



Toxicity assessment of garden soils in the vicinity of mining areas in Southern Morocco

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ABSTRACT

The aim of the present work is the assessment of the concentration, toxicity and phytoavailability of heavy metals in garden soils in the vicinity of three mines (A, B and C) in South of Morocco by using concurrently selective chemical extractions, MetPLATE™ a toxicity bioassay and plant growth experiments. The tailings materials containing very high concentrations of Mn, Cu and Co in mine A, Co, Mn, Cr and Ni in mine B and Cu and Zn in mine C. The high toxicity of tailings from mine C (86.7% inhibition) and moderate toxicity of tailings from mine B (51.0% inhibition) were mainly due to the relative high concentrations of soluble Cu and Zn. Nevertheless, the low metal toxicity observed in most garden soils was confirmed by the low metal concentrations in the soil water extracts. In all garden soils, *Lactuca sativa* L. and *Lolium multiflorum* L. contained in their shoots Cd, Co, Cr, Cu and Ni below toxic concentrations while Zn (in all soils) and Mn in two soils from mine A were accumulated at concentrations high enough to be considered phytotoxic. The low biomass produced on garden soils in the vicinity of mines B and C is explained by the relative low toxicity compared to mine A. Transfer factor values for Zn were higher than those found for Mn for both plant species, confirming that this element is present at lower bioavailable fraction in soil than Zn.

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1. Introduction

Anthropogenic activities, such as phosphate fertilizer application, irrigation with urban or industrial wastewater, mining operations are some of the main sources of heavy metal contamination in the environment [1]. Metal contamination in the vicinity of abandoned mining sites is an important problem in many countries [2–5]. Heavy metals (Cd, Cu, Pb, Cr and Hg) are major environmental pollutants, particularly in areas characterized by a strong anthropogenic activity.

Metals are quite persistent in the environment; non-biodegradable and are readily found in the atmosphere, aquatic environments and soils. Their presence in traces amounts may adversely impact the biota [5,6]. They can be accumulated on plants

via atmospheric deposition [7] and in plants [8], leading to potential serious health problems in humans and animals [5,9,10]. The accumulation of heavy metals in agricultural soils leads to the deleterious effects on plant growth and other biological processes in soils, as well as on the quality of agricultural products [10–13]. In addition to agriculture, edible plants are traditionally grown in home gardens [14,15]. Compared to forest or agricultural soils, the quality of garden soils is poorly known. There is a need for a better knowledge of characteristics of these soils to predict the risk of transfer of pollutants into the food chain, especially in urban and industrial environments.

Morocco, mainly the southern region, has a large number of metalliferous sites, some of which are being exploited while others have been abandoned [16]. These sites are concentrated in the region of Marrakech, Ouarzazate and in the North of the Anti-Atlas Mountains [17].

Both active and abandoned mines previously studied in Southern Morocco [3,16,18] contribute toxic metals (Cd, Cu, Zn, Pb, Mn) to surrounding soils, surface and/or underground water and thus may pose a potential risk to animal and human health. However, there is no data available on the impact of the mining activity on garden soils and agricultural crops grown in the affected area.

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The objective of this work was to assess the concentration, toxicity and phytoavailability of heavy metals in the garden soils in the vicinity of three mines in South of Morocco by using concurrently selective chemical extractions, toxicity using MetPLATE™ bioassay [16,19] and plant growth experiments.

2. Materials and methods

2.1. Sites description

The three mining sites (A, B and C) studied in this work are located in the South of Morocco in the region of Ouarzazate and Marrakech. They were selected to assess the concentrations and bioavailability of the heavy metals to plants from garden soils in the mining areas. The selection of the three mines was based on their ore composition and on our previous works [3,16] which showed that metal mining, a significant industrial activity in Southern Morocco, contributes toxic metals (Cd, Cu, Zn, Pb, Ag) to agricultural soils, surface and/or groundwater and thus may pose a potential risk to animal and human health. The mining sites A, B and C have been described in more details by Boularbah et al. [3] and Hakkou et al. [18].

2.2. Garden soils sampling

Soil samples were collected randomly in the cultivated upper horizon of garden soil profiles located in the vicinity of the three mining sites. Each soil sample was a composite of 10 subsamples collected in a given sector. In total, 16 garden soils were collected, six soils from mining area A, four from mining area B and six from mining area C. The garden soils were sampled in the village at different distances from the three mines (A, B and C) ranging from 10 to 800 m. 5–10 kg of soil samples were collected and transported to the laboratory. All samples were air-dried, homogenized and sieved through a 2 mm plastic screen before physicochemical analyses, toxicity assay and plant growth experiments.

2.3. Physicochemical characteristics and heavy metal concentrations of garden soils and their water extracts

Soils samples were analysed for particle size distribution, Kjeldahl total N, organic carbon and organic matter, Olsen-extractable phosphorus (P_2O_5) and pH in water (1:2.5 soil: water suspension). Total metals were extracted in 1 g soil with 7.5 ml HCl, 2.5 ml HNO_3 (NF IN ISO 11466). For heavy metals toxicity testing, 20 g of soil were extracted with 50 ml of deionised water and were shaken for 2 h at room temperature (25 °C). The soil samples were then centrifuged at $7000 \times g$ for 15 min. The soil extracts were stored in a refrigerator at 4 °C until chemical analysis and toxicity testing, using MetPLATE™ [16]. Concentrations of Cd, Co, Cr, Cu, Mn, Ni and Zn in soil and in water extracts were analysed by ICP-AES (inductively coupled plasma-atomic emission spectrometry).

2.4. Metal toxicity testing of garden soils extracts

The MetPLATE bioassay is specific for heavy metal toxicity and is based on the inhibition of the β -galactosidase of a mutant strain of *Escherichia coli* by the metallic pollutants [19]. Enzymatic activity was estimated according to the rate of degradation of the substrate Chlorophenol Red- β -D-Galacto Pyranoside (CPRG) to chlorophenol red the absorbance of which is measured at 575 nm. The test was applied to the garden soils and tailings water extracts. The MetPLATE™ methodology is described by El Khalil et al. [16].

2.5. Biomass and heavy metals uptake by plants

Plant growth experiments were conducted in five replicates pots containing 40 g of the garden soils sampled from the three mining sites. The pots were randomly arranged inside a greenhouse. Each pot received five lettuce (*Lactuca sativa* L. (*L. sativa* L.) or ryegrass (*Lolium multiflorum* L. (*L. multiflorum* L.) seeds. During the growth experiments, pots were maintained at 70% of water holding capacity with tap water and once every 15 days with Hoagland nutrient solution without metallic microelements (Cu, Mn and Zn). The crops were harvested after 2.5 months, rinsed thoroughly with deionised water, oven-dried (at 70 °C for 48 h) and weighed. The metal contents in the aerial part of the plants were determined as follows. The lettuce and ryegrass dried shoots were crushed in an agate mortar. A sample of 0.5 g of dry matter was then introduced into a mixture of 8 mL HNO_3 and 2 mL H_2O_2 , heated in a CEM type microwave oven (Mars 5). After filtration through ash-free filter paper (Prolabo) and adjustment in a 25 ml vial with HNO_3 0.1 M, the metals were analysed by inductive coupled plasma (ICP) and flame atomic absorption.

2.6. Analytical quality control and statistical analysis

Total metal concentrations were measured according to the common methods and following the standard (NF EN ISO 11 466). Quality control was based on the use of certified soil sample (BCR 141: Community Bureau of Reference, Commission of the European Communities-Brussels), samples from inter-laboratory comparisons, internal control samples and duplicates of the analysis. Quality control for plants was based on the use of certified samples (CRM 281 from BCR), samples from inter-laboratory comparisons, internal control samples, and duplicates of the analysis. A Pearson correlation index was determined to check the significance of the linear relations between obtained variables.

3. Results and discussion

3.1. Chemical properties of garden soils

The texture, physical and chemical characteristics of the 16 garden soils and the 3 tailings from the three mining areas are shown in Table 1. The garden soils were predominantly sandy soils and had a pH varying from 6.7 to 8.0. The pH values were in the range of those reported by Morel et al. [20] for urban garden soils in France and were similar to those reported by Boularbah et al. [3] for the agricultural soils (7.6 > pH < 8.8) sampled in the vicinity of five mines located in Southern Morocco. The high pH measured on garden soils was potentially limiting the risk of mobilization of metals.

The organic matter content varied between 2.5–7.3%, 2.7–6.6% and 2.5–4.3% for garden soils from mining areas A, B and C, respectively (Table 1). These values were generally higher in the garden soils than in most of local agricultural soils (mean value 1.8%), probably due to the manure inputs by gardeners [14]. The total N and available P contents in the garden soils were very low (<0.4% and mean value of 0.15% for total organic N and <1.7 mg g⁻¹ and mean value of 0.66 mg g⁻¹ for available P). This can be explained by the low inputs of mineral fertilization by the gardeners. However, the chemical fertility of garden soils largely exceeded the fertility of the mining soils and of the surrounding agricultural soils (0.08% for total organic N and 0.051 mg g⁻¹ for available P) [3] probably because gardening practices are more intensive than agriculture and are associated with waste recycling. As reported by Boularbah et al. [3], the tailings had lower content of organic matter A (0.6%), B (0.8%) and C (0.3%), total organic N and available P.

Table 1
Characterization of garden soils and tailings from three mining sites in South Morocco.

	Grain size distribution (%)			Organic C (%)	Organic matter (%)	Total organic N (%)	C/N	pH	P ₂ O ₅ Olsen (mg g ⁻¹)
	Clay	Silt	Sand						
Mine A									
GSA1	12.6	30.8	56.3	2.4	4.1	0.2	11.6	7.77	0.88
GSA2	12.8	30.8	56.0	2.4	4.1	0.2	11.9	7.76	0.49
GSA3	13.4	36.1	50.0	3.7	6.4	nd	ND	7.81	0.69
GSA4	12.2	36.0	51.3	4.2	7.3	nd	ND	7.94	1.70
GSA5	12.7	20.8	66.2	1.9	3.4	0.1	14.9	7.69	1.04
GSA6	13.4	21.3	65.1	1.5	2.5	0.1	9.6	7.84	0.86
TA	7.0	19.7	73.1	0.3	0.6	nd	ND	6.88	0.01
Mine B									
GSB1	13.6	12.9	73.3	2.1	3.7	0.1	33.3	7.70	0.25
GSB2	4.7	16.0	79.0	3.9	6.6	nd	ND	7.63	0.73
GSB3	13.2	9.3	77.4	3.5	6.0	0.4	9.2	7.53	1.23
GSB4	21.6	14.5	63.8	1.6	2.7	0.1	25.9	7.86	0.27
TB	1.8	4.0	94.2	0.4	0.8	nd	nd	7.97	1.78
Mine C									
GSC1	13.8	23.5	62.4	2.1	3.7	0.1	17.8	7.84	0.28
GSC2	9.7	16.7	73.3	2.1	3.6	nd	ND	6.72	0.62
GSC3	9.7	14.6	75.5	1.4	2.5	nd	ND	7.24	0.39
GSC4	10.9	16.4	72.5	2.1	3.7	0.1	17.8	7.17	0.48
GSC5	13.0	27.5	59.2	2.4	4.2	0.1	35.0	8.03	0.41
GSC6	12.5	23.2	64.1	2.5	4.3	0.1	22.9	7.30	0.19
TC	1.5	10.6	87.9	0.2	0.3	nd	ND	2.36	0.01

nd: not detected; ND: not Determined; GS: garden soil; T: tailing.

The heavy metal (Cd, Co, Cr, Cu, Mn, Ni and Zn) contents of tailings and garden soils in the three mining sites varied with the type of ore (Table 2). In general, the tailings had largely higher metal concentrations than the garden soils located in the vicinity of these sites. Tailings from Mn mine A contained high levels of Mn (4700 mg kg⁻¹), Cu (627.7 mg kg⁻¹) and Co (82.3 mg kg⁻¹). Tailings from Co–Ni mine B contained high levels of Co (1779 mg kg⁻¹), Mn (1396 mg kg⁻¹), Cr (667.6 mg kg⁻¹) and Ni (458.6 mg kg⁻¹). Tailings from mine C contained mostly Cu (1687 mg kg⁻¹) and Zn (513.2 mg kg⁻¹). The metal concentrations in the tailings (mine A, B and C) exceeded the European standards for Co, Cu and Mn in the tailing A; for Co, Cr, Cu, Mn and Ni in the tailings B; and for Cr,

Cu and Zn in the tailings C [21]. The industrial wastes are prone to wind and water erosion and are a source of metals for garden soils and edible vegetables through different exposure routes [2–4,7,16].

The garden soils from mine A (Mn mine) were highly contaminated with Mn (2179–3637 mg kg⁻¹) (Table 2). In this mine, all metals analysed (Cd, Co, Cr, Cu, Ni and Zn) had a lower metal concentration than the maximal tolerable values according to Kabata-Pendias and Pendias [21], except for Mn. The Mn concentrations in all garden soils largely exceeded the maximal value of 900 mg Mn kg⁻¹ [22]. The tailing dusts composed mainly by the black fine dry particles of the Mn oxides were disposed of near the mine. This ore is easily disseminated by wind erosion, leading to the contamination of the surrounding areas (soils, plants and vegetable gardens). The data published by Li et al. [12] in a Mn mine in Guangxi in South China showed that the Mn concentrations in the soils (2772 mg Mn kg⁻¹) were lower than the maximal values observed in the Moroccan Mn mine (3637 mg kg⁻¹). The high concentrations of Mn, measured in all garden soils (2179–3637 mg kg⁻¹) at the mining site A, may present a potential health risk for local human populations, as numerous studies have shown that Mn is potentially associated with the onset of Parkinson's disease [23,24]. Exposure to manganese dioxide from the tailings and contaminated garden soils via inhalation has been documented as regards miners and workers in smelting and alloy industries and could possibly affect the population exposed to the aerosols. The central nervous system (CNS) is a main target for manganese toxicity which leads to neurological disorders with symptoms (manganism) similar to those of Parkinson's disease [25]. Cell exposure to manganese dioxide nanomaterials causes cell death (apoptosis) due to the production of reactive oxygen species [26].

In (Co, Ni) mine B, the garden soils contained high concentrations of Co, Cr and Ni, exceeding the maximal tolerable values (40 mg Co kg⁻¹, 50 mg Cr kg⁻¹, and 60 mg Ni kg⁻¹) according to Kabata-Pendias and Pendias [21], except for the soil GSB1. The concentrations of Cd, Cu and Ni were higher than those in the garden soils studied by Morel and Schwartz [14] in France. The data published by Sipter et al. [27] in Gyöngyösoroszi, Hungary, near an abandoned Pb/Zn mine showed that the Cd and Zn concentrations

Table 2
Total metal concentrations in the garden soils and tailings of mining sites.

	Total metal (mg kg ⁻¹)						
	Cd	Co	Cr	Cu	Mn	Ni	Zn
Mine A							
GSA1	2.2	10.3	26.8	59.0	3 637	18.5	173.3
GSA2	1.2	8.9	21.5	45.3	2 179	15.0	95.5
GSA3	1.7	8.5	20.6	55.5	3 224	13.9	115.4
GSA4	0.8	9.5	20.0	55.9	3 428	13.3	156.0
GSA5	0.8	8.3	23.0	29.8	3 314	14.1	87.2
GSA6	0.7	9.0	23.8	25.4	2 340	14.1	83.8
TA	0.8	82.3	11.6	627.7	4 700	23.2	156.8
Mine B							
GSB1	0.1	35.6	6.7	31.5	336.8	82.8	53.9
GSB2	0.7	98.6	48.1	60.1	521.9	84.1	126.9
GSB3	0.9	109.1	67.3	67.1	559.9	53.1	182.1
GSB4	0.7	156.2	52.9	51.9	486.7	61.4	79.4
TB	0.7	1 779	667.6	166.9	1 396	458.6	146.6
Mine C							
GSC1	0.3	<ld	99.4	27.2	574.4	23.0	206.8
GSC2	0.2	7.6	78.1	282.4	618.2	32.0	327.9
GSC3	0.2	<ld	118.5	54.6	598.1	38.7	230.4
GSC4	0.3	<ld	115.5	175.5	557.4	32.7	322.5
GSC5	0.2	<ld	182.0	40.7	440.8	80.8	172.1
GSC6	0.2	<ld	115.3	33.1	666.9	41.1	186.3
TC	0.2	<ld	67.7	1 687	398.5	7.0	513.2

<ld: lower than detection limit; GS: garden soil; T: tailing.

Table 3
Toxicity and heavy metal content in the garden soils and tailings extracts from mining sites in South Morocco.

Garden soils	MetPLATE™ inhibition (%)	Water-extractable metal ($\mu\text{g l}^{-1}$)						
		Cd	Co	Cr	Cu	Mn	Ni	Zn
Mine A								
GSA1	Non-toxic	0.4	4.7	1.2	58.5	88.0	6.2	47.6
GSA2	8.6 ± 1.7	<ld	8.6	5.6	64.8	653.7	10.0	144.5
GSA3	4.6 ± 1.2	0.9	14.6	3.6	76.5	936.9	17.2	156.8
GSA4	Non-toxic	0.9	10.3	2.0	79.9	381.3	9.2	74.0
GSA5	3.8 ± 3.1	0.4	7.9	7.9	57.6	179.8	15.5	30.0
GSA6	Non-toxic	<ld	16.5	3.8	70.6	466.0	13.7	104.0
TA	Non-toxic	<ld	1.0	9.4	48.2	67.8	6.6	10.4
Mine B								
GSB1	15.4 ± 0.4	<ld	45.3	7.7	71.3	85.0	32.9	26.4
GSB2	22.7 ± 1.5	<ld	186.8	7.8	115.2	94.2	55.6	72.2
GSB3	6.4 ± 1.1	0.3	88.8	2.7	70.2	45.3	24.3	33.5
GSB4	6.0 ± 2.4	<ld	67.1	1.9	62.4	34.1	34.8	19.4
TB	51.0 ± 3.2	0.4	8 195	587.0	370.0	1 327	1 322	254.0
Mine C								
GSC1	16.8 ± 0.7	<ld	<ld	4.0	21.0	104.0	4.0	20.0
GSC2	38.8 ± 9.8	<ld	6.0	2.0	122.0	219.0	3.0	17.0
GSC3	22.3 ± 1.6	<ld	<ld	3.0	48.0	113.0	9.0	14.0
GSC4	29.7 ± 8.0	<ld	<ld	2.0	124.0	156.0	2.0	14.0
GSC5	12.7 ± 2.1	<ld	<ld	15.0	43.0	42.0	216.0	21.0
GSC6	31.2 ± 8.4	<ld	<ld	2.0	22.0	110.0	7.0	14.0
TC	86.7 ± 14.8	<ld	<ld	76.0	14 256	5 488	102.0	2 873

<ld: lower than detection limit; GS: garden soil; T: tailing.

in the garden soils (1.3 mg Cd kg⁻¹ and 366 mg Zn kg⁻¹) were higher than those found in mine B area.

As compared to the mine A and B, the garden soils from the mine C were moderately contaminated with Cu (up to 282.4 mg kg⁻¹) and Zn (up to 327.9 mg kg⁻¹) exceeding the maximal tolerable values for Cu (100 mg Cu kg⁻¹) and Zn (300 mg Zn kg⁻¹) according to Kabata-Pendias and Pendias [21]. The concentrations of Cu, Ni and Zn were much higher than those observed in the garden soils in urban and suburban areas in France [14]. All garden soils exceeded the maximal tolerable value for Cr (50 mg Cr kg⁻¹).

3.2. Heavy metal concentration and toxicity of garden soils water extracts

The MetPLATE™ toxicity tests [19] were conducted concurrently with metals (Cd, Co, Cr, Cu, Mn, Ni and Zn) analyses in water extracts of garden soils and tailings.

The percent inhibition, as determined by MetPLATE bioassay (Table 3), in the garden soils varied between 0% (non-toxic) and 8.6% in mine A, 6.0–22.7% in mine B and 12.7–38.8% in mine C. The results showed that only two (GSB1 and GSB2) out of the four soils from mine B and five (GSC1, GSC2, GSC3, GSC4 and GSC6) out of the six soils from mine C exhibited a relatively low toxicity (15–38% inhibition) whereas all soils from mine A and GSB3 and GSB4 from mine B were non-toxic. The comparison of the water metal concentrations to the MetPLATE™ EC₅₀s showed that the relatively low toxicity observed in the soils GSB2 (22.7% inhibition), GSC2 (38.8%) and GSC4 (29.7%) was mainly due to Cu concentrations lower than the EC₅₀ (220 $\mu\text{g Cu l}^{-1}$) determined by MetPLATE™ bioassay [19]. The tailings from mine C showed the highest percentage inhibition (86.7%) in comparison with tailings from mine A (non-toxic) and tailings from mine B (51%). This toxicity is confirmed by the high metal concentrations in the tailings C water extracts (76.0 $\mu\text{g l}^{-1}$ for Cr, 14,256 $\mu\text{g l}^{-1}$ for Cu, 5488 $\mu\text{g l}^{-1}$ for Mn, 102.2 $\mu\text{g l}^{-1}$ for Ni and 2873 $\mu\text{g l}^{-1}$ for Zn). In general, the low toxicity observed in most garden soils is confirmed by the low metal concentrations in the soil water extracts except soils GSA2 and GSA3 which contained Zn in their water extract exceeding the MetPLATE™ EC₅₀s. The MetPLATE™ EC₅₀s were obtained for free heavy metal cationic fractions,

generally considered to be more toxic [19]. According to Bitton et al. [19], Lin et al. [28] and Dam et al. [29], the non-toxicity of GSA2 and GSA3 could be attributed to the non-bioavailable chemicals species present in the water extract. Our results confirm those obtained by Boularbah et al. [3], which showed that the industrial soils from the Mn mine A showed a low toxicity using a semi-quantitative bioassay MetPAD™. This is explained by low metal concentrations in the water soil extracts.

The percent of extracted metals was low and did not exceeded 1% for all metals. The slightly basic pH of most soils (7.5–8) collected from three mines and the high organic matter contents due to gardening practices may reduce the mobility of heavy metals, thus reducing their bioavailability and toxicity [9,30].

3.3. Biomass and heavy metals uptake by plants growing in garden soils

In order to evaluate the suitability of using the garden soils from the three mining areas for growing crops. *L. sativa* L. (chosen as a model leafy vegetable) and *L. multiflorum* L. (chosen as model fodder grass) were grown in pots under controlled conditions. The aerial biomass produced showed a significant difference between the two plant species (Fig. 1). In general, most garden soils yielded a lower biomass for *L. multiflorum* L. than for *L. sativa* L. For *L. sativa* L., the plant biomasses obtained were lower than 42 mg dry matter plant⁻¹ in 2 soils out of the 6 from mine A (GSA3 and GSA4), 2 out of the 4 soils from mine B (GSB2 and GSB4) and 3 out of the 6 soils from mine C (GSC2, GSC4 and GSC6). The highest yield value (65.63 mg plant⁻¹ D.W) was obtained in the soil GSB3. For *L. multiflorum* L., the shoot biomass did not exceed 20 mg plant⁻¹ dry matter in 4 soils out of the 6 from mine C (SGC1, SGC2, SGC 4 and SGC5), 3 out of the 6 from mine A (SGA2, SGA4 and SGA5) and 3 out of the 4 from mine B (SGB1, SGB2 and SGB4); while the highest shoot dry weight (39.7–39.9 mg plant⁻¹ D.W) was observed in GSA6 and GSA3 from mine A.

The Cd, Co, Cr, Cu, Mn, Ni and Zn concentrations in the shoot of the two plant species grown on the garden soils were determined. In most soils, the Cd, Co, Cr, Cu and Ni displayed the lowest accumulation as compared to Mn and Zn (data not shown). The mean

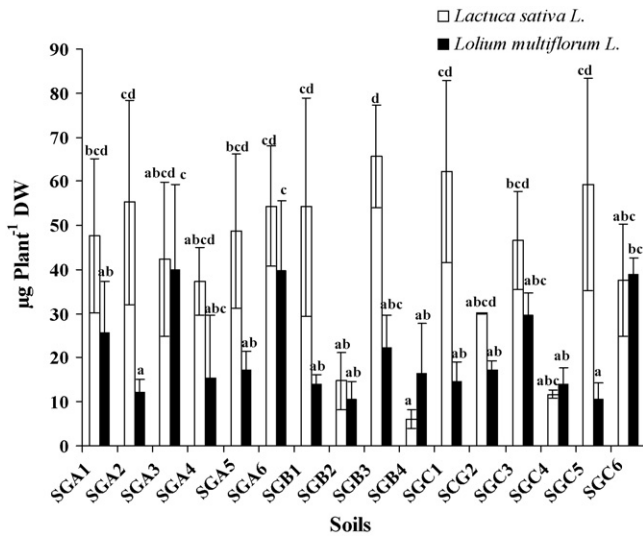


Fig. 1. Shoot biomass of two plant species grown on garden soils.

concentrations (mg kg^{-1} dry weight) in all the garden soils studied varied between < detection limit (dl) to 233 for Mn and < dl to 494 for Zn in *L. sativa* L. and between < dl to 780 for Mn and 155–354 for Zn in *L. multiflorum* L. (Fig. 2). In general, Zn exhibited a higher concentration in the shoots of the two plants than Mn. *L. sativa* L. is considered as accumulative for Cd and Zn [31]. The Cd, Co, Cr, Cu,

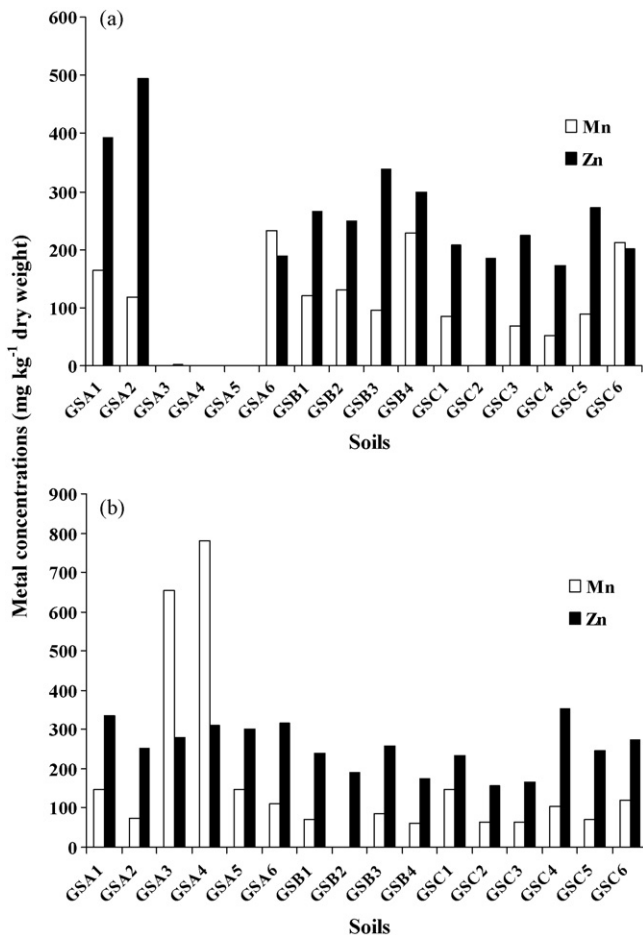


Fig. 2. Mn and Zn concentrations in leaves of *Lactuca sativa* L. (a) and *Lolium multiflorum* L. (b) growing on garden soils from the three mining areas.

Mn, Ni and Zn accumulations in *L. sativa* L. and *L. multiflorum* L. were compared to the concentrations of trace elements in mature leaf tissue for various species [21]. In all garden soils, the plants accumulated Cd, Co, Cr, Cu and Ni below the toxic concentrations (data not shown) while Zn (in all soils) and Mn (in soils GSA3 and GSA4 from mine A) was accumulated at concentrations high enough to be considered phytotoxic [21]. The comparison between the mean biomass produced for *L. sativa* L. in the three mining areas showed no significant difference. However, the mean biomass produced for *L. multiflorum* L. presented a significant difference between garden soils from the three mining areas. The biomass obtained for *L. multiflorum* L. followed the order: garden soils from mine A > mine C > mine B. As reported by previous works [32], our results showed that in general, the response of the MetPLATE bioassay was correlated with the biomass production; it is inversely proportional to the inhibition percentage given by the bioassay ($r = -0.37$ for *L. sativa* L. and $r = -0.36$ for *L. multiflorum* L.). The lower biomass produced in the garden soils from mines B and C than soils in mine A, is explained by the relative low toxicity (6–38% inhibition).

The high contents of Mn and Zn exceed the metal concentrations found in the literature for plants grown on polluted soils [9,12,30,31]. The mean concentration of Zn in *L. sativa* L. was higher than reported values (65 mg kg^{-1} DW) by Khan et al. [31] and (47 mg kg^{-1} DW) by Lim et al. [5]. Also the values of Mn and Zn in *L. sativa* L. and *L. multiflorum* L. were higher than those accumulated by the dominant species in Lipu Mn mineland. For example, *Curcuma aromatica* and *Imperata cylindrica* var. *major* accumulate $206 \text{ mg Mn kg}^{-1}$ and $260 \text{ mg Mn kg}^{-1}$, respectively [12].

The transfer factor (TF) from soil to plants is defined as the ratio of metal concentration in plant (dry weight) to the total metal concentration in soil (dry weight). The TF values for Mn and Zn in *L. sativa* L. and *L. multiflorum* L. are given in Fig. 3. TF values for Zn were higher than those observed for Mn. TF values for Mn were lower than 0.47 and 0.26 for *L. sativa* L. and *L. multiflorum* L., respectively. The relatively lower TF values for Mn confirm that this element ($34.1\text{--}936.9 \mu\text{g Mn l}^{-1}$ in soils water extract) is present at low bioavailable fraction in soils despite its high concentration in soils from mine A. The slightly basic pH of all garden soils may constitute the main factors controlling Mn bioavailability [9]. The highest TF obtained for Zn were 5.2 for *L. sativa* L. and 4.4 for *L. multiflorum* L., indicating that this element is more bioavailable than Mn in the mining garden soils in south of Morocco. This was confirmed by Zn concentrations in the shoots of the two plants, which is higher than Mn. The highest TF values for Mn are found in garden soil (GSB4) for *L. sativa* L. and garden soil (GSC1) for *L. multiflorum* L.; while the highest TF for Zn is observed in garden soil (GSA2) for *L. sativa* L. and garden soil (GSB1) for *L. multiflorum* L. In general, TF for Zn decreased with increasing total metal concentrations in soils, indicating an inverse relationship between transfer factor and total metal concentrations ($r = -0.92$ and -0.92 for *L. sativa* L. and $r = -0.59$, -0.82 and -0.71 for *L. multiflorum* L. respectively in mines A, B and C). Such inverse relationships were also reported by Khan et al. [31]. Absorption and accumulation of heavy metals in plant tissues depend upon many factors, which include temperature, moisture, organic matter, pH and nutrient availability. Soil properties influencing heavy metals availability varied significantly between the sites [9,10].

The relationships between the individual heavy metals in garden soils and metallic concentrations in the two studied plants; demonstrate positive correlations for Mn in garden soils from mine C for *L. sativa* L. and *L. multiflorum* L. ($r = 0.286$ and $r = 0.244$ respectively), in garden soils from mine A for two studied plants ($r = 0.746$ for *L. sativa* L. and $r = 0.767$ for *L. multiflorum* L.) and for Zn in garden soils from mines A and B for *L. sativa* L. ($r = 0.352$ and $r = 0.575$ respectively) and for *L. multiflorum* L. ($r = 0.499$ and $r = 0.388$ respectively). However, a negative correlation was found for Mn in garden

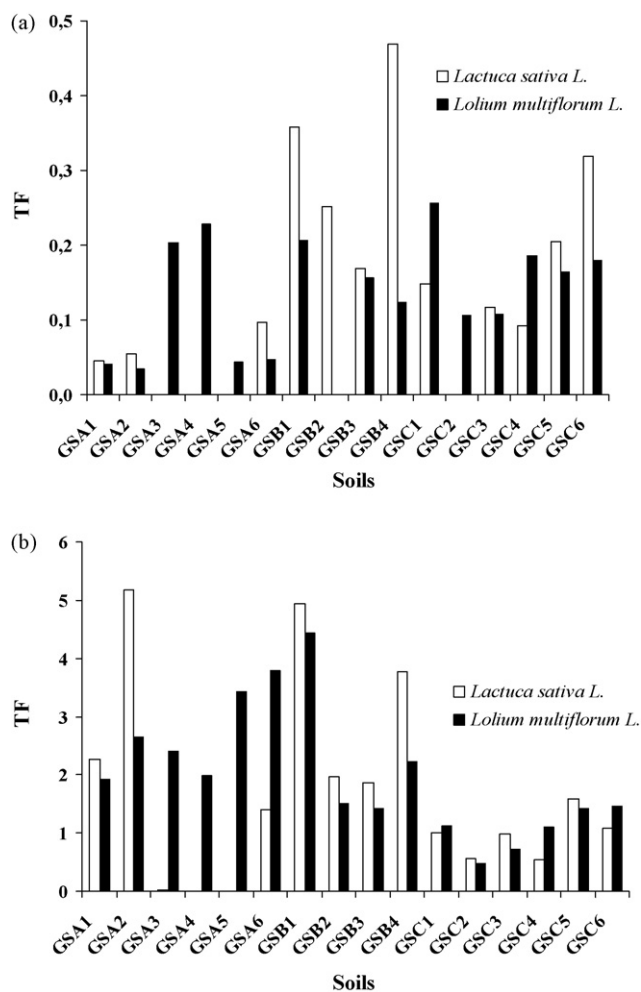


Fig. 3. Transfer factors (TF) for Mn (a) and Zn (b) in *Lactuca sativa* L. and *Lolium multiflorum* L. growing on garden soils from three mining areas.

soils from mine B for *L. multiflorum* L. ($r = -0.159$) and for Zn in garden soils from mine C for *L. sativa* L. ($r = -0.754$). The higher TF values for Zn and the relative high metal concentrations for Mn and Zn obtained in the two studied plants (*L. sativa* L. and *L. multiflorum* L.) in garden soils from three mining areas constitute a source of metallic pollution which may adversely affect human populations residing in the vicinity of the contaminated area.

4. Conclusion

The present study consisted in the assessment of metal contamination associated with garden soils in mining areas. This evaluation was based on the determination of total and water-extractable metal contents in garden soils from three mining sites, two sites localized in the region of Ouarzazate city and one in the region of Marrakech city, South of Morocco. The garden soils contained high total concentrations of Mn in mine A, Co and Ni in mine B and Cr, Cu and Zn in mine C. The application of the MetPLATE™ bioassay on the garden soils give values which do not exceed 38.8% inhibition. These low percentages are confirmed by the relatively low metal content obtained in aqueous extracts. However, the high heavy metals contents of Mn and Zn and their significant TF values for cultivated species (*L. sativa* L. and *L. multiflorum* L.) in the garden soils from three mining areas may be a health risk to the human population residing in the neighbourhood of those contaminated sites following consumption of the contaminated crops.

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