Mosses as a bioindicator of heavy metal pollution

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MOSSES AS A BIOINDICATOR OF HEAVY METAL POLLUTION

MASTER'S THESIS

Sven Bogdan

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Master's thesis written by **Sven Bogdan**, JMBAG 1003088425, under the title of
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For their advices, their patience, their faith,

Because they always understood.

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Summary

Of the master's thesis written by Sven Bogdan, under the title of

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Mountains are considered as one of the most sensitive ecosystems to climate changes which may be affected at a very faste rate. These changes may directly and/or indirectly affect organisms, thus native organisms may be used as indicators of different environmental alterations. Mosses are considered as effective air quality bioindicators because they have no roots, no cuticle, and acquire all their nutrients from direct exposure to the atmosphere. Thus, in this study mosses are used as an indicator of trace elements air pollution. Samples of *Brachythecium rutabulum* and *Hylocomium splendens* were collected during dry and rainy period in 2016 from 5 sampling sites in the Risnjak National Park. Cd, Cr, Pb and Zn concentrations in moss tissue were analysed by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES Vista MPX AX, Varian), and Hg with Cold Vapor Atomic Absorption Spectrophotometry (FIMS 400, Perkin-Elmer). The concentrations were in range: Cd from 0.2 to 0.58 mg kg^{-1}, Cr from 0.5 to 27 mg kg^{-1}, Hg from 0.036 to 0.136 mg kg^{-1}, Pb from 3.0 to 8.2 mg kg^{-1} and Zn from 8.6 to 82 mg kg^{-1}. Higher concentrations were measured in *B. rutabulum* and the samples collected in rainy season at higher altitude. Compared to the results from recent biomonitoring studies of trace metal pollution in the mountain ecosystems, deposition of atmospheric Cd, Cr, Hg, Pb and Zn in mosses in Risnjak National Park may be considered as minor to negligible.

Keywords: air quality, heavy metals, biomonitoring, Risnjak National Park

Sažetak

Diplomskog rada studenta Svena Bogdana, naslova

MAHOVINE KAO BIOINDIKATORI ONEČIŠĆENJA TEŠKIM METALIMA

Planinski ekosustavi smatraju se među najosjetljivijima na klimatske promjene te se taj utjecaj može očitovati i vrlo intenzivno. Svaka promjena u ekosustavima može izravno i/ili neizravno utjecati na organizme koji nastanjuju to područje te se stoga određeni organizmi mogu koristiti kao pokazatelji istih. Mahovine se smatraju učinkovitim bioindikatorima kakvoće zraka jer nemaju korijen i sve svoje hranjive tvari apsorbiraju izravno iz atmosfere. Stoga su u ovom istraživanju mahovine odabrane kao pokazatelj onečišćenja zraka metalima. Uzorci vrsta *Brachythecium rutabulum* i *Hylocomium splendens* prikupljeni su tijekom suhog i kišnog razdoblja 2016. godine s 5 mjesta u Nacionalnom parku Risnjak. Koncentracije Cd, Cr, Pb i Zn u tkivu prikupljenih mahovina analizirane su induktivno spregnutom optičkom emisijskom spektrometrijom (ICP-OES Vista MPX AX, Varian), dok je Hg određena metodom atomske apsorpcijske spektrofotometrije (FIMS 400, Perkin-Elmer). Koncentracija metala u tkivu mahovina varirale su u rasponima: Cd od 0.2 do 0.58 mg kg^{-1}, Cr od 0.5 do 27 mg kg^{-1}, Hg od 0.036 do 0.136 mg kg^{-1}, Pb od 3.0 do 8.2 mg kg^{-1} i Zn od 8.6 do 82 mg kg^{-1}. Više koncentracije izmjerene su u tkivu vrste *B. rutabulum* i uzorcima prikupljenim u kišnoj sezoni na višoj nadmorskoj visini. U usporedbi s rezultatima nedavnih studija biomonitoringa kvalitete zraka na planinskim područjima, taloženje atmosferskog Cd, Cr, Hg, Pb i Zn u mahovinama u Nacionalnom parku Risnjak može se smatrati minimalnim do zanemarivim.

Ključne riječi: kvaliteta zraka, teški metali, biomonitoring, Nacionalni park Risnjak

1. Introduction

Metals occur naturally in rocks and soils, but increasingly higher quantities of metals are being released into the environment by anthropogenic activities. Metals are chemically very reactive in the environment, which may result in their mobility and bioavailability to living organisms. People can be exposed to high levels of toxic metals by breathing air, drinking water, or eating food which contains them. Terms like heavy metals, metalloids and microelements are the most commonly encountered in ecological studies. Among the 96 known metals, 17 are semimetals or metalloids (B, Si, Ge, As, Sn, Te, Po ...) while the term heavy metal refers to a group of 53 metals with density higher than 5 g/cm^3 (Zovko and Romić, 2011).

The metals observed in this research cadmium (Cd), chromium (Cr), mercury (Hg), lead (Pb) and zinc (Zn) have been described by Halamić and Miko, (2009):

- Cadmium is a rare chalcophile trace element, 65th in abundance in the Earth's crust. In its characteristics, it is very similar to zinc. Cadmium mobility in the environment depends heavily on the pH value of the surrounding medium. It is resistant to atmospheric precipitations and other influences. It is dissolvable in acids; less so in an oxidizing, but more easily in acids that have oxidizing activity. Cadmium is brought into the environment through waste generated by disintegration of paints and protective substances.
- Chromium is a lithophile trace to accessory element, characteristic of high-temperature oxides. It is associated with Mg, Fe, Co, and Ni. It is an indicator of ultrabasic and basic rocks. It occupies 21st place in abundance in the Earth's crust. Th e mobility of chromium is low. The largest amounts of chromium occur in minerals that are not easily weathered (chromite, magnetite, ilmenite). Th is leads to its abundance in heavy mineral fraction in sands. It pollutes the environment mainly through industrial wastewaters (galvanization, textile and leather industry).
- Mercury is a trace element which occurs as native in the form of impregnation, but mostly as sulphide. Together with zinc and cadmium, mercury forms group 12 (IIB) of the Periodic System. It is the only naturally occurring mineral that is in liquid state under optimal conditions. Its main characteristics are that it is extremely halcophile and very mobile, as a result of a low melting point. Mercury is highly toxic, for both plants and animals. Its dispersion into the environment (especially in water) is mostly through the chemical industry waste.
- Lead is a trace oxiphile element and 36th in abundance in the Earth's crust. Its concentrationrises with the increase in magma alkalinity. In rock-forming alumosilicates it is associated with potassium as the main element, and is disguised by calcium. The mobility of lead in the environment is considered to be low. Sulphide ores oxidize into less soluble sulphate, carbonate, arsenate, vanadate, molybdate, and chromate. The mobility of Pb is limited by its

tendency to adsorption onto Mn-Fe oxy-hydroxides, clay minerals, and insoluble organic matter, and enhanced by formation of soluble organic complexes and anionic complexes. During metamorphism lead can be released and migrate.

- Zinc is an oxichalcophile trace element from the group of transitional elements. Zinc is relatively mobile at low pH values ($pH \leq 4.5$). During weathering, sulphide ores form easily soluble sulphates, poorly soluble carbonates, as well as hydrated silicates. Zinc is readily adsorbed onto Fe-Mn-oxihydroxides, clay minerals, and organic matter. In stream sediments, it amasses in the fi ne-grained fraction. Zinc is dispersed into the environment through pigments of paints, zinctreated iron, waste batteries, wastewaters, forges, old slag (cinder) on river banks etc.

Effects of pollutants in the environment may be estimated by using bioindicator organisms which reflect the state of the environment and are suitable for indicating pollution according to their occurrence, absence or presence, frequency, distribution, abundance, vitality, reactions and changes in responses under certain environmental conditions (Vargha et al., 2002).

Mosses are commonly used as indicators of heavy metal pollution because of their specific morphological and physiological properties (Tyler, 1990; Grodzińska and Szarek-Łukaszewska, 2001; Zechmeister et al., 2003):

- they have no epidermis or cuticle – cell walls are easily penetrable by metal ions;
- they have no organs for the uptake of minerals from the substrate – they obtain minerals mainly from precipitation and dry deposition;
- they accumulate metals in a passive way, acting as ion exchangers.

The unique positions of Risnjak National Park and the Velebit protected area, on the geomorphological boundary between the coastal Mediterranean part of Croatia and the continental hinterland have evidently caused a considerable atmospheric fallout impact on their soil cover. The sources of atmospheric pollution can be both local and regional, but in a case of Risnjak National Park, due to its unique location, local source of atmospheric pollution is hardly possible. The geomorphological boundary extends along the Dinaric mountain chain that arches along the Adriatic coast and as such, it is the first barrier to atmospheric pollutants from the whole north eastern part of Italy (Miko et al., 2000). During the last three decades the forests of this region have been declining due to the dry deposition of the acids under the influence of industrial and other soil, water and air pollution (Prpić, 1987; Prpić et al., 1991).

2. Aims and objectives

1. To evaluate the atmospheric deposition of the heavy metals (Cd, Cr, Hg, Pb and Zn) by using *Hylocomium splendens* species of pleurocarpous mosses, which natively grows in the Risnjak National Park (Croatia).

2. To evaluate the atmospheric deposition of the heavy metals (Cd, Cr, Hg, Pb and Zn) by using *Brachythecium rutabulum* species of pleurocarpous mosses, which natively grows in the Risnjak National Park (Croatia).

3. Literature overview

3.1. Heavy metals – definitions

Heavy metals are defined as metallic elements which have a relatively high density compared to water (Fergusson, 1990). Over the past two decades, the term "heavy metals" has been increasingly used in numerous publications and legislature related to chemical hazards and their safe use. The term is often used as a group name for metals and semimetals (metalloids) which have been associated with contamination and potential (eco)toxicity (Duffus, 2002).

The term trace elements describes elements present in low concentrations (1mg/kg or less) in ecosystems. Certain trace elements, including copper (Cu), zinc (Zn), manganese (Mn), iron (Fe), molybdenum (Mo), and boron (B), are essential for plant growth, thus considered as micronutrients. Except for B, these elements are also heavy metals, and may become toxic for plants at high soil concentrations. Some trace elements, such as cobalt (Co) and selenium (Se), are not essential for plant growth, but are required in small concentrations for certain physiological processes in animal and human organism. Other trace elements, such as cadmium (Cd), lead (Pb), chromium (Cr), nickel (Ni), mercury (Hg) and arsenic (As), have toxic effects on organisms and are often considered as contaminants (He et al., 2005).

3.2. Heavy metals in the environment

Although heavy metals are naturally occurring in soils and parent material and may be found throughout the Earth's crust, anthropogenic activities such as mining and smelting operations, industrial production and use, domestic and agricultural use of metals and metal-containing compounds are resulting in contamination of the environment, as well as human exposure to higher metal concentrations (Figure 3.2.1.) (Goyer and Clarkson, 2001). According to Begun and Huq (2016) these activities may be divided into point (located near the contaminated site) and non-point sources (contamination is a process of atmospheric transport and deposition) of metal contamination from which heavy metals are being released into the environment.

Environmental contamination can also occur because of metal corrosion, atmospheric deposition, soil erosion, leaching of heavy metals, sediment re-suspension and metal evaporation from water resources to soil and ground water (Nriagu, 1989).

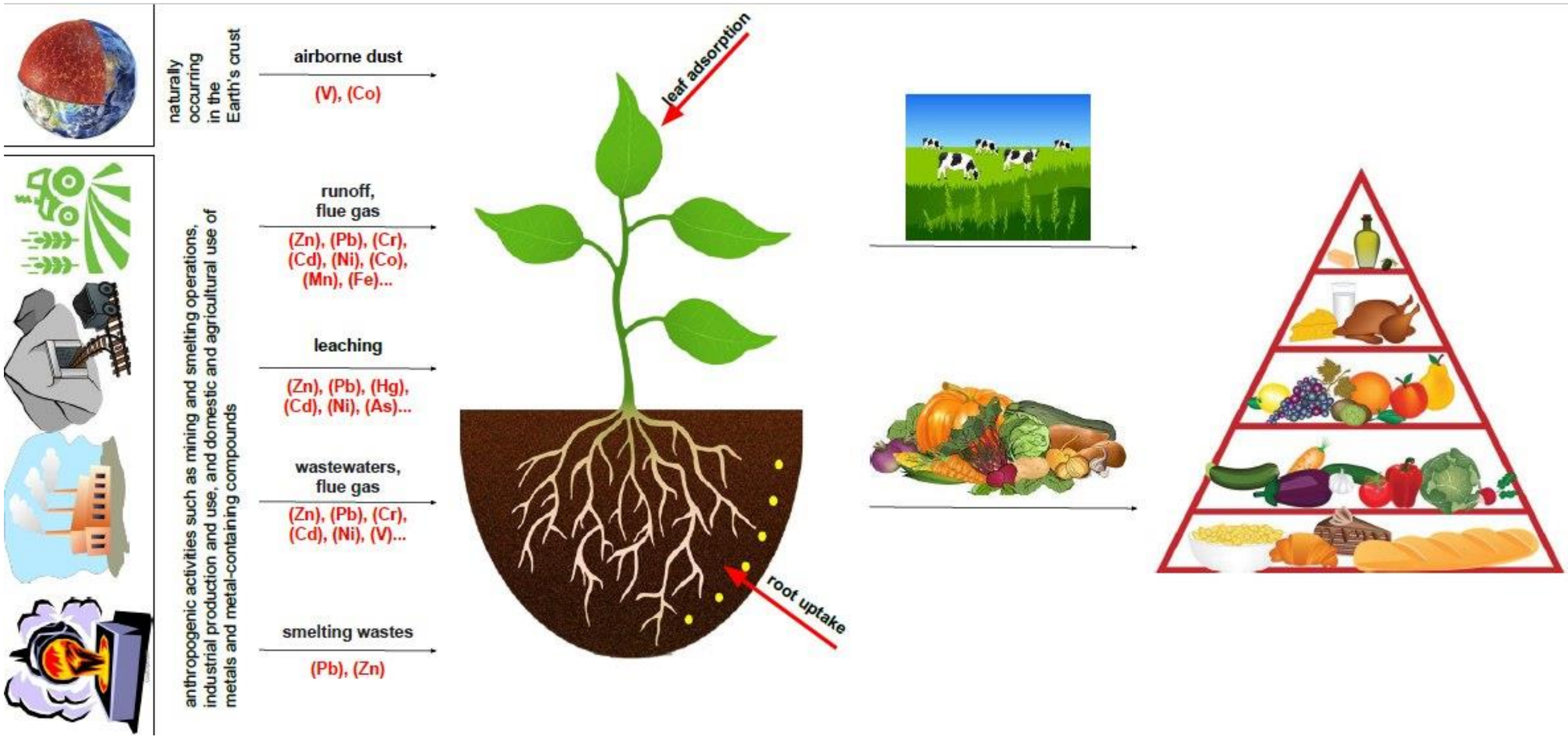

Figure 3.2.1. Heavy metals pathways into / throughout the food chain.

In recent years, there has been a growing concern associated with the environmental contamination by heavy metals. Also, human exposure has increased dramatically as a result of their elevated use in several industrial, agricultural and domestic applications. Therefore, numerous research has been focused on the human exposure to heavy metals, their potential sources and possible pathways of heavy metals transfer into the food chain (Begun and Huq, 2016).

3.2.1. Heavy metal transport in the environment

Heavy metals are involved in the *biogeochemical cycle*: the flow of the trophic chain elements in which the first link is a plant, the second link is entering an animal organism, and the last one is represented by metal moving to the next cell,often resulting in an increased cell concentration and their partial accumulation in the cell (Karczewska et al., 2001).

The amount of *heavy metals taken by plant root system from soil* mainly depends on the degree of individual element accumulation in soil and the soil adsorption complex immobilization capacity. Adsorption capacity of the soil is determined by the quantity and quality of soil colloids forming absorbent complex. As the soil pH and the content of floatable particles (mostly colloidal clay) and organic matter (especially decay) increase, the availability of heavy metals to plants is reduced (Hao and Chang, 2002).

Local point sources of contamination are those usually located near the contaminated site and often may be rather easily recognized as a source of the heavy metal pollution, e.g. pipes, ditches, ships or factory smokestacks (Hill et al., 1997).

On the other hand, *long-range atmospheric transport* of contaminants is a process of their atmospheric transport and deposition (Moser et al., 2002). Many anthropogenic contaminants such as industrial organics, pesticides and trace metals have become widely distributed around the globe. Even the most remote areas may not be out of range of contaminants emitted from distant anthropogenic sources. Any of these airborne contaminants such as carbon monoxide, ammonium chloride, asbestos and others are persistent in the environment, thus may become (eco)toxic, bioaccumulate and/or remain potentially harmful for organisms for long periods of time.

According to Shinn et al. (2000), Wang et al. (2007), Wang et al. (2013), Fang et al. (2012) and Leili et al. (2008) dust plays an important role in the atmosphere due to its potentially harmful effects on human health, environment and the climate conditions. *Airborne dust* is considered as a significant issue in carrying and distributing pathogens, pollutants and heavy metals.

Air emissions may generate multiple pathways of exposure and thus their assessment, which extends from the evaluation of the direct inhalation of contaminants (derived from air dispersion and deposition) to a variety of indirect pathways which include deposition of contaminants to soil surface, water and vegetation and their subsequent transfer and accumulation in the food-chain (De Lurdes Dinis and Fiúza, 2011). Higher rate of precipitation may affect the deposition of substances, in which case heavy metals are present in the atmosphere in the form of aerosols. Most of the trace elements are introduced into atmosphere in the form of fine aerosols by human activities and after travelling some distance are deposited in the natural way/or are scavenged out with rain, fog, etc. in both, proximate and remote areas (National Academy of Science, 1992). Concentrations of heavy metals in the rain water depends on several factors, such as vicinity of the sources, the amount of the precipitation and the direction of air masses (Koulousaris et al., 2009).

3.3. Impact of climate change on heavy metals fate in the environment

Definition of a climate change given by the United Nations Convention on Climate Change is: "a change of climate which is attributed directly or indirectly to human activity that alters the composition of the global atmosphere and which is in addition to natural climate variability observed over comparable time periods.". According to Easterling et al. (2000), the biggest concern about a climate change is an increase of the extreme events, together with the changes in total precipitation (Figure 3.3.1.).

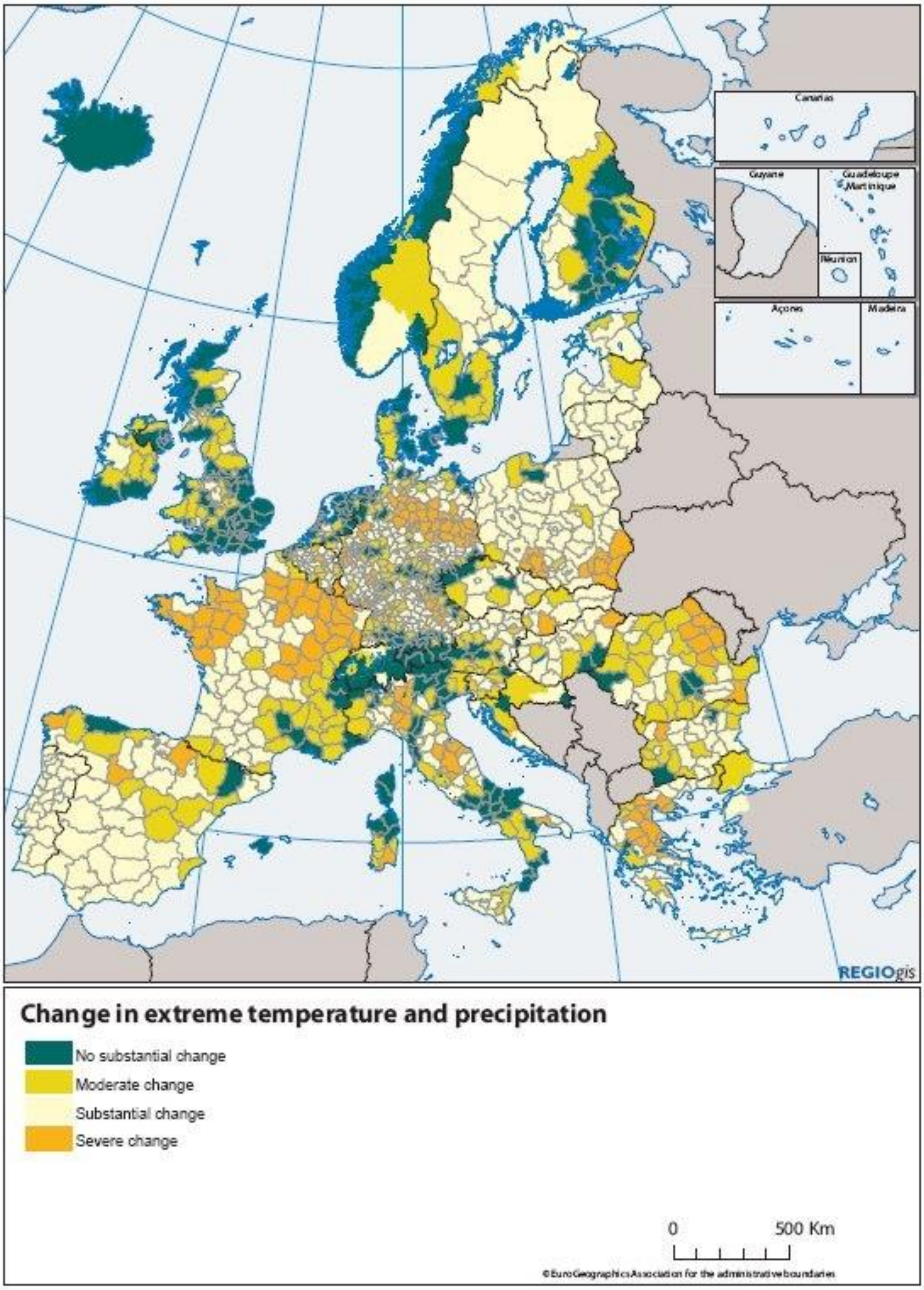

Figure 3.3.1. Change in extreme temperature and precipitation by 2080s relative to the period 1961 – 1990 under the A2 scenario reported by IPCC (1995).
Source: Dankers and Hiederer (2008)

Observations of surface temperature show that over the past one hundred years there has been a global mean warming of 0.3 – 0.6°C. The observed trend of a larger increase in minimum than the maximum temperatures is apparently linked to the associated increases in low cloud amount and aerosol, as well as to the enhanced greenhouse effect. There is a good evidence for decadal changes in the atmospheric circulation which contribute to regional effects, and some evidence for ocean changes. Changes in precipitation and other components of the hydrological cycle vary considerably geographically. Changes in climate variability and extremes are beginning to emerge, but global patterns are not yet apparent (Intergovernmental Panel on Climate Change, 1995).

A2 and B2 (IPCC, 1995) scenarios which are alternative images of how the future might unfold and are an appropriate tool with which to analyse how driving forces may influence future emission outcomes and to assess the associated uncertainties in climate change analysis, including climate modeling and the assessment of impacts, adaptation, and mitigation. The A2 storyline and scenario family describes a very heterogeneous world. The underlying theme is self-reliance and preservation of local identities. Fertility patterns across regions converge very slowly, which results in continuously increasing global population. Economic development is primarily regionally oriented and per capita economic growth and technological change are more fragmented and slower than in other storylines. The B2 storyline and scenario family describes a world in which the emphasis is on local solutions to economic, social, and environmental sustainability. It is a world with continuously increasing global population at a rate lower than A2, intermediate levels of economic development, and less rapid and more diverse technological change than in the B1 and A1 storylines. While this scenario is also oriented toward environmental protection and social equity, it focuses on local and regional levels. Modelling results (IPCC, 1995) show that the annual mean temperature in Europe is likely to increase more than the global mean temperature. Until the end of this century the average annual temperature in Europe is projected to increase for 2.5 – 5.5°C for the A2 scenario, and 1 – 4°C for the B2 scenario. Some regions may experience lower or higher temperature increases than the average. For the A2 scenario, temperature increase in some regions of Europe may be as low as 2°C or even higher than 7°C in both scenarios. Southern Europe will be the most affected part of Europe, with consistent temperature increases between 3°C and more than 7°C, or even greater warming during the summer. Northern Europe will experience temperature increases by less than 2°C and up to 4°C, depending on the scenario and the region, with mainly winters getting less cold. Temperature extremes will decrease in the winter, but increase in the summer. Annual average precipitation will increase in northern and North Central Europe, while it will decrease in Southern Europe. Annual precipitation patterns will also change. Southern Europe will experience less rainfalls compared to former average all year round. There will be less precipitation during summer time in Atlantic and Continental Europe, but more winter precipitation. Decreases in annual average precipitation in Southern and Central Europe can be as high as 30 – 45%, and as high as 70% in the summer in some regions. As a result of this, and warmer summer temperatures, the risk of summer drought is likely to increase in Central Europe and in the Mediterranean area (IPCC, 2007).

In the cold part of the year, the warming will slightly increase in the Northern (Continental) Croatia, while in the warm periods the warming will increase in the littoral Croatia. The decrease of total precipitation in future climate is expected in the large part of the year, primarily in the littoral Croatia and its hinterland. Such a decrease is, in relative terms, highest in summer because of pronounced climatological minimum in the annual cycle for total precipitation in this part of Croatia. In winter there will be a slight increase of precipitation, again in a narrow littoral zone, but such increase is not statistically significant. In the Northern Croatia no significant precipitation change in future climate is expected (Branković et al., 2009).

When talking about climate change, special attention needs to be given to ecosystems on higher altitudes. According to Rangwala and Miller (2012), it remains difficult to sufficiently assess whether mountains have warmed at a higher rate than the rest of the global land surface, primarily because we lack adequate observations to resolve it conclusively. However, available observations suggest that certain mountain regions may be experiencing higher warming rates on a seasonal time scale. On the other hand Gilbert and Vincent (2013) found the mean warming rate of 0.14°C/decade between 1900 and 2004 in high altitudes, similar to the observed regional low altitude trend in the northwestern Alps, suggesting that air temperature trends are not altitude dependent. Benisto et al. (1995) have studied the same subject from another perspective and in their paper ''Climatic change at high elevation sites'' concluded: "Mountains are also a key element of the hydrological cycle, being the source of many of the world’s major river systems. Shifts in climatic regimes, particularly precipitation, in space or seasonally in a changing global climate, would impact heavily on the river systems originating in mountain areas, leading to disruptions of the existing socio-economic structures of populations living within the mountains and those living downstream. "

3.4. Monitoring of heavy metals pollution

Environmental monitoring is a set of procedures and activities with an aim to monitor the quality of the environment. Commonly used environmental monitoring approaches are: air quality monitoring, soil quality monitoring and monitoring by using plants.

Biomonitoring by using plants has been shown in several countries as a cost-effective tool for assessing trace element atmospheric deposition (Rühling and Tyler, 1968; Steinnes, 1980; Zechmeister et al., 2003; Wolterbeek et al., 2003; Harmens et al., 2004).

Markert et al. (2002) agreed upon the following terminology :

- *Bioindicator* is an organism (or a part of an organism or the community of organisms) which contains the information about the quality of the environment (or a part of the environment).
- *Biomonitor* is an organism (or a part of an organism or the community of organisms) which contains the information about the quantitative aspects of the quality of the environment. A biomonitor is also always a bioindicator, but a bioindicator does not necessarily meet the requirements for a biomonitor.
- *Biomonitoring* is a continuous observation of an area with the help of bioindicators, e.g. by repeated measurement of their responses in a manner that reveals changes over space and time (for example: measuring the xenobiotics uptaken by plants). Active biomonitoring is characterized by bioindicators (biomonitors) bred in a laboratory or collected at a selected site which are then exposed in a standardized manner in an investigated area for a defined period of time. Passive biomonitoring is characterized with the examination of in-situ occurring organisms for their reactions.

Mosses and lichens are often used as bioindicators, mostly because both have a lack of roots and therefore obtain most of their nutrient supply directly from the precipitation and dry deposition of airborne particles and have no or only a very reduced cuticle so that ions retained on their surface have a direct access to the exchange sites on the cell walls (Garty, 1993; Zechmeister et al., 2003; Smodiš and Parr, 1999).

According to Galuszka (2006), because of their diversity of habitats, structural simplicity and rapid multiplication rate, mosses can be a useful tool for prospective research of the conditions and characteristics of the environment, and they are ideal to evaluate pollution in both, field and laboratory studies. There are a large number of species of mosses that have been used to determine the presence of pollutants including heavy metals and radioactive materials.

Several researches reported the use of different moss species as bioindicators of metal atmospheric deposition, such as *Pleurozium schreberi* (Markert et al., 1994; Galsomies et al., 1999; Grodzinska and Szarek-Lukaszewska, 2001; Galuszka, 2006), *Scleropodium purum* (Markert et al., 1994; Fernández et al., 1999; Galsomies et al., 1999; Fernández and Carballeira, 2001),*Hylocomium splendens* (Steinnes et al., 1994; Berg et al., 1995; Galsomies et al., 1999; Lucaciu et al., 2004; Galuszka, 2006), *Plagiothecium denticulatum*, *Bryum argentum* and *Sphagnum sp* (Gupta, 1995), *Hypnum cupressiforme* (Fernández et al., 1999; Galsomies et al., 1999; Fernández and Carballeira, 2001; Frontasyeva et al., 2004; Lucaciu et al., 2004; Pepi et al., 2006), *Thuidium tamariscinum* (Galsomies et al., 1999), *Brachytechium salebrosum* and *Brachytechium rutabulum* (Lucaciu et al., 2004), *Polytrichum formosum* (Markert and Weckert, 2008) and *Sphagnum girgensohnii* (Aničić et al., 2009).

According to the categories of the main source of pollution elements detected in *Hylocomium splendens* may be divided in a following way, (elements in the brackets also may originate from some other source) (Schaug et al., 1990; Steinnes et al., 1994; Berg et al., 1995):

- Long-range atmospheric transport: V, (Cu), Zn, (Ga), As, Se, Mo, Ag, Cd, Sn, Sb, Hg, TI, Pb, Bi.
- Local point sources: Co, Ni, Cu.
- Natural cycling processes, mainly atmospheric transport of seasalt and biogenic emission from the marine environment: (Li), B, Na, Mg, CI, Ca, Se, Br, Sr, I.
- Root uptake in vascular plants from soil and a subsequent transfer to the mosses by leaching from living or dead plant material: (Mg), (Ca), Mn, (Cu), (Zn), Rb, (Sr), Cs, Ba.
- Mineral particles, mainly windblown soil dust: Li, AI, (Ca), Sc, Ti, (V), Cr, Fe, (Co), Ga, Ge, Rb, (Sr), Y, Zr, Nb, La, Ce, Pr, Nd, Sin, Eu, Gd, Tb, Dy, Ho, Er, Tin, Yb, Lu, Hf, Ta, Ga, Th, U.

In addition, the following mechanisms can affect the metal concentration in mosses:

- Transport of soluble compounds from the soil into the moss tissue during the periods with extensive soil/ water contact, particularly during snowmelt. Although mosses do not have a root system, influence from this source cannot be disregarded, and particularly in areas with low atmospheric deposition (Ford et al., 1995).
- Ion exchange of metals with sea-salt cations and H^+ from acidic precipitation. In coastal areas far from local pollution sources elements in mosssuch as Br and Se, are likely to be originating mainly from this source (Gjengedal and Steinnes, 1990).
- Internal redistribution of elements within the moss plant the "vascular pump": root uptake of elements into higher plants (especially trees) and a subsequent leaching onto the moss from living or dead plant tissue (Brown and Brumelis, 1996).

The relative contribution from these additional sources will differ substantially among the elements. Table 3.4.1. presents the experience from the Norwegian moss surveys with the respect to the relative influence from different source categories for 15 most frequently studied elements in relation to the environmental pollution. From the Table 3.4.1. may be seen that the deposition of Mn cannot be studied by the moss method. Also, for elements such as Fe and Zn the contamination level must be very high in order to obtain accurate results (Steinnes et al., 1994).

Table 3.4.1. Different source factors relative influence on the elemental composition of mosses growing in rural and remote areas.
Source: Steinnes et al. (1994)

Element	Air pollution	Marine Factor	Vegetation factor	Soil factor
Al				+++
V	++			+
Cr	+			++
Mn		- -	+++	
Fe	+			+++
Ni	++			++
Cu	++		+	
Zn	++	-	++	
As	+++	-		+
Se	++	++		
Br		+++		
Sr		+++		+
Cd	++		+	
Sb	+++			
Hg	++			+*
Pb	+++			

+, ++, +++ : positive contribution, increasing importance
-,- -, - - - : negative contribution (removal), increasing importance
*Gaseous emission of HgO from the soil may be absorbed in the moss

Samecka-Cymerman et al. (2009) used the *B. rutabulum* moss species for the biomonitoring and the results indicated that the *B. rutabulum* may be a suitable ecological indicator of metal pollution originating from chlor-alkali industry.

4. Materials and methods

4.1. Site description

The survey was carried out in Risnjak National Park (45°25´42´´N, 14°44´42´´E), in Gorski kotar (Figure 4.1.1.). Gorski kotar is the most mountainous and heavily forested region of the country, about 15 km inland from the Adriatic Sea. Risnjak National Park and the Velebit protected area have the unique positions on the geomorphological boundary between the continental and Mediterranean part of Croatia.

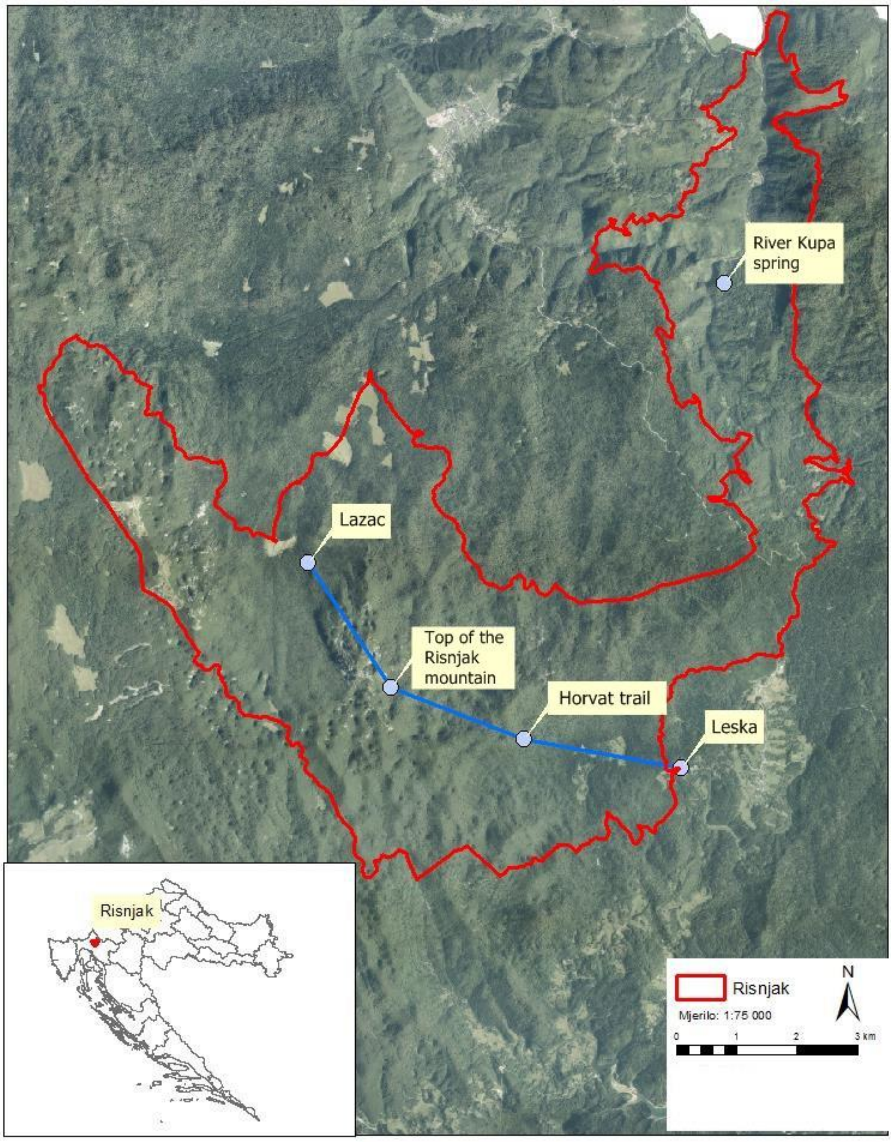

Figure 4.1.1. Locations of research in the area of Risnjak National Park and location of Risnjak National Park in the Republic of Croatia. The border of Risnjak National Park is marked by a red line.
Source: Zovko et al. (2017)

Risnjak National Park extends over 64 sq. km, including the massifs of Risnjak (1528 m) and Snježnik (1506 m), parts of the western branch of the Dinaric Mountains, and the source area of the Kupa River up to the village Hrvatsko (287 m). The area of the Risnjak massif was proclaimed a national park in 1953, being extended in 1997 to Snježnik and the source of the Kupa River (Ozimec et al. 2010).

According to Bajić and Peroš (2005) dominant wind directions for the nearest meteorological station Ogulin in period from 1995 – 2002 were west and northwest winds with maximum measured wind gusts of 26.4 m/s (Figure 4.1.2.).

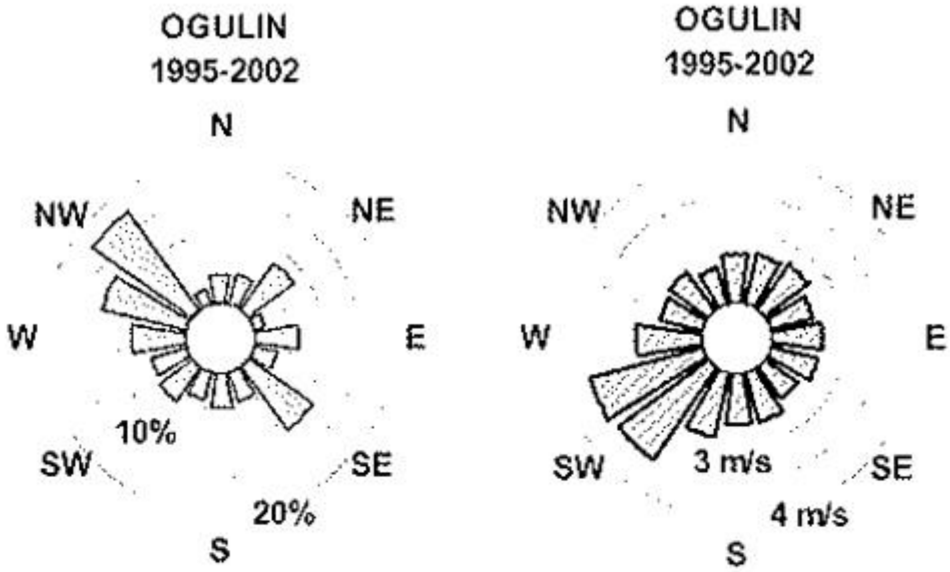

Figure 4.1.2. Relative frequency of wind direction (left) and average wind speed for each wind direction (right) for the meteorological station Ogulin in period 1995 – 2002.

Source: Bajić and Peroš (2005)

According to National Park Risnjak Management Plan from 2007 main forest vegetation types are beech forests, fir-tree forests, spruce forests with moss, mountain spruce forests with gavel, sub-mountain juniper forests, sub-mountain beech forests, prefabricated spruce forests with snow moss, snowdrifts with gooseberries, shrubbery hornbeam, beech forest with dead shrubbery, black hornbeam forest, hornbeam and beech forest, beech forest with fern, floodplain grey willow forest and black and white alder forest.

According to data from the meteorological station in Lividraga (939 m), the climate is perhumid and moderately cold. Mean annual temperature is 5.4 °C, the lowest in January (– 2.0 °C) and the highest, 14.2 °C, in July. Rainfall average is 3770 mm per year, with the highest amount occurring in November (488 mm), and the lowest in August (166 mm). Air humidity is high (94%). Due to the prevailing limestone bedrock a typical karst relief with its specific morphology has developed (Ozimec et al. 2010).

4.2. Field sampling

Samples of two different pleurocarpous mosses, *H. splendens* and *B. rutabulum*, were taken on five different altitudes in the forest ecosystems of Risnjak National Park. These two types of moss were selected because they were considered as suitable for later laboratory analysis, as well as their presence was abundant, so sampling of sufficient amount of samples was possible at all locations. Locations were selected according to the differences in altitude, geographic position and exposure and dominant wind directions (Table 4.2.1.). Sampling of mosses was carried out two times in 2016 taking into the account the amount of rainfall. First sampling was done in June 2016 which was considered as the end of the rainy season, and the second was carried out in August 2016, considered as the end of the dry season.

Table 4.2.1. Description of selected research locations in Risnjak National Park.

Location of field researches	Mark of the location and of the associated soil sample	Elevation (m)	Coordinates	Land cover / topography	Dominant type of mosses
River Kupa spring	M2, T2	325	5476175, 5038548	valley	*H. splendens*
Leska	M1, T1	694	5475606, 5030507	beech and fir forest with spruce / a small valley	*Asperula odorata, Sanicula europea*
North side of the mountain Risnjak – Lazac	M5, T5	1058	5469375, 5033803	moss (20% of cover), beech leaf litter, spruce needles	*H. splendens*
South side of the mountain Risnjak – Horvat trail	M3, T3	1094	5472986, 5030943	beech leaf litter / sinkhole	*B. rutabulum*
Top of the Risnjak mountain – Snježnik	M4, T4	1418	5470776, 5031760	grass vegetation / mountain top	*B. rutabulum*

4.2.1. Sampling of mosses for heavy metal analyses

Whenever it was possible the location of the moss sampling was at least 3 meters away from the higher trees in order to prevent possibly higher heavy metals concentrations due to potential leaching of contaminants from tree canopy. Mosses were sampled on the ground or on the surface of decaying stumps by avoiding the coarse contamination (litter, soil animals). Only the green parts of the moss which grow for two to three years and usually are less contaminated with soil were taken. A composite sample consisting of 3 sub-samples was taken for each species, *H. splendens* and *B. rutabulum*. Each sub-sample was packed separately in a plastic bag and appropriately marked.

4.2.2. Sampling of soil for heavy metal analyses

At the same time as the first moss sampling event, the soil samples (0 – 30 cm) were also collected at each moss sampling location. At each location, 2 kilos of soil surface layer were sampled, as well as the subsurface layer where possible. The samples were taken with the polished stainless steel probe (Eijkelkamp, Netherlands), marked and packed in plastic bags. To determine the exact sample coordinates, as well as for the better orientation in the field, the GPS Garmin type eTrax Vista device was used.

4.3. Analyses

4.3.1. Analyses of moss heavy metal concentrations

Moss samples were delivered to the Analytical laboratory of the Department of Soil Amelioration (University of Zagreb Faculty of Agriculture), where they were cleaned so that all the dead material and impurities were removed. Prepared moss samples were dried for 24 hours at 40°C. Total Cd, Cr, Pb and Zn concentrations in moss dry material were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) (ISO/DIS 22036:2008) on Vista MPX AX (Vista MPX AX, Varian, Palo Alto, Calif.) after extraction in HCl, HNO_3 and H_2O_2 in closed TFM vessels with automatic pressure and temperature control using microwave technique on MARS Xpress (CEM, Matthews, N.C.) (HRN ISO 11466: 2004). Hg concentration was determined by Cold Vapor Atomic Absorption Spectrophotometry (HRN ISO 16772:2009) in the same soil extracts.

4.3.2. Analyses of soil heavy metal concentrations

Soil samples were homogenized and dried at room temperature in the laboratory of the Department of Soil Amelioration (University of Zagreb Faculty of Agriculture). After the crushing of larger aggregates, soil was sieved through φ 2 mm. Each soil sample was divided into thirds: (i) one third of the sample was used as a sample in which standard chemical and physical indicators were examined, (ii) the second third was packed into plastic boxes as an archive sample, and (iii) the rest of the sample was sieved through φ 500 μm for the analyses of the soil chemical properties. Standard laboratory sieves DIN/ISO 3310 (Fritsch, Germany) were used. Preparation of soil samples was done according to the standard procedure for the preparation of soil samples for physical and chemical analyses (HRN ISO 11464: 2004).

In the soil fraction <2 mm, the following analyses were performed:

1) pH in 1 : 5 suspension of soil and: (i) H_2O, (ii) 0.01 M $CaCl_2$ and (iii) 1 M KCl, using the SCHOTT pH-meter Lab 870 (HRN ISO 10390:2004);
2) electrical conductivity (EC) in 1 : 5 suspension of soil and H_2O on SCHOTT conductometer Lab 970 (HRN ISO 11265:2004);
3) total $CaCO_3$ volumetrically after the 6 M HCl attack (HRN ISO 10693:2004);
4) the organic matter content using the modified Walkly-Black process (HRN ISO 14235:2004);
5) available phosphorus (P) and potassium (K) using the ammonium lactate (AL) method (Egner et al., 1960);
6) granulometric composition, fractions <0.002, 0.002-0.02, 0.02-0.05 , and 0.05-0.2 mm using the pipetting dissociation method in Na-pyrophosphate (HRN ISO 11277:2004);
7) residual moisture content gravimetrically, by drying the test samples at 105°C to constant mass and using data for the expression of the analytical results on a dry soil.

Soil samples (0.5 g) were subjected to microwave digestion with the addition of 6 ml of concentrated HCl and 2 ml of concentrated HNO_3 (HRN ISO 11466:2004). The resulting soil extract was filtered through the filter paper (blue ribbon) and supplemented with 1% v/v HNO_3 to a volume of 50 ml. The microwave extraction method was performed in closed TFM vessels on a MARS Xpress (CEM) with automatic pressure and temperature control. The total concentrations of cadmium, chromium, lead and zinc were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) (ISO/DIS 22036:2008) on Vista MPX AX (Varian). Mercury concentration was determined by Cold Vapor Atomic Absorption Spectrophotometry (HRN ISO 16772:2009).

4.4. Statistical analyses and data processing

A descriptive statistics was done for each analysed heavy metal; mean values, standard deviations and confidence limits (minimum and maximum values) were calculated. Mean values for the same moss species obtained from all locations and separated according to the dry and rainy criteria, were used as an indicator of an average central tendency for the certain metal concentration. Standard deviations were calculated to quantify the amount of variation or dispersion of a set of measured results. Confidence limits were calculated in order to illustrate the uncertainty. In order to separate the anthropogenic from natural contributions to metal concentrations in mosses, the enrichment factors (EF) were calculated according to Salomons and Förstner (1984). The next equation was used for the calculation of the enrichment factor (Poissant et al. 1994, Lawson and Winchester 1979):

$$EF = (C/R)\ moss\ /\ (C/R)\ soil,$$

where C is the concentration of the element and R is the concentration of the reference element (Zn) in the mosses and the soil. Several criteria have been used in order to determine the levels of metal enrichment in the mosses (Lantzy and Mackenzie 1979; Aničić et al. 2007; Dragovic and Mihailovic 2009; Zarazúa et al. 2013):

- enrichment factors ≤2 show that there is no enrichment of the element in the mosses and the element is coming from the soil (conservative);
- enrichment factors between 3 and 5 are considered slightly enriched;
- enrichment factors between 6 and 9 are considered moderately enriched;
- and enrichment factors ≥10 are considered highly enriched.

For the better understanding of the obtained results, all metal concentrations measured in soil were compared with the official median values of each metal for the croatian mountain region according to the Geochemical Atlas of the Republic of Croatia (Halamić and Miko, 2009). Measured metal concentrations in the moss tissue were also compared with median values from recent researches from neighboring countries (Vučković et al. 2012a; Vučković et al. 2012b; Vučković et al. 2013; Špirić et al. 2014; Špirić et al. 2016).

5. Results

5.1. Concentration of heavy metals in soil

5.1.1. Location: River Kupa spring (Risnjak National Park)

The physical and the chemical parameters of the soil in the surface layer (30 cm) at the location River Kupa spring (Risnjak National Park) are presented in Tables 5.1.1. and 5.1.2. Among locations examined in this study this soil sampling location is at the lowest altitude (325 m above sea level). At this location soil was acid with pH (H_2O) of 5.49 and poorly supplied with phosphorus and potassium. On the basis of the soil classification according to the humus content (Gračanin M., 1947) the soil belongs to the category of humus soils (3 – 5 %). Soil texture was sandy loam with sand as a dominant fraction (Table 5.1.2.).

Table 5.1.1. Soil surface layer (0 – 30 cm) chemical parameters at the location River Kupa spring (Risnjak National Park).

Parameter	Unit	Value
pH in H_2O	-	5.49
pH in 1N KCl	-	4.09
Specific electrical conductivity	dS m^{-1}	0.035
Organic matter content	%	3.42
P_2O_5	mg/100g	1.37
K_2O	mg/100g	6.71
NO_3-N	mg kg^{-1}	0.79
NH_4-N	mg kg^{-1}	7.99

Table 5.1.2. Soil surface layer texture (0 – 30 cm) at the location River Kupa spring (Risnjak National Park).

Particle diameter (mm)	Share (%)
2 – 0.2 mm	37
0.2 – 0.063 mm	21
0.063 – 0.02 mm	21
0.02 – 0.002 mm	15
<0.002 mm	6

Metal concentrations in the soil surface layer (0 – 30 cm) of at the location River Kupa spring (Risnjak National Park) are shown in Table 5.1.3. Metal concentrations in the soil surface layer had the following decreasing order: Zn > Cr > Pb > Cd > Hg.

Table 5.1.3. Metal concentrations in the soil surface layer (0 – 30 cm) at the location River Kupa spring (Risnjak National Park).

Metal	Concentration (mg kg^{-1})
Cd	0.27
Cr	32
Hg	0.06
Pb	27
Zn	53

5.1.2. Location: Leska (Risnjak National Park)

The physical and chemical parameters of the soil surface layer (0 – 30 cm) at the location Leska (Risnjak National Park) are shown in Tables 5.1.4. and 5.1.5. The soil was slightly alkaline with pH (H_2O) 7.38. Soil was poorly supplied with available phosphorus and potassium. According to Gračanin (1947) soil can be classified as very rich with organic matter (5 – 10 %). Soil texture was silty loam with silt as a dominant fraction (Table 5.1.5.).

Table 5.1.4. Soil surface layer (0 – 30 cm) chemical parameters at the location Leska (Risnjak National Park).

Parameter	Unit	Value
pH in H_2O	-	7.38
pH in 1N KCl	-	6.41
Specific electrical conductivity	dS m^{-1}	0.102
Organic matter content	%	5.64
P_2O_5	mg/100g	1.01
K_2O	mg/100g	7.84
NO_3-N	mg kg^{-1}	4.81
NH_4-N	mg kg^{-1}	7.94

Table 5.1.5. Soil surface layer texture (0 – 30 cm) at the location Leska (Risnjak National Park).

Particle diameter (mm)	Share (%)
2 – 0.2 mm	3
0.2 – 0.063 mm	5
0.063 – 0.02 mm	26
0.02 – 0.002 mm	52
<0.002 mm	14

Metal concentrations in the soil surface layer (0 – 30 cm) at the location Leska (Risnjak National Park) are shown in Table 5.1.6. Metal concentrations in the soil surface layer had the following decreasing order: Zn > Cr > Pb > Cd > Hg.

Table 5.1.6. Metal concentrations in the soil surface layer (0 – 30 cm) at the location Leska (Risnjak National Park).

Metal	Concentration (mg kg^{-1})
Cd	0.58
Cr	46
Hg	0.19
Pb	30
Zn	73

5.1.3. Location: Lazac (north side of the mountain Risnjak)

The physical and the chemical parameters of the soil surface layer (0 – 30 cm) at the location Lazac (south side of the mountain Risnjak) are presented in Tables 5.1.7. and 5.1.8. At this location the soil was very acid with pH (H_2O) of 3.95, poorly supplied with available phosphorus and potassium. Soil texture was silty clay loam with a silt content of 63% and clay content of 35% (Table 5.1.8.). According to Gračanin (1947) soil can be classified as very very rich with organic matter (>10 %).

Table 5.1.7. Soil surface layer (0 – 30 cm) chemical parameters at the location Lazac (north side of the mountain Risnjak).

Parameter	Unit	Value
pH in H_2O	-	3.95
pH in 1N KCl	-	3.28
Specific electrical conductivity	dS m^{-1}	0.083
Organic matter content	%	14.9
P_2O_5	mg/100g	1.47
K_2O	mg/100g	14.3
NO_3-N	mg kg^{-1}	10.4
NH_4-N	mg kg^{-1}	34.8

Table 5.1.8. Soil surface layer texture (0 – 30 cm) at the location Lazac (north side of the mountain Risnjak).

Particle diameter (mm)	Share (%)
2 – 0.2 mm	1
0.2 – 0.063 mm	1
0.063 – 0.02 mm	19
0.02 – 0.002 mm	44
<0.002 mm	35

Metal concentrations in the soil surface layer (0 – 30 cm) at the location Lazac (north side of the mountain Risnjak) are shown in Table 5.1.9. Metal concentrations in the soil surface layer had the following decreasing order: Zn > Cr > Pb > Cd > Hg.

Table 5.1.9. Metal concentrations in the soil surface layer (0 – 30 cm) at the location Lazac (north side of the mountain Risnjak).

Metal	Concentration (mg kg^{-1})
Cd	0.63
Cr	68
Hg	0.43
Pb	58
Zn	124

5.1.4. Location: Horvat trail (south side of the mountain Risnjak)

The physical and the chemical parameters of the soil surface layer (0 – 30 cm) at the location Horvat trail (south side of the mountain Risnjak) are presented in Tables 5.1.10. and 5.1.11. At this location the soil was acid with pH (H_2O) of 5.02, poorly supplied with available phosphorus and potassium. This is the location where the highest organic matter content was measured in the soil. Soil texture was silty clay loam with a silt content of 69% and clay content of 28% (Table 5.1.11.). According to Gračanin (1947) soil can be classified as very very rich with organic matter (>10 %).

Table 5.1.10. Soil surface layer (0 – 30 cm) chemical parameters at the location Horvat trail (south side of the mountain Risnjak).

Parameter	Unit	Value
pH in H_2O	-	5.02
pH in 1N KCl	-	4.22
Specific electrical conductivity	dS m^{-1}	0.093
Organic matter content	%	18.3
P_2O_5	mg/100g	3.07
K_2O	mg/100g	28.7
NO_3-N	mg kg^{-1}	17.6
NH_4-N	mg kg^{-1}	39.6

Table 5.1.11. Soil surface layer texture (0 – 30 cm) at the location Horvat trail (south side of the mountain Risnjak).

Particle diameter (mm)	Share (%)
2 – 0.2 mm	1
0.2 – 0.063 mm	2
0.063 – 0.02 mm	20
0.02 – 0.002 mm	49
<0.002 mm	28

Metal concentrations in the soil surface layer (0 – 30 cm) at the location Horvat trail (south side of the mountain Risnjak) are shown in Table 5.1.12. Metal concentrations in the soil surface layer had the following decreasing order: Zn > Pb > Cr > Cd > Hg.

Table 5.1.12. Metal concentrations in the soil at the location Horvat trail (south side of the mountain Risnjak).

Metal	**Concentration (mg kg^{-1})**
Cd	1.2
Cr	68
Hg	0.34
Pb	75
Zn	172

5.1.5. Location: Top of the Risnjak mountain – Snježnik (Risnjak National Park)

The physical and the chemical parameters of the soil surface layer (0 – 20 cm) at the location Top of the Risnjak mountain – Snježnik (Risnjak National Park) are presented in Tables 5.1.13. and 5.1.14. At this location the soil was alkaline with the pH (H_2O) of 7.26, poorly supplied with available phosphorus and potassium. According to Gračanin (1947) content of organic matter in soil was very very high (>10 %). Soil texture was silty loam with a silt content of 80% and clay content of 16% (Table 5.1.14.).

Table 5.1.13. Soil surface layer (0 – 20 cm) chemical parameters at the location Top of the Risnjak mountain – Snježnik (Risnjak National Park).

Parameter	Unit	Value
pH in H_2O	-	7.26
pH in 1N KCl	-	6.83
Specific electrical conductivity	dS m^{-1}	0.199
Organic matter content	%	10.8
P_2O_5	mg/100g	2.47
K_2O	mg/100g	25.1
NO_3-N	mg kg^{-1}	25.7
NH_4-N	mg kg^{-1}	24.3

Table 5.1.14. Soil surface layer texture (0 – 20 cm) at the location Top of the Risnjak mountain – Snježnik (Risnjak National Park).

Particle diameter (mm)	Share (%)
2 – 0.2 mm	2
0.2 – 0.063 mm	2
0.063 – 0.02 mm	28
0.02 – 0.002 mm	52
<0.002 mm	16

Metal concentrations in the soil surface layer (0 – 20 cm) at the location Top of the Risnjak mountain – Snježnik (Risnjak National Park) are shown in Table 5.1.15. Metal concentrations in the soil surface layer had the following decreasing order: Zn > Cr > Pb > Cd > Hg.

Table 5.1.15. Metal concentrations in the soil surface layer (0 – 20 cm) at the location Top of the Risnjak mountain – Snježnik (Risnjak National Park).

Metal	**Concentration (mg kg^{-1})**
Cd	1.3
Cr	65
Hg	0.24
Pb	51
Zn	196

5.2. Concentration of metal in mosses

Metal concentrations in moss tissue during field research in Risnjak National Park are presented in Tables 5.2.1., 5.2.3., 5.2.5., 5.2.7., 5.2.9. for the rainy and dry season separately.

In order to separate anthropogenic from natural contributions to metal concentrations in mosses, the enrichment factors (EF) were calculated according to Salomons and Förstner (1984). Calculated EFs are shown in Tables 5.2.2., 5.2.4., 5.2.6. and 5.2.8.

5.2.1. Cadmium

Species *B. rutabulum* was sampled during both sampling seasons at all locations. Cadmium concentrations varied from 0.27 mg kg^{-1} to 0.37 mg kg^{-1} in dry season, and from <0.20 mg kg^{-1} to 0.58 mg kg^{-1} in rainy season (Table 5.2.1.). In both sampling terms the highest concentration was measured at the location Lazac. In species *H. splendens* cadmium concentrations during the sampling in rainy season were <0.20 mg kg^{-1} on Lazac, River Kupa spring and Leska, while at other locations it was not possible to sample this species of moss. During the dry season highest concentration was 0.56 mg kg^{-1} at Top of the Risnjak mountain – Snježnik, concentrations at other locations were <0.20 mg kg^{-1} while on location Horvat trail sampling was not possible (Table 5.2.1.). Taking into account all the locations where the mosses were sampled, it is clear that the highest cadmium concentrations were measured in species *B. rutabulum*, while the maximum concentration in dry season was measured in species *H. splendens* at Top of the Risnjak mountain – Snježnik. If two sampling terms are observed separately, higher concentrations were measured during the rainy season in both moss species (Table 5.2.1.).

Table 5.2.1. Cadmium concentrations in mosses *B. rutabulum* and *H. splendens* on all sampling sites in the Risnjak National Park (River Kupa spring, Leska, Lazac, Horvat trail, Top of the Risnjak mountain – Snježnik) in dry and rainy season.

Location	**Cd concentration** [mg kg^{-1}]	
	B. rutabulum	*H. splendens*
	Dry season	
River Kupa spring	0.27 mg kg^{-1}	<0.20 mg kg^{-1}
Leska	0.32 mg kg^{-1}	<0.20 mg kg^{-1}
Lazac	0.37 mg kg^{-1}	<0.20 mg kg^{-1}
Horvat trail	0.27 mg kg^{-1}	*
Top of the Risnjak mountain – Snježnik	0.31 mg kg^{-1}	0.56 mg kg^{-1}
Summary statistic		
Minimum	**0.27**	**0.2**
Maximum	**0.37**	**0.56**
Mean value	**0.31**	**0.38**
Standard deviation	**0.04**	**0.16**
	Rainy season	
River Kupa spring	<0.20 mg kg^{-1}	<0.20 mg kg^{-1}
Leska	<0.20 mg kg^{-1}	<0.20 mg kg^{-1}
Lazac	0.58 mg kg^{-1}	<0.20 mg kg^{-1}
Horvat trail	0.21 mg kg^{-1}	*
Top of the Risnjak mountain – Snježnik	0.29 mg kg^{-1}	*
Summary statistic		
Minimum	**0.21**	**0.2**
Maximum	**0.58**	**0.2**
Mean value	**0.36**	**0.2**
Standard deviation	**0.19**	**0.0**

**no samples*

Wherever it was possible, the enrichment factor for all cadmium concentrations was calculated. The obtained values of enrichment factors were less than 2 in all measurements (Table 5.2.2.). Which means that there was no enrichment with cadmium in mosses.

Table 5.2.2. Enrichment factor calculated according to Salomons and Förstner (1984) for cadmium concentrations in mosses *B. rutabulum* and *H. splendens* on all sampling sites (River Kupa spring, Leska, Lazac, Horvat trail, Top of the Risnjak mountain – Snježnik) during the rainy (B) and dry season (A) in the mountain region of Croatia.

Location	**Moss species**			
	B. rutabulum		*H. splendens*	
	B	A	B	A
River Kupa spring	ND	0.01	ND	ND
Leska	ND	0.01	ND	ND
Lazac	0.04	0.14	ND	ND
Horvat trail	0.027	0.02	NS	NS
Top of the Risnjak mountain – Snježnik	0.002	0.02	NS	0.03
Average	0.023	0.04	ND	0.03

ND – not defined, NS – no sample

5.2.2. Chromium

Concentrations of chromium in moss *B. rutabulum* in dry season varied from <0.50 mg kg^{-1} to 27 mg kg^{-1} , while in rainy season concentrations were in range from 1.8 mg kg^{-1} to 16 mg kg^{-1} (Table 5.2.3.). During the dry season the highest concentration was measured at the location River Kupa spring, while during rainy season convincingly the highest concentrations of chromium were measured at the locations River Kupa spring and Top of the Risnjak mountain – Snježnik (Table 5.2.3.). In species *H. splendens* chromium was ranged from 0.61 mg kg^{-1} to 3.5 mg kg^{-1} for the dry season, while in rainy season concentrations were in range from 1.9 mg kg^{-1} at location Lazac to 2.8 mg kg^{-1} on location Leska (Table 5.2.3.). On location River Kupa spring the measured concentration was 2.5 mg kg^{-1}, while on the other two locations sampling was not possible. In both sampling terms the highest concentration was measured at the location Leska. Noticeably higher concentrations were measured in species *B. rutabulum* during both sampling terms. If the two sampling terms are observed separately higher concentrations were measured during the rainy season in both moss species (Table 5.2.3.), but the maximum concentrations were measured during the dry season in both moss species (Table 5.2.3.).

Table 5.2.3. Chromium concentrations in mosses *B. rutabulum* and *H. splendens* on all sampling sites in the Risnjak National Park (River Kupa spring, Leska, Lazac, Horvat trail, Top of the Risnjak mountain – Snježnik) in dry and rainy season.

Location	**Cr concentration** [mg kg^{-1}]	
	B. rutabulum	*H. splendens*
	Dry season	
River Kupa spring	27 mg kg^{-1}	1.5 mg kg^{-1}
Leska	5.0 mg kg^{-1}	3.5 mg kg^{-1}
Lazac	<0.50 mg kg^{-1}	0.61 mg kg^{-1}
Horvat trail	1.7 mg kg^{-1}	*
Top of the Risnjak mountain – Snježnik	2.4 mg kg^{-1}	1.8 mg kg^{-1}
	Summary statistic	
Minimum	**1.7**	**0.6**
Maximum	**27**	**3.5**
Mean value	**7.3**	**1.9**
Standard deviation	**11.92**	**1.2**
	Rainy season	
River Kupa spring	13 mg kg^{-1}	2.5 mg kg^{-1}
Leska	6.9 mg kg^{-1}	2.8 mg kg^{-1}
Lazac	4.5 mg kg^{-1}	1.9 mg kg^{-1}
Horvat trail	1.8 mg kg^{-1}	*
Top of the Risnjak mountain – Snježnik	16 mg kg^{-1}	*
	Summary statistic	
Minimum	**1.8**	**1.9**
Maximum	**16**	**2.8**
Mean value	**8.4**	**2.4**
Standard deviation	**5.89**	**0.45**

**no samples*

Table 5.2.4. Enrichment factor calculated according to Salomons and Förstner (1984) for chromium concentrations in mosses *B. rutabulum* and *H. splendens* on all sampling sites (River Kupa spring, Leska, Lazac, Horvat trail, Top of the Risnjak mountain – Snježnik) during the rainy (B) and dry season (A) in the mountain region of Croatia.

Location	**Moss species**			
	B. rutabulum		*H. splendens*	
	B	A	B	A
River Kupa spring	3.6	5.0	2.5	5.1
Leska	5.0	2.8	4.7	5.2
Lazac	3.2	ND	2.8	2.3
Horvat trail	4.0	2.6	NS	NS
Top of the Risnjak mountain – Snježnik	2.5	3.0	NS	1.9
Average	3.7	3.4	3.3	3.6

ND – not defined, NS – no sample

The calculated enrichment factor ranged between 3 and 5 on all samples. Which points to the fact that the slightly enrichment of mosses with chromium has occurred (Table 5.2.4.).

5.2.3. Mercury

Mercury concentrations in moss *B. rutabulum* were in range from 0.055 mg kg^{-1} to 0.075 mg kg^{-1} in dry season, while in rainy season they were in range from 0.041 mg kg^{-1} to 0.136 mg kg^{-1} (Table 5.2.5.). During the dry season the highest concentrations were measured at locations Lazac and Top of a Risnjak mountain – Snježnik, while the concentrations at the other locations were much lower. During the rainy season the highest concentration was measured at location Lazac where measured concentration was convincingly higher than at the other locations (Table 5.2.5.). In species *H. splendens* concentrations in dry season were in range from 0.036 mg kg^{-1} to 0.083 mg kg^{-1}, while in rainy season they were in range from 0.050 mg kg^{-1} to 0.107 mg kg^{-1} (Table 5.2.5.). In dry season the highest concentration was measured at the location Top of the Risnjak mountain – Snježnik, while in rainy season the highest concentration was measured at the location River Kupa spring. Higher concentrations were measured in species *B. rutabulum* in dry season, as well as in the rainy season. If the sampling terms are observed one apart from another higher concentrations as well as the maximum concentrations were measured during the rainy season (Table 5.2.5.).

Table 5.2.5. Mercury concentrations in mosses *B. rutabulum* and *H. splendens* on all sampling sites in the Risnjak National Park (River Kupa spring, Leska, Lazac, Horvat trail, Top of the Risnjak mountain – Snježnik) in dry and rainy season.

Location	**Hg concentration** [mg kg^{-1}]	
	B. rutabulum	*H. splendens*
	Dry season	
River Kupa spring	0.055 mg kg^{-1}	0.036 mg kg^{-1}
Leska	0.058 mg kg^{-1}	0.050 mg kg^{-1}
Lazac	0.074 mg kg^{-1}	0.051 mg kg^{-1}
Horvat trail	0.067 mg kg^{-1}	*
Top of the Risnjak mountain – Snježnik	0.075 mg kg^{-1}	0.083 mg kg^{-1}
	Summary statistic	
Minimum	**0.055**	**0.036**
Maximum	**0.075**	**0.083**
Mean value	**0.065**	**0.055**
Standard deviation	**0.009**	**0.019**
	Rainy season	
River Kupa spring	0.065 mg kg^{-1}	0.107 mg kg^{-1}
Leska	0.041 mg kg^{-1}	0.050 mg kg^{-1}
Lazac	0.136 mg kg^{-1}	0.074 mg kg^{-1}
Horvat trail	0.044 mg kg^{-1}	*
Top of the Risnjak mountain – Snježnik	0.088 mg kg^{-1}	*
	Summary statistic	
Minimum	**0.041**	**0.050**
Maximum	**0.136**	**0.107**
Mean value	**0.075**	**0.077**
Standard deviation	**0.039**	**0.028**

**no samples*

Table 5.2.6. Enrichment factor calculated according to Salomons and Förstner (1984) for mercury concentrations in mosses *B. rutabulum* and *H. splendens* on all sampling sites (River Kupa spring, Leska, Lazac, Horvat trail, Top of the Risnjak mountain – Snježnik) during the rainy (B) and dry season (A) in the mountain region of Croatia.

Location	**Moss species**			
	B. rutabulum		*H. splendens*	
	B	A	B	A
River Kupa spring	9.8	5.4	57.3	65.7
Leska	7.3	7.9	20.5	17.9
Lazac	15.3	41.9	17.1	30.1
Horvat trail	18.7	20.0	NS	NS
Top of the Risnjak mountain – Snježnik	3.7	25.0	NS	94.5
Average	11.0	20.0	31.7	52.0

NS – no sample

In all samples, the enrichment factor ranged in values greater than 6, and in many cases greater than 10 indicating that enrichment of mosses with mercury is moderated or high (Table 5.2.6.).

5.2.4. Lead

Concentrations of lead measured in species *B. rutabulum* were in range from <3 mg kg^{-1} to 4.4 mg kg^{-1} in dry season, while in rainy season were in range from <3 mg kg^{-1} to 8.2 mg kg^{-1} (5.2.7.). Highest concentration during dry season was measured at location River Kupa spring, while the highest concentration during the rainy season was measured at location Lazac (Table 5.2.7.). In species *H. splendens* concentrations in dry season were in range from 3.0 mg kg^{-1} to 6.3 mg kg^{-1}, while in rainy season they were in range from 3.0 mg kg^{-1} to 7.3 mg kg^{-1} (Table 5.2.7.). In dry season the highest concentration was measured at the location Top of the Risnjak mountain – Snježnik, while in the rainy season the highest concentration was measured at the location Lazac. Higher concentrations were measured in species *B. rutabulum* in dry season, as well as in the rainy season. If two sampling terms are observed separately higher concentrations were measured during the rainy season in both moss species (Table 5.2.7.), but the interesting thing is that the maximum concentrations were also measured during the rainy season in both moss species.

Table 5.2.7. Lead concentrations in mosses *B. rutabulum* and *H. splendens* on all sampling sites in the Risnjak National Park (River Kupa spring, Leska, Lazac, Horvat trail, Top of the Risnjak mountain – Snježnik) in dry and rainy season.

Location	**Pb concentration** [mg kg^{-1}]	
	B. rutabulum	*H. splendens*
	Dry season	
River Kupa spring	4.4 mg kg^{-1}	3.0 mg kg^{-1}
Leska	3.6 mg kg^{-1}	3.0 mg kg^{-1}
Lazac	<3 mg kg^{-1}	3.0 mg kg^{-1}
Horvat trail	3.1 mg kg^{-1}	*
Top of the Risnjak mountain – Snježnik	4.1 mg kg^{-1}	6.3 mg kg^{-1}
	Summary statistic	
Minimum	**3.1**	**3.0**
Maximum	**4.4**	**6.3**
Mean value	**3.6**	**3.8**
Standard deviation	**0.56**	**1.7**
	Rainy season	
River Kupa spring	4.9 mg kg^{-1}	3.0 mg kg^{-1}
Leska	6.7 mg kg^{-1}	3.0 mg kg^{-1}
Lazac	8.2 mg kg^{-1}	7.3 mg kg^{-1}
Horvat trail	<3 mg kg^{-1}	*
Top of the Risnjak mountain – Snježnik	7.8 mg kg^{-1}	*
	Summary statistic	
Minimum	**4.9**	**3.0**
Maximum	**8.2**	**7.3**
Mean value	**6.1**	**4.4**
Standard deviation	**1.47**	**2.5**

**no samples*

Table 5.2.8. Enrichment factor calculated according to Salomons and Förstner (1984) for lead concentrations in mosses *Brachythecium rutabulum* and *Hylocomium splendens* on all sampling sites (River Kupa spring, Leska, Lazac, Horvat trail, Top of the Risnjak mountain – Snježnik) during the rainy (B) and dry season (A) in the mountain region of Croatia.

Location	**Moss species**			
	B. rutabulum		*H. splendens*	
	B	A	B	A
River Kupa spring	1.7	1.0	ND	ND
Leska	7.6	3.2	ND	ND
Lazac	6.9	ND	12.7	ND
Horvat trail	ND	4.3	NS	NS
Top of the Risnjak mountain – Snježnik	1.6	4.7	NS	8.5
Average	4.4	3.3	12.7	8.5

ND – not defined, NS – no sample

Enrichment factor in moss *B. rutabulum* was in range from 3 to 5 which points to slightly enriched mosses with lead, while in moss *H. splendens* enrichment factor was higher than 10 which means that samples of these moss species were highly enriched with lead (Table 5.2.8.).

5.2.5. Zinc

Concentrations of zinc measured in species *B. rutabulum* were in range from 14 mg kg^{-1} to 37 mg kg^{-1} in a dry season, while in rainy season they were in range from 21 mg kg^{-1} to 82 mg kg^{-1} (Table 5.2.9.). The highest concentration during the dry season was measured at locations Leska and Lazac, while the highest concentration during the rainy season was measured at location Top of the Risnjak mountain – Snježnik. In species *H. splendens* the concentrations in dry season were in range from 8.6 mg kg^{-1} to 25 mg kg^{-1}, while in rainy season they were in range from 14 mg kg^{-1} to 32 mg kg^{-1} (Table 5.2.9.). In dry season the highest concentration was measured at the location Top of the Risnjak mountain – Snježnik, while in the rainy season the highest concentration was measured at the location Lazac. Higher concentrations were measured in species *B. rutabulum* in dry season, as well as in the rainy season. If two sampling terms are observed separately higher concentrations were measured during the rainy season in both moss species (Table 5.2.9.), but the interesting thing is that the maximum concentrations were also measured during rainy season in both moss species.

Table 5.2.9. Zinc concentrations in mosses *B. rutabulum* and *H. splendens* on all sampling sites in the Risnjak National Park (River Kupa spring, Leska, Lazac, Horvat trail, Top of the Risnjak mountain – Snježnik) in dry and rainy season.

Location	**Zn concentration** [mg kg^{-1}]	
	B. rutabulum	*H. splendens*
	Dry season	
River Kupa spring	21 mg kg^{-1}	8.6 mg kg^{-1}
Leska	37 mg kg^{-1}	15 mg kg^{-1}
Lazac	37 mg kg^{-1}	17 mg kg^{-1}
Horvat trail	30 mg kg^{-1}	*
Top of the Risnjak mountain – Snježnik	14 mg kg^{-1}	25 mg kg^{-1}
Summary statistic		
Minimum	**14**	**8.6**
Maximum	**37**	**25**
Mean value	**27.8**	**16.4**
Standard deviation	**10**	**6.6**
	Rainy season	
River Kupa spring	21 mg kg^{-1}	14 mg kg^{-1}
Leska	46 mg kg^{-1}	26 mg kg^{-1}
Lazac	65 mg kg^{-1}	32 mg kg^{-1}
Horvat trail	27 mg kg^{-1}	*
Top of the Risnjak mountain – Snježnik	82 mg kg^{-1}	*
Summary statistic		
Minimum	**21**	**14**
Maximum	**82**	**32**
Mean value	**48**	**24**
Standard deviation	**26**	**9.0**

**no samples*

6. Discussion

If the concentrations of metals in the soil are observed with the respect to the altitude, it may be noticed that Cd, Cr, Pb and Zn concentrations grow almost linearly with rising altitudes as shown in the Figures 6.1.1. and 6.1.2.

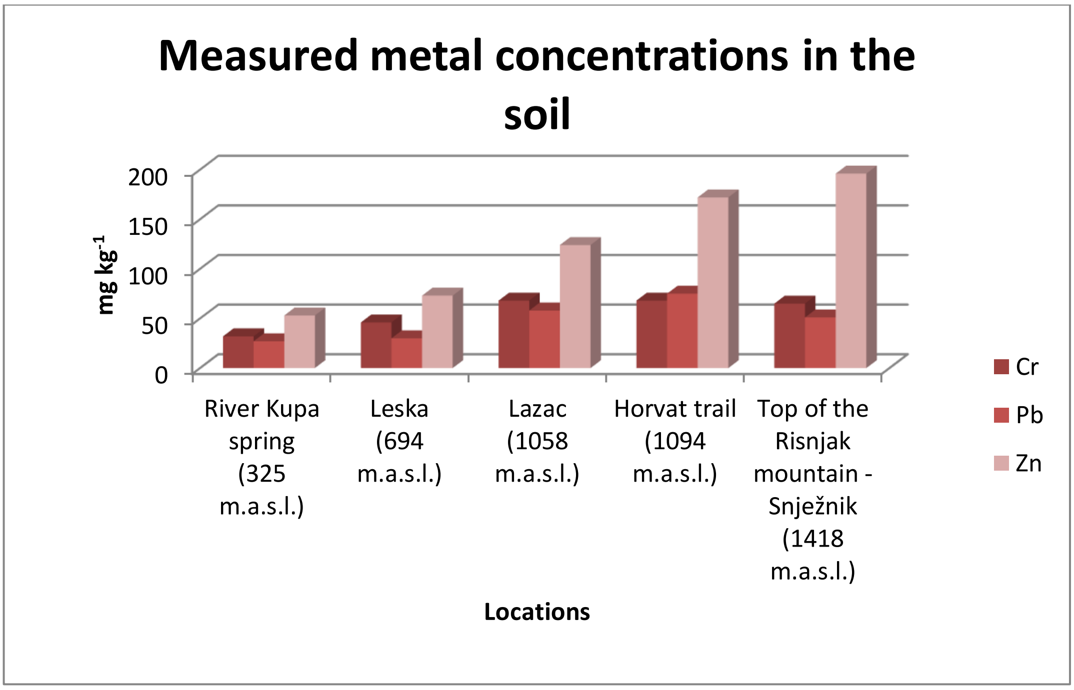

Figure 6.1.1. The concentrations of Cr, Pb and Zn measured in the soil with the respect to the rising altitude.

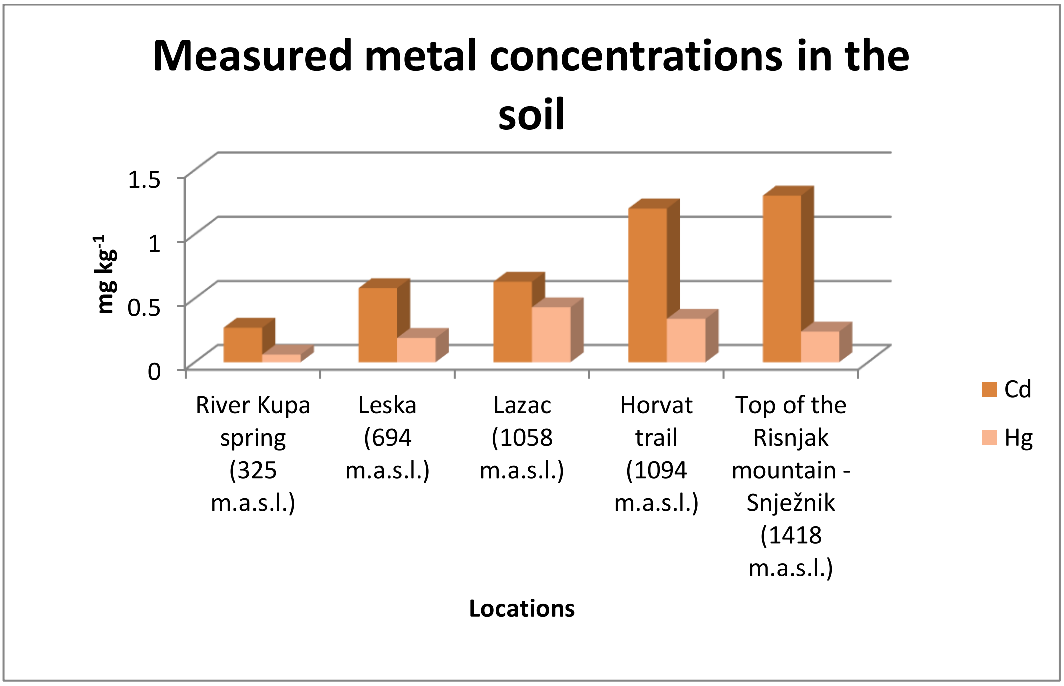

Figure 6.1.2. The concentrations of Cd and Hg measured in the soil with the respect to the rising altitude.

The cause of increased metal concentrations with rising altitudes may possibly be attributed to the air transport from surrounding areas. With rising altitude the intensity of wind increases as well, and such depositions have been described in other studies: Nybo et al. (1996) explained increased soil concentrations of cadmium, lead and zinc found in certain areas of Norway as possibly resulting from the long-range air transport.

6.1. Comparison between concentrations of Cd, Cr, Hg, Pb and Zn in soil samples and median values for Croatian mountain regions

6.1.1. Cadmium

The values of Cd concentrations in the investigated soils and the corresponding median values for the Croatian mountain region (Halamić and Miko, 2009) are shown in the Figure 6.1.3. The concentration of cadmium in soils at the investigated locations of the Risnjak National Park are in a range from 0.27 mg kg^{-1} (River Kupa spring) to 1.3 mg kg^{-1} (Top of the Risnjak mountain – Snježnik). On four out of five observed locations cadmium concentrations were higher than the median value of 0.6 mg kg^{-1} (Halamić and Miko, 2009). Data are suggesting that at certain areas of the Risnjak National Park, cadmium soil concentration should be more closely monitored because of the possible soil contamination.

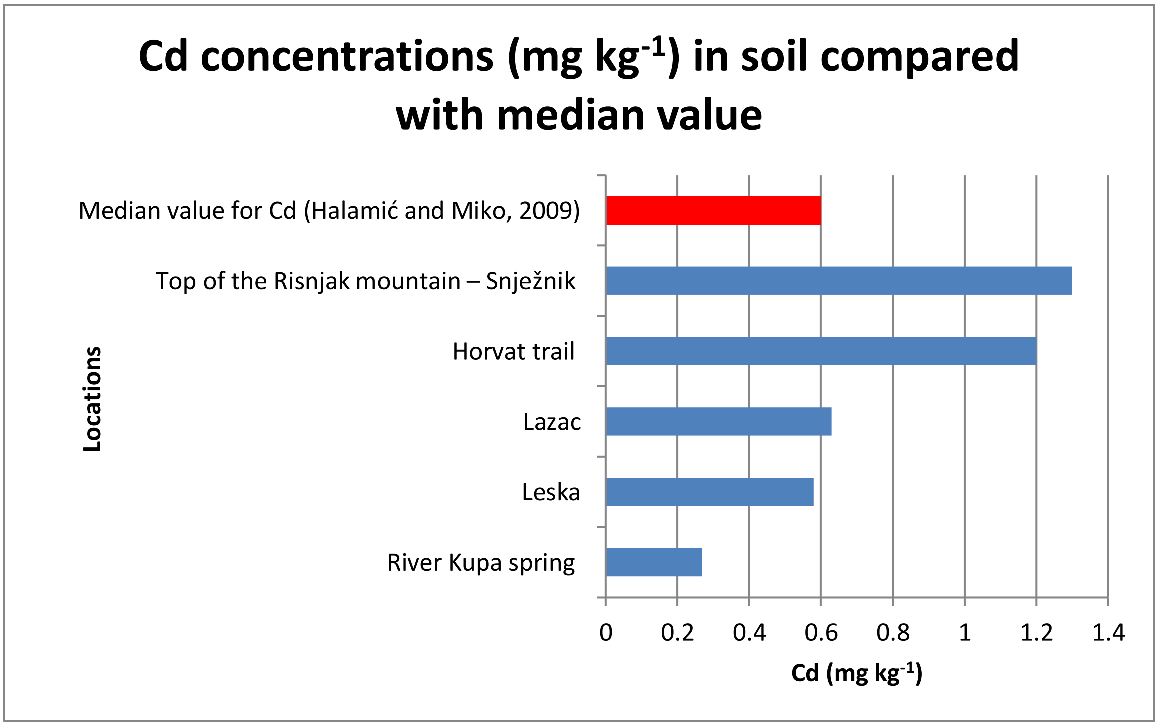

Figure 6.1.3. Cadmium concentrations at the sampling sites compared with the median value of cadmium concentration for the Croatian mountain region (Halamić and Miko, 2009).

6.1.2. Chromium

The values of Cr concentrations in the investigated soils and the corresponding median values for the Croatian mountain region (Halamić and Miko, 2009) are shown in the Figure 6.1.4. The concentration of chromium in soils at the investigated locations of the Risnjak National Park are in range from 32 mg kg^{-1} (River Kupa spring) to 68 mg kg^{-1} (Lazac and Horvat trail). All measured chromium values at the observed locations are lower than the median value of 86 mg kg^{-1} (Halamić and Miko, 2009), suggesting that soil chromium concentration in the Risnjak National Park is negligible and still at a lower level compared to the other Croatian mountain regions.

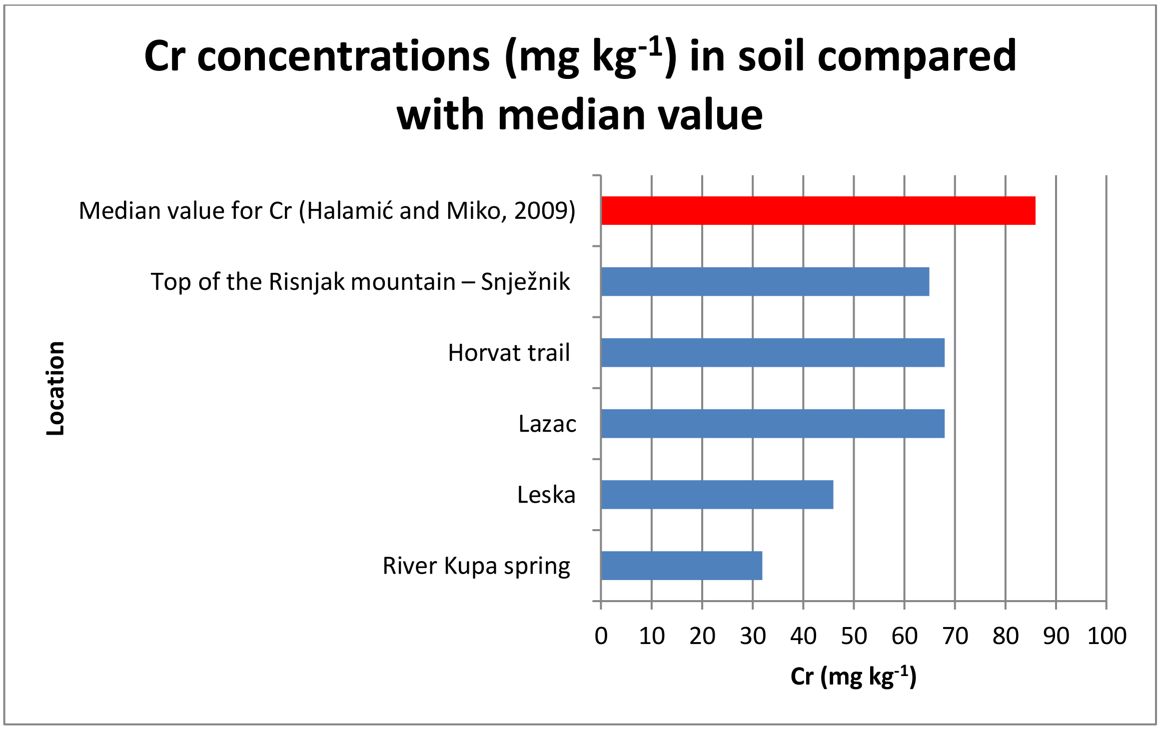

Figure 6.1.4. Chromium concentrations at the sampling sites compared with the median value of chromium concentration for the Croatian mountain region (Halamić and Miko, 2009).

6.1.3. Mercury

The values of Hg concentrations in the investigated soils and the corresponding median values for the Croatian mountain region (Halamić and Miko, 2009) are shown in the Figure 6.1.5. The concentration of mercury in soils at the investigated locations of the Risnjak National Park are in a range from 0.06 mg kg^{-1} (River Kupa spring) to 0.43 mg kg^{-1} (Lazac). All measured mercury values (except for the River Kupa spring) at the observed locations are higher than the median value of 0.105 mg kg^{-1} (Halamić and Miko, 2009). Data are suggesting that mercury soil concentration should be more closely monitored in the Risnjak National Park.

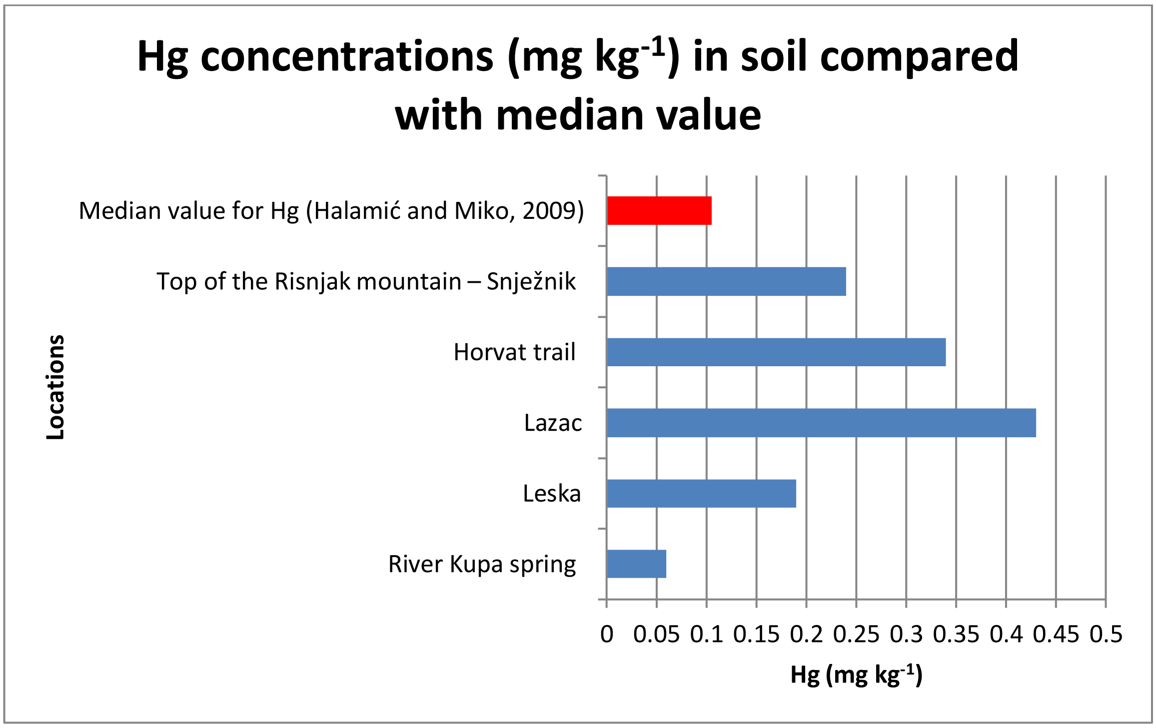

Figure 6.1.5. Mercury concentrations at the sampling sites compared with the median value of mercury concentration for the Croatian mountain region (Halamić and Miko, 2009).

6.1.4. Lead

The values of Pb concentrations in the investigated soils and the corresponding median values for the Croatian mountain region (Halamić and Miko, 2009) are shown in the Figure 6.1.6. The concentration of lead in soils at the investigated locations of the Risnjak National Park are in range from 27 mg kg^{-1} (River Kupa spring) to 75 mg kg^{-1} (Horvat trail). At the three observed locations (Horvat trail, Top of the Risnjak mountain – Snježnik and Lazac) the measured values were higher than the median value of 39 mg kg^{-1} (Halamić and Miko, 2009). Data are possibly suggesting that lead soil concentration should be more closely monitored in the certain areas of the Risnjak National Park.

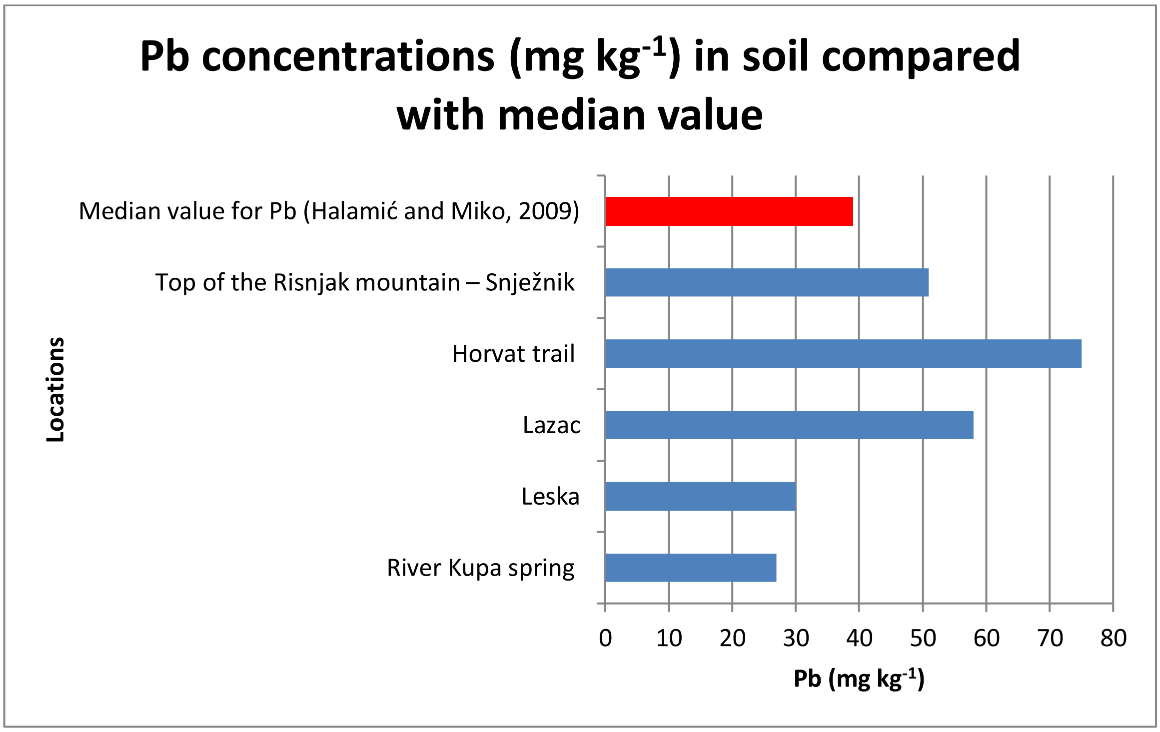

Figure 6.1.6. Lead concentrations at the sampling sites compared with the median value of lead concentration for the Croatian mountain region (Halamić and Miko, 2009).

6.1.5. Zinc

The values of Zn concentrations in the investigated soils and the corresponding median values for the Croatian mountain region (Halamić and Miko, 2009) are shown in the Figure 6.1.7. The concentration of zinc in soils at the investigated locations of the Risnjak National Park are in range from 53 mg kg^{-1} (River Kupa spring) to 196 mg kg^{-1} (Top of the Risnjak mountain – Snježnik). At the three observed locations (Horvat trail, Top of the Risnjak mountain – Snježnik and Lazac) the measured values were higher than the median value of 104 mg kg^{-1} (Halamić and Miko, 2009). Data are possibly suggesting that zinc may be found in increased concentrations in the soil at certain areas of the Risnjak National Park.

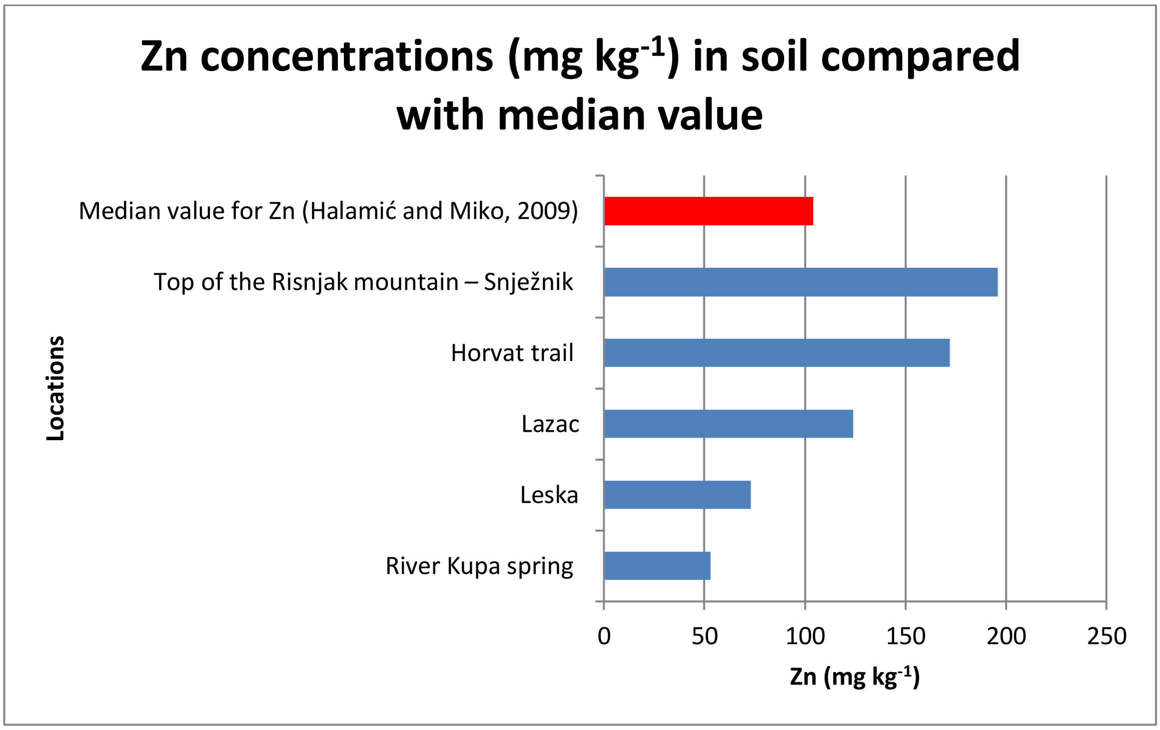

Figure 6.1.7. Zinc concentrations at the sampling sites compared with the median value of zinc concentration for the Croatian mountain region (Halamić and Miko, 2009).

6.2. Comparison of concentrations for each metal in mosses *B. rutabulum* and *H. splendens*

6.2.1. Cadmium

From Figure 6.2.1. it can be seen that the concentration of cadmium in mosses increases with the rise of the altitude, which may be related to the increased precipitation at higher elevations and a possible washout of the airborne Cd pollution.

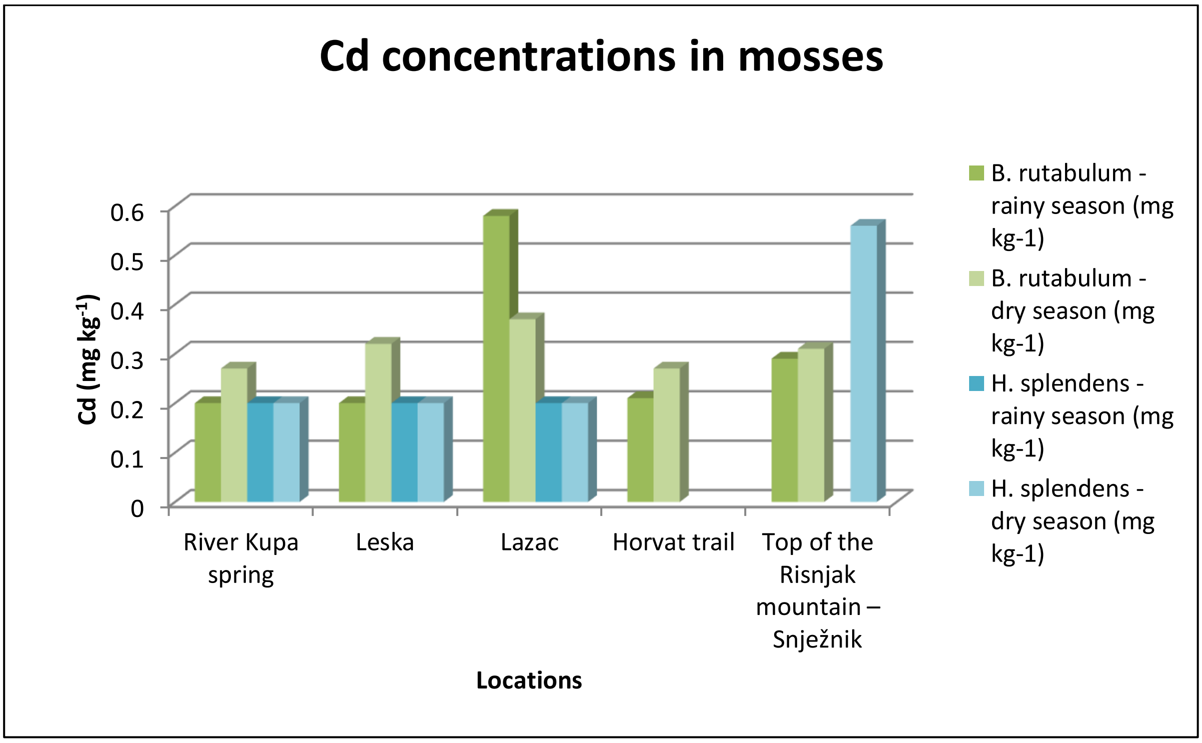

Figure 6.2.1. Comparison of cadmium concentrations in two moss species (*B. rutabulum* and *H. splendens*) collected from selected locations at Risnjak National Park during dry and rainy season (locations are presented from the lowest to the highest altitude).

Cadmium concentrations in moss tissue were first compared with the median values and ranges for cadmium concentrations in mosses in Croatia obtained from Špirić et al. (2012) when different species of moss samples (*Hypnum cupressiforme, Pleurozium schreberi, Brachythecium rutabulum* and *Homalothecium sericeum*) were collected from 94 locations evenly distributed over the country. Moss samples were collected during the summer and autumn of 2010. Collected moss samples were air dried, cleaned and totally digested by using microwave digestion system. Digests were analysed for cadmium by atomic emission spectrometry with inductively coupled plasma (ICP-AES) . Additional comparison was made with the median values and ranges for cadmium concentrations in mosses obtained from similar studies conducted in: (i) neighboring countries - Slovenia, Serbia (Harmens et al, 2008) and Hungary (Buse et al., 2003); (ii) other Balkan countries - Macedonia (Barandovski et al., 2012) and Bulgaria (Harmens et al., 2008); as well as (iii) Norway, as pristine area (Steinnes et al., 2011). Comparison of data is shown in Figure 6.2.2. and Table 6.2.1.

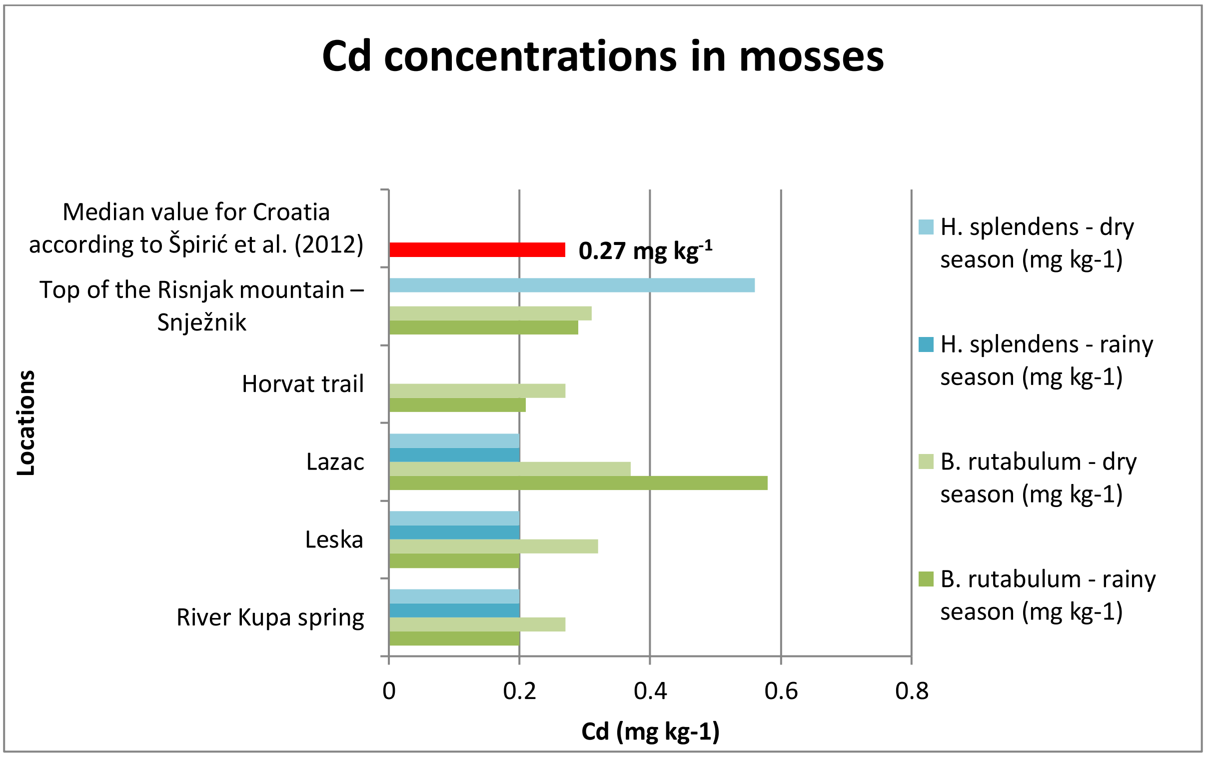

Figure 6.2.2. Comparison of cadmium concentrations in two moss species (*B. rutabulum* and *H. splendens*) collected from selected locations at Risnjak National Park during the dry and rainy season with the median value for cadmium concentration in mosses for Croatia according to Špirić et al. (2012).

Table 6.2.1. Comparison of median values and ranges between measured cadmium concentrations in mosses and cadmium concentrations in mosses obtained from the literature for Croatia, Slovenia, Serbia, Macedonia and Bulgaria for the year 2005, Hungary for the year 2000, and Norway for the year 2010.

		No. of samples	Median (mg kg^{-1})	Range (mg kg^{-1})
Measured Cd concentration in mosses	**RAINY SEASON**			
	B. rutabulum	5	**0.3**	0.2 – 0.58
	H. splendens	3	**0.2**	0.2
	DRY SEASON			
	B. rutabulum	5	**0.31**	0.27 – 0.37
	H. splendens	4	**0.29**	0.2 – 0.56
		No. of samples	**Median (mg kg^{-1})**	**Range (mg kg^{-1})**
Cd concentration in mosses according to the literature	Croatia, 2005 (Špirić et al., 2012)	94	**0.27**	0.07 – 1.9
	Slovenia, 2005 (Harmens et al., 2008)	56	**0.33**	0.13 – 1.21
	Serbia, 2005 (Harmens et al., 2008)	193	**0.26**	0.04 – 1.11
	Hungary, 2000 (Buse et al., 2003)	32	**0.55**	0.31 – 1.48
	Macedonia, 2005 (Barandovski et al., 2012)	72	**0.29**	0.015 – 3.0
	Bulgaria, 2005 (Harmens et al., 2008)	213	**0.31**	0.1 – 5.23
	Norway, 2010 (Steinnes et al., 2011)	464	**0.081**	0.009 – 1.87

Data show that cadmium concentrations in mosses measured in this study are higher than the median value of cadmium in mosses reported for Croatia (0.27 mg kg^{-1}) according to Špirić et al. (2012). If compared to neighbouring countries Slovenia and Hungary (Table 6.2.1.), Croatia has lower median values and possibly less Cd contamination in the environment. Thus, data may suggest that the concentrations of cadmium measured in mosses in this study in the Risnjak National Park can possibly be traced also to the industrial sources of pollution from the neighbouring countries. Accordnig to Vidič (2004) it is easily recognised that Italian emission sources creates considerable threat to natural ecosystems and environmental conditions of Croatian territory in general. Due to its position, distance and size long-range transport of pollutants and atmospheric deposition is possible on the territory of Croatia as well as on the territory of Risnjak National Park which is the first barrier to air currents from the direction of Italy.

6.2.2. Chromium

From Figure 6.2.3. it can be seen that the concentration of chromium in mosses has no strong connection with the rise of altitude.

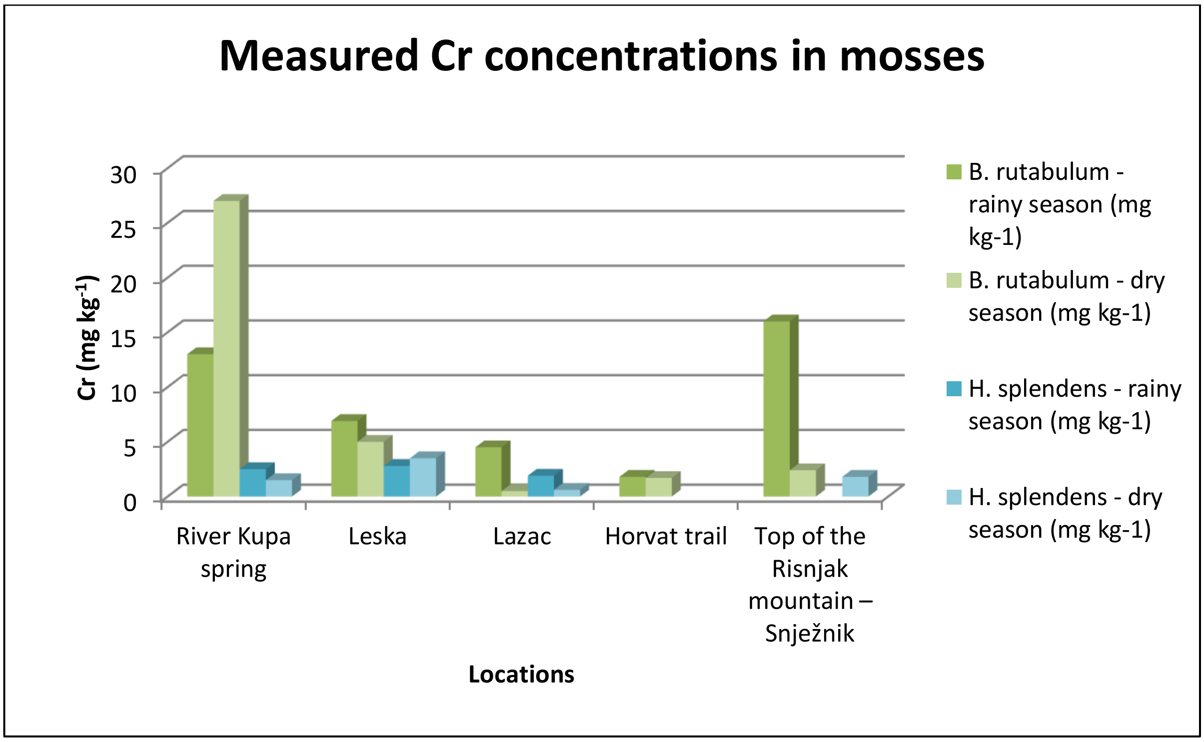

Figure 6.2.3. Comparison of chromium concentrations in two moss species (*B. rutabulum* and *H. splendens*) collected from selected locations at Risnjak National Park during the dry and rainy season (locations are presented from the lowest to the highest altitude).

Chromium concentrations in moss tissue were first compared with the median values and ranges for chromium concentrations in mosses in Croatia obtained from Špirić et al. (2012), in this research samples were collected from 94 sampling points during the summer and autumn of 2010. The most dominantmoss species in this study area were *Hylocomium splendens, Hypnum cupressiforme, Brachythecium rutabulum* and *Homalothecium sericeum.* Moss samples were homogenised by hands using nylon gloves. For digestion of moss samples, microwave digestion system was applied. Atomic emission spectrometer with inductively coupled plasma, AES-ICP (Varian, 715ES), was used for analyzing the content of chromium in moss samples. Additional comparison was made with the median values and ranges for cadmium concentrations in mosses obtained from similar studies conducted in: (i) neighboring countries - Slovenia, Serbia (Harmens et al, 2008) and Hungary (Buse et al., 2003); (ii) other Balkan countries - Macedonia (Barandovski et al., 2008) and Bulgaria (Harmens et al., 2008); as well as (iii) Norway, as pristine area (Steinnes et al., 2011). Comparison of data is shown in Figure 6.2.4. and Table 6.2.2.

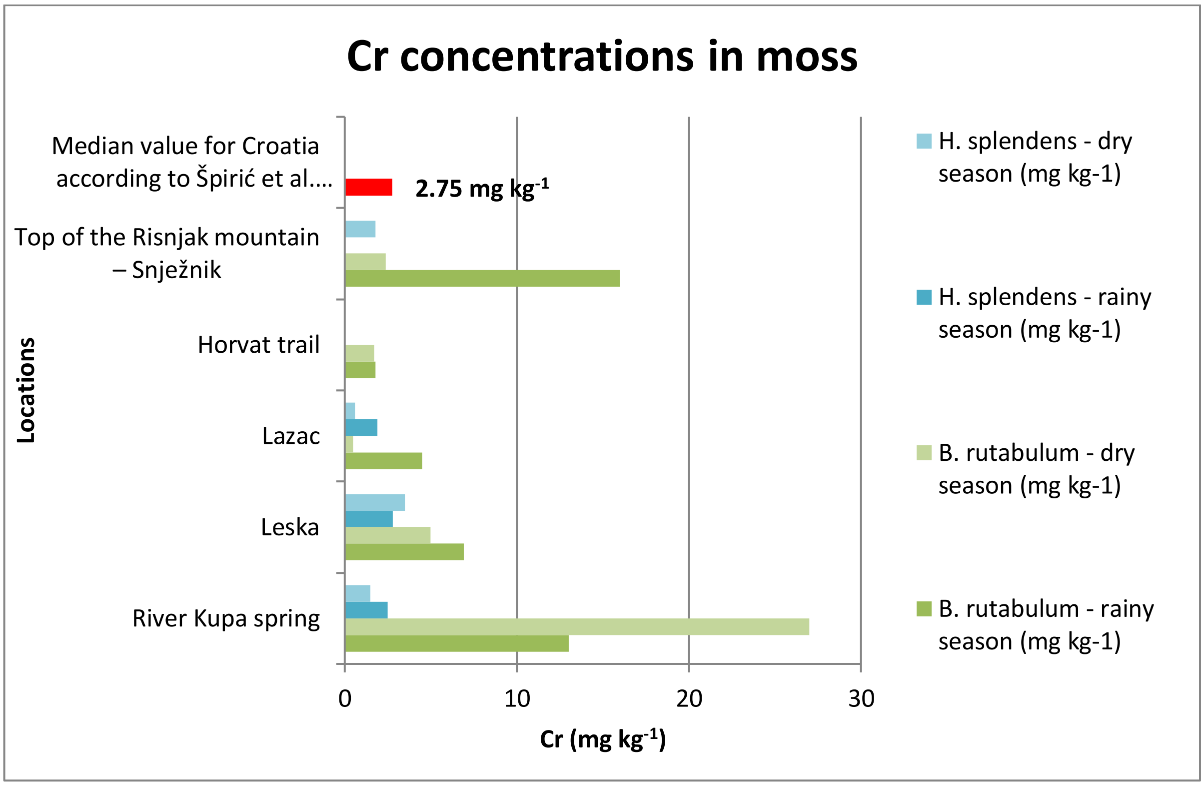

Figure 6.2.4. Comparison of chromium concentrations in two moss species (*B. rutabulum* and *H. splendens*) collected from selected locations at Risnjak National Park during the dry and rainy season with the median value for chromium concentration in mosses for Croatia according to Špirić et al. (2012).

Table 6.2.2. Comparison of median values and ranges between measured chromium concentrations in mosses and chromium concentrations in mosses obtained from the literature for Croatia, Slovenia, Serbia, Macedonia and Bulgaria for the year 2005, Hungary for the year 2000, and Norway for the year 2010.

		No. of samples	**Median (mg kg^{-1})**	**Range (mg kg^{-1})**
Measured Cr concentration in mosses	**RAINY SEASON**			
	B. rutabulum	5	**8.4**	1.8 – 16
	H. splendens	3	**2.4**	1.9 – 2.8
	DRY SEASON			
	B. rutabulum	5	**7.3**	0.5 – 27
	H. splendens	4	**1.85**	0.6 – 3.5
		No. of samples	**Median (mg kg^{-1})**	**Range (mg kg^{-1})**
Cr concentration in mosses according to the literature	Croatia, 2005 (Špirić et al., 2012)	94	**2.75**	0.76 – 33
	Slovenia, 2005 (Harmens et al., 2008)	57	**2.14**	0.85 – 10,3
	Serbia, 2005 (Harmens et al., 2008)	193	**6.44**	2 – 78.8
	Hungary, 2000 (Buse et al., 2003)	32	**6.4**	3 – 13.1
	Macedonia, 2005 (Barandovski et al., 2008)	72	**6.79**	2.09 – 82.0
	Bulgaria, 2005 (Harmens et al., 2008)	213	**2.43**	0.79 – 57.8
	Norway, 2010 (Steinnes et al., 2011)	464	**0.58**	0.099 – 65.5

Data show that chromium concentrations in mosses measured in this study are higher (for species *B. rutabulum*) than the median value of chromium in mosses reported for Croatia (2.75 mg kg^{-1}) according to Špirić et al. (2012). If compared to other Balkan countries and neighbouring countries (Table 6.2.2.), Croatia has lower median values and possibly less Cr contamination in the environment, however, much higher concentrations were measured in species *B. rutabulum* in this study. Thus, data may suggest that the concentrations of chromium measured in mosses in this study in the Risnjak National Park can possibly be traced also to the industrial sources of pollution from the neighbouring countries.

6.2.3. Mercury

From Figure 6.2.5. it can be seen that the concentration of mercury in mosses increases with the rise of the altitude, which may be related to the increased precipitation at higher elevations and a possible washout of the airborne Hg pollution.

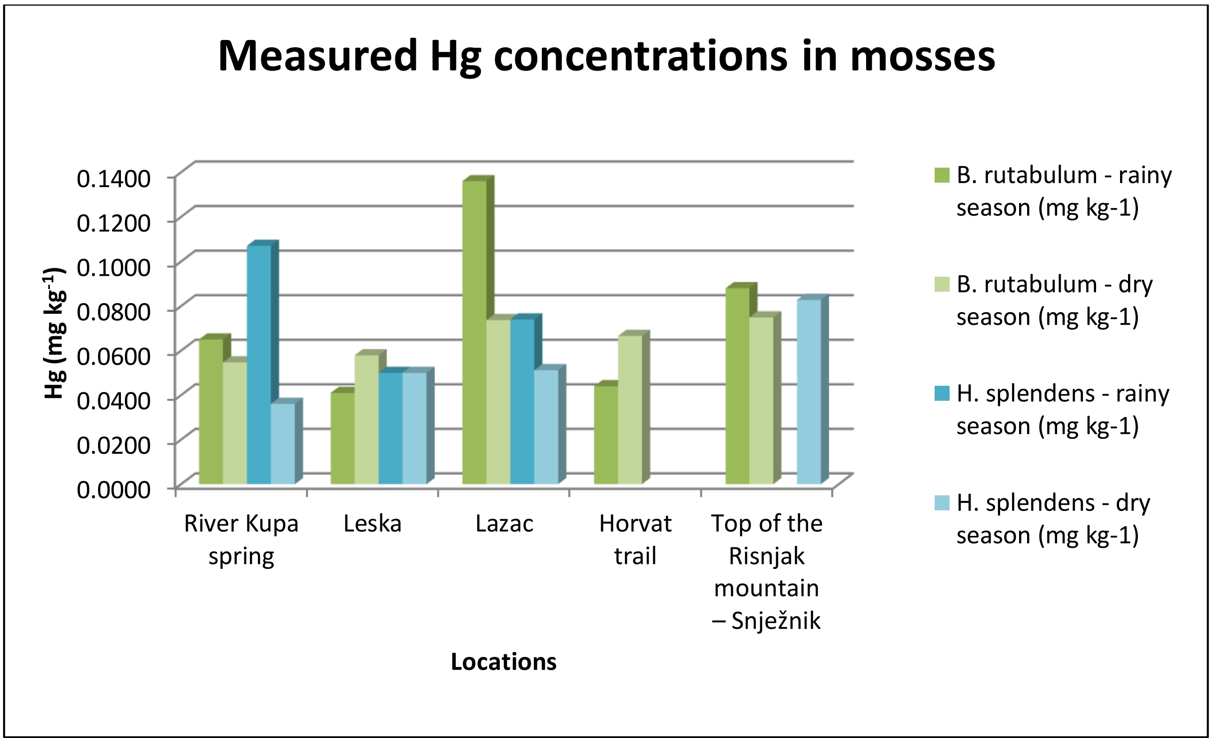

Figure 6.2.5. Comparison of mercury concentrations in two moss species (*B. rutabulum* and *H. splendens*) collected from selected locations at Risnjak National Park during the dry and rainy season (locations are presented from the lowest to the highest altitude).

Mercury concentrations in moss tissue were first compared with the median values and ranges for mercury concentrations in mosses in Croatia obtained from Špirić et al. (2012) when Moss samples from four dominant species (Hypnum cupressiforme, Pleurozium schreberi, Homalothecium sericeum and Brachythecium rutabulum) were collected during the summer and autumn of 2010 from 94 sampling sites evenly distributed over the territory of Croatia. Samples were totally digested by using microwave digestion system, whilst mercury was analysed by using cold vapour atomic absorption spectrometry (CV-AAS). Additional comparison was made with the median values and ranges for mercury concentrations in mosses obtained from similar studies conducted in: (i) neighboring countries - Slovenia (Harmens et al, 2013); (ii) other Balkan countries - Macedonia (Harmens et al, 2013); as well as (iii) Norway, as pristine area (Steinnes et al., 2011). Comparison of data is shown in Figure 6.2.6. and Table 6.2.3.

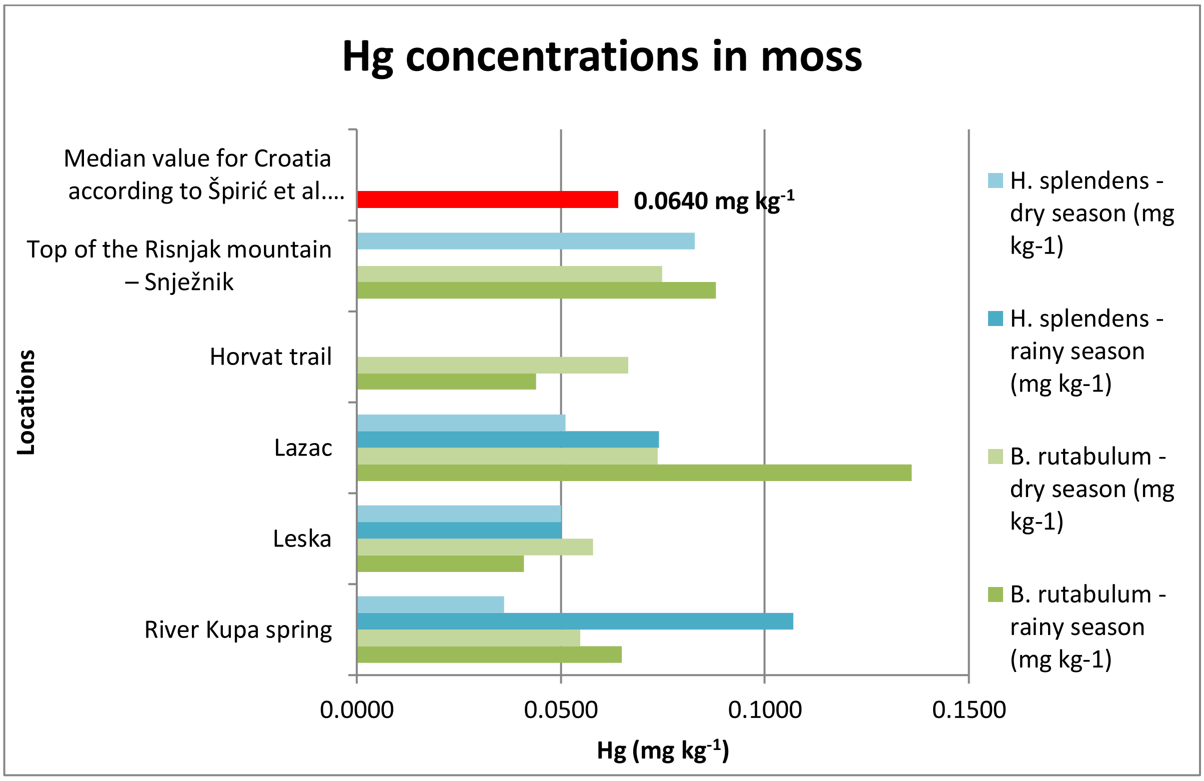

Figure 6.2.6. Comparison of mercury concentrations in two moss species (*B. rutabulum* and *H. splendens*) collected from selected locations at Risnjak National Park during the dry and rainy season with the median value for mercury concentration in mosses for Croatia according to Špirić et al. (2012).

Table 6.2.3. Comparison of median values and ranges between measured mercury concentrations in mosses and mercury concentrations in mosses obtained from the literature for Croatia for the year 2005, Slovenia, Macedonia and Norway for the year 2010.

		No. of samples	Median (mg kg^{-1})	Range (mg kg^{-1})
Measured Hg concentration in mosses	**RAINY SEASON**			
	B. rutabulum	5	**0.05**	0.014 – 0.088
	H. splendens	3	**0.045**	0.011 – 0.074
	DRY SEASON			
	B. rutabulum	5	**0.066**	0.055 – 0.075
	H. splendens	4	**0.044**	0.036 – 0.083
		No. of samples	**Median (mg kg^{-1})**	**Range (mg kg^{-1})**
Hg concentration in mosses according to the literature	Croatia, 2005 (Špirić et al., 2012)	94	**0.064**	0.007 – 0.301
	Slovenia, 2010 (Harmens et al., 2013)	63	**0.056**	0.030 – 0.16
	Macedonia, 2010 (Harmens et al., 2013)	72	**0.093**	0.010 – 0.60
	Norway, 2010 (Steinnes et al., 2011)	464	**0.060**	0.024 – 0.338

Data show that mercury concentrations in mosses measured in this study are lower than the median value of chromium in mosses reported for Croatia (0.064 mg kg^{-1}) according to Špirić et al. (2012). If compared to Slovenia and Croatia (Table 6.2.3.), lower median values and possibly less Hg contamination in the environment was measured in this investigation. Thus, data may suggest that the concentrations of mercury measured in mosses in this study in the Risnjak National Park can possibly be traced also to the industrial sources of pollution from the neighbouring countries and Croatia.

6.2.4. Lead

From Figure 6.2.7. it can be seen that the concentration of lead in mosses increases with the rise of the altitude, which may be related to the increased precipitation at higher elevations and a possible washout of the airborne Pb pollution.

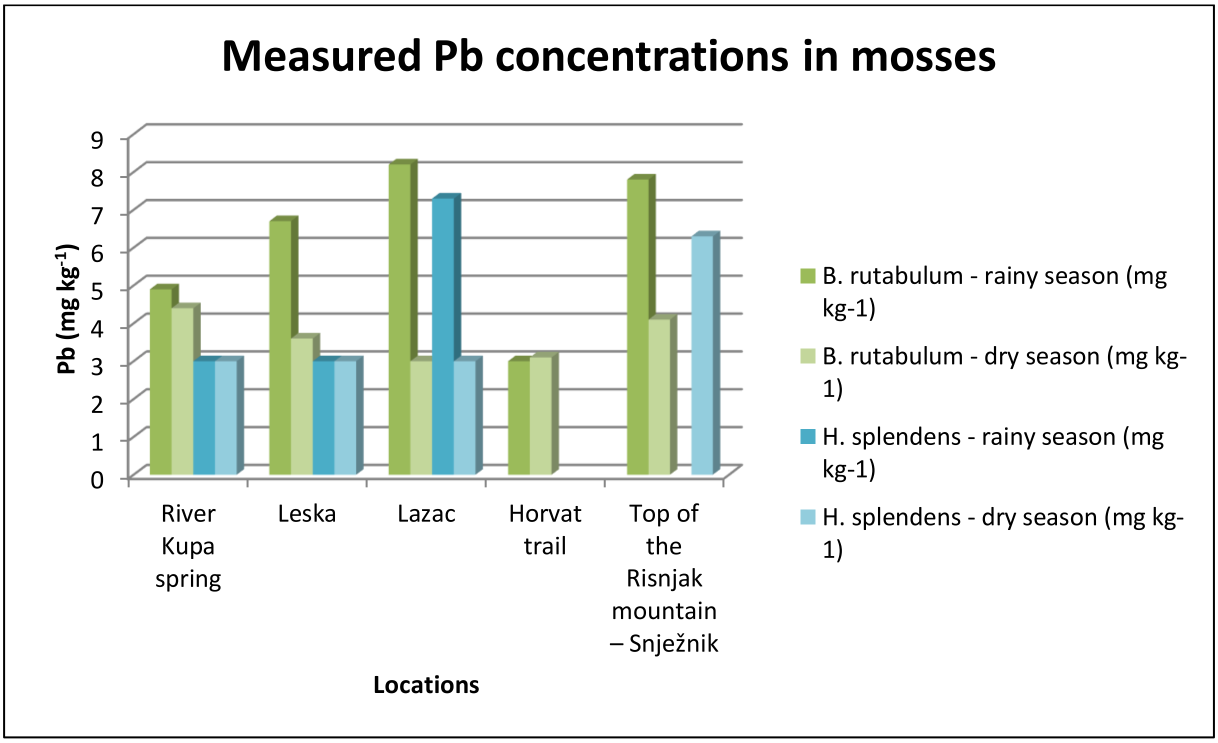

Figure 6.2.7. Comparison of lead concentrations in two moss species (*B. rutabulum* and *H. splendens*) collected from selected locations at Risnjak National Park during the dry and rainy season (locations are presented from the lowest to the highest altitude).

Lead concentrations in moss tissue were first compared with the median values and ranges for lead concentrations in mosses in Croatia obtained from Špirić et al. (2016) when moss samples from different species (Hypnum cupressiforme, Pleurozium schreberi, Homalothecium sericeum, Brachythecium rutabulum) were collected during the summer and autumn from 121 locations evenly distributed over the territory of Croatia. Moss samples were digested in microwave digestion system and then analysed by using atomic emission spectrometry with inductively coupled plasma (ICP-AES). Additional comparison was made with the median values and ranges for lead concentrations in mosses obtained from similar studies conducted in: (i) Europe in general (Harmens et al, 2008); (ii) other Balkan countries - Macedonia (Barandovski et al., 2008 and 2012); as well as (iii) Norway, as pristine area (Steinnes et al., 2011). Comparison of data is shown in Figure 6.2.8. and Table 6.2.4.

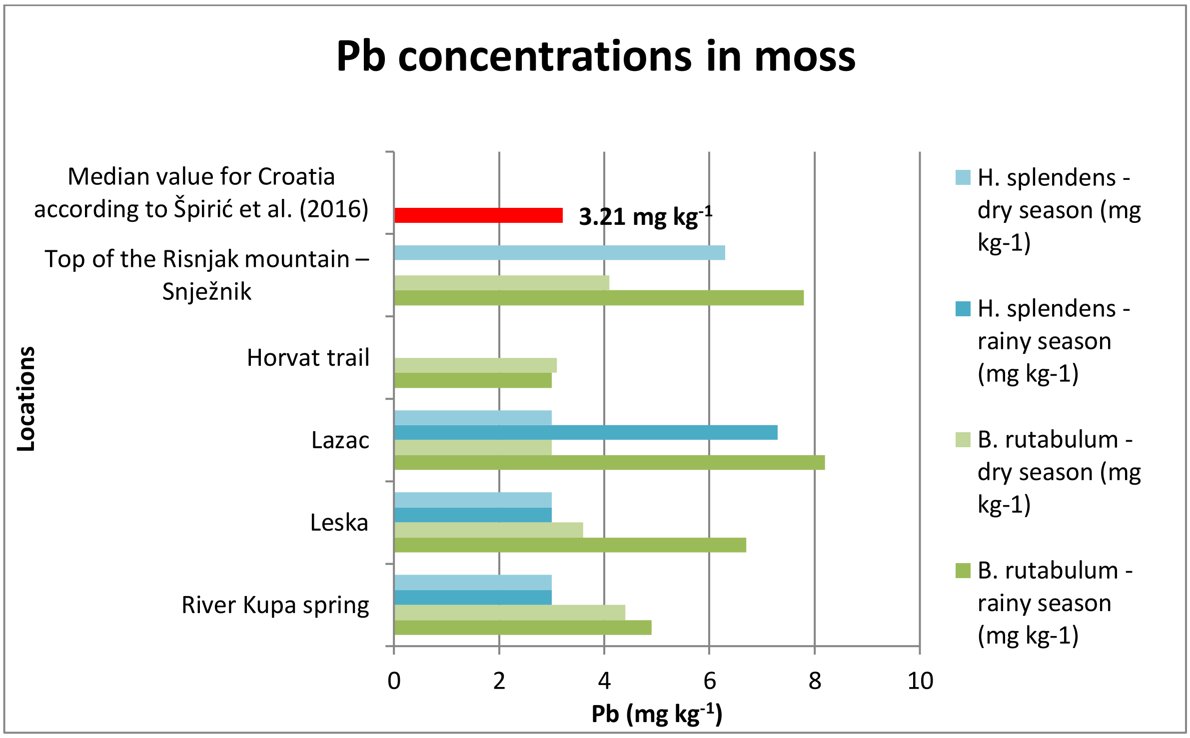

Figure 6.2.8. Comparison of lead concentrations in two moss species (*B. rutabulum* and *H. splendens*) collected from selected locations at Risnjak National Park during the dry and rainy season with the median value for lead concentration in mosses for Croatia according to Špirić et al. (2016).

Table 6.2.4. Comparison of median values and ranges between measured lead concentrations in mosses and lead concentrations in mosses obtained from the literature for Croatia for the year 2010, Macedonia for the years 2002 and 2005, Europe in general for the year 2005/2006 and Norway for the year 2010.

		No. of samples	Median (mg kg^{-1})	Range (mg kg^{-1})
Measured Pb concentration in mosses	**RAINY SEASON**			
	B. rutabulum	5	**6.21**	3 – 8.2
	H. splendens	3	**4.43**	3 – 7.3
	DRY SEASON			
	B. rutabulum	5	**3.64**	3 – 4.4
	H. splendens	4	**3.83**	3 – 6.3
		No. of samples	**Median (mg kg^{-1})**	**Range (mg kg^{-1})**
Pb concentration in mosses according to the literature	Croatia, 2010 (Špirić et al., 2016)	121	**3.21**	1.11 – 36.64
	Macedonia, 2005 (Barandovski et al., 2012)	72	**7.6**	0.1 – 46.1
	Macedonia, 2002 (Barandovski et al., 2008)	73	**6.0**	1.5 – 37.2
	Europe, 2005/2006 (Harmens et al., 2008)	6049	**1.76**	1.76 – 46.94
	Norway, 2010 (Steinnes et al., 2011)	464	**1.54**	0.33 – 20.83

Data show that lead concentrations in mosses measured in this study are higher than the median value of lead in mosses reported for Croatia (3.21 mg kg^{-1}) according to Špirić et al. (2016). If compared to Macedonia and Europe in general (Table 6.2.4.), lower median values and possibly less Hg contamination in the environment was measured in this investigation than in Macedonia, but much higher than median value for Europe. Thus, data may suggest that the concentrations of lead measured in mosses in this study in the Risnjak National Park can possibly be traced also to the industrial sources of pollution from the Balkan countries and Croatia.

6.2.5. Zinc

From Figure 6.2.9. it can be seen that the concentration of zinc in mosses increases with the rise of altitude, which may be related to the increased precipitation at higher elevations and a possible washout of the airborne Zn pollution.

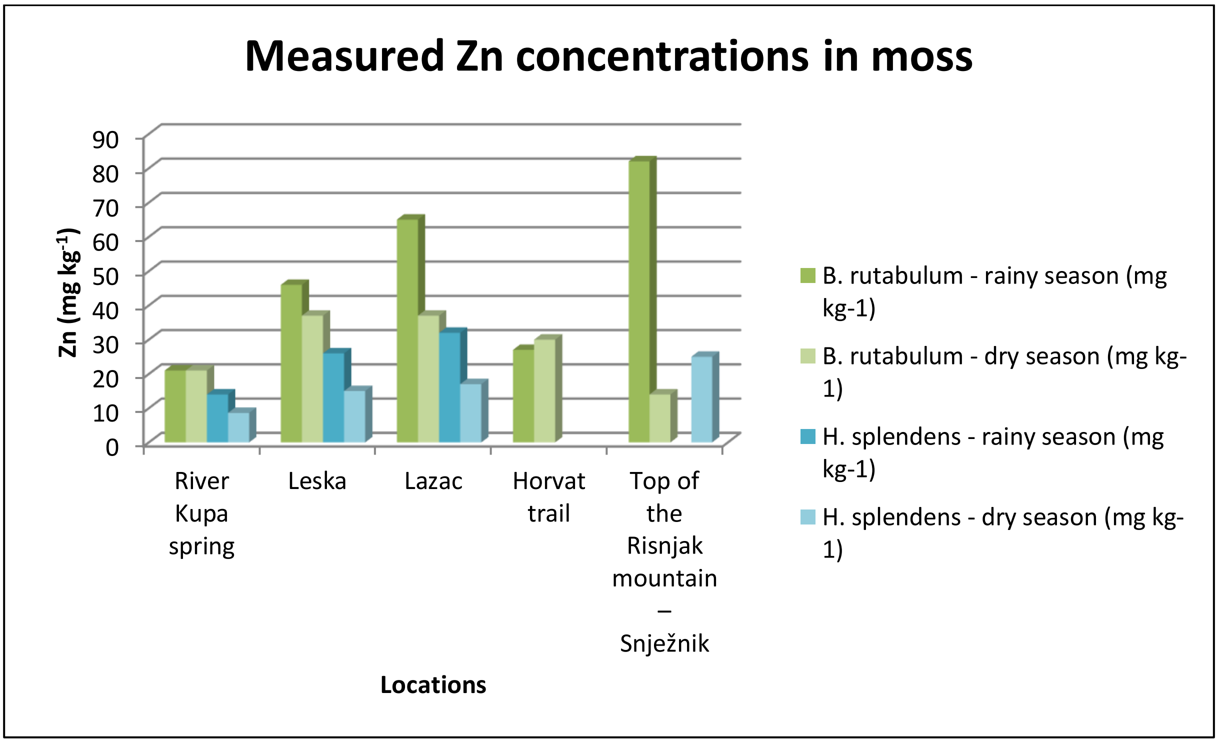

Figure 6.2.9. Comparison of zinc concentrations in two moss species (*B. rutabulum* and *H. splendens*) collected from selected locations at Risnjak National Park during the dry and rainy season (locations are presented from the lowest to the highest altitude).

Zinc concentrations in moss tissue were first compared with the median values and ranges for lead concentrations in mosses in Croatia obtained from Špirić et al. (2012) when amples from different moss species (*Hypnum cupressiforme, Pleurozium schreberi, Homalothecium sericeum and Brachythecium rutabulum*) were collected from 94 sampling sites evenly distributed over the territory of Croatia, during the summer and autumn of 2010. Moss samples were totally digested by using microwave digestion system and analysed by using atomic emission spectrometry with inductively coupled plasma (ICP-AES). Additional comparison was made with the median values and ranges for lead concentrations in mosses obtained from similar studies conducted in: (i) neighboring countries - Slovenia, Serbia (Harmens et al, 2008) and Hungary (Buse et al., 2003); (ii) other Balkan countries - Macedonia (Barandovski et al., 2008 and 2012) and Bulgaria (Harmens et al., 2008); as well as (iii) Norway, as pristine area (Steinnes et al., 2011). Comparison of data is shown in Figure 6.2.10. and Table 6.2.5.

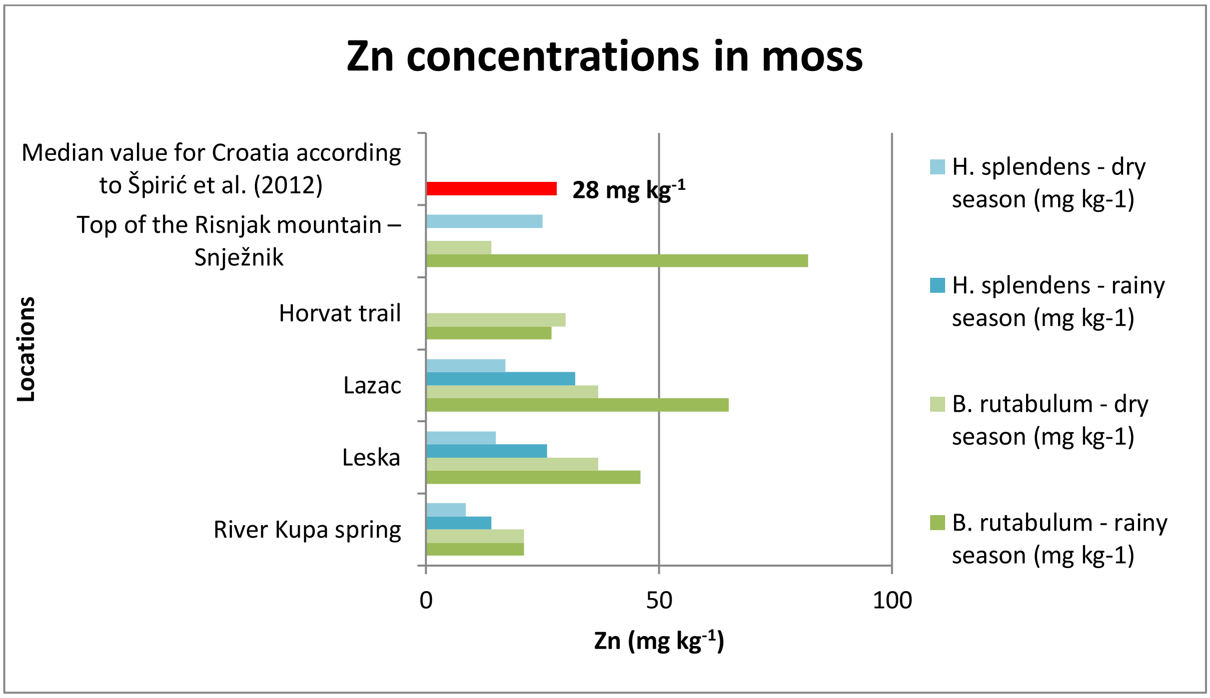

Figure 6.2.10. Comparison of zinc concentrations in two moss species (*B. rutabulum* and *H. splendens*) collected from selected locations at Risnjak National Park during the dry and rainy season with the median value for zinc concentration in mosses for Croatia according to Špirić et al. (2012).

Table 6.2.5. Comparison of median values and ranges between measured zinc concentrations in mosses and zinc concentrations in mosses obtained from the literature for Hungary for the year 2000, Macedonia for the year 2002 Croatia, Macedonia, Slovenia, Serbia and Bulgaria for the year 2005 and Norway for the year 2010.

		No. of samples	Median (mg kg^{-1})	Range (mg kg^{-1})
Measured Zn concentration in mosses	**RAINY SEASON**			
	B. rutabulum	5	**48.2**	21 – 82
	H. splendens	3	**24**	14 – 32
	DRY SEASON			
	B. rutabulum	5	**27.8**	14 – 37
	H. splendens	4	**16.4**	8.6 – 25
		No. of samples	**Median (mg kg^{-1})**	**Range (mg kg^{-1})**
Zn concentration in mosses according to the literature	Croatia, 2005 (Špirić et al., 2012)	94	**28**	12 – 283
	Slovenia, 2005 (Harmens et al., 2008)	57	**38.6**	16.5 – 99.3
	Serbia, 2005 (Harmens et al., 2008)	193	**29.0**	13.2 – 259
	Hungary, 2000 (Buse et al., 2003)	32	**29.9**	17.7 – 114
	Macedonia, 2002 (Barandovski et al., 2008)	73	**39.0**	14 – 203
	Macedonia, 2005 (Barandovski et al., 2012)	72	**36.0**	16 – 91
	Bulgaria, 2005 (Harmens et al., 2008)	213	**27.9**	9.38 – 366
	Norway, 2010 (Steinnes et al., 2011)	464	**30.7**	7.4 – 368

Data show that zinc concentrations in mosses measured in this study are lower than the median value of zinc in mosses reported for Croatia (28 mg kg^{-1}) according to Špirić et al. (2012), with the exception of species *B. rutabulum* in rainy season, when much higher concentrations were measured. If compared to neighbouring countries, other Balkan countries and even Norway (Table 6.2.5.), lower median values and possibly less Zn contamination in the environment was measured in this investigation, with the exception of species *B. rutabulum* in rainy season, when much higher concentrations were measured. Thus, data may suggest that the concentrations of zinc measured in species *B. rutabulum* in rainy season in this study in the Risnjak National Park can possibly be traced also to the industrial sources of pollution from other countries.

7. Conclusion

From the one-year study of heavy metal accumulation in mosses growing at the Risnjak National Park it may be concluded:

1. From the comparison of measured metal (Cd, Cr, Hg, Pb and Zn) concentrations in the soil at the selected locations in Risnjak National Park with the median values for the same metals for Croatian mountain region according to Halamić and Miko (2009), it may be concluded that at locations at higher altitudes, for all metals except for chromium, measured values were higher than the medians for the same metals for the Croatian mountain region. At River Kupa spring, located at the lowest altitude (325 m), all measured soil metal concentrations were lower than the soil median values for the same metals according to Halamić and Miko (2009), while at the location Leska, located at the next investigated altitude (694 m), measured Pb and Zn soil concentrations were also lower than their median values. Data suggest that the soil metal concentrations at the investigated locations at the Risnjak National Park are rising with the increase of the location altitude.

2. Species *B. rutabulum* is possibly more suitable for biomonitoring of atmospheric metal deposits than the species *H. splendens*. This conclusion arises from the fact that higher concentrations of all investigated metals were measured at almost all research locations in species *B. rutabulum.* This conclusion is additionally supported by higher concentrations of long-range transported metals such as Cd, Pb and Zn, also recorded in species *B. rutabulum*.

3. Higher metal concentrations in both moss species and at all investigated locations were measured in the rainy season, which may be related to the more intensive deposition of metals from the atmosphere due to the higher precipitation rate in period before moss sampling.

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9. Appendix

Figure	Title	Source
3.2.1.	Heavy metals pathways into / throughout the food chain.	Author
3.3.1.	Change in extreme temperature and precipitation by 2080s relative to the period 1961 – 1990 under the A2 scenario reported by IPCC (1995).	Dankers and Hiederer, (2008)
4.1.1.	Locations of research in the area of Risnjak National Park and location of Risnjak National Park in Republic of Croatia. The border of Risnjak National Park is marked by a red line.	Zovko et al. (2017)
4.1.2.	Relative frequency of wind direction (left) and average wind speed for each wind direction (right) for the meteorological station Ogulin in period 1995 – 2002.	Bajić and Peroš, (2005)
6.1.1.	The concentrations of Cr, Pb and Zn measured in the soil with the respect to the rising altitude.	Author
6.1.2.	The concentrations of Cd and Hg measured in the soil with the respect to the rising altitude.	Author
6.1.3.	Cadmium concentrations at the sampling sites compared with the median value of cadmium concentration for the Croatian mountain region (Halamić and Miko, 2009).	Author
6.1.4.	Chromium concentrations at the sampling sites compared with the median value of chromium concentration for the Croatian mountain region (Halamić and Miko, 2009).	Author
6.1.5.	Mercury concentrations at the sampling sites compared with the median value of mercury concentration for the Croatian mountain region (Halamić and Miko, 2009).	Author
6.1.6.	Lead concentrations at the sampling sites compared with the median value of lead concentration for the Croatian mountain region (Halamić and Miko, 2009).	Author
6.1.7.	Zinc concentrations at the sampling sites compared with the median value of zinc concentration for the Croatian mountain region (Halamić and Miko, 2009).	Author
6.2.1.	Comparison of cadmium concentrations in two moss species (*B. rutabulum* and *H. splendens*) collected from selected locations at Risnjak National Park during dry and rainy season (locations are presented from the lowest to the highest altitude).	Author
6.2.2.	Comparison of cadmium concentrations in two moss species (*B. rutabulum* and *H. splendens*) collected from selected locations at Risnjak National Park during the dry and rainy season with the median value for cadmium concentration in mosses for Croatia according to Špirić et al. (2012).	Author
6.2.3.	Comparison of chromium concentrations in two moss species (*B. rutabulum* and *H. splendens*) collected from selected locations at Risnjak National Park during the dry and rainy season (locations are presented from the lowest to the highest altitude).	Author

6.2.4.	Comparison of chromium concentrations in two moss species (*B. rutabulum* and *H. splendens*) collected from selected locations at Risnjak National Park during the dry and rainy season with the median value for chromium concentration in mosses for Croatia according to Špirić et al. (2012).	Author
6.2.5.	Comparison of mercury concentrations in two moss species (*B. rutabulum* and *H. splendens*) collected from selected locations at Risnjak National Park during the dry and rainy season (locations are presented from the lowest to the highest altitude).	Author
6.2.6.	Comparison of mercury concentrations in two moss species (*B. rutabulum* and *H. splendens*) collected from selected locations at Risnjak National Park during the dry and rainy season with the median value for mercury concentration in mosses for Croatia according to Špirić et al. (2012).	Author
6.2.7.	Comparison of lead concentrations in two moss species (*B. rutabulum* and *H. splendens*) collected from selected locations at Risnjak National Park during the dry and rainy season (locations are presented from the lowest to the highest altitude).	Author
6.2.8.	Comparison of lead concentrations in two moss species (*B. rutabulum* and *H. splendens*) collected from selected locations at Risnjak National Park during the dry and rainy season with the median value for lead concentration in mosses for Croatia according to Špirić et al. (2016).	Author
6.2.9.	Comparison of zinc concentrations in two moss species (*B. rutabulum* and *H. splendens*) collected from selected locations at Risnjak National Park during the dry and rainy season (locations are presented from the lowest to the highest altitude).	Author
6.2.10.	Comparison of zinc concentrations in two moss species (*B. rutabulum* and *H. splendens*) collected from selected locations at Risnjak National Park during the dry and rainy season with the median value for zinc concentration in mosses for Croatia according to Špirić et al. (2012).	Author

5.2.4.	Enrichment factor calculated according to Salomons and Förstner (1984) for chromium concentrations in mosses *B. rutabulum* and *H. splendens* on all sampling sites (River Kupa spring, Leska, Lazac, Horvat trail, Top of the Risnjak mountain – Snježnik) during the rainy (B) and dry season (A) in the mountain region of Croatia.	Author
5.2.5.	Mercury concentrations in mosses *B. rutabulum* and *H. splendens* on all sampling sites in the Risnjak National Park (River Kupa spring, Leska, Lazac, Horvat trail, Top of the Risnjak mountain – Snježnik) in dry and rainy season.	Author
5.2.6.	Enrichment factor calculated according to Salomons and Förstner (1984) for mercury concentrations in mosses *B. rutabulum* and *H. splendens* on all sampling sites (River Kupa spring, Leska, Lazac, Horvat trail, Top of the Risnjak mountain – Snježnik) during the rainy (B) and dry season (A) in the mountain region of Croatia.	Author
5.2.7.	Lead concentrations in mosses *B. rutabulum* and *H. splendens* on all sampling sites in the Risnjak National Park (River Kupa spring, Leska, Lazac, Horvat trail, Top of the Risnjak mountain – Snježnik) in dry and rainy season.	Author
5.2.8.	Enrichment factor calculated according to Salomons and Förstner (1984) for lead concentrations in mosses *B. rutabulum* and *H. splendens* on all sampling sites (River Kupa spring, Leska, Lazac, Horvat trail, Top of the Risnjak mountain – Snježnik) during the rainy (B) and dry season (A) in the mountain region of Croatia.	Author
5.2.9.	Zinc concentrations in mosses *B. rutabulum* and *H. splendens* on all sampling sites in the Risnjak National Park (River Kupa spring, Leska, Lazac, Horvat trail, Top of the Risnjak mountain – Snježnik) in dry and rainy season.	Author
6.2.1.	Comparison of median values and ranges between measured cadmium concentrations in mosses and cadmium concentrations in mosses obtained from the literature for Croatia, Slovenia, Serbia, Macedonia and Bulgaria for the year 2005, Hungary for the year 2000, and Norway for the year 2010.	Author
6.2.2.	Comparison of median values and ranges between measured chromium concentrations in mosses and chromium concentrations in mosses obtained from the literature for Croatia, Slovenia, Serbia, Macedonia and Bulgaria for the year 2005, Hungary for the year 2000, and Norway for the year 2010.	Author
6.2.3.	Comparison of median values and ranges between measured mercury concentrations in mosses and mercury concentrations in mosses obtained from the literature for Croatia for the year 2005, Slovenia, Macedonia and Norway for the year 2010.	Author
6.2.4.	Comparison of median values and ranges between measured lead concentrations in mosses and lead concentrations in mosses obtained from the literature for Croatia for the year 2010, Macedonia for the years 2002 and 2005, Europe in general for the year 2005/2006 and Norway for the year 2010.	Author

6.2.5.	Comparison of median values and ranges between measured zinc concentrations in mosses and zinc concentrations in mosses obtained from the literature for Hungary for the year 2000, Macedonia for the year 2002 Croatia, Macedonia, Slovenia, Serbia and Bulgaria for the year 2005 and Norway for the year 2010.	Author

Biography

Sven Bogdan was born in Đakovo, Croatia, on 22nd of October 1992. His high school education started in 2007 in town Osijek and he graduated from high school in 2011. In the same year, he applied for undergraduate studies in Sanitary engineering at University of Applied Health Sciences in Zagreb, where he obtained Bachelor‟s degree 4 years later. During his undergraduate studies, he spent one semester at Escola Superior de Tecnologia da Saúde de Coimbra in Porugal, via Erasmus+ programme. After he came back to Zagreb, he enrolled international graduate study programme Environmental, agricultural and resource management at University of Zagreb and during these three years, he spent one semester abroad, but this time at University of Sarajevo, Faculty of Agriculture and Food Science. While studying in Sarajevo, Sven wrote his graduate thesis and obtained Master's degree in June 2018.