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Benefits of the use of sewage sludge over EDTA to remediate soils polluted with heavy metals

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ABSTRACT

Sewage sludges from urban waste water treatment plants are often used to remediate degraded soils. However, the benefits of their use in metal-polluted soils remain unclear and need to be assessed in terms of factors besides soil fertility. This study examines the use of thermal-dried sewage sludge (TDS) as an amendment for heavy metal-polluted soil in terms of its effects on soil chemical properties, leachate composition and the growth of native plant communities. To assess the response of the soil and its plant community to an increase in metal mobilization, the effects of TDS amendment were compared with those of the addition of a chelating agent (EDTA). The experimental design was based on a real case scenario in which soils from of an abandoned mine site were used in a greenhouse bioassay.

Two doses of TDS and EDTA were applied to a soil containing high Pb, Zn, Cu and Cd levels (4925, 5675, 404 and 25 mg/kg respectively). Soil pH was 6.4 and its organic matter (OM)

content was 5.53 %. The factors examined after soil amendment were: soil fertility and heavy metal contents, leachate element losses, the plant community arising from the seed bank (plant cover, species richness and biodiversity, above/below ground biomass) and phytotoxic effects (chemical contents of abundant species). TDS emerged as a good phytostabilizer of Pb, Zn, Cu and Cd given its capacity to reduce the plant uptake of metals and achieve rapid plant cover. This amendment also enhanced the retention of other elements in the plant root system and overall showed a better capacity to remediate soils polluted with several heavy metals. EDTA led to plant productivity losses and nutritional imbalances as it increased the mobility of several elements in the soil and its leachates.

Keywords: polluted soils, ecological restoration, phytotoxicity, phytoremediation, plant community

INTRODUCTION

When trying to ameliorate the impacts of old abandoned mines on land ecosystems, the main problems faced are related to high concentrations of phytotoxic metals in top soil layers (Gutiérrez-Ginés et al., 2013), along with low soil nutrient contents. Some researchers have highlighted that soils containing heavy metals are among the most difficult to restore. This has led to a constant search for plant species capable of accumulating heavy metals as well as attempts to further our understanding of the effects of heavy metals on the plant species that spontaneously grow at these old mine sites (Moreno-Jiménez et al., 2009; Pratas et al., 2013). Another approach has been the use of soil amendments to help establish species targeted at phytoremediating soils (Mench et al., 2007; Walker et al., 2003) and improving soil fertility and structure.

Amendments composed of biowaste (unwanted material of biological origin, such as sewage sludge, animal effluent, wood waste, green waste and crop residues) are attractive due to the growing demand to incorporate waste in the ecological network while recovering waste waters (Hall, 1995). Organic amendments can increase the amount of organic matter and plant nutrients in soils, and act by retaining metals in the organic soil fraction (Park et al., 2011). The better nutrient conditions of soils and the reduction of metal mobility and bioavailability (thus toxicity) improve the growth of plants (Geebelen et al., 2002). However, these amendments can also increase the leaching of nitrogen (Knowles et al., 2011) or upset the balance of microflora and microfauna populations (Bright and Healey, 2003) with damaging effects in particular on the microarthropods responsible for recycling soil organic matter (OM) (Flores-Padarve et al., 2008; Flores-Padarvé and Hernández, 2012).

Other types of amendment, such as chelating agents, seek to increase the mobility of soil metals and thus promote the absorption of micronutrients via a process known as assisted phytoextraction. Plants grown in chelating agent-amended soils are able to accumulate higher concentrations of heavy metals. However, this technique has possible detrimental effects such as increasing the risk of leachates percolating to groundwater along with a loss of soil nutrients (Jiang et al., 2003; Mench et al., 2007; Pastor et al., 2007; Thalayakumaran et al., 2003). In spite of these drawbacks, some authors have continued to explore the use of chelating agents to improve the phytoextraction capacity of some plants (Gheju and Stelescu, 2013; Hadi et al., 2014; Kambhampati and Vu, 2013; Markovska et al, 2013; Suthar et al, 2014).

The main goal of ecological soil remediation is to restore its multifunctionality and fertility. This means that attention should be paid to the mobile heavy metal fraction both in terms of its stabilization/immobilization and of extraction by its accumulating plant biomass.

This study was designed to examine the effects of sewage sludge amendment of a heavy metal-polluted soil collected from an abandoned mine site. Apart from effects on soil properties and leachate composition, we tried to assess the development of the plant community that spontaneously thrives in the soil. We also compared the effects of this amendment with likely metal immobilization actions with those of a chelating agent known to increase the mobility and availability of metals.

To the best of our knowledge, this study is the first to address the issue of how soil amendments affect native plant communities able to withstand incredibly high concentrations of heavy metals in polluted environments.

MATERIALS AND METHODS

Polluted soil and amendments used in the bioassay

Soil was collected from an old silver mine in the province of Toledo (central Spain). This site has been the objective of numerous studies by our research group (Hernández and Pastor, 2007; 2008). The mine was abandoned in the 1980s and now consists of a landfill of tailings occupying some 20,000 m^3. The site was left untreated causing the transfer of heavy metals to neighbouring soils. The frequent use of this site for sheep grazing and as hunting grounds determines the need to assess possible remediation strategies to mitigate pollution risks. According to prior knowledge of the concentrations and distributions of pollutants at this site (Gutiérrez-Ginés et al., 2013), we collected soil from the most polluted zone for our experiment. The topsoil layer (0 – 20 cm) was carefully collected with the help of a hoe to avoid disrupting its structure after cutting all vegetation back to the soil surface. A further soil sample was obtained from the same zone for chemical determinations (see Table 1). The soil samples collected mainly showed high Pb and Zn concentrations but Cu and Cd were also present at concentrations higher than the permissible levels defined by Spanish law (BOE,

1990). Despite this, the soil sustains the growth of a plant community. The most common species of this community are listed in Table 2.

As an organic amendment, we used thermal-dried sewage sludge (TDS) from an urban waste water treatment plant. The chemical composition of the TDS used is detailed in Table 3. Its heavy metal concentration is lower than the maximum permissible level stipulated for sewage sludge used for agricultural purposes (BOE, 1990). The doses applied were 25 and 50 g of TDS for each kg of soil, corresponding to field doses of 100 t/ha and 200 t/ha respectively. Ethylenediaminetetraacetic acid (EDTA) was the chelating agent selected to assess phytoextraction by the plant community. This type of amendment is especially efficient at mobilizing bivalent ions and is used to increase the bioavailability of nutrients thus improving soil fertility (Li and Shuman, 1996). Results of our prior work (Pastor et al., 2007) suggest that EDTA produces fewer negative effects than DTPA on plant communities. EDTA is able to extract Zn, Cd and Ni from soils (Li and Shuman, 1996). EDTA was used in its acid form. The concentrations tested in this experiment were 0.5 g and 1 g of EDTA per kg of soil, corresponding to field doses of 2 t/ha and 4 t/ha respectively.

Experimental design and monitoring

The bioassay was conducted in a greenhouse with controlled temperature (24ºC) and humidity (60%). Microcosms were set up in plastic pots (surface area 14 cm x 19 cm, depth 7.5 cm) lined with black plastic to block the passage of light and thus avoid the growth of algae and moss within the soil. In each pot, a grid was positioned 1 cm from the bottom to collect any leached soil water. The pots were each filled with 1 kg of unsieved soil containing its autochthonous seeds.

The TDS was mixed with the soil introduced in the corresponding pots from the first day of the bioassay. EDTA was added 4 weeks later, after the first seeds had germinated to allow

sufficient time for a root system capable of absorbing mobilized metals to form. The chelating agent was diluted in 100 mL of deionised water and added gradually over 8 days given that the extracting effect of EDTA seems to improve when applied as several doses. This process also minimized the risk of the solution percolating through the system before contacting the soil particles containing the metals and trace elements. We are aware that the doses of both amendments used were high but this was designed to ensure marked differences between treatments and sufficient statistical power despite a fairly small number of replicates (three per treatment).

Initially, the microcosms were watered daily with 100 mL of deionised water to keep them wet until seedlings from the bank started to germinate. Once germinated, watering was reduced to 50 mL every two days since this amount of water proved to be sufficient for plant growth. When the microcosms became densely populated, the amount of water given was doubled.

Following germination, numbers of seedlings in each pot were recorded weekly. When these became so numerous they could not be counted without affecting the community, percentage cover values were recorded. Cover was measured visually with the help of the method of Emberger (1968). When the plants were mature, species were identified to estimate plant diversity (as Shannon indices). The bioassay was run for 30 weeks when plants started to lose their vigour.

Sample preparation and chemical analyses

Leachate collection started after the application of EDTA in the 4^{th} week. Leachates were collected in the 5^{th}, 7^{th} and 9^{th} week of the trial. No leachates were produced after that period, due to the development of the plant community. The leachate samples were filtered and stored

148 with a drop of HNO_3 at 4ºC until analysis. Nutrient and trace element concentrations in the
149 leachates were determined by ICP-OES.
150 At the end of the bioassay, the soil was separated from the plants, which were split into above
151 and below surface biomass. Total, above-ground and below-ground biomass were expressed
152 in mg/cm^2.
153 Original soil from the mine site, as well as soils from microcosms, were dried at room
154 temperature for one week and sieved through a 2 mm mesh. Tests conducted in each one
155 were: pH in slurry, organic matter by potassium dichromate reduction, total Kjeldahl nitrogen
156 (according to protocols described in Hernández and Pastor, 1989), metal concentration
157 determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES, Perkin-
158 Elmer 4300DV). Pseudo-total metal concentrations were determined after HNO_3 and $HClO_4$
159 digestion (Walsh and SSSA, 1971) and bioavailable metal concentrations after extraction with
160 DTPA 0.005 M at pH 8.3 (Lindsay and Norwell, 1978). All plant samples (separating roots
161 from shoots) were washed with tap water and rinsed two times with deionised water, oven-
162 dried at 70 ºC for 48 h, weighed and ground in a IKA-WERKE Yellow Line A10 grinder. The
163 procedure for determining total metal concentrations was the same as used on the soil (Walsh
164 and SSSA, 1971).
165
166 **Data treatment**
167 All soil data were analysed by unifactorial ANOVA ($p<0.05$) following their log and Box
168 Cox transformation to correct for deviations from normality and heteroscedasticity problems.
169 Leachate and biomass data were compared using the non-parametric Kruskal Wallis test at a
170 95% confidence level (indicated as * in the tables), given that the deviation from normality
171 and homoscedasticity hypotheses were too large for correction by transformation. All
172 statistical tests were performed using the software package STATISTICA 9.0.

RESULTS

Effects on soil variables

Soil properties related to fertility (Table 4) were modified by the two types of amendment. EDTA led to a slight decrease in pH, while TDS caused a marked drop in pH because it was more acidic than the soil. The addition of TDS also gave rise to an increase in total amounts of OM, N, Ca and P, but not their bioavailability. Only the bioavailability of Na was increased by soil amendment with TDS. In contrast, the use of EDTA besides lowering the total Mg concentration also affected the bioavailability of the remaining macronutrients.

Pseudo-total soil metal concentrations (Table 5) did not seem to be affected by either amendment, despite the presence of metals in the TDS or the expected reduction in metals due to their mobilization by EDTA. Only pseudo-total concentration of Al was lower in soils with the highest rate of EDTA application, although the difference with control was not significant. Neither was the difference produced in bioavailability very evident (Table 5). Only Zn showed a reduced bioavailable fraction in response to the higher TDS dose, while bioavailabilities of Fe and Cu increased, probably due to the drop in pH. Neither did EDTA notably affect the bioavailable fraction, which only showed an increase in Cu concentration.

Effects on the leachates produced

Although no substantial effects were recorded in the macronutrient and metal concentrations of the bioavailable fraction of the amended soils, their differing mobilities according to the amendment used were reflected by the leachate data (Figure 1). The addition of EDTA to the soil served to mobilize most of the elements examined (Ca, Mg, K, Na, Fe, Mn, Zn, Pb, Cu, Cd and Ni). Their concentrations in the leachates were always significantly higher than for the control treatment. Although the average concentration of Al in leachates was higher for the

198 highest EDTA dose, this difference was not significant. This could explain the lower pseudo-
199 total concentration of Al in soil treated with this amendment.
200 Although we expected the organic amendment to retain soil elements, their concentrations in
201 the leachates collected from the TDS treatments were somewhat higher than in controls yet
202 lower than in the case of EDTA. This difference with respect to the control was significant for
203 Ca, Mg, K, Na, Fe, Mn, Zn and Pb in the soils treated with the highest TDS dose, probably
204 due to the reduced soil pH and/or added metals in the TDS. The effect was more pronounced
205 in the leachates collected from the microcosms in Week 9.
206
207 **Effects on the plant community**
208 Changes produced during the growth of plants from the soil seed bank are shown in Figure 2.
209 The TDS amendment had a beneficial effect on the growth of species from the seed bank and
210 plant numbers were higher from the bioassay start in the microcosm soils treated with this
211 amendment than in untreated controls. This trend became more evident in the cover values
212 recorded after the third month of the bioassay. Thus, both TDS doses gave rise to cover values
213 of around 60% throughout the experiment compared to values of 20-30% observed for
214 controls. In contrast, the number of plants in the microcosms was unaffected by the addition
215 of EDTA. However, some two months after amendment, cover values gradually decreased
216 compared to controls with increasing concentrations of the chelating agent.
217 The remaining plant community factors measured at the end of the experiment (species
218 richness and diversity, soil cover, and above- and below ground phytobiomass) are provided
219 in Table 6. All factors except species richness were affected by the two soil amendments.
220 Despite no effects of TDS on plant number or biodiversity, plant growth was affected by this
221 treatment and both cover and biomass were significantly higher than in controls. Plants
222 growing in the EDTA-treated soils showed reduced growth in terms of significantly lower

cover, total phytobiomass, below-ground biomass and above-ground biomass values compared to the values recorded in controls. Species richness and Shannon indices were also lower. Finally, the most abundant species growing in the control microcosms were *Scirpoides holoschoenus* (L.) Soják, *Bartsia trixago* L., *Rumex bucephalophorus* L. and *Galium parisiense* L., reflecting the situation observed at the mine site. These species were less represented in the microcosms amended with EDTA, while grasses thrived mostly in the TDS treatments, especially *Agrostis castellana* Boiss. & Reut.

Phytotoxicity-related effects

The uptake of nutrients and metals from the soils in response to TDS or EDTA was assessed by examining the root system obtained from the microcosms as a whole along with the above-surface mass of species appearing in sufficient quantities for chemical analysis.

Despite expecting a reduction in the heavy metals absorbed by the plants in the TDS treatments, due to their retention by the OM, heavy metal concentrations (Fe, Mn, Zn, Pb, Cd, Ni) in the root biomass (Table 7) increased with TDS dose compared to the control treatments. In contrast, macronutrients did show a decreasing tendency in response to this treatment, especially in the case of P and K. EDTA significantly increased the entry of most of the elements (Na, Al, Fe, Mn, Zn, Pb, Cu, Cd, Ni) in the roots with respect to controls. Although both amendments led to greater concentrations of metals in the roots, levels detected in the above-ground mass of the most abundant species (Table 8) indicated their different translocation. Except in the case of *Vulpia myuros* (L.) C.C.Gmel., above-ground Zn concentrations were reduced in the species examined (*Agrostis castellana, Lolium rigidum* Gaudin, *Scirpoides holoschoenus*) when growing in soils amended with TDS. Lead levels also fell in the above-ground parts of *Agrostis castellana, Lolium rigidum* and *Vulpia myuros,* but

247 increased in *Scirpoides holoschoenus*. In the EDTA treatments, metal concentrations were
248 higher in the majority of species.
249 An overview of the effects produced by the two soil amendments is provided in Table 9.
250
251 **DISCUSSION**
252 Given the low N and nutrient contents of the polluted soil site examined in this study, the
253 addition of TDS induced a considerable change to this ecosystem. Unexpectedly, TDS,
254 especially when used at high concentrations, increased the mobility of soil metals, as
255 indicated by both leachate and root metal levels. This effect may be attributed to the
256 considerable drop in soil pH induced by this amendment. Antoniadis et al. (2008) similarly
257 detected a one-unit reduction in the pH of a soil treated with sewage sludge, and this gave rise
258 to a marked increase in the metal concentrations of both the soil's bioavailable fraction and
259 of the raygrass that grew in it. Chiu et al. (2006) reported similar findings. Some of the
260 negative effects of adding sewage sludge to soil can be mitigated by blending this waste with
261 other soil amendments such as Ca-carbonate (Siebielec and Chaney, 2012) or sawdust
262 (Ammari et al., 2012).
263 The increase in metal mobility do not, however, seem to affect the concentrations of heavy
264 metals in the shoots and leaves of plants, which in most cases were lower than in controls.
265 This possible reduction in metal toxicity along with the nutrients supplied by the TDS induced
266 the rapid growth of plants from the soil seed bank, increasing plant cover and biomass.
267 Although plant community diversity was unaffected, a change in floristic composition was
268 observed in favour of Gramineae. Moreno-Peñaranda et al. (2004) and Newman et al. (2014)
269 also addressed plant community dynamics in non-metal polluted degraded soils amended with
270 sewage sludge over a 5- or 6 year period. The results of the study by Moreno-Peñaranda et al.
271 (2004) revealed the more rapid initial growth of plant covers attributable to the amendment,

though this difference was lost over the years and similar covers to controls which grew at a slower rate were observed. This rapid expansion of cover does not necessary indicate an improved diversity or a floristic composition corresponding to a more advanced succession stage. Whichever the case, the large proportion of naked soil that exists at mine sites such as that examined here means that the rapid formation of a plant cover that will impair the erosion and transport of materials to adjacent zones is a priority of any remediation measure for polluted soils. In this regard, TDS seems to be an effective amendment for the remediation of soils such as the ones used here.

The application of EDTA to the soil gave rise to an impoverished soil due to a loss of macronutrients, as also observed by Wasay et al. (2001) and by Barona et al. (2001). The important loss of nutrients (Mg and K) using the higher EDTA dose suggests that, in the long term, phytoremediation would be ever less efficient due to cumulative plant nutritional deficiencies. EDTA persists in the environment (Adiloglu, 2002; Li and Shuman, 1996; Wu et al., 2004), such that nutrient loss might continue into the long term. This reduction in soil fertility means the soil cannot be considered recovered though heavy metal concentrations dropped to acceptable levels. In addition, the decrease in pH could also give rise to the greater mobility of metal ions and reduced fertility.

Changes produced in soil chemistry could produce effects on the biological activity of soil microorganisms (Sastre et al., 2007; Tiller, 1989). Despite the increased mobility of metals along with no marked change in soil chemical composition in the long term, this chelating agent cannot be recommended for the phytoremediation of soils with high concentrations of Pb, Zn, Cu, Cd. In effect, the levels of these heavy metals were practically identical to those of the untreated soils. It therefore seems that EDTA may not be useful for the recovery of some soils, since the loss of biomass of the more extracting species determines that the

quantity of metals removed from the system is similar in the long term to the amount present if the agent had not been added (Madrid et al., 2003).

The greater toxicity induced by this chelating agent seemed to manifest as a decrease in plant cover (though the number of seedlings germinating was unaffected). This indicates that this variable may be a good indicator of toxicity due to soil metals as observed by Pastor et al. (2003). In agreement with the findings of other authors (An, 2004; Landis and Yu, 1999; Newman and Jagoe, 1996), germination did not emerge as a reliable indicator of ecotoxicity. This amendment induced considerable reductions in plant cover and diversity along with a drop in productivity. This observation is consistent with the arguments of Landis and Yu, (1999) and highlights the idea that EDTA alone is not ideal for phytoextraction in a soil polluted with several heavy metals.

The growth and presence of *Scirpoides holoschoenus* and *Agrostis castellana* in most of the microcosms (control, TDS-amended and EDTA-amended) suggest the suitability of these species for further heavy metal phytoremediation studies. Despite the substantial levels of metals that can reach their above-ground parts, both species are considered metal excluders (Meharg et al., 1999; Pastor et al, 2015). This fact, together with the rhizomatous development of their below-ground phytomass, make them important constituents of plant covers to stabilize heavy metals and prevent soil erosion.

CONCLUSIONS

The ecological restoration of a site whose soils are polluted with several heavy metals using amendments cannot be assessed only in terms of effects produced on soil fertility. The transfer of metal ions through leachates or the incorporation of these ions in the system's autotrophic component is pivotal both for the health of the ecosystem being restored and to prevent pollutants from spreading to adjacent ecosystems.

When applied to a soil of these characteristics, EDTA seems to lead to plant productivity losses and nutritional imbalances, as it induces significant increases in the mobility of several elements both in the soil and its leachates. This gives rise to a real increase in toxicity along with soil impoverishment in the long term due to macronutrient loss.

TDS seems to be an interesting candidate for phytostabilizing soils polluted with Pb, Zn, Cu and Cd given its capacity to achieve a rapid soil cover as well as enhancing the retention of other elements in the plant root system, while avoiding their translocation to above-ground parts. This could help minimize soil erosion and the real toxicity posed to the plants of the ecosystem without a need to introduce allochthonous species. In future work, the optimal dose of this type of amendment will need to be determined.

This bioassay conducted under controlled conditions but simulating a real scenario seems a useful tool to assess the feasibility of strategies designed to remediate polluted soils.

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468 Figure 1. Mean concentrations (mg/L) of macroelements and metals in leachates from
469 microcosms collected in weeks 5, 7 and 9 of the experiment. Values along the vertical axes in
470 the Zn and Pb graphs are expressed as logs. Values of 0 mg/L are represented as 0.01. TDS 1
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474 Figure 2. Changes produced in the number of plants and plant cover throughout the bioassay.
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476 respectively) and EDTA 1 and 2 the results of the two EDTA treatments (0.5 g/kg and 1g/kg
477 respectively).

478 Table 1. pH, N (%), organic matter (OM, %), C (%), macroelements (mg/kg) and pseudo-
479 total and available metals (mg/kg) recorded in the mine soil used in the bioassay.

Parameter related to fertility		Metal concentration (mg/kg)			
		Pseudo-total		Available	
pH	6.4	Al	25100	Al	0.12
N	0.19	Fe	22600	Fe	6.4
OM	5.53	Mn	1190	Mn	7.4
C	3.21	Zn	5680	Zn	129
Ca	1930	Pb	4520	Pb	138
Mg	136	Cu	404	Cu	35
K	270	Cd	25	Cd	0.98
Na	10	Ni	30	Ni	0.36

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Table 2. Herb species that thrive in the grassland community where the experimental soil was collected.

GRAMINEAE	LEGUMINOSACEAE	OTHER	
Agrostis castellana	*Ornithopus compressus*	*Alyssum granatense*	*Sanguisorba minor*
Arrhenaterum album	*Trifolium arvense*	*Asparagus acutifolius*	*Scirpoides holoschoenus*
Avena barbata	*Trifolium gemellum*	*Bellardia trixago*	*Silene colorata*
Bromus diandrus	*Trifolium glomeratum*	*Capsella bursa-pastoris*	*Spergularia rubra*
Bromus madritensis	*Trifolium scabrum*	*Cerastium glomeratum*	*Thymus zigis*
Bromus rubens	*Trifolium suffocatum*	*Crassulla tillaea*	*Verbascum pulverulentum*
Bromus tectorum		*Diplotaxis catholica*	
Corynephorus fasciculatus	**COMPOSITAE**	*Echium plantagineum*	
Dactylis glomerata	*Anacyclus clavatus*	*Erodium cicutarium*	
Holcus setiglumis	*Andryala integrifolia*	*Gallium parisiense*	
Hordeum murinum	*Andryala laxiflora*	*Geranium dissectum*	
Lolium rigidum	*Carduus pynocephalus*	*Heliotropium europaeum*	
Melica ciliata	*Carduus tenuiflorus*	*Hirschfeldia incana*	
Mibora minima	*Centaurea melitensis*	*Juncus bufonius*	
Rostraria cristata	*Crepis vesicaria*	*Lamium amplexicaule*	
Stipa sp.	*Chondrilla juncea*	*Mentha rotundifolia*	
Vulpia bromoides	*Filago minima*	*Myosotis discolor*	
Vulpia ciliata	*Filago pyramidata*	*Neatostema apulum*	
Vulpia myuros	*Hypochoeris rostrata*	*Plantago afra*	
Vulpia unilateralis	*Leontodon saxatilis*	*Plantago coronopus*	
	Logfia arvensis	*Plantago lagopus*	
	Picnomon acarna	*Rumex bucephalophorus*	
	Tolpis barbata		

Table 3. pH, N (%), organic matter (OM, %), C (%), macroelements (mg/kg) and heavy metals (mg/kg) recorded in the thermally-dried sewage sludge used in the bioassay.

Fertility parameter	Value	Heavy metal	Value
pH	6.64	Zn	1120
N	3.94	Pb	398
OM	40.4	Cu	409
P	400	Cd	3.0
Ca	6360	Ni	175
Mg	1460		
K	1560		
Na	531		

490 Table 4. Soil pH, N (%), organic matter (OM, %) and pseudo-total (T) and available (A)
491 macroelements (mg/kg) recorded in the microcosms after the bioassay. *: significant
492 difference (95%) versus control, **: significant difference (95%) versus control and versus
493 the lower amendment dose.

Parameter	Control	TDS				EDTA			
		25 g/kg		50 g/kg		0.5 g/kg		1 g/kg	
pH	7.45 ± 0.05	7.23 ± 0.14	*	6.92 ± 0.03	**	7.27 ± 0.11	*	7.21 ± 0.10	*
N	0.23 ± 0.02	0.29 ± 0.01	*	0.32 ± 0.02	**	0.25 ± 0.03		0.25 ± 0.00	
OM	5.45 ± 0.07	6.08 ± 0.04	*	6.86 ± 0.18	**	5.41 ± 0.40		5.45 ± 0.38	
Ca T	4860 ± 467	5110 ± 102		5780 ± 245	*	4670 ± 123		4900 ± 307	
Mg T	2900 ± 316	2940 ± 235		2900 ± 290		2680 ± 264		2270 ± 154	*
P T	912 ± 167	1106 ± 91		1470 ± 90	*	926 ± 51		1098 ±140	
K T	10700 ± 2940	11200 ± 2030		10200 ± 3100		9940 ± 2320		5800 ± 1500	
Na T	225 ± 33	245 ± 14		219 ± 25		240 ± 14		249 ± 40	
Ca A	2220 ± 106	2140 ± 132		2100 ± 125		2560 ± 265	*	2520 ± 226	*
Mg A	151 ± 5.0	145 ± 6.1		137 ± 8.1		160 ± 3.0		182 ± 32	*
K A	145 ± 5.0	137 ± 5.8		163 ± 7.6		127 ± 20	*	116 ± 24	**
Na A	23 ± 6.0	62 ± 2.9	*	100 ± 18	**	30 ± 0.2	*	32 ± 3.0	*

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Table 5. Soil pseudo-total (T) and available (A) metal concentrations (mg/kg) recorded in the microcosms after the bioassay. *: significant difference (95%) versus control, **: significant difference (95%) versus control and versus the lower amendment dose.

Element	Control	TDS		EDTA	
		25 g/kg	50 g/kg	0.5 g/kg	1 g/kg
Al T	25600 ± 5060	27200 ± 3920	24200 ± 5800	23100 ± 4450	14700 ± 2910
Fe T	50700 ± 11700	50100 ± 6700	41800 ± 1750	45700 ± 2270	48600 ± 3760
Mn T	1120 ± 79	1040 ± 58	984 ± 104	988 ± 60	973 ± 74
Zn T	5780 ± 1840	5290 ± 264	5710 ± 848	5280 ± 487	5530 ± 733
Pb T	4020 ± 622	4050 ± 564	4400 ± 423	4470 ± 368	4140 ± 428
Cu T	117 ± 10	113 ± 3.7	114 ± 3.2	111 ± 5.2	109 ± 6.3
Cd T	28 ± 3.6	25 ± 1.2	27 ± 2.4	27 ± 1.8	28 ± 4.3
Ni T	29 ± 3.5	28 ± 1.7	28 ± 1.4	28 ± 0.8	27 ± 1.6
Al A	0.007 ± 0.01	0.003 ± 0.01	0.030 ± 0.02	0.060 ± 0.04	0.010 ± 0.02
Fe A	8.8 ± 5.6	18 ± 1.4 *	17 ± 0.3 *	12 ± 7.0	13 ± 3.1
Mn A	6.5 ± 4.2	11 ± 7.6	15 ± 14	14 ± 9.9	14 ± 5.6
Zn A	237 ± 11	224 ± 5.8	181 ± 14 *	235 ± 17	246 ± 6.1
Pb A	179 ± 8.5	180 ± 14	153 ± 11	157 ± 5.5 *	155 ±20
Cu A	2.3 ± 0.4	3.4 ± 0.3 *	3.1 ± 0.2 *	3.2 ± 0.30 *	3.7 ± 0.2 **
Cd A	1.8 ± 1.0	1.8 ± 0.2	1.6 ± 0.1	1.9 ± 2.4	2.2 ± 0.1
Ni A	0.9 ± 0.6	1.0 ± 0.1	0.9 ± 0.1	1.1 ± 1.8	1.3 ± 0.2

501 Table 6. Plant community indicators at the end of the bioassay. *: significant difference (95%)
502 versus control, **: significant difference (95%) versus control and versus the lower
503 amendment dose. T. biomass: total biomass, A.G biomass: above-ground biomass, B.G.
504 biomass: below-ground biomass.

Parameter		TDS			EDTA			
	Control	25 g/kg		50 g/kg		0.5 g/kg		1 g/kg	
# seedlings	112 ± 51	122 ± 48		126 ± 57		125 ± 50		115 ± 43	
Cover (%)	24 ± 4.9	61 ± 6.2	*	62 ± 7.9	*	24 ± 6.6		16 ± 6.9	
Species richness	9.3 ± 1.2	9.0 ± 2.6		8.3 ± 0.9		8.3 ± 0.6		5.0 ± 0.0	*
Shannon index	2.64 ± 0.21	2.30 ± 1.05		2.65 ± 0.16		2.53 ± 0.24		1.89 ± 0.22	*
T. biomass (mg/cm^2)	32 ± 9.0	41 ± 2.3	*	42 ± 5.1	*	19 ± 1.1	*	15 ± 2.0	*
A.G biomass	21 ± 6.0	25 ± 5.2		27 ± 3.9		12 ± 1.0	*	11 ± 1.9	*
B.G biomass	11 ± 1.0	16 ± 2.1	*	14 ± 0.9	*	7.1 ± 0.9	*	4.2 ± 0.9	**

505

506

507 Table 7. Macronutrient and metal concentrations (mg/kg) recorded in the below-ground
508 phytomass of the microcosms. *: significant difference (95%) versus control.

Element	Control	TDS			EDTA			
		25 g/kg	50 g/kg		0.5 g/kg		1 g/kg	
Ca	4550 ± 2740	4250 ± 748	4230 ± 825		4650 ± 994		4090 ± 893	
Mg	719 ± 58	538 ± 149	597 ± 82		772 ± 211		742 ± 354	
P	1140 ± 117	936 ± 587	833 ± 7.1	*	1380 ± 738		1750 ± 1710	
K	8050 ± 1070	4500 ± 2920	4720 ± 880		18700 ± 18500		7570 ± 3550	
Na	605 ± 38	461 ± 74	533 ± 50		1131 ± 323	*	1720 ± 707	*
Al	327 ± 194	1050 ± 726	495 ± 29		687 ± 374		1470 ± 547	*
Fe	913 ± 380	3370 ± 2380	8450 ± 3410	*	3520 ± 1920		4630 ± 219	*
Mn	211 ± 127	435 ± 276	1180 ± 448	*	604 ± 218		772 ± 351	*
Zn	342 ± 33	465 ± 188	715 ± 207	*	567 ± 127		797 ± 196	*
Pb	251 ± 219	960 ± 587	1760 ± 1130	*	1280 ± 559	*	1720 ± 220	*
Cu	23 ± 1.3	19 ± 4.0	21 ± 8.8		35 ± 5.9	*	41 ± 5.7	*
Cd	4.5 ± 1.4	9.4 ± 8.2	13 ± 5.3	*	11 ± 6.7		13 ± 2.3	*
Ni	0.9 ± 1.0	3.0 ± 2.2	2.5 ± 1.2		5.1 ± 1.4	*	7.9 ± 4.0	*

509

510

511 Table 8. Metal concentrations (mg/kg) recorded in the above-ground parts of some of the
512 species growing in the microcosms.

		Zn	Pb	Cu	Cd
Agrostis castellana					
Control		290	66	6.7	1.4
TDS	25 g/kg	218	4.10	43	2.5
	50 g/kg	203	3.30	6.6	n.d.
EDTA	0.5 g/kg	953	125	2.5	2.0
	1 g/kg	1170	145	5.0	2.0
Gallium parisiense					
Control		400	43	2.0	n.d
EDTA	0.5 g/kg	483	60	5.1	n.d
Lolium rigidum					
Control		277	0.80	0.8	n.d.
TDS	25 g/kg	108	0.70	1.0	n.d.
	50 g/kg	8.5	0.70	0.9	n.d.
Plantago coronopus					
Control		650	7.5	2.5	n.d
EDTA	0.5 g/kg	1250	118	255	3.0
	1 g/kg	973	150	5.0	2.0
Scirpoides holoschoenus					
Control		127	0.33	36	n.d.
TDS	25 g/kg	71	12	1.0	n.d.
	50 g/kg	69	7.2	5.4	n.d.
EDTA	0.5 g/kg	216	25	14	n.d.
Vulpia myuros					
Control		62	8.5	20	n.d.
TDS	25 g/kg	140	2.3	7.5	n.d.
	50 g/kg	165	2.5	6.2	n.d.

513

514

515 Table 9. Summary of the effects of the two amendments used in soils polluted

516 with several metals

Variable	TDS	EDTA
Soil pH	Lowered	Lowered but less than with TDS
OM and N	Increased	Unchanged
Soil nutrients	Null or beneficial effects	Null or negative effects
Soil heavy metals	Unchanged	Unchanged
Nutrients in the 3 leachates	Increasing trend	Increased
Metals in the 3 leachates	Somewhat increased but less than with EDTA	Increased, especially Zn and Pb
Species richness	Unchanged	Lowered
Plant cover	Positive effects	Decreasing trend
Above-ground biomass	Unchanged	Negative effects
Root biomass	Positive effects	Negative effects
Root metals	Generally beneficial	Beneficial effects

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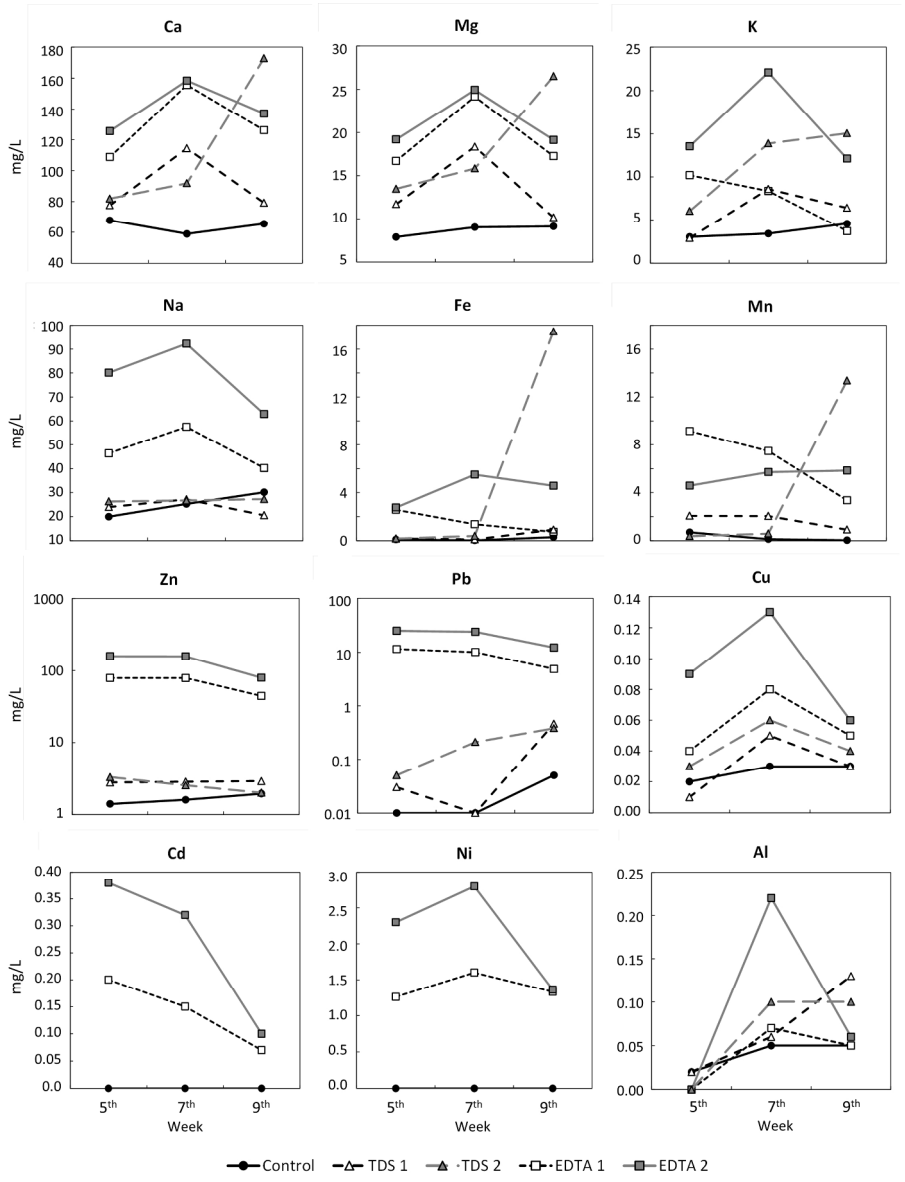

Figure 1. Mean concentrations (mg/L) of macroelements and metals in leachates from microcosms collected in weeks 5, 7 and 9 of the experiment. Values along the vertical axes in the Zn and Pb graphs are expressed as logs. Values of 0 mg/L are represented as 0.01. TDS 1 and 2 represent the results of the two TDS treatments (25 g/kg and 50 g/kg respectively) and EDTA 1 and 2 the results of the two EDTA treatments (0.5 g/kg and 1g/kg respectively).
190x254mm (300 x 300 DPI)

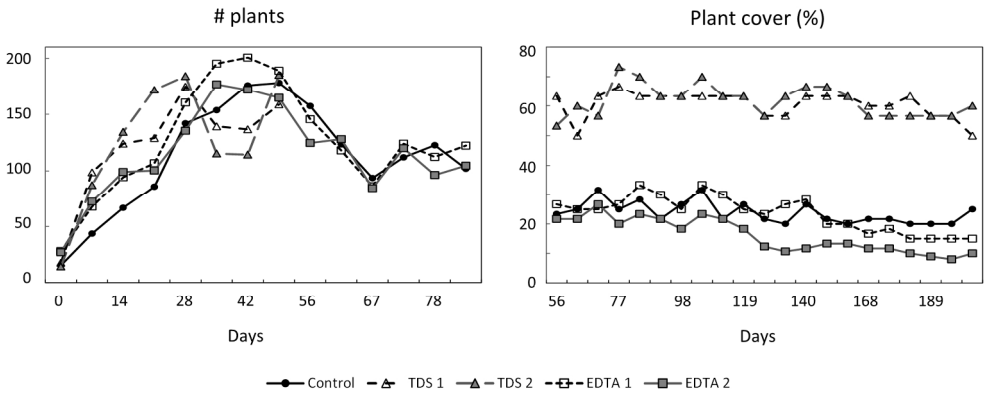

Figure 2. Changes produced in the number of plants and plant cover throughout the bioassay. TDS 1 and 2 represent the results of the two TDS treatments (25 g/kg and 50 g/kg respectively) and EDTA 1 and 2 the results of the two EDTA treatments (0.5 g/kg and 1g/kg respectively).
190x78mm (300 x 300 DPI)