Ecotoxicology and Environmental Safety

Chemical and ecotoxicological effects of the use of drinking-water treatment residuals for the remediation of soils degraded by mining activities

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The aim of this study was to evaluate the use of drinking-water treatment residuals (DWTR) in the amendment of a soil affected by mining activities (Aljustrel mine, Portuguese sector of the Iberian Pyrite Belt), considering the effects on its chemical, biochemical and ecotoxicological characteristics. The DWTR had neutral characteristics (pH 6.7) and an organic matter (OM) content of 575 g kg\(^{-1}\) dry matter (DM), which makes them a potential amendment for the remediation of mine degraded soils, as they may correct soil acidity and reduce the extractable metal fraction. An incubation assay, with soil and DWTR, with or without lime, was carried out to test the doses to be used in the assisted-phytostabilization experiment. Based on the results obtained, the doses of DWTR used were the equivalent to 48, 96, and 144 t DM ha\(^{-1}\), with and without lime application (CaCO\(_3\) 11 t DM ha\(^{-1}\)). Agrostis tenuis Sibth was used as the test plant. Some amendments doses were able to improve soil characteristics (pH and OM content), to decrease metal extractability by 0.01 M CaCl\(_2\) (especially for Cu and Zn), and to allow plant growth, that did not occur in the non-amended soil. Copper, Pb and Zn concentrations in the plant material were lower than the maximum tolerable level for cattle feed, used as an indicator of risk of entry of those metals into the human food chain. The simultaneous application of DWTR (96 and 144 t ha\(^{-1}\)), with lime, allowed a reduction in the mine soil ecotoxicity, as evaluated by some lethal and sub-lethal bioassays, including luminescence inhibition of Vibrio fischeri, Daphnia magna acute immobilization test, mortality of Thamnocephalus platyurus, and 72-h growth inhibition of the green microalga Pseudokirchneriella subcapitata. However, DWTR were unable to increase soil microbial activity, evaluated by dehydrogenase activity, an important soil-health indicator. Also, OM content and N\(_{\text{Kjeldahl}}\) concentrations increased slightly but remained low or very low (P and K extractable concentrations were not affected). In general, the bioassays highlighted a decrease in soil ecotoxicity with the presence of lime and DWTR (144 t DM ha\(^{-1}\)). In conclusion, DWTR are recommended to amend acidic soils, with high concentrations of trace elements, but an additional application of organic or mineral fertilizers should be considered.

I Introduction

In Portugal, mining exploration is ancient, at least since the Roman occupation of the peninsula (Alvarenga et al., 2004). Generally, the ore extraction and processing led to the production of large volumes of waste rocks and tailings, which were deposited in the surroundings of the mine (Matos and Martins, 2006). Nowadays, many of these mines are abandoned, but the erosion of these soils, by wind and water, contributes to the progressive enlargement of the contaminated area (Alvarenga et al., 2004; Matos and Martins, 2006). In Portugal, thousands of hectares of abandoned mine lands, affected by low pH, poor
to act as metal adsorbents in tailings contaminated with Pb, Zn and Cd, and found promising results with one of the combinations, which allowed the revegetation of the tailing (Ippolito et al., 2011; Brown et al., 2007).

The improvement in soil quality, because of its remediation, can be assessed by general physicochemical properties, which are related to soil fertility status, and using chemical extraction procedures, as surrogate measures of trace elements immobilization (Rao et al., 2008; Alvarenga et al., 2009a, 2013; Davidson, 2013). However, chemical data should be complemented with results from biochemical and ecotoxicological tests, which allow an integrated evaluation of the toxic effects of pollutants on organisms and the interactions between contaminants, matrix and biota (ISO 17402, 2006; Leitägib et al., 2007; Alvarenga et al., 2009b; Épédé et al., 2009; Épédé et al., 2014). Ecotoxicological tests using aqueous soil extracts can be used to assess soil toxicity, not only because chemical compounds present in the soil aqueous phase affect soil organisms, but also because they evaluate the impact of soil composition on water and runoff to surrounding receiving waters (i.e. soil retention function) (van Gestel et al., 2001; Loureiro et al., 2005; Leitägib et al., 2007; Antunes et al., 2008; Alvarenga et al., 2008b, 2009b, 2016).

Another important indicator of soil fertility and ecological status are soil enzymatic activities. Dehydrogenase is an intracellular oxidase-eductase, related to the oxidative phosphorylation process, which, because of that, can be used as an overall indicator of microbial activity in a soil (Tabatabai, 1994; Gil-Sotres et al., 2005; Izquierdo et al., 2005; Tejada et al., 2006). That is why the measurement of its activity has been used by several authors as an early and sensitive indicator of soil health recovery in remediation processes (Perez-de-Mora et al., 2005, 2006; Tejada et al., 2006; Hinojosa et al., 2008).

Taking all these facts into account, the aim of this study was to evaluate the effectiveness of DWTR, from the Roxo water treatment plant (Alentejo - Portugal), as the amendment of a soil affected by mining activities (Aljustrel mine, Iberian Pyrite Belt), in order to assess: (i) the effects of the amendments in soil chemical properties, (ii) the capacity of the soil to establish a plant cover using Agrostis tenuis Sibth., (iii) the capacity of DWTR to immobilize metals in the soil, avoiding their transference to the plant, and (iv) the effects of the amendments in the soil ecotoxicological and biochemical properties.

2. Materials and methods

2.1. Characterization of the drinking water treatment residuals

Drinking water treatment residuals were obtained from a water treatment plant (WTP) which is located at the Roxo dam in Alentejo (Portugal). The water is captured in the dam and submitted to a sequence of processes and operations to produce a safe drinking water. First, the water passes through a screen to remove coarse material, and then to a pre-chlorination, by the injection of ClO₂, for algae control and arresting biological growth. The addition of a polyelectrolyte, Al₂O₃, promotes the coagulation and flocculation steps, which neutralizes the negative charge of the suspended colloids, promoting coagulation and the formation of thicker flocs, which settle during the sedimentation. The clear water obtained on top, after the settlement, will pass through sand and activated carbon filters, to remove dissolved particles, and, afterwards it is submitted to a final disinfection, again with ClO₂, which has a strong oxidizing potential and will not only kill any remaining microorganism, but also allow the elimination of iron and manganese.

Drinking water treatment residuals are produced during the sedimentation and filtration stages and are submitted to thickening to increase their solid content. First, an organic floculant is added (Superfloc C-496®), which will promote the liquid-solid separation, and then they are mechanical dewatered in a belt-filter press. At the time of this experiment, all DWTR produced at Roxo WTP were sent to a municipal landfill.

The characterization of the DWTR was performed in accordance with the Decree-Law No. 276/2009, which regulates the use of sewage sludge, and similar sludges, in agricultural practices (Table 1), with techniques described elsewhere (Alvarenga et al., 2015). The DWTR have neutral characteristics (pH 6.7), which may contribute to the
The soil used in the study was collected in the Aljustrel mine (Alentejo), a state company, which has mostly used constructive techniques to dig and contain the contaminated tailings and other waste materials which were deposited in the area, but the soil that was left uncovered still needs to be ameliorated with the best available techniques.

The soil was collected from the 20-cm topsoil, air-dried and sieved through a 2-mm non-metallic sieve. Soil physicochemical analysis were performed using well described methodologies (Alvarenga et al., 2008a): particle-size distribution was determined by the pipet method (Gee and Bauder, 1986); soil pH (H₂O) 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 analysed by the Kjeldahl method (N₀(θ globae)); total oxidizable organic carbon (Corg) 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-Riethm method (Riehm, 1958); and pseudo-total metal concentrations (Cd, Cr, Cu, Ni, Pb and Zn) were determined by flame atomic absorption spectrometry 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 used in the study can be classified as a loamy-sand soil (76% sand, 18% silt and 6% clay), very acidic (pH 3.05), with high electrical conductivity (EC 3.65 mS cm⁻¹), low in OM content (5.3 g kg⁻¹ DM), in essential nutrients (0.6 g kg⁻¹ DM N₀(θ globae), 32.2 mg P₂O₅ kg⁻¹ DM, and 12.4 mg K₂O kg⁻¹ DM), and with high levels of some trace elements (296.9 mg Cu kg⁻¹, 1269.4 mg Pb kg⁻¹ and 801.0 mg Zn kg⁻¹, all in a DM basis).

### 2.3. Incubation assay

An incubation assay was conducted to evaluate the ability of the DWTR to correct soil acidity and to decrease trace elements extractability. The results from this incubation assay will allow the selection of the conditions to be used in the phytostabilization assay (Section 2.4). Different doses of DWTR: 0, 4, 8, 16, 32 and 64 g DM kg⁻¹ (equivalent, approximately, to 0, 6, 12, 24, 48 and 96 t DM ha⁻¹), considering 20 cm depth, 1.5 t m⁻³ bulk density, and 50% soil fraction < 2-mm, were tested, with and without lime application (13.3 g DM kg⁻¹ CaCO₃, equivalent approximately to 11 t ha⁻¹), using 200 g of soil, three replicates per treatment. Mixtures were maintained at 60% water-holding capacity, in aerated boxes, at 20 ± 2 °C, for one month.

The effects of the amendments on the soil physicochemical characteristics were assessed by measuring different soil properties: pH (1:2.5 w/v, in deionised water), EC (1:5 w/v, in deionised water), and OM content, using the methods referred in 2.2 sub-chapter. Copper, Pb and Zn in a readily available fraction were assessed by extraction using a 0.01 M CaCl₂ solution (Alvarenga et al., 2009c). Extractions were performed for 2 h, in a horizontal reciprocate shaking, on a 1:10 (w/v) soil to solution ratio, at room temperature. The extract was separated from the solid residue by filtration through a Whatman-40 filter, acidified to pH < 2 with HNO₃, and kept at 4 °C until analysis (Alvarenga et al., 2009c). Copper, Pb and Zn were analysed using a Varian apparatus (SpectrAA 220FS). Three independent replicates were performed for each sample and blanks were measured in parallel.

The highest dose of sludge (96 t DM ha⁻¹), caused a two-fold increase in the soil OM content and a decrease in its salinity (Table 2). The increase in the soil pH was more pronounced with the simultaneous application of CaCO₃. A significant decrease in the Cu and Zn CaCl₂:0.01 M extractable concentrations was observed: extractable Cu decreased from 111.4 to 0.2 mg kg⁻¹ DM, and Zn from 712.0 to 8.1 mg kg⁻¹ DM, respectively, with the simultaneous application of 96 t DM ha⁻¹ DWTR and CaCO₃ (Table 2). Lead extractable content was very low, even for the non-amended soil, lower that the detection limit of the technique (< 1.67 mg kg⁻¹, data not shown).
2.4. Assisted-phytostabilization experiment

Considering the results from the previous incubation assay, a pot experiment was assembled to evaluate the effects of the DWTR on soil properties in a less controlled scenario. As the acidity correction and OM content improvement were not very effective following the application of 12 and 24 t DWTR ha\(^{-1}\), they were not used in the pot experiment, and a higher application dose was tested: 144 t DWTR ha\(^{-1}\). In this experiment, the pots were filled with 3 kg of soil, amended with different doses of DWTR: 0, 32, 64, and 96 g DM kg\(^{-1}\) (equivalent, approximately, to: 0, 48, 96, and 144 t DM ha\(^{-1}\)), with and without lime application (13.3 g DM kg\(^{-1}\) CaCO\(_3\), equivalent approximately to 11 t ha\(^{-1}\)), four replicates per treatment. Pots were watered \textit{ad libitum} and maintained outdoors. \textit{Agrostis tenuis} Sibth (equivalent to 250 kg seeds ha\(^{-1}\)) was seeded one week after amendments application, and the pots were left outdoors for three months. \textit{A. tenuis} has been used because it is considered suitable for the reclamation of metaliferous sites, due to its tolerance to acid lead/zinc wastes, and copper tolerances (Alvarenga et al., 2014; Williamson and Johnson, 1981). Its metal tolerance may result from a metal exclusion strategy, comprising avoidance digestion), and extractable trace elements in soils (Cu, Pb and Zn; extracted at 500 °C for 6 h, dissolved with 10 mL of 3 M HCl and evaporated to near dryness twice, dissolved again with the same acid solution, (Whatman-40), and adjusted to a volume of 100 mL with ultra-pure water. The digested samples were analysed for total Cu, Pb and Zn using the procedures described previously by Alvarenga et al. (2008a).

The effects of the amendments on the soil chemistry were assessed by measuring the properties already referred: pH(H\(_2\)O), EC, OM, \(N\_\text{NKR}a\text{lab}\), extractable P and K, total trace elements in soils (Cu, Pb and Zn; aqua-regia digestion), and extractable trace elements in soils (Cu, Pb and Zn; extracted by 0.01 M CaCl\(_2\)).

2.5. Soil biochemical and ecotoxicological characterization

Biochemical status of the soil was assessed by measuring dehydrogenase activity, according to Tabatabai (1994), with modifications.

Soils sub-samples, at their “field moisture content” were passed through a 2-mm sieve and incubated for 16 h, at 25 °C, with 0.1% (w/v) triphenyltetrazolium chloride (TTC) in a Tris-buffer (0.1 M, pH 7.8 for acid soils, pH 7.6 for neutral soils), which allows the reduction of TTC to triphenylformazan (TPF), which is measured spectrophotometrically at 546 nm. Dehydrogenase activity was expressed in g TPF g\(^{-1}\) h\(^{-1}\), on an oven-dried soil weight basis (105 °C, 48 h).

The effect of the DWTR application on the soil retention function was evaluated using the soil water-extract, obtained according to the DIN 38414-S4 (1984) methodology. Briefly, leaching was carried out using a batch test, with a single leaching cycle, at room temperature, during 24 h, under constant agitation, using deionised water in a solid-to-liquid ratio of 1:10 (w/v). The leachate was separated by centrifugation (3000g, 30 min), and filtered through a membrane filter Whatman-40 of pore size 0.45 μm. The soil water-extracts obtained were analysed for pH and EC and used in the different bioassays with their intrinsic pH values.

Four different bioassays were carried out 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-h growth inhibition of the green microalgae Pseudokirchneriella subcapitata (OECD 201, 1984).

### Table 2

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Soil properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>DWTR (t ha(^{-1}))</td>
<td>CaCO(_3) (t ha(^{-1}))</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>11</td>
</tr>
<tr>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td>48</td>
<td>11</td>
</tr>
<tr>
<td>96</td>
<td>0</td>
</tr>
<tr>
<td>96</td>
<td>11</td>
</tr>
</tbody>
</table>

DWTR: drinking-water treatment residuals; EC: electrical conductivity; DM: dry matter.
The inhibition of algal growth was estimated as percentage of reduction of growth rate with respect to the control:

\[ \% I = \left[ \frac{\rho_c - \rho_f}{\rho_c} \right] \times 100 \]  

(2)

Where: \( \% I \) is the mean percentage of inhibition for specific growth rate; \( \rho_c \) is the mean value for the growth rate in the control, and \( \rho_f \) is the mean value for the growth rate in the water samples.

2.6. Statistical treatment of data

All physicochemical data were subjected to one-way ANOVA to evaluate statistical differences between tested treatments. Whenever significant differences were found (\( p < 0.05 \)), a post hoc Tukey HSD test was used to further elucidate differences among means (\( p < 0.05 \)).

The EC_{50} values corresponding to \( T. platyurus \) mortality and to \( D. magna \) immobilization tests were calculated using the Probit Method (Finney, 1971). For the \( V. fischeri \) bioluminescence inhibition test EC_{50} values were determined using LUMISSoft 4 Software™. The effects on growth inhibition of algae were checked for normality by the Kolmogorov-Smirnov test and variance homogeneity (Levene's tests). As the ANOVA assumptions were not met, data were analysed non-parametrically using Kruskal-Wallis ANOVA by ranks test. When significant differences were found (\( p < 0.05 \)), a post-hoc Dunn's test was used to compare sampling stations with the control with a \( p \)-value of 0.05 as the minimum significant level (Zar, 1996). All statistical analyses were performed with the STATISTIC 6.0 Software™ (StatSoft, Inc., 2001).

3. Results and discussion

3.1. Effects on physicochemical soil properties

The amendments led to a significant increase in soil pH (Fig. 1-A), especially with the simultaneous application of lime. The application of DWTR alone, caused an increment in soil pH from 3.3 to 4.9, for the highest application dose, but the application of 11 t CaCO₃ ha⁻¹ allowed an increase in soil pH of about 1.2-1.5 units, in addition to the pH increment brought by the DWTR application, for each treatment. This seems an important contribution, since, considering the results, the increment of the DWTR application rate by itself, from 48 to 96, and from 96 to 144, was not able to promote a proportional significant increase in the soil pH, which remained acid.

Electrical conductivity values were not affected by the DWTR application, that is, the treatments did not increase soil salinity (Fig. 1-B). In fact, DWTR salinity (EC 0.92 mS cm⁻¹) is lower than the soil salinity itself (EC 3.65 mS cm⁻¹). This is an interesting characteristic, since most of the soil conditioners, both organic and inorganic, can promote and undesired increase in soil secondary salinity and, because of that, their application....
doses need to be restricted (Alvarenga et al., 2015). This is an extra concern when the salinity of the soil to be remediated is already high, which is the case.

Soil OM content increased significantly: the highest dose of sludge (144 t DM/ha), caused a three-fold increase in the soil OM content (Fig. 1-C), while N\textsubscript{extractable} content (Fig. 1-D) doubled with the same application rate of sludge. However, despite this high increase, the classification of the OM content only turned from a ‘very low’ to ‘low’ OM content classification, in accordance with LQARS (2000), for the highest dose of sludge (144 t DM/ha). Elliott and Dempsey (1991), have already alerted for that, soil conditioning effects, resulting from increased organic matter content through DWTR application, are quite small. The same is true for N in DWTR, although its agronomic benefits depend more on its availability than on the total amount present, which is governed by the mineralization rate (the rate at which waste-borne nutrients are converted into forms available to plants; Elliott and Dempsey, 1991). Several studies proved that the addition of DWTR to soil did not increase N mineralization rate, and that a low percentage of N in DWTR is available to crops in the first growing season (Elliott and Dempsey, 1991).

As for the K\textsubscript{extractable} Content (Fig. 1-E), it did not increase after DWTR application, while K\textsubscript{extractable} content increased significantly only for the application of 96 and 144 t DM/ha of DWTR with lime (Fig. 1-F). Moreover, both P and K extractable concentrations are still considered as ‘very low’ or ‘low’ (LQARS, 2000) in the amended soils, indicating that additional mineral fertilization should be considered when DWTR are applied, to establish a wealthy plant cover. The results for the low content of P in the amended soils could be expected, because there are studies showing that DWTR have a strong capacity to adsorb P, and that the adsorption process is fast and nearly irreversible (Elliott and Dempsey, 1991; Ippolito et al., 2011). In fact, Elliott and Dempsey (1991) stated that the reduced availability of P to crops is a main disadvantage of DWTR application to agronomic soils. Therefore, since DWTR application can induce P deficiency in crops, further studies need to ascertain application doses and the need for additional fertilization with phosphate salts when DWTR are used in mine soil remediation.

3.2. Effects on trace elements extractable fractions

As expected, total metal concentrations, extracted by aqua regia, did not change because of amendments application, maintaining values that were not statistically different for each treatment: 269 ± 89 mg kg\textsuperscript{-1} DM for Cu; 1479 ± 443 mg kg\textsuperscript{-1} DM for Pb; and 576 ± 99 mg kg\textsuperscript{-1} DM for Zn, mean values ± standard deviation, n = 32. Similar results have been obtained by Garau et al. (2014) when applying 3% w/w Fe-DWTR to a circumneutral contaminated soil.

In contrast, a significant decrease in the Cu and Zn CaCl\textsubscript{2}-extractable content was observed (Figs. 2-A and 2-B, respectively), emphasizing the ability of the DWTR to immobilize trace elements by adsorption/occlusion. That ability was higher for Cu, which CaCl\textsubscript{2}-extractable content decreased from 75.2 to 2.0 mg kg\textsuperscript{-1} DM, with the application of 144 t DM/ha DWTR. The decrease in Zn CaCl\textsubscript{2}-extractable content was more pronounced with the simultaneous application of lime, allowing a further decrease in its extractability to less than half of the value that was accomplished by the single application of DWTR. In fact, as previously discussed, the increase in soil pH was more efficient by the simultaneous application of a liming agent, and not by the sole application of DWTR, which has a pH of 6.7. In fact, the significant increase in soil pH, relatively to the mine soil, by the application of the DWTR, was only possible because the soil was very acidic. Consequently, DWTR oxides played an important role on metal adsorption/occlusion, but that effect would be more pronounced if higher soil pH values were obtained, that is, if higher CaCO\textsubscript{3} application rates were applied.

Lead CaCl\textsubscript{2}-extractable content was determined in the sequence of the amendments. The values were very low (< LD =1.67 mg kg\textsuperscript{-1}), even before the amendments application, and despite the high total Pb concentration in the non-amended soil. The authors have also noticed that fact in previous studies in mines from the Iberian Pyrite Belt, where Pb was pointed as the trace element with the lower bioavailable values (Alvarenga et al., 2012).

Plants germinated but died in the non-amended pots and in the pots amended with 48 t ha\textsuperscript{-1} DWTR, without lime (Fig. 2-C). The other combinations of amendments allowed the establishment of a plant cover, however, with high differences between replicates.

Copper, Pb and Zn concentrations in the aboveground plant material were measured and the concentrations found with the application of 144 t ha\textsuperscript{-1} DWTR were used to evaluate the possibility of using the plant in a phytostabilization strategy (Table 3). Considering the concentrations in contaminated plants from Kabata-Pendias and Pendias (2001), Pb concentrations found in the plant material were lower than the typical values for contaminated plants, which could be expected, taking in consideration the low Pb CaCl\textsubscript{2}-extractable concentrations found in the soils. The same was not true for Cu and Zn, especially for Zn in the higher application rate (Table 3), with concentrations which are typical of contaminated plants. However, Cu, Pb and Zn concentrations in the plant material were still lower than the maximum tolerable level for cattle feed, according to the National Research Council (2005). Those limit values can be used as an indicator of risk of entry of those metals into the human food chain (Table 3), which suggests that there is a low risk of their transference if this phytotechnology were to be applied.

3.4. Effects on soil biochemical and ecotoxicological properties

There are several microbial endpoints (e.g. microbial biomass-C, dehydrogenase activity, N-mineralization, number of culturable heterotrophic bacteria, soil basal and substrate-induced respiration, soil exoenzymes activities) which can be used to assess the ability of different amendments to improve soil health in remediation studies (Lombi et al., 2004; Pardo et al., 2011; Perez-de-Mora et al., 2011; Alvarenga et al., 2014; Garau et al., 2014; Galende et al., 2014). It is commonly acknowledged that, since dehydrogenase is an oxidoreductase, which is only present in viable cells, the results from dehydrogenase activity represent a good indicator of the active microbial population of a soil (Tabatabai, 1994; Izquierdo et al., 2005), and, consequently, that microbial endpoint was selected to be used in this study. In this case, the treatments did not affect dehydrogenase activity, which remained with very low activities, lower than the quantification limits, QL, of the technique (< QL = 1 ^g TPF g\textsuperscript{-1} DM h\textsuperscript{-1}). Because of that, it is possible to conclude that DWTR were not able to improve the soil overall microbial activity, which is partly a consequence of their own low microbial activity. Garau et al. (2014) also found that the level of soil microbial biomass-C remained the same after the application of 3% Fe-DWTR to a circumneutral trace element contaminated soil, which indicates a deficient ability of DWTR to improve soil microbial activity.
activity. However, the same authors found a significant increase in the number of culturable heterotrophic bacteria after the application of Fe-DWTR, suggesting that the number of culturable bacteria can be a more sensitive indicator of the microbial status of a polluted soil than microbial biomass-C (Garau et al., 2014).

Considering the results from the ecotoxicological tests, some amendment doses of DWTR, especially with the simultaneous application of CaCO₃, led to a reduction in the mine soil water-extract ecotoxicity (Table 4 and Fig. 3).

Soil water-extract toxicity towards V. fisheri decreased in the higher DWTR application rates, 96 and 144 t ha⁻¹, with or without lime (Table 4). For D. magna, the toxicity decreased with the application of the higher application rates of DWTR, 96 and 144 t ha⁻¹, and the toxic response disappeared with the simultaneous application of DWTR and lime (Table 4). The toxicity towards T. platyurus also decreased as a response to DWTR application, especially with the simultaneous application of lime. The bioassay with T. platyurus was the most sensitive, when we evaluate the acute toxicity (EC₅₀ values), induced by the soil water-extractable trace elements fraction, with and without amendments. The sensitivity of this crustacean already has been reported by the authors, in other liquid matrices, such as domestic wastewaters and irrigation waters (Palma et al., 2016). The growth inhibition of the microalga P. subcapitata was only positively reverted with the simultaneous application of DWTR and lime, with values of 27% reduction in the growth inhibition, comparing with the control, for the higher application rate.

Considering these results, it is possible to conclude that the application of the DWTR, especially for the higher application rates, 96 and 144 t ha⁻¹, positively affected mine soil water-extract composition, by correcting its acidity and trace elements extractability - the soil was effectively chemically stabilized.

### Table 3

Trace elements concentration in the aboveground plant material with the application of 144 t ha⁻¹ of DWTR (mean ± standard deviation, n = 8), typical concentrations found in the literature for contaminated plants, and limit values for trace elements in amendment doses of DWTR, especially with the simultaneous application.

<table>
<thead>
<tr>
<th>Element</th>
<th>Concentration in the plants (mg kg⁻¹ DM)</th>
<th>Concentrations in contaminated plants (mg kg⁻¹ DM)</th>
<th>Maximum tolerable level for cattle (mg kg⁻¹ DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>34 ± 5</td>
<td>20 - 100</td>
<td>40</td>
</tr>
<tr>
<td>Pb</td>
<td>24 ± 12</td>
<td>30 - 300</td>
<td>100</td>
</tr>
<tr>
<td>Zn</td>
<td>477 ± 259</td>
<td>100 - 400</td>
<td>500</td>
</tr>
</tbody>
</table>

*Kabata-Pendas and Pendas (2001); *National Research Council (2005); DWTR: drinking-water treatment residuals; DM: dry matter.

### Table 4

Soil water-extract characteristics: pH, electrical conductivity (EC; mS cm⁻¹) (mean ± standard-deviation, n = 3), and ecotoxicological responses calculated as EC₅₀ values (mean values % v/v; 95% confidence interval, CI; n = 2 for V. fisheri, and n = 3 for T. platyurus and D. magna).

<table>
<thead>
<tr>
<th>DWTR (t ha⁻¹)</th>
<th>CaCO₃ (t ha⁻¹)</th>
<th>Soil water-extract pH</th>
<th>Soil water-extract EC</th>
<th>V. fisheri 30 min-EC₅₀</th>
<th>T. platyurus 24h-EC₅₀</th>
<th>D. magna 48h-EC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>2.8 ± 0.0</td>
<td>2.14 ± 0.16</td>
<td>43.2 (42.4-44.0)</td>
<td>0.5 (0.4-0.6)</td>
<td>5.1 (3.0-7.0)</td>
</tr>
<tr>
<td>0</td>
<td>11</td>
<td>4.9 ± 0.5</td>
<td>1.35 ± 0.06</td>
<td>nt</td>
<td>2.2 (1.8-2.8)</td>
<td>4.0 (1.8-5.7)</td>
</tr>
<tr>
<td>48</td>
<td>0</td>
<td>4.2 ± 0.0</td>
<td>1.90 ± 0.17</td>
<td>27.2 (27.0-27.5)</td>
<td>0.4 (0.3-0.5)</td>
<td>4.7 (2.6-6.6)</td>
</tr>
<tr>
<td>48</td>
<td>11</td>
<td>5.9 ± 0.4</td>
<td>1.40 ± 0.03</td>
<td>53.6 (53.4-53.8)</td>
<td>21.6 (9.9-31.8)</td>
<td>nt</td>
</tr>
<tr>
<td>96</td>
<td>0</td>
<td>4.8 ± 0.3</td>
<td>1.35 ± 0.06</td>
<td>nt</td>
<td>12 (7.7-16.2)</td>
<td>22.0 (17.0-19.5)</td>
</tr>
<tr>
<td>96</td>
<td>11</td>
<td>6.1 ± 0.4</td>
<td>1.57 ± 0.11</td>
<td>nt</td>
<td>19.7 (15.4-24.5)</td>
<td>nt</td>
</tr>
<tr>
<td>144</td>
<td>0</td>
<td>4.7 ± 0.0</td>
<td>1.59 ± 0.07</td>
<td>95.6 (94.4-96.8)</td>
<td>4.4 (2.6-6.1)</td>
<td>31.2 (25.7-39.4)</td>
</tr>
<tr>
<td>144</td>
<td>11</td>
<td>6.1 ± 0.2</td>
<td>1.71 ± 0.01</td>
<td>nt</td>
<td>51.0 (36.1-57.0)</td>
<td>nt</td>
</tr>
</tbody>
</table>

DWTR: drinking-water treatment residuals; EC₅₀: soil water-extract concentration, % v/v, at which a toxic effect on 50% of the exposed organisms can be observed; nt: non-toxic.
Fig. 3. Growth rate (d⁻¹) from the microalga P. subcapitata after 3 days exposed to the treatments with different doses of DWR, with and without lime (mean ± SD; n = 6; * p < 0.05. Dunnett's test with a control (MBL).}

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