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Impact of rootstock on heavy metal bioaccumulation in apple plant grown near an industrial source in Obiliq, Kosovo

R. Imeri[1], E. Kullaj[1], E. Duhani[2] and L. Millaku[3,*]

[1]Agricultural University of Tirana, Faculty of Agriculture and Environment, Department of Horticulture and Landscape Architecture, Koder-Kamez, AL1029 Tirana, Republic of Albania
[2]University of Prishtina, Faculty of Agriculture and Veterinary, Department of Agriculture, George Bush, nr. 31, XK10000 Prishtine, Republic of Kosovo
[3]University of Prishtina, Faculty of Mathematics and Natural Sciences, Department of Biology, George Bush, nr. 31, XK10000 Prishtine, Republic of Kosovo
[*]Correspondence: lulzimmillaku@hotmail.com

Abstract. Food exposure to heavy metals such as Pb, Cd, Cr, Ni, As, Zn, Cu and Fe is considered a risk to human health. This study analyzes the level of heavy metals in soil and delicious apple tissues (fruit, leaf, shoot) in three different rootstocks: mm106, m26 and m9 grown in the Obiliq region (considered as a polluted region). The data obtained from the Obiliq areas are compared with those grown in reference clear area. Individual soil samples were collected from each plant to assess metal content in the immediate plant environment. Samples of soil, fruit, leaf and shoot have been analyzed for heavy metals (Pb, Cd, Ni, As, Zn, Cu, Cr and Fe) using atomic absorption spectrophotometry (AAS).

The results indicated that the average concentrations of Pb, Cd, Ni, As, Zn, Cu, Cr and Fe in soil of Obiliq areas were 2.03, 0.15, 6.99, 12.4, nd, 12.3, 4.68, 5.32 mg kg^{-1} d.w. respectively. The concentration of metals in the apple tissue increased with the increase of heavy metals in soil from polluted area. The accumulation ratios of heavy metals were calculated to assess the potential health risks. The mean concentrations of the heavy metals in the soil were in order of magnitude Ni > Zn > Cr > Cu > Fe > Pb > Cd > As while that in the fruits of apple were in order of magnitude Cr > Fe > Cu > Ni > Pb > Zn > Cd > As; in the leaves were Fe > Zn > Cu > Cr > Pb > Ni > Cd > As; in shoots were Zn > Fe > Cu > Pb > Ni > Cr > Cd > As.

Mobility of heavy metals and potentially hazardous in studied lands threatens the quality of apple fruit consumption, with a real risk that these elements (Cd, Pb, Ni and Cr) can enter the food chain.

Key words: pollution areas, apple, rootstock, accumulation, fruit consumption safety.

INTRODUCTION

Metals are present in the soil crust at different levels. Extraction of fuels, metallurgical minerals, municipal waste and excessive use of pesticides and fertilizers has affected accelerated release of metals and metals into various components of ecosystems (Arya & Roy, 2011). High concentrations of metals, either of natural origin or of anthropogenic activity, can become toxic to soil microflora. The toxicity of heavy metals depends on the type of metal element and its bioavailability on the soil. Some metals such as Zn, Cu are essential for plants with low concentrations, but become toxic in increasing concentrations, while some metals such as Pb, Cd, Cr have never been shown to be essential to the development of living organisms and are toxic even at very low concentrations (Todeschini et al., 2011).

Regardless of the source of heavy metals on the ground; higher concentrations of some metals degrade soil quality and reduce crop production, including poor quality products, pose risks to ecosystems and human and animal health (Blaylock & Huang, 2000). Heavy metals into the plant through the intake of water, which are then consumed by animals. The ingestion of these cplant and animal products is the main source of accumulation of heavy metals in humans because they are barely metabolised. Different plants exhibit different tolerance for heavy metals collection (Yang & Chu, 2011). In most cases, heavy metals are not easily absorbed from the ground because they have low mobility on the ground. Their absorption from the plant depends on pH, organic substances, water content, metal content and other elements in the rhizosphere.

Mining operation, grinding, concentrating ores and disposal of tailings, provide obvious sources of contamination in the environment, along with mine and mill wastewater. This will lead to the release and migration of heavy metals thus cause heavy metals pollution of soil near the mining area. Heavy metals can spread from soil to other ecosystem components, such as groundwater, plants, thus affecting human health through drinking water and food chain, so the evaluation of heavy metal pollution in soil is very important (Adamsa et al., 2004). Native plants are good indicators of ambient air quality and its growth. Plants absorb airborne anthropogenic pollutants, and their chemical composition may be a good indicator for contaminated areas when it is assessed against background values obtained for unpolluted vegetation. Optimum quantity of Mn, Zn, Ni, Cu and other elements is the main precondition for the proper growth and development of plants. However, high concentrations of these elements can have a negative and toxic effect on plants (Cairns, 1980). Seasonal fruits represent a source of nutrients and can contain toxic elements as well, which can cause the appearance of some chronic diseases in humans. Emission from anthropogenic pollution sources increases the concentration of pollutants in the environment, which poses a potential threat to fruit grown in polluted areas (Müller & Anke, 1994; Ramadan & Al-Ashkar, 2007). Consumption of contaminated food with heavy metals can be the cause of the reduction of immune defense and the high prevalence of the appearance of gastrointestinal cancer (Turkdogan et al., 2003).

This study analysed the heavy metal pollution in soil and apple (malus sp.) tissue of Obiliq area in Kosovo. The purpose of this research was to determine the distribution of selected heavy metals (Pb, Cd, As, Ni, Zn, Cu, Cr and Fe) in different fractional parts of agricultural land and apple tissues. This study was conducted in order to better understand the chemical fractionation and mobility of heavy metals selected in agricultural lands and their transfer to the food chain through apple fruit widely.

MATERIALS AND METHODS

Description of the Study area

Coal and lignite-mining, coal burning, industry, a nearby thermal power plant ‘Kosova’, traffic, and farming left ecotoxicological burdens in Kosovo industrial – rural region of Obiliq municipality, and surrounding regions. The newest data shows that lignite resources in Kosovo reach 15 billion tons (Rizaj et al., 2008). Lignite is the most important energy resource in Kosovo, providing about 87% of electric energy production in two thermo-power plants (6 units with 1,470 MW installed capacity). The discharges of liquid organic waste (measured as chemical oxygen demand, COD), from the industrial thermo-power complex in Obiliq, 5–10 tons per day, are, however, largely exceeded by the discharges of untreated urban sewage from the city of Prishtina: up to 700 tons per day. In addition to the huge modification of the landscape due to the open-cast mining and large dumps for overburden and solid wastes (ash and sludge), the Obiliq industries emit important quantities of air pollutants (dust, sulphur dioxide, and nitrogen oxides), and cause considerable pollution.

Figure 1. Area in this study – Obiliq, Kosovo.

Sample collection

Individual soil samples were collected from each plant to assess metal content in the immediate plant environment. Samples of fruit, leaf, shoot and soil have been analysed for eight heavy metals (Pb, Cd, As, Ni, Zn, Cu, Cr and Fe). Twenty soil samples and 90 apple samples were collected in the Obiliq region and reference area during September–November 2017 (Fig. 1). Three kinds of apple tree tissues were collected during the harvest period, including fruit, leaf and shoot. To reduce the effect of other agro environmental factors on the issue analysed, the experiment included a row of fruit trees with the same age (6 years), grafted on the same rootstock (mm106, m26, m9). Soil samples were taken from the surface layer (0–20 cm). All samples were sealed in polyethylene bags and transported to the laboratory within 6 h of collection. The soil samples were air-dried at room temperature, with impurities manually removed. Then, the soils were ground and sieved through 80 meshes (0.2 mm). For all samples, the decay and withered tissues were removed and the edible parts were washed with tap water to remove surface dirt. The edible parts of fruits or leaf were repeatedly rinsed with deionised water and dried at 60 °C to a constant weight.

Sample analysis

For the research needs of the metal content: Lead (Pb), Cadmium (Cd), Chromium (Cr), Nickel (Ni), Arsenic (As), Zinc (Zn), Copper (Cu), Iron (Fe) field samples were obtained in accordance with the respective protocols and consisted in sampling soil from apple planted surfaces within the respective localities. Samples received are labelled with all relevant data (locality, sampling date and other notes). Concentration of metals in soil samples was determined by the atomic absorption spectroscopy (AAS) of the Perkin-Elmer model 1200 mark. Work samples (2.0 gr of soil sample) were treated with a 1:3 aqueous regia mixture (4 mL HNO3 + 12 mL of concentrated HCl) in an electrical reso at a temperature of 200 °C for 60 minutes. Prior to mineralization with aqua regia, the organic matter was disintegrated with hydrogen peroxide concentrated (35% H_2O_2). Then the mineralized samples were mixed with distilled water and filtered with Whatman 0.45 μm filtration paper. The filter is placed on a volumetric balloon of 50 cm^3 and is levelled up to the mark with distilled water. Such samples are read with AAS and spectrophotometer. The AAS calibration is done with the standard reference material of 1,000 ppm (mg kg^{-1}) from which the respective metal standards are prepared.

Statistical analysis

The data were statistically analyzed using GraphPad Prism – version 7.05 and Microsoft Excel 2010 computer package. The level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Concentrations of heavy metals Pb, Cd, Cr, Ni, As, Zn, Cu and Fe in the apple tissues varies depending on the type of rootstock. Table 1 shows the distribution values of the heavy metals in soils and the analyzed tissues (fruit, leaves and shoots) of the apple sp. with rootstock mm106, m26, m9 in the region of Obiliq. The average content in plants of each studied heavy metal was compared with the content of the same metal in the plants collected from the reference area, Table 2. Accumulation and distribution of heavy metals on soil depends on many different factors such as: chemical form of elements, pH, organic matter content, etc. Ph values in all analyzed soil samples were distributed from 5.8 to 7.5.

According to our results, the highest and lowest values of heavy metal accumulation in apple plant fruit with mm106 rootstock varied between 0.32–3.52 mg kg^{-1} d.w for Pb; 0.04–0.85 mg kg^{-1} d.w for Cd; 1.32–10.7 mg kg^{-1} d.w for Cr; 0.22–5.36 mg kg^{-1} d.w for Ni; **nd** mg kg^{-1} d.w for As; 0.39–3.06 mg kg^{-1} d.w for Zn; 0.54–5.35 mg kg^{-1} d.w for Cu; 1.27–7.32 mg kg^{-1} d.w for Fe.

Apple plant fruit with m26 rootstock varied between 0.27–4.16 mg kg^{-1} d.w for Pb; 0.04–1.03 mg kg^{-1} d.w for Cd; 1.42–11.1 mg kg^{-1} d.w for Cr; 0.36–4.75 mg kg^{-1} d.w for Ni; **nd** mg kg^{-1} d.w for As; 0.14–2.99 mg kg^{-1} d.w for Zn; 0.52–3.65 mg kg^{-1} d.w for Cu; 2.33–9.36 mg kg^{-1} d.w for Fe.

Apple plant fruit with m9 rootstock varied between 0.15–3.06 mg kg^{-1} d.w for Pb; 0.03–0.42 mg kg^{-1} d.w for Cd; 1.41–9.36 mg kg^{-1} d.w for Cr; 0.11–2.74 mg kg^{-1} d.w for Ni; **nd** – mg kg^{-1} d.w for As; 0.13–2.78 mg kg^{-1} d.w for Zn; 0.32–4.05 mg kg^{-1} d.w for Cu; 0.74–8.56 mg kg^{-1} d.w for Fe.

The results of the present study show that Pb, Cd and Fe were accumulated in the largest quantity of m26 rootstock, while Cr, Ni, Zn and Cu accumulated more in mm106 rootstock (Table 1). The m9 rootstocks have been found to be the lowest values of heavy elements compared to mm106 and m27. At the reference site, the amount of the heavy metals concentration was the lowest and the pollution rate was also very low (Table 2).

While significant differences in concentrations of metals in plant tissues mean that different apple rootstock had different abilities and capacities to take up and accumulate different metals. The average values of Pb, Cd, Cr, Ni, As, Zn, Cu, Fe in apple plant tissues with different rootstock in the Obiliq area are given in Figs 2, 3, 4.

Therefore, to determine the amount of accumulation of heavy metals in selected plant tissues, the transfer factor (TF) was determined. TF is an index for estimating the possible transfer of a metal from soil to plants versus the ability of fruit trees to accumulate a particular metal with respect to its concentration on the substrate of the earth (Adamsa et al., 2004).

Table 1. Heavy metal concentration (mg kg^{-1}) in soil, shoot, leaf and fruit of apple species depending on rootstocks type from Obiliq region

			Heavy metals (mg kg^{-1})							
Rootstock	Tissues	Level	Pb	Cd	Cr	Ni	As	Zn	Cu	Fe
M106	Shoot	Mean	5.19	0.18	1.46	2.53	0.01	52.1	7.39	19.4
		SD (±)	3.13	0.31	0.63	1.69	0.01	8.94	3.01	7.09
		CV (%)	60.3	172.2	43.1	66.7	100	17.1	40.7	36.5
	Leaf	Mean	2.21	0.01	3.03	0.71	0.05	46.1	11.8	51.1
		SD (±)	1.26	0.01	0.93	0.81	0.07	10.4	6.25	7.33
		CV (%)	57.1	100	30.6	114.1	140	22.5	52.9	14.3
	Fruit	Mean	1.41	0.23	5.47	1.96	Nd	1.49	2.54	4.31
		SD (±)	0.99	0.26	3.16	1.61		0.94	1.59	1.97
		CV (%)	70.2	113.1	57.7	82.1		63.1	62.5	45.7
M26	Shoot	Mean	3.98	0.13	1.35	1.74	0.01	43.1	9.11	33.3
		SD (±)	2.32	0.11	0.46	0.77	0.01	9.02	5.79	10.4
		CV (%)	58.3	84.6	34.1	44.2	100	20.9	63.5	31.2
	Leaf	Mean	2.47	0.01	2.63	0.44	0.02	24.2	15.2	63.1
		SD (±)	1.53	0.02	1.11	0.31	0.02	6.72	4.99	8.11
		CV (%)	40.4	200	42.2	70.4	100	27.7	32.8	12.8
	Fruit	Mean	1.56	0.29	3.95	1.36	Nd	1.22	2.36	5.02
		SD (±)	1.09	0.33	3.08	1.32		1.14	0.97	2.16
		CV (%)	69.8	113.7	77.9	97.1		93.4	41.1	43.1
M9	Shoot	Mean	3.49	0.09	0.94	2.29	Nd	39.2	8.83	28.1
		SD (±)	2.01	0.09	0.32	1.09		8.42	4.48	6.35
		CV (%)	57.5	100	34.1	47.5		21.4	50.7	22.5
	Leaf	Mean	1.77	0.003	1.59	0.61	Nd	15.3	10.5	45.8
		SD (±)	0.89	0.002	0.61	0.36		7.51	2.77	10.8
		CV (%)	50.2	66.6	38.3	59.1		49.1	26.3	23.5
	Fruit	Mean	1.12	0.14	4.23	0.88	Nd	0.88	1.22	3.29
		SD (±)	0.99	0.13	2.74	0.81		1.07	1.21	2.31
		CV (%)	88.3	92.8	64.7	92.1		121.5	99.1	70.2
Soil		Mean	2.03	0.15	6.99	12.4	Nd	12.3	4.68	5.32
		SD (±)	1.32	0.11	4.61	4.03		4.27	3.86	3.09
		CV (%)	65.1	73.3	65.9	32.5		34.7	82.4	58.1

Note: Values are expressed as means X and ± SD.

TF values are affected by several factors as: metal chemistry, type of soil, soil characteristics and also the plant species. On average, the transfer of heavy metals from soil to shoot, leaf and fruit of the apple with the rootstock mm106 was in the order:

TF=C(shoot)/C(soil) Zn (4.23) > Fe (3.64) > Pb (2.55) > Cu (1.57) > Cd (1.21) > Ni (0.22) > Cr (0.21) > As (nd).
TF=C(leaf)/C(shoot) As(5) > Fe (2.63) > Cr (2.07) > Cu (1.59) > Zn (0.88) > Pb (0.42) > Ni (0.28) > Cd (0.05).
TF=C(fruit)/C(leaf) Cd (23.1) > Ni (2.76) > Cr (1.81) > Pb (0.63) > Cu (0.21) > Fe (0.08) >Zn (0.03) > As(nd).

Transfer factor to apple tissues with rootstock m26 was in the order:
TF=C(shoot)/C(soil) Fe (6.25) > Zn (3.51) > Pb (1.96) > Cu (1.94) > Cd (0.86) > Cr (0.19) > Ni (0.14) > As (nd);
TF=C(leaf)/C(shoot) As (2) > Cr (1.94) > Fe (1.89) > Cu (1.66) > Pb (0.62) > Zn (0.56) > Ni (0.25) > Cd (0.07);
TF=C(fruit)/C(leaf) Cd (29.2) > Ni (3.09) > Cr (1.51) > Pb (0.63) > Cu (0.15) > Zn (0.05) > Fe (0.01).

Table 2. Heavy metal concentration (mg kg^{-1} d.w.) in soil, shoot, leaf and fruits of apple species depending on rootstocks type from Reference site

			Heavy metals (mg kg^{-1})							
Rootstock	Tissues	Level	Pb	Cd	Cr	Ni	As	Zn	Cu	Fe
M106	Shoot	Mean	3.23	0.23	0.26	3.76	nd	56.17	5.12	34.05
		SD (±)	1.09	0.17	0.18	0.54		10.3	0.91	4.16
		CV (%)	33.7	73.9	69.2	14.3		18.3	17.7	12.2
	Leaf	Mean	1.69	0.16	1.13	0.93	nd	23.2	8.55	79.14
		SD (±)	0.81	0.15	0.19	0.47		4.67	2.63	4.51
		CV (%)	47.9	93.7	16.8	50.5		20.1	30.7	5.69
	Fruit	Mean	0.51	0.009	1.27	0.28	nd	0.88	0.98	3.36
		SD (±)	0.34	0.001	0.63	0.07		0.44	0.32	1.79
		CV (%)	66.6	11.1	49.6	25		50	32.6	53.2
M26	Shoot	Mean	2.65	0.17	0.18	2.44	nd	48.02	6.31	41.03
		SD (±)	1.69	0.24	0.11	1.08		11.03	1.41	4.37
		CV (%)	63.7	141.1	61.1	44.2		22.9	22.3	10.6
	Leaf	Mean	1.38	0.19	1.06	0.41	nd	41.15	11.7	104.3
		SD (±)	0.7	0.16	0.43	0.33		6.66	3.92	9.55
		CV (%)	50.7	84.2	40.5	80.4		16.1	33.5	9.15
	Fruit	Mean	0.38	0.08	0.99	0.18	nd	0.74	0.78	3.23
		SD (±)	0.26	0.05	0.58	0.17		0.36	0.56	0.74
		CV (%)	68.4	62.5	58.5	94.4		48.6	71.7	22.9
M9	Shoot	Mean	2.12	0.13	0.42	3.33	nd	62.11	4.97	36.14
		SD (±)	1.05	0.09	0.14	1.38		12.17	1.65	6.09
		CV (%)	49.5	69.2	33.3	41.4		19.5	33.1	16.8
	Leaf	Mean	0.85	0.06	0.86	0.31	nd	29.2	9.84	94.9
		SD (±)	0.82	0.04	0.28	0.28		6.96	2.93	11.24
		CV (%)	96.4	66.6	32.5	90.3		23.8	29.7	11.8
	Fruit	Mean	0.31	0.003	0.49	0.19	nd	0.31	0.93	4.13
		SD (±)	0.21	0.001	1.37	0.27		0.13	0.87	2.59
		CV (%)	67.7	33.3	279.5	142.1		41.9	93.5	62.7
Soil		Mean	1.03	0.05	0.39	1.99	nd	4.72	1.92	9.22
		SD (±)	0.66	0.009	0.31	1.43		1.14	0.39	3.58
		CV (%)	64.1	18	79.4	71.8		24.1	20.3	38.8

Note: Values are expressed as means X and ± SD. nd- not detected.

Transfer factor to apple tissues with rootstock m9 was in the order: **TF=C(shoot)/C(soil)** Fe (5.28) > Zn (3.18) > Cu (1.88) > Pb (1.71) > Cd (0.61) > Ni (0.18) > Cr (0.13) > As (nd); **TF=C(leaf)/C(shoot)** Cr (1.69) > Fe (1.62) > Cu (1.18) > Pb (0.51) > Zn (0.39) > Ni (0.26) > Cd (0.03) > As (nd); **TF=C(fruit)/C(leaf)** Cd (46.6) > Cr (2.66) > Ni (1.44) > Pb (0.56) > Cu (0.11) > Fe (0.07) > Zn (0.05) > As (nd).

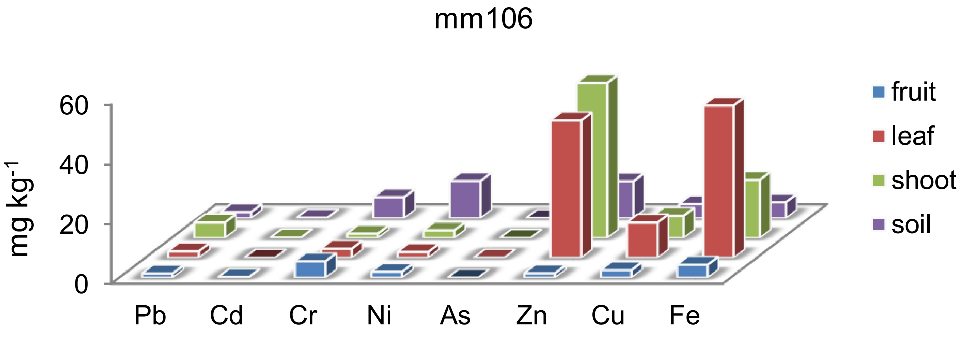

Figure 2. Heavy metals levels in soil and apple tissues with mm106 rootstock in Obiliq region.

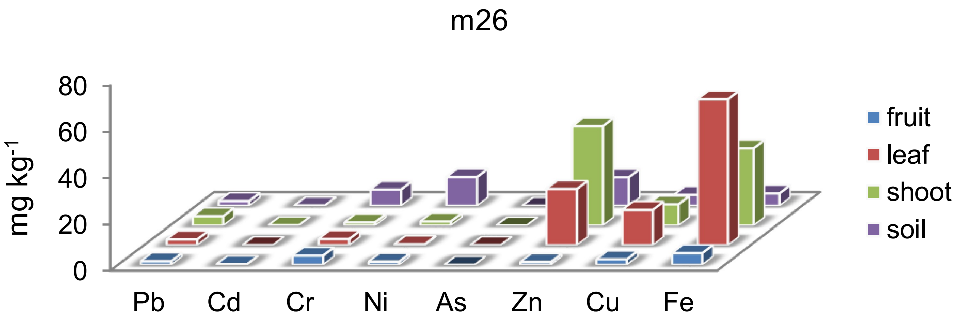

Figure 3. Heavy metals levels in soil and apple tissues with m26 rootstock in Obiliq region.

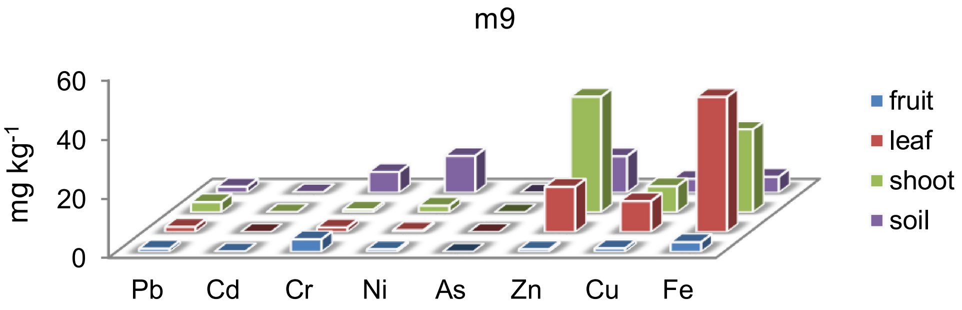

Figure 4. Heavy metals levels in soil and apple tissues with m9 rootstock in Obiliq region.

In this study, we analysed the level of heavy metals in soil and plant tissues of apple tree with different rootstock grown near the industrial area. Our main objective was to evaluate the availability of heavy metals from soil to fruit, leaves and shoot of apple tree. During the study, we have chosen some types of rootstock, and we have analysed their role in the transfer of heavy metals from soil to tissues of apple tree.

Our study analyzes showed that concentrations of heavy metals (Pb, Cd, Cr, Ni, As, Cu, Zn and Fe) in the analyzed tissue samples were higher in areas near the industrial zone of Obiliq region (Table 1) compared to control area (Table 2). Concentrations of heavy metals varied between different rootstocks due to their different absorption capacity and the level of soil pollution and atmospheric pollution (Roba et al., 2016). Previous studies showed that the higher the concentration of heavy metals on soil, its probability in plant tissue would be greater (Mapanda et al., 2007).

Cadmium is considered as being one of the most ecotoxic metals that exhibit adverse effects on all biological processes of humans, animals, and plants. This metal reveals its great adverse potential to affect the environment and the quality of food. Although Cd is considered to be a nonessential element for metabolic processes, it is effectively absorbed by both root and leaf systems. The distribution of Cd within plant organs is quite variable and clearly illustrates its rapid transport from roots to tops (Table 3).

Table 3. Transfer factor of heavy metals (Pb, Cd, Cr, Ni, As, Zn, Cu, Fe) in the Obiliq region

Rootstock	Transfer factor (TF)	Pb	Cd	Cr	Ni	As	Zn	Cu	Fe
M106	TF=C(shoot)/C(soil)	2.55	1.21	0.21	0.22	-	4.23	1.57	3.64
	TF=C(leaf)/C(shoot)	0.42	0.05	2.07	0.28	5	0.88	1.59	2.63
	TF=C(fruit)/C(leaf)	0.63	23.1	1.81	2.76	-	0.03	0.21	0.08
M26	TF=C(shoot)/C(soil)	1.96	0.86	0.19	0.14	-	3.51	1.94	6.25
	TF=C(leaf)/C(shoot)	0.62	0.07	1.94	0.25	2	0.56	1.66	1.89
	TF=C(fruit)/C(leaf)	0.63	29.2	1.51	3.09	-	0.05	0.15	0.01
M9	TF=C(shoot)/C(soil)	1.71	0.61	0.13	0.18	-	3.18	1.88	5.28
	TF=C(leaf)/C(shoot)	0.51	0.03	1.69	0.26	-	0.39	1.18	1.62
	TF=C(fruit)/C(leaf)	0.56	46.6	2.66	1.44	-	0.05	0.11	0.07

The interaction of Cd and Zn has received much study, and all findings may be summed up by stating that, in most cases, Zn reduces the uptake of Cd by both root and foliar systems. Chaney & Hornick (1977) suggested that when the Cd:Zn ratio in plant tissues is limited to 1%, the Cd content is restricted to below 5 mg kg^{-1}, thus below its phytotoxic level. Cd–Cu interactions are also complex. The inhibitory effect of Cu on Cd absorption is reported most often. Cd–Ca relationship seems to be highly cross-linked with variation in the soil pH. It cannot be precluded, however, that Ca^{2+} ions are able to replace Cd^{2+} in carrier mechanisms and thus Cd absorption by plants may be inhibited by an excess of Ca cations. In our study, fruit analysis from all sampling points was contaminated by an excessive amount of Cd compared to the permitted limit (0.05 mg kg^{-1}) proposed by FAO/WHO (1995). Cadmium can accumulate in the human body and can cause kidney dysfunction, skeletal damage and reproductive deficiency. The cadmium content in the literature was reported in the range of and 0.0002 0.527 mg kg^{-1} in fruit foods from the Greek market (Karavoltsos et al., 2002).

Toxic effects of Cr on plant growth and development include alterations in the germination process as well as in the growth of roots, stems and leaves. Hence, exposure to high level of Cr affected total dry matter production and yield of plants (Shanker et al., 2005). The mechanism of absorption and translocation of Cr in plants is apparently similar to those of Fe. There is some evidence that easily available Cr^{6+} is transformed into Cr^{3+} form in plant cells which readily interact with DNA and protein compounds

(Zayed et al., 1998). The concentration of Cr in delicious apple cultivars in three rootstock mm106, m26 and m9 in the contaminated studied fields was greater than in the reference area. The content of chromium in the literature was reported in the range of 1.48–6.43 mg kg^{-1} in wet weight in various summer fruits from Pakistan (Zahoor et al., 2003).

High level of Pb also causes inhibition of enzyme activities, water imbalance, alterations in membrane permeability and disturbs mineral nutrition. Pb inhibits the activity of enzymes at cellular level by reacting with their sulfhydryl groups. High Pb concentration also induces oxidative stress by increasing the production of ROS in plants (Reddy et al., 2005). Our results are in line with the results reported by Zhen et al. (2008), Xiao et al. (2010) in China. Zhen (2008) reported that cultivated fruits near the Shenyang-Dalian highway were polluted with Pb and Cd with average concentrations of 0.082 and 0.010 mg kg^{-1} in apple fruit.

Copper (Cu) is considered as a micronutrient for plants (Thomas et al., 1998) and plays important role inCO_2 assimilation and ATP synthesis. Exposure of plants to excess Cu generates oxidative stress and ROS. Oxidative stress causes disturbance of metabolic pathways and damage to macromolecules (Hegedus et al., 2001). Ni levels in fruit were many times higher than the maximum allowed limit 0.3 mg kg^{-1} (Liu et al., 2012). The content of nickel in literature was reported in the range of 1.0–8.9 mg kg^{-1} in some fruits from Pakistan (Zahoor et al., 2003).

Table 4. Transfer factor of heavy metals (Pb, Cd, Cr, Ni, As, Zn, Cu, Fe) to the reference site

Rootstock	Transfer factor (TF)	Pb	Cd	Cr	Ni	As	Zn	Cu	Fe
	TF=C(shoot)/C(soil)	3.13	4.61	0.66	1.88	nd	11.9	2.66	3.69
M106	TF=C(leaf)/C(shoot)	0.52	0.69	4.34	0.24	nd	0.41	1.66	2.31
	TF=C(fruit)/C(leaf)	0.31	0.05	1.12	0.31	nd	0.03	0.11	0.04
	TF=C(shoot)/C(soil)	2.57	3.41	0.46	1.22	nd	10.1	3.28	4.44
M26	TF=C(leaf)/C(shoot)	0.52	1.11	5.88	0.16	nd	0.85	1.85	2.53
	TF=C(fruit)/C(leaf)	0.27	0.42	0.93	0.43	nd	0.01	0.06	0.03
	TF=C(shoot)/C(soil)	2.05	2.63	1.07	1.67	nd	13.1	2.58	3.91
M9	TF=C(leaf)/C(shoot)	0.41	0.46	2.04	0.09	nd	0.47	1.97	2.62
	TF=C(fruit)/C(leaf)	0.36	0.05	0.56	1.18	nd	0.01	0.09	0.04

The obtained results showed that the fruits were powerful accumulators of heavy metals, considering that for some types of rootstocks the concentrations of heavy metals in the samples have exceeded the allowed values. High TF values (≥ 1) show the largest absorption of metals from soil to plants (Tables 3 and 4), while lower values indicate low levels of metal absorption from the plant that can be used for human consumption (Rangnekar et al. 2013). High values of transfer factors for Cd and Zn were reported (Lăcătuşu et al., 2012) in particular soil-plants systems with plants grown in the mud pond with sludge from municipal wastewater having high trace metal content and high moisture content. The highest TF was obtained for Cd in Obiliq areas. In fact, Cd is already known for its mobility from soil to plants (Kirkham, 2006). TF are even higher than 1, which means plants are accumulating Cd and should be recommended to not use them as food especially when the plants are grown in soil with high contamination factors. Thus, TF value of 23.1; 29.2 and 46.6 for Cd represent a concern for the health of the consumers.

CONCLUSIONS

The concentration of metals in the apple tissue increased with the increase of heavy metals in soils from polluted area. The accumulation ratios of heavy metals were calculated to assess the potential health risks. The mean concentrations of the heavy metals in the soil were in order of magnitude Ni > Zn > Cr > Cu > Fe > Pb > Cd > As while that in the fruits of apple were in order of magnitude Cr > Fe > Cu > Ni > Pb > Zn > Cd > As; in the leaves were Fe > Zn > Cu > Cr > Pb > Ni > Cd > As; in shoots were Zn > Fe > Cu > Pb > Ni > Cr > Cd > As.

The data of the present study showed that Cd, Cr and Ni had the highest transfer factor. The results of the recent study will help to assess the long-term impact on human health caused by the heavy metals released by mining and smelting activities in Obiliq region (Kosovo). However, much study is needed in this respect such as metal uptake studies at cellular level including efflux of different metal ions by different cell organelles and membranes. It is therefore, suggested that regular monitoring of heavy metals in plant tissues is essential in order to prevent excessive build up of these metals in the human food chain.

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