



## Study of the bioavailability of trace metals (As, Sb, Ni and Cd) in root soils and their transfer in the soil-plant system in the vicinity of the Sabodala mine tailings pond

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**Abstract:** Bio-oxidation mining of the Massawa refractory gold deposit generates large quantities of mine tailings contaminated with trace metals (arsenic, antimony, nickel and cadmium). Biotic environmental matrices are exposed to these toxic and non-biodegradable contaminants. The overall objective of this study is to investigate the bioavailability of trace metals and their transfer in the soil-plant system in the vicinity of the Sabodala-Massawa mine tailings pond. As part of this study, we sampled three plants (*Boscia angustifolia*, *Pterocarpus erinaceus*, *Hexalobus monopetalus* and *Combretum nigricans*) and their root soils. After sampling, we assessed the levels of trace metals in root soils, their bioavailability and the ability of plants to accumulate trace metals. This study clearly demonstrated that root soils (0-20cm horizon) have high average levels of trace metals (As, Sb, Ni and Cd) compared with the upper continental crust (UCC), in the following order: nickel > antimony > arsenic > cadmium. Sequential extractions using Tessier's procedure show that arsenic and antimony in root soils are not bioavailable. Chemical fractionation shows that nickel and cadmium associate preferentially with the residual fraction (50% for cadmium and 27% for nickel) and the sulfide-bound fraction (34% for cadmium and 26% for nickel). Soil-plant transfer of cadmium is very low, mainly due to its low bioavailability in soils. Nickel is found at high levels in some plants, far exceeding the permissible limit values (50 ppm). However, none of these species can be described as hyperaccumulative plants, since the ETMs content in their aerial parts does not exceed 1000 ppm. However, on the basis of analyses, it can be recognized that it has certain accumulative capacities, especially for nickel. The moderate levels of TMEs in plants are due to their low bioavailable fractions.

## 1. Introduction

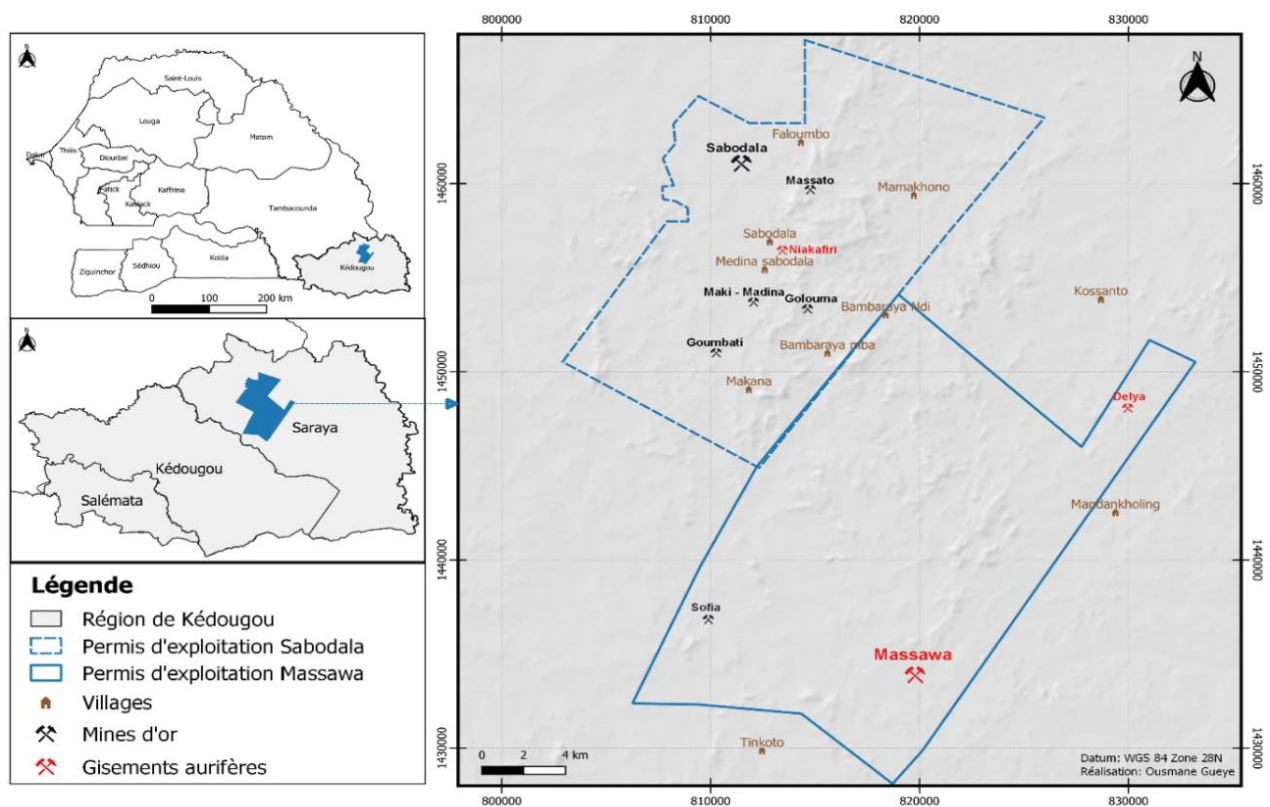
The exploitation of natural resources, especially mining, is at the root of environmental degradation that can locally disrupt the living conditions of local communities, in the absence of integrated waste management. Mining is generally detrimental to the environment and becomes one of the main sources of contamination and nuisance. In other words, the mining industry appears to be destruction of land and pollution of air and water (Belbachir *et al.* 2013; Hirwa *et al.*, 2019; Bazzi *et al.*, 2020; Dehkordi *et al.*, 1999). Mining extraction and crushing operations, the concentration of minerals and the disposal of mining waste are obvious sources of environmental contamination in all

its components (EL Hachimi, 2013). High concentrations of Trace Metal Elements (TMEs) such as arsenic, antimony, cadmium etc. in soils influence the uptake of these elements by plants (Lee *et al.* 2001; Fares 2021; Ben Ghnaya *et al.*, 2007, 2009; Küpper *et al.*, 1999). Sabodala, located in the Kédougou region of Senegal, is well known for its mining activities, particularly gold mining. Once the gold has been recovered, the tailings, which are likely to release trace metal elements into the environment, are discharged into a tailings pond. Studies have shown that mine tailings can contain high concentrations of heavy metals, which, without proper management, can contaminate soil, surface water and groundwater (Lottermoser, 2010; Errich *et al.*, 2023; Li *et al.*, 2024). These toxic trace metals can contaminate plant species, thus affecting their sanitary quality. Against this backdrop, we set out to assess the bioavailability of trace metals (As, Sb, Ni and Cd) and their transfer to the soil-plant system in the vicinity of the Sabodala-Massawa mine tailings pond. This approach will enable us to qualitatively and quantitatively estimate the degree of metal contamination of the surrounding soils and its impact on plant species: *Boscia angustifolia*, *Pterocarpus erinaceus*, *Hexalobus monopetalus* and *Combretum nigricans*.

## 2. Methodology

### 2.1 Geographical location of the Sabodala-Massawa mine

Situated in eastern Senegal, around 700 km from the capital, the Sabodala deposit is located in the Saraya department of the Kédougou region (Figure 1).



**Figure 1:** Location of the Sabodala mine

### 2.2 Sampling

In our study, both abiotic (root soils) and biotic (roots and aerial parts) environmental matrices were sampled. The sampling mission was carried out from 20 to 24-09-2023 during the wintering period.

The samples were taken in the vicinity of the tailings pond. Soil samples were taken using a shovel and a pickaxe, which allows us to dig and remove plant roots. The aerial and root parts of the plants were separated manually. In total, we obtained five plant species and their root soils. The species sampled were: *Boscia angustifolia*, *Pterocarpus erinaceus*, *Hexalobus monopetalus* and *Combretum nigricans*.

### 2.3 Treatment of root soils

Sample preparation is necessary for any chemical analysis. The samples were air-dried at room temperature. The samples were ground using an agate mortar and sieved using a < 63µm mesh sieve. The fine fraction was recovered for the various laboratory analyses.

### 2.4 Treatment of anatomical plant parts (leaves and roots)

The aerial and root parts of the plants are separated by hand. Both parts were rinsed well with demineralized water to eliminate any contaminants adsorbed on the surface. The roots and leaves are air-dried for around 48 hours. They are then crushed and sieved to a mesh size of less than 63µm. The fine part is kept in well-labelled plastic bags for analysis.

### 2.5 Analysis of soil physico-chemical parameters (pH, EC)

The principle consists of stirring a mass of soil in distilled water for two (2) hours with a ratio of 1/2.5 and 1/5 to measure pH and electrical conductivity respectively (Bocoum 2004). After stirring, the solution is filtered through 0.45µm filter paper. A HANNA multi-parameter was used to measure the physical parameters. We will use the Boccoum scale to better interpret the data (Table 1).

**Table 1:** Assessment of soil acidity and salinity based on pH and electrical conductivity (Bocoum, 2004)

Ph		Electrical conductivity	
Soil type		Type of soil	
< 4.5	Extremely acidic	< 250	Non-saline
4.6-5.2	Very acidic	250-500	Slightly saline
5.3-5.5	Acid	500-1000	Saline
5.6-6.0	Moderately acidic	1000-2000	Very saline
6.1-6.6	Slightly acidic	>2000	Extremely saline
6.7-7.2	Neutral		
7.3-7.9	Slightly alkaline		
8.0-8.5	Alkaline		
>8.6	Very alkaline		

### 2.6 Extraction of heavy metals from soils

Total TMEs were extracted from the soil using a mixture of nitric acid (HNO<sub>3</sub>), hydrofluoric acid (HF) and perchloric acid (HClO<sub>4</sub>) in accordance with standard *NF X 31-147*, *NF ISO 14869-1*. The principle

consists of digesting 1.2 g of soil in 2 mL 1 nitric acid (HNO<sub>3</sub>, 65%), 10 mL hydrofluoric acid (HF, 40%) and 2 mL perchloric acid (HClO<sub>4</sub>, 60%) in a 100 mL glass conical flask on a hot plate at 100°C for 2 hours. The hot solution was cooled and filtered through 0.45µm filter paper. The resulting solution was diluted with 50 mL of distilled water and stored in a polyethylene container. Trace metals were analysed by inductively coupled plasma optical emission spectrometry (ICP-OES) to determine the content of trace metals.

## 2.7 Metal bioavailability

In order to study the affinity of heavy metals with the different soil phases, sequential extractions were carried out. Sequential extraction of metals was carried out according to the procedure of (Tessier, et al. 1979). The procedure is based on the use of a set of chemical reagents selected for their ability to react with the different major parts of the soil and release the metals that accompany them (Table 2).

**Table 2:** Sequential extraction of trace metals

Fraction	Extraction solvent	Masse (g)	Stirring time	Temp (°)	Phase
F1	40ml 1M of MgCl <sub>2</sub> (pH=7)	5	1h	25	Exchangeable
F2	40ml 1M CH <sub>3</sub> COONa pH=5 with CH <sub>3</sub> COOH	5	5h	25	Carbonates
F3	20 ml 0.04 M of Chlorhydrate d'hydroxylamine NH <sub>2</sub> OH.HCl in 25% (v/v) CH <sub>3</sub> COOH, pH	1	5h	96	Fe-Mn oxide
F4	6ml of HNO <sub>3</sub> 0,02 M ; 10ml H <sub>2</sub> O <sub>2</sub> 30 %, pH 2 avec HNO <sub>3</sub> à 85 °C during 3h, and 10ml CH <sub>3</sub> OONH <sub>4</sub> in 20 % (v/v) HNO <sub>3</sub> , diluted in 10 ml, shake for 30 minutes	2	3h	85	Organique / sulphite
F5	10 ml HF + 2 ml HClO <sub>4</sub> + 2 ml HCl 12 N	1	2h	100	Residues

The extractions were carried out in five successive stages in 50 ml batches, on a mass (Table 2) of the granulometric fraction smaller than 0.63 µm of a dried and finely ground soil sample. The fractions extracted were as follows

- ✓ Fraction F1 ('exchangeable'): possible solubilisation of metals during sorption-desorption processes. Extracted with 8 ml 1M MgCl<sub>2</sub> at pH7; after 1 hour of agitation.
- ✓ Fraction F2 ('carbonate-bound'): results from possible solubilisation during pH change by 8 ml 1M CH<sub>3</sub>COONa, pH 5 with CH<sub>3</sub>COOH; stirring for 5h;
- ✓ Fraction F3 ('easily reducible'): liberation of metals bound to Mn oxides and Fe hydroxides: this consists of dissolving these oxides and hydroxides in the presence of a moderate reducing agent, in our case hydroxylamine hydrochloride NH<sub>2</sub>OH.HCl 0.04 M in 25% (v/v) and CH<sub>3</sub>COOH 96°C for 6 hours;
- ✓ Fraction F4 ('linked to organic matter and sulphides'): resulting from possible solubilisation during changes in redox conditions (oxidation) 3ml of 0.02 M HNO<sub>3</sub>; 5ml H<sub>2</sub>O<sub>2</sub> 30%, pH 2 with HNO<sub>3</sub> at 85°C for 3h, then 5ml CH<sub>3</sub>OONH<sub>4</sub> in 20% (v/v) HNO<sub>3</sub>, diluted in 20 ml, stirring for 30 mins;

- ✓ Fraction F5 ('residue' or 'clay fraction'; metals closely linked to the structure of the crystals, some of which may be well-crystallised sulphide crystals).

These different extraction stages provide a better understanding of the potential mobility and bioavailability of metals.

## 2.8 Extraction of heavy metals from plants (leaves and roots)

For the mineralisation of plant samples (leaves and roots), a mass of approximately 1g (powder) of each sample is first attacked with 10mL of oxygenated water (H<sub>2</sub>O<sub>2</sub>) to which 5mL of distilled water has been added. The sample was then left to stand for 24 hours to allow slow oxidation of the organic matter. Next, 10mL of nitric acid HNO<sub>3</sub> 65%, 10mL of perchloric acid HClO<sub>4</sub> 60% and 5mL of distilled water were gradually added. The mixture was stirred for 1 hour and filtered through 0.45µm filter paper. The resulting solution was diluted with 50mL of distilled water and stored in a polyethylene container. Finally, the trace metals were analysed by inductively coupled plasma optical emission spectrometry (ICP-OES) after extraction to determine the trace metal content.

## 2.9 Calculation of metal content in soils and anatomical parts of plant species

The TME content is calculated using the following formula:  $T(\text{ppm}) = (C \times V) / S$

- ✓ T = Trace metal content in mg/kg or ppm ;
- ✓ C = Concentration of the element in mg/l determined by ICP-OES ;
- ✓ S = Weight of the sample in g of matrix;
- ✓ V = Final volume in ml (50mL).

The root bioconcentration factor BFC is the ratio between the ETM concentration in the root parts (PR) and the ETM concentration in the soil. The FT is the ratio between the concentration of ETM in the aerial parts and the concentration of ETM in the root parts. The translocation factor is a parameter that can be used to check whether metals are stored preferentially in the aerial parts (FT > 1) or in the roots (FT < 1) (Deng et al., 2004).

## 3. Results and Discussion

### 3.1 Presentation of the plant species studied

Species collected in the vicinity of the Sabodala mine tailings pond. Table 3 shows the species and their families.

**Table 3:** Presentation of the plant species studied

N°	Plant species	Families
1	<i>Boscia angustifolia</i>	<i>Capparaceae</i>
2	<i>Pterocarpus erinaceus</i>	<i>Fabaceae</i>
3	<i>Hexalobus monopetalus</i>	<i>Annonaceae</i>
4	<i>Combretum nigricans</i>	<i>Combretaceae</i>

### 3.2 Physical and chemical soil parameters (pH and electrical conductivity)

The pH of the soils under vegetation is neutral according to Boccoum's acidity scale (2004). The electrical conductivity values of the root soils at the stations sampled ranged from 315 to 351  $\mu\text{s}/\text{cm}$ . According to the salinity scale, the electrical conductivity values in the under-vegetation soils are below 500  $\mu\text{s}/\text{cm}$ , so they are classified as non-salty to slightly salty (table 4).

**Table 4:** Physical parameters of root soils (pH and E.C)

Plant species	pH	C.E
<i>Boscia angustifolia</i>	6,84	351
<i>Pterocarpus erinaceus</i>	6,78	338
<i>Hexalobus monopetalus</i>	6,73	315
<i>Combretum nigricans</i>	6,79	322

### 3.3 Metal levels (As, Cd, Ni and Sb) in root soils

Table 5 Shows the levels of TMEs found in root soils, the average levels in the upper continental crust (UCC) and the World Health Organisation (WHO) target values.

**Table 5:** Trace metal levels (As, Cd, Ni, Sb) in root soil samples.

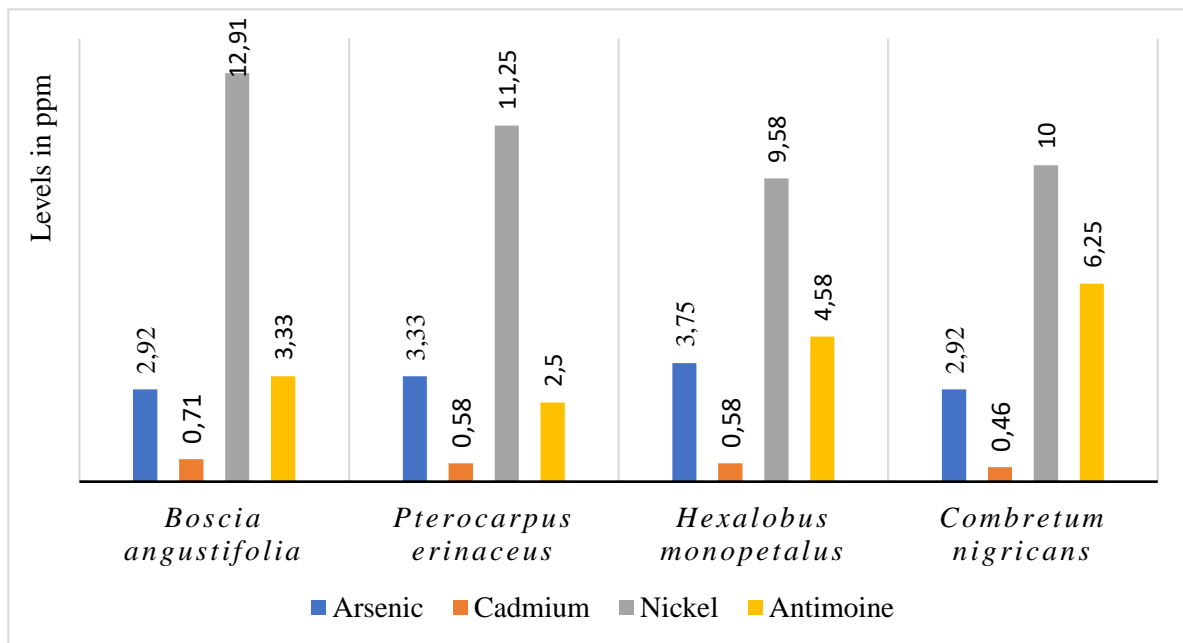
Plant root soils	Arsenic	Cadmium	Nickel	Antimoine
<i>Boscia angustifolia</i>	2.92	0.710	12.91	3.33
<i>Pterocarpus Erinaceus</i>	3.33	0.580	11.25	2.50
<i>Hexalobus monopetalus</i>	3.75	0.580	9.58	4.58
<i>Combretum nigricans</i>	2.92	0.460	10.00	6.25
CCS*	1.50	0.102	20	0.20
VC**	20 <sup>a</sup> ; 5 <sup>b</sup>	2.00	20 <sup>a</sup> ; 50 <sup>b</sup>	2.00

\*World average upper continental crust (UCC);

\*\*Target values, refer to maximum desirable levels of elements in unpolluted soils, Sources of Permissible Limits in Soils, WHO.

Analysis of root soils (0-20cm horizon) gave an idea of the levels of polluting metals (As, Cd, Ni and Sb). The results of these analyses reveal high quantities of trace metals compared with the upper continental crust (CCS). With the exception of antimony, the average concentrations of all the metals are below the maximum levels of metals desirable in unpolluted soils, as laid down by the WHO (Adagunodo, L.A. Sunmonu and M.E. Emeteri 2018). Trace metal levels in root soils vary in the

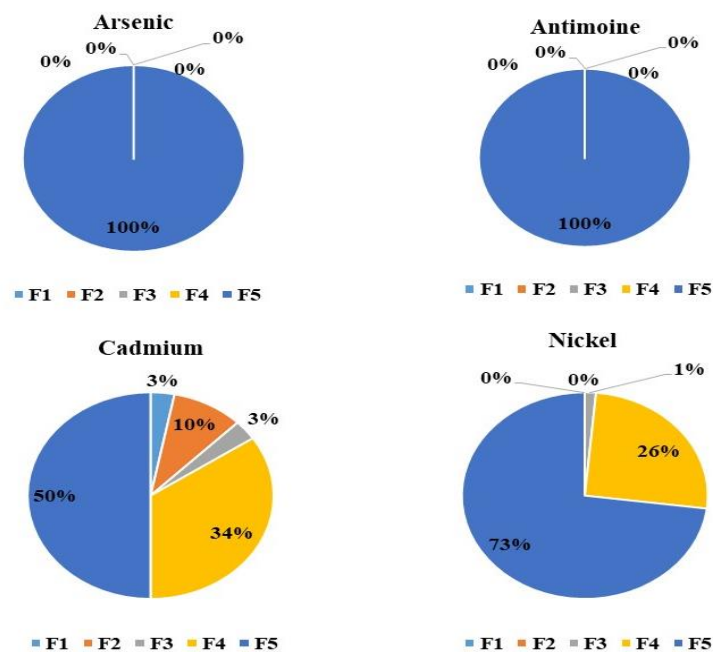
following order: nickel > antimony > arsenic > cadmium. The content of each metal is almost evenly distributed (Figure 2). This means that metals in root soils do not vary widely.



**Figure 2:** Comparative study of TME (Ni, Sb, As and Cd) levels in the root soils of the plant species studied

### 3.4 Study of the bioavailability of metals (As, Sb, Ni and Cd) in root soils

Figure 3 shows the distribution of arsenic, antimony, cadmium and nickel levels in the different fractions of under-plant soils.



**Figure 3:** Distribution of arsenic, antimony, cadmium and nickel content in the different fractions of soils under vegetation. F1: Exchangeable fraction, F2: Carbonate fraction, F3: Iron oxide and manganese fraction, F4: Sulphide fraction, F5: Residual fraction.

The study of the bioavailability of metals in soils makes it possible to predict potential metals for plant pollution. Metals that are often attributed to a geogenic origin are generally associated with the residual fraction, while metals derived from anthropogenic activities are generally distributed between the other fractions, namely organic matter, carbonates/sulphides, Fe/Mn oxy-hydroxides and phyllosilicates. In root soils, arsenic and antimony are not bioavailable (100% in the residual phase). There is no risk of contamination of biotic species. The presence of arsenic and antimony in soils is of geochemical origin. Cadmium is 50% in the residual phase. Nickel is preferentially associated with the residual fraction (Figure 3), with an average rate of around 50%. This association has been highlighted by several authors (Added, 1981, Lopez-Sanchez et al., 1996). Chemical fractionation (Figure 3) shows that nickel and cadmium are preferentially associated with the residual fraction and the fraction linked to sulphides (34% for cadmium and 26% for nickel). The association of these elements with the residual and sulphide fractions has been reported by several authors (Ramos et al., 1994, 1999; Zhou et al., 1998).

### 3.4 Assessment of plant contamination by trace metals (As, Sb, Cd and Ni)

The results show that the levels of arsenic and antimony in the anatomical parts of the plants (leaves and roots) are below the detection limit of the measuring device. These results confirm the non-bioavailability of these metalloids (As, Sb) in root soils. The results for nickel and cadmium are presented in Table 6.

**Table 6:** Comparative study of TME (Cd, Ni) levels in soils and anatomical parts of plants and metal bioconcentration and translocation factors

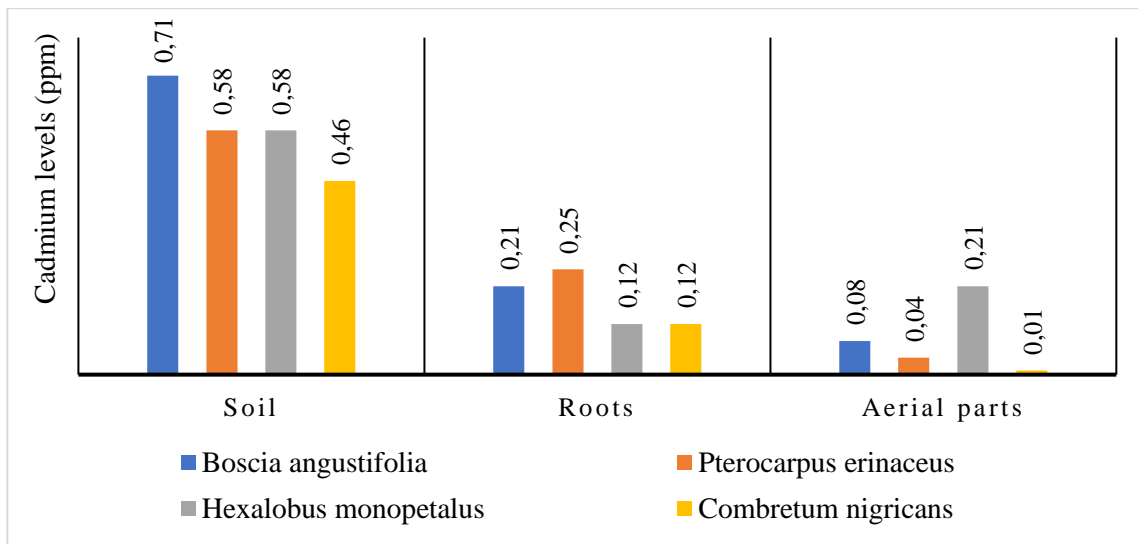
Nom	Leaves		Roots		Sol		FBC		FT	
	Ni	Cd	Ni	Cd	Ni	Cd	Ni	Cd	Ni	Cd
<i>Boscia angustifolia</i>	158,49	0,08	<0,01	0,21	12,90	0,71	0	0,29	15 849	0,40
<i>Pterocarpus erinaceus</i>	87,78	0,04	<0,01	0,25	11,23	0,58	0	0,43	8778	0,17
<i>Hexalobus monopetalus</i>	247,10	0,21	131,87	0,12	9,57	0,58	13,78	0,21	1,87	1,68
<i>Combretum nigricans</i>	146,43	<0,01	<0,01	0,12	9,98	0,46	0	0,27	14643	1

FBC= CR/CS : CR= Root concentration, CS= Soil concentration ; FT= CL/CR: CL= Leaves concentration; CR= root concentration

### 3.5 Comparative analysis of cadmium in the different matrices (soil, roots and aerial parts of plants)

The comparative study in the different matrices shows that cadmium levels in root soils are higher than those in the anatomical parts of plants (Figure 4). In plants, cadmium accumulates mainly in the roots, with the exception of *Hexalobus monopetalus*. Soil-plant transfer of cadmium is very low and is mainly due to its low bioavailability in soils (only 50% is bioavailable). Referring to the work of (Cottenie 1983), who set the maximum Cd content in plants at 0.01 ppm, we can report cadmium contamination in the plants studied.



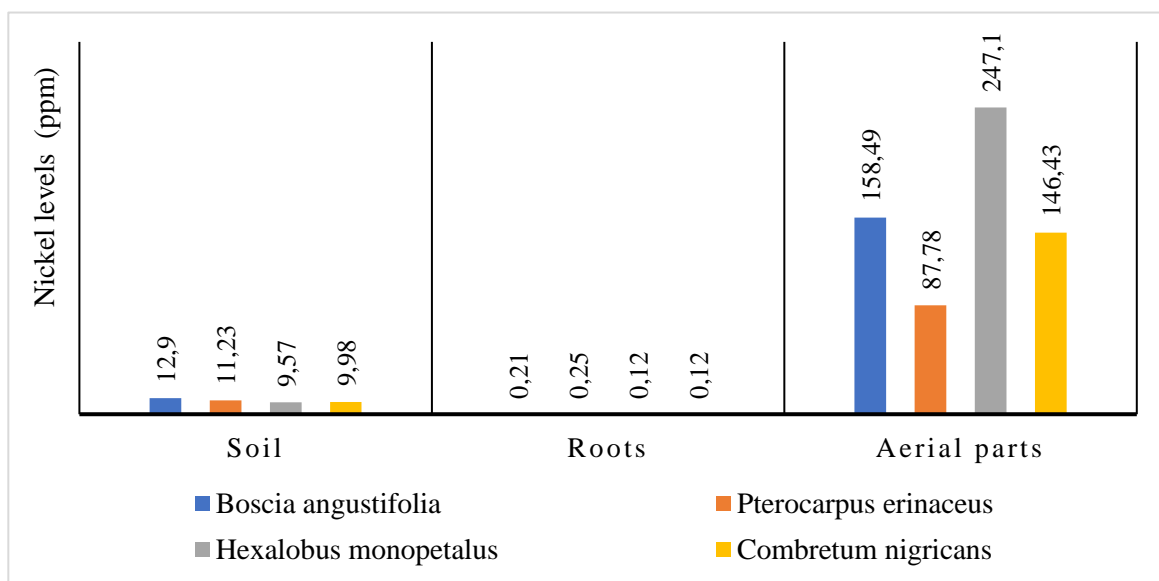


**Figure 4:** Changes in Cd levels in soils, aerial parts and roots

The bioaccumulation factors calculated for cadmium are less than unity, showing that this metal is not stored in plant roots. The translocation factors found are higher than those found for the plants *Asphodelus microcarpus* and *Juncus acutus* for the same metal growing near the Jin Chuantang mine, Huna province, (China) and the Jebel Hallouf-Sidi Bou Aouane mine, Bou Salem, Tunisia (Deng *et al.*, 2004; Chakroum *et al.*, 2006). The bioaccumulation of heavy metals can also affect the marine fishes (Karim *et al.*, 2016; Emon *et al.*, 2023; Wang *et al.*, 2024)

### 3.6 Comparative analysis of nickel in different matrices (root soils, roots and aerial parts)

Figure 5 shows the comparative study of Ni content in soils, aerial parts and roots. For nickel, plant species accumulate high levels in their aerial parts. Plants translocate the metal into their aerial parts to varying degrees. The plant *Hexalobus monopetalus*, with a storage rate of 247.1ppm in its aerial part, can be called a nickel-accumulating plant and a candidate for the treatment of nickel-contaminated soils by phytoremediation.



**Figure 5:** Comparison of Ni levels in soils, aerial parts and roots

For nickel (Ni), the normal value is set in the range 1-50 ppm (Mihali, *et al.* 2012); our results show that the plant species studied have nickel levels above the permissible limit. The translocation factors are greater than 1, showing that plants phyto-extract nickel. Nickel bioconcentration factors (BCFs) in plants are very high, with a maximum of 15849 (takarina, *et al.* 2017; Kanwar, *et al.* 2023). This reflects the high mobility of this metal in plants. These results are still much higher than the data reported by Kouakou *et al.* (2008): 11.98 to 41.69 ppm Ni in *Spinacia oleracea* leaves.

## Conclusion

In this work we assessed the bioavailability of trace metals (As, Sb, Ni and Cd) in root soils and the contamination of certain plant species by these elements, which are considered toxic. Trace metal levels in root soils vary in the following order: nickel > antimony > arsenic > cadmium. Sequential extractions using the Tessier procedure show that arsenic and antimony in root soils are not bioavailable. Chemical fractionation shows that nickel and cadmium are preferentially associated with the residual fraction and the fraction linked to sulphides. Plants in the vicinity of the tailings pond do not run any risk of being contaminated by arsenic and antimony, unlike cadmium and nickel. The species studied, which also grow in the vicinity of the tailings pond, have the capacity to store these pollutants, but to varying degrees from one species to another and between different parts of the same species. Cadmium is mainly found in the root parts of plants, while nickel is accumulated in the aerial parts. The low levels of TMEs in plants are due to their bioavailability.

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