



Evaluation of the suitability of three Mediterranean shrub species for phytostabilization of pyritic mine soils



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ABSTRACT

A greenhouse pot experiment was conducted to evaluate the potential use of *Nerium oleander*, *Cistus albidus* and *Pistacia lentiscus* for phytostabilization of acidic mine soils. The selected species were grown in mine tailing soil, unamended (TS) and amended with calcium carbonate and pig manure (ATS), and in a reference unpolluted substrate for control (CT); plant growth, root characterization, soil trace element contents, and their accumulation in plants were measured. Results indicated that seed emergence was independent of the substrate characteristics, but seedlings were seriously affected and died in TS, with survival of 5–40% in ATS. Only stem biomass of *P. lentiscus* and root volume of *N. oleander* were negatively affected when grown in TS, but without differences between ATS and CT. There were significant negative correlations between soil and plant trace element concentrations and plant biomass and root development, indicating the effect of trace elements on plant growth. The application of amendments reduced the soil exchangeable and extractable fraction concentrations of trace elements in ATS compared with TS, mainly for As, Cd and Pb. The tested species directly contributed to the immobilization of trace elements in the soil. As a general pattern, trace element concentrations in plants grown in the unamended tailing soil were similar to those reported for the amended soil, with levels below toxicity thresholds. Thus, these species fulfill the criteria to be used for phytostabilization purposes, aided by application of amendments to increase plant growth.

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1. Introduction

Heavy metal and metalloid contamination of soils is a major environmental problem that can reduce the productivity of the ecosystems and enhance environmental risk for humans and other organisms (Kabata-Pendias and Pendias, 1991). In mining areas, most of the mine tailings, which may extend in some cases over hundreds of hectares, are now abandoned without any particular safety measures and with a high environmental impact on the surrounding ecosystems and populations (Bradshaw, 1997). In these tailing ponds, waste usually contains acid materials rich in Fe-oxyhydroxides, sulphides, sulphates, and trace elements. As a consequence, these soils remain bare of vegetation and have low fertility (Acosta et al., 2011; Martínez-Pagán et al., 2011). Therefore, it is necessary to take actions towards the remediation of these sites, since environmental hazards, especially water and wind erosion, stand out with propensity to adversely affect both human health and the functioning of ecosystems (Kabas et al., 2012).

Phytomanagement takes its place as a feasible reclamation technique in the removal or stabilization of soil trace elements by the use of vegetation and soil amendments. The reclamation of abandoned

mine sites relies on achieving optimal conditions for plant growth by improving the soil's physical, chemical and biological characteristics by using different amendments (Forján et al., 2014; Hattab et al., 2015; Li et al., 2015). Nevertheless, after the implementation of any environmental reclamation project, monitoring is essential in order to find out if main objectives have been reached (Pastorok et al., 1997). Incorporation of organic residues as amendments into mine soils has been proposed as a feasible, inexpensive and environmentally sound disposal practice as such residues can improve the soil's physical and chemical properties, and contain nutrients beneficial to microorganisms and plants (Zornoza et al., 2012, 2013). Alkaline materials are commonly used as an amendment for ameliorating the acidic conditions of many acid-generating mine wastes and for immobilizing metals in the form of carbonates, mitigating metal toxicity (Pardo et al., 2011; Zornoza et al., 2012, 2013).

As a general rule, for the creation of a self sustaining vegetation cover through the phytomanagement of a contaminated site, native species that are adapted to the specific conditions are preferred, as their use prevents the introduction of non-native and potentially invasive species that may result in decreasing local plant diversity and endangering of the harmony of the ecosystem (Kabas et al., 2011; Mendez and Maier, 2008). Hence, plant screening is a prerequisite for successful revegetation. Numerous studies have reported the ability of herbaceous species

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to potentially phytoremediate mine soils (e.g. Arnetoli et al., 2008; Boojar and Tavakkoli, 2011; Conesa et al., 2007; Kabas et al., 2012; Ye et al., 1999), with fewer studies evaluating the efficiency of woody species (Domínguez et al., 2008; Shi et al., 2011; Tapia et al., 2011; Trigueros et al., 2012; Yanqun et al., 2004). Compared to herbaceous species, woody species constitute a major part of total plant biomass in native Mediterranean shrub lands (Domínguez et al., 2008). In addition, most studies have tested the accumulation of trace elements by crop species, and few species that would be planted in an ecological restoration project have been evaluated regarding their phytoremediation potential.

In this study, three Mediterranean shrub species (*Nerium oleander* L., *Cistus albidus* L. and *Pistacia lentiscus* L.) were tested as potential metal-tolerant plants for use in phytostabilization of contaminated mine soils. The selected plants were grown in a mine soil, unamended and amended, and in a reference unpolluted substrate used as positive control; plant growth, root characterization, soil trace element contents, and their translocation in plants were measured. The objectives of this study were 1) to assess if these species are able to emerge and grow in contaminated mine soils; 2) determine the efficiency of the amendments used (calcium carbonate and pig manure) for the improvement of growth and reduction of the metal uptake by native plants; and 3) to assess the potential use of these species for phytoremediation.

2. Material and methods

2.1. Soil, amendments and plant material

Soil from a tailing pond at the Mining District of Cartagena-La Unión (SE Spain) (37° 35' 38" N, 0° 53' 11" W) was selected. It was characterized by absence of vegetation, high trace element concentrations, low organic carbon content and affection by wind and water erosion. The climate of the area is semiarid Mediterranean, with a mean annual temperature of 18 °C and mean annual rainfall of 275 mm. Soil is classified as a Spolic Technosol (Toxic) (IUSS, 2007), with a sandy loam texture. Soil was collected from the top 20 cm, air-dried for 7 days, and sieved <2 mm for pot experiments and analyses. Substrate was prepared in the laboratory containing (by volume) sphagnum peat (40%), coconut fibre (40%) and perlite (20%), which was used as unpolluted control growing medium. We used two different amendments (pig manure and marble waste (CaCO₃)) for reclamation purposes, in order to increase soil organic matter and soil nutrients, decrease heavy metals availability, ameliorate soil structure and neutralize acidity. The pig manure came from a pig farm in Pozo Estrecho (SE Spain) and the marble

Table 1
Main properties and total concentration of trace elements for soils and amendments used.

Property	Mine tailing soil	Control soil	Pig manure	Marble waste
pH	3.5	7.2	9.1	8.0
Electrical conductivity (dS m ⁻¹)	2.3	1.6	10.2	2.2
CaCO ₃ (%)	<d.l.	<d.l.	n.d.	99.0
Total organic carbon (g kg ⁻¹)	1.78	172.92	170.81	n.d.
Total N (g kg ⁻¹)	0.21	3.91	13.6	n.d.
C/N	8	44	13	n.d.
Available P (g kg ⁻¹)	<d.l.	224	9.64	<d.l.
As (mg kg ⁻¹)	177.91	2.23	6.35	<d.l.
Cd (mg kg ⁻¹)	13.28	0.29	0.08	0.05
Cu (mg kg ⁻¹)	42.31	8.97	157	0.36
Pb (mg kg ⁻¹)	1141.98	6.22	7.99	<d.l.
Zn (mg kg ⁻¹)	3877.69	2091.0	732	0.26
Exchangeable Ca (g kg ⁻¹)	8.97	4.14	0.85	2.19
Exchangeable Mg (mg kg ⁻¹)	321	568	802	347
Exchangeable Na (mg kg ⁻¹)	174.22	919.90	4.24	0.06
Exchangeable K (mg kg ⁻¹)	36.04	2935.92	15.66	0.05

n.d.: not determined.

<d.l.: below detection limit (CaCO₃: 0.07%; P: 60 µg kg⁻¹; As: 7 µg kg⁻¹; Pb: 30 µg kg⁻¹).

waste was collected from quarries in Cehegín (SE Spain). Soil, substrate and amendment characteristics are shown in Table 1.

The experiments were performed with three different substrates: unamended tailing soil (TS), amended tailing soil (ATS) and unpolluted substrate (control, CT). For ATS, marble waste was added in a rate of 10 g kg⁻¹. This rate was calculated considering the quantity of lime required to neutralize all the potential acid according to the percentage of sulphides present in the mine soil, to reach a final pH of 7. Pig manure was added at 23 g kg⁻¹. This dose was calculated on the basis of its organic carbon content to increase soil organic carbon by 4 g kg⁻¹, which is the organic carbon content of the soils from the area.

Seeds from *N. oleander* L., *C. albidus* L. and *P. lentiscus* L. were obtained from the storehouse of seeds of the Plant Production Department, Universidad Politécnica de Cartagena. Seedlings from the same species were acquired from local nurseries. These species were selected because they are native Mediterranean shrubs that grow in the study area, and their adoption for reclamation strategies hinders the introduction of non-native and potentially invasive species in the ecosystem.

The experiments were conducted at "Tomás Ferro" Experimental Agro-Food Station, Universidad Politécnica de Cartagena (UPCT; 37° 41' N; 0° 57' W).

2.2. Emergence assay

The target species *N. oleander* L., *C. albidus* L. and *P. lentiscus* L. were sown in trays of alveoli of 10 × 6 units, with twelve replications per species and soil (TS, ATS and CT). Each alveolus had a volume of 150 cm³, where one seed was separately introduced. They were placed in a greenhouse covered with thick thermic polyethylene and the environmental conditions were not artificially modified. It had only natural light and controlled nebulisation irrigation was daily used, 15 min per day.

The seeding was effectuated on 25th July 2011. During 50 days emergence and mortality of seedlings were recorded weekly. After that, the survival percentage of emerged seedlings was recorded up to 30th January 2012 (6 months after seeding).

2.3. Plant growth experiment

Six units of *N. oleander*, *C. albidus* and *P. lentiscus* seedlings (15–20 cm in height) were transplanted into 4500 cm³ plastic pots, and filled up with the three different substrates: TS, ATS and CT. For transplanting, the growing medium was not eliminated from the roots to avoid the loss of small roots and simulate the real scenario in case this reclamation strategy is carried out large-scale in the field. The plants were grown in the same greenhouse as the emergence experiment from 24th February 2012 to 4th June 2012 (120 days). The plants were irrigated daily with controlled nebulization irrigation (15 min per day). The environmental conditions of the greenhouse were not artificially modified. Plant height, stem number and leaf colour (Hue angle) were measured every 15 days.

At the end of the experiment, the 54 plants were carefully removed, washed with tap water to remove any attached particles and rinsed with deionized water, and then divided into roots, stems and leaves. The roots were carefully washed by hand with ample water to clean off soil but to preserve as many roots as possible. Analyses of root lengths (RL), areas (RA), and volumes (RV) were determined using a Winrhizo LA 1600 root counter (Regent Inc., Quebec, Canada) from pictures taken of each root system by a double-pass scanner incorporated in the counter. The roots, stems and leaves were oven-dried at 55 °C to a stable weight, and the dry biomass was recorded. The dried material was then ground using a mill (A11 Basic, IKA). For each sample, 0.7 g was incinerated prior to a metal redilution using 6 N HNO₃. Plant extracts were stored at 4 °C until analyzed. Soil from each pot was also removed for analysis.

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