As) determined by week 14 were first reduced to uncorrelated variables, and then both chemical parameters and experimental treatments were classified in order to highlight their relationships.

When needed, data were corrected for non-normality using logarithmic transformations ($x=\log10(x+1)$; Zar 1984) for the valid application of the parametric analyses described above. All statistical analyses were performed on Statistica software, version 6.0 (StatSoft Inc., U.S.A.).

3 Results and discussion

3.1 Physicochemical characteristics of experimental substrates

Values at the beginning of the assay (week 1) are shown in Table 3. Incorporation of amendments into soil C− did not change total Cu, Zn, and As concentrations (no dilution effect by amendment mixing with soil; Table 3). Incorporation of SG into C− allowed acidity neutralization to a pH value of 6.5, as expected (Table 3). However, further addition of natural zeolites or biosolids, either alone or mixed, resulted in higher values (7.0 to 8.0; Table 3), due to the alkaline characteristics of these materials (U.S. EPA 2007; Kumpiene et al. 2008). Indeed, an increase from 1.3 to 1.9 times in total Ca concentrations was detected when seashell grit, natural zeolite, and/or biosolids were incorporated into soil C− (Table 3), as these are Ca-rich materials. Soil neutralization has been commonly used to reduce metal solubility in polluted soils (i.e., Simon 2005; Bes and Mench 2008; Lee et al. 2011), but excessive increases of soil pH may induce As solubilization (Adriano 2001; U.S. EPA 2007), an aspect evaluated and further discussed in the next section.

Addition of biosolids into SG-amended soil increased FC, PWP, OM content, and available levels of N and P in experimental substrates, in a dose-dependent form, but not CEC (Table 3). These results confirm previous findings.

Table 3 General physicochemical parameters of experimental substrates at the beginning of the assay (week 1)

Treatment code	pH	EC (dS/L)	CEC (cmol+/kg)	FC100% (g water)	PWP (% w.w.)	OM (%)	Available macronutrients (mg/kg)			Total metal (mg/kg)			
							N	P	K	Cu	Zn	As	Ca
C+	6.30	0.17	8.55	24.3	4.6	1.25	15.3	33.1	267	209.0	115.4	20.7	4,392
C-	5.58	0.14	9.07	25.2	4.7	2.10	29.4	42.5	216	684.6	158.6	51.1	4,391
SG	6.47	0.16	8.04	25.6	4.5	1.93	31.1	41.1	218	619.5	146.8	50.9	5,624
SGAZ	6.21	0.18	8.30	22.9	4.8	1.77	35.2	39.7	202	629.8	146.0	46.2	5,858
SGZ	7.39	0.59	11.7	27.1	7.5	2.21	28.6	39.7	216	633.2	147.6	38.0	6,040
SGAZZ	7.17	0.55	17.3	27.9	8.1	1.93	52.6	35.7	226	603.9	144.1	42.8	8,205
SGB50	7.32	1.04	9.33	30.1	5.8	2.97	427	96.0	216	710.5	197.1	51.3	7,460
SGB100	7.01	1.48	10.6	38.7	7.1	4.28	457	152	233	649.1	196.0	53.4	7,629
SGB50AZ	7.50	0.86	9.33	30.3	5.6	2.61	387	87.9	236	721.7	183.8	44.3	7,076
SGB50Z	7.95	1.07	15.8	34.0	7.7	2.32	413	60.1	233	665.2	182.2	50.7	8,059
SGB50AZZ	8.02	0.88	15.8	33.5	7.0	2.15	414	73.8	230	667.0	180.2	54.0	6,378
SGB100AZ	7.39	1.32	11.4	42.3	6.8	4.14	593	147	254	687.2	216.8	45.6	7,483
SGB100Z	7.90	1.56	15.8	39.5	19.4	4.00	796	152	267	616.8	207.7	52.5	8,407
SGB100AZZ	7.92	1.53	16.6	39.5	21.0	4.01	833	147	267	658.3	206.8	47.7	8,205

Treatment codes follow Table 2

EC electric conductivity, *CEC* cation exchange capacity, *FC100%* field capacity at 100%, *PWP* permanent wilting point

Even though biosolids have been used for their liming and metal cation sorbent properties (i.e., Brown et al. 2003; U.S. EPA 2007), they have been most frequently used to provide essential nutrients (such as N and P) and to rebuild soil organic matter content (i.e., Haering et al. 2000; Brown et al. 2003). Benefits directly associated with improved organic matter content in soils are enhanced water infiltration and moisture-holding capability, aggregation, aeration, and nutrient supply for plant growth (i.e., Wallace and Terry 1998; Stofella and Kahn 2001). All of these parameters are relevant for improving the quality of contaminated and degraded soils under semiarid Mediterranean climate that is characteristic of the Puchuncaví valley. Besides these positive effects, biosolids increased EC of experimental substrates, in a dose-dependent form (Table 3). Biosolids have high EC values (14 dS m^{-1}), thus explaining their impact on this parameter when added to soil at high loading rates (i.e., Santibáñez et al. 2007; Verdugo et al. 2011). This effect is aggravated under pot cultivation conditions, where soil salinization problems frequently occur, particularly when no water lixiviation is allowed from experimental pots, as in this study. Therefore, the washout effect of salts due to natural precipitation under field conditions does not occur in laboratory experimental conditions.

Incorporation of zeolites into SGB-amended soils increased CEC of experimental substrates (Table 3), as expected due to its high CEC (80–100 cmol+kg^{-1}). However, it resulted on excessive increases in PWP values (>19% w.w.), particularly at a rate of 100 t ha^{-1} (Table 3), which may limit plant growth under semiarid field conditions.

3.2 Pore water chemistry of experimental substrates

Chemical characteristics of pore water samples are shown in Tables 4 and 5. Significant differences among experimental treatments, week of pore water sampling, and the interaction factor (treatment×week) were found for pH, EC, and pCu^{2+} (Table 5). Seashell grit significantly increased pore water pH of the soil C−, and this effect remained constant throughout the experimental time (Table 4). Addition of AZ, Z, biosolids (B50 and B100), or a mixture of Z and B to SG-amended soils further increased pore water pH to neutral or slightly alkaline values (Table 4). However, while pore water pH of SGAZ, SGZ, and SGAZZ treatments significantly increased from weeks 1 to 9, this parameter significantly decreased in treatments SGB100 and SGB100AZ in the same period of time

Table 4 Temporal evolution of pH, electric conductivity (EC) and copper activity (pCu^{2+}) in pore water samples taken from experimental substrates

Treatment code	pH			EC (dS/m)			pCu^{2+}		
	Week 1	Week 9	Week 14	Week 1	Week 9	Week 14	Week 1	Week 9	Week 14
C+	4.7a	4.8ad	5.1ad	2.4a	1.2a	0.7a	7.08b	8.35c	8.21c
C-	4.6ab	4.9ab	3.9b	1.7a	1.1a	1.1a	4.86a	6.44b	6.50b
SG	6.0c	7.0eg	7.0ce	1.3a	1.1a	0.2a	6.44b	9.03c	9.72ed
SGAZ	5.4d	7.0ceg	6.8ce	1.6a	1.1a	0.4a	5.67ab	9.14c	9.90ed
SGZ	7.0ce	7.7f	7.7f	9.3cd	1.7a	2.0a	8.82c	10.75df	11.16f
SGAZZ	6.3c	7.8f	7.6f	11.8d	1.3a	0.6a	8.20c	10.92df	11.15f
SGB50	7.3ef	6.9eg	7.0eg	5.6b	6.3be	3.8b	9.03c	9.61ce	9.76ed
SGB100	7.2ef	6.6cg	6.5cg	7.3ce	6.6bc	5.3b	8.86c	9.18ce	9.34ed
SGB50AZ	7.2ef	6.9ceg	6.6cg	4.1b	6.0be	5.3b	8.46c	9.35ce	8.56c
SGB50Z	7.6ef	7.1efg	7.0eg	8.7ce	8.3c	8.1ce	10.40df	10.13de	10.29def
SGB50AZZ	7.5ef	7.0eg	6.9eg	9.0c	9.4c	9.0ce	10.45df	10.05de	10.15def
SGB100AZ	7.4ef	6.9cg	6.6cg	7.6ce	6.2be	4.7b	9.30ce	9.28ce	9.61e
SGB100Z	7.5ef	7.1efg	6.9ceg	9.7cd	9.7cd	8.4ce	10.22df	10.05de	10.36def
SGB100AZZ	7.6ef	7.1efg	7.0eg	8.4ce	10.0cd	9.4ce	10.64df	10.18def	10.24def
Factors	F ANOVA	df	P	F ANOVA	df	P	F ANOVA	df	P
Week	5.8	2	<0.05	79.62	2	<0.05	182.2	2	<0.05
Treatment	184.7	13	<0.05	147.44	13	<0.05	1153.6	13	<0.05
Week×treatment	17.7	26	<0.05	19.41	26	<0.05	21.4	26	<0.05

Treatment codes follow Table 2. Different letters among treatments and weeks per chemical parameter indicate significant differences (factorial ANOVA and Fisher's LDS tests)

df degrees of freedom

Table 5 Pore water chemistry of experimental substrates by the end of the experimental assay (week 14)

Treatment code	SAR (meq/L)$^{0.5}$	DOC (mg/L)	Total dissolved		
			Cu (mg/L)	Zn (mg/L)	As (μg/L)
C+	3.0	88	0.36	0.78	12.1
C−	0.8	97	4.81	6.36	38.2
SG	0.4	95	0.81	0.10	39.6
SGAZ	0.5	117	0.73	0.12	37.6
SGZ	25.3	378	6.91	0.31	275.9
SGAZZ	11.6	120	1.37	0.33	102.1
SGB50	0.6	365	1.58	0.38	63.8
SGB100	0.6	490	1.82	0.89	95.9
SGB50AZ	0.5	352	1.56	0.70	45.8
SGB50Z	18.3	394	2.30	0.33	103.7
SGB50AZZ	19.7	548	2.16	0.32	72.2
SGB100AZ	0.8	582	2.24	0.88	70.5
SGB100Z	16.4	464	1.98	0.35	109.0
SGB100AZZ	14.6	538	2.17	0.41	77.8

Treatment codes follow Table 2

SAR sodium adsorption ratio, *DOC* dissolved organic carbon

(Table 4). In all cases, no further pH variation was detected after week 9. Pore water pH of all amended soils was significantly higher than the values of C− by the end of the assay (week 14).

Soil acidity neutralization with inorganic liming agents (i.e., CaCO$_3$, Ca(OH)$_2$, CaO) is an effective traditional practice in acidic agricultural soils (Simon 2005). However, industrial alkaline by-products (i.e., fly ash, red mud, furnace slag) have been also considered for neutralization of acidic metal-polluted soils (i.e., U.S. EPA 2007; Kumpiene et al. 2008; Lee et al. 2011). Biosolids may also have soil neutralizing capabilities (Brown et al. 2003; U.S. EPA 2007), but several factors can modify the pH of the substrate in long-term experiments, such as the microbial breakdown of organic matter and the nitrification process, among others. In the present study, application of high rates of biosolids to SG-amended soils resulted in a significant decrease of pH values in pore water in time, which agrees with results reported by Tsadilas et al. (1995) and Santibáñez et al. (2008).

In terms of pore water EC, incorporation of Z, B, or their mixture into SG-amended soils significantly increased this parameter when compared to control soils (C+ and C−; Table 4). In the case of zeolite (SGZ and SGAZZ), EC dramatically decreased from weeks 1 to 9, reaching values below 2 dS m^{-1} by week 14 and similar to control soils (C+ and C−) and SG-amended soil. On the other hand, even though pore water EC on B- and BZ-amended soils tended to slightly decrease from weeks 1 to 14, this parameter remained significantly high throughout the assay with values above 4 dS m^{-1} (Table 4). Soil salinization is expected after application of biosolids, particularly at high doses, as biosolids have high EC values (i.e., Brown et al. 2003; Santibáñez et al. 2008; Verdugo et al. 2011).

The pCu^{2+} on C− was 1.3 to 1.5 times lower than in the C+, thus meaning that activity of free Cu ions (Cu^{2+}) is much higher in C− soil than in C+ soil (Table 4). Furthermore, pCu^{2+} on control soils (C+ and C−) significantly increased from weeks 1 to 9 and then remained constant until week 14 (Table 4), a phenomenon explained by metal aging in soils (Lock et al. 2006; Rooney et al. 2007), as no leaching occurred from experimental pots. Incorporation of all amendments significantly increased pCu^{2+} values by week 1 to values similar or even higher than C+, thus effectively and rapidly decreasing the concentration of free Cu^{2+} ions in pore water (short-term equilibration time). However, they vary in terms of their efficacy to reduce the concentration of free Cu^{2+} ions in pore water (Table 4), being the order of treatments from low to high efficacy SG=SGAZ<SGZ=SGAZZ=SGB= SGBAZ<SGBZ=SGBAZZ. Addition of SG to C− reduced the activity of free copper ions measured in soil pore water to levels even lower than C+, an expected result as activity of free metal cations decreases as soil pH increases (Bourg 1995). Addition of Z and/or B to SG-amended soils further reduced free Cu^{2+} ion values, as they have high cation exchange capacities due to negative charged surface (Sauvé et al. 2000; Simon 2005; Janos et al. 2010).

By the end of the assay (week 14), those treatments where Z was added to both SG- and SGB-amended soils resulted in marked increases in the SAR (Table 5) of experimental substrates. The SGZ treatment also increased pore water DOC (Table 5), a phenomenon explained by dissolution of soil OM due to both soil sodification and the slightly alkaline conditions (pore water pH of 7.74 by week 14; Table 4) produced by zeolite. Furthermore, a dose-dependent increase of DOC resulted from incorpora-

tion of biosolids to the polluted study soil (Table 5). This finding has been described on biosolid- and other organic-amended soils (i.e., Ruttens et al. 2006) and hard rock mine wastes (i.e., Santibáñez et al. 2008; Verdugo et al. 2011). In some cases, increased amount of soluble organic ligands can increase metal mobility in the substrate (i.e., Santibáñez et al. 2008; Neaman et al. 2009; Verdugo et al. 2011), a phenomenon that was not detected in the present study; indeed, no significant correlations were found among pore water DOC and total dissolved Cu ($r=0.18$), total dissolved Zn ($r=-0.30$), and pCu^{2+} ($r=0.35$).

In the case of total dissolved metals (Cu and Zn), addition of all amendments and their mixtures resulted in reduced concentrations by week 14 when compared to concentrations present in the soil C−. The exception was the treatment SGZ for total dissolved Cu where an increase occurred; this result may be explained by both the elevated SAR values ((25.3 meq L^{-1})$^{0.5}$; Table 5) and DOC values reached in this particular treatment. The effective increase in both pore water (Table 4) and soil pH (Table 3) values after amendment additions may account for detected reductions in total dissolved metal concentrations (Table 5) and free Cu^{2+} ion activities (Table 4) in soil pore water, as largely demonstrated by others when acidic metal-polluted soils or acidic hard rock mine wastes are neutralized (i.e., Bourg 1995; Sauvé et al. 2000; Ginocchio et al. 2002, 2009; Simon 2005; U.S. EPA 2007). In the present study, a significant negative correlation ($r=-0.85$, $P<0.05$) occurred between total dissolved Zn and pH in pore water of experimental treatments while a significant positive correlation was found between pCu^{2+} and pore water pH ($r=0.95$, $P<0.05$) by week 14.

By week 14, total dissolved As showed increases from 1.2 to 7.2 times in all amended soils when compared to the soil C−, with the exception of treatments SG and SGAZ (Table 5). This response was significant and positively correlated to soil pore water pH ($r=0.56$, $P<0.05$) and also occurred in treatments where AZ was incorporated into experimental soil with other amendments. Increased As solubilization has been also demonstrated on amended Cu mine tailings (Verdugo et al. 2011) and on beringite amended As-enriched soils near an As smelter in Belgium (Mench et al. 2006), particularly when substrate neutralization reached pH values higher than 6.5. Therefore, soil neutralization has to be carefully controlled to the target soil pH of 6.5, as suggested by U.S. EPA (2007). In the present study, most treatments reached pHs higher than 7.0 (Table 3).

Pearson correlation analysis across pore water chemical parameters at week 14 showed significant correlations between DOC and EC ($r^2=0.85$, $P<0.05$) and pCu^{2+} and pH ($r^2=0.95$, $P<0.05$); therefore, EC and pH were excluded from the PCCA, but indirectly considered through

their correlated parameters. The PCCA for pore water chemical parameters of experimental substrates (week 14) showed that 100% of the variance was explained by considered parameters. Specifically, SAR, DOC, pCu^{2+}, and total dissolved Cu explained 51%, 29%, 13%, and 5% of the variance of the data, respectively. The first three factor coordinates explained 98% of the total variance; SAR, pCu^{2+}, and total dissolved As were strongly correlated to factor 1, total dissolved Cu and Zn were strongly correlated to factor 2, and DOC was strongly correlated to factor 3. The PCCA ordination diagram (Fig. 1) shows the relative distribution of experimental treatments in direct relation to pore water chemical parameters. Specifically, pore water chemical parameters of C− and SGZ treatments differed from the rest due to their higher values of total dissolved Cu (Table 5), and from each other, due to opposite trends for SAR, pCu^{2+}, and total dissolved As values (see Tables 4 and 5). Pore water chemical parameters of SG-amended soils either alone (SG) or mixed with activated zeolite (SGAZ) and/or biosolids (SGB50, SG100, SGB50AZ, SGB100AZ) were the best correlated treatments to C+. However, addition of Z to SGB-amended soils (SGB50Z, SGB50AZZ, SGB100Z, SGB100AZZ) resulted in higher values of SAR and total dissolved As in pore water, far above the values of C+ (Tables 4 and 5).

3.3 Plant responses to experimental substrates

Significant differences were found across experimental treatments for all plant variables assessed (Figs. 2 and 3). Soil C− has adverse effects on the growth of ryegrass plants, reducing both the shoot biomass production and the root cover by 71% and 61% (Fig. 2), respectively, when compared to the productivity reached in soil C+. The same phytotoxicity results have been described for other plant species grown on soils artificially spiked with copper smelter dust (Sauvé et al. 2000; Ginocchio et al. 2006, 2009).

Shoot and root parameters of ryegrass plants showed the same trends to experimental treatments (Fig. 2); indeed, shoot biomass was significantly and positively correlated to root cover ($r=0.92$, $P<0.05$). Addition of SG, AZ, Z, and B to C− resulted in significant increases in both shoot biomass production and root cover, reaching values even higher (125–280%) than in soil C+ (Fig. 2). The same positive responses on ryegrass productivity have been demonstrated for other inorganic and/or organic amendments incorporated into highly polluted soils of the Puchuncaví valley (i.e., Stuckey et al. 2009; Goecke et al. 2011). Exceptions were treatments SGB50Z, SGB50AZZ, SGB100Z, and SGB100AZZ where no significant differences of shoot productivity and root cover with C− were

Fig. 1 Ordination diagram for the PCCA illustrating the distribution of pore water chemical parameters (*solid circles*; *N*=6) and experimental treatments (*squares*; *N*=14). The first two axes explain 80% of total variance of the chemical parameter–experimental treatment relationship. *Dotted lines* show different groups of experimental treatments. Numbers represent experimental treatments. *1* C+, *2* C−, *3* SG, *4* SGAZ, *5* SGZ, *6* SGAZZ, *7* SGB50, *8* SHB100, *9* SGB50AZ, *10* SGB50Z, *11* SGB50AZZ, *12* SGB100AZ, *13* SGB100Z, *14* SGB100AZZ (codes follow Table 2)
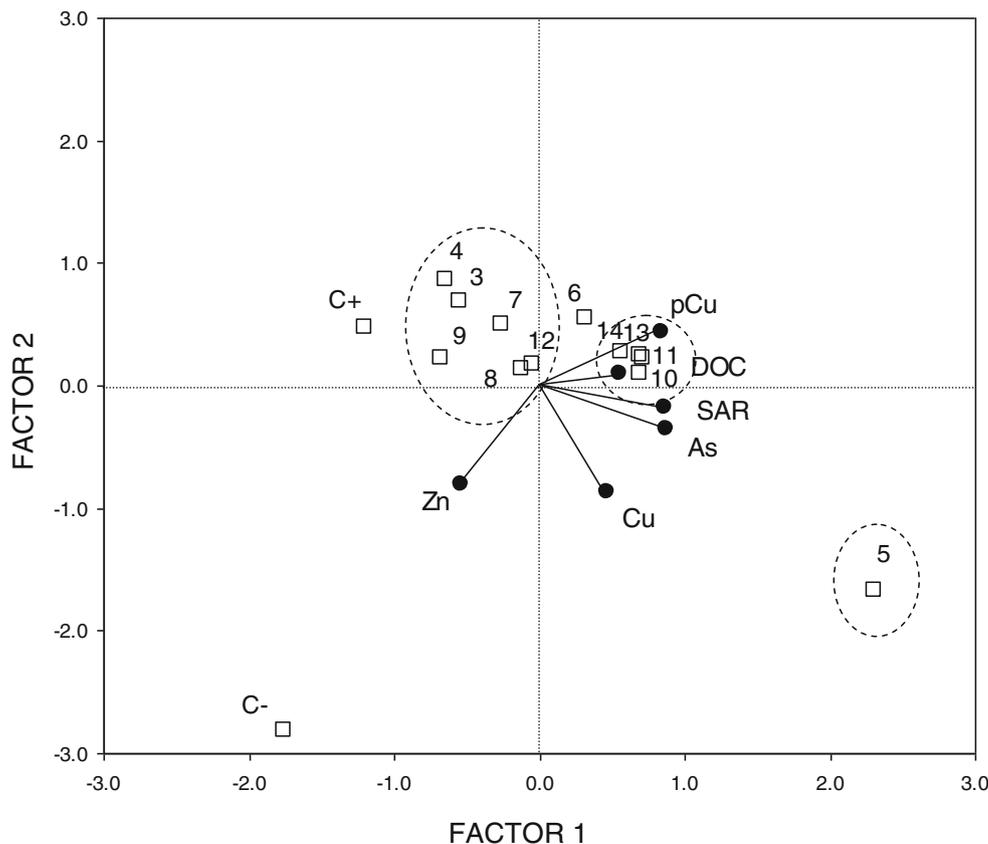
found (Fig. 2). Soil sodification and elevated contents of both free Cu ions and total dissolved As in pore water samples resulting from addition of Z to SGB-amended soils (Tables 4 and 5) could explain these results. Specifically, SAR values above 12 (meq L^{-1})$^{0.5}$, as the values resulting from these experimental treatments, restrict plant growth due to elevated concentrations of exchangeable sodium (Na^+ toxicity to plants); besides this, in nonsaline sodic soils, with pH lower than 8.5, Ca^{2+} and/or Mg^{2+} concentrations are often nutritionally inadequate (Arshad 2008).

Shoot biomass and root cover were significantly and negatively correlated to shoot Cu concentrations ($r=-0.70$ and -0.67, respectively; $P<0.05$) and to shoot As concentrations ($r=-0.60$ and -0.53, respectively; $P<0.05$). Therefore, experimental treatments that significantly reduced shoot biomass and root cover (C−, SGB50Z, SGB50AZZ,

Fig. 2 Variation of shoot biomass (dry weight basis) and root cover of *L. perenne* (ryegrass) plants grown on experimental substrates for 15 weeks. Mean and standard deviation values are given. *Different letters* indicate significant differences (one-way ANOVA and Fisher's LSD; *P*<0.05) among experimental treatments (*capital letters* for root cover and *lowercase letters* for shoot biomass). Experimental treatment codes follow Table 2
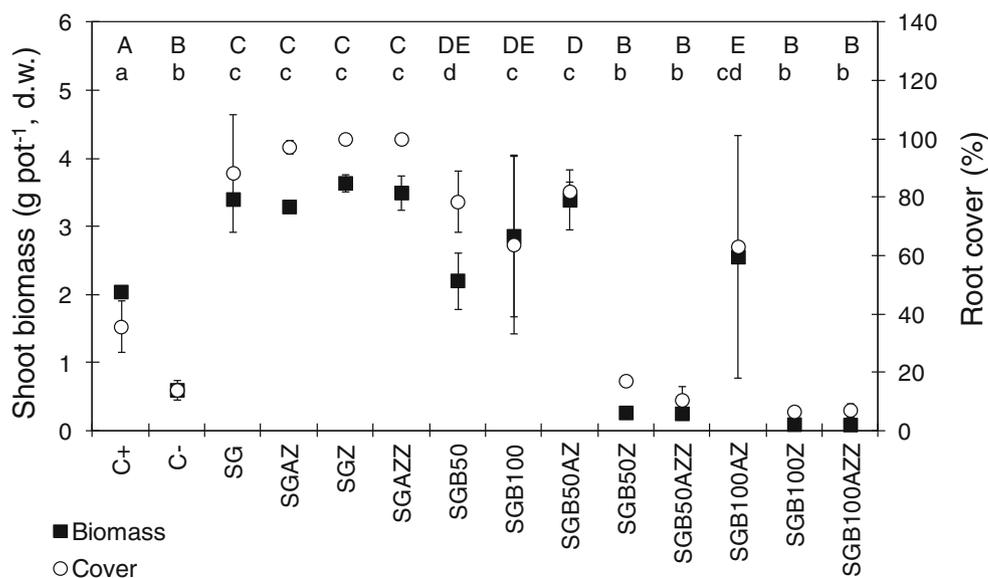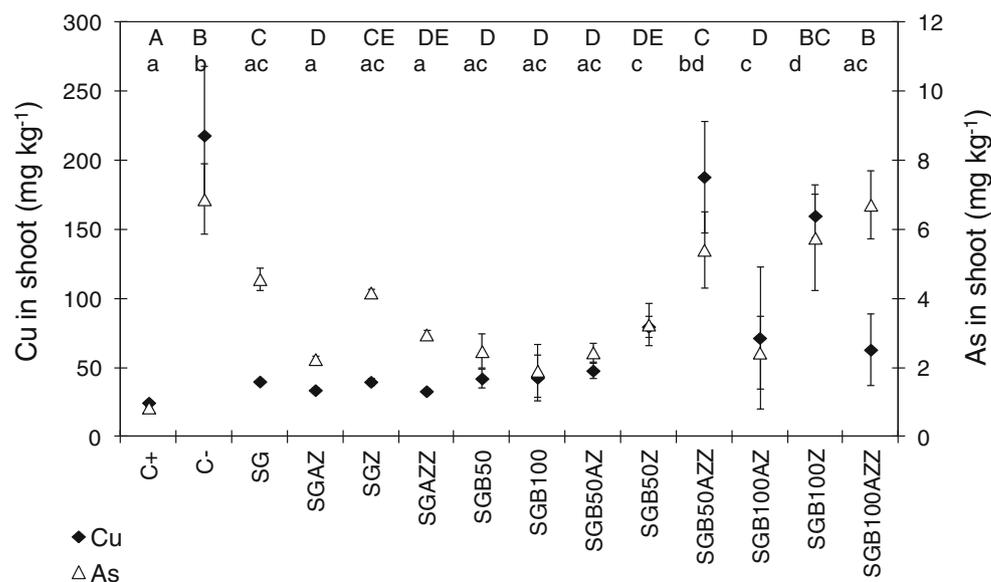
Fig. 3 Variation of shoot Cu and As concentrations of *L. perenne* (ryegrass) plants grown on experimental substrates for 15 weeks. Mean and standard deviation values are given. *Different letters* indicate significant differences (one-way ANOVA and Fisher's LSD; $P < 0.05$) among experimental treatments (*capital letters* for As in shoots and *lowercase letters* for Cu in shoots). Experimental treatment codes follow Table 2

SGB100Z, SGB100AZZ) also showed significant increases in both shoot Cu and As concentrations (Fig. 3), and thus resulted in metal toxicity. This adverse effect was reflected in typical metal toxicity visual symptoms, such as stunted growth with chlorotic and reddish leaves. Shoot Cu and As concentrations of ryegrass plants grown in all the other experimental treatments did not differ from values of the soil C+ and were below 50 and 4 mg kg^{-1}, respectively (Fig. 3).

The typical shoot Cu concentration range in perennial ryegrass is 7.3–13.4 mg kg^{-1}, and 20 mg kg^{-1} is often considered the threshold of excessive shoot Cu content in plants (excluding highly sensitive and tolerant species; Kabata-Pendias and Pendias 1992). However, the majority of plant species can accumulate more than 20 mg kg^{-1} of Cu when growing in soils with elevated Cu levels (Kabata-Pendias and Pendias 1992). The phytotoxic threshold for shoot Cu concentration in ryegrass is approximately 21–22 mg kg^{-1} d.w. and is relatively independent of growing conditions (Adriano 2001). In the present study, the nonamended polluted soil (C−) resulted in shoot Cu concentrations higher than 200 mg kg^{-1} (Fig. 3), a value well above the phytotoxic threshold of 21–22 mg kg^{-1}.

The incorporation of B into SG-amended soils did not resulted in any further significant increase in shoot biomass (Fig. 2), even though available macronutrient contents, among other soils factors, were clearly improved (Table 3). The significant increase in pore water EC values resulting from incorporation of biosolids into SG-amended soil may explain this result. Although soil salinization did not occur (EC values <4 dS m^{-1}, Arshad 2008), perennial ryegrass has been found to be sensitive to soil salinity (Marcar 1987). On the other hand, root cover was significantly reduced by incorporation of B into SG-amended soils (Fig. 2), as expected; this effect was probably due to

increased pore water EC values (Table 5) but also due to a change in the "foraging" strategy of the plant root system when exposed to improved availability of macronutrients in soils (Table 4). It is well established that in low-nutritional substrates, such as the soil C−, plants show long and thin roots as resources are mainly allocated to root growth due to the need to "forage" for nutrients (Ginocchio 1994). However, under increased level of nutrients in the substrate, plants may allocate more resources to the development of aerial structures (i.e., Noble and Marshall 1983; Ginocchio 1994), thus showing short and thick roots.

Even though the question is often raised whether results from pot experiments are applicable to events in the field (i.e., Friesl et al. 2006; Mench et al. 2006), the present study adds further knowledge on the importance of not only using proper amendments but also for controlling key chemical characteristics of resulting soils, such as pH, for reaching the goal of simultaneous in situ immobilization of metals and As in polluted soils.

We are aware that results from pot assays are not equivalent to field trials when assessing the efficacy of soil amendments; however, from the results of the present study, we can now plan better field trials in polluted soils of the Puchuncaví valley. Furthermore, long-term field studies will be also needed in order to better assess chemical mechanisms in amended soils under field conditions, ecological consequences, and potential remobilization of metals and As, among other factors.

4 Conclusions

Effective in situ immobilization of metals and As in polluted soils of the Puchuncaví valley is possible through

the incorporation of some specific amendments, at least in the short time (14 weeks) and under controlled laboratory conditions. Specifically, soil neutralization to a pH of approximately 6.5 with seashell grit is enough for reducing metal solubilization without affecting As solubilization into soil pore water. Furthermore, it eliminates phytotoxicity and excessive metal(oid) accumulation in aerial plant tissues, allowing adequate plant yields.

Contrary to expected, addition of iron-activated zeolite to seashell grit-amended soil does not further reduce As solubilization into soil pore water, but it is effective for further reducing As uptake and translocation to aerial plant tissues. However, its costs may be elevated for large-scale field applications. Identification of locally available low-price, iron-rich amendments, such as the fly ash generated by a thermoelectric plant located next to the Ventanas copper smelter, may be a feasible alternative, but this has to be evaluated.

Addition of biosolids and/or natural zeolites to seashell grit-amended soil does not further reduce metal solubilization into soil pore water, at least at the rates used in the present study, but increased As solubilization into soil pore water. However, no significant As increase occurs in aerial plant tissues. Therefore, particular care has to be taken with excessive soil neutralization (pH>6.5) when using a mixture of rather alkaline materials as amendments, to avoid excessive arsenic solubilization in soil, translocation to aerial plant tissues, and accumulation through food webs.

Biosolids are effective for increasing water retention capability, organic matter content, and available concentrations of N and P of the polluted soil of Los Maitenes, all positive factors for improving plant productivity, particularly under Mediterranean semiarid climate field conditions. However, these positive effects are likely counteracted by the soil salinization produced by this amendment, at least under laboratory conditions (no leaching allowed) and at high doses (100 t ha^{-1} of biosolids). In the case of natural zeolites, soil sodification may result when incorporated to neutralized soils that have been simultaneously amended with biosolids, limiting plant productivity. Soil salinization problems may, however, be controlled in field trials with excess irrigation.

In conclusion, for simultaneous in situ immobilization of metals and As in polluted soil of the Puchuncaví valley through aided phytostabilization, we suggest neutralization of topsoil to a pH up to 6.5 with an alkaline material, such as seashell grit, and simultaneous addition of biosolids, or any other organic matter-rich material, to improve physical and nutritional limiting conditions of soils. Metal phytotoxicity will be eliminated, and accumulation of metal(oid) in aerial plant tissues will be avoided.

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