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Original Research

Hyperaccumulator Plants of the Keban Mining District and Their Possible Impact on the Environment

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Abstract

Metal intake abilities of *Euphorbia macroclada, Verbascum cheiranthifolium Boiss and Astragalus gummifer*, which are common and native throughout Turkey and similar locations, were studied in the heavily polluted Keban mining district in Elazig, Turkey. For this aim metal contents of dried plants and soil were determined and correlated. Soils of Keban area have higher than average values for soil, Mo, Cu, Pb, Zn, Ag, As and Cd contents.

All the studied plants take up metals in high amounts - as high as hundreds of times more than averages for non-hyperaccumulator plants. Usually, higher plant metal contents are attained where higher soil metal contents exist. Enrichment factors, which are calculated by dividing metal contents of plant by metal contents of soil (= metal content of plant/metal content of soil), are higher in lower soil metal contents.

Maximum metal contents in the shoots (as mg kg^{-1}) and enrichment factors for *Euphorbia* are: Mo 260-1.28, Cu 33-0.18, Pb 76-0.09, Zn 190-0.51, Ag 0.53-1.1, Mn 276-0.28, As 10.2-0.08, and Cd 0.20-0.13. For *Verbascum*: Mo 80-0.83, Cu 27-2.87, Pb 295-1.57, Zn 254-1.78, Ag 0.37-0.92, Mn 627-0.58, As 63.5-0.50 and Cd 0.59-1.25. For *Astragalus's gummufer*: Mo 402-0.98, Cu 30-0.95, Pb 552-0.82, Zn 241- 0.31, Ag 0.54-0.64, Mn 1072-0.34, As 45.4-0.34 and Cd 0.34-0.44.

All of the three plant species have enrichment factors exceeding hyperaccumulating criterion >1 for most of the elements investigated. Most of the hyperaccumulator values belong to *Verbascum cheiranthifolium Boiss*.

Hyperaccumulating properties have been considered for reclamation of contaminated lands. This study claims that plants with high metal intake abilities escalate mobility of metals and increase contaminations on surface and subsurface.

Keywords: contaminated lands, heavy metal, hyperaccumulator plants, Keban, Turkey

Introduction

The root system of plants acts as a powerful sampling mechanism as they collect solutions from a large volume of moist ground. Inorganic salts contained in the solutions are usually deposited in the upper parts of the plant. Therefore, plants realize two important functions in the environment where they live: they solve and intake metals and other constituents of the ground. As they concentrate metals and other inorganic substances in their bodies, plants have been used as a useful tool for biogeochemical exploration of subsurface sources since the pioneering works of V.M. Goldschmit at early 1930s.

Metal intake abilities of plants vary in large intervals and the plants which take up high amounts of metals are defined as "hyperaccumulator plants.'' Criteria for "hyperaccumulator plants'' are described as metal contents in shoot dry matter (Cd >100 mg kg^{-1}, Cu >1000 mg kg^{-1},

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Pb>1000 mg kg^{-1}, Zn>10000 mg kg^{-1}), the ability to store heavy metals in above-ground parts 10-500 times more than in usual plants, and an enrichment coefficient >1 [1, 2, 3].

The inorganic substance intake ability of plants is also considered for the rehabilitation of contaminated environments due to industrial and mining activities. This relatively new approach is called phytoremediation, which is defined as the use of plants to remove, destroy or sequester hazardous substances from the environment [4]. Phytoremediation has become a topical research field in the last decades as it has emerged as a cheap and effective natural way of rehabilitation of the environment [5-8]. Many metal hyper accumulators have so far been discovered as a result of scientific work on the subject [9-15].

This study investigates metal intake capabilities of three common plant species: *Euphorbia, Astragalus, and Verbascum* in the Keban mining district (Fig. 1), and discuss the impacts of hyper accumulation on environment.

Material and Methods

The Study Area

This study was carried out in the Keban mining district of Elazig, a province in Eastern Turkey (E38°40’502” and N38°47’52”) (Fig. 1). The study area consists of Paleozoic-Mesozoic metamorphic lithologies; marble, calc-shists and mica schists: and subvolcanics of trachyite, trachilatite and alkali trachytie [16]. The age of subvolcanics is given as 74± 3 my. according to K/Ar absolute age determinations of [17]. The subvolcanics consist of the lithologies of four different phases, each of them with a distinct ore mineral suite of pyrometasomatic and vein type ore depositions. The economic concentrations are Pb-Zn-Ag, Magnetite-Cu, W, Mn-Ag and Flourite-Mo ores (Fig. 1).

Argentiferous Pb-Zn ores were the main economic sources of the Keban area and have been mined for 6000 years (^{14}C absolute age determinations on wooden mining tools discovered in ancient mining cavities by Seeliger et al. [18]. Cu, Fe and Flourite ores were mined only in short intervals. Abandoned mining sites, wastes, overburden, slugs from ancient smelters and flotation discharges have contaminated the area heavily in the absence of any reclamation. Rough surface morphology might have played an important role in the secondary dispersion of the metallic patterns.

The Plants Studied

The investigated plants were chosen among those which are native and common in Anatolia and in the regions with similar morphology and climate. The ability to colonize and thrive in heavily contaminated soil and semi-arid areas and deep-reaching root systems were other criteria used for choosing the plants. The three studied plants met all the criteria. The plants and their brief biological features are as follows:

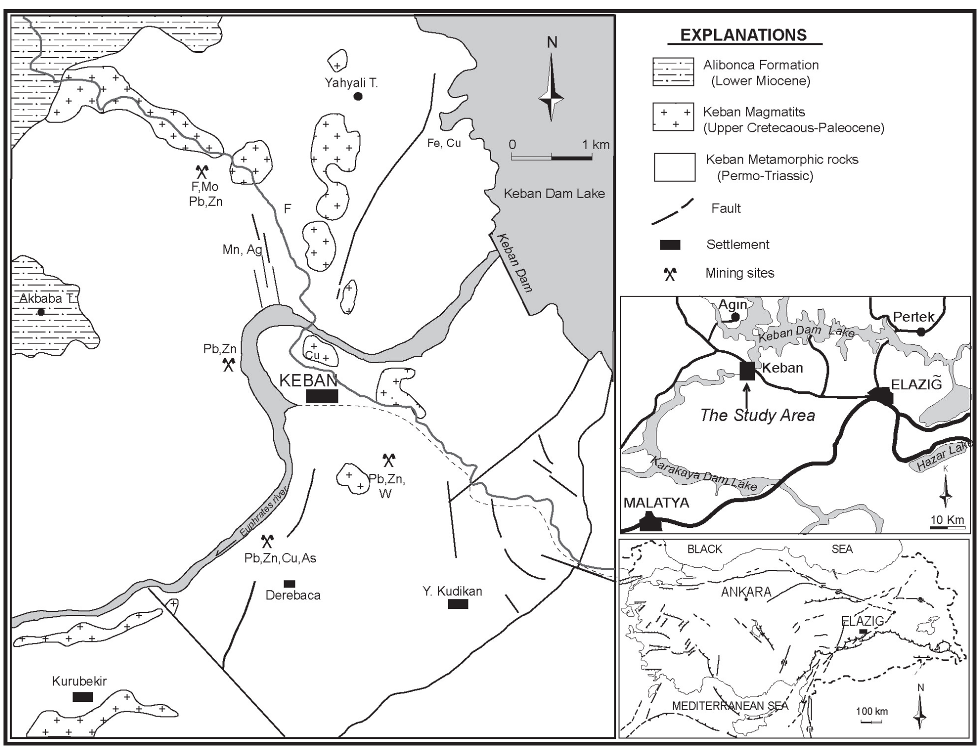

Fig. 1. Geology of the study area (simplified from [27]).

Euphorbia macroclada Boiss
(Common name: Spurge, Local name: Sütlegen)

The *Euphorbia* family of plants are annual, biennial and perennial herbs and sub shrubs with milky latex [21]. There are about 2,000 species of *Euphorbia* and the species range from weeds to trees. Studied *Euphorbia macroclade Boiss* is a very common weed in Anatolia. All the species have latex. The latex of *Euphorbia* has been intensively studied for medical and fuel yield purposes. Its poisonous contents have also been studied. Most of the members of the *Euphorbia* species blossom as buckets of long-lasting flowers. These features make *Euphorbia* a very popular garden plant [19]. *Euphorbia* prevents erosion effectively because of its expansive root system, ability to live in unfavorable terrains and dense shoot growth. However, *Euphorbia* has a very bad reputation of inducing digestive maladies and exerting allelopathic effects on other plants [20].

Verbascum cheiranthifolium Boiss
(Common name: Mullein, Local name: Sigir Kuyrugu)

These are annual, biennial or perennial herbs and are 30-120 cm. tall shrubs (rarely small), with hairy leaves [21]. Common Mullein (*Verbascum)* is a medicinal plant that has been used for the treatment of inflammatory diseases, asthma, spasmodic coughs, diarrhea and other pulmonary problems [22].

Astragalus gummifer
(Common name: Milkvetch, Local name Keven)

These plants are annuals, herbaceous perennials, and unarmed or spiny shrubs. There are 380 species of *Astragalus* in Turkey [21]. With the deep (up to 20-30 m) reaching massive root system and low-surface covering umbraciform shrub shape, the *Astragalus* species prevents soil erosion effectively and as they accumulate metals of thick zones via their massive root system, have the ability to reflect the geochemistry of thick soil zones and fractured and altered rocks.

Sampling

Plant Samples

Plant samples were not collected systematically. Instead, collection sites were determined in accordance with a pattern which represents a whole of the Keban mining area. Shoot and root samples were taken at each plant sampling site. As the root system of plant species are deep reaching, the root samples were not collected as a whole of root system but only from shallow parts (up to 30-40 cm). Therefore, root samples do not represent the whole of the root system.

Soil Samples

Soil samples were collected at 30-40 cm depths and from immediate surrounding of the roots of sampled plants. The soil samples were not sieved as described in some geochemical prospecting studies for yielding more readily extractable fractions. For metal intake studies such sieving is not needed for the soil samples as the extremely corrosive environment near the root tips of plants can extract mineral matter not only from readily exchangeable forms but even from silicates [23].

Soil samples were sent to ACME Analytical Labs, Vancouver, Canada for analysis. The samples were analyzed as a whole (without screening to fractions) and using total digestion methods. Elements were determined by using ICP /AES and MS analytical techniques.

Plant Sample Processing

Shoot-leaf and root samples of plants were carefully washed with deionized water and oven-dried at 100^{0}C for 30 minutes and 60^{0}C for 24 hours. For ashing up or flameless burning of the plant samples, higher temperatures were required and previous studies recommended temperatures varying between 475^{0}C [24] and 600^{0}C [25]. They also pointed out that although high temperatures (475-500^{0}C) are necessary for removing the carbon, high temperatures also cause some trace element volatilization. Therefore, plant samples started to ash up at 250^{0}C and the temperature was ramped up to 500^{0}C in 24 hours. Ashed samples were grounded using hand mortars, labeled and analyzed using ICP/AES and MS techniques at ACME Analytical Labs, Vancouver, Canada.

As most of the evaluations in biogeochemistry are made using metal contents of dry plant matter; ashed/dried matter ratios were determined for each plant species and analysis data converted to dry matter contents. To avoid the effect of contamination, metal contents of above ground plant parts (shoot-leaves) are taken for correlation with soil contents.

Results and Discussion

The metal accumulations in the three plants and the associated soils from different sites in the Keban Mining District are presented in Table 1. In addition, data is also presented as histograms and correlation diagrams. Metal uptake by plants are discussed on the basis of the enrichment factor which is formulated as: Enrichment Factor= metal content in dry matter/metal content in soil. The Mo, Cu, Pb, Zn, Ag, As, and Cd contents of soil and plants were evaluated. These are the elements that have probably originated from mineralizations in Keban mining district. The contents of the other elements analyzed are very low and not related to the mineralizations.

Table1. Metal contents in soil and roots and twigs of studied *Euphorbia m., Verbascum c.,* and *Astragalus g.*, (all values are in mg/kg^{-1}). Soil and plant average values (as mg/kg^{-1}) are from [23] (EF: Enrichment factor).

	Mo				Cu				Pb				Zn				Ag				Mn				As				Cd			
Samp.No	soil	root	twig	E.F.	soil	root	twig	E.F	soil	root	twig	E.F	soil	root	twig	E.F	soil	root	twig	E.F	soil	root	twig	E.F	soil	root	twig	E.F	soil	root	twig	E.F
EU-21	136	85	43	0.32	27. 2	12	3.2	0.12	654	243	13	0.02	188	104	95	0.51	0.66	0.25	0.17	0.26	1246	396	53	0.04	111	35	2.6	0.02	0.82	0.41	0.11	0.13
EU-24	127	78	137	1.08	56.7	15	4.9	0.09	687	152	16	0.02	292	100	100	0.34	1.15	0.21	0.53	0.46	944	174	67	0.07	130	21	2.8	0.02	1.21	0.36	0.01	0.01
EU-26	313	55	23	0.07	48.7	30	9	0.18	773	242	14	0.02	449	126	53	0.12	0.75	0.19	0.39	0.52	730	388	109	0.15	380	42	5.2	0.01	1.30	0.19	0.01	0.01
EU-29	2636	686	124	0.05	5412	949	33	0.01	7089	1985	68	0.01		3600	190	0.01	24.1	3.12	0.12	0.01	2995	700	168	0.06	1806	332	10.2	0.01	63	6.22	0.13	0.01
EU-31	423	68	260	0.61	134	15	10	0.07	1352	211	32	0.02	631	180	70	0.11	1.93	0.18	0.11	0.06	1579	151	121	0.08	117	13	2.9	0.02	2. 20	0.71	0.01	0.01
EU-34	94	60	68	0.72	34.5	13	6.3	0.18	815	277	76	0.09	821	269	92	0.11	0.88	0.25	0.97	1.10	477	171	132	0.28	75	20	5.9	0.08	2.26	0.72	0.20	0.09
EU-41	466	171	39	0.08	61.2	26	4.8	0.08	1600	557	21	0.01	411	186	83	0.20	1.46	0.31	0.52	0.36	2929	818	106	0.04	185	39	2.9	0.02	2.03	0.34	0.01	0.01
EU-44	578	273	75	0.13	145	44	4.5	0.03	2802	1179	49	0.02	1585	436	155	0.10	4.49	1.46	0.14	0.03	6151	1499	276	0.04	278	63	3.7	0.01	2.98	0.56	0.14	0.05
EU-45	22.3	11	3	0.13	65	19	4.7	0.07	1301	338	13	0.01	305	81	15	0.05	1.15	0.25	0.34	0.30	2262	718	83	0.04	96	29	1.3	0.01	1.40	0.41	0.02	0.01
VR-25	115	81	80	0.70	41	61	27	0.66	549	16	295	0.54	592	194	254	0.43	0.57	0.10	0.37	0.65	894	36	234	0.26	423	3	63.5	0.15	2.71	0.62	0.37	0.14
VR -25Y	115	26	28	0.24	41	40	22	0.54	549	79	140	0.26	592	238	202	0.34	0.57	0.12	0.22	0.38	894	107	140	0.16	423	24	35.8	0.08	2.71	0.48	0.25	0.09
VR-27	15. 6	9	13	0.83	3.49	9	10	2.87	25.5	27	40	1.57	23.6	26	42	1.78	0.09	0.55	0.08	0.92	251	138	146	0.58	56	24	28.1	0.50	0.08	0.14	0.10	1.25
VR-35	94.3	16	16	0.17	66.4	25	20	0.30	815	138	195	0.24	820	147	237	0.29	0.88	0.20	0.32	0.36	477	177	228	0.48	75	11	22.7	0.30	2.26	0.65	0.57	0.25
VR-47	48	23	13	0.27	33	18	12	0.36	651	240	218	0.33	671	222	224	0.33	0.67	0.20	0.29	0.44	3038	787	627	0.21	69	18	18.6	0.27	2.80	0.84	0.59	0.21
AG-22	114		95	0.83	80.4		13	0.16	1205		156	0.13	529		102	0.19	2.15		0.26	0.12	860		282	0.33	164		18.3	0.11	1.69		0.15	0.09
AG-28	21.6		26	1.20	4.96		4.7	0.95	57.1		47	0.82	307		70	0.23	0.11		0.07	0.64	1066		306	0.29	95		32	0.34	0.25		0.11	0.44
AG-32	411		402	0.98	127		27	0.21	1364		395	0.29	680		208	0.31	1.89		0.43	0.23	1516		517	0.34	104		25	0.24	2.17		0.17	0.08
AG-36	1243		362	0.29	240		30	0.13	3640		419	0.12	1190		241	0.20			0.54	0.04	1567		351	0.22	128		27.1	0.21	5.51		0.34	0.06
AG-40	589		375	0.64	75.8		20	0.26	1843		552	0.30	407		108	0.27	1.20		0.31	0.26	3300		953	0.29	142		39.2	0.28	2.23		0.10	0.04
AG-42	342		303	0.89	95.6		28	0.29	1713		528	0.31	669		171	0.26	1.50		0.39	0.26	3695		1072	0.29	219		45.4	0.21	2.21		0.14	0.06
Plant average	ash ave. > 5				ash med 130				ash med. 30				ash 570				ash 0.1-1				ash med. 670				< 0.25 dry				ash 4.3			
Soil	2.5				15				17				36				> 0.1				320				7.5				0.1-0.5			

Soil Metal Contents

The metal contents of the soil samples are hundreds and even thousands of times higher than the standard values given for soils (Table 1). This is expected as the Keban area is heavily polluted by mining activities and secondary dispersions. Metal contents of some soil samples (e.g. EU-29) are exceptionally high, indicating closeness to the ore bodies. None of the soil sample metal content is lower than soil standard value and soil metal contents vary greatly. Therefore, metal uptake by plants is not hindered due to lack of metal. In other words, the Keban area is a very suitable natural environment to study the metal uptake abilities of plants.

Euphorbia - Soil

Euphorbia was sampled at 9 sites and samples were collected as root and shoot-leaves. Soil samples were also collected from the same site and next to the root system. The analysis data is presented in Table 1, Figs. 2 and 3.

As can be seen in Table 1 and Figs. 2 and 3, Euphorbia accumulated the metals hundreds of times more than the standard values for plants. At lower soil metal contents, metal uptakes are at high ratios and low at very high soil metal contents. Enrichment factors (EF) for studied elements vary widely among the metals. For only one sample, EU 24, EF exceeds "hyperaccumulation" definition criterion value of EF >1.0 for Mo (1.08) and one sample EU-34 for Ag (1.1).

Root-soil metal content correlation paths differ from shoot-soil paths. However, because of problems arising in the analysis of root samples, root-soil metal contents correlations are not evaluated and discussed in detail.

Verbascum cheiranthifolium Boiss - Soil

Root-leaf samples of *Verbascum cheiranthifolium Boiss* and soil samples were collected from 5 locations. Metal contents of plant and soil samples are given in Table 1. In general, *Verbascum c*. has higher intake ability than *Euphorbia* and has EF values exceeding "hyperaccumulator" criterion for Cu (2.87), Pb (1.57), Zn (1.78) and Cd (1.25). In addition, EF values for Mo (0.83) and Ag (0.92) are close to criterion value. Statistics on the analytical values of this study (Table 1) indicate that the correlation relations between dry plant metal contents and soil metal contents are similar to those of *Euphorbia* (Fig. 2 and 3).

Astragalus gummifer - Soil

Seven *Astragalus gummifer* samples representing varied environments and soil samples from the same sites were collected. Plant samples were collected as shoot-leaf samples but root samples could not be collected because of the plant's root morphology; this plant has a single thick root which extends 20-30 meters down and it was not convenient to collect samples representing the whole root system. This plant has only one EF value for Mo (1.2) exceeding the criterion value. However, EF values close to 1 are present for Mo (0.83, 0.89 and 0.98), Ag (0.64), Cu (0.95) and Pb (0.82). Soil-plant correlation paths are similar to those of *Euphorbia* and *Verbascum* (Table 1, Figs. 2 and 3).

Conclusions

The sudied plants *Euphorbia macroclada Boiss, Astragalus gummifer,* and *Verbascum cheiranthifolium Boiss* are native and widely distributed in the Keban mining area, Turkey and Euro-Asia. Their well-developed and deep-reaching root system, low–surface covering umbraciform nature and ability to live in severe arid and hot conditions make these plants very effective at erosion control.

Biogeochemical functions of these plants are also extraordinary as they accumulate metals Mo, Cu, Zn, Pb, Ag, As and Cd by enrichment factors up to 2.87. Plants, which accumulate high amounts of metals in their tissue, are classified as hyper accumulator plants. Hyper accumulator plants have been considered for the reclamation of contaminated lands due to mining and other industrial activities [9, 13, 14, 15]. Scientists in favor of this view claim that plants with metal accumulation ability can extract metals from the contaminated media. However, this claim does not explain the harmful consequences of metals converted to readily soluble organic compounds. The metal-organic compounds are either digested by animals (thus playing a more harmful role in organic cycle than their relatively stable form of inorganic compounds as silicates, sulphides or oxides) or decomposed, releasing metals in readily soluble ionic forms which can be easily taken into bodies of organisms. In other words, hyper accumulator plants increase migrated metal amounts and migration speeds, in organic cycles. In fact this is well manifested by *Euphorbia,* as inducing digestive maladies and exerting allelopathic effects on other plants, possibly through chemicals leached from decomposing leaf, stem and root tissues [20]. Furthermore, as more evidence of their allelopathic effects on the environment, studied plants (especially *Euphorbia*, [26]) when consumed by domestic and wild animals may cause mortality. Therefore, hyper accumulator plants should be considered and treated as plants that extract metals from stable or semi-stable phases at depths and introduce them to the surface as mobile forms and phases. A good example could be Pb, which has very low mobility at surface and subsurface conditions (e.g. pH: 6-8. and Eh 0.00- (+ 0.01); average soil Pb content is a mere 17 mg kg^{-1} and the studied accumulator plants extract Pb from soil and accumulate it (enrichment factor of 1.57). Without plant contribution, Pb mobility would be limited to only very strong acidic

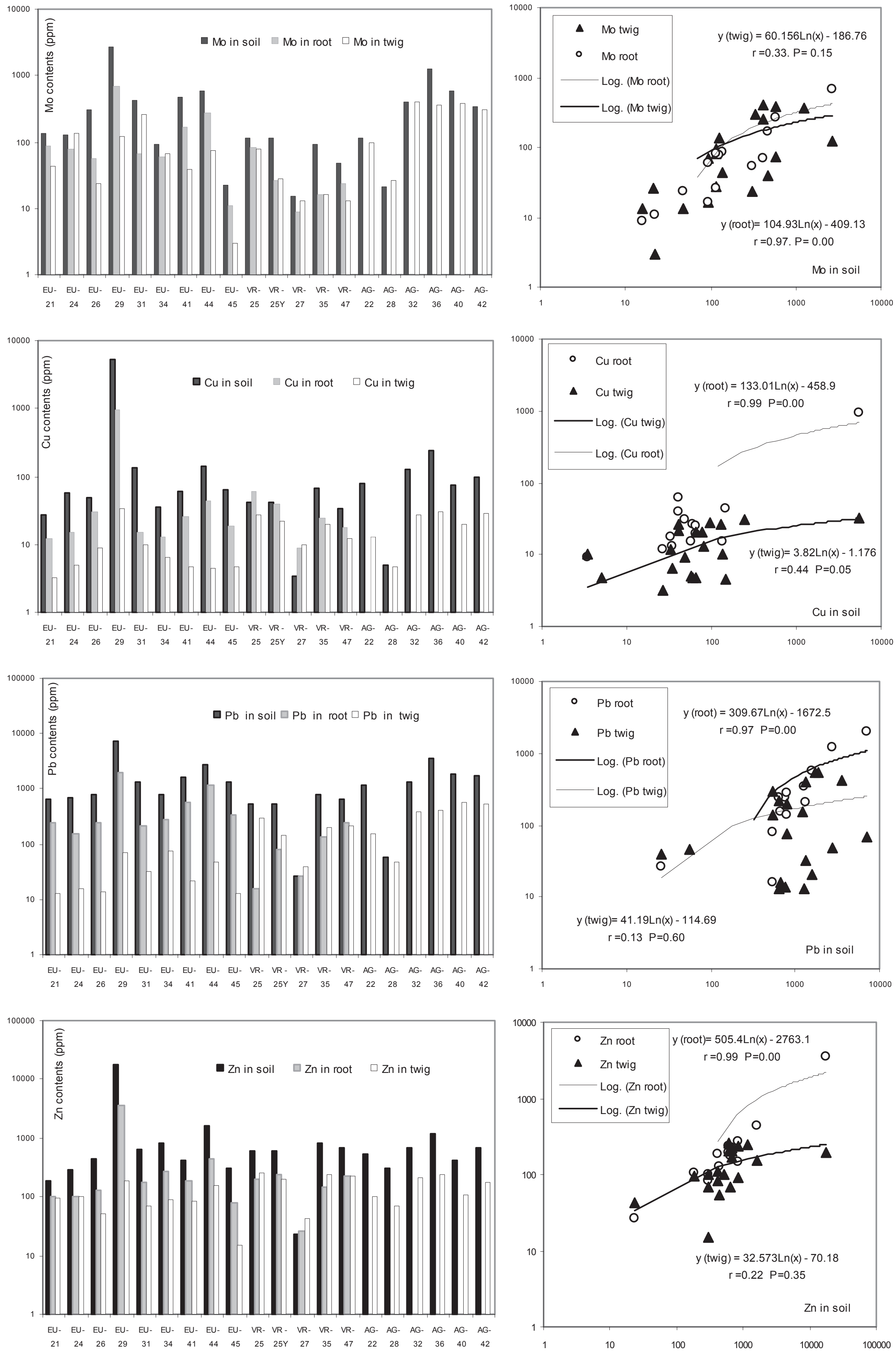

Fig. 2. Mo, Cu, Zn and Pb contents of plants (as histograms) and correlations between metal contents of soil and plant ash (EU:*Euphorbia*, VR:*Verbascum* AG: *Astragalus*).

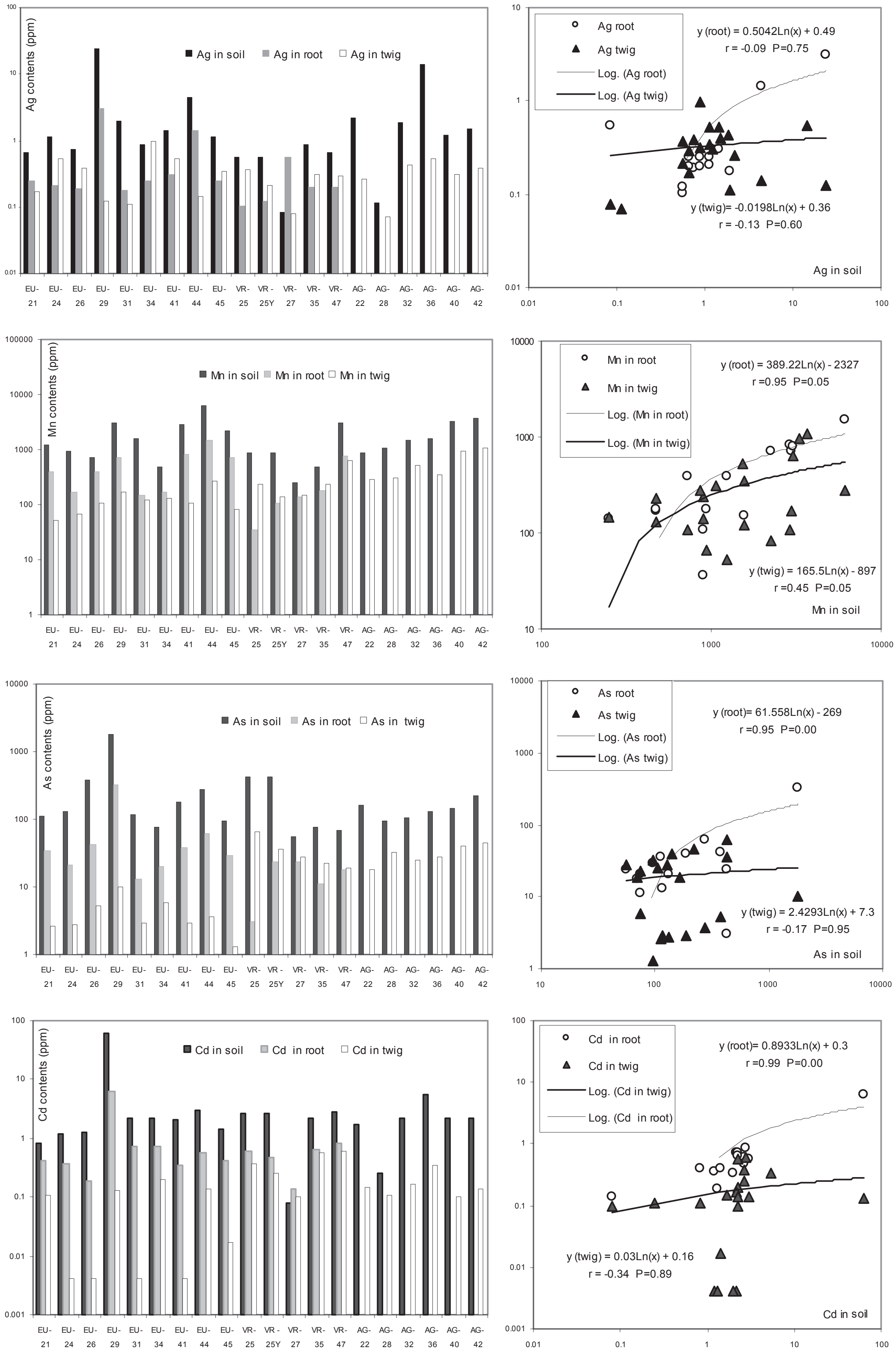

Fig. 3. Ag, Mn, As and Cd contents of plants (as histograms) and correlations between metal contents of soil and plant ash (EU: *Euphorbia,* VR:*Verbascum,* AG: *Astragalus*).

and reducing conditions. Copper also has moderate mobility at surface and subsurface conditions (15 mg kg^{-1} for average soil content) but can be accumulated in plants up to 2.87 times more than the soil content. Similar arguments for Zn, As and mobile elements Ag, Mo and Cd are also true.

These adverse effects of hyperacccumulation on the environment can be avoided by removing plant matter from the environment. This is a costly procedure, therefore the phytoremediation concept should be carefully reviewed before implementation.

Nonetheless, this study shows that the metal accumulation abilities of *Euphorbia macroclada, Verbascum cheiranthifolium Boiss and Astragalus gummifer* could be useful for biogeochemical prospecting of unsurfaced mineralization because they accumulate metals as high as 3-4 times more than soil metal contents. *Euphorbia M.* can be especially useful for the exploration of Mo, Pb, Zn, Ag and As, *Verbascum c. B.* for Mo, Cu, Pb, Zn, Ag and As, and *Astragalus g.* for Mo, Pb, Zn, Ag and As mineral contents.

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