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Environmental impact statement

Laboratory-based research has shown that the industrial by-products (sweet sorghum vinasse, medicinal herb residues and spent mushroom compost) held promise in reducing the metal bioavailability and restoring the ecological functions of Pb/Zn mine tailings. However, field evaluation of the effectiveness of these by-products is necessary before they can be applied at full scale. In the present field study, we found that the three organic-rich industrial by-products were approximately equally effective at reducing the levels of bioavailable metals in the mine tailings, increasing soil nutrient status, and enhancing soil respiration, microbial biomass and enzyme activities. In addition, the application of these amendments increased the vegetation cover and biomass, and reduced the metal concentrations in plant tissues. Our results provided strong evidence that the three industrial by-products could be used as organic amendments for aided phytostabilization of the mine tailings.

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2 **Field evaluation of the effectiveness of three industrial**
3 **by-products as organic amendments for phytostabilization of a**
4 **Pb/Zn mine tailings**

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3 22 **Abstract:** Although the potential of industrial by-products as organic amendments for
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5 23 phytostabilization has long been recognized, most previous studies addressing this
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7 24 issue have been laboratory-based. In this study, a field trial was conducted to evaluate
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9
10 25 the effectiveness of three industrial by-products [sweet sorghum vinasse (SSV),
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12 26 medicinal herb residues (MHR) and spent mushroom compost (SMC)] as organic
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14 27 amendments for phytostabilization of an abandoned Pb/Zn mine tailings. Our results
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16 28 showed the following: (i) when compared to the control tailings, the mean
17
18 29 concentrations of diethylene-triamine-pentaacetic acid (DTPA)-extractable Cd, Cu, Pb
19
20 30 and Zn in SSV, MHR and SMC treatments decreased by 20.8%-28.0%, 41.6%-49.1%,
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22 31 17.7%-22.7% and 9.5%-14.7%, respectively; (ii) the mean values of organic C,
23
24 32 ammonium-N and available P in SSV, MHR and SMC treatments increased by 1.7-2.8,
25
26 33 10.8-14.9 and 3.9-5.1 times as compared with the mine tailings; and (iii) the addition
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28 34 of SSV, MHR and SMC significantly enhanced soil respiration and microbial biomass
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30 35 being 1.5-1.8 and 1.3-1.6 fold higher than that in the control tailings. There were no
31
32 36 significant differences in soil biochemical properties among the plots amended with
33
34 37 these by-products, suggesting that they were almost equally effective in improving
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36 38 biochemical conditions of the tailings. In addition, the application of these
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38 39 amendments promoted seed germination, seedling growth, and consequently
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40 40 increased the vegetation cover and its biomass. Moreover, concentrations of Cd, Cu,
41
42 41 Pb and Zn in above-ground parts of the plants were below the toxicity limit levels for
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44 42 animals. The results obtained in this field study confirmed that the three organic-rich
45
46 43 industrial by-products could be used as amendments for phytostabilization of some
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48 44 types of mine tailings.
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45 Keywords: Pb/Zn mine tailings, industrial by-products, phytostabilization

1 Introduction

Mine tailings are of environmental concern in post-mining landscapes since they present a permanent threat to surrounding populations and ecosystems. The remediation of mine tailings remains a challenge globally. Traditional remediation practices used in mining areas, such as excavation, transport and landfilling are not feasible and appropriate for mine tailings due to their frequent high concentrations of heavy metals and the scale of storage facilities (area, depth, volume).¹ The *in situ* stabilization technique is currently being explored with the aim of minimizing adverse environmental impacts of the tailings by increasing the stability of land surfaces, impairing mobility and toxicity of metals and reducing wind and water erosion.²

Stabilization techniques are generally based on physical, chemical or phytostabilization.³ Physical stabilization refers to the use of innocuous materials to cover the unstable mine tailings. Chemical stabilization aims to form a crust or surface layer over the tailings by adding chemical agents. Phytostabilization establishes a permanent vegetation cover on the mine tailings to reduce surface erosion and to prevent contaminant dispersion. In many cases, the widespread application of physical or chemical stabilization techniques is limited by the availability of suitable materials and high costs.^{2,4} In contrast, phytostabilization has received a growing amount of interest and is emerging to be a promising solution for mine tailings due to its several advantages such as stabilization, pollution control and visual improvement.^{5,6}

Although phytostabilization is desirable, direct establishment of vegetation on the barren mine tailings is limited due to unfavorable conditions – particularly high concentrations of metals, lack of normal soil structure, low/no organic matter or macronutrients.⁶ To overcome these limitations, amendments may be added. Among

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2
3 71 suitable amendments for phytostabilization, organic-rich materials appear to be
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5 72 excellent candidates. Use of organic amendments in mine tailings reclamation offers
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7 73 the following advantages: (1) improvement of the physical and chemical nature of the
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10 74 tailings, especially improving the water- and nutrient- holding capacities; (2) supply
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12 75 of plant nutrients in a slow-release form, facilitating early plant establishment; (3) *in*
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14 76 *situ* immobilization of heavy metals by reducing their bioavailability and
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16 77 phytotoxicity; and (4) re-establishment of microbial populations, eventually restoring
17
18 78 the ecological function to the tailings.⁴

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21 79 Various organic materials have been proposed and tested for phytostabilization of
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23 80 heavy metals in contaminated soils, including agricultural and industrial by-products.
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25 81 Alvarenga *et al.*^{7,8} employed three industrial by-products (sewage sludge, municipal
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27 82 solid waste compost and garden waste compost) as immobilizing agents in
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29 83 phytostabilization of a highly acidic metal-contaminated soil, showed that all three
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31 84 residues significantly corrected soil acidity, decreased Cu, Pb and Zn mobile fractions,
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33 85 enhanced soil enzyme activities and increased plant biomass. Chiu *et al.*⁹ reported
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35 86 that the application of industrial by-products (manure compost and sewage sludge) to
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37 87 Pb/Zn and Cu mine tailings increased N, P and K contents of the tailings, decreased
38
39 88 DTPA-extractable Pb, Zn concentrations in Pb/Zn tailings and DTPA-extractable Cu
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41 89 concentrations in Cu tailings, and ultimately led to a reduction in heavy metal uptake
42
43 90 and accumulation by the grasses *Vetiveria zizanioides* and *Phragmites australis*. Lee
44
45 91 *et al.*² found that the addition of iron-rich industrial by-products (limestone, red mud
46
47 92 and furnace slag) to arsenic and metal-contaminated agricultural soils not only
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49 93 lowered the availability of trace elements, but also improved soil microbial
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51 94 community structure and function. Our earlier attempt to remediate Pb/Zn tailings by
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53 95 adding industrial by-products (sweet sorghum vinasse, medicinal herb residues and
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3 96 spent mushroom compost) proved efficient in decreasing extractable metal
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5 97 concentrations, enhancing enzyme activities and reducing metal concentrations in
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7 98 plant tissues.¹⁰ However, in most cases, the effectiveness of industrial by-products
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9 99 proposed in the literature was tested under laboratory conditions. Many studies have
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11 100 noted that the greenhouse scenario cannot represent the real field condition and it is
12
13 101 difficult to apply the results into practice.^{11,12} Therefore, field evaluations are essential
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15 102 before these materials can be applied at the field-scale or to real situations.
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19 103 The present work focused on the restoration of a vegetation cover for a Pb/Zn mine
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21 104 tailings through phytostabilization combining organic industrial by-products and
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23 105 metal-tolerant plants. A field study was carried out at an abandoned Pb/Zn mine
24
25 106 tailings pond to evaluate the effectiveness of three freely-available industrial
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27 107 by-products (SSV: sweet sorghum vinasse; MHR: medicinal herb residues and SMC:
28
29 108 spent mushroom compost) for phytostabilization of Pb/Zn mine tailings. We
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31 109 hypothesized that the three amendments might improve the physico-chemical
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33 110 properties of the tailings, reduce heavy metal availability and enhance microbial
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35 111 activity, thereby enhancing plant establishment and growth. To test our hypothesis, we
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37 112 studied soil chemical properties (available heavy metals, macronutrients), microbial
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39 113 activities, plant growth and metal accumulation in plant tissues. Relationships
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41 114 between soil properties and plant parameters were further analyzed to demonstrate the
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43 115 remediation efficacy of these industrial by-products in the phytostabilization.
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48 116 **2 Materials and methods**

49 117 **2.1 Study site**

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55 118 The Huayuan Pb/Zn mine (28°06'N, 109°11'E) is located in Xiangxi Tujia and Miao
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57 119 Autonomous District, Hunan Province, China. This mining area has a middle
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3 120 subtropical mountainous climate with a mean annual temperature of 16.7 °C and an
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5 121 annual average precipitation of 1421 mm. The region has an abundant reserve of
6
7 122 manganese and zinc. Mining and processing activities have generated large quantities
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10 123 of tailings deposited on the ground with over 100 tailings sites abandoned (data from
11
12 124 the local Environmental Protection Bureau). The tailings pond studied had been
13
14 125 abandoned for about two years. The tailings surface was dry and completely devoid of
15
16 126 vegetation. The main physico-chemical properties of the tailings were detailed in our
17
18 127 previous study.¹⁰

128 **2.2 Experimental design**

129 The experiment was performed in a plot (30 m × 20 m) in the center of the abandoned
130 Pb/Zn tailings pond. The experimental plot was divided into 20 subplots of 4 m² (2 m
131 × 2 m), leaving a corridor of 2 m between subplots as a barrier to avoid interactions
132 between them. Three industrial by-products (SSV, MHR and SMC) were used as
133 organic amendments and detailed information about them was given in our previously
134 reported pot experiment.¹⁰ The three specific industrial by-products were chosen since
135 they are widely available at a local level, have a low cost and are easy to apply. These
136 were applied at 12 kg subplot⁻¹ (equivalent to 30 t ha⁻¹, a rate based on the results of
137 our pot experiment)¹⁰ and incorporated manually with a hoe into the upper layer of
138 tailings (0-30 cm depth). The subplots were arranged according to a complete
139 randomized block design with four replicates per treatment. Two control treatments
140 were also established: tailings without amendment (Tailings) and tailings with a local
141 ‘normal’ topsoil (30 t ha⁻¹, NTS). Topsoil was selected for comparison because it has
142 been used as an amendment by local residents to cover mine tailings. Sowing took
143 place after the amendments addition and equilibration for two months. A grass seed

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3 144 mix of *Lolium perenne*, *Cynodon dactylon*, *Medicago sativa* and *Dendranthema*
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5 145 *indicum* (5/g m² for *L. perenne*, *C. dactylon* and *M. sativa*; 2.5/g m² for *D. indicum*,
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7 146 according to the seed germination in the pot experiment) was sown by hand. Plants
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9 147 were grown under natural conditions and neither agricultural practices nor irrigation
10
11 148 were employed.

149 **2.3 Soil sampling and analysis**

150 Soil samples were collected from all experimental subplots at the time of sowing (0)
151 and then at 3, 6, 9 and 12 months after planting. In each case, 5 regularly distributed
152 soil cores (5 cm diameter, 25 cm depth, using a manual stainless steel soil auger) were
153 taken from each subplot to give a composite sample. The soil samples were
154 immediately transported to the laboratory and divided into two subsamples. One
155 subsample was air-dried, passed through a 2 mm sieve and subjected to chemical
156 analysis; the other fresh subsample was sieved to 2 mm and then stored at 4 °C for
157 microbial analysis.

158 **2.3.1 Chemical analysis**

159 Organic C (OC) was analyzed by dichromate oxidation and titration with ferrous
160 sulphate.¹³ Ammonium-N (NH₄⁺-N) was extracted with potassium chloride and
161 measured by colorimetric N determination.¹⁴ Available P (AP) was extracted with
162 sodium bicarbonate and estimated according to the molybdenum blue method.¹⁵ Soil
163 bioavailable metals were extracted using a mixture of 5 mM DTPA
164 (diethylene-triamine-pentaacetic acid), 10 mM CaCl₂ and 100 mM triethanolamine at
165 pH 7.3¹⁶ and determined by Inductively-coupled Optical Emission Spectrometry
166 (ICP-OES, iCAP6300, Thermo Electron, USA). Quality assurance of metal analysis
167 was performed using added certified reference materials (provided by Beijing

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3 168 Shijiao Biotechnology, China) in the extracted solution (recovery ratio 84-116%).
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5 169 The detection limits of Cd, Pb and Zn were 0.001, 0.001 and 0.003 mg L⁻¹,
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7 170 respectively.
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10 171 **2.3.2 Microbial analysis**

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12 172 Basal soil respiration was measured according to the method of Anderson:¹⁷ the
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14 173 field-moist soil was incubated in an air-tight sealed jar at 25°C for 24 h in the dark.
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16 174 CO₂ produced during the test was absorbed in 0.05 M NaOH and quantified by
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18 175 titration with 0.1 M HCl. Soil microbial biomass C was determined based on a
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20 176 modification of the chloroform fumigation extraction method of Vance *et al.*¹⁸ Ten
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22 177 gram samples of field moist soil were fumigated with chloroform for 7 days and
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24 178 extracted for 1 h in 50 mL K₂SO₄. Organic C in the extracts was oxidized by
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26 179 dichromate and titration with FeSO₄. Microbial C was calculated as the difference
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28 180 between fumigated and non-fumigated samples and corrected with a K_{EC} value of 0.38.
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30 181 Dehydrogenase activity was measured using the method of Thalmann.¹⁹
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32 182 β -Glucosidase activity was determined as proposed by Hoffmann and Dedeke.²⁰
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34 183 Urease activity was measured using a buffered method described by Kandeler and
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36 184 Gerber.²¹ Phosphatase activity was determined following the method of Tabatabai and
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38 185 Bremner.²² The detailed experimental procedures for soil enzyme analysis were
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40 186 described previously in our earlier study.¹⁰
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47 187 **2.4 Plant sampling and analysis**

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49 188 Plant investigations and samplings were taken on two occasions, at 6 and 12 months
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51 189 after sowing. Vegetation cover was estimated by the percentage of the subplots
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53 190 covered by grasses. Plant sampling involved clipping grass at 5 mm above ground
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55 191 within a quadrat (0.5 m × 2 m) in each subplot. Root material was not removed in
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3 192 order not to disrupt the established vegetation. All plant species were listed, washed
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5 193 thoroughly with tap water, rinsed three times with deionized water and, finally
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7 194 oven-dried at 80 °C for 48 h for determining biomass production and metal
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9 195 concentrations. Approximately 0.5 g of finely-ground plant samples were digested
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11 196 with a mixture of concentrated HNO₃ and concentrated HClO₄ at 5:1 (v/v).²³ The
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13 197 concentrations of Cd, Cu, Pb and Zn in the plant materials were determined by
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15 198 ICP-OES analysis of the digests. A certified reference plant material (GBW07405)
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17 199 obtained from the National Center for Standard Materials was used to verify the
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19 200 accuracy of metal determination. The recovery ratios were: 93±6% for Cd, 89±7%
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21 201 for Cu, 88±5% for Pb and 97±3% for Zn, respectively.

202 **2.5 Statistical analysis**

203 All statistical analyses were conducted using the SPSS 16.0 for Windows. Datasets
204 were checked for homogeneity of variance and normality (Kolmogorov-Smirnov test)
205 and, when necessary, log-transformed. One-way ANOVA was carried out to compare
206 the means of different treatments, followed by multiple comparisons using the least
207 significant difference test. The level of significance was set at $p < 0.05$. Pearson's
208 correlation coefficients were calculated between plant parameters and soil
209 biochemical properties. Two levels of significance were considered, $p < 0.05$ and $p <$
210 0.01 . A multivariate approach was applied to explore the relationships between soil
211 biochemical properties and plant parameters using canonical correspondence analysis
212 (Canoco 4.5 for Windows).²⁴ A summary diagram was prepared on which soil
213 biochemical variables were represented as arrows: the length of these arrows indicated
214 the relative importance of that soil factor in explaining variation in plant development
215 and the angle between arrows indicated the degree to which they were correlated.

216 **3 Results**

217 **3.1 Effects of industrial by-products on heavy metal availability**

218 The DTPA-extractable metal concentrations with different industrial by-products are
219 presented in Fig. 1. In the tailings, the DTPA-extractable metal concentrations were
220 within the range 0.97-0.99 mg kg⁻¹ for Cd, 6.11-6.78 mg kg⁻¹ for Cu, 46.36-47.89 mg
221 kg⁻¹ for Pb and 96.12-100.17 mg kg⁻¹ for Zn, respectively. The addition of SSV, MHR
222 and SMC significantly decreased DTPA-extractable metal concentrations. The mean
223 values of DTPA-extractable Cd, Cu, Pb and Zn in SSV, MHR and SMC treatments
224 decreased by 20.8%-28.0%, 41.6%-49.1%, 17.7%-22.7% and 9.5%-14.7% as
225 compared to the control tailings. No significant differences were found between the
226 two controls (Tailings and NTS). In addition, DTPA-extractable Cd, Cu, Pb and Zn in
227 all the treatments remained constant over the one-year of remediation.

228 **3.2 Effects of industrial by-products on nutrient accumulation**

229 Fig. 2 shows the accumulation of major nutrients in different treatments. As expected,
230 the addition of SSV, MHR and SMC significantly increased OC, NH₄⁺-N and AP
231 relative to the Tailings and NTS treatments. When compared to the control tailings,
232 the mean values of OC, NH₄⁺-N and AP in SSV, MHR and SMC treatments increased
233 by 1.7-2.8, 10.8-14.9 and 3.9-5.1 times, respectively. A slightly increasing trend in
234 OC, NH₄⁺-N and AP in SSV, MHR and SMC treatments was observed as the
235 remediation time progressed. For example, OC contents in SSV, MHR and SMC
236 treatments were 7.18, 7.02 and 7.29 g kg⁻¹ at the initial sampling and increased to 9.36,
237 9.55 and 10.35 g kg⁻¹ at the last sampling. In contrast, the contents of OC, NH₄⁺-N
238 and AP in Tailings and NTS remained unchanged.

239 **3.3 Effects of industrial by-products on microbial activity**

240 The soil respiration, microbial biomass and enzyme activities with different industrial
241 by-products are shown in Fig. 3 and 4. In all cases, the two controls (Tailings and
242 NTS) showed very low mean values for soil respiration and microbial biomass and the
243 added industrial by-products (SSV, MHR and SMC) significantly enhanced soil
244 respiration and microbial biomass, being 1.5-1.8 and 1.3-1.6 fold higher than that in
245 the control tailings. An appreciable trend of increasing microbial biomass with time
246 was observed, whereas mean values of soil respiration remained constant (Fig. 3).
247 Similarly, the addition of SSV, MHR and SMC also significantly increased soil
248 enzyme activities in comparison to the two controls. Overall, the levels of the four soil
249 enzyme activities were similar in the three organic treatments at each sampling time
250 and there was an appreciably increasing trend in dehydrogenase, β -glucosidase, urease
251 and phosphatase activities with time in SSV, MHR and SMC treatments (Fig. 4).

252 **3.4 Effects of industrial by-products on vegetation cover, biomass and heavy** 253 **metal accumulation in the plant tissues**

254 The vegetation cover was assessed as the average of the two investigations and the
255 biomass yield of each species was the sum of the two harvests. Fig. 5a shows that the
256 vegetation cover was about 25% and 35% for the two control subplots, and reached
257 84%, 79% and 86% at SSV, MHR and SMC subplots, respectively. For *L. perenne* and
258 *C. dactylon*, the addition of SSV, MHR and SMC led to significant increases in the
259 shoot biomass yields with 4.2-5.6 and 15.7-17.3 fold greater than those in the tailings
260 (Fig. 5b). No seeds of *M. sativa* and *D. indicum* could germinate in the control
261 subplots (Tailings and NST); therefore, there were no biomass data for them. The
262 addition of SSV, MHR and SMC triggered the seed germination and seeding growth

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3 263 of *M. sativa* and *D. indicum*. Due to similarity of metal concentrations in shoots of *L.*
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5 264 *perenne* and *C. dactylon* in the two harvests of each harvest, only data from the
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7 265 second harvest (12 month) are presented (Table 1). The addition of SSV, MHR and
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9 266 SMC significantly reduced the concentrations of Cd, Cu, Pb and Zn in the shoots of *L.*
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11 267 *perenne* and *C. dactylon* in comparison with the tailings and NTS treatments.
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13
14 268 However, no significant differences were found in shoot metal concentrations between
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16 269 the three organic treatments. The metal concentrations (Cd, Cu, Pb and Zn) in the
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18 270 shoots of *M. sativa* and *D. indicum* are presented in Table S1.

21 271 **3.5 Relationship between plant parameters and soil biochemical properties**

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25 272 Pearson's correlation coefficients between plant parameters and soil biochemical
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27 273 properties are listed in Table 2. The vegetation cover and biomass were positively
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29 274 correlated with soil nutrient elements (OC, NH₄⁺-N and AP) and microbial parameters
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31 275 (soil respiration, microbial biomass and enzyme activities). Significant negative
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33 276 correlations were observed between DTPA-extractable metal concentrations and
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35 277 vegetation cover and biomass. The metal concentrations in plants were positively
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37 278 correlated with soil DTPA-extractable metal concentrations and negatively correlated
38
39 279 with soil nutrient elements and microbial parameters. In general, the significances
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41 280 were at lower significance levels ($p < 0.01$). Canonical correspondence analysis (CCA)
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43 281 was carried out to determine how soil biochemical properties influenced plant
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45 282 development. CCA revealed that all the tailings subplots clustered into two groups
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47 283 attributed to the variation in soil and plant parameters (Fig. 6). Axes 1 and 2 were
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49 284 found to explain 76.6% and 17.6% of the overall variance and plant-soil correlations
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51 285 for both axes were 0.96, indicating a strong correlation between plant parameters and
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53 286 soil biochemical properties. The two control subplots were located on the negative
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3 287 side of Axis 1 which indicated that they had a strong correlation with soil
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5 288 DTPA-extractable metal concentrations. In contrast, the subplots added SSV, MHR
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7 289 and SMC all positioned in the positive side of Axis 1, suggesting that they were
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10 290 strongly correlated with soil nutrient elements and microbial parameters.

11 12 13 291 **4 Discussion**

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16 292 Phytostabilization is recognized as a potentially cost-effective, ecologically sound and
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18 293 sustainable solution for the remediation of heavy metal-contaminated soils and mine
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20 294 tailings.² The success of phytostabilization on the mine tailings mainly depends on the
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22 295 improvement of the substrate. Organic amendment is a major requirement which can
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24 296 provide essential nutrients, rebuild soil structure, re-establish microbial populations,
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26 297 and eventually allow plant establishment and subsequent vegetation development.²⁵

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29 298 As other mine tailings,^{2,26,27} heavy metal toxicity is the major constraint for
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31 299 ecological restoration of this Pb/Zn mine tailings. In the present field trial, the
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33 300 application of industrial by-products (SSV, MHR and SMC) as organic amendments
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35 301 significantly reduced DTPA-extractable Cd, Cu, Pb and Zn compared to the control
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37 302 tailings (Fig. 1), which was consistent with the results of our earlier pot study.¹⁰
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39 303 DTPA-extractable metal concentrations usually well predict the metal fraction readily
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41 304 available to plants and microorganisms (i.e. metal bioavailability) and this reduction
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43 305 in DTPA-extractable metal concentrations may be attributed to the immobilization
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45 306 processes by the industrial by-product amendments. The main mechanisms by which
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47 307 they immobilize metals are probably based on adsorption, complexation and/or
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49 308 precipitation reactions.⁴ It has been reported that SMC has a very high sorption
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51 309 capacity for Cd, Pb and Cr owing to the presence of hydroxyl, phosphoryl and
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53 310 phenolic functional groups on its surface.²⁸ In addition, no significant changes were
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3 311 found in DTPA-extractable Cd, Cu, Pb and Zn concentrations within the one-year
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5 312 remediation period. Similar results were also reported by other authors,^{29,30} which can
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7 313 be related to the plant rhizospheric processes since root exudates and microbial
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9 314 activity can increase solubility of metal ions in the rhizosphere.

10
11 315 Apart from reducing metal bioavailability, another important aim of
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13 316 phytostabilization is to restore the ecological function and health of mine tailings. As
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15 317 expected, the addition of SSV, MHR and SMC led to significant increases in soil
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17 318 nutrient elements such as OC, NH₄⁺-N and AP (Fig. 2). This improvement of the
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19 319 tailings conditions with the application of these amendments allowed microbial
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21 320 community development and facilitated soil-forming processes.^{1,31} In recent years,
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23 321 microbial parameters have increasingly been used as indicators of soil quality to
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25 322 evaluate the success of remediation efforts.^{32,33} In the present study, the addition of
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27 323 SSV, MHR and SMC significantly enhanced soil respiration, microbial biomass and
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29 324 soil enzyme activities when compared to the controls (Fig. 3 and 4). This is in
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31 325 accordance with the findings of Kumpiene *et al.*³⁴ who found that phytostabilization
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33 326 significantly increased soil respiration, microbial biomass and the activity of key soil
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35 327 enzymes, indicating a clear enhancement of soil function. In addition, we also
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37 328 observed a slightly increasing trend in nutritional status (OC, NH₄⁺-N and AP) and
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39 329 microbial parameters (microbial biomass and enzyme activities) in SSV, MHR and
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41 330 SMC treatments (Figs. 2, 3 and 4). This result supported the conclusion that the
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43 331 addition of organic amendments led to a larger and more active microflora and
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45 332 nutrient accumulation in the mine tailings.^{10, 34}

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47 333 Beneficial effects of the three industrial by-products were also observed in
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49 334 vegetation characteristics, as reflected by the vegetation cover, biomass and heavy
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51 335 metal accumulation in plant tissues (Fig. 5 and Table 1). In the present field trial, the
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3 336 application of these amendments promoted seed germination, seedling growth, and
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5 337 subsequently increased the vegetation cover and biomass on SSV, MHR and SMC
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7 338 subplots (Fig. 5). Plant growth may improve soil nutrient accumulation and
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9 339 microbiological function, which was evidenced by the significant positive correlations
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11 340 between the vegetation cover and biomass and soil nutrient status (OC, NH_4^+ -N and
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13 341 AP) and microbial parameters (soil respiration, microbial biomass and enzyme
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15 342 activities) (Table 2). Another particular concern associated with the revegetation of
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17 343 mine tailings is the accumulation of heavy metals in the above-ground parts of plants.
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19 344 From the viewpoint of stabilizing metals in the mine tailings, the desirable species
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21 345 should always absorb or transport as low as possible heavy metals from the tailings,
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23 346 thereby limiting the propagation of metals into the food chain.^{2,3} Moreover, the
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25 347 advantages of using native plant species are generally considerable since they are
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27 348 ecologically adapted to the local environmental conditions.³⁵ The species selected (*L.*
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29 349 *perenne*, *C. dactylon*, *M. sativa* and *D. indicum*) for this study are native and have
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31 350 been reported as metallophytes, which have all been widely employed as pioneer
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33 351 species for phytostabilization of metal-contaminated soils.^{5,7,8} Almost all
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35 352 concentrations of Cd, Cu, Pb and Zn in above-ground parts of the plants were below
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37 353 toxicity limits of the US toxicity limits for cattle ($\text{Cd} \leq 10 \text{ mg kg}^{-1}$, $\text{Cu} \leq 40 \text{ mg kg}^{-1}$,
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39 354 $\text{Pb} \leq 100 \text{ mg kg}^{-1}$ and $\text{Zn} \leq 500 \text{ mg kg}^{-1}$),³⁶ and the addition of SSV, MHR and SMC
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41 355 significantly decreased the shoot metal concentrations (Cd, Cu, Pb and Zn) of *L.*
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43 356 *perenne* and *C. dactylon* compared to the tailings and NTS treatments (Table 1). This
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45 357 was consistent with the results of significant negative correlation between the metal
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47 358 concentrations in plants and soil nutrient elements and microbial parameters (Table 2).
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49 359 However, we did not ascertain how much of the improvement in the tailings
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51 360 conditions was due to the addition of organic amendments and how much of it was
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3 361 due to the plant development. The strong correlation between plant parameters and
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5 362 soil biochemical properties (Fig. 6) demonstrated that these factors are likely to be
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7 363 synergistic in phytostabilization of this Pb/Zn mine tailings.
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10 364 **5 Conclusions**

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14 365 The results obtained from the present field experiment indicate that phytostabilization
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16 366 (plants together with the application of amendments) can be a promising strategy for
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18 367 the restoration of mine tailings. The three industrial by-products (SSV, MHR and
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20 368 SMC) were equally effective at reducing the levels of bioavailable metals in the mine
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22 369 tailings, increasing soil nutrient status (organic C, ammonium-N and available P) and
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24 370 enhancing soil respiration, microbial biomass and enzyme activities. The addition of
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26 371 SSV, MHR and SMC promoted plant growth (vegetation cover and biomass) and
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28 372 decreased heavy metal uptake and accumulation in harvestable plant tissues. Although
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30 373 caution must be used when extrapolating from controlled laboratory experiments to
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32 374 field conditions, our work showed the similar results in both the pot and field trials.
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54 383 paper.
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Table 1 Concentrations of Cd, Cu, Pb and Zn in the shoots of *L. perenne* and *C. dactylon* grown in different substrata (mean \pm SE, $n = 4$)^a

Treatments	<i>L. perenne</i>				<i>C. dactylon</i>			
	Cd	Cu	Pb	Zn	Cd	Cu	Pb	Zn
CK	16.94 \pm 3.06 ^a	16.44 \pm 0.97 ^a	101.06 \pm 22.29 ^a	357.66 \pm 39.42 ^a	10.36 \pm 2.65 ^a	20.44 \pm 1.62 ^a	93.82 \pm 12.92 ^a	418.76 \pm 28.21 ^a
NTS	12.03 \pm 1.5 ^b	14.53 \pm 1.61 ^a	88.14 \pm 11.25 ^a	242.52 \pm 45.07 ^b	7.09 \pm 2.56 ^a	17.03 \pm 1.4 ^b	89.62 \pm 6.6 ^b	330.88 \pm 34.22 ^b
SSV	2.77 \pm 0.84 ^c	8.91 \pm 0.55 ^b	29.33 \pm 5.72 ^b	52.79 \pm 8.4 ^c	3.19 \pm 0.51 ^b	10.02 \pm 0.94 ^c	20.77 \pm 4.50 ^c	78.58 \pm 9.55 ^c
MHR	2.42 \pm 0.34 ^c	8.42 \pm 1.05 ^b	24.37 \pm 5.58 ^b	68.8 \pm 1.09 ^c	4.30 \pm 0.88 ^b	9.17 \pm 0.98 ^c	20.06 \pm 2.33 ^c	86.89 \pm 17.13 ^c
SMC	2.08 \pm 0.45 ^c	9.58 \pm 1.32 ^b	23.15 \pm 5.29 ^b	62.93 \pm 4.97 ^c	4.93 \pm 0.64 ^b	10.83 \pm 1.13 ^c	17.28 \pm 1.17 ^c	87.73 \pm 18.12 ^c

^a Different letters in the same column indicate a significant difference at $p < 0.05$ according to LSD tests.

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Table 2 Pearson's correlation coefficients between plant parameters and soil biochemical properties ($n = 56$)^a

Plant parameters	OC	NH ₄ ⁺ -N	AP	Soil respiration	Microbial biomass	Soil enzymes				DTPA-extractable metals			
						dehydrogenase	β-glucosidase	urease	phosphatase	Cd	Cu	Pb	Zn
Cover	0.916** ^a	0.922**	0.944**	0.847**	0.707**	0.944**	0.847**	0.948**	0.883**	-0.799**	-0.905**	-0.745**	-0.605**
Biomass	0.830**	0.928**	0.926**	0.839**	0.653**	0.948**	0.858**	0.849**	0.866**	-0.719**	-0.893**	-0.670**	-0.633**
Cd	-0.795**	-0.845**	-0.840**	-0.820**	-0.741**	-0.870**	-0.784**	-0.835**	-0.802**	0.706**	0.845**	0.543*	0.631**
Cu	-0.779**	-0.789**	-0.786**	-0.719**	-0.596**	-0.808**	-0.628**	-0.776**	-0.689**	0.806**	0.775**	0.806**	0.476*
Pb	-0.842**	-0.872**	-0.875**	-0.847**	-0.699**	-0.855**	-0.806**	-0.883**	-0.785**	0.645**	0.812**	0.654**	0.484*
Zn	-0.823**	-0.867**	-0.858**	-0.814**	-0.677**	-0.863**	-0.772**	-0.867**	-0.799**	0.708**	0.861**	0.546*	0.486*

^a * $p < 0.05$, ** $p < 0.01$.

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Table S1 Concentrations of Cd, Cu, Pb and Zn in the shoots of *M. sativa* and *D. indicum* grown in different substrata (mean \pm SE, $n = 4$)^a

Treatments	<i>M. sativa</i>				<i>D. indicum</i>			
	Cd	Cu	Pb	Zn	Cd	Cu	Pb	Zn
CK	- ^a	-	-	-	-	-	-	-
NTS	-	-	-	-	-	-	-	-
SSV	3.30 \pm 0.37	6.44 \pm 0.97	10.74 \pm 0.83	124.72 \pm 19.47	1.61 \pm 0.17	7.94 \pm 0.67	6.13 \pm 0.91	150.13 \pm 23.76
MHR	2.13 \pm 0.39	5.53 \pm 1.02	11.3 \pm 0.43	130.51 \pm 3.43	2.46 \pm 0.86	6.78 \pm 1.23	4.82 \pm 0.37	147.6 \pm 27.39
SMC	2.05 \pm 0.46	5.08 \pm 0.8	15.79 \pm 3.34	123.26 \pm 12.88	2.94 \pm 0.54	7.08 \pm 0.86	4.54 \pm 0.3	138.4 \pm 26.44

^a - : not detected.

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3 452 **Figure captions**

4
5 453 **Fig. 1** DTPA-extractable Cd, Cu, Pb and Zn concentrations in the tailings with
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7 454 different amendments and remediation time (mean \pm SE, $n = 4$).

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10 455 **Fig. 2** The accumulation of major nutrients in the tailings with different amendments
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12 456 and remediation time (mean \pm SE, $n = 4$).

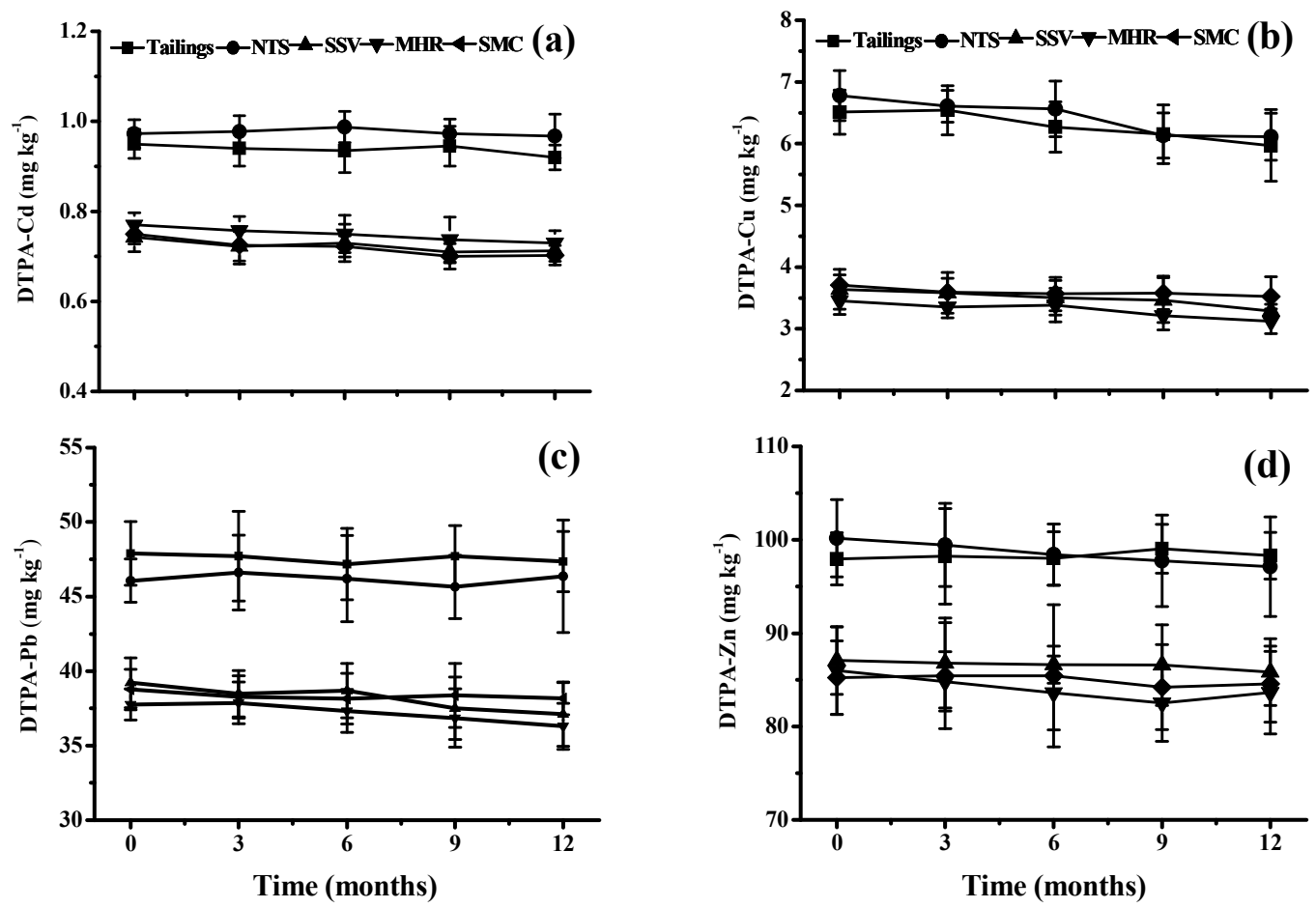
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14 457 **Fig. 3** Soil microbial activity and biomass in the tailings with different amendments
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16 458 and remediation time (mean \pm SE, $n = 4$).

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19 459 **Fig. 4** Soil enzyme activity in the tailings with different amendments and remediation
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21 460 time (mean \pm SE, $n = 4$).

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23 461 **Fig. 5** Vegetation cover and plant biomass in the tailings with different amendments
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25 462 and remediation time (mean \pm SE, $n = 4$). Different letters in the bar indicate a
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27 463 significant difference at $p < 0.05$ according to LSD tests.

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29 464 **Fig. 6** Ordination biplot of the canonical correspondence analysis ($n = 380$).
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31 465 Symbols: Tailings (\bullet), NTS (\blacksquare), SSV (\blacktriangle), MHR (\blacktriangledown), SMC (\blacklozenge).

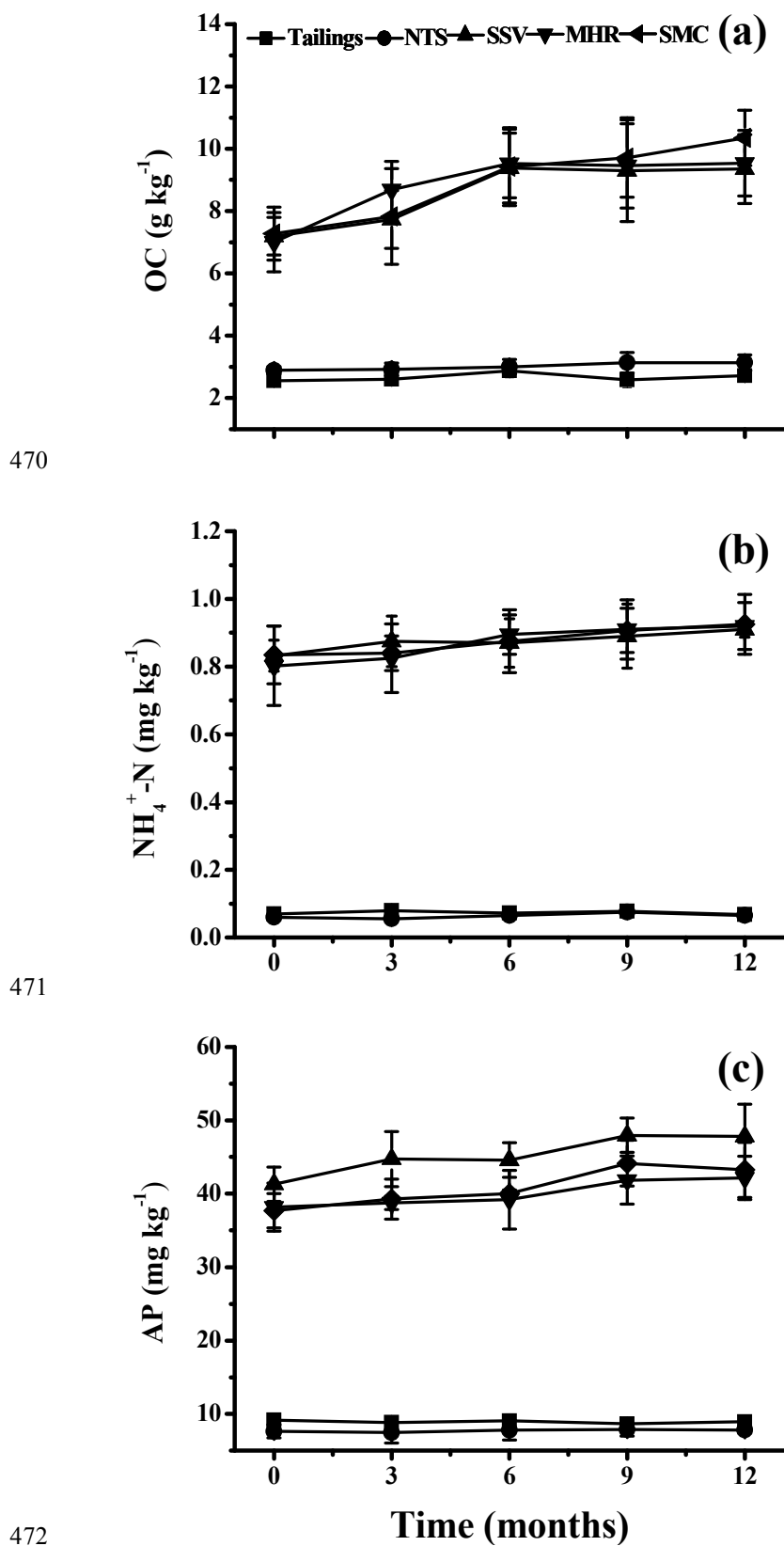
466 Fig.1



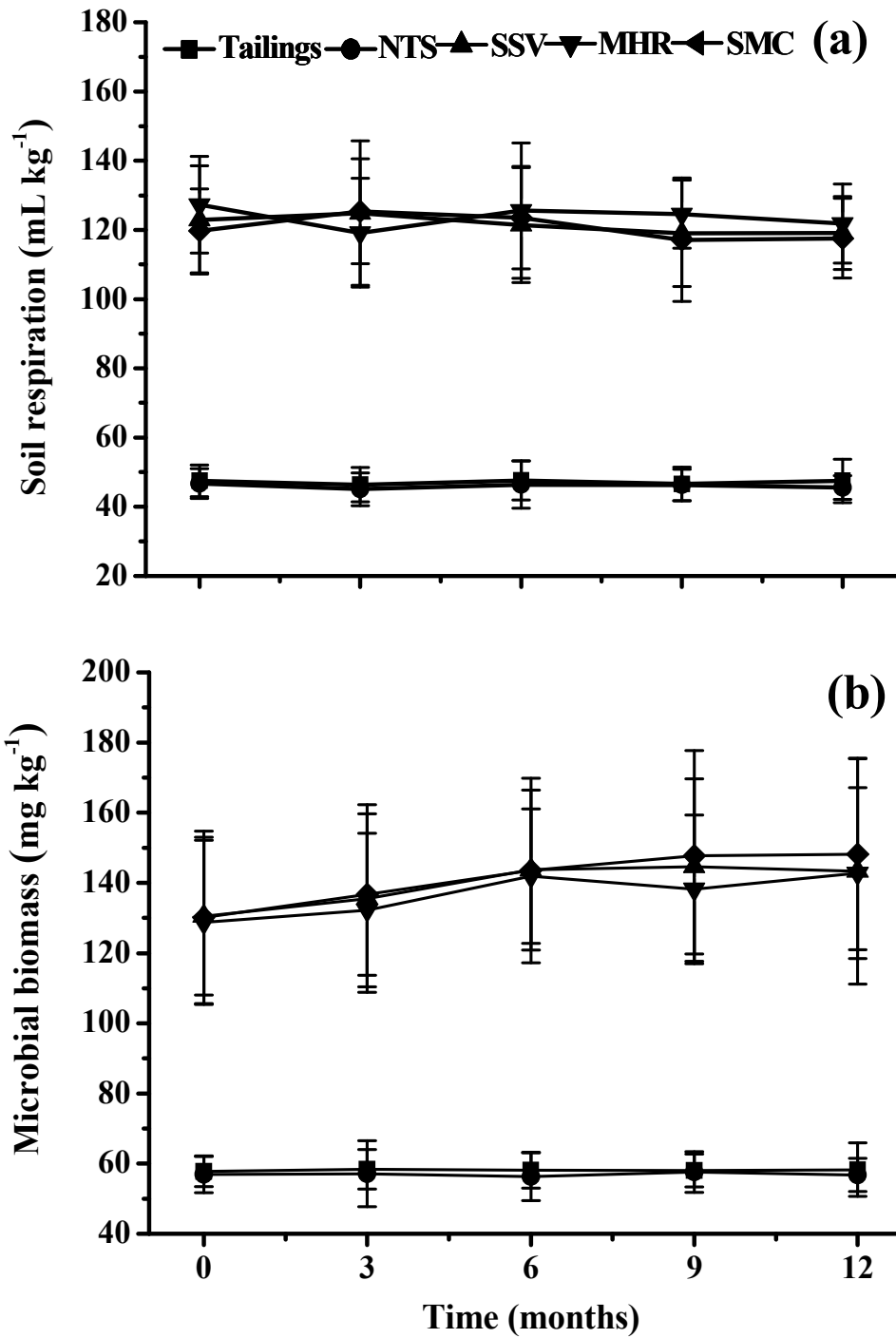
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469 Fig. 2



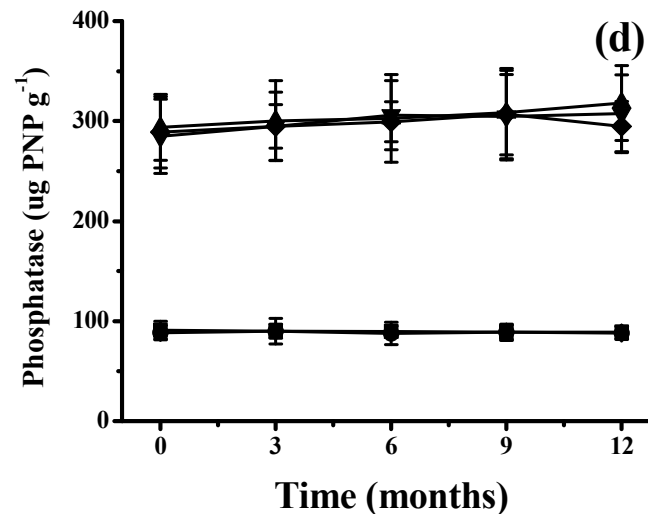
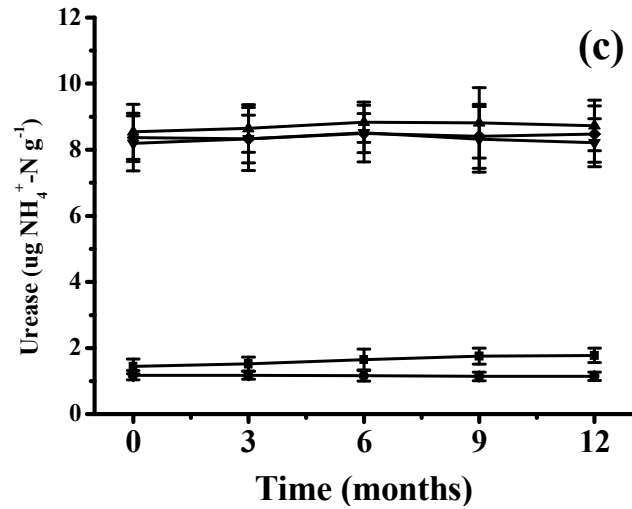
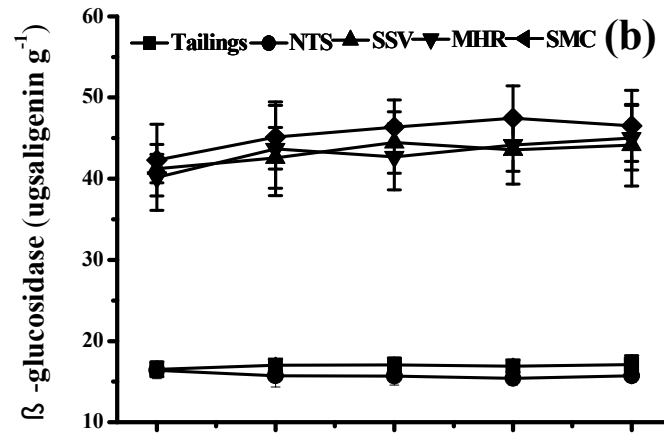
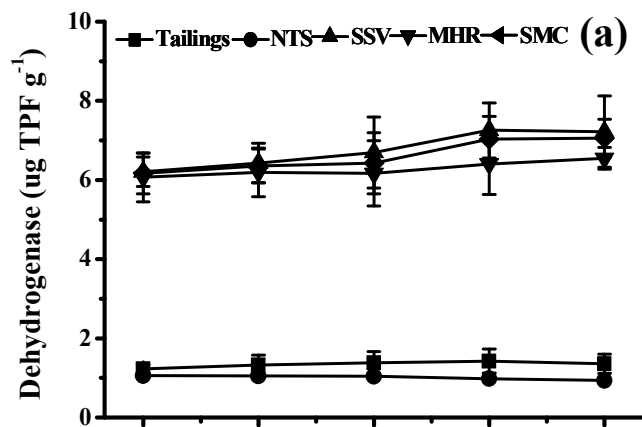
473 Fig. 3



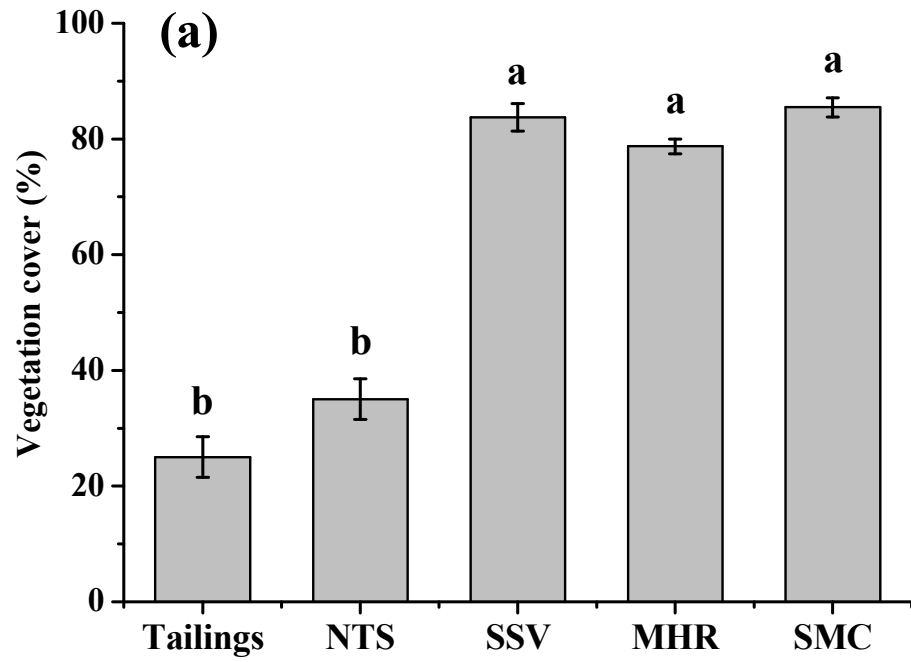
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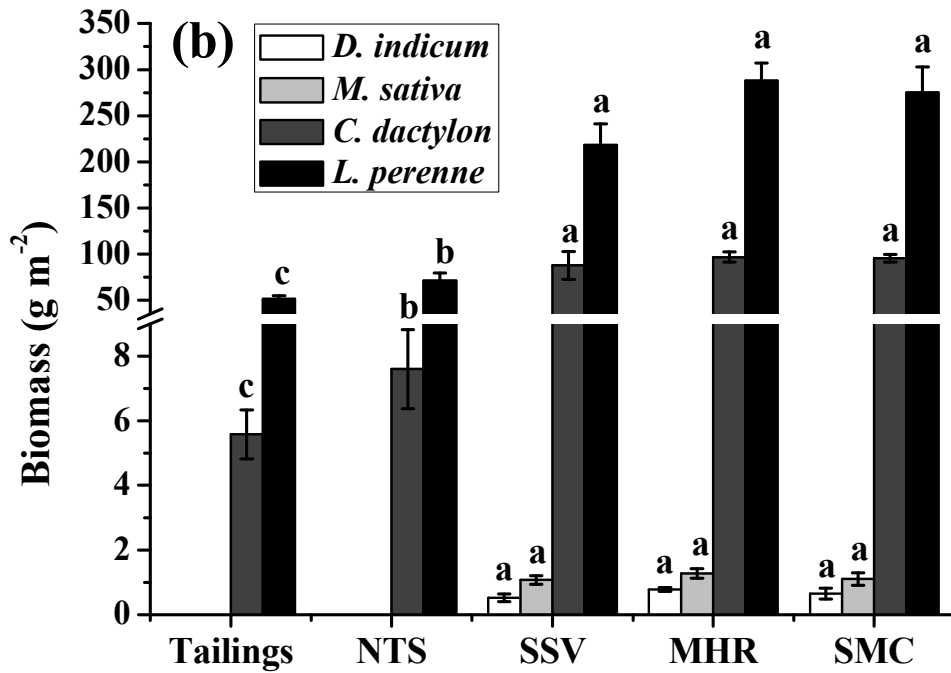
477 **Fig. 4**



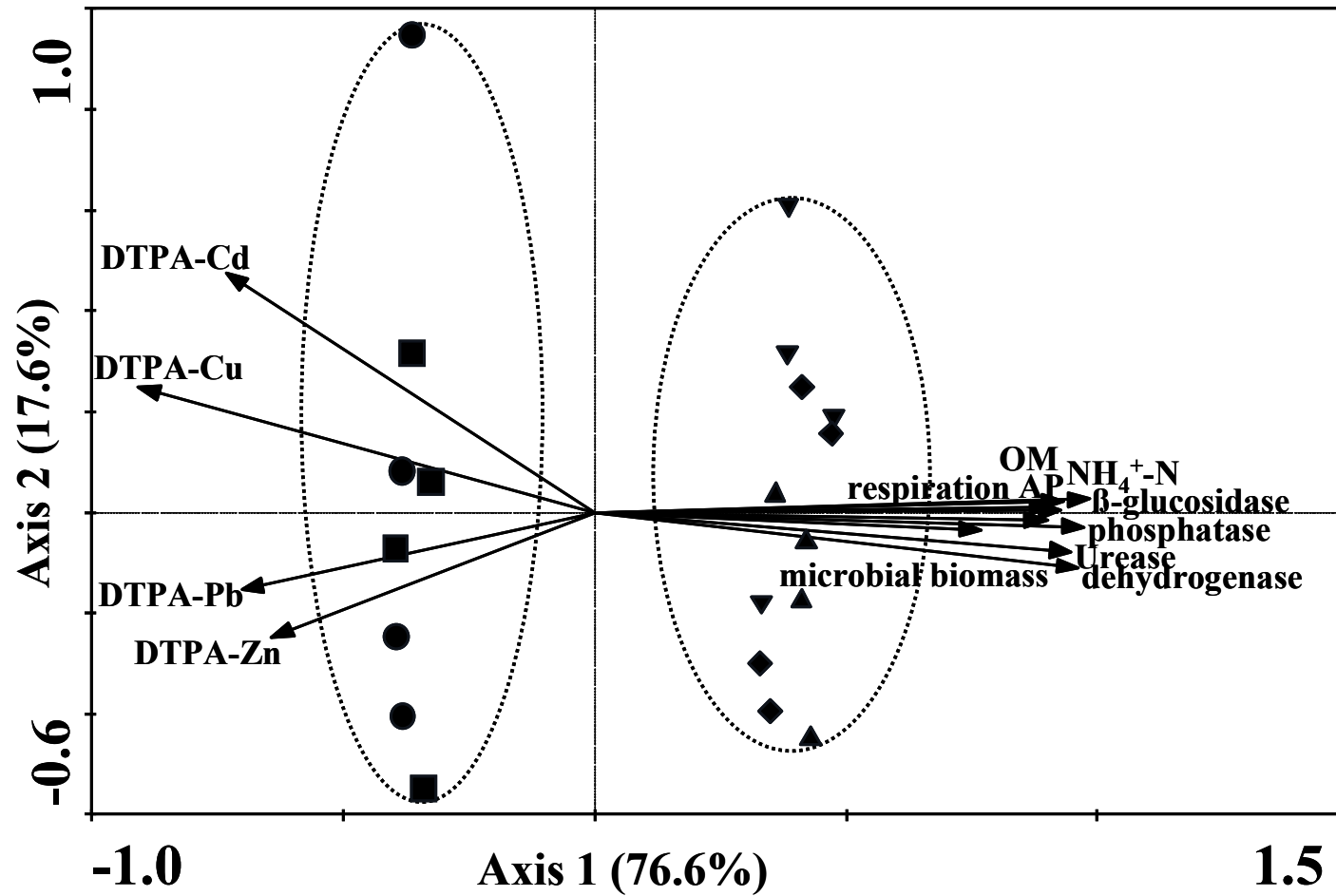
480 Fig. 5



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484 Fig. 6



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