Research Paper

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Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) Approach for the Determination of Heavy Metals, Metalloid and Trace Element in Soil and Vegetables

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ABSTRACT

The quantitative analysis of selected toxic heavy metals (Cd and Pb) and metalloid (As) as well as the trace element (Zn) in few vegetables and soil has been carried out by specific method-inductively coupled plasma-optical emission spectroscopy (ICP-OES) in our recent research work. Samples of soil and some vegetables were taken close to our four cities/regions in Macedonia: Skopje, Veles, Ohrid and Kocani, in the time period/duration of November to December 2012. We have illustrated the concentration of Cd, Pb, As and Zn for our samples and found in such orders, the highest concentrations of Pb in cauliflower is 1.222 mg/kg, Cd in carrot is 0.192 mg/kg, Zn in leek is 0.319 mg/kg and As in onion is 0.228 mg/kg in the Veles (Bashino village/region), and the highest concentration values of Pb in leek is 1.261 mg/kg, Cd in carrot is 1.027 mg/kg, Zn in carrot 0.251 mg/kg and As in cabbage is 0.057 mg/kg in the region of Skopje (near cement Usje), respectively. All these concentrations are in relation to the prescribed criteria of WHO-World health organization and FAO-Food and Agriculture Organization. Finally, we get that, there is a strong correlation between the concentration of Zn in vegetables and soil in our present analytical work.

Key words: Crops/vegetables, Soil, Inductively coupled plasma – Optical emission spectroscopy (ICP-OES)

Human exposure to heavy metals increased dramatically in the last 50 years. The rapid extension of world population and its needs for nourishment are highly extensive, but the anthropogenic metals maturities in the soil may reduce as much as crops yield and as same as the food quality. Heavy metals are usually and/or most generally found in crops, since the concentrations of heavy metals in the crops are directly related to concentration in the soil in which they grow (Martinez 2001, Chen 2005). The soil pollution with toxic significant elements like Cadmium (Cd), Lead (Pb), metalloid Arsenic (As) and trace element Zinc (Zn) is one's of the most serious environmental problems throughout the world today, in which thousands of millions of the mankind population suffer from the health problems. By using newly conductive techniques in the environmental pollutions might be good public awareness and scientific temperament in the Republic of Macedonia. The Republic of Macedonia is settled in the Balkan Peninsula, southeastern Europe, and it is characterized with the Mediterranean and continental climate, and generally the territory is surrounded with the mountains, basins and valleys. It encompasses approximately 67,000 square kilometers and has a population of 4.76 millions. And also, here the soil is highly operative. Hence, the pollution of soil, water, and air became inevitable as a result of the anthropogenic influences. The presence of the toxic metals in plants and soil are as a result of advancement of industries in large numbers and in the age of modern technology. As we know, since the advent of the industrial age, the environment has been exposed to emission and deposition of anthropogenic chemicals, organic and inorganic. Heavy metal recruitment in the biosphere by human activities has become a significant process in the geochemical cyrcling of such toxic metals (Martinez 2001). This is evident in the industrial vicinities where stationary and itinerant sources release huge quantities of heavy metals into the atmosphere, soil and vegetation exceeding the natural emission levels (Chen 2005).

Heavy metal distribution between soil and vegetation is an important issue in evaluating environmental effects of the toxic metals (Bilos 2001). Heavy metal toxicity effects on plant growth, photosynthetic activity, enzymatic activity and accumulation of other nutrient elements, and also damages the root system. The determination of some selective heavy metals Pb and Cd, metalloid As and trace element Zn have been carried out by Duffus (2002), that gives a superior awareness in the address of environment pollution. And later on, such study of the toxic heavy metals in the diets leads towards the improvement of diet safety and quality (Bilos 2001, Chen 2005, Abulude 2006). Recent studies that have been made based on experiments for animals, which indicate that the results obtained by consuming diets with increased amounts of specific heavy metals causes severe diseases in very short time (Joseph 2009, Czeczot and Skrzycki 2010). On behalf of the literature survey (Osma *et al*. 2012), the present work has been carried out on the pollution, i e toxic heavy metals in vegetables and soil of some regions in the Republic of Macedonia which are settled in the Balkan Peninsula.

MATERIALS AND METHODS

The study area is located in the Republic of Macedonia is a country settled in the south-eastern Europe with geographic coordinates latitude 41°50'N and longitude 22.00°E. No other industrial developments exist within the region. The material applied and its processing has been described in three parts, namely (i) samples collected from four cities/regions in Macedonia, (ii) sample preparation for the analysis and (iii) instrumentation/apparatus analysis. The samples of few vegetables and soil have been collected from the Republic of Macedonia, strictly specified or to cover the whole territory. So, these regions/sites are Skopje, Veles, Ohrid and Kocani respectively in Macedonia. These entire samples have been collected during the time period of November to December 2012, in the amount of 1 kg of each sample. Soil has been taken at a depth of 10 cm below the surface of vegetables and also is being taken within a radius of 300 cm from the place on the ground. All samples were collected in transparent plastic bags, under the standard criteria for conducting pedagogical analysis, and were sealed in double bags and also took precaution from metal tools. Results are obtained out of the mean of 3 samples of each vegetable or plant sample, as well as from the soil.

Samples collected from Skopje (near cement Usje)

The *Daucus carota* (carrot), *Petroselinum crispum* (parsley), *Brassica oleracea* var. capitata var. alba (cabbage), *Brassica oleracea* (cauliflower) and *Alium porum* (leek) and also a representative sample of soil has been collected in December, 2012.

Samples collected from Skopje (Jurumleri)

We have collected the *Daucus carota* (carrot), *Beta vulgaris* var. vulgaris (beetroot), *Brassica oleracea* var. capitata var. alb (cabbage), *Petroselinum crispum* (parsley), *Alium cepa* (onion), *Brassica oleracea* (cauliflower), *Alium porum* (leek) along with a representative sample of soil in December, 2012.

Samples collected from Ohrid (road-side)

Here we have taken the *Daucus carota* (carrot), *Pastinaca sativa* (parsnips), *Brassica oleracea* var. capitata var. alba (cabbage), *Beta vulgaris* var. vulgaris (beetroot), *Brassica oleracea* (cauliflower) and *Alium porum* (leek), along with a representative sample of soil in November, 2012.

Samples collected from Kocani (road-side)

Daucus carota (carrot), *Petroselinum crispum* (parsley), *Brassica oleracea* var. capitata var. alba (cabbage), *Brassica oleracea* (cauliflower), *Alium cepa* (onion) and *Alium porum* (leek) and also a soil sample have been taken in November, 2012.

Samples taken in Veles (Basino village)

Daucus carota (carrot), *Petroselinum crispum* (parsley), *Brassica oleracea* var. capitata var. alba (cabbage), *Alium cepa* (onion) and *Brassica oleracea* (cauliflower) and a soil sample in December, 2012.

Sample preparation for analysis

The collected samples of vegetables have been washed twice under a strong jet of water, then again washed with de-ionized water and later on dried on the filter paper. After this, the samples have been ground in a blender. Finally, both the prepared samples of vegetables and soil were dried in a special type of drier at temperature 85°C until 10 to 12 hours in our laboratory. We have repeated this analysis three times with each sample collected from each site/region to get much more precision and less errors in our results. The same pattern of sample preparation has been followed by other works (Dilek and Aksoy 2006). After the sample preparations we followed next step to mineralization of vegetables and soil in a special Microwave Oven (MO) Sutjeska, Yugoslavia with Automatic pipettes FINNPIPETTE also with volume 5-40 µL, 40-200 µL and 200-l000 µL. The mixing of chemical reagents in samples has been done by Standard Glass Laboratory Inventory (SGLI) and that are reagents HNO_3 (69%)- Tracepur, MERCK, Darmstadt, Germany, H_2O_2 (28%-31%), Alkaloid-Skopje with maximum pressure of 75 bar, maximum temperature 300°C, strength 1000W, 15 mL quartz kivet and rotor with 6 bearings.

Mineralization of agriculture vegetables

First, the vegetable samples were cut in into small pieces and dried at 115°C for 15 hours. After that for mineralization, we took 1.0 gram of each vegetable / dried sample and placed it into a quartz kivet, then add 4 mL of HNO_3 (63%) and 1 mL of H_2O_2 and after that burned it into a microwave oven under the appropriate conditions (Table 1). Finally, the mineralization samples quantitatively transferred through a filter (Filtrak 388) to a 25 mL and then supplemented with de-ionized water to the mark.

Table 1 Analysis procedure for the mineralization of vegetables (samples) in microwave oven

Steps	Power (Watt)	Time (min)	Dryer	Pressure (bar)	Temperature (°C)
1	100-500	5.0	1	75	max 300
2	800	15.0	1	75	max 300
3	0	15.0	3	75	max 300

Table 2 Analysis procedure for the mineralization of soil (samples) in microwave oven

Steps	Power (Watt)	Time (min)	Dryer	Pressure (bar)	Temperature (°C)
1	700-1000	5.0	0	30	max 300
2	1000	15.0	0	30	max 300
3	0	15.0	3	30	max 300

Soil mineralization

Soil after drying is homogenized in a mortar and sieved through a sieve with square openings with page size of 2 mm. A weighted amount of 0.3 grams of sieved soil has been placed in quartz kivet and burned in a microwave oven with 4 mL HNO_3 (63%) and 1 mL H_2O_2. Burned samples are quantitatively transferred through a filter (Filtrak 388) to a 100 mL and supplemented with de-ionized water to the mark. Much more description in about the mineralization of the soil in the microwave oven under appropriate conditions has been given in (Table 2).

Instrumental analysis

We have done instrumentation analysis by using the most suitable technique inductively coupled plasma-optical emission spectroscopy (ICP-OES) and ICP mass spectrometry (ICP-MS) for our present research requirements. The ICP-OES (Gabriella 2010, Limmatvapirat 2012) is a good match with the productivity requirements of many laboratories and requires only a moderate investment. Toxic heavy metals were determined by “Perkin Elmer OPTIMA 2000DV”, Shelton, CT, USA, with ultrasonic sprayer “CETAC- Ultrasonic Nebulizer U5000AT+. For analytical quality assurance, after each six sample readings, standards were run to make sure that the approximate error in within limits of 7%. An analytical balance Mettler H10, (d= 0.1 mg) made from Germany and Homogenizer BLENDOR from New Hartford, Conn, USA were used. The wavelengths in nanometre for the toxic metals lead (Pb) and Cadmium (Cd), the metalloid Arsenic (As) and the trace element zinc (Zn) are found to be 220.35 nm, 214.44 nm, 188.98 nm and 206.20 nm, respectively. All statistical analyses were performed using Microsoft Excel 2007 and Statistic 6.0.

RESULTS AND DISCUSSION

All obtained values of allowable concentration of heavy metals, metalloid and trace element in a vegetable and soil, comparatively processed in line with standards prescribed by Regulations of the Republic of Macedonia (Anonymous 2012), also from world health organization and food and agriculture organization (FAO). The concentration of heavy metals Pb and Cd, and the concentration for metalloid Arsenic (As) as well as the concentration of and trace element zinc (Zn) in the soil for all above said regions revealed that the highest concentrations measured in all the samples of soil for lead Pb and Zn (Fig 1-5). The concentrations of the heavy metals, metalloid and trace element were determined by using inductively coupled plasma – optical emission spectroscopy (ICP-OES) methods in our laboratory at Bitola, R. Macedonia. Specified amounts of toxic heavy metals Pb and Cd, metalloid As and trace element Zn in the present analytical work of vegetables, taken from the Skopje region (the cement Usje and Jurumleri surrounding) (Table 3, 4). Also the concentration values for heavy metals Pb and Cd and metalloid As and also the trace element Zn in the vegetable samples obtained from other regions (Ohrid, Kocani, Veles,) are depicted in (Tables 5 - 7) and the description and/or outcomes of all these have been described in the following:

The findings of results (Table 3) has been shown for the vegetable samples in the region of Skopje. One can find that the maximum concentration of the toxic heavy metals in our present work for Pb is 1.261 mg/kg in leek; Cd is 1.027 mg/kg in carrot and metalloid As is 0.057 mg/kg in cabbage and the trace element Zn is 0.251 mg/kg in carrot (Bielińska and Agnieszka 2010, Osma *et al*. 2012). We are also reporting the minimum concentration of such metals/elements first time and these are in such orders; Pb is 0.031 mg/kg in parsley; Cd is 0.053 mg/kg in parsley, As is 0.012 mg/kg in parsley and Zn is 0.026 mg/kg in cauliflower. Finally, the average concentrations and standard deviation (S.D.) for all toxic heavy metals/elements also have been reported.

Table 3 Concentrations of heavy metals, metalloid and trace element (mg/kg) at Skopje (the cement Usje surroundings)

Vegetables samples		Concentrations in mg/kg			
Scientific name (Latin)	Local name (Macedonian)	Pb	Cd	As	Zn
Daucus carota	Carrot	0.093	(1.027)	0.022	(0.251)
Petroselinum crispum	Parsley	0.031*	0.053*	0.012*	0.157
Brassica oleracea var. Capitata var. alba	Cabbage	0.061	0.142	(0.057)	0.132
Brassica oleracea	Cauliflower	0.037	0.212	0.019	0.026*
Alium porum	Leek	(1.261)	0.073	0.019	0.151
Average concentration	Over all samples	0.296	0.301	0.025	0.143
Standard deviation (S.D.)	Over all samples	0.539	0.410	0.017	0.080

Date in parenthesis indicate maximum concentration (mg/kg) of metals/elements to their respective samples
*Minimum concentration (mg/kg) of metals/elements to their respective samples

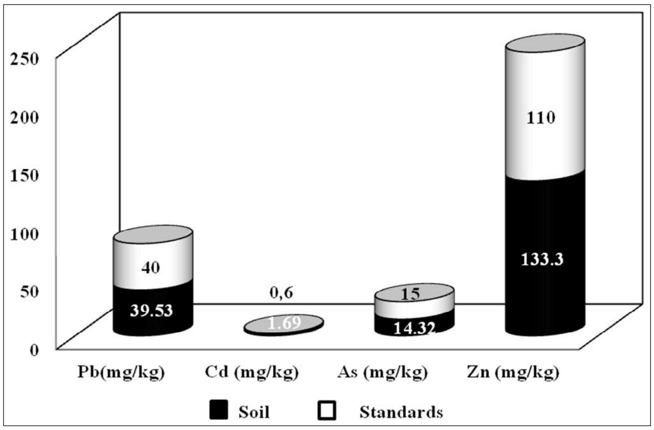

Fig 1 Obtained values of heavy metals, metalloid and trace element: Pb, Cd, As and Zn in samples of soil in Skopje (the cement Usje surrounding)

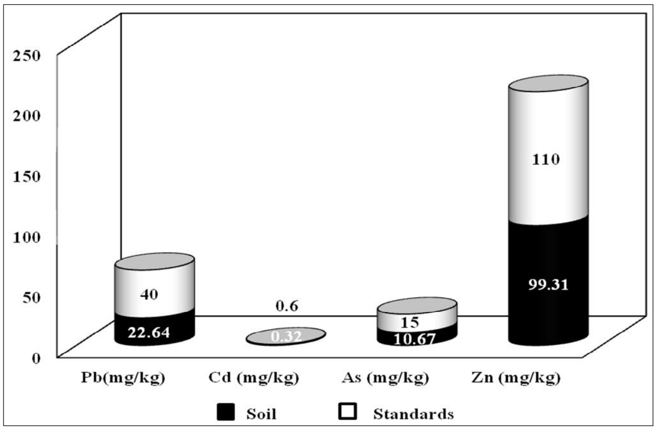

Fig 2 Obtained values of heavy metals, metalloid and trace element: Pb, Cd, As and Zn in samples of soil in (Jurumleri)

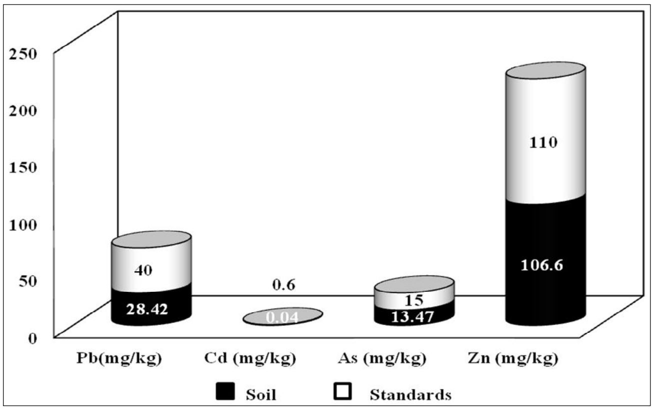

Fig 3 Obtained values of heavy metals, metalloid and trace element: Pb, Cd, As and Zn in samples of soil in Ohrid

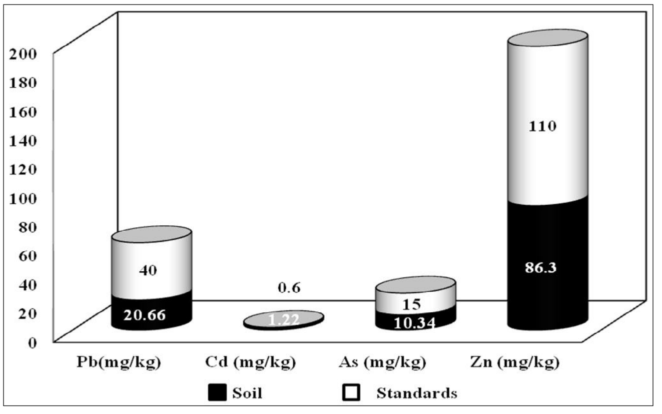

Fig 4 Obtained values of heavy metals, metalloid and trace element: Pb, Cd, As and Zn in samples of soil in Kocani

The concentrations of toxic heavy metals, metalloid and trace element in the region of Skopje (Jurumleri) revealed that the maximum concentration of the toxic heavy metals, metalloid and trace element are found such as: Pb is 0.078 mg/kg in onion; Cd is 0.029 mg/kg in carrot, As is 0.021 mg/kg in parsley and Zn is 0.133 mg/kg in beets (Table 4). These results represented a good agreement with the results find by other researchers in the same field (Bielińska and Agnieszka 2010, Osma *et al.* 2012a). The minimum concentrations of such metals/elements are also evaluated and in such orders: Pb is 0.023 mg/kg in cauliflower; Cd is 0.013 mg/kg in cauliflower, As is 0.012 mg/kg in two samples (onion and leek) and Zn is 0.009 mg/kg in carrot. Finally, the average concentrations and standard deviation for all toxic heavy metals/elements also have been reported (Suruchi and Khanna 2011).

Table 4 Concentrations of heavy metals, metalloid and trace element (in mg/kg) in Skopje (Jurumleri)

Vegetables samples		Concentrations in mg/kg			
Scientific name (Latin)	Local name (Macedonian)	Pb	Cd	As	Zn
Daucus carota	Carrot	0.039	(0.029)	0.015	0.009*
Beta vulgaris var. vulgaris	Beets	0.030	0.016	0.014	(0.133)
Brassica oleracea var. Capitata var. alba	Cabbage	0.025	0.019	0.019	0.011
Petroselinum crispum	Parsley	0.036	0.027	(0.021)	0.061
Alium cepa	Onion	(0.078)	0.015	0.012*	0.131
Brassica oleracea	Cauliflower	0.023*	0.013*	0.015	0.121
Alium porum	Leek	0.033	0.013	0.012*	0.086
Average concentration	Over all samples	0.037	0.018	0.015	0.078
Standard deviation (S.D.)	Over all samples	0.018	0.006	0.003	0.053

Date in parenthesis indicate maximum concentration (mg/kg) of metals/elements to their respective samples
*Minimum concentration (mg/kg) of metals/elements to their respective samples

Similarly, we have described the concentrations of toxic heavy metals, metalloid and trace element for all vegetable samples including with the maximum, minimum concentration values of Pb, Cd, As and Zn for three different regions Ohrid, Kocani and Veles (Basino village) respectively (Table 5-7). On the basis of the present study, we only can say that the findings of our results are worthy due to the point of health safety for all human beings. The agricultural vegetables obtained the heavy metals from the soils at high concentrations may result in a great health risk taking in reflection food-chain implications. Consumption of agricultural vegetables contaminated with heavy metals is a major food chain route of human exposure. The agricultural vegetables whose examination system is based on extensive and incessant cultivation have enormous competence of extracting elements from the soils. The cultivation of such agricultural vegetables in contaminated soil represents a hazard since the vegetal tissues can accumulate heavy metals (Jordao *et al.* 2006, Singh and Kalamdhah 2011). Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues (Singh and Kalamdhah 2011, Sobha *et al*. 2007). Chronic level ingestion of toxic metals has undesirable impacts on humans and the associated harmful impacts become perceptible only after several years of exposure (Singh and Kalamdhah 2011, Khan *et al*. 2008).

Table 5 Concentrations of heavy metals, metalloid and trace element (in mg/kg) in the city Ohrid

Vegetables samples		Concentrations in mg/kg			
Scientific name (Latin)	Local name (Macedonian)	Pb	Cd	As	Zn
Daucus carota	Carrot	(0.093)	0.022	0.023	0.040*
Pastinaca sativa	Parsnips	0.032*	0.013	0.033	0.085
Brassica oleracea var. Capitata var. alba	Cabbage	0.055	0.010*	0.012	0.060
Beta vulgaris var. vulgaris	Beets	0.081	0.014	(0.066)	0.052
Brassica oleracea	Cauliflower	0.041	0.016	0.019	(0.095)
Alium porum	Leek	0.063	(0.091)	0.011*	0.065
Average concentration	Over all samples	0.060	0.027	0.027	0.066
Standard deviation (S.D.)	Over all samples	0.023	0.031	0.020	0.020

Date in parenthesis indicate maximum concentration (mg/kg) of metals/elements to their respective samples
*Minimum concentration (mg/kg) of metals/elements to their respective samples

Table 6 Concentrations of heavy metals, metalloid and trace element (in mg/kg) in the city Kocani

Vegetables samples		Concentrations in mg/kg			
Scientific name (Latin)	Local name (Macedonian)	Pb	Cd	As	Zn
Daucus Carota	Carrot	0.088	0.011*	0.045	0.112*
Petroselinum crispum	Parsley	0.036	0.016	0.031	0.131
Brassica oleracea var. Capitata var. alba	Cabbage	0.025*	0.016	0.042	0.117
Brassica oleracea	Cauliflower	0.046	0.021	0.029	0.121
Alium cepa	Onion	(1.052)	0.011	0.027*	(0.213)
Alium porum	Leek	0.621	(0.058)	(0.057)	0.145
Average concentration	Over all samples	0.311	0.022	0.038	0.139
Standard deviation (S.D.)	Over all samples	0.429	0.017	0.011	0.037

Date in parenthesis indicate maximum concentration (mg/kg) of metals/elements to their respective samples
*Minimum concentration (mg/kg) of metals/elements to their respective samples

Table 7 Concentrations of heavy metals, metalloid and trace element (in mg/kg) in Veles (Basino village)

Vegetables samples		Concentrations in mg/kg			
Scientific name (Latin)	Local name (Macedonian)	Pb	Cd	As	Zn
Daucus carota	Carrot	0.240	(0.192)	0.033	0.131*
Petroselinum crispum	Parsley	0.155	0.082	0.031	0.231
Brassica oleracea var. Capitata var. alba	Cabbage	0.592	0.041	0.029	0.252
Brassica oleracea	Cauliflower	0.530	0.026	0.032	0.232
Alium cepa	Onion	(1.222)	0.019*	(0.228)	0.312
Alium porum	Leek	0.126*	0.052	0.023*	(0.319)
Average concentration	Over all samples	0.477	0.068	0.062	0.246
Standard deviation (S.D.)	Over all samples	0.413	0.064	0.081	0.068

Date in parenthesis indicate maximum concentration (mg/kg) of metals/elements to their respective samples
*Minimum concentration (mg/kg) of metals/elements to their respective samples

Further, we have performed a statistical analysis so called ANOVA variance; a Turkey's test to determine the multiple statistical comparisons in our results. This analysis were used to evaluate heavy metals mean differences between the sites/regions and between the samples of soil and vegetables and already has been tested and proved by various workers (Afshin and Farid 2007). In the present work, the statistical variance or ANOVA test was used to examine statistically significant differences in the mean concentrations of heavy metals between the groups of soils and vegetables. It is observed that variables of different regions showed no such significant variation between the groups of sites/regions and between the samples of soil and vegetables in the Republic of Macedonia. In general, our results indicated that all studied heavy metals concentrations were significantly lower ($p > 0.05$, two ways ANOVA test). Similar work has been drawn by Afshin and Farid (2007), Madejon *et al.* (2013), for the monitoring of heavy metals in vegetables and soil.

It is very important to display a conclusion whether there is some correlation between the concentration of heavy metals in vegetable and soil in the same regions of the country. The given results show that we have a positive coefficient and medium correlation for Pb, Cd and As heavy metals, whereas there is a strong correlation for Zn which is depicted in Table 9. Similar results have been obtained by others Lars (2003), Dospatliev *et al.* (2012).

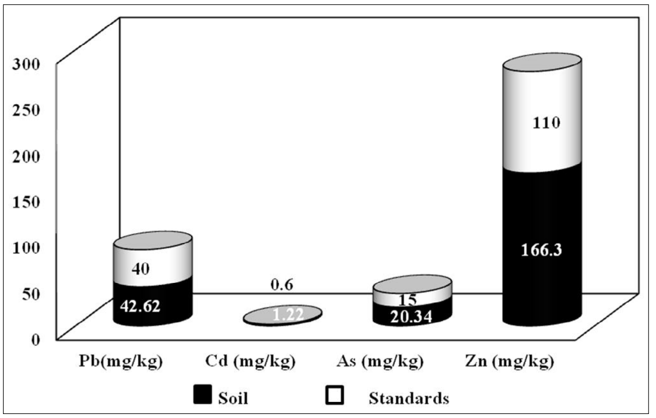

Fig 5 Obtained values of heavy metals, metalloid and trace element: Pb, Cd, As and Zn in samples of soil in Veles (Basino village)

Finally, the studied average values of heavy elements have been found on the order of (Pb-0.478 mg/kg) in Basino village >(Cd-0.301 mg/kg) in Usje >(As-0.063 mg/kg) in Basino village >(Zn-0.246 mg/kg) in Basino village (Ehsanul *et al*. 2012, Chutima *et al*. 2012, Etem *et al*. 2012).

Table 8 ANOVA analysis for concentration of heavy metals between the regions

Source of variations	S S	D. F	M. S	F	P-Values	F crit
Between Groups	0.105	4	0.026	1.758	0.190*	3.056
Within Groups	0.225	15	0.015	-	-	-
Total	0.330	19	-	-	-	-

*($p > 0.05$)
S S: type III sum square, D. F: degree of freedom, M. S: Mean Square, F: test in one way analysis of variance and F crit: F-test critical value of extracted from F-distribution

Table 9 Correlation coefficients between heavy metals concentration in soil and in vegetables

Heavy metals concentration in vegetable and soil	Correlation coefficients values
Correlation coefficients of Pb (mg/kg)	0.635*
Correlation coefficients of Cd (mg/kg)	0.699*
Correlation coefficients of As (mg/kg)	0.780*
Correlation coefficients of Zn (mg/kg)	0.802**

*represented to medium correlation
**represented to strong correlation

The concentration of the accumulation level of the studied elements in both targets for this experimental research was: Pb >Zn >Cd >As. As an outcome of the research, using the inductively coupled plasma – optical emission spectroscopy, the agricultural region of Veles (Bashino village) and Skopje region (surrounding the cement Usje) are polluted. Expected results confirmed data obtained that require a serious approach for making further eco-monitoring (Anita *et al.* 2007, Pragati and Simerjit 2012) for this precision regions as well as on a national level.

Heavy metals effects are not limited up to the soil, vegetables, and human health, but also affect the fertilizing process by changing biodiversity. So, on the basis of the above experimental work, can be drawn the following conclusions. Heavy metals contained in manure can change the physical, chemical and biological properties of soil. Agricultural vegetables/crops, that have received these heavy metals from such soil of that regions / sites, may reduce the capacity of soil productivity. Heavy metals obtained via consumption of agricultural vegetables, causing both, human health and environment concerns through the food chain. Heavy metals contained in agricultural runoff, enter in the aquatic environment (get into the water), and do damage to crops and aquatic animals. Therefore, if the fertilizer has to be applied in agriculture it should be free from the heavy metals. The high levels of toxic heavy metals, metalloid and the trace element took place in the consumer's area that might become health risk with time. Therefore, we recommended an urgent step/attempt for precautions.

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