



Use of combined tools for effectiveness evaluation of tailings rehabilitated with designed Technosol

Diego Arán · Erika S. Santos · Maria Manuela Abreu · Juan Antelo · Felipe Macías

Received: 28 February 2021 / Accepted: 27 September 2021
© The Author(s), under exclusive licence to Springer Nature B.V. 2021

Abstract Soil and water characteristics and biogeochemical processes can be improved by the application of an integrated technology based on circular economy: designed Technosol. The evaluation of the effectiveness of the superficial application of a designed Technosol, with andic and eutrophic properties, on the rehabilitation of sulfide tailings of a uranium mine (Fé mining area, Spain) was the aim of this study. After 20 months of the Technosol application, the tailing rehabilitation status (Rehabilitated

tailing) was compared to a non-rehabilitated tailing (Tailing). To assess the rehabilitation of these systems, several properties were analyzed: chemical characteristics of the materials and their leachates, soil enzymatic activities (dehydrogenase, β -glucosidase, acid phosphatase and urease), basal respiration and several plant endpoints from direct and indirect bioassays and pot experiment using *Lolium perennse* L. and *Trifolium pratense* L.. Potentially toxic concentrations of Co, Mn and Ni were identified in both available fraction and leachates, pointing out the serious environmental risk posed by the tailing. The improvement of overall physicochemical properties in the rehabilitated tailing materials (e.g., decrease of the hazardous element concentrations in leachates and available fraction, and improvement of the fertility and structure) allowed a quick plant cover with pasture species and provided a suitable habitat for active microbial community (evaluated by increasing dehydrogenase activity and basal respiration). This improvement in the rehabilitated tailing contributed to a significant decrease in the ecotoxicological risk and the spread of hazardous elements. The field application of this specific Technosol was a promising and lasting solution for rehabilitation of this type of tailings.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10653-021-01118-3>.

D. Arán · J. Antelo · F. Macías
Instituto de Investigaciones Tecnológicas, Universidad de Santiago de Compostela, Santiago de Compostela, Spain

Present Address:

D. Arán (✉)
Inproyen Consulting, C/Lugo 18, Santa Comba,
A Coruña, Spain
e-mail: diegoaran@inproyen.com

D. Arán · E. S. Santos · M. M. Abreu
Universidade de Lisboa, Instituto Superior de Agronomia,
LEAF—Linking Landscape, Environment, Agriculture
and Food—Research Center, Tapada da Ajuda,
1349-017 Lisbon, Portugal

F. Macías
Departamento de Edafología y Química Agrícola,
Facultad de Biología, Universidad de Santiago de
Compostela, Santiago de Compostela, Spain

Keywords Bioassays · Biological soil indicators · Chemical soil indicators · Environmental risk · Environmental rehabilitation · Sulfide tailings

Introduction

Abandoned and active mines have several environmental problems due to the physicochemical characteristics of mine wastes and their leachates (Santos et al., 2018; Wong, 2003). Mining areas of coal, metals and radionuclides extraction have additional problems because their mineralization can be associated with reactive solid phases such as sulfides. These minerals are easily weathered by oxidation processes generating acid mine drainage (AMD) rich in potentially hazardous elements (PHEs; e.g., As, Cu, Pb, and Zn) (Abreu et al., 2010; Johnson & Hallberg, 2005; Sánchez-España et al., 2005).

A well-designed rehabilitation strategy is necessary to decrease the environmental and human risks and recover the ecological and geochemical functions of mining soils and tailings. This would increase the water quality and promote the pedogenesis in mine wastes. Nowadays a recovery plan is already a key component of any mining and processing operation. In general, a rehabilitation strategy should include low-cost technologies based on sustainability and circular economy and, if possible, those which promote environmental services. The negative effects of the environmental problems associated with mining activity will remain, or even increase, when the selection of the rehabilitation and management practices is poor (Rivas-Pérez et al., 2016). Ecological rehabilitation of sulfide-rich mining areas is achieved by minimizing the oxidation processes through the improvement of the chemical characteristics in the mine wastes (Johnson & Hallberg, 2005). Consequently, the leaching of PHEs to surface and groundwater will be minimized.

Surface coverage of this type of mine wastes with designed Technosols (IUSS Working Group WRB, 2015), a man-made soil with more than 20% artifacts (e.g., wastes or materials from anthropic activities), is considered a promising and eco-friendly rehabilitation technology rather than amendments which need successive applications. In fact, the use of some designed Technosols reduces the generation of AMD and improves the physicochemical and biological quality of mine wastes and their leachates allowing the vegetation establishment and sustainability of ecosystem services (Arán et al., 2016; Asensio et al., 2013a, 2013b; Monterroso et al., 1998; Santos et al., 2014, 2016).

The assessment of the entire system at field scale and long term is essential to verify the effectiveness of the rehabilitation technologies applied. Although monitoring and evaluation at short term are important, the sustainability of the rehabilitation strategy in the rehabilitated area is only confirmed at medium- to long-term range, especially in sulfide-rich mining areas. In general, the soil quality is evaluated by analysis of different physicochemical, biological and biochemical characteristics (Kumar et al., 2013; Martínez-Salgado et al., 2010). However, the functions and services of the soils are the result of combined interactions among these characteristics. Hence, the single use of chemical, biological or ecotoxicological analysis is insufficient to evaluate the potential environmental risk of contaminated soils/tailings, leachates and rehabilitated materials (Santos et al., 2017a).

The interaction among PHEs, matrix and organisms and consequent effects cannot be assessed only with a physicochemical study (ISO, 17402 2006; Leitgib et al., 2007; van Gestel et al., 2001). Moreover, ecotoxicity tests by itself cannot provide an integrated evaluation of the availability and toxic effects of the PHEs on organisms and ecosystem, since some recommended species do not show sensitiveness to hazardous elements (Abreu et al., 2014; Santos et al., 2013). Thus, the main objective of the present study was to evaluate, at physicochemical, biological and ecotoxicological level and under field conditions, the effectiveness of a designed Technosol with andic and eutrophic properties on the rehabilitation of sulfide tailings.

Materials and methods

Site characterization

Fé mining area is a uranium mine located at Saelicies el Chico (29 T 702,113 4,501,299) (Salamanca–Spain). It was the most important U deposit in Spain with a reserve exceeding 16,000 Mg of U₃O₈ with a hydrothermal system origin 34.8 ± 1.6 Ma (Both et al., 1994). In this deposit, the U mineralization contains sulfides, mainly pyrite. The surface extraction and concentration of U occurred between 1975 and 2000 having been carried out by ENUSA company.

The region is under a hot-summer Mediterranean climate (CSA climate according to Köppen classification) with annual temperatures ranging between 4 and 30 °C and average annual rainfall of ≈ 503 mm, which frequently occurs only in winter months. Soil moisture and temperature regimes are xeric and mesic, respectively.

A dumping zone was built, and radioactive wastes were encapsulated under a layer of arkose material. The remaining mine wastes without radioactive risk, mainly constituted by sulfide-rich materials and host rocks, were used to recover the previous cover. Although this management practice allowed the isolation of radioactive mine wastes and minimized the radioactive risk, the environmental problems associated with the sulfide-rich materials were not solved.

Pilot assay and sampling

A pilot assay (625 m²) was implemented within the mine area at 2014 in order to rehabilitate a tailing, composed of a mixture of sulfide-rich materials and host rocks, and its leachates by a green technology solution: designed Technosol application. For this, a 20-cm-layer of a designed Technosol, derived from organic and inorganic wastes from agroforestry and industrial sectors, and with andic and eutrophic properties (confirmed by chemical analysis; data not shown) was applied over the tailing. In general, these soil properties were selected for immobilization of cations and anions, and fertility supply (macro- and micro-nutrients availability and stable organic matter) to support, at long-term, plant and microbiota development. As control was used a similar area without Technosol application (Tailing without recuperation). In both areas of the assay (with and without Technosol application), a commercial mixture of several herbaceous species was sown.

After 20 months of this application, composite samples were collected from Technosol (0–20 cm depth), rehabilitated tailing below the Technosol (20–40 cm depth) and tailing from control area (0–20 cm depth). These samples were air-dried and homogenized for physicochemical, biological and ecotoxicological analysis and to obtain simulated leachates. During the field assay, mean temperature varied between 4.5 and 24.2 °C and precipitation 8.6 and 71.2 mm.

Physicochemical and biological characterization

The materials (fraction < 2 mm of Technosol, Rehabilitated tailing and Tailing) were characterized for: particle size by sieving and sedimentation; pH in H₂O and 1 mol/L KCl (1:2.5 m:V); extractable P (Olsen method); total CNS (analyzed by combustion with a LECO analyzer), organic C (Sauerlandt method), and effective cation exchange capacity (CEC) (1 M NH₄Cl method; Peech et al., 1947). Major and trace elements in pseudototal fraction were obtained by microwave-assisted digestion with aqua regia, while for the available fraction (water soluble + exchangeable complex) was used the rhizosphere-based method (Feng et al., 2005).

The same materials (Technosol, Rehabilitated tailing and Tailing) were also used to obtain simulated leachates (DIN 38,414-S4, 1984). Following the extraction of simulated leachates, pH and electrical conductivity (EC) were measured. Simulated leachates and extracts corresponding to the pseudototal and available fractions were filtrated (0.45 μ m) and stored at -18 °C until multielemental determination. Chloride, nitrate and sulfate content was determined by ion chromatography; fluoride concentration by ion selective electrode; metals/metalloids by ICP-MS; and ammonium by continuous flow analyzer. Nitrite and phosphate were determined following Zambelli method (Rodier, 1976) and molybdenum blue method (Murphy & Riley, 1962), respectively.

Soil basal respiration and four enzymatic activities (total fraction) were evaluated as biological indicators. Dehydrogenase (Tabatabai, 1994) was used as an indicator of overall microbial activity, while β -glucosidase (EC 3.2.1.21; Eivazi & Tabatabai, 1988), acid phosphomonoesterase (acid phosphatase EC 3.1.3.2; Eivazi & Tabatabai, 1977) and urease (EC 3.5.1.5; Kandeler & Gerber, 1988) are associated with C, P and N cycling, respectively. Before the determination of enzymatic activities, the materials were incubated in a growth chamber under controlled conditions (25 ± 1 °C; 16 h light/8 h darkness) at $\approx 70\%$ water-holding soil capacity for 12 days.

Quality and metabolic capacity of the different microbial communities were determined with a respirometer with sensors of CO₂ and CH₄ under controlled conditions for 450 h. For this analysis, samples were moistened ($\approx 70\%$ of the water-holding

capacity) and pre-incubated under controlled conditions (30 ± 1 °C and in the darkness) for 24 h.

Ecotoxicological characterization

Ecotoxicological evaluation of the materials and their leachates was performed using two plant species, *Lolium perenne* L. (monocot) and *Trifolium pratense* L. (dicot). Plant species selection was based on ISO recommendations (ISO, 11269–2, 1995). These species present a rapid development under field conditions being used, frequently, in the seeds mixtures from revegetation projects.

Three bioassays were carried out under growth chamber conditions to evaluate the ecotoxicological effect of the whole materials (direct bioassay) and their leachates (indirect bioassays): filter paper test (Salvatore et al., 2008), hydroponic test (Santos et al., 2013), and soil test (ISO, 15799, 1999; Martí et al., 2007). Ecotoxicological effect of the materials for the same plant species was also evaluated through a microcosm assay (pot experiment) for a longer period under greenhouse conditions.

For filter paper test, three layers of filter paper were placed at the bottom of a petri-box, which was moistened with 5 mL of simulated leachate from each material. Twenty-five seeds of each plant species were placed per replicate ($n = 4$). In soil test, 15 g of each material (fraction < 2 mm) at 70% of the water-holding capacity was placed in glass beakers. Seven seeds of *L. perenne* and 10 seeds of *T. pratense* were used per replicate ($n = 5$). Deionized water and a sandy soil were used as negative control. Both tests were incubated in a growth chamber under controlled and moisture conditions (25 ± 1 °C; 16 h light/8 h darkness). Germinated seeds were periodically counted during 10 and 12 days for *L. perenne* and *T. pratense*, respectively. Germination criterion was the emergence of a radicle through the seed coat.

For hydroponic test, both species seeds were germinated in the dark at 25 ± 1 °C for seven days. Seedlings were measured before their use in the test. Four seedlings of each species were used per replicate ($n = 5$) to grow in beakers filled with 50 mL of simulated leachates. The seedlings were supported by a thin and flexible plastic net placed on the top of each beaker, so only the roots were immersed in the leachate. After 16 days of growth in the same conditions as before, plant endpoints were evaluated.

Ecotoxicity evaluation on plants was determined through visual aspects, germination rate, root and shoot elongation and dry biomass after exposure of the seeds and seedlings to moistened soil or leachates (OECD, 2006; Salvatore et al., 2008). Plants survival was also evaluated in hydroponic test.

Microcosm assay was carried out in pots containing 500 g of each studied material. One gram of *L. perenne* seeds (≈ 410 seeds) or 1.0 g of *T. pratense* seeds (≈ 520 seeds) was sown in each pot. All materials were incubated at 70% of water-holding capacity under greenhouse-controlled conditions for one month. At the end of the experiment, visual aspects, plant cover and dry biomass of aerial part of the plants were evaluated.

Data analysis

Statistical analysis was performed using SPSS v18.0. All data were checked for homogeneity of variance (Levene's test) and normality (Shapiro–Wilk test). If possible, one-way ANOVA and post hoc Duncan test ($p < 0.05$) were applied. When the data did not satisfy assumptions for ANOVA, non-parametric Kruskal–Wallis test was used. For statistical purposes, results below detection limit were assumed as half of the detection limit. Correlations between materials characteristics were determined by Pearson's correlation ($r > 0.8$). Principal component analysis (PCA) was applied to the data set to identify possible relations among chemical characteristics, biological and ecotoxicological parameters of the materials and/or leachates.

Quality control of the analysis was made by replicate samples ($n = 3$ – 5 depending on assay), use of certified standard solutions and reference material, and laboratory standards.

Toxicity indexes [$(\% \text{ germination}_{\text{sample}} - \% \text{ germination}_{\text{control}}) / \% \text{ germination}_{\text{control}}$ and $(\text{Root elongation}_{\text{sample}} - \text{Root elongation}_{\text{control}}) / \text{Root elongation}_{\text{control}}$; Bagur-González et al., 2011] were calculated for both plant species. These indexes were also calculated for the controls (deionized water and sandy soil). The indexes can vary from -1 (maximum phytotoxicity) to > 0 (no toxicity).

Results and discussion

Physicochemical characterization of the materials

The chemical characteristics of the tailing materials indicate a considerable environmental risk (Tables 1, 2). These materials are moderately acid, possibly due to small proportion of sulfide-rich materials, with unfavorable physical structure and very low fertility (non-detectable organic C and small concentrations of extractable P and exchangeable Ca, K and Mg). Furthermore, these tailings present high pseudototal concentrations of several PHEs (e.g., As, Mn and Ni) compared to soil guidelines from Galician where similar geological materials and formation exist (DOGA, 2009). The pH values in KCl indicated a slight tendency of the tailing materials to acidification, while CEC indicated their weak capacity to retain the elements. This low CEC is related to the small organic C concentration and clay fraction.

The Technosol, after 20 months, still presented adequate chemical characteristics to stabilize the pH and provide nutrients and organic C (and other C forms as carbonates, which represent between 10–23% of the total C content; Table 1) ensuring

biogeochemical processes involved in the rehabilitation process (e.g., immobilization of PHEs, minimization of acid leachates generation, development of microbiota and plant cover). In fact, these results show that the effect can be lasting. The Technosol showed the highest values of pH, total and organic C, total N, extractable P, CEC and concentration of exchangeable cations, when compared to the Tailing and Rehabilitated tailing. This material also presented high total concentrations of some nutrients (Ca, Cu and P) and low concentrations of PHEs (e.g., As, Co, Cr, Ni).

In general, the application of the designed Technosol with andic and eutrophic properties over the tailing materials contributed to a significant improvement of their physicochemical characteristics (Rehabilitated tailing, Tables 1, 2, 3). Similar results were obtained in other rehabilitation process with Technosols (Asensio et al., 2013b; Santos et al., 2016). Values of pH, total and organic C, extractable P and CEC increased significantly compared to those in the tailings without rehabilitation, although no differences were found for the total N content (Table 1) possibly due to its use by the organisms during 20 months. The CEC in the Rehabilitated tailing may be considered high and is correlated to the increase in organic matter

Table 1 Characteristics of the studied samples (mean ± SD; n = 4, except for particle size distribution n = 2)

	Tailing	Rehabilitated tailing	Technosol
pH (H ₂ O)	4.3 ± 0.01*	4.5 ± 0.02*	7.7 ± 0.01
pH (KCl)	3.4 ± 0.02*	4.1 ± 0.02*	7.6 ± 0.01
Total C (g/kg)	0.8 ± 0.1*	4.3 ± 0.7*	48.2 ± 1.9
Organic C (g/kg)	< DL*	3.3 ± 0.7*	40.4 ± 1.2
Total N (g/kg)	0.4 ± 0.2	0.4 ± 0.2	2.6 ± 1.0
Extractable P (mg/kg)	1.0 ± 0.1*	16.2 ± 0.1*	97.9 ± 1.2
<i>Effective cation exchange capacity (cmol_c/kg)</i>			
pH	3.5 ± 0.1*	3.7 ± 0.1*	7.3 ± 0.2
CEC*	14.2 ± 6.7*	26.1 ± 3.8*	41.0 ± 2.3
H ⁺	0.3 ± 0.1*	0.1 ± 0.1*	—
Al	1.0 ± 0.02	1.0 ± 0.1	< 0.1
Ca	6.8 ± 1.4*	20.5 ± 3.8*	34.6 ± 2.3
K	0.1 ± 0.02*	0.5 ± 0.02*	1.4 ± 0.1
Mg	5.8 ± 8.1	3.3 ± 0.1	3.8 ± 0.4
Na	0.3 ± 0.1*	0.7 ± 0.2*	1.3 ± 0.4
<i>Particle size distribution (g/kg)</i>			
Coarse sand	156.2 ± 10.8*	187.1 ± 5.4*	269.2 ± 5.0
Fine sand	357.4 ± 12.2*	396.1 ± 22.3*	384.4 ± 8.0
Silt	436.5 ± 21.7	372.1 ± 11.4	339.9 ± 14.8
Clay	50.0 ± 1.3	44.7 ± 5.8	6.4 ± 4.9

DL: detection limit of the methodology; CEC: cation exchange capacity. Values in each parameter followed by an asterisk indicate significant differences between Tailing and Rehabilitated tailing (p < 0.05)

Table 2 Pseudototal concentration of the elements (mg/kg) in the collected materials (mean ± SD; *n* = 3)

Elements	Tailing	Rehabilitated tailing	Technosol
Al	$(46.2 \pm 10.2) \times 10^3$	$(43.3 \pm 0.5) \times 10^3$	$(46.1 \pm 1.3) \times 10^3$
As	23.2 ± 1.0	26.4 ± 3.0	11.6 ± 1.9
Ca	$(1.1 \pm 0.2) \times 10^3*$	$(3.8 \pm 0.4) \times 10^3*$	$(24.7 \pm 0.5) \times 10^3$
Cd	< 0.1	< 0.1	< 0.1
Co	29.8 ± 3.5*	19.5 ± 1.9*	10.6 ± 2.8
Cr	60.5 ± 9.9	50.2 ± 3.8	28.6 ± 1.4
Cu	47.3 ± 3.1*	66.9 ± 8.5*	116.1 ± 1.5
Fe	$(63.2 \pm 2.0) \times 10^3$	$(61.4 \pm 2.0) \times 10^3$	$(25.4 \pm 1.0) \times 10^3$
K	$(4.2 \pm 2.9) \times 10^3$	$(4.6 \pm 0.1) \times 10^3$	$(2.9 \pm 0.2) \times 10^3$
Mg	$(17.8 \pm 0.3) \times 10^3*$	$(13.0 \pm 0.3) \times 10^3*$	$(6.8 \pm 1.2) \times 10^3$
Mo	< 0.5	< 0.5	2.1 ± 0.3
Mn	1349 ± 145*	620 ± 58.2*	663 ± 138
Na	499 ± 284	728 ± 125	893 ± 105
Ni	103 ± 10.5*	59.0 ± 4.3*	33.8 ± 3.6
P	392 ± 13.0*	1063 ± 7.6*	7230 ± 373
Pb	17.0 ± 2.1*	11.7 ± 0.6*	14.0 ± 1.6
S	$(3.0 \pm 0.1) \times 10^3$	$(3.2 \pm 0.3) \times 10^3$	$(3.0 \pm 0.4) \times 10^3$
Sb	< 1.0	< 1.0	< 1.0
Se	< 2.0	< 2.0	< 2.0
Zn	202 ± 9.2*	224 ± 5.3*	280 ± 77.3

Values in each parameter followed by an asterisk indicate significant differences between Tailing and Rehabilitated tailing (*p* < 0.05)

Table 3 Concentration of elements (mg/kg) in the available fraction of the collected materials (mean ± SD; *n* = 4)

Element	Tailing	Rehabilitated tailing	Technosol
Al	138 ± 1.2*	523 ± 71.6*	62.1 ± 1.1
As	< 0.1	< 0.1	0.1 ± 0.02
Ca	582 ± 27.3*	2642 ± 489*	2449 ± 89.1
Cd	$(17.3 \pm 4.9) \times 10^{-2}$	$(15.3 \pm 1.0) \times 10^{-2}$	$(0.6 \pm 0.3) \times 10^{-2}$
Co	5.3 ± 0.9*	1.2 ± 0.2*	$(13.8 \pm 0.5) \times 10^{-2}$
Cr	$(11.8 \pm 1.0) \times 10^{-2*}$	$(15.5 \pm 2.4) \times 10^{-2*}$	$(13.8 \pm 0.5) \times 10^{-2}$
Cu	0.4 ± 0.1*	2.1 ± 0.6*	0.6 ± 0.02
Fe	24.7 ± 1.1*	71.4 ± 1.9*	23.5 ± 0.9
K	18.0 ± 0.5*	209 ± 3.1*	352 ± 20.5
Mg	1215 ± 148*	578 ± 84.5*	514 ± 24.2
Mo	$< 5.0 \times 10^{-2}$	$(4.3 \pm 1.5) \times 10^{-2}$	$(28.3 \pm 1.9) \times 10^{-2}$
Mn	227 ± 63.1*	53.8 ± 8.2*	1.0 ± 0.04
Na	67.3 ± 8.5*	194 ± 20.0*	197 ± 20.9
Ni	10.7 ± 2.4*	5.1 ± 0.6*	$< 5.0 \times 10^{-2}$
Pb	$< 5.0 \times 10^{-2}$	$< 5.0 \times 10^{-2}$	$< 5.0 \times 10^{-2}$
Sb	< 1.0	< 1.0	< 1.0
Se	$< 20.0 \times 10^{-2}$	$< 20.0 \times 10^{-2}$	$< 20.0 \times 10^{-2}$
Zn	5.1 ± 0.7*	11.1 ± 1.1*	0.1 ± 0.02

Values in each parameter followed by an asterisk indicate significant differences between Tailing and Rehabilitated tailing (*p* < 0.05)

(*r* = 0.87). High CEC values favor higher immobilization of most PHEs and, consequently, decrease in the contamination spreading. The texture and structure

were also improved (compared with the Tailing) due to the increase in the sand fractions (coarse and fine) and organic matter. According to the pseudototal

concentrations of elements found in the Rehabilitated tailing (Table 2), positive effects of the Technosol application were also observed. Thus, eluviation of particles from Technosol to the tailing (located under the Technosol) contributed to the decrease of the pseudototal concentrations of Co, Mg, Mn, Ni and Pb, between 17 and 55% depending on element, while macronutrients and micronutrients, as Ca, Cu and P increased their concentration by 3.5-, 1.4- and 2.7-fold, respectively. For the remaining elements, no significant differences were observed between Tailing and Rehabilitated tailing.

The availability of PHEs in the tailing materials (Table 3) corresponds to < 2.5% of the pseudototal concentrations, except for Co, Mo, Mn and Ni. For these elements, the available concentration corresponded to 10–19% of their total concentrations. No significant differences were found between Tailing and Rehabilitated tailing for available concentration of As and Cd. Besides, the concentration of Co, Mg, Mn and Ni in the Rehabilitated tailing diminished compared to the Tailing (Table 3).

Although the pseudototal concentration of these elements in the Rehabilitated tailing also decreased, the decrease in their availability is related to different immobilization mechanisms (and not by a dilution factor). In fact, the decrease in the availability of Co, Mg and Mn is related to the increase in CEC ($r = \approx -0.8$), while in the case of Ni, it is related to the pH and organic C content ($r = -0.83$ and -0.86 , respectively).

The available concentrations of Al and Fe were higher for Rehabilitated tailing (Table 3), although the content of exchangeable Al was similar for the Tailing and Rehabilitated tailing (Table 1). This result reflects mainly: i) the eluviation of Al and Fe forms from Technosol to Rehabilitated tailing, and ii) the significant effect of plants exudates on the availability of these elements. The andic properties of the soils are mainly provided by the presence of nanosized Al and Fe oxyhydroxides (IUSS Working Group WRB, 2015). Generally, these nanosized oxyhydroxides present large specific surface area, variable surface charge, high affinity for metals/metalloids and, consequently, high adsorption capacity (Barrón & Torrent, 2013). Thus, in a leaching environment, the superficial application of a Technosol with andic properties allowed the migration of these nanosized particles to the tailing materials (under the Technosol),

leading to PHEs immobilization in the Rehabilitated materials. The high concentrations of Al and Fe extracted by low-molecular weight organic acids, analogous to the root exudates in the rhizosphere, on Rehabilitated tailing reflect not only the presence of these Fe and Al oxyhydroxides but also the effect that plant exudates may have on these particles. In fact, the dissolution of Fe and Al oxyhydroxides can be greatly increased with some organic acids (Jones et al., 1996; Wang et al., 2015). The available concentrations of Al and Fe in the Rehabilitated tailings corresponded to 1% of the pseudototal concentration, which does not constitute any environmental risk.

A significant enhancement of the availability of some nutrients, such as Ca, Cu, Fe, K, Mo and Zn, occurred in the Rehabilitated tailings due to the Technosol application (Table 3). In the case of K and Mo, as well as extractable P (Table 1), the increase in these elements availability is associated with the increase in organic matter content ($0.86 < r < 0.99$). The increase in the available fraction of the nutrients (Table 3), extractable P and organic matter content (Table 2) is considered an advantage for the improvement of biological properties in the system facilitating the establishment and development of a plant cover.

Biological characteristics of the materials

The tailing rehabilitation was also evaluated by biological indicators, which reflect soil functional diversity, changes in microbial community composition and microbial status (Kumar et al., 2013; Martinez-Salgado et al., 2010; Romero-Freire et al., 2016). The higher enzymatic activities and basal respiration found in the Technosol (Figs. 1, 2A) indicated the good performance of the overall microbial communities involved in the organic matter degradation, mineralization process, and nutrient cycling. Moreover, the accumulation curve of the CO₂ released from the Technosol presents two well-defined stages of the growth of the active aerobic microorganisms (beginning at hour 32th and 150th). This high basal respiration (similarly to dehydrogenase activity) can be associated with the organic C concentration. A large content of organic matter can supply enough substrate and energy for microbial growth and enzyme production (Romero-Freire et al., 2016). Neutral pH values and high nutrient concentration in available forms can also promote the activity

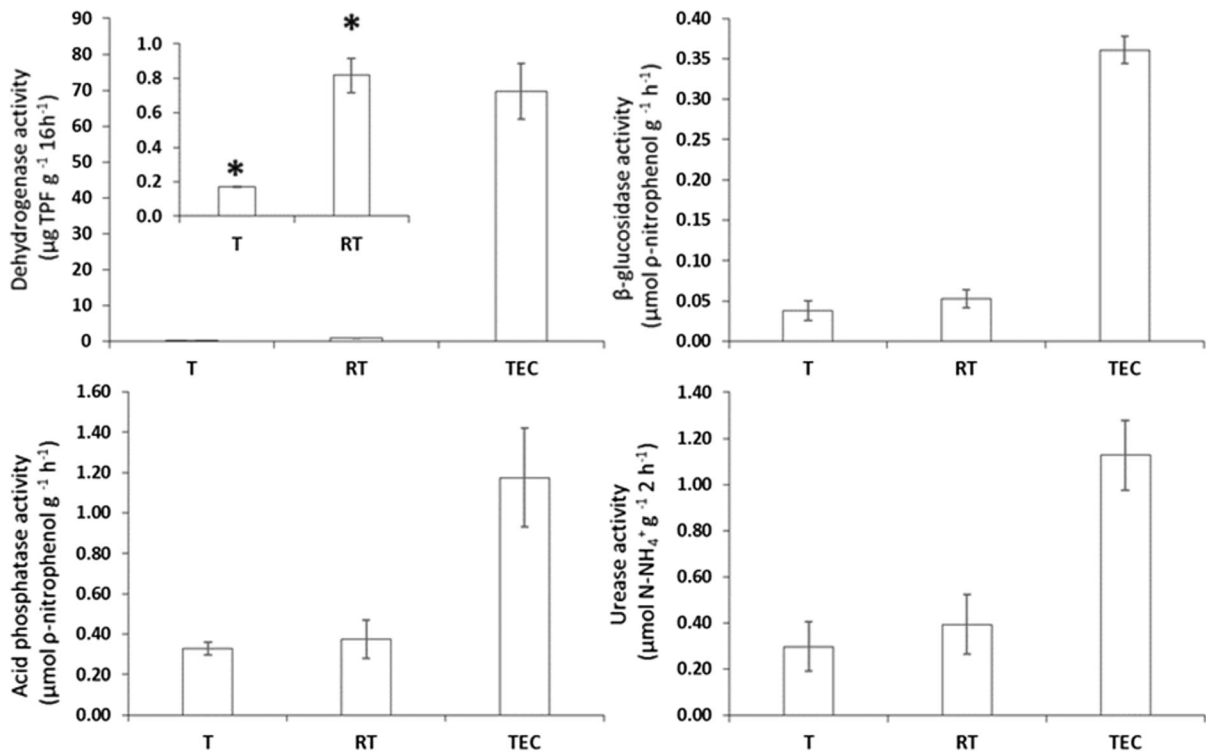


Fig. 1 Enzymatic activities (dehydrogenase, β -glucosidase, acid phosphatase and urease) in the materials from the Tailing (T), Rehabilitated tailing (RT) and Technosol (TEC) (Mean \pm

SD; $n = 3$). Values followed by an asterisk indicate significant differences between the Tailing and Recovered tailing ($p < 0.05$)

of the microbial communities in the Technosol and, consequently, a great CO_2 release (Oertel et al., 2016). Such conditions limited the proliferation of the microbial CH_4 generators (Fig. 2B).

The dehydrogenase activity and basal respiration in Rehabilitated tailing were higher than in Tailing (Figs. 1, 2A), indicating the stimulation of the overall active microbial community. However, no significant differences between Rehabilitated tailing and Tailing were found for the enzymatic β -glucosidase, acid phosphatase and urease activities. Similar results were obtained by Asensio et al. (2013a) for urease and acid phosphatase activities. According to these authors, the activity of the enzymes involved in N and P cycling increases significantly only during the first stage of the ecological rehabilitation process, when the substrate presents great availability. These enzymes have great sensitivity to changes in the physicochemical characteristics (Kumar et al., 2013; Santos et al., 2016), although the microorganism activities tend to balance if conditions in the materials are maintained.

PCA analysis (PC1 with 73.94% of the data variation; Figure S1) showed that dehydrogenase activity is positively related to the pH, organic C content and some nutrients in available fraction (e.g., Ca, Cu, Fe, Na and Zn), while it is negatively influenced by the available concentrations of Co, Mg, Mn and Ni. However, the activities of other enzymes were not justified by any studied chemical characteristics of the materials.

It is possible to assess the functionality of different microbial communities and to establish the gaseous equilibria by analyzing the amounts of CO_2 and CH_4 released by the tailing materials (Fig. 2). In the Tailing, the absence of organic matter as C source (Table 1) may lead to a minimum presence of CO_2 producers. This sample seems to have CH_4 producers (methanogenic community), which are able to use rapidly the initial CO_2 existent in box to produce CH_4 . The increase in this compound stimulates the methanotrophic community (CH_4 users), establishing the equilibrium between both microbial communities. The activity of the methanogenic community requires acid

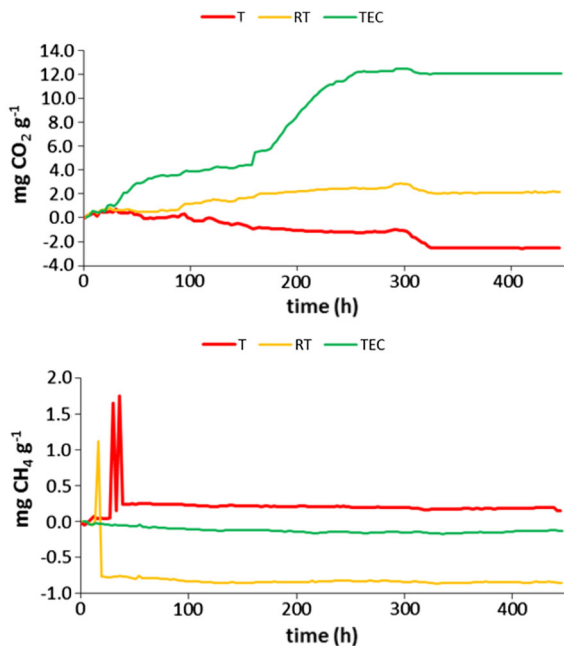


Fig. 2 Concentrations of a) CO₂ (basal respiration) and b) CH₄ released from the Tailing (T), Rehabilitated tailing (RT) and Technosols (TEC), as a consequence of microbial activity (mean \pm SD; $n = 3$)

and anaerobic conditions. The weak structure and moisture of the tailing materials favors the later conditions (Smith et al., 2003). However, these anaerobic conditions were temporary, since methanotrophic community is a CH₄ sink under aerobic conditions (Fiedler et al., 2005).

In the Rehabilitated tailing, the methanogenic and methanotrophic communities coexist until total consume of CH₄. The main microbial communities in this Rehabilitated tailing were CO₂ producers, which are associated with organic matter decomposition and mineralization processes.

Chemical characterization of the simulated leachates

In general, the element concentrations in the leachates were smaller than the corresponding concentrations in available fraction in the solid samples. Leachates from Technosol had neutral pH values, significant nutrient concentrations and small PHEs concentrations.

Leachates from Tailings presented moderately acid pH values, indicating a low sulfide reactivity of the mine wastes. Moreover, high concentrations of

sulfates and several PHEs (e.g., Al, Cd, Co, Cr, Ni, Zn) were determined, which agrees with the high EC (Table 4). The chemical composition of these leachates showed lower pH values and PHEs concentrations (> 10 fold) than runoff waters from tailings collected in field (Arán et al., 2016). In fact, under field conditions, elements leaching from wastes and soils can vary due to several factors (e.g., climatic conditions, heap design) (Chezom et al., 2013). Moreover, percolated leachates from mine wastes presented higher concentrations than simulated leachates, even for microcosm assays under greenhouse conditions (Santos et al., 2017b). The large PHEs concentrations found in leachates from the present study indicated a potential spread of toxic elements from tailings to the adjacent areas.

The leachates from Rehabilitated tailing showed significant enhancement of the chemical quality compared with the Tailing (Table 4), minimizing the potential spread of contaminants. No significant differences were observed between leachates of the Rehabilitated and non-rehabilitated tailings for pH, Cu, Mo, PO₄, Sb and Se. However, the concentrations of Ca, Fe, K and NO₃ in Rehabilitated tailing leachates increased between 2- and 41-fold depending on element (Table 4). The concentrations of F, Cl and Na were higher in Rehabilitated tailing leachates, compared to Tailing leachates. However, these concentrations are still below the legislation limits established for several water uses (BOE, 2003, 2015). Concentrations of K and NO₃ in the leachates are correlated with the organic matter ($r = 0.90$ and 0.85), but for the other elements no significant correlations were obtained.

The sulfate concentration in Rehabilitated tailing leachates showed a decrease of 24%, while a larger decrease, between 38 and 92%, was found for several PHEs (Al, Cd, Co, Cr, Mn, Ni and Zn). The pH of the system and the formation of organometallic complexes justify the decrease of Mg in the leachates ($r \approx -0.80$), whereas the increase in CEC found in the Rehabilitated tailing led to the decrease in Cd, Co, Cr, Mg, Mn, Ni and Zn concentrations in the leachates ($-0.81 < r < -0.92$). The presence of Al and Fe oxyhydroxides also contributes to the immobilization of most of these PHEs (Kumpiene et al., 2008). The formation of sulfate solid phases explains the decrease in this anion (Santos et al., 2014).

Table 4 Chemical characteristics of the simulated leachates from collected samples (mean \pm SD; $n = 4$)

	Tailing	Rehabilitated tailing	Technosol
pH	4.7 \pm 0.4	5.0 \pm 0.3	7.8 \pm 0.3
EC (mS/cm)	1.5 \pm 0.3	1.4 \pm 0.2	1.0 \pm 0.1
<i>Elements concentration (mg/kg)</i>			
Al	4.8 \pm 0.1*	0.8 \pm 0.1*	1.5 \pm 0.1
As	< 0.1	< 0.1	0.1 \pm 0.03
Ca	695 \pm 46.8*	2987 \pm 89.0*	1021 \pm 48.0
Cd	(12.7 \pm 0.6) $\times 10^{-2}$ *	(4.0 \pm 1.0) $\times 10^{-2}$ *	< 1.0 $\times 10^{-3}$
Cl	49.4 \pm 8.2*	106 \pm 6.2*	104 \pm 4.7
Co	3.9 \pm 0.1*	0.3 \pm 0.01*	0.02 \pm 0.01
Cr	(4.3 \pm 0.6) $\times 10^{-2}$ *	(2.7 \pm 0.6) $\times 10^{-2}$ *	(2.0 \pm 0.1) $\times 10^{-2}$
Cu	(14.0 \pm 4.6) $\times 10^{-2}$	(14.3 \pm 1.5) $\times 10^{-2}$	(12.7 \pm 1.2) $\times 10^{-2}$
F	1.6 \pm 0.5*	3.2 \pm 0.4*	22.5 \pm 4.1
Fe	< 1.0 $\times 10^{-2}$ *	(20.3 \pm 16.2) $\times 10^{-2}$ *	(4.0 \pm 1.0) $\times 10^{-2}$
K	35.9 \pm 15.6*	191 \pm 8.6*	302 \pm 0.6
Mg	1105 \pm 24.1*	613 \pm 11.6*	331 \pm 2.2
Mo	< 5.0 $\times 10^{-2}$	(3.7 \pm 1.2) $\times 10^{-2}$	(36.3 \pm 1.2) $\times 10^{-2}$
Mn	167 \pm 5.2*	30.1 \pm 0.7*	0.1 \pm 0.01
Na	63.9 \pm 0.6*	201 \pm 4.5*	186 \pm 2.4
Ni	7.3 \pm 0.2*	1.8 \pm 0.1*	< 0.05
NH ₄	16.3 \pm 1.4*	5.6 \pm 3.1*	7.7 \pm 2.4
NO ₂	< 0.4	< 0.4	2.7 \pm 0.1
NO ₃	17.2 \pm 6.1*	446 \pm 36.6*	879 \pm 22.1
Pb	< 0.1	< 0.1	< 0.1
PO ₄	< 0.5	0.5 \pm 0.4	1.9 \pm 2.5
SO ₄	(8.1 \pm 0.7) $\times 10^3$ *	(6.1 \pm 0.3) $\times 10^3$ *	(2.8 \pm 0.2) $\times 10^3$
Sb	(8.7 \pm 1.5) $\times 10^{-2}$	(11.7 \pm 1.5) $\times 10^{-2}$	(16.7 \pm 1.5) $\times 10^{-2}$
Se	0.2 \pm 0.1	0.3 \pm 0.1	0.2 \pm 0.02
Zn	4.0 \pm 0.2*	0.8 \pm 0.03*	0.1 \pm 0.1

EC: Electrical conductivity. Values in each parameter followed by an asterisk indicate significant differences between Tailing and Rehabilitated tailing ($p < 0.05$)

In the Rehabilitated tailing, the significant decrease in NH₄ in its leachates, along with the increase in NO₃, can be related to the promotion of nitrifying microbial community in the materials. The increase in the activity for this microbial community results in higher rates of net nitrification over ammonification.

Ecotoxicological characterization

Ecotoxicological risk of the Rehabilitated tailing and Tailing combined different matrices: assays (OECD bioassays and a microcosm test), plant species and plant endpoints, to provide a more rigorous assessment of the system and the rehabilitation process. Through bioassays using leachates (filter and hydroponic bioassays), the ecotoxicological risk of materials to

adjacent areas can be evaluated by acting as a source of contamination (van Gestel et al., 2001).

The organisms can be affected not only by the presence of PHEs in soluble forms but also by those elements associated with exchangeable complex of inorganic and organic colloids (available fraction). Hence, the bioassays using the solid matrix are essential (soil test). Ecotoxicological effects of the solid matrix on the same plant species were also evaluated through a microcosm assay for a slight longer period, which can simulate more real conditions (e.g., light intensity, relative air humidity and temperature).

The different sensitiveness depended on assay (between type, matrix, time period and growth conditions) and/or plant species (*L. perenne* and *T.*

pratense). Similar results were reported in bioassays conducted with other plant species (*Lactuca sativa* L., *Avena sativa* L. and *Zea mays* L.) to assess the ecotoxicological effect of rehabilitated materials (gossan mine wastes and contaminated soils) by Technosols and amendments (Abreu et al., 2014; Santos et al., 2013). According to the same authors, the plant response to elements depends on biological tolerance of the plant group (dicot Vs monocot) and species.

No visual signs of nutritional deficiency and phytotoxicity were observed in both species from three bioassays. In general, Technosol did not show any ecotoxicological risk since several endpoints of both species in the three bioassays were similar compared to negative control. Even plants growing in its leachates and microcosm assay reached the greatest survival and development (Figs. 3, 4 and Table 5). The only exception was observed for the *T. pratense* in the soil bioassay where the germination in Technosol was slightly lower than those in the negative control (Sand: $92 \pm 13\%$). The slight reduction of the *T. pratense* germination can be related to its sensitivity to the higher EC in the Technosol, as a result of high nutrient concentration (Tables 2 and 3). In fact, *T. pratense* presented the highest sensitivity, compared to other Leguminosae species, in its germination by the increase in the application rate of municipal waste compost, which always increases the elements content and EC in soil solution (Marchiol et al., 1999).

Similar results were obtained using the toxicity indexes RE and SG (Bagur-González et al., 2011) for both plant species growing in the Technosol leachates (filter bioassay) and materials (soil bioassay). Thus, the indexes calculated for the Technosol show low ecotoxicity, between -0.14 and -0.24 , depending on index and species, while its leachates did not represent any ecotoxicological effect since the germination and roots elongation were stimulated (indexes > 0).

For both species, the germination percentage, aerial part elongation and dry biomass weight in filter paper and soil bioassays did not show differences between Tailing and Rehabilitated tailing, and even with Technosol (Fig. 3). However, root elongation showed more sensitivity to the potential toxic effect of the materials and its leachates. Thus, the elongation of roots in the Rehabilitated tailing materials and their

leachates (except for *L. perenne* in filter paper) presented higher values than in the Tailing (Fig. 3). Moreover, the root elongation was similar between Rehabilitated tailing and Technosol.

The toxic effect, of the Tailing and Rehabilitated tailing materials and their leachates, evaluated by toxicity indexes, depended on plant species, plant endpoint (germination and root elongation) and bioassay (filter paper and soil). Leachates from Tailing and Rehabilitated tailing indicated low or no toxicity ($0 < \text{index values} < 0.42$, depending on species and endpoint). However, evaluating the solid materials, the Tailing presents moderate (SG ≈ -0.3 for both species) and very high toxicity (RE ≈ -0.8), while the Rehabilitated tailing had low or no toxicity ($-0.25 < \text{index values} < 0.16$ depending on species and endpoint).

In the hydroponic bioassay, the plant survival percentage in the Tailing and Rehabilitated tailing leachates depended on plant species. Survival rate of *T. pratense* reached 85% in both leachates, whereas for *L. perenne* in the Rehabilitated tailing was higher (100%; similar to Technosol) than in Tailing (90%).

Ecotoxicological effect of the Tailing leachates was observed in the aerial part and dry biomass of *T. pratense* but not in the root elongation (Fig. 3). The Tailing leachates reduced the aerial part elongation and consequently the dry biomass. The reduction of the root–aerial part ratio (root elongation/shoot elongation) from 1.7 in the Tailing to 1.2 in the Rehabilitated tailing suggests a positive response of *T. pratense* and more favorable growing conditions. Nevertheless, ecotoxicological effect of the Rehabilitated tailing leachates on same species, compared to the Tailing leachates, was not clear. The aerial part and root elongations were smaller compared to those found with Tailing leachates, but no significant differences were obtained for dry biomass. The lower *L. perenne* survival in the Tailing leachates can explain its higher development (biomass and aerial part and roots elongations) due to small competition among individuals. For the same reason, the root–shoot ratios (≈ 1.4) were similar between both Rehabilitated and non-rehabilitated tailings.

In the microcosm assay, the results were different to those compared to the soil bioassay and varied with plant species. In this assay, *T. pratense* showed strong sensitivity (at germination and dry biomass weight) to the Tailing conditions compared to *L. perenne*. As

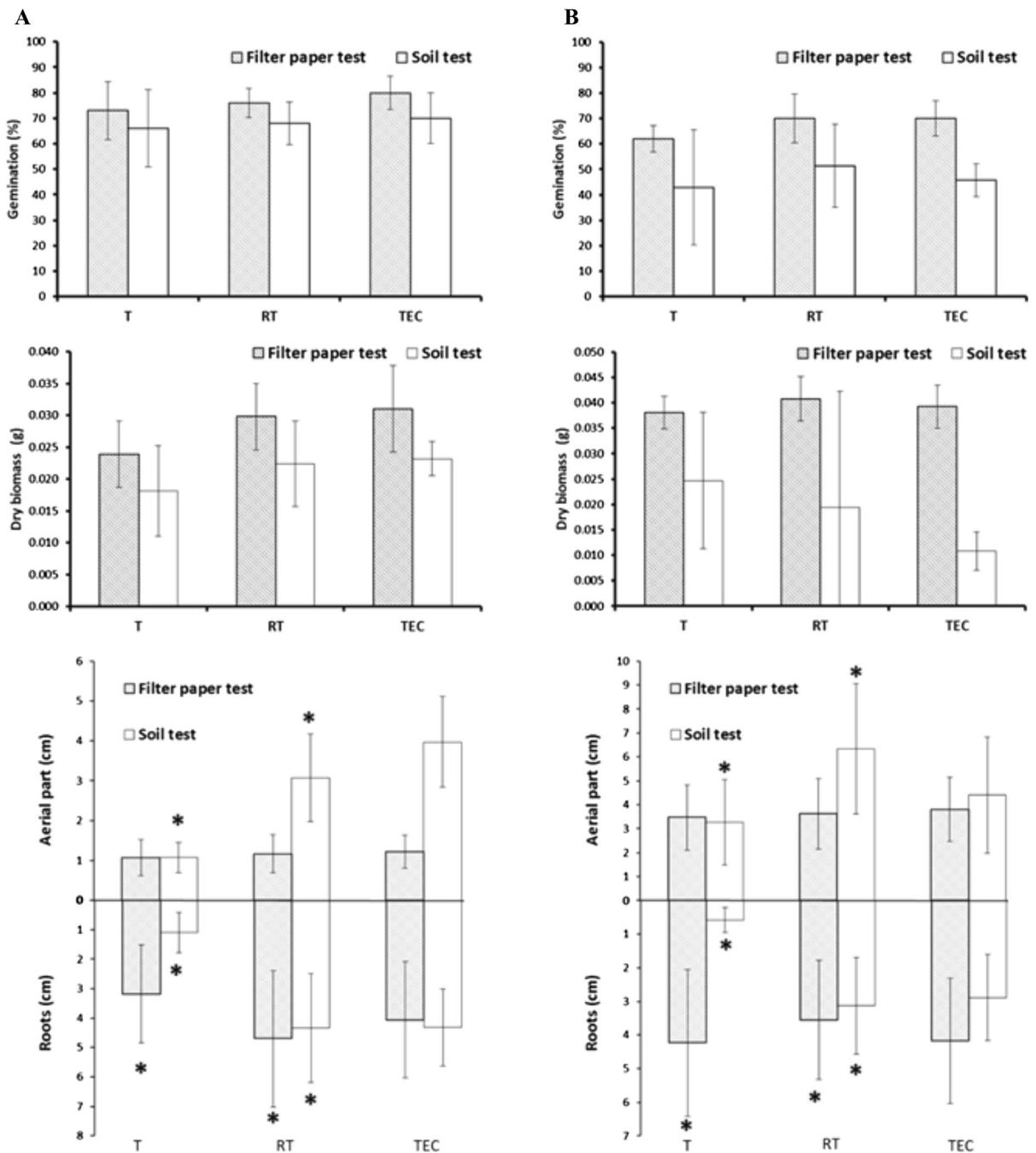


Fig. 3 Plant endpoints obtained in the filter paper and soil bioassays with *Trifolium pratense* (A) and *Lolium perenne* (B) in Tailing (T), Rehabilitated tailing (RT) and Technosol

(TEC) (mean \pm SD; $n = 4$ and 5 for each bioassay, respectively). Values followed by an asterisk indicate significant differences between Tailing and Recovered tailing ($p < 0.05$)

mentioned above, this was not clear in the soil bioassay. Plant cover and dry biomass of both species depend on the material, reaching the highest values in the Technosol. Even after 20 months of application,

the Technosol still showed adequate physicochemical characteristics, ensuring a significant germination, plant cover and biomass production. Therefore, this may lead in the future to the establishment of a pasture

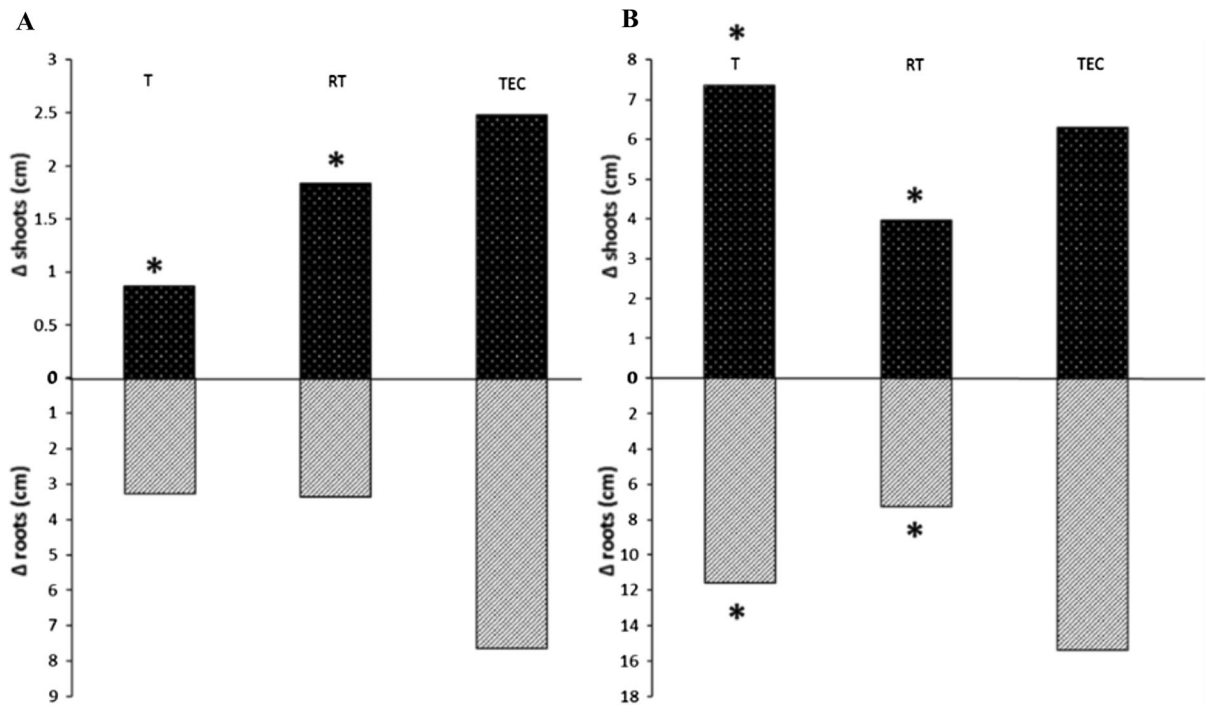


Fig. 4 Plant endpoints obtained in the hydroponic bioassay with *Trifolium pratense* and *Lolium perenne* in Tailing (T), Rehabilitated tailing (RT) and Technosol (TEC) (mean ± SD;

n = 5). Values followed by an asterisk indicate significant differences between Tailing and Recovered tailing (*p* < 0.05)

Table 5 Plant cover and dry biomass of *Lolium perenne* and *Trifolium pratense* growing in the studied samples, after one month from sowing (mean ± SD; *n* = 4)

Plant parameters	Tailing	Rehabilitated tailing	Technosol
<i>Lolium perenne</i>			
Plant cover (%)	< 1	65 ± 4.08	92 ± 4.01
Dry biomass (g/pot)	0.020 ± 0.008*	0.852 ± 0.084*	1.687 ± 0.091
<i>Trifolium pratense</i>			
Plant cover (%)	–	90 ± 9.01	100 ± 0.00
Dry biomass (g/pot)	–	1.014 ± 0.542*	2.533 ± 0.754

Values in each parameter followed by an asterisk indicate significant differences between Tailing and Rehabilitated tailing (*p* < 0.05)

in the mining site (and surroundings) providing a suitable habitat and economic land use.

In the Tailing materials, a total inhibition of *T. pratense* germination and decrease in *L. perenne* germination were found. This inevitably affects the plant cover, with significant erosion risk and spreading of contaminated particulate material in the Tailing (Table 5). Moreover, the few plants growing in this system showed visible symptoms of nutritional deficiency or phytotoxicity in the aerial part (e.g.,

chlorosis and narrow leaves; Kabata-Pendias, 2011). The combined effect of the poor structure, extreme chemical characteristics (e.g., high total and available concentrations of PHEs and low fertility; Tables 1, 2) and emergence of significant amounts of salt efflorescence explains the total inhibition of seeds germination of *T. pratense* and low plant development of *L. perenne* (evaluated by dry biomass weight) in the Tailing materials.

The germination and seedlings growth of both species were stimulated in the Rehabilitated tailing (Table 5). The improvement of the structure and water holding capacity (data not shown) can explain the higher germination of both species in this sample. These characteristics are key factors for the stimulation of the germination and guarantee the seedlings growth during the first stage of the rehabilitation process (Santos et al., 2016; Wang et al., 2008). The Rehabilitated tailing allowed a quick plant cover. In fact, after 30 days of the sowing, no significant differences were obtained for the plant cover between the Rehabilitated tailing and Technosol (Table 5). Nonetheless, the biomass produced by both plant species was smaller in the Rehabilitated tailing (Table 5). In the beginning of the plant growth, no visual signs of nutritional deficiency or phytotoxicity were observed (as for bioassays), but at the end of the experiment the leaves of both species showed a light green color (possibly due to N deficiency).

In order to determine the ecotoxicological influence of the chemical characteristics of the materials and their leachates, a PCA analysis was conducted. For all ecotoxicological assays (bioassays and microcosm assay), PC1 explained more than 66.47% of data variance and the separation of the materials was clearly observed (Figures S2–S4). For both species, the germinations from filter paper and soil bioassays, as well as dry biomasses from three bioassays, were not affected by the studied characteristics of the materials, indicating low ecotoxicological sensitivity of these plant endpoints. However, in microcosm assay the plant endpoints showed a clear ecotoxicological response related to the available concentrations of Co, Mg, Mn and Ni. The same result was obtained in the aerial part and roots elongations from soil bioassay.

In leachates, the sensitiveness to PHEs depended on plant species. According to PCA analysis, the ecotoxicological effects of the leachates (filter and hydroponic bioassays) on aerial part and roots elongations of the *T. pratense* were associated with the concentrations of Co, Mg, Mn and Ni (as observed for soil bioassay and microcosm assay) as well as the concentration of Al, Cd, SO₄, NH₄ and Zn. However, these PHEs did not lead to ecotoxicological effects on *L. perenne*. Only the concentrations of some nutrients, such as Ca, NO₃, K and Na, can limit the plant elongations of this species.

In general, the ecotoxicological effect evaluated by plant bioassays was not clear. However, the evaluation using pot experiments under greenhouse conditions showed an evident and strong ecotoxicological effect of the Tailings materials and a significant reduction of this effect in the Rehabilitated tailing. Differences between OECD bioassays and microcosm assay are related to time of contact and assay conditions.

Conclusions

The tailings present in the Fé mining area can lead to significant environmental risk to adjacent areas, due to PHE's concentrations (especially by the high concentrations of Co, Mg, Mn and Ni) in the leachates and available fraction, and the total mass of the mine wastes. Therefore, it is essential the rehabilitation of the tailings to minimize the contamination effects.

The application of a designed Technosol with andic and eutrophic properties significantly improved the physicochemical and biological characteristics of the tailing materials and their leachates. Also, the use of the Technosol decreased the ecotoxicological impact of the mine tailings. Additional advantages in the rehabilitation process were the increase in organic matter content and nutrient concentrations in available forms and leachates as well as stimulation of the microbial activity (evaluated by dehydrogenase activity and basal respiration). After 20 months of application over the tailing, the Technosol still presents efficient characteristics to continue the tailing rehabilitation process and ensure the ecological functions and a stable vegetation cover. Moreover, no ecotoxicological effects were observed. Microcosm assay results evidenced the effectiveness of the Technosol and its potential for pasture revegetation. Thus, it is essential to assess the PHEs concentration in these plants in order to evaluate the potential risk to domestic animals.

Acknowledgements The authors thank: ENUSA for technical cooperation and for providing access to the study area and field samples; Carmen Pérez and David Romero for technical support; Fundação para a Ciência e a Tecnologia for financial support of Linking Landscape, Environment, Agriculture and Food Research Centre (UID/AGR/04129/2020); and Xunta de Galicia for financial support of Group of Excellence GI-1245, AMBIOSOL (GRC2014/003). The authors from the USC belong to the CRETUS Strategic Partnership (AGRUP2015/02), co-funded by FEDER.

References

Abreu, M. M., Batista, M. J., Magalhães, M. C. F., & Matos, J. X. (2010). Acid mine drainage in the Portuguese Iberian Pyrite Belt. In C. R. Brock (Ed.), *Mine drainage and related problems* (pp. 71–118). Nova Science Publishers Inc.

Abreu, M. M., Lopes, J., Santos, E. S., & Magalhães, M. C. F. (2014). Ecotoxicity evaluation of an amended soil contaminated with uranium and radium using sensitive plants. *Journal of Geochemical Exploration*, *142*, 112–121. <https://doi.org/10.1016/j.gexplo.2014.01.029>

Arán, D., Antelo, J., Macías, F., 2016. Uso de Tecnosuelos para la mejora en la calidad química de aguas de escorrentía de la mina Fé (Cuidad Rodrigo, Salamanca), in: Sociedade Portuguesa de Ciência do Solo (Ed.), Livro de Actas do VII Congresso Ibérico das Ciências do Solo/VI Congresso Nacional de Rega e Drenagem, pp. 337–340.

Asensio, V., Covelo, E. F., & Kandeler, E. (2013a). Soil management of copper mine tailing soils — Sludge amendment and tree vegetation could improve biological soil quality. *Science of the Total Environment*, *456–457*, 82–90. <https://doi.org/10.1016/j.scitotenv.2013.03.061>

Asensio, V., Vega, F. A., Andrade, M. L., & Covelo, E. F. (2013b). Technosols made of wastes to improve physico-chemical characteristics of a copper mine soil. *Pedosphere*, *23*, 1–9. [https://doi.org/10.1016/S1002-0160\(12\)60074-5](https://doi.org/10.1016/S1002-0160(12)60074-5)

Bagur-González, M. G., Estepa-Molina, C., Martín-Peinado, F., & Morales-Ruano, S. (2011). Toxicity assessment using *Lactuca sativa* L. bioassay of the metal(loid)s As, Cu, Mn, Pb and Zn in soluble-in-water saturated soil extracts from an abandoned mining site. *J. Soil. Sediment.*, *11*, 281–289. <https://doi.org/10.1007/s11368-010-0285-4>

Barrón, V., & Torrent, J. (2013). Iron, manganese and aluminium oxides and oxyhydroxides. *EMU Notes in Mineral.*, *14*, 297–336. <https://doi.org/10.1180/EMU-notes.14.9>

BOE. (2003). Real Decreto 140/2003, de 7 de febrero, por el que se establecen los criterios sanitarios de la calidad del agua de consumo humano. *Boletín Oficial Del Estado*, *45*, 7228–7245.

BOE, 2015. Real Decreto 817/2015, de 11 de septiembre, por el que se establecen los criterios de seguimiento y evaluación del estado de las aguas superficiales y las normas de calidad ambiental. *Boletín Oficial del Estado* 219, 80582–80677.

Both, R. A., Arribas, A., & de Saint-Andre, B. (1994). The origin of breccia-hosted uranium deposits in carbonaceous metasediments of the Iberian Peninsula; U-Pb geochronology and stable isotope studies of the Fe Deposit, Salamanca Province Spain. *Economic Geology Bulletin Society*, *89*, 584–601. <https://doi.org/10.2113/gsecongeo.89.3.584>

Chezom, D., Chimi, K., Choden, S., Wangmo, T., & Gupta, S. K. (2013). Comparative study of different leaching procedures. *International Journal of Engineering Research*, *1*, 1–5.

DIN 38414-S4, 1984. Schlamm und Sedimente, Bestimmung der Eluierbarkeit mit Wasser. DIN Deutsches Institut für Normung, Berlin.

Eivazi, F., & Tabatabai, M. A. (1977). Phosphatases in soils. *Soil Biology & Biochemistry*, *9*, 167–172. [https://doi.org/10.1016/0038-0717\(77\)90070-0](https://doi.org/10.1016/0038-0717(77)90070-0)

Eivazi, F., & Tabatabai, M. A. (1988). Glucosidases and galactosidases in soils. *Soil Biology & Biochemistry*, *20*, 601–606. [https://doi.org/10.1016/0038-0717\(88\)90141-1](https://doi.org/10.1016/0038-0717(88)90141-1)

Feng, M., Shan, X., Zhang, S., & Wen, B. A. (2005). A comparison of rizosphere-based method with DTPA, EDTA, CaCl₂ and NaNO₃ extraction methods for prediction of bioavailability of metals in soil to barley. *Environmental Pollution*, *137*, 231–240. <https://doi.org/10.1016/j.envpol.2005.02.003>

Fiedler, S., Höll, B. S., & Jungkunst, H. F. (2005). Methane budget of a black forest spruce ecosystem considering soil pattern. *Biogeochem.*, *76*, 1–20. <https://doi.org/10.1007/s10533-005-5551-y>

ISO 11269–2. (1995). Soil quality: Determination of the effects of pollutants on soil flora. Part 2. Effects of chemicals on the emergence and growth of higher plants. International Organization for Standardization, Switzerland.

ISO 15799 (1999). Soil quality: Guidance on the ecotoxicological characterization of soils and soil materials. Annex A.1.2.2 Determination of the effects of pollutants on soil flora - part 2: effects of chemicals on the emergence and growth of higher plants. International Organisation for Standardisation, Switzerland.

ISO 17402 (2006). Soil Quality: Guidance for the selection and application of methods for the assessment of bioavailability in soil and soil materials. International Organisation for Standardisation, Switzerland.

IUSS Working Group WRB (2015). World reference base for soil resources 2014: International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports No. 106. FAO, Rome.

Johnson, D. B. & Hallberg, K. B. (2005). Acid mine drainage remediation options: a review. *Science of The Total Environment*, *338*(1-2), 3–14. <https://doi.org/10.1016/j.scitotenv.2004.09.002>

Jones, D. L., Darrah, P. R., & Kochian, L. V. (1996). Critical-evaluation of organic-acid mediated iron dissolution in the rhizosphere and its potential role in root iron uptake. *Plant and Soil*, *180*, 57–66. <https://doi.org/10.1007/BF00015411>

Kabata-Pendias, A. (2011). *Trace Elements in Soils and Plants*. CRC Press.

Kandeler, E., & Gerber, H. (1988). Short-term assay of soil urease activity using colorimetric determination of ammonium. *Biology and fertility of Soils*, *6*, 68–72. <https://doi.org/10.1007/BF00257924>

Kumar, S., Chaudhuri, S., & Maiti, S. K. (2013). Soil dehydrogenase enzyme activity in natural and mine soil: a review. *Middle-East Journal of Scientific Research*, *13*, 898–906. <https://doi.org/10.5829/idosi.mejsr.2013.13.7.2801>

Kumpiene, J., Lagerkvist, A., & Maurice, C. (2008). Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments — a review. *Waste Management*, *28*, 215–225. <https://doi.org/10.1016/j.wasman.2006.12.012>

Leitgib, L., Kálmán, J., & Gruiz, K. (2007). Comparison of bioassays by testing whole soil and their water extract from contaminated sites. *Chemosphere*, *66*, 428–434. <https://doi.org/10.1016/j.chemosphere.2006.06.024>

- Marchiol, L., Mondini, C., Leita, L., & Zerbi, G. (1999). Effects of Municipal waste leachate on seed germination in soil-compost mixtures. *Restoration Ecology*, *7*, 155–161. <https://doi.org/10.1046/j.1526-100X.1999.72007.x>
- Martí, E., Sierra, J., Sánchez, M., Cruañas, R., & Garau, M. A. (2007). Ecotoxicological tests assessment of soils polluted by chromium (VI) or pentachlorophenol. *Science of the Total Environment*, *378*, 53–57. <https://doi.org/10.1016/j.scitotenv.2007.01.012>
- Martínez-Salgado, M.M., Gutiérrez-Romero, V., Janssens, M., Ortega-Blu, R., 2010. Biological soil quality indicators: a review, in: Mendez-Vilas, A. (Ed.), *Current research, technology and education topics in applied microbiology and microbial biotechnology*, pp. 319–328.
- Monterroso, C., Macías, F., Gil Bueno, A., & Val Caballero, C. (1998). Evaluation of the land reclamation project at the As Pontes Mine (NW Spain) in relation to the suitability of the soil for plant growth. *Land Degradation & Development*, *9*, 441–451. [https://doi.org/10.1002/\(SICI\)1099-145X\(199809/10\)9:5%3c441::AID-LDR299%3e3.0.CO;2-U](https://doi.org/10.1002/(SICI)1099-145X(199809/10)9:5%3c441::AID-LDR299%3e3.0.CO;2-U)
- Murphy, J., & Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, *27*, 31–36. [https://doi.org/10.1016/S0003-2670\(00\)88444-5](https://doi.org/10.1016/S0003-2670(00)88444-5)
- OECD 208 (2006). OECD guidelines for the testing of chemicals/Section 2: effects on biotic systems, test No. 208: terrestrial plant test: seedling emergence and seedling growth test. Organization for Economic Co-operation and Development, Paris.
- Oertel, C., Matschullat, J., Zurba, K., Zimmermann, F., & Erasmí, S. (2016). Greenhouse gas emissions from soils: A review. *Chemie Der Erde*, *76*, 327–352. <https://doi.org/10.1016/j.chemer.2016.04.002>
- Peech, M., Alexander, L.T., Dean, L.A., Reed, J.F., 1947. Methods of soil analysis for soil fertility investigations. USDA 575. U.S.Gov. Print. Office, Washington.
- Rivas-Pérez, I. M., Fernández-Sanjurjo, M. J., Núñez-Delgado, A., Monterroso, C., Macías, F., & Álvarez-Rodríguez, E. (2016). Evolution of chemical characteristics of Technosols in an afforested coal mine dump over a 20-year period. *Land Degradation & Development*, *27*, 1640–1649. <https://doi.org/10.1002/ldr.2472>
- Rodier, J., 1976. L'analyse de L'eau: Eaux Naturelles, Eaux Résiduaires, Eau de Marée. T. II. Bordas, France.
- Romero-Freire, A., Sierra Aragón, M., Martínez Garzón, F. J., & Peinado, F. J. M. (2016). Is soil basal respiration a good indicator of soil pollution? *Geoderma*, *263*, 132–139. <https://doi.org/10.1016/j.geoderma.2015.09.006>
- Salvatore, M. D., Carafa, A. M., & Carratù, G. (2008). Assessment of heavy metals phytotoxicity using seed germination and root elongation tests: A comparison of two growth substrates. *Chemosphere*, *73*, 1461–1464. <https://doi.org/10.1016/j.chemosphere.2008.07.061>
- Sánchez-España, J., Pamo, E. L., Santofimia, E., Aduvire, O., Reyes, J., & Baretino, D. (2005). Acid mine drainage in the Iberian Pyrite Belt (Odiel river watershed, Huelva, SW Spain): Geochemistry, mineralogy and environmental implications. *Applied Geochemistry*, *20*, 1320–1356. <https://doi.org/10.1016/j.apgeochem.2005.01.011>
- Santos, E., & S., Abreu, M.M., de Varennes, A., Macías, F., Leitão, S., Cerejeira, M.J., (2013). Evaluation of chemical parameters and ecotoxicity of a soil developed on gossan following application of polyacrylates and growth of *Spergularia purpurea*. *Science of the Total Environment*, *461–462*, 360–370. <https://doi.org/10.1016/j.scitotenv.2013.05.003>
- Santos, E. S., Magalhães, M. C. F., Abreu, M. M., & Macías, F. (2014). Effects of organic/inorganic amendments on trace elements dispersion by leachates from sulfide containing tailings of the São Domingos mine Portugal. *Time Evaluation Geoderma*, *226–227*, 188–203. <https://doi.org/10.1016/j.geoderma.2014.02.004>
- Santos, E. S., Abreu, M. M., Macías, F., & de Varennes, A. (2016). Chemical quality of leachates and enzymatic activities in Technosols with gossan and sulfide wastes from the São Domingos mine. *Journal of Soils and Sediments*, *16*, 1366–1382. <https://doi.org/10.1007/s11368-015-1068-8>
- Santos, E. S., Abreu, M. M., & Magalhães, M. C. F. (2017a). Hazard assessment of soils and spoils from the Portuguese Iberian Pyrite Belt mining areas and their potential reclamation. In J. Bech, C. Bini, & M. Pashkevich (Eds.), *Assessment, restoration and reclamation of mining influenced soils* (pp. 63–88). Elsevier.
- Santos, E. S., Abreu, M. M., Macías, F., & Magalhães, M. C. F. (2017b). Potential environmental impact of Technosols composed of gossan and sulfide-rich wastes from São Domingos mine: Assay of simulated leaching. *Journal of Soils and Sediments*, *17*, 1369–1383. <https://doi.org/10.1007/s11368-016-1518-y>
- Santos, E.S., Arán, D., Abreu, M.M., & de Varennes, A. (2018). Engineered soils using amendments for in situ rehabilitation of mine lands. In: Prasad, M.N.V., Favas, P.J.C., & Maiti, S.K. (Eds.), *Bio-geotechnologies for mine site rehabilitation*. Elsevier, 131–146. <https://doi.org/10.1016/B978-0-12-812986-9.00008-7>
- Smith, K. A., Ball, T., Conen, F., Dobbie, K. E., Massheder, J., & Rey, A. (2003). Exchange of greenhouse gases between soil and atmosphere: Interactions of soil physical factors and biological process. *European Journal of Soil Science*, *54*, 779–791. <https://doi.org/10.1046/j.1351-0754.2003.0567.x>
- Tabatabai, M.A. (1994). Soil enzymes. In Mickelson, S.H., & Bigham, J.M. (Eds.), *Methods of soil analysis, part 2: microbiological and biochemical properties. Soil science society of America book series 5*. Soil Science Society of America, USA, pp. 775–833.
- van Gestel, C. A. M., van der Waarde, J. J., Derksen, J. G. M., van der Hoek, E. E., Veul, M. F. X. W., Bouwens, S., Rusch, B., Kronenburg, R., & Stokman, G. N. M. (2001). The use of acute and chronic bioassays to determine the ecological risk and bioremediation efficiency of oil-polluted soils. *Environmental Toxicology and Chemistry*, *20*, 1438–1449. <https://doi.org/10.1002/etc.5620200705>
- Wang, X., Liu, Y., Zeng, G., Chai, L., Xiao, X., Song, X., & Min, Z. (2008). Pedological characteristics of Mn mine tailings and metal accumulation by native plants. *Chemosphere*, *72*, 1260–1266. <https://doi.org/10.1016/j.chemosphere.2008.05.001>

Wang, Z., Schenkeveld, W. D. C., Kraemer, S. M., & Giammar, D. E. (2015). Synergistic effect of reductive and ligand-promoted dissolution of goethite. *Environmental Science and Technology*, *49*, 7236–7244. <https://doi.org/10.1021/acs.est.5b01191>

Wong, M. H. (2003). Ecological restoration of mine degraded soils with emphasis on metal contaminated soils.

Chemosphere, *50*, 775–780. [https://doi.org/10.1016/S0045-6535\(02\)00232-1](https://doi.org/10.1016/S0045-6535(02)00232-1)

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.