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Comparative Assessment of Mono-Cropping and Co-Cropping of Three Phytoremediation Halophytes in Mitigating Polymetallic Mining Pollution in M'Dhilla Area

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Abstract Soil pollution emerging from mining activities is a worldwide environmental concern. Finding biological solutions to this problem is of paramount importance in reducing metal toxicity and limiting its spread. In this context, halophytes-assisted phytoremediation has been widely considered as a promising alternative to remediate both salt-affected and heavy metal-contaminated soils. In this study, three halophytes *Sesuvium portulacastrum*, *Carpobrotus edulis* and *Mesembryanthemum cordifolium* have been evaluated for their metal phytoextraction potential performed on a mining soil collected from the mining phosphate basin of Gafsa. A 7-month greenhouse pot experiment was conducted under two cultivation systems: mono-cropping and co-cropping. The effect of co-cropping on the

phytoextraction efficiency was highlighted and the associated phytoremediation indexes were assessed. Results showed that the crude collected soil revealed strong Cd and Sr contamination, moderate Cr and Ni levels, and low Cu and Zn concentrations. Compared to mono-cropping, co-cropping enhanced Cd uptake efficiency achieving a ~40% reduction in initial soil Cd level. For both cropping systems, the extraction efficiencies of the other metals were less than 1% of their initial values. Fe and Mn levels remained nearly unchanged across cultivation systems. The decline in pollution indices and metal concentrations confirmed the phytoremediation potential of the investigated species in co-cropping. This approach shows promise for in-situ remediation of mining-affected soils.

Keywords Halophytes · Co-cropping · Phytoextraction · Polymetallic soil pollution · Mining area

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1 Introduction

Soil contamination by heavy metals has become one of the major environmental problems worldwide. Anthropogenic activities such as mining and industrial processing are the most important sources of heavy metals entering the environment (Afonso et al., 2022). Natural phosphates contain many metallic elements, some of them could negatively affect plants and human (Çiner et al., 2024; Mabrouk et al.,

2020). In Tunisia, phosphate industry plays a major role in the country's development and its integration into the world economy (Hamed et al., 2022). Despite its strategic importance for economy, mining and ore-processing activities become a critical problem due to the release of heavy metals and dust inducing serious health issues in Gafsa region (Khelifi et al., 2020). The Company of Phosphates of Gafsa (CPG), operates seven open-pit quarries and one underground mine scattering around the mining basin of Gafsa (Mekki & Sayadi, 2017). After being restricted to phosphate rock production and exportation during the first fifty years, CPG upgraded its activity to phosphoric acid and mineral fertilizers production to reach the processing of more than 80% of the national need to phosphate (Mekki et al., 2017). Inevitably, the enrichment of phosphates by a humid process is accompanied with the reject of mud poured in the hydrographic network of the mining basin region (Bezzeghoud et al., 2024). These releases are rich in phosphate and trace elements (Cd, Zn, Cr, Cu, Ni and Sr) (Galfati et al., 2011; Khelifi et al., 2021). Moreover, water used in phosphate processing was evacuated wildly in nature, which results in harmful consequences on the receiving medium (Sharifi & Safari Sinegani, 2012). Soils adjoining the mining rejects demonstrated total concentrations in Cd, Zn and Cr higher than the maximum tolerated concentrations (Galfati et al., 2011; Zaghdoudi et al., 2025). Overall, Gafsa mining district is exposed to water, soil and air pollution resulting from extraction, treatment, and transport of phosphates. Further constraints facing the region is soil and water salinization caused by aridity, irregular rainfall and over-exploitation of water reserves (Chenchouni et al., 2024). Previously, Hamed and co-workers reported a deteriorated water quality (salinity higher than 5 g/L) in the mining basin of Gafsa (Hamed et al., 2022). Thus, over-exploitation of groundwater to wash phosphate caused water-level decline in the main aquifers, loss of artesian condition as well as water and soil quality degradations (Mokadem et al., 2018). The immense quantity of metals generated from mine wastes makes the cleaning up and rehabilitation of these contaminated soils a fundamental challenge with urgent necessity (Martins et al., 2022; Zerizghi et al., 2022). Although it is difficult to entirely degrade heavy metals from ecosystems, many conventional approaches including physicochemical methods are acknowledged

to reduce their spreading in such ecosystems (Chen et al., 2000; Dhaliwal et al., 2020; C. Li et al., 2019). However, these techniques have many limits (Roy-Chowdhury et al., 2018). In fact, despite their high efficiency to extremely contaminated sites, they are only applicable for small areas (Lambert et al., 2000; Sharma et al., 2018). Moreover, these methods, which need high energy and harmful chemicals, are expensive and non-environmental friendly (Sharma et al., 2018). Based on the previously reported limits of the physicochemical approaches and in line with sustainable development, nature-based solutions using biological tools notably plant-assisted metal remediation referred to as phytoremediation, offer an eco-friendly and low-cost solution to remove metals or at least limit their spread in the environment. Phytoremediation is an in-situ process that uses plants to degrade, extract, or immobilize contaminants from soil and water (Pirzadah et al., 2015). Several plant species were evaluated based on their capability to extract metals from contaminated soils (Caparrós et al., 2022; Nedjimi, 2021). To be suitable for phytoremediation, plants should fulfil the following requirements: (i) high tolerance to the target pollutants, (ii) elevated root and shoot biomasses (iii), fast growing rate leading to the production of elevated biomass during relatively reduced period, and (iv) high potential to translocate metals from root to the shoot (Siyar et al., 2022). Halophytes, known to be not only tolerant to salt and heavy metals but also to other stresses such as chilling, freezing, heat, and drought comply well with the above requirements (Zhihua Zhang et al., 2025). Besides, many of these plant species can grow on land of marginal quality which support their use for phytoremediation in both low fertility and poor structure soils with a low operating cost (Manousaki & Kalogerakis, 2011; Wiszniewska et al., 2018). The efficiency of halophytes species for phytoextraction or phytostabilization applications in heavy metal polluted environments (Amari et al., 2016; Hajri et al., 2023) notably saline contaminated soils (Bonaventure et al., 2023; C. Y. Li et al., 2023; S. Zhang et al., 2023) has been widely studied. *Aizoaceae* is a very large family of dicotyledonous flowering plants containing 135 genera and about 1900 species endemic to Southern Africa (Bruckmann et al., 2001). Most members of that family are ornamental, annual or perennial and form part of specialist succulent collections (Klak et al., 2007). *Sesuvium portulacastrum*,

Carpobrotus edulis also called *Mesembryanthemum edule* and *Mesembryanthemum cordifolium* (formerly known as *Aptenia cordifolia*) are widely used as ground covers and ornamentals as well as phytoremediators for metal-contaminated saline environments (Navarro-Torre et al., 2023; Singh et al., 2023). However, although specific plant species may have extraordinary tolerance to specific contaminants, the range and diversity of pollutants present in the soil could impede crop establishment or effective phytoremediation (Azizi et al., 2023; Ghafouri et al., 2022). Halophytes are potential sources of salt-tolerant microorganisms with plant growth-enhancing abilities (Bonaventure et al., 2023; Teotia & Chaudhary, 2024). Microorganisms associated with halophytes such as endophytic bacteria and microbes might boost the ability of these plants to remediate saline and polluted soils (Szymańska et al., 2016; Yasseen & Al-Thani, 2022). Inspired by this previous natural effect in halophytes, various bioremediations approaches were suggested to boost phytoremediation process on diversely contaminated soils including the application of phytohormones, microbes, bacteria and co-cropping of different species with potentially complementary functions (Brereton et al., 2020; Mehmood et al., 2021; Singh et al., 2023). This study aims to:

- (i) Investigate the pollution status of a polymetallic mining soil before and after a 7-months duration of phytoremediation experiment.
- (ii) Evaluate the potential of extraction of different metal using three fast-growing halophytes from the *Aizoaceae* family (*Sesuvium portulacastrum* L., *Carpobrotus edulis* L. and *Mesembryanthemum cordifolium* L.) mono or co-cropped.
- (iii) Determine the appropriate combination between species and cropping system for each metal that yield the more effective phytoremediation.

2 Material and Methods

2.1 Soil Sampling and Analysis

The soil used throughout this study was collected from the upper horizon (0–25 cm depth) with a hand auger from M'Dhilla sector close to the second

phosphate laundry (34°17'21"N 8°44'24"E), and near the Tunisian Chemical Group (TCG), on February 2022.

Soil Samples were air-dried and crushed to pass through a 10 mm stainless steel sieve. Soil texture was analyzed by X-ray diffraction (XRD with a Pan-Analytical brand X' PERT PRO type) (Malvern Instrument Ltd) carried out at Georesources Laboratory of Water Research and Technology Centre from Tunisia. The percentage of organic matter (OM) content was determined according to the method described by Brown (Brown 1998). Soil pH and electrical conductivity (EC) were determined at a soil-to-distilled water suspension ratio of 1:5, with a shaking time of 5 min, using glass electrodes (ISO 10390; 2005).

Major cation concentrations were determined by the loss on ignition method at 950 °C of soil samples with addition of lithium metaborate, and lithium tetraborate. Heavy metals concentrations were obtained after an acid attack of soil samples with HNO₃ 68% and HF 40% as previously detailed (Lambrechts et al., 2011). Samples were prepared using ultrapure water with a resistivity of 18 MΩ cm (Merck-Millipore, Germany). Measurements were performed with an inductively plasma emission spectrometry ICP-OES (Thermo Jarrel Ash Iris Advantage) by using Merck ICP standards solutions (quality: traceable to SRM from NIST) with standard concentrations ranging from 0.01 to 0.2 ppm for Cd, Sr, Zn, Cu, Cr, Ni and Pb and from 0.1 to 10 ppm for Fe. SLRS-6 was used as reference material. Soil characterization was analyzed before planting (T0) and after plant harvest (Tf) at the end of each experiment.

2.2 Plant Material and Growth Conditions

The experiment was conducted in a greenhouse under sunlight condition at 23/25 °C in the Centre of Biotechnology of Borj- Cedria (North-East of Tunisia, 36°42'32.9"N, 10°25'40.9"E). Three species belonging to *Aizoaceae* family are used in this study. *Sesuvium portulacastrum* L., *Carpobrotus edulis* L. and *Mesembryanthemum cordifolium* L. seedlings, were obtained by stem cuttings taken from mother plants. Stem segments with one node and two opposite leaves were planted in pots containing distilled water. After 20 days, uniform and rooted cuttings were transferred to 5 L plastic pots filled with 5 kg sieved and homogenized soil pre-described above and collected from

M'Dhilla site. The density of plantation was three plants per pot. Two growing systems were applied in this experiment. For monoculture system, in each pot the three-planted seedlings were issued from the same species. While in co-cropping system, in each pot three different seedlings were planted, each one is representative of one species. For each treatment, we considered 5 distinguished pots (15 plants per treatment). Five pots were filled as previously described, where unplanted are considered as a control. Irrigation took place twice a week with an equal volume of distilled water for a 7-months duration experiment. To minimize nutrient depletion, starting from the third month a 1/5 diluted Hoagland solution was applied once a month. At the harvest, each plant has been divided into roots, stems and leaves. The roots were immediately washed with a 0.01 M HCl solution in order to eliminate all traces of metals adsorbed on the surface, then washed with cold distilled water for three times.

2.3 Plant Element Content

Mineral concentrations were quantified separately in roots and leaves. Samples of dry matter were digested by incubation in 68% HNO₃ at 80 °C in a sand bath. After complete evaporation, minerals were re-dissolved in a mix of 68% HNO₃ and 37% HCl (3.1) and filtered on a Whatman n°1 filter paper. Elements were quantified by ICP-OES inductively plasma emission spectrometry (Thermo Jarrel Ash Iris Advantage). Samples were injected through a Mainhard nebulizer and carried through an argon gas plasma with reflected power of 7 W and forward power of 1300 W. Gas flow rates of plasma, auxiliary, and nebulizer were set at 16.0, 1.0 and 1.0 L.min⁻¹, respectively. The internal standard multi-element stock solution obtained from Agilent (USA) allowed us to control the stability of the instrument, while certified reference material ERM-CA713 was obtained from Sigma-Aldrich (Germany). After determining metals' concentrations in roots, shoots, and soils, the phytoremediation indexes were carried out to evaluate the ability of plants to remediate the contaminated soil.

Metal quantities accumulated in different organs were measured as follows:

The metal quantity in the shoot ($\mu\text{g}\cdot\text{plant}^{-1}$) which represents the product of metal shoot concentration

($\mu\text{gMetal}/\text{g}_{\text{shoot}}\text{DW}$) by the shoot biomass ($\text{g}\cdot\text{plant}^{-1}$) was defined as:

$$Q\text{Metal}_{\text{shoot}} = [\text{Metal}]_{\text{shoot}} (\mu\text{g}\cdot\text{g}^{-1}\text{DW}) \times \text{DW}_{\text{shoot}} (\text{g}\cdot\text{plant}^{-1})$$

The metal quantity in the root ($\mu\text{g}\cdot\text{plant}^{-1}$) that represents the product of metal root concentration ($\mu\text{g Metal}\cdot\text{g}_{\text{root}}\text{DW}$) by the root biomass ($\text{g}\cdot\text{plant}^{-1}$) was determined by:

$$Q\text{Metal}_{\text{root}} = [\text{Metal}]_{\text{root}} (\mu\text{g}\cdot\text{g}^{-1}\text{DW}) \times \text{DW}_{\text{root}} (\text{g}\cdot\text{plant}^{-1})$$

The metal quantity accumulated in the whole plant (WP) was calculated as follows:

$$Q\text{Metal}_{\text{WP}} = Q\text{Metal}_{\text{root}} + Q\text{Metal}_{\text{shoot}}$$

The metal acquisition efficiency (MAE) reflects the capacity of root to absorb the target metal from the soil solution. It was estimated by rationing the amount of metal taken up during the experiment to the mean root biomass and expressed in ($\mu\text{mol metal mg}^{-1}\text{ root DW}$) (Zribi et al., 2014).

$$\text{MAE} = \frac{Q\text{Metal}_{\text{WP}}}{\text{meanDW}_{\text{root}}}$$

2.4 Estimating Pollutant Impact

The enrichment factor (EF), the geo-accumulation index (I_{geo}) and the contamination factor (CF) are indices used for quantifying the level of metal enrichment in soil. Pollution impact scales were proposed to convert the calculated numerical results into ranges spanning from unpolluted to strong polluted intensity (Bern et al., 2019; Wu Jin et al., 2014).

2.4.1 Geo-Accumulation Index (I_{geo})

I_{geo} Index is calculated using the following equation (Muller, 1969): $I_{\text{geo}} = \text{Log}_2\left(\frac{C_i}{1.5B_i}\right)$, where C_i represents the concentration of the examined metal in soil and B_i is the geochemical background concentration of the same metal (Kabata-Pendias, 2004). The factor 1.5 is introduced to minimize the effect of any possible variations in the background or control values, which may be attributed to lithogenic variations in the sediment (Lizarraga Mendiola et al., 2008). The degree of metal pollution is assessed in terms of seven contamination classes based on the increasing

numerical value of the index as follows: uncontaminated soil ($I_{geo} \leq 0$), uncontaminated to moderately contaminated soil ($0 < I_{geo} \leq 1$), moderately contaminated soil ($1 < I_{geo} \leq 2$), moderately to strongly contaminated soil ($2 < I_{geo} \leq 3$), strongly contaminated soil ($3 < I_{geo} \leq 4$), strongly to extremely contaminated soil ($4 < I_{geo} \leq 5$) and extremely contaminated soil ($I_{geo} > 5$) (Ho et al., 2010; Muller, 1969).

2.4.2 Enrichment Factor (EF)

The enrichment factor (EF) is calculated by the following equation: (Wu Jin et al., 2014) $EF = \left(\frac{C_i}{C_{ref}}\right)_{sample} / \left(\frac{C_i}{C_{ref}}\right)_{background}$, where $\left(\frac{C_i}{C_{ref}}\right)_{sample}$ is the ratio of the content of the target element to the reference element in the soil sample, and $\left(\frac{C_i}{C_{ref}}\right)_{background}$ is the ratio of the content of the target element to the reference element in natural background (average crust) (Kabata-Pendias, 2004). Since heavy metal background values of Tunisian soils are not available, alternatively those of earth's crust were used. In this study, iron (Fe) was selected as a reference element. The EF values were classified according to (Bern et al., 2019; Sutherland, 2000) as: $EF < 1$ (no enrichment), $1 < EF < 3$ (minor enrichment), $3 < EF < 5$ (moderate enrichment), $5 < EF < 10$ (moderately severe enrichment), $10 < EF < 25$ (severe

enrichment), $25 < EF < 50$ (very severe enrichment) and $EF > 50$ (extremely severe enrichment).

2.4.3 Contamination Factor (CF)

The contamination factor (CF) was calculated according to the following equation (Hakanson, 1980): $CF = \frac{X}{X_{ref}}$, where X is the concentration of the metal in soil (mg kg^{-1}) and X_{ref} is the background concentration of metal (mg kg^{-1}). The background soil concentrations of the studied heavy metals were adopted from world agencies such as World Health Organization (WHO), Food and Agricultural Organization (FAO) and European community (Chiroma et al., 2014). Soil contamination was evaluated according to CF values as follows: low contamination ($CF < 1$), moderate contamination ($1 \leq CF < 3$), considerable contamination ($3 \leq CF < 6$), and very high contamination ($CF \geq 6$).

2.4.4 Soil Pollution Index (PI)

Soil pollution index of the was calculated by dividing metals' concentrations in soil (mg kg^{-1}) by the background metal concentration according to Wu et al. (Wu Jin et al., 2014). Background concentrations correspond to standard levels in uncontaminated soil (Chiroma et al., 2014; Kabata-Pendias, 2004). The pollution index is calculated using the following equation:

$$PI = \left(\frac{[Cd]_{soil}}{3} + \frac{[Cu]_{soil}}{100} + \frac{[Cr]_{soil}}{100} + \frac{[Mn]_{soil}}{2000} + \frac{[Ni]_{soil}}{50} + \frac{[Fe]_{soil}}{50000} + \frac{[Zn]_{soil}}{300} \right) / 7$$

where $[Metal]_{soil}$: concentration in mg kg^{-1} .

$PI > 1$ corresponds to a polluted soil.

2.5 Phytoremediation Factors

To evaluate the metal enrichment characteristics of the different plant species, the following factors were calculated:

2.5.1 Biological Concentration Factor (BCF)

BCF is a factor that reflects the accumulation ability of root from the soil (Usman et al., 2019). It was calculated as the ratio of the trace metal concentration in plant tissue (root) to that in soil, where C_{root} and C_{soil} are the concentrations given in units of mg kg^{-1} of dry weight.

$$BCF = \frac{C_{root}}{C_{soil}}$$

2.5.2 Translocation Factor (TF)

The translocation factor is the metal ratio transfer capacity from the soil to shoots. It was calculated as the ratio of metal concentration in the aerial parts to that in the roots, where C_{aerial} and C_{roots} are the concentrations given in mg kg^{-1} of dry weight in aerial parts of the plant and root, respectively (Lago-Vila et al., 2019).

$$TF = \frac{C_{aerial}}{C_{roots}}$$

Considering the abovementioned factors, the following criteria apply: if $TF > 1$ and $BCF > 1$, phytoextraction occurs; if $TF < 1$ and $BCF > 1$, phytostabilization is attained (Ghazaryan et al., 2019).

2.5.3 Biological Accumulation Coefficient (BAC)

The biological accumulation coefficient is defined as the concentration of metal in plant shoots divided by the metal concentration in rhizospheric soils (Nazir et al., 2011; Yu et al., 2022). It is calculated using the following formula:

$$BAC = \frac{C_{aerial}}{C_{soil}}$$

2.5.4 Metal Extraction Ratio (MER)

Expressing the extraction capacity as a ratio between the produced biomass and the soil volume to be cleaned would be more informative (Afonso et al., 2022; Liu et al., 2011). Therefore, the metal extraction ratio (MER) is proposed and defined as the ratio of metal accumulation in shoots to that in soil, calculated as:

$$MER(\%) = \left[\frac{C_{shoot} \times M_{shoot}}{C_{soil} \times M_{rootedzone}} \right] \times 100, \text{ where } C_{shoot} \text{ is the metal concentration in the harvested component of the plant biomass, } M_{shoot} \text{ is the mass of the harvestable aboveground biomass produced in one harvest, } C_{soil} \text{ is the metal concentration in the soil volume and } M_{rootedzone} \text{ is the mass of the soil volume rooted by the species under study.}$$

2.5.5 Plant Effective Number (PENs) and Plant Effective Number Total (PENt)

PEN is defined as the number of plants needed to extract 1.0 g of metal when the biomass of shoots or total plant is considered. Plant effective number shoot (PENs) is defined as the number of plant shoots needed to extract 1.0 g of metal, while PENt (plant effective number total) is considered as the total biomass of the plant (shoots and roots). Both PENs and PENt were determined considering the concentration of the target element present in the dry biomass of the plant as well as the dry weight biomass (Afonso et al., 2022; Garcia et al., 2004).

The phytoremediation potential (g ha^{-1}) was estimated as reported by (Afonso et al., 2022). Typically, Cd contamination was recognised to occur only in the rooting zone (0–20 cm) with soil bulk density of 1.3 t/m^3 .

2.6 Statistical Analysis

Reported values for all parameters represent the means of five individual replicates ($n=5$), corresponding to five pots for each treatment. The unplanted soil control (blank) was also represented by five replicates. ANOVA was applied, followed by Duncan's multiple range test to assess mean differences at a 5% significance level. No significant outliers were detected in the dataset; hence, no data points were excluded. All statistical analyses were performed using XLSTAT software, version 2014 (Addinsoft, France).

3 Results

3.1 Site Description

The studied area is located in Gafsa, southern Tunisia (Fig. 1A). The region has an arid climate and experiences temperature variation throughout the year, with hot summers and mild winters. Annual rainfall rates range from 150 to 200 mm per year (Mokadem et al., 2018). Gafsa basin has a complex geological history that includes various phases of sedimentation, deformation, and tectonic

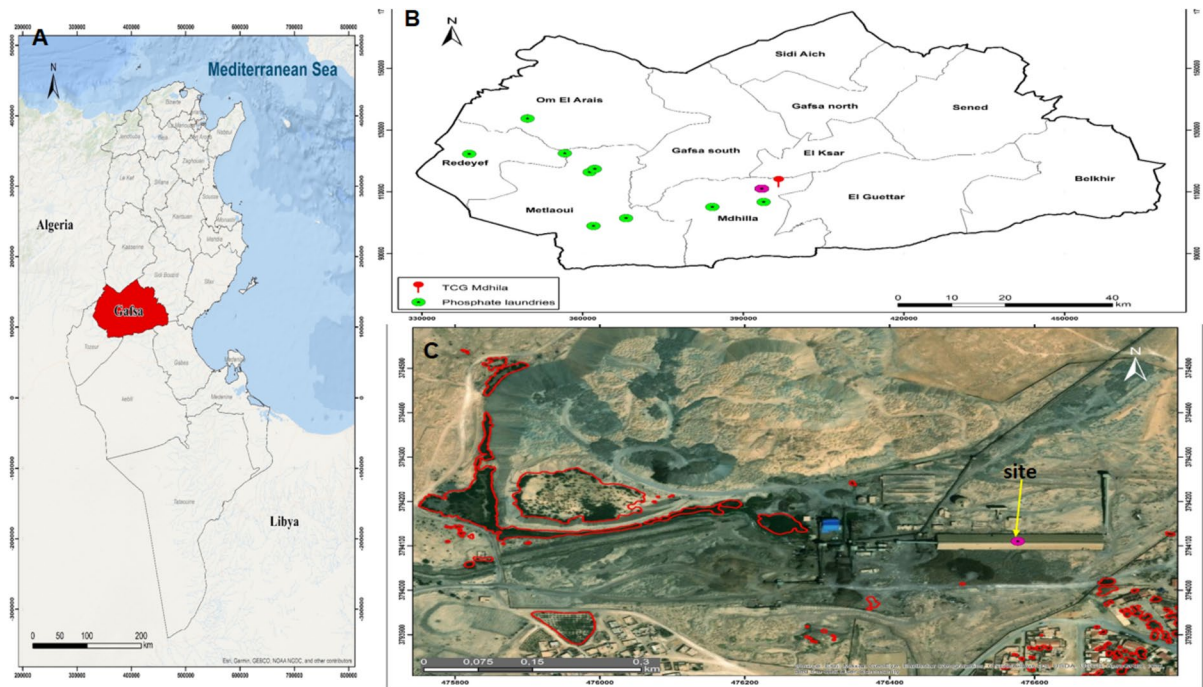


Fig. 1 Location of the study area: soil-sampling area at M'Dhilla site. **A** Gafsa region, **B** mining area of Gafsa **C** M'Dhilla sector and mining tailing area

activity (Ahmadi et al., 2013). This basin is characterized by the deposition of sedimentary rocks including carbonate fluorapatite, carbonates, quartz, gypsum, clays, and clinoptilolite, during the Cenozoic era (Kocsis et al., 2014; Messadi et al., 2019). The basin was underwater during the Mesozoic era, with the Tethys sea expanding and depositing limestone and clay along the coasts. The end of the Cretaceous period saw the beginning of oceanic closure, with Europe coming closer to Africa and the Tethys narrowing again (Kocsis et al., 2014; Messadi, 2022). This geological history makes of Gafsa mining basin the most important phosphate-mining center in Tunisia, with areas of exploitation including Mélaoui, Redeyef, Kef eddour, Moularès, and M'Dhilla (Fig. 1B). The basin is home to the GAFSA Phosphates Company (CPG), which operates several laundries and processing units in the region (Fig. 1B). In particular, M'Dhilla sector has a rich history of phosphate mining industry since M'Dhilla mine was inaugurated in 1920 by CPG to exploit phosphate reserves in Tunisia. The three laundries in M'Dhilla belong to the ten washing units in Gafsa-Mélaoui Basin including four

laundries in Mélaoui sector, one Laundry in Moularès sector, one Laundry in Kef Eddour sector, and one Laundry in Redeyef sector (Fig. 1B). Besides, M'Dhilla city include the Tunisian Chemical Group (TCG), producer of phosphoric acid where phosphogypsum releases are stored near the site which contributes soil pollution (Fig. 1B and 1C). M'Dhilla sector's geological composition and history reflect its importance in Tunisian phosphate industry, however the ongoing activities in the region affect the environment and the mineralogical composition of the soil (Khelifi et al., 2021). Sampling conducted in this area revealed the presence of varying mineralogical compositions in mud and soil (Khelifi et al., 2020). Soils in M'Dhilla exhibit different levels of elements like P_2O_5 , CaO, MgO, Cd, Cr, Zn, Cu, Ni, and Sr, underlining the impact of phosphate industry activities on the environment in this region (Khelifi et al., 2020; Smida et al., 2021).

3.2 Soil Properties and Metal Concentration

Soil collected from the studied area had a loamy sand texture with a neutral pH; about 7.02. The

Table 1 Properties of the collected mining soil in terms of metal contaminants. All values are given by the mean of five independent repetitions \pm SD (standard error deviation) of ($p \leq 0.5$)

Metal	Cd	Zn	Cu	Mn	Cr	Ni	Fe	Al	Sr
Concentration (mg Kg ⁻¹) DW	20.4 \pm 0.3	366.1 \pm 2.3	80.0 \pm 3	80.4 \pm 1.3	248.0 \pm 2	120 \pm 1.9	5.2 \pm 0.03	10.0 \pm 1	4.1 \pm 0.06
<i>I_{geo}</i>	2.2 \pm 0.02	-0.3 \pm 0.01	-0.9 \pm 0.05	-5.2 \pm 0.02	0.72 \pm 0.01	0.7 \pm 0.02	-3.8 \pm 0.01	-	-
<i>EF</i>	64.6 \pm 0.8	11.6 \pm 0.08	7.6 \pm 0.3	0.38 \pm 0.004	23.6 \pm 0.2	23 \pm 0.03	ns	-	-
<i>CF</i>	6.8 \pm 0.1	1.2 \pm 0.1	0.8 \pm 0.3	0.04 \pm 0.001	2.5 \pm 0.02	2.4 \pm 0.03	0.105 \pm 0.001	-	-

I_{geo}: Geo-accumulation Index, *EF*: Enrichment Factor, *CF*: contamination Factor

soil sample presented a high organic matter (OM) content reaching 4% and an electrical conductivity (EC) of about 3.2 mS. Heavy metals' concentrations in soil sampled from M'Dhillia site are presented in Table 1. The soil showed a polymetallic pollution featured by the presence of nine metals (Cd, Zn, Cu, Mn, Cr, Ni, Fe, Al, Sr) with concentrations ranging from mg Kg⁻¹ to g Kg⁻¹. Specifically, the studied area has a content of Cd 20.4 mg Kg⁻¹ DW, Zn 366.1 mg Kg⁻¹ DW, Cu 80 mg Kg⁻¹ DW, Mn 80.4 mg Kg⁻¹ DW, Cr 248 mg Kg⁻¹ DW and Ni 120 mg Kg⁻¹ DW. The metal distribution showed a dominance of Sr, Fe and Al at concentrations 4.1 g Kg⁻¹, 5.2 g Kg⁻¹ and 10 g Kg⁻¹, respectively. Hence, the soil collected from M'Dhillia can be considered as highly contaminated with the presence of high levels of Cd, Cr, Ni, and Sr compared to the maximum allowed limit in soil. Pollution index recorded in this site was about 13.84, significantly higher than one defined as the threshold soil pollution, reflecting the extremely deleterious effects of mining activities and abandoned wastes in the environment (Table 1).

The highest *I_{geo}* value was 2.2 recorded for Cd ($2 < I_{geo} \leq 3$) while those of Cr and Ni were about 0.7 ($0 < I_{geo} \leq 1$) (Table 1.). According to *I_{geo}* categories, the obtained data indicates a strong contamination of the soil by Cd and a moderate contamination by Cr and Ni. On the other hand, the *I_{geo}* values for Zn, Cu, Mn, and Fe were less than 0 (ranging from -5.2 to -0.3) suggesting the absence of soil contamination by those metals. Concerning Al and Sr, *I_{geo}* calculus was not possible due to the unavailability of background values for these two elements. Earlier, (Lindsay, 1979) and (Dragun, 1988) suggested an average concentration of Al in the earth crust varying from 1 to 30% equivalent to (10000–300000 ppm). Natural

strontium (not radioactive) exist in four stable isotopic forms representing 0.02–0.03% of the earth's crust with an average of 200–300 mg kg⁻¹ (Edwards et al., 1998). Referred to the above Simmons's estimation, the studied soil is strongly polluted by Sr detected at 4.1 g Kg⁻¹.

Enrichment factor (*EF*) defines the concentration of a given element at a specific location compared to the average natural occurrence. In M'Dhillia, the highest *EF* value was 64.6 for Cd, followed by Cr (23.6) and Ni (23) (Table 1.) against 11.6 and 7.6 for Zn and Cu, respectively. According to *EF* categories all metals except Mn (*EF* < 1, no enrichment), were affected by human activities. Being in the category (*EF* > 50), the enrichment factor of Cd indicates an extremely severe enrichment of soil. *EF* values for Cr and Ni situated in the category ($10 < EF < 25$) indicate severe soil enrichment by these metals. Zn and Cu are in the categories of severe enrichment ($10 < EF < 25$) and moderately severe enrichment ($5 < EF < 10$), respectively. Altogether, these results indicate that heavy metals contents are predominantly affected by anthropogenic activities since the sampling site is near M'Dhillia second laundry and TCG (Fig. 1).

Similar to *EF* and *I_{geo}*, the highest *CF* value was recorded to be (6.8) for Cd, followed by Cr (2.5), then Ni (2.4) (Table 1.). Lower contamination factors were registered for Zn and Cu at 1.2 and 0.8 respectively. According to *CF* categories, the contamination factor of Cd belonging to category (*CF* \geq 6) reflects a very high contamination, while *CF* values for Cr and Ni belong to the category ($1 \leq CF < 3$) indicating a moderate contamination. *CF* values for Zn and Cu are in the categories moderate contamination ($1 \leq CF < 3$) and low contamination (*CF* < 1), respectively.

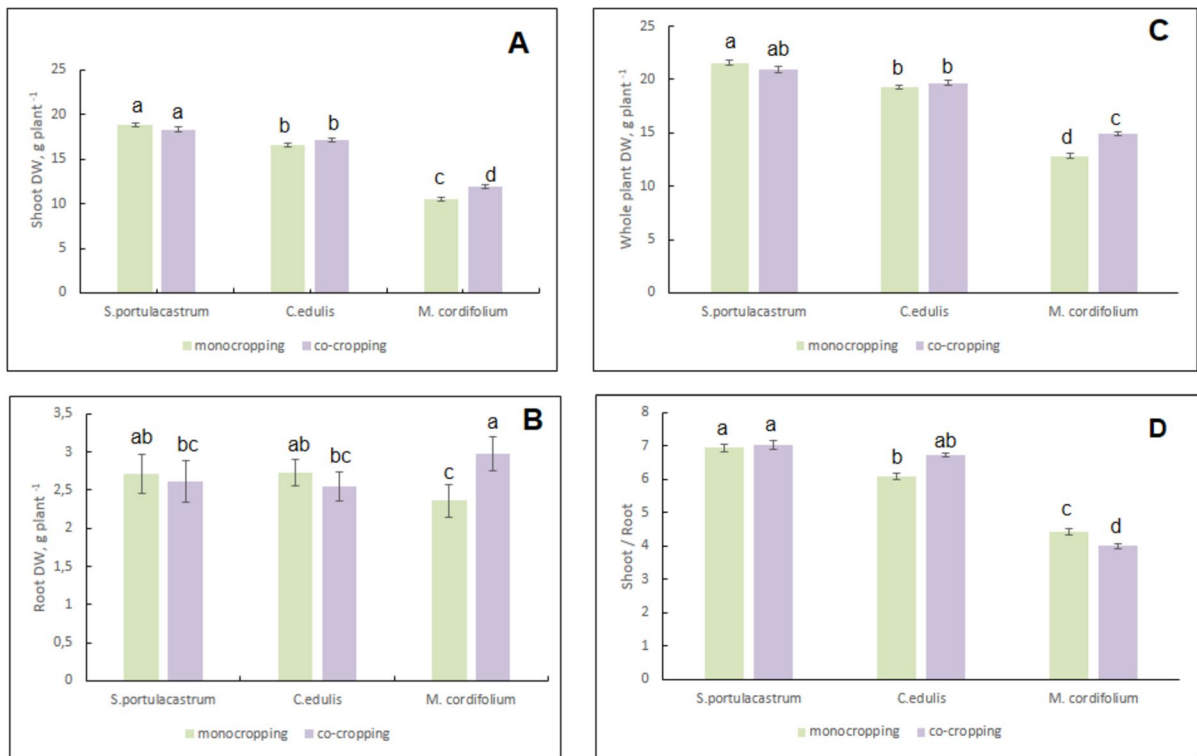


Fig. 2 Variation of shoot **A**, root **B**, the whole plant biomass per plant ($\text{g}\cdot\text{plant}^{-1}$) **C** and shoot root ratio **D** in three halophytes: *S. portulacastrum*, *C. edulis* and *M. cordifolium* cultivated for 7-month growth-chamber pot experiment on the

mining soil originated from M'Dhilla area, under mono or co-cropping systems. The values are means of five replicates for each species and each treatment. Bars marked with at least the same letter are not significantly different at the level of 5%

3.3 Polymetallic Effect on Plant Growth Parameters Under Mono-Cropping and Co-Cropping

The variation of root, shoot and whole biomasses measured in the three halophytes subjected to a long-term polymetallic exposure (7-months duration) under two growing systems is shown in Fig. 2. Results revealed that cropping systems affect differently the growth of the studied species and biomass repartition between organs (shoot and root). Therefore, the variation of shoot biomass showed a significant increase of 14% in co-cropped *M. cordifolium* with respect to mono-cropped (Fig. 2A). In contrast, *S. portulacastrum* and *C. edulis* shoot biomasses remain almost unchanged, around 18 g DW and 17 g DW respectively, regardless the cropping system. Moreover, the association of three halophytes stimulated significantly roots development of *M. cordifolium* and enhanced significantly dry

biomass of this organ (+26%) compared to monoculture condition (Fig. 2B). Concerning the two other halophytes, the decrease in root growth was not significant. The effect of polymetallic stress, on the whole biomass production showed approximately the same variation as that found in the shoots (Fig. 2C). At this level, *S. portulacastrum* presented the highest biomass in both growing systems, followed by *C. edulis* and *M. cordifolium*. For *S. portulacastrum* and *C. edulis*, the whole biomass production did not vary significantly depending less on the cropping systems to be around 21 g DW and 18 g DW, respectively (Fig. 2C). When co-cropped with the other two halophytes, *M. cordifolium* whole plant exhibited an increase of 16% with respect to monoculture. The decrease in Shoot/Root mass ratio revealed that root development was favored under co-cropping conditions in *M. cordifolium* (Fig. 2.D). It seems that *M. cordifolium*

Table 2 Heavy metal (Cd, Zn, Cu, Cr, Ni, Fe, Al and Sr) concentration ($\mu\text{g g}^{-1}$ DW) in shoots and roots of three halophytes: *S. portulacastrum*, *C. edulis* and *M. cordifolium* cultivated during seven months under mono or co-cropping system on a min-

ing soil originating from M'Dhilla sector. Values are expressed as the average of five replicates. For each metal, those marked by the same letter are not significantly different at $p=5\%$

		<i>S. portulacastrum</i>		<i>C. edulis</i>		<i>M. cordifolium</i>	
Cropping mode							
Metal	Plant part	Mono-Cropping	Co-Cropping	Mono-Cropping	Co-Cropping	Mono-Cropping	Co-Cropping
Cd	Shoot	422.7 (fg)	568.0 (ef)	380.0 (fg)	527.8 (efg)	337.2 (g)	380.2 (fg)
	Root	1593.6 (c)	2469.2 (a)	1834.4 (b)	2356.4 (a)	696.2 (e)	1311.7 (d)
Zn	Shoot	36.0 (h)	84.4(f)	122.7 (d)	133.5 (bc)	126.6 (cd)	130.6 (cd)
	Root	101.5 (e)	66.0 (g)	139.3 (b)	65.8 (g)	198.6 (a)	124.7 (cd)
Cu	Shoot	7.7 (de)	13.0 (b)	7.8 (de)	7.2 (de)	7.5 (de)	6.8 (e)
	Root	13.7 (b)	8.5 (cd)	3.7 (f)	6.5 (e)	18.0 (a)	9.6 (c)
Cr	Shoot	12.8 (h)	15.9 (gh)	16.0 (gh)	20.0 (fg)	21.2 (fg)	23.0 (f)
	Root	48.0 (d)	36.0 (e)	145.6 (a)	62.4 (b)	44.8 (d)	56.8 (c)
Ni	Shoot	9.9 (fg)	11.2 (f)	9.0 (g)	15.3 (e)	17.2 (d)	10.5 (f)
	Root	23.1 (b)	14.7 (e)	26.7 (a)	23.6 (b)	20.5 (c)	17.2 (d)
Fe	Shoot	178.3 (hi)	142.3(i)	201.1(h)	273.8 (g)	402.7 (f)	312.3 (g)
	Root	967.7 (d)	663.2 (e)	2393.0 (a)	1038.2 (c)	1194.5 (b)	1031.6 (c)
Al	Shoot	131.4 (h)	226.0 (g)	133.1 (h)	156.8 (gh)	828.0 (f)	1105.6 (d)
	Root	1187.3 (c)	766.7 (f)	2625.8 (a)	925.2 (e)	972.6 (e)	1293.1 (b)
Sr	Shoot	171.0 (i)	221.5 (h)	225.0 (h)	236.2 (h)	488.0 (b)	279.3 (g)
	Root	405.7 (d)	390.2 (e)	548.7 (a)	386.3 (e)	429.2 (c)	367.8 (f)

Manganese was present below the maximum permissible levels in soils. Despite its presence in the mining soil, this metal was not detected in the vegetal material, thus not mentioned in the following table

benefited from the simultaneous presence of the two other halophytes. For the two other halophytes, the Shoot/Root mass ratio was unchanged in *S. portulacastrum*, while a slight increase was observed in *C. edulis* (Fig. 2D).

3.4 Heavy Metals Concentration and Accumulation in Plants

The variation of metal concentration in roots and shoots of three halophytes cultivated on a polymetallic-polluted soil is given in Table 2. Results showed that all heavy metals present in the soil sampled from M'Dhilla site (plant substrate) are detected in plant tissues except Mn. Since Mn concentration is below the maximum allowed level in soil and co-exists in minority with other 8 metals, its uptake by roots is likely hindered due to a competition with these elements as confirmed by ICP-OES (Table 2). Metal repartition between shoot and root differed

over the studied halophyte species and depended on the adopted growing system. Generally, cadmium is preferentially accumulated in plant roots (Siyar et al., 2022). In this study, Cd concentrations were considerably higher in roots than in shoots for all the studied species especially in *S. portulacastrum* and *C. edulis*.

The variation of metal acquisition efficiency (MAE) for the investigated halophytes cultivated on a polymetallic-mining soil is summarized in Table 3. Results demonstrated that the association of these species enhanced Cd content in shoots and roots (Table 2.) and also raised its acquisition efficiency (Table 3). In fact, with respect to mono-cropping, in co-cropping shoot Cd concentration increased by 34%, 39% and 13% in *S. portulacastrum*, *C. edulis* and *M. cordifolium*, respectively. Similarly, Cd concentration in roots showed an important increase estimated by 55% and 28% in *S. portulacastrum* and *C. edulis* respectively. For the two previous species, Cd acquisition efficiency also experienced an increase of 40%. Interestingly,

Table 3 Metal acquisition efficiency (MAE, (μmole metal mg⁻¹ roots)), bioconcentration factor (BCF), Translocation factor (TF) and biological accumulation coefficient (BAC) for three halophytes: *S. portulacastrum*, *C.edulis* and *M. cordifolium* cultivated during seven months under mono-cropping

or co-cropping systems on a mining soil originating from M’Dhilla sector. Each value is the mean of five replicates. For the same parameter, means marked with the same letter are not significantly different at *p*=0.05

Parameters	<i>S. portulacastrum</i>		<i>C. edulis</i>		<i>M. cordifolium</i>	
	<i>Mono- Cropping</i>	<i>Co- Cropping</i>	<i>Mono- Cropping</i>	<i>Co- Cropping</i>	<i>Mono- Cropping</i>	<i>Co- Cropping</i>
MAE (Cd)	508 (c)	726 (a)	454 (d)	656 (b)	252 (f)	322 (e)
MAE (Zn)	23 (p)	42 (o)	60 (mn)	66 (m)	51 (no)	42 (o)
MAE (Cu)	4.3 (q)	6.2 (q)	3.3 (q)	3.5 (q)	3.3 (q)	2.3 (q)
MAE (Cr)	6 (q)	6.4 (q)	11 (q)	8.5 (q)	6.30 (q)	6.5 (q)
MAE (Ni)	5.4 (q)	5.4 (q)	4.7 (q)	7 (q)	5.5 (q)	3.4 (q)
MAE (Fe)	123 (k)	93 (l)	203 (h)	158 (i)	172 (i)	129 (jk)
MAE (Al)	57 (mn)	64 (m)	93 (l)	49 (no)	130 (jk)	163 (i)
MAE (Sr)	137 (j)	166 (i)	167 (i)	171 (i)	229 (g)	133 (jk)
BCF (Cd)	59 (d)	91 (a)	68 (c)	87 (b)	26 (f)	48 (e)
BCF (Zn)	0.28 (g)	0.18 (g)	0.38 (g)	0.18 (g)	0.54 (g)	0.34 (g)
BCF (Cu)	0.17 (g)	0.11 (g)	0.05 (g)	0.08 (g)	0.22 (g)	0.12 (g)
BCF (Cr)	0.19 (g)	0.14 (g)	0.59 (g)	0.25 (g)	0.18 (g)	0.23 (g)
BCF (Ni)	0.19 (g)	0.12 (g)	0.22 (g)	0.2 (g)	0.17 (g)	0.14 (g)
BCF (Fe)	0.18 (g)	0.13 (g)	0.45 (g)	0.2 (g)	0.23 (g)	0.2 (g)
BCF (Al)	0.12 (g)	0.08 (g)	0.26 (g)	0.10 (g)	0.10 (g)	0.13 (g)
BCF (Sr)	0.10 (g)	0.10 (g)	0.13 (g)	0.10 (g)	0.10 (g)	0.1 (g)
TF (Cd)	0.26 (qrst)	0.23 (rstuv)	0.21 (stuv)	0.22 (rstuv)	0.48 (kl)	0.29 (pqrstu)
TF (Zn)	0.35(mnopq)	1.3 (d)	0.9 (f)	2.0 (b)	0.63 (ij)	1.0 (e)
TF (Cu)	0.56 (jk)	1.54 (c)	2.1 (a)	1.1 (e)	0.42 (lmno)	0.7 (hi)
TF (Cr)	0.26 (qrst)	0.44 (lmn)	0.1 (vw)	0.32 (nopqrs)	0.47 (klm)	0.4 (lmnop)
TF (Ni)	0.43 (lmn)	0.76 (gh)	0.34 (nopqr)	0.64 (ij)	0.84 (fg)	0.61 (ij)
TF (Fe)	0.18 (tuvw)	0.21 (rstuv)	0.08 (wx)	0.26 (qrst)	0.34 (nopqr)	0.3 (opqrst)
TF (Al)	0.11 (vw)	0.3 (pqrst)	0.05 (x)	0.17 (uvw)	0.85 (fg)	0.85 (fg)
TF (Sr)	0.42 (lmno)	0.57 (jk)	0.41 (lmnop)	0.61 (ij)	1.13 (e)	0.76 (gh)
BAC (Cd)	15.5 (c)	21 (a)	14 (d)	19.4 (b)	12.4 (e)	14 (d)
BAC (Zn)	0.1 (f)	0.23 (f)	0.33 (f)	0.36 (f)	0.34 (f)	0.36 (f)
BAC (Cu)	0.1 (f)	0.16 (f)	0.10 (f)	0.09 (f)	0.1 (f)	0.08 (f)
BAC (Cr)	0.05 (f)	0.06 (f)	0.06 (f)	0.08 (f)	0.08 (f)	0.09 (f)
BAC (Ni)	0.43 (f)	0.76 (f)	0.34 (f)	0.64 (f)	0.84 (f)	0.61 (f)
BAC (Fe)	0.03 (f)	0.02 (f)	0.04 (f)	0.05 (f)	0.07 (f)	0.06 (f)
BAC (Al)	0.013 (f)	0.02 (f)	0.013 (f)	0.015 (f)	0.082 (f)	0.11 (f)
BAC (Sr)	0.04 (f)	0.05 (f)	0.05 (f)	0.05 (f)	0.12 (f)	0.07 (f)

Bold values indicate BCF, TF and BAC values greater than 1.0

in *M. cordifolium*, cadmium acquisition efficiency decreased by 28% despite the increase of Cd root concentration associated with the enhancement of the root biomass of this species under co-cropping.

The prominent increase of Cd concentration in roots under co-cropping system is accompanied by

a net decrease in the level of some metal elements. Thus, in *C. edulis*, the accumulation of Cd induced a concentration drop of 53% in Zn, 77% in Cu, 57% in Cr, 57% in Fe, 36% in Ni, 65% in Al and 30% in Sr (Table 2.). This antagonistic effect was also identified in *S. portulacastrum* but to lower extent.

In such species, a 56% increase in Cd root concentration was accompanied with a decrease of 35% in Zn, of 38% in Cu, of 25% in Cr, of 31% in Fe, of 36% in Ni, of 35% in Al and 4% in Sr (Table 2). Unlike, *S. portulacastrum* and *C. edulis*, in *M. cordifolium* the increase of Cd content was accompanied by an increase of 27% in Cr and 33% in Al, against a decrease of 37% in Zn, of 46% in Cu, of 14% in Fe, of 16% in Ni, and 14% in Sr (Table 2).

In terms of metal acquisition efficiency, a high Cd root uptake reduced the uptake of the other metals present in the soil except Cr and Ni (Table 3).

On the other hand, MAE values varied over the three halophytes cultivated either alone or together. Under co-cropping conditions, Zn, Cu and Sr acquisition efficiency increased in *S. portulacastrum*, while Al acquisition efficiency was increased only in *C. edulis*. *M. cordifolium* has experienced a decrease in Sr acquisition efficiency and an augmentation in MAE (Al) (Table 3).

In shoots, the metal concentration displayed a high variation depending on both the halophyte species and the applied growth system. Thus, under co-cropping, *C. edulis* showed a concentration increase by 39% in Cd, 9% in Zn, 25% in Cr, 70% in Ni, and 36% in Fe, while those of Cu, Al and Sr remain unaffected. An increase in shoot of Al content (34%) was observed in *M. cordifolium* under association against a decrease in Sr 42%, Fe 22% and Ni 39%. Co-cropped *S. portulacastrum* showed a prominent increase in shoot concentrations of Zn 135%, Cu 24%, Al 72% and Sr 29%.

The soil used for phytoremediation experiments was qualified to be strongly contaminated by Cd with a severe enrichment. The variations in the bioconcentration factor (BCF), the biological accumulation coefficient (BAC) and the translocation factor (TF) are summarized in Table 3, where values higher than 1.0 are highlighted in bold. The comparison of BCF and BAC for the eight identified metals revealed that the highest values are attributed to Cd.

C. edulis and *S. portulacastrum* manifested the highest BCF of Cd under mono and co-cropping. In both growing systems, *M. cordifolium* showed the lowest BCF for Cd. The biological accumulation coefficient (BAC) increased under co-cropping for all species. *S. portulacastrum* and *M. cordifolium* displayed the highest and the lowest BAC values, respectively regardless of the growing method. It is worth to mention that in all conditions the translocation factor

(TF) for Cd was less than 1 revealing the phytostabilization potential of the three halophytes towards this element. In contrast, for Zn, Cu and Sr, TF reached or exceeds 1 suggesting the phytoextraction role of these metal elements. The association of the three halophytes enhanced significantly Zn TF in all species compared to monoculture. The transfer factor of Cu was higher in mono-cropped (2.1) than in co-cropped (1.1) *C. edulis*. In *S. portulacastrum*, Cu TF increased under co-cropping reaching 1.54. For *M. cordifolium*, the translocation factor was 1.13 for Sr in mono-cropping and about 1 for Zn in co-cropping.

3.5 Heavy Metals' Amounts and Experimental Estimation of Metal Extraction Capacities by the Halophytes

The variation of metal amounts in roots and shoots for the three halophytes cultivated on a polymetallic-mining soil is presented in Table 4. Unlike Cd concentration tendency, Cd amount was rather different considering the metal repartition between shoots and roots. In fact, the experiment duration was 7 months and the biomass allocation was mainly concentrated in shoots. Hence, the conversion of concentrations in quantities or amounts involved biomass accumulation during 7 months for each organ, which yields an important increase of Cd amount in shoots to exceed, sometimes, that accumulated in roots. In terms of quantities, Cd amount in *S. portulacastrum* was the highest among all species regardless the growing system. The association between halophytes indicated an increase of Cd amount in shoots by 31% in *S. portulacastrum*, 47% in *C. edulis* and 26% in *M. cordifolium*. Although Cd translocation factor was below 1, the quantities of extracted Cd in shoots are considered important and reached 7952 $\mu\text{g plant}^{-1}$ in *S. portulacastrum*, 6014 $\mu\text{g plant}^{-1}$ in *C. edulis* and 3650 $\mu\text{g plant}^{-1}$ in *M. cordifolium* (Table 4). A similar effect was underlined for Cd concentration and Cd acquisition efficiency as mentioned before. Taking into account that one whole plant (root + shoot) of *S. portulacastrum* is able to accumulate 12,277 μg of Cd (7952 μg in the shoot and 4325 μg in the roots) in mono-cropping, 3 plants per pot absorb 36,831 μg Cd. Given that the pot was filled by 5 kg of soil with Cd concentration 20 mg Kg^{-1} (equivalent to 100 mg Cd pot^{-1}), mono-cropped *S. portulacastrum* (3 plants pot^{-1}) was able to extract 36.83% of Cd initially

Table 4 Variation of heavy metals quantities ($\mu\text{g plant}^{-1}$) for (Cd, Zn, Cu, Cr, Ni, Fe, Al and Sr) accumulated in shoots and in roots of *S. portulacastrum*, *C. edulis* and *M. cordifolium* cultivated during seven months in monoculture or co-cropped

on a mining soil originated from M'Dhilla sector. Each value is the mean of five biological replicates. For each metal, those marked by the same letter are not significantly different at $p=5\%$

		<i>Sesuvium portulacastrum</i>		<i>Carpobrotus edulis</i>		<i>Mesembryanthemum cordifolium</i>	
		Mono- Cropping	Co- Cropping	Mono- cropping	Co- Cropping	Mono- cropping	Co- Cropping
Cd	Shoot	7952 (c)	10,438 (a)	6014 (d)	8870 (b)	3650 (h)	4622 (ef)
	Root	4325 (fg)	6449 (d)	5013 (e)	6000 (d)	1643 (i)	3909 (gh)
Zn	Shoot	675.4 (e)	1508 (c)	2150 (b)	2425 (a)	1369 (d)	1556 (c)
	Root	275.6 (h)	172.4 (i)	380.8 (g)	167.6 (i)	469 (f)	371.6 (g)
Cu	Shoot	145.4 (b)	232.4 (a)	134.1 (bc)	122.1 (c)	79 (d)	81.6 (d)
	Root	37.3 (ef)	22.1 (fgh)	10.05 (h)	16.6 (gh)	42.51 (e)	28.7 (efg)
Cr	Shoot	241.8 (e)	289 (c)	265 (d)	337.2 (b)	234 (e)	274 (cd)
	Root	130.2 (g)	94 (h)	398 (a)	159 (f)	105.8 (h)	169.4 (f)
Ni	Shoot	185.4 (c)	204.6 (b)	148.7 (e)	243.5 (a)	173.8 (d)	119.6 (f)
	Root	62.6 (h)	38.6 (k)	72.8 (g)	60.2 (hi)	48.5 (j)	51.3 (ij)
Fe	Shoot	1604.4 (d)	1644 (g)	2254 (d)	2728 (b)	3207 (b)	2563.5 (c)
	Root	2626.7 (g)	1732 (h)	6540 (a)	2643.6 (g)	2820 (f)	3074.4 (e)
Al	Shoot	2503.4 (g)	4239.3 (d)	2222.8 (gh)	2280.8 (gh)	9086.6 (b)	14,180.8 (a)
	Root	3222.6 (f)	2002.5 (h)	7176.3 (c)	2356 (g)	2296 (gh)	3853.4 (e)
Sr	Shoot	3193.1 (e)	3921.7 (b)	3725.8 (c)	3976.1 (b)	5166.4 (a)	3424.5 (d)
	Root	1101.3 (g)	1019.1 (gh)	1499.6 (f)	983.7 (h)	1013.1 (gh)	1096.1 (g)

Manganese was present in the soil below the maximum permissible levels in soils. Despite its presence in the mining soil, this metal was not detected in the vegetal material, thus not mentioned in the following table

present in the soil. Following to the same calculation, mono-cropped *C. edulis* and *M. cordifolium* absorbed about 33.08% and 15.88% of Cd, respectively. In co-cropping, given that the number of plants per pot was kept 3 with one from each species, the determination of the extracted Cd per pot was performed using the above protocol described for mono-cropping by considering the sum of the quantity extracted by each plant species in co-cropping. Co-cropped *S. portulacastrum* was able to accumulate 16,887 μg of Cd (10,438 μg in the shoot and 6449 μg in the roots), *C. edulis* was able to accumulate 14,870 μg of Cd (8870 μg in the shoot and 6000 μg in the roots), and *M. cordifolium* was able to accumulate 8531 μg of Cd (4622 μg in the shoot and 3909 μg in the roots). Therefore, in co-cropping the total extracted quantity of Cd was 40,288 μg . Considering that one pot contains 100 mg Cd, the association of three halophytes was able to extract 40.28% of the initial Cd present in one pot.

Whatever the growing system, in shoots, *C. edulis* was the highest accumulators of both Zn and Cr, *S.*

portulacastrum was the highest accumulator for Cu and *M. cordifolium* was the highest Al accumulator.

On the other hand, whole plant (shoots + roots) displayed an apparent extraction efficiency in monocultured species for all identified metals except Cd. *M. cordifolium* cultivated separately extracted 0.45% and 0.07% of Sr and Al, respectively. *C. edulis* extracted 0.41% and 0.16% of Zn and Cr, respectively. *S. portulacastrum* extracted predominantly Cd against only 0.12% of Ni and 0.14% of Cu. The variation of some phytoextraction indices calculated based on metal extracted quantities by shoots or whole plants is summarized in Table 5. In terms of metal extraction ratio (MER), the highest values were registered in *M. cordifolium* for both Al and Sr, while the lowest values correspond to Zn, Cu, Cr and Ni. In both cropping systems, *S. portulacastrum* demonstrated the highest MER for Cd, underlying an increase of 31% under co-cropping versus mono-cropping. For Fe, the highest MER was found in *M. cordifolium* in monoculture and *C. edulis* under co-cropping (Table 5). The highest MER for Sr was observed in mono-cropped *M. cordifolium* and co-cropped *S. portulacastrum* and *C.*

Table 5 Phytoretraction indices: metal extraction ratio (MER), plant effective number of shoots (PENs), plant effective number total plants (PENT), potential of phytoremediation g ha^{-1} (P.p.) for the three halophytes: *S. portulacastrum*, *C. edulis* and *M. cordifolium* cultivated during seven months

under mono-cropping or co-cropping systems on a mining soil originating from M'Dhilla area. Each value is the mean of five replicates. For the same parameter, means marked with the same letter are not significantly different at $p=0.05$

Parameters	<i>S. portulacastrum</i>		<i>C. edulis</i>		<i>M. cordifolium</i>	
	Mono- Cropping	Co- Cropping	Mono- Cropping	Co- Cropping	Mono- Cropping	Co- Cropping
MER Cd (%)	7.81 (n)	10.22 (k)	6.19 (o)	8.88 (l)	3.46 (s)	4.45 (r)
MER Zn (%)	0.04 (t)	0.08 (t)	0.11 (t)	0.12 (t)	0.07 (t)	0.08 (t)
MER Cu (%)	0.04 (t)	0.06 (t)	0.03 (t)	0.03 (t)	0.02 (t)	0.02 (t)
MER Cr (%)	0.02 (t)	0.02 (t)	0.02 (t)	0.03 (t)	0.02 (t)	0.02 (t)
MER Ni (%)	0.03 (t)	0.03 (t)	0.02 (t)	0.04 (t)	0.03 (t)	0.02 (t)
MER Fe (%)	12.78 (j)	9.93 (k)	12.70 (j)	17.86 (e)	16.04 (gh)	14.20 (i)
MER Al (%)	4.94 (q)	8.27 (m)	4.41 (r)	5.36 (p)	17.3 (f)	26.37 (a)
MER Sr (%)	15.74 (h)	19.81 (c)	18.25 (d)	19.73 (c)	25.0 (b)	16.3 (g)
PENs Cd	127 (lm)	96 (lm)	159 (lm)	111 (lm)	284 (lm)	220 (lm)
PENs Zn	1480 (k)	647 (lm)	491 (lm)	438 (lm)	755 (l)	642 (lm)
PENs Cu	6960 (c)	4207 (gh)	7701 (b)	8166 (b)	12685 (a)	12351 (a)
PENs Cr	4145 (gh)	3435 (ij)	3784 (hi)	2925 (j)	4499 (fg)	3657 (hi)
PENs Ni	5378 (de)	4877 (ef)	6725 (c)	3823 (hi)	5551 (d)	7942 (b)
PENs Fe	298 (lm)	385 (lm)	300 (lm)	213 (lm)	237 (lm)	268 (lm)
PENs Al	404 (lm)	242 (lm)	453 (lm)	372 (lm)	115 (lm)	76 (m)
PENs Sr	310 (lm)	246 (lm)	268 (lm)	247 (lm)	195 (lm)	300 (lm)
PENt Cd	46 (qr)	31 (qr)	47 (qr)	35 (qr)	151 (qr)	79 (qr)
PENt Zn	676 (o)	635 (op)	395 (pq)	510 (op)	480 (op)	525 (op)
PENt Cu	4333 (fg)	4464 (f)	8988 (a)	7400 (c)	6104 (d)	8196 (br)
PENt Cr	1529 (m)	1846 (l)	641 (op)	1235 (n)	2361 (k)	1688 (lm)
PENt Ni	2818 (ij)	3685 (h)	2903 (i)	2612 (j)	4130 (g)	4834 (e)
PENt Fe	81 (r)	119 (r)	40 (r)	77 (r)	97 (r)	99 (r)
PENt Al	70 (r)	96 (r)	37 (r)	94 (r)	86 (r)	56 (r)
PENt Sr	161 (qr)	156 (qr)	134 (qr)	163 (qr)	170 (qr)	207 (qr)
P.p. Cd	6209.52 (e)	8120.52 (b)	4918.12 (f)	7052.39 (c)	2751.88 (jk)	3541.3 (h)
P.p. Zn	527.15 (t)	1207.28 (r)	1587.93 (q)	1784.2 (op)	1033.67 (s)	1216.13 (r)
P.p. Cu	113.36 (uv)	185.71 (uv)	101.61 (uv)	96.63 (uv)	61.59 (v)	63.51 (v)
P.p. Cr	188.51 (uv)	227.16 (uv)	206.36 (uv)	266.86 (uv)	173.42 (uv)	213.29 (uv)
P.p. Ni	145.21 (uv)	160.16 (uv)	116.54 (uv)	204.02 (uv)	140.65 (uv)	98.28 (uv)
P.p. Fe	2619.0 (kl)	2034.0 (mn)	2602.1 (kl)	3658.28 (h)	3286.54 (i)	2908.24 (j)
P.p. Al	1931.1 (no)	3230.1 (i)	1723.2 (pq)	2096.0 (m)	6756.91 (d)	10296.6 (a)
P.p. Sr	2512.18 (l)	3166.7 (i)	2911.67 (j)	3155.82 (i)	3982.59 (g)	2601.38 (kl)

MER: metal extraction ratio, **PENs:** plant effective number of the shoots, **PENT:** plant effective number total plants, P.p.: Potential of phytoremediation (g ha^{-1})

edulis. *M. cordifolium* exhibits the highest MER for Al in both growing systems. Remarkably, Zn, Cu, Cr and Ni presented the lowest MER and MAE values, due to the hindrance of their uptake and accumulation

likely attributed to competing effects with highly absorbed metals, notably Cd.

The lower PENs and PENT values, the lesser the plant number needed to extract 1.0 g and the better

the extraction potential of plants. Table 5 showed the variation of PENs and PENT values depending of the target metal, species and cropping system (Table 5). The highest PENs value was attributed to *M. cordifolium* for Cu extraction. Thus, considering shoot, in *M. cordifolium*, 12,685 and 12,351 plants are needed to extract 1.0 g of Cu under mono and co-cropping, respectively (Table 5). The lowest PENs value matches with *M. cordifolium* for Al extraction, hence a smaller number of plants is required for Al decontamination. Consequently, only 76 plants of co-cropped *M. cordifolium* are required to extract 1.0 g

of Al. Now, since the total biomass (shoot + root) was involved in the evaluation of PENT, it's expected the latter will behave differently than PENs. The highest PENT was registered in mono-cropped *C. edulis* for Cu. Thus, an effective number of 8988 plants of mono-cropped *C. edulis* are required to extract 1.0 g of Cu (Table 5). Regardless the growing system, the three halophytes demonstrated the lowest PENT values for Fe and Al extraction.

In order to extrapolate the greenhouse pot experiment results to in-field application, the potential of metal extraction per hectare was evaluated below.

Table 6 Evaluation of soil contamination indices: (Geo-accumulation factor (Igeo), Enrichment factor (EF), contamination factor (CF) and Soil Pollution Index (PI)) and final metal concentrations in the mining soil subjected or not (unplanted control) to phytoremediation growing three halophytes: *S. portulacastrum*, *C. edulis* and *M. cordifolium* for seven months duration under mono or co-cropping systems. Each value is the mean of five replicates. For the same parameter, means marked with the same letter are not significantly different at $p=0.05$. **Note:** n.a signify not applicable

Parameters	Unplanted control	Monoculture <i>S. portulacastrum</i>	Mono-culture <i>C. edulis</i>	Monoculture <i>M.cordifolium</i>	Co-cropping
Igeo (Cd)	2.10 (a)	1.48 (cd)	1.57 (c)	1.9 (b)	1.42 (d)
Igeo (Zn)	-0.31 (f)	-0.34 (f)	-0.38 (f)	-0.35 (f)	-0.36 (f)
Igeo (Cu)	-0.91 (g)	-4.49 (o)	-2.56 (l)	-1.73 (j)	-3.45 (m)
Igeo (Mn)	-5.22 (r)	-5.22 (r)	-5.22 (r)	-5.22 (r)	-8.31 (t)
Igeo (Cr)	0.72 (e)	0.70 (e)	0.73 (e)	0.72 (e)	0.67 (e)
Igeo (Ni)	0.68 (e)	-2.2 (k)	-1.07 (h)	-0.36 (f)	-1.34 (i)
Igeo (Fe)	-3.84 (n)	-4.71 (q)	-5.70 (s)	-4.57 (op)	-4.66 (pq)
EF (Cd)	62.0 (c)	73.9 (c)	158.2 (a)	89.2 (b)	68 (c)
EF (Zn)	11.6 (efgh)	20.7 (ef)	40.5 (d)	18.6 (efg)	19.6 (efg)
EF (Cu)	7.6 (efgh)	1.2 (h)	8.8 (efgh)	7.1 (fgh)	2.3 (h)
EF (Mn)	0.38 (h)	0.70 (h)	1.41 (h)	0.64 (h)	0.08 (h)
EF (Cr)	23.6 (e)	43.03 (d)	88.04 (b)	39.4 (d)	40.5 (d)
EF (Ni)	23.06 (e)	5.72 (gh)	24.8 (e)	18.5 (efg)	9.95 (efgh)
EF (Fe)	n.a	n.a	n.a	n.a	n.a
CF (Cd)	6.45 (a)	4.21 (d)	4.47 (c)	5.60 (b)	4.02 (d)
CF (Zn)	1.21 (f)	1.18 (f)	1.15 (f)	1.17 (f)	1.16 (f)
CF (Cu)	0.79 (g)	0.06 (klm)	0.25 (jkl)	0.45 (ij)	0.13 (klm)
CF (Mn)	0.04 (klm)	0.04 (lm)	0.04 (lm)	0.04 (lm)	0.04 (m)
CF (Cr)	2.47 (e)	2.45 (e)	2.50 (e)	2.48 (e)	2.40 (e)
CF (Ni)	2.40 (e)	0.32 (jk)	0.71 (gh)	1.16 (f)	0.58 (hi)
CF (Fe)	0.1 (klm)	0.05 (klm)	0.02 (lm)	0.06 (klm)	0.05 (klm)
PI	13.5 (a)	8.38 (d)	9.16 (c)	11.0 (b)	8.33 (d)
[Cd] mg Kg ⁻¹	19.36 (a)	12.63(c)	13.42(c)	16.81(b)	12.01 (c)
[Zn] mg Kg ⁻¹	363.4 (a)	354.0 (a)	345.2 (a)	351.1 (a)	349.0 (a)
[Cu] mg Kg ⁻¹	79.8 (a)	6.67 (e)	25.28 (c)	45.1 (b)	13.72 (d)
[Mn] mg Kg ⁻¹	80.63 (a)	80.26 (a)	80.26 (a)	80.35 (a)	80.35 (a)
[Cr] mg Kg ⁻¹	247.6 (a)	245.1 (a)	250.3 (a)	248.1 (a)	240.1 (a)
[Ni] mg Kg ⁻¹	120.4 (a)	16.31 (d)	35.52 (c)	58.25 (b)	29.46 (c)
[Fe] g Kg ⁻¹	5.22 (a)	2.84 (b)	1.44 (c)	3.14 (b)	2.96 (b)
[Al] g Kg ⁻¹	10.25 (a)	7.89 (b)	6.44 (c)	6.92 (c)	6.75 (c)
[Sr] g Kg ⁻¹	4.06 (a)	2.22 (bc)	2.03 (c)	2.42 (b)	2.22 (bc)

n.a signify not applicable

Thus, the highest Cd extraction efficiency consists of co-cropping of the three halophytes. The association of three species could remove 8120.5 g Cd ha⁻¹ by *S. portulacastrum*, 7052.4 g Cd ha⁻¹ by *C. edulis* and 3541.3 g Cd ha⁻¹ by *M. cordifolium* (Table 5). The maximum extraction of Sr was obtained by applying a monoculture of *M. cordifolium* and estimated to 3982.6 g Sr ha⁻¹. Co-cropped *M. cordifolium* is expected to provide the best Al extraction with 10,296.6 g Al ha⁻¹, while co-cropped *C. edulis* is expected to achieve the highest Fe extraction estimated to 3658.28 g Fe ha⁻¹ (Table 5).

3.6 Evaluation of Soil Pollution Status and Metal Content in Soil after a 7-Months Phytoremediation Period

The variation of soil contamination indices and final metal concentrations in the mining soil before and after being subjected to cropping or not (unplanted control) evaluated at the end of the phytoremediation period is presented in Table 6. Results showed that pollution indices and metal concentrations in the unplanted soil were almost identical to the initial crude soil (T₀). Indeed, the pollution index was 13.5 in the unplanted soil, against 13.8 in crude soil (T₀). Similarly, geo-accumulation, enrichment and contamination factors values of Cd, Cu, Zn, Mn, Cr, Ni and Fe remain unchanged. For planted soils, the pollution indexes were obviously decreased with respect to the unplanted soil (Table 6). This decrease depended closely on the plant species and the adopted cropping system. Thus, PI was reduced in *S. portulacastrum*, *C. edulis* and *M. cordifolium* by 38%; 32% and 19%, respectively (Table 6). In co-cropping system PI was diminished by 38.3%. The pollution indices I_{geo} and CF decreased also in the planted soil compared to the unplanted. Geo-accumulation index of Cd decreased by 29%, 25% and 10% in mono-cropped *S. portulacastrum*, *C. edulis* and *M. cordifolium*, respectively compared to the unplanted soil (Table 6). The depollution effect was higher under association, since I_{geo} value falls to 1.42 equivalent to 32% reduction versus the control. According to I_{geo} categories, these data indicate a decrease in Cd pollution level throughout this experiment since the unplanted control indicated a strong contamination $I_{geo} = 2.1$ ($2 < I_{geo} \leq 3$), (Table 6). Crude soil (T₀) was initially

moderately contaminated with Ni (I_{geo} value = 0.68; $0 < I_{geo} \leq 1$), showing negative value of I_{geo} ($I_{geo} \leq 0$) at the end of experiment so it becomes uncontaminated. For Cr, the pollution status remain moderately polluted ($0 < I_{geo} \leq 1$). Furthermore, the mean I_{geo} values for Zn, Cu, Mn, and Fe were lower than 0 suggesting an absence of soil contamination by those metals (Table 6).

After 7 months phytoremediation experiment a significant decrease in CF values was observed, notably for Cd. A reduction of 35%, 31% and 13% in CF values of Cd was recorded in mono-cropped *S. portulacastrum*, *C. edulis* and *M. cordifolium*, respectively. The depollution effect was higher under association, since CF value of Cd falls to 4.05 ($3 \leq CF < 6$), reflecting a considerable contamination soil corresponding to 38% decrease (Table 6). Based on CF categories, these data indicate a decrease in Cd pollution level compared to the unplanted control classified as very highly contaminated; $CF = 6.45$ ($CF \geq 6$).

For the other identified metals, moderately contaminated initial soil with Ni ($I_{geo} = 0.68$), showed negative values of I_{geo} at the end of experiment to become uncontaminated ($I_{geo} \leq 0$).

For Cr, the pollution status remain moderately polluted ($0 < I_{geo} \leq 1$) (Table 6). The mean I_{geo} values for Zn, Cu, Mn, and Fe were negative suggesting the absence of a soil contamination by those metals.

In mono-cropped *S. portulacastrum*, initial soil moderately contaminated with Ni (CF value = 2.40; $1 \leq CF < 3$), becomes low contaminated (CF value = 0.32 < 1) (Table 6). For Zn and Cr, the pollution status remain moderately polluted ($1 \leq CF < 3$). The mean CF values for Cu, Mn, and Fe were lower than 1 suggesting a low contamination by those metals ($CF < 1$).

The enrichment factor values showed a high variability since in this study, iron (Fe) was selected as a reference element in the calculation formula and the final Fe content changed over the species and cropping system (Table 6).

In general, a decrease in the final concentrations of the extracted metals namely, Cd, Ni, Fe, Al and Sr in the planted soil was observed. The final concentration of Zn, Mn and Cr did not vary compared to the unplanted control. For Cd, the mono-cropped *S. portulacastrum* (12.63 mg Kg⁻¹) and the association of three halophytes (12.01 mg Kg⁻¹) shared the lowest Cd concentration in treated soil.

In mono-cropping, *S. portulacastrum* and *C. edulis* presented the lowest concentration of (Cu, Ni) and Sr in soil, respectively. For Al, mono-cropped *C. edulis* and *M. cordifolium* as well as the association of the three halophytes shared the lowest Al concentration in the treated soil.

Taking into account that one whole plant (root + shoot) of *S. portulacastrum* was able to accumulate 12277 μg of Cd (7952 μg in the shoot and 4325 μg in the roots) when cultivated in mono-cropping, hence we estimate that 3 plants per pot are able to absorb 36831 μg Cd. Since the pot was initially filled with 5 kg of soil and the remaining Cd concentration was 12.63 mg Kg^{-1} , then the equivalent quantity per pot was 63.15 mg Cd pot^{-1} . Summing the absorbed Cd quantity 36831 μg Cd; equivalent to 36.83 mg with the remaining Cd quantity in the pot gives: $36.83 + 63.15 = 99.981 \sim 100$ mg, representing the initial Cd quantity in the pot (Table 6). Mono-cropped *C. edulis* was able to extract 33083 $\mu\text{g pot}^{-1}$ equivalent to 33.0 mg and the final Cd concentration in soil was 13.42 mg Kg^{-1} equivalent to 67.1 mg Cd pot^{-1} . Summing the absorbed Cd quantity 33.083 mg with the remaining Cd quantity in the pot at the end of experiment yields: $33.08 + 67.1 = 100.1 \sim 100$ mg. Likewise, mono-cropped *M. cordifolium* was able to extract 15.88 mg of Cd initially present in the soil and the final Cd concentration was 16.81 mg Kg^{-1} equivalent to 84.05 mg Cd pot^{-1} . The sum of absorbed Cd quantity 16.81 mg with the remaining Cd quantity in the pot at the end of experiment provides: $15.88 + 84.05 = 99.93 \sim 100$ mg (Table 6). Under co-cropping and given that one whole plant of *S. portulacastrum* is able to accumulate 16,887 μg of Cd, one whole plant of *C. edulis* is able to accumulate 14870 μg of Cd and one whole plant of *M. cordifolium* is able to accumulate 8531 μg of Cd, when co-cropped, the three plants are able to absorb 40288 μg Cd. The final Cd concentration in the soil was 12.01 mg Kg^{-1} equivalent to 60.05 mg Cd pot^{-1} . The sum of absorbed Cd quantity 40.28 mg with the remaining Cd quantity in the pot gives: $40.28 + 60.05 = 100.3 \sim 100$ mg (Table 6).

4 Discussion

Mining is of key importance for the socio-economic development of several countries. Unfortunately,

mining operations generate large quantities and diverse types of residues that cause significant environmental damage, affecting the quality of water, soil, and air. Among these issues, soil pollution is the most critical problem (Parraga-Aguado et al., 2014; Rodríguez-Tapia & Morales-Novelo, 2017). In Tunisia, as in other mining regions, phosphate industry generates various types of waste throughout the treatment process, particularly between extraction and commercialization phases (Zaghdoudi et al., 2025). To evaluate the resulting soil contamination, assessment methods are generally categorized as either qualitative or quantitative. Among the quantitative approaches, the most widely used indices include the Contamination Factor (CF), the Enrichment Factor (EF), and the Geo-accumulation Index (Igeo). These indices play a crucial role in determining both the extent and severity of soil contamination (Wu et al., 2021; Wu Jin et al., 2014). Moreover, they have been extensively applied in studies of mining and industrial sites, where they not only quantify contamination levels but also help to identify the potential sources of pollution anomalies, thereby providing valuable insights for environmental monitoring and remediation efforts (Gao et al., 2022). In this study, the mean concentrations of metals (Table 1), particularly Cd, Cr, and Ni, exceeded the environmental background levels established by international organizations such as the World Health Organization (WHO), the Food and Agriculture Organization (FAO), and the European Community (Chiroma et al., 2014). Besides, referring to Igeo, CF and EF categories, soil collected from M'Dhilla sector is strongly contaminated with Cd, moderately contaminated with Cr and Ni, and presents low contamination with Cu and Zn (Table 1). The Sr concentration in M'Dhilla soil (4.1 g kg^{-1}) was approximately 13 times higher than the reported global average (Edwards et al., 1998), highlighting severe polymetallic pollution likely resulting from extensive mining activities in the area. To address this environmental challenge, three halophytes from the family *Aizoaceae*, namely *Sesuvium portulacastrum*, *Carpobrotus edulis*, and *Mesembryanthemum cordifolium*, were selected for a phytoremediation trial M'Dhilla mining sector. Despite being non-native to the Gafsa Basin, these species are particularly well adapted to arid and desert environments like the Gafsa region, as they can withstand high salinity, drought, and extreme temperatures (Caparrós et al.,

2022). In addition to their resilience, these halophytes are recognized for their potential in heavy metal and salt phytoextraction (Ghafouri et al., 2022). They also offer significant benefits for soil stabilization, erosion control, and have ornamental use, further enhancing their suitability for land restoration (C. Y. Li et al., 2023). Their combined tolerance to harsh conditions and remediation capabilities make them promising candidates for restoring degraded soils affected by mining activities (Manousaki & Kalogerakis, 2011; Zaghoudi et al., 2025). To assess their effectiveness, the metal phytoextraction potential of these halophytes was evaluated using two cultivation systems: monocropping and co-cropping. After seven months, the quantities of extracted Cd in the shoots are considered important and reached in *S. portulacastrum* 7952 $\mu\text{g plant}^{-1}$, in *C. edulis* 6014 $\mu\text{g plant}^{-1}$ and 3650 $\mu\text{g plant}^{-1}$ in *M. cordifolium* (Table 4). Notably, co-cropping these species enhanced Cd accumulation in shoots by 31%, 47%, and 26%, respectively, compared to monoculture systems; a synergy likely driven by interspecific root interactions. Despite translocation factors (TF) remaining below 1, all three species exceeded the 100 mg kg^{-1} shoot concentration threshold for Cd hyperaccumulators (Reeves et al., 2018), underscoring their ability to compartmentalize metals in aerial tissues despite limited root-to-shoot transfer efficiency. These observed variations in metal uptake arise from species-specific physiological adaptations to metal stress, including differences in root architecture (e.g., depth and branching patterns) and exudate profiles, which directly influence rhizosphere conditions and metal bioavailability (Mehmood et al., 2021; Q. Zhang et al., 2020). In this experiment, monocultured *S. portulacastrum* and *C. edulis* developed more extensive root systems, which correlated with their higher metal acquisition efficiency compared to *M. cordifolium*. Additionally, metabolic adaptations in *S. portulacastrum* including enhanced vacuolar sequestration, accumulation of osmolytes, secretion of organic acids, and strengthened antioxidant defences enable it to sustain growth under high metal exposure (Van Oosten & Maggio, 2015; Zaier et al., 2010). These physiological mechanisms not only facilitate efficient metal uptake but also mitigate metal toxicity, contributing to its superior tolerance and accumulation capacity. The co-cropping of three halophytes significantly enhanced Cd extraction capacities compared to individual species. Cultivation

systems further modulate these dynamics; monocropping relies primarily on intrinsic plant tolerance and root-soil interactions, whereas co-cropping alters the rhizosphere through interspecies interactions, stimulating root proliferation and microbial activity (Breerton et al., 2020; Thomas et al., 2024). For instance, co-cropping increased root biomass and metal uptake efficiency in *M. cordifolium* compared to monoculture. Additionally, soil properties such as pH, organic matter content, and contaminant speciation critically influence metal bioavailability and uptake (Bonaventure et al., 2023). Notably, *S. portulacastrum* demonstrated hyperaccumulator potential for multiple metals, Cu (Lokhande et al., 2020), Pb (Zaier et al., 2010), Ni (Fourati et al., 2016), and Cd (Ghnaya et al., 2007), meeting or exceeding established hyperaccumulation thresholds while maintaining stable biomass; a critical indicator of stress tolerance. Likewise, *S. portulacastrum* accumulated 22.10 mg kg^{-1} Cd alongside Cr (49.82 mg kg^{-1}), Cu (35.10 mg kg^{-1}), and Zn (70.10 mg kg^{-1}) in tannery effluent contaminated soil within 125 days (Ayyappan et al., 2016). The species also exhibited polycyclic aromatic hydrocarbon (PAH) remediation potential, retaining 94% of absorbed phenanthrene in roots when grown in seawater (Zhenhua Zhang et al., 2012). Similar findings have emerged in related halophyte species, further highlighting their phytoremediation potential. For instance, *Carpobrotus rossii* accumulated 162 mg kg^{-1} of cadmium (Cd) in shoots after just 63 days of treatment (C. Zhang et al., 2016), and more recent studies have endorsed its effectiveness for Cd removal in arid regions (Miranzadeh et al., 2023). Also, *Carpobrotus edulis* exhibited dose-dependent Cd accumulation when exposed to concentrations of 50–100 μM (Hajri et al., 2023), demonstrating its capacity to tolerate and extract Cd under varying contamination levels. Additionally, the facultative halophyte *Salicornia persica* has shown promising arsenic (As) phytoextraction capabilities in saline soils (Patel & Parida, 2021), which reinforces the broader applicability of salt-tolerant species for heavy metal remediation. Collectively, these results underscore the exceptional adaptability of *Aizoaceae* halophytes in extracting both metallic and organic pollutants under saline conditions, positioning them as strategic and effective tools for rehabilitating industrially degraded ecosystems (S. Zhang et al., 2023). Although non-indigenous halophytes show

promise for phytoremediation in mining areas, native plants are more commonly used due to their better adaptation to local conditions. In Ecuador, two native woody plants, *Erato polymnioides* and *Miconia sp.*, from an artisanal gold mine were assessed for Cd, Pb, Zn, and Hg accumulation, indicating that *Miconia sp.* is suitable for Cd and Zn phytostabilization, *E. polymnioides* for Cd and Zn phytoextraction, and both species show potential for Hg phytoextraction (Chamba-Eras et al., 2022). While in Peruvian Andes, evaluating native plants in polluted post-mining soils of the Cajamarca region indicated that *Pernettya prostrata* and *Gaultheria glomerata* were suitable for Zn phytostabilization, and *Gaultheria glomerata* and *Festuca sp.* were efficient for Cd. While several native plants showed potential for Cd and Zn phytoremediation, none were effective for Pb, Cu, As, or Ag (Cruzado-Tafur et al., 2021). Not far in Brazil, the evaluation of the phytoremediation potential of *Baccharis dracunculifolia* and *Baccharis trimera*, two plants naturally growing in a copper mining tailings area in southern Brazil, showed a strong potential for phytoextraction of Zn, Cd, Cr, and Pb and phytostabilization of Ba and Ni in *B. trimera*, outperforming *B. dracunculifolia*, which was effective for phytoextraction of Pb and phytostabilization of Cu, Zn, and Ba (Afonso et al., 2022). In Morocco, the assessment of heavy metal tolerance and accumulation in native plants from an abandoned Pb/Zn mining site in eastern Morocco allows the identification of *Reseda alba*, *Cistus libanotis*, *Stipa tenacissima*, and *Artemisia herba-alba* as strong Pb hyperaccumulators, while *Stipa tenacissima* and *Artemisia herba-alba* showed promise phytostabilization effect for Cd/Cu and Cu/Zn, respectively (Hasnaoui et al., 2020). The efficiency in controlling heavy metal uptake, mobilization, and activity is a critical factor in the high accumulation capacity of halophytes. Many halophytes exhibit remarkable heavy metal accumulation due to their ability to sequester toxic ions in specialized structures such as trichomes or vacuoles, perform effective osmotic adjustment, and mitigate oxidative stress (Singh et al., 2023). Consequently, essential cytosolic processes are protected from the harmful effects of heavy metal-induced reactive oxygen species (ROS) through the production of ROS-scavenging proteins and molecular chaperones. Sulfur metabolism further supports heavy metal detoxification by enhancing the biosynthesis of sulfur-rich compounds,

including cysteine and other chelators (Sghayar et al., 2015; Van Oosten & Maggio, 2015). In addition to phytochelators, halophytes are rich in other chelators such as polyphenols and proline, which contribute significantly to protecting cell membranes from heavy metal toxicity (Kafle et al., 2022). A key strategy for detoxification involves the transport and sequestration of chelated metals, particularly through vacuolar compartmentalization in leaves, which effectively removes both non-essential and excess essential metals from the cytosol (Van Oosten & Maggio, 2015). This process depends on the high expression of membrane transporters, notably ABC transporters, which facilitate the movement of phytochelated metals like arsenic, cadmium, and mercury into vacuoles (Zhao et al., 2022). Moreover, osmoprotectants play an important role in heavy metal tolerance; similar to responses under water deficit and salinity stress, plants exposed to heavy metals accumulate significantly higher levels of proline up to five to six times more (S. Zhang et al., 2023). Studies on *Atriplex halimus L.* revealed that Cd exposure enhances osmotic adjustment by inducing the synthesis of compatible solutes such as glycine betaine (Singh et al., 2023), while free polyamines like spermidine and spermine increase under Cd stress, contributing further to osmotic balance and protection (Ghuge et al., 2023).

The integration of phyto-combined remediation strategies, such as the addition of exogenous substances, genetic modification of plants and enhancement by rhizosphere microorganisms, may overcome limitations associated with phytoremediation itself (Deng et al., 2024). Therefore, co-cropping appeared to be promising as phytoremediation tool for profitable land use either through the production of high biomass for biofuel, the extraction of valuable elements through phytoextraction, or the safe production of other valuable crops (Wang et al., 2018). The co-cropping of two plant species, *Chrysopogon zizanioides* (vetiver grass) and the legume *Medicago truncatula* (barrel clover) with a wheat straw biochar amendment was applied in an acid mine drainage-impacted soil from a gold mining region in South Africa and the influence of co-cropping was investigated on the phytostabilization and phytoextraction of Cr, Zn, and As (Thomas et al., 2024). For purifying vanadium-containing wastewater co-polluted by V, Cr, Cd and Pb, (Wang et al., 2018) used different cropping patterns of four terrestrial plants (*Artemisia*

seleniumensis, *Trifolium repens*, *Houttuynia cordata* and *Medicago sativa*). The co-planting of selected plants enhanced phytoremediation of polymetallic-contaminated wastewater suggesting that by reasonable co-planting involved mechanisms that would improve rhizosphere environment, such as the activities of rhizosphere microbes, root exudates, pH and redox potential (Wang et al., 2018). Yang and collaborators applied five cropping patterns for Cd remediation in farmland soils and concluded that the best phytoremediation potential goes to (oilseed sunflower–rape) and (peanut–oilseed rape) rotations which extracted 73.31 and 54.63 g/ha Cd, respectively (Yang et al., 2022). In the same context, other studies reported the importance of crop rotation and inter-cropping in phytoremediation of contaminated or saline lands (Deng et al., 2024; Zhou et al., 2020). The *Pteris. Vittata* crop intercropping with maize, ramie and castor crops had a positive significance for improving arsenic (As) remediation effect of *P. vittata* and reducing the As content in crops. Therefore, those modes could be applied in the practice of heavy metal contamination remediation along with crop production in farmland (Wan et al., 2021). The oil crop rotation, such as sunflower-sesame rotation, is reported a cost-effective for phytoremediation of heavy metals (Zhou et al., 2020). New cotton/halophytes intercropping systems such as cotton/*Suaeda salsa* intercropping (CSSI) and cotton/*alfalfa* intercropping (CAI) systems, are proposed to improve soil salinization and crop production based on the traditional monoculture cotton (MC) (Liang & Shi, 2021). In this study, phytoremediation assisted by *S. portulacastrum*, *C. edulis*, and *M. cordifolium* shows great promise for restoring mining sites, particularly in arid and saline environments. However, the broader global application of this approach faces several challenges, mainly due to the highly site-specific nature of soil and contamination conditions. The effectiveness of phytoremediation depends heavily on factors such as local soil composition, contaminant types and concentrations, climate, and edaphic characteristics (Dhaliwal et al., 2020). These factors vary widely across mining sites and can significantly influence plant survival, growth, and contaminant uptake (Deng et al., 2024). Plant adaptability is another critical factor for successful phytoremediation. Although these halophytes are naturally tolerant to salinity, drought, and extreme temperatures, their ability to thrive in

diverse mining soils and under different contaminant profiles may be limited (Ghughe et al., 2023). Therefore, successful replication of this method requires careful matching of plant species to the specific environmental and contamination conditions of each site (Martins et al., 2022). Additional challenges include slow remediation timelines, effective management of contaminated biomass, and limited agronomic knowledge regarding cultivation practices for these species. To overcome these obstacles, site-specific trials, integration with complementary remediation techniques, and rigorous monitoring are essential (Nedjimi, 2021). Despite these challenges, the use of these species can be successfully replicated in other mining sites globally under certain conditions. Their inherent tolerance to harsh environments makes them suitable candidates for arid and saline mining areas worldwide. Field trials and real-scale validations are crucial to adapt protocols to local conditions and optimize species selection, planting systems (monocropping versus co-cropping), and soil amendments (Parraga-Aguado et al., 2014). Multidisciplinary collaboration and standardized monitoring will support the development of best practices for their application across diverse geographic and climatic zones (Siyar et al., 2022). Furthermore, integrating these halophytes with local native species and microbial inoculants may improve plant establishment and enhance remediation outcomes. With these considerations in mind, phytoremediation using *S. portulacastrum*, *C. edulis*, and *M. cordifolium* can be effectively adapted and applied to a variety of mining sites worldwide, offering a sustainable and environmentally friendly solution for soil restoration in harsh environments.

5 Conclusion

Long term mining activities in M'Dhilla area in southwestern Tunisia has caused strong heavy metal contamination related to massive hazardous and poorly managed waste deposits. Soil is characterized by high level of contamination especially Cd, Sr, Cr, and Ni. Among different plants utilized for phytoremediation, halophytes are the promising candidates not only for the management of salinity affected ecosystems, but also for the remediation of heavy metal contaminated sites. The outcome of this work demonstrated the efficiency of the use of three halophytes

mono or co-cropped in a polymetallic mining soil to extract simultaneously several metals in particular cadmium. Hence, *S portulacastrum* and *C edulis* were able to survive and produce biomass on a highly contaminated soil. *M. cordifolium* benefited from association with the previous species to improve shoot and root growth. The association of three halophytes improved metal acquisition efficiency and phytoremediation potential in particular for Cd. This capability is concomitant to high potential of metal accumulation in tissues showed in these species. Based on Cd concentration and the biomass production, *S portulacastrum*, *C edulis* and *M. cordifolium* were able to extract 36.83%, 33.08% and 15.88% of Cd initially existing in the soil, respectively. The extraction potential of Cd was higher under co-cropping condition attaining 40.28%. The evaluation of soil pollution status and the final metal concentration at the end of experiment confirmed a decrease in soil contamination. Future study will be devoted to assess the ability of these halophytes for the in-field depollution of heavy metals contaminated mining areas.

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Data Availability All data included in this study are available upon request by contacting the corresponding author.

Declarations

Ethical Approval Not applicable.

Consent to Publish All authors have read and complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors.

Conflict of Interest The authors declare that they have no competing interests.

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