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ESTIMATION OF HEAVY METAL LEVELS IN GREEN LEAFY VEGETABLES PURCHASED FROM SUCEAVA

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Abstract: *In the present study the levels of five important heavy metals were identified in vegetable samples purchased from Suceava local markets. The concentrations of Cadmium (Cd), Lead (Pb), Iron (Fe), Zinc (Zn) and Copper (Cu) were analysed using a mass spectrometer with inductively coupled plasma (ICP-MS) from the Instrumental Analysis Laboratory within the Faculty of Food Engineering Suceava. The mean levels of heavy metals examined in spinach (Spinacia oleracea), ramsons (Allium ursinum), lettuce (Lactuca sativa), orache (Atriplex hortensis) and nettle (Urtica dioica) were found to be in the order: Fe (13.52 µg/g) > Cu (4.83 µg/g) > Zn (3.623 µg/g) > Cd (1.890 µg/g)> Pb (0.290 µg/g). The highest concentration of heavy metal was identified in the case of Fe (51.333 µg/g in ramsons), whereas the lowest amount was identified for Pb (0.227 µg/g orache). The estimated daily intake for Cd is above 60 µg/kg b.w./day. The levels of the other metals are lower than the safe limits predicted by the FAO/WHO.*

Keywords: heavy metals, green leafy vegetables, daily intake

1. Introduction

Green leafy vegetables are an important ingredient of human diet that contains essential nutrients like vitamins, minerals, dietary fiber and anti oxidants [1].
Leaves from different plant species such as perennial and annuals are consumed especially in rural areas and there has been observed an increased trend of the consumption among the urban community. Green leafy vegetables are an economic source to ensure the micronutrient intake.
Rapid industrialization and the use of natural resources have increased the accumulation of toxic substances like heavy metals in the soil. Heavy metal contamination is a major environmental problem because they are harmful to humans [2]. Plants accumulate these toxic substances in their edible parts [3]. Zn, Cu, and Fe are essential for various biological activities within human body, but elevated concentrations can have bad consequences on people [4, 5].
Pb and Cd are non essential and toxic elements associated with many chronic diseases [6, 7]. This study aimed the determination of the concentration of Cd, Pb, Fe, Zn, and Cu in five different types of green leafy vegetables collected randomly from two markets sites located in Suceava in order to estimate daily intake of heavy metals through consumption.

2. Materials and Methods

2.1. Materials

Green leafy vegetables were purchased from two markets sites from Suceava city. The vegetables were spinach (*Spinacia oleracea*), ramsons (*Allium ursinum*),

lettuce (*Lactuca sativa*), orache (*Atriplex hortensis*) and nettle (*Urtica dioica*), collected in clean polyethylene bags and brought to the laboratory for the analysis.
Green leafy vegetables samples were washed with distilled water to remove soil and dirt.

2.2. Methods

Moisture determination in samples was performed according to the European Standard EN ISO 665/2000 by the drying process in a drying chamber at the temperature of 103 °C [8].
Total ash composition was determined by calcinations of 5g of sample at 600 °C for 240 min (SR ISO 763: 2008) [9].
For the determination of heavy metal concentration 3 grams of green leafy vegetables were mineralized in an electric furnace at a temperature of 600°C, for 6 hours. The resulted ash was transferred into a 25 mL volumetric flask, where it was dissolved by adding a mixture of nitric acid 65% and deionized water. All solutions were prepared with reagent grade chemicals and ultra-pure water (18 MΩ cm). Nitric acid was purchased from Sigma Aldrich. The determination of five heavy metals was performed in a mass spectrometer with inductively coupled plasma (ICP-MS) Agilent Technologies 7500 Series (Agilent, USA).

Calculation of results

Concentration (C) of the heavy metals in samples is expressed in *µg/g* sample and is calculated using the formula (eq. 1):

$$C = a * \frac{V}{m} \quad (1)$$

where:
a - concentration value measured by the device, [ppb];
V - volume of acid dissolving the sample, [ml];
m - mass of sample mineralized, [g].

2.3. Data analysis

All analyses were carried out in triplicates with replication. The statistical data processing was done by the program XLSTAT 2013.

2.4. Daily intake of heavy metals from vegetables

Daily intake of heavy metals was calculated according to the equation 2 [10]:

$$DIM = DVC \times VMC \quad (2)$$

where:
DIM = daily intake of metals;
DVC = daily vegetable consumption;
VMC = mean vegetable metal concentrations (mg/day, fresh weight).
Daily vegetable consumption was 98 g of vegetables per person per day as set by FAO/WHO (1999), for heavy metal intake based on body weight for an average adult (60 kg body weight) [11].

3. Results and discussion

Samples were always analyzed in triplicates. Moisture and ash content of the samples are shown in Figure 1.
From the data presented we can observe that lettuce has recorded the highest moisture and ash content (94.44%, respectively 2.82%). The lowest level of moisture and ash content was obtained in the case of nettle (86.81%, respectively 2.44%). The metal content from studied vegetables from the market sites of Suceava are listed in Table 1. Among the five leafy vegetables studied, spinach recorded highest concentrations of Cu and Zn which are 7.50 µg/g and 4.233 µg/g, respectively. Ramsons recorded highest level of Fe which is 59.333 µg/g, lettuce recorded highest level of Pb, 0.318 µg/g and spinach the highest concentration of Cd (2.117 µg/g). Similarly, orache recorded the lowest mean levels of Pb (0.227 µg/g) and Zn (2.667 µg/g).
The mean levels of the metals examined in nettle, orache, lettuce, ramsons and spinach samples were found to be in the order: Fe

> Cu > Zn > Cd > Pb. Regarding plant ability to retain Pb we can notice that the lowest level was obtained in the case of orache, followed by nettle, ramsons, spinach and lettuce. In the case of Cd retention the hierarchy in increasing order is as follows: orache, ramsons, lettuce, nettle and spinach. These trends suggest that nettle, orache, lettuce, ramsons and spinach have a high retention capacity for Fe followed by Cu and then Zn, Cd and Pb.

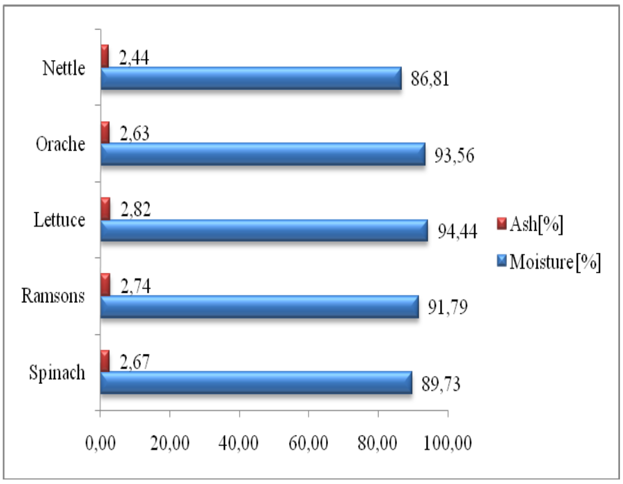

Fig. 1 Moisture and ash content of green leafy vegetables

Table 1

Concentrations of heavy metals (µg/g) in green leafy vegetables

Plant sample	Pb	Cd	Fe	Zn	Cu
Nettle	0.300 ± 0.0632	2.083 ± 0.0753	2.283 ±0.0983	3.767 ±0.0516	3.550 ±0.0837
Orache	0.227 ±0.0137	1.550 ±0.1049	1.433 ±0.1033	2.667 ±0.0816	7.367 ±0.0816
Lettuce	0.318 ±0.0117	2.050 ±0.1049	2.400 ±0.1414	4.033 ±0.1862	3.000 ±0.1414
Ramsons	0.302 ±0.7091	1.650 ±0.9316	59.333 ±1.7512	3.417 ±0.3793	2.733 ±1.1009
Spinach	0.302 ±0.0643	2.117 ±0.3189	2.150 ±0.2429	4.233 ±0.2422	7.500±0.2757
Mean ± SD	0.290 ±0.1724	1.890 ±0.3071	13.520 ±0.4674	3.623 ±0.1882	4.830 ±0.3367
Range	0.227-0.381	1.550-2.117	1.433-59.333	2.667-4.233	2.733-7.500
FAO/WHO Safe limit (2001) [a]	0.300	0.2	425.00	99.40	73.00

SD = standard deviation; source: a = Adu et al. (2012) [11]

The mean concentrations in increasing order of the metals in spinach are found to be in the following order: Pb > Cd > Fe > Zn > Cu. This indicates that spinach has a high retention capacity for Pb followed by Cd, Fe and then Zn and Cu. It could be observed that majority of studied vegetables have higher concentration of toxic elements (Pb and Cd) than Cu, Zn and Fe.

This study showed that, in the plants, Pb content exceeded the permissible limit in the case of lettuce, ramsons and spinach. Pb is a toxic element for the plants and human body and high levels can be attributed to pollutants from irrigation water, farm soil and traffic [12]. Cd levels detected in all vegetable samples examined in this study were above safe limits. In the case of spinach determined values for all the heavy metals were bellow the results obtained in other studies [13, 14]. Related health risks are usually expressed as provisional tolerance daily intake. FAO/WHO (1999) have set a limit for the heavy metal intake based on the body weight for an average adult, 60 kg body weight. The average diet per person per day of vegetables is 98 g.

If the mean levels of Pb (0.290 µg/g), Cd (1.890 µg/g), Fe (13.520 µg/g), Zn (3.623 µg/g) and Cu (4.830 µg/g) found in this study are consumed daily, the contribution of heavy metal intake for an average adult from the vegetable diets were calculated and presented in Table 2.

It can be concluded that our estimated daily intakes for heavy metals studied are above those reported by the FAO/WHO in the case of zinc and copper, which had set a limit for heavy metal intake based on body weight for an average adult (60 kg body weight) for Pb, Cd, Zn and Cu.

Table 2

Estimation of heavy metal intake through consumption of green leafy vegetables

Heavy metal	Mean conc. (µg/g)	Daily intake (µg/day)	WHO/FAO limit (µg)[b]
Pb	0.290	28.42	214
Cd	1.890	185.22	60
Fe	13.520	1324.96	-
Zn	3.623	355.054	60000 (60 mg)
Cu	4.830	473.34	3000 (3 mg)

Source: b = Elbagermi et al. (2012) [15]

Principal Component Analysis was carried out according to the mineral concentrations, moisture content and ash in different samples green leafy vegetables and to determine the significant differences among vegetables. The correlation value of the parameters measured in selected green leafy vegetables is presented in Table 3. The highest negatively correlation has been observed in the case of total moisture with the level of Cd (r = -0.488) this can be explained as follows: when the product moisture content is low, the content of Cd increases.

Table 3

Correlation matrix for the variables measured in green leafy vegetables

Variables	Pb	Cd	Fe	Zn	Cu	Moisture (%)	Ash (%)
Pb	1	0.721	0.201	0.883	-0.634	-0.252	0.257
Cd	0.721	1	-0.488	0.918	-0.115	-0.488	-0.155
Fe	0.201	-0.488	1	-0.174	-0.499	0.091	0.315
Zn	0.883	0.918	-0.174	1	-0.220	-0.340	0.149
Cu	-0.634	-0.115	-0.499	-0.220	1	0.017	-0.167
Moisture (%)	-0.252	-0.488	0.091	-0.340	0.017	1	0.824
Ash (%)	0.257	-0.155	0.315	0.149	-0.167	0.824	1

Significance level alpha=0,05

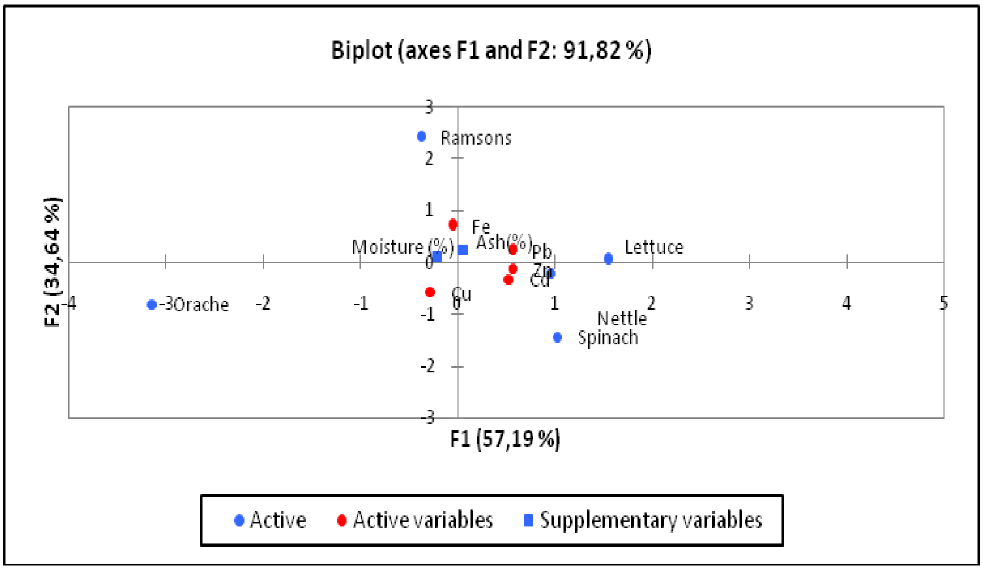

Fig. 2. Principal Component Analysis of samples

The influence of chemical composition of the main component analysis is presented in the Figure 2. Principal component 1 (PC1) explained 57.19% of variance, while component 2 (PC2) explains 34.64% of variation, the overall percentage of variation of the two main components being 91.82%. PCA grouped the studied parameters according to their chemical composition. In the first quadrant there are ramsons, in the second orache, in the third there are grouped nettle and spinach and in the fourth lettuce.

4. Conclusions

The results reported confirm that leafy green vegetables purchased from the market sites of Suceava contained substantial amounts of heavy metals. Levels of the metals are found to be below the safe limits prescribed by the FAO/WHO. These are important results as heavy metal intake through the consumption of vegetables may possibly cause numerous health hazards in people.

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