

Original Research

Heavy Metals in Mine-Tailing Soil Mixtures Cultivated with *Ricinus communis* L.

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Abstract

Ricinus communis L. was found to vegetate mine tailings with high concentrations of Al (4,456 mg kg⁻¹), As (3,473 mg kg⁻¹), Cd (120 mg kg⁻¹), Cr (14 mg kg⁻¹), Cu (1,147 mg kg⁻¹), and Pb (910 mg kg⁻¹). We investigated how this plant responded to increased heavy metal concentrations by mixing mine tailing at 0%, 50%, 70%, and 100% with soil at 100%, 50%, 30%, and 0%, while metal concentrations in the rhizosphere, roots, and aboveground parts of *R. communis* were monitored. *Ricinus communis* shoots were 19% smaller and roots 8% in soil mixed with an equal amount of mine tailings compared to plants cultivated in soil and 33% and 54%, respectively, when cultivated in mine tailings. The ratio of As, Cd, Cu, and Pb in the aboveground plant parts to the concentration in soil remained <0.12, while that of the roots <0.25. The As concentration was 35% lower in the bulk soil than in the rhizosphere. We found that *R. communis* growth was inhibited strongly when cultivated in mine tailings, but less so when mixed with soil, and metals did not accumulate in the roots and aboveground plant parts. These characteristics make *R. communis* ideal to vegetate metal-contaminated soil, thereby reducing the environmental hazards of mine tailings.

Keywords: aboveground plant parts, bulk soil, exclusion or accumulation of metals, rhizosphere

Introduction

Mining activities generate large amounts of metal-rich waste materials and are considered a major cause of soil contamination [1]. In Mexico, mining for gold,

silver, and copper started 500 years ago, which has created huge amounts of mine waste, or tailings [2]. These tailings contaminate soil, subsoil, aquifers, surface and ground water, the atmosphere, flora, and fauna. Drinking water is contaminated and animals left to graze accumulate the heavy metals taken up by plants [3-4]. This dispersion of these heavy metals in the environment and their bioaccumulation poses a serious risk to humans [4].

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There are plants that resist high concentrations of heavy metals in the soil [5-6]. Some plants incorporate the metals, but are not affected metabolically by them, while others exclude them [7]. The efficiency of these processes depends on the type of plant, soil characteristics, and microbiota in the rhizosphere [8]. Consequently, some plants can grow on heavily contaminated mine tailings [9]. In a previous study, Ortega et al. [10] found species of the *Euphorbiaceae* family, e.g. *Acalypha monostachya* Cav., *Euphorbia* sp. L., *Jatropha* sp. L., and *Ricinus communis* L. growing on mine tailings in Zimapan (Hidalgo, Mexico). These plants, particularly *Ricinus communis*, grew in soils with high concentrations of heavy metals, such as Al, As, Cd, CO, Cr, Cu, and Pb [10]. These characteristics would make them ideal to vegetate mine tailings, thereby limiting the environmental hazards of mine waste.

Ricinus communis is a plant adapted to a wide range of climates and can be found now in most tropical and subtropical parts of the world [11], and is cultivated in countries such as India, China, Brazil, Argentina, Thailand, and the Philippines [12-13]. *Ricinus communis* is a multipurpose crop of interest because of its commercial importance as a non-food tree for biodiesel production [14-15], and unique biochemistry and valuable biomaterials, such as, castor oil, ricinoleic acid, ricinoleyl-sulfate, lithium grease (lithium hydroxystearate), 10-undecylenic acid, and 11-amino-un-decanoic acid [16]. It is used widely in traditional medicine and has been used against constipation, stomach disorders, swelling fever, and scorpion stings [17-18].

Several studies have found that *R. communis* grows in metal-contaminated soil with a potential for phytoremediation [14]. It is not known, however, how much the growth of *Ricinus communis* is affected when cultivated in mine tailings with high concentrations of heavy metals and if the plant accumulates or excludes them. Therefore, in this study *R. communis* plantlets were cultivated in mixtures of soil (100%, 50%, 30%, and 0%) and mine tailings (0%, 50%, 70%, and 100%), while plant growth was monitored and the roots and shoots were analyzed for metals after 90, 120, and 270 days.

Material and Methods

Sampling Mine Tailings and Soil Collection

Mine tailing No. 9 (20°49'9"N, 99°22'46"W) at the San Francisco Mine in Zimapan (state of Hidalgo, Mexico) was sampled (Fig. 1). Three 400 m² plots were outlined and the top 20 cm layer was spade-sampled 20 times. The 20 samples taken in each plot were pooled so that 3 samples were obtained, each of approximately 150 kg. The collected mine tailings were transported to the laboratory and characterized (Table 1). The mine tailing sampling procedure is schematized in supplementary Fig. 1.

A soil was sampled at Cinvestav, Mexico D.F. (S1: 19°30'42"N, 99°7'49"W; S2: 19°30'41"N, 99°7'46"W and S3: 19°30'38"N, 99°7'48"W). Three 400 m² plots were outlined and the 0-15 top soil layer was

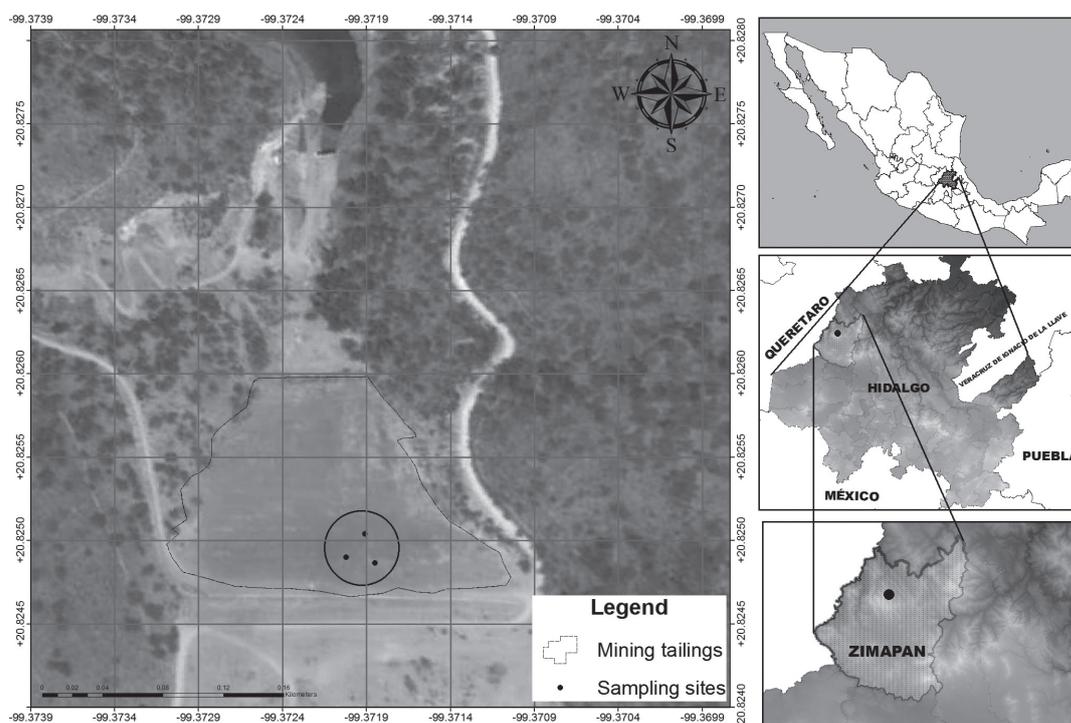
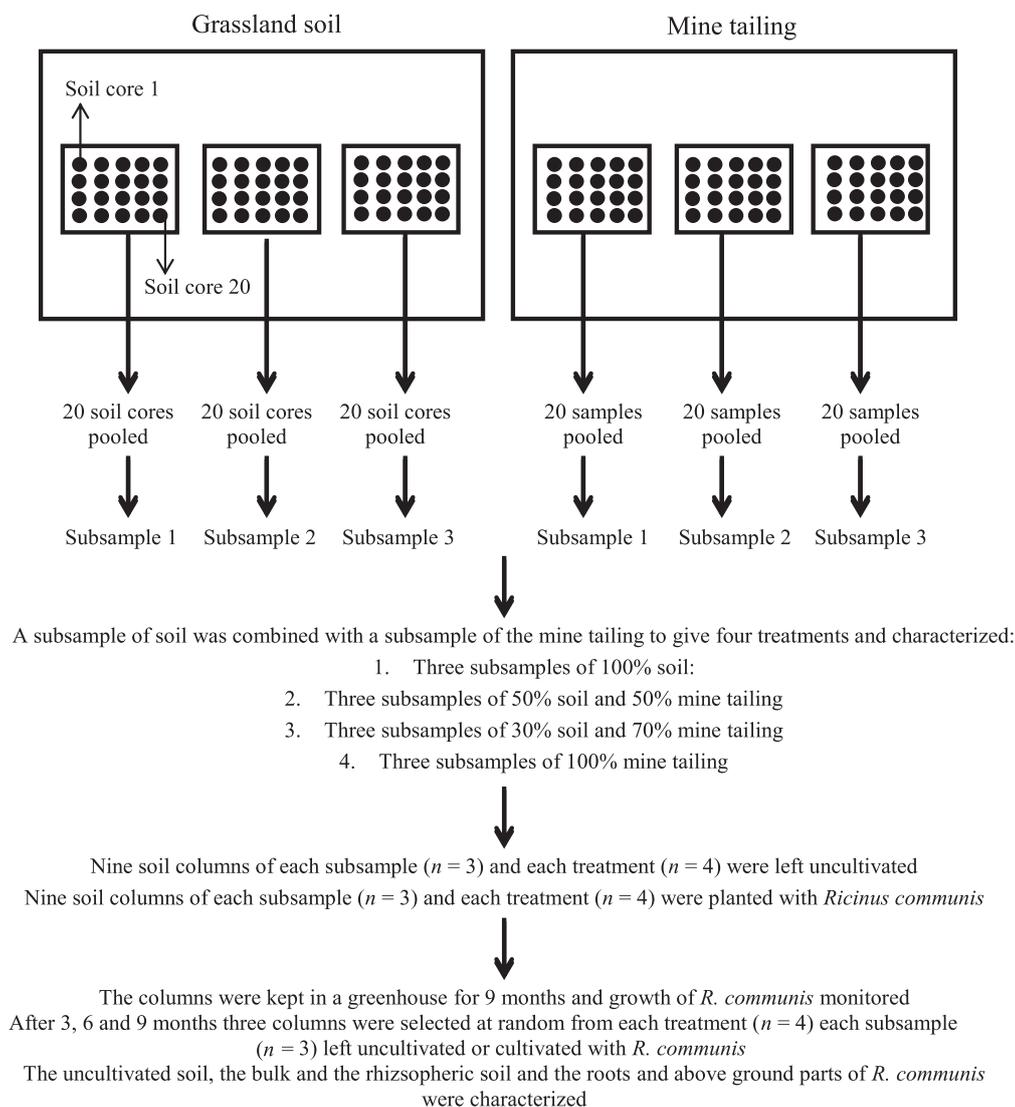


Fig 1. Sampling Sites final.

Table 1. Characteristics of soil, soil mixed with 50% mine tailings, 30% mine tailings, and 100% mine tailings at the onset of the experiment.

Mixture ^e	EC ^f (dS m ⁻¹)	pH	Particle size distribution (g kg ⁻¹ soil)					
			C _{org} ^g	N _{tot} ^h	WHC ⁱ	Clay	Loam	Sand
A	1.55 ^j b ^k	8.9 a	8.53 a	1.02 a	540	420 a	270 a	310 b
B	2.75 ab	8.4 ab	8.05 a	0.54 bc	520	120 b	110 b	770 a
C	3.78 a	8.4 ab	9.17 a	0.30 c	520	50 b	80 b	870 a
D	3.03 ab	8.0 b	4.86 a	0.13 cd	0	40 b	60 b	900 a
<i>F</i> value	10.74	9.59	0.21	28.86	1.35	24.89	15.02	57.65
<i>P</i> value	0.008	0.011	0.889	<0.001	0.344	<0.001	0.003	<0.001
MSD ^l	1.68	0.6	16.71	0.38	860	200	140	200

^eMixture A: 100% soil, Mixture B: 50% soil and 50% mine tailing, Mixture C: 30% soil and 70% mine tailing, Mixture D: 100% mine tailing, ^fEC: Electrolytic conductivity, ^gC_{org}: Organic carbon, ^hN_{tot}: Total nitrogen, ⁱWHC: Water holding capacity, ^jMean of three plots (*n* = 3), ^kValues with the same capital letter are not significantly different between the mixtures (i.e. within the lines), ^lMSD: Minimum significant difference at *P*<0.05 (SAS Institute 1989)



Supplementary Fig. 1 Sampling procedure at the mine tailing, collection of the soil, mixture of soil and mine tailing and treatments applied.

Table 2. Characteristics of *Ricinus communis* L. cultivated in different mixtures of soil and mine tailings in a greenhouse.

Mixture ^c	Number	Leaves		Shoot	Root
		Length	Width		
(cm)					
A	16.9 ^{f a} ^g	8.1 a	9.0 a	32.9 a	61.4 a
B	6.4 b	6.7 ab	7.6 ab	26.6 ab	56.4 a
C	6.2 b	7.0 a	7.6 ab	28.2 ab	48.7 a
D	5.7 ^h b	5.3 b	6.6 b	22.1 b	28.0 b
<i>F</i> value	7.87	6.61	4.36	4.63	9.32
<i>P</i> value	< 0.001	< 0.001	0.007	0.005	< 0.001
MSD ⁱ	8.2	1.5	1.8	7.3	14.9

^c Mixture A: 100% soil, Mixture B: 50% soil and 50% mine tailing, Mixture C: 30% soil and 70% mine tailing, Mixture D: 100% mine tailing, ^f Mean of 27 plants ($n = 27$), i.e. three sampling points, i.e. 3, 6 and 9 months, three plots and three plants per plot,

^g Values with the same capital letter are not significantly different between the mixtures (i.e. within the columns),

^h mean of 6 surviving plants, ⁱ MSD: Minimum significant difference at $P < 0.05$ (SAS Institute, 1989)

spade-sampled 20 times. The 20 samples from each plot were pooled so that 3 different soil samples were obtained of approximately 150 kg. The soil was 2 mm-sieved and characterized (Table 1). The soil sampling procedure is schematized in supplementary Fig. 1.

Cultivation of *Ricinus communis* in the Greenhouse

Seeds of *R. communis* were collected along the edge of the Rio de los Remedios in the State of México (México). The seeds were placed in small pots with soil and the emerged plantlets collected after 20 days. Four different treatments were applied in the experiment. In the first treatment, *R. communis* was cultivated in 100% soil. In a second treatment mine tailings were mixed with an equal amount of soil, in a third treatment soil (30%) was mixed with mine tailings (70%), and in a fourth treatment plants were cultivated in 100% mine tailings. Growth of *R. communis* in the 100% mine tailings was much slower than in the other treatments and most of the plants died. The surviving plants were collected after 9 months and included in the analysis.

The cultivation procedure of *R. communis* is schematized in supplementary Fig. 1. A sub-sample of soil of one of the three plots was mixed with one sample of the mine tailing so that 3 different replicates were obtained and these 3 replicates ($n = 3$) were used in each treatment ($n = 4$).

The experiment was conducted in a greenhouse. Polyvinyl chloride (PVC) tubes (length 50 cm and diameter (\varnothing) 16 cm) were filled at the bottom with 7 cm gravel topped with 3 cm sand [19]. A total of 6.5 kg of the different mixtures of soil and mine tailings were transferred to the PVC tubes. As such, a layer of

30 cm soil was obtained. Nine *R. communis* plantlets were planted separately in each of the 3 replicates of the 4 treatments. As such, 27 *R. communis* plants were used in each treatment. Every 7 days, 500 ml water was added to each column. The temperature, relative humidity, and light intensity in the greenhouse were monitored.

After 3, 6, and 9 months, 3 columns were selected at random from each replicate and each treatment, and the plant and soil were removed from the PVC tubes. As such, 9 plants and soil samples were obtained. The soil was separated from the roots and the soil attached to the roots was brushed off and collected [20]. Root and shoot length and number, and width and length of the leaves were determined. The roots, leaves, and shoots were washed with distilled water, and the roots were separated from the shoots and analyzed separately for total metals.

Analysis of Plant and Soil

The shoots + leaves, roots, and soil were analyzed as described by Franco-Hernández, (2010). The heavy and total metals were measured by inductively coupled plasma-optical emission spectrometry (ICP-OES) spectrometer (4300DV-Perkin Elmer, USA). Montana soil standard reference materials were obtained from the National Institute of Standards and Technology (USA) and served as control. Quality control was done for each batch of 50 samples. The plastic material used for analysis of metals were treated with 2% HNO₃ before use. The bulk soil samples were analyzed for total carbon and nitrogen, electrolytic conductivity (EC), pH, clay content, and water-holding capacity (WHC) as described by Aguilar-Chavez et al. [21] (Table 1). All chemicals used in this study were of laboratory grade and purchased at Sigma Aldrich (St. Louis, Missouri, USA).

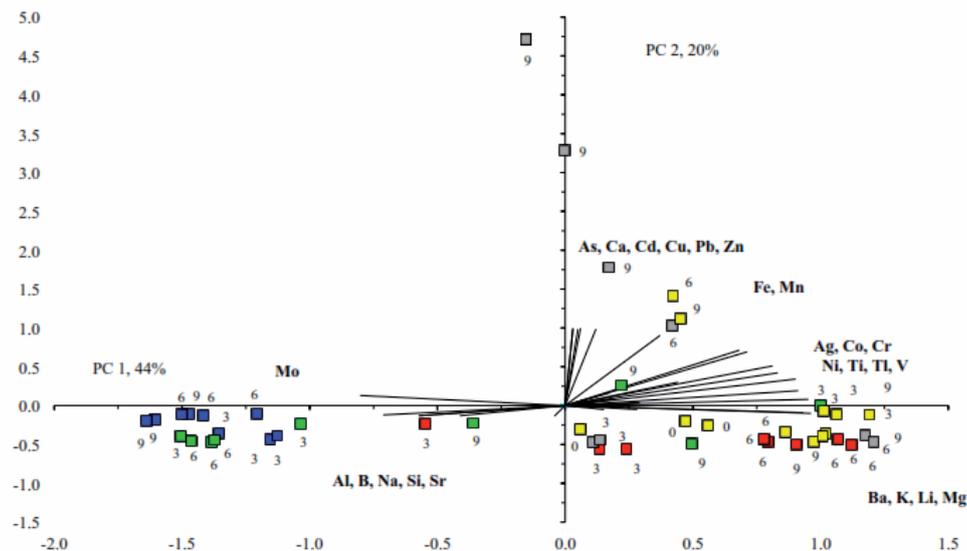


Fig. 2. Principal component analysis with the concentration of the different elements measured in the roots (■), and aboveground parts of *Ricinus communis* L. (■), and in the uncultivated soil (■), the non-rhizosphere soil (■), and the rhizosphere (■). Samples were taken at the onset of the experiment (0) and after 3, 6, or 9 months. Principal component 1 (PC1) explained 44% of the variation and PC2 20%.

Statistical Analysis

Significant differences between the soil characteristics and the plant characteristics as a result of the different treatments were determined by analysis of variance (ANOVA) and based on the minimum significant difference using the general linear model procedure (PROC GLM) [22]. This procedure can be used for an analysis of variance (ANOVA) for unbalanced data, i.e., when data are missing. Significant differences between the metal concentrations in the uncultivated, bulk, and rhizospheric soil and the aboveground plant parts and the roots as a result of the different treatments were determined by ANOVA and based on the least significant difference using the general linear model procedure (PROC GLM) [22].

Results

Characteristics of Soil and Mine Tailings

The soil was characterized by an alkaline pH of 8.9 and an organic C content of 8.53 g kg⁻¹ dry soil (Table 1). The total N content of the clayey soil was 1.02 g kg⁻¹ dry soil and the EC 1.55 dS m⁻¹, while the WHC was 540 g kg⁻¹. The mine tailings had pH 8.0 and EC 3.03 dS m⁻¹. The total N content of 0.13 g kg⁻¹ was low compared to the total C content of 4.86 g kg⁻¹ resulting in a C-to-N ratio of 37. The mine tailings were sandy, but did not retain water so it was impossible to determine the WHC. The characteristics of the mixture (50% soil and 50% mine tailings, and 30% soil and 70% mine tailings) were between those found in the soil or mine tailings.

The organic C content in soil, the mine tailings, and the mixtures of both cultivated with *R. communis* was significantly higher after 9 months than at the onset of the experiment, but no differences were found in the other soil characteristics (data not shown). After 9 months, the organic C content had increased 2.8 times in the soil cultivated with *R. communis* and the mixture of 50% soil with 50% mine tailings, 2.5 times in the mixture of 30% soil with 70% mine tailings and 1.2 times in the mine tailings compared to the onset of the experiment.

Growth of *R. communis*

Ricinus communis grew best in soil and its development was inhibited strongly when cultivated in the mine tailings (Table 2). Most of the *R. communis* plants cultivated in the mine tailings died and only 6 of the 27 plants survived for 9 months. The root length of *R. communis* was significantly reduced when cultivated in the mine tailings compared to plants cultivated in soil or soil mixed with mine tailings ($p < 0.05$). The number of leaves was significantly higher when plants were cultivated in soil compared to plants cultivated in the mine tailings or mine tailings mixed with soil ($p < 0.001$). Shoot length and the width and length of the leaves of *R. communis* were significantly lower when cultivated in mine tailings compared to plants cultivated in soil ($p < 0.01$).

Metal Concentrations when *Ricinus communis* was Cultivated in 100% Soil

The concentrations of As, Cd, Co, Cr, Cu, and Pb were higher in the rhizosphere than in the

Supplementary Table 1. Metal concentrations in bulk soil, the rhizosphere, roots, and aboveground plant material of *Ricinus communis* cultivated in soil, soil mixed with 50% of mine tailings, 30% of mine tailings, and 100% mine tailings and kept in a greenhouse for 270 days.

A. Uncultivated soil						
Metal	A ^c	B	C	D	MSD ^f	P value
	mg Kg ⁻¹ soil					
Ag	3.06 ^g a ^h	1.26 a	3.22 a	1.36 ⁱ a	3.96	0.559
Al	ND ^j	ND	437 a	7399 a	1519	<0.001
As	167 b	2335 a	2740 a	3274 a	550	<0.001
B	5.06 b	ND b	ND	ND	6.04	0.215
Ba	224 a	122 a	51.5 a	26.7 a	49.2	<0.001
Be	0.09 a	0.09 b	0.11 b	ND	0.24	0.448
Ca (x10 ³)	16 b	47 ab	54 a	57 a	8.3	<0.001
Cd	5.90 b	76.2 a	96 a	110 a	18.4	<0.001
Co	10.0 ab	17.5 a	18.5 a	18.0 b	3.0	<0.001
Cr	24.9 a	26.1 a	21.0 a	15.2 ab	9.3	0.098
Cu	66.2 b	718 a	881 a	1058 ab	159	<0.001
Fe (x10 ³)	21 a	50 a	54 a	57 a	8.0	<0.001
K	1459 a	1465 a	867 a	1929 a	1612	0.704
Li	11.4 a	25.0 a	331 a	5.20 a	570	0.582
Mg	7956 a	5460 a	3582 ab	2583 a	1315	<0.001
Mn	339 b	714 ab	709 ab	834 a	158	<0.001
Mo	1.05 b	2.29 bc	3.20 ab	4.3 ab	3.0	0.005
Na	929 a	550 ab	169 b	183 b	644	0.0426
Ni	16.0 a	26.3 a	76.1 a	24.0 a	93	0.511
Pb	65.2 b	634 a	664 a	839 ab	165	<0.001
Se	2.35 a	1.00b	55.2 a	ND	93	0.549
Si	283 a	251 a	92 b	279 ab	241	0.281
Sr	ND	2.07 a	3.23 a	20.3 b	10.2	0.01
Ti	1007 a	784 a	600 a	508 a	183	<0.001
V	33.3 a	47.0 a	49.3 a	47.9 a	9.2	<0.001
Zn	228 b	2662 a	3342 a	4142 a	820	<0.001

^cA: 100% soil, B: Mixture of 50% soil and 50% mine tailings, C: Mixture of 30% soil and 70% mine tailings, D: 100% mine tailings, ^fMSD: Minimum significant difference at 5% (SAS Institute 1989), ^g Mean of 27 plants ($n = 27$), i.e. three sampling points, i.e. 3, 6, and 9 months, three plots and three plants per plot, ^h Values with the same letter are not significantly different between the mixtures (i.e. within the columns), ⁱ mean of 6 surviving plants, ^j ND: Not detected

non-rhizospheric and uncultivated soil, and the roots and aboveground parts of *R. communis* (Supplementary Table 1). The concentrations of As, Cd, Co, Cr, Cu, and Pb were lower in the aboveground parts of *R. communis* than in its roots when cultivated in 100% soil. The PCA confirmed that the metal concentrations in the aboveground parts and roots of the *R. communis* plants were clearly different from those found in uncultivated

soil, the non-rhizospheric and rhizospheric soil (Fig. 2, Supplementary Table 1).

Metal Concentrations when *Ricinus communis* was Cultivated in 50% Soil and 50% Mine Tailings

The concentrations of As, Cd, Co, Cr, Cu, and Pb were generally lower in the rhizosphere than in

Supplementary Table 1. Continued.

B. Non Rhizosphere						
Metal	A ^e	B	C	D	MSD ^f	P value
	mg Kg ⁻¹ soil					
Ag	0.09 ^g a ^h	1.42 a	2.84 a	ND ^j	2.14	<0.001
Al	ND	ND	ND	3878 ⁱ a	ND	<0.001
As	11.0 b	2278 a	2768 a	3909 a	886	< 0.001
B	4.40 b	5.30 b	ND	ND	10.0	0.389
Ba	183 ab	90.0 a	69.0 a	23.0 a	83.0	<0.001
Be	0.07 a	0.53 a	0.10 b	0.60c	0.23	<0.001
Ca (x10 ³)	13 b	51 a	55 a	63 a	13	<0.001
Cd	2.10 b	76.4 a	97.1 a	131 a	31.0	0.08
Co	9.00 ab	16.3 a	18.1 a	25.0 a	5.9	< 0.001
Cr	25.1 a	24.9 a	24.1 a	22.9 a	12	0.986
Cu	24.1 b	718 a	917 a	1162 a	272	< 0.001
Fe (x10 ³)	18 ab	51 a	53 a	61 a	12	<0.001
K	ND	247 b	142 b	751 b	859	0.236
Li	ND	ND	ND	6 a	1	<0.001
Mg	8118 a	5089 a	4085 ab	2621 a	2407	<0.001
Mn	270 b	754 a	810 a	883 a	212	<0.001
Mo	1.05 b	3.00 b	1.42 b	3.00 b	2.3	<0.001
Na	262 b	177 b	457 ab	143 b	688	<0.001
Ni	15.0 a	22.1 a	25.1 ab	29.3 a	9.3	0.001
Pb	24.9 b	600 a	682 a	954 a	186	< 0.001
Se	2.00 a	2.30 a	2.00 a	ND	3.0	0.767
Si	ND	ND	ND	ND	ND	<0.001
Sr	ND	ND	ND	19.1 b	ND	<0.001
Ti	921 a	614 b	632 a	533 a	461	0.146
V	35.9 a	48.6 a	50.2 a	53.9 a	16	<0.001
Zn	85.0b	2101 b	3688 a	4367 a	1193	<0.001

^eA: 100 % soil, B: Mixture of 50 % soil and 50 % mine tailing, C: Mixture of 30% soil and 70% mine tailing, D: 100 % mine tailing, ^fMSD: Minimum significant difference at 5 % (SAS Institute 1989), ^g Mean of 27 plants ($n = 27$), i.e. three sampling points, i.e. 3, 6 and 9 months, three plots and three plants per plot, ^h Values with the same letter are not significantly different between the mixtures (i.e. within the columns), ⁱ mean of 6 surviving plants, ^j ND: Not detected.

the uncultivated and non-rhizosphere and generally higher than in the roots and aboveground parts of *R. communis* (Supplementary Table 1). The concentrations of As, Cd, Co, Cr, Cu, and Pb were lower in the aboveground parts of *R. communis* than in its roots when cultivated in a mixture of 50% mine tailings and 50% soil. The aboveground parts of *R. communis* accumulated B and Mo, while the roots accumulated Be and Sr ($p < 0.05$) (Supplementary Table 1). The metal concentrations in the aboveground plant parts and roots of *R. communis* cultivated in the mixture of 50% soil and 50% mine tailings were also clearly different from

those in the bulk, rhizospheric, and uncultivated mixture of 50% soil and 50% mine tailings (Fig. 3, Supplementary Table 1).

Metal Concentrations when *R. Communis* was Cultivated in 30% Soil and 70% Mine Tailings

The concentrations of As, Cd, Co, Cr, Cu, and Pb were lower in the rhizosphere than in the uncultivated and non-rhizosphere and generally higher than in

Supplementary Table 1. Continued.

C. Rhizosphere						
Metal	A ^e	B	C	D	MSD ^f	P value
	mg Kg ⁻¹ soil					
Ag	2.04 ^g a ^h	1.03 a	1.31 b	0.81 ⁱ a	3.08	0.616
Al	ND ^j	20.0 c	2.00 a	5654 a	1965	<0.001
As	662 a	795 b	662 b	2527 b	1132	0.014
B	0.87 b	0.96 b	ND	ND	2.17	0.345
Ba	151 b	128 a	94.2 a	71 0 a	89.4	0.107
Be	0.10 a	0.10 b	0.09 b	ND	0.31	0.854
Ca (x10 ³)	25 a	42 b	40 b	49 a	21	0.053
Cd	24.0 a	59.1 b	86 a	59.0 b	40	0.022
Co	11.2 a	15.7 a	14.4 a	17.1 b	4.7	0.054
Cr	29.5 a	28.6 a	17.4 a	19.2 a	15	0.012
Cu	253 a	543 b	570 b	836 b	379	0.033
Fe (x10 ³)	27 a	44 b	40 b	50 b	18	0.034
K	ND	1768 a	ND	2815 a	1878	0.025
Li	ND	14.0 a	ND	15.1 a	14	0.058
Mg	6515 a	5404 a	4523 a	3147 a	1582	0.024
Mn	443 a	629 b	605 b	772 a	265	0.069
Mo	1.00 b	1.96 bc	2.00 b	4.05 ab	2.2	0.012
Na	ND	513 ab	ND	980 a	540	0.024
Ni	18.2 a	26.2 a	17.5 ab	23.3 a	12	0.05
Pb	201 a	501 b	459 b	679 b	290	0.01
Se	5.96 a	7.00 a	3.02 a	ND	13	0.742
Si	4.00 b	262 a	12 .05b	517 a	399	0.009
Sr	ND	ND	15.7 a	91.6 a	187	0.288
Ti	864 a	760 ab	646 a	662 a	428	0.378
V	38.6 a	41.5 a	39.4 b	47.2 a	17	0.869
Zn	1079 a	1953 b	1790 b	3391 a	1565	0.147

^eA: 100 % soil, B: Mixture of 50 % soil and 50 % mine tailing, C: Mixture of 30% soil and 70% mine tailing, D: 100 % mine tailing, ^fMSD: Minimum significant difference at 5 % (SAS Institute 1989), ^g Mean of 27 plants ($n = 27$), i.e. three sampling points, i.e. 3, 6 and 9 months, three plots and three plants per plot, ^h Values with the same letter are not significantly different between the mixtures (i.e. within the columns), ⁱ mean of 6 surviving plants, ^j ND: Not detected.

the roots and aboveground parts of *R. communis* (Supplementary Table 1). The concentrations of As, Cd, Co, Cr, Cu, and Pb were lower in the aboveground parts of *R. communis* than in its roots when cultivated in a mixture of 30% mine tailings and 70% soil. The metal concentrations in the aboveground plant parts and roots of *R. communis* cultivated in the mixture of 30% soil and 70% mine tailings were different from those in the non-rhizosphere, rhizosphere, and uncultivated mixture of 30% soil and 70% mine tailings (Fig. 4, Supplementary Table 1).

Metal Concentrations when *R. Communis* was Cultivated in 100% Mine Tailings

The concentrations of As, Cd, Co, Cu, and Pb were lower in the rhizosphere than in the uncultivated and non-rhizosphere, and generally higher than in the roots and aboveground parts of *R. communis* (Supplementary Table 1). The concentrations of As, Cd, Co, Cr, Cu, and Pb were lower in the aboveground parts of *R. communis* than in its roots when cultivated in a mixture of 30%

Supplementary Table 1. Continued.

D. Plant						
Metal	A ^e	B	C	D	MSD ^f	P value
	mg Kg ⁻¹ soil					
Ag	0.30 ^g a ^h	0.40 a	0.40 b	0.96 ⁱ a	0.9	<0.001
Al	469 a	574 b	1252 a	758 a	1697	0.191
As	23.0 b	6.20 d	155 b	458 c	485	0.179
B	312 a	327 a	277 a	47.2 a	299	0.538
Ba	19.0 c	97.0 a	97.1 a	17.0 a	363	0.719
Be	0.10 a	0.10 b	0.64 b	3.70 a	0.90	<0.001
Ca (x10 ³)	8 b	10 c	13 c	18 b	8	0.011
Cd	1.00 b	1.00 d	6.10 b	13.0 c	16	0.164
Co	1.00 c	1.00 b	2.10 b	8.20 c	4.0	0.004
Cr	5.00 b	7.02 b	7.10 b	16.4 ab	8.0	0.174
Cu	13.8 b	12.1 c	61.0 c	129 c	192	0.255
Fe (x10 ³)	1 c	1 d	3 c	11 c	8	0.036
K	136 b	ND ^j	80.0 b	ND	577	0.756
Li	4.05 a	5.99 ab	5.90 a	4.00 a	6.78	0.826
Mg	2761 b	2685 c	3262 b	3218 a	1426	0.408
Mn	37.0 c	24.6 d	65.0 c	230 b	128	0.022
Mo	3.89 a	5.00 a	4.60 a	7.80 a	3.0	0.16
Na	656 ab	946 a	820 a	ND	1382	0.6786
Ni	5.80 b	6.04 b	9.00 b	10.89 b	7.4	0.306
Pb	9.01 b	5.10 d	47.2 c	93.0 c	124	0.23
Se	3.04 a	3.20 ab	4.42 a	13.3 a	4.0	0.022
Si	451 a	450 a	404 a	14.0 b	444	0.682
Sr	7.98 a	4.03 a	1.93 a	28.1 b	18	0.125
Ti	23.0 c	23.2 d	54.6 b	23.2 b	96	0.401
V	0.10 b	ND	2.40 c	8.90 b	7.80	0.069
Zn	80.0 b	71.0 c	260 c	867 b	562	0.027

^eA: 100 % soil, B: Mixture of 50 % soil and 50 % mine tailing, C: Mixture of 30% soil and 70% mine tailing, D: 100 % mine tailing, ^fMSD: Minimum significant difference at 5 % (SAS Institute 1989), ^g Mean of 27 plants ($n = 27$), i.e. three sampling points, i.e. 3, 6 and 9 months, three plots and three plants per plot, ^h Values with the same letter are not significantly different between the mixtures (i.e. within the columns), ⁱ mean of 6 surviving plants, ^j ND: Not detected.

mine tailings and 70% soil. The metal concentrations in the roots and aboveground parts of plants cultivated in 100% mine tailings were clearly different from the concentration of the metals in the rhizosphere and the mine tailings cultivated or uncultivated (Fig. 5, Supplementary Table 1).

Apart from Be, Mo, Se, and Si, none of the concentrations of the metals monitored was higher in the aboveground plant parts than in the non-rhizosphere, mine tailings, and their mixtures (Supplementary Table 1). The ratio of the heavy metals As, Cd, Cu, and

Pb in the aboveground plant parts of *R. communis* to the concentration in the non-rhizosphere soil increased with increased amount of mine tailings in the mixture, but remained <0.12. For instance, the ratio of Cd increased from 0.01 in the mixture 50% soil and 50% mine tailings to 0.06 in the 30% and 70% mixture and increased to 0.10 in the 100% mine tailings. The ratio of the heavy metals As, Cd, Cu, and Pb in the rhizosphere of *R. communis* to the concentration in the non-rhizosphere fluctuated between 0.6 and 0.8.

Supplementary Table 1. Continued.

E. Root						
Metal	A ^e	B	C	D	MSD ^f	P value
	mg Kg ⁻¹ soil					
Ag	1.37 ^g a ^h	1.30 a	1.31b	ND ^j	2.03	0.574
Al	781 a	966 a	1188 a	3051 ⁱ a	1512	0.152
As	55 b	366 c	504 b	146 c	682	0.18
B	50.6 b	40.8 b	53.6 b	30.3 a	55.4	0.83
Ba	58.0 c	40.0 a	22.1 a	18.0 a	72.2	0.516
Be	0.50 a	0.20 b	0.09b	2.30 b	1.21	0.102
Ca (x10 ³)	8 b	12 c	12 c	7 b	15	0.733
Cd	2.00 b	13.0 c	19.3 b	17.0 c	22	0.08
Co	8.00 b	3.30 b	3.50 b	8.0 c	6	0.413
Cr	10.5 b	10.6 b	5.6 0b	4.70 b	12	0.54
Cu	28.5 b	106 c	163 c	157 c	278	0.159
Fe (x10 ³)	6 c	11 c	13 c	6 c	16	0.572
K	2.30 b	13.1 b	11.0 b	34.4 b	24	0.188
Li	6.06 a	1.0 b	2.48 a	ND	4	0.524
Mg	3541 b	3527 b	3210 b	3173 a	2225	0.962
Mn	95.0 c	113 c	137 c	190 b	205	0.856
Mo	1.39 b	1.43 c	1.90 b	4.10 ab	2	0.092
Na	143 b	ND	ND	ND	4	0.5737
Ni	7.00 b	9.20 b	10.0 b	12.3 b	9.8	0.578
Pb	28 b	111 c	168 c	123 c	177	0.093
Se	9.00 a	2.30 ab	4.29 a	6.00 b	14	0.725
Si	288 a	411 a	434 a	407 ab	382	0.669
Sr	12.7 a	20.4 a	22.4 a	32.7 b	24	0.489
Ti	280 b	208 c	142 b	99.0 b	357	0.716
V	8.30 b	3.50 b	3.06 c	ND	11	0.5
Zn	92.0 b	168 c	389 c	296 b	387	0.046

^eA: 100 % soil, B: Mixture of 50 % soil and 50 % mine tailing, C: Mixture of 30% soil and 70% mine tailing, D: 100 % mine tailing, ^fMSD: Minimum significant difference at 5 % (SAS Institute 1989), ^g Mean of 27 plants ($n = 27$), i.e. three sampling points, i.e. 3, 6 and 9 months, three plots and three plants per plot, ^h Values with the same letter are not significantly different between the mixtures (i.e. within the columns), ⁱ mean of 6 surviving plants, ^j ND: Not detected.

Metals in the Uncultivated, Rhizosphere, and Non-Rhizosphere Soil-Mine Tailing Mixtures, and in the Roots and Aboveground Parts of *R. communis*

The PCA to metals in the uncultivated soil-mine tailing mixtures, separated clearly uncultivated soil from the mixtures of soil and mine tailings, and the mine tailings (Fig. 6). The uncultivated soil had a negative PC1 (loaded by Ba, Mg, and Ti) while the mixtures of soil and mine tailings and the mine

tailings were characterized by a positive PC1 (loaded by Al, As, Ca, Cd, Co Cu, Fe, Li, Mn, Mo, Ni, Pb, V, Zn). The mixtures of soil and mine tailings were separated and the mixture of 30% soil and 70% mine tailings was characterized mostly by a negative PC2 and the mixture of 50% soil and 50% mine tailings.

The PCA to the metals in the non-rhizosphere soil-mine tailing mixtures separated clearly the soil, mine tailings, and their mixtures (Fig. 7). The concentrations of Ag, As, Ca, Cd, Co, Cr, Cu Fe, Mn, Mo, Ni, Pb, V,

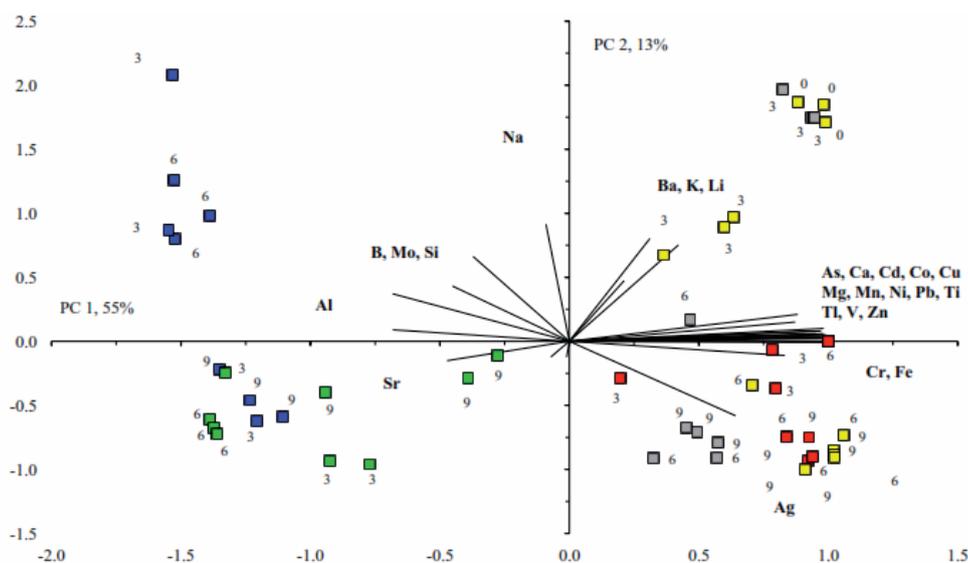


Fig. 3. Principal component analysis with the concentration of the different elements measured in the roots (■) and aboveground parts of *Ricinus communis* L. (■), and in the uncultivated mixture of soil (at 50%) and mine tailings (at 50%) (■), the non-rhizosphere mixture (■), and the rhizosphere (■). Samples were taken at the onset of the experiment (0) and after 3, 6, or 9 months. Principal component 1 (PC1) explained 55% of the variation and PC2 13%.

and Zn was lowest in the 100% soil and increased with an increased percentage of mine tailings (Fig. 7). The soil was characterized by a larger concentration of B, Ba, Mg, and Ti than the mine tailings and their mixtures.

The concentrations of As, Cd, Co, Cr, Cu, and Pb in the rhizosphere of *R. Communis* cultivated in soil-mine tailing mixtures increased generally with the increased amount of mine tailings in the mixture, but the effect was less accentuated than in the non-rhizosphere soil (Supplementary Table 1). Additionally, the rhizosphere of *R. communis* cultivated in the mixtures of mine

tailings and soil was not as clearly separated in the PCA as the non-rhizosphere (Figs 7-8). The concentrations of Al, As, Ca, Cd, Co, Cu, Fe, Mn, Mo, Pb, Tl, V, and Zn (loaded PC1) did not separate the rhizosphere of the different mixtures, but the concentrations of Cr, K, Li, Na, Ni, and Si did (loaded PC2). The soil and the mixture 50% soil and 50% mine tailings had generally a larger concentration of Cr, K, Li, Na, Ni, and Si than the 30% soil 70% mine tailings, but not the 100% mine tailings.

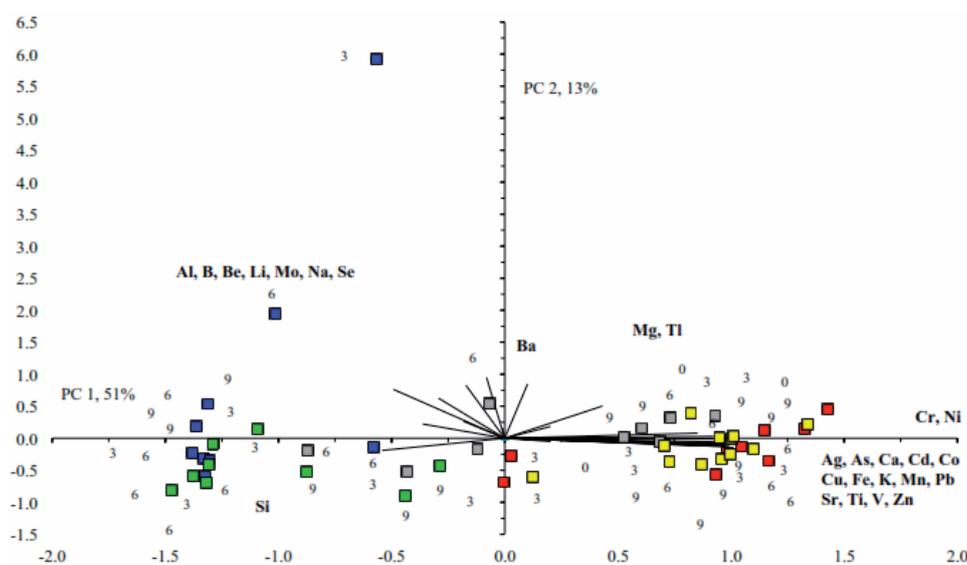


Fig. 4. Principal component analysis with the concentration of the different elements measured in the roots (■) and aboveground parts of *Ricinus communis* L. (■), and in the uncultivated mixture of soil (at 30%) and mine tailings (at 70%) (■), the non-rhizosphere mixture (■), and the rhizosphere (■). Samples were taken at the onset of the experiment (0) and after 3, 6, or 9 months. Principal component 1 (PC1) explained 51% of the variation and PC2 13%.

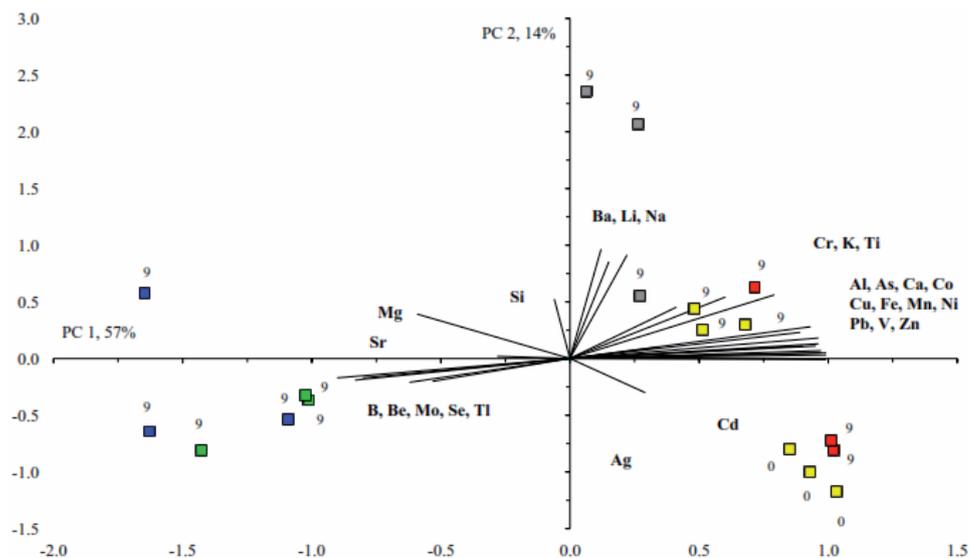


Fig. 5. Principal component analysis with the concentration of the different elements measured in the roots (■) and aboveground parts of *Ricinus communis* L. (■), and in the uncultivated mine tailings (■), the non-rhizosphere mine tailings (■), and the rhizosphere (■). Samples were taken at the onset of the experiment (0) and after 3, 6, or 9 months. Principal component 1 (PC1) explained 57% of the variation and PC2 14%..

The concentration of metals in the roots of *R. communis* cultivated in soil-mine tailing mixtures was affected less by the mixture in which the plant grew, but more by the age of the roots (Fig. 9). The roots of 9-month old plants were characterized by a larger concentration of As, Ca, Cd, Co, Cu, Fe, K, Mn, Ni, Pb, Si, and Zn than the amounts found in the roots of younger plants. The roots of 9-month old *R. communis* plants grown in the soil were characterized by a larger concentration of Ag, Ba, Cr, Mg, Ti, and V than the younger other roots, i.e., a larger positive PC2.

For metals concentration in the aboveground parts of *R. communis* cultivated in soil-mine tailing mixtures, the PCA did not separate the aboveground parts of *R. communis* when the plant was cultivated in soil, the mixture of 30% or 50% soil with 70% or 50% mine tailings (Fig. 10). However, the metal concentrations in the aboveground material of *R. communis* when cultivated in 100% mine tailings was separated clearly and the amount of Ag, As, Ca, Cd, Co, Cr, Cu, Fe, Mn, Mo, Pb, V, and Zn was higher than when plants were cultivated in the soil,

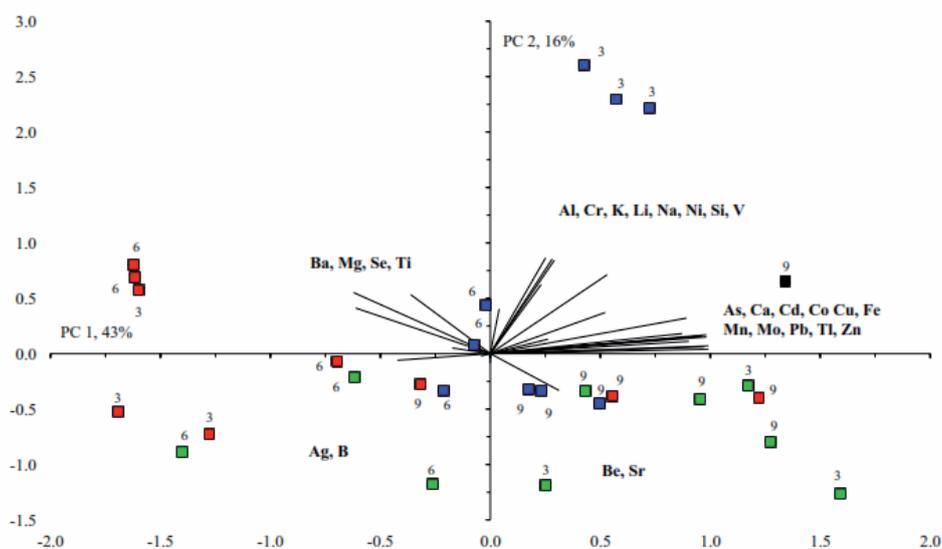


Fig. 6. Principal component analysis with the concentrations of the different elements in the rhizosphere of *Ricinus communis* L. cultivated in soil (■), in a mixture of 50% soil and 50% mine tailings (■), in a mixture of 30% soil and 70% mine tailings (■), and mine tailings (100%) (■). Samples were taken at the onset of the experiment (0) and after 3, 6, or 9 months. Principal component 1 (PC1) explained 43% of the variation and PC2 16%.

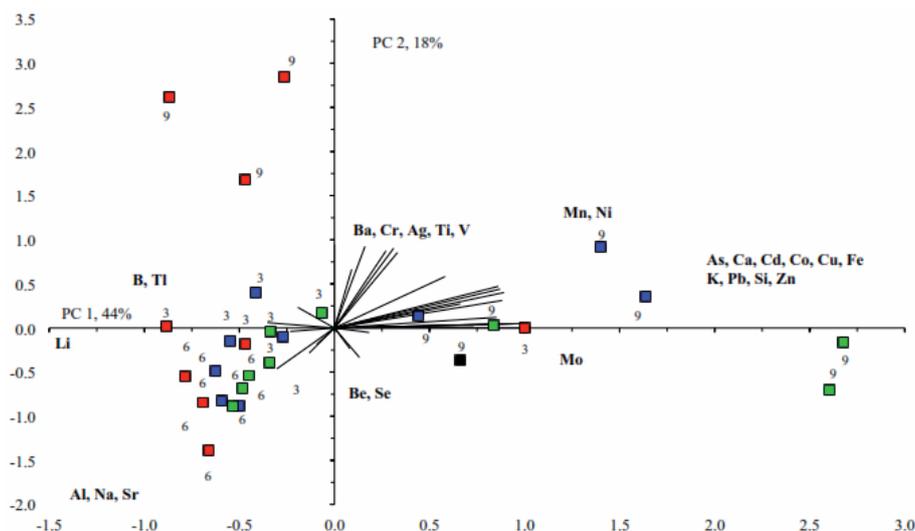


Fig. 7. Principal component analysis with the concentrations of the different elements in the roots of *Ricinus communis* L. cultivated in soil (■), in a mixture of 50% soil and 50% mine tailings (■), in a mixture of 30% soil and 70% mine tailings (■), and mine tailings (100%) (■). Samples were taken at the onset of the experiment (0) and after 3, 6, or 9 months. Principal component 1 (PC1) explained 44% of the variation and PC2 18%.

the mixture of 30% or 50% soil with 70% or 50% mine tailings.

Discussion

The organic carbon content in all treatments was higher at the end of the experiment (>1.2 times) than at the beginning. Wu et al. [23] found that bulk density and soil nutrients increased in waste land soil when cultivated with *R. communis* for 2 years.

Concentrations of some heavy metals found in this study were higher than values reported for other mine wastes in Mexico and high compared to those reported for normal soil. For instance, the concentration of 17 mg Co kg⁻¹ was much higher than that reported for a normal soil (0.1 mg Co kg⁻¹) or that (4 mg Co kg⁻¹) found in a mine tailing in San Luis Potosi (Mexico), active since 1800 [24]. The concentrations of 120 mg Cd kg⁻¹ and 910 mg Pb kg⁻¹ were higher than those reported for mine tailings in San Luis Potosi (81 mg Cd kg⁻¹ and 754 mg Pb kg⁻¹) [24] and much

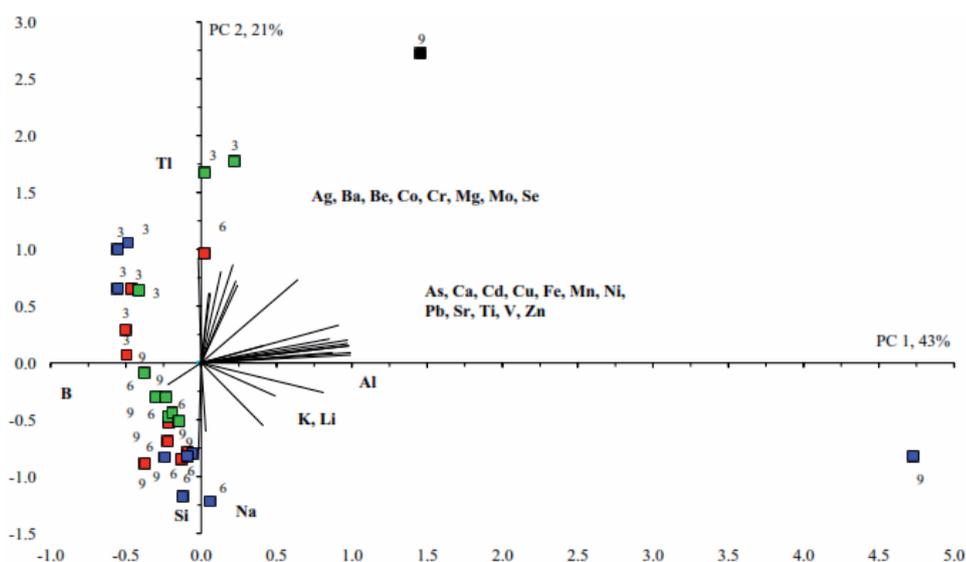


Fig. 8. Principal component analysis of the concentrations of the different elements in the aboveground parts of *Ricinus communis* L. cultivated in soil (■), in a mixture of 50% soil and 50% mine tailings (■), in a mixture of 30% soil and 70% mine tailings (■), and mine tailings (100%) (■). Samples were taken at the onset of the experiment (0) and after 3, 6, or 9 months. Principal component 1 (PC1) explained 43% of the variation and PC2 21%.

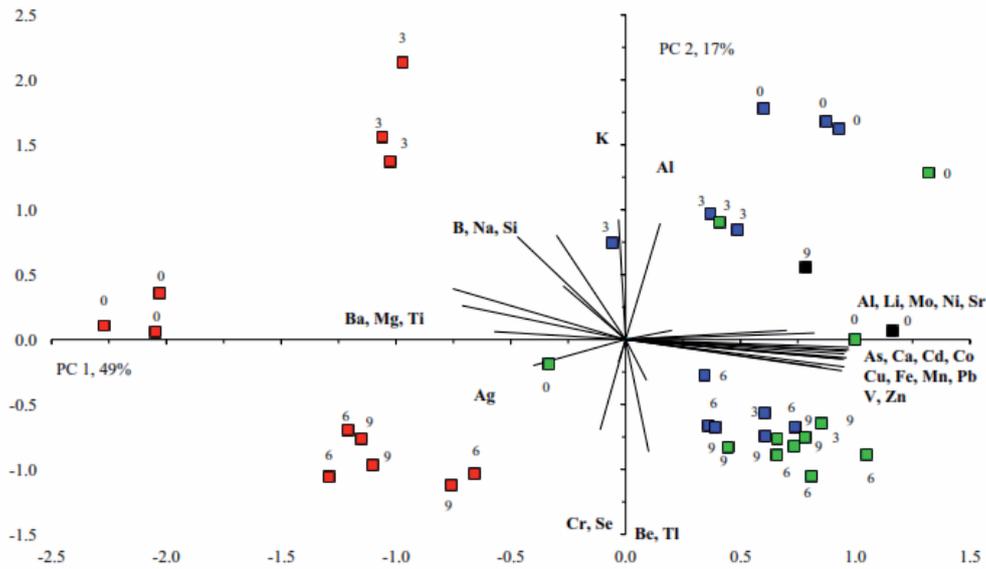


Fig. 9. Principal component analysis of the concentrations of the different elements in uncultivated soil (■), in a mixture of 50% soil and 50% mine tailings (■), a mixture of 30% soil and 70% mine tailings (■), and mine tailings (100 %) (■). Samples were taken at the onset of the experiment (0) and after 3, 6, or 9 months. Principal component 1 (PC1) explained 49% of the variation and PC2 17%.

higher than reported in normal soil, i.e., 2 mg Cd kg⁻¹ and 200 mg Pb kg⁻¹ [25]. Concentrations of Cu in soil are normally <100 mg kg⁻¹, but reached 1,147 mg kg⁻¹ in the mine tailings used in this study, similar to values reported for the mine tailings in San Luis Potosi (1,154 mg kg⁻¹). Concentrations of As are normally <50 mg kg⁻¹ soil [26], but they reached 3,473 mg kg⁻¹ in the mine tailings used in this study. In a previous study, however, we found concentrations of 8,420 mg As kg⁻¹ in mine tailings from San Luis Potosi [24].

Plants can survive contaminated sites by excluding heavy metals or by accumulating them. Some plants accumulate actively metals and some are even called hyper-accumulators, i.e., the concentration of the metal in the shoot is higher than in the root and much higher than in the soil [27]. *Ricinus communis* did not accumulate heavy metals and excluded them all (i.e., As, Co, Cd, Cr, Cu, Ni, Pb, and Zn) from its roots and aboveground parts. Only Mo was accumulated in both the roots and aboveground plant parts and the

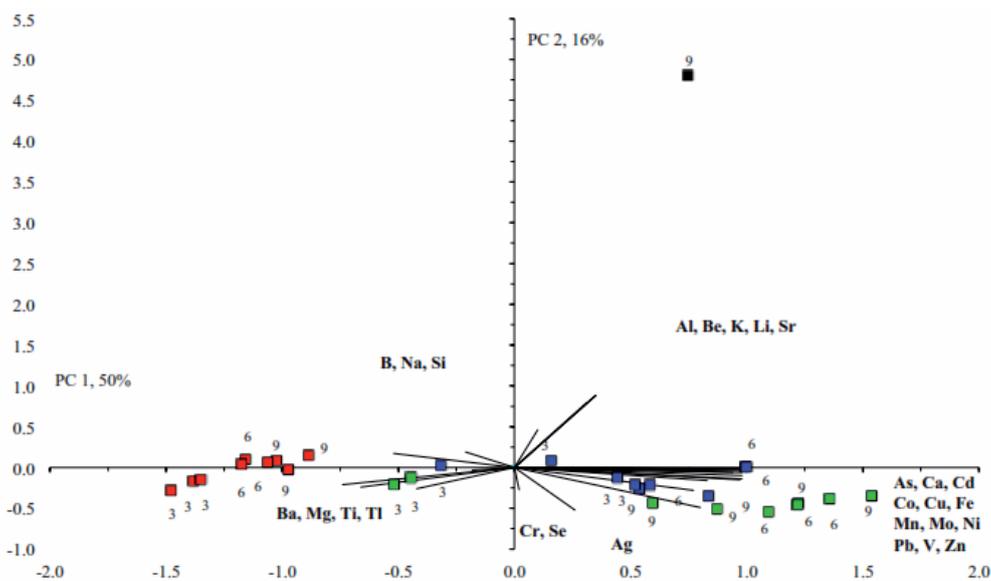


Fig. 10. Principal component analysis of the concentrations of the different elements in the non-rhizosphere when *Ricinus communis* L. was cultivated in soil (■), in a mixture of 50% soil and 50% mine tailings (■), in a mixture of 30% soil and 70% mine tailings (■), and mine tailings (■). Samples were taken at the onset of the experiment (0) and after 3, 6, or 9 months. Principal component 1 (PC1) explained 50% of the variation and PC2 16%.

concentration was higher in the latter than in the first. This indicated that *R. communis* was an accumulator of Mo, but not yet a super-accumulator. Molybdenum was presumably accumulated as it is part of the enzyme nitrate reductase that catalyzes the reduction of nitrates and subsequent formation of amino acids (mainly glutamic acid and glutamine).

Some metals are toxic to plants or plants need them at only low concentrations (e.g., As, Cd, Cr, Cu, Ni, Pb, and Zn) [28-29]. Mine tailings, even at 50%, strongly reduced the number of leaves and reduced leaf area. Toward the end of the experiment, the plants that survived in the mine tailings had leaves, but they were dwarfed severely presumably due to the oxidative stress caused by the high concentration of the heavy metals and lack of water [6]. The mine tailings reduced plant growth, and root length was nearly halved compared to plants cultivated in 30% soil. Large amounts of heavy metals in soil can increase the concentration of heavy metals in the root system, which inhibit normal plant growth as plant physiology is altered [30-31]. Plants might stimulate defensive mechanisms when they are exposed to Cd, Cu, Zn, Pb, Ag, and As. They synthesize enzymes that can bind to metal ions via sulfhydryl (-SH) and carboxyl (-COOH) links. Plants also release root exudates – primarily consisting of low molecular weight organic acids – in response to these metal ions in soils. These substances through chelation form complex metal compounds (chelates) avoiding metal intoxication in the plant. These compounds are transported and stored in specialized compartments in the rhizosphere allowing soluble Fe, P, and other micronutrients present in the rhizosphere to be available for plant growth.

Nickel, Mo, Cu, Zn, Mn, Fe, and B are micronutrients of plants and considered beneficial for plant growth at low concentrations, i.e., as trace elements [32]. These elements are essential for growth and metabolic activity, and found mostly in proteins and as activators of enzymatic reactions. Boron is involved in reproductive growth and found in the membrane and plasmatic wall, while Fe is associated with the production of chlorophyll [33].

The heavy metal tolerance of plants depends not only on the concentration of the contaminants, but also on other factors. For instance, it has been reported that high Ca concentrations reduces the toxicity of heavy metals. The high Ca concentration in the mine tailings used in this study might have helped *R. communis* survive in the adverse conditions.

The mine tailings in this study did retain little water, e.g., it was impossible to determine the WHC, and this might have inhibited growth of *R. communis* in the mine tailings. This suggests that mixing soil into the mine tailings did not only reduce the negative effect of the heavy metals, but it also increased water retention (e.g., the WHC was 520 g kg⁻¹ in the mixture 30% soil and 70% mine tailings). Consequently, vegetating the mine tailings would require some soil to be mixed in the mine

tailings, but wind erosion and runoff would be reduced strongly from the vegetated mine tailings, although metal leaching might also be stimulated.

Conclusions

Ricinus communis grew in 100% mine tailings although it was strongly inhibited and not all plants survived. *Ricinus communis* shoots were 19% smaller and roots 8% in soil mixed with an equal amount of mine tailings compared to plants cultivated in soil and 33% and 54%, respectively, when cultivated in mine tailings. *Ricinus communis* did not accumulate metals, but excluded them to survive with higher concentrations found in the roots than in the aboveground parts. The ratio of As, Cd, Cu, and Pb in the aboveground plant parts to the concentration in soil remained <0.12, while that of the roots <0.25. Concentrations of heavy metals were generally lower in the rhizosphere than the non-rhizosphere soil of *R. communis*. The As concentration was 35% lower in the bulk soil than in the rhizosphere. Cultivation of *R. communis* on mine tailings could be used to reduce wind and runoff, but some soil will have to be mixed into the top-layer of the mine tailings to promote water retention (which might stimulate infiltration), reduce metal concentrations, and improve plant growth.

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References

1. MONTERROSO C., RODRÍGUEZ F., CHAVES R., DIEZ J., BECERRA-CASTRO C.C., KIDD P.S., MACIAS F. Heavy metal distribution in mine-soils and plants growing in a Pb/Zn-mining area in NW Spain. *Appl. Geochem.* **44**, 3, 2014.
2. SECRETARÍA DE ECONOMÍA. Reporte de coyuntura de la minería 2011. Available online at <http://www.economia.gob.mx> (accessed on 30 March 2013).
3. VÁSQUEZ-MURRIETA, M.S., MIGUELES-GARDUNNO I., FRANCO-HERNANDEZ O., GOVAERTS B., DENDOOVEN L. C and N mineralization and microbial biomass in heavy-metal contaminated soil. *Eur. J. Soil. Biol.* **42** (2), 89, 2006.

4. HERNÁNDEZ-HERNÁNDEZ A., LÓPEZ-LUNA J., GONZÁLEZ-TERREROS E. Heavy metals quantification in community soils impacted by mining activities in the northern mountains of Oaxaca, México. *Environ. Sci. Ind. J.* **7**, 343, **2012**.
5. PHAENARK C., POKETHITIYOOK P., KRUATRACHUE M., NGERNSANSARUAY C. Cd and Zn accumulation in plants from the Padaeng zinc mine area. *Int. J. Phytoremediat.* **11** (5), 479, **2009**.
6. KUMAR G.H., KUMARI J.P. Heavy metal lead influent toxicity and its assessment in phytoremediating plants a review. *Water Air Soil Poll.* **226** (10), 324, **2015**.
7. GIORDANIC., CECCHI S., ZANCHI C. Phytoremediation of soil polluted by nickel using agricultural crops. *Environ. Manage.* **36** (5), 675, **2005**.
8. MELO E.E.C., GUILHERME L.R.G., NASCIMENTO W.A.C., PENHA, H.G.V. Availability and accumulation of arsenic in oilseeds grown in contaminated soils. *Water Air Soil Poll.* **223** (1), 233, **2012**.
9. WEERADEJ M., PRAYAD P., MALEEYA K., PHANWIMOL T., RATTANAWAT C. Phytostabilization of a Pb-contaminated mine tailing by various tree species in pot and field trial experiments. *Int. J. Phytoremediat.* **14** (9), 925, **2012**.
10. ORTEGA-LARROCEA M.P., XOCONOSTLE-CÁZARES B., MALDONADO-MENDOZA I.E., CARRILLO-GONZÁLEZ R., HERNÁNDEZ-HERNÁNDEZ J., DÍAZ-GARDUÑO M., LÓPEZ-MEYER M.M., GÓMEZ-FLORES L., GONZÁLEZ-CHÁVEZ C.A. Plant and fungal biodiversity from metal mine wastes under remediation at Zimapán, Hidalgo, Mexico. *Environ. Pollut.* **158** (5), 1922, **2010**.
11. LIM T.K. *Ricinus communis*. In *Edible Medicinal And Non-Medicinal Plants: Fruits*, 1st ed.; Springer Science and Business Media B.V., Heidelberg: platz 3, d-14197 Berlin, Germany, **2**, 484, **2012**.
12. LAVANYA C., MURTHY I.Y.L.N., NAGARAJ G., MUKTA N. Prospects of castor (*Ricinus communis* L.) genotypes for biodiesel production in India. *Biomass Bioenerg.* **39**, 204, **2012**.
13. PERDOMO F.A., ACOSTA-OSORIO A.A., HERRERA G., VASCO-LEAL J.S., MOSQUERA-ARTAMONOV J.D., MILLAN-MALO B., RODRIGUEZ-GARCÍA M.D. Physicochemical characterization of seven Mexican *Ricinus communis* L. seeds and oil contents. *Biomass Bioenerg.* **48**, 17, **2013**.
14. JABEEN S., TAHIR S.M., KHAN S., QASIM H.M. Determination of major and trace elements in ten important folk therapeutic plants of Haripur basin, Pakistan. *J. Med. Plants Res.* **4** (7), 559, **2010**.
15. BODA R.K., PRASAD M.N.V. *Ricinus communis* L. (Castor bean), a potential multi-purpose environmental crop for improved and integrated phytoremediation. *The Euro. Biotech. Journal.* **1** (2), 101, **2017**.
16. KNOTHE G.H. Biodiesel and its properties. In *Industrial Oil Crops*, McKeon, T.A., Hayes, D.G., Hildebrand, D.F., Weselake, R.J., Eds. Urbana, IL: AOCS Press, **15**, **2016**.
17. ALGUACIL M.M., TORRECILLAS E., HERNÁNDEZ G., ROLDÁN A. Changes in the diversity of soil arbuscular mycorrhizal fungi after cultivation for biofuel production in a Guantanamo (Cuba) tropical System. *Plos ONE.* **7** (4), e34887, **2012**.
18. BAUDDH K., SINGH K., BHASKAR S., SINGH R.P. *Ricinus communis*: A robust plant for bio-energy and phytoremediation of toxic metals from contaminated soil. *Ecol. Eng.* **84**, 640, **2015**.
19. BELLINI G., SUMNER M.E., RADCLIFFE D.E., QAFOKU N.P. Anion transport through columns of highly weathered acid soil: Adsorption and retardation. *Soil Sci. Soc. Am. J.* **60** (1), 132, **1996**.
20. CLEMENSSON L., PERSSON H. Effects of freezing on rhizosphere and root nutrient content using two soil sampling methods. *Plant Soil.* **139** (1), 39, **1992**.
21. AGUILAR-CHÁVEZ A., DÍAZ-ROJAS M., CÁRDENAS-AQUINO M.D., DENDOOVEN L., LUNA-GUIDO G.M. Greenhouse gas emissions from a wastewater sludge-amended soil cultivated with wheat (*Triticum spp.* L.) as affected by different application rates of charcoal. *Soil Biol. Biochem.* **52**, 90, **2012**.
22. SAS INSTITUTE. *Statistic Guide for Personal Computers*. Version 6.0. (eds.). SAS Institute, Inc., Cary, **1989**.
23. WU X.H., ZHANG H.S., GANG L., LIU X.C., QIN P. Ameliorative effect of castor bean (*Ricinus communis* L.) planting on physicochemical and biological properties of seashore saline soil. *Ecol. Eng.* **38**, 97, **2012**.
24. FRANCO-HERNÁNDEZ M.O., VASQUEZ-MURRIETA M.S., PATIÑO-SICILIANO A., DENDOOVEN L. Heavy metals concentration in plants growing on mine tailings in Central México. *Bioresource Technol.* **101** (11), 3864, **2010**.
25. LINDSAY W L. *Chemical Equilibria in Soil*. Wiley Interscience, New York, USA., Pp. 315-326, **1979**.
26. MITCHELL P., BARR D. The nature and significance of public exposure to arsenic a review of its relevance to South-West England. *Environ. Geochem. Hlth.* **17** (2), 57, **1995**.
27. OLOWU R.A., ADEWUYI G.O., ONIPEDE O.J., LAWAL O.A., SUNDAY O.M. Concentration of heavy metals in root, stem and leaves of *Acalypha indica* and *Panicum maximum* Jacq from three major dumpsites in Ibadan metropolis, South West Nigeria. *Am. J. Chem.* **5** (1), 40, **2015**.
28. SAGHALI M., HOSEINI S.M., HOSSEINI S.A., BAQRAF R. Determination of heavy metal (Zn, Pb, Cd and Cr) concentration in benthic fauna tissues collected from the southeast Caspian Sea, Iran. *B. Environ. Contam. Tox.* **92** (1), 57, **2014**.
29. SANCHEZ-LÓPEZ A.S., CARRILLO-GONZÁLEZ R., GONZÁLEZ-CHÁVEZ M.D.A., ROSAS-SAITO G.H., VANGRONSVELD J. Phytobarrriers: Plants capture particles containing potentially toxic elements originating from mine tailings in semiarid regions. *Environ. Pollut.* **205**, 33, **2015**.
30. MASSAS I., KALIVAS D., EHALIOTIS C., GASPARATOS D. Total and available heavy metal concentrations in soils of *Thriassio plain* (Greece) and assessment of soil pollution indexes. *Environ. Monit. Assess.* **185** (8), 6751, **2013**.
31. WANG S., ZHAO Y., GUO J., ZHOU L. Effects of Cd, Cu and Zn on *Ricinus communis* L. Growing in single element or co-contaminated soils: Pot experiments. *Ecol. Eng.* **90**, 347, **2016**.
32. EPSTEIN E., BLOOM J. *Inorganic Components of Plants*. In *Mineral Nutrition of Plants: Principles and Perspectives*, 2nd. Sunderland, Sinauer Associates. Sunderland, Massachusetts, USA, **41**, **2004**.
33. KIRKBY E., RÖMHELD V. *Micronutrients in Plant Physiology: Functions, Up-take and Mobility*. Proceedings 543, The International Fertilizer Society, P.O. Box, York, United Kingdom, **52**, **2007**.