applied sciences

MDPI

Article

Propagation of *Atriplex halimus* (Mediterranean Saltbush) in Multi-Contaminated Mine Tailings by Unrooted Cuttings

Marta Canu [1], Stefano Milia [2,*], Stefano Ubaldini [2], Elena Tamburini [3], Alessandra Carucci [2,4] and Giovanna Cappai [2,4]

[1] Agricultural Research Agency of Sardinia (AGRIS), Viale Trieste 111, 09123 Cagliari, Italy; mcanu@agrisricerca.it
[2] Institute of Environmental Geology and Geoengineering, National Research Council (IGAG-CNR), Via Marengo 2, 09123 Cagliari, Italy; stefano.ubaldini@cnr.it
[3] Department of Biomedical Sciences, University of Cagliari (DiSB-UniCA), Cittadella Universitaria Sp. 8, 09042 Monserrato, Italy; etamburini@unica.it
[4] Department of Civil, Environmental Engineering and Architecture, University of Cagliari (DICAAR-UniCA), Via Marengo 2, 09123 Cagliari, Italy; carucci@unica.it (A.C.); gcappai@unica.it (G.C.)
* Correspondence: stefano.milia@cnr.it

Featured Application

This study demonstrates the potential of using unrooted cuttings of *Atriplex halimus* directly in metal-contaminated mine tailings as a cost-effective strategy for phytoremediation. The approach simplifies plant propagation, reducing both time and resource demands while maintaining metal uptake efficiency. Direct propagation by unrooted cuttings can be implemented at mining sites to foster clonal selection and, therefore, enhance in situ remediation. Plants' behaviour under field conditions and over longer durations is worth further investigation.

Abstract

Phytotechnologies offer sustainable solutions for remediating mine residues by combining site rehabilitation with the potential recovery of secondary and critical raw materials (SRMs and CRMs, respectively), contributing to resource efficiency strategies. This study explored the direct propagation of *Atriplex halimus* unrooted cuttings into metal-contaminated mine tailings, assessing survival, biomass production, and trace metal accumulation. Treatments were carried out on mine tailings, with and without the addition of organic and inorganic amendments, and on commercial soil as a control. After an 8-week preliminary trial, *Atriplex halimus* demonstrated moderate survival and growth without phytotoxic symptoms, despite elevated trace metal concentrations. Significant accumulation of zinc, lead, and cadmium as model contaminants in the biomass of *Atriplex halimus* (up to 495.4, 31.9, and 1.2 mg kg^{-1}, respectively), as well as magnesium and manganese as model CRMs (2081 and 87.8 mg kg^{-1}, respectively) was observed in aerial tissues, comparable with traditional, though more labor-intensive propagation methods. Plants' ability to accumulate metals was high in the presence of amendments added to promote biomass growth. These results highlight the significance of direct propagation by unrooted cuttings as a promising, low-cost strategy to initiate site restoration in metal-contaminated areas and warrant further investigation under field conditions and over longer durations.

Keywords: heavy metals; mine tailings; phytoremediation; raw materials

check for updates

Received: 26 May 2025
Revised: 16 June 2025
Accepted: 19 June 2025
Published: 22 June 2025

Citation: Canu, M.; Milia, S.; Ubaldini, S.; Tamburini, E.; Carucci, A.; Cappai, G. Propagation of *Atriplex halimus* (Mediterranean Saltbush) in Multi-Contaminated Mine Tailings by Unrooted Cuttings. *Appl. Sci.* **2025**, *15*, 7027. https://doi.org/10.3390/app15137027

1. Introduction

Metal pollution is a widespread environmental problem that affects both rural and urban areas, causing harm to environmental matrices and posing serious risks to human health [1,2]. Several strategies exist to control metal contamination; among them, phytoremediation stands out as a reliable and cost-effective approach [3]. In addition to site restoration, phytoremediation biomass can serve as a valuable source for secondary raw materials (SRMs) recovery, supporting sustainable resource management and contributing to circular economy initiatives [4–6].

In this context, the bio-ore technology, which involves recovering SRM from biomass used for phytomining, has gained interest as a viable solution for contaminated sites [7,8]. However, in arid and semi-arid regions, successful phytotechnological applications require the selection of plant species highly adapted to environmental stresses, such as salinity and drought. In this respect, halophytic and xerophytic species represent promising candidates for the ecological rehabilitation of degraded lands [9–12].

Among halophytes, the genus Atriplex has been extensively studied for its ability to grow in soils polluted with trace metals [13–16]. *Atriplex halimus* L. (Mediterranean saltbush), a perennial shrub of the Chenopodiaceae (Amaranthaceae) family, is native to Mediterranean regions and well adapted to arid, semiarid, and saline environments [17,18]. This species can propagate both by seeds and by vegetative means, through stem cuttings.

In standard practices, vegetative propagation involves the rooting of stem segments under controlled conditions before transplanting the young plants into the target soil [18] Direct planting of unrooted stem cuttings could provide a more economical alternative, particularly for the revegetation of mining areas, where surface conditions (e.g., stony substrates, steep slopes) often hinder the establishment of seedlings.

Furthermore, in practical applications, the transport and handling of stem cuttings are easier and cheaper than for rooted plants, making this method attractive for large-scale environmental restoration activities. Indeed, the use of unrooted cuttings has been successfully implemented in other species used for phytoremediation, such as *Salix* spp. and *Populus* spp., also planting the unrooted cuttings in contaminated soils [19–22].

The propagation of Atriplex by stem cuttings is straightforward and may be a valuable strategy for ecosystem restoration [23]. However, phytoremediation studies have typically used seedlings or rooted cuttings rather than unrooted cuttings in polluted soils [3,17,24–27].

The success of phytotechnologies not only depends on the plant's ability to tolerate and accumulate metals but also on substrate characteristics. Mine residues typically have high metal concentrations and poor nutrient content, often requiring the use of organic and/or inorganic amendments to facilitate plant growth [27,28]. In the case of *Atriplex halimus*, several studies have demonstrated the positive effects of amendments in improving biomass production and phytoremediation performance in contaminated soils [25–27,29].

Despite the growing interest in phytoremediation strategies using halophytic shrubs, most previous studies have concentrated on the use of seedlings or pre-rooted cuttings, overlooking the potential of directly implanting unrooted cuttings into contaminated substrates. This approach remains underexplored, particularly in the context of harsh and nutrient-poor environments such as mine tailings, where traditional propagation methods may be impractical or cost-prohibitive.

While related species like *Halimione portulacoides* (formerly *Atriplex portulacoides*) have shown successful rooting and growth in metal-contaminated sediments [23,30], data on *Atriplex halimus* remain scarce. To date, very few studies have examined its performance when propagated directly from unrooted cuttings in polluted substrates [31], and none, to our knowledge, have assessed this strategy in multi-metal contaminated mine tailings.

In particular, there is a lack of information on (i) the rooting success and establishment of *Atriplex halimus* unrooted cuttings in such challenging substrates, (ii) the potential role of soil amendments in improving cutting viability and plant performance, and (iii) the associated patterns of trace metal accumulation. Addressing these gaps is essential for advancing low-cost and scalable phytomanagement practices.

Based on its physiological traits and previous applications in phytoremediation, we hypothesize that *Atriplex halimus* unrooted cuttings may be able to establish and grow in multi-metal contaminated mine tailings, and that the use of amendments could further enhance this process. Therefore, the aim of this preliminary investigation is to: (i) evaluate the feasibility of propagating *Atriplex halimus* via direct unrooted stem cutting in heavily polluted mine residues, (ii) investigate the effects of amendments on plant survival, initial biomass development, and trace metal accumulation in aerial tissues. The results are expected to contribute to the development of more efficient phytomanagement practices for the restoration of contaminated mining environments, offering flexible options for large-scale plantation projects.

2. Materials and Methods

2.1. The Study Area

The experimental tailings were collected from the Campo Pisano flotation dump, located in the historical Sulcis-Iglesiente mining district (Sardinia, Italy), where intense Pb-Zn mining activities were carried out between the 1850s and 1990s. The site is part of the National Interest Sites (SIN, [32]) due to severe environmental degradation. Sampling was conducted at a depth of 20 cm (after discarding the top 2 cm), and samples were air-dried at 50 °C for 48 h and sieved to <2 mm to obtain a homogeneous material. The sampling area is shown in Figure S1 (Supplementary Materials).

2.2. Substrate Preparation

To the aim of the study, three experimental substrates were prepared using mine tailings, with and without the addition of compost plus sand/gravel, and commercial soil as a control. Compost derived from the organic fraction of municipal solid waste was supplied by "Tecnocasic S.p.A." (Cagliari, Italy), and contained 24% carbon, 1.9% nitrogen, and 0.8% phosphorus, with a pH of 7.8. Cation exchange capacity (CEC) was 62 meq 100 g substrate^{-1}.

More specifically, the three experimental substrates consisted of:

- CP: mine tailings from Campo Pisano.
- CP + CI: mixture of Campo Pisano mine tailing (6.8 L), compost (2.2 L), sand (9 L), and gravel (9 L) in a 25:8:33:33% volume ratio (CP + CI).
- B (Blank control): commercial horticultural substrate mixed with sand and gravel in a 33:33:33% volume ratio.

Each 27 L experimental unit consisted of one pot (0.40 × 0.60 × 0.15 m) with a 3 L gravel drainage layer at the bottom and 24 L of test substrate.

2.3. Plants and Experimental Design

Stem cuttings (20 cm in length) were collected from mature *Atriplex halimus* plants growing spontaneously in Cagliari (Sardinia, Italy). A total of 35 unrooted cuttings were randomly planted per pot. Spontaneous *Atriplex halimus* plants used for stem cutting propagation, and stem cutting plantation in an experimental pot are shown in Figure S2 (Supplementary Materials).

The bioclimate of the area is defined as Upper Thermomediterranean, Lower Dry, Euoceanic Weak [33], and it is characterized by mild to warm winters and very hot summers, with rainfalls concentrated in the cooler months and long summers with little rainfall.

The experiment was conducted under natural light and temperature in an open greenhouse (March–April), with temperatures ranging from 8.4 to 21.2 °C (nighttime and daytime, respectively).

Cuttings were irrigated daily with fine-mist deionized water for the first week to prevent desiccation. After root emergence, irrigation was reduced to 2–3 times per week.

2.4. Analytical Methods

2.4.1. Substrate Characterization

Substrates were treated and analysed according to the Italian Ministry of Agriculture and Forestry guidelines and methods [34]. For each substrate, three samples were analysed (particle diameter <2 mm). Substrate pH was measured in $CaCl_2$ solution, and Cation Exchange Capacity (CEC) was measured using $BaCl_2$ and triethanolamine. According to the CEC method, the CP substrate was previously treated with several washing cycles using ammonium acetate to reduce the magnesium (Mg) ions and limestone contents. Carbon (C) and nitrogen (N) content were determined by the flash combustion method, followed by gas chromatography separation and thermal conductivity detection using an elemental analyser (CHN 1000, LECO Corp., St Joseph, MI, USA). The available phosphorus (P) was determined following the Olsen procedure, which is usually adopted for neutral to alkaline soils, as described by Method XV.3 [34].

For the quantification of total content of trace metals (Cd, Cu, Mg, Mn, Pb, Zn), substrates were digested by aqua regia in hot plate, while the bioavailable fractions were extracted as described by [35], a multi-step sequential extraction procedure, where metals were extracted, separately, by water, KNO_3 and EDTA. The concentration of metals in the extracts was determined using an inductively coupled plasma optical emission spectrometer (Optima DV 7000 ICP/OES, Perkin Elmer Inc., Waltham, MA, USA).

2.4.2. Plant Sampling and Analysis

For each pot, plant survival was determined 5 weeks after plantation (mid-term) and at the end of the experiment (week 8), according to equation (Equation (1)):

$$Survival_{mid,\ end}\ (\%) = \frac{n_{Tmid,Tend}}{n_{T0}} \times 100 \quad (1)$$

where:

n_{T0}: number of steam cuttings planted at the beginning of the experiment;

n_{Tmid}: number of surviving plants (i.e., with viable sprouts) at week 5 (mid-term);

n_{Tend}: number of surviving plants (i.e., with viable sprouts) at the end of the experiment (week 8).

For each treatment, measurements were carried out considering all plants, without subsampling. Therefore, results are presented as cumulative values per treatment, and no variability measures (e.g., standard deviation or standard error) were calculated.

Plant growth was evaluated after harvesting (week 8), considering new tissues produced by stems (Figure S3 Supplementary Materials). More specifically, the aerial growth was quantified considering sprout weight, and root growth was quantified considering root weight. The stems' weight was also evaluated. Plant specimens were harvested and rinsed with tap and distilled water. Fresh weight (FW, after carefully absorbing the rinsing water with paper) and dry weight (DW, after stove drying at 60 °C for 72 h) of roots, stems, and sprouts were determined. Trace metal content in stems and sprouts was determined according to standard methods [34]. Plant tissues were pretreated with oxygen peroxide and digested with aqua regia, and metal concentration was determined by ICP/OES (Optima DV 7000 ICP/OES, Perkin Elmer Inc.).

The stage and the quality of rooting and sprouting were evaluated for each harvested plant, using a 0–4 scale. As reported in the literature, rooting quality is assessed by visual parameters for different species and using various attributing quality scores [36–38]. In this study, both for roots and sprouts, the evaluation scale was adapted to the plant studied (Table 1).

Table 1. Scores and criteria to evaluate the stage and quality of rooting and sprouting.

Tissue	Scores 0	1	2	3	4
Roots	No visible roots, no callus.	Presence of root callus	Roots visible but mainly less elongated. Poor branching of rooting system.	Roots in elevated number and good health status. Medium branching of root system.	Roots in high number, long and healthy. Well-developed, branched root system.
Sprouts	No visible sprouts, no buds.	Start of bud sprouting.	All buds open and sprouts developed, but less elongated and poorly branched.	Sprouts in elevated number and good health status. Sprouts medium branched.	Sprouts in high number, long and healthy. Sprouts well branched.

At the end of the experiment (week 8), the content of photosynthetic pigments such as chlorophylls and carotenoids in leaves was determined and considered as an indicator of plants' health and tolerance to high metal concentration in substrates. Samples of leaves were randomly cut from plants (2 or 3 plants for each substrate), a quantity of 0.02–0.05 g (FW) was collected per plant and subsequently ground and mixed with ethanol (96%) in darkness for 12 h. The supernatant was analysed using a spectrophotometer (U-2000, Hitachi Ltd., Tokyo, Japan) at wavelengths of 470, 648.6 and 664.2 nm to determine the content of chlorophyll a (Chla) and chlorophyll b (Chlb), carotenes (Cc) and xanthophylls (Cx) according to the equations proposed by [39]. The concentration of total chlorophylls Chla+b was calculated as the sum of Chla and Chlb concentrations, and the ratio between chlorophyll types (Chla/Chlb) was also determined. The total concentration of carotenoids (Cx+c) was calculated as the sum of xanthophylls and carotenes. The ratio between total carotenoids and chlorophylls (Cx+c/Chla+b) was also calculated.

2.5. Statistical Analysis

The experimental data were statistically analysed to find significant differences among the experimental substrates. The one-way ANOVA (Analysis Of Variance) was applied when data normal distribution and homoscedasticity of the variances were respected. When ANOVA assumptions were not respected, after data transformation, the Kruskal-Wallis non-parametric test was applied. To define differences between the groups, post hoc analyses were performed using the Tukey test (after ANOVA) or the Dunn test with Bonferroni p-value correction (after the Kruskal-Wallis test). Pearson or Spearman correlations were applied in the case of normal or abnormal data distribution, respectively. Data were statistically analyzed at a 0.05 significance level ($p = 0.05$) using the Paleontological Statistics (PAST) Software version 4.06b package [40].

3. Results and Discussion

3.1. Substrate Properties

Table 2 shows the main physical and chemical properties of the substrates before plantation. The pH and total carbon (TC) values observed in the mine district align with the dominant carbonate lithology described by [41], where carbon was primarily present in inorganic forms such as calcite (48.0 ± 4.4 g kg^{-1}) and dolomite (430.0 ± 10.1 g kg^{-1}). These findings are further supported by XRD analyses performed in [42]. The addition of compost improved the CEC, total nitrogen, and bioavailable phosphorus content.

Table 2. Physical and chemical properties of the substrates before the plantation (Means ± SE, *n* = 3).

Substrates	pH	CEC	C	N	P (Bioavailable)
		[meq 100 g $Substrate^{-1}$]	[%]	[%]	[mg kg^{-1}]
B	5.5 ± 0.1	23.2 ± 0.5	3.9 ± 0.5	0.07 ± 0.01	29.0 ± 1.08
CP	7.3 ± 0.1	nd *	6.5 ± 0.1	nd	nd
CP + CI	7.5 ± 0.1	11.0 ± 0.7	7.1 ± 0.3	0.08 ± 0.01	13.6 ± 0.8

* nd: data below the detection limits.

These results confirm previous findings [43,44] and highlight the positive effect of compost as an amendment in degraded substrates.

The results of the preliminary characterization (Table 3) confirmed that total concentrations of Cd, Pb, and Zn in the Campo Pisano mine residues exceeded the threshold levels established by Italian legislation for industrial sites (15, 1000, and 1500 mg kg^{-1}, respectively) [45]. In the CP + CI substrate (Campo Pisano mine residues with compost and inert amendments), the observed reduction in total metal concentrations is attributable to a dilution effect caused by the added materials.

Table 3. Concentration of Cd, Pb, and Zn (i.e., model contaminants), and Cu, Mg, and Mn (i.e., model SRM) in the experimental substrates at the beginning of the tests, with different extraction methods. (Means ± SE, *n* = 3).

Substrates	Aqua Regia–Extractable (mg kg^{-1})			EDTA–Extractable (mg kg^{-1})			KNO_3–Extractable (mg kg^{-1})			H_2O–Extractable (mg kg^{-1})		
	Cd	**Pb**	**Zn**	**Cd**	**Pb**	**Zn**	**Cd**	**Pb**	**Zn**	**Cd**	**Pb**	**Zn**
B	nd *	6.36 ± 2.24	36.5 ± 1.3	nd	nd	8.4 ± 0.8	nd	nd	8.8 ± 3.4	nd	nd	nd
CP	70.1 ± 2.7	3041 ± 44.9	12,779 ± 33.4	33.2 ± 2.0	1066 ± 56.1	2407 ± 59.1	nd	nd	7.1 ± 0.8	nd	nd	6.6 ± 1.4
CP + CI	31.6 ± 2.5	1236 ± 102	5268 ± 513	13.5 ± 6.1	361 ± 88.6	1003 ± 497	nd	nd	4.6 ± 0	nd	nd	5 ± 0.3
Substrates	Cu	Mg	Mn	Cu	Mg	Mn	Cu	Mg	Mn	Cu	Mg	Mn
B	11.8 ± 0.8	1460 ± 83.1	280 ± 10.4	3.33 ± 0.2	330 ± 14.9	106 ± 18.8	nd	515 ± 10.5	26.3 ± 1.8	nd	31.1 ± 1.04	1.47 ± 0.07
CP	55.4 ± 5.3	60,194 ± 729	1316 ± 29.6	14.5 ± 0.8	1970 ± 58.7	115 ± 6.8	nd	149 ± 6.9	0.35 ± 0.02	nd	581 ± 31	0.5 ± 0.06
CP + CI	34.6 ± 1.9	31,795 ± 2732	761 ± 46.1	6.43 ± 1.6	1799 ± 120	133 ± 20.6	nd	228 ± 3.8	1.15 ± 0.7	1.08 ± 0.1	713 ± 131	2.03 ± 0.4

* nd = data below the detection limits. Cd: cadmium; Pb: lead; Zn: zinc; Cu: copper; Mg: magnesium; Mn: manganese.

Among the extractable fractions, EDTA-extractable metals were lower in the amended substrate, indicating a potentially lower bioavailability. The values detected for Mn and Mg extracted with KNO_3 or H_2O were higher in the amended substrate. As observed from Table 2, the addition of compost enhances the soil's CEC due to its high organic matter content. This increase in CEC provides more weakly bound adsorption sites, facilitating the retention and exchange of divalent cations such as Mg^{2+} and Mn^{2+}. As a result, the extractability of these elements using neutral salt solutions like KNO_3 is increased, reflecting their higher availability in the amended substrates.

3.2. Plant Survival

Figure 1 presents the survival percentages of stem cuttings at week 5 (mid-term) and at the end of the experiment (week 8). The highest survival rate was recorded in the control substrate (B) at 48.5%, while the lowest occurred in CP + CI (17.4%). Notably, survival in CP (42.8%) was comparable to that in the uncontaminated control, suggesting that high trace metal concentration in the mine tailings did not prevent rooting and sprout development.

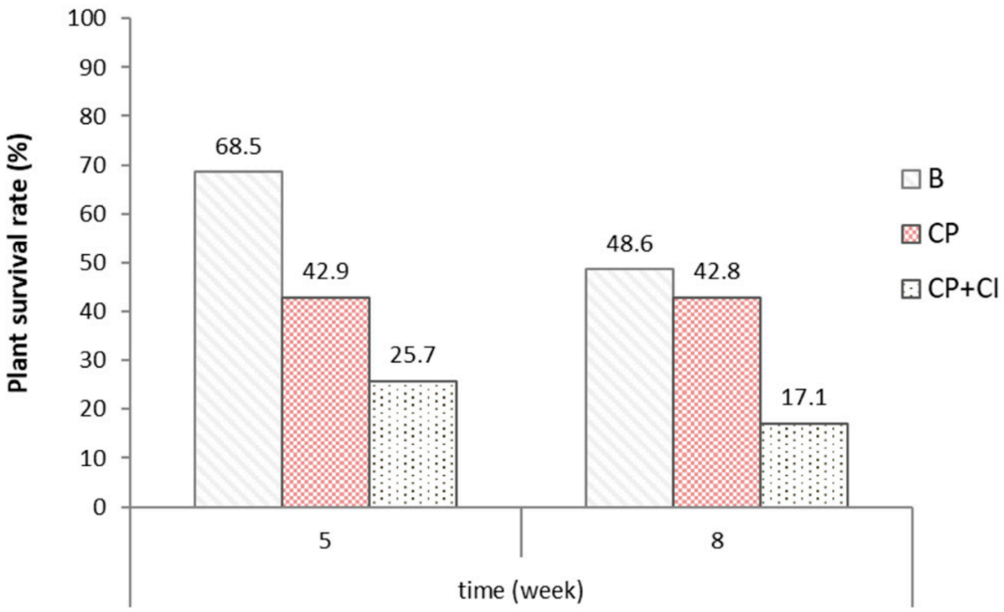

Figure 1. Plant survival in the tested substrates at week 5 (mid-term) and week 8 (end).

The limited survival observed in CP + CI may be attributed to the proliferation of Sciaridae larvae (dark-winged fungus gnats), which developed in the humid, organic-rich compost. The insect infestation likely contributed to early root damage, particularly during the first weeks, and was mitigated by the application of a pest control treatment (avermectins) in all pots.

Stem cuttings rooting was successfully applied by [31] with the same species in vermiculite soil artificially contaminated with copper, and by [23] and [30] with another *Amaranthaceae* halophytic species, i.e., *Halimione portulacoides*, in marsh sediments polluted with different heavy metals and in perlite substrates artificially polluted with Zn solutions, respectively.

Though the plant survival observed in our study was lower than that reported by [31] and [30], a direct comparison may be misleading since different types of contaminations (multi- vs. single-contamination) and/or plant species (*Atriplex halimus* vs. *Halimione portulacoides*) were considered.

In general, the percentage of viable stem cuttings was in accordance with [46], which reported high variability in the rooting capability of Sardinian *Atriplex halimus* plants.

Despite these biotic and environmental stresses, *Atriplex halimus* successfully formed roots and sprouts in highly contaminated mine residues, indicating an interesting potential for direct cutting propagation even in substrates poor in nutrients and rich in trace elements.

3.3. Biomass Growth

Atriplex halimus plant cuttings were evaluated for root and sprout quality and for biomass growth at the end of the experiment (week 8). The rooting and sprouting quality scores are reported in Table 4, where the best scores were observed in plants grown in the control soil (B) and in the polluted substrate amended (CP + CI), highlighting that amendments improve rooting and sprouting performance. However, sprout scores in both contaminated substrates remained lower than those in the control, indicating that shoot development was overall more affected by substrate conditions.

Table 4. Evaluation of stage and quality of rooting and sprouting (Means ± SE, n = 5). Different letters indicate significant differences among substrates at $p \leq 0.05$.

	Roots			Sprouts		
	B	**CP**	**CP + CI**	**B**	**CP**	**CP + CI**
Score	2.8 ± 0.2 (a)	1.6 ± 0.2 (b)	2.8 ± 0.2 (a)	4.0 ± 0 (a)	1.8 ± 0.4 (b)	2.4 ± 0.2 (b)

Dry biomass measurements further clarified the observed differences in sprout and root performance. As shown in Figure 2A, sprout dry weight was significantly higher in the control substrate (B) compared to both CP and CP + CI, confirming the trend already observed in the sprouting quality scores (Table 4). In contrast, root biomass was higher in CP and CP + CI (49.3 mg and 64.9 mg, respectively), while the control (B) recorded the lowest value (13.7 mg). This result diverges from previous findings [23,31], where root biomass was typically reduced in contaminated soils. In our study, root development seemed to be enhanced in polluted substrates, possibly reflecting a plastic response to environmental stress [47,48], whereby the plant allocates more biomass to root systems to improve resource acquisition under suboptimal conditions. Importantly, despite its lower biomass, the control substrate showed the highest root quality score (Table 4), indicating the formation of healthy and branched roots. As per the evaluation criteria (Table 1), such scores reflect morphological features (number, length, health, branching) rather than mass. This supports the idea that root quality and biomass may reflect distinct aspects of plant adaptation, with *A. halimus* developing structurally functional roots in optimal conditions (B) and more massive root systems in stressed environments (CP, CP + CI).

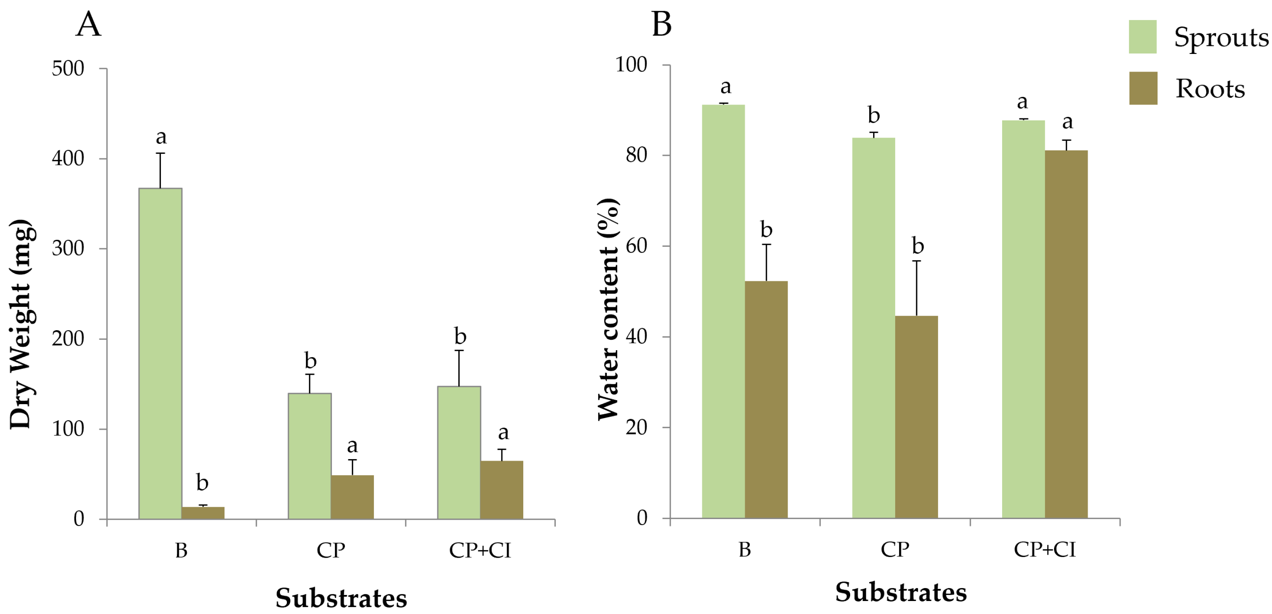

Figure 2. Dry weight (**A**) and water content of roots and sprouts (**B**) in the tested substrates. Bars indicate SE (5 replicates). Different letters indicate, separately for roots and sprouts, significant differences among substrates at $p \leq 0.05$.

In contrast, sprout biomass was significantly higher in the control substrate (B) (367 mg dry weight) compared to 147 mg in CP + CI and 139 mg in CP. These results suggest that metal contamination may have hindered aerial growth, while root development was not equally affected. Similar effects were reported in previous studies with *Atriplex halimus* [16,31,49] and *Halimione portulacoides* [23].

No statistically significant correlations were detected between stem biomass and either sprout or root biomass (assuming Spearman coefficient of 0.4 and 0.01, respectively), confirming that the original stem did not influence the production of new biomass/tissues, as already reported by [50] for willow and poplar, and by [51] for willow. This suggests that cutting performance was mainly determined by the interaction with the substrate.

Amendments improved root growth, especially in CP + CI, but did not significantly enhance sprout biomass within the 8-week experimental period. It is conceivable that a longer experimental duration may be required to observe the positive effects of compost on shoot development. Indeed, in field trials conducted by [25] with *Atriplex halimus* over 16 months, organic amendments significantly increased shoot and root biomass. Similarly, [27] reported better growth of *Atriplex halimus* after one year when manure was used as an amendment compared to pine bark compost. An increased shoot and root biomass was also reported after 95 days in contaminated soils amended with spent mushroom compost [26].

The water content analysis (Figure 2B) showed that roots in CP + CI had significantly higher water content than those in the other substrates. This differs from the findings of [10,52], who reported no significant differences in root water content across treatments. In sprouts (Figure 2B), water content was higher in B and CP + CI compared to CP. This pattern may reflect the positive effect of compost on water retention, consistent with the known ability of compost to increase substrate moisture [53].

Increased water content in halophyte tissues under moderate salinity has been previously reported for *Atriplex halimus* [54–56]. Furthermore, [54] observed that moderate salinity stimulated rooting in *Atriplex halimus*, which could explain the improved root water content in CP + CI.

Overall, the results suggest that *Atriplex halimus* can successfully establish roots and produce new biomass in contaminated mine tailings. The presence of amendments—particularly compost—appears to enhance root development and water retention, although a longer growth period may be required to maximize shoot biomass production.

3.4. Chlorophyll and Carotenoids

Plants showed good health conditions during the entire experimental period, as no visual stress symptoms such as chlorosis or leaf necrosis were observed, unlike the symptoms reported by [31] in plants exposed to high Cu concentrations.

The contents of chlorophyll a (Chla), chlorophyll b (Chlb), and total chlorophylls (Chla+b), as reported in Table 5, showed significant differences among the treatments. The highest pigment concentrations were observed in plants grown on the unamended contaminated substrate (CP). In contrast, plants grown on CP + CI exhibited the lowest Chla and Chla+b values, suggesting that amendments may have influenced pigment production.

Table 5. Plant chlorophyll and carotenoid content and plant chlorophyll and carotenoid relationships in the tested substrates. (Means ± SE, n = 3). Different letters indicate significant differences among substrates at $p \leq 0.05$.

Substrates	Ca (mg g^{-1})	Cb (mg g^{-1})	Cx+c (mg g^{-1})	Ca+Cb (mg g^{-1})	Ca/Cb	(Cx+c)/(Ca+b)
B	1.11 ± 0.03 b	0.4 ± 0.02 b	0.37 ± 0.01 b	1.52 ± 0.05 b	2.81 ± 0.1 a	0.24 ± 0.01 c
CP	1.46 ± 0.05 a	0.52 ± 0.02 a	0.5 ± 0.02 a	1.99 ± 0.07 a	2.81.0 ± 2 a	0.25 ± 0 c
CP + CI	0.87 ± 0.04 c	0.32 ± 0.02 b	0.38 ± 0.01 b	1.19 ± 0.06 c	2.75 ± 0.04 a	0.32 ± 0.01 a

Carotenoid contents (Cx+c) followed a similar trend to Chlb, with higher values in CP compared to the other treatments. Carotenoids, including carotenes and xanthophylls, are known to contribute to the protection of photosynthetic structures under conditions of oxidative stress [57].

Under environmental stress, chlorophyll content often decreases, while carotenoid content tends to increase [58]. An increase in the Cx+c/Chla+b ratio is typically associated with stress responses [59]. However, in our study, this ratio was significantly lower in CP, suggesting no evident oxidative stress, despite the high metal concentrations.

The Chla/Chlb ratio remained similar across all treatments, indicating no major alteration in pigment composition, in agreement with [39]. This suggests that the photosynthetic apparatus was not negatively affected by metal contamination.

Metal stress typically leads to chlorophyll degradation and photosynthesis inhibition. For example, Ref. [60] described the substitution of Mg^{2+} in chlorophyll molecules by toxic metals such as Cd, Pb, or Zn, which can damage the photosynthetic system.

However, responses vary across species. Some halophytes, such as *Tamarix gallica* exposed to As and NaCl [61], showed no significant changes in chlorophyll and carotenoid content, similar to what was observed in this study.

Ref. [62] reported no Cd-induced reduction in chlorophyll in *Atriplex nummularia*, while [10] found that Pb reduced chlorophyll in *Atriplex halimus*, but Zn had no effect. Similarly, Ref. [63] observed a reduction in chlorophyll with increasing Cd concentrations, and [64] reported chlorophyll degradation under Cu stress.

In contrast, Ref. [31] observed no significant changes in photosynthetic pigments in *Atriplex halimus* grown in Cu-polluted substrate. This variability suggests that chlorophyll content alone may not be a reliable stress indicator in this species, particularly during early growth stages.

In our study, the high chlorophyll content observed in CP plants aligns with the results of [16], who reported increased chlorophyll levels in *Atriplex halimus* grown in As-contaminated substrates. Additionally, Ref. [10] suggested that Pb may have a stimulatory effect on chlorophyll synthesis, consistent with the higher pigment concentrations observed in CP in this experiment.

The findings of this study, combined with those reported by [31], indicate that during the early growth stages analyzed, high metal concentrations did not compromise the photosynthetic apparatus. The stability of the Chla/Chlb and Cx+c/Chla+b ratios further supports the tolerance of *Atriplex halimus* to multi-metal contamination under the conditions tested.

3.5. Plants Metals Content

At the end of the experiment, the concentrations of Zn, Pb, and Cd were determined in stems and sprouts across all treatments (CP, Control, and CP + CI), while root biomass was insufficient for metal quantification, preventing a complete mass balance assessment. Additionally, with a view toward long-term cultivation, the contents of Cu, Mg, and Mn were specifically evaluated in the CP + CI substrate.

As shown in Figure 3A, Zn was the principal element accumulated in plant tissues, with concentrations significantly higher in sprouts than in stems across all treatments. The highest Zn concentration in sprouts (379.7 mg kg^{-1}) was observed in CP, while lower but substantial concentrations were found in CP + CI (291.2 mg kg^{-1}) and B (132.6 mg kg^{-1}). In stems, Zn levels ranged from 170.6 mg kg^{-1} in CP to 17.4 mg kg^{-1} in B.

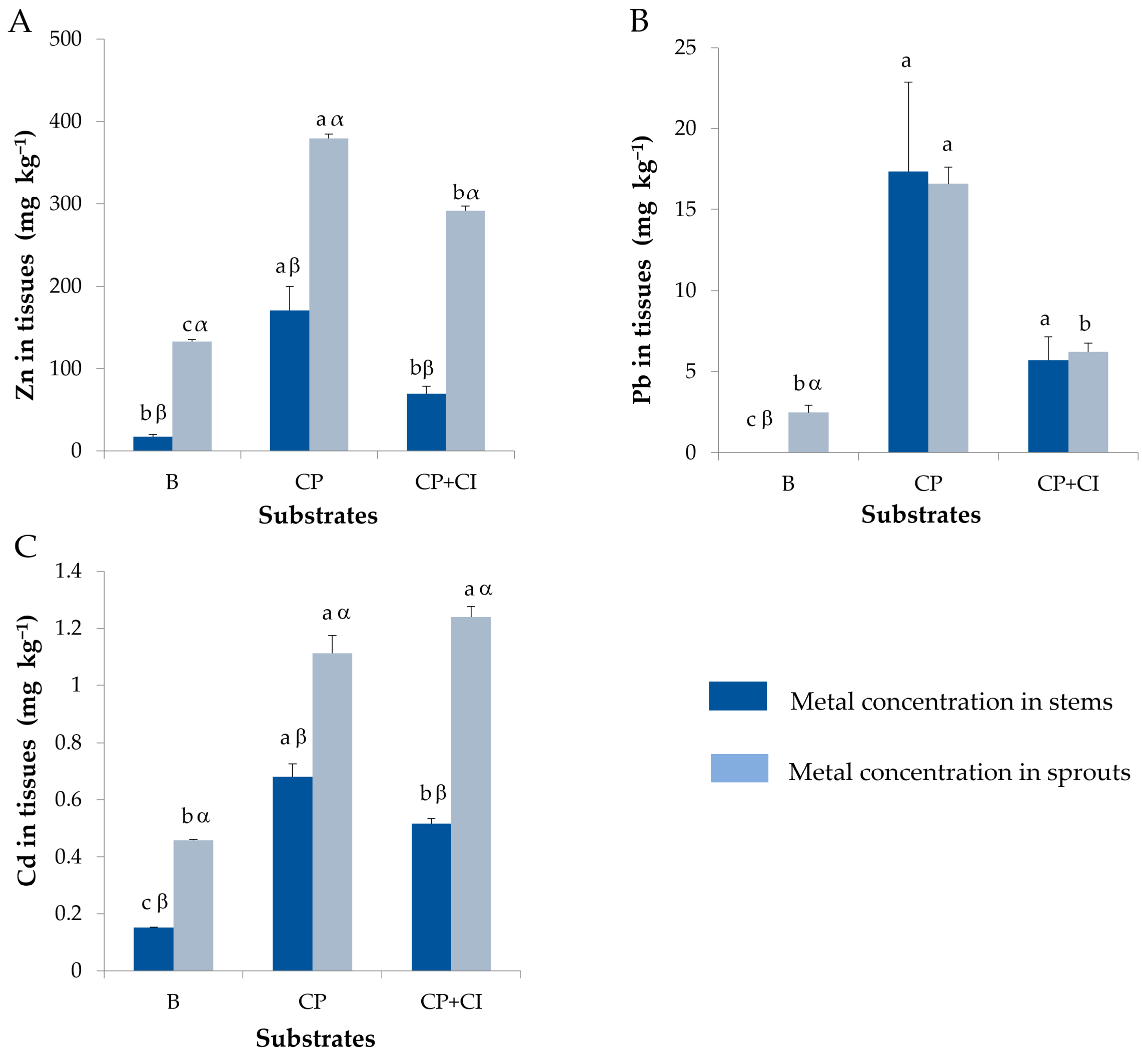

Figure 3. Metal accumulation in plant tissues for the four treatments applied: Zn (**A**), Pb (**B**), and Cd (**C**). Different Greek letters indicate statistically significant differences between plant tissues for a specific substrate ($p < 0.05$); different Latin letters indicate statistically significant differences among substrates for a specific plant tissue ($p < 0.05$); bars indicate SE (3 replicates).

For Pb, the trend was similar (Figure 3B), with the highest sprout concentration (16.6 mg kg^{-1}) recorded in CP, followed by CP + CI (6.2 mg kg^{-1}), and B (2.5 mg kg^{-1}). In stems, Pb concentrations were highest in CP (17.4 mg kg^{-1}), with lower values in CP + CI (7.1 mg kg^{-1}), and B (2.4 mg kg^{-1}).

Cadmium concentrations (Figure 3C) were much lower than Zn and Pb, but significantly higher in sprouts than in stems in all treatments. The highest Cd concentration (1.24 mg kg^{-1}) was found in sprouts from CP + CI, while in stems, Cd remained below 0.5 mg kg^{-1} in all treatments.

The use of amendments influenced metal accumulation in *Atriplex halimus* aerial tissues. The highest Zn and Pb uptakes were observed in CP, while the presence of compost in CP + CI likely had an immobilization effect. This partially differs from [52], who reported no significant amendment effects for Zn, Pb, or Cd. However, regarding Cd, our results agree with both [25,52], as no clear amendment effect was observed in this study.

These patterns are consistent with previous studies on *Atriplex halimus*, where higher metal accumulation was often reported in sprouts than in stems. For example, Ref. [23] reported lower Zn (40–52 mg kg^{-1}) and Pb (<3 mg kg^{-1}) concentrations in *Atriplex portula-*

coides, while [3,10,26] observed comparable or higher values in *Atriplex halimus* grown from seeds or rooted cuttings.

In field conditions, Ref. [65] reported Pb concentrations ranging from 12 to 40 mg kg^{-1} in leaves and stems, similar to the values observed here. Higher Pb concentrations (over 200 mg kg^{-1}) were reported by [29] in leaves of *Atriplex halimus* after a 5-year phytoremediation trial, and Zn values up to 1200 mg kg^{-1} were observed by [25] in field-grown plants.

Regarding medium- and long-term experiments, Ref. [25] observed increasing Cd and Zn concentrations over two years, while [26] reported Pb increases over time. Refs. [10,29] also highlighted temporal variability in metal uptake, depending on soil properties and amendment use.

High genetic variability characterizes *Atriplex halimus* species, and, for field application, using cutting to select clones represents a feasible option to standardize the plant material [3].

The short experimental duration (8 weeks) limits the assessment of long-term accumulation trends; however, selecting clones during sensitive stages, such as cuttings and young plants, could speed up the selection.

Our findings suggest that direct cutting propagation does not compromise the plant's ability to accumulate trace metals in aerial tissues, supporting its potential to boost clonal screening for SRM recovery in phytomanagement schemes.

With the perspective of long-term cultivation, Cu, Mg, and Mn contents were evaluated in CP + CI substrate. Stems contained 18.5 mg kg^{-1} of Cu, 2081 mg kg^{-1} of Mg, and 37.7 mg kg^{-1} of Mn, while sprouts had 67.1 mg kg^{-1} of Cu, 1411 mg kg^{-1} of Mg, and 87.8 mg kg^{-1} of Mn. Mg data align with [24], who reported up to 0.6% Mg in shoots and leaves, noting Zn and Pb exposure could decrease Mg concentration. In 30-month-old *Atriplex halimus* clones, Ref. [66] found Cu, Mg, and Mn mainly in aerial biomass, with the highest amounts in leaves, averaging 1.4% Mg and about 450 mg kg^{-1} Mn. Mg concentrations in leaves seem positively related to soil content [67]. Cu and Mn values can be compared with [52]. Cu values align with [29] and [26], who reported increasing metal content in tissues over time. An increase of Mn over time with compost, boosting shoot translocation, was reported by [17].

The literature is divided regarding the phytoremediation role of *Atriplex halimus*. While some authors emphasize its phytostabilization potential [18], others report significant metal translocation to shoots, supporting a phytoextraction role for Cd and Pb [10,29]. Therefore, while the early accumulation patterns observed here are promising, longer-term studies are needed to assess the maximum uptake capacity of *Atriplex halimus* and to validate its phytoextraction potential.

4. Conclusions

This preliminary short-term study demonstrated, for the first time, the feasibility of propagating *Atriplex halimus* through direct planting of unrooted stem cuttings in multi-metal contaminated mine tailings. Despite moderate survival rates and limited aerial biomass production after eight weeks, the species exhibited a consistent ability to root and develop in substrates characterized by high concentrations of Zn, Pb, and Cd, thus confirming its tolerance to extreme conditions.

Two key findings emerge from our work: (*i*) direct propagation does not impair the species' capacity to accumulate trace metals in aerial tissues, with concentrations comparable to those reported for seedlings or pre-rooted cuttings in longer trials; and (*ii*) the use of compost and inert amendments improved rooting and moisture retention, though their effect on shoot biomass and metal uptake requires further investigation.

The absence of data on root metal content limits the current ability to classify the remediation behavior of *Atriplex halimus* as either phytoextraction or phytostabilization. Nevertheless, the accumulation of metals in above-ground biomass highlights its potential for phytomanagement applications, especially considering the possibility of recovering secondary raw materials.

To fully assess the long-term viability and effectiveness of this approach, further field studies are needed to evaluate plant survival, growth dynamics, and metal uptake over extended periods. Future research should also explore the optimization of amendment types and doses, particularly with low-cost or site-available materials, and investigate clonal selection to enhance metal accumulation performance. Such efforts would support the development of cost-effective, scalable solutions for the ecological restoration and resource recovery of degraded mining sites.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app15137027/s1.

Author Contributions: Conceptualization: M.C., A.C. and G.C. Methodology: M.C., A.C. and G.C. Validation: S.M., S.U. and G.C. Investigation: M.C. Resources: G.C. and A.C. Data curation: M.C., S.M., E.T. and G.C. Writing—original draft preparation: M.C. Writing–review and editing: S.M., S.U., E.T., A.C. and G.C. Supervision: A.C. and G.C. Funding acquisition: G.C. and A.C. All authors have read and agreed to the published version of the manuscript.

Funding: This work is part of the research project "REMINE-REstoration and remediation of abandoned MINE sites", funded by the Fondazione di Sardegna and the Regional Sardinian Government (Grant CUP F72F16003160002).

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ANOVA	Analysis of variance
Cc	Carotenes
CEC	Cation exchange capacity
Chla	Chlorophyll a
Chlb	Chlorophyll b
CRM	Critical raw materials
Cx	xanthophylls
DW	Dry weight
EDTA	Ethylene-diamine-tetra-acetic acid
FW	Fresh weight
PAST	Paleontological statistics
SE	Standard error
SIN	National Interest Site
SRM	Secondary raw materials

References

1. WHO. *Human Health Effects of Benzene, Arsenic, Cadmium, Nickel, Lead and Mercury: Report of an Expert Consultation*; WHO: Geneva, Switzerland, 2024.
2. Wang, Z.; Luo, P.; Zha, X.; Xu, C.; Kang, S.; Zhou, M.; Nover, D.; Wang, Y. Overview Assessment of Risk Evaluation and Treatment Technologies for Heavy Metal Pollution of Water and Soil. *J. Clean. Prod.* **2022**, *379*, 134043. [CrossRef]

3. Zaghdoudi, R.; Sghayar, S.; Necib, M.; Dekaezmaeker, P.; Dailly, H.; Zorrig, W.; Abdelly, C.; Debez, A.; Lutts, S. Screening for Heavy Metal-Resistant Clones in the Xero-Halophyte *Atriplex halimus* L.: A Prerequisite for Phytoremediation of Polymetallic Mining Pollution in Arid Areas. *Int. J. Environ. Res.* **2025**, *19*, 72. [CrossRef]
4. Dang, P.; Li, C. A Mini-Review of Phytomining. *Int. J. Environ. Sci. Technol.* **2022**, *19*, 12825–12838. [CrossRef]
5. Han, Z.; Guo, Z.; Zhang, Y.; Xiao, X.; Xu, Z.; Sun, Y. Adsorption-Pyrolysis Technology for Recovering Heavy Metals in Solution Using Contaminated Biomass Phytoremediation. *Resour. Conserv. Recycl.* **2018**, *129*, 20–26. [CrossRef]
6. Liu, Z.; Tran, K.-Q. A Review on Disposal and Utilization of Phytoremediation Plants Containing Heavy Metals. *Ecotoxicol. Environ. Saf.* **2021**, *226*, 112821. [CrossRef]
7. Moreira, H.; Pereira, S.I.A.; Mench, M.; Garbisu, C.; Kidd, P.; Castro, P.M.L. Phytomanagement of Metal(Loid)-Contaminated Soils: Options, Efficiency and Value. *Front. Environ. Sci.* **2021**, *9*. [CrossRef]
8. Vázquez-Núñez, E.; Fernández-Luqueño, F.; Peña-Castro, J.M.; Vera-Reyes, I. Coupling Plant Biomass Derived from Phytoremediation of Potential Toxic-Metal-Polluted Soils to Bioenergy Production and High-Value by-Products—A Review. *Appl. Sci.* **2021**, *11*, 2982. [CrossRef]
9. Ding, Z.; Alharbi, S.; Almaroai, Y.A.; Eissa, M.A. Improving Quality of Metal-Contaminated Soils by Some Halophyte and Non-Halophyte Forage Plants. *Sci. Total Environ.* **2021**, *764*, 142885. [CrossRef]
10. Manousaki, E.; Kalogerakis, N. Phytoextraction of Pb and Cd by the Mediterranean Saltbush (*Atriplex halimus* L.): Metal Uptake in Relation to Salinity. *Environ. Sci. Pollut. Res.* **2009**, *16*, 844–854. [CrossRef]
11. Van Oosten, M.J.; Maggio, A. Functional Biology of Halophytes in the Phytoremediation of Heavy Metal Contaminated Soils. *Environ. Exp. Bot.* **2015**, *111*, 135–146. [CrossRef]
12. Waris, M.; Baig, J.A.; Talpur, F.N.; Afridi, H.I.; Kazi, T.G.; Yousaf, H. Evaluation of Selected Halophytes for Phytoextraction of Co, Cu, Zn and Capability of Desalination of Saline Soil. *Int. J. Environ. Sci. Technol.* **2022**, *19*, 2737–2746. [CrossRef]
13. Kachout, S.S.; Mansoura, A.B.; Mechergui, R.; Leclerc, J.C.; Rejeb, M.N.; Ouerghi, Z. Accumulation of Cu, Pb, Ni and Zn in the Halophyte Plant Atriplex Grown on Polluted Soil. *J. Sci. Food Agric.* **2012**, *92*, 336–342. [CrossRef] [PubMed]
14. Mann, E.; Rutter, A.; Zeeb, B. Evaluating the Efficacy of *Atriplex* Spp. in the Phytoextraction of Road Salt (NaCl) from Contaminated Soil. *Environ. Pollut.* **2020**, *265*, 114963. [CrossRef]
15. Orrego, F.; Ortíz-Calderón, C.; Lutts, S.; Ginocchio, R. Effect of Single and Combined Cu, NaCl and Water Stresses on Three Atriplex Species with Phytostabilization Potential. *South Afr. J. Bot.* **2020**, *131*, 161–168. [CrossRef]
16. Tapia, Y.; Diaz, O.; Pizarro, C.; Segura, R.; Vines, M.; Zúñiga, G.; Moreno-Jiménez, E. *Atriplex Atacamensis* and *Atriplex halimus* Resist as Contamination in Pre-Andean Soils (Northern Chile). *Sci. Total Environ.* **2013**, *450–451*, 188–196. [CrossRef]
17. Ellili, A.; Rabier, J.; Prudent, P.; Salducci, M.-D.; Heckenroth, A.; Lachaâl, M.; Laffont-Schwob, I. Decision-Making Criteria for Plant-Species Selection for Phytostabilization: Issues of Biodiversity and Functionality. *J. Environ. Manag.* **2017**, *201*, 215–226. [CrossRef]
18. Walker, D.J.; Lutts, S.; Sánchez-García, M.; Correal, E. *Atriplex halimus* L.: Its Biology and Uses. *J. Arid Environ.* **2014**, *100–101*, 111–121. [CrossRef]
19. Babi, K.; Guittonny, M.; Bussière, B.; Larocque, G.R. Effect of Soil Quality and Planting Material on the Root Architecture and the Root Anchorage of Young Hybrid Poplar Plantations on Waste Rock Slopes. *Int. J. Min. Reclam. Environ.* **2023**, *37*, 1–20. [CrossRef]
20. Labrecque, M.; Hu, Y.; Vincent, G.; Shang, K. The Use of Willow Microcuttings for Phytoremediation in a Copper, Zinc and Lead Contaminated Field Trial in Shanghai, China. *Int. J. Phytoremediat.* **2020**, *22*, 1331–1337. [CrossRef]
21. Ruttens, A.; Boulet, J.; Weyens, N.; Smeets, K.; Adriaensen, K.; Meers, E.; Van Slycken, S.; Tack, F.; Meiresonne, L.; Thewys, T.; et al. Short Rotation Coppice Culture of Willows and Poplars as Energy Crops on Metal Contaminated Agricultural Soils. *Int. J. Phytoremediat.* **2011**, *13*, 194–207. [CrossRef]
22. Saulino, L.; Allevato, E.; Rossi, S.; Minotta, G.; Fiorentino, N.; Saracino, A. Lengthening Single-Stem Rotation Improves Biomass Yield and Water Use Efficiency in Black Poplar Genotype Multi-Stem Rotation Coppice Plantations. *Biomass Bioenergy* **2021**, *154*, 106256. [CrossRef]
23. Andrades-Moreno, L.; Cambrollé, J.; Figueroa, M.E.; Mateos-Naranjo, E. Growth and Survival of *Halimione Portulacoides* Stem Cuttings in Heavy Metal Contaminated Soils. *Mar. Pollut. Bull.* **2013**, *75*, 28–32. [CrossRef] [PubMed]
24. Bankaji, I.; Pérez-Clemente, R.; Caçador, I.; Sleimi, N. Accumulation Potential of *Atriplex halimus* to Zinc and Lead Combined with NaCl: Effects on Physiological Parameters and Antioxidant Enzymes Activities. *South Afr. J. Bot.* **2019**, *123*, 51–61. [CrossRef]
25. Clemente, R.; Walker, D.J.; Pardo, T.; Martínez-Fernández, D.; Bernal, M.P. The Use of a Halophytic Plant Species and Organic Amendments for the Remediation of a Trace Elements-Contaminated Soil under Semi-Arid Conditions. *J. Hazard. Mater.* **2012**, *223–224*, 63–71. [CrossRef]
26. Frutos, I.; García-Delgado, C.; Cala, V.; Gárate, A.; Eymar, E. The Use of Spent Mushroom Compost to Enhance the Ability of *Atriplex halimus* to Phytoremediate Contaminated Mine Soils. *Environ. Technol.* **2017**, *38*, 1075–1084. [CrossRef]
27. Pérez-Esteban, J.; Escolástico, C.; Ruiz-Fernández, J.; Masaguer, A.; Moliner, A. Bioavailability and Extraction of Heavy Metals from Contaminated Soil by *Atriplex halimus*. *Environ. Exp. Bot.* **2013**, *88*, 53–59. [CrossRef]

28. Kidd, P.; Mench, M.; Álvarez-López, V.; Bert, V.; Dimitriou, I.; Friesl-Hanl, W.; Herzig, R.; Olga Janssen, J.; Kolbas, A.; Müller, I.; et al. Agronomic Practices for Improving Gentle Remediation of Trace Element-Contaminated Soils. *Int. J. Phytoremediation* **2015**, *17*, 1005–1037. [CrossRef]
29. Acosta, J.A.; Abbaspour, A.; Martínez, G.R.; Martínez-Martínez, S.; Zornoza, R.; Gabarrón, M.; Faz, A. Phytoremediation of Mine Tailings with *Atriplex halimus* and Organic/Inorganic Amendments: A Five-Year Field Case Study. *Chemosphere* **2018**, *204*, 71–78. [CrossRef]
30. Cambrollé, J.; Mancilla-Leytón, J.M.; Muñoz-Vallés, S.; Cambrón-Sena, A.; Figueroa, M.E. Advances in the Use of *Halimione portulacoides* Stem Cuttings for Phytoremediation of Zn-Polluted Soils. *Estuar. Coast. Shelf Sci.* **2016**, *175*, 10–14. [CrossRef]
31. Mancilla-Leytón, J.M.; Navarro-Ramos, M.J.; Muñoz-Vallés, S.; Figueroa, M.E.; Cambrollé, J. Evaluation of the Potential of *Atriplex halimus* Stem Cuttings for Phytoremediation of Metal-Polluted Soils. *Ecol. Eng.* **2016**, *97*, 553–557. [CrossRef]
32. Ministerial Decree Perimetrazione Del Sito Di Interesse Nazionale Del Sulcis-Iglesiente-Guspinese. Decreto 12 Marzo 2003. GU Serie Generale n.121 Del 27-05-2003-Suppl. Ordinario n. 83. 2003. Available online: https://www.gazzettaufficiale.it/eli/gu/2003/05/27/121/so/83/sg/pdf (accessed on 10 June 2025).
33. Canu, S.; Rosati, L.; Fiori, M.; Motroni, A.; Filigheddu, R.; Farris, E. Bioclimate Map of Sardinia (Italy). *J. Maps* **2015**, *11*, 711–718. [CrossRef]
34. Ministerial Decree Approvazione Dei "Metodi Ufficiali Di Analisi Chimica Del Suolo" (GU Serie Generale n.248 Del 21-10-1999—Suppl. Ordinario n. 185). 1999. Available online: https://www.gazzettaufficiale.it/eli/gu/1999/10/21/248/so/185/sg/pdf (accessed on 10 June 2025).
35. Barbafieri, M.; Lubrano, L.; Petruzzelli, G. Characterization of pollution in sites contaminates by heavy metals: A proposal. *Ann. Chim.* **1996**, *86*, 585–594.
36. Caplan, D.; Stemeroff, J.; Dixon, M.; Zheng, Y. Vegetative Propagation of Cannabis by Stem Cuttings: Effects of Leaf Number, Cutting Position, Rooting Hormone, and Leaf Tip Removal. *Can. J. Plant Sci.* **2018**, *98*, 1126–1132. [CrossRef]
37. Gilliam, C.H.; Eakes, D.J.; Olive, J.W. Herbicide Use During Propagation Affects Root Initiation and Development. *J. Environ. Hortic.* **1993**, *11*, 157–159. [CrossRef]
38. Hou, P.-C.; Lin, K.-H.; Huang, Y.-J.; Wu, C.-W.; Chang, Y.-S. Applications of Vegetation Indices and Biostimulators to the Rooting of Camellia Cuttings. *J. Appl. Hortic.* **2019**, *21*, 111–115. [CrossRef]
39. Lichtenthaler, H.K. Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes. *Methods Enzymol.* **1987**, *148*, 350–382.
40. Hammer, Ø.; Harper, D.A.T.; Ryan, P.D. PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontol. Electron.* **2001**, *4*, 9. Available online: http://palaeo-electronica.org/2001_1/past/past.pdf (accessed on 10 June 2025).
41. Bacchetta, G.; Cappai, G.; Carucci, A.; Tamburini, E. Use of Native Plants for the Remediation of Abandoned Mine Sites in Mediterranean Semiarid Environments. *Bull. Environ. Contam. Toxicol.* **2015**, *94*, 326–333. [CrossRef]
42. Bacchetta, G.; Boi, M.E.; Cappai, G.; De Giudici, G.; Piredda, M.; Porceddu, M. Metal Tolerance Capability of *Helichrysum microphyllum* Cambess. Subsp. *Tyrrhenicum Bacch., Brullo & Giusso: A Candidate for Phytostabilization in Abandoned Mine Sites. Bull. Environ. Contam. Toxicol.* **2018**, *101*, 758–765. [CrossRef]
43. Bacchetta, G.; Cao, A.; Cappai, G.; Carucci, A.; Casti, M.; Fercia, M.L.; Lonis, R.; Mola, F. A Field Experiment on the Use of *Pistacia lentiscus* L. and *Scrophularia canina* L. Subsp. Bicolor (Sibth. et Sm.) Greuter for the Phytoremediation of Abandoned Mining Areas. *Plant Biosyst.-Int. J. Deal. All Asp. Plant Biol.* **2012**, *146*, 1054–1063. [CrossRef]
44. Kharazian, P.; Bacchetta, G.; Cappai, G.; Piredda, M.; De Giudici, G. An Integrated Geochemical and Mineralogical Investigation on Soil-Plant System of *Pinus Halepensis* Pioneer Tree Growing on Heavy Metal Polluted Mine Tailing. *Plant Biosyst.-Int. J. Deal. All Asp. Plant Biol.* **2022**, *157*, 272–285. [CrossRef]
45. Decree Law Norme in Materia Ambientale. GU n.88 Del 14-04-2006-Suppl. Ordinario n. 96. 2006. Available online: https://www.gazzettaufficiale.it/dettaglio/codici/materiaAmbientale (accessed on 10 June 2025).
46. Dessena, L.; Mulas, M. Cultivar Selection from Spontaneous Population of *Atriplex halimus* L. In Proceedings of the ISHS Acta Horticulturae 937, Lisbon, Portugal, 30 September 2012; Volume 2, pp. 205–210.
47. Francis, B.; Aravindakumar, C.T.; Brewer, P.B.; Simon, S. Plant Nutrient Stress Adaptation: A Prospect for Fertilizer Limited Agriculture. *Environ. Exp. Bot.* **2023**, *213*, 105431. [CrossRef]
48. Shahzad, Z.; Amtmann, A. Food for Thought: How Nutrients Regulate Root System Architecture. *Curr. Opin. Plant Biol.* **2017**, *39*, 80–87. [CrossRef] [PubMed]
49. Mateos-Naranjo, E.; Andrades-Moreno, L.; Redondo-Gómez, S. Comparison of Germination, Growth, Photosynthetic Responses and Metal Uptake between Three Populations of *Spartina densiflora* under Different Soil Pollution Conditions. *Ecotoxicol. Environ. Saf.* **2011**, *74*, 2040–2049. [CrossRef]
50. Dos Santos Utmazian, M.N.; Wieshammer, G.; Vega, R.; Wenzel, W.W. Hydroponic Screening for Metal Resistance and Accumulation of Cadmium and Zinc in Twenty Clones of Willows and Poplars. *Environ. Pollut.* **2007**, *148*, 155–165. [CrossRef]

51. Weih, M.; Nordh, N.-E. Characterising Willows for Biomass and Phytoremediation: Growth, Nitrogen and Water Use of 14 Willow Clones under Different Irrigation and Fertilisation Regimes. *Biomass Bioenergy* **2002**, *23*, 397–413. [CrossRef]
52. Martínez-Fernández, D.; Walker, D.J. The Effects of Soil Amendments on the Growth of *Atriplex halimus* and *Bituminaria bituminosa* in Heavy Metal-Contaminated Soils. *Water Air Soil Pollut.* **2012**, *223*, 63–72. [CrossRef]
53. Reddy, N.; Crohn, D.M. Compost Induced Soil Salinity: A New Prediction Method and Its Effect on Plant Growth. *Compos. Sci. Util.* **2012**, *20*, 133–140. [CrossRef]
54. Belkheiri, O.; Mulas, M. The Effects of Salt Stress on Growth, Water Relations and Ion Accumulation in Two Halophyte *Atriplex* Species. *Environ. Exp. Bot.* **2013**, *86*, 17–28. [CrossRef]
55. Bendaly, A.; Messedi, D.; Smaoui, A.; Ksouri, R.; Bouchereau, A.; Abdelly, C. Physiological and Leaf Metabolome Changes in the Xerohalophyte Species *Atriplex halimus* Induced by Salinity. *Plant Physiol. Biochem.* **2016**, *103*, 208–218. [CrossRef]
56. Nedjimi, B. Seasonal Variation in Productivity, Water Relations and Ion Contents of *Atriplex halimus* Spp. *Schweinfurthii* Grown in Chott Zehrez Wetland, Algeria. *J. Saudi Soc. Agric. Sci.* **2012**, *11*, 43–49. [CrossRef]
57. Miazek, K.; Ledakowicz, S. Chlorophyll Extraction from Leaves, Needles and Microalgae: A Kinetic Approach. *Int. J. Agric. Biol. Eng.* **2013**, *6*, 107–115.
58. Richardson, A.D.; Duigan, S.P.; Berlyn, G.P. An Evaluation of Noninvasive Methods to Estimate Foliar Chlorophyll Content. *New Phytol.* **2002**, *153*, 185–194. [CrossRef]
59. Peñuelas, J.; Filella, I. Visible and Near-Infrared Reflectance Techniques for Diagnosing Plant Physiological Status. *Trends Plant Sci.* **1998**, *3*, 151–156. [CrossRef]
60. Küpper, H.; Küpper, F.; Spiller, M. Environmental Relevance of Heavy Metal-Substituted Chlorophylls Using the Example of Water Plants. *J. Exp. Bot.* **1996**, *47*, 259–266. [CrossRef]
61. Sghaier, D.B.; Duarte, B.; Bankaji, I.; Caçador, I.; Sleimi, N. Growth, Chlorophyll Fluorescence and Mineral Nutrition in the Halophyte *Tamarix Gallica* Cultivated in Combined Stress Conditions: Arsenic and NaCl. *J. Photochem. Photobiol. B Biol.* **2015**, *149*, 204–214. [CrossRef]
62. Badache, H.; Sbartai, H.; Djebar, M.; Manousaki, E.; Bourguignon, J. Phytoextraction of Cd by *Atriplex nummularia* L., a Xero-Halophyte Species. *Int. J. Biosci. (IJB)* **2015**, *6*, 130–139.
63. Nedjimi, B.; Daoud, Y. Cadmium Accumulation in *Atriplex halimus* Subsp. Schweinfurthii and Its Influence on Growth, Proline, Root Hydraulic Conductivity and Nutrient Uptake. *Flora-Morphol. Distrib. Funct. Ecol. Plants* **2009**, *204*, 316–324. [CrossRef]
64. Lotmani, B.; Mesnoua, M. Effects of Copper Stress on Antioxidative Enzymes, Chlorophyll and Protein Content in *Atriplex halimus*. *Afr. J. Biotechnol.* **2011**, *10*, 10143–10148. [CrossRef]
65. Acuña, E.; Castillo, B.; Queupuan, M.; Casanova, M.; Tapia, Y. Assisted Phytoremediation of Lead Contaminated Soil Using *Atriplex halimus* and Its Effect on Some Soil Physical Properties. *Int. J. Environ. Sci. Technol.* **2021**, *18*, 1925–1938. [CrossRef]
66. Dessena, L.; Mulas, M. Mineral Composition of *Atriplex halimus* Plant as Influenced by Genotype and Thermal Regime. *Int. J. Phytoremediat.* **2021**, *23*, 548–558. [CrossRef] [PubMed]
67. Shahrokh, V.; Perez, V.; Zornoza, R.; Acosta, J.A.; Faz, A.; Martinez-Martinez, S. Soil Sodium, Magnesium and Potassium Contents Contribute to Metals Uptake and Accumulation in Leaves of *Atriplex halimus* in Tailings Ponds. *J. Environ. Chem. Eng.* **2022**, *10*, 107948. [CrossRef]