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## Metal immobilization and soil amendment efficiency at a contaminated sediment landfill site: a field study focusing on plants, springtails, and bacteria

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3 **Title:** Metal immobilization and soil amendment efficiency at a contaminated sediment  
4 landfill site: a field study focusing on plants, springtails, and bacteria

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24 **Capsule**

25 In-situ incorporation of Thomas Basic Slag into a landfilled metal-contaminated  
26 sediment decreases metal mobility and ecotoxicity and increases bacterial activity.

27

28 **Abstract**

29 Metal immobilization may contribute to the environmental management strategy of  
30 dredged sediment landfill sites contaminated by metals. In a field experiment,  
31 amendment effects and efficiency were investigated, focusing on plants, springtails and  
32 bacteria colonisation, metal extractability and sediment ecotoxicity. Conversely to  
33 hydroxylapatite (HA, 3 % DW), the addition of Thomas Basic Slag (TBS, 5 % DW) to a  
34 5-yr deposited sediment contaminated with Zn, Cd, Cu, Pb and As resulted in a decrease  
35 in the 0.01M Ca(NO<sub>3</sub>)<sub>2</sub> extractable concentrations of Cd and Zn. Shoot Cd and Zn  
36 concentration in *Calamagrostis epigejos*, the dominant plant species, also decreased in  
37 the presence of TBS. The addition of TBS and HA reduced sediment ecotoxicity and  
38 improved the growth of the total bacterial population. Hydroxylapatite improved plant  
39 species richness and diversity and decreased antioxidant enzymes in *C. Epigejos* and  
40 *Urtica dioica*. Collembolan communities did not differ in abundance and diversity  
41 between the different treatments.

42

43 **Keywords :** basic slag; *Calamagrostis epigejos*; dredged sediment; hydroxylapatite;  
44 ecotoxicity

45

46

## 47 **1. Introduction**

48 Human activities during the last decades have contaminated canal sediments  
49 with various organic and inorganic pollutants. Those of most concern are metal(loid)s,  
50 polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and mineral  
51 oils. Polluted sediments in canals may be disposed on land. Indeed, the maintenance of  
52 waterways requires dredging on a regular basis to prevent flooding, facilitate  
53 navigation, and allow for use of a given water system. European Community policy  
54 encourages sediment recovery (Directive 2008/98/EC). Nevertheless, due to the high  
55 concentration of pollutants and their potential toxicity, contaminated dredged sediments  
56 cannot be used in civil engineering as raw material or the deposit cannot be used to  
57 produce valuable biomass. Many treatments are available for contaminated sediments,  
58 but relatively few are applicable to metal(loid) pollution. Currently, treatment and reuse  
59 of heavily contaminated dredged materials is not a cost-effective alternative to disposal  
60 landfill sites (Bert et al., 2009). In the Nord-Pas-de-Calais region (France), which is  
61 affected by intensive industrial activities, local authorities are required to manage  
62 contaminated landfill sites where large volumes of polluted dredged materials were  
63 deposited. The regional division of Voies Navigables de France (VNF) developed a  
64 management strategy for its disposal sites. This strategy includes the implementation of  
65 an environmental management system which aims to meet best practices and comply  
66 with environmental regulation in the field of human health and environment. The VNF  
67 is involved in the reclamation of its disposal sites into 'natural' and 'green' zones  
68 (Prevost, 2008). Metal immobilization in metal-contaminated sediments at some landfill  
69 sites may contribute to this environmental management strategy.

70 Metal immobilization aims at (1) changing speciation of trace elements (TE) in  
71 the substrate to reduce their soluble and exchangeable fractions, (2) limiting TE-uptake  
72 by plants, (3) reducing the direct exposure through soil by reducing metal availability to  
73 heterotrophic organisms, resulting in enhanced biodiversity (Vassilev et al., 2004). In  
74 situ immobilization of metal(loid)s is achieved by incorporating amendments into the  
75 soil, promoting their sorption and precipitation. In addition to immobilization effects,  
76 soil amendment can improve soil fertility by increasing pH, organic matter content,  
77 microbial activity, and moisture retention, and reducing soil compaction (Vangronsveld  
78 et al., 1995, 1996).

79 The efficiency of amendments is site-specific, depending on various factors  
80 among which pollutant types and soil properties (Kumpiene et al., 2008). Numerous soil  
81 amendments have been tested in pots, at pilot and field scale, for remediation purposes  
82 (Bes and Mench, 2008; Knox et al., 2001; Kumpiene et al., 2008). The effects of  
83 Thomas Basic Slag (TBS) and hydroxylapatite (HA) incorporation have only been  
84 investigated in batch and pot experiments (Bes and Mench, 2008; Boisson et al., 1999;  
85 Friesl et al., 2006; Mench et al., 1994a, 1994b; Mench et al., 2000; Misra and  
86 Chaturvedi, 2007; Negim et al., 2010; Panfili et al., 2005). Hydroxylapatite, a mineral  
87 from the phosphate group, is the major component of tooth enamel and bone mineral. It  
88 is mainly used for medical purposes. Hydroxyapatite powders can be synthesised via  
89 numerous production routes, using a range of different reactants. Some processing  
90 techniques include wet chemical methods (precipitation), hydrothermal techniques,  
91 hydrolysis of other calcium phosphates and sol-gel. Due to its particular properties  
92 including the sorption of metallic ions, HA can be useful for the management of  
93 contaminated groundwater and soil. The application of HA  $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$  to

94 contaminated soil immobilizes dissolved Pb (Ma et al., 1993). Other studies on metal  
95 stabilization by phosphorous compounds focused on reduction of plant uptake of  
96 metals, as well as reduction of their solubility and mobility (Knox et al., 2001). After  
97 HA incorporation into a contaminated soil, the concentrations of exchangeable metals  
98 decreased and plant uptake of these elements was reduced (Boisson et al., 1999).  
99 However, arsenic uptake by plants increased and there were nutrient deficiencies.  
100 Therefore it was concluded that HA application could be effective to immobilize Zn,  
101 Pb, Cu and Cd but was inappropriate in the case of mixed metal-arsenic pollution and  
102 when potential nutrient deficiencies may occur. Thomas Basic Slag (TBS) is an alkaline  
103 by-product of steel industry used as a fertilizer by farmers. It usually increases soil pH  
104 (Bes and Mench, 2008) and contains P, Mn, Mg, and Fe (Panfili et al., 2005). Soil  
105 treatment with TBS decreased Cd and Zn-soluble and  $(Ca(NO_3)_2)$  exchangeable  
106 fractions and reduced Cd concentrations in tobacco shoots (Mench et al., 1994a, 1994b).  
107 Zinc availability decreased after TBS addition and this persisted over 5 months without  
108 any phytotoxicity (Mench et al., 2000). Bes and Mench (2008) studied the incorporation  
109 of 0.25% and 3.9% (DW) TBS into Cu-contaminated soils. Based on soil phytotoxicity,  
110 3.9% TBS was one of the most efficient amendments even though high levels of Cu  
111 remained in the soil solution. Panfili et al. (2005) studied the effect of HA and TBS on  
112 Zn in a metal-contaminated sediment and suggested that the formation of Zn phosphate  
113 contributed to Zn immobilization.

114         Plants, bacteria and soil fauna, notably springtails (Collembola), can develop  
115 tolerant populations on metal-contaminated soils (Bes et al., 2010; Escarré et al., 2011;  
116 Gillet and Ponge, 2003; Lors et al., 2005; Ryan et al., 2004; Tyler et al., 1989;  
117 Vangronsveld et al., 1995, 1996). Vegetation reduces contaminant mobility by reducing

118 leaching, soil erosion, and improves the aesthetic value of formerly barren areas  
119 (Ruttens et al., 2006). The vegetation itself may contribute to metal immobilization in  
120 the rhizosphere and through the production of litter (Bouwman and Vangronsveld,  
121 2004). When metal bioavailability and exposure decrease, due to vegetation or  
122 amendment or both, sensitive plants, bacteria and soil fauna can develop and participate  
123 in turn to the restoration of an ecosystem on contaminated soils (Bouwman et al., 2001;  
124 Lock et al., 2003; Lock and Janssen, 2003, 2005; Vangronsveld et al., 1995, 1996).

125         This field-scale work was performed at an experimental metal-contaminated  
126 sediment landfill site. The aim was to evaluate the efficiency of TBS and HA  
127 incorporation on metal(oid) immobilization. Efficient soil amendments may decrease  
128 metal(oid) extractability and shoot metal concentrations. In parallel, the potential  
129 adverse or beneficial effects of TBS and HA incorporation were investigated on an  
130 array of biological parameters related to plant and springtail communities composition  
131 and diversity, total and metal specific bacterial populations (anaerobic sulphate-  
132 reducing bacteria and aerobic sulphur-oxidising bacteria), springtail trophic status (gut  
133 contents), sediment ecotoxicity and sublethal effects in plants (maximum photochemical  
134 efficiency of photosystem II and antioxidant enzymes activity levels). This battery  
135 might be relevant to assess the efficiency of soil additives.

136         This is the first time that a full-scale study of metal immobilization combined  
137 with ecosystem monitoring is reported on a contaminated dredged sediment disposal  
138 site.

139

## 140 **2. Materials and methods**

### 141 *2.1. Description of experimental site and sediment treatment*

142 In May 2002, a field trial was set up in an agricultural area in Lallaing (North of  
143 France, 50°23'17''N and 3°11'59''E). Three plots of 60 m<sup>2</sup> and 40 cm depth were dug  
144 in an uncontaminated soil. These plots were filled with freshly dredged sediments from  
145 the nearby Scarpe canal (Pont de Râches). These sediments were contaminated due to  
146 past and present non-ferrous metal processing and smelting activities. Two plots were  
147 used to assess soil additives. Thomas Basic Slag (TBS) and a synthetic hydroxylapatite  
148 (HA), both in powdered forms, were singly incorporated into the sediment at a rate of  
149 5% and 3% DW, respectively. TBS was obtained from Cedest<sup>®</sup> (Mâcon, France) and  
150 HA was from Brenntag<sup>®</sup> (Mülheim/Ruhr, Germany). The purity of HA was certified to  
151 99% by the supplier. The third plot remained untreated (NT). After TBS and HA  
152 incorporation, the treated and untreated plots were mechanically homogenized with a  
153 crane shovel for two hours to ensure that the mixture was homogeneous. The plots were  
154 air dried for two months to reduce the sediment water content. The three plots were  
155 further subdivided into 9 sub-plots of 20 m<sup>2</sup> each, resulting in 3 untreated plots (NT), 3  
156 HA-treated plots and 3 TBS-treated plots. Six of these plots were further sown with  
157 grasses (*Deschampsia cespitosa* and *Festuca rubra*) while the 3 remaining plots  
158 remained unplanted. This study reported the work performed on these unsown plots.  
159 From mid-spring 2003, spontaneous vegetation started to develop on these plots.  
160 Vegetation management (i.e. removal of selected species, harvest, fertilizer addition,  
161 and irrigation) was not carried out from this time to March 2007.

## 162 2.2. Collection of sediment and soil samples

163 In March 2007, top-sediments (0-20 cm depth) of the three tested plots (NT, HA,  
164 TBS) were sampled. Three surface sediment samples per plot were randomly collected  
165 with a hand auger to determine (pseudo)-total metal(oid) and extractable concentrations,



166 pH<sub>water</sub> and sediment ecotoxicity. Six additional randomly collected samples were taken  
167 on each of the plots at a depth of about 20 cm using a hand auger for bacterial analyses  
168 (3 samples/plot) and a corer of 5 cm diameter for collembolan analyses (3 samples /  
169 plot). One composite sample per plot was formed with 5 sub-samples randomly  
170 collected to determine the other physico-chemical characteristics (particle size, organic  
171 carbon, total nitrogen, carbonates and cation exchange capacity). In parallel, an  
172 uncontaminated soil (0-20 cm depth), located outside the plots, was collected. This  
173 control area (CA) was selected because it had similar spontaneous vegetation to the  
174 plots. Physicochemical properties were determined on a composite sample made of 5  
175 sub-samples collected below the vegetation of interest. Three additional soil samples  
176 were collected for collembolan analyses.

### 177 *2.3. Sediment and soil characteristics*

178 All samples were air dried until constant weight and sieved through 2 mm mesh  
179 size. All analyses on composites have been performed at the Laboratoire Départemental  
180 d'Analyses et de Recherche (LDAR), Laon, France using standard methods (particle  
181 size (NF X 31 – 107, 2003), organic carbon (NF ISO 14 235, oxidation method, 1998),  
182 total N (NF ISO 11261, colorimetric method, 1995), carbonates (NF ISO 10693, 2004),  
183 cation exchange capacity (CEC, NF X31-130, 1999).

184 A sample of 0.5 g of air-dried and sieved sediment and soil was weighed into 70  
185 mL teflon microwave tubes .Eight mL of *aqua regia* containing 2 mL 67% concentrated  
186 HNO<sub>3</sub> and 6 mL 36% concentrated HCl (NF EN 13657, 2003) was added. Samples  
187 were heated in a microwave digester (MARS Xpress, CEM Corporation<sup>®</sup>, Matthews,  
188 NC) to 180 °C for 20 min, with a 30 minute ramp time. After filtration through a 0.45  
189 μm Whatman filter, the pseudo-total metal(oid) concentrations (As, Cd, Pb, Zn, Cu)

190 were determined using an Inductively Coupled Plasma Atomic Emission Spectrometer  
191 ICP-AES (Jobin Yvon<sup>®</sup>, Longjumeau, France). To assess the analytical quality, a  
192 standard reference sediment material SRM 2704 (Buffalo River Sediment, Standard  
193 Reference Material) was subjected to the same protocol. Recoveries were: 100 % for  
194 Cd, 101% for Zn, 104% for Pb, 90% for Cu and 94% for As.

195 Soil pH was measured in 1:5 sediment/soil:water suspension using a glass  
196 electrode pH meter (NF ISO 10390, 1994).

197 Table 1 lists physico-chemical properties of contaminated sediments (NT, HA,  
198 TBS) and the uncontaminated soil (CA).

#### 199 *2.4. Extractable trace element analysis and sediment ecotoxicity*

200 Extractable sediment metal(oid) concentrations of treated and untreated plots  
201 were determined after extraction with 0.01 M Ca(NO<sub>3</sub>)<sub>2</sub>. Prior to analysis, 20 g of air-  
202 dried and sieved sediment were shaken for 48 h with 40 mL of 0.01 M Ca(NO<sub>3</sub>)<sub>2</sub>  
203 solution. Extracts were filtered through a 0.45 μm cellulose membrane and metal  
204 concentrations were measured using ICP-AES.

205 Ecotoxicity was assessed by two standardized bioassays: the bacterial Microtox<sup>®</sup>  
206 assay and the algal test, using *Vibrio fischeri* and *Pseudokirchneriella subcapitata* as  
207 test organisms, respectively. Those two assays were previously shown to be highly  
208 sensitive to leachates obtained from dredged materials (Piou et al., 2009). Prior to the  
209 bioassays, soil leachates were prepared according to the French standard AFNOR X31-  
210 210 (1991) and diluted (10 to 90%) with demineralised water. Ecotoxicity assays were  
211 run according to AFNOR T90-320 (1991) and ISO 8692 (1989) standards for  
212 Microtox<sup>®</sup> and algal bioassays, respectively. The half maximum effective concentration

213 (EC<sub>50</sub>, Microtox<sup>®</sup> bioassay) and the 50% inhibitory concentration (IC<sub>50</sub>, algal bioassay)  
214 were derived from dilution-effect curves. When the toxicity of leachates was not  
215 sufficient to reach 50% of effect, EC<sub>50</sub> or IC<sub>50</sub> values were indeterminable and the %  
216 of effect observed at the 90% dilution was then reported.

## 217 *2.5. Plant survey, biodiversity indices and plant analysis*

### 218 *2.5.1. Plant survey and similarity index*

219 Vascular plants were surveyed in May and June 2007 in all plots using  
220 Lambinon et al. (2004) as a standard reference book for plant taxonomy. The total  
221 vegetation cover and the cover rate of mosses, herbaceous plants, shrubs and trees were  
222 assessed on each plot. Individuals per species and per plot were counted and numbers  
223 were related to plot area. Species richness corresponded to the number of species per  
224 plot.

225 A similarity index (Sørensen, 1948) was calculated to compare the composition  
226 of vegetation between (i) experimental plots (influence of treatments) and (ii)  
227 experimental plots and surrounding biotopes (identification of putative biotopes that  
228 may contribute to plot colonization).

### 229 *2.5.2. Biodiversity indices*

230 The diversity of vascular plants was assessed using the Shannon-Weaver index  
231 H' (Shannon and Weaver, 1949), calculated as

$$232 \quad H' = - \sum_{i=1}^s p_i * \log_2 p_i$$

233 where  $p_i$  is the species proportion in a community composed of  $s$  species. The  
234 homogeneity of species distribution was assessed through the regularity index  $J$  of  
235 Piélou (1966).

### 236 2.5.3. *Plant analyses*

237 *Calamagrostis epigejos*, a grass, and *Urtica dioica*, a forb, were chosen for  
238 phytotoxicity and metal content analyses as both herb species were present in all plots  
239 and in the control area in sufficient abundance to sample 5 individuals per species of  
240 approximately similar size and sufficient biomass to perform analyses. Plant sampling  
241 occurred in June 2007.

#### 242 2.5.3.1 *Chlorophyll fluorescence*

243 The fluorescence of chlorophyll  $a$  was measured on dark-adapted leaves of *C.*  
244 *epigejos* and *U. dioica* submitted to a saturating light pulse using a portable chlorophyll  
245 fluorimeter (Handy-PEA, Hansatech Instruments<sup>®</sup>, Norfolk, UK). Basal fluorescence  
246 ( $F_0$ ) and maximum fluorescence ( $F_m$ ) values were used to derive  $F_v/F_m$  ratio  
247 (maximum photochemical efficiency of PSII), with the variable fluorescence  $F_v = F_m -$   
248  $F_0$ .

#### 249 2.5.3.2 *Antioxidant enzymes*

250 Leaves from both plant species were collected from CA, NT, HA and TBS plots  
251 and immediately transferred to polypropylene tubes then frozen in liquid nitrogen. At  
252 the laboratory, leaf samples were crushed manually in a 125 mM phosphate buffer at pH  
253 7.8 by using a porcelain mortar placed on ice. After centrifugation (15 000 g for 10 min  
254 at 4° C), the supernatant was used to determine antioxidant enzyme activities.

255 Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol  
256 peroxidase (GPX), and glutathione reductase (GRD) activities were measured according  
257 to methods described by Dazy et al. (2008). The protein concentration was determined  
258 in plant extracts (Bradford, 1976); enzyme activity levels were expressed in  
259  $\mu$ moles/min/mg protein excepted for SOD for which one unit of activity corresponded  
260 to the amount of enzyme causing 50% inhibition of absorbance readings by comparison  
261 to control tubes lacking enzymes.

#### 262 2.5.3.3 *Elemental concentrations in plant shoots*

263 Plant shoots were carefully washed (3 times) with distilled water and oven-dried  
264 at 40°C until constant weight. Weighed aliquots (0.5 g DW) were wet digested in 10 mL  
265 HNO<sub>3</sub> (65%) using a microwave digester (Mars Xpress) according to the following  
266 program: 3 min to reach 100 °C, 2 min to reach 140 °C, 2 min to reach 160 °C, 2 min to  
267 reach 180 °C and 20 min at 180 °C. Solutions were filtered to <0.45  $\mu$ m (Whatman  
268 filter) and concentrations of Cd, Zn, Pb, Cu, As, Ca, Fe, Mg, Mn, P, Na, K and Al were  
269 analyzed using ICP- AES (VARIAN 720 AES-ICP, Ullys<sup>®</sup>, France). Blanks and  
270 certified reference material (trace elements in hay, IAEA V10, International Atomic  
271 Energy Agency, Vienna, Austria) were included for quality control. Recoveries were:  
272 100% for Ca, 91% for Cu, 79% for Fe, 113% for Mg, 91% for As, 89% for Mn and Pb,  
273 105% for K, 89% for Na, 118% for P, 87% for Zn, 93% for Cd and 67% for Al.

274

#### 275 2.6 *Collembola identification and gut content observation*

276 After sampling at a depth of 20 cm, considering that the whole collembolan  
277 community was sampled on a surface of  $\sim 20$  cm<sup>2</sup>, sediments (NT, HA, TBS) and soil

278 (CA) samples were embedded in polythene bags then transported to the laboratory. The  
279 extraction of arthropods started on the same day using the dry funnel (Berlese) method  
280 (Edwards and Fletcher, 1971) and was completed within 7-10 days. The daytime  
281 maximum temperature averaged 25-30 °C. Animals were collected and preserved in  
282 ethyl alcohol until sorting and identification. Sorting was done in alcohol under a  
283 dissecting microscope at x 20 magnification. All springtails were mounted in chloral-  
284 lactophenol then observed in a light microscope under phase contrast at x 400  
285 magnification. Identification was done at the species level using keys by Bretfeld  
286 (1999), Fjellberg (1998), Gisin (1960), Hopkin (2000), Jordana et al. (1997), Potapov  
287 (2001) and Zimdars and Dunger (1994). Gut contents were observed by transparency  
288 and classified in 8 categories using morphological features observable in phase contrast  
289 light microscopy at x 400 magnification, and were counted according to the method  
290 devised by (Gillet and Ponge, 2003, 2005): algae, animal remains, bacteria, melanised  
291 and hyaline hyphae, fungal spores, holorganic humus, hemorganic humus, to which  
292 empty guts were added. The examination of gut contents may elucidate the composition  
293 of horizons in which the animals were living (Ponge, 2000). We sought to determine  
294 when toxicity forced the organisms to move to horizons in which they do not normally  
295 live or delete some food resources from their current diet (Gillet and Ponge, 2003).

## 296 *2.7 Bacterial analyses*

297 Sediment samples (NT, HA and TBS) were collected in polythene bags then  
298 transported to the laboratory. After homogenization, samples were maintained at 4°C  
299 before performing bacterial analyses. The enumeration of total sulphur-oxidising  
300 bacteria (SOB) was carried out on Nunc<sup>®</sup> Delta (Nalge Nunc International<sup>®</sup>, Roskilde,  
301 Denmark) microplates of 96 wells (8 lines x 12 columns), on which each well contained

302 250  $\mu\text{L}$  of specific medium. Nutrient Broth<sup>®</sup> (Difco<sup>®</sup>, Detroit, USA) medium (diluted to  
303 1/10), added to fungicidal cyclohexamide solution ( $0.2 \text{ g L}^{-1}$ ), was used for total  
304 bacterial count. The medium used to enumerate neutrophilic SOB contained 5 g  
305  $\text{Na}_2\text{SO}_3 \cdot 5\text{H}_2\text{O}$ , 2 g  $\text{K}_2\text{HPO}_4$ , 0.1 g  $(\text{NH}_4)_2\text{SO}_4$ , 0.1 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.1 g  $\text{CaCl}_2$ , 0.002 g  
306  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ , 0.005 g  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$  and 3 ml bromocresol purple ( $c = 0.02 \text{ g L}^{-1}$ ). The  
307 pH of the medium was initially adjusted to pH 6.5 with NaOH. For acidophilic SOB,  
308 the medium, which contained 1.5 g  $\text{KH}_2\text{PO}_4$ , 0.1 g  $\text{CaCl}_2$ , 0.1 g  $\text{MgCl}_2 \cdot \text{H}_2\text{O}$ , and 0.1 g  
309  $\text{NH}_4\text{Cl}$ , was combined with 3 ml of bromocresol green ( $c = 0.02 \text{ g L}^{-1}$ ). The pH was  
310 initially adjusted to 4.5 with HCl. A suspension containing 10 g of dry soil and 50 ml of  
311 Ringer solution was mixed in a Waring blender for 1.5 min at high speed. After  
312 decantation, the solution was successively diluted with Ringer solution to  $10^{-1} - 10^{-8}$   
313 (Ramsay, 1984). Each well was inoculated with 25  $\mu\text{l}$  of solution. The wells of the eight  
314 lines corresponded to the eight tested dilutions. Each sample was inoculated in the wells  
315 of three columns and a column was not inoculated to control the sterility of the medium.  
316 As each microplate had twelve columns, only one microplate was used for the three  
317 samples of a given plot. The inoculated plates were incubated at  $30^\circ\text{C}$  for 15 days and  
318 then the coloured products were measured. Bacteria were counted using the Most  
319 Probable Numbers (MPN) method (De Man, 1977). Sulphate-reducing bacteria (SRB)  
320 were counted in tubes containing a solid medium specific to SRB (American Petroleum  
321 Institute, Washington, DC) (Lors et al., 2004).

## 322 *2.8 Statistical analyses*

323 A one-way ANOVA was used to detect treatment effects on metal  
324 concentrations, Fv/Fm ratio, antioxidant enzyme activities, species distribution,  
325 biodiversity indices, distribution of gut contents and bacterial numbers. When ANOVA

326 was significant, mean values were compared with post-hoc multiple-comparison  
327 procedures. When the sample weight was too low and variance homogeneity was not  
328 fulfilled, a non-parametric statistical test followed by a multiple-comparison procedure  
329 was performed. Correlation analysis, using Pearson product-moment correlation  
330 coefficient, was performed between metal concentrations and antioxidant enzymes  
331 activities in leaves. Statistica v7 (StatSoft Inc.) was used for bacterial calculations  
332 whereas XLSTAT<sup>®</sup> (Addinsoft<sup>®</sup>, Paris, France) was used for springtail calculations. All  
333 other statistics were made with PASW statistics 18.

334         A semi-quantitative method was specially devised to make an overall assessment  
335 of beneficial or adverse effects of soil amendment (HA and TBS) addition on dredged  
336 sediments. For this purpose, the treatments (NT, HA, TBS) were ranked 1 to 3, a value  
337 of 1 being attributed to the treatment that maximized variables describing optimal  
338 features of plant, faunal and microbial communities (i.e. vegetation succession state,  
339 species richness, species diversity, equitability, abundance) and plant Fv/Fm ratio, or  
340 minimized those describing metal mobility, ecotoxicity, plant metal concentration and  
341 antioxidant enzyme activity,. Some variables cannot be taken into account in these  
342 calculations, either because they may respond to other factors than soil toxicity (acid vs  
343 neutral sulphur-oxidizing bacteria, collembolan gut contents, vegetation composition,  
344 pH), or because they do not differ significantly among treatments (plant Fv/Fm ratio for  
345 *C. epigejos*). Ranks were then averaged among the different variables which had been  
346 kept for the calculations. Overall differences between the three treatments NT, HA and  
347 TBS were then tested by a sign-test, using ranks as unit values.

### 348 **3 Results**



349           3.1.    *Metal(oid) mobility and sediment ecotoxicity*

350           Extractable Pb and As concentrations in all plots were below quantification  
351 limits (0.07 and 0.1 mg kg<sup>-1</sup> DW for Pb and As, respectively). Cadmium and Zn showed  
352 a similar pattern with higher 0.01 M Ca(NO<sub>3</sub>)<sub>2</sub>-extractable concentrations in the NT plot  
353 than in the TBS-treated plot, while intermediate values were found in the HA-treated  
354 plot (Table 2). Expressed as percentages of the total metal concentrations, extractable  
355 Cd and Zn represented 0.14 and 0.07%, respectively, in the TBS-treated plot, whereas  
356 they represented 0.37 and 0.76% in the NT plot and 0.31 and 0.61% in the HA-treated  
357 plot. For Cu, no significant differences occurred across the plots.

358           Sediment leachates were moderately toxic to the bacteria *V. fischeri* since no  
359 EC<sub>50</sub> could be determined. Considering the % of effect measured at the 90% dilution,  
360 the ecotoxicity of sediment leachates could be ranked in the following order NT > HA >  
361 TBS (Table 2). Sediment leachates were more toxic to Algae, especially those prepared  
362 from NT and HA (IC<sub>50</sub> values being 12.8 and 19.2 %, respectively). TBS sediment  
363 leachate had only a slight inhibitory effect on Algae.

364

365           3.2.    *Plant survey, plot similarity, source of colonization and element*  
366                    *concentrations*

367           The composition of the plant community was influenced by the treatments of the  
368 plots as shown by plant survey and the number of individuals per species and per  
369 surface (Table 3). The TBS plot was mainly colonized by herbaceous plants while HA  
370 and NT plots were dominated by shrubs. The maximum height of shrubs was higher in  
371 HA plot than in NT plot. The TBS plot had the lowest plant species richness (n=14).

372 Based on Shannon-Weaver diversity and Pielou equitability indices, the HA plot had the  
373 highest plant diversity with a more homogeneous species distribution while TBS and  
374 NT plots showed a lower diversity and at least one species that dominated the overall  
375 community (Table 3).

376 In all plots, the dominant family was Poaceae with 5 species for HA, 4 species  
377 for TBS and 3 species for NT. Among Poaceae, only *C. epigejos* was present on all  
378 plots. *Calamagrostis epigejos* was more present on the NT plot (46.9%), than on TBS  
379 (30%) and HA plots (19.9%). On all plots, other plant species were abundant: *Carex*  
380 *hirta* (11.5%) and *Glechoma hederacea* (11.7%) on the NT plot, *Betula pendula*  
381 (12.7%) and *G. hederacea* (22.8%) on the HA plot, and *Phalaris arundinacea* (32.1%),  
382 *Galium aparine* (16.4%) and *Epilobium angustifolium* (11.7%) on the TBS plot. The  
383 second most abundant family was *Asteraceae* with three species. At least two  
384 *Asteraceae* were present on all plots. Perennial species dominated the three group  
385 communities. Plant species were native to the Nord-Pas-de-Calais region with no clear  
386 specificity linked to sediment traits except for some plants showing hygrophilic  
387 characteristics (e.g. *Eupatorium cannabinum*, *C. hirta*, *Carex cuprina*, *P. arundinacea*,  
388 *Salix alba*).

389 The Sørensen index, comparing vegetation composition between plots and  
390 between plots and surrounding biotopes, indicated that 85% of plant species were  
391 common between NT and HA plots whereas the TBS plot showed 60% and 57%  
392 species similarity with HA and NT plots, respectively (data not shown). The  
393 surrounding biotopes consisted of a slag heap, a ruderal zone, a canal bank, and an  
394 agricultural land. A similarity index indicated that the plant colonizers of the NT and  
395 HA plots mainly originated from the slag heap, with 45 and 41% of species similarity.

396 This result agreed with the direction of the south-easterly prevailing wind and the seed  
397 shadow colonization hypothesis (Clark et al., 2005), as the slag heap was situated south-  
398 east from the plots. The TBS plot showed less similarity with the slag heap (33%).

399 The concentrations of the chemical elements were measured in *C. epigejos* and  
400 *U. dioica* as both species were sampled in all plots (Table 4). Their shoot Pb and As  
401 concentrations were below quantification limits (1 and 0.5 mg kg<sup>-1</sup> for Pb and As,  
402 respectively). Shoot Cd and Zn concentrations for both species and Cu concentration for  
403 *U. dioica* were lower in CA than in the three treatments. The shoot Cd concentration  
404 was the lowest in *C. epigejos* grown on TBS whereas for *U. dioica* it did not differ  
405 across treatments. Shoot Zn concentration was the lowest in *C. epigejos* grown on TBS.  
406 The highest shoot P and K concentrations were measured in *U. dioica* grown on TBS.  
407 No other significant effect of TBS treatment on metal concentration was observed. The  
408 HA treatment did not decrease shoot Cd and Zn concentrations of *C. epigejos* and *U.*  
409 *dioica* compared to NT but increased shoot Cu in *C. epigejos*. Shoot Mg, Na, and Al  
410 concentrations in *C. epigejos* and shoot Fe concentration in *U. dioica* were higher in the  
411 HA plot than in the NP plot. The HA treatment did not influence other shoot element  
412 concentrations.

413

### 414 3.3. Antioxidant enzyme activities and chlorophyll fluorescence of leaves

415 Unstressed plants typically exhibit Fv/Fm ratios of 0.8-0.83 (Laposi et al., 2009),  
416 which was the case for *C. epigejos* on all plots (Fig. 1). For all antioxidant enzymes,  
417 lowest activities were determined in CA plants. In untreated sediment (NT), *C. epigejos*  
418 enzymes were significantly increased 1.2 to 3 fold depending on the enzyme considered.

419 For plants collected on HA plots, the activity of SOD, CAT, and GRD were similar to  
420 CA values, except APX which remained higher. For GPX, the HA treatment had no  
421 significant effect. This enzyme was less influenced than the others by the composition  
422 of the NT sediment since its activity only increased by a factor of 1.2 compared to  
423 plants harvested on CA plots. Plant enzymes responded differently to the TBS  
424 treatment. For SOD, CAT and APX, activities levels were not influenced since their  
425 values did not differ significantly from corresponding values in NT plants. GPX and  
426 GRD were both affected by TBS treatment, but in opposite ways, since GPX increased  
427 by comparison to NT values while GRD decreased.

428 *Urtica dioica* collected from CA and HA plots, and to a lesser extent NT plots,  
429 had all Fv/Fm ratios around 0.8 (Fig. 2). For TBS-plants, the Fv/Fm ratios were  
430 significantly lower. As noted for *C. epigejos*, for all antioxidant enzymes, the lowest  
431 activities were found for CA plants. For the untreated sediment (NT), *U. dioica*  
432 enzymes significantly increased by a factor 3 to 5. The HA and TBS treatments had  
433 opposite effects on antioxidant enzymes. All tested enzyme activities in *U. dioica* leaves  
434 decreased for HA compared to NT and tended to recover CA values. However,  
435 differences between HA and CA plants remained significant for SOD, APX and GPX  
436 activities. In contrast, TBS plants still exhibited high enzyme activities, similar to NT  
437 values (e.g. APX and CAT) or even higher (e.g. SOD, GPX, and GRD).

#### 438 3.4. *Correlations between shoot element concentrations and antioxidant* 439 *enzyme activities*

440 A correlation analysis was performed, between shoot element concentrations in  
441 *C. epigejos* and *U. dioica* and their antioxidant enzyme activities in leaves, to reveal  
442 significant linear relationships (Table 5). All enzyme activities negatively correlated

443 with shoot Cd concentration in *C. epigejos*. Manganese concentrations were positively  
444 correlated with 4 out of the 5 enzyme activities whereas Cu, Zn, Mg, and Na  
445 concentrations were correlated with GRD. For *U. dioica*, no relationship was found  
446 between shoot Cd concentration and any of the enzyme activities, but all enzyme  
447 activities were positively correlated with shoot Cu, Zn, P, and K concentrations.

### 448 3.5. *Collembolan diversity and gut contents*

449 A total of fourteen species were found in the four areas (Table 6). Neither total  
450 abundance nor biodiversity indices (species richness, Shannon index, equitability)  
451 showed significant differences between treatments. However, the TBS plot tended to  
452 harbour more specimens and species than NT and HA plots and CA. In the NT plot,  
453 there was no decrease in any of the four biodiversity indices when compared to CA,  
454 with a mean abundance of 20 specimens per core sample, shared between four species.  
455 At the species level, *Friesea truncata* was only present in the TBS samples.

456 The only significant effect on the gut contents of Collembola (Fig. 3) was an  
457 increase in the percent of specimens with hyaline fungal hyphae, which increased in all  
458 cores sampled in the HA plot, reaching an average of 16% compared to less than 3% for  
459 the other treatments. Hemorganic humus (amorphous organo-mineral matter) was  
460 dominant everywhere, indicating that the sediments (treated or not) were consumed by  
461 springtails, in particular by the dominant species *Mesaphorura florum*.

### 462 3.6. *Total and specific bacterial numbers*

463 The total bacterial population in all plots varied from  $10^7$  to  $10^8$  bacteria  $g^{-1}$  dry  
464 sediment, and was similar to that usually found in unpolluted topsoils (Robert, 1996;

465 Taylor et al., 2002) (Table 7). However, in the TBS plot, the total bacterial population  
466 (in  $10^8$  bacteria.g<sup>-1</sup> dry soil) was significantly higher (1.7) than in the NT plot (0.21).

467 In all plots, neutrophilic sulphur-oxidising bacteria (NSOB) were the most  
468 abundant group, with about  $10^5$  bacteria g<sup>-1</sup> dry sediment, whereas acidophilic sulphur-  
469 oxidising bacteria (ASOB) were the least abundant group with about  $10^3$ - $10^4$  bacteria g<sup>-1</sup>  
470 dry sediment. The amendments did not modify the size of these bacterial populations.

471 The sulphate-reducing bacterial microflora (SRB) was about  $10^1$ - $10^2$  bacteria g<sup>-1</sup>  
472 dry sediment and was of the same order of magnitude in all plots.

473

### 474 3.7. *Integration of data from the ecotoxicity battery*

475 Averaging ranks of all variables selected for the synthetic assessment of  
476 treatment effect (see Material and methods) indicated that TBS and HA exhibited  
477 similar favourable effects on the ecosystem when compared to the untreated sediment  
478 (NT) (Table 8). HA and TBS treatments ranked significantly above NT (one-sided t-  
479 tests, P = 0.001 and 0.011, respectively) while they did not differ from each other (two-  
480 sided t-test, P = 0.797).

## 481 4. Discussion

482 Although the TBS application rate and the soil type used in this study differed  
483 from those investigated in other works, our results are broadly similar for Zn and Cd.  
484 The incorporation of TBS (5% DW) into the metal contaminated sediment decreased the  
485 0.01 M Ca(NO<sub>3</sub>)<sub>2</sub>-extractable Cd and Zn fractions and shoot Cd and Zn concentrations  
486 of *C. Epigejos* (Tables 2 and 4), confirming previous findings (Mench et al., 1994a,  
487 1994b, 2000). The TBS incorporation into a Cd-Zn-contaminated soil, reduced both the

488  $\text{Ca}(\text{NO}_3)_2$  extractability of Cd and Zn and their contents in tobacco shoots (Mench et al.,  
489 1994a, 1994b). In addition, TBS added into a Zn-contaminated soil increased soil pH,  
490 decreased extractable Zn and shoot Zn concentration in ryegrass, 3 and 5 months after  
491 soil treatment (Mench et al., 2000). Contrary to TBS, HA incorporation (3% DW) into  
492 the sediment did not decrease significantly Cd and Zn extractability nor Cd and Zn  
493 concentrations in plants (Tables 2 and 4). These results were not consistent with those  
494 of Boisson et al. (1999) who found a decrease of extractable Cd and Zn and metal  
495 concentrations in maize when compared to the untreated soil and with increasing HA  
496 application rate (0.5, 1 and 5% DW).

497         In contrast to other works (Boisson et al., 1999; Friesl et al., 2006), As-  
498 mobilization was not observed either in the presence of HA or TBS (Table 2). No  
499 amendment effect was recorded for Cu (Tables 2 and 4) whereas TBS incorporation  
500 (0.25 and 3.9% DW) into a Cu contaminated soil decreased Cu concentrations in the  
501 primary leaves of bean (Bes and Mench, 2008).

502         In our field study, TBS was the most efficient amendment to decrease Zn and Cd  
503 mobility and transfer to the plants. We hypothesise that Zn- and Cd-phosphates were  
504 precipitated, thereby immobilizing these elements in a non-available form as suggested  
505 by Panfili et al. (2005) who studied Zn speciation in the same metal-contaminated  
506 sediment treated with TBS. In our field experiment, the low efficiency of HA compared  
507 to TBS may be explained by the low solubility of HA at neutral pH (Table 1). As  
508 suggested by Miretzky and Fernandez-Cirelli (2007), the solubility of the phosphate  
509 phase is necessary for successful in situ treatment, metal immobilization by phosphorus  
510 being limited or inefficient when the matrix is neutral or alkaline.

511 As the essential nutrient concentrations did not decrease in plant shoots (Table  
512 4), our results suggested that neither TBS nor HA led to deficiency problems in the  
513 tested plant species. In the presence of HA (5% DW), Boisson et al. (1999) reported a  
514 Mn-deficiency in maize. Neither treatment promoted the uptake of essential nutrients  
515 such as Ca except in the case of *U. dioica* where shoot P and K concentrations were  
516 increased by TBS addition. In primary leaves of bean, Ca concentration was enhanced  
517 in the presence of TBS whereas P concentration was not promoted (Bes and Mench,  
518 2008; Negim et al., 2010).

519 In our study, sediment ecotoxicity was reduced by the incorporation of TBS and  
520 HA, TBS being the most efficient amendment.

521 At excessive concentrations in the soil solution, Cd, Cu, Pb and Zn exert toxic  
522 effects on plants, including alterations in photosynthetic and respiration processes or  
523 inhibition of plant growth. They may also stimulate the formation of reactive oxygen  
524 species (Dazy et al., 2009). To prevent oxidative damage, plant cells usually use  
525 enzymatic protection mechanisms, such as superoxide dismutase, catalase, and  
526 peroxidases, and metabolites such as ascorbate and glutathione (Dazy et al., 2008).  
527 Following amendment of the soil, a decrease in phytotoxicity and a recovery in plant  
528 antioxidant defence to control values are expected as a result of metal-immobilization  
529 processes (Ruttens et al., 2006; Vangronsveld et al., 1995, 1996). In the NT plot, all  
530 antioxidant enzyme activities of both plant species were increased compared to the CA  
531 plot, indicating that the plants suffered from oxidative stress. The increase in shoot Zn  
532 and Cu concentrations were consistent with enzymatic activity increases, suggesting  
533 that these metals accounted for the stress response recorded in plants growing on  
534 untreated dredged sediment. This was not the case for Cd, as no correlation for *U.*



535 *dioica* and no positive correlation for *C. epigejos* were found. The addition of HA  
536 permitted an almost total recovery of antioxidant enzyme activities to CA values,  
537 whereas the addition of TBS did not. Thus, HA was a better amendment for keeping the  
538 stress responses of plants at a low level. Levels of chlorophyll fluorescence (Fig. 1 and  
539 Fig. 2) indicated that the plants were not stressed on NT, HA and TBS plots. There are  
540 few studies that report the effect of TBS on phytotoxicity and an absence of studies on  
541 the effect of HA on phytotoxicity. After the incorporation of TBS (0.25% and 3.9%)  
542 into a Cu-contaminated soil, the chlorophyll density of primary leaves of bean  
543 decreased and guaiacol-peroxidase activity in roots fell to control level (Bes and Mench,  
544 2008). In another experiment, TBS added to a Zn-contaminated soil restored guaiacol-  
545 peroxidase activity of the primary leaves in beans to control values (Mench et al., 2000).

546         Most of the plant species present on the NT plot were also observed on the  
547 surrounding uncontaminated biotopes (slag heap and ruderal zone), which were  
548 similarly firstly devoid of vegetation. Thus, colonization of the NT plot was not limited  
549 by the total metal concentration in the sediment, which is consistent with previous  
550 findings (Escarré et al., 2011). Madejon et al. (2006) showed that the application of  
551 amendments to a metal contaminated soil improved spontaneous colonization and  
552 establishment of early-successional plants. In our study, all plots were covered by dense  
553 vegetation (close to 100%) but differed in plant community composition and  
554 biodiversity descriptors. This may indicate that the amendment application was not a  
555 key factor for colonization and establishment of spontaneous vegetation but an  
556 important factor that could modify vegetation composition and dynamics. The addition  
557 of TBS or HA to the sediment influenced the dynamic process of plant colonization, the  
558 three plots showing differences in composition of the plant community. The large

559 increase in pH following addition of TBS to the sediment (7.4 to 10.2 in May 2002),  
560 compared to the modest increase (7.4 to 7.7 in May 2002) in the presence of HA, may  
561 be responsible for the main changes in plant community in the TBS plot compared to  
562 other plots. Only spontaneous vegetation that is able to tolerate alkaline pHs could  
563 colonize this plot. Elevated pH favours the establishment of herbaceous rather than  
564 woody species (Skousen et al., 1994). Five years after the single application, the pH in  
565 the TBS-treated sediment decreased to 7.7 while pHs of NT and HA plots were 7.0 and  
566 7.3, respectively (Table 1). This decrease may be sufficient to favour the establishment  
567 of *B. pendula* in the next few years, as two seedlings were noticed in the TBS plot.  
568 *Calamagrostis epigejos* is known as a competitive social grass able to arrest plant  
569 succession by forming extended dense areas (Prach, 2003). At maturity, it can reach 1.5  
570 m in height thereby shading smaller or slowly-growing plant species (Rebele and  
571 Lehmann, 2001). As the higher rate of *C. epigejos* was recorded on the TBS plot, this  
572 plant would contribute to the maintenance of early stage succession vegetation on this  
573 plot.

574         The highest level of plant species richness was found in the presence of HA (26  
575 species out of 16 families) compared to the NT plot (21 species out of 14 families). In  
576 contrast, TBS did not favour species richness as it accounted for the least value (14  
577 species out of 9 families). This is paradoxical since the TBS plot had the lowest  
578 concentrations of extractable Zn and Cd and the lowest sediment ecotoxicity, but the  
579 competitive exclusion of plants by *C. epigejos* possibly outweighed effects of decreased  
580 toxicity.

581         Concerning diversity indices, our H' values were much higher than those given  
582 by Conesa et al. (2007) and Dazy et al. (2008) who studied plant communities growing

583 respectively on Zn-Pb-contaminated tailings and experimental plots filled with a  
584 multicontaminated soil. However, such comparisons of H' values may be confounded  
585 by different soil and pollution parameters as well as the time elapsed after colonization..  
586 For Dazy et al. (2008), the survey was performed after 14 months of colonization  
587 whereas in this study the survey was done 5 years after colonization.

588         The influence of metal contamination on natural springtail communities has  
589 been documented (Bengtsson and Rundgren, 1988; Chauvat and Ponge, 2002; Gillet  
590 and Ponge, 2002, 2003; Lock et al. 2003; Strojan, 1978). In these works, no major  
591 adverse effects of metal contamination were recorded. Springtails were abundant even  
592 in highly contaminated sites, which indicates the resistance of this group to toxic effect  
593 of metals and other environmental stresses (Hopkin, 1997), exemplified by high EC<sub>50</sub>  
594 values in *Folsomia candida* reproduction test at 20 °C for Cd (590 µg.g<sup>-1</sup>), Cu (700  
595 µg.g<sup>-1</sup>), Zn (900 µg.g<sup>-1</sup>) and Pb (2790 µg.g<sup>-1</sup>) (Sandifer and Hopkin, 1997). In our study,  
596 neither total abundance nor biodiversity indices showed significant differences between  
597 NT plot and CA, showing the normal colonization by springtails of the NT plot. The  
598 addition of TBS and HA did not change the abundance or biodiversity indices, showing  
599 no effect of these amendments on the springtail community. Some effect may have been  
600 expected on the TBS plot as Lock et al. (2003) reported a significant negative  
601 relationship between the metal extractable fraction (0.01 M CaCl<sub>2</sub> solution) and the  
602 number of springtail species. In our study, the gut content analyses showed that  
603 untreated and amended dredged sediments were consumed by springtails (Fig. 3).

604         In soils that are highly contaminated with metal(loid)s, a lower number of  
605 bacteria than in uncontaminated soil could be expected, as a result of the harmful effects  
606 of the contaminants on soil bacterial activity, especially bacterial respiration and soil

607 bacterial biomass (Renella et al., 2005; Sobolev and Begonia, 2008). In our study, the  
608 total bacterial population in the NT plot was similar to that usually found in unpolluted  
609 topsoils (Taylor et al., 2002).

610 The addition of TBS to the soil improved the total bacterial population, probably  
611 as a consequence of the low Cd-Zn-extractable fraction on this plot.

612 Bacteria play an important role in increasing or decreasing metal availability,  
613 notably in sediments (Bert et al., 2009). Bioleaching of metals is caused by sulphur-  
614 oxidising bacteria (SOB) whereas anaerobic sulphate-reducing bacteria (SRB) can  
615 precipitate metals. Neutrophilic and acidophilic SOB were found in similar numbers in  
616 all plots, showing no amendment effect. In all sediment plots, neutrophilic SOB were  
617 more numerous than acidophilic SOB, due to soil pH close to neutrality (Table 1). The  
618 moderate development of acidophilic SOB was consistent with the results of Lors et al.  
619 (2004) who reported an acidophilic sulphur-oxidising population of about  $10^4$  bacteria  
620  $g^{-1}$  dry soil in a similar sediment. These results indicated that metals might be released  
621 in the case of metal sulphide availability.

622 Sulphate-reducing bacteria were present in the same low range in the three  
623 sediment plots, showing no amendment effect (Table 7). The low amount of SRB may  
624 be explained by the sampling performed on the first 20 cm depth layer corresponding to  
625 aerobic conditions, not favourable to the growth of SRB that are strictly anaerobic.  
626 However, during wet periods, the saturation of the soil pore system might induce anoxic  
627 conditions that would promote the size of the SRB community, leading to increased  
628 metal sulphide content.

629

## 630 **5. Conclusions**

631 In this paper, TBS and HA were investigated in an experimental metal-  
632 contaminated sediment landfill site to evaluate their effects on metal extractability,  
633 sediment ecotoxicity and on living organisms such as plants, bacteria and springtails.

634 The incorporation of TBS into the metal contaminated sediment decreased the  
635 extractable fraction of Cd and Zn. In contrast, HA did not decrease Cd and Zn  
636 extractability. TBS at a rate of 5% DW was effective in Cd and Zn immobilization, did  
637 not cause any nutrient imbalance or sediment ecotoxicity, and improved the bacterial  
638 activity.

639 However, TBS application had either no influence or a negative effect, on  
640 maximum photochemical efficiency of PSII and antioxidant enzymes in plants. The  
641 bioavailability of the compound(s) responsible for those biological responses was not  
642 decreased by TBS treatment. Even if plants successfully colonized the TBS plot, they  
643 remained under stress.

644 Our study revealed (i) the favourable influence of both HA and TBS compared  
645 to NT, (ii) the better influence of HA on vegetation development and physiological  
646 welfare (as measured by PSII measurements and antioxidant enzymes), (iii) the better  
647 influence of TBS on sediment ecotoxicity, metal immobilization, microbial population  
648 and Cd-Zn accumulation in *C. epigejos*. The choice of using metal immobilization with  
649 TBS rather than HA as a tool in the management of dredged sediment landfill sites will  
650 depend on the objectives of such strategy, i.e. to limit metal transfer and sediment  
651 ecotoxicity or to favour plant diversity and welfare. Realising both objectives requires a  
652 study of a mixture of both soil amendments.

653

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662

663 **References**

664 AFNOR T90-320, 1991. Testing water-determination of the inhibition of the light  
665 emission of *Photobacterium phosphoreum*. Association Française de  
666 Normalisation, Paris.

667 AFNOR X31-210, 1992. Waste – Leaching of waste. Association Française de  
668 Normalisation, Paris.

669 Bengtsson, G., Rundgren, S., 1988. The Gusum case : a brass mill and the distribution  
670 of soil Collembola. Canadian Journal of Zoology 66, 1518-1526.

671 Bert, V., Seuntjens, P., Dejonghe, W., Lacherez, S., Thi Thanh Thuy, H.,  
672 Vandecasteele, B., 2009. Phytoremediation as a management option for  
673 contaminated sediments in tidal marshes, flood control areas and dredged sediment  
674 landfill sites. Environmental Science and Pollution Research 16, 745–764.

675 Bes, C., Mench, M., 2008. Remediation of copper-contaminated topsoils from a wood  
676 treatment facility using in situ stabilization. Environmental Pollution 156, 1128-  
677 1138.

678 Bes, M.C., Mench, M., Aulen, M., Gaste, H., Taberly, J., 2010. Spatial variation of  
679 plant communities and shoot Cu concentrations of plant species at a timber  
680 treatment site. *Plant and Soil* 330, 267-280.

681 Boisson, J., Ruttens, A., Mench, M., Vangronsveld, J., 1999. Evaluation of  
682 hydroxyapatite as a metal immobilizing soil additive for the remediation of  
683 polluted soils. I. Influence of hydroxyapatite on metal exchangeability in soil,  
684 plant growth and plant metal accumulation. *Environmental Pollution* 104, 225-  
685 233.

686 Bouwman, L.A., Bloem, J., Römkens, P.F.A.M., Boon, G.T., Vangronsveld, J., 2001.  
687 Beneficial effects of the growth of metal tolerant grasses on biological and  
688 chemical parameters in copper and zinc contaminated sandy soils. *Minerva*  
689 *Biotechnologica* 13, 19-26.

690 Bouwman, L.A., Vangronsveld, J., 2004. Rehabilitation of the nematode fauna in a  
691 phytostabilized heavily zinc-contaminated, sandy soil. *Journal of Soils and*  
692 *Sediments* 4, 17-23.

693 Bradford, M.M., 1976. A rapid and sensitive method for the quantification of  
694 microgram quantities of protein utilizing the principle of protein-dye binding.  
695 *Analytical Biochemistry* 72, 248-254.

696 Bretfeld, G., 1999. Synopses on Palaearctic Collembola. II. Symphypleona.  
697 *Abhandlungen und Berichte des Naturkundemuseums Görlitz* 71, 1-318.

698 Chapin, F.S. III, Walker, L.R., Fastie, C.L., Sharman, L.C., 1994. Mechanisms of  
699 primary succession following deglaciation at Glacier Bay, Alaska. *Ecological*  
700 *Monographs* 64, 149-175.

701 Chauvat, M., Ponge, J.F., 2002. Colonization of heavy metal-polluted soils by  
702 collembola : preliminary experiments in compartmented boxes. *Applied Soil*  
703 *Ecology* 21, 91-106.

704 Clark, C.J., Poulsen, J.R., Bolker, B.M., Connor, E.F., Parker, V.T., 2005. Comparative  
705 seed shadows of bird-, monkey-, and wind-dispersed trees. *Ecology* 86, 2684-  
706 2694.

707 Conesa, H., M., Garcia, G., Faz, A., Arnaldos, R., 2007. Dynamics of metal tolerant  
708 plant communities' development in mine tailings from the Cartagena-La Union  
709 Mining District (SE Spain) and their interest for further revegetation purposes.  
710 *Chemosphere* 68, 1180–1185.

711 Dazy, M., Jung, V., Férard, J.F., Masfaraud, J.F., 2008. Ecological recovery of  
712 vegetation at a former coke-factory industrial wasteland: role of plant  
713 antioxidant enzymes and possible implications in site restoration. *Chemosphere*  
714 74, 57–63.

715 Dazy, M., Masfaraud, J.F., Férard J.F., 2009. Induction of oxidative stress biomarkers  
716 associated with heavy metal stress in *Fontinalis antipyretica* Hedw.  
717 *Chemosphere* 75, 297–302.

718 De Man, J.C., 1977. MPN tables for more than one test. *European Journal of Applied*  
719 *Microbiology* 4, 307-316.

720 Edwards, C.A., Fletcher, K.E., 1971. A comparison of extraction methods for terrestrial  
721 arthropods. In: Phillipson, J. (Ed.), *Methods of Study in Quantitative Soil*  
722 *ecology: Population, Production and Energy Flow*. Blackwell, Oxford, pp. 150-  
723 185.



- 724 Escarré, J., Lefèbvre Cl., Raboyau, S., Dossantos, A., Gruber, W., Cleyet Marel, J., Cl.,  
725 Frérot, H., Noret, N., Mahieu., S., Collin, Ch., Van Oort F., 2011. Heavy metal  
726 concentration survey in soils and plants of the Les Malines mining district  
727 (Southern France): implications for soil restoration. *Water, Air and Soil*  
728 *Pollution* 216, 485-504.
- 729 Fjellberg, A., 1998. *The Collembola of Fennoscandia and Denmark. I. Poduromorpha.*  
730 Brill, Leiden.
- 731 Friesl, W., Friedl, J., Platzer, K., Horak, O., Gerzabek, M.H., 2006. Remediation of  
732 contaminated agricultural soils near a former Pb/Zn smelter in Austria: Batch,  
733 pot and field experiments. *Environmental Pollution* 144, 40-50.
- 734 Gillet, S., Ponge, J.F., 2002. Humus forms and metal pollution in soil. *European Journal*  
735 *of Soil Science* 53, 529-539.
- 736 Gillet, S., Ponge, J.F., 2003. Changes in species assemblages and diets of Collembola  
737 along a gradient of metal pollution. *Applied Soil Ecology* 22, 127-138.
- 738 Gillet, S., Ponge, J.F., 2005. Species assemblages and diets of Collembola in the organic  
739 matter accumulated over an old tar deposit. *European Journal of Soil Biology*  
740 41, 39-44.
- 741 Gisin, H., 1960. *Collembolenfauna Europas.* Muséum d'Histoire Naturelle, Geneva.
- 742 Hopkin, S.P., 1997. *Biology of the Springtails.* Oxford University Press, Oxford.
- 743 Hopkin, S.P., 2000. *A key to the Collembola (springtails) of Britain and Ireland.* Field  
744 Studies Council AIDGAP Guides 111, 1-245.

745 ISO 8692, 1989. Water quality – Freshwater algal growth inhibition test with  
746 *Scenedesmus subspicatus* and *Selenastrum capricornutum*. International  
747 Organization for Standardization, Geneva.

748 Jordana, R., Arbea, J.I., Simón, C., Luciáñez, M.J., 1997. Fauna Iberica. VIII.  
749 Collembola Poduromorpha. CSIC, Madrid.

750 Knox, A.S., Seaman, J.C., Mench, M., J., Vangronsveld, J., 2001. Remediation of  
751 metal- and radionuclides-contaminated soils by in situ stabilization techniques,  
752 in: Iskandar, I.K. (Ed), Environmental Restoration of Metal-contaminated Soils.  
753 Lewis Publishers, Boca Raton, pp. 21-60.

754 Kumpiene, J., Lagerkvist, A., Maurice, C., 2008. Stabilization of As, Cr, Cu, Pb and Zn  
755 in soil using amendments – a review. Waste Management 28, 215-225.

756 Lambinon, J., Delvosalle, L., Duvignaud, J., 2004. Nouvelle Flore de la Belgique, du  
757 Grand-Duché de Luxembourg, du Nord de la France et des Régions Voisines  
758 (Ptéridophytes et Spermatophytes), 5th éd. Jardin Botanique de Belgique, Meise.

759 Laposi, R., Veres, S., Lakatos, G., Olah, V., Fieldsend, A., Meszaros, I., 2009.  
760 Responses of leaf traits of European beech (*Fagus sylvatica* L.) saplings to  
761 supplemental UV-B radiation and UV-B exclusion. Agricultural and Forest  
762 Meteorology 149, 745-755.

763 Lock, K., Janssen, C.R., 2003. Effect of new soil immobilizing agents on metal toxicity  
764 to terrestrial invertebrates. Environmental Pollution 121, 123-127.

765 Lock, K., Janssens, F., Janssen, C.R., 2003. Effects of metal contamination on the  
766 activity and diversity of springtails in an ancient Pb-Zn mining area at  
767 Plombières, Belgium. European Journal of Soil Biology 39, 25-29.

768 Lock, K., Janssen, C.R., 2005. Influence of soil zinc concentration on zinc sensitivity  
769 and functional diversity of microbial communities. *Environmental Pollution* 136,  
770 275-281.

771 Lors, C., Tiffreau, C., Laboudigue, A., 2004. Effects of bacterial activities on the release  
772 of heavy metals from contaminated dredged sediments. *Chemosphere* 56, 619-  
773 630.

774 Ma, Q.Y., Traina, S.J., Logan, T.J., 1993. In situ lead immobilization by apatite.  
775 *Environmental Science and Technology* 27, 1803-1810.

776 Madejon, E., Perez de Mora, A., Felipe, E., Burgos, P., Cabrera, F., 2006. Soil  
777 amendments reduce trace element solubility in a contaminated soil and allow  
778 regrowth of natural vegetation. *Environmental Pollution* 139, 40-52.

779 Mench, M., Vangronsveld, J., Didier, V., Clijsters, H., 1994a. Evaluation of metal  
780 mobility, plant availability and immobilization by chemical agents in a limed-  
781 silty soil. *Environmental Pollution* 86, 279-286.

782 Mench, M.J., Didier V.L., Löffler M., Gomez, A., Masson, P., 1994b. A mimicked in-  
783 situ remediation study of metal-contaminated soils with emphasis on cadmium  
784 and lead. *Journal of Environmental Quality* 23, 58-63.

785 Mench, M.J., Manceau, A., Vangronsveld, J., Clijsters, H., Mocquot, B., 2000. Capacity  
786 of soil amendments in lowering the phytoavailability of sludge-borne zinc.  
787 *Agronomie* 20, 383-397.

788 Miretzky, P., Fernandez-Cirelli, A., 2007. Phosphates for Pb immobilization in soils: a  
789 review *Environmental and Chemical Letters* 6, 121-133.

790 Misra, V., Chaturvedi, P. K., 2007. Plant uptake/bioavailability of heavy metals from  
791 the contaminated soil after treatment with humus soil and hydroxyapatite  
792 Environmental and Monitoring Assessment 133, 169–176.

793 Negim, O, Eloifi, B., Mench, M., Bes, C., Gaste, H., Motelica-Heino, M., Le  
794 Coustumer, P., 2010. Effect of basic slag addition on soil properties, growth and leaf  
795 mineral composition of beans in a Cu-contaminated soil. Journal of Soil and  
796 Sediment Contamination 19, 174-187.

797 NF EN 13657, 2003. Characterization of waste - Digestion for subsequent  
798 determination of aqua regia soluble portion of elements. AFNOR, Association  
799 française de normalisation, Paris.

800 NF ISO 10693, 1995. Soil quality - Determination of carbonate content - Volumetric  
801 method. AFNOR, Association Française de Normalisation, Paris.

802 NF ISO 11261, 1995. Soil quality - Determination of total nitrogen - Modified Kjeldahl  
803 method. AFNOR, Association Française de Normalisation, Paris.

804 NF X 31-107, 2003. Soil Quality - Particle size determination by sedimentation - Pipette  
805 method. AFNOR, Association Française de Normalisation, Paris.

806 NF X 31-130, 1999. Soil quality - Chemical methods - Determination of cationic  
807 exchange capacity (CEC) and extractible cations. AFNOR, Association  
808 Française de Normalisation, Paris.

809 NF ISO 14235, 1998. Soil quality - Determination of organic carbon by sulfochromic  
810 oxidation. AFNOR, Association Française de Normalisation, Paris.

811 NF ISO 10390, 1994. Soil quality - Determination of pH. AFNOR, Association  
812 Française de Normalisation, Paris.

813 Panfili, F.R., Manceau, A., Sarret, G., Spadini, L., Kirpichtchikova, T., Bert, V.,  
814 Laboudigue, A., Marcus, M.A., Ahamdach, N., Libert, M.F., 2005. The effect of  
815 phytostabilization on Zn speciation in a dredged contaminated sediment using  
816 scanning electron microscopy, X-ray fluorescence, EXAFS spectroscopy, and  
817 principal components analysis. *Geochimica et Cosmochimica Acta* 69, 2265-2284.

818 Piélou, E.C. 1966. The measures of diversity in different types of biological collections.  
819 *Journal of Theoretical Biology*, 13, 131-144.

820 Piou, S., Bataillard, P., Laboudigue, A., Férard, J.F., Masfaraud, J.F., 2009. Changes in  
821 the geochemistry and ecotoxicity of a Zn and Cd contaminated dredged  
822 sediment over time after land disposal. *Environmental Research* 109, 712–720.

823 Ponge, J.F., 2000. Vertical distribution of Collembola (Hexapoda) and their food  
824 resources in organic horizons of beech forests. *Biology and Fertility of Soils*, 32,  
825 508-522.

826 Potapov, M., 2001. Synopses on Palaearctic Collembola. III. Isotomidae. *Abhandlungen  
827 und Berichte des Naturkundemuseums Görlitz* 73, 1-603.

828 Prach, K., 2003. Spontaneous succession in Central-European man-made habitats: what  
829 information can be used in restoration practice? *Applied Vegetation Science* 6,  
830 125-129.

831 Prevost, O., 2008. Destination des sédiments fluviaux contaminés retirés des canaux:  
832 optimisation des filières et possibilités de valorisation. *Techniques Sciences  
833 Méthodes* 9, 71-79.

834 Ramsay, A.J., 1984. Extraction of bacteria from soil: efficiency of shaking or  
835 ultrasonification as indicated by direct counts and autoradiography. *Soil Biology*  
836 *and Biochemistry* 16, 475-481.

837 Rebele, F., Lehmann, C., 2001. Biological flora of Central Europe, *Calamagrostis*  
838 *epigejos* (L.) Roth. *Flora*, 196, 325–344.

839 Renella, G., Mench, M., Gelsomino, A., Landi, L., Nannipieri, P., 2005. Functional  
840 activity and microbial community structure in soils amended with bimetallic  
841 sludges. *Soil Biology and Biochemistry* 37, 1498-1506.

842 Robert, M., 1996. *Soil: Interface in the Environment, Resource for the Development.*  
843 Masson, Paris (in French).

844 Ruttens, A., Mench, M., Colpaert, J., V., Boisson, J., Carleer, R., Vangronsveld, J.,  
845 2006. Phytostabilization of a metal contaminated sandy soil. I. Influence of  
846 compost and/or inorganic metal immobilizing soil amendments on phytotoxicity  
847 and plant availability of metals. *Environmental Pollution* 144, 524-532.

848 Ryan, R.P., Ryan, D.J., Dowling, D.N., 2005. Multiple metal resistant transferable  
849 phenotypes in bacteria as indicators of soil contamination with heavy metals.  
850 *Journal of Soils and Sediments* 5, 95-100.

851 Sandifer, R.D., Hopkin, S.P., 1997. Effects of temperature on the relative toxicities of  
852 Cd, Cu, Pb, and Zn to *Folsomia candida* (Collembola). *Ecotoxicology and*  
853 *Environmental Safety*, 37, 125-130.

854 Shannon, C.E., Weaver, W., 1949. *The Mathematical Theory of Communication.*  
855 University of Illinois Press, Urbana.

856 Skousen, J.G., Johnson, C.D., Garbutt, K., 1994. Natural revegetation of 15 abandoned  
857 mine land sites in West Virginia. *Journal of Environmental Quality* 23, 1224-  
858 1230.

859 Sobolev, D., Begonia, M., F., T., 2008. Effects of heavy metal contamination upon soil  
860 microbes: Lead-induced changes in general and denitrifying microbial  
861 communities as evidenced by molecular markers. *International Journal of*  
862 *Environmental Research and Public Health* 5, 450-456.

863 Sørensen, T., 1948. A method of establishing groups of equal amplitude in plant  
864 sociology based on similarity of species and its application to analyses of the  
865 vegetation on Danish commons. *Biologiske Skrifter* 5, 1-34.

866 Strojan, C.L., 1978. The impact of zinc smelter emissions on forest litter arthropods.  
867 *Oikos* 31, 41-46.

868 Tyler, G., Balsberg Pahlsson, M., Bengtsson, G., Baath, E., Tranvik, L., 1989. Heavy-  
869 metal ecology of terrestrial plants, microorganisms and invertebrates – a review.  
870 *Water, Air and Soil Pollution* 47, 189-215.

871 Taylor, J.P., Wilson, B., Mills, M.S., Burns, R.G., 2002. Comparison of microbial  
872 numbers and enzymatic activities in surface soils and subsoils using various  
873 techniques. *Soil Biology and Biochemistry* 34, 387-401.

874 Vangronsveld, J., Van Assche, F., Clijsters, H., 1995. Reclamation of a bare industrial  
875 area contaminated by non-ferrous metals: in situ metal immobilization and  
876 revegetation. *Environmental Pollution* 87, 51-59.

877 Vangronsveld, J., Colpaert, J., Van Tichelen, K., 1996. Reclamation of a bare industrial  
878 area contaminated by non-ferrous metals: physico-chemical and biological

879 evaluation of the durability of soil treatment and revegetation. Environmental  
880 Pollution 94, 131-140.

881 Vassilev, A., Schwitzguébel, J., P., Thewys, T., van der Lelie, D., Vangronsveld, J.,  
882 2004. The use of plants for remediation of metal-contaminated soils.  
883 ScientificWorld Journal 4, 9-34.

884 Zimdars, B., Dunger, W., 1994. Synopses on Palaearctic Collembola. I. Tullbergiinae.  
885 Abhandlungen und Berichte des Naturkundemuseums Görlitz 68, 1-71.

886



887 **Figure captions**

888 Figure 1.

889 Fv/Fm ratios and antioxidant enzyme activities ( $\mu$ moles/min/mg proteins excepted for  
890 SOD expressed as U/mg proteins) in *C. epigejos* leaves collected from control area  
891 (CA), untreated plot (NT), plots treated with hydroxylapatite (HA) or Thomas basic slag  
892 (TBS). Different letters indicate significant differences between plots according to post-  
893 hoc Tukey HSD tests run when ANOVA was significant.

894 Figure 2.

895 Fv/Fm ratios and antioxidant enzyme activities ( $\mu$ moles/min/mg proteins excepted for  
896 SOD expressed as U/mg proteins) in *U. dioica* leaves collected from control area (CA),  
897 untreated plot (NT), plots treated with hydroxylapatite (HA) or Thomas basic slag  
898 (TBS). Different letters indicate significant differences between plots according to post-  
899 hoc Tukey HSD tests run when ANOVA was significant.

900 Figure 3.

901 Distribution of gut contents among the 403 collembolan specimens collected in three  
902 treatments and in the control area (CA). Letters indicate significant differences among  
903 means at 0.05 level (ANOVA followed by SNK procedure).

904

905 **Table 1**

906 Main characteristics and (pseudo)-total metal concentrations of sediments (NT,  
 907 untreated; HA, hydroxylapatite; TBS, Thomas Basic Slag) and the control area (CA).  
 908 For trace elements (except for CA) and pH, values are means of triplicate measurements  
 909 and their standard deviation.

	CA	NT	HA	TBS
<b>Particle size distribution (%)</b>				
<b>Sand</b>	13.2	69.6	60.1	65.4
<b>Silt</b>	70.3	14	21.1	19.1
<b>Clay</b>	16.5	16.4	18.8	15.5
<b>Organic C (%)</b>	1.64	3.2	4.1	3.7
<b>N (g kg<sup>-1</sup>)</b>	1.6	1.62	1.67	2.04
<b>CaCO<sub>3</sub> (g kg<sup>-1</sup>)</b>	63	76	76	105
<b>CEC (cmol kg<sup>-1</sup>)</b>	13.3	7.2	8.3	11.3
<b>pH<sub>water</sub></b>	7.6 ± 0.1	7.0 ± 0,1	7.3 ± 0,1	7.7 ± 0,3
<b>Zn (mg kg<sup>-1</sup> DW)</b>	75	3250 ± 319	2521 ± 461	3029 ± 217
<b>Cd (mg kg<sup>-1</sup> DW)</b>	0.4	84 ± 2	67 ± 11	86 ± 8
<b>Pb (mg kg<sup>-1</sup> DW)</b>	27	448 ± 82	389 ± 96	413 ± 57
<b>Cu (mg kg<sup>-1</sup> DW)</b>	17	93 ± 13	94 ± 11	123 ± 29
<b>As (mg kg<sup>-1</sup> DW)</b>	8	53 ± 4	48 ± 4	45 ± 2

910

911 **Table 2**

912 Concentrations of trace elements extracted from soils by 0.01 M Ca(NO<sub>3</sub>)<sub>2</sub> (means of  
 913 triplicate values ± standard deviation) and corresponding leachate ecotoxicity.  
 914 Significant differences among means at 0.05 level were indicated by different letters. nr  
 915 = not reached

	<b>NT</b>	<b>HA</b>	<b>TBS</b>
<b>Zn (mg kg<sup>-1</sup> DW)</b>	12.05a ± 0.96	7.77ab ± 0.33	2.07b ± 2.73
<b>Cd</b>	0.64a ± 0.12	0.41ab ± 0.04	0.12b ± 0.13
<b>Pb</b>	<0.07	<0.07	<0.07
<b>Cu</b>	0.12a ± 0.01	0.13a ± 0.02	0.21a ± 0.14
<b>As</b>	<0.1	<0.1	<0.1
<b>Microtox EC<sub>50</sub> (%)</b>	nr	nr	nr
<b>Microtox (% luminescent inhibition at 90%)</b>	44	20.5	6.3
<b>Algae test IC<sub>50</sub> (%)</b>	12.8	19.2	nr
<b>Algal test (% inhibition at 90%)</b>	100	100	36.4

916

917

918 **Table 3**

919 Composition of the plant community in the three treatment plots. Species richness is the  
 920 number of species.  $H'$  is the Shannon-Weaver index.  $J$  is the Pielou equitability index.

Family	Species	Plant type	Plant life cycle	NT	HA	TBS
Apiaceae	<i>Pastinaca sativa</i>	Herbaceous	Annual	0.47	0.46	0
Asteraceae	<i>Eupatorium cannabinum</i>	Herbaceous	Perennial	0	3.15	0.29
	<i>Achillea millefolium</i>	Herbaceous	Annual	0.12	7.59	0
	<i>Cirsium arvense</i>	Herbaceous	Perennial	0.47	0	1.39
Brassicaceae	<i>Cardamine hirsuta</i>	Herbaceous	Annual	6.18	1.76	0.19
Boraginaceae	<i>Myosotis arvense</i>	Herbaceous	Annual	0.12	0.09	0
Convolvulaceae	<i>Convolvulus arvensis</i>	Herbaceous	Perennial	0	0.46	0
Cyperaceae	<i>Carex hirta</i>	Herbaceous	Perennial	11.55	1.85	0.33
	<i>Carex cuprina</i>	Herbaceous	Perennial	0.12	0.09	0
Lamiaceae	<i>Glechoma hederacea</i>	Herbaceous	Perennial	11.67	22.85	7.08
	<i>Lycopus europaeus</i>	Herbaceous	Perennial	0.70	1.57	0
Onagraceae	<i>Epilobium angustifolium</i>	Herbaceous	Annual	0.35	1.2	11.76
Poaceae	<i>Deschampsia cespitosa</i>	Herbaceous	Perennial	1.52	1.76	0
	<i>Festuca rubra</i>	Herbaceous	Perennial	3.73	6.85	0
	<i>Phalaris arundinacea</i>	Herbaceous	Perennial	0	0.93	33.09

	<i>Calamagrostis epigejos</i>	Herbaceous	Perennial	46.91	19.98	30.00
	<i>Holcus lanatus</i>	Herbaceous	Perennial	0	2.22	0.1
	<i>Poa trivialis</i>	Herbaceous	Perennial	0	0	0.14
Ranunculaceae	<i>Ranunculus repens</i>	Herbaceous	Perennial	0.12	1.11	0
Rubiaceae	<i>Galium aparine</i>	Herbaceous	Annual	2.22	2.13	16.40
Scrophulariaceae	<i>Linaria vulgaris</i>	Herbaceous	Perennial	0	1.67	0
Urticaceae	<i>Urtica dioica</i>	Herbaceous	Perennial	2.33	4.63	0.86
Betulaceae	<i>Betula pendula</i>	Tree	Perennial	8.05	12.67	0.05
	<i>Betula pubescens</i>	Tree	Perennial	1.87	2.31	0.05
Fagaceae	<i>Quercus robur</i>	Tree	Perennial	0.12	0.28	0
Salicaceae	<i>Salix sp.</i>	Tree	Perennial	0	0.46	0
	<i>Salix alba</i>	Tree	Perennial	0.12	1.02	0
	<i>Salix capraea</i>	Tree	Perennial	1.40	0.93	0
<b>Species richness</b>				21	26	14
<b>H'</b>				2.7	3.6	2.35
<b>J</b>				0.62	0.77	0.62

921 For species, values were percent numbers of individuals per species and per unit  
922 surface.

923

924 **Table 4**925 Concentrations (mg/kg DW) in shoots of *Calamagrostis epigejos* and *Urtica dioica*.

	CA	NT	HA	TBS
<i>Calamagrostis epigejos</i>				
<b>Cd</b>	< 0.05	2.16 (a) ± 1.4	2.51 (a) ± 1.1	0.34 (b) ± 0.18
<b>Zn</b>	23 (a) ± 9	121 (c) ± 58	98 (bc) ± 32.6	44 (ab) ± 20
<b>Pb</b>	<1	<1	<1	<1
<b>Cu</b>	4.5 (ab) ± 0.9	3.5 (a) ± 0.3	5.1 (b) ± 0.7	4.3 (ab) ± 0.2
<b>As</b>	< 0.5	< 0.5	< 0.5	< 0.5
<b>Ca</b>	405 ± 331	617 ± 115	769 ± 273	791 ± 243
<b>Fe</b>	23 ± 2	52 ± 56	33 ± 5	23 ± 1
<b>Mg</b>	453 (b) ± 121	251 (a) ± 83	532 (b) ± 147	446 (ab) ± 62
<b>Mn</b>	16 (a) ± 5	51 (b) ± 8	13 (a) ± 3	15 (a) ± 1
<b>P</b>	1570 ± 247	1530 ± 189	1863 ± 875	1175 ± 235
<b>Na</b>	123 (ab) ± 4	115 (a) ± 4	131 (b) ± 12	119 (ab) ± 13
<b>K</b>	12407 (a) ± 1329	14523 (ab) ± 1543	16648 (b) ± 2620	13074 (a) ± 1288
<b>Al</b>	n.d	5 (a) ± 2	8.5 (b) ± 1.6	24 (c) ± 1
<i>Urtica dioica</i>				
<b>Cd</b>	0.06 (a) ± 0.01	1.5 (b) ± 1.3	0.9 (ab) ± 0.3	0.36 (ab) ± 0.18
<b>Zn</b>	12 (a) ± 1	210 (b) ± 104	184 (b) ± 28	145 (b) ± 21
<b>Pb</b>	<1	<1	<1	<1
<b>Cu</b>	4.0 (a) ± 0.5	6.9 (b) ± 1.9	6.8 (b) ± 1.9	7.4 (b) ± 0.9
<b>As</b>	<0.5	<0.5	<0.5	<0.5
<b>Ca</b>	19719 ± 8227	20945 ± 3292	24398 ± 2407	23901 ± 3334
<b>Fe</b>	35 (a) ± 6	51 (ab) ± 10	60 (b) ± 12	43 (ab) ± 11
<b>Mg</b>	1726 ± 1753	1278 ± 433	2176 ± 285	1722 ± 241
<b>Mn</b>	13 ± 3	12 ± 6	11 ± 2	12 ± 2
<b>P</b>	2314 (a) ± 267	4229 (b) ± 465	4310 (b) ± 841	6096 (c) ± 563
<b>Na</b>	136 ± 38	177 ± 41	162 ± 20	149 ± 12
<b>K</b>	17355 (a) ± 4593	19029 (ab) ± 1434	23699 (bc) ± 1721	27963 (c) ± 2117
<b>Al</b>	n.d	20 ± 8	32 ± 10	28 ± 12

926 Mean values and standard deviations (n=5; mg kg<sup>-1</sup> DW). Different letters indicate

927 significant differences among plots for α =0.05.

928

929 **Table 5.** Correlation analysis between shoot element concentrations and antioxidant  
 930 enzyme activities in leaves (n=20).

<i>C. epigejos</i>					
	<b>SOD</b>	<b>CAT</b>	<b>APX</b>	<b>GPX</b>	<b>GRD</b>
<b>Cd</b>	-0.68**	-0.67**	-0.79***	-0.61**	-0.58**
<b>Zn</b>	0.31ns	0.35ns	0.56**	0.01ns	0.52*
<b>Cu</b>	-0.27ns	-0.48*	-0.38ns	0.00ns	-0.56*
<b>Mn</b>	0.55*	0.60**	0.53*	-0.11ns	0.80***
<b>P</b>	0.09ns	-0.02ns	-0.18ns	0.13ns	-0.23ns
<b>Ca</b>	0.37ns	0.18ns	0.38ns	0.44ns	0.08ns
<b>K</b>	0.06ns	-0.01ns	0.22ns	-0.03ns	0.14ns
<b>Mg</b>	-0.30ns	-0.43ns	-0.52*	-0.32ns	-0.56*
<b>Na</b>	-0.33ns	-0.54*	-0.19ns	-0.21ns	-0.50*
<i>U. dioica</i>					
	<b>SOD</b>	<b>CAT</b>	<b>APX</b>	<b>GPX</b>	<b>GRD</b>
<b>Cd</b>	0.22ns	0.24ns	0.30ns	0.32ns	0.11ns
<b>Zn</b>	0.48*	0.47*	0.56**	0.61**	0.45*
<b>Cu</b>	0.51*	0.49*	0.56*	0.60**	0.70**
<b>Mn</b>	-0.07ns	-0.02ns	-0.08ns	-0.04ns	0.08ns
<b>P</b>	0.79***	0.74***	0.79***	0.85***	0.82***
<b>Ca</b>	0.10ns	0.12ns	0.18ns	0.21ns	0.26ns
<b>K</b>	0.54*	0.45*	0.48*	0.56*	0.60**
<b>Mg</b>	-0.20ns	-0.21ns	-0.10ns	-0.07ns	-0.11ns
<b>Na</b>	0.22ns	0.24ns	0.28ns	0.22ns	0.02ns

931 ns: not significant, \*\*\*, \*\*, \*, significant at a probability level P< 0.001, 0.01, 0.05,  
 932 respectively.

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**Table 6.** Species distribution and biodiversity indices of collembolan species.

ANOVA: NS = not significant; \*\* P < 0.01. Post-hoc tests (SNK procedure):

significant differences indicated by different letters

	CA	NT	HA	TBS	F value
<i>Dicyrtomina minuta</i>	0	0	0	0.33±0.27	1 NS
<i>Entomobrya sp.</i>	0	1.33±1.09	0	0	1 NS
<i>Folsomia candida</i>	0.33±0.27	0.33±0.27	0	1±0.82	0.58 NS
<i>Friesea truncata</i>	0 <sup>b</sup>	0 <sup>b</sup>	0 <sup>b</sup>	4±1.25 <sup>a</sup>	6.86**
<i>Hererosminthurus claviger</i>	0.33±0.27	0	0	0	1 NS
<i>Isotomodes productus</i>	0.67±0.54	0	0	2.67±1.78	1.22 NS
<i>Lepidocyrtus lignorum</i>	0	0	0	0.33±0.27	1 NS
<i>Mesaphorura florum</i>	1.33±0.72	6.33±2.13	21.67±7.92	25.33±8.57	2.56 NS
<i>Mesaphorura macrochaeta</i>	3.33±2.72	0	0.67±0.27	0.67±0.54	0.75 NS
<i>Mesaphorura pongei</i>	0	0	0	1.33±1.09	1 NS
<i>Orchesella sp.</i>	0	0.33±0.27	0.33±0.27	0	0.67 NS
<i>Parisotoma notabilis</i>	6.67±1.96	4.67±1.66	5.67±2.13	0.33±0.27	1.85 NS
<i>Proisotoma minima</i>	7±2.45	9±2.94	0.67±0.54	7±2.36	1.71 NS
<i>Tomocerus vulgaris</i>	0	0	0.33±0.27	0	1 NS
<b>Total abundance</b>	19.67±3.07	22±5.44	29.33±6.4	43±10.6	1.53 NS
<b>Species richness</b>	4±0.47	4	3.67±0.27	5.67±1.19	1.28 NS
<b>Shannon Index</b>	1.44±0.21	1.65±0.07	1.09±0.32	1.78±0.26	1.09 NS
<b>Equitability Index</b>	0.72±0.04	0.82±0.04	0.56±0.15	0.75±0.03	1.25 NS

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946 **Table 7. Total and specific bacterial numbers**

947 Counts of total bacteria, acidophilic sulphur-oxidising bacteria (ASOB), neutrophilic  
948 sulphur-oxidising bacteria (NSOB) and sulphate-reducing bacteria (SRB) (expressed as  
949 bacteria  $\text{g}^{-1}$  dry soil) collected from untreated plot (NT), plot treated with  
950 hydroxylapatite (HA) or Thomas basic slag (TBS). Values are expressed as means  $\pm$   
951 standard deviation in triplicate samples. Values with the same letters are not  
952 significantly different according to ANOVA used Newman-Keuls (SNK) test at  $P \leq$   
953 0.05.

	<b>NT</b>	<b>HA</b>	<b>TBS</b>
<b>Total bacteria</b>	$2.12 \cdot 10^7$ $\pm 5.09 \cdot 10^5$ c	$5.58 \cdot 10^7$ $\pm 7.90 \cdot 10^6$ b	$1.75 \cdot 10^8$ $\pm 1.86 \cdot 10^6$ a
<b>ASOB</b>	$3.69 \cdot 10^3$ $\pm 2.31 \cdot 10^1$ a	$7.36 \cdot 10^3$ $\pm 3.19 \cdot 10^3$ a	$2.93 \cdot 10^3$ $\pm 1.29 \cdot 10^3$ a
<b>NSOB</b>	$1.00 \cdot 10^5$ $\pm 6.78 \cdot 10^4$ a	$3.05 \cdot 10^5$ $\pm 1.15 \cdot 10^5$ a	$1.45 \cdot 10^5$ $\pm 4.62 \cdot 10^4$ a
<b>SRB</b>	$6.97 \cdot 10^1$ $\pm 4.04 \cdot 10^1$ a	$5.97 \cdot 10^1$ $\pm 2.89 \cdot 10^1$ a	$5.97 \cdot 10^1$ $\pm 2.89 \cdot 10^1$ a

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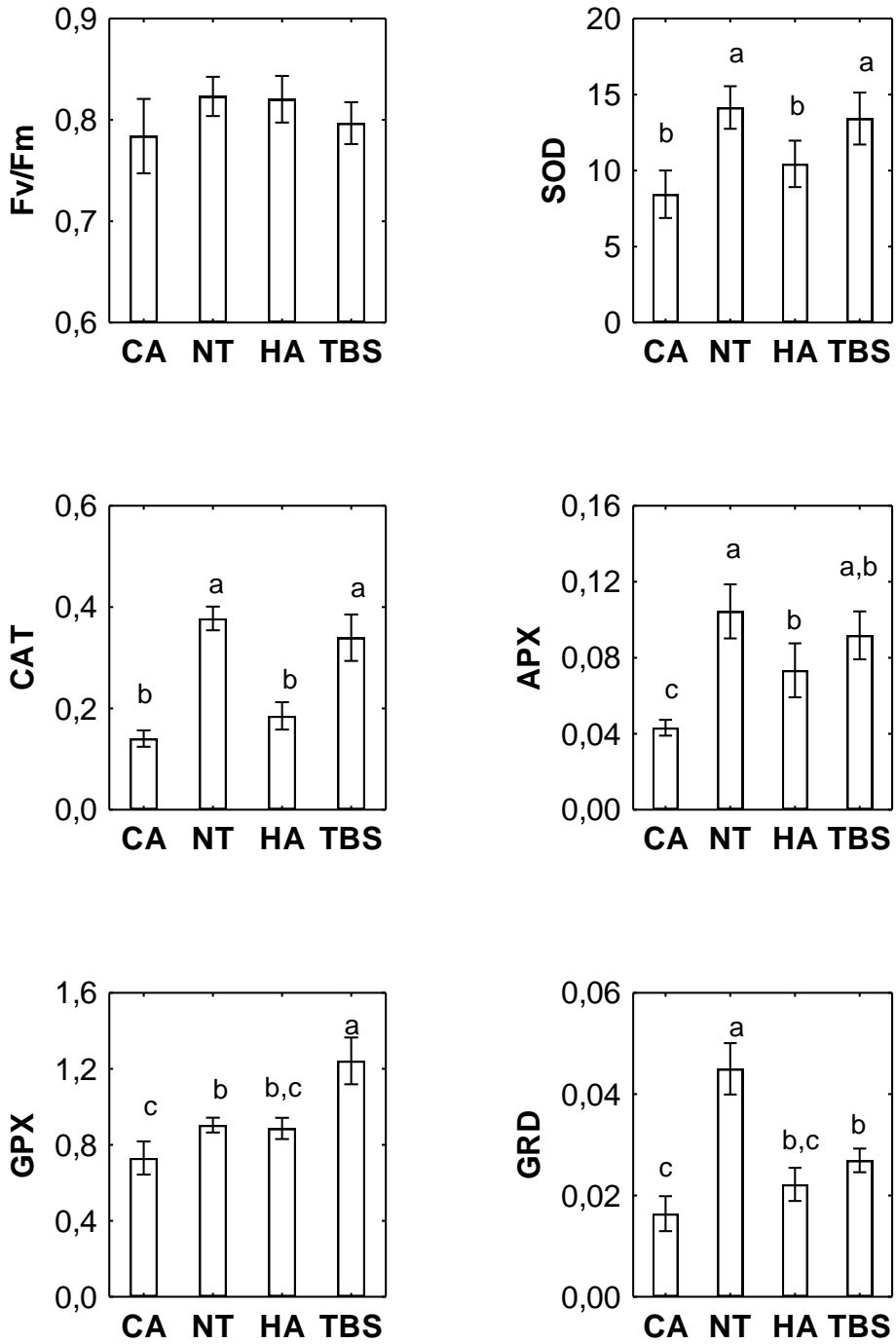
956 **Table 8**

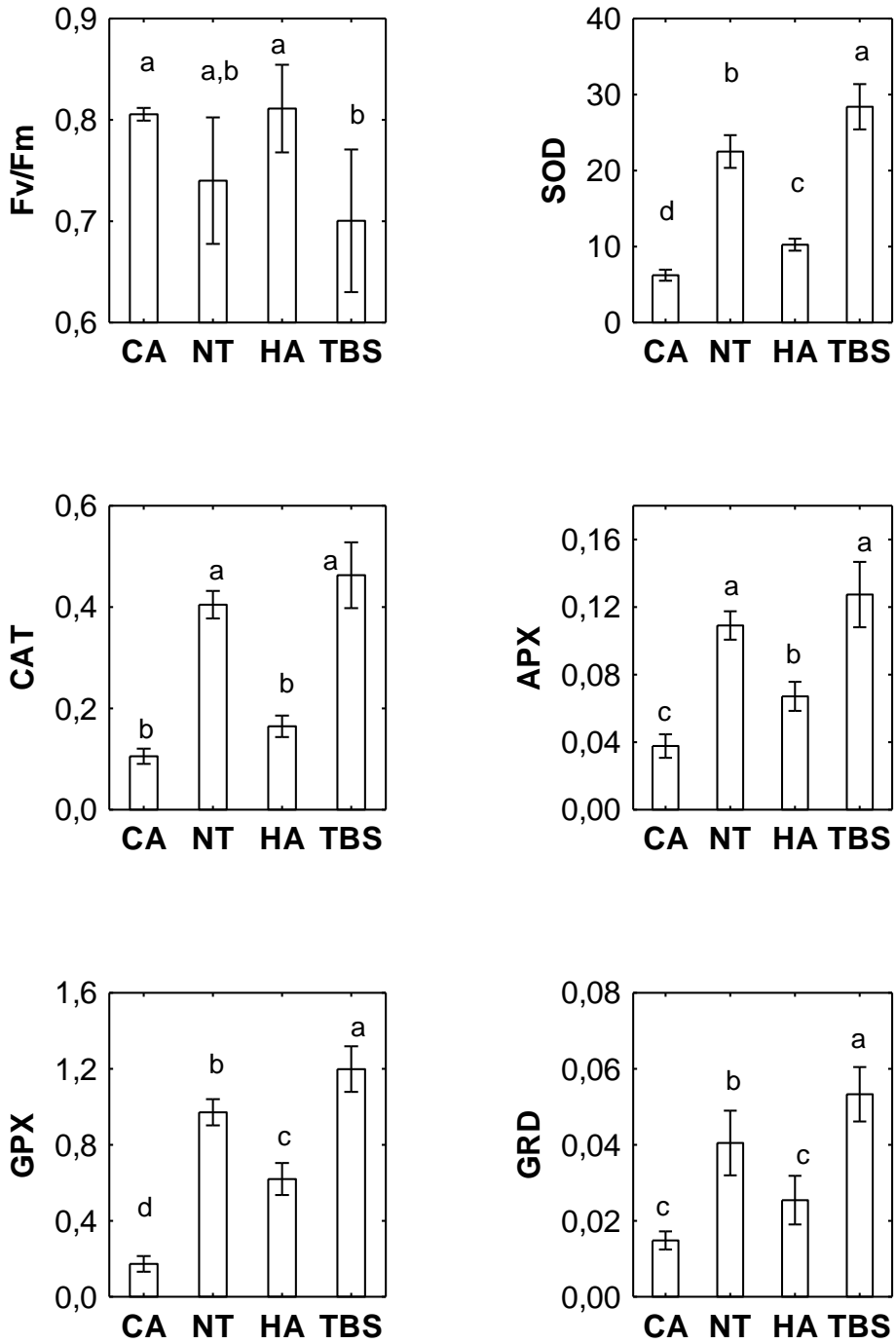
957 Treatments (NT = Untreated, HA = Hydroxyapatite, TBS = Thomas Basic Slag) ranked  
 958 according to 24 variables describing optimal features of plant, faunal and microbial  
 959 communities and sediment toxicity. Average ranks are indicated by mean  $\pm$  standard  
 960 deviation.

<b>Variable</b>	<b>NT</b>	<b>HA</b>	<b>TBS</b>
<b>Plant, faunal and microbial communities good health</b>			
Vegetation succession state	2	1	3
Plant species richness	2	1	3
Plant species diversity	2	1	3
Plant species equitability	2.5	1	2.5
Fv/Fm in <i>U. dioica</i>	2	1	3
Collembola abundance	3	2	1
Collembola species richness	2	3	1
Collembola species diversity	2	3	1
Collembola species equitability	1	3	2
Total bacteria abundance	3	2	1
<b>Sediment toxicity</b>			
Cd mobility	3	2	1
Zn mobility	3	2	1
Microtox	3	2	1
Algal test	2.5	2.5	1
Cd in <i>C. epigejos</i>	2	3	1
Zn in <i>C. epigejos</i>	3	2	1
Cd in <i>Urtica</i>	3	2	1
Zn in <i>Urtica</i>	3	2	1
SOD in <i>C. epigejos</i>	3	1	2
CAT in <i>C. epigejos</i>	3	1	2
APX in <i>C. epigejos</i>	3	1	2
SOD in <i>U. dioica</i>	2	1	3
CAT in <i>U. dioica</i>	2	1	3
APX in <i>U. dioica</i>	2	1	3
<b>Average rank</b>	<b>2.46<math>\pm</math>0.57</b>	<b>1.73<math>\pm</math>0.76</b>	<b>1.81<math>\pm</math>0.9</b>

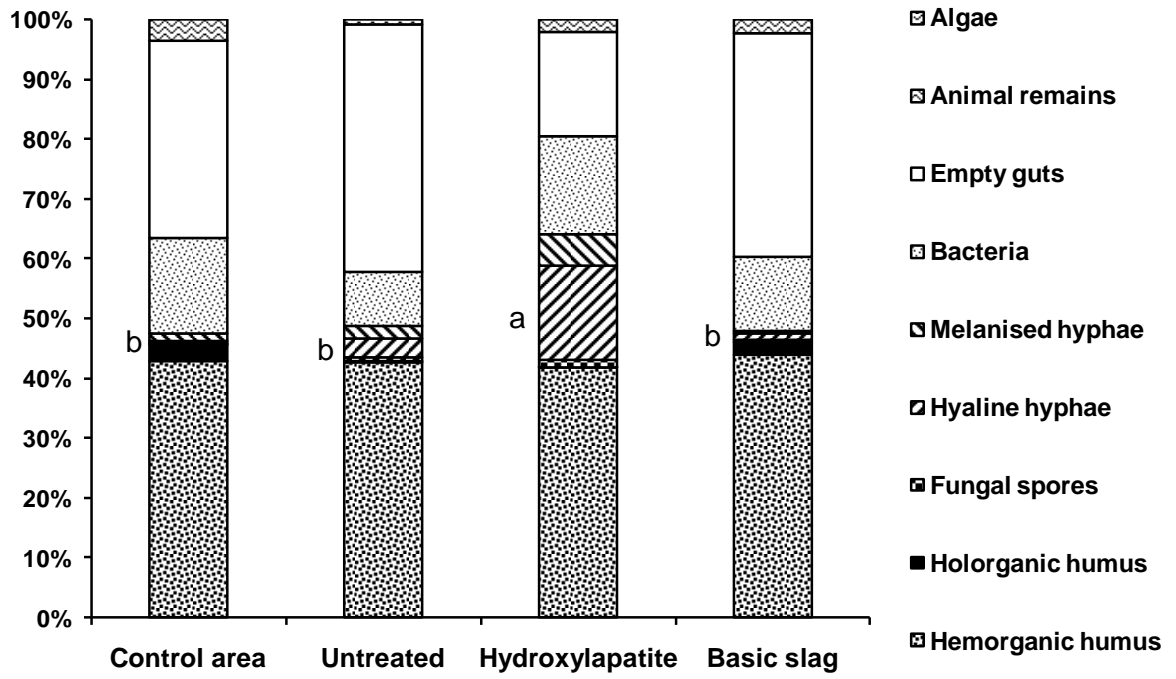
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969 **FIGURE 3**



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