Selenium and trace metal content in selected vegetables from Raša and Opatija towns, and their estimated daily intakes

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Diplomski rad
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SADRŽAJ SELENA I METALA U TRAGOVIMA U ODABRANOM POVRĆU S PODRUČJA RAŠE I OPATIJE TE NJIHOVI PROCIJENJENI DNEVNI UNOSI

Tina Zorić

Rad je izrađen: Institut Ruđer Bošković, Bijenička cesta 54, 10000 Zagreb, Republika Hrvatska

Sažetak: Nedostatak zakonodavnih mjera i naprednih tehnologija ima za posljedicu nepovoljne učinke rudarstva, izgaranja ugljena i neprikladnog odlaganja otpada po okoliš. Primjer je Raški visokosumporni selenozni ugljen. Ispitano je 12 uzoraka tla i 22 uzorka povrća s područja Raše, Krapna i Opatije. Potonja lokacija je odabrana kao područje koje predstavlja prirodne okolišne uvjete. Ukupne koncentracije elemenata u uzorcima određene su spektrometrijom masa visoke razlučivosti uz induktivno spregnutu plazmu nakon raščinjavanja u specijaliziranoj mikrovalnoj pećnici. Svi su uzorci analizirani na ukupne koncentracije 32 elementa (Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cs, Cu, Fe, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, S, Sb, Sc, Se, Sn, Sr, Ti, Tl, U, V, Zn). Rezultati analiza ukazuju na onečišćenje tla u Raši i Krapnu sljedećim elementima: Cd, Mo, Pb i Se. Na temelju dobivenih koncentracija elemenata u uzorcima povrća izračunat je kvocjent opasnosti za elemente: As, Cd, Cr, Cu, Fe, Mn, Mo, Ni, Se, Sn i Sr, te procjena dnevnih unosa za sljedeće elemente: As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Se i Zn u uzorcima povrća s područja Raše, Krapna i Opatije. Kvocjent opasnosti nije prešao 1 ni za jedan element uključen u izračun, dok je procijenjeni dnevni unos veći od maksimalnog dozvoljenog izračunat za sve elemente, osim Zn, na svim lokacijama. Rezultati pokazuju da su tlo i povrće na području Raše i Krapna opterećeni metalima i Se, no isto vrijedi i za Opatiju.

Ključne riječi: visokosumporni selenozni ugljen, povrće, kvocjent opasnosti, procijenjeni dnevni unos, Raša, Krapan, Opatija

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SELENIUM AND TRACE METAL CONTENT IN SELECTED VEGETABLES FROM RAŠA AND OPATIJA TOWNS, AND THEIR ESTIMATED DAILY INTAKES

Tina Zorić

Thesis completed in: Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia

Abstract: The lack of legislative measures and advanced technologies results in adverse effects of mining, coal combustion and inappropriate waste disposal on the environment. An example is high-sulphur seleniferous Raša coal. Total of 12 soil samples and 22 vegetable samples from the area of Raša, Krapan and Opatija, the latter used as an area representing natural environmental conditions, were examined. Total element concentrations in the samples were determined by HR-ICP mass spectrometry after digestion in a specialized microwave oven. All samples were analysed for total concentrations of 32 elements (Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cs, Cu, Fe, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, S, Sb, Sc, Se, Sn, Sr, Ti, Tl, U, V, and Zn). The obtained results showed that the soil of Krapan and Raša is contaminated with Cd, Mo, Pb and Se. Based on the measured element levels in vegetable samples, the hazard quotient for the elements: As, Cd, Cr, Cu, Fe, Mn, Mo, Ni, Se, Sn, and Sr, and the estimated daily intake for the following elements: As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Se, and Zn in vegetable samples from the area of Raša, Krapan and Opatija were calculated. The hazard quotient did not exceed 1 for any element included in the calculation, while the estimated daily intake higher than the maximum allowed was calculated for all elements, except Zn, at all locations. The results show that in the area of Raša and Krapan the soil and vegetables are contaminated with metals, but the same is true for Opatija.

Keywords: high-sulphur selenifeorus coal, vegetables, hazard quotient, estimated daily intake, Raša, Krapan, Opatija

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1. INTRODUCTION

Humans are constantly changing ecosystems, directly or indirectly affecting the living world. Antropogenic activities like traffic, agriculture, industry, and waste management are large contributors to soil contamination whereas energy production has the largest contribution in this regard. Namely, these activities often cause elevated levels of different types of pollutants in the environment, including the trace elements. Furthermore, some of these activities can also enhance the mobilization of trace metals due to which they pass through the soil, enter the groundwater, or by air enter the biota. The increasing intake of toxic substances is of particular concern because they accumulate in plant tissue and can enter the food chain.

The concentration of trace elements in plants depends on many factors, the concentration of trace elements in soil, soil pH values (Kabata-Pendias, 2001), but also on the species, growth stage, cultivar, organ of the plant, and most importantly on the environmental conditions (Stančić et al., 2016). Although environmental factors can have a detrimental effect on plants and cause different effects in multiple plant generations, some of the plants have developed mechanisms by which they select elements needed to maintain homeostasis or even eliminate toxic elements. Some plants also have the ability to accumulate high concentrations of metals. Such plants are called hyperaccumulators. They can live in soils enriched in metals due to both the natural or anthropogenic factors. Due to special adaptations to high concentrations of metals in tissues, such plants can be used in phytoremediation (Kabata-Pendias, 2001).

Many trace elements such as zinc (Zn), copper (Cu), iron (Fe), and nickel (Ni) are needed by plants because they are included in various processes required for the normal functioning. They participate in the process of photosynthesis, redox reactions, breathing, expression, and regulation of genes and protein synthesis. But it is a fine line between utility and toxicity of some metals (Stančić et al., 2016). The problem appears when elevated concentrations of heavy metals are found in plants used for nutrition, e.g. vegetables. To avoid excessive build-up of metals in food chains, it is very important to monitor their concentrations in such plants. Croatian law sets maximum permitted values for heavy metals in soil and food (http://www.propisi.hr ; http://narodne-novine.nn.hr). In order to make the land favourable for the production of healthy food, the ordinance defines substances that above certain levels can be considered contaminants of agricultural land. Accordingly, the maximum values are established for the following metals: cadmium (Cd), chromium (Cr), copper (Cu), mercury

(Hg), nickel (Ni), lead (Pb), zinc (Zn), molybdenum (Mo), arsenic (As) and cobalt (Co) while for foodstuffs maximum values are prescribed only for lead (Pb), arsenic (As), iron (Fe) and nickel (Ni). However, to assess the impact of consumption of certain agricultural products on humans, more potentially toxic elements need to be measured. For instance, selenium is an essential element but potentially also very dangerous (Reilly, 1996).

Centuries of mining activity had been present in the area of the town Labin and Istrian coal mines and are considered the economic generator of this region from the beginning of the 20th century to the early 70s (Matošević, 2011). For decades, coal from this area was used to obtain energy in local households but also in industry in both Croatia and Italy. Coal combustion and associated industry have undoubtedly left a negative impact on the environment, visible not only in elevated metal concentrations in the local soil (Medunić et al.,2018a), but also in biota and local watercourses (Medunić et al., 2018b, 2018c).

Coal and metal ore mining, with the accompanying industry of combustion and metal processing, have adverse effects on surrounding water, soil, people and ecosystems in general (Oreščanin et al., 2009; Ćujić et al., 2014; Hower et al., 2005; Kumar et al., 2015; Spadoni et al., 2014). However, coal is still one of the essential sources of energy and it is mostly used as a major source of energy in Germany, Russia, China, USA and India (Verma et al., 2015). Thermal power plants that use coal are the major polluters of the environment. Study of Fiket et al. (2016) showed that TPP Plomin affected the local soil composition due to the long-term combustion of Raša coal. Studies in other parts of the world showed increased contaminations of sulphur (Chou, 2012), radionuclides (Clarke et al., 1992), polycyclic aromatic hydrocarbons (PAHs) (Mastral et al., 1996), mercury (White et al., 2009) and other metals in soil and aquatic ecosystems.

Soil is a multifunctional good with many important roles: ecological regulation, filtration of water, universal buffering, regulation of climate, as a source of biodiversity, and, most importantly, in supplying plants with water, air and nutrients, therefore enabling the production of organic compounds in the process of photosynthesis (Stančić et al, 2016). Heavy metals are a common occurrence in the environment and have resulted in human exposure for the entire history of mankind. However, anthropogenic activities such as mining have resulted in elevated levels of these contaminants in the environment (Kamunda et al., 2016).

Opatija town, although geographically located on the Istrian peninsula, does not belong to real Istria, but it is included in the Kvarner. Učka mountain is taken as a natural demarcation. Since there was no industry in this area and Opatija has been known as a tourist-recreational centre and health resort from the 19th century until today (Turk, 1996), this city is considered an area without significant anthropogenic influence.

The aim of this study is threefold: i) to determine the total concentrations of selenium and trace metals in vegetable samples from Raša, an area where elevated concentrations of some metals in the soil have been reported as a result of centuries of mining activity; ii) to compare them with those from Opatija town, an area avoid of significant anthropogenic influence; and iii) to assess the daily intake of selected trace elements due to consumption of vegetables grown in both described areas, and their potential impact on human health.

2. PREVIOUS GEOLOGICAL AND ENVIRONMENTAL RESEARCH

2.1. Coal mining in Raša

The history of the Labin region is imbued with mining, a key determinant of social, economic, and political activity for centuries. Namely, Labin basin is one of the six coal basins of the Istrian coal syncline (Medunić et al., 2018a). It is believed that a hundred million years ago Istria was a lagoon of rich flora and fauna, and from the organic material deposited at the end of the Cretaceous and Palaeocene layers of coal were formed (Fonović, 2000). Mining in the area of Labin began in 1420 in today´s municipality of Raša under the administration of Venice. The appearance of mines was of great importance in the poorly developed economic structure of this part of Istria. The immigration of miners from other parts of Austro-Hungarian Empire initiated the development of Krapan as a mining centre. After the fall of Austria, the commercial bank of Trieste took over the coal mine and, despite the general crisis, had a positive effect on its development. Changes began in 1935 when the state, wanting to take control of all energy production in the country, founded „Azienda Carboni Italiani", which also managed the Raša coal mines.

In order to make life easier for miners and to provide them with all the necessary services, such as medical care, accommodation and food, several cities were planned specifically for miners, and the city of Raša was built first. Until 1935 there was a barren swamp and an unorganised mouth of the river Raša. In the same year, reclamation interventions began, organized by the Italian Ministry of Agriculture and Forestry. The valley was drained and healed, and the riverbed of the river Raša was arranged. The new settlement soon became the municipality.

Due to Italy's accelerated preparations for World War II, coal exploitation increased. Although Liburnian lignite was not particularly expensive, the state successfully sold it to the railways and the army, and in the period from 1935 to 1940 the mine reached its peak of production. In addition to the former miners from the surrounding areas, miners from Sicily, Sardinia and Friuli were also employed. However, they were first to leave the country with the fall of Italy in 1943.

After the recovery of the economy following the Second World War, Raša had several more successful periods, but the mines were nevertheless gradually closed (Radović Mahečić, 2000; Medunić et al., 2015).

2.2. Raša coal

Raša coal is a superhigh-organic-sulphur (SHOS) coal. It is characterized by an unusually high amount of sulphur, largely present in the form of an anomalously high level of organic sulphur (S) (Medunić et al., 2018a). In addition, it is characterized by high concentrations of selenium (Se), uranium (U), vanadium (V) and molybdenum (Mo) (Medunić et al., 2019). High sulphur content in Raša coal is connected to the environmental conditions during the formation of Raša coal beds which were anaerobic and alkaline (Hamrla, 1960). Sulphur fixation was induced by the alkaline marine environment which fostered bacterial growth. Calcite and dolomite are major mineral phases in Raša coal (White et al., 1990). Sinninghe Damsté et al. (1999) identified the organosulfur compounds as well as the high abundance of polyaromatic sulphur compounds but also a very low abundance of lignin-derived compounds and low oxygen values which indicated that organic matter in Raša coal had not been predominantly derived from the higher terrestrial plants. Bauman and Horvat (1981) reported natural uranium ranges from 14.0 mg/kg to 100 mg/kg and Valković et al. (1984) reported the following element levels: Fe 3300 mg/kg, K 532 mg/kg, Sr 412 mg/kg, Ti 380 mg/kg, Mo 94 mg/kg, U 55 mg/kg, V 43 mg/kg, Se 43 mg/kg, Zn 41 mg/kg, Cu 25 mg/kg, As 25 mg/kg, Cr 23 mg/kg, Ni 23 mg/kg, S 13,05 mg/kg, Rb 13 mg/kg, Ca 1,8 mg/kg. Marović et al. (2004) discovered increased radioactivity of Raša coal. The radioactivity of ^{238}U was 500-1200 Bq/kg, 10-15 times higher than the average of other types of coal in the world. Stergašek et al. (1988) reported increased levels of S, Ca, U and V. They showed that Raša coal had 15.0% of ash, which was alkaline and distributed as 70:30 fly ash:bottom ash. Average composition of the Raša coal ash was CaO 64.2, SO_3 20.5, Fe_2O_3 4.59, MgO 3.79, Al_2O_3 3.27, SiO_2 1.90, Na_2O 1.42, K_2O 0.19. The main minerals in Raša coal are calcite ($CaCO_3$), gypsum ($CaSO_4$ x $2H_2O$) and dolomite ($CaMg(CO_3)_2$), while rozenite ($FeSO_4$ x $4H_2O$) and siderotil ($FeSO_4$ x $4H_2O$) are found in traces. Main mineral composition in ash were anhydrite ($CaSO_4$), aragonite and calcite, gypsum and bassanite ($CaSO_4 \times 0.5H_2O$) with small fractions of portlandite, calcite, anhydrite and ternesite ($Ca_5(SiO_4)_2SO_4$) were found (Peco, 2018).

2.3. Environmental impact

The environmental impact of Raša coal mining and combustion began to be explored at the end of the 20th century. The focus was on thermal power plant (TPP) Plomin, the only thermal power plant in Croatia at that time. Several papers have been published on the impact of Raša coal exploitation on flora. Komlenović and Pezdirc (1987) noticed mechanical damage of the needles and leaves of species of trees like Aleppo pine (*Pinus halepensis*), Austrian pine (*Pinus nigra*), flowering ash (*Fraxinus ornus*) and pubescent oak (*Quercus pubescens*). They compared concentrations of SO_2 in Austrian pines (*Pinus nigra*) in the area of the thermal power plant Plomin with those 22 kilometres away and registered higher concentrations in Austrian pines near TPP Plomin. The most damaged was the culture of *Pinus nigra* (Komlenović, 1989). Komlenović et al. continued research in 1990 when they reported elevated levels of sulphur in needles of *Pinus nigra* and elevated levels of sulphur and lead in the first five centimetres of the humus-accumulating soil horizon. Prohić et al. (1998) studied the distribution of selected elements in soils in the vicinity of the coal-burning power plant Plomin. They found the highest content of selenium and chromium in the soil around the power plant while the content of nickel, vanadium and manganese was only moderately elevated. Lead and zinc concentrations were present with the background values. Fiket et al. (2016) studied the distribution of rare earth elements (REE) in coal ash and slag as well as in soils sampled in the vicinity of coal-fired power plant Plomin. They reported highest REE levels in samples of recent ash, while concentrations of REE in soil samples decrease with distance from the TPP. Radić et al. (2018) demonstrated that Plomin soil extracts induced phytotoxic effects. Medunić et al. (2016a) shifted the focus of research from pollution caused by TPP Plomin to pollution caused by mining. They reviewed historical, geological, geochemical and environmental aspects of the Raša coal mines and generated waste. It was also shown that the soil in the area of Raša town is contaminated with sulphur, polycyclic aromatic hydrocarbons (PAHs), selenium and cadmium (Medunić et al. 2016b).

Medunić et al. (2018b) analysed sulphur, selenium, vanadium, uranium, mercury, strontium, cadmium, chromium, lead, copper, and zinc levels in Raša coal and imported coal, their bottom ash, seawater but also plant specimens collected in the Labin city area. According to ecological indices, the values of previously mentioned trace elements fell into the category of an extremely high level of soil pollution, while the values of selenium were found increased in both aquatic and herbal plant specimens. Elevated total selenium values in surface water were ascribed to polluted soil (Medunić et al., 2018b). Furthermore, selenium, sulfur, trace metals and BTEX (benzene, toluene, ethylbenzene and xylene) in soil, surface water, and lettuce in Raša Bay were investigated by Medunić et al. (2018a). Their results point to elevated levels of sulphur, selenium, vanadium, and uranium in soil, surface water, and lettuce. Medunić et al. (2018c) again reported total selenium, arsenic, cadmium, copper, chromium, mercury, lead, strontium, uranium, vanadium and zinc values in vegetables (lettuce, potato) but also in tissues of three non-migratory bird species (pigeon, jay, and black coot) from Raša Bay. Values of molybdenum, strontium, vanadium and uranium were found increased in the majority of water samples, soil, aquatic sediments and vegetables, while zinc, lead, copper and vanadium were found only slightly increased in liver samples of birds. Fiket et al. (2018) showed that, although some of the plants and factories closed decades ago in this area, the REE fingerprint of soils near such facilities reflects their influence, observable even to the present day. Moreover, they provided an insight into the processes influencing Raša coal formation, asserting that the formation of superhigh-organic-sulphur Raša coal was previously attributed to the seawater percolation but also influenced by hydrothermal solutions which led to sulphur enrichment and REE accumulation.

2.4. Metal intake in plants

Plants reveal various tendencies in the uptake of trace elements (Kabata-Pendias, 2001). There are two main pathways through which elements accumulate in plants: i) transfer from the soil through the root system and ii) atmospheric deposition. Their ratio, however, depends on the plant physiology (Ivanić et al., 2019). The main sources of trace elements in plants are their growth media and one of the most important factors that determine the biological availability of a trace element is its binding to soil constituents. Plants take up trace elements that are dissolved in the soil solutions in ionic, chelated, and complexed forms.

Although absorption by roots is the main pathway of trace elements to plants, absorption by other tissues has also been observed. The uptake of trace elements by plants is affected by pH, Eh, water regime, clay content, organic matter content, cation exchange capacity, the concentration of other trace elements, and nutrient balance. Climatic conditions are also shown to influence the rate of trace metal uptake which may be an indirect impact due to the water flow phenomenon (Kabata-Pendias, 2001). In general, a higher ambient temperature influences a greater uptake of trace elements by plants. Plant ability to take up chemical elements from growth media is evaluated by a ratio of element concentration in plants to element concentration in soils and it is called Biological Absorption Coefficient (BAC). It is also known as the Index of Bioaccumulation (IBA) or Transfer Factors (TF).

The absorption of trace elements by roots can be passive or nonmetabolic but also active or metabolic. In each case the rate of trace element uptake will positively correlate with its available pool at the root surface. Passive uptake includes the diffusion of ions from the external solution into the root endodermis. Active uptake requires metabolic energy and takes place against a chemical gradient. The absorption is controlled by metabolic processes within roots while the ion activity in the solution is believed to be one of the significant factors that influence plant uptake of ions, but only when the uptake is active. Mechanisms of uptake depends on the given element. For instance, lead and nickel are preferably absorbed passively, while copper, molybdenum, and zinc are preferably absorbed actively (Kabata-Pendias, 2001). When biological and structural properties of root cells are altered or when concentrations of elements pass over a threshold value for a physiological barrier, all elements are taken up passively. Roots and associated microorganisms produce various organic compounds that are very effective in releasing the trace elements from firmly fixed species in soil.

The trace elements most readily available to plants are those that are absorbed on clay minerals, while those fixed by oxides and bound onto microorganisms are much less readily available (Kabata-Pendias, 2001). The mechanisms of uptake of trace elements by roots involve several processes: i) cation exchange by roots, ii) transport inside cells by chelating agents or other carriers, and iii) rhizosphere effects. Root uptake is controlled by root exudates which are composed mainly of amino acids and vary with plant species and varieties, microorganism association, and conditions of plant growth. It is believed that cation oxidation states around roots are of great importance in these processes as well as changes in pH of the root ambient solution. Although the absorption of trace elements varies greatly among plant species, when compared on a large scale the index of bioaccumulation illustrates some general trends. Elements such as cadmium (Cd), boron (B), bromine (Br), caesium (Cs), and rubidium (Rb) are extremely easily taken up, while barium (Ba), titanium (Ti), zirconia

(Zr), scandium (Sc), bismuth (Bi), gallium (Ga), iron (Fe), and selenium (Se) are less available to plants. The bioavailability of trace elements from aerial sources through leaves may have a significant impact on plant contamination but it is also of practical importance in foliar applications of fertilizers, especially of elements such as iron, manganese, zinc, and copper. Foliar uptake is believed to consist of two phases: i) nonmetabolic cuticular penetration - the major route of entry, ii) metabolic mechanisms - element accumulation against a concentration gradient. Trace elements taken up by leaves can be translocated to other plant tissues including roots. The rate of trace element movement among tissues varies greatly, depending on the plant organ, its age and the element involved. Also, the morphology of the surface of leaves is an important factor governing foliar uptake of trace elements. Some plants like mosses and lichens are especially susceptible to absorb elements and some compounds from aerial sources. Those plants are very suitable for the phytoindication of atmospheric pollution.

The transport of ions within plant tissues and organs involves many processes like: i) movement in xylem, ii) movement in phloem, iii) storage, accumulation, and immobilization. The chelating ligands are most important in the control of cation translocation in plants. However, numerous other factors such as pH, the oxidation-reduction state, competing cations, polymerization, the formation of insoluble salts and hydrolysis govern metal mobility within plant tissues. The electrochemical variables of elements also play important role in the transport among plant organs. For example, silver (Ag), boron (B), lithium (Li), molybdenum (Mo), and selenium (Se) are easily transported from roots to above-earth parts, while manganese (Mn), nickel (Ni), cadmium (Cd), and zinc (Zn) are moderately mobile. On the other hand, cobalt (Co), copper (Cu), chromium (Cr), lead (Pb), mercury (Hg), and iron (Fe) are strongly bound in root cells. Long-distance transport in higher plants depends on the vascular tissues (xylem and phloem) and it is partly related to the transpiration intensity. Generally, the distribution and accumulation patterns of trace elements vary considerably for each element, growth season and the plant species (Kabata-Pendias, 2001).

2.5. ICP-MS multielement analysis in environmental research

In environmental research and management, trace elements have to be determined over a wide range of atomic number, down to very low concentrations and in quite different matrices. One of the commonly used techniques for this purpose is Inductively coupled plasma mass spectrometry (ICP-MS), a technique in which inductively coupled plasma is used as an ionization source, and detection is performed by mass spectrometry. High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS) uses a high-resolution mass spectrometer which, by combining the physical restriction of the ion beam by passing through a narrow slit of different dimensions and specific constructions of MS (double-focusing in electrostatic and magnetic fields) allows precise isotope determination and the use of three different resolutions (low, medium, and high). By selecting the appropriate resolution for each element, the maximum separation of the isotopes we want to measure from possible interferences is achieved. The instrument is characterized by high sensitivity, large linear range, and the possibility of simultaneous determination of more than 50 elements (multielement analysis). The instrument is usually equipped with an autosampler so that it has the ability to automate and measure a large number of samples.

3. TOXICITY OF THE ELEMENTS

Toxicity is the degree to which a chemical substance or a particular mixture of substances can damage an organism. All metals are persistent but not necessarily bioaccumulative and toxic like iron, bismuth, aluminum, manganese and zinc. Toxicity does not depend only on the element, but also on its valence and the form in which it is found in the environment. The biggest problem represents lead, mercury, and cadmium, but also antimony, cobalt, arsenic, beryllium, copper, manganese, molybdenum, nickel, selenium, vanadium, and zinc. The main anthropogenic sources of metals in the environment are mining, burning fossil fuels and industrial processes, while their natural sources are volcanoes and forest fires. They are emitted into the air mostly in the form of small particles and by their deposition (dry or wet), they get into the soil.

Trace metals are common soil constituents, but their elevated concentrations are most often the result of pollution. By the term trace metal we imply chemical elements present in trace concentrations (ppb range to less than 10 ppm). Their share in the crust does not exceed 1% (Siegel, 2002). In the soil, metals can bind to the absorption complex of the soil or be found in ionic form, and became accessible to plants (Kabata-Pendias, 2007).

3.1. Properties of potentially toxic elements present in soil

Potentially toxic elements in the environment occur mostly as a result of human action and consequently reach water bodies and soil by direct discharges or by evaporation into the atmosphere followed by precipitation and sedimentation. Because of such a biochemical cycle in the environment, we find different forms of metals with different physico-chemical properties, which are then reflected in their varying persistence and toxicity. They reach the body by inhalation, absorption through intestines and absorption through the skin, depending on the chemical form (Nordberg, 2007).

- **Arsenic (As)**

  Arsenic is distributed rather uniformly in major types of rocks and its common concentrations in most rocks range from 0.5 to 2.5 mg kg^{-1}. Only in argillaceous sediments is As, on the average, concentrated as high as 13 mg kg^{-1}. Significant anthropogenic sources of As are related to industrial activities (metal processing, chemical works based on S and P minerals, coal combustion, and geothermal power plants) and to the use of arsenical sprays, particularly in orchards. As is a constituent of most plants, but little is known about its biochemical role. Concentrations of As in plants grown on uncontaminated soils vary from 0.009 to 1.5 mg kg^{-1} DW, with leafy vegetables being in the upper range, and fruits in the lower range. Surface contamination with insecticides may increase the concentrations of arsenic in plants (Nordberg et al., 2007).

- **Cadmium (Cd)**

Among heavy metals, cadmium is considered the most harmful and toxic. It accumulates rapidly in crops, especially in acidic soils. Globally, the range of cadmium concentrations in soils ranges from 0.06 to 1.1 mg kg^{-1}. In slightly acidic soils there is a high risk of cadmium leaching into the ground and groundwater. Due to its similarity, it can easily replace zinc in various chemical compounds. It is especially dangerous that plants do not distinguish it from zinc, and so it enters the human food chain. Cadmium toxicity is associated with the displacement of calcium at a number of receptor sites in an organism. It also displaces calcium in some proteins. Sources of cadmium pollution can be fertilizers, lead and zinc mines and pesticides (Kabata-Pendias, 2007; Wright et al., 2002). According to the Ordinance on the protection of agricultural land from pollution by harmful substances in Croatia, soil is considered contaminated if it contains more than 2 mg kg^{-1} of cadmium (texturally heavy and heavy soils rich in humus) or 1 mg kg^{-1} soils, (skeletal soils and soils poor in humus). Municipal sludge and compost from municipal sludge and waste used on agricultural soil may contain up to 10 mg kg^{-1} of cadmium (http://narodne-novine.nn.hr). The range of cadmium concentrations in soil of coastal Croatia is 0.2 to 9.5 mg kg^{-1}, which is less than in the mountainous region. In contrast, twice the median value of 1.1 mg kg^{-1} indicates that most of the area of coastal Croatia is burdened with higher concentrations than average. Low concentrations of cadmium are characteristic of almost the whole of Istria, not only for areas covered with flysch deposits and are often lower than 0.4 mg kg^{-1}, and areas more exposed to cadmium are most likely caused by local pollution (Halamić et al., 2009).

- **Chromium (Cr)**

The mean chromium concentration in soils is 54 mg kg^{-1}, and the concentration is thought to be due to lithogenic concentrations of parent rocks. Since its concentration is related to the source rocks, soils bound to basic and ultrabasic rocks generally have elevated concentrations. Chromium concentrations are higher in soils that are richer in finer fractions (fine silt and clay) where natural concentrations can reach 1100 mg kg^{-1} (Kabata-Pendias, 2007). According to the Ordinance on the protection of agricultural land from pollution by harmful substances, the soil is considered contaminated if it contains more than 100 mg kg^{-1} of chromium (texturally heavy soils rich in humus) or 60 mg kg^{-1} (skeletal soils and soils poor in humus). Municipal sludge and compost from municipal sludge and waste used on agricultural soil may contain up to 500 mg kg^{-1} of chromium (http://narodne-novine.nn.hr). The range of chromium concentrations in the northern Pannonian part of Croatia ranges from 32 mg kg^{-1} to 524 mg kg^{-1}, and its mean value is 83 mg kg^{-1}. In Dalmatia, chromium concentrations range from 15 to 2200 mg kg^{-1} (mean 126 mg kg^{-1}). In the Coastal Croatia, high concentrations of chromium are limited to soils developed on flysch, Quaternary sediments and *terra rossa*, which may also have precipitated bauxite fragments (Miko et al., 2001).

- **Copper (Cu)**

Copper is an essential element, but depending on the concentration, it is also very toxic. It is found in soils in concentrations between 20 and 30 mg kg^{-1} (Kabata-Pendias, 2007). There are indications that even moderate soil pollution can cause acute poisoning in children due to the ingestion of the soil. Prolonged intake of more than 200 mg leads to various symptoms such as confusion, nausea, vomiting, low blood pressure, abnormal kidney function, and muscle pain (Wright et al., 2002). The range of copper concentrations in soils in the Republic of Croatia ranges from 5 to 248 mg kg^{-1} in the northern Pannonian part of Croatia, and from 6 to 923 mg kg^{-1} in Dalmatia. The increased concentrations are the result of agricultural activities, especially near vineyards due to the use of copper sulphate (Miko et al., 2001; Miko et al. 2003).

- **Iron (Fe)**

Fe is one of the major constituents of the lithosphere and comprises approximately 5%, being concentrated mainly in the mafic series of magmatic rocks. The mechanisms of Fe uptake and transport by plants have received much study because they are the key processes in the supply of Fe to plants. The metabolic functions of Fe in green plants are relatively well-understood, and Fe is considered the key metal in energy transformations needed for syntheses and other life processes of the cells. Edible parts of vegetables appear to contain fairly similar amounts of Fe, ranging from 29 to 130 mg kg^{-1} (DW), with lettuce being in the upper range and onion in the lower range (Kabata-Pendias, 2007). Too much of a good thing can be harmful, and this is exemplified by iron overload. Iron metabolism in humans is characterized by a limited external exchange and by an efficient reutilization from internal sources. There is no uniform agreement regarding the toxic dose (Nordberg et al., 2007).

- **Lead (Pb)**

The natural ranges of lead concentrations in soils are primarily due to lithological concentrations in parent rocks. Soils with a higher proportion of clay show the most common and elevated lead concentrations. The average total concentration of lead in soils is about 25 mg kg^{-1} (Kabata-Pendias, 2007). Lead is concentrated in the surface layer of the soil because it is deposited in the humus layer, and the iron horizon of laterite soils. Many studies have shown a correlation of lead with mercury and zinc, especially in urban environments. Lead is a toxic metal associated with emissions in urban areas and results in damage to almost all organs, especially affecting the central nervous system, kidneys and blood. Lead, like cadmium, in human organisms tends to replace calcium, so we find it in bones and teeth. Lead poisoning has a negative effect on the central nervous system, and children's exposure to lead, up to three years of age, results in a decrease in IQ (Wright et al., 2002). Coastal Croatia is most burdened by the concentration of lead in the soil. Although the concentrations in the Dinarides are in smaller ranges than in the northern part of the country and for coastal Croatia do not exceed 177 mg kg^{-1} (while the minimum is 10 mg kg^{-1}), the lead concentration is generally between 46 and 60 mg kg^{-1} (Halamić et al., 2009).

- **Manganese (Mn)**

Mn is one of the most abundant trace elements in the lithosphere, and its common range in rocks is 350 to 2000 mg kg^{-1}. Numerous studies have been carried out on Mn uptake by plants and on Mn distribution among plant tissues. All findings give ample evidence that Mn uptake is metabolically controlled, apparently in a way similar to that of other divalent cation species such as Mg^{2+} and Ca^{2+}. Concentrations of Mn in plants range from 1.3 to 1000 mg kg^{-1}. Generally, most plants are affected by Mn content around 500 ppm (DW) (Kabata-Pendias, 2007).

- **Molybdenum (Mo)**

The terrestrial abundance of Mo, estimated at 3 mg kg^{-1}, shows its association with granitic and other acid magmatic rocks. The common range of Mo in these rocks is 1 to 2 mg kg^{-1}, while in organic-rich argillaceous sediments, the Mo content may be above 2 mg kg^{-1}. The solubility, and thus the availability of Mo to plants, is highly governed by soil pH and drainage conditions. Mo from wet alkaline soils is the most easily taken up, but the geochemical processes involved in this phenomenon are not completely understood. Industrial pollution (mining, smelting, processing of metals, and oil refining) may be responsible for elevated Mo concentrations in soils. Plant foodstuffs contain variable amounts of Mo within the range from 0.07 to 1.75 mg kg^{-1} (DW). The ready availability of Mo causes a great increase in uptake when plants are grown in contaminated sites. Hornick et al. (1975) reported that plants grown on the Mo-polluted soil near a Mo processing plant accumulated this element in concentrations ranging from 124 to 1061 mg kg^{-1} (DW) in lettuce and cabbage, respectively (Kabata-Pendias, 2007).

- **Nickel (Ni)**

There is a general similarity between the distribution of Ni, Co, and Fe in the Earth's crust. Thus, Ni contents are highest in ultramafic rocks (1400 to 2000 mg kg^{-1}), and its concentrations decrease with increasing acidity of rocks down to 5 - 15 mg kg^{-1} in granites. Sedimentary rocks contain Ni in the range from 5 to 90 mg kg^{-1}, with the highest range being for argillaceous rocks and the lowest for sandstones. Geochemically, Ni is siderophilic and will join metallic Fe wherever such a phase occurs. The Ni status in soils is highly dependent on the Ni content of parent rocks.

However, the concentration of Ni in surface soils also reflects soil-forming processes and pollution. Soils throughout the world contain Ni within the broad range from 0.2 to 450 mg kg^{-1}. Ni recently has become a serious pollutant that is released in the emissions from metal processing operations and the increasing combustion of coal and oil. The application of sludges and certain phosphate fertilizers also may be important sources of Ni. Anthropogenic sources of Ni, from industrial activity in particular, have resulted in a significant increase in the Ni content of soils. Ni is easily extracted from soils by plants, especially by hyperaccumulator plants. The mean levels of Ni in grasses range from around 0.1 to 1.7 mg kg^{-1} (DW) and in clovers range from 1.2 to 2.7 mg kg^{-1} (DW). There is not much information on Ni in vegetables (Kabata-Pendias, 2007). IARC, the International Agency for Cancer Research, concluded in 1990 that nickel compounds were human carcinogens (Nordberg et al., 2007).

- **Selenium (Se)**

The average global concentration in soils is 0.33 mg kg^{-1}, while in sandy soils of northern Europe concentrations range from 0.2 to 0.3 mg kg^{-1} (Kabata-Pendias, 2007). Higher selenium concentrations occur in forest soils, while very high concentrations are present in wetlands. Too high selenium concentrations in soil can be harmful to plants and crops in the area. In the United States, moderate toxicity was found at 8 mg kg^{-1} and severe toxicity at 80 mg kg^{-1} in soil (Frankenberger, 1998; Fordyce, 2005). The mean Se contents of all food plants do not exceed 0.1 mg kg^{-1} (DW) (Kabata-Pendias, 2007). Selenium is an essential element (10 to 40 μg ml^{-1}), present in serum and urine (0.1 μg ml^{-1}) and toxic if it exceeds these concentrations. It is also thought to be an important agent in inhibiting cancer (Wright, 2002). It is a rare element and it is often accompanied with sulphur. When frying sulphides or burning sulphur, selenium is oxidized to SeO_2 and a high correlation with SO_2 emissions has been reported (Richter et al., 1998; Fordyce, 2005; Fan et al., 2007).

- **Strontium (Sr)**

Strontium is a lithophilic trace element, covered with calcium and barium, and in the igneous environment it is bound to calcium. It is not a biogenic element for most organisms. It replaces calcium, and in its presence is almost non-toxic. The element itself is non-toxic, but the Sr - isotope ^{90}Sr is very radiotoxic. The anthropogenic impact on the environment is less significant. Increased concentrations of strontium were recorded in the internal Istria (gray Istria) where its concentration is up to 588 mg kg^{-1}, and such increased concentrations are related to the spread of flysch zones (Halamić et al., 2009).

- **Sulphur (S)**

Sulphur is found in organic and inorganic form in the soil. Of the total amount of sulphur in soil (0.01 to 0.25 %), 80 to 90 % is in organic form, and 10 to 20 % is in mineral form. The mineral form of sulphur is represented by sulphur in minerals, in easily soluble and sparingly soluble salts, adsorbed sulphur and sulphur in aqueous soil solution. The amount of sulphur in the soil depends on the balance between immobilized soluble sulphur and organic mineralization sulphur fractions. Moisture, pH reaction of soil, presence of vegetation cover, agricultural production and especially microbiological activity and diversity are factors influencing mobilization and mineralization of sulphur in organic matter. Sulphur is found in nature in different oxidation states between -2 and +6, where HS is the most reduced form, while SO_4^{2-} is the most oxidized form of sulphur. Hydrogen sulphide (H_2S) is the most stable form (Lucheta et al., 2012). Sulphur is one of the main undesirable constituents of coal because it contributes to environmental pollution and causes corrosion of devices and equipment. SO_2 emissions into the atmosphere during the coal combustion processes are a serious environmental problem. It is estimated that one third of the total sulphur in the atmosphere is due to the combustion of fossil fuels, of which the largest contribution is sulphur from coal. Coal contains both inorganic and organic sulphur. Pyrite is the main inorganic contaminant in most coals (Rađenović, 2004).

- **Tin (Sn)**

The abundance of Sn in rocks shows an increased concentration in argillaceous sediments (6 to 10 mg kg^{-1}) and lower amounts in ultramafic and calcareous rocks (0.35 to 0.5 mg kg^{-1}). Although Sn in soils is largely derived from Sn in the bedrock, all soil surface horizons contain fairly similar amounts of this element, averaging 1.1 mg kg^{-1}. There is no evidence that Sn is either essential or beneficial to plants, although plants may easily take up Sn, if present in the nutrient solution, but most of the absorbed Sn remains in roots. Concentrations of Sn in plants range from 0.2 to

2000 mg kg^{-1} (Kabata-Pendias, 2007). Tin is not an essential metal and is widely used in the industry. In nature, it occurs both in inorganic and organic forms. In chronic exposures, bone is the major storage organ for tin. The average concentration of tin in fresh vegetables, meat, milk, and fish is <1 mg kg^{-1} and often below the limit of determination (Nordberg et al., 2007).

- **Uranium (U)**

Uranium is a naturally occurring radioactive element, which in nature is a mixture of three isotopes: ^{234}U, ^{235}U, and ^{238}U. The most common isotope, ^{238}U, makes up about 99% of natural uranium, and due to that predominance, is thought to be primarily responsible for the chemical toxicity of uranium. Uranium is naturally present in many soils with an average concentration in the United States of about 3 mg kg^{-1}; some areas, particularly in the western United States, have higher concentrations. Uranium mining, milling, and processing operations have released uranium into the environment leading to elevated levels of uranium in affected soils and dust. Uranium from soil is adsorbed onto the roots of plants; root crops including potatoes, radishes, and other root vegetables are a source of uranium in the diet. Environmental exposures to uranium from contaminated sites can involve multiple pathways including ingestion of soil, foods, surface water, or groundwater as well as consumption of locally grown or foraged food (http://hero.epa.gov).

- **Vanadium (V)**

Vanadium is a lithophilic trace element. It is enriched with iron in basic and intermediate magmas. It separates from iron in the sedimentation cycle. For some plants, it is important for nitrogen binding (legumes). It is widespread in most organisms. It stimulates the production of chlorophyll and iron metabolism in some plants. For plants in higher concentrations than 10 mg kg^{-1}, it is poisonous. It is an important part of the diet for many animals and some of its preparations can significantly increase growth. It also affects biomass production. In industry, it serves as a steel refiner. It does not disperse to a significant extent into the environment, except by burning oil derivatives. Coastal Croatia contains the highest concentrations of vanadium in the soil. The concentration range is from 26 to 473 mg kg^{-1} with a median of 148 mg kg^{-1} which is above twice the European average (60 mg kg^{-1}). The highest concentrations were recorded on the Učka mountain, where they exceed 240 mg kg^{-1}, and the lowest concentrations are related to the flysch zones of Istria and Dalmatia, where they can be less than 30 mg kg^{-1}, but their spatial distribution is very limited (Halamić et al., 2009).

4. MATERIALS AND METHODS

4.1. Study area

The research area includes the municipality of Raša and the city of Opatija. Raša is situated in the southeast part of the Istrian peninsula, the westernmost part of Croatia. The town of Opatija is located in the Kvarner Bay, where the Mediterranean cuts into the mainland of Central Europe at the foot of Mount Učka. The geological setting of the study area consists of Lower and Upper Cretaceous and Paleogene deposits. It is a karst region composed predominantly of limestones and other kinds of acid-vulnerable carbonate rocks, covered with thin layers of *terra rossa* and brown soils (Medunić et al., 2018a). The municipality of Raša is special because of the occurrence of hard coal beds (Medunić et al. 2016b), that are not present in the city of Opatija.

In Fig. 1 basic geological map of the wider area is given.

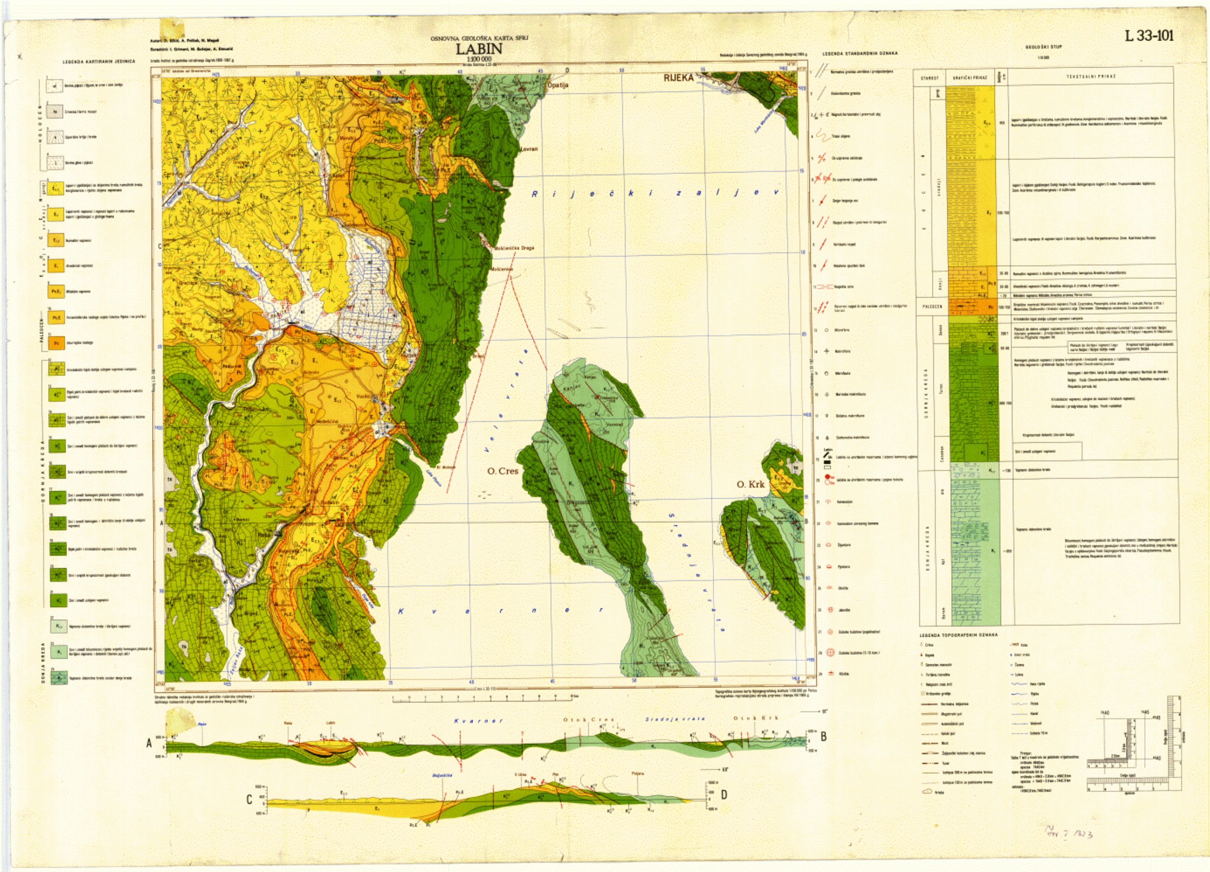

Fig. 1. Basic geological map 1 : 100 000 L 33 – 101 Labin (Šikić and Polšak, 1973)

4.2. Sampling and sample description

Samples of soils and vegetables were taken from five locations in Krapan and two locations in Raša, both included in the Raša municipality, and four locations in Opatija, the reference location, in November 2019 (Fig. 2). Total of 22 plant samples and 12 soil samples were taken. All sampling areas, except one in Krapan, were private gardens where locals grow vegetables for their own needs. In that one location in Krapan, samples were collected along the channel through which the water flows from the mine. While sampling plants, special attention was paid to that, if possible, the plant has all plant parts (root, stem, leaf, fruit). Samples were collected with a shovel, washed with distilled water immediately after sampling, and stored in plastic bags with appropriate labels.

Figure 2. Satellite image of the investigated area.

4.3. Sample preparation

Sample preparation and analysis were performed in Laboratory for inorganic environmental geochemistry and chemodynamics of nanoparticles, Division for Marine and Environmental Research, Ruđer Bošković Institute.

Samples of plants were washed with plain, then Milli-Q water, and then parts of plants (root (R), stem (S), leaf (L), bulb (B)) were separated and air-dried. Soil samples were placed in petri dishes and also allowed to air dry. All dried samples were homogenised in an agate mill.

For total element analysis, plant sub-samples (~0,07g) were subjected to total digestion in the microwave oven (Multiwave 3000, Anton Paar, Graz, Austria) in a one-step procedure consisting of digestion with a mixture of 6 mL nitric acid (HNO_3, 65%, pro analysi, Kemika, Zagreb, Croatia) and 0.1 mL hydrofluoric acid (HF, 48%, pro analysi, Kemika, Zagreb, Croatia). Soil sub-samples (~0.05 g) were subjected to total digestion in the microwave oven in a two-step procedure consisting of digestion with a mixture of 4 mL nitric acid (HNO_3, 65%, pro analysi, Kemika, Zagreb, Croatia), 1 mL hydrochloric acid (HCl) and 1 mL hydrofluoric acid (HF, 48%, pro analysi, Kemika, Zagreb, Croatia), followed by addition of 6 mL of boric acid (H_3BO_3, Fluka, Steinheim, Switzerland). Prior to analysis, soil digests were 10-fold diluted, acidified with 2% (v/v) HNO_3 (65%, supra pur, Fluka, Steinheim, Switzerland) and In (1µg/L) as internal standard was added. Plant digests were only acidified with 2% (v/v) HNO_3 (65%, supra pur, Fluka, Steinheim, Switzerland) without further dilution and In (1µg/L) as internal standard was added.

4.4. Multielement analysis

Multielement analysis of samples was performed by High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS) using an Element 2 instrument (Thermo, Bremen, Germany). All samples were analysed for a total concentration of 32 elements (Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cs, Cu, Fe, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, S, Sb, Sc, Se, Sn, Sr, Ti, Tl, U, V, Zn). Quality control of analytical procedure was performed by simultaneous analysis of the blank and certified reference material for plants (NCS Certified Reference Material, ZC73018, Citrus leaves) and soil (NCS DC 77302, China National Analysis Center for Iron and Steel, Beijing, China). Good agreement between the analyzed and the certified concentrations within their analytical uncertainties for all elements was obtained (~ 10%).

4.5. Health risk assessment

Health risk assessment is used to determine the impact of certain harmful conditions on human health or the quality of the environment in general. The scientific aspects of risk assessment relate to the preparation of the basis on which such an assessment can be made. The application of risk assessment is carried out through risk management, i.e. by adopting measures to reduce risk, adopting regulations, and making decisions on the acceptability of risk. Risk assessment itself ensures the transition from scientific knowledge and societal needs to risk control aimed at maintaining its acceptable level. Risk assessment is closely related to reliability problems that limit it. It is based on the probability of an adverse event and the intensity of the consequences of such an event. Public perceptions of risk often have a decisive influence on society´s response (Ružić, 1998). According to the Croatian Agency for Agriculture and Food, risk analysis is divided between politics and science for the purpose of their independent but mutual action for the benefit of the entire community related to human safety; and this safety mainly refers to food that people take into their bodies. Risk management in the Republic of Croatia is carried out by the bodies responsible for carrying out official controls. The bodies responsible for the implementation of official controls, decrees and decisions from the basis of the Food Act are the Ministry responsible for agriculture, the Ministry responsible for health, and the State inspectorate. The Center for Food Safety within the Croatian Agency for Agriculture and Food deals with activities in accordance with the provisions of the Food Act, the Food Hygiene Act and microbiological criteria for food, the Act on official controls carried out in accordance with the food, animal health, health and welfare regulations, the Law on the Croatian Agency for Agriculture and Food and other regulations in this area. The Center performs a risk assessment of food-borne diseases and informs about the results of risk assessment (https://www.hapih.hr). The Human Health Risk (HHRA), according to the Environmental Protection Agency (EPA), is targeted to improve understanding of the key effects of exposure to pollutants on biological, chemical and physical processes that affect human health. What EPA scientists and their partners represent, provides the basis for the operation of the Public Health Agency and environment. The EPA generates health assessment that are used to determine the potential risk for public health from exposure to environmental pollutants. HHRA is based on planning, hazard identification, dose-toxicity relationship, exposure assessment, and risk characterization (https://www.epa.gov).

4.5.1. Planning

When planning, the following questions should be answered:

1) Who/what/where is the danger? (e.g. individual, children, general population, teenagers, pregnant women, sensitive subgroups of the population)
2) What is the risk of environmental impact? (e.g. chemicals, radiation, physical, microbiological or biological, nutritional, socioeconomic)
3) Where do these ecological dangers come from? (one-point source or sources with more than one point)
4) How does exposure occur? (e.g. routes such as air, surface water, groundwater, soil, solid waste, food, medicines)

5) What does the organism do with the danger and how are factors such as age, race, gender, genetics etc. affecting the environment? (e.g. absorption, distribution, metabolism, excretion)

6) What are the health effects? (cancer, heart disease, liver disease, nerve disease, etc.)

7) How long does the hazard last to cause a toxic effect? (e.g. acute, subchronic, chronic, or occasionally)

4.5.2. Hazard identification

It is a process of determining whether exposure to a potential environmental hazard may cause an increase in the frequency of specific adverse health effects. Based on the quality and adequacy of the carcinogenicity data, the EPA places each chemical in one of the following five weight categories: 1) carcinogenic to humans, 2) probably carcinogenic, 3) possibly carcinogenic, 4) not classified, 5) evidence of non-carcinogenicity.

4.5.3. Dose-toxicity relationship

The likelihood and severity of adverse health effects (responses) relate to the amount and state of exposure to a potential hazard and the information obtained is called "Concentration-response". There is a nonlinear and linear assessment of the dose-response: The nonlinear assessment has its origin in the threshold hypothesis, which says that the organism can tolerate the range of exposure from zero to some final value, with no possibility of expression of the toxic effect and toxicity threshold is where the effects (or their predecessors) begin to appear. It is often wise to focus on the most vulnerable members of the population because this results in regulatory processes generally below the population threshold. A linear assessment is taken when toxicity has no threshold. In the case of carcinogens, if the mode of action of the information is insufficient, then linear extrapolation is usually used as the default approach to dose estimation. Linear assessment is performed using the Guidelines for Carcinogen Risk Assessment (USEPA, 2005).

4.5.4. Exposure assessment

It is the process of measuring or estimating the magnitude, frequency and duration of human exposure to a potential environmental hazard. The exposure assessment shall include consideration of the size, nature and types of human populations at risk, as well as a discussion of the uncertainties of the information provided. An exposure assessment is performed with the help of the Guidelines for Exposure Assessment (USEPA, 1992).

4.5.5. Risk characterization

Risk characterization makes an assessment by the risk assessor of the nature and presence or absence of risk, together with information on the risk assessment, where there are assumptions and uncertainties. Risk characterization takes place in human health risk assessment and environmental risk assessments. Risk characterization is performed using the Guidelines for Risk Characterization (Fowle and Dearfield, 2000).

4.5.6. Calculating human health risk assessment

The health risk associated with metals ingested through vegetable consumption is assessed using hazard quotient (HQ) (Bermudez et al., 2011). It is a ratio of determined dose to the referent dose:

$$\mathbf{HQ= (Div) \times (C_{metal}) / R_fD \times B_o}$$

Div represents the daily intake of vegetables ($kg person^{-1} day^{-1}$), C_{metal} is the concentration of metal in vegetable samples ($mg kg^{-1}$), R_fD stands for oral reference dose for the metal ($mg kg^{-1} day^{-1}$) and B_o is the human body mass (kg). The EPA defines oral reference dose as an estimation of a daily oral exposure to which the human population is likely to be without an appreciable risk of deleterious effects during a lifetime. The values of R_fD for heavy metals were taken from the Integrated Risk Information System (IRIS). The HQ is a very relative and conservative index. When HQ is <1, there is no obvious risk from the substance over a lifetime of exposure, while HQ is > 1, the toxicant may produce an adverse effect. The probability of experiencing long-term carcinogenic effects increases with the HQ value (Gupta et al., 2013).

4.5.7. Estimated Daily Intake (EDI)

The estimated daily intake in this study was determined based on the mean concentration of metals in vegetables so as the estimated daily consumption of vegetables and was calculated according to the following equation (Copat et al., 2013):

EDI (mg day^{-1}) = (IR x C) / BW

IR represents the ingestion rate daily or meal size (g day^{-1}), C is the metal concentration (mg kg^{-1}) and BW is the body weight (kg).

5. RESULTS

5.1. Total concentrations

Total concentrations of 32 elements (Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cs, Cu, Fe, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, S, Sb, Sc, Se, Sn, Sr, Ti, Tl, U, V, Zn) in soil and plant samples are shown in Table 1 and Table 2, respectively.

Element concentrations in soil samples from all three locations range from 215682 to 0.12 mg kg^{-1}. The highest element concentrations were observed in Krapan 4, and the lowest in Opatija 1. However, at some locations highest concentration was measured for several elements. For example, at the location **Opatija 4,** highest concentrations in soil sample were measured for as many as 12 elements: Al (94064 mg kg^{-1}), As (35.7 mg kg^{-1}), Bi (0.89 mg kg^{-1}), Co (27.7 mg kg^{-1}), Cr (172 mg kg^{-1}), Fe (55281 mg kg^{-1}), Li (202 mg kg^{-1}), Ni (137 mg kg^{-1}), Sc (17.8 mg kg^{-1}), Ti (5850 mg kg^{-1}), Tl (2.62 mg kg^{-1}) and V (227 mg kg^{-1}). Then follows the location **Krapan 2**, where the highest concentrations of the following 5 elements were measured: Ba (1052 mg kg^{-1}), Mn (2339 mg kg^{-1}), P (4446 mg kg^{-1}), Sr (352 mg kg^{-1}) and Zn (797 mg kg^{-1}).

In general, element concentrations in soil samples were more than three orders of magnitude higher compared to plant samples. Concentrations of elements in plant samples, on the other hand, vary widely between localities and it is difficult to determine the general trend. They range from 68262 to 0.2 mg kg^{-1}. Comparing plant samples with each other, we notice that in lettuce samples there are always higher concentrations of elements than in other plant species. Also, the concentration of the elements decreases from the root through the stem towards the leaf of plant samples.

Table 1. The concentration of elements (expressed in mg kg^{-1}) in soil samples from Raša, Krapan, and Opatija.

Sample	Al	As	Ba	Be	Bi	Ca	Cd	Co	Cr	Cs	Cu
Krapan 1	40111	13.6	277	1.69	0.57	163861	1.42	10.4	91.2	3.55	60.5
Krapan 2	35552	9.65	1052	1.47	0.30	181823	1.13	10.9	71.7	3.58	69.7
Krapan 3	77178	1.30	413	3.01	0.53	40028	1.34	18.1	151	3.35	50.0
Krapan 4	20046	6.67	363	0.93	0.32	215682	1.14	5.87	47.6	1.66	64.3
Krapan 5	74702	2.20	412	3.00	0.65	44628	1.61	20.2	161	7.19	85.3
Raša 1	40597	11.7	322	1.68	0.44	154070	0.97	18.5	111	4.13	57.9
Raša 2	68110	20.7	370	2.60	0.52	91660	1.01	17.1	134	5.91	51.2
Opatija 1	51377	17.4	253	2.25	0.57	96906	1.31	20.1	101	5.54	65.8
Opatija 1	58498	21.2	244	2.46	0.58	85164	0.86	14.1	111	6.42	61.3
Opatija 2	79006	26.2	393	3.56	0.75	54518	1.25	21.8	149	8.71	84.7
Opatija 3	85497	24.1	312	3.40	0.77	20121	1.16	20.0	149	9.33	85.9
Opatija 4	94064	35.7	318	3.32	0.89	10408	1.9	27.7	172	8.45	64.4
	Fe	**Li**	**Mg**	**Mn**	**Mo**	**Na**	**Ni**	**P**	**Pb**	**Rb**	**S**
Krapan 1	26900	32.3	8800	633	17.7	3035	71.4	1219	100	53.2	4997
Krapan 2	22683	27.1	7414	2339	3.43	2540	73.7	4446	54.9	68.9	1713
Krapan 3	43432	56.2	8657	1021	3.92	6002	107	898	37.7	117.9	1019
Krapan 4	18538	14.7	7972	466	3.16	2639	60.4	1912	408	28.9	3701
Krapan 5	48550	63.9	7913	1190	9.00	4039	115	2054	59.1	108	2470
Raša 1	27981	33.6	6580	1133	4.01	3054	94.6	1945	99.0	64.7	4606
Raša 2	38070	50.1	7361	1067	9.81	4453	110	1710	39.3	91.5	1852
Opatija 1	31186	76.9	10198	1296	2.25	1853	91.0	1784	73.8	67.6	851
Opatija 1	34849	95.1	5789	961	1.97	1547	90.9	1817	43.5	89.4	588
Opatija 2	46801	131	6495	1129	3.76	2450	110	1984	80.3	91.9	481
Opatija 3	48424	88.3	9429	1427	5.24	3028	119	2229	44.5	125	502
Opatija 4	55281	202	8161	1372	3.40	2734	137	1895	60.7	93.9	405
	Sb	**Sc**	**Se**	**Sn**	**Sr**	**Ti**	**Tl**	**U**	**V**	**Zn**	
Krapan 1	1.83	7.7	4.86	7.89	224	2398	0.61	4.23	118	279	
Krapan 2	1.39	6.19	1.85	5.04	352	2466	0.59	1.75	84.2	797	
Krapan 3	1.25	14.2	4.19	5.27	115	4883	1.23	3.51	179	144	
Krapan 4	5.62	3.68	3.94	9.67	298	1203	0.27	3.21	55.4	570	
Krapan 5	2.13	13.8	3.97	6.12	139	4550	1.26	4.40	193	222	
Raša 1	1.53	7.89	6.29	7.79	311	2729	0.54	3.45	111	209	
Raša 2	2.02	12.2	7.31	6.14	204	4092	0.91	5.20	165	217	
Opatija 1	1.66	9.38	4.63	6.60	118	3208	1.28	3.47	131	442	
Opatija 1	1.73	10.3	0.12	7.23	121	3288	1.40	3.47	159	192	
Opatija 2	2.62	15.0	1.94	7.20	121	5240	2.08	6.23	224	331	
Opatija 3	2.01	15.9	2.09	5.11	94.2	5162	2.25	5.84	197	233	
Opatija 4	2.41	17.8	1.70	6.72	84.8	5850	2.62	5.96	227	248	

Concentrations of some elements are higher in samples of vegetables from the area of Raša and Krapan (S, Se, Mo, Cd and Cu) than in Opatija, but the opposite is also true (U, V, Pb, Cr, Sr, Ni, Fe, Sn, As and Mn).

Table 2. The concentration of elements (expressed in mg kg^{-1}) in plant samples (dry weight) from Raša, Krapan, and Opatija.

			Al	As	Ba	Be	Bi	Ca	Cd	Co
Location	**Sample**									
Krapan 1	parsley	L	510	0.27	33.3	0.02	0.01	8837	0.14	0.24
		R	1483	0.51	74.4	0.05	0.02	4470	0.18	0.35
		S	197	0.14	125	0.01	0.01	13049	0.18	0.12
	onion	L	460	0.21	7.36	0.01	0.01	9881	0.36	0.22
		R	3828	2.34	53.3	0.21	0.08	11213	1.50	1.66
		B	135	0.12	11.1	0.01	<0.002	7255	0.31	0.06
Krapan 2	fennel	L+S	428	0.19	16.2	0.02	0.01	12906	0.25	0.17
		R	2160	1.73	124	0.08	0.02	23090	0.78	0.65
Krapan 3	onion	L	870	0.29	5.50	0.03	0.01	2225	0.20	0.26
		R	4156	1.56	42.8	0.30	0.06	7509	2.07	2.34
		B	66,5	0.05	2.30	0.01	<0.002	3984	0.50	0.09
Krapan 4	moss		6129	1.44	108	0.25	0.13	68262	0.71	1.95
	fern	L	730	0.36	30.1	0.03	0.04	9029	0.20	0.31
		R	1766	0.68	63.5	0.08	0.07	35000	0.57	0.72
Krapan 5	lettuce	L	1169	0.50	12.8	0.04	0.02	12817	0.57	0.42
		R	11164	3.18	67.7	0.41	0.09	9428	0.63	2.83
	garlic	L	701	0.29	5.96	0.02	0.01	3632	0.14	0.22
		R	6783	2.62	34.2	0.21	0.05	8523	0.67	1.53
		B	48.8	0.09	3.48	<0.002	<0.002	2657	0.25	0.05
Raša 1	lettuce	L1	377	0.16	7.97	0.01	0.01	10208	0.22	0.27
		L2	822	0.40	14.1	0.03	0.01	10050	0.27	0.46
		R1	2610	1.13	28.3	0.10	0.03	6201	0.47	0.78
		R2	8117	2.17	69.2	0.30	0.09	23158	0.59	3.30
	garlic	L	639	0.30	5.82	0.02	0.01	3892	0.14	0.26
		R	5085	1.34	33.4	0.15	0.05	9272	0.43	1.48
		B	47.2	0.05	4.04	<0.002	<0.002	2791	0.47	0.04
Raša 2	lettuce	L	824	0.64	11.8	0.03	0.01	7607	0.38	0.40
		R	7006	2.66	56.7	0.28	0.07	9748	0.54	1.98
	radicchio	L	752	0.37	11.5	0.03	0.01	7891	0.19	0.34
		R	1114	0.42	17.0	0.03	0.02	4920	0.32	0,45
Opatija 1	parsley	L	1119	0.55	33.8	0.04	0.01	14481	0.14	0.30
		R	3428	1.64	77.3	0.18	0.05	7300	0.39	0.97
		S	609	0.36	115	0.03	0.01	22817	0.20	0.20
	radicchio	L	941	0.49	16.6	0.04	0.01	16726	0.65	0.32
		R	2533	1.11	30.7	0.10	0.05	6305	0.50	0.81
	fennel	L+S	441	0.26	6.99	0.01	0.01	19656	0.07	0.14
		R	3709	1.25	53.3	0.16	0.04	16278	0.51	1.51
Opatija 2	lettuce	L	984	0.40	13.1	0.05	0.02	6344	0.52	0.36
		R	16394	6.96	114	0.88	0.20	12594	0.65	5.60
	onion	L	812	0.46	30.5	0.04	0.01	24374	0.14	0.35
		R	6241	4.54	53.3	0.33	0.09	9128	1.16	2.37
		B	345	0.19	25.9	<LOD	0.07	11337	0.14	0.21
Opatija 3	lettuce	L	1373	0.54	9.80	0.05	0.02	7425	0.13	0.44
		R	19953	6.20	47.9	0.79	0.19	15363	0.62	5.65
	parsley	L	1009	0.35	18.1	0.03	0.01	11818	0.12	0.24
		R	3422	1.22	87.8	0.15	0.04	5287	0.32	0.94
		S	1008	0.83	89.6	0.04	0.01	17081	0.25	0.61
Opatija 4	lettuce	L	920	0.49	12.1	0.03	0.02	12286	0.44	0.34
		R	6899	3.79	58.9	0.43	0.12	6858	0.94	3.58
	radicchio	L	2149	0.87	20.1	0.08	0.03	18944	0.49	0.65
		R	6569	2.89	36.8	0.26	0.07	5478	0.41	2.38

L-leaf; R-root; S-stem; B-bulb.

Table 2. *extension*

			Cr	**Cs**	**Cu**	**Fe**	**Li**	**Mg**	**Mn**	**Mo**
Location	**Sample**									
Krapan 1	parsley	L	1.41	0.06	18.1	355	0.48	2000	40.3	1.94
		R	2.80	0.14	15.0	736	1.01	2162	28.6	1.01
		S	0.40	0.02	7.33	132	0.19	1141	14.2	0.54
	onion	L	1.09	0.05	8.07	947	0.41	2230	35.4	6.05
		R	13.8	0.60	140	3369	5.38	1764	86.5	8.80
		B	0.47	0.02	1.67	163	0.21	1221	8.48	4.24
Krapan 2	fennel	L+S	1.11	0.13	21.9	293	2.55	2285	63.3	0.74
		R	4.12	0.27	23.8	1032	1.67	3181	175	0.32
Krapan 3	onion	L	2.00	0.09	14.0	534	0.69	1852	45.2	0.89
		R	12.1	0.63	27.0	3297	5.00	1860	119	6.02
		B	0.94	0.01	7.84	59.1	0.24	893	11.1	0.67
Krapan 4	moss		14.6	0.46	24.5	4917	3.36	32736	257	1.65
	fern	L	2.73	0.13	12.3	508	0.55	2288	73.7	2.28
		R	7.79	0.21	25.2	1470	1.22	2434	130	4.50
Krapan 5	lettuce	L	2.71	0.12	13.5	768	1.02	1959	55.7	3.25
		R	21.5	1.08	31.0	6221	10.1	2403	159	2.24
	garlic	L	1.57	0.08	7.71	448	0.60	2116	31.6	2.66
		R	12.5	0.60	12.7	3136	5.49	4971	91.0	4.72
		B	0.10	0.01	1.82	43.6	0.27	1185	6.86	5.85
Raša 1	lettuce	L1	1.02	0.06	11.9	300	0.34	2774	52.5	1.37
		L2	2.38	0.09	10.3	569	0.68	1483	45.7	0.96
		R1	5.07	0.29	21.1	1384	4.58	1749	65.3	0.44
		R2	19.9	0.93	20.1	4824	7.75	2485	185	2.87
	garlic	L	1.85	0.07	10.4	443	0.54	2249	33.0	2.01
		R	10.2	0.51	14.6	2521	3.97	3940	76.9	4.49
		B	0.15	0.01	7.25	49.2	0.21	1451	7.90	6.14
Raša 2	lettuce	L	1.94	0.09	20.1	534	0.66	2396	48.4	0.79
		R	15.2	0.72	16.7	4101	6.75	2109	122	3.02
	radicchio	L	1.61	0.08	15.4	448	0.63	2623	45.2	1.13
		R	3.73	0.12	9.31	633	0.64	1946	33.0	0.99
Opatija 1	parsley	L	2.22	0.13	11.7	654	1.84	3185	58.4	1.39
		R	7.69	0.53	17.0	2390	8.72	3703	89.1	1.50
		S	1.18	0.07	5.28	351	0.91	2497	25.7	0.45
	radicchio	L	1.66	0.11	15.5	560	1.53	2949	80.3	0.93
		R	5.26	0.32	23.6	1456	5.10	1212	69.0	0.48
	fennel	L+S	1.03	0.06	14.3	229	0.61	3132	63.7	0.82
		R	8.59	0.49	22.5	2365	5.70	4797	103	0.55
Opatija 2	lettuce	L	3.01	0.12	17.5	644	1.75	1927	43.2	1.07
		R	34.1	2.30	41.8	10808	39.5	2932	260	2.93
	onion	L	1.88	0.09	7.38	505	1.39	1397	35.7	5.16
		R	16.4	0.88	28.4	4233	14.9	1685	96.2	3.56
		B	0.73	0.04	8.91	198	0.62	1709	25.1	2.37
Opatija 3	lettuce	L	2.66	0.15	13.0	791	1.49	2692	43.4	0.82
		R	35.9	2.35	34.9	10891	24.7	4114	368	2.48
	parsley	L	2.08	0.12	7.20	633	1.15	1993	57.1	3.70
		R	7.24	0.47	11.8	2154	5.00	2413	65.3	1.55
		S	1.69	0.10	12.9	588	0.83	1437	38.3	0.64
Opatija 4	lettuce	L	2.06	0.09	9.86	610	2.09	2471	41.8	0.80
		R	22.1	1.09	16.7	6152	28.0	1089	178	1.67
	radicchio	L	3.99	0.21	7.82	1241	5.18	2029	80.3	4.36
		R	13.0	0.69	12.6	3828	18.0	1987	105	1.49

L-leaf; R-root; S-stem; B-bulb.

Table 2. *extension*

			Na	Ni	P	Pb	Rb	S	Sb	Sc
Location	**Sample**									
Krapan 1	parsley	L	413	4.02	6194	1.30	14.7	3329	0.04	0.09
		R	2517	3.13	2083	4.55	8.13	1214	0.06	0.27
		S	548	1.69	5586	0.75	16.0	1455	0.02	0.03
	onion	L	316	2.14	4274	1.06	6.94	7499	0.05	0.08
		R	1611	8.68	10331	15.5	13.7	6980	0.35	1.05
		B	999	0.56	1821	0.51	1.81	1702	0.07	0.03
Krapan 2	fennel	L+S	9785	3.40	6112	1.18	40.4	4265	0.03	0.08
		R	3509	6.93	3471	5.65	32.2	1085	0.29	0.38
Krapan 3	onion	L	263	3.21	4631	0.68	13.0	7365	0.03	0.17
		R	5907	8.43	5527	4.04	22.5	9429	0.12	1.04
		B	1401	1.38	3914	0.56	11.0	6946	0.02	0.01
Krapan 4	moss		1557	9.92	2136	42.6	13.7	1457	1.30	0.99
	fern	L	206	4.76	3032	4.13	21.3	2013	0.11	0.14
		R	1320	5.29	2303	27.8	7.93	2014	0.42	0.41
Krapan 5	lettuce	L	4827	5.00	5902	1.11	8.26	4665	0.04	0.22
		R	2895	13.1	3559	8.27	20.1	3579	0.47	2.30
	garlic	L	160	2.82	4336	0.85	9.03	8753	0.03	0.13
		R	1713	7.56	3455	5.00	16.0	12387	0.15	1.26
		B	33.8	0.34	2789	0.11	5.03	5022	0.01	0.01
Raša 1	lettuce	L1	5354	3.54	5204	0.54	14.0	3345	0.01	0.07
		L2	986	5.67	4911	1.33	10.2	2217	0.04	0.16
		R1	1761	4.71	1414	2.30	21.5	1261	0.12	0.47
		R2	2515	14.5	3686	10.7	22.3	3626	0.38	1.76
	garlic	L	255	3.38	6035	0.80	5.74	14064	0.03	0.13
		R	2493	8.22	3701	4.96	12.7	8506	0.15	1.04
		B	1026	0.48	4566	0.22	3.37	9489	0.01	0.01
Raša 2	lettuce	L	581	9.77	9114	0.82	16.9	3441	0.04	0.16
		R	1654	9.68	7013	5.08	20.9	1542	0.53	1.28
	radicchio	L	841	6.18	5926	0.86	10.2	4076	0.03	0.15
		R	3052	2.93	3250	1.34	6.91	2030	0.13	0.21
Opatija 1	parsley	L	737	3.34	3737	0.98	41.2	3395	0.04	0.19
		R	1483	5.97	2177	3.72	48.7	2233	0.12	0.91
		S	820	3.34	1578	0.73	80.4	1312	0.03	0.12
	radicchio	L	1209	2.75	4713	0.93	28.0	4066	0.03	0.17
		R	1856	5.00	1390	2.58	23.8	1250	0.12	0.62
	fennel	L+S	8900	1.27	3530	0.89	28.3	4675	0.04	0.08
		R	12819	10.6	3383	5.65	21.1	1631	0.17	0.74
Opatija 2	lettuce	L	1365	4.50	7903	1.37	12.3	4234	0.05	0.20
		R	2531	22.3	3554	22.1	29.1	4990	0.71	3.64
	onion	L	444	2.83	5309	1.05	7.51	5268	0.03	0.15
		R	4149	12.1	5372	8.39	14.7	4366	0.25	1.38
		B	597	1.33	4854	0.75	5.00	4565	0.03	0.06
Opatija 3	lettuce	L	2681	4.40	5778	0.97	16.0	2787	0.04	0.26
		R	5173	24.6	2060	11.5	35.7	2689	0.48	3.82
	parsley	L	532	1.68	4817	0.61	15.3	3211	0.03	0.19
		R	1450	8.13	2729	2.23	18.8	1396	0.09	0.77
		S	731	26.7	2465	2.04	31.8	1287	0.04	0.19
Opatija 4	lettuce	L	999	2.19	7889	0.89	12.3	3369	0.04	0.17
		R	1242	17.2	4007	9.46	19.7	1431	0.35	2.07
	radicchio	L	1292	3.67	3065	1.57	8.07	2815	0.07	0.39
		R	2076	8.96	3634	5.11	11.0	1391	0.29	1.52

L-leaf; R-root; S-stem; B-bulb.

Table 2. *extension*

			Se	Sn	Sr	Ti	Tl	U	V	Zn
Location	**Sample**									
Krapan 1	parsley	L	1.59	0.19	14.2	33.8	0.01	0.03	1.39	77.6
		R	0.95	0.29	26.9	62.0	0.04	0.11	3.74	44.4
		S	1.18	0.12	45.0	5.43	0.01	0.02	0.48	28.5
	onion	L	5.51	0.16	9.77	28.0	0.01	0.04	1.23	101
		R	2.01	1.27	40.4	254	0.13	0.60	32.7	172
		B	0.87	0.11	19.5	8.50	0.01	0.06	0.61	31.2
Krapan 2	fennel	L+S	0.59	0.16	30.5	33.5	0.01	0.05	1.13	94.9
		R	0.58	0.32	112	124	0.05	0.16	4.82	243
Krapan 3	onion	L	0.49	0.19	4.09	51.5	0.02	0.04	2.09	32.7
		R	0.52	0.57	49.4	265	0.14	0.30	15.6	117
		B	0.38	0.22	13.6	4.03	0.02	<0.002	0.12	46.5
Krapan 4	moss		0.92	7.54	127	137	0.08	0.52	9.88	169
	fern	L	0.23	0.66	41.3	48.4	0.02	0.07	1.81	78.8
		R	1.09	1.14	97.6	102	0.04	0.69	12.9	216
Krapan 5	lettuce	L	0.48	0.22	24.2	67.1	0.02	0.07	2.92	55.8
		R	0.67	0.82	56.5	459	0.21	0.71	29.9	78.8
	garlic	L	3.67	0.17	4.11	36.9	0.01	0.04	1.83	69.3
		R	1.28	0.47	32.7	267	0.11	0.44	26.2	67.4
		B	1.85	0.09	6.68	2.72	<0.002	<0.002	0.16	29.1
Raša 1	lettuce	L1	1.11	0.14	21.0	25.8	0.01	0.03	1.01	61.8
		L2	0.83	0.22	25.7	46.8	0.02	0.07	2.20	55.6
		R1	< 0.002	0.30	27.4	119	0.07	0.22	7.92	81.3
		R2	1.68	1.03	75.4	384	0.20	0.98	28.6	84.0
	garlic	L	14.3	0.20	5.42	39.5	0.01	0.04	1.64	75.2
		R	4.31	0.54	35.3	202	0.08	0.35	18.3	66.3
		B	6.70	0.15	8.25	2.60	<0.002	0.01	0.19	61.6
Raša 2	lettuce	L	2.37	0.19	16.3	51.0	0.01	0.07	2.15	84.8
		R	1.67	1.69	48.4	358	0.14	0.86	23.1	82.5
	radicchio	L	2.77	0.23	19.3	71.2	0.02	0.08	1.97	75.5
		R	1.69	0.35	33.0	56.8	0.03	0.27	5.66	73.4
Opatija 1	parsley	L	0.16	0.20	16.0	55.7	0.03	0.07	2.90	61.5
		R	<0.002	0.36	30.6	202	0.13	0.29	12.6	50.9
		S	0.11	0.13	46.2	32.2	0.02	0.04	1.62	34.7
	radicchio	L	0.12	0.16	27.7	19.9	0.03	0.05	2.41	68.2
		R	0.04	0.33	29.9	131	0.08	0.25	8.49	74.9
	fennel	L+S	0.21	0.16	18.9	25.5	0.02	0.03	1.07	61.5
		R	0.68	0.54	70.0	218	0.12	0.29	11.0	106
Opatija 2	lettuce	L	0.35	0.19	7.78	66.7	0.03	0.09	2.83	81.4
		R	0.45	3.12	448	801	0.63	1.59	64.1	145
	onion	L	0.20	0.21	43.0	46.7	0.03	0.07	2.56	34.7
		R	0.15	0.81	33.2	366	0.22	0.71	49.3	144
		B	<LOD	0.24	35.7	19.0	0.02	0.04	1.11	38.8
Opatija 3	lettuce	L	0.11	0.17	13.0	77.3	0.04	0.11	3.24	48.4
		R	0.35	1.08	52.2	907	0.61	1.54	52.7	106
	parsley	L	0.10	0.17	15.1	56.2	0.03	0.07	2.42	31.6
		R	0.23	0.36	30.0	199	0.12	0.29	10.4	69.0
		S	0.27	0.17	46.4	28.5	0.02	0.08	2.37	71.5
Opatija 4	lettuce	L	0.17	0.17	17.1	55.5	0.04	0.07	2.30	50.4
		R	0.31	0.85	30.6	516	0.48	0.86	28.2	91.1
	radicchio	L	0.17	0.22	26.6	116	0.08	0.15	5.28	37.9
		R	0.23	0.55	23.5	318	0.28	0.49	19.3	45.7

L-leaf; R-root; S-stem; B-bulb.

The highest concentrations of as many as nine elements (Al 19953 mg kg^{-1}, Co 5.65 mg kg^{-1}, Cr 35.9 mg kg^{-1}, Cs 2.35 mg kg^{-1}, Fe 10891 mg kg^{-1}, Mn 368 mg kg^{-1}, Ni 26.7 mg kg^{-1}, Sc 3.82 mg kg^{-1}, Ti 907 mg kg^{-1}) were found at the location **Opatija 3** in a sample of lettuce root and parsley stem. At the location **Opatija 2**, the highest concentrations of eight elements were found in the lettuce root sample: As (6.96 mg kg^{-1}), Be (0.88 mg kg^{-1}), Bi (0.20 mg kg^{-1}), Li (39.5 mg kg^{-1}), Sr (448 mg kg^{-1}), Tl (0.63 mg kg^{-1}), U (1.59 mg kg^{-1}), V (64.1 mg kg^{-1}). At the location **Krapan 4** the highest concentrations of five elements were found in the moss sample: Ca (68262 mg kg^{-1}), Mg (32736 mg kg^{-1}), Pb (42.6 mg kg^{-1}), Sb (1.30 mg kg^{-1}), Sn (7.54 mg kg^{-1}).

Table 3. Comparison of the mean values of the concentration (in mg kg^{-1}) of selected elements in vegetables (dry weight) from Raša and Krapan with those from Opatija.

	Krapan, Raša	**Opatija**
S	5601	2970
Se	2.45	0.22
U	0.10	0.34
V	7.74	13.6
Mo	2.97	1.84
Pb	2.83	3.98
Cd	0.49	0.42
Cu	19.4	16.2
Cr	4.93	8.31
Sr	28.5	50.55
Ni	4.91	8.23
Fe	1316	2442
Sn	0.38	0.49
As	0.84	1.69
Mn	71.6	91.7

5.2. Hazard quotient (HQ)

Hazard quotient for the oral route uptake for adults was calculated for As, Cd, Cr, Cu, Fe, Mn, Mo, Ni, Se, Sn, Sr, U and V for those parts of the plant that are eaten e.g. lettuce leaves, onion bulbs. Obtained HQ values are shown in Table 4. There are no RfD values for S and Pb, therefore, due to the unavailability of data for these elements, the HQ cannot be calculated

(USEPA, 2017). For no element was the HQ greater than 1 which means that there is no high chronic risk of carcinogenic effects on human health.

Table 4. Hazard Quotient (HQ) for the oral route uptake for adults.

Location	Sample		As	Cd	Cr	Cu	Fe	Mn	Mo
Krapan 1	parsley	L	0.20	0.03	0.11	0.10	0.12	0.07	0.00
		S	0.11	0.04	0.03	0.04	0.04	0.02	0.00
	onion	B	0.09	0.07	0.04	0.00	0.05	0.01	0.02
Krapan 2	fennel	L+S	0.14	0.06	0.08	0.12	0.10	0.10	0.00
Krapan 3	onion	B	0.04	0.11	0.07	0.04	0.02	0.02	0.00
Krapan 5	lettuce	L	0.38	0.13	0.21	0.08	0.25	0.09	0.01
	garlic	B	0.07	0.06	0.00	0.01	0.01	0.01	0.03
Raša 1	lettuce	L1	0.12	0.05	0.08	0.07	0.10	0.09	0.00
		L2	0.30	0.06	0.18	0.06	0.18	0.07	0.00
	garlic	B	0.04	0.10	0.01	0.04	0.02	0.01	0.03
Raša 2	lettuce	L	0.49	0.07	0.15	0.11	0.17	0.08	0.00
	radicchio	L	0.28	0.04	0.12	0.09	0.15	0.07	0.00
Opatija 1	parsley	L	0.42	0.03	0.17	0.07	0.21	0.09	0.00
		S	0.27	0.05	0.09	0.03	0.11	0.04	0.00
	radicchio	L	0.37	0.15	0.13	0.09	0.18	0.13	0.00
	fennel	L+S	0.20	0.02	0.08	0.08	0.07	0.10	0.00
Opatija 2	lettuce	L	0.30	0.12	0.23	0.10	0.21	0.07	0.00
	onion	B	0.14	0.03	0.06	0.05	0.06	0.04	0.01
Opatija 3	lettuce	L	0.41	0.03	0.20	0.07	0.26	0.07	0.00
	parsley	L	0.27	0.03	0.16	0.04	0.21	0.09	0.02
		S	0.63	0.10	0.13	0.07	0.19	0.06	0.00
Opatija 4	lettuce	L	0.37	0.10	0.16	0.06	0.20	0.07	0.02
	radicchio	L	0.67	0.11	0.30	0.04	0.40	0.13	0.00

			Ni	Se	Sn	Sr	U	V
Krapan 1	parsley	L	0.05	0.07	0.14	0.00	0.00	0.04
		S	0.02	0.00	0.09	0.02	0.00	0.01
	onion	B	0.00	0.00	0.08	0.00	0.00	0.02
Krapan 2	fennel	L+S	0.04	0.03	0.12	0.01	0.00	0.03
Krapan 3	onion	B	0.02	0.02	0.17	0.00	0.00	0.00
Krapan 5	lettuce	L	0.06	0.02	0.17	0.00	0.00	0.07
	garlic	B	0.00	0.08	0.07	0.00	0.00	0.00
Raša 1	lettuce	L1	0.04	0.05	0.11	0.00	0.00	0.03
		L2	0.06	0.04	0.17	0.00	0.00	0.06
	garlic	B	0.00	0.30	0.11	0.00	0.00	0.00
Raša 2	lettuce	L	0.11	0.11	0.14	0.00	0.00	0.05
	radicchio	L	0.07	0.13	0.17	0.00	0.00	0.05
Opatija 1	parsley	L	0.04	0.00	0.15	0.00	0.00	0.07
		S	0.04	0.00	0.10	0.02	0.00	0.04
	radicchio	L	0.03	0.00	0.12	0.01	0.00	0.06
	fennel	L+S	0.01	0.00	0.12	0.00	0.00	0.03
Opatija 2	lettuce	L	0.05	0.02	0.14	0.00	0.00	0.07
	onion	B	0.02	0.00	0.18	0.00	0.00	0.03
Opatija 3	lettuce	L	0.05	0.00	0.13	0.00	0.00	0.08
	parsley	L	0.02	0.00	0.13	0.00	0.00	0.06
		S	0.30	0.01	0.13	0.00	0.00	0.06
Opatija 4	lettuce	L	0.02	0.00	0.13	0.00	0.00	0.06
	radicchio	L	0.04	0.00	0.17	0.00	0.00	0.13

L-leaf; S-stem; B-bulb.

5.3. Estimated Daily Intake (EDI) of trace elements in vegetable samples

The values of the Maximum Tolerable Daily Intake (MTDI) (mg day^{-1}) are given in Table 5. EDI was calculated for As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Se and Zn for those parts of the plant that are eaten (Table 6).

Table 5. Maximum Tolerable Daily Intake (MTDI) (mg day^{-1}) of selected elements.

	MTDI (mg day^{-1})
As	0.13[a]
Cd	0.02-0.07[abc]
Co	0.05[c]
Cr	0.035-0.2[ac]
Cu	2.5-3[bc]
Fe	15[c]
Mn	2-5[ac]
Ni	0.1-0.3[ac]
Pb	0.21[a]
Se	0.4[d]
Zn	60-65[ab]

a- Shaheen et al., 2016

b- Zheng et al., 2007

c- Basha et al., 2014

d- WHO, 1996

Table 6. Estimated Daily Intake (mg day^{-1}) for selected elements in vegetables (dry weight) from Krapan, Raša and Opatija (red values are above MTDI values of certain element).

Location	Sample		As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Se	Zn
Krapan 1	parsley	L	0.06	0.03	0.05	0.32	4.12	80.8	9.17	0.91	0.30	0.36	17.7
		S	0.03	0.04	0.03	0.09	1.67	30.0	3.23	0.38	0.17	0.00	6.48
	onion	B	0.03	0.07	0.01	0.11	0.38	37.1	1.93	0.13	0.12	0.00	7.10
Krapan 2	fennel	L+S	0.04	0.06	0.04	0.25	4.98	66.7	14.4	0.77	0.27	0.13	21.6
Krapan 3	onion	B	0.01	0.11	0.02	0.21	1.78	13.4	2.53	0.31	0.13	0.09	10.6
Krapan 5	lettuce	L	0.11	0.13	0.10	0.62	3.07	175	12.7	1.14	0.25	0.11	12.7
	garlic	B	0.02	0.06	0.01	0.02	0.41	9.92	1.56	0.08	0.03	0.42	6.62
Raša 1	lettuce	L1	0.04	0.05	0.06	0.23	2.71	68.3	11.9	0.81	0.12	0.25	14.1
		L2	0.09	0.06	0.10	0.54	2.34	129	10.4	1.29	0.30	0.19	12.7
	garlic	B	0.01	0.11	0.00	0.03	1.65	11.2	1.80	0.11	0.05	1.52	14.0
Raša 2	lettuce	L	0.15	0.09	0.09	0.44	4.57	121	11.0	2.22	0.19	0.54	19.3
	radicchio	L	0.08	0.04	0.08	0.37	3.50	102	10.3	1.41	0.20	0.63	17.2
Opatija 1	parsley	L	0.13	0.03	0.07	0.51	2.66	149	13.3	0.76	0.22	0.04	14.0
		S	0.08	0.05	0.05	0.27	1.20	79.9	5.85	0.76	0.17	0.03	7.90
	radicchio	L	0.11	0.15	0.07	0.38	3.53	127	18.3	0.63	0.21	0.03	15.5
	fennel	L+S	0.06	0.02	0.03	0.23	3.25	52.1	14.5	0.29	0.20	0.05	14.0
Opatija 2	lettuce	L	0.09	0.12	0.08	0.68	3.98	147	9.83	1.02	0.31	0.08	18.5
	onion	B	0.04	0.03	0.05	0.17	2.03	45.1	5.71	0.30	0.17	0.00	8.83
Opatija 3	lettuce	L	0.12	0.03	0.10	0.61	2.96	180	9.88	1.00	0.22	0.03	11.0
	parsley	L	0.08	0.03	0.05	0.47	1.64	144	13.0	0.38	0.14	0.02	7.19
		S	0.19	0.06	0.14	0.38	2.94	134	8.71	6.08	0.46	0.06	16.3
Opatija 4	lettuce	L	0.11	0.10	0.08	0.47	2.24	139	9.51	0.50	0.20	0.04	11.5
	radicchio	L	0.20	0.11	0.15	0.91	1.78	282	18.3	0.84	0.36	0.04	8.62

6. DISCUSSION

6.1. Selenium and trace metal content in soil and vegetables of the study area

Raša coal is known as coal that is rich in sulphur (up to 14%), and contains elevated concentrations of Se, V and U (Medunić et al., 2016a; Medunić et al., 2017). This is certainly a consequence of the long Istrian industry (Rađenović et al., 2017). In the period from 1970 to 2000, this coal was used in the only Croatian coal-fired power plant (TPP Plomin). The latter resulted in local soil pollution with sulphur, selenium, cadmium and polycyclic aromatic hydrocarbons (Medunić et al., 2016b). In this work, selenium concentrations were also elevated for the above reasons up to 7.31 mg kg^{-1}.

Furthermore, elevated values of Cd, Cu, Pb and Zn in potentially contaminated soil (in Raša and Krapan) are typical of sites contaminated by the mining and metallurgical industries (Davies and Ballinger, 1990; Helios Rybicka, 1996; Verner et al., 1996; Kierczak et al., 2008; Sofilić et al., 2013). In this study, concentrations of Pb (with an average of 127 mg kg^{-1} in Krapan and Raša and 60.6 mg kg^{-1} in Opatija) were above the highest value expected for silty and loamy soil (1.5 to 70 mg kg^{-1}, Kabata-Pendias, 2007). The concentrations of Cd (1.71 mg kg^{-1}) were above Croatian legislative values for medium-textured soils (0.5 to 1.0 mg kg^{-1}; www.propisi.hr) and also slightly above the highest value expected for silty and loamy soil (0.08 to 1.61 mg kg^{-1}; Kabata-Pendias, 2007). The value of Mo (7.85 mg kg^{-1}) was slightly above the value for silty and loamy soil (0.1 to 7.2 mg kg^{-1}; Kabata-Pendias, 2007).

In Table 7, comparison of levels for selected set of elements between locations where we expect elevated concentrations (Raša and Krapan) and the reference site (Opatija).

Table 7. Comparison of obtained values (in mg kg^{-1}) in soil samples from Raša, Krapan and Opatija with Croatian legislation[a] and average soil values[b] (the values marked in red are above the prescribed or average soil values)

	Krapan, Raša	Opatija	a	b
S	3223	471	-	-
Se	4.70	1.17	-	0.02-1.9
U	3.71	4.99	-	-
V	121	187.6	-	15-330
Mo	7.85	3.32	-	0.1-7.2
Pb	127	60.56	50-100	1.5-70
Cd	1.71	1.13	0.5-1.0	0.08-1.61
Cu	64.8	72.42	60-90	4-100
Cr	103	136.4	40-80	4-1100
Sr	255	107.8	-	15-1000
Ni	87.5	109.58	30-50	3-110
Fe	30454	43308.2	-	-
Sn	7.11	6.57	-	-
As	14.3	25.08	-	1.3-27
Mn	1138	1237	-	45-9200

a- Croatian legislative values for medium-textured soils (silty and loamy) (www.propisi.hr)

b- Ranges of total concentrations of trace elements in silty and loamy soils calculated on the world scale (Kabata-Pendias, 2007).

Comparing values from Krapan and Raša with those from Opatija, it is evident that some values are higher in the area of Krapan and Raša compared to Opatija (S, Se, Mo, Pb, Sr, Sn), but also vice versa (U, V, Cu, Cr, Ni, Fe, As, Mn).

Assessment of the toxicity of selenium is complicated by its occurrence in many different chemical forms (Ohlendorf et al., 2011). In the recent study of Raša Bay, results reported by Medunić et al. (2018b) point to necessity of new studies on selenium distribution in the environment, food, and animals in this area. Lettuce as a plant can be considered a good accumulator of metals in general, since the maximum concentrations of U, V, Pb, Cr, Sr, Fe, Sn, As, and Mn were found in lettuce (Table 3). Medunić et al. (2018a) also noted that lettuce proved to be a higher accumulator of selenium than the potato at the Raša study locality.

The highest obtained values for Se (14.3 mg kg^{-1}), V (64.1 mg kg^{-1}), Cu (140 mg kg^{-1}) are higher than those published by Medunić et al. (2018a) where maximum values of Se, V, and Cu in lettuce from Raša Bay were 2.53 mg kg^{-1}, 8.33 mg kg^{-1} and 12.8 mg kg^{-1}, respectively. Additionally, observed element concentrations differ significantly from those published by Stančić et al. (2016) where heavy metals were detected in vegetables from Varaždin City Market, especially for As (0.005 to 0.13 mg kg^{-1}), Cd (0.01 to 0.12 mg kg^{-1}), Mn (0.2 to 5.4 mg kg^{-1}), and Pb (0.02 to 1.15 mg kg^{-1}). In this study, maximum concentrations for these elements in different vegetables were: As (6.96 mg kg^{-1}) in Opatija, Cd (2.07 mg kg^{-1}) in Krapan, Mn (368 mg kg^{-1}) in Opatija and Pb (22.1 mg kg^{-1}) in Opatija.

In some samples from the area of the Opatija, the concentrations of certain metals (As, Fe, Cr, Sr, and Ni) were higher than those in Raša and Krapan, which was unexpected considering that Opatija is an area without significant anthropogenic influence. A possible explanation is the proximity of regional pollutants (NE Italy) and local (oil industry in Rijeka, thermal power plant in Plomin) which leads to dry deposition of substances. According to the Agricultural Land Agency (2015), the leading role of the Primorje-Gorski Kotar County in carcinogenic diseases is the result of the highest soil load in Croatia with acid rain, i.e. deposits of nitrates, sulphates and heavy metals, and Gorski kotar is particularly endangered. According to the measurements of Vrbek and Pilaš (2001) at five localities in the continental and hilly-mountainous part of Croatia, most nitrogen, sulphur and chlorine are deposited in Gorski kotar (Lividraga), at an altitude of slightly more than 900 m. In this region, Miko et al. (2000) also identified areas polluted by air currents from the industry of NE Italy but also local industries (Petroleum Industry in Rijeka and TPP Plomin).

6.2. Health assessment and estimated daily intake

The importance of the various risks to the environment and humans posed by metals and metalloids is increasingly recognised. Covello and Merkhofer (1993) defined risk assessment as a systematic process for describing and quantifying risk associated with hazardous substances, processes, actions and events. In the context of environmental risk assessment, researchers apply different indices. Human health risk assessment is a model for calculating metal exposure in the urban environment. However, the calculation of the risk due to exposure to potentially toxic soil elements can be influenced by several uncertainties. In this study, exposure factors and parameters for health risk assessment were derived from the

USEPA manual (USEPA, 2011), which is valid for the USA and may not be suitable for risk assessment in Croatia, although it is used in many countries such as Poland, Spain, China and India. EPA has collected numerous data from various sources that can be used in the calculation of human health risk assessment and all data related to potential contaminants can be found on the website https://www.epa.gov.

Heavy metals in urban areas come from various sources such as car exhaust, various industries and similar activities. Essential micronutrients such as Zn and Cu or toxic elements such as Hg, Cd and Pb in the environment can accumulate to elevated toxic concentrations which then lead to environmental disasters (Zheng et al., 2007). For example, recent studies have been conducted on soil, air, dust and water, human hair, nails and serum (Mohmand et al., 2015), but also on food available in supermarkets (Xia et al., 2010). By calculating the hazard quotient (HQ), estimates can be made for many situations.

In this study, a risk assessment analysis for the following elements (As, Cd, Cr, Cu, Fe, Mn, Ni, Se, Sn, Sr, U and V) in vegetable samples from the area of Raša, Krapan and Opatija was made. The average body weight used was 65 kg while 174 g (FW) was taken as the daily dose of vegetable intake according to EFSA Europa (2011) and converted to (DW) using a conversion factor of 0.085 (Sajjad et al., 2009). For no element was the HQ greater than 1 which means that there is no risk of carcinogenic effect on the health of people consuming the analyzed vegetables. The closest value to 1 was calculated for As (0.67) in the sample of radicchio leaf from Opatija 4 sampling site.

Estimated Daily Intake (EDI) of eleven elements (As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se and Zn) in different vegetables from Raša, Krapan and Opatija was calculated. The average body weight used for calculation was 65 kg, while 174 g (FW) was taken as the daily dose of vegetable intake (EFSA Europa, 2011) but was converted to DW using the conversion factor 0.085 (Sajjad et al., 2009). Future studies should include surveying residents about their eating habits so that we can get the real picture of the dangers of consuming contaminated vegetables. All analysed elements except Zn showed higher values than MTDI in vegetables not only from Raša and Krapan, but also from Opatija. EDI of Fe was significantly above MTDI value (15 mg day^{-1}; Basha et al., 2014), since the values in most samples from Raša, Krapan and Opatija range from 30.0 to 282 mg day^{-1}. EDI od Mn was also increased in almost all samples with the highest value (18.3 mg day^{-1}) in radicchio leaf from Opatija 1 and Opatija 4 sampling sites. EDI of Se was higher than MTDI (0.4 mg day^{-1}) for vegetables in three samples: garlic bulb from Krapan 5 (0.42 mg day^{-1}), garlic bulb from Raša 1 (1.52 mg day^{-1}), lettuce leaf (0.54 mg day^{-1}) and radicchio leaf (0.63 mg day^{-1}) from Raša 2. Medunić et al. (2020) calculated EDI for Se for mixed Raša vegetables (0.055 mg day^{-1}) which was almost equal to the Recommended Dietary Allowance (RDA) for adults (Institute of Medicine, 2000). Another Croatian study, carried in the Eastern Croatia, found that the average daily Se intake in the study area was only 0.027 mg day^{-1}, as a consequence of low environmental Se levels there. However, the authors noted that there was no evidence of health problems connected to low Se status though (Klapec et al., 1998). EDI of other elements were elevated but not markedly significantly above their MTDI.

Various agencies, both in the world and in Croatia, should encourage harmonized data collection, research, legislation and regulations. Measurements of exposure to heavy metals are necessary to protect plant and animal populations and humans. Furthermore, the possible exposure and vulnerability of children and adults should be taken into account when setting acceptable levels or criteria for element concentrations (Morais et al., 2012).

7. CONCLUSION

This study showed that the soil of Krapan and Raša is contaminated with Cd, Mo, Pb and Se. In vegetables grown on that soil, high concentrations of As, Cu, Mn, Pb, V and Se were recorded. Surprisingly, some vegetable samples from Opatija showed higher levels of trace metals compared to those from Raša and Krapan.

Most of the values from Raša and Krapan were increased compared to previously published data. Sulphur and selenium stand out again, which proves the lasting influence of TPP Plomin, which has used Raša coal as a raw material for years.

The hazard quotient for the oral route of uptake was used to assess human health risk for the analysed elements in vegetables, but all values were below 1 indicating that there is no risk of chronic carcinogenity in adults.

Estimated Daily Intake (EDI) of trace metals in vegetable samples from Krapan, Raša and Opatija showed that consumption of analysed vegetables may pose a health risk to the consumers. Due to the appearance of elevated concentrations of certain metals in Opatija, the causes should be investigated in more detail.

The investigation of Raša Bay should also be continued since environmental contamination remains persistent.

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