Use of wastes from the pulp and paper industry for the remediation of soils degraded by mining activities: Chemical, biochemical and ecotoxicological effects

Paula Alvarenga, Débora Rodrigues, Clarisse Mourinha, Patrícia Palma, Amarilis de Varennes, Nuno Cruz, Luís A.C. Tarelho, Sónia Rodrigues

PII: S0048-9697(19)32598-7
DOI: https://doi.org/10.1016/j.scitotenv.2019.06.038
Reference: STOTEN 32692

To appear in: *Science of the Total Environment*

Received date: 22 October 2018
Revised date: 30 May 2019
Accepted date: 3 June 2019

Please cite this article as: P. Alvarenga, D. Rodrigues, C. Mourinha, et al., Use of wastes from the pulp and paper industry for the remediation of soils degraded by mining activities: Chemical, biochemical and ecotoxicological effects, Science of the Total Environment, https://doi.org/10.1016/j.scitotenv.2019.06.038

Use of wastes from the pulp and paper industry for the remediation of soils degraded by mining activities: Chemical, biochemical and ecotoxicological effects

Paula Alvarenga[1], Débora Rodrigues[2], Clarisse Mourinha[2], Patrícia Palma[2,3,*], Amarilis de Varennes[1], Nuno Cruz[4], Luís A.C. Tarelho[5], Sónia Rodrigues[4]

[1]Linking Landscape, Environment, Agriculture, and Food Research Unit (LEAF), Instituto Superior de Agronomia, University of Lisbon, Tapada da Ajuda 1349-017, Lisboa, Portugal

[2]Department of Applied Sciences and Technologies, Polytechnic Institute of Beja, Rua Pedro Soares S/N, Apartado 6155, 7800-295 Beja, Portugal

[3]ICT, Institute of Earth Sciences, University of Évora, Rua Romão Ramalho 59, Évora, Portugal

[4]CESAM & Chemistry Department, University of Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal

[5] CESAM & Department of Environment and Planning, University of Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal

*Corresponding author:

Patrícia Palma
Escola Superior Agrária - Instituto Politécnico de Beja
Rua Pedro Soares S/N, Apartado 6155
7800-295 Beja, Portugal
Phone: +351 284 314 300 (ext. 01067)
Fax: +351 284 388 207
E-mail address: ppalma@ipbeja.pt

Abstract

Fly ash (FA) from biomass combustion and biological sludge (S), both wastes from the pulp and paper industry, were granulated in different proportions (90% FA + 10% S, and 70% FA + 30% S w/w, dry weight basis: dw) and used to recover the functionality of soils affected by mining activities (Aljustrel, Iberian Pyrite Belt), with and without the application of municipal solid waste compost (MSWC). Application doses of both mixtures were 2.5, 5.0 and 10% (w/w, dw). These materials corrected soil acidity to circumneutral values and increased extractable P and K concentrations. A significant increase in soil organic matter (from 0.6 to 0.8 - 1.5% w/w, dw) and N content (from 0.04 to 0.09 - 0.12% w/w, dw) was also observed, but only when MSWC was applied. The soil was already heavily contaminated with Cu, Pb and Zn and the application of amendments did not increase their pseudo-total concentrations. The $CaCl_2$ extractable fractions of both Cu and Zn decreased to very low values. The improvement in soil quality, compared to fertilizer only treatment, was further evidenced by the increase in some soil enzymatic activities (dehydrogenase, β-glucosidase and cellulase), with a better response for the granules with the higher proportion of biological sludge, as well as by the decrease in the soil-water extract toxicity towards different organisms (*Daphnia magna*, *Thamnocephalus platyurus*, and *Pseudokirchneriella subcapitata*). *Agrostis tenuis* germinated and grew during the first month only in the amended pots, but, after that, a considerable phytotoxic effect was evident. This was mainly attributed to salt stress or to some specific ionic toxicity. In conclusion, to establish a long-term plant cover in mining soils amended with biomass ash-based materials, the selection of plants with higher resistance to salinity and/ or the stabilization of the amendments, to reduce their soluble salt content, is recommended.

Keywords: Biomass ash; biological sludge; mine contaminated soil; soil enzymatic activities; soil-water extract ecotoxicity; phytotoxicity.

1. Introduction

Soils affected by mine exploration have usually low pH, poor nutritional conditions, high concentrations of potentially toxic trace elements (PTEs) and scarce vegetation (Matos and Martins, 2006; Abreu et al., 2010; Silva et al., 2009; Alvarenga et al., 2012; 2103). To restore these derelict environments, it is important to consider gentle-remediation options, because they tend to have a low-cost and low-impact, and are considered environmentally-sustainable technologies that can be applied to large areas. One possible approach is i*n situ* chemical immobilization, which uses soil amendments, organic and/or inorganic, to restrain contaminants via adsorption and/or precipitation reactions (Adriano et al., 2004). The benefits of using amendments are important, not only regarding PTEs immobilization, but also because they can add essential nutrients for plant growth, increase soil organic matter content, correct its acidity and influence their physical properties (Alvarenga et al., 2008a; 2009a; 2014; Peña et al., 2015). All these factors may improve soil water retention and habitat functions, enhancing its capacity to bear a plant cover and to sustain microbial activity, which will, ultimately, contribute to its full recovery as a natural ecosystem (Pardo et al., 2011; Pérez-de-Mora et al., 2011; Galende et al., 2014; Peña et al., 2015).

The European Waste Framework Directive (2008/98/EC; European Commission, 2008), considers that landfill waste disposal should be the last option to be considered in waste management, and that the integration of certain wastes in the productive system must be promoted. Another important goal to achieve a sustainable growth in European Union (EU) by 2020 is that 20% of energy consumption must come from renewable sources (European Commission, 2010). When combined, these EU directives and strategies will lead to an increment in the combustion of biomass in the EU in the near future, with a concomitant increase in the production of biomass ashes, for which landfill disposal should be avoided (Demeyer et al., 2001; Modolo et al., 2015; Tarelho et al., 2015; Pesonen et al., 2016).

Biomass ashes may be used for geotechnical purposes (Ribbing, 2007; Modolo et al., 2015; Cruz et al., 2017), applications which will only use a small part of the quantity of ash that is produced. On the contrary, high quantities of ash could be recycled as soil ameliorant or fertilizer in agriculture and forestry (Demeyer et al., 2001; Saarsalmi et al., 2012; Cruz et al., 2017). In fact, biomass ash application to soil is considered a sustainable way to recycle exported nutrient elements such as K, P, Mg and Ca, saving non-renewable sources of minerals (Demeyer et al., 2001; Saarsalmi et al., 2012; Pesonen, et al., 2016; Cruz et al., 2017). Ashes from biomass combustion are very alkaline (pH ranging from 9 to 13) and can be used as a liming agent to increase the pH and the buffering capacity of acid soils (Demeyer et al., 2001; Ram and Masto, 2014), thus avoiding the use of conventional agricultural lime (Basu et al., 2009). In addition, these ashes can be involved in carbon sequestration processes (Pandey and Singh, 2010; Ukwattage et al., 2013).

The processing of the biomass ash prior to its land application (*e.g.*, granulation), needs to be evaluated, not only because high initial pH values can compromise its direct application to soil, but also because the application of fine powdered ash to soil may lead to phytotoxic effects (Nieminen et al., 2005; Brännvall et al., 2015; Pesonen et al., 2016). Pre-treatment processes might turn the biomass ash into a product with lower pH and lower reactivity compared to the initial ash material, with the possibility of easier handling and application to soils, preventing dust problems during transportation and application (Brännvall et al., 2015). Granules can be prepared with ash only, by rolling a mixture of ashes and water in a drum (Nieminen et al., 2005), or take advantage of the adhesive properties and the water content of organic waste materials to improve the granulation process (Brännvall et al., 2015, Pesonen et al., 2016). The use of organic waste materials could also be important to supply organic C to soil and to overcome N, P and K deficiencies of the biomass ash (Saarsalmi et al., 2012; Brännvall et al., 2014; Pesonen, et al., 2016; Nurmesniemi et al., 2012). The co-granulation of these materials facilitates their use as fertilizers. In the case of pulp and paper industry, one option is the use of the biological sludge produced in the wastewater treatment plant. This sludge, referred to as biologic or cellulosic sludge, has a high organic matter and N content, which may compensate for their low content in the biomass ash. The valorization of this industrial waste would be an important alternative to its landfill disposal.

The composition of biomass ash depends primarily on the raw biomass used as fuel and on the combustion technology (Girón et al., 2013; Freire et al., 2015; Cruz et al., 2017). Nevertheless, bottom ashes (BA) are predominantly composed by silicate minerals, while FA are mainly composed by Ca minerals and, because of that, most of the inorganic nutrients and PTEs are also found in the FA (Dahl et al., 2009). In Portugal, due to the lack of a regulatory framework guiding the re-use or recycling of these materials, biomass ashes are considered a solid waste, according to the European List of Wastes (Commission Decision 2000/532/EC) and are mostly disposed of in landfills (Freire et al., 2015; Cruz et al., 2017). However, biomass ashes produced in Portugal have very low PTEs contents, below the usual concentrations found in other countries (Dahl et al., 2009; Nurmesniemi et al., 2012; Freire el al., 2015; Cruz et al., 2017) and low concentrations of persistent organic pollutants, like polycyclic aromatic hydrocarbons (PAH) or polychorinated dibenzodioxines and polychorinated dibenzofuranes (PCDD/F), below the national limit levels for sewage sludge application to agricultural soils (Decree-Law No. 276/2009) or the guideline values for ash in north European countries (Freire et al., 2015).

Rodríguez-Jordá et al. (2010b) reported the ability of biomass ashes to diminish the leachability of metals like Pb, Zn and Ni, as well as of some metalloids, like As and Se in contaminated acid soils (Rodríguez-Jordá et al., 2010a). Ribbing (2007) reported results from a Swedish study, where mine tailings were simply covered by a mixture of ash and digested sludge, this reducing metal leaching through the surface into the mound and acidity, this allowing the vegetation to grow on the surface of the mine tailings. Liu et al. (2017) were able to phytostabilize an As, Cd and Pb contaminated soil using giant reed and a combination of an organic complex fertilizer and FA, diminishing the availability of those elements and increasing the activity of some soil enzymes. Garcia-Sanchéz et al. (2015) studied the application of a digestate and FA to a metal (Pb-Ag-Zn) polluted soil and found that both amendments were effective in metal immobilization and that the application of FA had a positive effect on soil microbial biomass (Garcia-Sánchez et al., 2015).

In this context, the aim of this study was to develop new knowledge describing the capacity of biomass ash and biological sludge, both wastes from the pulp and paper industry, granulated in different proportions, to improve the quality of a soil affected by mining activities from Aljustrel mine in the Iberian Pyrite Belt. To the best of our knowledge, it is the first study that evaluates the co-granulation of these materials and their ability to amend acid mine-contaminated soil, assessing: (i) the effects of the amendments on soil physicochemical properties; (ii) the capacity to immobilize PTEs in soil; (iii) the capacity to establish a plant cover with *Agrostis tenuis* Sibth; (iv) the effects of the amendments on soil-water extract ecotoxicity; and (v) the effects of the amendments on soil biochemical properties, namely on enzymatic activities (dehydrogenase, β-glucosidase, acidphosphatase, cellulase and protease).

Our hypotheses were that: (i) granular combinations of biomass ash and biological sludge would be able to improve the physicochemical, ecotoxicological and biochemical properties of this type of mine-contaminated soil and, eventually, make it possible to establish a plant cover, and (ii) the surplus application of compost could overcome the organic matter and N deficiency of the soil, which may not be sufficiently provided by the biological sludge used in the co-granulation process of the biomass ash.

2. Materials and methods

2.1. Amendments characterization

2.1.1. Residues from the pulp and paper industry

Biomass ash and biological sludge were provided by a Portuguese pulp and paper company. Previous studies from Cruz et al. (2017) fully characterized BA and FA collected at this industry. The ashes were obtained from the combustion of residual forest biomass (*e.g.* branches, bark) of *Eucalyptus globulus* and *Pinus pinaster* obtained from local forestry harvesting companies. The careless biomass handling led to its mixture with exogenous inorganic material, such as soil particles and stones (Tarelho et al., 2015). Most of the exogenous coarse textured inorganic materials ended up in the bottom ashes (Cruz et al., 2017). Hence, only FA were used in this study. The material was collected at the biomass thermal power plant, with an installed thermal power of 50 MW_{th}, using bubbling fluidized bed combustion (BFBC). This BFBC technology is commonly applied, because it allows low processing temperatures, fuel flexibility, and low NO_x emissions (Tarelho et al., 2015, Cruz et al., 2017).

Considering that FA are poor in organic C, and in some other essential macronutrients, notably N (Supplementary Material, Table S1), the simultaneous application of biological sludge from the industrial wastewater treatment plant (Supplementary Material, Table S2) was evaluated, and the co-granulation of these two materials was performed, as suggested by other authors (Brännvall et al., 2015; Pesonen et al., 2016). Two different ratios (% w/w, dry weight basis (dw)) of biological sludge (S) and fly ashes (FA) were used to prepare the granules: 10% S + 90% FA, codified as 10S90A, and 30% S + 70% FA, codified as 30S70A. In the co-granulation process, the materials were mixed in the abovementioned dry weight basis, but with the sludge with the original water content (80 to 90% w/w), which favoured the mixing with the ash and the formation of a paste. The mixtures were manually agitated in a closed plastic container until they formed granules, which were then left to air dry and stabilize for approximately two months. The process led stable granules with a 10-15 mm diameter (Supplementary Material, Fig. S1).

2.1.2. Mixed municipal solid waste compost

Municipal solid waste compost (MSWC), was obtained in a composting plant near Setúbal (Portugal), which serves about 113,000 inhabitants. The unsorted municipal solid waste is mechanically segregated and biologically treated at the composting plant. The compost produced is commercialized in bulk, mainly to be land applied in vineyards in the Alentejo region. This compost was already characterized (Alvarenga et al., 2015; 2016) and has value as fertilizer: pH 7.8 (H_2O 1:5 w/w ratio), 39.5% OM content, 2.1% $N_{Kjeldahl}$, 15.1 g kg^{-1} for P, 8.7 g kg^{-1} for K, 83.4 g kg^{-1} for Ca, 14.0 g kg^{-1} for Mg, and 9.3 C/N w/w ratio. In agreement with Portuguese legislation (Decree-Law No. 103/2015), the MSWC is classified as a Class IIA compost, considering its trace elements content (3.0 mg kg^{-1} for Cd, 14.5 mg kg^{-1} for Cr, 179.5 mg kg^{-1} for Cu, 0.63 mg kg^{-1} for Hg, 29.2 mg kg^{-1} for Ni, 202.3 mg kg^{-1} for Pb, and 473.5 mg kg^{-1} for Zn, dw), which allows its application in tree and shrub agricultural crops, namely orchards, olive groves and vineyards.

Previous studies evaluated the use of this MSWC as an organic amendment in a mining contaminated soil, and the equivalent to an application rate of 50 t ha^{-1} was recommended to correct soil acidity and nutritional deficiencies (Alvarenga et al., 2009a; 2009b; 2014).

2.2. Soil characterization

The soil was collected at the Aljustrel mine, in the Portuguese sector of the Iberian Pyrite Belt. This mine was intensively exploited from 1850 to 1991, when the production was discontinued until 2008, when it was re-established by the Almina Company. The main mineral in the ore deposit is pyrite (FeS_2), associated with other sulfides, like chalcopyrite

($CuFeS_2$), sphalerite (ZnS) and galena (PbS) (Alvarenga et al., 2004). Despite some rehabilitation works done by EDM (http://edm.pt/) to contain the contaminated tailings and other waste materials that were deposited in the area, the soils are still deeply affected by years of acid mine drainage from old tailings and acid water dams and lagoons (Abreu et al., 2010).

Ten subsamples from the 20 cm topsoil were randomly collected in an area of about 80 m^2, around a site with the geographical coordinates 37°51.889 North, 008°09.390 West, and mixed together to obtain a composite sample. Soil was air-dried and sieved through a 2 mm nonmetallic sieve. Soil physicochemical analysis were performed according to methodologies described by Alvarenga et al. (2008a): particle-size distribution was determined by the pipet method (Gee and Bauder, 1986); soil pH (H_2O) was determined in a soil to deionized water suspension of 1:2.5 (w/v); electrical conductivity (EC) was determined in a soil to deionized water suspension of 1:5 (w/v); total nitrogen was analyzed by the Kjeldahl method ($N_{Kjeldahl}$); total oxidizable organic carbon (C_{org}) was determined according to Walkley and Black (1934), and converted to organic matter content (OM) by multiplying by a factor of 1.72; extractable P and K were determined using the Egner-Riehm method (Riehm, 1958); and pseudo-total metal concentrations (Cd, Cr, Cu, Pb and Zn) were determined by flame atomic absorption spectrometry (FAAS) after digestion of the samples with *aqua regia* according to ISO 11466 (1995), using a Varian apparatus (SpectrAA 220FS, 220Z, and 110Z). Three independent replicates were performed for each sample and blanks were measured in parallel.

The soil was classified as sandy loam (61% sand, 19% silt and 20% clay), was highly acidic (pH 4.24), with high electrical conductivity (EC 2.41 mS cm^{-1}), low OM (0.70 %, dw) and essential nutrients (0.07 % $N_{Kjeldahl}$, 5.3 mg P_2O_5 kg^{-1}, and 8.0 mg K_2O kg^{-1}, dw) content, and high concentrations of potentially toxic trace elements (1315 mg Cu kg^{-1}, 1150 mg Pb kg^{-1}

and 678 mg Zn kg^{-1}, dw), surpassing the Canadian Soil Quality Guideline Values for soils from industrial sites (CCME, 2018). These Guideline Values are usually recommended by the Portuguese Environmental Agency for soil risk evaluations while national legislation is not implemented.

2.3. Preliminary incubation experiment

A preliminary incubation experiment was conducted to select the conditions to be used in the pot experiment and to evaluate the ability of the amendments to correct soil acidity and to increase OM and nutrient content, without increasing soil salinity.

The amendments were tested in their bulk state, as they were prior to the granulation process, or as granules (10S90A and 30S70A, diameter of about 10-15 mm) to evaluate the influence of the granulation of the material on the amendment performance. The amendments were applied at doses of 1.25, 2.5 and 5% (w/w, dw), to 200 g of soil, three replicates per treatment, and a control without amendments. Amendments were applied at the soil surface, without mixing, and watered to achieve 60% of each mixture water-holding capacity (WHC). In the following day, the amendments were roughly mixed with the soil, with a spatula, simulating what would be a mechanical plowing procedure in the field. Despite their physical stability when dried, the moistened granules were easily fragmented and incorporated into the soil. The mixtures were incubated in aerated boxes, watered when needed to maintain 60% WHC, and kept at 20±2ºC for one month.

At the end of the incubation soil pH (1:2.5 w/v, in deionized water), EC (1:5 w/v, in deionized water), OM content, $N_{Kjeldahl}$, and extractable P and K were evaluated using the methods already described.

2.4. Pot experiment

Based on the results from the incubation experiment the doses and type of amendments to be used in the pot experiment were chosen: 2.5, 5.0 and 10% (w/w, dw) of the same granules formulations (10S90A and 30S70A) and the application of mineral fertilizers to provide additional N and P. The simultaneous application of the equivalent to 50 t ha^{-1} of a mixed municipal solid waste compost (MSWC) was evaluated to assure the increase in OM and N content in the pots; this dose had been previously tested in similar soils (Alvarenga et al., 2009a; 2009b; 2014).

The pots were filled with 1 kg of soil, amended with the abovementioned doses of the two different granules with four replicates per treatment. Non-amended soil (S) and soil with mineral fertilizers only (SF) were used as controls. The same number of pots were prepared and supplemented with the equivalent to 50 t ha^{-1} of MSWC (31.5 g compost kg^{-1} of soil, w/w, dw). The amendments application to soil was performed as described for the incubation experiment, adding water in one day, and mixing the materials with the soil the day after. Following an incubation period of 20 days, pots were sown with *Agrostis tenuis* Sibth, at 0.5 cm depth (0.31 g seeds per pot, approximately the equivalent to 250 kg seeds ha^{-1}). All pots, except the non-amended soil (S), received a basal dressing of N and P, considering the recommendations of LQARS (2006) for soils with very low levels of N and P: the equivalent to 200 kg N ha^{-1} and 180 kg P_2O_5 ha^{-1} (1.2 g of N fertilizer, with 27% w/w N, per pot, and 1.1 g P fertilizer, with 26.5% w/w P_2O_5, per pot). According to these recommendations, N fertilizer was applied twice, half the dose immediately after sowing and the other half one month later. Pots were left outdoors for two months and watered when needed. Two months later, plant aboveground material was collected, washed thoroughly with tap water to remove any attached particles, and then rinsed three times with deionized water. The samples were dried at 70 ºC for 48 h, and shoot dry biomass was determined.

2.4.1. Effects on soil physicochemical properties and trace elements concentrations

The effects of the amendments on soil characteristics were assessed by measuring: pH, EC, OM content, $N_{Kjeldahl}$, and extractable P and K, using the methods already described. The concentrations of pseudo-total trace elements in soils (Cd, Cr, Cu, Pb and Zn), and the same trace elements in a readily available fraction (0.01M $CaCl_2$, 2 h, 1:10 (w/v) soil to solution ratio at room temperature; Alvarenga et al., 2009c) were measured. Trace metals were analyzed using FAAS (Varian SpectrAA 220FS). Three independent replicates were performed for each sample and blanks were measured in parallel.

2.4.2. Effects on soil enzymatic activities

Biochemical status of the soil was assessed by measuring different soil enzymatic activities, namely: dehydrogenase, β-glucosidase, acid-phosphatase, cellulase, protease and urease. A thorough description of the methodology used is described in Alvarenga et al. (2009b).

2.4.3. Effects on soil water-extract properties and ecotoxicological status

The soil retention function, *i.e.*, the capacity to immobilize pollutants and reduce the eluate/leachate toxicity, was evaluated using a soil-water extract, solid-to-liquid ratio of 1:10 (w/v), obtained with the DIN 38414-S4 (1984) methodology, as described in Alvarenga et al. (2016; 2018).

The soil-water extracts were analyzed for pH, EC, and sodium and chloride concentrations (Na^+, emission photometry; Cl^-, Mohr's volumetric method), which could potentially cause phytotoxic problems. A fraction of the soil-water extract was acidified to pH<2 with HNO_3 and kept at 4 ºC until the analysis of Cu, Pb and Zn, the trace elements that had pseudo-total concentrations above the guideline values for industrial soils (CCME, 2018), using FAAS

(Varian SpectrAA 220FS). Three independent replicates were performed for each sample and blanks were measured in parallel. The ecotoxicity of the soil-water extracts was evaluated by four different bioassays with organisms representative of different trophic levels: (i) luminescence inhibition of *Vibrio fischeri* (ISO 11348-2, 2007); (ii) *Daphnia magna* acute immobilization test (ISO 6341, 2012); (iii) 24 h mortality test with *Thamnocephalus platyurus* (Persoone, 1999), and (iv) 72 hours population growth of the green microalgae *Pseudokirchneriella subcapitata* (OECD 201, 2006). A thorough description of the methodology used is described in Alvarenga et al. (2018). Whenever possible, the EC_{50} values (soil-water extract concentration, % v/v, at which a toxic effect on 50% of the exposed organisms can be observed) were calculated.

2.5. Statistical treatment of data

The results obtained in the different physicochemical and biochemical analyses were subjected to one-way ANOVA to evaluate statistical differences between the tested treatments ($p \geq 0.05$). For the incubation test, a one-way ANOVA was performed, with one factor grouping three factors (type of granule/dose/formulation), while for the pot experiment, a one-way ANOVA was performed, with one factor grouping three factors (type of granule/dose/compost). Whenever significant differences were found ($p \geq 0.05$), a post hoc Tukey HSD (Honest Significant Difference) test was used to further elucidate differences among means ($p \leq 0.05$).

The effects on growth inhibition of the microalgae *P. subcapitata* were checked for normality by the Kolmogorov–Smirnov test and variance homogeneity (Levene´s tests). As the ANOVA assumptions were not met, data were analyzed non-parametrically using the Kruskal–Wallis test by ranks. When significant differences were found ($p \leq 0.05$), a post-hoc Dunnet´s test was used to compare the results obtained for each treatment with the control, with a p-value of 0.05 as the minimum significant level (Zar, 1996). For the *V. fischeri* bioluminescence inhibition, test EC_{50} values were determined using the LUMISsoft 4 Software™. The EC_{50} values obtained for the *T. platyurus* mortality and the *D. magna* immobilization bioassays were calculated using the Probit Method (Finney, 1971).

All statistical analyses were performed with the STATISTICA 7.0 (Software™ Inc., PA, USA, 2004).

3. Results and discussion

3.1. Results from the preliminary incubation experiment

Granulation of the materials did not significantly affect the performance of the ash amendment (Table 1), since the differences between the results obtained for the soils amended with the bulk *versus* the granulated materials were not significantly different, except for extractable P and K. In fact, once moistened, the granules were very easily disintegrated and mixed with the soil, allowing pH and nutritional corrections similar to those of bulk materials. Moreover, the results obtained with the different formulations, 10S90A and 30S70A, were not significantly different, showing no benefits of increasing the ratio of biological sludge/biomass ash of the granules for the soil properties that were measured, and in the time frame of the experiment.

The application of variable granules doses induced a small variation on soil properties (Table 1). The change in soil pH was lower than expected considering the highly alkaline nature of the materials. After amendment, the soils were still acidic (pH 4.6-5.5) or slightly acidic (pH 5.6-5.5; LQARS, 2006). Soil OM content was also poorly affected, with an increase of +/- 0.2% (w/w) at the highest granules dose, with values still classified as "low" (between 0.6 and 1.5%; LQARS, 2006). There were also no clear effects on $N_{keldahl}$ content after the addition of the amendments, either bulk or granulated.

For the doses tested, there was no impact of the amendments on soils EC (Table 1), which was a relevant result, since one of the potential problems often associated with the application of biomass ashes is the increase of soil salinity.

The granulation of the material only significantly affected the Egner-Rhiem extractable P and K (Table 1). Their concentrations were always lower when the amendments were applied in a granulated form compared to bulk materials. The ash granules tested doses were not enough, on their own, to correct soil P deficiency, with the highest values still considered low (26-50 mg P_2O_5 kg^{-1}) (LQARS, 2006), while K extractable content increased to high (101-200 mg K_2O kg^{-1}) or very high values (>200 mg K_2O kg^{-1}) (LQARS, 2006).

Considering the results of this preliminary experiment, the dose 1.25% w/w was excluded from the test, and a 10% w/w application dose was selected for use in the pot experiment. Moreover, a mineral fertilization with N and P, and the application of an organic amendment (compost) was considered necessary to obtain the target improvement on soils physicochemical characteristics, and which could have a significant effect on soil biochemical and ecotoxicological characteristics.

3.2. Pot experiment: effects of the amendments on soil physicochemical properties

Both amendments, granules and compost, were able to increase soil pH relatively to nonamended soil (Fig. 1A), reaching pH values between 6-7 when those materials were applied alone or in combination. The pH values achieved in the soil amended with ash granules or amended with ash granules combined with compost were not significantly different ($p>0.05$). Therefore, in the test, the inclusion of compost in the ash granules did not bring any additional benefit for acidity correction relative to ash granules.

The soil had a high EC, which, generally, increased upon amendments application (Fig. 1B). That increase was less than 15% of the initial value, but was statistically significant in most of the cases ($p<0.05$). The application of compost further increased soil salinity, relatively to the same assay without compost. This high soluble salt content could be a constraint for the re-vegetation of these soils (Brännvall et al., 2014). Ram and Masto (2014) had already stated that the most limiting factor for the use of FA as soil amendment is the excessive concentration of soluble salts, a problem which can be minimized by their weathering in ash ponds.

This soil had very low OM content (about 0.6% w/w), and that value only significantly increased after amendment with the higher dose (10% w/w) of 30S70A (Fig. 1C), or with the simultaneous application of compost with the granules, which allowed about a two-fold increase in the OM content relatively to the same assay without compost.

The effect of amendments on $N_{Kjeldahl}$ concentrations in soil was similar to those observed for OM (Fig. 1D), although, in this case, the conventional N-fertilizer contributed to its increase, relatively to the mine non-amended soil, but the increase was more marked when compost application was included, which doubled the N concentration in the soil. The application of the granules did not significantly increase the $N_{Kjeldahl}$ concentration, relatively the fertilizer only treatment (SF), except for the application of 10% (w/w) 30S70A. The fact that biomass ash cannot be considered a N fertilizer for agricultural soils has been previously reported (Sajwan et al., 2003; Saarsalmi et al., 2012; Brännvall et al., 2014; Ram and Masto, 2014; Cruz et al., 2017), and co-application of other amendments such as sewage sludge (Sajwan et al., 2003; Pesonen et al., 2016), biosolids (Brännvall et al., 2014), conventional inorganic N-fertilizers (Saarsalmi et al., 2012), or other different organic and inorganic amendments (Ram and Masto, 2014), is required to meet N requirements.

Soil amendment with granules produced from wastes from the pulp and paper industry led to an increase on P content (Egner-Rhiem extractable, Fig. 1E), relatively to the SF treatment, after the application of 10% (w/w) of the 10S90A granules, or after the application of 5 and

10% (w/w) of 30S70A granules. The extractable P content remained low for the lowest application dose of both granules, but it raised to sufficient (51-100 mg P_2O_5 kg^{-1}; LQARS, 2006) after the application of 10% (w/w) 30S70A, showing the importance of increasing the ratio sludge/ashes for this parameter. However, compost was a more relevant source of available P, often doubling its extractable content compared to the application of granules alone.

Contrastingly, the extractable K concentration (Egner-Rhiem extractable, Fig. 1F) became very high upon amendment (>200 mg K_2O kg^{-1}; LQARS, 2006) for all application rates. In consequence, co-application of an organic amendment to provide K was not necessary.

3.3. Effects of the amendments on pseudo-total and trace elements extractable fractions

The mining soil was contaminated with Cu, Pb and Zn (Table 2). The pseudo-total concentrations for those elements were generally higher than the values from the Canadian Soil Quality Guideline Values for soils from industrial sites (91 mg kg^{-1} for Cu, 600 mg kg^{-1} for Pb and 410 mg kg^{-1} for Zn; CCME, 2018). Although the pseudo-total concentrations of Cd, Cr and Ni in the soil were below the referred guideline values (22 mg kg^{-1} for Cd, 87 mg kg^{-1} for Cr and 89 mg kg^{-1} for Ni; CCME, 2018), their concentrations were also measured to monitor any possible input from soil amendments.

Pseudo-total concentrations of Cd, Cr, Cu, Ni, Pb and Zn remained constant in the amended soils, or even decreased slightly for the higher application rates, due to the dilution effect (Table 2). In fact, several authors have mentioned that the content of PTEs is not a limiting factor for the application of biomass ashes to soils, due to the low concentrations of these metals in the ashes resulting from forest biomass combustion (Dahl et al., 2009; Nurmesniemi et al., 2012; Girón et al., 2013; Freire et al., 2015; Cruz et al., 2017). The MSWC produced from mechanical and biological treatment of unsorted municipal solid waste exhibited pseudo-total PTE concentrations of: 3.2 mg Cd kg^{-1}, 17.8 mg Cr kg^{-1}, 167.9 mg Cu kg^{-1}, 179.9 mg Pb kg^{-1}, and 383.3 mg Zn kg^{-1} (dry weight basis; Alvarenga et al., 2015). Nevertheless, those values were lower than those found in the mining soil (or similar, in the case of Cd) and therefore no PTEs enrichment in the soil was observed and the potential risk of contamination with PTEs from soil amendments was considered negligible.

The $CaCl_2$ 0.01M solution extractable Cr and Pb levels were low in the original mine soil, with values below the analytical detection limits (DL) (Table 1), and their extractable concentrations did not increase above those values upon soil amendment. That was an important finding, particularly for Pb, because its $CaCl_2$-extractable concentration represents less than 0.1% of its pseudo-total concentration (approximately 1600 mg Pb kg^{-1}, dw), suggesting that the risk of PTEs' plant uptake or mobility in amended soil was low. The beneficial effect of Cu and Zn immobilization in soil was confirmed by a reduction of 0.1% to 0.03% in their $CaCl_2$-extractable concentrations relative to the pseudo-total concentration (for Cu), and from 1.6% to less than 0.02% of the pseudo-total concentration for Zn (extractable Zn concentrations were often below the analytical DL (Table 2).

3.4. Effects of the amendments on soil biochemical properties

Soil enzymatic activities are relevant indicators of soil fertility and of their ecological status, providing useful information to evaluate the improvement of soil quality in a remediation process. It is not consensual that biomass FA are beneficial to soil microbial parameters: some authors obtained an increase in several soil microbial indicators, including soil enzymatic activities (Ram and Masto, 2014; Pandey and Singh, 2010), while others did not observe significant improvements (García-Sánchez et al., 2015a). Nevertheless, soil microbial biomass can be positively affected because of the macro- and micro-nutrients contained in FA.

In this study, the fertilizer only treatment did not increase soil enzymatic activities, when compared with the non-amended soils (Figure 2A). For some application doses, FA application increased dehydrogenase activity, compared to fertilizer only treatment, which is an important indicator of soil health restoration, since its activity is representative of the active overall microbial population of a soil (Tabatabai, 1994; Izquierdo et al., 2005). The soil dehydrogenase activity was higher in the case of dosing with granules with the highest dose of biological sludge (30S70A), suggesting that co-granulation of ash with sludge is positive for the overall soil microbial activity, especially for the highest proportion of biological sludge (30% w/w) in the granule. The additional application of compost increased dehydrogenase activity even further, relatively to the pots with the application of granules alone.

Exoenzymes activities, such as hydrolases can also be used as early-stage indicators of the improvement of soil nutritional status in soil remediation processes, since they are related to the mineralization of important nutrients such as N, P, and C (Caravaca et al., 2005; GilSotres et al., 2005; Izquierdo et al., 2005). The responses to treatments were different for the several enzymes tested in this work. Both enzymes related to the C-cycle, β-glucosidase (Fig. 2B) and cellulase (Fig. 2C), with major importance in OM degradation (Tabatabai, 1994; Eivazi and Tabatabai, 1988), responded positively to soil amendment. Again, dosing with granules (particularly 30S70A), led to a better response of β-glucosidase activity when compared to the fertilizer only treatment (SF), which evidences the beneficial effect of the biological sludge on the microbial activity. Moreover, as the biological sludge used in the co-granulation process was produced in a wastewater treatment plant from a pulp and paper industry, the C-content of the biological sludge was high, and the enzymes responsible for their degradation were very active. Compost application was not responsible for a significant increase in cellulase activity, compared to the soils without compost, which means that the effect observed can be mainly attributed to the granules application.

Acid phosphatase is related to the P-cycle, largely responsible for the mineralization of organic phosphate compounds in acid soils (Eivazi and Tabatabai, 1977). The effects of the application of the different amendments on its activity were not clear, with significant increases in activity observed only after the application of the highest doses of the granules (10% w/w of 10S90A or 30S70A). Taking into consideration that this enzyme is more active in acid soils, the pH correction towards more alkaline values may have prevented a further response on acid phosphatase activity. In this case, as for the cellulase activity, compost application did not induce a positive effect compared to the pots without compost.

Protease and urease activities are both related to the N-cycle (Ladd and Butler, 1972; Kandeler and Gerber, 1988; Alef and Nannipieri, 1995b). Their activities were very low, as usual in derelict soils from mine areas, below the quantification limit (QL) of the techniques (QL(protease) = 0.56 µmol tyrosine g^{-1} h^{-1}, and QL(urease) = 0.14 µmol NH_4^+-N g^{-1} h^{-1}, dw). Soil amendment with granules or compost, was not able to increase their activities to quantifiable values (data not shown). In a different study, also in mine soils, Alvarenga et al. (2009b) observed that the enzymatic activities of urease and protease increased upon the application of urban sewage sludges, still in a very active state of decomposition, while their activities were not affected by the application of a mature green-waste derived compost, and the application of MSWC only positively affected protease activity.

3.5. Effects of the amendments on soil-water extract composition and ecotoxicity

Soil-water extracts were used to evaluate the effects of the amendments on the soil retention function. The retention function of a soil is related to its capacity to immobilize pollutants, in this case PTEs, avoiding their impact on soil porewater, surface and groundwaters

(Alvarenga et al., 2008b; 2016; 2018). This can be confirmed by evaluating extracts toxicity towards some aquatic organisms, representative of different trophic levels, and using standardized bioassays (Alvarenga et al., 2016; 2018).

The effects of the applied amendments on soil-water extract composition, namely on pH (Fig. 3A), EC (Fig. 3B), and water extractable Cu and Zn (Fig. 3C and 3D), were in accordance with the soil data (Fig. 1), leading to a decrease in the toxicity that was observed towards some of the aquatic organisms used (Table 3), notably towards *T. platyurus* and *D. magna* (the most sensitive organisms to this type of matrix). The toxicity decreased to nondetectable values following amendments application, even for the application of mineral fertilizers alone (suggesting that N and P are limiting soil functions); the exception was the higher dose (10% w/w) of application of the 10S90A granules, although the remaining toxicity was small. The toxicity response of *V. fischeri* was different, and the only toxic response to the soil-water extract was observed after the application of the N mineral fertilizer to soil, an effect which decreased with compost application and was not observed when the N mineral fertilizer was applied together with the granules, presumably due to sensitivity of the organisms to mineral N.

The growth rate of the microalgae *P. subcapitata* after three days of contact with the extract increased in treatments with the different doses of granules, without compost (Fig. 4A), and with compost application (Fig. 4B). Again, the ecotoxicity effect became marginal and it was only possible to quantify the EC_{50} value for the mine soil (EC_{50} = 76.0±6.2% v/v). In this case, a significant relationship between the decrease in the growth and the increment in the concentration of soil-water extract was observed. The supplementary application of compost to the mine soil (Fig. 4B) had a positive effect on the microalgae growth rates, but with small differences and some variability of the results.

The differences observed among the controls (MBL, *P. subcapitata* growth medium; OECD 201, 2006), may be explained by the variability of the algae culture's inoculum used to run the test for each sample. The algae culture's inoculum used to begin the tests were in the same time of exponential growth phase (± four days), but some differences were observed between cellular growth obtained from different cultures. Because of that culture variability, the results for each sample can only be compared to the negative control (MBL) run with that sample.

3.6. Effects of the amendments on the establishment of a plant cover

In contrast to non-amended control pots, *A. tenuis* seeds germinated in amended soil and the plants grew apparently well during the first month. The best results (both in number of plants per pot, and their height) were obtained for the pots amended with 10% (w/w) of the 30S70A granules, supplemented with compost: plants had 3-4 cm height, and between ¾ to the whole pot surface was covered with plants. Moreover, it was also possible to observe that plant growth improved with increasing granules dosing, from 2.5%, to 5% and to 10% (w/w), with better plant establishment and growth with the application of the 30S70A granules, relatively to the 10S90A granules. However, after the first month, necrosis of leaf margins and tips begun to occur in the plants growing in the pots with soil amendment, first in older leaves, and then plant death at the end of the second month, suggesting a strong phytotoxic effect.

Phytotoxicity was attributed to salt stress (Brännvall et al., 2014). In fact, salt deposition in the surface of soil after water evaporation (including the non-amended controls) between watering procedures was observed. Previous studies suggested low salt tolerance in *A. tenuis*, particularly low tolerance to high Mg concentrations (Wu, 1981), which can explain the phytotoxic effects observed here. In fact, EC values in the range 2 to 2.5 mS/cm in the 1:10 (w/v) soil-water extract can be considered high, only tolerable by resistant plants (LQARS, 2006). However, EC did not increase because of the amendments application (Fig. 3B). Therefore, to understand what could have caused the toxicity, Na^+ and Cl^- concentrations in the soil-water extract were determined (Fig. 3E and 3F). These ions are often the cause of specific toxicity to plants, and it was noticed that biomass ash exhibited high chloride concentration (Cruz et al., 2017).

In contrast to the overall soluble salt content, the concentrations of both Na^+ and Cl^- ions increased significantly with the increase of the dosing of the granules (about 30x and 10x times, respectively, for the highest application rates), suggesting that the application of granules, without a preliminary leaching process, contributed to the increase in concentrations of these ions in the soil. Moreover, for Cl^- (Fig. 3F), the concentrations obtained by the application of the 10S90A granules were higher than those obtained by the same dose of the 30S70S granules, indicating that Cl^- ions are mainly coming from the FA. The same could not be concluded for Na^+ ions (Fig. 3E). It was clear that the compost contributed markedly to the increase in Na^+ concentration in the amended soils. This can also induce structural problems in soils, contributing to the de-flocculation of the soil aggregates.

4. Conclusions

In this study, wastes from the pulp and paper industry were co-granulated and used in a pot experiment to improve the quality of acid mine-contaminated soils, with high Cu, Pb and Zn concentrations. The granules obtained were able to correct soil acidity and to increase extractable P and K. A significant increase in the soil OM and N content were only obtained by the simultaneous application of MSWC and mineral fertilizers.

The improvement in soil quality, in comparison to the non-amended soil or with the soil with N and P mineral fertilization, was further evidenced by the increase of soil enzymatic activities (dehydrogenase, β-glucosidase and cellulase), with the best response observed in the case of dosing with granules with the highest proportion of biological sludge (30% w/w).

Upon the soil acidity correction and the consequent improvement in the soil retention function, Cu- and Zn-$CaCl_2$ extractable fractions decreased to residual values. This also had a positive effect on the soil-water extract toxicity, which decreased, as determined by different aquatic bioassays.

Agrostis tenuis germinated and grew in pots where amendments were applied but, after 1 month, a considerable phytotoxic effect was evident. This phytotoxicity could be attributed to low salt tolerance of *A. tenuis*, or to specific toxicity promoted by the increase in the concentration of Cl^- ions (from granules application), and/ or to the increase of Na^+ ions (as consequence of compost application).

In conclusion, the amendments improved soil quality, as evaluated by physicochemical, biochemical and ecotoxicological parameters, but they were not able to support the establishment of a permanent plant cover with *A. tenuis*. For that purpose, and to extend the experiment to a larger scale, different plant species, with higher resistance to salinity, should be considered. The ageing (stabilization) and leaching of the granules to decrease their soluble salt concentration before application, could be also be considered.

Acknowledgements

This study was supported by the project: LIFE No_Waste - LIFE14 ENV/PT/000369 – "Management of biomass ash and organic waste in the recovery of degraded soils: a pilot project set in Portugal", and through the research unit UID/AGR/04129/2013 (LEAF).

Thanks are due to FCT/MCTES for the financial support to CESAM (UID/AMB/50017/2019), through national funds. S. M. Rodrigues acknowledges the financial support from FCT (Project

IF/01637/2013). Nuno M. C. Cruz acknowledges financial support from FCT through Ph.D. grant SRFH/BD/115115/2016.

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: Robinson, B.C. (Ed.), Mine Drainage and Related Problems. Nova Science Publishers, Inc., New York, USA, pp. 71-118.

Alef, K., Nannipieri, P., 1995a. □-Glucosidase activity, in: Alef, K., Nannipieri, P. (Eds.), Methods in Applied Soil Microbiology and Biochemistry. Academic Press, London, pp. 350-352.

Alef, K., Nannipieri, P., 1995b. Protease activity, in: Alef, K., Nannipieri, P. (Eds.), Methods in Applied Soil Microbiology and Biochemistry. Academic Press, London, pp. 313315.

Alef, K., Nannipieri, P., Trasar-Cepeda, C., 1995. Phosphatase activity, in: Alef, K., Nannipieri, P. (Eds.), Methods in Applied Soil Microbiology and Biochemistry. Academic Press, London, pp. 335-344.

Alvarenga, P.M., Araújo, M.F., Silva, J.A.L., 2004. Elemental uptake and root-leaves transfer in *Cistus ladanifer* L. growing in a contaminated pyrite mining are (Aljustrel – Portugal). Water Air Soil Pollut. 152, 81-96.

Alvarenga, P., Gonçalves. A.P., Fernandes, R.M., de Varennes, A. Vallini, G., Duarte, E., Cunha-Queda, A.C., 2008a. Evaluation of composts and liming materials in the phytostabilization of a mine soil using perennial ryegrass. Sci. Total Environ. 406, 43–56.

Alvarenga, P., Palma, P., Gonçalves, A.P., Fernandes, R.M., de Varennes, A., Vallini, G., Duarte, E., Cunha-Queda, A.C., 2008b. Evaluation of tests to assess the quality of mine contaminated soils. Environ. Geochem. Hlth. 30, 95-99.

Alvarenga, P., Gonçalves, A.P., Fernandes, R.M., de Varennes, A., Vallini, G., Duarte, E. Cunha-Queda, A.C., 2009a. Organic residues as immobilizing agents in aided phytostabilization: (I) Effects on soil chemical characteristics. Chemosphere 74, 12921300.

Alvarenga, P., Palma, P., Gonçalves, A.P., Fernandes, R.M., de Varennes, A., Vallini, G., Duarte, E., Cunha-Queda, A.C., 2009b. Organic residues as immobilizing agents in aided phytostabilization: (II) Effects on soil biochemical and ecotoxicological characteristics. Chemosphere 74: 1301-1308.

Alvarenga, P., Gonçalves, A.P., Fernandes, R.M., de Varennes, A., Vallini, G., Duarte, E., Cunha-Queda, A.C., 2009c. Reclamation of a mine contaminated soil using biologically reactive organic matrices. Waste Manage. Res. 27, 101-111.

Alvarenga, P., de Varennes, A., Cunha-Queda, A.C., 2014. The Effect of Compost Treatments and A Plant Cover with *Agrostis tenuis* on the Immobilization/Mobilization of Trace Elements in a Mine-Contaminated Soil. Int. J. Phytoremediation 16(2), 138-154. Alvarenga, P., Mourinha, C., Farto, M., Santos, T., Palma, P., Sengo, J., Morais, M.- C., CunhaQueda, C., 2015. Sewage sludge, compost and other representative organic wastes as agricultural soil amendments: Benefits versus limiting factors. Waste Manag. 40, 44–52.

Alvarenga, P., Mourinha, C., Farto, M., Palma, P., Sengo, J., Morais, M.- C., Cunha-Queda, C., 2016. Ecotoxicological assessment of the potential impact on soil porewater, surface and groundwater from the use of organic wastes as soil amendments. Ecotoxicol. Environ. Saf. 126, 102–110.

Alvarenga, P., Ferreira, C., Mourinha, C., Palma, P., de Varennes, A., 2018. Chemical and ecotoxicological effects of the use of drinking-water treatment residuals for the remediation of soils degraded by mining activities. Ecotoxicol. Environ. Saf. 161, 281289.

Basu, M., Pande, M., Bhadoria, P.B.S., Mahapatra, S.C., 2009. Potential fly-ash utilization in agriculture: A global review. Prog. Nat. Sci. 19, 1173-1186.

Brännvall, E., Nilsson, M., Sjöblom, R., Skoglund, N., Kumpiene, J., 2014. Effect of residue combinations on plant uptake of nutrients and potentially toxic elements. J. Environ. Manage. 132, 287-295.

Brännvall, E., Wolters, M., Sjöblom, R., Kumpiene, J., 2015. Elements availability in soil fertilized with pelletized fly ash and biosolids. J. Environ. Manage. 159, 27-36.

CCME, 2018. Soil Quality Guideline Values for Protection of the Environment and Human Health, Canadian Council of Ministers of the Environment. https://www.ccme.ca/en/resources/canadian_environmental_quality_guidelines/ (accessed 9 July 2018).

Commission Decision 2000/532/EC, of 3 May 2000, establishing a list of wastes and of hazardous waste. Official Journal of the European Communities. I. 226/3. https://publications.europa.eu/en/publication-detail/-/publication/239a2785-9115-4e06adae-66c8e08a5a42 (accessed 20 March 2014).

Cruz, N.C., Rodrigues, S.M., Carvalho, L., Duarte, A.C., Pereira, E., Römkens, P.F.A.M., Tarelho, L.A.C., 2017. Ashes from fluidized bed combustion of residual forest biomass: recycling to soil as a viable management option. Environ. Sci. Pollut. R. 24, 14770-14781.

Dahl, O., Nurmesniemi, H., Pöykiö, R., Watkins, G., 2009. Comparison of the characteristics of bottom ash and fly ash from a medium-size (32 MW) municipal district heating plant incinerating forest residues and peat in a fluidized-bed boiler. Fuel Process. Technol. 90, 871-878.

Decree-Law No. 276/2009, on the sewage sludge application to soil. Ministério do Ambiente, do Ordenamento do Território e do Desenvolvimento Regional. Diário da República, 1.ª série - N.º 192 - 2 de Outubro de 2009. (in Portuguese)

Decree-Law No. 103/2015, of 15 June, on fertilizing materials. Diário da República, 1.ª série - N.º 114 - 15 de junho de 2015, pp 3756- 3788. (in Portugese).

Demeyer, A., Nkana, J.C.V., Verloo, M.G., 2001. Characteristics of wood ash and influence on soil properties and nutrient uptake: an overview. Bioresour. Technol. 77, 287-295.

Eivazi, F., Tabatabai, M.A., 1977. Phosphatases in soils. Soil Biol. Biochem. 9, 167-172.

Eivazi, F., Tabatabai, M.A., 1988. Glucosidases and galactosidases in soils. Soil Biol. Biochem. 20, 601-606.

European Commission, 2008. Council Directive 2008/98/EC of 19 November 2008: on waste and repealing certain Directives. OJ L 312, 22.11. pp: 3-30.

European Commission, 2010, Europe 2020 - A European strategy for smart, sustainable and inclusive growth. https://ec.europa.eu/info/business-economy-euro/economic-andfiscal-policy-coordination/eu-economic-governance-monitoring-preventioncorrection/european-semester/framework/europe-2020-strategy_en (accessed 25 February 2018).

DIN 38414-S4, 1984. Determination of leachability by water (S4). German standard methods for the examination of water, wastewater and sludge. Sludge and Sediments (group S).

Finney, D.J., 1971. Probit Analysis. Cambridge University Press. Cambridge, UK.

Freire, M., Lopes, H., Tarelho, L.A.C., 2015. Critical aspects of biomass ashes utilization in soils: Composition, leachability, PAH and PCDD/F. Waste Manag. 46, 304-315.

Galende, M.A., Becerril, J.M., Barrutia, O., Artetxe, U., Garbisu, C., Hernández, A., 2014. Field assessment of the effectiveness of organic amendments for aided phytostabilization of a

Pb-Zn contaminated mine soil. J. Geochem. Explor. 145, 181-189.

García-Sánchez, M., Siles, J.A., Cajthaml, T., García-Romera, I., Tlustos, P., Száková, J., 2015a. Effect of digestate and fly ash applications on soil functional properties and microbial communities. Eur. J. Soil Biol. 71, 1-12.

Garcia-Sánchez, M., Garcia-Romera, I., Cajthaml, T., Tlustos, P., Száková, J., 2015b. Changes in soil microbial community functionality and structure in a metal-polluted site: The effect of digestate and fly ash applications. J. Environ. Manage. 162, 63-73.

Gee, G.W., Bauder, J.W., 1986. Particle-size analysis, in: Klute, A. (Ed.), Methods of Soil Analysis. Part 1. Physical and Mineralogical Methods. Soil Science Society of America. Madison, Wisconsin, USA. pp. 383-412.

Gil-Sotres, F., Trasar-Cepeda, C., Leirós, M.C., Seoane, S., 2005. Different approaches to evaluating soil quality using biochemical properties. Soil Biol. Biochem. 37, 877-887.

Girón, R.P., Ruiz, B., Fuente, E., Gil, R.R., Suárez-Ruiz, I., 2013. Properties of fly ash from forest biomass combustion. Fuel, 114, 71-77.

Hope, C.F.A., Burns, R.G., 1987. Activity, origins and location of cellulase in a silt loam soil. Biol. Fert. Soils. 5, 164-170.

ISO 11466, 1995. Soil Quality - Extraction of Trace Elements Soluble in Aqua Regia. International Organisation for Standardisation, Genève, Switzerland.

ISO 11348-2, 2007. Water quality - Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test) - Part 2: Method using liquid-dried bacteria. International Organisation for Standardisation, Genève, Switzerland.

ISO 6341, 2012. Water quality -- Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea) - Acute toxicity test. International Organisation for Standardisation, Genève, Switzerland.

Izquierdo, I., Caravaca, F., Alguacil, M.M., Hernández, G., Roldán, A., 2005. Use of microbiological indicators for evaluating success in soil restoration after revegetation of a mining area under subtropical conditions. Appl. Soil Ecol. 30, 3-10.

Kandeler, E., Gerber, H., 1988. Short-term assay of soil urease activity using colorimetric determination of ammonium. Biol. Fert. Soils. 6, 68-72.

Ladd, J.N., Butler, J.H.A., 1972. Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. Soil Biol. Biochem. 4, 19-30.

Liu, Y.-N., Guo, Z.-H., Xiao, X.-Y., Wang, S., Jiang, Z.-C., Zeng, P., 2017. Phytostabilisation potential of giant reed for metals contaminated soil modified with complex organic fertiliser and fly ash: A field experiment. Sci. Total Environ. 576, 292-302.

LQARS, 2006. Manual de Fertilização das Culturas. INIAP – Instituto Nacional de Investigação Agrária e das Pescas, Laboratório Químico Agrícola Rebelo da Silva, Ministério da Agricultura, do Desenvolvimento Rural e das Pescas. (in Portuguese)

Matos, J.X. Martins, L.P., 2006. Reabilitação ambiental de áreas mineiras do sector português da Faixa Piritosa Ibérica: estado da arte e perspectivas futuras. Boletín Geológico y Minero 117(2), 289-304. (in Portuguese)

Modolo, R.C.E., Silva, T., Senff, L., Tarelho, L. A. C., Labrincha, J. A., Ferreira, V. M., Silva, L., 2015. Bottom ash from biomass combustion in BFB and its use in adhesive-mortars. Fuel Process. Technol. 129, 192-202.

Nieminen, M., Piirainen, S., Moilanen, M., 2005. Release of mineral nutrients and heavy metals from wood and peat ash fertilizers: Field studies in Finnish forest soils. Scand. J. Forest

Res. 20, 146-153.

Nurmesniemi, H., Mäkela, M., Pöykiö, R., Manskinen, K., Dahl, O., 2012. Comparison of the forest fertilizer properties of ash fractions from two power plants of pulp and paper mill incinerating biomass-based fuels. Fuel Process. Technol. 104, 1-6.

OECD 201, 2006. Freshwater Alga and Cyanobacteria, Growth Inhibition Test, OECD Guideline for Testing of Chemicals. Organisation for Economic Cooperation and Development (OECD), Paris.

Pandey, V.C., Singh, N., 2010. Impact of fly ash incorporation in soil systems. Agri. Ecos. Environ. 136, 16-27.

Pardo, T., Clemente, R., Bernal, M.P., 2011. Effects of compost, pig slurry and lime on trace element solubility and toxicity in two soils differently affected by mining activities. Chemosphere 84, 642-650.

Peña, A., Mingorance, M.D., Guzmán-Carrizosa, I., Fernández-Espinosa, A., 2015. Improving the mining soil quality for a vegetation cover after addition of sewage sludges: Inorganic ions and low-molecular-weight organic acids in the soil solution. J. Environ. Manage. 150, 216-225.

Pérez-de-Mora, A., Madejón, P., Burgos, P., Cabrera, F., Lepp, N.W., Madejón, E., 2011. Phytostabilization of semiarid soils residually contaminated with trace elements using byproducts: Sustainability and risks. Environ. Pollut. 159, 3018-3027.

Persoone, G., 1999. THAMNOTOXKIT F^{TM} - Crustacean toxicity screening test for Freshwater. Standard Operational Procedure. Belgium.

Pesonen, J., Kuokkanen, V., Kuokkanen, T., Illikainen, M., 2016. Co-granulation of bio-ash with sewage sludge and lime for fertiliser use. J. Environ. Chem. Eng. 4, 4817-4821.

Ram, L.C., Masto, R.E., 2014. Fly ash for soil amelioration: A review on the influence of ash blending with inorganic and organic amendments. Earth Sci. Rev.128, 52-74.

Riehm H., 1958. Die ammoniumlaktatessigsaure-methode zur bestimmung der leichtoeslichen phosphosaure in karbonathaltigen boden. Agrochimica 3, 49-65.

Ribbing, C., 2007. Environmentally friendly use of non-coal ashes in Sweden. Waste Manag. 27, 1428-1435.

Rodríguez-Jordá, M.P., Garrido, F., García-González, M.T., 2010a. Assessment of the use of industrial by-products for induced reduction of As, and Se potential leachability in an acid soil. J. Hazard. Mater. 175, 328-335.

Rodríguez-Jordá, M.P., Garrido, F., García-González, M.T., 2010b. Potential use of gypsum and lime rich industrial by-products for induced reduction of Pb, Zn and Ni leachability in an acid soil. J. Hazard. Mater. 175, 762-769.

Saarsalmi, A., Smolander, A., Kukkola, M., Moilanen, M., Sramäki, J., 2012. 30-Year effects of wood ash and nitrogen fertilization on soil chemical properties, soil microbial processes and stand growth in a Scots pine stand. For. Ecol. Manag. 278, 63-70.

Sajwan, K.S., Paramasivam, S., Alva, A.K., Adriano, D.C., Hooda, P.S., 2003. Assessing the feasibility of land application of fly ash, sewage sludge and their mixtures. Adv. Environ. Res. 8, 77-91.

Silva, E.F., Bobos, I., Matos, J.X., Patinha, C., Reis, A.P., Fonseca, E.C., 2009. Mineralogy and geochemistry of trace metals and REE in volcanic massive sulfide host rocks, stream sediments, stream waters and acid mine drainage from the Lousal mine area (Iberian Pyrite Belts, Portugal). Appl. Geochem. 24, 383-401.

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, Madison, Wisconsin, pp. 775-833.

Tarelho, L.A.C., Teixeira, E.R., Silva, D.F.R., Modolo, R.C.E., Labrincha, J.A., Rocha, F.J.F.T. 2015. Characteristics of distinct ash flows in a biomass thermal power plant with fluidised bed combustor. Energy, 90, 387-402.

Ukwattage, N.L., Ranjith, P.G., Bouazza, M., 2013. The use of coal combustion fly ash as a soil amendment in agricultural lands (with comments on its potential to improve food security and sequester carbon). Fuel, 109, 400-408.

Walkley, A., Black, J.A., 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. 37, 29-38.

Wu, L., 1981. The potential for evolution of salinity tolerance in *Agrostis stolonifera* L. and *Agrostis tenuis* Sibth. New Phytol. 89, 471-486.

Zar, J.H., 1996. Biostatistical analysis. Prentice-Hall International, Englewood Cliff. USA, DC.

Figure Captions:

Figure 1. Effects of the different doses of biological sludge and biomass ash granules, with and without mixed municipal solid waste compost, on: (A) soil pH, (B) electrical conductivity (EC), (C) organic matter content (OM), (D) $N_{Kjeldahl}$, (E) extractable P, and (F) extractable K (mean ± standard deviation, n=4). A one-way ANOVA was performed ($p \leq 0.05$), with one factor grouping three factors (type of granule/dose/compost), followed by a post hoc Tukey HSD test whenever significant differences were found. Columns marked with the same letter are not significantly different (Tukey HSD test, $p > 0.05$). S: soil; SF: soil + fertilizer; 10S90A: granules with 10% biological sludge + 90% biomass ash, applied in the doses 2.5, 5 and 10% (w/w) to soil; 30S70A: granules with 30% biological sludge + 70% biomass ash, applied in the doses 2.5, 5 and 10% (w/w) to soil.

Figure 2. Effects of the different doses of biological sludge and biomass ash granules, with and without mixed municipal solid waste compost on the activities of: (A) dehydrogenase (DHA), (B) β-glucosidase, (C) cellulase, and (D) acid-phosphatase activities (mean ± standard deviation, n=8). A one-way ANOVA was performed ($p \leq 0.05$), with one factor grouping three factors (type of granule/dose/compost), followed by a post hoc Tukey HSD test whenever significant differences were found. Columns marked with the same letter are not significantly different (Tukey HSD test, $p > 0.05$). S: soil; SF: soil + fertilizer; 10S90A: granules with 10% biological sludge + 90% biomass ash, applied in the doses 2.5, 5 and 10% (w/w) to soil; 30S70A: granules with 30% biological sludge + 70% biomass ash, applied in the doses 2.5, 5 and 10% (w/w) to soil.

Figure 3. Effects of the different doses of biological sludge and biomass ash granules, with and without mixed municipal solid waste compost, on soil-water extract characteristics: (A) pH; (B) electrical conductivity (EC); (C) extractable Cu; (D) extractable Zn; (E) extractable sodium (Na^+), and (F) extractable chloride (Cl^-) (mean ± standard deviation, n=4). A one-way ANOVA was performed ($p \leq 0.05$), with one factor grouping three factors (type of granule/dose/compost), followed by a post hoc Tukey HSD test whenever significant differences were found. Columns marked with the same letter are not significantly different (Tukey HSD test, $p > 0.05$). S: soil; SF: soil + fertilizer; 10S90A: granules with 10% biological sludge + 90% biomass ash, applied in the doses 2.5, 5 and 10% (w/w) to soil; 30S70A: granules with 30% biological sludge + 70% biomass ash, applied in the doses 2.5, 5 and 10% (w/w) to soil.

Figure 4. Growth rate (per day) of the microalgae *P. subcapitata* after 3 days of exposure to water extracts from the different doses of biological sludge and biomass ash granules: (A) without, and (B) with application of municipal solid waste compost (mean ± SD; n= 6; bars marked with an asterisk (*) are significantly different from their own negative control (MBL, *P. subcapitata* growth medium; OECD 201, 2006), $p \leq 0.05$, Dunnett's test with a control).

Table 1. Results obtained in the incubation experiment of the mine soil with two types of biological sludge and biomass ash formulations (10S90A: 10% biological sludge + 90% biomass ash, and 30S70A: 30% biological sludge + 70% biomass ash), in bulk or as granules, applied in the doses 1.25, 2.5 and 5% (w/w), on: soil pH, electrical conductivity (EC), organic matter content, $N_{Kjeldahl}$, extractable P, and extractable K (mean, n=3). A one-way ANOVA was performed ($p \leq 0.05$), with one factor grouping three factors (type of granule/dose/formulation), followed by a post hoc Tukey HSD test whenever significant differences were found. Results marked with the same letter are not significantly different (Tukey HSD test, $p > 0.05$).

	Treatments		Soil properties					
Type of granule	Application dose (% w/w)	Formulation	pH	EC (mS cm^{-1})	Organic matter (%, g 100 g^{-1} dw)	$N_{Kjeldahl}$ (%, g 100 g^{-1} dw)	Extractable P (mg kg^{-1} dw)	Extractable K (mg kg^{-1} dw)
10S90A	0	-	4.29ab	2.34	0.98de	0.072	8.7a	9.6a
	0	-	4.10a	2.44	0.65ab	0.068	9.4ab	10.4a
	1.25	Granular	4.87df	2.37	0.93cde	0.074	14.2ab	75.7cd
	1.25	Bulk	4.34abc	2.40	0.82abcd	0.072	11.2ab	45.3bc
	2.5	Granular	5.21e	2.38	0.94de	0.074	17.8ab	137.4e
	2.5	Bulk	4.70cd	2.34	0.88abcde	0.069	23.2abc	77.3cd
	5	Granular	5.64g	2.41	1.10e	0.082	32.0bcd	324.1g
	5	Bulk	5.22ef	2.38	0.93cde	0.072	41.6cd	179.9f
30S70A	0	-	4.24ab	2.43	0.64a	0.072	5.3a	8.0a
	0	-	4.24ab	2.43	0.66ab	0.072	11.1ab	10.1a
	1.25	Granular	4.64bcd	2.39	0.69abc	0.072	14.3ab	37.4ab
	1.25	Bulk	4.51bcd	2.38	0.91bcde	0.075	13.5ab	37.7ab
	2.5	Granular	5.25e	2.41	0.89abcde	0.082	26.1abc	95.3d
	2.5	Bulk	4.88def	2.35	0.81abcd	0.071	20.2abc	104.8d
	5	Granular	5.75g	2.32	0.88abcde	0.082	52.5d	186.0f
	5	Bulk	5.66g	2.41	0.89abcde	0.082	41.0cd	179.4f

Table 2. Effects of the different doses of biological sludge and biomass ash granules, with and without mixed municipal solid waste compost (MSWC), on total heavy metal concentrations (digestion with *aqua regia*), and $CaCl_2$ 0.01M extractable concentrations (mean, n=4). A one-way ANOVA was performed ($p \leq 0.05$), with one factor grouping three factors (type of material/dose/compost), followed by a post hoc Tukey HSD test whenever significant differences were found. Results for the same metal marked with the same letter are not significantly different (Tukey HSD test, $p > 0.05$). S: soil; SF: soil + fertilizer; 10S90A: granules with 10% biological sludge + 90% biomass ash, applied in the doses 2.5, 5 and 10% (w/w) to soil; 30S70A: granules with 30% biological sludge + 70% biomass ash, applied in the doses 2.5, 5 and 10% (w/w) to soil.
DL: detection limit; DL(Cd) = 0.08 mg kg^{-1} dw; DL(Cr) = 1.67 mg kg^{-1} dw; DL(Ni) = 0.33 mg kg^{-1} dw; DL(Pb) = 1.67 mg kg^{-1} dw; DL(Zn) = 0.17 mg kg^{-1} dw.

Type of material	Application dose (% w/w)	MSWC (t ha^{-1} dw)	Cd (mg kg^{-1} dw)		Cr mg kg^{-1} dw)		Cu (mg kg^{-1} dw)		Ni (mg kg^{-1} dw)		Pb (mg kg^{-1} dw)		Zn (mg kg^{-1} dw)	
			Total	$CaCl_2$ Extractable	Total	$CaCl_2$ Extractable	Total	$CaCl_2$ Extractable	Total	$CaCl_2$ Extractable	Total	$CaCl_2$ Extractable	Total	$CaCl_2$ Extractable
S	0	0	3.27ab	0.27d	11.03ab	<DL	1640.8ab	1.70d	17.0a	0.42b	1598.6ab	<LD	941.4d	14.72c
	0	50	3.75e	0.21bc	12.84bc	<DL	1825.4b	0.88bc	20.2b	0.38ab	1835.6ab	<DL	922.0cd	9.03bc
SF	0	0	3.27ab	0.25cd	10.89ab	<DL	1662.7ab	1.19cd	16.4a	0.34a	1544.5a	<DL	930.6cd	8.60bc
	0	50	3.60cde	0.17b	9.20a	<DL	1697.4ab	0.51ab	16.9a	0.40ab	1735.4ab	<DL	870.5bcd	5.13ab
10S90A	2.5	0	3.19a	0.10a	10.39ab	<DL	1616.7ab	0.22a	16.9a	<DL	1660.5ab	<DL	885.4bcd	0.21a
	2.5	50	3.70e	0.09a	10.59ab	<DL	1671.3ab	0.27ab	17.5ab	0.37ab	1781.4ab	<DL	832.6abcd	0.28a
	5	0	3.34abc	<DL	11.09ab	<DL	1553.8a	0.25ab	16.4a	0.35a	1604.7ab	<DL	922.1cd	<DL
	5	50	3.74e	<DL	11.18ab	<DL	1695.8ab	0.29ab	16.6a	0.39ab	1903.1b	<DL	816.2abcd	<DL
	10	0	3.37abcd	0.09a	12.17abc	<DL	1521.7a	0.33ab	15.4a	0.38ab	1528.6a	<DL	862.1abcd	<DL
	10	50	3.52bcde	<DL	12.42bc	<DL	1561.7a	0.34ab	16.8a	0.34a	1760.8ab	<DL	759.5ab	<DL
30S70A	2.5	0	3.56bcde	<DL	10.32ab	<DL	1616.6ab	0.24ab	16.3a	0.43b	1701.4ab	<DL	933.4cd	<DL
	2.5	50	3.68de	<DL	11.27ab	<DL	1715.4ab	0.28ab	16.5a	<DL	1644.6ab	<DL	894.9cd	<DL
	5	0	3.54bcde	<DL	10.33ab	<DL	1638.2ab	0.28ab	16.8a	0.41b	1659.7ab	<DL	847.3abc	<DL

													d	
	5	50	3.75e	<DL	14.96c	<DL	1729.1ab	0.32ab	17.3ab	<DL	1680.8ab	<DL	804.4abc	<DL
	10	0	3.47abcde	<DL	10.18ab	<DL	1567.8a	0.46ab	17.1a	0.43b	1676.8ab	<DL	817.9abcd	<DL
	10	50	3.56bcde	<DL	11.01ab	<DL	1578.6a	0.48ab	16.8a	<DL	1672.3ab	<DL	736.2a	<DL

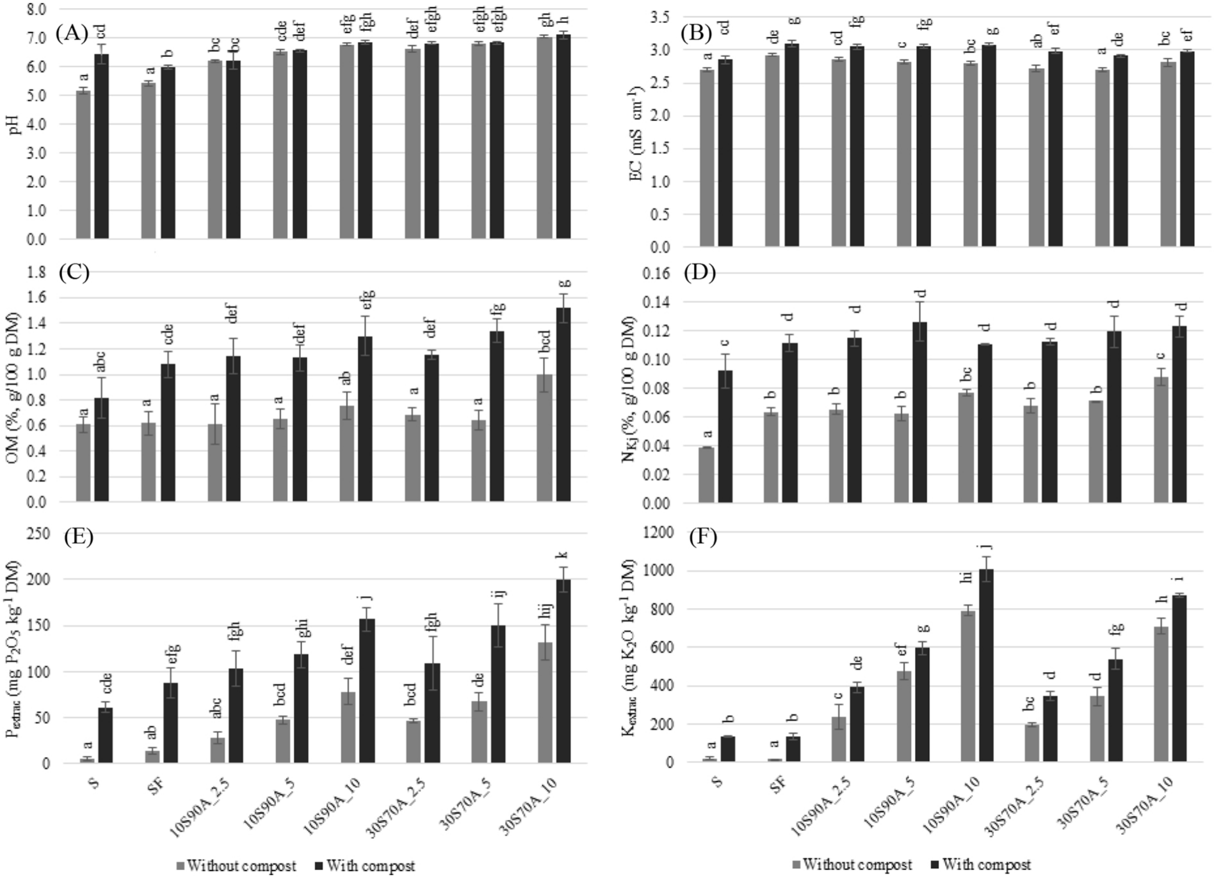
Table 3. Effects of the different doses of cellulosic sludge and biomass ash granules, with and without mixed municipal solid waste compost (MSWC), on soil-water extract ecotoxicological responses calculated as EC_{50} values (mean values % v/v (95% confidence interval); n=2 for *V. fischeri*, and n=3 for *T. platyurus* and *D. magna*). S: soil; SF: soil + fertilizer; 10S90A: granules with 10% biological sludge + 90% biomass ash, applied in the doses 2.5, 5 and 10% (w/w) to soil; 30S70A: granules with 30% biological sludge + 70% biomass ash, applied in the doses 2.5, 5 and 10% (w/w) to soil.

EC_{50}: soil water-extract concentration, % v/v, at which a toxic effect on 50% of the exposed organisms can be observed; nt: non-toxic.

Type of material	Application dose (% w/w)	MSWC (t ha^{-1} dw)	*V. fischeri* 30 min-EC_{50}	*T. platyurus* 24h-EC_{50}	*D. magna* 48h-EC_{50}
S	0	0	nt	35.1 (31.2-39.7)	27.9 (23.2-36.9)
	0	50	nt	54. 1 (47.1-63.9)	44.5 (37.8-53.7)
SF	0	0	53.9 (53.4-54.1)	nt	nt
	0	50	76.1 (75.9-76.2)	nt	nt
10S90A	2.5	0	nt	nt	nt
	2.5	50	nt	nt	nt
	5	0	nt	nt	nt
	5	50	nt	nt	nt
	10	0	nt	95.0 (79.9-120.4)	75.1 (62.8-88.0)
	10	50	nt	97.8 (82.3-124.2)	nt
30S70A	2.5	0	nt	nt	nt
	2.5	50	nt	nt	nt
	5	0	nt	nt	nt
	5	50	nt	nt	nt
	10	0	nt	nt	nt
	10	50	nt	nt	nt

Highlights

- Biomass ash and biological sludge from a paper mill were used to amend mine soils
- Soil pH increased to neutral values and Cu and Zn $CaCl_2$ extractability decreased
- Compost application was essential to increase soil organic matter, N and P content
- Some soil enzymatic activities increased, and soil-water extract toxicity decreased
- However, high salinity promoted phytotoxicity to *Agrostis tenuis* Sibth.



(A)
pH
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
a
cd
a
b
bc
bc
cde
def
efg
fgh
def
efgh
efgh
efgh
gh
h
(B)
EC (mS cm^{-1})
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
a
cd
de
g
cd
fg
c
fg
bc
g
ab
ef
a
de
bc
ef
(C)
OM (%, g/100 g DM)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
a
abc
a
cde
a
def
a
def
ab
efg
a
def
a
fg
bcd
g
(D)
N$_{Kj}$(%, g/100 g DM)
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
a
c
b
d
b
d
b
d
bc
d
b
d
b
d
c
d
(E)
P$_{extrac}$ (mg P$_2$O$_5$ kg^{-1} DM)
0
50
100
150
200
250
a
cde
ab
efg
abc
fgh
bcd
ghi
def
j
bcd
fgh
de
ij
hij
k
(F)
K$_{extrac}$ (mg K$_2$O kg^{-1} DM)
0
200
400
600
800
1000
1200
a
b
a
b
c
de
ef
g
hi
j
bc
d
d
fg
h
i
S
SF
10S90A_2.5
10S90A_5
10S90A_10
30S70A_2.5
30S70A_5
30S70A_10
Without compost
With compost

Figure 1

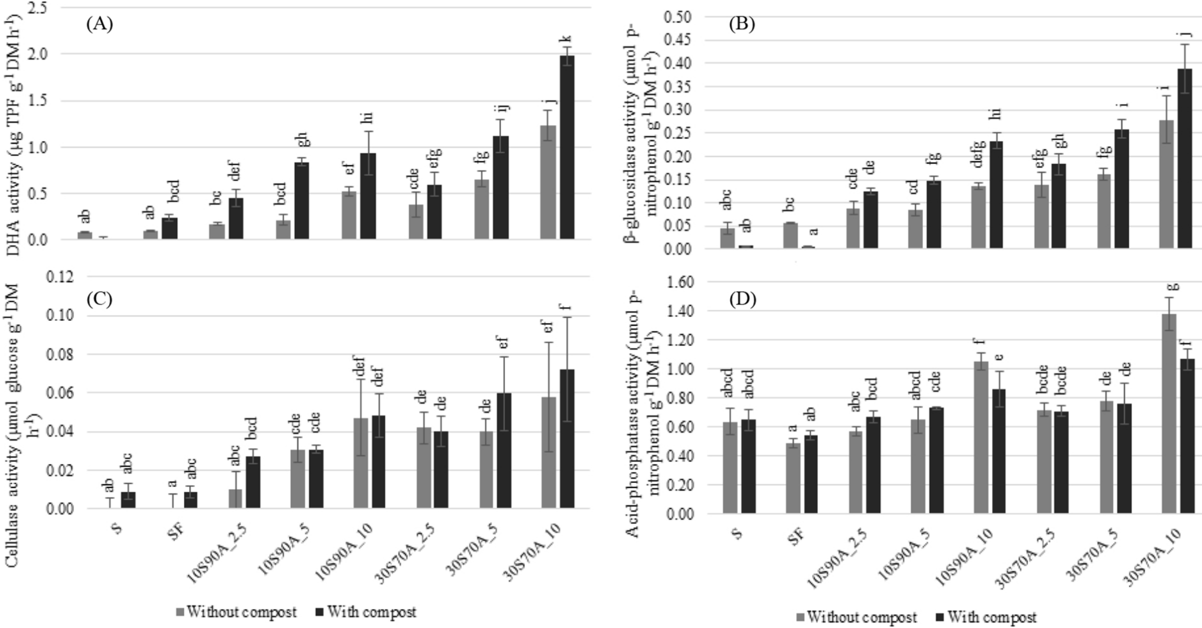

(A)
DHA activity (µg TPF g-1 DM h-1)
2.5
2.0
1.5
1.0
0.5
0.0
ab
ab
bcd
bc
def
bcd
gh
ef
hi
cde
efg
fg
ij
j
k
(B)
β-glucosidase activity (µmol p-nitrophenol g-1 DM h-1)
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
abc
ab
bc
a
cde
de
cd
fg
defg
hi
efg
gh
fg
i
i
j
(C)
Cellulase activity (µmol glucose g-1 DM h-1)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
ab
abc
a
abc
abc
bcd
cde
cde
def
def
de
de
de
ef
ef
f
(D)
Acid-phosphatase activity (µmol p-nitrophenol g-1 DM h-1)
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
abcd
abcd
a
ab
abc
bcd
abcd
cde
f
e
bcde
bcde
de
de
g
f
S
SF
10S90A_2.5
10S90A_5
10S90A_10
30S70A_2.5
30S70A_5
30S70A_10
■ Without compost
■ With compost

Figure 2

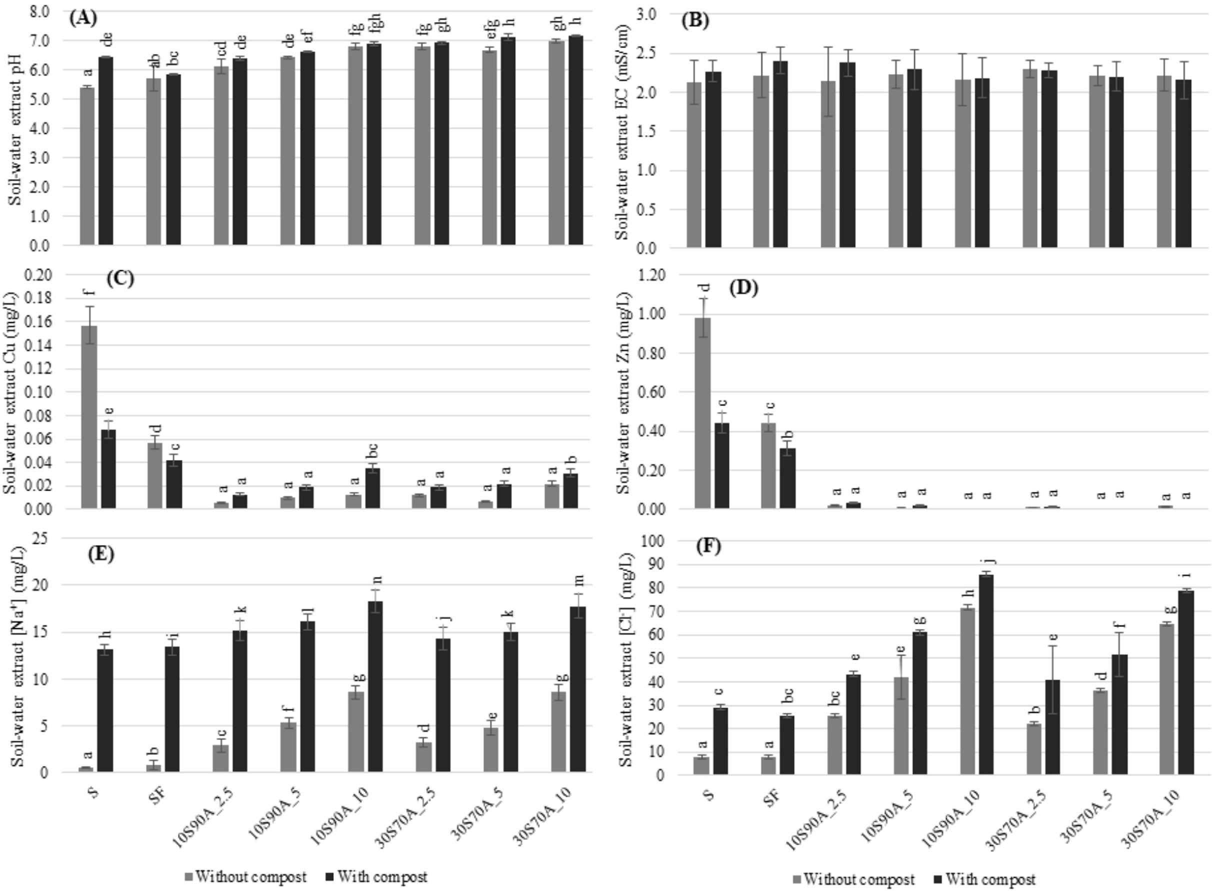

(A)
Soil-water extract pH
(B)
Soil-water extract EC (mS/cm)
(C)
Soil-water extract Cu (mg/L)
(D)
Soil-water extract Zn (mg/L)
(E)
Soil-water extract [Na⁺] (mg/L)
(F)
Soil-water extract [Cl⁻] (mg/L)
S
SF
10S90A_2.5
10S90A_5
10S90A_10
30S70A_2.5
30S70A_5
30S70A_10
Without compost
With compost

Figure 3

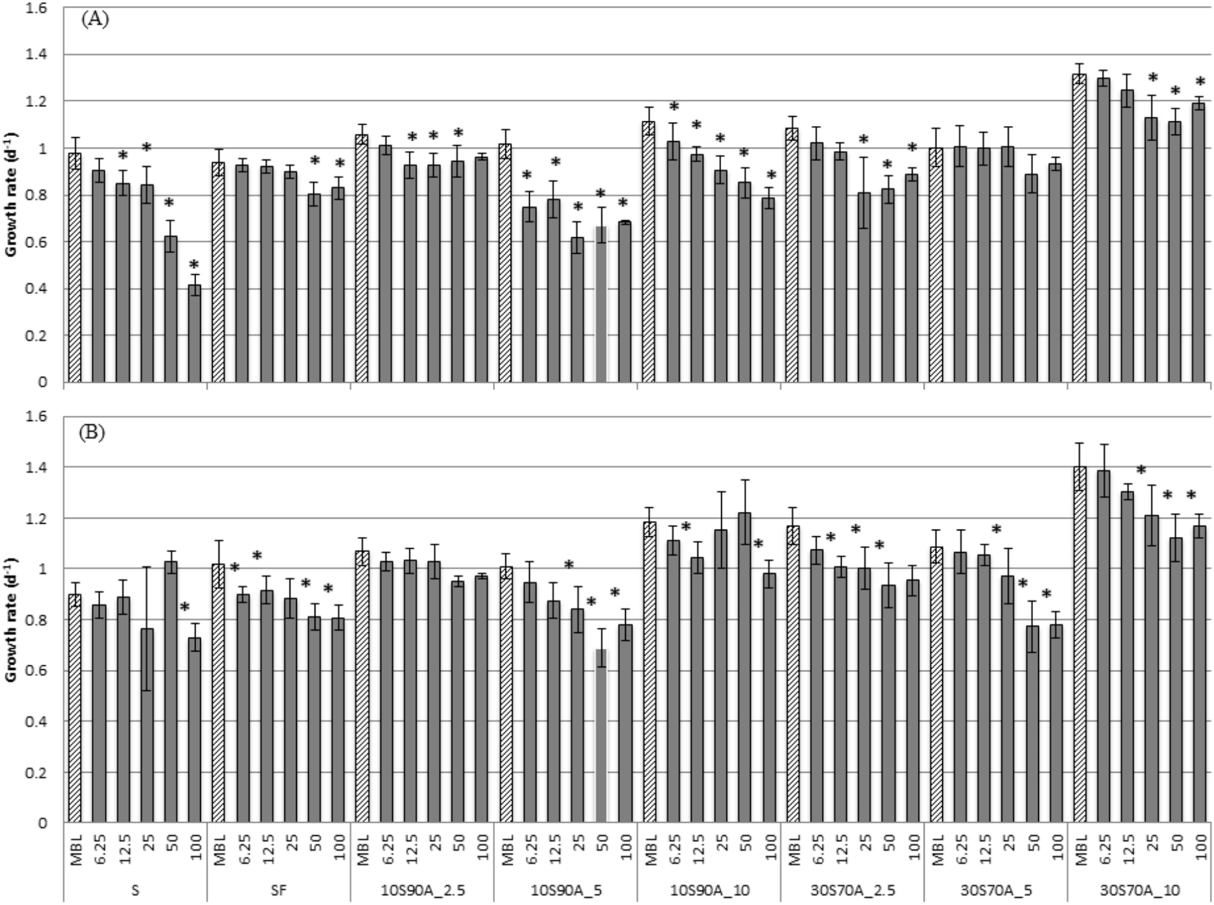

(A)
(B)
Growth rate (d⁻¹)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
MBL
6.25
12.5
25
50
100
S
SF
10S90A_2.5
10S90A_5
10S90A_10
30S70A_2.5
30S70A_5
30S70A_10
*

Figure 4