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Metal extraction capacities of the two halophytes *Sesuvium portulacastrum* and *Suaeda australis* from New Caledonian estuaries contaminated with metals

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Abstract :

The increase in population and its needs have worsened the issue of metal contamination of the environment, negatively impacting plants, animals, and human health. New Caledonia, a biodiversity hotspot, faces a significant source of metals from mining activity and erosion that affect unique ecosystems that require protection. In this study, we explore the potential of halophytes to remediate metals, with the aim of safeguarding seashores and lagoons. We examine the optimal growth and metal bioaccumulation conditions of two halophytic species, Sesuvium portulacastrum (L.) L. and Suaeda australis (R.Br.) Moq, which dominates the salt marshes of New Caledonian coasts. These species were subjected to two levels of metal concentration and two levels of irrigation frequency, which mimic the fluctuation in soil water content due to seasonal changes and tidal irregularities. The results showed that S. portulacastrum growth was enhanced when the irrigation frequency was lowered, while S. australis preferred constant soil moisture. S. australis accumulated more Ni, Co, and Cr than S. portulacastrum, especially in the roots. However, S. portulacastrum transported more metals in the aerial parts than S. australis. This work showed promising abilities of both species to accumulate metals and suggests further research to improve their metal phytoremediation potential in New Caledonian salt marshes.

Introduction

Metal pollution causes major ecological and economic issues for most countries and often results from anthropogenic activities such as intensive farming, mining, and many other industrial fields. While most studies related to environmental metal contaminations have focused on cultivable lands, coastal environments are often affected by several pollutions, as the economic development near coastal zones implies the establishment of industrial estates (Qian et al. 2015; Han et al. 2021). Although coastal lands represent a buffering zone between terrestrial and marine ecosystems (Oyetibo et al. 2017), the amounts of metals spreading into these environments are usually so grave that they cannot be fully retained and are released in marine trophic networks, thus representing a threat for human health and ecosystems (Briand et al. 2018; Han et al. 2021; Yousif et al. 2021). In this context, while most technologies employed in decontaminating metals require important financial and technical resources, phytoremediation of metals has received great attention in the last two decades because of its low cost and environmental impact (Origo et al. 2012). This latter technology using plants to extract or stabilize metals in the soil has already proven itself as a reliable and ecological alternative (Liang et al. 2016; Sarwar et al. 2017; Saxena et al. 2019).

Halophytes are plants particularly adapted to saline environments. Their potential for the phytoremediation of metals has been reviewed many times (Van Oosten and Maggio 2015; Liang et al. 2016; Nikalje et al. 2018). In tidal environments, plants face several stresses, such as salinity elevation and floodings, causing hypoxia episodes (Colmer et al. 2013), particles' sedimentation, and modifications in soil porosity and aeration (Schwarz et al. 2015). Hence, these stressors should be considered in the design of efficient phytoremediation strategies. Soil salinity is linked to soil humidity and fluctuates with tides and across seasons, decreasing in rainy periods and increasing at the surface in dry periods (Fu et al. 2020). Moreover, tides can also impact plant growth. For Silvestri, Defina, and Marani (2005), subsurface water movements combined with evapotranspiration patterns of plants influence their distribution by creating variations in aerobic/anaerobic conditions and soil porosity. Fluctuations of such parameters can be determinant in the metal bioavailability and thus can influence the phytoremediation process (Peijnenburg and Jager 2003).

In New Caledonia, mining activities, in combination with soil erosion, lead to metal pollution, mainly represented by high Ni, Co, Cr, and Mn concentrations due to the transport of metals-rich sediments from the mining sites to the seashore (Briand et al. 2018). New Caledonia is also affected by a wet season characterized by intense rains that increase the transport of sediments. For example, the recorded annual rainfall between 2009 and 2013 varied from 1460 mm to 2720 mm (Allenbach et al. 2015).

Here, we studied the phytoremediation potential of the two halophytes, *Sesuvium portulacastrum* (L.) L., and *Suaeda australis* (R.Br.) Moq., to assess their potential in removing or stabilizing metals in the polluted estuaries of New Caledonia. *S. portulacastrum* has been reported as a suitable metal phytoremediator (Mnasri et al. 2015; Ayyappan et al. 2015; Fourati et al. 2020), and *S. australis* has been studied for its metal resistance (Alam et al. 2021). According to Poupart et al. (2013), these two species share the same habitat in the tidal transect from Kone in the north to the south of the west coast in New Caledonia mainland ("Grande Terre"). However, their metal accumulation behavior under soil humidity fluctuation has not yet been studied. We hypothesized that these two species could have interesting extraction capacities, and in the present study, we aim to determine the impact of humidity fluctuation on their metal bioaccumulation in greenhouse conditions. To this end, the two native halophytes were grown in pots at two metal concentration levels corresponding to the average and maximum metal concentrations found at the contaminated marshland site studied. Two soil moisture conditions were performed: the first corresponded to pots permanently humid on the soil surface, and the second to pots with soil surface periodically dried. These conditions reproduced those affecting the two species in the studied coastal site. We aim to elucidate their potential in the phytoremediation of metal-contaminated estuaries in New Caledonia and, consequently, in the protection of one of the most important lagoons in the world.

Materials and Methods

Soil collection and characterization

The soil used for the experiment was collected on a contaminated (red) saline tidal flat located on the estuary of the Baie de Saint-Vincent, on the west coast of New Caledonia (21°59' and 166°9') on June 12th, 2020 (Figure 1). The soil was recovered at three different points situated between tufts of plants, about 10 m apart. The soil surface was scraped to eliminate the excess salt and compacted pellicle of fine particles before collecting it with a clean spade. The soil, relatively poor in roots, was homogenized by hand before use.

Three samples were used for physicochemical characterization and were oven-dried at 45°C, ground, and passed through 2-mm mesh sieves for the following analyses performed by the Laboratoire des Méthodes Analytiques of Noumea (LAMA-US IMAGO IRD, New Caledonia). Total nitrogen was measured according to Kjeldahl's method. Total organic carbon content was determined using Walkley and Black's method. Exchangeable cations and cation exchange capacity (CEC) were determined using the cobalt hexamine chloride method (Ciesielski et al. 1997). Carbonate content was determined using Petard's method using a calcimeter (Bernard type). Chloride content was determined with the colorimetric method (SEAL Analytical Method, G-133-95). Exchangeable P was determined using the Olsen method. Total Ni, Fe, Mn, Co, Cr, P, Mg, Ca, Na, and K extracted by alkaline fusion and DTPA-extractable fractions for Ni, Cr, Co, Mn, and Fe were determined using an Inductively Coupled Plasma Optical Emission Spectroscopy (ICP OES, Varian, Varian 730-ES, Palo Alto, USA). Electrical conductivity (EC) and soil pH were measured according to the standard methods ISO 11265:1997, and ISO 10390:2005, respectively. All these soil characteristics are summarized in Table 1.

Plant material

Two common halophyte species of New Caledonia were selected: *Suaeda australis* (R.Br.) Moq. (Amaranthaceae) and *Sesuvium portulacastrum* (L.) L. (Aizoceae) (Della Patrona 2016). Plants used in this work were obtained by cutting propagation on plants sampled in a non-polluted tidal flat. Medium-sized, healthy and green tufts of each of the two species were selected and removed with their root system. The plants were cultivated in 50/50 potting soil/sand under greenhouse conditions and watered twice a week with 50/50 sea water/tap water, until they form new branches. They were then multiplied by cutting. For this experiment, cuttings were taken from one unique plant for each of the two species, and rooted in a mixture of 50/50 potting soil/2 mm-mesh sieved coral sand in 250 mL pots and watered 2 min twice daily. After four weeks, cuttings were transplanted in 1 L pots (one plant per pot) containing a mixture of the sampled soil/potting soil (70/30). At the beginning of the experiment, plant heights were roughly homogeneous between treatments.

Pot experiment in greenhouse

Because of its low fertility, inducing growth difficulties, the soil was mixed with 30 % of potting soil. The total and DTPA extractable metal contents (Fe, Ni, Co, Cr, Mn) of the mix of contaminated and potting soil were assessed using the same methods described previously. DTPA extractable concentrations were 8.298 ± 0.874 mg/kg Fe, 6.742 ± 0.268 mg/kg Ni, 2.301 ± 0.212 mg/kg Co, 0.025 ± 0.003 mg/kg Cr, and 33.143 ± 2.353 mg/kg Mn.

Cuttings transplanted in the studied soil were then subjected to the 4 following treatments:

- A: Low metals' concentration level, high irrigation frequency;
- B: Low metals' concentration level, low irrigation frequency;
- C: High metals' concentration level, high irrigation frequency;
- D: High metals' concentration level, low irrigation frequency.

The low metals' concentration level was the level of metal concentrations already present in the contaminated soil (no metal addition). The high metals' concentration level has been defined to correspond approximately to the maximum metal concentrations at the contaminated marshland site studied (Bonaventure, 2023). Considering that the concentrations of available metals could vary in relation to metal binding capacity of the soil, we have beforehand tested the addition of different concentration supplies of the mixed metals, followed by the analysis of the DTPA-extracted metal concentrations, which allowed us to determine the amounts of each metal to be added to obtain the expected concentrations. The concentrations of the mixed metals chosen corresponded then to 150 mL of a mix of $NiSO_4.6H_2O$ ([Ni] =50 mg/L), $CoCl_2.6H_2O$ ([Co] = 10 mg/L), $CrCl_3.6H_2O$ ([Cr] = 5 mg/L), $MnSO_4.H_2O$ ([Mn] = 100 mg/L). To reduce metal toxicity at the beginning of the experiment, when plants were still fragile, this amount of metal solution was added in 3 times (50 mL for each supply), with an interval of two days. Saucers were added under the pots to avoid metal leaching.

Given the difficulty of regularly measuring fluctuations in soil moisture over time, we have only sought to approximate the effects of large fluctuations in moisture, as it can be observed in the natural environment of these plants, compared to relatively stable moisture. Thus, the high irrigation frequency level was defined so that the soil moisture at the pot surface was constantly maintained: twice a week with tap water until saturation. For the low irrigation frequency level, plants were watered only when a crust of desiccated soil was formed at the pot surface, without plant wilting: about once a week until saturation. For the two species, each treatment contained 10 replicates. The temperature in the greenhouse fluctuated between 24-28 °C during the day and 19-23°C at night, and the relative humidity was about 65-75%. The experiment was followed for 6 months.

Growth parameters using images processing

Ten images of each plant were taken at the beginning (the first day after transplantation of cuttings in final pots) and at the end of the experiment using a camera Canon 600D, with a 30 cm-ruler positioned next to the plant to standardize the scale, and with a red background behind the objects (Supplementary data Figure S1). Because of various contrasts among images, we used a machine-learning-based segmentation tool, Ilastik vers. 1.0 (https://www.ilastik.org/) (Berg et al. 2019) to extract in batch mode the images of every plant from the background and the pot zone and calculate plant area. We also used Fiji vers. 1.42 (https://imagej.net/software/fiji/) to compare raw and segmented images to identify and correct potential segmentation errors. We call "aerial relative growth" the difference between the values of initial and final measurements, expressed in cm^2. Only segmented objects with a probability of 80 % were kept for the analysis.

Biomass and metal contents in plants

At the end of the experiment, roots, shoots, and soil were gently separated from each other, weighted, and oven-dried at 45°C. The dry mass of roots and shoots was determined, and each part was ground into a fine powder. The succulence index or saturated water content (SWC) was calculated according to Ogburn and Edwards Ogburn and Edwards 2012) :

SWC = (shoots fresh weight – shoots dry weight)/(shoots dry weight)

The 0.5 mm sieved fraction was subjected to acid digestion: 100 mg of the powdered root and shoots material were put into 15 mL vials containing 1 mL of 70% HNO_3 and were heated for 2 h at 100°C. After cooling to room temperature, 1 mL of 30% H_2O_2 was added and left on for 30 min. Then, vials were once more heated at 100°C for 2 h. Non-completely digested samples were separated from the others, supplemented with 1 % NH_4F, and heated at 100°C for 2 h. Therefore, both matrices (HNO_3/H_2O_2 and

HNO3/H2O2/NH4F) were analyzed using an Inductively Coupled Plasma Optical Emission Spectroscopy (ICP OES, Varian, Varian 730-ES, Palo Alto, USA) with 2 distinct standard curves using the corresponding standard solutions. The translocation factor (TF) was calculated for Ni, Co, Cr, and Mn using the following formula (Mujeeb et al. 2020) :

TF = concentrations in the shoots/concentrations in the roots

Concentrations correspond to the final state of the experiment.

Soil extractable metal concentrations and Bioconcentration factor

At the end of the experiment, the soil of each pot was dried at 45°C until the weight was stabilized. It was subsequently grounded and passed through a 2 mm mesh sieve. For each pot, 10 g of sieved soil was supplemented with 20 mL of DTPA, shaken for 1 hour at 75 rpm, and then centrifuged at 3000 g for 10 min. The supernatants were recovered in 15 mL tubes, and extractable fractions for Ni, Cr, Co, and Mn were obtained using an ICP-OES (ICP OES, Varian, Varian 730-ES, Palo Alto, USA).

The bioconcentration factor (BCF) was defined as follows, based on the calculation of BCF in Zhang et al. (2020) :

BCF = concentrations in the roots/extractable concentrations in the soil

Concentrations correspond to the final state of the experiment. Metal concentrations in the soil correspond to the DTPA-extractable fraction.

The calculation of the bioaccumulation factor (BAF) was based on and modified according to Khaokaew and Landrot (2015) as follows :

BAF = concentrations in the whole plant/extractable concentrations in the soil

Concentrations correspond to the final state of the experiment. Metal concentrations in the soil correspond to the DTPA-extractable fraction.

Statistical analysis

All statistical analyses were performed using R software version 3.6.2 (http://www.r-project.org) with R Studio version 1.2.5033. Prior to statistical comparisons, outliers in each variable were detected according to the Grubbs test from the package {outliers} and were removed if the values were possibly related to an experimental mistake. A two-way analysis of variance or a non-parametric Kruskal-Wallis ranks test was used to assess the differences between treatments within each species. When the test was significant, we performed a pairwise comparison using the post-hoc Tukey's HSD test or a non-parametric pairwise Conover–Iman test.

Results

Aerial relative growth

The aerial relative growth is highly variable within treatments (figure 2). In *S. portulacastrum,* no significant difference was observed between treatments. On the contrary, *S. australis* aerial parts were significantly less developed in treatments C (p=0.0092) and D (p=0.0095) compared with treatment A. *S. australis* resulted in significantly more aerial surface than *S. portulacastrum* only in treatment A (p=0.0212), with 16.00 ± 6.78 cm² in *S. australis* and 1.94 ± 5.31 cm² in *S. portulacastrum*.

Final dry biomass and shoots/roots biomass ratio

Results on the final total plant, roots and shoot dry weight, and shoots to roots ratio are presented in Figure 3.

The total dry weight of *S. portulacastrum* fluctuated between 2.9 and 3.2 g per plant and between 1.5 and 2.2 g for *S. australis*. The total dry weight and shoot dry weight of *S. portulacastrum* were significantly greater than those of *S. australis* in treatments A (respectively, p=0.0003 and p=0.0004), B (respectively, p=0.0000 and p<0.0000) and D (p<0.0000 for both treatments). *S. portulacastrum* shoot biomass was also significantly higher than *S. australis* in treatment C (p=0.0137). Treatments had no significant effect on the root biomass of *S. portulacastrum*, whereas *S. australis* root biomass was significantly affected by the reduction of the irrigation frequency when metals were added (p=0.0401) (Figure 3c). *S. portulacastrum* had significantly higher root biomass than *S. australis* only in treatments A (p=0.0367) and B (p=0.0274). The ratio between shoot and root biomass (Figure 3d) was significantly higher in treatment D than in treatment C in both species (for *S. portulacastrum*, p=0.0082; for *S. australis*, p=0.0023).

Succulence index (SWC)

As shown in Figure 4, no significant difference on the succulence index (SWC) between treatments was observed in *S. portulacastrum,* while in *S. australis*, SWC was significantly higher in treatment C than in treatment B (p=0.0010). The difference between treatments C and D was near the significance level (p=0.0519). SWC in *S. portulacastrum* was significantly higher than in *S. australis* in treatments A (p=0.0015), B (p< 0.0010), and D (p=0.0142).

Metal accumulation

Concentrations in plant organs

Results on the metal concentrations in the shoots and roots of the two halophytes are presented in Table 2. In both plant parts, *S. australis* accumulated significantly more metals in total than *S. portulacastrum* in almost every treatment (in the shoots, B: p=0.0078, C: p<0.0000, D: p<0.0000. In the roots, A: p=0.0011, B: p<0.0000, C: p=0.0031, D: p=0.0122). The highest values of total metals accumulated were observed in *S. australis* in treatment C (high metal level and high irrigation frequency), with 173.6 mg/kg in shoots and 495.4 mg/kg in roots. However, the amounts of total metals accumulated per plant did not show significant differences between the two plants for each of the 4 treatments considered separately. These amounts varied from 830.5 mg/plant (treatment A for *S. portulacastrum*) to 1458.4 mg/plant (treatment C for *S. portulacastrum)*.

The average values of Ni concentrations vary between 8.8 and 31.7 mg/kg in the shoots and 55.4 to 245.8 mg/kg in the roots. Ni concentrations in the roots of *S. australis* were higher than *S. portulacastrum* in every treatment (A: p=0.0052; B: p<0.0000; C: p=0.0003; D: p=0.0017) whereas in the shoots, *S. australis* accumulated significantly more Ni than *S. portulacastrum* only when metals were added to the pot (C: p=0.0079; D: p=0.0088).

Regarding Co, concentrations range on average from 1.0 to 2.6 mg/kg in shoots and from 5.1 to 23.6 mg/kg in the roots. Co concentrations in the shoots were also higher for *S. australis* than *S. portulacastrum* in treatments B (p=0.0074), C (p=0.0006), and D (p=0.0000). In the roots, these concentrations were higher for *S. australis* in all treatments (A: p=0.0004; for B, C, and Dp<0.0000). A maximal average was found in *S. australis* in treatment C, which was 2.6 times higher than in *S. portulacastrum*.

The average values of Cr concentrations vary between 2.6 and 21.3 mg/kg in the shoots and 19.5 to 88.3 mg/kg in the roots. The concentrations in *S. australis* shoots were lower than in *S. portulacastrum* in treatment A (p=0.0067). In the roots, *S. australis* accumulated more Cr than *S. portulacatrum* only in treatments A (p=0.0491) and B (p=0.0000), respectively 2.2 and 4.5 times more than in *S. portulacastrum*.

Mn was the major metal accumulated in both species compared with the other metals. Plants accumulated in average concentrations ranging from 72.4 to 130.4 mg/kg in the shoots and from 74.9 to 147.5 mg/kg in the roots. Concentrations in the shoots were higher in *S. australis* than in *S. portulacastrum* in the treatments B (p=0.0033), C

($p<0.0000$), and D ($p<0.0000$). In the roots, Mn concentrations were higher in *S. australis* in the treatment B ($p=0.0067$).

As the Anova was not applicable to the full dataset, including both species, we tried to do so on each species' sub-dataset to evaluate the effect of treatments on metal accumulation patterns and identify whether the metal concentration level, the irrigation frequency level, or both factors affect metal bioaccumulation. These results are presented in Tables 3 and 4.

For *S. australis*, Mn, Co, and total metal accumulation in the shoots was significantly higher at high metal concentration levels (Table 3). Moreover, the interaction of irrigation frequency and metal addition significantly impacted Ni, Co, and Cr accumulation in the shoots of this species. In particular, Ni and Co concentrations were higher in treatment C than in treatment A. Ni concentration in its shoots was also higher in treatment C than in treatment D. Regarding Cr, *S. australis* accumulated it more in treatment B than in treatment A. In the presence of a low irrigation frequency level, the addition of metals significantly decreased the Cr concentration in the shoots. Metal addition significantly increased Co concentrations in the roots (Table 3). The irrigation frequency level or the interaction of both factors had no significant influence on metal accumulation.

Regarding *S. portulacastrum*, the irrigation frequency level significantly impacted Cr and total metal concentrations in the shoots (Table 4), with a higher concentration of Cr and total metals in the presence of a high irrigation frequency level. The addition of metals also significantly affected the accumulation of Co, Cr, Mn, and total metals in the shoots, with a decrease in metal concentrations when metals were added to the soil. For

Ni, the non-parametric test was significant, with higher concentrations in treatments B and C than in treatment D. The addition of metals also significantly impacted the accumulation of Ni, Co, Cr, and total metals in the roots (Table 4). Moreover, the non-parametric test performed on Mn concentrations was also significant. In particular, Ni, Co, Cr, and total metals were higher in the high metal concentration level. In the presence of a low irrigation frequency level, adding metals to the pot significantly increased the Mn accumulation in the roots of *S. portulacastrum*.

Translocation factor (TF), bioconcentration (BCF) and bioaccumulation factor (BAF)

The translocation factor values of Ni, Co, Cr, and Mn are shown in Figure 5. Both species have relatively low TF (generally under 1) for Ni, Co, and Cr (Figure 5d). Except for Mn, TF significantly differed within species and treatments (Ni Treatments x species: p=0.0173; Co Kruskal-Wallis: p=0.0000; Cr Treatments x Species: p=0.0000). Indeed, Ni and Co TF (Figure 5a, b) were significantly higher in *S. portulacastrum* than in *S. australis* for the treatments A (respectively, p=0.0016, p=0.0023) and B (p=0.0038, p=0.0026). The same was observed for Cr in the treatment A (p< 0.001). While treatments had no significant impact on metal translocation in *S. australis*, the TF for Ni, Co, and Cr was significantly reduced by the addition of metals in *S. portulacastrum* (respectively, p=0.0000; p=0.0000; p<0.0000).

BCF and BAF were always greater than 1 for both species, (Table 5). Treatments had no significant impact on either BCF or BAF in both species. For Ni, Co, and total metals, BCF was significantly higher in *S. australis* than in *S. portulacastrum* in all treatments (Ni in A: p=0.0100; in B: p=0.0000; in C: p=0.0021; in D: p=0.0054. Co in A: p=0.0003, in B, C, and D p<0.0000. Total metals in A: p=0.0059, in B: p=0.0000, in C: p=0.0013, in D: p=0.0145). BCF for Cr was significantly higher in *S. australis* than in *S. portulacastrum* only in treatment B (p=0.0000) and for Mn in treatment C (p=0.0061). BAF was significantly higher in *S. australis* than in *S. portulacastrum* in all treatments for Co (A: p=0.0007; for B, C, and D p<0.0000), Mn (A: p=0.0006; B: p=0.0005; for C and D p<0.00001), and total metals (A: p=0.0441; B, C, and D p<0.0000). It was also the case for Ni (B: p=0.0000; C: p=0.00001; D: p=0.0002), except in treatment A for Cr, a significant difference was only found in treatment B (p=0.0007).

DTPA-extractable metals, final soil pH and electrical conductivity

Final DTPA-extractable metal concentrations in the soil

Final DTPA-extractable metal concentrations in the soil are presented in Figure 6. Final DTPA-extractable Ni concentrations in the soil (Fig. 6a) were significantly impacted by the treatments (p<0.0000), but no difference was found between species. In both species, final Ni concentrations were significantly higher in the treatment C and D enriched with metals (for *S. australis* respectively, p=0.0063 and p< 0.0010. for *S. portulacastrum*: both p<0.0010)

Regarding Co (Fig. 6b), no significant difference was found between species. *S. portulacastrum* was significantly impacted by both the irrigation frequency (p=0.00284), with higher Co concentrations in the high irrigation frequency level (p=0.00249) and the metal concentration (p=0.0006) with higher Co concentrations in the high metal concentration level (p=0.0005). *S. australis* was also significantly impacted by both factors (irrigation frequency level: p=0.0059. metal concentration level: p=0.0094), with higher Co concentrations in the high metal concentration level (p=0.0085) and in the high irrigation frequency level ($p=0.0052$). For Cr (Fig. 6c), neither the treatments nor the species significantly impacted DTPA-extractable concentrations. Finally, Mn concentrations (Fig. 6d) were significantly impacted by treatments ($p<0.00001$) and species ($p=0.0000$) with a greater Mn concentration in the treatment C than in the treatments D ($p=0.0331$) and B ($p=0.0047$) for *S. australis* and a greater concentration in the treatment C compared to B ($p< 0.0010$) for *S. portulacastrum*.

Final soil pH and electrical conductivity

The final soil pH and electrical conductivity (EC) results are presented in the Supplementary Data Table S1. In both species, the soil in the pot remained alkaline in every treatment, with values varying between 8.1 and 8.5 without significant differences. The electrical conductivity was not significantly different between treatments and species despite high variations in the values.

Discussion

In this study, a greenhouse experiment allowed us to evaluate the ability of two halophytes to grow and accumulate metals under two irrigation conditions and two metal concentration levels.

Effect of irrigation frequency on plant growth

The growth rate results for the two species showed considerable variability. Therefore, only large differences between growth rates appeared to be significant. Thus, there were no significant differences between the tested treatments for *S. postulacastrum*. This species can be considered to be adapted to large humidity fluctuations. *S. portulacastrum* is known as a psammophyte (eHALOPH | eHALOPH - Show Plant), i.e., a plant adapted to life in sandy soils with low water retention capacities. However, it can also be found in estuarine mudflats, salt marshes, and habitats associated with mangroves (Lonard and Judd 1997; Singh 2020). In New Caledonia, *S. portulacastrum* can be found in tidal muddy soils that frequently dry out in the dry season, with sometimes a visible salt crust forming on the soil surface (Figure 1c, d). This species is known for its resistance to several stresses. It has been recommended in the phytodesalinisation of arid regions owing to its ability to accumulate salt ions compared with other species (Rabhi et al. 2008). In their experiment, Slama et al. (2006) demonstrated that *S. portulacastrum* could tolerate a reduction of the water supply up to 50% of the field capacity of the used soil. The study of Winter et al. (2019) showed that *S. portulacastrum* can use a crassulacean acid metabolism at low level, especially when plants are exposed to drought, while most of the species of this genus are known as C4 plants. Moreover, according to Lokhande et al. (2013), drought causes similar deleterious morphological and physiological effects than salt and metal stress on this species. However, under moderate stress, this species can modulate its succulence, antioxidant systems, water potential, and morphological traits to enhance its response to those stressors. Besides, to our knowledge, this species has not been reported in highly and long-time flooded areas and is present mostly in rarely flooded zones. Additionally, few studies (Morandeira and Kandus 2016; Nikalje et al. 2019) classified halophytes according to their functional adaptations, and *S. portulacastrum* was always out of the anoxia or flood-resistant species categories. Several studies have demonstrated the link between the adaptation to salinity of this species and its resistance to drought (Fan et al. 2009; Yang et al. 2015). In this study, we evaluated the saturated water content that can be used to describe tissue succulence (Ogburn and Edwards 2012;

Carvalho et al. 2022) and found that succulence was not influenced significantly by reducing the irrigation frequency.

Several studies highlighted how soil humidity and salinity, as two common edaphic stressors in tidal environments, can shape the species distribution, acting with other parameters, such as soil oxygen availability, organic matter, sand, silt, and clay contents. (Yilmaz et al. 2020; Sarika and Zikos 2021; Chung et al. 2021; Mumcu et al. 2023). As for *S. portulacastrum*, our treatments had little effects on *S. australis*, which suggests that this species can also bear high fluctuations of soil humidity. According to the eHALOPH database (eHALOPH | eHALOPH - Show Plant), *S. australis* is considered a hydrohalophyte, thus growing in soils with a high water retention capacity or in constant presence of water. Certain (2021) asserted that *S. australis* in New Caledonia colonizes coastal zones that are frequently flooded. If the impacts of soil humidity and salinity on *S. australis* growth have received little research attention, other *Suaeda* species are documented and are considered to be well adapted to anoxia (Colmer et al. 2013). Baoshan, Qiang, et Xinsheng (2008) assumed that *Suaeda salsa* (L.) Pall. could switch from a xerophytic strategy to a limnophytic strategy along environmental gradients as in its environment, the plant community is structured by the water table depth and soil salinity gradients, which are negatively correlated. According to Wang et al. (2019), the biomass of *S. salsa* was the highest in plants from the site that received the slightest flooding per month. In our work, the tidal flat zone from where *S. australis* was collected (figure 1) is not frequently flooded. The sun can quickly dry the soil surface in the summer, suggesting a relative adaptation of this species to low humidity levels. Despite variations between species, *Suaeda* is known to have diverse adaptations to face salinity and water stress, such as enzymatic and non-enzymatic adaptations, the production of amino and organic acids and soluble sugars or the compartmentation of toxic ions (Shang et al. 2020), and the excretion of root mucilage (Jaiswar et Kazi, 2016).

Effect of metals on plant growth

Whereas DTPA extractable Co, Cr, and Mn were reduced in the soil at the end of the experiment, compared with the initial concentrations, the final DTPA-extractable Ni concentrations increased. This increase, despite the plant's absorption of a part of the Ni during the experiment, can be explained by the effect of bacterial activity on ultramafic soil particles that contaminated the estuary. Indeed, it has been demonstrated that bacteria regularly release Ni from minerals in ultramafic soils (Amir and Pineau 2003).

In the high metal concentration level, DTPA-extractable Ni concentrations reached around 13 mg/kg in *S. portulacastrum* pots without affecting its growth. This species is known for its high resistance to metal stress. As shown in the study of Mnasri et al., (2015), *S. portulacastrum* growth was not impacted in the presence of 50 µM Cd (=5.62 mg/L), while in the presence of 100 µM Ni (=5.87 mg/L) or the combination of 50 µM Cd and 100 µM Ni, growth was severely impacted. Moreover, Fourati et al. (2020) showed that this species could grow normally while accumulating 500 mg/kg DW Ni in the shoots. According to Fourati et al. (2016), *S. portulacastrum* tolerates Ni better than *Cakile maritima* Scop. Plants of *S. portulacastrum* subjected to 0, 25, 50, and 100 µM of Ni (respectively 0, 1.47, 2.93, and 5.87 mg/L), in hydroponics showed better photosynthesis activity, chlorophyll content, and photosystems II integrity than other species, while accumulating more Ni in the shoots than *C. maritima*.

In *S. australis*, only the relative aerial growth is significantly impacted by the high concentrations level of metals and only if the plants are frequently irrigated. The absence of effect on final biomass parameters could be explained by the fact that the values of these parameters include the initial weight of the plants before their transfer into the pots, whereas the aerial relative growth measured only the growth from the beginning of the treatments to the end of the experiment. Bankaji et al. (2015) showed a high oxidative stress in *Suaeda fructicosa Forssk. ex J.F.Gmel.* in the presence of 400 µM Cd (44,96 mg/L) and 400 µM Cu (25,42 mg/L). However, the growth conditions, the species, and the tested metals differ from those in our experiment, and the concentrations are globally higher than those tested here. In our experiment, *S. australis* water content (succulence) was enhanced in pots added with metals and frequently irrigated. This could then be a mechanism to reduce metal toxicity. Indeed, plants did not show any toxicity symptoms compared to plants with no metal added (Supplementary Data, Figure S2).

Effects of irrigation frequency on the accumulation of metals in plant organs

The accumulation pattern in response to treatments was generally the same for the four metals in each species. For *S. portulacastrum*, a global negative effect of the low irrigation on Cr and total metal concentrations was detected. Regarding *S. australis*, the bioaccumulation of metals in shoots and roots was generally not significantly impacted by the irrigation level. However, Cr concentration in the shoots was lower at low irrigation level, as detected by factorial ANOVA, with a clear interaction between irrigation frequency and metal concentration level. As shown on *Suaeda salsa* by Song and Sun (2014), metal mobility and, thus plant extraction can differ between the low and high marsh zones that are impacted differently by tides. These authors showed that the population from the low marsh translocated more Ni and Cr than plants from the middle marsh. This could be due to greater mobility of metals in the low marsh that was more affected by tides and where the salinity was higher.

The accumulation of metals also depends on the soil waterlogging conditions, with anoxia in roots increasing metal translocation (Song and Sun 2014; Alhdad et al. 2015). The soil used in our case was sampled in a tidal flat; it was pretty dense and muddy, and greenhouse conditions, with high atmospheric humidity, did not allow rapid evaporation from the soil. Although the level of oxygen in soil has not been measured, we could expect that this could have fluctuated between treatments, which could explain the greater accumulation of metals by *S. australis* on pots more frequently irrigated. The effect of irrigation frequency was more significant for Cr than Ni in our experiment, as Song and Sun (2014) also showed. Alhdad et al. (2015), working on *S. maritima*, measured Mn concentrations in shoots and roots in normal aeration and hypoxic growth conditions in artificial seawater and a solid substrate made of sand and mud. In this case, also, hypoxia increased metal translocation.

Effects of metal concentrations on the accumulation of metals in plant organs

S. portulacastrum limited the accumulation in the shoots when metals were added, but this increased the metal concentration in the roots, resulting in a reduced TF. The reduction of metal translocation in response to high metal concentrations in soil is known as one of the metal tolerance mechanisms (Van Oosten and Maggio 2015). For *S. australis*, a global positive effect of metal addition on metal accumulation in shoots was detected by the factorial ANOVA, but it is relatively weak. Song and Sun (2014) reported that metal accumulation in plant organs of *Suaeda salsa* depends on the considered metal and its concentrations in the substrate. Alhdad et al. (2015) showed that increasing Mn concentrations in the growth medium of *Suaeda maritima* (L.) Dumort. enhanced Mn concentrations in shoots in the hypoxic conditions more than in normal aeration, which agrees with our results, particularly for Cr in *S. australis*.

Phytoremediation potential of S. australis and S. portulacastrum

As for most halophytes, TF was lower than 1 in both species, indicating that they accumulated metals preferentially in the roots rather than in their aerial parts. TF values of *S. portulacastrum* were higher than *S. australis* in pots non-added with metals. However, S. australis concentrations in the shoots and roots remained higher than in *S. portulacastrum*. Considering all treatments, the total concentrations of the four metals in *S. australis* ranged from 366.5 to 495.3 mg/kg DW in the roots, and from 497.3 to 669.0 mg/kg DW in the whole plant. In *S. portulacastrum*, average values ranged from 154.9 to 292.3 mg/kg DW in the roots, and from 263.0 to 390.6 mg/kg DW in the whole plant. Similarly to us, Samundeeswari and Lakshmi (2018) compared the Pb, Cr, Cd, Cu, and Zn phytoremediation capacities of *S. portulacastrum* to another *Suaeda* species, treated with various metal concentrations in open-air conditions. The study also concluded a better accumulation in the *Suaeda* species. However, unlike our study, *S. maritima* and *S. portulacastrum* generally accumulated more metals in the shoots than in the roots. Kaewtubtin et al. (2016) studied the heavy metal phytoremediation potential of 18 species associated with mangroves by analyzing samples of soils and plants taken in their natural environment. *S. portulacastrum* accumulated more Ni than 15 of the other species (30.6 mg/kg in the roots and 32.8 in the leaves). These concentrations, clearly lower than ours, cannot be compared with our results because the quantities of extractable Ni are lower in the mangrove sediments. Indeed, BCF values (10.6 for the roots) were a little higher than ours. *S. portulacastrum* also fixed Mn and Cr but with lower performances. In another study (Kaewtubtin et al. 2018), the authors analyzed the metal bioaccumulation potential of *Avicennia marina* (tree) and *Pluchea indica* (shrub) in the field and in the greenhouse. The amounts of Mn, Ni, and Cr taken together accumulated in six months were similar to those of our experiment (BCF values were variable but not different on average than ours). They considered these species as promising for metal phytoremediation.

Several *Suaeda* species have been studied for their metal phytoremediation potential. Li et al. (2006) showed that *S. australis* had different bioaccumulation capacities for Cu, Zn, Cd, Pb, Cr and Hg. Metal concentrations in roots were positively correlated to their bioavailable fractions and depended on the chemical form of the metals. Conversely, in the present study, the DTPA-extractable fractions in soil were similar among treatments and species. Ayyappan and Ravindran (2014) studied plants of *Suaeda monoica* treated for 6 months with a paper mill effluent containing 142.4 mg/L of Cr. At the end of the experiment, the hole of the plant accumulated 40.89 mg/kg of Cr, while in this work, *S. australis* accumulated up to 11.22 mg/kg Cr in the shoots and up to 88.35 mg/kg Cr in the roots.

Concerning *S. portulacastrum*, the maximal mean values for Cr concentrations were 21.3 mg/kg in shoots and 67.0 mg/kg in roots; for Ni, it was respectively 27.7 mg/kg and 112.1 mg/kg DW for shoots and roots. This halophyte is considered a good phytoremediator for several metals in numerous studies. However, most of these studies were conducted in hydroponics, where plants can freely absorb metal ions in solutions. For example, Fourati et al. (2016) tested *S. portulacastrum* in hydroponic conditions and measured 1050 mg/kg shoot DW of Ni. In soil, the absorption of metal ions by plants can be limited by their adsorption on clay and humic particles, influencing their bioavailability. Moreover, polluted soils are generally characterized by multi-metal contamination, which can reduce the accumulation capacities of one metal species compared with controlled experiments focusing on one or two metals. To assess the phytoremediation potential of plants, BCF or BAF values higher than 1 are considered a reliable criterion (Shmaefsky 2020). In our study, *S. portulacastrum* showed BAF values of total metals ranging from 3.5 to 4.9. For *S. australis*, these values ranged from 7.2 to 12.9. However, definitions and calculations vary from one study to another (Subhashini and Swamy 2014; Mujeeb et al. 2020) or need to be more precisely calculated (Lokhande et al. 2011). This leads to confusion and misinterpretation in data comparison, sometimes with a wide variation of values (Buscaroli, 2017). However, BAF and BCF traduce the balance of metal concentrations between plants and soil. In our case, Total metals mean concentrations range from 366.5 to 495.4 mg/kg for *S. australis* and from 154,9 to 292.4 mg/kg compared with the mixed soil, in which the mean concentration was 42,211 mg/kg (13,027 mg/kg in the non-mixed soil). Thus, both species concentrated metals more than 2-fold the concentrations in the soil, with high accumulation in *S. australis*.

The phytoremediation potential of the plants also needs to consider their biomass productivity. As halophytes, the two plants studied are perennial herbaceous species. The total maximal amount of the four metals accumulated per plant in 6 months was 1458.4 mg for *S. australis* and 1191.3 for *S. portulacastrum*. A field experiment is now necessary to better determine the potential of these accumulated metals. This will allow us to know the biomass productivity per ha and, consequently, the amounts of metals accumulated per ha.

Conclusions

In conclusion, the growth of *S. australis* and *S. portulacastrum* differed depending on irrigation frequency and metal concentration levels. *S. portulacastrum*, preferentially accumulated metals in the roots, protecting its aerial parts under the most stressful conditions, despite a higher translocation capacity than *S. australis*. *S. australis* accumulates and stores metals in higher concentrations than *S. portulacastrum*, to the detriment of its growth. *S. australis* had then greater bioaccumulation capacities than *S. portulacastrum* and could be a better candidate for the phytostabilization of metals. If we base our interpretation on the ability of plants to extract metals at a BCF greater than 1, as has been done in numerous publications, the two species accumulate enough metals to be considered relevant candidates for the phytoremediation of metals in saline soils. However, field experiments are needed to determine their potential in relation to their biomass productivity in natural conditions. Moreover, both species accumulated metals in a small period in natural soil, reinforcing the confidence in their potential to remediate *in-situ* metal-contaminated soils from estuaries of New Caledonia. Further investigation should thus bring new information on how to improve their phytoremediation abilities in new Caledonian salt marsh soils in combination with other techniques, such as the addition of microbial inoculants or chelating agents.

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Figure captions:

Figure 1. Sampling site of contaminated soil for the pot experiment located in the estuary of the Baie de St Vincent, La Tontouta. (a) Site location to the active mine upstream; (b) Tidal flat where soil was collected, colonized by *Suaeda australis*, *Sarcocornia quinqueflora* and *Sesuvium portulacastrum;* (c) Visible salt crust appearing at the soil surface near *S. portulacastrum* plants; (d) *S. portulacastrum* plants.

Figure 2. Effect of irrigation frequency and metal concentration level on the aerial relative growth (cm²) in each species, estimated *via* image computation. Boxplot middle bars represent medians while dark dots are mean values. Pairwise comparisons with $p<0.05$ are represented by different letters for each plant species separately.

Figure 3. Effect of irrigation frequency and metal concentration level on plant growth. Boxplot middle bars represent medians while dark dots are mean values. Pairwise comparisons with $p<0.05$ are represented by different letters for each plant species separately.

Figure 5. Effect of irrigation frequency and metal concentration levels on metals' translocation factors in *S. portulacastrum* and *S. australis*. Boxplot middle bars represent medians while dark dots are mean values. Pairwise comparisons with $p<0.05$ are represented by different letters.

Figure 6. Effect of treatments on final DTPA-extractable concentrations of Ni, Co, Cr and Mn in soil in the pots of *S. portulacastrum* and *S. australis* (mg/kg). Pairwise comparisons

with $p<0.05$ are represented by different letters. Boxplot middle bars represent medians while dark dots are mean values.

Table captions :

Table 1. Chemical characteristics of the contaminated soil used in the pot experiment

Table 2. Comparison of metal accumulation in shoots and roots of *S. australis* and *S. portulacastrum* under two irrigation frequency levels and two metal concentration levels. Concentrations are expressed in mg/kg DW (means and standard deviations). Pairwise comparisons with $p<0.05$ are represented by different letters based on results from one-tailed post-hoc Conover-Iman tests. A = low metal concentration level, high irrigation frequency; B = low metal concentration level, low irrigation frequency; C = high metal concentration level, high irrigation frequency; D = high metal concentration level, low irrigation frequency.

Table 3. Statistics and p-values of the effects of treatments on metal accumulation in the shoots and the roots of *S. australis*, on the basis of factorial ANOVA or the non-parametric Kruskal-Wallis test and cross comparisons using Tukey'HSD or Conov-Iman test.

Table 4. Statistics and p-values of effects of treatments on metal accumulation in the shoots and the roots of *S. portulacastrum*, on the basis of factorial ANOVA or the non-parametric Kruskal-Wallis test and cross comparisons using Tukey'HSD or Conover-Iman test.

Table 5. Comparisons of BCF and BAF of *S. australis* and *S. portulacastrum* under two irrigation frequency levels and two metal concentration levels. Results are means ±

standard deviations. Pairwise comparisons with $p<0.05$ are represented by different letters based on results from one-tailed post-hoc Conover-Iman tests. A = low metal concentration level, high irrigation frequency; B = low metal concentration level, low irrigation frequency; C = high metal concentration level, high irrigation frequency; D = high metal concentration level, low irrigation frequency.

Appendix Supplementary data :

Figure S1. Non-destructive method used to determine plant shoots area at the beginning and the end of the experiment using the image segmentation tool Ilastik vers. 1.0. 1. Images of the initial and final state of the aerial part of plants. 2. A sub-sample of images is used to train the segmentation tool to distinguish plant from background and pot area. 3. Batch object segmentation and plant area calculation are processed on all images. The output contains the segmentation file and a csv file with the size of every detected object in each image. 4. Segmentation files are converted into png format in Image J and are compared with their respective original picture to detected any wrong results. 5. Csv files are combined and total plant area is calculated for each sample. This value is then converted into cm ^{2} with respective scale of each image.

Figure S2. Aerial parts of *S. portulacastrum* and *S. australis* at the end of the experiment.

Table S1. Effect of treatments on final pH and electrical conductivity (EC) in pots and comparison between *S. portulacastrum* and *S. australis*. Presented values are means ± SD. Pairwise comparisons with $p<0.05$ are represented by different letters based on results from one-tailed post-hoc Conover-Iman tests. A = low metal concentration level, high irrigation frequency; B = low metal concentration level, low irrigation frequency; C = high metal concentration level, high irrigation frequency; D = high metal concentration level, low irrigation frequency.

Metal extraction capacities of the two halophytes *Sesuvium portulacastrum* and *Suaeda australis* from New Caledonian estuaries contaminated with metals

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Short title: Phytoremediation potential of two halophytes

Metal extraction capacities of the two halophytes *Sesuvium portulacastrum* and *Suaeda australis*~~: new insights of the phytoremediation possibilities in~~from New Caledonian estuaries contaminated with metals

Abstract

The increase in population and its needs have worsened the issue of metal contamination of the environment, negatively impacting plants, animals, and human health. New Caledonia, a biodiversity hotspot, faces a significant source of metals from mining activity and erosion that affect unique ecosystems that require protection. In this study, we explore the potential of halophytes to remediate metals, with the aim of safeguarding seashores and lagoons. We examine the optimal growth and metal bioaccumulation conditions of two halophytic species, *Sesuvium portulacastrum* (L.) L. and *Suaeda australis* (R.Br.) Moq, which dominates the salt marshes of New Caledonian coasts. These species were subjected to two levels of metal concentration and two levels of irrigation frequency, which mimic the fluctuation in soil water content due to seasonal changes and tidal irregularities. The results showed that *S. portulacastrum* growth was enhanced when the irrigation frequency was lowered, while *S. australis* preferred constant soil moisture. *S. australis* accumulated more Ni, Co, and Cr than *S. portulacastrum*, especially in the roots. However, *S. portulacastrum* transported more metals in the aerial parts than *S. australis*. This work showed promising abilities of both species to accumulate metals and suggests further research to improve their metal phytoremediation potential in New Caledonian salt marshes. Mining activities with natural soil erosion lead to the contamination of watersheds in New Caledonia with metal-rich sediments. As metal contaminations represent a major threat to populations and ecosystems, we aim to develop an ecological and economical solution using the phytoremediation potential of halophytes to protect seashores and the lagoon from the metals' spread. In this perspective, the current study intends to explore the optimal growth and metal bioaccumulation conditions of two halophytic species dominating the salt marshes of the New Caledonian coasts. *Sesuvium portulacastrum* and *Suaeda australis* (R.Br.) Moq. were subjected to two levels of metal concentration and two levels of irrigation frequency, which tended to mimic the fluctuation in soil water content as a function of the seasons and the irregularity of the tides. Results showed that *S. portulacastrum* growth was enhanced when the irrigation frequency was lowered, while *S. australis* preferred constant soil moisture. *S. australis* accumulated more Ni, Co, and Cr than *S. portulacastrum*, especially in the roots. However, *S. portulacastrum* translocated metals in the aerial parts more than *S. australis*. This work presents promising abilities of both species to accumulate metals. Further research will improve their potential for phytoremediation of metals in New Caledonian salt marshes.

1. Introduction

Metal pollution causes major ecological and economic issues for most countries and often results from anthropogenic activities such as intensive farming, mining, and many other industrial fields. While most studies related to environmental metal contaminations have focused on cultivable lands, coastal environments are often affected by several pollutions, as the economic development near coastal zones implies the establishment of industrial estates (Qian et al. 2015; Han et al. 2021). Although coastal lands represent a buffering zone between terrestrial and marine ecosystems (Oyetibo et al. 2017), the amounts of metals spreading into these environments are usually so grave that they cannot be fully retained and are released in marine trophic networks, thus representing a threat for human health and ecosystems (~~Hedfi et al. 2007; ~~Briand et al. 2018; Han et al. 2021; Yousif et al. 2021). In this context, while most technologies employed in decontaminating metals require important financial and technical resources, phytoremediation of metals has received great attention in the last two decades because of its low cost and environmental impact (Origo et al. 2012). This latter technology using plants to extract or stabilize metals in the soil has already proven itself as a reliable and ecological alternative (~~Alkorta et al. 2004; ~~Liang et al. 2016; Sarwar et al. 2017; Saxena et al. 2019).

~~The potential of H~~Halophytes~~,~~ are plants particularly adapted to saline environments. Their potential ~~,~~ for the phytoremediation of metals has been reviewed many times (Van Oosten and Maggio 2015; Liang et al. 2016; Nikalje et al. 2018). In tidal environments, plants face several stresses, such as salinity elevation and floodings~~ (Pennings and Callaway 1992)~~, causing hypoxia episodes (Colmer et al. 2013), particles' sedimentation,~~,~~ and modifications in soil porosity and aeration (Schwarz et al. 2015). Hence, these stressors should be considered in the design of efficient phytoremediation strategies. ~~Seasons affect edaphic factors such as pH and electrical conductivity (EC), which in turn influence plant metal accumulation (Bai et al. 2014; Mujeeb et al. 2021). ~~Soil salinity is linked to soil humidity and fluctuates with tides and across seasons, decreasing in rainy periods and increasing at the surface in dry periods (Fu et al. 2020). Moreover, tides can also impact plant growth~~ (Mahall and Park 1976)~~. For Silvestri, Defina, and Marani (2005), ~~the distribution of halophytes in the environment is complex and related to spatial~~

and time fluctuations of soil parameters. sSubsurface water movements combined with evapotranspiration patterns of plants influence their distribution by creating variations in aerobic/anaerobic conditions and soil porosity. Fluctuations of such parameters can be determinant in the metal bioavailability and thus can influence the phytoremediation process (Peijnenburg and Jager 2003).

In New Caledonia, mining activities, in combination with soil erosion, lead to metal pollution, mainly represented by high Ni, Co, Cr, and Mn concentrations due to the transport of metals-rich sediments from the mining sites to the seashore (Briand et al. 2018). New Caledonia is also affected by a wet season characterized by intense rains that increase the transport of sediments. For example, the recorded annual rainfall between 2009 and 2013 varied from 1460 mm to 2720 mm (Allenbach et al. 2015).

Here, we studied the phytoremediation potential of the two halophytes, *Sesuvium portulacastrum* (L.) L.,, and *Suaeda australis*, (R.Br.) Moq., -to assess their potential in removing or stabilizing metals in the polluted estuaries of New Caledonia. *S. portulacastrum* has been reported as a suitable metal phytoremediator (Nouairi et al. 2006; Mnasri et al. 2015; Ayyappan et al. 2015; Fourati et al. 2020), and *S. australis* has been studied for its metal resistance (Alam et al. 2021). According to Poupart et al. (2013), these two species share the same habitat in the tidal transect from Kone in the north to the south of the west coast in New Caledonia mainland ("Grande Terre"). However, their metal accumulation behavior under soil humidity fluctuation has not yet been studied. We hypothesized that these two species could have interesting extraction capacities, and in the present study, we aim to determine the impact of humidity fluctuation on their metal bioaccumulation in greenhouse conditions. To this end, the two native halophytes were grown in pots -at two metal concentration levels corresponding to the average and maximum metal concentrations found at the contaminated marshland site studied. Two soil moisture conditions were performed: the first corresponded to pots permanently humid on the soil surface, and the second to pots with soil surface periodically dried. These conditions reproduced those affecting the two species in the studied coastal site. We aim to elucidate their potential in the phytoremediation of metal-contaminated estuaries in New Caledonia and, consequently, in the protection of one of the most important lagoons in the world.

2. Materials and Methods

2.1. Soil collection and characterization

The soil used for the experiment was collected on a contaminated (red) saline tidal flat located on the estuary of the Baie de Saint-Vincent, on the west coast of New Caledonia (21°59' and 166°9') on June 12th, 2020 (Figure 1 near here). The soil was recovered onat three different points situated between tufts of plants, about 10 m aparts. The soil surface was scraped to eliminate the excess salt and compacted pellicle of fine particles before collecting it with a clean spade. The soil, relatively poor in roots, has beenwas homogenized by hand before use.

The soil has been homogenized by hand before use. Three samples were used for physicochemical characterization and were oven-dried at 45°C, ground, and passed through 2-mm and 0.1-mm mesh sieves for the following analyses performed by the Laboratoire des Méthodes Analytiques of Noumea (LAMA-US IMAGO IRD, New Caledonia). Total nitrogen was measured according to Kjeldahl's method (Kirk 1950). Total organic carbon content was determined using Walkley and Black's method. Exchangeable cations and cation exchange capacity (CEC) were determined using the cobalt hexamine chloride method (Ciesielski et al. 1997). Carbonate content was determined using Petard's method using a calcimeter (Bernard type). Chloride content was determined with the colorimetric method (Pétard 1993; SEAL Analytical Method, G-133-95). Exchangeable P was determined using the Olsen method. Total Ni, Fe, Mn, Co, Cr, P, Mg, Ca, Na, and K extracted by alkaline fusion and DTPA-extractable fractions for Ni, Cr, Co, Mn, and Fe were determined using an Inductively Coupled Plasma Optical Emission Spectroscopy (ICP OES, Varian, Varian 730-ES, Palo Alto, USA). Electrical conductivity (EC) and soil pH were measured according to the standard methods ISO 11265:1997, and ISO 10390:2005, respectively. All these soil characteristics are summarized in Table 1(Table 1 near here).

2.2. Plant material

We selected Ttwo common halophyte species of New Caledonia were selected: *Suaeda australis* (R.Br.) Moq. (Amaranthaceae) and *Sesuvium portulacastrum* (L.) L. (Aizoceae) -(Della Patrona 2016). Plants used in this work were obtained by cutting propagation on plants sampled in a non-polluted tidal flats. Medium-sized, healthy and green tufts of each of the two species were selected- and removed with their root system. They The plants were cultivated in 50/50 potting soil/sand under greenhouse conditions and watered twice a week with 50/50 sea water/tap water, until they form new branches. They were then multiplied by cutting. For this experiment, Cuttings cuttings of S. portulacastrum and S. australis plants were taken from one unique plant for each of the two species, and rooted in a mixture of 50/50 potting soil/2 mm-mesh sieved coral sand in 250 mL pots and watered 2 min twice daily. After four weeks, cuttings were transplanted in 1 L pots (one plant per pot) containing a mixture of the sampled soil/potting soil (70/30). At the beginning of the experiment, plant heights were roughly homogeneous between treatments.

Pot experiment in greenhouse

Because of its low fertility, inducing growth difficulties, the soil was mixed with 30 % of potting soil. The total and DTPA extractable metal contents (Fe, Ni, Co, Cr, Mn) of the mix of contaminated and potting soil were assessed using the same methods described previously. DTPA extractable concentrations were 8.298 ± 0.874 mg/kg Fe, 6.742 ± 0.268 mg/kg Ni, 2.301 ± 0.212 mg/kg Co, 0.025 ± 0.003 mg/kg Cr, and 33.143 ± 2.353 mg/kg Mn.

Cuttings transplanted in the studied soil were then subjected to the 4 following treatments:

- A: Low metals' concentration level, high irrigation frequency;
- B: Low metals' concentration level, low irrigation frequency;
- C: High metals' concentration level, high irrigation frequency;
- D: High metals' concentration level, low irrigation frequency.

The low metals' concentration level was defined aswas the level of metal concentrations already present in the contaminated soil (no metal addition). The high metals' concentration level has been defined to correspond approximately to the maximum metal concentrations at the contaminated marshland site studied (Bonaventure, 2023). Considering that the concentrations of available metals could vary in relation to metal binding capacity of the soil, we have beforehand tested the addition of different concentration supplies of the mixed metals, followed by the analysis of the DTPA-extracted metal concentrations, which allowed us to determine the amounts of each metal to be added to obtain the expected concentrations. The concentrations of the mixed metals chosen The high metals' concentration level correspondsed then to the addition in 3 times of 150 mL of a mix of NiSO4.6H2O ([Ni] =50 mg/L), CoCl2.6H2O ([Co] = 10 mg/L), CrCl3.6H2O ([Cr] = 5 mg/L), MnSO4.H2O ([Mn] = 100 mg/L). To reduce metal toxicity at the beginning of the experiment, when plants were still fragile, this amount of metal solution was added in 3 times (50 mL for each supply), with an interval of two days. Saucers were added under the pots to avoid metal leaching.

Given the difficulty of regularly measuring fluctuations in soil moisture over time, we have only sought to approximate the effects of large fluctuations in moisture, as it can be observed in the natural environment of these plants, compared to relatively stable moisture. Thus, the high irrigation frequency level (twice a week with tap water) was defined so that the soil moisture at the pot surface was constantly maintained: twice a week with tap water until saturation. For the low irrigation frequency level, plants were watered only when a crust of desiccated soil was formed at the pot surface, without plant wilting: about once a week until saturation. For the two species, each treatment contained 10 replicates. The temperature in the greenhouse fluctuated between 24-28 °C during the day and 19-23°C at night, and the relative humidity was about 65-75%. The experiment was followed for 6 months.

2.4. *Growth parameters using images processing*

Ten images of each plant were taken at the beginning (the first day after transplantation of cuttings in final pots) and at the end of the experiment using a camera Canon 600D, with a 30 cm-ruler positioned next to the plant to standardize the scale, and with a red background behind the objects (Supplementary data Figure S1). Because of various contrasts among images, we used a machine-learning-based segmentation tool, Ilastik vers. 1.0 (https://www.ilastik.org/) (Berg et al. 2019) to extract in batch mode the images of every plant from the background and the pot zone and calculate plant area. We also used Fiji vers. 1.42 (https://imagej.net/software/fiji/) to compare raw and segmented images to identify and correct potential segmentation errors. We call "aerial relative growth" the difference between the values of initial and final measurements, expressed in cm^2. Only segmented objects with a probability of 80 % were kept for the analysis. Consequently, there were 8 replicates left for *S. australis* in treatment A, 6 in treatment B, 9 in treatment C, and 5 in treatment D. For *S. potulacastrum*, there were 7 replicates left in the treatment A, 5 in the treatment B, 9 in the treatment C and 5 in the treatment D.

2.5. *Biomass and metal contents in plants*

At the end of the experiment, roots, shoots, and soil were gently separated from each other, weighted, and oven-dried at 45°C. The dry mass of roots and shoots was determined, and each part was ground into a fine powder. The succulence index or saturated water content (SWC) was calculated according to Ogburn and Edwards (Ogburn and Edwards 2010; Ogburn and Edwards 2012) :

SWC = (shoots fresh weight – shoots dry weight)/(shoots dry weight)

The 0.5 mm sieved fraction was subjected to acid digestion: 100 mg of the powdered root and shoots material were put into 15 mL vials containing 1 mL of 70% HNO3 and were heated for 2 h at 100°C. After cooling to room temperature, 1 mL of 30% H2O2 was added and left on for 30 min. Then, vials were once more heated at 100°C for 2 h. Non-completely digested samples were separated from the others, supplemented with 1 % NH4F, and heated at 100°C for 2 h. Therefore, both matrices (HNO3/H2O2 and HNO3/H2O2/NH4F) were analyzed using an Inductively Coupled Plasma Optical Emission Spectroscopy (ICP OES, Varian, Varian 730-ES, Palo Alto, USA) with 2 distinct standard curves using the corresponding standard solutions. The translocation factor (TF) was calculated for Ni, Co, Cr, and Mn using the following formula (Mujeeb et al. 2020) :

TF = concentrations in the shoots/concentrations in the roots

Concentrations correspond to the final state of the experiment.

~~2.6.~~ *Soil extractable metal concentrations and Bioconcentration factor*

At the end of the experiment, the soil of each pot was dried at 45°C until the weight was stabilized. It was subsequently grounded and passed through a 2 mm mesh sieve. For each pot, 10 g of sieved soil was supplemented with 20 mL of DTPA, shaken for 1 hour at 75 rpm, and then centrifuged at 3000 g for 10 min. The supernatants were recovered in 15 mL tubes, and extractable fractions for Ni, Cr, Co, and Mn were obtained using an ICP-OES (ICP OES, Varian, Varian 730-ES, Palo Alto, USA).

The bioconcentration factor (BCF) was defined as follows, based on the calculation of BCF in Zhang et al. (2020) :

BCF = concentrations in the roots/extractable concentrations in the soil

Concentrations correspond to the final state of the experiment. Metal concentrations in the soil correspond to the DTPA-extractable fraction.

The calculation of the bioaccumulation factor (BAF) was based on and modified according to Khaokaew and Landrot (2015) as follows :

BAF = concentrations in the whole plant/extractable concentrations in the soil

Concentrations correspond to the final state of the experiment. Metal concentrations in the soil correspond to the DTPA-extractable fraction.

~~2.7.~~ *Statistical analysis*

All statistical analyses were performed using R software version 3.6.2 (http://www.r-project.org) with R Studio version 1.2.5033. Prior to statistical comparisons, outliers in each variable were detected according to the Grubbs test from the package {outliers} and were removed if the values were possibly related to an experimental mistake. A two-way analysis of variance or a non-parametric Kruskal-Wallis ranks test was used to assess the differences between treatments within each species. When the test was significant, we performed a pairwise comparison using the post-hoc Tukey's HSD test or a non-parametric pairwise Conover–Iman test.

3. Results

3.1. Aerial relative growth

The variation of the aerial relative growth by treatment indicated how plant growth is affected by metals and irrigation frequency in the two species (figure 2 near here).

The aerial relative growth is highly variable within treatments (figure 2). In *S. portulacastrum,* no significant difference was observed between treatments. On the contrary, *S. australis* aerial parts were significantly less developed in treatments C ($p=0.0092$) and D ($p=0.0095$) compared with treatment A. Comparing species within treatments, we found that *S. australis* resulted in significantly more aerial surface than *S. portulacastrum* only in treatment A ($p=0.0212$), with 16.00 ± 6.78 cm² in *S. australis* and 1.94 ± 5.31 cm² in *S. portulacastrum*.

3.2. Final dry biomass and shoots/roots biomass ratio

Results on the final total plant, roots and shoot dry weight, and shoots to roots ratio are presented in Figure 3.

The total dry weight of *S. portulacastrum* fluctuated between 2.9 and 3.2 g per plant and between 1.5 and 2.2 g for *S. australis*. The total dry weight and shoot dry weight of *S. portulacastrum* were significantly greater than those of *S. australis* in treatments A (respectively, p=0.0003 and p=0.0004), B (respectively, p=0.0000 and p<0.0000) and D (p<0.0000 for both treatments). *S. portulacastrum* shoot biomass was also significantly higher than *S. australis* in treatment C (p=0.0137). Treatments had no significant effect on the root biomass of *S. portulacastrum*, whereas *S. australis* root biomass was significantly affected by the reduction of the irrigation frequency when metals were added (p=0.0401) (Figure 3c). *S. portulacastrum* had significantly higher root biomass than *S. australis* only in treatments A (p=0.0367) and B (p=0.0274). The ratio between shoot and root biomass (Figure 3d) was significantly higher in treatment D than in treatment C in both species (for *S. portulacastrum*, p=0.0082; for *S. australis*, p=0.0023).

~~3.3.~~ *Succulence index (SWC)*

~~The treatments and the two species were compared for their succulence index (SWC) (Figure 4 near here).~~

~~No~~ As shown in Figure 4, no significant difference on the succulence index (SWC) between treatments was observed in *S. portulacastrum,* while in *S. australis*, SWC was significantly higher in treatment C than in treatment B (p=0.0010). The difference between treatments C and D was near the significance level (p=0.0519).

SWC in *S. portulacastrum* was significantly higher than in *S. australis* in treatments A (p=0.0015), B (p< 0.0010), and D (p=0.0142).

3.4. Metal accumulation

3.4.1. Concentrations in plant organs

Results on the metal concentrations in the shoots and roots of the two halophytes are presented in Table 2.

In both plant parts, *S. australis* accumulated significantly more metals in total than *S. portulacastrum* in almost every treatment (in the shoots, B: p=0.0078, C: p<0.0000, D: p<0.0000. In the roots, A: p=0.0011, B: p<0.0000, C: p=0.0031, D: p=0.0122). The highest values of total metals accumulated were observed in *S. australis* in treatment C (high metal level and high irrigation frequency), with 173.6 mg/kg in shoots and 495.4 mg/kg in roots. However, the amounts of total metals accumulated per plant did not show significant differences between the two plants for each of the 4 treatments considered separately. These amounts varied from 830.5 mg/plant (treatment A for *S. portulacastrum*) to 1458.4 mg/plant (treatment C for *S. portulacastrum*).

The average values of Ni concentrations vary between 8.8 and 31.7 mg/kg in the shoots and 55.4 to 245.8 mg/kg in the roots. Ni concentrations in the roots of *S. australis* were higher than *S. portulacastrum* in every treatment (A: p=0.0052; B: p<0.0000; C: p=0.0003; D: p=0.0017) whereas in the shoots, *S. australis* accumulated significantly more Ni than *S. portulacastrum* only when metals were added to the pot (C: p=0.0079; D: p=0.0088).

Regarding Co, concentrations range on average from 1.0 to 2.6 mg/kg in shoots and from 5.1 to 23.6 mg/kg in the roots. Co concentrations in the shoots were also higher for

S. australis than *S. portulacastrum* in treatments B (p=0.0074), C (p=0.0006), and D (p=0.0000). In the roots, these concentrations were higher for *S. australis* in all treatments (A: p=0.0004; for B, C, and Dp<0.0000). A maximal average was found in *S. australis* in treatment C, which was 2.6 times higher than in *S. portulacastrum*.

The average values of Cr concentrations vary between 2.6 and 21.3 mg/kg in the shoots and 19.5 to 88.3 mg/kg in the roots. The concentrations in *S. australis* shoots were lower than in *S. portulacastrum* in treatment A (p=0.0067). In the roots, *S. australis* accumulated more Cr than *S. portulacatrum* only in treatments A (p=0.0491) and B (p=0.0000), respectively 2.2 and 4.5 times more than in *S. portulacastrum*.

Mn was the major metal accumulated in both species compared with the other metals. Plants accumulated in average concentrations ranging from 72.4 to 130.4 mg/kg in the shoots and from 74.9 to 147.5 mg/kg in the roots. Concentrations in the shoots were higher in *S. australis* than in *S. portulacastrum* in the treatments B (p=0.0033), C (p<0.0000), and D (p<0.0000). In the roots, Mn concentrations were higher in *S. australis* in the treatment B (p=0.0067).

As the Anova was not applicable to the full dataset, including both species, we tried to do so on each species' sub-dataset to evaluate the effect of treatments on metal accumulation patterns and identify whether the metal concentration level, the irrigation frequency level, or both factors affect metal bioaccumulation. These results are presented in Tables 3 and 4 ~~(Table 3 is near here)~~.

For *S. australis*, Mn, Co, and total metal accumulation in the shoots was significantly higher at high metal concentration levels (Table 3). Moreover, the interaction of irrigation frequency and metal addition significantly impacted Ni, Co, and Cr accumulation in the shoots of this species. In particular, Ni and Co concentrations were higher in treatment C than in treatment A. Ni concentration in its shoots was also higher in treatment C than in treatment D. Regarding Cr, *S. australis* accumulated it more in treatment B than in treatment A. In the presence of a low irrigation frequency level, the addition of metals significantly decreased the Cr concentration in the shoots.

Metal addition significantly increased Co concentrations in the roots (Table 3). The irrigation frequency level or the interaction of both factors had no significant influence on metal accumulation.

Regarding *S. portulacastrum*, the irrigation frequency level significantly impacted Cr and total metal concentrations in the shoots (Table 4), with a higher concentration of Cr and total metals in the presence of a high irrigation frequency level. The addition of metals also significantly affected the accumulation of Co, Cr, Mn, and total metals in the shoots, with a decrease in metal concentrations when metals were added to the soil. For Ni, the non-parametric test was significant, with higher concentrations in treatments B and C than in treatment D. ~~(Table 4 near here).~~

The addition of metals also significantly impacted the accumulation of Ni, Co, Cr, and total metals in the roots (Table 4). Moreover, the non-parametric test performed on Mn concentrations was also significant. In particular, Ni, Co, Cr, and total metals were higher in the high metal concentration level. In the presence of a low irrigation frequency level, adding metals to the pot significantly increased the Mn accumulation in the roots of *S. portulacastrum*.

3.4.2. Translocation factor (TF), bioconcentration (BCF) and bioaccumulation factor (BAF)

The translocation factor values of Ni, Co, Cr, and Mn are shown in Figure 5. (near here).

Both species have relatively low TF (generally under 1) for Ni, Co, and Cr (Figure 5d). Except for Mn, TF significantly differed within species and treatments (Ni Treatments x species: $p=0.0173$; Co Kruskal-Wallis: $p=0.0000$; Cr Treatments x Species: $p=0.0000$). Indeed, Ni and Co TF (Figure 5a, b) were significantly higher in *S. portulacastrum* than in *S. australis* for the treatments A (respectively, $p=0.0016$, $p=0.0023$) and B ($p=0.0038$, $p=0.0026$). The same was observed for Cr in the treatment A ($p< 0.001$).

Effects of the irrigation frequency and metal concentration levels were also evaluated on species separately. While treatments had no significant impact on metal translocation in *S. australis*, the TF for Ni, Co, and Cr was significantly reduced by the addition of metals in *S. portulacastrum* (respectively, $p=0.0000$; $p=0.0000$; $p<0.0000$).

BCF and BAF were calculated to evaluate the balance of metal concentrations between the soil and the plant and reflect the ability of plants to remove metals from the soil. For both species, BCF and BAF were always greater than 1 for both species, (Table 5 near here). Treatments had no significant impact on either BCF or BAF in both species. For Ni, Co, and total metals, BCF was significantly higher in *S. australis* than in *S.*

portulacastrum in all treatments (Ni in A: p=0.0100; in B: p=0.0000; in C: p=0.0021; in D: p=0.0054. Co in A: p=0.0003, in B, C, and D p<0.0000. Total metals in A: p=0.0059, in B: p=0.0000, in C: p=0.0013, in D: p=0.0145). BCF for Cr was significantly higher in *S. australis* than in *S. portulacastrum* only in treatment B (p=0.0000) and for Mn in treatment C (p=0.0061).

BAF was significantly higher in *S. australis* than in *S. portulacastrum* in all treatments for Co (A: p=0.0007; for B, C, and D

p<0.0000), Mn (A: p=0.0006; B: p=0.0005; for C and D p<0.00001), and total metals (A: p=0.0441; B, C, and D p<0.0000). It was also the case for Ni (B: p=0.0000; C: p=0.00001; D: p=0.0002), except in treatment A for Cr, a significant difference was only found in treatment B (p=0.0007).

~~3.5.~~ ***DTPA-extractable metals, final soil pH and electrical conductivity***

~~3.5.1.~~ *Final DTPA-extractable metal concentrations in the soil*

Final DTPA-extractable metal concentrations in the soil are presented in Figure 6 ~~(near here)~~.

Final DTPA-extractable Ni concentrations in the soil (Fig. 6a) were significantly impacted by the treatments (p<0.0000), but no difference was found between species. In both species, final Ni concentrations were significantly higher in the treatment C and D enriched with metals (for *S. australis* respectively, p=0.0063 and p< 0.0010. for *S. portulacastrum*: both p<0.0010)

Regarding Co (Fig. 6b), no significant difference was found between species. *S.*

portulacastrum was significantly impacted by both the irrigation frequency (p=0.00284), with higher Co concentrations in the high irrigation frequency level (p=0.00249) and the metal concentration (p=0.0006) with higher Co concentrations in the high metal concentration level (p=0.0005). *S. australis* was also significantly impacted by both factors (irrigation frequency level: p=0.0059. metal concentration level: p=0.0094), with higher Co concentrations in the high metal concentration level (p=0.0085) and in the high irrigation frequency level (p=0.0052).

For Cr (Fig. 6c), neither the treatments nor the species significantly impacted DTPA-extractable concentrations. Finally, Mn concentrations (Fig. 6d) were significantly impacted by treatments (p<0.00001) and species (p=0.0000) with a greater Mn concentration in the treatment C than in the treatments D (p=0.0331) and B (p=0.0047) for *S. australis* and a greater concentration in the treatment C compared to B (p< 0.0010) for *S. portulacastrum*.

~~3.5.2.~~ *Final soil pH and electrical conductivity*

The final soil pH and electrical conductivity (EC) results are presented in the Supplementary Data Table S1. In both species, the soil in the pot remained alkaline in every treatment, with values varying between 8.1 and 8.5 without significant differences. The electrical conductivity was not significantly different between treatments and species despite high variations in the values.

Discussion

In this study, a greenhouse experiment allowed us to evaluate the ability of two halophytes to grow and accumulate metals under two irrigation conditions and two metal concentration levels.

~~4.1.~~ *Effect of irrigation frequency on plant growth*

The growth rate results for the two species showed considerable variability. Therefore, only large differences between growth rates appeared to be significant. Thus, there were no significant differences between the tested treatments for *S. postulacastrum*. This species can be considered to be adapted to large humidity fluctuations. *S. portulacastrum* is known as a psammophyte (eHALOPH | eHALOPH - Show Plant), i.e., a plant adapted to life in sandy soils with low water retention capacities. However, it can also be found in estuarine mudflats, salt marshes, and habitats associated with mangroves

(Lonard and Judd 1997; Singh 2020). In New Caledonia, *S. portulacastrum* can be found in tidal muddy soils that frequently dry out in the dry season, with sometimes a visible salt crust forming on the soil surface (Figure 1c, d). This species is known for its resistance to several stresses. It has been recommended in the phytodesalinisation of arid regions owing to its ability to accumulate salt ions compared with other species (Rabhi et al. 2008). In their experiment, Slama et al. (2006) demonstrated that *S. portulacastrum* could tolerate a reduction of the water supply up to 50% of the field capacity of the used soil. The study of Winter et al. (2019) showed that *S. portulacastrum* can use a crassulacean acid metabolism at low level, especially when plants are exposed to drought, while most of the species of this genus are known as C4 plants.

Moreover, according to Lokhande et al. (2013), drought causes similar deleterious morphological and physiological effects than salt and metal stress on this species. However, under moderate stress, this species can modulate its succulence, antioxidant systems, water potential, and morphological traits to enhance its response to those stressors. Besides, to our knowledge, this species has not been reported in highly and long-time flooded areas and is present mostly in rarely flooded zones. Additionally, few studies (Morandeira and Kandus 2016; Nikalje et al. 2019) classified halophytes according to their functional adaptations, and *S. portulacastrum* was always out of the anoxia or flood-resistant species categories. Several studies have demonstrated the link between the adaptation to salinity of this species and its resistance to drought (Fan et al. 2009; Yang et al. 2015). In this study, we evaluated the saturated water content that can be used to describe tissue succulence (~~Ogburn and Edwards 2010;~~ Ogburn and Edwards 2012; Carvalho et al. 2022) and found that succulence was not influenced significantly by reducing the irrigation frequency.

Several studies highlighted how soil humidity and salinity, as two common edaphic stressors in tidal environments, can shape the species distribution, acting with other parameters, such as soil oxygen availability, organic matter, sand, silt, and clay contents. (Yilmaz et al. 2020; Sarika and Zikos 2021; Chung et al. 2021; Mumcu et al. 2023). A~~a~~s for *S. portulacastrum*, our treatments had little effects on *S. australis*, which suggests that this species can also bear high fluctuations of soil humidity. According to the eHALOPH database (eHALOPH | eHALOPH - Show Plant), *S. australis* is considered a hydrohalophyte, thus growing in soils with a high water retention capacity or in constant presence of water. Certain (2021) asserted that *S. australis* in New Caledonia colonizes coastal zones that are frequently flooded. ~~Several authors (Pennings and Callaway 1992; YILMAZ et al. 2020; Sarika and Zikos 2021; Chung et al. 2021; Mumcu et al. 2023) highlighted how soil humidity and salinity as two common edaphic stressors in tidal environments can shape the species distribution in salt marshes as other parameters such as soil oxygen availability, organic matter, sand, silt, and clay contents.~~ If the impacts of soil humidity and salinity on *S. australis* growth have received little research attention, other *Suaeda* species are documented~~. According to the eHALOPH database (eHALOPH | eHALOPH - Show Plant), *S. australis* is considered a hydrohalophyte, thus growing in soils with a high water retention capacity or in constant presence of water. We can expect a reduction of its growth under low irrigation frequency. However, as for *S. portulacastrum*, our treatments had little effects on *S. australis*, which suggests that this species can also bear high fluctuations of soil humidity. *Suaeda* species~~ and are considered to be well adapted to anoxia (Colmer et al. 2013). Baoshan, Qiang, et Xinsheng (2008) assumed that *Suaeda salsa* (L.) Pall. could switch from a xerophytic strategy to a limnophytic strategy along environmental gradients as in its environment, the plant community is structured by the water table depth and soil salinity gradients, which are negatively correlated. ~~(Certain 2021) asserted that *Suaeda australis* in New Caledonia colonizes coastal zones that are frequently flooded. On the contrary,~~ According to ~~in the study of~~ Wang et al. (2019), the biomass of ~~*Suaeda*~~ *S. salsa* was the highest in plants from the site that received the slightest flooding per month. In our work, the tidal flat zone from where *S. australis* was collected (figure 1) is not frequently flooded. The sun can quickly dry the soil surface in the summer, suggesting a relative adaptation of this species to low humidity levels. Despite variations between species, *Suaeda* is known to have diverse adaptations to face salinity and water stress, such as enzymatic and non-enzymatic adaptations, the production of amino and organic acids and soluble sugars or the compartmentation of toxic ions (Shang et al. 2020), and the excretion of root mucilage (Jaiswar et Kazi, 2016).

4.2. *Effect of metals on plant growth*

Whereas DTPA extractable Co, Cr, and Mn were reduced in the soil at the end of the experiment, compared with the initial concentrations, the final DTPA-extractable Ni concentrations increased. This increase, despite the plant's absorption of a part of the Ni during the experiment, can be explained by the effect of bacterial activity on ultramafic soil particles that contaminated the estuary. Indeed, it has been demonstrated that bacteria regularly release Ni from minerals in ultramafic soils (Amir and Pineau 2003).

In the high metal concentration level, DTPA-extractable Ni concentrations reached around 13 mg/kg in *S. portulacastrum* pots without affecting its growth. This species is known for its high resistance to metal stress. As shown in the study of Mnasri et al., (2015), *S. portulacastrum* growth was not impacted in the presence of 50 µM Cd (=5.,62 mg/L), while in the presence of 100 µM Ni (=5.,87 mg/L) or the combination of 50 µM Cd and 100 µM Ni, growth was severely impacted. Moreover, Fourati et al. (2020) showed that this species could grow normally while accumulating 500 mg/kg DW Ni in the shoots. This species has been compared several times to other metal phytoremediation candidates and was successfully the best one in several diverse experimental conditions and with several metal species. According to Fourati et al. (2016), *S. portulacastrum* tolerates Ni better than *Cakile maritima* .Scop. Plants of *S. portulacastrum* subjected to 0, 25, 50, and 100 µM of Ni (respectively 0, 1.,47, 2.,93, and 5.,87 mg/L), in hydroponics showed better photosynthesis activity, chlorophyll content, and photosystems II integrity than other species, while accumulating more Ni in the shoots than *C. maritima*.

In *S. australis*, only the relative aerial growth is significantly impacted by ~~adding~~ the high concentrations level of metals ~~to the pot~~ and only if the plants are frequently irrigated. ~~This~~ The absence of effect ~~for~~ on final biomass parameters could be explained by the fact that the values of these parameters include the initial weight of the plants before their transfer into the pots~~.~~, ~~Conversely,~~whereas the aerial relative growth measured only the growth from the beginning of the treatments to the end of the experiment. Bankaji et al. (2015) showed a high oxidative stress in *Suaeda fructicosa Forssk. ex J.F.Gmel.* in the presence of 400 µM Cd (44,96 mg/L) and 400 µM Cu (25,42 mg/L). However, the growth conditions, the species, and the tested metals differ from those in our experiment, and the concentrations are globally higher than those tested here. In our experiment, *S. australis* water content (succulence) was enhanced in pots added with metals and frequently irrigated. This could then be a mechanism to reduce metal toxicity. Indeed, plants did not show any toxicity symptoms compared to plants with no metal added (Supplementary Data, Figure S2).

~~4.3.~~ *Effects of irrigation frequency on the accumulation of metals in plant organs*

The accumulation pattern in response to treatments was generally the same for the four metals in each species. ~~The effect of low irrigation frequency on metal accumulation was weak for the two species.~~ For *S. portulacastrum*, a global negative effect of the low irrigation on Cr and total metal concentrations was detected. Regarding *S. australis*, the bioaccumulation of metals in shoots and roots was generally not significantly impacted by the irrigation level. However, Cr concentration in the shoots was lower at low irrigation level, as detected by factorial ANOVA, with a clear interaction between irrigation frequency and metal concentration level. As shown on *Suaeda salsa* by Song and Sun (2014), metal mobility and, thus~~,~~ plant extraction can differ between the low and high marsh zones that are impacted differently by tides. ~~In this study, they~~These authors ~~compared the responses of two populations of this species in the Yellow River estuary, one from a middle marsh and one from a low marsh that was more affected by tides and where the salinity was higher than the middle marsh. The results~~ showed that the population from the low marsh translocated more Ni and Cr than plants from the middle marsh~~, and~~. T~~t~~his could be due to greater mobility of metals in the low marsh that was more affected by tides and where the salinity was higher.~~.~~

~~Moreover, the~~The accumulation of metals also depends on the soil waterlogging conditions, with anoxia in roots increasing metal translocation (Song and Sun 2014; Alhdad et al. 2015). The soil used in our case was sampled in a tidal flat; it was pretty dense and muddy, and greenhouse conditions, with high atmospheric humidity, did not allow rapid evaporation from the soil. Although the level of oxygen in soil has not been measured, we could expect that this could have fluctuated between treatments, which could explain the greater accumulation of metals by *S. australis* on pots more frequently irrigated. The effect of irrigation frequency was more significant for Cr than Ni in our experiment, as Song and Sun (2014) also showed. Alhdad et al. (2015),~~,~~ working on *S. maritima*, measured Mn concentrations in shoots and roots in normal aeration and hypoxic growth conditions in artificial seawater and a solid substrate made of sand and mud. In this case, also, hypoxia increased metal translocation.

4.4. Effects of metal concentrations on the accumulation of metals in plant organs

S. portulacastrum limited the accumulation in the shoots when metals were added, but this increased the metal concentration in the roots, resulting in a reduced TF. The reduction of metal translocation in response to high metal concentrations in soil is known as one of the metal tolerance mechanisms (Van Oosten and Maggio 2015). For *S. australis*, a global positive effect of metal addition on metal accumulation in shoots was detected by the factorial ANOVA, but it is relatively weak. Song and Sun (2014) reported that metal accumulation in plant organs of *Suaeda salsa* depends on the considered metal and its concentrations in the substrate. Alhdad et al. (2015) showed that increasing the metal concentrations that increasing Mn concentrations in the growth medium of *Suaeda maritima* (L.) Dumort. influenced the accumulation in shoots and roots differently according to the metal species. They found that increasing Mn concentrations in the growth medium enhanced Mn concentrations in shoots in the hypoxic conditions more than in normal aeration, which agrees with our results, particularly for Cr in *S. australis*.

4.5. Phytoremediation potential of S. australis and S. portulacastrum

As for most halophytes, TF was lower than 1 in both species, indicating that they accumulated metals preferentially in the roots rather than in their aerial parts. TF values of *S. portulacastrum* were higher than *S. australis* in pots non-added with metals. However, S. australis concentrations in the shoots and roots remained higher than in *S. portulacastrum*. Considering all treatments in this present work, the total concentrations of the four metals in *S. australis* ~~in the roots~~ ranged from 366.5 to 495.3 mg/kg DW in the roots~~in *S. australis*~~, and from 497.3 to 669.0 mg/kg DW in the whole plant. ~~whereas~~ I~~i~~n *S. portulacastrum*, average values ranged from 154.9 to 292.3 mg/kg DW in the roots, and from 263.0 to 390.6 mg/kg DW in the whole plant. Similarly to us, Samundeeswari and Lakshmi (2018) compared the Pb, Cr, Cd, Cu, and Zn phytoremediation capacities of *S. portulacastrum* to another *Suaeda* species, treated with various metal concentrations in open-air conditions. The study also concluded a better accumulation in the *Suaeda* species. However, unlike our study, *S. maritima* and *S. portulacastrum* generally accumulated more metals in the shoots than in the roots. Kaewtubtin et al. (2016) studied the heavy metal phytoremediation potential of 18 species associated with mangroves by analyzing samples of soils and plants taken in their natural environment. *S. portulacastrum* accumulated more Ni than 15 of the other species (30.6 mg/kg in the roots and 32.8 in the leaves). These concentrations, clearly lower than ours, cannot be compared with our results because the quantities of extractable Ni are lower in the mangrove sediments. Indeed, BCF values (10.6 for the roots) were a little higher than ours. *S. portulacastrum* also fixed Mn and Cr but with lower performances. In another study (Kaewtubtin et al. 2018), the authors analyzed the metal bioaccumulation potential of *Avicennia marina* (tree) and *Pluchea indica* (shrub) in the field and in the greenhouse. The amounts of Mn, Ni, and Cr taken together accumulated in six months were similar to those of our experiment (BCF values were variable but not different on average than ours). They considered these species as promising for metal phytoremediation.

Several *Suaeda* species have been studied for their metal phytoremediation potential. Li et al. (2006) showed that *S. australis* had different bioaccumulation capacities for Cu, Zn, Cd, Pb, Cr and Hg. Metal concentrations in roots were positively correlated to their bioavailable fractions and depended on the chemical form of the metals. Conversely, in the present study, the DTPA-extractable fractions in soil were similar among treatments and species. Ayyappan and Ravindran (2014) studied plants of *Suaeda monoica* treated for 6 months with a paper mill effluent containing 142.4 mg/L of Cr. At the end of the experiment, the hole of the plant accumulated 40.89 mg/kg of Cr, while in this work, *S. australis* accumulated up to 11.22 mg/kg Cr in the shoots and up to 88.35 mg/kg Cr in the roots.

Concerning *S. portulacastrum*, the maximal mean values for Cr concentrations were 21.3 mg/kg in shoots and 67.0 mg/kg in roots; for Ni, it was respectively 27.7 mg/kg and 112.1 mg/kg DW for shoots and roots. This halophyte is considered a good phytoremediator for several metals in numerous studies. However, most of these studies were conducted in hydroponics, where plants can freely absorb metal ions in solutions. For example, Fourati et al. (2016) tested *S. portulacastrum* in hydroponic conditions and measured 1050 mg/kg shoot DW of Ni. In soil, the absorption of metal ions by plants can be limited by their adsorption on clay and humic particles, influencing their bioavailability. Moreover, polluted soils are generally characterized by multi-metal contamination, which can reduce the accumulation capacities of one metal species compared with controlled experiments focusing on one or two metals. To assess the phytoremediation potential of plants, BCF or BAF values higher than 1 are considered a reliable criterion (Shmaefsky 2020). In our study, *S. portulacastrum* showed BAF values of total metals ranging from 3.5 to 4.9. For *S. australis*, these values ranged from 7.2 to 12.9. However, definitions and calculations vary from one study to another (Subhashini and Swamy 2014; Mujeeb et al. 2020) or need to be more precisely calculated (Lokhande et al. 2011). This leads to confusion and misinterpretation in data comparison, sometimes with a wide variation of values (Buscaroli, 2017). However, BAF and BCF traduce the balance of metal concentrations between plants and soil. In our case, Total metals mean concentrations range from 366.5 to 495.4 mg/kg for *S. australis* and from 154,9 to 292.4 mg/kg compared with the mixed soil, in which the mean concentration was 42,211 mg/kg (13,027 mg/kg in the non-mixed soil). Thus, both species concentrated metals more than 2-fold the concentrations in the soil, with high accumulation in *S. australis*.

The phytoremediation potential of the plants also needs to consider their biomass productivity. As halophytes, the two plants studied are perennial herbaceous species. The total maximal amount of the four metals accumulated per plant in 6 months was 1458.4 mg for *S. australis* and 1191.3 for *S. portulacastrum*. A field experiment is now necessary to better determine the potential of these accumulated metals. This will allow us to know the biomass productivity per ha and, consequently, the amounts of metals accumulated per ha.

5. Conclusions

In conclusion, the growth of *S. australis* and *S. portulacastrum* differed depending on irrigation frequency and metal concentration levels. *S. portulacastrum*, preferentially accumulated metals in the roots, protecting its aerial parts under the most stressful conditions, despite a higher translocation capacity than *S. australis*. *S. australis* accumulates and stores metals in higher concentrations than *S. portulacastrum*, to the detriment of its growth. *S. australis* had then greater bioaccumulation capacities than *S. portulacastrum* and could be a better candidate for the phytostabilization of metals. If we base our interpretation on the ability of plants to extract metals at a BCF greater than 1, as has been done in numerous publications, the two species accumulate ~~more than~~ enough metals ~~in this experiment~~ to be considered relevant candidates for the phytoremediation of metals in saline soils~~, but~~. However, field experiments are needed to determine their potential in relation to their biomass productivity in natural conditions. Moreover, both species accumulated metals in a small period in natural soil, reinforcing the confidence in their potential to remediate *in-situ* metal-contaminated soils from estuaries of New Caledonia. Further investigation should thus bring new information on how to improve their phytoremediation abilities in new Caledonian salt marsh soils in combination with other techniques, such as the addition of microbial inoculants or chelating agents.

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Figure captions:

Figure 1. Sampling site of contaminated soil for the pot experiment located in the estuary of the Baie de St Vincent, La Tontouta. (a) Site location to the active mine upstream; (b) Tidal flat where soil was collected, colonized by *Suaeda australis*, *Sarcocornia quinqueflora* and *Sesuvium portulacastrum;* (c) Visible salt crust appearing at the soil surface near *S. portulacastrum* plants; (d) *S. portulacastrum* plants.

Figure 2. Effect of irrigation frequency and metal concentration level on the aerial relative growth (cm^2) in each species, estimated *via* image computation. Boxplot middle bars represent medians while dark dots are mean values. Pairwise comparisons with $p<0.05$ are represented by different letters for each plant species separately.

Figure 3. Effect of irrigation frequency and metal concentration level on plant growth. Boxplot middle bars represent medians while dark dots are mean values. Pairwise comparisons with $p<0.05$ are represented by different letters for each plant species separately.

Figure 5. Effect of irrigation frequency and metal concentration levels on metals' translocation factors in *S. portulacastrum* and *S. australis*. Boxplot middle bars represent medians while dark dots are mean values. Pairwise comparisons with $p<0.05$ are represented by different letters.

Figure 6. Effect of treatments on final DTPA-extractable concentrations of Ni, Co, Cr and Mn in soil in the pots of *S. portulacastrum* and *S. australis* (mg/kg). Pairwise comparisons

with $p<0.05$ are represented by different letters. Boxplot middle bars represent medians while dark dots are mean values.

Table captions :

Table 1. Chemical characteristics of the contaminated soil used in the pot experiment

Table 2. Comparison of metal accumulation in shoots and roots of *S. australis* and *S. portulacastrum* under two irrigation frequency levels and two metal concentration levels. Concentrations are expressed in mg/kg DW (means and standard deviations). Pairwise comparisons with $p<0.05$ are represented by different letters based on results from one-tailed post-hoc Conover-Iman tests. A = low metal concentration level, high irrigation frequency; B = low metal concentration level, low irrigation frequency; C = high metal concentration level, high irrigation frequency; D = high metal concentration level, low irrigation frequency.

Table 3. Statistics and p-values of the effects of treatments on metal accumulation in the shoots and the roots of *S. australis*, on the basis of factorial ANOVA or the non-parametric Kruskal-Wallis test and cross comparisons using Tukey'HSD or Conov-Iman test.

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Table 5. Comparisons of BCF and BAF of *S. australis* and *S. portulacastrum* under two irrigation frequency levels and two metal concentration levels. Results are means ±

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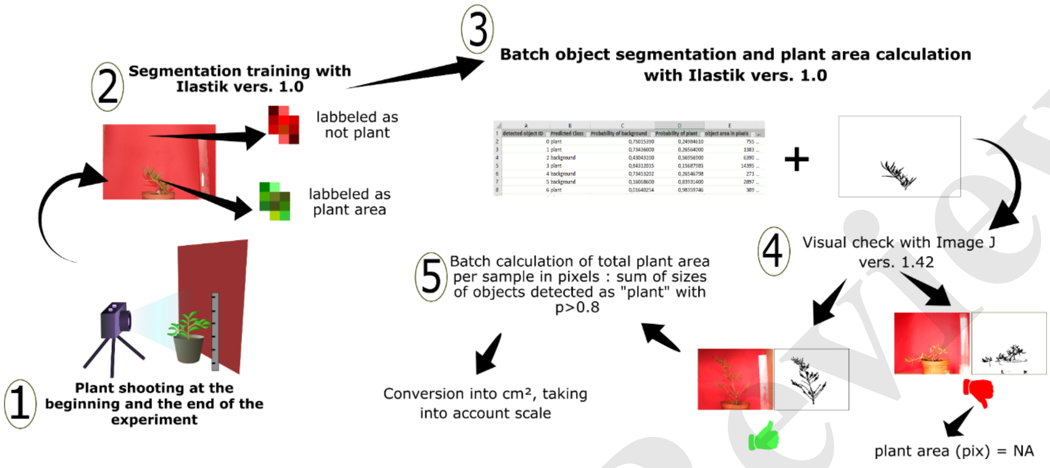
Appendix Supplementary data :

Figure S1. Non-destructive method used to determine plant shoots area at the beginning and the end of the experiment using the image segmentation tool Ilastik vers. 1.0. 1. Images of the initial and final state of the aerial part of plants. 2. A sub-sample of images is used to train the segmentation tool to distinguish plant from background and pot area. 3. Batch object segmentation and plant area calculation are processed on all images. The output contains the segmentation file and a csv file with the size of every detected object in each image. 4. Segmentation files are converted into png format in Image J and are compared with their respective original picture to detected any wrong results. 5. Csv files are combined and total plant area is calculated for each sample. This value is then converted into cm ² with respective scale of each image.

Figure S2. Aerial parts of *S. portulacastrum* and *S. australis* at the end of the experiment.

Table S1. Effect of treatments on final pH and electrical conductivity (EC) in pots and comparison between *S. portulacastrum* and *S. australis*. Presented values are means ± SD. Pairwise comparisons with $p<0.05$ are represented by different letters based on results from one-tailed post-hoc Conover-Iman tests. A = low metal concentration level, high irrigation frequency; B = low metal concentration level, low irrigation frequency; C = high metal concentration level, high irrigation frequency; D = high metal concentration level, low irrigation frequency.

Appendix Supplementary data:



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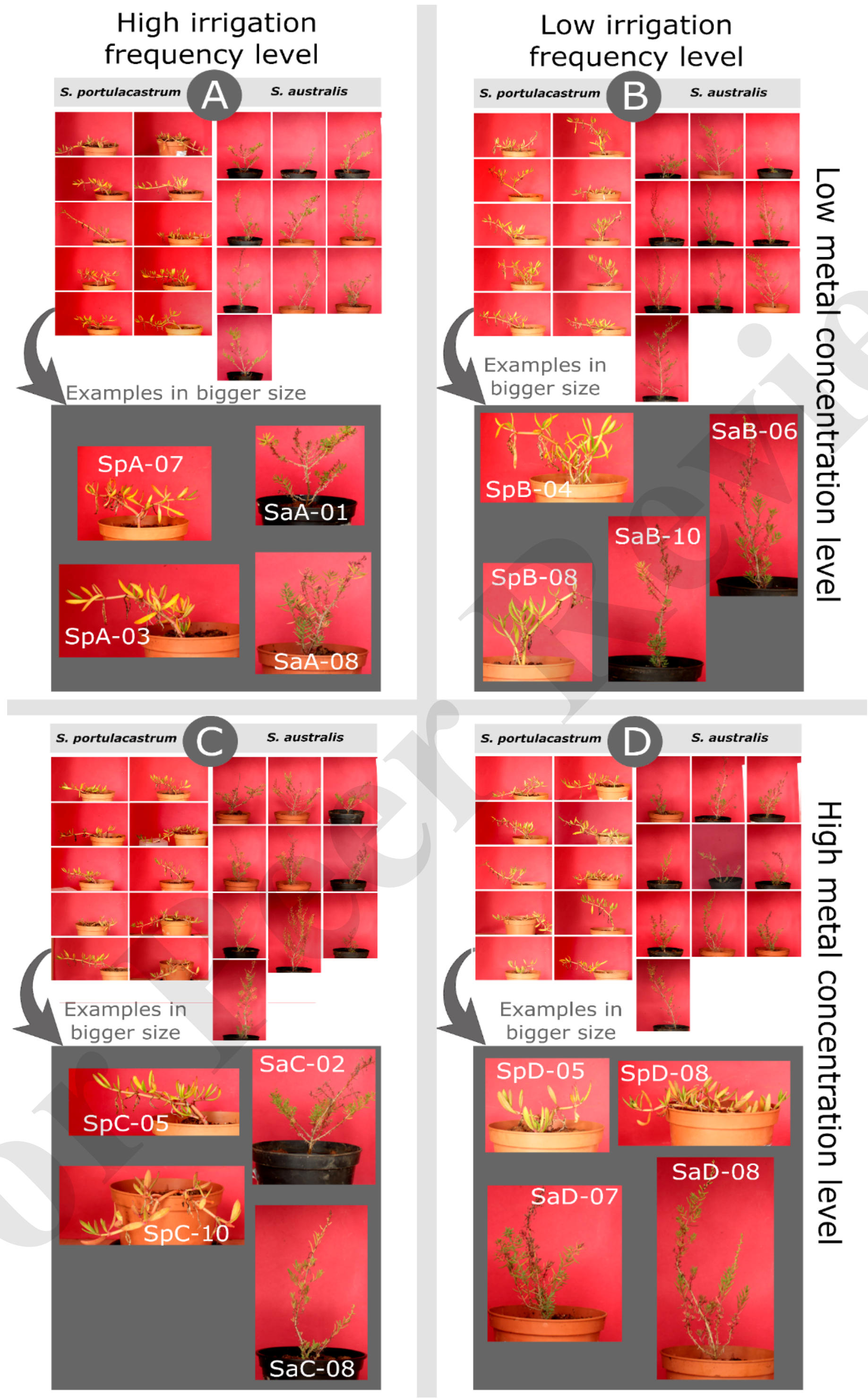
High irrigation frequency level
Low irrigation frequency level
S. portulacastrum
A
S. australis
S. portulacastrum
B
S. australis
Low metal concentration level
Examples in bigger size
SpA-07
SaA-01
SpA-03
SaA-08
Examples in bigger size
SpB-04
SaB-06
SaB-10
SpB-08
S. portulacastrum
C
S. australis
S. portulacastrum
D
S. australis
High metal concentration level
Examples in bigger size
SpC-05
SaC-02
SpC-10
SaC-08
Examples in bigger size
SpD-05
SpD-08
SaD-07
SaD-08

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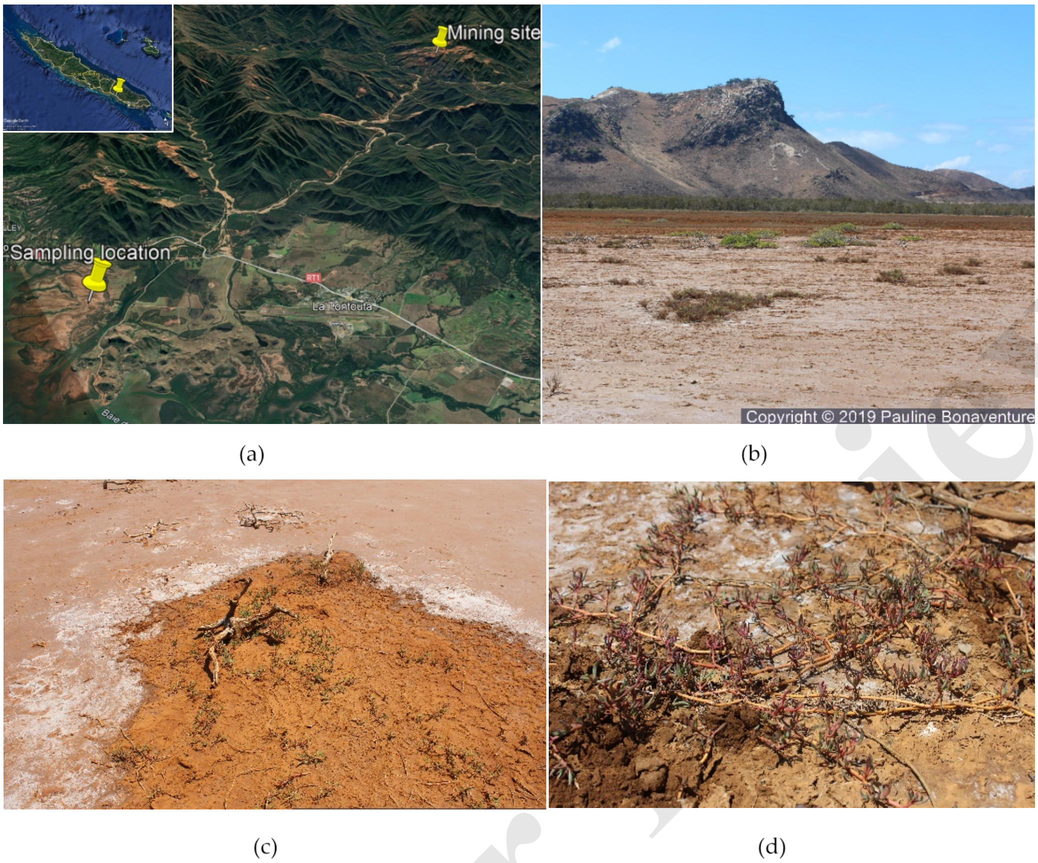
	S. australis				***S. portulacastrum***			
	A	**B**	**C**	**D**	**A**	**B**	**C**	**D**
pH	8,55 ± 0,20 [a]	8,20 ± 0,42 [a]	8,30 ± 0,30 [a]	8,13 ± 0,30 [a]	8,42 ± 0,22 [a]	8,30 ± 0,33 [a]	8,24 ± 0,40 [a]	8,28 ± 0,27 [a]
EC (dS/m)	1,612 ± 1,600 [a]	5,479 ± 4,905 [a]	3,279 ± 3,237 [a]	4,766 ± 5,222 [a]	0,997 ± 0,519 [a]	3,571 ± 4,986 [a]	1,623 ± 1,325 [a]	2,123 ± 3,737 [a]



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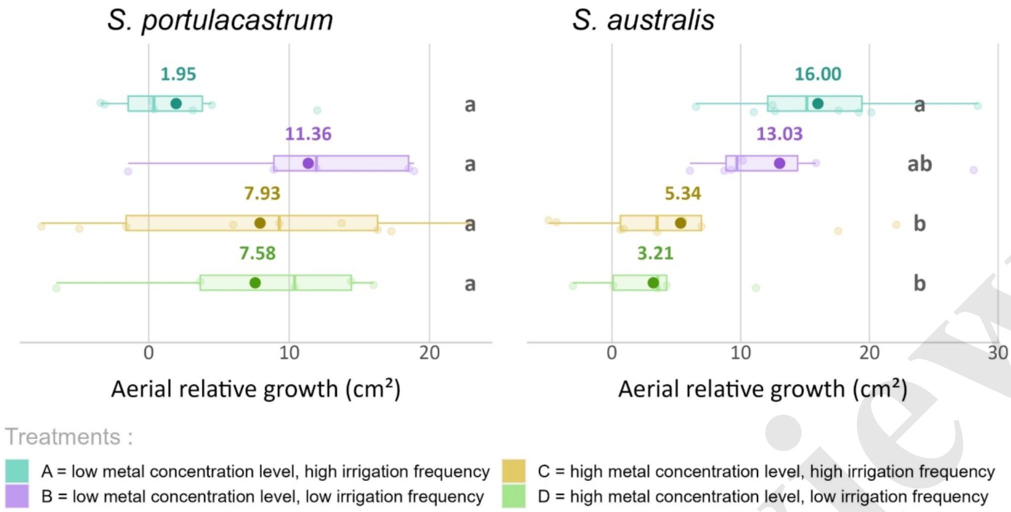


Figure 2. Effect of irrigation frequency and metal concentration level on the aerial relative growth (cm²) in each species, estimated *via* image computation. Boxplot middle bars represent medians while dark dots are mean values. Pairwise comparisons with $p<0.05$ are represented by different letters for each plant species separately.

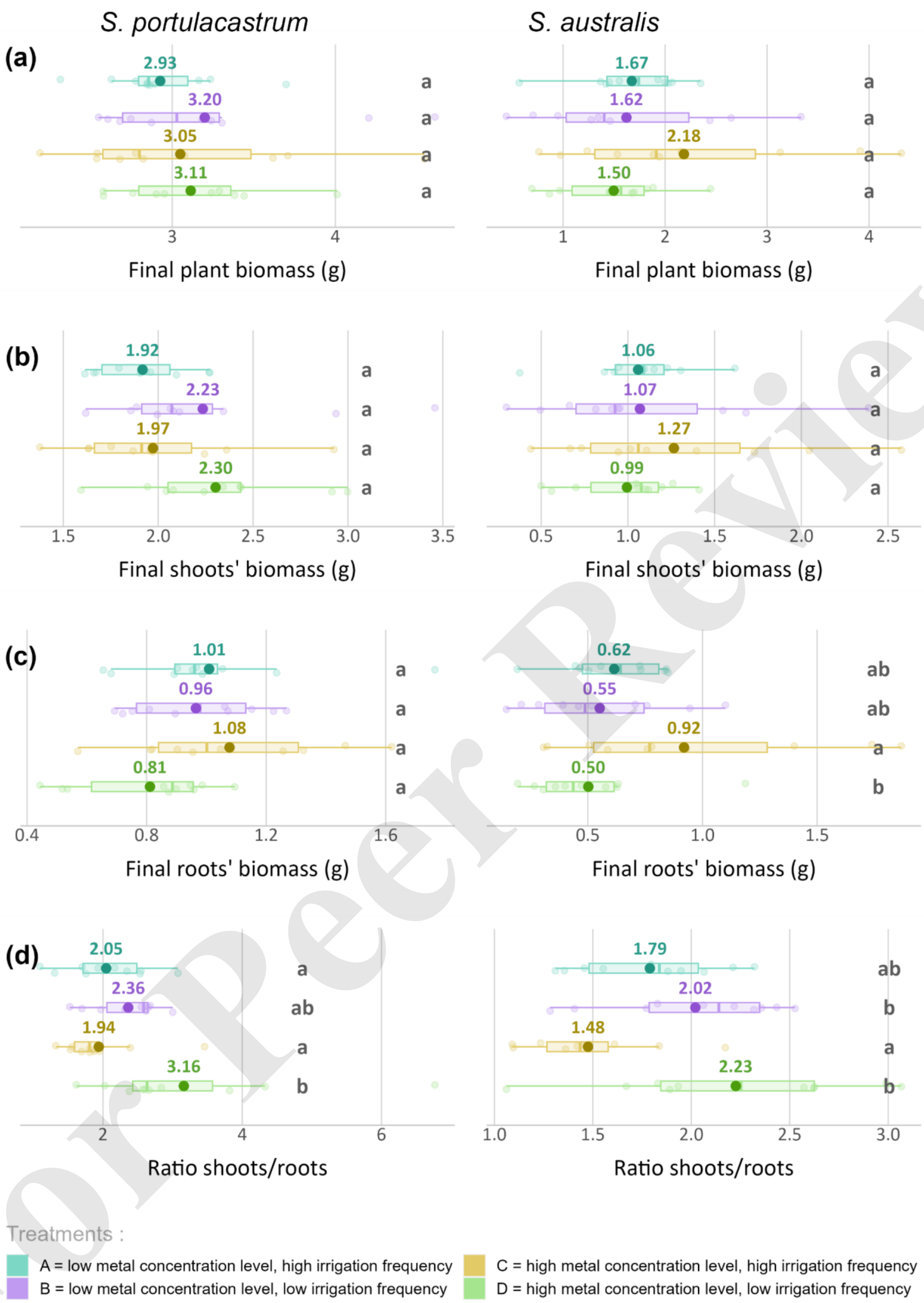


Figure 3. Effect of irrigation frequency and metal concentration level on plant growth (total dry weight). Boxplot middle bars represent medians while dark dots are mean values. Pairwise comparisons with p<0.05 are represented by different letters for each plant species separately.

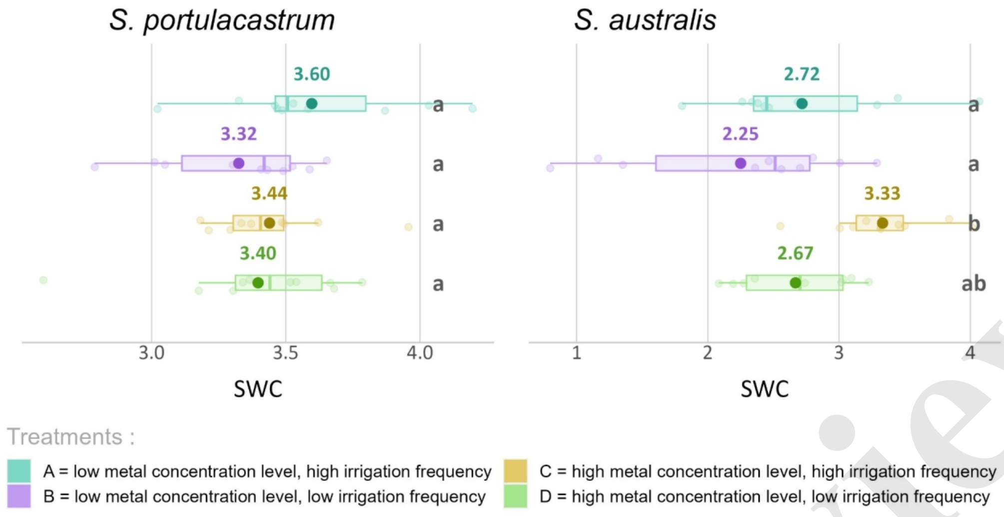
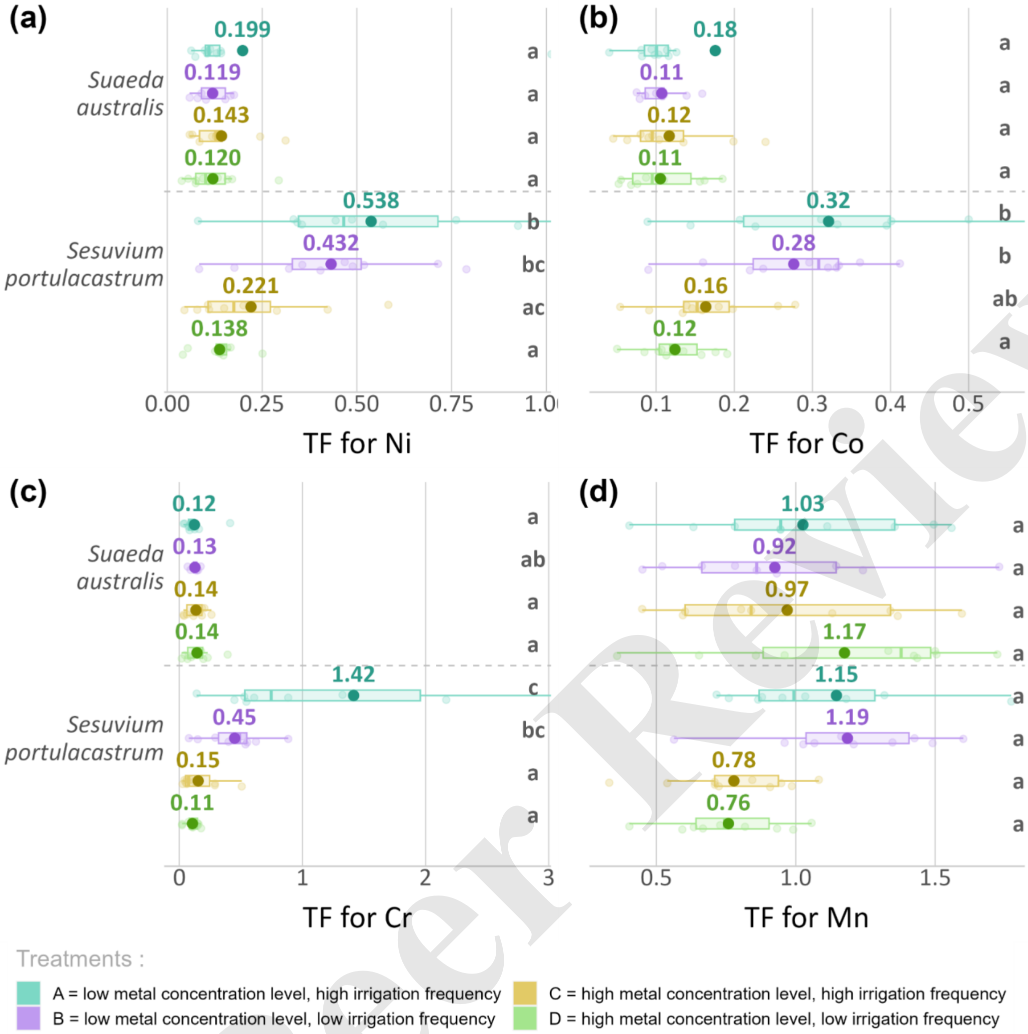


Figure 4. Effect of irrigation frequency and metal concentration level on plant SWC. Boxplot middle bars represent medians while dark dots are mean values. Pairwise comparisons with $p<0.05$ are represented by different letters based on results from a one-tailed Tukey's HSD test for each plant species separately.



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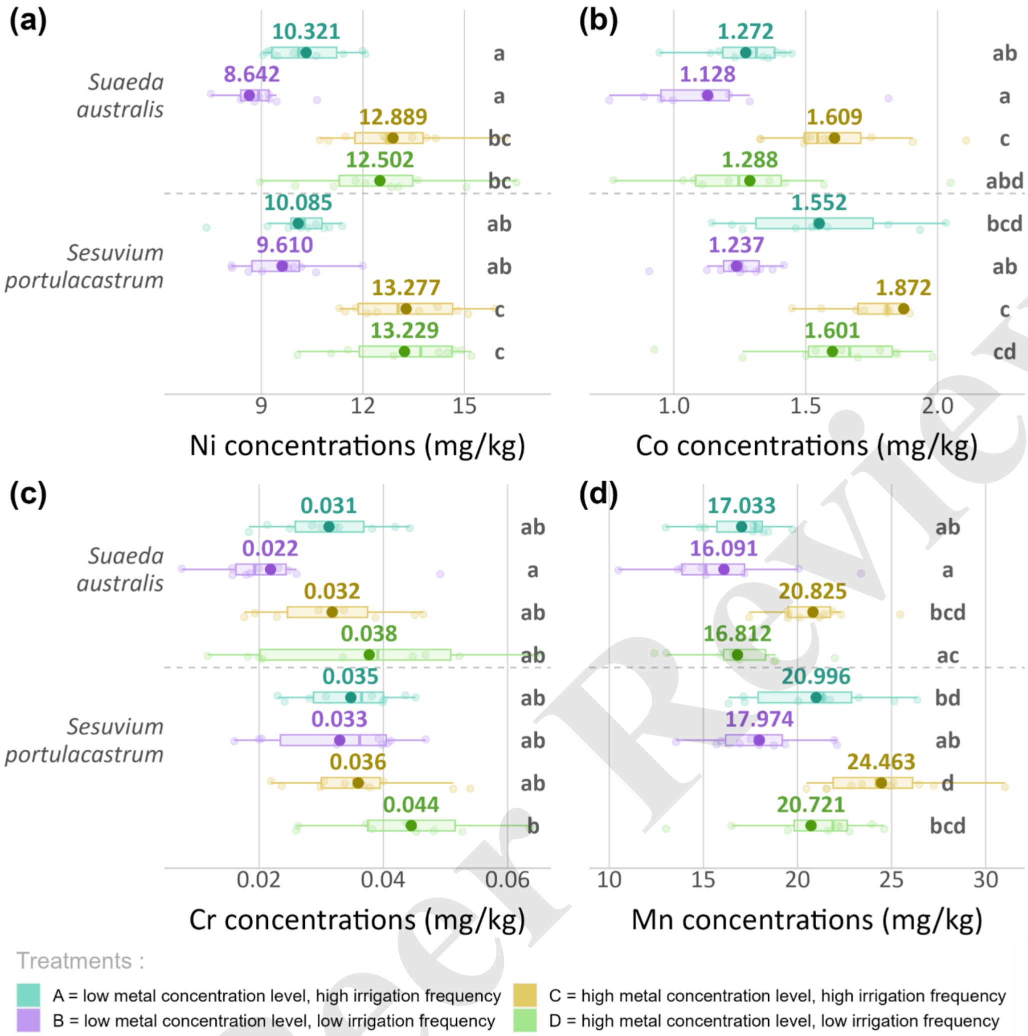

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Table 1. Chemical characteristics of the contaminated soil used in the pot experiment

Chemical characteristics	***Means and standard deviations***		
CaCO3%	10,86	±	0,02
Total N (mg/g)	0,51	±	0,04
Total Organic Carbon (mg/g)	9,68	±	0,59
Exchangeable P mg/kg	0,26	±	0,11
Cl (méq%)	72,38	±	11,67
Ca (méq%)	7,38	±	0,55
Mg (méq%)	16,97	±	0,25
Na (méq%)	69,20	±	10,14
K (méq%)	3,03	±	0,34
Cation exchange capacity (meq%)	33,12	±	3,09
Total Ca (mg/kg)	19960,70	±	1080,10
Total Mg (mg/kg)	161675,45	±	1140,73
Total Na (mg/kg)	8731,90	±	1055,81
Total K (mg/kg)	1562,88	±	138,91
Total P (mg/kg)	129,74	±	14,52
Total S (mg/kg)	1312,31	±	42,93
Total Mn (mg/kg)	1758,65	±	91,77
Total Co (mg/kg)	216,89	±	22,23
Total Cr (mg/kg)	8750,05	±	328,30
Total Ni (mg/kg)	3819,24	±	367,31
Total Fe (mg/kg)	113377,45	±	4809,75
Fe DTPA (mg/kg)	36,51	±	2,50
Ni DTPA (mg/kg)	4,71	±	0,33
Co DTPA (mg/kg)	0,27	±	0,04
Cr DTPA (mg/kg)	0,033	±	0,006
Mn DTPA (mg/kg)	8,00	±	0,67
Total metals (without Fe) DTPA (mg/kg)	13,02	±	1,04
pH	7,22	±	0,12
EC (dS/m)	22,78	±	5,73

Table 2. Comparison of metal accumulation in shoots and roots of *S. australis* and *S. portulacastrum* under two irrigation frequency levels and two metal concentration levels. Concentrations are expressed in mg/kg DW (means and standard deviations). Pairwise comparisons with $p<0.05$ are represented by different letters based on results from one-tailed post-hoc Conover-Iman tests. A = low metal concentration level, high irrigation frequency; B = low metal concentration level, low irrigation frequency; C = high metal concentration level, high irrigation frequency; D = high metal concentration level, low irrigation frequency.

	S. australis				***S. portulacastrum***			
	A	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Ni								
Shoots	18.8 ± 5.5ab	27.0 ± 11.1ab	31.7 ± 14.0a	20.2 ± 8,9ab	27.7 ± 17;9ab	16.8 ± 4.3bc	16.0 ± 8.7bc	8.8 ± 4.5c
Roots	156.8 ± 66.9ab	235.5 ± 110.0a	245.8 ± 53.0a	200.6 ± 94,9a	64.0 ± 48.5c	55.4 ± 44.6c	112.1 ± 111.4bc	72.6 ± 43.9bc
Co								
Shoots	1.5 ± 0.3ab	2.1 ± 0.6a	2.6 ± 1.1a	2.1 ± 0.5a	1.8 ± 0.9ab	1.2 ± 0.2bc	1.2 ± 0.4bc	1.0 ± 0.4c
Roots	15.9 ± 9.5ab	19.4 ± 5.9a	23.6 ± 5.7a	22.3 ± 7.6a	6.1 ± 2.5c	5.1 ± 2.8c	8.9 ± 5.9bc	8.3 ± 2.4bc
Cr								
Shoots	5.1 ± 1.5ab	11.2 ± 4.8c	8.9 ± 4.8ac	5.5 ± 3.4ab	21.3 ± 14.0c	5.9 ± 1.6ac	7.0 ± 7.5ab	2.6 ± 1.6b
Roots	59.0 ± 31.8ab	88.3 ± 39.7a	78.5 ± 30.2a	57.1 ± 51.2abcd	27.5 ± 28.2cd	19.5 ± 16.3c	67.0 ± 56.3abd	32.5 ± 25.5bcd
Mn								
Shoots	105.4 ± 22.5ab	110.8 ± 16.1a	130.4 ± 29.0a	128.2 ± 30.6a	88.3 ± 17.5bc	84.1 ± 11.3bc	74.0 ± 13.3c	72.4 ± 11.5c
Roots	114.3 ± 45.1abc	136.5 ± 65.8ab	147.5 ± 46.4a	128.8 ± 62.5ab	82.2 ± 21.0bc	74.9 ± 19.4c	104.4 ± 48.6abc	100.1 ± 23.9abc
Total metals[1]								
Shoots	130.8 ± 23.5ab	151.0 ± 28.2a	173.6 ± 43.9a	155.9 ± 38.2a	139.1 ± 34.8ab	108.1 ± 13.9bc	98.2 ± 21.6c	84.8 ± 14.8c
Roots	366.5 ± 109.0ab	479.7 ± 211.6a	495.4 ± 106.0a	408.7 ± 203.8ab	179.9 ± 92.8c	154.9 ± 81.2c	292.4 ± 219.2bc	213.5 ± 91.7c
Total metals/plant	830.5 ± 224.3b	1021.7 ± 322.1ab	1458.4 ± 356.6a	846.9 ± 195.5b	934.7 ± 207.4ab	841.6 ± 197.7b	1191.3 ± 389.2ab	927.7 ± 237.4ab

[1] Total metals is the sum of the 4 metal concentrations.

Table 3. Statistics and p-values of the effects of treatments on metal accumulation in the shoots and the roots of *S. australis,* on the basis of factorial ANOVA or the non-parametric Kruskal-Wallis test and cross comparisons using Tukey'HSD or Conov-Iman test.

Shoots		**Ni**		**Co**		**Cr**			**Mn**		**Total metals**	
ANOVA		**F**	**p**	**F**	**p**	**F**	**p**		**F**	**p**	**F**	**p**
Humidity		0.26	0.61	0.37	0.54	0.75	0.39		0.03	0.85	0.01	0.90
Metals		0.56	0.45	5.75	0.02 *	0.83	0.36		6.77	0.01 *	4.61	0.03 *
Humidity x Metals		10.01	0.00 **	4.76	0.03 *	17.17	0.00 ***		0.22	0.64	2.93	0.09
Tukey												
Groups		**Diff**	**p**	**Diff**	**p**	**Diff**	**p**	**Groups**	**Diff**	**p**	**Diff**	**p**
A	B	0.32	0.15	0.27	0.13	0.73	0.00 **	High - Low metal conc. level	21.31	0.00 **	24.33	0.01 *
	C	0.47	0.02 *	0.44	0.00 **	0.47	0.06					
	D	0.03	0.91	0.29	0.08	-0.00	0.95					
B	C	0.15	0.59	0.17	0.39	-0.26	0.40					
	D	-0.29	0.21	0.02	0.92	-0.74	0.00 **					
C	D	-0.45	0.03 *	-0.15360	0.99	-0.48	0.05					

Roots	**Co**		**Mn**		**Total metals**	
ANOVA	**F**	**p**	**F**	**p**	**F**	**p**
Humidity	0.20	0.65	0.036	0.85	0.06	0.81
Metals	5.00	0.03 *	0.64	0.43	0.28	0.59
Humidity x Metals	1.05	0.31	1.57	0.21	3.34	0.07
Tukey						
Groups	**Diff**	**p**				
High metal conc. level - Low metal conc. level	5.22	0.01 *				

	Ni		**Cr**	
Kruskal-Wallis	**Chi**	**p**	**Chi**	**p**
	6.98	0.07	6.97	0.07

[1] Total metals is the sum of the 4 metal concentrations.
* Significance at $p < 0.05$; **significance at $p < 0.01$; ***significance at $p < 0.001$.

Table 4. Statistics and p-values of effects of treatments on metal accumulation in the shoots and the roots of *S. portulacastrum,* on the basis of factorial ANOVA or the non-parametric Kruskal-Wallis test and cross comparisons using Tukey'HSD or Conover-Iman test.

Shoots	**Co (1/x)²**		**Cr**		**Mn**		**Total metals**	
ANOVA	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**
Humidity	3.54	0.06	19.15	0.00 ***	0.44	0.51	7.24	0.01 *
Metals	5.38	0.02 *	25.44	0.00 ***	9.07	0.00 **	19.08	0.00 ***
Humidity x Metals	0.13	0.71	0.33	0.56	0.09	0.76	0.47	0.49
Tukey **Groups**	**Diff**	**p**	**Diff**	**p**	**Diff**	**p**	**Diff**	**p**
High - Low metal conc. Level	0.22	0.01 *	-1.04	>0.00***	-12.99	0.00 **	-0.29	0.00 ***
Low - high irrigation level			-0.90	0.00 ***			-0.18	0.00 **

		Ni	
Kruskal-Wallis		**Chi**	**p**
		14.06	0.00 **
Conover-Iman **Groups (j-i)**		**Diff**	**p**
A (i)	B (j)	-3.70	1.00
	C (j)	-6.60	0.41
	D (j)	-18.50	0.00 ***
B (i)	C (j)	-2.90	1.00
	D (j)	-14.80	0.00 **
C (i)	D (j)	-11.90	0.02 *

Roots	**Ni**		**Co (1/x)²**		**Cr**		**Total metals (1/x)**	
ANOVA	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**
Humidity	0.83	0.36	0.86	0.36	2.37	0.13	1.06	0.31
Metals	4.53	0.04 *	15.14	0.00 ***	9.73	0.00 **	9.86	0.00 **
Humidity x Metals	0.09	0.76	1.58	0.21	1.09	0.30	0.00	0.91
Tukey **Groups**	**Diff**	**p**	**Diff**	**p**	**Diff**	**p**	**Diff**	**p**
High - Low metal conc. level	0.44	0.01 *	-0.08	0.00 *	0.79	0.00 **	-0.00	0.00 **

		Mn	
Kruskal-Wallis		**Chi**	**p**
		11.12	0.01 *
Conover-Iman **Groups (j-i)**		**Diff**	**p**
A (i)	B (j)	-5.00	0.85
	C (j)	9.00	0.17
	D (j)	9.60	0.13
B (i)	C (j)	14.00	0.01 *
	D (j)	14.60	0.00 **
C (i)	D (j)	0.60	1.00

[1] Total metals is the sum of the 4 metal concentrations.
[2] As data presented a severe positive skew, Co data are 1/x-transformed to normalize the data distribution.
* Significance at $p < 0.05$; **significance at $p < 0.01$; ***significance at $p < 0.001$.

Commented [PB1]: En me relisant, c ela me semblait bizarre que ça ne soit pas une fonction 1/x pour « total metals » vu qu'on a des concentrations plus élevées dans le niveau « high » pour chaque métal. J'ai vérifié et en effet j'avais oublié de le mentionner.

Table 5. Comparisons of BCF and BAF of *S. australis* and *S. portulacastrum* under two irrigation frequency levels and two metal concentration levels. Results are means ± standard deviations. Pairwise comparisons with $p<0.05$ are represented by different letters based on results from one-tailed post-hoc Conover-Iman tests. A = low metal concentration level, high irrigation frequency; B = low metal concentration level, low irrigation frequency; C = high metal concentration level, high irrigation frequency; D = high metal concentration level, low irrigation frequency.

	S. australis				***S. portulacastrum***			
	A	**B**	**C**	**D**	**A**	**B**	**C**	**D**
BCF	11.6 ± 4.6abc	19.1 ± 10.3a	16.7 ± 8.6a	13.5 ± 6.6ab	5.8 ± 3.6d	5.5 ± 3.1d	7.6 ± 5.9bcd	6.2 ± 3.4cd
BAF	7,2 ± 1,6a	12,9 ± 8,3a	9,8 ± 3,6a	8,1 ± 3,3a	4,9 ± 1,9b	4,3 ± 0,9b	4,4 ± 2,5b	3,5 ± 1,1b