

Phytoremediation potential of native plants: Biomonitoring approach in contaminated soils

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Abstract

Phytoremediation has been used to remediate contaminated site. This study aims to investigate the potential of the selected native plants to accumulate and tolerate chemical pollutants. Two sites were selected in Southern Tunisia lands (Gabès), i) Gannouch located near the chemical industrial complex and ii) Zerkineis around 35 kilometres far from the industrial activities. Soil and plants were sampled and analysed for cadmium (Cd), lead (Pb), copper (Cu), zinc (Zn) and fluorine (F) concentrations. The native plants were *Bassia indica* and *Chenopodium album*, with no obvious morphological toxic symptoms. Results indicated that the upper soil layers were alkaline and salt affected with low organic matter content. The highest levels of fluoride and heavy metal were found in Ghannouch. The contents of Cd, Cu, Pb, Zn and F in the roots, stem and leaves of the native plants exceed the normal ranges and the phytotoxic level. Bioconcentration factor (BCF), translocation factor (TF), and biological accumulation factor (BAF) were determined for each element. Based on these factors the selected plants were classified as excluders, indicators, and accumulators for each element. The native plants, studied herein, seems to have significant potential for phytoremediation, thus they may be used as biomonitors in contaminated soils. *Bassia indica* identified as Cd, Cu and Pb accumulator, could be an excellent candidate for phytoextraction and phyto stabilization because of its F excluder performance.

Keywords: environmental pollutants; phytoextraction; phytostabilisation; soil contamination

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Introduction

The pollution of heavy metals is one of the major environmental issues nowadays. Through human activities like mining, farming, and industry operations, they can infiltrate the environment. Heavy metal soil pollution may harm crops, get into the food chain, and endanger human health. As a result, one of the most crucial environmental challenges is the removal of these heavy metals from the soil (Pouresmaieli *et al.*, 2022). Since 1960 the industrialization has been developing in Tunisia. The major industrial activities in Tunisia are textiles, building materials, electrical and chemical factories, about 1.200 of this industrial units are polluting ones (Henchi, 2019). Gabes city is the most contaminated zone in the country because of huge industrial complex activity; heavy metals and fluorides are the common inorganic pollutants which are found in Gabes lands environment. They are whispered through the emission of gas and particles into the atmosphere, solid waste: the phosphor-gypsum and by the industrial effluent (Ben Amor *et al.*, 2021; Ben Amor *et al.*, 2023).

Heavy metals including Pb, Cd, Cu, Zn, and fluorides are present in nature in low scales (20, 0.4, 20, 50 and 300 mg kg⁻¹ respectively) (Hong *et al.*, 2016; Saha *et al.*, 2017). They are extremely stable in the soil in Cd/Pb-phosphate minerals (e.g., pyromorphite) forms (Jiang *et al.*, 2023), or Cu and Pb organo-mineral complexes (Raj *et al.*, 2020) and Cd, Zn dissolved organic matter (such as humic acid) complex (Wong *et al.*, 2007). The incorporation of these pollutants can have detrimental effects on plant physiological and biochemical function (reduction in seed germination, plant nutrient content and inhibition of shoot and root length as well as decrease in plant protein content and regression of enzymatic activities (Kumar *et al.*, 2019)). Moreover, heavy metals enter food chains directly (by discharging effluents into soils) or indirectly (by using polluted water to irrigate crops) (Madhav *et al.*, 2020; Vardhan *et al.*, 2019), and consequently cause food contamination, thus posing a threat to human and animal health (renal dysfunction, liver and kidneys damage, impair lung function and increase the risk of lung cancer (Kumar *et al.*, 2019)). Globally, more than 10 million sites of soil pollution have been reported, with >50% of the sites contaminated with heavy metals and/or metalloids (such as arsenic, selenium...) (He *et al.*, 2015). Heavy metal pollution has a combined worldwide economic impact estimated to be in excess of US \$10 billion per year (He *et al.*, 2015).

There are many methods for soil remediation that can remove and reduce the dispersion of pollutants in the polluted sites (Li *et al.*, 2019; Vardhan *et al.*, 2019; Chen *et al.*, 2019); such conventional treatment techniques, called physicochemical methods have several limitations: high cost, low efficiency and possibilities of secondary contamination (Wang *et al.*, 2022). In contrast the bioremediation techniques such as phytoremediation have several advantages: more sustainable, economical and environmentally friendly approaches (Sharma, 2020).

The process of phytoremediation includes rhizo-filtration, rhizo-degradation, phyto-evaporation, phyto-extraction, phyto-stabilization, phyto-volatilization, phyto-degradation, phyto-desalination, and phyto-degradation. However, a number of variables, including the plant species, the characteristics of the medium, the bioavailability of the metal, and the inclusion of a chelating agent, had an impact on these pathways (Awa and Hadibarata, 2020).

Phytoextraction is a kind of phytoremediation technology that can be used to remediate contaminated soil with inorganic contaminants such as heavy metals, inorganic salts, and radionuclides. It requires plants that can uptake contaminants by the roots, then translocate and accumulate them to aerial parts: leaves and/or shoots (Parvez, 2022). Plants improve also the soil structure by increasing aeration, humidity, as well as promoting microbial growth (Chen *et al.*, 2019). Phyto-stabilization, which is also referred to phyto-immobilization or phyto-restoration prevents the dispersion of pollutants in the environment. The suitable plants species for phytoremediation should exhibit well developed root system and large biomass. Besides it should be able to tolerate high levels of inorganic pollutants. Native halophyte plant species such as *Phragmites australis*, *Phragmites karka*, *Aeluropus lagopoides*, and *Sporobolus virginicus* are interesting species for phyto-stabilization (Aziz and Mujeeb, 2022).

To cope with the pressure of the harmful components plant exhibit avoidance strategies by restriction of toxic metals uptake by roots and/or by the tolerance approach allowing plants to detoxify elements that invade cytoplasm (Skuza *et al.*, 2022). Based on these strategies, plants can be classified as indicators (growth decreased and showed toxic symptoms), excluders (restrict metals into the roots) and accumulators (accumulate metals in non-toxic forms with metalphytochelatins / metallothioneins complex, within their tissues without showing toxic symptoms) or hyper-accumulators (accumulate metals at levels 100-fold greater than those typically measured in common non-accumulator plants) (Lam *et al.*, 2022).

The main objectives of this study were to determine the ability of native plants to accumulate the chemical pollutants and select the best phytoextracted specie and the suitable phytoremediation technique.

Materials and Methods

Study area description:

The study area was Gabes city, located in the South-East of Tunisia. It is characterized by an arid climate; the monthly average temperature is around 15.8 °C in winter and 33.4 °C in summer. The precipitation is irregular, intense and the rainfall generally ranges between 112.2 and 290.5 mm. years⁻¹. Two fields were selected for this study based on the distance to the chemical industrial zone. Site 1: Ghannouch (33° 54' 59" N, 10° 05' 43" E) in the vicinity of the industrial complex which represents the main source of chemical pollution in the region. These phosphoric acid, di-ammonium phosphate, ammonitrate, and fluorine production factories cause a serious environmental problem by producing different forms of waste: industrial effluent, which is discharged into seawater, emission of gaseous and particulate pollutants. Phosphogypsum (PG) is the major solid waste that is stored near the factories (Elloumi *et al.*, 2017). Site 2: Zerkine located at 35 km South from the industrial activities (unpolluted site) (Figure 1).

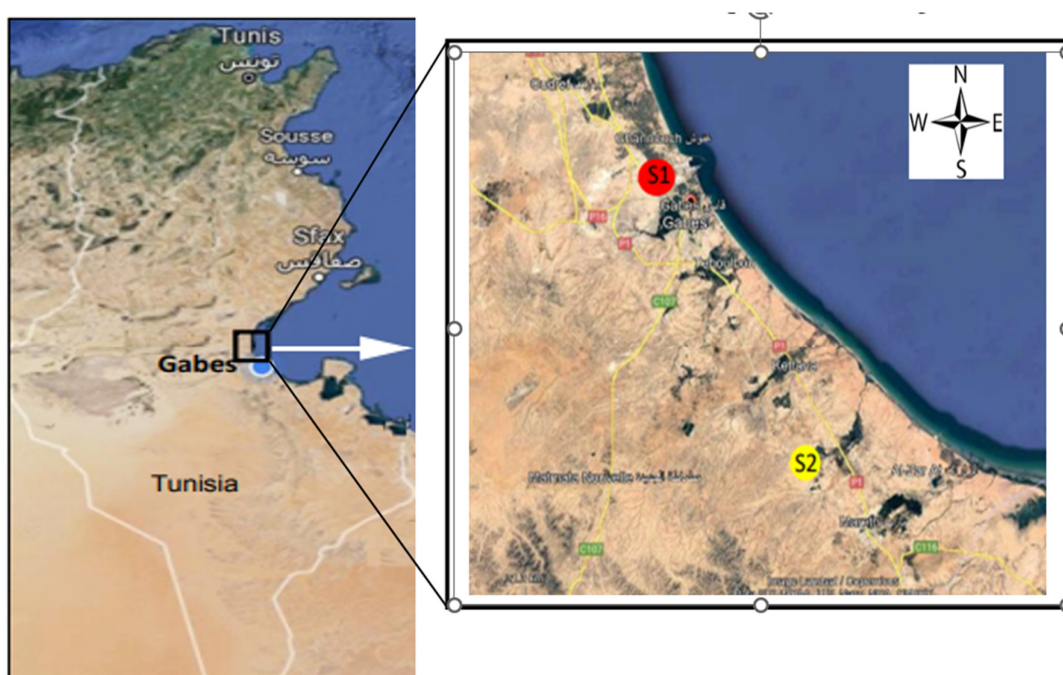


Figure 1. Location of the sampling sites in the South-East of Tunisia (map redrawn from <http://www.d-maps.com>). S1, Ghannouch; S2, Zerkine.

Plant sampling and chemical analysis:

Plant species were selected based on their abundance (number of plants per unit area), occurrence, (probability of encountering plants) and availability in the polluted zone. The two native species belongs to the Amaranthaceae family: *Bassia indica*, and *Chenopodium album*. Voucher specimens of plants were identified by professor Lotfi Abdallah from the Department of Life Sciences, Faculty of Sciences, at the University of Gabes, and kept at the herbarium of the Laboratory of Biodiversity and Valorization of Bioresources in Arid Zones (Faculty of Sciences, University of Gabes, Gabes, Tunisia) with the references LBVAZ-01/021 (*B. indica*) and LBVAZ-02/021 (*C. album*).

Completely randomized sampling of entire plants was collected from the studied sites. All plant samples were rinsed thoroughly in distilled water to remove dust contaminating their surfaces. Then after, stems, leaves and roots of each plant were separated and were oven dried in paper bags at 60 °C for 48 hours. Plant samples were crushed to powders with an electrical blender and stored at 4 °C. The concentrations of heavy metals in plant tissues were determined according to Buszewski *et al.* (2000). Briefly, 1 g of powdered sample was mineralized for 3 hours at 550 °C in muffle furnaces then digested with 50 ml of 1M nitric acid (HNO₃) and filtered through membrane of 0.45 µm cellulose acetate. Metal concentrations in each extract were measured by atomic absorption spectrophotometer (model Avanta GBC GF 3000 system, GBC Scientific Equipments, Australia). All samples were digested and analysed in 3 replicates. To determine fluoride concentrations, 1g of the grounded sample was incinerated with 8 g of mixture of carbonate potassium and carbonate sodium at 550 °C for 3 hours and digested with 50 ml of 1M hydrochloric acid (HCl) (Borjigin *et al.*, 2009). All digested samples were analysed by potentiometry and ion chromatography (Metrohm, 850 Professional IC, Herisau, Switzerland) (Zhao *et al.*, 2015).

Soil sampling and analysis

Soil samples from 0 to 20 cm depth were collected from both sites and sealed properly then transported to the laboratory. All soils oven dried (60 °C) during 72 h, to remove moisture. The pH measurement was carried out according to the international standard ISO 10390:2005 (Aydi *et al.*, 2023). Briefly, a suspension of the substrate in five times its volume of distilled water was prepared, stirred for 1 hour, then allowed to precipitation for at least two hours. The pH is then measured using a portable multi-parameter meter HI9828 (Hanna Instruments US Inc., USA). The electrical conductivity (EC) was determined according to the ISO 11265:1994 standard which consists of dissolving the electrolytes from substrates with water in a proportion of 1/5. After agitation and filtration, the EC (dS m⁻¹) was measured in the extract using multi-parameter (Hanna instruments) at 25 °C (Aydi *et al.*, 2023). The texture (particle size distribution) was estimated according to the ISO 11277 standard by the sieve–pipette method (Svensson *et al.*, 2022).

The soil organic carbon (SOC) content was analysed using Walkley–Black wet oxidation method (Tuffour *et al.*, 2014).

The percentage of the total calcium CaCO₃ (%) is estimated by the calcimeter Bernard method (Lamas *et al.*, 2005). Heavy metals were extracted by Aqua regia method (Venkateswaran *et al.*, 2007). 5 g of dried soil sample was weighed then 20 ml of concentrated HCl and HNO₃ (3:1, v/v) was added. The sample was placed on a heating plate at 150 °C and taken to dryness. Next, the residue was leached with 100 ml of 5% HNO₃. The extracts were filtered through filter paper (membrane of 0.45 µm cellulose acetate). The digestion and extracts were performed in triplicate. Heavy metals were determined spectro-chemically using atomic absorption spectrophotometer (Avanta GBC 3000)(Kenawy *et al.*, 2000). Fluoride content was determined using the potentiometric technique (Mezghani *et al.*, 2005).

Water analysis

pH was determined using pH meter; EC was measured using conductivity meter. The fluorine analysis was achieved by ion chromatography (Metrohm, 850). The determination of heavy metals in water via atomic

absorption spectrometry (Avanta GBC 3000). Briefly, 10 ml of samples was digested with concentrated 5 ml HNO₃ and 5 ml HCl. The mixture was gently stirred for homogenization, covered with watch glass and kept for one hour at room temperature. The samples were then heated on a hot plate until yellow fumes are released and the solution becomes clear. After cooling, at room temperature, the acid solution was filtered through the 0.45 µm cellulose acetate membrane, and volume was supplemented to 50 ml with deionized water (Fong *et al.*, 2006).

Phytoremediation factors

To evaluate the phytoremediation potential (phytoextraction/ phyto-stabilization) of the studied plant species, the following parameters were considered: Biological accumulation factor (BAF), Translocation factor (TF) and Bio concentration factor (BCF) (Saha *et al.*, 2017). These factors were calculated as shown below:

Biological accumulation factor (BAF) = Metal concentration in shoots / Metal concentration in soil

Translocation Factor (TF) = Metal concentration in shoots / Metal concentration in roots

Biological concentration Factor (BCF) = Metal concentration in roots / Metal concentration in soil

Statistical analysis

Data were expressed as the mean value ± standard deviation (SD) of tree replicate samples. The statistical analyses were done using the two-ways analysis of variance (ANOVA) procedure with the Statistical Package for the Social Sciences (SPSS) version 20.0 software (IBM Corp, released 2011, Armonk, NY, USA). The significance of variation in soil quality parameters between the polluted site and reference one was assessed using paired t-test. The significant differences between means were assessed by the Tukey's test at $P < 0.05$. Correlations between the concentrations of the elements in soil samples and between those concentrations in plants tissues and soils were evaluated using the Pearson's r coefficient at a significance level of $P < 0.01$ and $P < 0.05$.

Results and Discussion

Soil characterization

The retention and mobility of metals in soil are greatly influenced by its physico-chemical properties, which also impact the soil's overall quality (Hechmi *et al.*, 2020).

Granulometric results in the texture triangle showed that both soils were found to be sandy-loam with small amount of clay. The pH analysis revealed that all soil samples were mainly alkaline in nature. The carbonate percentages ranged from 10.77% to 15.86%. The mean soils EC varied from 2.6 to 3.02 mS cm⁻¹, which confirmed salt-affected soils (Alaya *et al.*, 2014). The studied soils displayed low organic matter content (0.98-1.47%) which reflect low biological activities and plant developments, moreover it assist the metal absorption by the plant species (Nasfi *et al.*, 2018). The findings of a paired sample t-test (Table 1) support the idea that the levels of all elements in the polluted site (Ghannouch) are statistically significantly higher than the levels of these elements in the non-polluted site (Zerkine). The concentrations in both studied soils have the following sequence: F > Zn > Pb > Cu > Cd.

Table 1. Soil characterization and metallic trace elements in the soils of both studied sites

Properties	Ghannouch	Zerkine
Texture	Sandy loam	Sandy loam
pH	8.00 ± 0.12 b	8.38 ± 0.17 a
EC (mS cm ⁻¹)	2.60 ± 0.30 b	3.02 ± 0.36 a
CaCO ₃ (total carbonate, %)	15.86 ± 0.95 a	10.77 ± 0.86 b
SOC (%)	0.98 ± 0.15 b	1.47 ± 0.21 a
Cd (mg kg ⁻¹)	2.38 ± 0.38 a	1.27 ± 0.15 b
Pb (mg kg ⁻¹)	38.34 ± 4.24 a	9.47 ± 2.82 b
Zn (mg kg ⁻¹)	154.00 ± 18.97 a	132.77 ± 9.37 b
Cu (mg kg ⁻¹)	4.30 ± 0.29 a	3.34 ± 0.2 b
F (mg kg ⁻¹)	1017.70 ± 173.46 a	246.89 ± 1.52 b

EC= electrical conductivity. SOC= soil organic carbon. Results are the means of three replicates ± SD. Numbers followed by a different letter within a line are significantly different at $p < 0.05$ according to least significant difference (LSD) analysis.

The high concentration of F indicates severe fluoride pollution (Table 1), which is caused by anthropogenic inputs such as phosphate fertilizer (Luo *et al.*, 2018). Actually, fluoride is typically found in soils together with micas and other clay minerals (García and Borgnino, 2015). Without naturally occurring phosphate and fluoride deposits, the total fluoride concentration in soils ranges from 20 to 1,000 $\mu\text{g g}^{-1}$ although organic soils often have lower F contents (Choudhary *et al.*, 2019). Similar average fluoride concentration were found by (Boukhris *et al.*, 2018); these results highlighted that the surrounding soils of the industrial phosphate factory (1017.7 mg kg⁻¹ F) are the most polluted due to the emissions of fluoride from the industry and through the untreated phosphogypsum (PG) which is stored in the vicinity of the factory (Ben Amor *et al.*, 2021). The level of fluoride in Zerkine is 246.89 mg kg⁻¹, it can be considered as geochemical background level compared to F average concentration in many soils of the world that ranged from 150 to 400 mg kg⁻¹ F (Kabata-Pendias and Szteke, 2015). High soil pH in this study may increase the availability of F. This was previously showed that alkaline pH due to precipitation of calcium carbonate, favoured release of fluoride (Maurya *et al.*, 2020). The high amount of fluoride increase the accumulation of organic matter content in surface soil as a result of the inhibition of the microbiological activity, thus reducing organic matter breakdown (Cronin *et al.*, 2000). Principal component analysis suggested that alkaline pH due to precipitation of calcium carbonate, favoured release of fluoride.

Concentrations of Pb (9.47 - 38,34 mg kg⁻¹), Cd (1.27 - 2,38 mg kg⁻¹) and Zn (132.77 - 154 mg kg⁻¹) were high in both sites (Table 1), while the average worldwide should not be higher than 0.53 mg kg⁻¹ for Cd, 64 mg kg⁻¹ for Zn and 20 mg kg⁻¹ for Pb in the topsoil (Kabata-Pendias and Szteke, 2015). However, Cu concentration varied between 3.3 to 4.3 mg kg⁻¹ in the Zerkine and Ghannouch soil respectively, it was lower than the average worldwide (20 mg kg⁻¹) (Kabata-Pendias and Szteke, 2015). Heavy metals contents corroborate findings of (Dahri *et al.*, 2018) and (Ben Chabchoubi *et al.*, 2021) who noted that the possible reasons for high concentrations of heavy metals within the studied areas (Gabes) might be the industrial and heavy traffic activities and agricultural practices. The alkaline soil pH and the carbonate abundance contents that promoted the metal and anions complexing decreased the metal solubility and bioavailability (Bashir *et al.*, 2020). Enya *et al.* (2020) indicated that high amount of heavy metals in soils especially Zn may inhibit the decomposition of soil organic matter by negatively affecting the microbial activities, even at low level. Results herein confirm that the soil of the first site (Ghannouch) was more polluted as compared to the second site (Zerkine). This pollution was mainly due to fluorine deposition as well as Pb and Cd.

Quality parameters of waters used for irrigation

The pH values ranged from 7.71 to 8.14, this shows that the North and south groundwater of the study area is generally alkaline. The EC of groundwater varied between 3.05 and 4.17 (Table 2), which is classified as very high salinity water as previously mentioned by Alaya *et al.* (2014). The increase of salinity of the groundwater is due to the prevalent geochemical processes in the region and to excessive groundwater pumping for irrigation (Alaya *et al.*, 2014). Leaching and good drainage are the basic management tools for controlling this type of salinity. The fluoride concentration in Ghannouch site (2.15 mg L^{-1}) was higher than in Zerkine site (1.66 mg L^{-1}), these findings exceeded the allowable limit of the World Health Organization (WHO) and Tunisian National Standard T09.14 ($F < 1.5 \text{ mg L}^{-1}$). According to (Agoubi and Gzam, 2016) the high level of fluoride in groundwater of Ghannouch in North Gabes is due to the natural origin through the rock-water interaction and anthropogenic sources such as agricultural activities and industrial activities mostly from phosphate industries, while in South Gabes the increase of Fluoride of the groundwater derived from the limestone and marno-gypsum formations and their interaction with water (Ketata *et al.*, 2011). The concentration of Cu, Pb and Zn did not exceed the guideline values of the Tunisian norm NT 106-002 and the WHO, respectively: Cu ($0.5 - 2 \text{ mg L}^{-1}$), Pb ($0.1 - 0.01 \text{ mg L}^{-1}$) and Zn ($5 - 3 \text{ mg L}^{-1}$). However, the level of Cd reaches 0.035 mg L^{-1} (Table 2) exceeding the admissible values ($0.005 - 0.003 \text{ mg L}^{-1}$). This indicate that the shallows aquifers are very vulnerable to anthropogenic pollution such as agriculture fertilizer.

Table 2. Chemical properties of the water of the studied sites

Properties	Ghannouch	Zerkine
pH	$7.710 \pm 0.27 \text{ b}$	$8.140 \pm 0.04 \text{ a}$
EC (dS m^{-1})	$4.170 \pm 0.30 \text{ a}$	$3.050 \pm 0.61 \text{ b}$
Cd (mg L^{-1})	$0.035 \pm 0.01 \text{ a}$	$0.031 \pm 0.01 \text{ b}$
Pb (mg L^{-1})	BDL	BDL
Zn (mg L^{-1})	BDL	BDL
Cu (mg L^{-1})	$0.083 \pm 0.01 \text{ a}$	$0.076 \pm 0.01 \text{ b}$
F (mg L^{-1})	$2.150 \pm 0.06 \text{ a}$	$1.660 \pm 0.02 \text{ b}$

EC= electrical conductivity. Results are the means of three replicates \pm SD. Numbers followed by a different letter within a line are significantly different at $P < 0.05$ according to least significant difference (LSD) analysis.

* BDL: Below detection limit.

Heavy metals contents in plants

The accumulation of Cd, Zn, Cu, Pb and F by native plants species growing in the industrial zone in Ghannouch were assessed. Each plant species was compared to the same species collected from the agricultural area in Zerkine (Table 3) Both plant species *Bassia indica* and *Chenopodium album* grown at the industrial site showed higher concentration of heavy metals as compared to those grown at the reference site (Table 3). The metals concentrations of plants grown in Zerkine area ranged from $0 - 1.93 \text{ mg kg}^{-1}$ for Cd, $0.9 - 5.63 \text{ mg kg}^{-1}$ for Pb, $28.16 - 84.33 \text{ mg kg}^{-1}$ for Zn, and $1.5 - 7.43 \text{ mg kg}^{-1}$ for Cu, whereas the plants grown in polluted exhibited concentrations reaching $0.35 - 5.58 \text{ mg kg}^{-1}$ for Cd, $2.8 - 67 \text{ mg kg}^{-1}$ for Pb, $49.67 - 117 \text{ mg kg}^{-1}$ for Zn, and $3.43 - 9.37 \text{ mg kg}^{-1}$ for Cu. The highest Cd concentration were found in leaves and roots of *Bassia indica* grown in Ghannouch, respectively 5.58 mg kg^{-1} and 3.63 mg kg^{-1} . While *Chenopodium album* showed only 0.44 mg kg^{-1} Cd in leaves and 0.56 mg kg^{-1} in roots. According to WHO, (Kabata-Pendias and Szteke, 2015), both collected plants species that exceeded the maximum values of 0.02 mg kg^{-1} and $0.1 - 3 \text{ mg kg}^{-1}$ respectively, which would be considered as toxic to plants. However, none of them showed Cd hyper-accumulation significant higher ($> 100 \text{ mg kg}^{-1}$) in their tissue. Therefore both studied species could not be identified as Cd hyper-accumulator (Krämer, 2010). Cd hyper-accumulating species have been recorded in various families such as *Thlaspi caerulescens* (2130 mg kg^{-1}), *Azolla pinnata* (740 mg kg^{-1}) (Reeves, 2006).

The Pb accumulation reached significant high levels in stem of *Bassia indica* (67 mg kg^{-1}) followed by leaves (56.58 mg kg^{-1}) while the maximum Pb concentration in *Chenopodium album* has been reported in leaves (6.37 mg kg^{-1}). The Pb concentration levels recorded were above the maximum allowable values by the Food and Agriculture Organization (FAO)/WHO (0.3 mg kg^{-1}) and (Krämer, 2010) ($0.6 - 28 \text{ mg kg}^{-1}$) but did not exceed the allowable threshold for Pb hyper-accumulation ($>1000 \text{ mg kg}^{-1}$). Some species are known to hyper-accumulate Pb, as *Deschampsia cespitosa* (966.5 mg kg^{-1}), *Thlaspi rotundifolium* (8200 mg kg^{-1}) (Midhat *et al.*, 2019).

Table 3. Heavy metal accumulation (mg kg^{-1}) in different organs of the native studied plants among sites

Site	Plant organ	Cd	Pb	Zn	Cu
<i>Bassia indica</i>					
Ghannouch	Roots	3.63 ± 0.2	54.16 ± 1.2	76.91 ± 3.4	4.61 ± 0.3
	Stems	2.41 ± 0.1	67 ± 1	95.56 ± 0.8	3.53 ± 0.1
	Leaves	5.58 ± 0.15	56.58 ± 0.47	117 ± 8.2	9.37 ± 1.5
	Accumulation	L > R > S	S > R > L	L > S > R	L > R > S
Zerkine	Roots	1.93 ± 0.12	1.57 ± 0.2	59.5 ± 1.3	3.3 ± 0.25
	Stems	1.38 ± 0.12	5.63 ± 0.15	84.33 ± 2.8	2.3 ± 0.2
	Leaves	1.08 ± 0.14	1.28 ± 0.07	76.5 ± 1.5	7.43 ± 0.2
	Accumulation	R > S > L	S > L > R	S > L > R	L > R > S
Site X Organ	<i>p</i>	<.001	<.001	0.069 (NS)	<.001
<i>Chenopodium album</i>					
Ghannouch	Roots	0.56 ± 0.04	2.8 ± 0.2	80.67 ± 0.6	3.43 ± 0.4
	Stems	0.35 ± 0.11	3.85 ± 0.15	59.33 ± 2.0	4.87 ± 0.1
	Leaves	0.44 ± 0.07	6.37 ± 0.12	49.67 ± 1.6	7.81 ± 0.5
	Accumulation	R > L > S	L > S > R	R > S > L	L > S > R
Zerkine	Roots	BDL	0.9 ± 0.06	62.5 ± 1.0	1.5 ± 0.1
	Stems	BDL	1.15 ± 0.1	32.16 ± 1.2	2.6 ± 0.2
	Leaves	BDL	2.03 ± 0.02	28.16 ± 1.5	4.1 ± 0.6
	Accumulation	---	L > S > R	R > S > L	L > S > R
Site X Organ	<i>p</i>	NS	<.001	0.004 (NS)	<.001

Results are the means of three replicates \pm SD. R = roots, S = stems, L = leaves.

*BDL: Below detection limit.

NS: not significant.

Zn concentration in leaves and stems ranged from 28.16 mg kg^{-1} to 117 mg kg^{-1} and from 32.16 mg kg^{-1} to 95.56 mg kg^{-1} respectively, with the highest level in leaves and stems of *Bassia indica* while in plants roots it varies from 59.5 mg kg^{-1} to 80.67 mg kg^{-1} , with the highest level in roots of *Chenopodium album*. The level of Zn in plant samples grown in both sites exceed the tolerable limit according to (WHO 1996) which is 0.60 mg kg^{-1} . The concentration of Zn in *Bassia indica* tissues reached the phytotoxic level ($100-300 \text{ mg kg}^{-1}$), suggesting it as a Zn tolerant plant. According to (Krämer, 2010) none of the collected plant species can be considered as Zn hyper-accumulator because the level of Zn in their aboveground tissue did not exceed the value of Zn hyper-accumulation ($> 10.000 \text{ mg kg}^{-1}$). Nowadays, few plants are considered as Zn hyper-accumulator such as *Thlaspi caerulescens* and *Thlaspi chroleucum* with 8.000 mg kg^{-1} and 2.000 mg kg^{-1} , respectively (McGrath *et al.*, 1997).

Copper concentration in both native plants were low, in the aerial parts but remains higher than root contents. The highest Cu content was observed in *Bassia indica*'s leaves and roots at the polluted site, reaching respectively 9.37 mg kg^{-1} and 4.61 mg kg^{-1} , while the lowest content was observed in *Chenopodium album* roots (1.5 mg kg^{-1}) at the reference site (Zerkine). The values of Cu in tissues of the aerial parts were less than 30 mg kg^{-1} considered as phytotoxic Cu concentration in plants. None of the studied plants species reached Cu hyper-

accumulation level ($>1.000 \text{ mg kg}^{-1}$). Many studies reported Cu hyper-accumulator plants, such as *Pandia kametallorum* (6.270 mg kg^{-1}), *Vigna dolomitica* (3.000 mg kg^{-1}) and *Actiniopteris sp* (3.535 mg kg^{-1}) (Krämer, 2010; Kabata-Pendias and Szteke, 2015).

Generally, the heavy metals accumulation depends on plant species and metal concentration in soils. In order to avoid toxicity of high level of heavy metals, plants develop tolerance mechanisms such as sequestration of metals in roots endodermal cells or the soluble metals accumulated in root symplasm may translocate into shoots through xylem by binding with organic acids and amino acids in the xylem vessels (Sheoran *et al.*, 2009). In addition, the plant roots release exudates that alter the chemical features in the rhizosphere and can influence the availability of metals in that zone or site. Root exudations, low molecular weight compounds (i.e.: citrate) (Lutts and Lefèvre, 2015) decrease the soil pH, thus, the acidification of rhizosphere increase heavy metals solubility. Subsequently the free heavy metals ions enter the roots cells and transport to aboveground tissues (Xu *et al.*, 2019). This was reported that a total of 313 heavy metal associated transporters (HMATs) widely distributed in 17 transporter (membrane proteins) families were found to be responsible for heavy metal uptake, transport, and translocation in plants (Yang *et al.*, 2022).

Zn and Cu are essential elements for plants, both metals have specific membrane transporters as zinc-regulated transporters (ZRT) of the ZIP (ZRT / IRT-like proteins) family, and heavy metal ATPases (HMA) proteins (Claus *et al.*, 2013; Printz *et al.*, 2016) in plant cells that enhance Zn and Cu uptake by plants and make evident their high concentration in the selected plants of this study. In contrast, Cd and Pb are not essential and do not have specific membrane transporters in plants (Yan *et al.*, 2020). The absorption and the translocation of these elements could be achieved by cation/ H^+ antiporter (CAXs) and ATPases that promote the passage of cations through the membrane system, changing the location of different ions including H^+ , Na^+/K^+ , H^+/K^+ , Ca^{2+} and lipids, transporting Cu, Ag, Cu, Zn, Cd, Pb and Co (Zuran *et al.*, 2021). Furthermore, the bioavailability of metals for plant uptake is also affected by the environmental factors such as heavy metal concentrations, organic matter and particularly soil pH and redox potential (Eh). This was previously claimed that, in alkaline soils (pH within the range of 7.1–8.1), the bioavailability of metals to plants are lower, and the presence of organic matter can inhibit metals uptake from the soil solution (Zwolak *et al.*, 2019; Peng *et al.*, 2020). Eid and Shaltout, (2016) reported that *Bassia indica* could accumulate Pb and Zn in large quantities and was identified as Pb hyper-accumulator in soil with acidic nature but not in alkaline soils reported herein. Hashem *et al.* (2019) and Zulfiqar *et al.* (2021) proved that *Bassia indica* and *Chenopodium album* have an important Cd tolerance. Moreover, the salt affected soil increase the Cd uptake by plants is due to the formation of a Cl-Cd complex (Smolders, 2001), and the disruption of metabolic activities by the generation of reactive oxygen species, which cause damage to membrane lipids and proteins (Abdal *et al.*, 2023). In this study Zn concentrations in *Chenopodium album* were higher than those indicated by (Malik *et al.*, 2010). Results herein corroborate findings of this native plant growing on industrial sludge (Chandra *et al.*, 2020).

Fluoride concentration in plants

The fluoride content in plants tissues varied from 44.11 mg kg^{-1} to 603 mg kg^{-1} (Table 4). Plants F accumulation was higher in Ghannouch site than in Zerkine one, these results were comparable to those of soil pollution (Table 1). The maximum F concentrations were observed in *Bassia indica* roots (603 mg kg^{-1}) and leaves ($370.33 \text{ mg kg}^{-1}$) at Ghannouch site, while in *Chenopodium album* these concentrations reached $178.67 \text{ mg kg}^{-1}$ in roots and 91 mg kg^{-1} in leaves at the same site. Whereas in Zerkine areas the highest levels of F were found in leaves of *Bassia indica* ($127.42 \text{ mg kg}^{-1}$) followed by stems of *Chenopodium album* ($119.58 \text{ mg kg}^{-1}$). For the roots, F contents reached 104.7 mg kg^{-1} and 99.67 mg kg^{-1} respectively in Ghannouch and Zerkine sites.

Table 4. Fluoride accumulation (mg kg^{-1}) in roots, stems and leaves of the studied plants in among sites

Plant specie	Site		
	Organs	Ghannouch	Zerkine
<i>Bassia indica</i>	Roots	603 \pm 63.6	104.7 \pm 4.3
	Stems	148.67 \pm 48.0	54.84 \pm 5.5
	Leaves	370.33 \pm 26.7	127.42 \pm 1.2
	Accumulation	R > L > S	L > R > S
<i>Chenopodium album</i>	Roots	178.67 \pm 19.7	99.67 \pm 6.0
	Stems	51.33 \pm 14.7	119.58 \pm 6.9
	Leaves	91 \pm 22.6	44.11 \pm 13.65
	Accumulation	R > L > S	S > R > L
Site X Organ	<i>p</i>	<.001	<.001

Results are the means of three replicates \pm SD.

R = roots, S = stems, L = leaves.

According to Kabata-Pendias and Szteke (2015), F levels in plant tissues range from 10 to 30 mg kg^{-1} . It seems that the alkaline conditions in soil increase F solubility, then its plant uptake which explain the highest roots accumulation in the two studied plants in Ghannouch site. Our results corroborate the findings of Hong *et al.* (2016). At high pH, an increasingly unfavourable electrostatic potential decreases the retention of the fluoride ion to soil and increases the F concentration in soil solution (Jha *et al.*, 2011). The increase of fluoride in the soil solution may also result from displacement of adsorbed fluoride, i.e., by the increased concentration of OH^- in soil solution at higher pHs (Yadav *et al.*, 2018). In addition, the atmospheric F deposition from the industrial emission could be another source of F to plant, thus the airborne F can reach the shoots tissues through the open stomata and cuticle at their surface and lenticels (Silva and Ferraz-Almeida, 2023). In this study both native plants exceed the phytotoxic values of F contents which indicates their high tolerance to F and their bioaccumulation abilities in contaminated environment. This could be an adaptative tool to sequester F in root tissues and event in accumulation in the photosynthetic organs. This was described the harmful effects of F on foliar water status, photosynthetic parameters, photosynthetic pigments, and cell membranes (Elloumi *et al.*, 2017).

Relationship between plants and soil

To assess the connection between plants and soil variables, correlation coefficients were determined between metal concentrations in above and below ground tissues and soil parameters. The data of correlation coefficients between heavy metals (pollutants) concentrations (Cd, Pb, Zn, Cu and F) in the studied native plants *Bassia indica* and *Chenopodium album* and those in soil are presented in Table 5. A highly significant positive correlation was detected between Pb, Cu and F in all the tissues (Cd except for *B. indica* stems) of both studied plants and the soil in the study areas. However, Zn concentrations in roots, stems and leaves of these native plants were poorly correlated with soil concentration.

Table 5. Correlation analysis of pollutants in the soil and plant tissues

Plant organ	Cd	Pb	Zn	Cu	F
<i>Bassia indica</i>					
Roots	0.83*	0.98**	0.56	0.96**	0.96**
Stems	0.69	0.97**	0.76	0.85*	0.90*
Leaves	0.88*	0.98**	0.48	0.81*	0.98**
<i>Chenopodium album</i>					
Roots	0.93**	0.99**	0.66	0.96**	0.94**
Stems	0.97**	0.96**	0.60	0.88*	0.89*
Leaves	0.95**	0.98**	0.59	0.89*	0.78

* correlation is significant at the 0.01 level;

** correlation is significant at the 0.05 level

The significant correlation in this study indicate that the capability of heavy metals accumulation of the studied plants could be related to the soil's heavy metals concentration. As the soil's pollutants increase, the plant's ability to accumulate them increase as well. Actually, different amounts of metal ions are absorbed by plants growing on metal-enriched substrates (Sharma *et al.*, 2023). This absorption is significantly controlled by the metals' bioavailability, which is in turn influenced by both internal and external (soil and plant-related) variables (Nouri *et al.*, 2009).

Phytoremediation factors

The data for BCF, TF and BAF are summarized in Table 6. These factors are usually used to evaluate a plant's ability for phytoremediation (Hossain *et al.*, 2022). BCF refers to the ratio of the element concentration in root to that in soil, it represents the ability of plant root to absorb the element from soil (Siyar *et al.*, 2022). The values of BCF in the plants ranged from 0.07 to 1.53. The order BCF values for various element in *Bassia indica* were as Cd > Pb > Cu > F > Zn, while their values in *Chenopodium album* were in the following order: Cu > Zn > Cd > F > Pb.

TF is an indicator of the capability of the plant species to translocate the pollutant from roots to shoots organs of plant, it defined as the ratio of the pollutant concentration of in stem and leaves to the roots (Mahamood *et al.*, 2023). The maximum TF was measured in *Chenopodium album*'s leaves (2.3) for Cu while the minimum was detected in *Bassia indica*'s stems (0.25) for F (Table 6).

Table 6. Phytoremediation factors of the selected plants

Plant specie	Factors	Cd	Pb	Zn	Cu	F
<i>Bassia indica</i>	BCF	1.53	1.41	0.5	1.07	0.6
	TF Stems	0.66	1.24	1.24	0.77	0.25
	TF Leaves	1.54	1.04	1.52	2.03	0.6
	BAF Stems	1.01	1.74	0.62	0.82	0.15
	BAF Leaves	2.34	1.47	0.76	2.18	0.36
<i>Chenopodium album</i>	BCF	0.24	0.07	0.52	0.80	0.18
	TF Stems	0.63	1.38	0.74	1.42	0.29
	TF Leaves	0.79	2.28	0.62	2.3	0.51
	BAF Stems	0.15	0.10	0.39	1.13	0.05
	BAF Leaves	0.18	0.17	0.32	1.82	0.09

The BAF are also called the enrichment factor, it indicates the ability of plants to accumulate the pollutant in their tissues faster than it is metabolized by the plant defence mechanisms (Chandra *et al.*, 2020). It is calculated as the ratio of the concentration of the pollutant in plant shoots to its concentration in soil. The

BAF values ranged from 0.05 to 2.34. The BAF in stems and leaves were in the following decreasing order: Cd > Cu > Pb > Zn > F (Table 6).

Based on these factors the selected plants can be divided into three categories excluders (BCF, TF and BAF) (Ahmad *et al.*, 2022); thus, the selection of the phytoremediation strategies is determined by the accumulation and exclusion abilities of plants to clean up the contaminated area.

Native plants classification and their use in phytoremediation strategies for Cd

The highest BCF for Cd was found in *Bassia indica* (1.53) while in *Chenopodium album* BCF= 0.24. The values of TF and BAF higher than one is recorded only in *Bassia indica* leaves. According to the criteria of phytoextraction (BCF, TF and BAF > 1) and plant classification, *Bassia indica* is identified as Cd accumulator and it has the potential for Cd phytoextraction. However, *Chenopodium album* is classified as Cd excluder and unsuitable for phytoextraction, thus it can be used as phytostabilizer in Cd contaminated soils (Figure 2). In contrast Eid and Shaltout, (2016) reported that *Bassia indica* is an indicator. The difference between the two findings can be explained by the fact that the contamination sources are different in both studies: a sewage sludge dump and an industrial complex. In the present study the industrial and vehicle emissions may be the major sources of the Cd deposition. Furthermore, the airborne Cd can be absorbed into the stem and leaves through apoplastic pathway (Yan *et al.*, 2020).

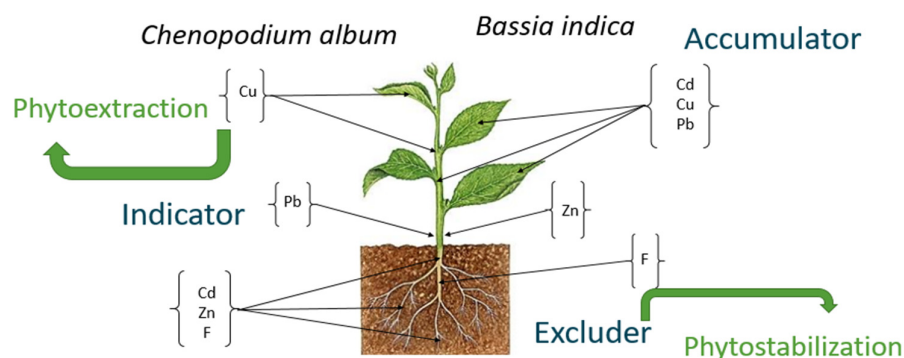


Figure 2. Phytoremediation strategies adopted by the two species among chemical pollution

Native plants classification and their use in phytoremediation strategies for Pb

The BCF values for Pb of *Bassia indica* were higher compared to BCF values of *Chenopodium album*. All the TF values in both plants were noted as > 1, this indicates the strong ability of these plants for Pb accumulation in their shoot tissues and therefore their phytoextraction ability (Egendorf *et al.*, 2020). The BAF values were recorded higher in *Bassia indica*. This later is classified as Pb accumulator because it reaches three criteria of Pb accumulator thus, it can be used to extract Pb from contaminated soil (Figure 2). These finding are similar to those reported by Eid and Shaltout (2016) who identified *Bassia indica* as Pb hyperaccumulator when grown at a sewage sludge dump site in Egypt. Whereas *Chenopodium album* reach only one criterion for phytoextraction (TF>1) and it is classified as Pb indicator. Similar results (BCF < 1, TF >1) were shown in studies by Malik *et al.* (2010) on wild plant species from industrial area of Islamabad, Pakistan. In comparison the study of Chandra *et al.* (2020) have reported that *Chenopodium album* growing on paper mill effluent sludge, is suitable for phytostabilization with BCF > 1 and TF <1.

Native plants classification and their use in phytoremediation strategies for Zn

Both plant species exhibited a BCF and BAF less than one. However only *Bassia indica* showed a TF > 1 in stems and leaves, it has a good capacity to translocate Zn from roots to the shoot's parts (Table 6).

Therefore, it is considered as Zn indicator and a good candidate for phytoextraction. Since the roots of *Chenopodium album* accumulated the highest amount of Zn in plant tissues (80.67 mg kg^{-1}) and were higher than those reported by Ugulu *et al.* (2022) when irrigated with wastewater in field conditions in Khushab, Pakistan. Thus, this native plant is described as a root accumulator, otherwise put, a Zn excluder and is then can be a suitable candidate for Zn phyto-stabilization.

Native plants classification and their use in phytoremediation strategies for Cu

The maximum BCF value for Cu was observed in *Bassia indica* (>1) and the lowest was measured in *Chenopodium album* (0.80). Both plants have recorded the highest TF and BAF values in their leaves (2.03, 2.3, and 2.18, 1.82 respectively). The accumulation factors values in the current study were higher than previously reported values (Nazir *et al.*, 2011) in plant species growing on contaminated sites in industrial areas in Pakistan. *Bassia indica* represent the three criteria (BCF, TF and BAF >1) which indicate that this plant is an accumulator of Cu, while *Chenopodium album* satisfied just two criteria, hence it is considered as a potential accumulator. Thereupon these native plants have the potential for phytoextraction of Cu (Figure 2).

Native plants classification and their use in phytoremediation strategies for F:

The BCF values of the two species were less than one, whereas *Bassia indica* characterized by high amount accumulation of F in the roots (603 mg kg^{-1}) and BCF = 0.6 compared to *Chenopodium album* which had BCF = 0.18 (Table 6). All TF and BAF values of plants samples were less than unity, this indicates that both plants have the capacity to restrict translocation of F from roots to shoots. Based on these results *Bassia indica* and *Chenopodium malbum* are considered as excluders of F. Therefore, they are an excellent plant for F phyto-stabilization to minimize this contaminate distribution in soils (Zhu *et al.*, 2018).

Conclusions

The findings in the present field investigation demonstrated that surface soils in Gabes area were alkaline and salt affected. Soils characterized by high level of contamination especially F, Cd, Pb, and Zn in the two sampling sites, which suggests that the long term of the anthropogenic activities such as industrial emissions and road traffic activities have caused Fluoride and heavy metal contamination. *Bassia indica* and *Chenopodium album* present a high ability to tolerate and accumulate several toxic elements in their tissues. *Bassia indica* was classified as Cd, Cu and Pb accumulator, a Zn indicator and an F excluder while *Chenopodium album* was classified as a Cu accumulator, a Pb indicator and Cd, Zn and F excluder. Hence the studied plants in this investigation may be used as biomonitors in contaminated site and may have the potential for phytoremediation.

Authors' Contributions

Conceptualization: SA and SSA; Data curation: AD and SA; Formal analysis: AD, SA and SSA; Funding acquisition: SA, NAM and G.B.S; Investigation: SA, AD and SSA; Methodology: SA, AD, AB and SSA; Project administration: SA; Resources: SA, G.B.S and NAM; Software: AD and RR; Supervision: CA; Validation: SSA, NAM and G.B.S; Visualization: AB and CA; Writing - original draft: AD and SA; Writing - review and editing: SA and SSA. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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