



Salt glands enable salt but not metal(loid)s excretion in *Limonium daveaui* under amended saline and contaminated soils

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Abstract

Soil–plant interactions are essential for understanding adaptive responses to abiotic stressors such as salinity and metal(loid)s contamination. This study analyzed plant behavior of the recretohalophyte *Limonium daveaui* Erben when cultivated in saline and in gossan mine soils, both with and without organic–inorganic amendments. Analysis included soil physicochemical and biological parameters, multielemental composition of plant tissues, and the cationic profile of salts excreted by foliar salt glands. All plants grown in unamended gossan soil died, whereas amendment addition substantially improved both gossan and saline Fluvisol properties, raising pH in gossan soil, reducing salinity, increasing nutrient availability, generally stimulating microbial enzymatic activity, thereby allowing normal plant development. In amended gossan soil, plants accumulated high concentrations of As and Pb and moderate levels of other potentially hazardous elements (PHE) in roots and shoots without visible toxicity symptoms. Transfer coefficients revealed low PHE translocation but high biological absorption, indicating efficient uptake. Microscopic analyses showed the presence of the typical *Limonium* salt glands on both leaf surfaces through which plants excreted salts with macroelements but not As and Pb. The findings indicate that plant's primary tolerance strategy is based on accumulation of metal(loid)s in roots, rather than exclusion or excretion through foliar salt glands.

Keywords *Limonium* spp. · Potentially hazardous elements · Soil enzymatic activities · Tailored soils

Introduction

Halophyte species are adapted to a wide range of saline habitats and can survive in the presence of high concentrations of soluble salts in the medium (Aronson 1989; Flowers and Colmer 2008, 2015). These species possess specific functional traits that allow them to tolerate extreme environmental conditions, relying on both tolerance and avoidance mechanisms to cope with saline stress. These mechanisms

include a variety of biochemical, physiological, anatomical, and morphological adaptations (Flowers and Colmer 2015; Yuan et al. 2019a, b; Rahman et al. 2021; Mann et al. 2023; Navarro-Torre et al. 2023). The most common traits include succulence, salt excretion through specialized salt glands, Na and Cl exclusion at the root level, compartmentalization of Na and Cl in vacuoles, maintenance of Ca and K uptake, and the synthesis of osmolyte compounds (Flowers and Colmer 2015; Dassanayake and Larkin 2017; Yuan et al. 2019a; Caperta et al. 2020; Mann et al. 2023). When exposed to elevated NaCl concentrations, some halophytes can enhance succulence by increasing leaf thickness (Debez et al. 2004; Yepes et al. 2018), thereby improving leaf water content and diluting salts (e.g., NaCl) in tissues, which mitigates salt-induced damage (Rangani et al. 2016; Yepes et al. 2018). Salt-secreting halophytes (recretohalophytes) belong to five families that comprise approximately 90% of known species with this trait (Plumbaginaceae, 28%; Poaceae, 21% Amaranthaceae 20%; Tamaricaceae, 15% and Frankeniaceae, 6%) (Yuan et al. 2016; Dassanayake and Larkin 2017; Caperta et al. 2020). The Plumbaginaceae family has diversified across coastal, inland-saline, and gypsum soils

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(Hernandez-Ledesma et al. 2015). The genus *Limonium* (Plumbaginaceae) includes at least 45 halophytic species that possess leaf glands, specific foliar structures from which salts can be excreted (Caperta et al. 2020).

Halophytes can be cultivated under saline conditions, where they often exhibit increased flowering, reduced sterility, and improved seed quality (Debez et al. 2004; Yuan et al. 2019b; Cortinhas et al. 2020). Soil management strategies can incorporate integrated applications of organic and inorganic amendments combined with native substrates to enhance the physicochemical properties, fertility, and structural integrity of salt-affected soils and sediments, and to facilitate halophyte cultivation under saline irrigation regimes (Navarro-Torre et al. 2023). These technologies can also be employed to remediate contaminated soils, including those affected by mining activities (Santos et al. 2014, 2017, 2019; Arán et al. 2021, 2025). Organic and inorganic amendments addition to soils improve the physicochemical conditions and stimulate biogeochemical processes mitigating potentially hazardous elements (PHE) bioavailability through mechanisms such as adsorption, complexation, and immobilization. These changes facilitate vegetation establishment and promote plant development in contaminated or degraded environments (Adriano et al. 2004; Asencio et al. 2013; Aguilar-Garrido et al. 2023).

Soil-plant interactions are important for understanding plant behavior and stress tolerance in extreme environments. The present study hypothesizes that the recretohalophyte *Limonium daveau* Erben can secrete chemical elements through its leaves when grown in both native saline habitat and non-saline, contaminated mining soils. Given that both soil types exhibit physicochemical limitations and poor structural conditions for plant growth, they were amended with a mixture of organic and inorganic wastes. The objectives of this study were to: (1) evaluate the physicochemical properties, multielemental composition, and microbial activity (via enzymatic indicators) of the saline and mining soils collected in 2020, in saltmarsh area of Tagus estuary and in São Domingos mine area (Portugal); (2) determine the multielemental concentrations in *L. daveau* plants grown in unamended and amended saline and contaminated soils at Instituto Superior de Agronomia; (3) assess plant uptake behavior using soil-to-plant transfer coefficients; and (4) characterize the chemical composition of salts excreted from leaves.

Materials and methods

Study species

Limonium daveau Erben is a hemicryptophyte, chamaephyte endemic to thermo-Mediterranean dry salt marshes,

occurring in areas only occasionally exposed to estuarine water during equinoctial tides (Costa et al. 2014). It is a perennial species with a basal and glabrous rosette, leaves with parallel veins, inflorescences (scapes) branched in the lower third, with bluish-violet flowers that bloom from June to August (Franco 1984).

Its distribution has been severely reduced by coastal urbanization and industrial activities and now it is classified as Critically Endangered in the Portuguese Red Data Book and is protected within the special area of conservation SIC PTCON00099 (Tagus Estuary, western Portugal) (Caperta and Carapeto 2020).

Study soils

Two soils were studied: (i) a saline soil from the degraded estuarine area of “Praia do Sobralinho” in the Tagus Estuary (Vila Franca de Xira municipality (38° 54' 16'' N, 9° 01' 09'' W), (ii) a mine soil from an abandoned mining area contaminated by potentially hazardous elements (PHE), the São Domingos mine.

The estuarine area is periodically subjected to saltwater intrusion due to tidal influence extending ~50 km upstream (Cortinhas et al. 2021). The São Domingos mine, situated in the northern Iberian Pyrite Belt (37° 40' 28'' N, 7° 30' 01'' W), is one of the largest historical mining sites, with the exploitation of Ag, Cu and Au mainly in the gossan resulting from the massive sulfide oxidation orebody (Quental et al. 2002; Álvarez-Valero et al. 2008). The mining activities in the modern times exploitation left behind extensive and different mining wastes, unevenly distributed across the area, with significant environmental risks due to its chemical and mineralogical properties (Pérez-López et al. 2008).

Microcosm assay

Previous cultivation studies on *Limonium* species revealed that saline soils exhibited physicochemical properties and structural constraints to plant growth (Cortinhas et al. 2020, 2021). The application of organic and inorganic waste-derived amendments can significantly improve soil quality and facilitate plant growth and development (Cortinhas et al. 2020, 2021; Nogales et al. 2023; Aguilar-Garrido et al. 2024). In this study the assay consisted of four treatments (hereafter, substrata), prepared in pots with an approximate volume of 1.5 dm³, with four replicates each, as in Aguilar-Garrido et al. (2024). The substrates were: (i) gossan soil from the São Domingos mine (G) (control for PHE contamination); (ii) gossan amended soil (AG); (iii) Salic Fluvisol (Eutric) (WRB 2022) from the Sobralinho salt marsh area



(VF) (control for salinity); and (iv) amended saline Fluvisol (AVF) (Cortinhas et al. 2021; Aguilar-Garrido et al. 2024).

The AG consisted of 95% G and 5% (by mass) of a waste mixture comprising biomass pruning, gravel limestone ($\varnothing < 5$ mm), coffee grounds, sludge, and brewery kieselguhr in a ratio of 25:20:20:25:10, respectively, as in (Aguilar-Garrido et al. 2024). The AVF substrate consisted of 85% VF and 15% (by mass) of a waste mixture composed of sludge, brewery kieselguhr, medium sand (0.25–0.5 mm), gravel limestone ($\varnothing < 5$ mm), and biomass pruning in a ratio of 15:5:30:20:30, respectively (details in Cortinhas et al. 2021). All substrates were incubated for 30 days at 75% water-holding capacity and maintained at room temperature (20–25 °C).

Seedlings (five replicates) were raised from seeds germinated on *jiffy* pots wet with deionized water, which were then transplanted to the substrata after 2.5 months. Plantlets were irrigated with deionized water throughout the experimental assay, keeping the substrata at 75% of the maximum water-holding capacity (400 ml). The complete assay was carried out in a greenhouse under natural sunlight and controlled aeration conditions.

Sample analysis

Soils

Composite substrate samples ($n=5$ per pot) were collected at the beginning and end of the assay. Subsamples for enzymatic activity were stored at 4 °C in sterile opaque containers; the rest were air-dried at room temperature, homogenized, and sieved (2 mm) for physicochemical and elemental analysis.

The characterization of all substrates (G, AG, VF, and AVF) was realized at the beginning and at the end of the assay, following the procedures outlined in Póvoas and Baral (1992). These included assessing the following physicochemical parameters: pH and electrical conductivity (EC) in water (1:2.5 m/V), total organic carbon (Corg) via wet combustion, total N (N_T) using the Kjeldahl method, and extractable P and K (P_{Ext} and K_{Ext}) through the Egner–Riehm method.

Total macro- (Ca, Mg, Na, K) and micronutrients (Fe, Mn, Zn, Cu) were measured using flame atomic absorption spectroscopy after extraction as in Lakanen and Erviö (1971). In the soil bioavailable fraction, the elements concentrations were measured via inductively coupled plasma mass spectrometry (ICP-MS) at Activation Laboratories Lda (Activation Laboratories 2024a, 2024b), using rhizosphere-based organic acid extraction (Feng et al. 2005).

To assess soil biological quality, the enzymatic activities were analyzed across all substrates. Dehydrogenase was utilized as an indicator of total microbial activity (Tabatabai 1994; Kumar et al. 2013; Dotaniya et al. 2019); β -glucosidase (Eivazi and Tabatabai 1988) and cellulase linked to the C cycle (Jecu 2000; Hope and Burns 1987); acid phosphatase (Eivazi and Tabatabai 1977) and urease (Kandeler and Gerber 1988), associated with the P and N cycles, respectively (Krämer and Green 2000; Liang et al. 2003; Bai et al. 2013), were also measured. Protease activity related to the N cycle (Ladd and Butler 1972) was determined based on Adetunji et al. (2017).

Plants

Leaf imprints from the abaxial and adaxial surfaces of young, fully expanded leaves were collected from three *L. daveaui* plants following the method described by Balasooriya et al. (2009). The imprints were obtained using colorless nail polish and transparent adhesive tape, then mounted on microscope slides for observation under a light microscope (Leitz Dialux 20 EB) at a total magnification of 125x. Images of five randomly selected fields per imprint were captured using a digital camera (Leica EC3) and analyzed with LAS software (v.2.13). The number of salt glands, stomata, and pavement cells were determined using ImageJ software (v. 1.49 s). The density of each cell type was calculated as the number of cells or structures per mm² of leaf area. Salts on leaf surfaces were photographed using a VMS-004 USB microscope, with 10× objective.

The concentration of PHE and nutrients in salts excreted was evaluated using one leaf per plant per pot, which was collected and submerged in 10 mL of 0.1% (V/V) HNO₃ for 2 min (Hagemeyer and Waisel 1988), to dissolve salts accumulated on the leaf surfaces. The resulting solution was analyzed using ICP-MS (Activation Laboratories 2014c).

At the end of the experiment, plant aerial parts (shoots and flowers) and root samples were collected and rinsed with tap water, followed by distilled water. The roots were also sonicated (after washing) in distilled water in an ultrasound bath for 30 min. The fresh biomass was oven-dried at 70 °C until a constant weight was reached. The shoots, flowers, and roots dry weight were determined using a digital analytical balance (Sartorius ENTRIS124-1S), and ground into a fine powder. Multi-elemental chemical analyses of aerial parts and root samples from each substrate were conducted using ICP-MS following digestion with HNO₃ (Activation Laboratories Lda 2024a, 2024b). The Activation Laboratories Lda



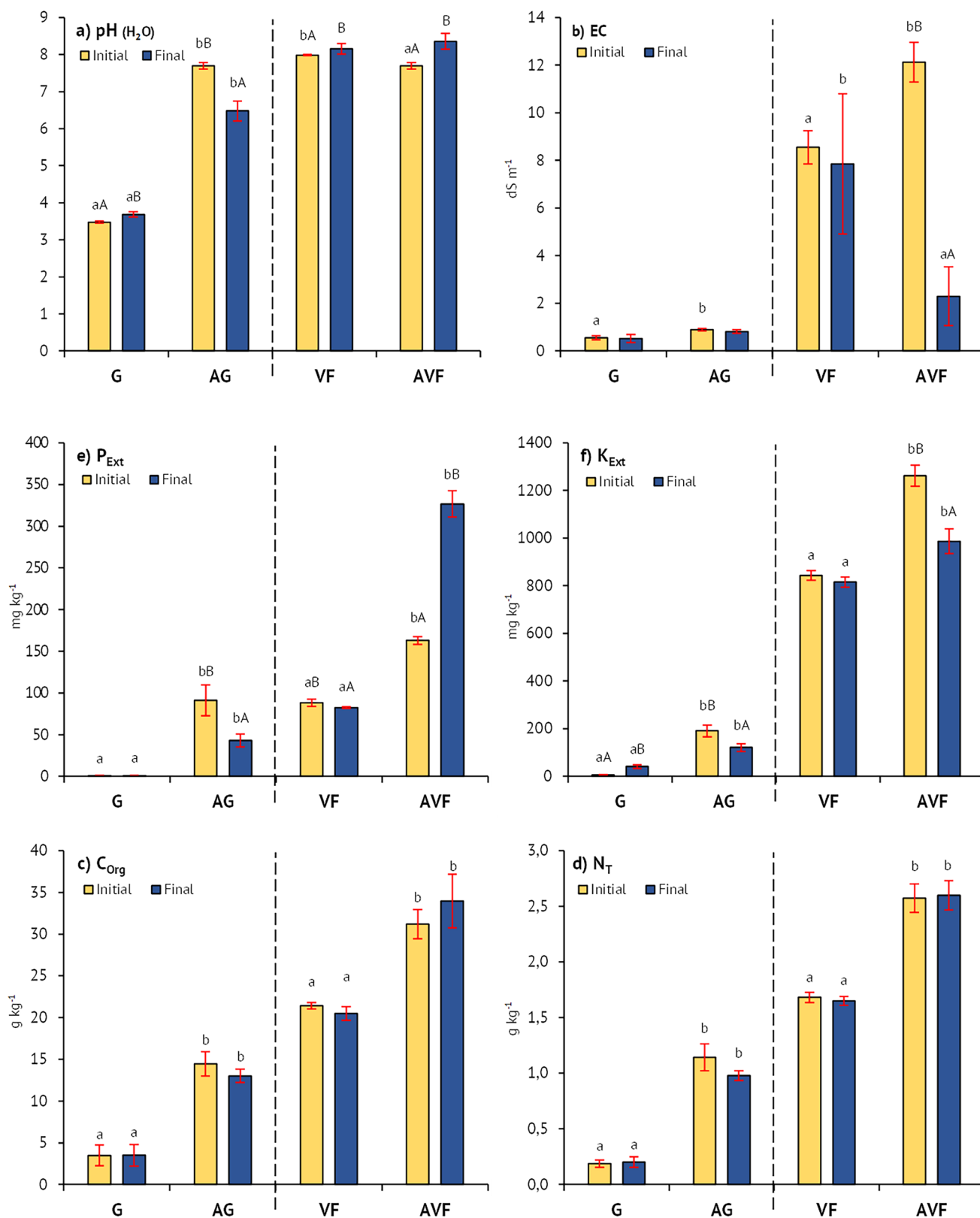


Fig. 1 Physicochemical characteristics [a pH, b Electrical conductivity (EC), c Organic carbon (C_{Org}), d Total nitrogen (NT), e Extractable phosphorus (P_{Ext}), f Extractable potassium (K_{Ext}) of the gossan mine waste, saline-affected Fluvisol and Amended soils. G: Gossan waste; AG: Amended-Gossan; VF: Fluvisol; AVF: Amended-Fluvisol. Values correspond to average of four biological replicates ($\pm$ SD). Lowercase letters indicate significant differences between the control (G or VF) and the respective Amended soil (AG or AVF) in each time; and capital letters indicate significant differences in each treatment with respect to time (initial and final cultivation) (U test Mann–Whitney)

is a certified laboratory (ISO/IEC 2017) that ensures quality control of the multi-elemental analyses for both substrates and plant samples according to their standards. Quality control for all other analyses was maintained through technical replicates, the use of certified standard solutions, and method reagent blanks.

Data analysis

Data normality (Kolmogorov Smirnov) and variance homogeneity test (Levene) were tested. When assumptions were not met, even after transforming, non-parametric tests (Mann–Whitney U) ($p < 0.05$) were used for multiple comparisons. Furthermore, Spearman correlations were applied to analyze the influence of physicochemical characteristics, nutrient, and available PHE concentrations on soil enzymatic activities, as well as to explore the relationships between element concentrations in the soil and their accumulation in different plant compartments (aerial part, root, and excreted salts). All analyses were made with a confidence level of 95% by using RStudio software (RStudio Inc., 250 Northern Ave, Boston).

To evaluate soil–plant behavior, the translocation coefficient and biological absorption coefficient of each element and nutrient analyzed were calculated. The translocation coefficient ($\text{TranslC} = [\text{total element in the shoots}] / [\text{total element in the roots}]$) assesses the plant's capacity to translocate an element from the roots to the shoots (Abreu et al. 2014). The biological absorption coefficient ($\text{BAC} = [\text{total element in the roots}] / [\text{element in the available fraction of the soil}]$) evaluates the uptake capacity of an element by the plant. This latter coefficient was divided into five groups considering the Perelman classification, which indicates the intensity of an element absorption by plant roots (intensive BAC, 10–100; strong BAC, 1–10; intermediate BAC, 1–0.1; weak BAC, 0.1–0.01; very weak BAC, 0.01–0.001) (Perelman 1966).

Results and discussion

Physicochemical characterization of gossan mine soil and saline-affected fluvisol

The gossan soil (G) was highly acid ($\text{pH} \approx 3.5$), with low electrical conductivity, very low levels of C_{Org} ($< 4 \text{ g kg}^{-1}$), total N ($< 0.2 \text{ g kg}^{-1}$), extractable P ($< 1.0 \text{ mg kg}^{-1}$) and extractable K ($< 7.5 \text{ mg kg}^{-1}$), indicating very poor fertility (Fig. 1). Macronutrients and micronutrients availability was also low (Fig. 2), while total concentrations of several PHE were high such as, in g kg^{-1} : As (9.13), Cu (0.22), Hg (0.03), and Pb (29.63) (Aguilar-Garrido et al. 2024). These levels classify the G substrate as contaminated for agricultural, residential/parkland, commercial, and industrial uses in most cases, as they exceeded the most restrictive regulatory thresholds by factors of 830, 3.5, 169, and 658, respectively (CCME 2007; APA 2019). Such conditions are highly adverse for plant establishment, explaining the observed mortality of all plants in this substrate.

Soil amendments addition is a widely recognized strategy to reduce PHE availability or even immobilize these elements in soils or wastes by improving soil properties by rising soil pH, increasing organic C content, enhancing nutrient availability, soil structure and water holding capacity (Adriano 2004; Abreu and Magalhães 2009; Madejón 2021). Although, the effectiveness of these amendments may decline over time, amended soils promote and maintain biogeochemical processes and decrease PHE availability for plants (Macías 2004; Macías et al. 2011).

The Salic Fluvisol from Vila Franca de Xira (VF) was slightly alkaline ($\text{pH} \approx 8$) and saline ($\text{EC} > 8 \text{ dS m}^{-1}$). Organic carbon (21 g kg^{-1}) and total N (1.7 g kg^{-1}) were in the low to medium range, whereas extractable P (0.09 g kg^{-1}) and K (0.8 g kg^{-1}) were relatively high (Fig. 1). Macronutrients and micronutrient concentrations were very high (Veloso et al. 2022), specially for Fe, Mn, and Zn, and medium for Cu (Fig. 2). The presence of approximately 25% exchangeable Na resulted in soil structure degradation, blocking soil pores and hampering root penetration (Cortinhas et al. 2021, 2023). Furthermore, a [Ca]/[Mg] ratio of approximately two indicated adverse physical conditions, such as aggregate destabilization and colloids dispersion, which impair plants development (Veloso et al. 2022). In VF, the total concentrations of PHE (Aguilar-Garrido et al. 2024) were below the maximum permissible values for agricultural use, except for As (15.6 mg kg^{-1}), which exceeded the limit of 11 mg kg^{-1} (CCME 2007; APA 2019).



Fig. 2 Total concentration of macronutrients **a** and micronutrients **b** in the soil fraction extracted by Lakanen and Ervö (1971) method of gossan mine waste, saline-affected Fluvisol and Amended soils. G: Gossan waste; AG: Amended-Gossan; VF: Fluvisol; AVF: Amended-Fluvisol. Lowercase letters indicate significant differences between the control (G or VF) and the respective Amended soils (AG or AVF) in each time; and capital letters indicate significant differences in each treatment with respect to time (initial and final cultivation) (U test Mann–Whitney)

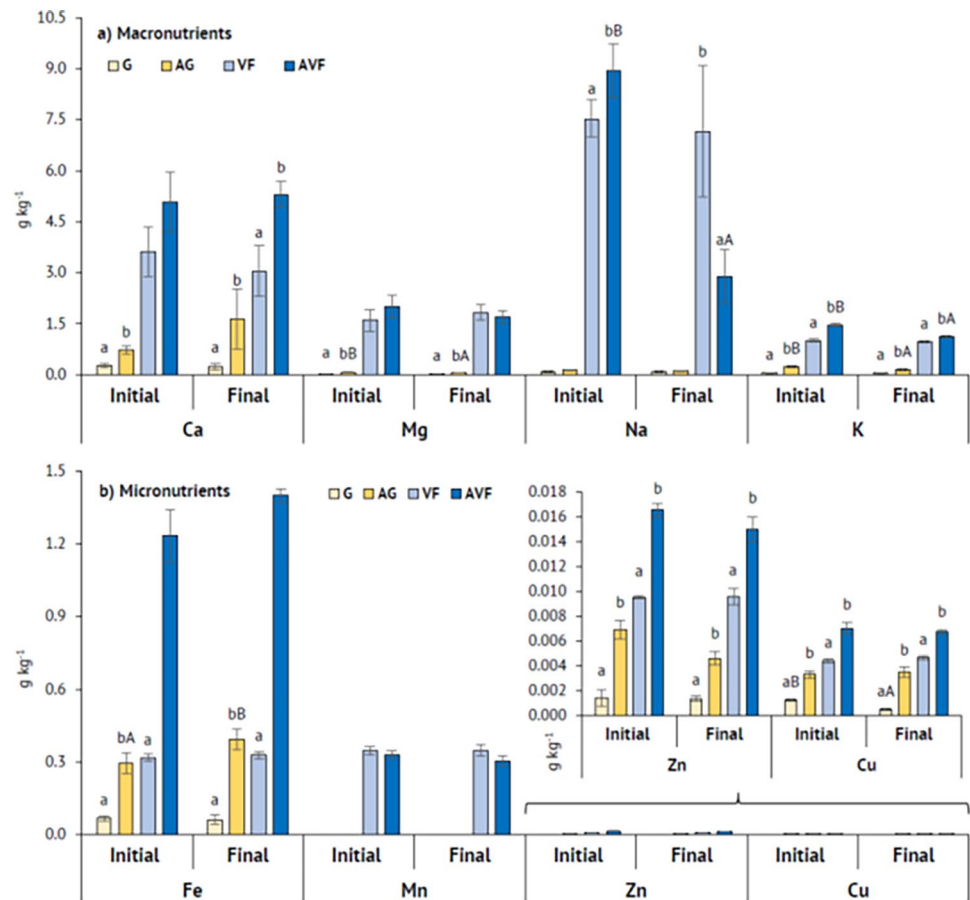


Table 1 Concentration of chemical elements in the available fraction of gossan mine waste, saline-affected Fluvisol and Amended soils at the beginning of the microcosm assay

Elements	G _i	AG _i	VF _i	VFA _i
As (mg kg ⁻¹)	0.31 ± 0.12 a	2.20 ± 0.11 b	bdl	0.40 ± 0.03
Mn (mg kg ⁻¹)	0.36 ± 0.08 a	3.27 ± 0.08 b	20.55 ± 2.09	21.69 ± 3.90
Na (mg kg ⁻¹)	606.06 ± 38.97 b	113.98 ± 5.61 a	5724.03 ± 387.91 a	7377.62 ± 854.72 b
Pb (mg kg ⁻¹)	2.64 ± 0.71 b	0.45 ± 0.09 a	bdl	bdl
Zn (mg kg ⁻¹)	3.57 ± 1.09 a	7.46 ± 0.88 b	0.37 ± 0.08	0.46 ± 0.12

G: Gossan waste; AG: Amended-Gossan; VF: Fluvisol; AVF: Amended-Fluvisol; bdl: below detection limit. Letters indicate significant differences between the control (G or VF) and the respective Amended soil (AG or AVF) (U test Mann–Whitney)

Physicochemical characterization of the amended soils

The amended gossan soil (AG) showed a marked improvement in soil physicochemical properties, with pH rising above 6.5 and macro- and micronutrients enrichment relative to unamended gossan (Figs. 1 and 2). Total N (1.1 g kg⁻¹)

increased 5–6 times, and Corg (14 g kg⁻¹) 3–4 times, while extractable P and K also rose remarkably (Fig. 1).

Following plants growth in AG, extractable P and K and most PHE concentrations in the available fraction decreased (Fig. 1, Table 1, Table SM1), with the exception of As and Zn (Table 1), consistent with previous studies (Beesley et al. 2010; Paradelo et al. 2011; Santos et al. 2019). The observed



decline in Pb availability in AG was likely linked to increase of organic matter by amendments addition, whereas availability of other PHE like As increased. The higher AG pH (> 6.5) can facilitate As desorption from iron oxides and/or organic matter (Wang and Mulligan 2006; Aguilar-Garrido et al. 2020; Paniagua-López et al. 2021). In AG, the phosphate anions can also compete with arsenic anions for soil components binding sites (Bolan et al. 2014) increasing both As availability and extractable P concentration (Table 1, Fig. 1).

In the amended saline Fluvisol (AVF), EC decreased by ~81% (12.13 to 2.3 dS m⁻¹) compared to the initial substrate, whereas in unamended VF it decreased by ~8% (from 8.55 to 7.85 dS m⁻¹) (Fig. 1). In line with previous findings, amendments addition (biomass pruning, coffee grounds, sludge, waste kieselguhr) increased Corg, total N, and extractable P and K (Cortinhas et al. 2020, 2021) by factors of 1.5, 1.6, 4.6, and 1.2 compared to VF, respectively (Fig. 1). Macro- and micronutrients significantly enhanced in AVF, especially Ca and Fe (Fig. 2). The organic and mineral amendments effectively reduced salinity while enhancing nutrient availability (Cortinhas et al. 2020, 2021) in the end of the assay.

Biological characterization of substrata

Soil enzymatic activities were assessed as bioindicators of microbial function, focusing on key enzymes involved in C, N and P cycles (Sardans et al. 2008; Aragón et al. 2014). Dehydrogenase activity, a marker of the soil's microbiological redox system and microbial oxidative processes (Casida et al. 1964) was nearly absent in G (2.66 µg TPF g⁻¹ 16 h⁻¹), and low in VF (11.18 µg TPF g⁻¹ 16 h⁻¹) (Fig. 3a). In contrast, amended soils (AG and AVF) showed significantly higher enzymatic activity (Fig. 3a), suggesting improved microbial community function supporting organic matter decomposition, mineralization, and nutrient cycling processes (Arán et al. 2022).

After plants growth, dehydrogenase, B-glucosidase, cellulase and acid phosphatase activities decreased in AG, while these enzymes increased in AVF substrate. These findings are probably a consequence of Corg, total N and macro- and micronutrients highest availability for microorganisms during plant development in AVF substrata, stimulating those enzymes activity (Figs. 1 and 2). Spearman correlations indicated clear relationships

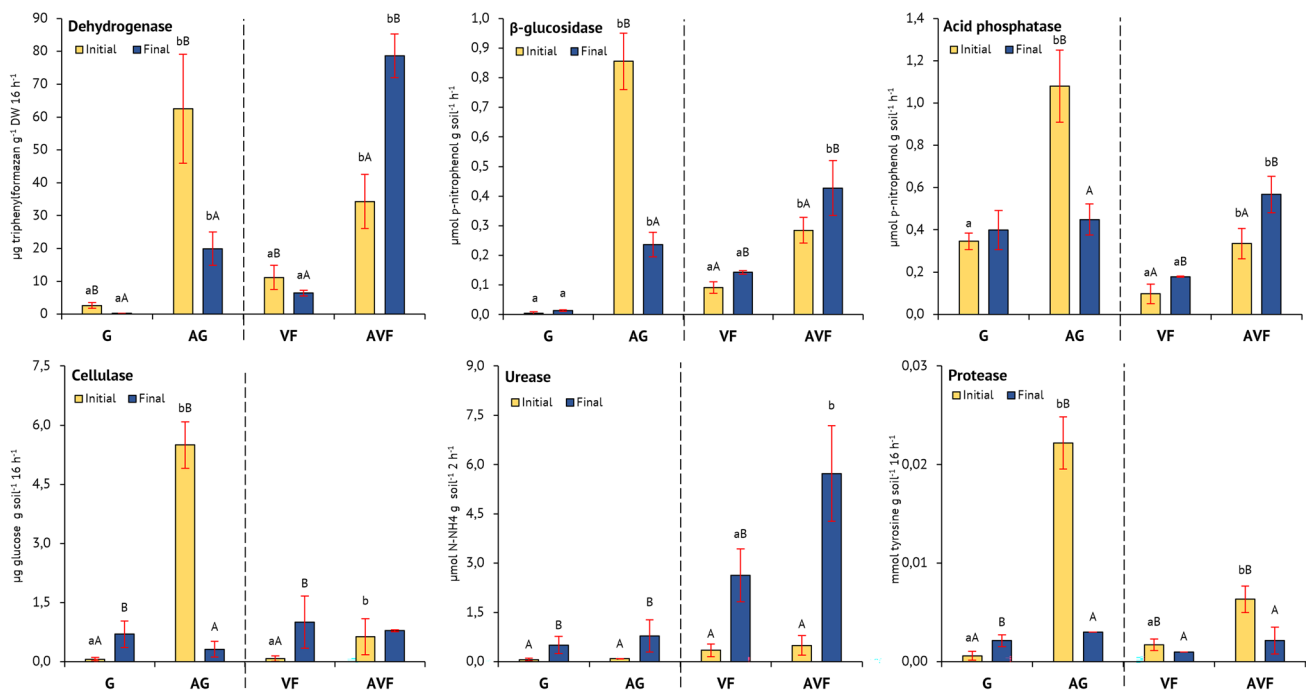


Fig. 3 Enzymatic activities [a dehydrogenase, b β-glucosidase, c acid phosphatase, d cellulase, e urease, f protease] in the soil fraction of gossan mine waste, saline-affected Fluvisol and Amended soils. G – Gossan waste; AG – Amended-Gossan; VF –Fluvisol; AVF – Amended-Fluvisol. Lower case letters indicate significant differences

between the control (G or VF) and the respective Amended soil (AG or AVF) in each time; and capital letters indicate significant differences in each treatment with respect to time (initial and final cultivation) (U test Mann–Whitney)



between soil properties and enzymatic activities (Fig. SM1a). Organic carbon and total N were positively correlated with enzymatic activities revealing that nutrient availability supported microbial functioning. In contrast, EC was negatively correlated with enzymatic activity in saline substrates, suggesting salinity inhibition of microbial activity.

Enzymes implicated in N cycling, such as urease and protease showed an opposite trend. After plant growth, urease activity increased in all soils (G, AG, VF, AVF), while protease activity decreased in AG, VF, and AVF. The higher total N in VF and AVF is strongly correlated with higher Corg in these soils ($r = 0.96$, $p < 0.05$). According to Koçak (2020) Corg regulates microbial abundance and activity in the rhizosphere, thereby influencing urease activity. Protease increased in G, where all plants died, but decreased in AG, where plant survival was observed. These findings may suggest that *L. daveaui* roots release protease inhibitors into the rhizosphere as found for other species (Rudrappa et al. 2008; Parisi et al. 2024) or elevated urease activity may indirectly suppress protease activity, as enzymatic interactions are often modulated by substrate competition and microbial community shifts.

Plant growth and behavior

In the G substrate, characterized by low pH (3.5), very low Corg content, total N, and extractable P and K, all plants died within 15 days after transplantation. Amendment addition to G (AG) improved these properties (e.g. pH increased up to 7.7) as well as soil structure and water holding capacity, allowing plant establishment and growth, as previously for São Domingos mine amended substrate (Santos et al. 2014).

Plant biomass increased significantly in both amended substrates (AG and AVF), with shoots and roots dry



Fig. 5 *Limonium daveaui* plants after growth on the different substrata. From left to right, plants cultivated in saline-Fluvisol (left) and Amended soils from saline-Fluvisol (centre) and gossan soil (right) at the end of the experiment

weights approximately doubling relatively to VF (Fig. 4). Growth stimulation reflected improved physicochemical properties and biological conditions and reduced PHE bio-availability. The VF plants' root system was predominantly concentrated at the bottom of pots, whereas plants in AVF were uniformly distributed, due to better substrate conditions (Cortinhas et al. 2020, 2021). Plants growing in AVF also produced more flowers and shoot biomass (Fig. 4) exceeds root biomass in all treatments.

Fig. 4 Development of *Limonium* plants (dry biomass of shoots, roots and flowers) in the gossan mine waste, saline-affected Fluvisol and Amended soils. G – Gossan waste; AG – Amended-Gossan; VF – Fluvisol; AVF – Amended-Fluvisol. Letters indicate significant differences between the control (G or VF) and the respective Amended soils (AG or AVF) (U test Mann–Whitney)

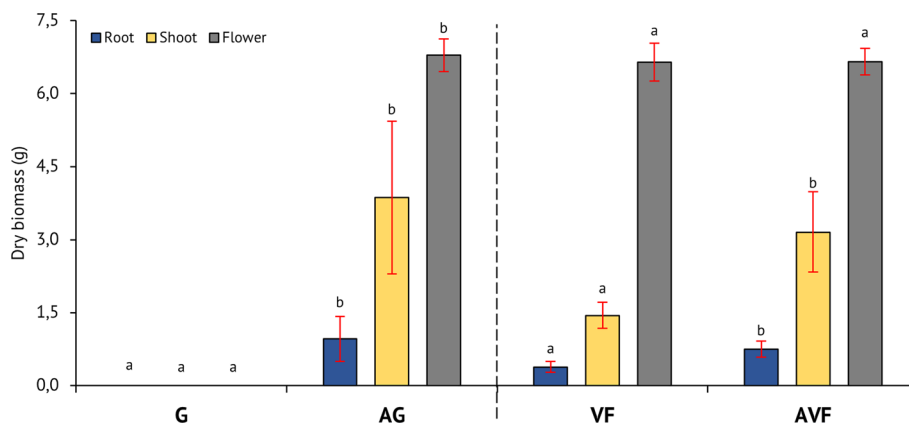


Table 2 Total concentration of potential hazardous elements (PHEs) and nutrients in shoots and roots. *Limonium* plants grown in gossan mine waste, saline-affected Fluvisol and Amended soils at the end of the assay

Elements	Shoots				Roots			
	G _f	AG _f	VF _f	AVF _f	G _f	AG _f	VF _f	AVF _f
As (mg kg ⁻¹)	–	56.38 ± 19.65	bdl	bdl	–	294.98 ± 171.42	bdl	bdl
Cu (mg kg ⁻¹)	–	6.93 ± 1.95	9.02 ± 0.72	8.01 ± 0.67	–	23.44 ± 8.13 B	10.70 ± 5.20	10.04 ± 0.79 A
Pb (mg kg ⁻¹)	–	132.23 ± 48.23 B	0.50 ± 1.00	0.50 ± 1.00 A	–	532.35 ± 67.16 B	3.51 ± 1.02	2.00 ± 1.63 A
Zn (mg kg ⁻¹)	–	45.03 ± 11.75 B	37.96 ± 1.33 b	27.97 ± 4.29 aA	–	41.08 ± 13.97 B	22.86 ± 3.62	20.42 ± 1.10 A
Fe (g kg ⁻¹)	–	1.03 ± 0.37 B	0.31 ± 0.08 a	0.36 ± 0.10 bA	–	6.37 ± 3.19 B	0.39 ± 0.16	0.56 ± 0.25 A
K (g kg ⁻¹)	–	14.53 ± 1.46 B	10.57 ± 1.48	10.32 ± 2.01 A	–	4.46 ± 1.04	4.50 ± 0.79	6.09 ± 1.07
Mn (mg kg ⁻¹)	–	46.89 ± 19.03	20.74 ± 2.18 a	38.01 ± 9.01 b	–	17.48 ± 1.92 A	14.00 ± 1.67 a	34.43 ± 11.60 bB
Na (g kg ⁻¹)	–	5.03 ± 0.27 A	26.14 ± 3.83 b	18.09 ± 1.56 aB	–	1.22 ± 0.18 A	8.74 ± 1.73 b	4.60 ± 0.71 aB
S (g kg ⁻¹)	–	12.71 ± 0.98	10.51 ± 1.27	12.76 ± 1.99	–	3.00 ± 0.16 B	2.10 ± 0.19	2.23 ± 0.11 A
Si (g kg ⁻¹)	–	0.45 ± 0.05	0.33 ± 0.11	0.61 ± 0.17	–	0.42 ± 0.15	0.52 ± 0.23	0.54 ± 0.09

G: Gossan waste; AG: Amended-Gossan; VF: Fluvisol; AVF: Amended-Fluvisol; bdl: below detection limit. Lowercase letters indicate significant differences between the control (VF_f) and the respective Amended soil (AVF_f); and capital letters indicate significant differences between the two Amended soils (AG_f vs. AVF_f) (U test Mann–Whitney)

Table 3 Translocation coefficient (TransIC) and biological absorption coefficient (BAC) of potential hazardous elements (PHE) and nutrients of *Limonium* plants grown in Amended gossan mine waste (AG), saline-affected Fluvisol (VF) and Amended soils (AVF) at the end of the assay

Elements	TransIC			BAC		
	AG	VF	AVF	AG	VF	AVF
As	0.25 ± 0.11	–	–	134.50 ± 60.64	–	–
Cu	0.34 ± 0.12	0.98 ± 0.34	0.80 ± 0.09	25.57 ± 7.04	49.50 ± 22.53	34.42 ± 2.59
Pb	0.23 ± 0.10	0.12 ± 0.19	–	1665.81 ± 721.28	–	–
Zn	1.21 ± 0.43	1.69 ± 0.17	1.36 ± 0.11	5.59 ± 1.62	63.16 ± 9.09	46.66 ± 7.37
Fe	0.20 ± 0.08	0.90 ± 0.29	0.70 ± 0.18	31.13 ± 11.95	5.64 ± 1.72	8.17 ± 3.41
K	3.42 ± 0.78	2.40 ± 0.36	1.70 ± 0.19	27.20 ± 5.08	15.11 ± 1.88	10.16 ± 1.18
Mn	2.78 ± 1.03	1.48 ± 0.06	1.13 ± 0.13	5.35 ± 0.42	0.69 ± 0.09	1.67 ± 0.61
Na	4.21 ± 0.58	3.05 ± 0.41	3.97 ± 0.29	10.69 ± 1.07	1.53 ± 0.23	0.63 ± 0.10
S	4.26 ± 0.39	5.01 ± 0.29	5.55 ± 0.60	6.76 ± 0.56	5.69 ± 0.43	2.62 ± 0.35
Si	1.13 ± 0.24	0.68 ± 0.16	1.13 ± 0.14	12.20 ± 3.23	3.14 ± 1.03	5.19 ± 0.86

AG: Amended-Gossan; VF: Fluvisol; AVF: Amended-Fluvisol

In AG plants PHE accumulation (except Zn and Mn) was higher in roots, with limited translocation to shoots (TransIC < 1; Table 3). Despite high As (56.4 mg kg⁻¹) and Pb (132.3 mg kg⁻¹) concentrations in AG plant shoots, no toxicity symptoms were observed, and flower biomass remained unaffected (Fig. 5). Both VF and AVF substrates showed low total and available PHE levels. Consequently, low PHE concentrations were found in both shoots and roots from plants in such substrates (Table 2, Table SM2).

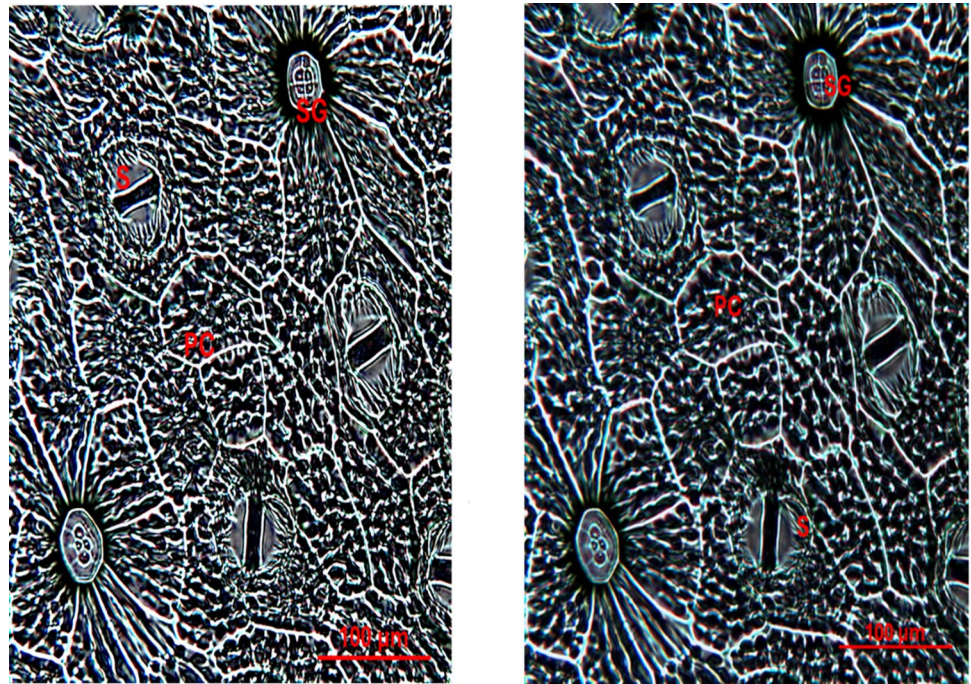
Zinc displayed a consistent translocation pattern (TransIC > 1) across all treatments, remaining within the sufficiency range for most plants (25–150 mg kg⁻¹; Kabata-Pendias 2011). The high BAC values in AG plants, particularly for As and Pb (often between 10–100

or > 100), confirm *L. daveaui* intensive uptake capacity for PHE under contaminated conditions (Table 3, Table SM3).

Macronutrients translocation followed expected patterns: most elements moved from roots to shoots (TransIC > 1), except for P (TransIC < 1; Table SM3), which presents relatively low concentration in shoots (1–2 g kg⁻¹; Mengel and Kirkby 2012). Potassium concentration in shoots was below the optimal range for healthy plant development (20–25 g kg⁻¹; De Varennes 2003), but its high uptake (BAC: 10–27; Table 3) suggests active root sequestration. Iron also had a TransIC < 1 (Table 3), although shoots Fe concentration was within the sufficiency range (50–250 mg kg⁻¹; Mengel and Kirkby 2012). Despite the suboptimal K content, plants showed no apparent deficiency



Fig. 6 Plants leaf epidermis. Scale Bar = 100 μm . Salt gland (SG), stomata (S) and pavement cells (PC)



symptoms, which may be due to the beneficial effect of Na (Mengel and Kirkby 2012). Sodium had high concentration in both VF and AVF substrates ($7.5\text{--}8.9\text{ g kg}^{-1}$, Fig. 2); however plants Na uptake capacity in these substrates was intermediate (VF: $\text{BAC} = 1.5$; AVF: $\text{BAC} = 0.6$), whereas in AG soil (0.13 g Na kg^{-1} , Table SM3), plants uptake capacity is stronger ($\text{BAC} = 10.7$). Salt positively influences halophytes, enabling them to grow larger and more quickly

when exposed to optimal salt concentration (Song and Wang 2015). The sulfur content in all plant shoots exceeded the normal range for plants ($2\text{--}5\text{ g kg}^{-1}$ (Table 2); Mengel and Kirkby 2012) with a $\text{TransIC} > 1$ and a strong uptake capacity ($\text{BAC}: 2.6\text{--}6.8$) (Table 3), but without any apparent influence in plants growth.

Strong positive correlations were found between elements concentrations in plants shoots and soil availability (notably

Table 4 Total concentration of potential hazardous elements (PHE) and nutrients in leaf excreted salts. *Limonium* plants grown in gossan mine waste, saline-affected Fluvisol and Amended soils at the end of the assay

Elements	Excreted salts			
	G_f	AG_f	VF_f	AVF_f
As (mg kg^{-1})	–	bdl	bdl	bdl
Cu (mg kg^{-1})	–	0.93 ± 0.82	1.01 ± 0.81	0.67 ± 0.25
Pb (mg kg^{-1})	–	bdl	bdl	bdl
Zn (mg kg^{-1})	–	21.87 ± 37.41	$13.36 \pm 3.05\text{ b}$	$4.29 \pm 1.74\text{ a}$
Fe (mg kg^{-1})	–	$6.56 \pm 1.74\text{ A}$	34.58 ± 6.34	$50.77 \pm 22.08\text{ B}$
K (g kg^{-1})	–	0.23 ± 0.09	$1.87 \pm 0.55\text{ b}$	$0.60 \pm 0.42\text{ a}$
Mn (mg kg^{-1})	–	1.40 ± 0.50	1.51 ± 0.57	3.00 ± 1.40
Na (g kg^{-1})	–	$0.09 \pm 0.03\text{ A}$	$15.03 \pm 3.76\text{ b}$	$8.10 \pm 4.48\text{ aB}$
S (mg kg^{-1})	–	$202.03 \pm 1.67\text{ A}$	555.84 ± 132.51	$527.35 \pm 230.27\text{ B}$
Si (mg kg^{-1})	–	$35.33 \pm 5.63\text{ A}$	63.11 ± 12.59	$102.57 \pm 33.17\text{ B}$

G: Gossan waste; AG: Amended-Gossan; VF: Fluvisol; AVF: Amended-Fluvisol; bdl: below detection limit. Lowercase letters indicate significant differences between the control (VF_f) and the respective Amended soil (AVF_f); and capital letters indicate significant differences between the two Amended soils (AG_f vs. AVF_f) (U test Mann–Whitney)



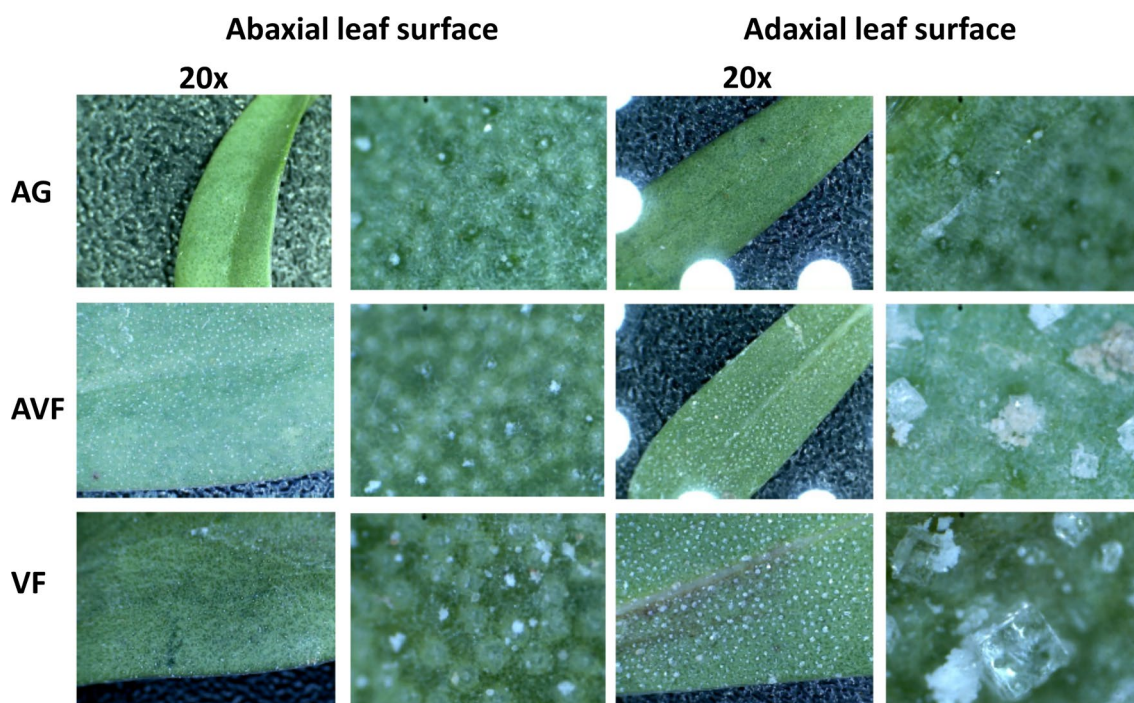


Fig. 7 Salt crystals' excretion on leaf surfaces. Leaves from plants grown in Amended- Gossan soil (AG) and in Amended-Fluvisol (AVF), or in saline-Fluvisol (VF) at the end of the experiment. Plants

from AVF and VF presented their leaves epidermal surfaces fully covered with salt crystals

for Na, K, and P), confirming efficient uptake from enriched substrates (Fig. SM2). However, PHE showed a contrasting behavior (Fig. SM2), with significant correlations between their concentrations in roots and the soil bioavailable fraction (e.g., Pb and As), supporting the hypothesis of preferential root sequestration.

Leaf salts excretion

Limonium daveaui showed stomata and salt glands on both adaxial and abaxial surfaces of the leaf blades, consistent with previous findings in other *Limonium* species (Cortinhas et al. 2020). The salt glands were embedded within the epidermis, as reported in related taxa (Akhani et al. 2013; Yuan et al. 2016; Caperta et al. 2020), and were separated by at least two epidermal cells, with four being the most common configuration (Fig. 6). Gland-to-gland contiguity was never

observed, reinforcing their structured distribution pattern. There were no significant differences between the adaxial and abaxial epidermis in terms of cell densities of the three cell types observed. The density of stomata and salt glands was 8.46 and 32.59 mm⁻², respectively, being stomata 3.7 times more abundant than salt glands, and 8.5 less abundant than pavement cells.

In agreement with other halophytic species in the genus (Caperta et al. 2020), *L. daveaui* excreted salts via foliar salt glands (Table 4, Table SM4). A higher Na concentration was observed in leaf salt excretions from VF plants, approximately double than that observed in AVF plants (Table 4), a trend that aligns with the relative quantity of salt crystals on the leaf surfaces (Fig. 7). Moreover, Na in salts was highly correlated to Na in shoots ($r=0.95$) and roots ($r=0.93$) (Fig. SM3), reinforcing the role of salt glands in regulating internal Na levels as previously observed (Caperta et al. 2020). Calcium, K, Mn and P concentrations in foliar excretions



were similar between AG and AVF plants (Table 4 and Table SM4). However, salts from AVF plants contained a higher Mg and S levels than salts from AG, reflecting the relative increase of these elements in the shoot tissues. Notably, Si and Fe concentrations were also higher in AVF salt excretions than in AG. While shoot Si concentrations (Table 2) were similar between the two treatments, Fe accumulation in AG plant shoots was three times higher than in AVF. These findings suggest a specialized regulatory mechanism in *L. daveau*, wherein the plant excretes excess Si through salt glands to maintain internal Si homeostasis.

Unexpectedly, *L. daveau* did not excrete PHE such as Cd, Co, Mo, As, or Pb, through foliar salt glands when grown in AG soil (Table 4, Table SM4). However, As and Pb concentrations in shoots were elevated and within toxic ranges (Table 2). Salt excretions were positively correlated with macronutrients shoot concentrations such as Na and Si (Fig. SM3), suggesting active regulation through salt glands. However, no significant correlations were observed between salt excretions and PHE concentrations in shoots or roots (Fig. SM3), consistent with the undetectable levels of these elements in the excreted salts.

Our findings contrast with observations in other members of the Plumbaginaceae family. For instance, in *Armeria maritima* secretory glands are found in both halophytic and metallophyte populations, and can excrete metals such as Cu, Zn, Ni, Fe, and Mn when grown in contaminated soils (Ruhland 1915; Neumann et al. 1995; Baumbach and Hellwig 2007; Nikalje and Suprasanna 2018). Similarly, in the related Tamaricaceae family, *Tamarix africana* plants growing in a contaminated soil excreted salts containing As, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, and Zn through salt glands (Santos et al. 2017). While *Tamarix myrrensensis*, exhibit opposite behavior when exposed to Pb different concentrations and does not excrete this element by salt glands (Kadukova and Kalogerakis 2007).

The use of soil amendments is an important tool to enhance the soil's physical, chemical, and biological conditions, ultimately supporting plants' growth. Since the studied plants do not excrete metal(loid)s they do not constitute an environmental risk.

Limonium daveau shares with other halophytes structural salt gland features and ionic excretion capacity, but it appears to rely on root sequestration rather than in salt gland excretion as its primary strategy to cope with PHE under metal-contaminated conditions. Nevertheless, such mechanisms remain to be tested in the future.

Conclusion

This study lays the groundwork for further investigation into the adaptive responses of *Limonium* species growing in estuarine environments in non-contaminated and PHE contaminated soils and/or sediments. Since contaminated environments showed adverse physicochemical and biological conditions for plant growth, the addition of amendments enhanced substrate properties allowing *L. daveau* growth and development.

In saline Fluvisols, plants actively extrude salts via salt glands, whereas in non-saline soils contaminated with PHE, they sequestered PHE in the roots, rather than eliminating them through glandular excretion.

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Author contribution Caperta AD: Conceptualization, methodology, investigation, formal analysis, resources, writing—original draft preparation, writing—review and editing, supervision, project administration, funding acquisition, Aguilar-Garrido A: Investigation, formal analysis, validation, writing—original draft preparation, writing—review and editing, Vidigal P: Investigation, formal analysis, writing—review and editing, Costa MCSC: Formal analysis, Abreu MM: Conceptualization, methodology, investigation, formal analysis, resources, writing—original draft preparation, writing—review and editing, supervision, project administration, funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Data availability Contact the corresponding author for data requests.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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