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NEW MOLYBDENUM-HYPERACCUMULATOR AMONG PLANT SPECIES GROWING ON MOLYBDENUM MINE- A BIOCHEMICAL STUDY ON TOLERANCE MECHANISM AGAINST METAL TOXICITY

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□ *The aim of this work was to determine metal accumulation by plants growing on three molybdenum-mine zones and their tolerance strategies. The plants from tailing, extracting and non-contaminated zones were sampled with their corresponding soils. The results show that molybdenum (Mo) and copper (Cu) were at toxic levels in soils and their levels varied in 44 collected species from 21 families.* Ajuga chamaecistus *and* Cramb orientalis *L. excluded Mo and Cu, respectively.* Achilla tenuifollia *as Mo-hyperaccumulator with total Mo (1979 mg* kg^{-1}*) and then* Erodium ciconium *with 1308 mg* kg^{-1} *Mo and* Conyza Canadensis *with 618 mg* kg^{-1} *Cu were moderate metal accumulators. They stored considerable levels of metals in their leaves vacuoles and elevated the levels of phytochelatins, cysteine and glutathione and induced antioxidant enzymes. In conclusion, this study indicated that some collected plants excluded metals. In metal-accumulators, antioxidant enzymes, phytochelatins and sequestration of excess metals were involved in their tolerance mechanism.*

Keywords: tolerance strategies, phytochelatins, Mo-hyperaccumulator, antioxidant enzymes, oxidative damage

INTRODUCTION

Molybdenum (Mo) is an essential nutrient element, functions as an electron transport agent in flavo-enzymes including xanthine oxidase, sulfite oxidase, aldehyde oxidase (Hille, 1999). Lack of this micronutrient may result accumulation of nitrate and decrease of amino acids and levels of vitamin metabolism in plant tissues (Wang, 1991). Excess Mo may inhibit enzyme system such as succinic acid oxidase and glutaminase (Stokinger, 1981). On the other hand, industries need a great deal of this metal for different purposes.

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Molybdenum use in ferric and manganese alloys, catalysts, ceramics, electronic parts and welding electrodes steadily increase its demand around the world. Mining, as the main source of Mo extraction, unavoidably covers a relatively large mineland area with metalliferous soils. Molybdenum is one of the main compounds generally accompanied by mixture of other heavy metals in Mo-ore extracted and release into the environment. Surface exposure of metalliferous ores and the spoils associated with Mo-mining exerts a pressure on plant communities. This unfavorable condition can support the growth of tolerant plant species called metalophytes, refers to specific individuals of a metal-tolerant plant species which are able to withstand greater amount of toxicity than their immediate relatives on normal soil (Shu et al., 2002; Wong, 2003). These plants develop specific physiological and biochemical mechanisms that enable them to function normally in lands polluted with heavy metals, forming heavy metal resistant populations (Baker et al., 1999).

Some species may have developed avoidance strategy to high level of toxic metals via exclusion or binding of metal to ligands renders them harmless. On the other hand, some plants may reveal high metal accumulation capacity varies greatly between different species and varieties. They can be used in phytoremediation technology to clean-up contaminated soils with toxic metals (Lasat, 2002). In this case, biochemical defense strategies may include complexation of ions, storage at subcellular organelles, and enhanced production of antioxidants that detoxify reactive oxygen species (ROS). Phytochelatins (PCs) and its homologuses activate by various heavy metals, immobilize and sequestrate them. On the other hand, antioxidant enzymes generate the active form of antioxidants and eliminate ROS. Superoxide dismutase (SOD) is a metalloenzyme, functioning at first step of ROS formation (Alscher et al., 2002), and its conversion to hydrogen peroxide (H_2O_2). Catalase (CAT) achieves removal of H_2O_2. Glutathione peroxidase (GPX) protects the membrane lipids from oxidative damage and detoxified the organic peroxides (Kantol et al., 1998). Glutathione reductase (GR) regulates the redox state of the glutathione pool that play important role in antioxidative defense (Mrittunjai et al., 2005). When plants cope with metal-induced oxidative stress, ROS damage membrane lipids and proteins as well as nucleic acids, which result increase in oxidative damage biomarkers including malondialdehyde (MDA) and dityrosine.

There is very little information recognizing Mo-tolerant species and mechanism usually underlying their tolerance and protective strength. So far, few Mo-hyperaccumulator as a subset of tolerant group have been known worldwide. In this work, field survey has been carried out on plant communities growing on molybdenum mineland in their natural habitats. Accordingly, the aim of this work was to acquire information about the flora and to assess the extent of metal accumulation by plants, their tolerance and

protective strategies against metal toxicity in mineland with the ultimate goal of finding Mo-hyperaccumulating species.

MATERIALS AND METHODS

Site Description

Sanj molybdenum mine is located in Tehran state at 57 km away from Karaj city and 2236 m elevation (Lat. 36° 01′ 927″ N, Long. 50° 58′ 990″ E). Molybdenum minerals in this mine were in molybdenit that appeared at the surface, all over the mine area. In addition there were many tunnels and extraction activities. Tailing resulted from continues ore extraction and deposition of packed contaminated soils around this area. The climate of the mine district was generally mild and characterized as semi-arid with maximum temperature occurring in June and July ranging in 27°C–38°C. The annual average total precipitation was 41.4 cm. This study was carried out in mine area (extracting zone) and tailing as metal contaminated fields and in another area in mine vicinity as non-contaminated filed.

Plant and Soil Sampling

Plant samples were collected at a determined time of single growing season, according to the actual landform of Mo-mine and the distribution of vegetation at same times and age at flowering period. Expert botanist personally identified plant species. For each species five to eight plants were collected within the sampling region and their fresh tissues, including roots, stems and mature leaves, were used for analysis. Prior to analysis, plant samples were carefully washed with tap water and thoroughly rinsed with deionized water. Each collected plant was considered for three replicate analyses. Corresponding soil samples were collected at the location of plant sampling (maximum sampling depth about 20–30 cm). These samples were then air-dried and sieved through a 2 mm plastic screen.

Determination of Metals in Plants Leaves

Dry plant material (0.1g) was separately ashed at 550°C and the residue was brought to standard volume with 20% hydrochloric acid (HCl). Each of metal content was determined directly by atomic absorption spectrometry (SP 191, Unicam, Portsmouth, NH, USA).

Soil Analysis

All soil samples were air-dried, sieved to <2 mm and then oven-dried at 70°C. A hydrometric method was used to analyze soil particle- size (Day, 1965). Water-holding capacity (WHC) an atmospheric pressure of 1/3 bar

was determined as the amount of water held in soil in the upper ring 24 h after drainage of water (Wang, 1989). The total Kjeldahl nitrogen (TKN) was determined by the method outlined in Bremner and Mulvaney (1982). A further sub-sample of 0.5 g was transferred to a Kjeldahl digestion tube for extraction with 10 mL of a 3:1 hydrochloric acid (HCl)/nitric acid (HNO_3) mixture and the metals in soils were sequentially extracted (Yuan, 1988).

Dried soil samples were digested with HCl + HNO_3 + perchloric acid ($HClO_4$) (3:1:1, v/v) (Yuan, 1988). Mo and other metals in this acid digested extract were determined by atomic absorption spectrometry (Analyst 100, Perkin Elmer, Waltham, MA, USA). The metals in soils were sequentially extracted following a slight modification of the method described by Tessier et al. (1979). The modification consisted of initially extracting with double-distilled water (2g of soil shaken for 4 h in distilled water of electric conductivity <0.02 dS m^{-1}, followed by centrifugation during 10 min at 3000 rpm). This step represents the fraction that is water soluble and most easily available to plants and easily leacheable into the groundwater (Siebe, 1995).

The pH and electrolytic conductivity (EC) were determined in a water:soil extract 1:1 using a Beckman pH-meter and a conductivity meter model HI8633(Hanna Instruments Co., Woonsocket, RI, USA), respectively. Electric conductivity of soil samples were classified by Boulding criteria in which non-saline <2; moderately saline 2–8; very saline 8–16; extremely saline >16, (Boulding, 1994).

Protein content was determined by the method of Bradford (1976), with standard curves prepared using bovine serum albumin (BSA).

Vacuole Isolation

Isolation was achieved by the method of Kringstad et al. (1980). Leaves were washed in distilled H_2O, sliced and incubated in medium contained 0.7 mannitol, 50 mm NaOH (pH 5.5), 5 mM magnesium chloride ($MgCl_2$), 2.0% (w/v) Cellulysin (Calbiochem, Gibbstown, NJ, USA), 1% (w/v) Pectinase (Sigma, St. Louis, MO, USA), and 0.1% (w/v) BSA. Tissue digestion was carried out at 30°C for 30 to 45 min. Digested leaf tissue was filtered and washed with 25 mL of a buffer containing 0.5 mannitol, 25 mm Tris-HCl, and 5 mm EGTA (ethylene glycol tetraacetic acid). The protoplasts were collected and resuspended in 40 mL of resuspension buffer. Vacuoles were isolated from protoplast on discontinuous Ficoll-400 gradient. One ml of the protoplast suspension was gently layered onto the top of a Ficoll-400 gradient containing 10 mL each of 5, 10, and 15% (w/v) Ficoll-400. Each Ficoll solution was made up in 0.5 mannitol and 25 mm Tris- HCl (pH 8.0). The gradients were spun at 26,000 rpm l00,000 *g* ultracentrifuge. The vacuoles were removed from the gradient with a 16 gauge cannula attached to a l-mL syringe and setting in standard hypotonic solution.

Chloroplast Isolation

Fresh and mature leaves (5 g) were homogenized for 15s with a homogenizer in 50 ml ice-cold grinding medium containing: 0.33 M sorbitol, 1mM ethylenediaminetetraacetic acid (EDTA), 0.1% bovine serum albumin (BSA), 2 mM sodium ascorbate and 50 mM potassium phosphate (K_2HPO_4), pH 7.5. The homogenate was filtrated through Miracloth and centrifuged for 1 min at 1000 *g* at 4°C to remove whole cells and cell debris. The intact chloroplasts were pelleted through centrifugation at 4500 *g* for 30s and were gently resuspended in the same buffer without BSA and centrifuged again at the same conditions. This washing procedure was repeated twice and pelleted chloroplasts were isolated (Rusina et al., 2004).

Chlorophyll and Biomass Determination

Fresh and mature leaves (0.5 g) were extracted with 10 ml 80% acetone as described by Alan (1994). The absorbance of extract was measured at 663 and 645 nm in the UV-Vis light spectrophotometer. The chlorophyll content was calculated using the equation $C_T = 20.2\ A_{645} + 8.02\ A_{663}$. The washed plants were separated into roots and shoots, and dried in an oven at 60°C for 48 h, then biomass (DW) was measured and recorded as mg/g. fresh weight (mg g^{-1} fw).

Preparation of Enzyme Extracts

Whole tissue (leaves, stems and/or roots) were homogenized (1:5 w/v) separately in an ice cold mortar using 50 mM sodium phosphate buffer, pH 7.0, containing 1 M sodium chloride (NaCl), 1% polyvinylpyrrolidone and 1 mM EDTA. After centrifugation (20,000 *g*, 15 min), the supernatant (crude extract of leaves) was used to determine enzyme activities, which were measured at 25°C.

Catalase (EC: 1.11.1.6) activity was determined by following the consumption of H_2O_2 (extinction co-efficient 0.0394 mM. cm^{-1}) at 240 nm for 30 s (Aebi, 1984). The assay mixture containing 100 mM potassium phosphate buffer (pH 7.0), 15 mM hydrogen peroxide (H_2O_2) and 50 μL leaf extract in a 3 mL volume. Unit was defined as nmol H_2O_2 decomposed per 1 min.

To detect glutathione peroxidase [EC: 1.11.1.9 (GPX)] activity, the method of Hopkins and Tudhope (1973), with t -butyl hydroperoxide as a substrate was used. The reaction mixture comprised 50 mM potassium phosphate buffer, pH 7.0, 2 mM EDTA, 0.28 mM nicotinamide adenine dinucleotide phosphate-oxidase (NADPH), 0.13 mM GSH, 0.16 U glutathione reductase (GR), 0.073 mM t-butyl hydroperoxide and enzyme extract (50 mg protein). One unit of GSH-Px activity was defined as the amount

of enzyme that catalyzed the oxidation of NADPH [mmol. min^{-1} mg^{-1} protein].

Glutathione Reductase (GR)

The activity was assayed by following the method of Smith et al. (1988). The reaction mixture contained 1.0 mL of 0.2 M potassium phosphate buffer (pH 7.5) containing 1 mM EDTA, 0.5 mL, 3 ml 5, 5′-dithiobis (2-nitrobenzoic acid) in 0.01 M phosphate buffer (pH 7.5), 0.25 ml H_2O, 0.1 mL 2 mM NADPH, 0.05 mL enzyme extract and 0.1 mL 20 mM GSSG. The component was added in the order as above directly to a cuvette and the reaction was started by the addition of GSSG. The increase in absorbance was monitored for 5 min at 412 nm. The rate of enzyme activity was calculated using standard curve prepared by known amounts of GR (Sigma-Aldrich Chemie Gmbh, Munich, Germany). Activity of enzyme was expressed as μmoles of GSSG reduced min^{-1} g^{-1} fw.

Ascorbate Peroxidase (APX)

The activity of APX was measured according to the method of Nakano and Asada (1981) by estimating the rate of ascorbate oxidation (extraction coefficient 2.8 mM-1 cm^{-1}). The 3 mL reaction mixture contained 50 mM phosphate buffer (pH 7.0), 0.1 mM H_2O_2, 0.5 mM sodium ascorbate, 0.1 mM EDTA and a suitable aliquot of enzyme extract. The change in absorbance was monitored at 290 nm and enzyme activity was expressed as μmoles of ascorbate oxidized min^{-1} g^{-1} fw.

SOD activity was determined by the method of Minami and Yoshikawa (1979) with 50 mM Tris-ca-codylic sodium salt buffer, pH 8.2, containing 0.1 mM EDTA. The reaction mixture was composed of 1.42% Triton X-100, 0.055 mM nitroblue tetrazolium (NBT), 16 mM pyrogallol and enzyme extract (50 mg protein). The unit (50% inhibition) was established according to the definition of McCord and Fridovich (1969). Unit was defined as the quantity of enzyme required to inhibit the reduction of NBT by 50% per 1 min.

Measurement of Dityrosine

1.2 grams of fresh tissue material were homogenized with 5 ml of ice-cold 50 mM HEPES-KOH, pH 7.2, containing 10 mM EDTA, 2 mM PMSF, 0.1 mM p-chloromercuribenzoic acid, 0.1 mM DL-norleucine and 100 mg polyclar AT. The plan tissue homogenate was centrifuged at 5000 *g* for 60 min to remove debris. Purification of *o,o′*_dityrosine in the clear tissue homogenized supernatant fluid was accomplished by preparative HPLC.

*o,o′*_Dityrosine was recovered by gradient elution from the C-18 column (Econosil C18, 250 mm × 10 mm, Alltech Associates, Deerfield, IL, USA) (Orhanl et al., 2004). The composition of eluent varied linearly from acetonitrile–water–TFA (1:99:0.02) to acetonitrile–water–TFA (20:80:0.02) over 25 min. The gradient was started 5 min after the injection. A flow rate of 4 ml/min was used. *o,o′*_Dityrosine was analyzed by reversed-phase HPLC with simultaneous UV-detection (280 nm) and fluorescence-detection (ex. 280 nm, em. 410 nm). A phenomenex inertsil ODS 2 (150 mm × 4.6 mm, 5 μm) HPLC column (Bester, Amsterdam, the Netherlands) equipped with a guard column was used for these analyses. A gradient was formed from 10 mM ammonium acetate, adjusted to pH 4.5 with acetic acid, and methanol, starting with 1% methanol and increasing to 10% over 30 min. The flow rate was 0.8 mL min^{-1}. A standard dityrosine sample was prepared according to Amado et al. (1984). Dityrosine was quantified by assuming that its generation from the reaction of tyrosine with horseradish peroxidase in the presence of H_2O_2 was quantitative (using the extinction coefficient e_{315} = 4.5 mM^{-1} cm^{-1} at pH 7.5).

Malondialdehyde (MDA) Analysis

Proteins of tissue homogenate were precipitated with 40% trichloracetic acid (TCA), w/v. The MDA assay was based on the condensation of one molecule malondialdehyde with two molecules of thiobarbituric acid (TBA) in the presence of reduced reagent volumes to increase sensitivity, generating a chromogen with UV absorbance. The TBA + MDA complex was analyzed by HPLC essentially as described by Bird et al. (1983). Briefly, the HPLC system consisted of a Hewlett + Packard 1050 gradient pump (Avondale, PA, USA) equipped with an automatic injector, a 1050 diode-array absorption detector and a personal computer using Chem Station Software from Hewlett + Packard (Palo Alto, CA, USA). Aliquots of the TBA + MDA samples were injected on a 5 mm Supelcosil LC-18 (Sigma Aldrich) reversed phase column (30 × 4.6 mm). The mobile phase consisted of 15% methanol in double-distilled water degassed by filtering through a 0.5 μm filter (Millipore, Bedford, MA, USA). The flow rate was 2 mL min^{-1}. The MDA + TBA standards were prepared using tetraethoxypropane. The absorption spectra of standards and samples were identical with a characteristic peak at 540 nm. Measurements were expressed in terms of malondialdehyde (MDA) normalized to the sample protein content.

Determination of PCs and Glutathione (GSH)

Extraction and analysis of PCs and GSH were performed according to the method described by Sneller et al. (2000) with a slight modification. Frozen plant tissues were homogenized in a mortar and a pestle with

quartz sand in 2 ml of 6.3 mM diethylenetriaminepentaacetic (DTPA) with 0.1% trifluoroacetic acid (TFA) at 4°C. The homogenate was centrifuged at 14,000 *g* at 4°C for 12 min. The clear supernatants were collected for the assay by high-performance liquid chromatography (HPLC), using pre-column derivatization with a fluorescent probe, monobromobiane (mBrB). 250 μL of supernatant was mixed with 450 μL of 200 mM HEPPS (3-[4-(2 Hydroxyethyl)-1-piperazinyl] propanesulfonic acid) at pH 8.2, with 6.3 mM DTPA, and 10 μl of 25 mM (mBrB). Derivatization was carried out in the dark at 45°C for 30 min. The reaction was terminated by adding 300 μL of 1 M methanesulfonic acid (MSA). The samples were stored in the dark at 4°C until HPLC analysis. Blank samples were used to identify the reagent peaks. The bimane derivatives were separated using a binary gradient of mobile phase A (0.1% TFA) and B (100% acetonitrile) at room temperature (22 ± 2°C). Fluorescence was detected at 380 nm excitation and 470 nm emission wavelengths. The flow rate was 0.5 mL/min. Fifty μL of the derivatives sample was run in a linear gradient from 12% to 25% B for 15 min, then 25% to 35% B for 14 min and next 35% to 50% B for 21 min. Before injecting a new sample, the column was cleaned (5 min, 100% B) and equilibrated (10 min, 12% B) and post-time was 5 min. Total analysis time was 70 min. Analytical data were integrated by using the HP ChemStation. Retention times of PCs and GSH in biological samples were checked with PCs and GSH standards, respectively. Individual PC subtypes were quantified by using the relationship peak vs. concentrations of GSH standard solutions. Corrections for differential derivatization efficiencies were made according to the method stated by Sneller et al. (2000).

Glutathione

Plant material (500 mg) was frozen in liquid nitrogen, homogenized in 0.1 sodium phosphate buffer (pH 8.0) containing 25% meta-phosphoric acid. The homogenate was centrifuged at 20,000 *g* for 20 min at 4°C and total glutathione (GSSG and GSH) content was determined fluorometrically in the supernatant after 15 min incubation with *o*-phthaldialdehyde (OPT; Hissin and Hilf, 1976). Fluorescence intensity was recorded at 420 nm after excitation at 350 nm on a Perkin-Elmer LS 55 fluorescence spectrophotometer.

Cysteine

Plant tissue was homogenized in 5% chilled perchloric acid and centrifuged at 10,000 *g* for 10 min at 4°C. Cysteine level was measured in supernatant using acid-ninhydrin reagent at 560 nm according to the method of Gaitonde (1967).

Enrichment Coefficient, Translocation and Accumulation Factors

The translocation factor (TF), enrichment coefficient (EC) and accumulation factor (AF) of heavy metals (Zu et al., 2005) were calculated as follows:

AF: [element in shoot]/[normal level in plant]
TF: [element in shoot]/[element in root]
EC: [element in shoot]/[available level in soil]

Statistical Analysis

All statistical analysis was carried out by using procedure available in the SPSS v.10 (SPSS Inc., Chicago, IL, USA) statistical package. Each experiment was run on each sample at least in three replicate, then we calculated mean ± SD of test results obtained from all samples collected from all parts for each zone and the data presented are given as mean ± SD. Student's t-test was applied to determine the significance of results between different samples. Statistical significance was set at the $P < 0.05$ confidence level. When testing for relationships, the sample parameters at each zone were considered separately and Pearson's correlation coefficient were calculated.

RESULTS

Quantitative data of physical and chemical characteristics of soils collected from three zones in Mo-mine are given in Table 1. As EC levels revealed, its variations were lower than 2, indicated that the soils of studied zones were non-saline (Boulding, 1994) and pH indexes were at neutral range (Tanji, 1990). Other physical characteristics did not differ remarkable. A range of elements with variable quantities was identified in the soils that covered tailing and extracting zones. Except copper (Cu) and Mo, the levels of nickel (Ni), lead (Pb), and zinc (Zn) were generally below the maximum allowable concentrations of heavy metals in normal soils Ni (750), Pb (300), and Zn (900) mg kg^{-1} (Alloway, 1995; Bowen, 1979). Total and available levels of Mo and Cu in high-contaminated zones were considerably higher as compared with other heavy metals. High concentrations of Mo and then Cu were observed in extracting and tailing zones respectively in comparison with non-contaminated zone. Available levels of these two metals exceeded their maximum concentrations in normal soil; Cu (250), Mo (40) mg kg^{-1} (Bowen, 1979). The ratios of total and available levels in extracting zone to those in non-contaminated zone for Mo were about 19 and 21, respectively.

The levels of Mo and Cu values in different plant tissues collected from studied zones were exhibited in Table 2. There were 44 species from 21 families of vascular plants distributed on three studied zones. Most of them were herbaceous annuals, biennials or perennials. Major plant families were

TABLE 1 Chemical composition and physical characteristics of soils of the studied zones*

Zone	Soil texture % Sand	% Slit	% Clay	Mo (mg kg^{-1}.dw) Total	A.C.	Cu (mg kg^{-1}dw) Total	A.C.	Ni (mg kg^{-1}dw) Total	A.C.	Pb (mg kg^{-1}dw) Total	A.C.	Zn (mg kg^{-1} dw) Total	A.C.	EC (dS m^{-1})#	pH (H_2o)	W.H.C. (m^3 m^{-3})	Total nitrogen g kg^{-1}
Extracting	28	32	40	448 ± 35¶	169 ± 14¶	610 ± 54¶	183 ± 16.2¶	117 ± 9.5¶	27.1 ± 2.3¶	219 ± 17.3¶	41.3 ± 4.2¶	431 ± 39.4¶	112 ± 10¶	1.18 ± 0.15	6.1	0.26 ± 0.04	1.77 ± 0.1
Tailing	32	35	33	57.4 ± 5.1¶	18.7 ± 1.5¶	286 ± 25¶	83 ± 7.4¶	82.8 ± 6.9	17.4 ± 1.5	156 ± 14¶	27.1 ± 2.1¶	310 ± 28	84 ± 7.8	0.91 ± 0.1	7.1	0.41 ± 0.06	2.33 ± 0.2
Non-contaminant	38	28	34	23.2 ± 2.1	7.8 ± 0.5	189 ± 14.5	49 ± 3.2	71.2 ± 6.5	15 ± 1.2	106 ± 11.5	19.3 ± 1.4	275 ± 24	72 ± 6.9	1.48 ± 0.2	7.4	0.20 ± 0.02	2.71 ± 0.2

*Data were presented as mean ± SD.

¶: Significant difference as compared with Non-contaminant zones ($P < 0.05$).

EC: Electrolytic conductivity in water: soil extract (1:1).

A. C.: Available concentration. W.H.C., water-holding capacity.

TABLE 2 The concentration of metals (mg kg^{-1} dw) in tissues of plant species grown on studied zones

	Plant tissue						Zone		
	Leaf		Stem		Root				
Taxa	Mo	Cu	Mo	Cu	Mo	Cu	E	T	N
Achilla tenuifolia	1024 ± 70.3	18.1 ± 1.8	745 ± 37.2	3.4 ± 0.2	210 ± 18.4	1.6 ± 0.1	*		
	682 ± 45.8	15.2 ± 1.2	306 ± 20.3	2.2 ± 0.2	81.5 ± 6.1	1.8 ± 0.1		*	
	36.5 ± 3.7	8.3 ± 0.5	18.2 ± 1.7	0.8 ± 0.1	5.1 ± 0.5	1.1 ± 0.2			*
Erodium ciconium (Jusl) L.	725 ± 51.2	14.2 ± 1.2	411 ± 33.4	5.1 ± 0.3	72.5 ± 6.4	2.4 ± 0.16	*		
	472 ± 38.6	8.7 ± 0.71	305 ± 21	3.2 ± 0.24	39.1 ± 3.3	1.5 ± 0.08		*	
	19.3 ± 1.5	4.2 ± 0.31	6.6 ± 0.45	1.8 ± 0.12	2.8 ± 0.11	2.3 ± 0.13			*
Conyza Canadensis (L.)Cornq.	82.3 ± 7.1	461 ± 35	36.4 ± 3.7	136 ± 11.1	7.3 ± 0.54	21.5 ± 2.1	*		
	31.5 ± 2.8	394 ± 36	17.3 ± 1.9	120 ± 11.3	3.5 ± 0.22	15.2 ± 1.2		*	
	2.1 ± 0.01	6.4 ± 0.44	1.2 ± 0.01	2.8 ± 0.12	0.7 ± 0.06	1.5 ± 0.1			*
Ajuga chamaecistus	0.77 ± 0.04	5.72 ± 0.32	0.91 ± 0.07	1.8 ± 0.08	0.66 ± 0.04	091 ± 0.08	*		
	1.8 ± 0.06	3.51 ± 0.27	0.8 ± 0.05	2.1 ± 0.16	0.52 ± 0.04	1.4 ± 0.09		*	
	2.83 ± 0.18	2.44 ± 0.21	1.10 ± 0.08	1.93 ± 0.07	0.61 ± 0.05	0.67 ± 0.04			*
Cramb orientalis L.	2.88 ± 0.18	1.60 ± 0.07	1.71 ± 0.06	2.1 ± 0.14	0.30 ± 0.02	0.7 ± 0.05	*		
	1.21 ± 0.06	2.2 ± 0.14	0.62 ± 0.04	1.82 ± 0.06	0.21 ± 0.03	0.92 ± 0.07		*	
	0.93 ± 0.07	4.79 ± 0.32	1.04 ± 0.015	5.20 ± 0.41	1.06 ± 0.04	1.12 ± 0.09			*
Crepis sancta (L.) Babcock	5.12 ± 0.42	17.2 ± 0.12	2.10 ± 0.13	7.15 ± 0.61	0.82 ± 0.05	3.51 ± 0.24	*		
	4.24 ± 0.32	13.5 ± 1.41	1.85 ± 0.12	3.21 ± 0.28	0.32 ± 0.03	1.92 ± 0.08		*	
Zigophyllum fabago	8.18 ± 0.62	47.10 ± 3.21	2.16 ± 0.18	16.83 ± 1.5	2.81 ± 015	4.41 ± 0.32	*		
	3.49 ± 0.31	31.60 ± 2.41	1.85 ± 0.13	12.51 ± 1.10	0.72 ± 0.05	2.03 ± 0.13		*	
	1.16 ± 0.12	10.13 ± 1.24	1.37 ± 0.12	5.10 ± 0.41	0.52 ± 0.03	3.42 ± 0.21			*
Chenopodium ambrosioides	14.7 ± 1.32	11.3 ± 1.21	8.51 ± 0.65	4.2 ± 0.32	1.15 ± 0.10	1.60 ± 0.09	*		
	10.31 ± 1.21	16.21 ± 1.23	4.26 ± 0.32	6.74 ± 0.51	1.31 ± 0.11	1.21 ± 0.10		*	
	0.81 ± 0.06	2.24 ± 0.19	0.23 ± 0.04	1.93 ± 0.08	0.14 ± 0.03	1.04 ± 0.07			*
Astragalus ardehalicus parsa	4.91 ± 0.31	9.71 ± 0.62	1.38 ± 0.09	3.54 ± 0.28	2.18 ± 0.19	1.82 ± 0.17	*		
	1.40 ± 0.12	2.91 ± 0.24	0.85 ± 0.06	1.72 ± 0.11	1.20 ± 0.13	0.91 ± 0.06		*	
Mediceae sative L.	3.10 ± 0.21	6.61 ± 0.52	0.72 ± 0.05	4.22 ± 0.32	0.51 ± 0.029	1.10 ± 0.08	*		
	2.24 ± 0.19	4.15 ± 0.30	0.53 ± 0.04	2.55 ± 0.21	0.37 ± 0.05	1.29 ± 0.14		*	
	1.73 ± 0.12	3.32 ± 0.25	0.82 ± 0.07	1.80 ± 0.16	0.23 ± 0.04	0.82 ± 0.08			*

Gaillonia bruguieri A. Rich	12.4 ± 1.11	6.42 ± 0.51	8.85 ± 0.65	7.32 ± 0.45	4.1 ± 0.32	3.15 ± 0.25	*		
	3.84 ± 0.26	2.54 ± 0.19	1.54 ± 0.11	1.76 ± 0.12	2.31 ± 0.17	1.25 ± 0.10		*	
	0.93 ± 0.09	1.42 ± 0.12	0.61 ± 0.07	0.83 ± 0.08	0.52 ± 0.04	0.91 ± 0.07			*
Anethum graveolens L.	2.18 ± 0.15	9.31 ± 0.65	0.92 ± 0.07	3.12 ± 0.26	1.14 ± 0.08	0.53 ± 0.04	*		
	1.22 ± 0.010	6.52 ± 0.44	0.67 ± 0.08	2.33 ± 0.19	0.86 ± 0.07	0.76 ± 0.05		*	
Descuraninia Sophia (L.) Schur	13.11 ± 0.12	23.17 ± 2.015	4.15 ± 0.38	7.42 ± 0.51	1.09 ± 0.08	3.10 ± 0.22		*	
Parietaria judaica	8.43 ± 0.66	56.61 ± 4.47	2.17 ± 0.16	19.10 ± 1.5	1.26 ± 0.12	4.55 ± 0.33	*		
	1.15 ± 0.10	10.20 ± 1.07	1.42 ± 0.09	3.66 ± 0.25	0.77 ± 0.08	1.14 ± 0.10			*
Datura stramonium	39.56 ± 3.24	81.18 ± 7.14	13.71 ± 1.22	20.14 ± 2.10	3.51 ± 0.26	3.32 ± 0.24	*		
	16.13 ± 0.12	52.07 ± 4.23	4.22 ± 0.31	31.80 ± 3.12	1.73 ± 0.19	6.18 ± 0.52		*	
	2.47 ± 0.19	7.51 ± 0.66	1.03 ± 0.09	2.16 ± 0.15	1.46 ± 0.10	0.89 ± 0.05			*
Malva sylvestris	6.20 ± 0.49	18.19 ± 1.52	4.91 ± 0.33	5.20 ± 0.44	1.77 ± 0.12	2.16 ± 0.19	*		
	2.06 ± 0.19	10.21 ± 1.08	0.081 ± 0.05	2.91 ± 0.15	0.52 ± 0.10	0.74 ± 0.07		*	
	1.19 ± 0.08	4.55 ± 0.35	0.70 ± 0.06	1.08 ± 0.09	0.36 ± 0.07	0.83 ± 0.7			*
Centaurea virgata	12.03 ± 1.21	39.06 ± 3.5	8.16 ± 0.54	21.50 ± 2.06	2.41 ± 1.85	4.17 ± 0.36	*		
Heterocaryum szovitsianum (Fish. & Mey.) DC.	82.41 ± 7.05	112.65 ± 9.5	89.14 ± 6.44	41.20 ± 3.21	3.13 ± 0.41	7.11 ± 0.52	*		
	23.02 ± 2.10	50.33 ± 4.14	11.19 ± 1.032	12.16 ± 1.05	2.18 ± 0.19	2.21 ± 1.85		*	
	2.03 ± 0.15	7.17 ± 5.12	1.51 ± 0.10	5.32 ± 0.38	0.74 ± 0.08	1.16 ± 0.12			*
Rapistrum rugosum (L.) All.	7.13 ± 0.52	30.42 ± 3.29	8.49 ± 0.71	36.70 ± 2.58	2.10 ± 0.11	5.50 ± 0.41	*		
	3.39 ± 0.25	19.61 ± 1.55	2.91 ± 0.21	22.61 ± 1.95	0.88 ± 0.08	3.17 ± 0.250		*	
	2.19 ± 0.16	10.40 ± 1.23	1.05 ± 0.06	6.18 ± 0.52	0.53 ± 0.06	2.92 ± 0.21			*
Euphorbia helioscopia L.	5.81 ± 0.44	40.16 ± 0.35	3.92 ± 0.28	21.20 ± 0.25	1.63 ± 0.10	6.73 ± 0.52	*		
	6.19 ± 0.52	44.50 ± 4.12	2.70 ± 0.19	23.47 ± 2.16	1.44 ± 0.17	4.18 ± 0.35		*	
	3.15 ± 0.29	9.30 ± 0.66	1.18 ± 0.06	5.61 ± 0.46	0.81 ± 0.05	1.33 ± 0.09			*
Rosa perisica Michx. ex. juss.	27.3 ± 2.65	172.5 ± 12.35	19.61 ± 1.65	43.72 ± 4.1	3.70 ± 0.29	9.18 ± 0.68	*		
	12.16 ± 1.33	93.72 ± 8.84	7.30 ± 0.66	31.50 ± 3.25	1.65 ± 0.12	6.82 ± 0.52		*	
	1.50 ± 0.16	8.16 ± 0.77	1.21 ± 0.10	5.13 ± 0.41	0.59 ± 0.04	1.60 ± 0.06			*
Peganum harmala L.	9.15 ± 0.76	219.1 ± 16.3	3.35 ± 0.24	88.52 ± 6.25	1.92 ± 0.12	10.41 ± 1.21	*		
	4.03 ± 0.32	127.2 ± 10.26	1.30 ± 0.12	33.81 ± 2.65	0.83 ± 0.08	7.72 ± 0.55		*	
	1.67 ± 0.12	14.32 ± 1.28	1.03 ± 0.08	5.52 ± 0.37	0.60 ± 0.07	2.18 ± 0.19			*
Filago vulgaris Lam.	10.2 ± 1.32	41.91 ± 3.05	7.11 ± 0.66	18.80 ± 1.55	1.20 ± 0.25	3.19 ± 0.24	*		
	1.31 ± 0.09	9.16 ± 0.47	1.04 ± 0.08	3.72 ± 0.26	0.66 ± 0.07	2.03 ± 0.15			*
Salsola incanesens C. A. Mey.	151.3 ± 14.20	79.51 ± 5.62	41.24 ± 3.30	23.10 ± 2.15	8.20 ± 0.60	9.16 ± 0.55	*		
	103.4 ± 9.58	40.12 ± 3.23	27.11 ± 2.06	18.38 ± 1.65	5.32 ± 0.44	7.35 ± 0.60		*	
Cichorium intybus L.	26.14 ± 2.1	29.53 ± 2.8	20.25 ± 1.8	12.17 ± 1.1	3.17 ± 0.24	4.60 ± 0.27	*		
	15.23 ± 1.3	11.39 ± 1.20	8.10 ± 0.45	6.64 ± 0.52	2.66 ± 0.18	3.15 ± 0.29		*	
	2.12 ± 0.20	4.51 ± 0.36	1.83 ± 0.14	3.19 ± 0.22	0.94 ± 0.09	1.70 ± 0.10			*

(Continued on next page)

TABLE 2 The concentration of metals (mg kg^{-1} dw) in tissues of plant species grown on studied zones*(Continued)*

	Plant tissue								
	Leaf		Stem		Root		Zone		
Taxa	Mo	Cu	Mo	Cu	Mo	Cu	E	T	N
Eryngium billardieri F. Delar.	3.14 ± 0.29	92.10 ± 8.45	5.23 ± 0.44	70.36 ± 6.28	1.35 ± 0.10	9.66 ± 0.77	*		
	2.41 ± 0.18	63.47 ± 5.12	3.15 ± 0.22	31.86 ± 2.85	0.90 ± 0.11	7.59 ± 0.49		*	
Anagallis arvensis	0.85 ± 0.08	8.17 ± 0.46	0.59 ± 0.06	5.10 ± 0.33	0.42 ± 0.05	2.36 ± 0.10	*		
	6.11 ± 0.41	13.2 ± 1.12	3.28 ± 0.26	6.14 ± 0.42	1.10 ± 0.12	1.85 ± 0.55		*	
	0.74 ± 0.10	2.16 ± 0.15	0.55 ± 0.08	1.71 ± 0.19	0.22 ± 0.07	0.47 ± 0.06			*
Descuraina Sophia (L.) Schur	212.5 ± 16.8	55.32 ± 4.21	52.10 ± 4.16	19.51 ± 1.5	18.52 ± 1.66	8.14 ± 0.51	*		
	92.1 ± 6.52	21.17 ± 16.8	30.22 ± 2.94	7.72 ± 0.62	7.31 ± 0.49	3.03 ± 0.21		*	
	3.06 ± 0.17	5.30 ± 0.67	1.14 ± 0.09	4.10 ± 0.33	0.73 ± 0.09	2.46 ± 0.15			*
Caspella bursa-pastoris (L.) Medicus	2.17 ± 0.16	114.5 ± 10.3	1.02 ± 0.05	88.51+6.52	0.70 ± 0.08	14.31 ± 1.24	*		
	1.39 ± 0.12	17.35 ± 1.44	0.85 ± 0.08	3.92 ± 0.22	0.51 ± 0.07	2.15 ± 0.16			*
Stachys inflata Benth.	18.10 ± 0.12	61.31 ± 0.55	6.52 ± 0.45	27.07 ± 1.88	2.18 ± 0.19	4.47 ± 0.39		*	
Reseda lutea L.	6.11 ± 0.46	42.10 +4.31	1.88 ± 0.32	27.51 ± 2.41	0.62 ± 0.08	8.19 ± 0.52		*	
	3.90 ± 0.23	32.04 ± 3.51	1.31 ± 0.16	21.20 ± 2.34	0.50 ± 0.08	5.30 ± 0.29			*
Centaurea cyanus L.	10.33 ± 1.21	254.6 ± 24.3	3.17 ± 0.21	133.5 ± 10.62	1.80 ± 0.12	17.42 ± 1.22	*		
	6.52 ± 0.42	191.7 ± 14.2	2.23 ± 0.15	107.4 ± 8.52	2.78 ± 0.19	13.60 ± 1.54		*	
	2.16 ± 0.16	18.50 ± 1.62	1.34 ± 0.18	8.34 ± 0.51	1.59 ± 0.14	3.12 ± 0.28			*
Anchusa itallica Retz.	33.27 ± 3.10	25.19 ± 2.42	8.80 ± 0.51	20.15 ± 2.05	4.33 ± 0.32	6.73 ± 0.41	*		
	15.82 ± 1.23	31.24 ± 3.06	5.13 ± 0.41	16.09 ± 1.24	3.61 ± 0.28	7.14 ± 0.39		*	
	2.38 ± 0.14	7.59 ± 0.60	2.08 ± 0.15	4.16 ± 0.31	0.53 ± 0.08	2.69 ± 0.28			*
Cirsium acarna Moench	15.28 ± 1.22	33.63 ± 3.11	3.63 ± 0.21	11.40 ± 1.24	2.50 ± 0.19	4.21 ± 0.33	*		
Cleome coluteoides Boiss.	8.04 ± 0.28	41.51 ± 0.33	3.62 ± 0.21	18.32 ± 1.42	1.86 ± 0.12	6.30 ± 0.51	*		
	9.46 ± 0.55	29.66 ± 2.15	5.15 ± 0.42	14.80 ± 1.21	2.10 ± 0.18	4.15 ± 0.28		*	
	1.80 ± 0.30	11.13 ± 0.12	1.35 ± 0.10	7.50 ± 0.52	0.42 ± 0.05	2.40 ± 0.15			*
Centaura cyanus L.	6.91 ± 0.52	44.31 ± 0.24	5.20 ± 0.42	29.69 ± 2.10	1.85 ± 0.12	10.16 ± 1.25	*		
	5.25 ± 0.42	18.80 ± 1.52	2.91 ± 0.46	11.35 ± 1.21	1.42 ± 0.13	7.20 ± 0.52		*	
	2.08 ± 0.15	5.17 ± 0.41	1.72 ± 0.51	3.60 ± 0.21	0.59 ± 0.08	1.75 ± 0.12			*
Medicago lupulina L.	15.35 ± 1.66	29.66 ± 2.21	3.85 ± 0.25	20.16 ± 2.10	1.80 ± 0.18	6.40 ± 0.42	*		
	10.20 ± 1.21	19.18 ± 2.10	2.40 ± 0.19	14.33 ± 1.21	1.25 ± 0.10	8.20 ± 0.51		*	

	1.85 ± 0.33	10.32 ± 1.24	1.15 ± 0.10	5.18 ± 0.24	0.81 ± 0.16	2.77 ± 0.41			*
Gypsophila pilosa Huds.	72.18 ± 5.31	67.51 ± 4.24	23.10 ± 2.11	38.42 ± 2.85	5.18 ± 0.42	12.30 ± 1.54	*		
	44.06 ± 4.10	32.10 ± 2.55	15.26 ± 1.32	19.46 ± 1.21	3.81 ± 0.24	7.15 ± 0.62		*	
	3.21 ± 0.24	11.62 ± 2.12	1.06 ± 0.08	5.81 ± 0.44	0.62 ± 0.05	2.36 ± 0.21			*
Glaucium elegans Fisch. & C. A. Mey.	94.50 ± 7.51	139.25 ± 11.20	32.61 ± 2.88	56.36 ± 4.10	5.20 ± 0.41	12.15 ± 1.14	*		
	61.16 ± 4.55	107.12 ± 8.15	17.80 ± 1.22	23.15 ± 2.14	3.69 ± 0.21	10.70 ± 1.29		*	
	4.22 ± 0.33	15.05 ± 1.12	1.60 ± 0.21	5.22 ± 0.41	0.80 ± 0.10	3.12 ± 0.21			*
Prangos ferulacea (L.) Lindl.	9.10 ± 0.45	82.37 ± 7.65	12.74 ± 1.33	50.71 ± 4.15	3.52 ± 0.35	6.69 ± 0.42	*		
	7.27 ± 0.55	47.15 ± 3.55	8.52 ± 0.65	21.60 ± 2.15	2.80 ± 0.43	4.10 ± 0.33		*	
	3.02 ± 0.22	9.27 ± 0.55	1.54 ± 0.10	3.85 ± 0.21	0.41 ± 0.06	2.25 ± 0.19			*
Xanthium strumarium L.	11.51 ± 1.21	37.11 ± 3.02	4.35 ± 0.33	20.35 ± 2.15	2.80 ± 0.41	6.61 ± 0.38	*		
	6.70 ± 0.52	15.92 ± 2.21	3.10 ± 0.21	6.47 ± 0.51	2.15 ± 0.31	3.80 ± 0.40		*	
	2.12 ± 0.15	6.50 ± 0.35	1.26 ± 0.12	4.81 ± 0.39	0.91 ± 0.22	2.30 ± 0.24			*
Eurotia ceratoides (L.) C. A. Mey.	5.23 ± 0.44	59.12 ± 4.40	2.90 ± 0.31	37.14 ± 3.22	1.55 ± 0.13	8.81 ± 0.61	*		
	3.78 ± 0.25	40.50 ± 4.32	2.59 ± 0.42	28.21 ± 2.23	1.21 ± 0.20	6.30 ± 0.42		*	
	0.86 ± 0.12	19.15 ± 1.55	0.52 ± 0.06	10.16 ± 1.21	0.41 ± 0.05	4.27 ± 0.33			*
Polygonum hydropiper L.	14.21 ± 1.22	37.11 ± 3.24	5.26 ± 0.42	19.03 ± 1.51	2.12 ± 0.21	9.10 ± 0.54	*		
	6.52 ± 0.43	20.52 ± 2.19	3.10 ± 0.21	11.23 ± 1.54	1.63 ± 0.22	5.42 ± 0.36		*	
	2.41 ± 0.30	8.35 ± 0.62	0.90 ± 0.08	4.02 ± 0.31	0.50 ± 0.04	2.91 ± 0.32			*
Echium amoneum Fisch. et Mey	3.21 ± 0.23	6.88 ± 0.55	0.72 ± 0.06	4.31 ± 0.34	0.54 ± 0.03	1.12 ± 0.08	*		
	2.14 ± 0.19	4.24 ± 0.31	0.56 ± 0.04	2.61 ± 0.21	0.38 ± 0.05	1.21 ± 0.14		*	
	1.78 ± 0.13	3.21 ± 0.27	0.84 ± 0.07	1.86 ± 0.18	0.25 ± 0.04	0.81 ± 0.08			*

*E: extraction zone, T: tailing zone, N: non-contaminated zone.

Asteraceae (10 species), Brassicaceae (four species) and three species in each family of Boraginaceae, Fabaceae, Lamiaceae and Chenopodiaceae. The levels of Mo and Cu in leaves, stems and roots varied with plant species. High metal contents were observed in aerial parts than roots of studied plants collected from different zones. In general all plants collected from extracting and/or tailing zones contained higher Mo and Cu concentrations in their tissues than those growing on non-contaminated soils, except *Ajuga chamaecistus* and *Cramb orientalis* L.

A. chamaecistus from extracting (0.66–0.91 mg kg^{-1} dw) and tailing zones (0.5–1.8 mg kg^{-1} dw) showed the lowest Mo in its tissues and *C. orientalis* L. revealed the lowest Cu contents (0.7–2.2 mg kg^{-1} dw). The species *Achilla tenuifollia* showed Mo concentration above 1500 mg/kg, the level above which a species could be considered as Mo-hyperaccumulator. Molybdenum and Cu contents were greater than their normal levels (5 mg kg^{-1} Mo and 25 mg kg^{-1} Cu) in 36 species from extracting zone and in 33 species from tailing zone among which *Erodium ciconium* with 1308 mg kg^{-1} Mo, and *Conyza Canadensis* with 618 mg kg^{-1} Cu were tops. The indexes of metal uptake for Mo and Cu in five selected plant species with highest and lowest metal accumulating levels were shown in Table 3. The accumulation indexes of Mo in shoots of *Achilla tenuifollia* with AF; 354 and EC; 10.4 were tops of the list followed by 227 and 6.7 of these indexes for *Erodium ciconium*. In these two species, AF and EC were lower than 1 for Cu. The species *Conyza Canadensis* showed AF, 23, and EC, 3.2, for Cu in extracting zone. These indexes were below 1 for *Cramb orientalis* L. and *Ajuga chamaecistus*. Two species, *Ajuga chamaecistus* and *Cramb orientalis* L., had EC values for Cu and Mo below 1, indicated that the levels of these metals in their shoots were lower than the soils that they were grown on.

The TF values for Mo and Cu in plants of this table were greater than 1, indicating that these metals move from their roots to their shoots. The

TABLE 3 The indexes levels of metal accumulation and translocation in tissues of plant species collected from metal contaminated zones

	Accumulation factor				Translocation factor				Enrichment coefficient			
	Extracting zone		Tailing zone		Extracting zone		Tailing zone		Extracting zone		Tailing zone	
Plant species	Mo	Cu	Mo	Cu	Mo	Cu	Mo	Cu	Mo	Cu	Mo	Cu
Achilla tenuifolia	354	0.85	197	0.7	8.4	13.4	12.1	9.6	10.4	0.11	52.8	0.20
Erodium ciconium (Jusl) L.	227	0.76	155	0.48	15.6	8.0	20	7.9	6.7	0.10	41.5	0.14
Conyza Canadensis (L.)Cornq.	24	23	9.7	20.5	16.2	27.7	13.9	33.4	0.7	3.2	2.6	6.1
Ajuga chamaecistus	0.62	0.30	0.52	0.23	2.8	8.3	5.2	4.0	0.01	0.04	0.14	0.06
Cramb orientalis L.	0.32	0.14	0.36	0.16	5.4	5.2	8.6	4.4	0.01	0.02	0.09	0.04

TABLE 4 The leaf chlorophyll and biomass contents of aerial parts of metal accumulator and excluder plants collected from studied zones

Plant species	Zone	Chlorophyll (mg g^{-1} FW)	Biomass (mg g^{-1} FW)	
			Shoot	Root
Achilla tenuifolia	Extracting	3.17 ± 0.18	63.7 ± 7.20	25.17 ± 2.66
	Tailing	2.72 ± 0.12	72.14 ± 4.32	27.3 ± 2.55
	Non-contaminant	3.26 ± 0.21	76.2 ± 6.18	31.4 ± 4.42
Erodium ciconium (Jusl) *L.*	Extracting	2.12 ± 0.34	93.15 ± 7.2	41.52 ± 3.88
	Tailing	2.51 ± 0.19	84.80 ± 9.3	50.32 ± 6.10
	Non-contaminant	2.74 ± 0.38	105.3 ± 12.5	56.71 ± 8.78
Conyza Canadensis (L.)Cornq.	Extracting	1.64 ± 0.23	142.9 ± 15.8	91.52 ± 8.56
	Tailing	1.93 ± 0.16	158.1 ± 12.7	83.52 ± 9.27
	Non-contaminant	2.08 ± 0.27	171.4 ± 14.51	102.6 ± 12.10
Ajuga chamaecistus	Extracting	4.21 ± 0.30	39.15 ± 5.58	21.22 ± 3.12
	Tailing	3.81 ± 0.28	30.22 ± 3.74	24.77 ± 3.64
	Non-contaminant	4.49 ± 0.35	36.1 ± 2.84	26.14 ± 2.31
Cramb orientalis L.	Extracting	2.65 ± 0.26	59.7 ± 5.21	36.20 ± 4.15
	Tailing	3.04 ± 0.29	66.1 ± 6.22	31.19 ± 4.52
	Non-contaminant	3.18 ± 0.41	70.4 ± 6.45	29.31 ± 3.84

highest TF levels of Mo were obtained in *Erodium ciconium* and the lowest were observed in *Ajuga chamaecistus* from extracting zone.

Table 4 shows the chlorophyll content and biomass values of selected plant species. The factors in these plants varied insignificantly between contaminated and non-contaminated zones.

The rate of metal storage, metal chelator protein levels and non-enzymatic antioxidant concentrations in leaves were shown in Table 5. Vacuole storage of Mo and/or Cu in metal accumulators was considerably greater than chloroplast storage of these metals. Chloroplast metal accumulation rates in plant species from contaminated zones were not considerable (4.5 to 12.5%) and they did not differ remarkably with respect to the same plants from non-contaminated zone. The rates of Mo vacuole storage were the highest for *Achilla tenuifollia* and *Erodium ciconium* among plant species from contaminated zones. This rate was also the highest for Cu in *Conyza Canadensis* and it was about three folds of the same plant species collected from non-contaminated zone. Two other species, *Ajuga chamaecistus* and *Cramb orientalis* L., showed low metal storage in their studied organelles (about 5 to 12%). In addition the values of phytochelatins; PC_2 and PC_3, and the levels of Cys and GSH in *Achilla tenuifollia*, *Erodium ciconium* and *Conyza canadensis* from high-contaminated zones were significantly higher than the tissues of the same plants collected from non-contaminated zone. These parameters did not differ significantly in *Ajuga chamaecistus* and *Cramb orientalis* L. as they compared between studied zones.

The levels of antioxidative enzyme activities in leaves of studied plant species are shown in Table 6. All enzyme activities in leaf tissues, particularly

TABLE 5 The levels of non-enzymatic antioxidant parameters, the rate of metal storage in leaf organelles and metal chelators of plant species from studied zones

		High contaminant								Non-contaminant							
		% in vacuoles		% in chloroplasts						% in vacuoles		% in chloroplasts					
Plant species	Zone	Mo	Cu	Mo	Cu	Cys	GSH	PC_2	PC_3	Mo	Cu	Mo	Cu	Cys	GSH	PC_2	PC_3
Achilla tenuifolia	E	38.1	10.2	7.6	5.8	273 ± 21¶	481 ± 45¶	216 ± 18¶	122 ± 10¶	9.3	9.6	4.2	7.5	81.2 ± 6.2	88.3 ± 7.4	91.5 ± 8.5	46.2 ± 3.8
	T	33.2	8.8	6.7	6.2	215 ± 17¶	392 ± 33¶	181 ± 16¶	105 ± 8¶								
Erodium ciconium	E	30.7	11.4	5.7	4.4	439 ± 36¶	715 ± 65¶	515 ± 48¶	196 ± 16¶	12.4	8.8	10.4	6.9	172 ± 15.2	228 ± 21	214 ± 19.5	70.5 ± 6.3
(Jusl) L.	T	36.2	9.7	7.3	8.6	385 ± 31¶	872 ± 81¶	430 ± 39¶	162 ± 14¶								
Conyza Canadensis	E	7.7	38.5	10.2	12.2	517 ± 41¶	239 ± 20¶	819 ± 77¶	310 ± 24¶	11.5	13.3	5.5	8.8	177 ± 15.2	127 ± 11	330 ± 29	206 ± 19
(L.) Cornq.	T	8.9	34.3	5.3	9.4	641 ± 60¶	315 ± 23¶	1027 ± 94¶	396 ± 41¶								
Ajuga chamaecistus	E	4.6	8.3	10.8	12.5	126 ± 14.2	257 ± 17	154 ± 18.5	92 ± 9.5	7.3	10.4	5.1	11.8	109 ± 12.6	284 ± 21	196 ± 23.2	77.5 ± 7.2
	T	9.1	11.5	6.1	5.7	92 ± 8	302 ± 24	104 ± 9	74 ± 6								
Cramb orientalis L.	E	5.5	9.4	10.6	6.3	184 ± 19.2	152 ± 12.5	226 ± 21	139 ± 11	9	6.5	12	8	156 ± 16.6	181 ± 16	237 ± 19.5	115 ± 10.2
	T	8.8	5.3	4.8	11.2	109 ± 10	81 ± 7	171 ± 15	118 ± 9								

*Cys: Cysteine (nmol g^{-1} FW), GSH: reduced glutathione (nmol g^{-1} FW), PC_2: phytochelatin (nmol g^{-1} FW), E: extracting zone, T: tailing zone.

¶: significant difference as compared with plants of non-contaminated zone.

TABLE 6 Activities of antioxidative enzymes (U/mg protein) in leaves of plant species collected from studied zones

	Contaminated zone											
	GR		APX		CAT		SOD		Non-contaminated zone			
Species	Extracting	Tailing	Extracting	Tailing	Extracting	Tailing	Extracting	Tailing	GR	APX	CAT	SOD
Achilla tenuifolia	2.81 ± 0.21¶	1.92 ± 0.13¶	13.7 ± 1.5¶	10.8 ± 1.3¶	141 ± 12.3¶	130 ± 11.1¶	312 ± 28¶	262 ± 24.1¶	0.63 ± 0.05	5.2 ± 4.4	82.5 ± 7.7	143 ± 10.2
Erodium ciconium (Jusl) L.	2.10 ± 0.3¶	1.71 ± 0.12¶	9.4 ± 0.8¶	8.1 ± 0.7¶	118 ± 9.5¶	103 ± 8.5¶	204 ± 19.1¶	193 ± 15.2¶	0.59 ± 0.04	6.1 ± 0.54	69 ± 6.2	121 ± 8.8
Conyza Canadensis (L.) Cornq.	3.40 ± 0.4¶	2.21 ± 0.18¶	8.2 ± 0.7¶	7.1 ± 0.6¶	173 ± 18.2¶	126 ± 12.3¶	362 ± 31¶	286 ± 25.3¶	1.12 ± 0.1	5.4 ± 4.9	102 ± 9.5	171 ± 14.2
Ajuga chamaecistus	1.24 ± 0.19	1.11 ± 0.1	6.8 ± 0.4	6.38 ± 0.5	82.5 ± 8.1	71 ± 6.6	111.2 ± 10.2	128 ± 9.4	0.98 ± 0.10	7.25 ± 0.7	68.4 ± 6.2	123 ± 11.6
Cramb orientalis L.	4.15 ± 0.3	3.71 ± 0.28	10.3 ± 1.5	8.9 ± 0.7	53.2 ± 6.1	62.1 ± 5.5	89.3 ± 8.2	71.5 ± 7.6	4.40 ± 0.5	8.1 ± 0.8	64.5 ± 6.6	76.2 ± 8.1

*GR: glutathione reductase, APX: ascorbate peroxidase, SOD: superoxide dismutase, CAT: catalase.

¶: significant difference as compared with plants of non-contaminated zone.

glutathione reductase, were significantly higher in *Achilla tenuifollia*, *Erodium ciconium* and *Conyza canadensis* from high-contaminated zones with respect to the non-contaminated zone. In these three species, enzyme activities were higher in extracting zone with respect to tailing zone. Two species, *Ajuga chamaecistus* and *Cramb orientalis* L., from high-contaminated zones revealed insignificant variations in their antioxidant enzyme activities with respect to the same plants collected from non-contaminated zone.

Table 7 illustrates the levels of oxidative damage biomarkers. The species *Achilla tenuifollia*, *Erodium ciconium* and *Conyza canadensis* from extracting zone showed higher MDA than non-contaminated zone among which only the increase in *Achilla tenuifollia* was significant. The levels of dityrosine varied insignificantly in studied plant species as compared between high-contaminated and non-contaminated zones.

DISCUSSION

Mining activity in Mo-mineland generates large amounts of particulate emissions and metalloids that can contaminate the surrounding soils. Mo-ore also occurs naturally at surface soils covered the mine area. Our soil analysis revealed low levels of Ni, Pb, and Zn, the concentrations which did not meet pollution warning threshold for plant growth; Ni: 750, Pb: 300, Zn: 900 mg/kg, (Alloway, 1995). Total levels of Mo and Cu were at toxic values in soils of tailing and extracting zones. High levels of these metals maybe related to the fact that Mo in the form of molybdenit is naturally found and co-extracted with copper (Poorkani and Banisi, 2005). Phyto-availability of these metals in solid-phase fractions from soils refers to the degree of metal association with roots readily bio-available to plants and thus are a better indicator of immediate phytotoxicity. Aqueous extraction as a portion of total Mo and/or Cu provided an estimate of the amount of metal available in the soil. In tailing and extracting zones of Mo-mineland the rate of available to total concentration of these two metals were greater than 30%, the levels that exceeded from their normal values; Mo (40), Cu (25) mg kg^{-1} (Alloway, 1995; Bowen, 1979).

Therefore, the soils were sources of available Mo and Cu metals for potential toxic and stress effects on plants. This condition could support the growth of specific plant communities which most of them revealed wide variations of Mo and Cu within their tissues. Although we had many species with low levels of Mo and Cu, among them two species; *A. chamaecistus* and C. *orientalis* L. revealed these metals at least of evaluated list on contaminated zones and didn't exhibit morphological toxicity signs of Mo and/or Cu. Because metal exclusion is a more common strategy than metal accumulation, we selected these two species to clarify their tolerance mechanism. In our species accumulation factor (AF) and enrichment coefficient (EC) were

TABLE 7 The levels of oxidative damage biomarkers (nmol/mg protein) in leaf of plant species from different zones

Species	Malondialdehyde			Dityrosine		
	Extracting zone	Tailing zone	Non-contaminant zone	Extracting zone	Tailing zone	Non-contaminant zone
Achilla tenuifolia	247 ± 20.5¶	224 ± 23.7	183.5 ± 19.3	93.7 ± 7.4¶	80.6 ± 6.1	71.3 ± 8.9
Erodium ciconium (Jusl) L.	332 ± 31.5	256 ± 18.1	281.6 ± 24.7	131.7 ± 14.2	102.5 ± 8.8	113.7 ± 10.8
Conyza Canadensis (L.) Cornq.	92.7 ± 11.8	60.1 ± 6.2	72.8 ± 8.5	123.9 ± 8.3	156.5 ± 12.3	136.4 ± 10.1
Ajuga chamaecistus	36.5 ± 2.1	30.7 ± 4.1	41.6 ± 5.9	53.7 ± 6.6	74.6 ± 7.9	90.3 ± 8.6
Cramb orientalis L.	132.7 ± 10.6	201 ± 17.4	123.7 ± 11.2	146.2 ± 12.5	246 ± 23.5	214 ± 17.5

¶: significant difference as compared with plants of non-contaminated zone.

below 1 for these metals referred to exclusion mechanism in which they avoid metal influx into their roots and then their aerial parts. In addition, metal ion stabilization in soil by mechanism in roots can restrict metal mobility and uptake by plants (Salt et al., 1995). Therefore, we can consider these plants as hypertolerant, in contrast to hyperaccumulator plants. In agreement with our finding, the study of Jie et al. (2004) on plant population from Cu-mine showed the lowest Cu level in *Rumex dentatus* species, indicated on as excluder species. Basically, metal excluder species cannot be used as means to clean-up contaminated soil but may be considered for phyto-stabilization to prevent off-site movement (Cunningham et al., 1996).

Low incidence of Mo in *A. chamaecistus* and C. *orientalis* L. restricted intrinsic exposure of these plants to metal toxicity and leaded to insignificant biomass and chlorophyll variations with respect to non-contaminated zone. Other consequences of conservation of these metals at low levels in these plants were the lack of induction in antioxidant enzyme activities and in levels of GHS, Cys and phytochelatins. Decline or slight induction in these parameters have also been confirmed in many studies on plant species with low levels of toxic heavy metals (Mishra, et al., 2006; Nicotio et al., 2002; Inouth, 2005).

In this study, extracting and tailing zones had higher levels of Mo and Cu available for plants respectively. Most of plants collected from these contaminated zones showed the same pattern of metal distribution in their tissues. On this basis, concentration of heavy metals in plant specimens reflected mainly the concentration of available metals in soils. Our analysis showed the highest levels of Mo in *Achilla tenuifolia* and then in *Erodium ciconium* (Jusl) L. respectively. *Achilla tenuifolia* can be considered as Mo-hyperaccumulator because the level of Mo exceeded 1500 mg kg^{-1} that is prescribed for hyperaccumulation of this metal in plants (Lombi et al., 2001). In addition, these two species had the highest values of AF, TF and EC parameters for Mo among studied plant species. Accordingly, Mo concentration in their shoots was greater than their roots, demonstrating a special ability of these plants to absorb and transport the metal from soil and store it in their aboveground components (Baker and Brooks, 1989; Wei et al., 2002). In spite of high levels of Mo in *Achilla tenuifolia* and *Erodium ciconium* (Jusl) L. from contaminated zone, Cu concentration fit in the range considered as normal (Reeves and Baker, 2000a). This may be attributed to the antagonistic effect of Mo on Cu uptake by these plants (Pyatt, 2001), leaded to obtain AF and EC lower than 1 for Cu. In *Conyza Canadensis* (L.) Cornq., the level of Cu was at the highest levels among studied plants, however Mo increased moderately. The level of Cu did not meet the threshold limit (1000 $\mu g\ g^{-1}$ dw) that could be considered as Cu-hyperaccumulator (Reeves and Baker, 2000b).

We observed variations in metal accumulation level among all plant species that may rise from co-tolerance against multi-metal presence in contaminated zones. This pattern has also been indicated by other studies that

focused on metal distribution levels in plants growing on contaminated soils with various heavy metals (Shu et al., 2002; Brun et al., 2001). In study of Boularbah et al. (2006), on plant species grown in poly-metallic mine sites, the main tolerant plants accumulated different amount of Pb, Cu, and Zn. Our results are also in line with the view that tolerance to more than one metal is dependent on the presence of these metals at elevated levels in contaminated zones (Wong, 1982). Due to the fact that tailing and extracting zones contained more than one metal at toxic levels, metal co-tolerance genotypes showed greater tolerance levels to multi-metals than their normal counterparts. In agreement to these documents, plants co-tolerant to more than one metal has been reported by other authors (Von-Frenckell-Insam and Hutchinson, 1993). Based on TF index, our metal-accumulator plants were able to transport the absorbed metal into the above ground parts particularly into the leaves. To avoid metal toxicity in the leaves, a part of excess metals may store at cell walls (Hughes and Williams, 1988), or accumulate in vacuoles (McCain and Markley 1989). With regard to these documents, we found that accumulated percentage of Mo was about 35 in *Achilla tenuifolia*, 33 in *Erodium ciconium (Jusl)* L. and for Cu was about 36 in *Conyza Canadensis* (L.) Cornq. that were collected from contaminated zones. Metal stabilization and chelating via binding to ligands is another strategy against toxicity of excess metals within cells and tissues (Rauser, 1995). Accumulation of various metals in plant tissues has been shown to induce Cys, GSH and then phytochelatins as thiol-rich amino acids and peptides with high affinity to free metal ions (Zenk, 1996). Metals are firstly chelated by GSH and then transferred to phytochelatins for eventual sequestration to render them harmless (Gupta et al., 1998). Accordingly, the considerable elevations in these parameters are involved in tolerance mechanism of our metal accumulators. On the other hand, metal accumulation plays stressor role in plants and may prone them to ROS production.

It has been indicated in many studies that metal phyto-toxicity is mediated by ROS generation (Lynch and Clair, 2004). One of the most important mechanisms of metal-mediated free radical generation is via a Fenton-type reaction in which a transition metal ion reacts with H_2O_2 to form hydroxide radical (Devi and Prasad, 1998). In addition, Mo is the cofactor of xanthine oxidase and sulfate oxidase which their catalysis produce ROS (Hille, 1999). These oxygen species are very reactive and causes severe damage to membrane lipids and cell proteins. They also induce activity of antioxidant enzymes as defense system to restrict biomolecules damage by ROS (Lombardi and Sebastiani, 2005). To test the scavenging potential of ROS in metal-accumulator plants, we evaluated the levels of antioxidant enzymes and biomarkers of oxidative damages; MDA and dityrosine. Our data showed that considerable elevation in antioxidant enzymes in metal-accumulator plants from contaminated zones were capable of restricting MDA and dityrosine levels within the range of their variations in same plants from un-contaminated

zone. Another consequence of well regulated of scavenging and production of ROS may be the insignificant variations in chlorophyll and biomass contents of metal-accumulator leaves. Accordingly, the induction effects of accumulated metals on the enzymatic and non-enzymatic defense parameters in metal-accumulator and hyperaccumulator plants were sufficient to protect them against ROS attack.

CONCLUSION

This study presented *Achilla tenuifolia* as a new Mo-hyperaccumulator growing on Mo-mineland contaminated with toxic levels of Mo and Cu. We showed different tolerance strategies in studied plants. In *Ajuga chamaecistus* and *Cramb orientalis* L. exclusion of metals from roots or its stabilization in the soil limited metal toxicity effects. *Erodium ciconium* (Jusl) L. and *Conyza Canadensis* (L.) Cornq. as moderate metal accumulators with *Achilla tenuifolia*, all responded positively by induction in synthesis of metal chelators and antioxidant enzyme activities, thought played roles for detoxification of Mo and Cu. They also stored a portion of metals in their leaves vacuoles. These processes involved in studied plants tolerance and protected them against metal toxicity. However, plants accumulated high amount of Mo and/or Cu can be used as phyto-remediators species.

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