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CHEMICAL ANALYSIS OF HEAVY METALS IN LEAF OF GREEN SALAD AND ASSESSMENT OF CONTAMINATION

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ABSTRACT
The paper presents the results of an analysis of the content of heavy metals (Pb, Cd, Zn, Ni, Cu and Fe) in a leaf of green salad from six locations which gravitate towards the city of Zenica. After sampling from the defined locations, samples of green salad were prepared for analysis in the laboratory of the Faculty of Metallurgy, by dry digestion procedure. Analysis of heavy metals was performed on device "Atomic Absorption Spectrophotometer, PerkinElmer AAnalyst 800", using two techniques, flame technique and technique of graphite cuvette (TGHA). The results show that the concentration of lead in leaf of green salad from one location exceeds the maximum allowable concentration (MDK) defined by the national regulations of BiH. Concentrations of other analyzed heavy metals in the leaf of green salad are below MDK at all defined locations.

1. INTRODUCTION
The green salad is from the family of Asteraceae and belongs to hyper accumulator species. It is a one-year-old herbaceous plant of Latin name Lactuca sativa. It has a well-developed root. As a hyperaccumulator species, green salad adopts heavy metals through roots from where they are further transported to the leafs depending on their contents in the soil. As there is no protection mechanism (tree), heavy metals are transported into the leafs where accumulate in certain concentrations. In addition to adoption from the land, heavy metals comes in leafs of green salad from the environment too. The adoption of heavy metals by plants can be active (metabolic) and passive (non-metabolic) [1]. Passive adoption is a diffusion of the ions from the outer solution into the endoderm of root and so are adopted the ions of lead, sulfur and other elements. Active adoption takes place with the help of metabolic energyand flows in the opposite direction to the chemical gradient of the concentration of elements, and in this way are adopted: cadmium, zinc, copper and some other metals [1]. Heavy metals are classified as essential trace elements in which they are counted Cu, Fe, Mn, Zn, Mo, Ni and potentially toxic or non-essential elements which includes those metals that are not biogenic and act only as toxic as Pb, Cd, Cr, Hg, and as As which belong to semimetals [2]. Lead is a heavy metal that comes to nature primarily through exhaust gases from the car. It's not know that lead has

any essential function in metabolism, although a lead in nature can be found in all plants. Lead is known as a major pollutant in the environment and an element toxic to plants [5]. Toxic effect of lead is reflected in processes such as photosynthesis, mitosis and water absorption.

Cadmium is an element with very toxic effects on plants, animals and humans. The natural content of cadmium in plants is between 0.05 and 0.20 mg/kg [3]. The greatest influence on cadmium input into a plant is pH soil and concentration of this metal in the soil, then the concentration of Zn in the soil,cation exchange complex of soil, content of organic matter in the soil,as well as the texture of the land. The use of artificial fertilizers and waste sludge is the primary reason for the increased concentration of cadmium in the soil over the last 20 to 30 years in Europe [4]. Cadmium is a metal very similar to zinc and zinc deficiency increases the intake of cadmium in plants.

Cadmium unlike as lead is very intensely absorbed through the roots and is transported to vegetative aboveground organs of plant [6]. General opinion is that the lead in its inorganic form poorly and slowly adopted and transmitted to the aboveground organs of plant, but organic compounds are very mobile and therefore often accumulate in reproductive organs of plants [6]. If concentrations of toxic heavy metals in plants are greater than the limit values, then they can exhibit different toxic effects, which depends on the level of concentration, the type of metal, the organism, its ontogenetic development phase and other ecological factors.

2. EXPERIMENTAL PART

The samples of green salads were collected in March, April and May 2017 from six locations that gravitate toward the city of Zenica. These are the following locations: Raspotočje, Janjići, Donja Broda, Dolača, Tetovo and Brist. Samples of green salads were first washed in water and then in distilled water. The washed samples were dried in air, then in oven at a temperature of 105 ˚C and after which they are a chipped by crushing. For the determination of metal content is weighed 1.000 g plant material and transferred to a platinum pot in which was carefully burned without flames. Carbonized residue was burned in a fired furnace. The samples were annealed for one hour at a temperature of 450 ˚C. After cooling, 5 cm^3 of distilled water and 2 cm^3 of concentrated nitric acid are added to the pot, and with gentle heating, the contents of the pot are converted into the solution. The heating was carried out until the solution became clear. The resulting solution is then quantitatively transferred to a volumetric flask of 25 cm^3, which upon cooling to room temperature, complemented with distilled water up to the mark and intermingled. For the purpose of testing heavy metals in the root and the list of green salads, six metals were analyzed: lead, cadmium, zinc, nickel, copper and iron. Analysis of heavy metals was performed on device "Atomic Absorption Spectrophotometer, PerkinElmer AAnalyst 800", using two techniques, flame technique and technique of graphite cuvette (TGHA). Zinc, copper, iron and nickel are analysed by flame technique, whereas the determination of content of lead and cadmium used the technique of graphite cuvette.

3. RESULTS AND DISCUSSION

The results of the tested heavy metals in the leaf of green salads are shown in Table 1, converted from units mg/dm^3 and $\mu g/dm^3$ to mg/kg, which is common for solid samples.

Table 1. The content of the tested heavy metals in the leaf of green salad

Location	Concentration of the elements, mg/kg					
	Pb	Cd	Zn	Ni	Cu	Fe
Raspotočje	-	0.089	51.050	0.050	2.975	274.75
Janjići	2.028	0.019	50.975	0.475	12.175	255.00
Donja Broda	0.045	0.075	49.800	0.600	1.500	314.00
Dolača	-	0.089	36.125	0.025	3.850	210.88
Tetovo	-	0.101	42.975	-	0.875	302.00
Brist	-	0.126	40.875	0.300	0.975	187.25

The highest content of lead was found in the leaf of green salads from the location Janjići and is 2.028 mg/kg. In other samples, except for the sample collected from the location Donja Broda, lead in the green salad list was not registered. According to the Ordinance on maximum allowed quantities for certain contaminants in food, „Službeni glasnik BiH“, number 37/09, maximum allowable quantity (MDK) of lead in the leaf of green salad is 0.3 mg/kg [7]. Due to the high exceeding MDK, with goal to determine entry of lead in leaf of green salad, lead analysis was carried out and in the root of green salad from the mentioned locations. The content of lead in the root of green salads was 0.18 mg/kg. Comparing the concentration of lead in the leaf and root of green salad can be concluded that the main reason for this high exceeding MDK for lead in the leaf of green salad could be contamination of the leaf of green salad from the atmosphere. The location is near the Zenica-Sarajevo highway, as well as the main road M17, so the contamination of the green salad with lead was most likely to came from exhaust gases of motor vehicles with gasoline engines. In support of this claim is report on the analysis of gasoline BMB 95 which is produced in the oil refinery "Brod", where it is stated that the concentration of lead in BMB 95 is less than 2.5 mg/dm^3 and that MDK is 5 mg/dm^3 [8].

The highest content of cadmium was found in the leaf of green salad from the location Brist and is 0.126 mg/kg. According to the Ordinance on maximum allowed quantities for certain contaminants in food, „Službeni glasnik BiH“, number 37/09, the maximum allowable quantity (MDK) of cadmium in the leaf of green salad is 0.2 mg/kg [7]. The results of the analysis shown in Table 1 show that none of the sample does not exceed MDK for cadmium.

The highest content of zinc was found in leaf of green salad from the location Raspotočje and is 51.050 mg/kg. The literature states that the average natural content of zinc in plants is 20-100 mg/kg of dry matter [9]. Harmful effects of zinc on plants occurring when the contents of the plants over 100 mg/kg dry matter. Based on the obtained results it can be concluded that the content of zinc in the leaf of green salad does not exceed the limit value in any locality. However, unlike lead and cadmium, zinc is an essential element. The necessity of Zn in the nutrition and his deficiency in humans has been recognized in 1963. During the past 50 years, it became apparent that zinc deficiency in humans is prevalent and that almost 40% of the world's population suffering of Zn deficiency [10]. The recommended daily intake for Zn is in ranges from 3 to 16 mg per day, depending on the age, gender, type of nutritions and other factors.

The highest content of nickel was found in leaf of green salad from the location Donja Broda and is 0.6 mg/kg. Natural value content of nickel in plants is 1-10 mg/kg [9]. Based on these results it can be concluded that the content of nickel in the leaf of green salad does not exceed natural value in any locality.

The highest content of copper was found in leaf of green salad from the location Janjići and amounts to 12.175 mg/kg. The natural content of copper in plants is in the range between 1 and 12 mg/kg dry matter [11]. The average content of copper in herbaceous plants is between 2 and 20 mg/kg [9]. Based on these results it can be concluded that the content of copper in the leaf of green salad does not exceed natural value in any locality.

The highest content of iron was found in leaf of green salad from the locality Donja Broda and is 314 mg/kg. The average content of iron in plants is 100 mg/kg and varies within wide limits of 50 to 3000 mg/kg [9]. Plants much more absorb iron from other heavy metals. This shows that the adoption and accumulation of certain metals in plants depends on the type of metal and its physiological functions [5].

4. CONCLUSION

Analyzing the content of lead, cadmium, zinc, nickel, copper and iron in a leaf of green salad from six locations which gravitate towards the city of Zenica, results show that the content of lead from the site Janjići exceeds MDK for almost seven times. Namely, the content of lead in the leaf of green salad from the site Janjići is 2.028 mg/kg and MDK for lead in green salad is 0.3 mg/kg. In other samples, except for samples collected from the locality Donja Broda, the lead in the leaf of green salad was not registered. The location "Janjići" is near the highway Zenica-Sarajevo, as well as the main road M17, so excessive contamination of the green salad with lead was most likely to occur through the exhaust gases of motor vehicles with gasoline engines. In support of this claim is report on the analysis of gasoline BMB 95 which is produced in the oil refinery "Brod", where it is stated that the concentration of lead in BMB 95 is less than 2.5 mg/dm^3 and that MDK is 5 mg/dm^3 [8]. Concentrations of other analyzed heavy metals in the leaf of green salad are below MDK at all defined locations.

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