

## **BIOMONITORING OF AIR POLLUTION WITH ZINC IN CROATIA STUDIED BY MOSS SAMPLES AND ICP-AES**

**Ivana Vučković, Zdravko Špirić, Trajče Stafilov, Vladimir Kušan**

**A b s t r a c t:** Samples from different moss species were collected from 121 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). Descriptive statistics were made from analyses of zinc in all moss samples. The content of Zn varies between  $11.6 \text{ mg kg}^{-1}$  and  $77.1 \text{ mg kg}^{-1}$ . The median value of Zn content is  $24.8 \text{ mg kg}^{-1}$  which is similar to the median value obtained in investigation in 2005 ( $29.0 \text{ mg kg}^{-1}$ ). Map of distribution of zinc shows the sites of the country with higher levels of this element. High contents of Zn were found in moss samples collected from the vicinity of Sisak, region of Podravina and Rijeka as a result of anthropogenic pollution. By the comparison of median value found in moss samples from neighboring and Balkan countries as well as Norway, it can be noted that the median for Zn in Croatia has the lowest value.

**Key words:** air pollution, zinc, moss biomonitoring, ICP-AES, Croatia

### **INTRODUCTION**

Environmental pollution is a worldwide problem and its potential to influence the health of human populations is great [1]. Pollution reaches its most serious proportions in the densely settled urban-industrial centers of the more developed countries [2]. In most of the undeveloped countries, more than 80% of polluted water is used for irrigation, which seriously affects human health in

industrial urban and semi urban areas [3]. Environmental pollution is tangled with the unsustainable anthropogenic activities, resulting in substantial public health problems. The air we breathe is an essential ingredient for our wellbeing and healthy life. Unfortunately, polluted air is common throughout the world especially in developed countries from 1960s [4]. Heavy metals are widespread pollutants of great concern as they are nondegradable and thus persistent. These metals are used in various industries from which effluents are consequently discharged into the environment. Common sources of heavy metal pollution include discharge from industries such as electroplating, plastics manufacturing, fertilizer producing plants and wastes left after mining and metallurgical processes [5]. Heavy metals occur in the atmosphere, basically in particulate form. Hence, the transfer of air borne particles to land or water surfaces by dry, wet and occult deposition [6] constitutes the first stage of accumulation of atmospheric heavy metals.

Zinc is ubiquitous in the environment and occurs in the earth's crust at an average concentration of about  $70 \text{ mg kg}^{-1}$  [7]. Zinc is an essential human nutrient, a cofactor for over 300 enzymes, and is found in all tissues. In humans, the highest concentrations of zinc have been found in bone, muscle, prostate, liver, and kidneys [8, 9]. The primary anthropogenic sources of zinc in the environment are from metal smelters and mining activities [10]. The production and use of zinc in brass, bronze, die castings metal, alloys, rubbers, and paints may also lead to its release to the environment through various waste streams. There are three major routes of entry for zinc into the human body; by inhalation, through the skin, or by ingestion [11]. The most widely known effect of inhaling zinc-containing smoke is the so-called metal fume fever (MFF), which is mainly caused by inhalation of zinc oxide. This acute syndrome is an industrial disease which mostly occurs by inhalation of fresh metal fumes with a particle size  $<1 \mu\text{m}$  in occupational situations such as zinc smelting or welding [12]. Zinc is a well-known supplement for topical treatment of wounds and several dermatological conditions [13–16]. Based on the existing data, it can be concluded that dermal exposure to zinc does not constitute a note worthy toxicological risk. Immediate symptoms after uptake of toxic amounts of zinc include abdominal pain, nausea, and vomiting. Additional effects include lethargy, anemia, and dizziness [17].

Air quality can be monitored by measuring the pollutants directly in the air or in deposition, by constructing models depicting the spread of pollutants, or by using biomonitors [18]. Biomonitors provide information on both the quantity of pollutants and their effect on the occurrence and condition of biomonitors. Although the methods are fast and inexpensive, they only provide a

relatively approximate picture of air quality and the deposition of pollutants. The term bioindicator/biomonitor is used to refer to an organism, or a part of it, that depicts the occurrence of pollutants on the basis of specific symptoms, reactions, morphological changes or concentrations [19]. Biomonitoring species for trace-element air pollution are selected on the basis of specificity (accumulation is considered to occur from the atmosphere only) [20]. In practice, to be a suitable biomonitor some specific requirements have to be met: (1) the organism has to be common in the area of interest, (2) it has to be available for sampling during all seasons, (3) if not, then some simple special devices have to be developed to grow it in all seasons, (4) the monitor should be tolerant of pollutants at the relevant levels. Mosses are cryptogams that thrive in a humid climate. They possess many properties that make them suitable for monitoring air pollutants [21, 22]. The use of mosses as biomonitors for atmospheric pollution became common after suitable methods for sampling and analysing mosses had been developed in Sweden [23, 24]. Mosses have especially been used in regional heavy metal surveys. The first extensive survey was carried out in Scandinavia as early as the end of the 1960s [25]. During the 1980s the surveys expanded to cover all of the Nordic countries [26, 27] and, in the 1990s, to most of the countries in Europe [20, 28, 29].

The aim of this study is to present the content of zinc in 121 moss samples collected from the territory of Croatia, for the moss survey in 2010. The descriptive statistics with arithmetical mean, geometrical mean, median, range, 10 percentile, 90 percentile, variance, standard deviation, skewness and kurtosis will be presented from the analyses of all moss samples. The map of distribution for zinc will show the sites of the country with higher levels of this element and also will be identified the main anthropogenic and geological sources of this element. The data obtained in this study will be compared with the results from a previous study in 2006 in Croatia and with the results from similar studies in some neighboring Balkan countries and Norway.

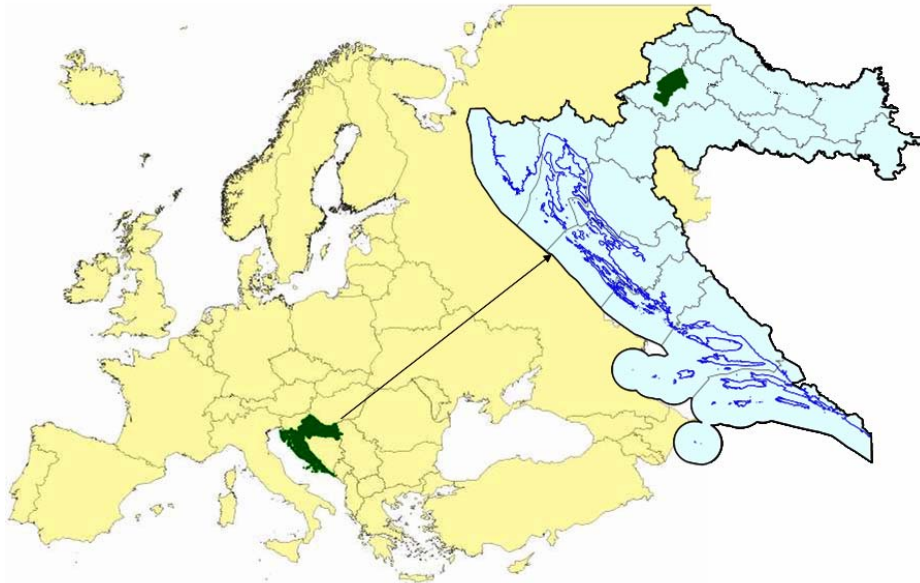
## EXPERIMENTAL SECTION

### *Study area*

The Republic of Croatia is a Central European and is a Mediterranean country that lies mostly between 13.5° and 19.5° eastern longitudes and 42.5° and 46.5° northern latitudes extending from the vast Pannonian plain across the narrow Dinaric mountain range to the Adriatic coast. The territory of Croatia

covers an area of 87.677 km<sup>2</sup> of which 56.610 km<sup>2</sup> is landmass and is located between Bosnia-Herzegovina and Serbia in the east, Slovenia in the west, Hungary in the north and Montenegro and the Adriatic Sea in the south (Fig. 1). Croatia is naturally divided into three large, geographical entities: half of its territory belongs to the Pannonian–Peripannonian region (54%), one third to the Adriatic part (31.6%) while the rest belongs to Dinaric region (14%).

The general state of the environment in Croatia is relatively good compared to the situation in EU industrial countries. In the 1990s, heavy industries experienced a collapse and they had low detrimental impact to the environment. Heavy industry, light industry, metallurgy, chemical, steel, and textile industry are mostly developed in larger cities like Zagreb, Sisak, Kutina, Osjek, Split, Šibenik and their vicinity. Additional information about the study area is given in the previous study [30, 31].



**Fig. 1.** Geographical position of Croatia

### *Sampling*

Different species of recommended moss samples (*Hypnum cupressiforme*, *Pleurozium schreberi*, *Homalothecium sericeum* and *Brachythecium rutabulum*) [32], were collected from 121 sampling sites evenly distributed

over the territory of Croatia during the summer and autumn of 2010 (Fig. 2). Sampling was carried out according to international standards and guidelines of international associations. Samples were taken with a special pair of polyethylene gloves without talc in order to avoid contamination. From each location 5–10 sub-samples were taken within an area of 50 x 50 meters which are mixed in one sample. Locations are at least 300 meters from the main roads and settlements 100 meters from the smaller local roads and individual houses. Samples were stored and transported in a tightly closed paper bags.

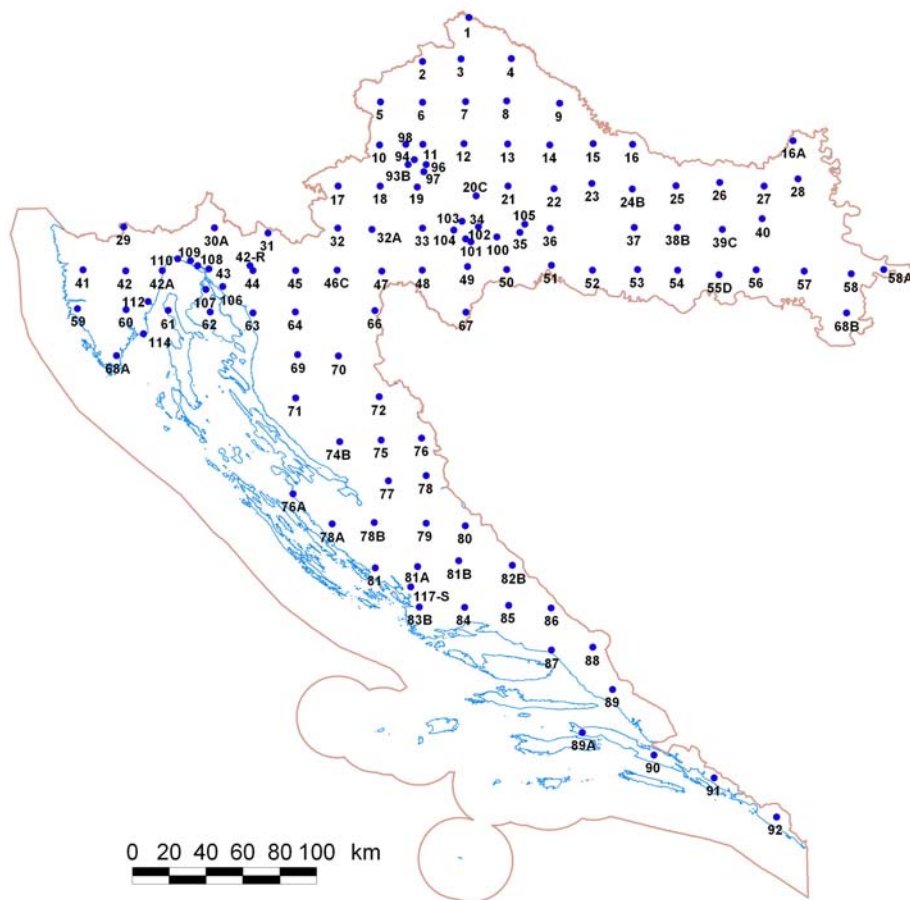


Fig. 2. Map of the locations of sampling sites

### Sample preparation

Microwave digestion system (Mars, CEM, USA) was applied for digesting the moss samples. First of all moss samples were air dried. Then they were cleaned from some contaminants like soil particles. Only the last three years growths of cleaned moss samples were separated. Further on separated moss samples were homogenised and powdered by hands using nylon gloves. About 0.5 g from powdered material, were measured and placed in specialized teflon digestion vessels. Then, 7 ml of concentrated HNO<sub>3</sub> and 2 ml of H<sub>2</sub>O<sub>2</sub> (30%), were added and thus prepared moss samples stayed overnight. Digestion continued the next day and was carried out with digestion program in two steps that are given in Table 1.

Table 1

*Parameters of the two steps from program for digesting moss samples with microwave digestion system (Mars, CEM, USA)*

Step	Temperature, °C	Time, min	Power, W	Pressure, bar
1	180	20	400	20
2	180	10	400	20

### Reagents and standards

All of the reagents that were used for the preparation of the moss samples were with analytical grade: nitric acid, trace pure (Merck, Germany), hydrogen peroxide, p.a. (Merck, Germany), and redistilled water. The solution used for preparing standard solutions was with a concentration of 1000 mg L<sup>-1</sup> (Merck, ICP-Multielement standard solution IV).

### Instrumentation

The content of zinc in moss samples was analyzed by using atomic emission spectrometer with inductively coupled plasma, AES-ICP (Varian, 715ES), Optimal operating instrument parameters are given in previous study (Table 1) [30, 31]. Analyses of zinc in moss samples were performed at a wavelength of 213.857 nm.

### Mapping

For the preparation of spatial distribution of zinc content in mosses in Croatia was used Universal kriging with linear interpolation method (ARC MAP 9.x). The basic grid cell size for interpolation was 0.5 x 0.5 km, number of neighboring points were 8 and maximum distance 200 km (distance between sampling points were 23 x 23 km). Irregular distribution of represented classes was used for best presentation of zinc distribution.

## RESULTS AND DISCUSSION

Descriptive statistics and map of distribution of zinc was made, from data obtained from analyses of zinc in moss samples. Data from the descriptive statistics are given in Table 2 and the map of distribution is presented on Fig. 3.

Table 2

*Data from the descriptive statistics of the results from analyses of Zn in moss samples*

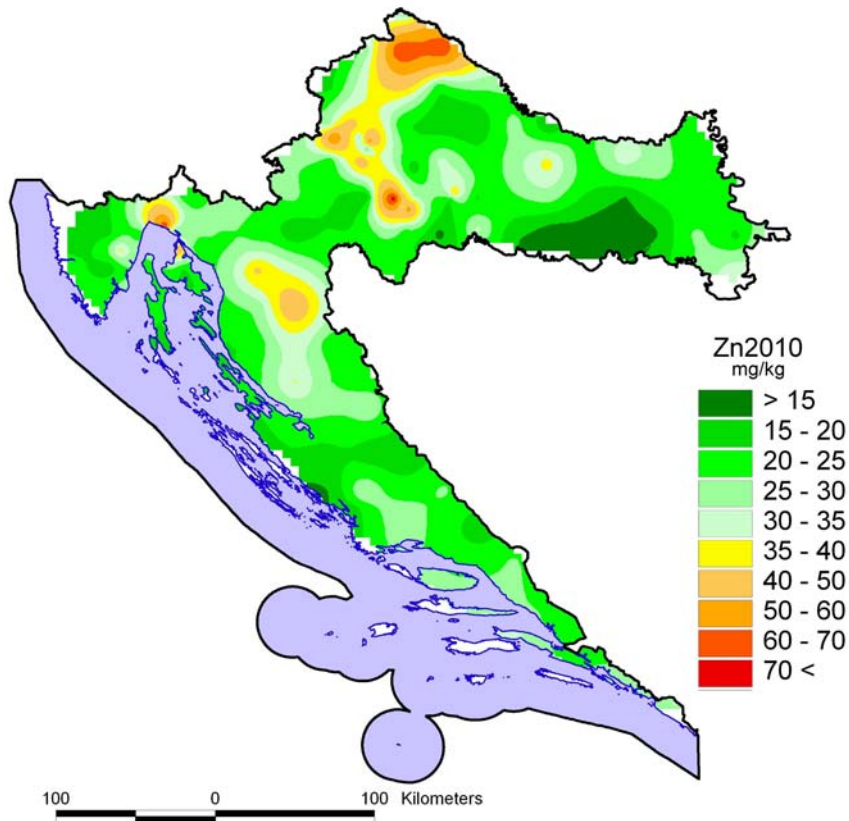
n	$X_a$	$X_g$	Md	Min	Max	P <sub>10</sub>	P <sub>90</sub>	Var	s	A	E
121	27.2	25.2	24.8	11.6	77.1	16.3	41.6	141	11.8	1.91	4.39

*n* – number of moss samples;  $X_a$  – arithmetical mean;  $X_g$  – geometrical mean; Md – median; min – minimum; max – maximum; P<sub>10</sub> – 10 percentile; P<sub>90</sub> – 90 percentile; Var – variance; s – standard deviation; A – skewness; E – kurtosis

The content of zinc in moss samples from the territory of Croatia vary between 11.6 mg kg<sup>-1</sup> and 77.1 mg kg<sup>-1</sup>. The median value of zinc content is 24.8 mg kg<sup>-1</sup>. The highest content of zinc, 77.1 mg kg<sup>-1</sup>, is found in the moss sample collected from sampling point 104 in the vicinity of Sisak. This high content is a result of anthropogenic pollution caused by developed chemical industry and metallurgy.

In the region of Podravina, which is located in central northern part of the country, high contents of zinc were found in the moss samples collected from sampling points 3 (69.9 mg kg<sup>-1</sup>) and 4 (66.9 mg kg<sup>-1</sup>). It should be noted that in this region zinc concentration in soil is the highest in Croatia [33]. The highest concentrations occur in soils above the alluvial deposits of the Drava,

Mura and Danube rivers, particularly in the river Drava Valley from the border with Slovenia to Novo Virje in Podravina [34, 35]. High content of zinc in soil and moss samples from this region is a consequence of long-standing mining activities in Slovenia (Mežica) and Austria (Bleiberg).



**Fig. 3.** Map of geographical distribution of Zn in moss samples

Median value of zinc in soils from the region of Coastal Croatia is  $108 \text{ mg kg}^{-1}$ , which is above the Croatian average ( $88 \text{ mg kg}^{-1}$ ) [33]. Higher contents in moss samples collected from this region are the result of geological source, except the content of zinc in sample collected from the sampling site 109 ( $66.5 \text{ mg kg}^{-1}$ ) near Rijeka. This high content is caused by anthropogenic source from industries that are present near this town.



Heavy metal industry, metallurgy, chemical and textile industry, are the reason for high content of zinc in moss samples collected near the capital city Zagreb.

Comparison of median values and ranges for zinc with the values obtained in previous study in 2005 [34] was made, in order to be seen the trend of atmospheric pollution in the last five years. Additionally, there is a comparison of median values and ranges for zinc obtained in this study with the median values and ranges obtained in similar studies in some neighbouring (Slovenia, Serbia and Hungary), other Balkan countries (Macedonia and Bulgaria) and Norway. These comparisons are shown in Table 3.

Table 3

*Comparison of median values and ranges for Zn obtained in present study with those obtained in previous study in 2005, neighboring and other Balkan countries and Norway*

	No. of samples	Median (mg kg <sup>-1</sup> )	Range (mg kg <sup>-1</sup> )	Reference
Croatia	121	24.8	11.6–77.1	Present work
Croatia, 2005	94	29.0	12–283	[36]
Slovenia, 2005	57	38.6	16.5–99.3	[32]
Serbia, 2005	193	29.0	13.2–259	[32]
Hungary, 2000	32	29.9	17.7–114	[29]
Macedonia, 2002	73	39.0	14–203	[37]
Macedonia, 2005	72	36.0	16–91	[38]
Bulgaria, 2005	213	27.9	9.38–366	[32]
Norway, 2010	464	30.7	7.4–368	[39]

From data presented in Table 3 it can be seen that during the last five years the median value of zinc, 24.8 mg kg<sup>-1</sup> in 2010 and 29.0 mg kg<sup>-1</sup> in 2005 [36], was not changed significantly. It decreased 1.4 times and the reason for this is probably reduced influence from closed mines in Slovenia and Austria and introducing of some safety measures to preserve the environment. When

we see the comparison with neighboring and Balkan countries, it can be noted that Croatia in the present work has the lowest median value. Even when we see the comparison with Norway as a pristine area, it is noticeable that the median value for zinc in Norwegian moss samples ( $30.7 \text{ mg kg}^{-1}$ ) [39] is 1.24 times higher than the median value obtained in Croatian moss samples. Median values obtained in the previous investigations in Serbia ( $29.0 \text{ mg kg}^{-1}$ ) [32], Hungary ( $29.9 \text{ mg kg}^{-1}$ ) [29] and Bulgaria ( $27.4 \text{ mg kg}^{-1}$ ) [32] are very similar to the median value of zinc obtained in present work. Slovenia and Macedonia are the most polluted with zinc in comparison with other countries, probably as a result of the presence of Pb-Zn mines in Veles, Macedonia and Mežica in Slovenia. Macedonia had similar median values in the last two investigations ( $36 \text{ mg kg}^{-1}$  in 2002 and  $39 \text{ mg kg}^{-1}$  in 2005) [37, 38]. From the previous discussion we can conclude that Croatia has relatively insignificant pollution with zinc and is one of the less polluted countries in its surroundings.

## CONCLUSION

The content of zinc was determined in moss samples by using moss biomonitoring technique and atomic emission spectrometry with inductively coupled plasma (ICP-AES). The highest content of zinc was found in moss sample collected near Sisak, caused by anthropogenic influence of chemical industry and metallurgy. As a result of long-standing mining activities in Slovenia (Mežica) and Austria (Bleiberg), high contents of zinc were found in moss samples from the region of Podravina. High values for content of zinc were also found in moss samples collected near Zagreb and Rijeka as a consequence of developed industries near these cities. Median value of zinc is very similar with those from the previous investigation in 2005. From the comparison of median value of zinc with data obtained in some neighbouring, other Balkan countries and Norway, it can be observed that Croatian moss samples have the lowest median value for zinc. It can be concluded that Croatia has is one of the less polluted countries with zinc in its surrounding.

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#### Резиме

#### **БИОМОНИТОРИНГ НА ЗАГАДУВАЊЕТО НА ВОЗДУХОТ СО ЦИНК ВО ХРВАТСКА СО УПОТРЕБА НА ПРИМЕРОЦИ ОД МОВ И АЕС-ИСП**

Различни видови на примероци од мов се собрани од 121 локација соодветно распределени на целата територија на Хрватска во периодот на летото и есента во 2010 година. Примероците од мов се целосно растворени со употреба на микробранов систем и анализирани со примена на атомска емисиона спектрометрија со индуктивно спрегната плазма (АЕС-ИСП). Од добиените резултати од определувањето на цинк во сите примероци од мов извршена е дескриптивна статистичка анализа. Содржината на Zn се движи од  $11,6 \text{ mg kg}^{-1}$  до  $77,1 \text{ mg kg}^{-1}$ . Вредноста на медијаната на Zn изнесува  $24,8 \text{ mg kg}^{-1}$  која е слична на онаа добиена од испитувањата реализирани во 2005 година ( $29,0 \text{ mg kg}^{-1}$ ). Карта на дистрибуција на цинк ги покажува областите во земјата со зголемена содржина на овој елемент. Високи содржини на Zn се утврдени во примероци од мов земени од околии-

ната на Сисак, регионот на Подравина и Риека што се должи на антропогено загадување. Во споредба со вредностите на медијаната за Zn во примероци од мов од соседните и од балканските земји како и со Норвешка, се забележува дека оваа вредност во Хрватска е најниска.

**Клучни зборови:** загадување на воздухот; цинк; биомониторинг со мов; ИСП-АЕС; Хрватска

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