

# Trace Metals in the Environment and Population as Possible Long Term Consequence of War in Osijek-Baranja County, Croatia

Domagoj Vidosavljević<sup>1,2</sup>, Dinko Puntarić<sup>2</sup>, Vlatka Gvozdić<sup>3</sup>, Matijana Jergović<sup>4</sup>, Anamarija Jurčev-Savičević<sup>5</sup>, Ida Puntarić<sup>6</sup>, Eda Puntarić<sup>7</sup> and Marina Vidosavljević<sup>8</sup>

<sup>1</sup> General Hospital Vukovar, Vukovar, Croatia

<sup>2</sup> »J. J. Strossmayer« University, School of Medicine, Department of Public Health, Osijek, Croatia

<sup>3</sup> »J. J. Strossmayer« University, Department of Chemistry, Osijek, Croatia

<sup>4</sup> »Dr. Andrija Štampar« Public Health Institute, Department of Health Ecology, Zagreb, Croatia

<sup>5</sup> Split Public Health Institute, Department for Epidemiology, Split, Croatia

<sup>6</sup> Zagreb County Institute for Emergency Medicine, Zagreb, Croatia

<sup>7</sup> University of Zagreb, Faculty of Science, Biology Department, Environmental Sciences Direction, Zagreb, Croatia

<sup>8</sup> General Hospital Vinkovci, Department for Internal Medicine, Vinkovci, Croatia

## ABSTRACT

*War in Croatia resulted with a significant release of contaminants into the environment as a result of the use of combat assets, mainly conventional, mostly aimed at civilian targets. The aim of the study was to investigate the concentration of metals and metalloids in the soils, water, plants (*Taraxacum officinale*), serum, urine and hair of the inhabitants in eastern Croatia. Overall results show minor abnormalities in presence of some trace metals in soil (As, Hg, Pb, Sb), water (As, Fe) and dandelion leaves (As) in some locations. Compared with soil samples from areas exposed to low intensity combat activity soil samples from areas exposed to heavy fighting had higher concentrations of As, Hg and Pb than allowed by national legislation for ecologic farming. Drinking water with the exceptions of the concentrations of Fe, As and Mn was in accordance with national legislation. Examinees from Dalj had mean hair Sb level 10–19x higher than examinees from any other location. However, when these data are correlated through methods of Principal Component Analysis, presence of trace metals in some war affected areas can be followed from soil, through plants up to population proving that intense combat activities over small area leave metal presence that can be followed even 15 years after the war.*

**Key words:** population, water, soil, dandelion, hair, urine, serum, metals, metalloids, environment, war, Croatia

## Introduction

The war in former Yugoslavia (1991–1999), the first in Europe since World War II, is one of the bloodiest chapters in recent European history. Direct results of the war were thousands of dead and wounded and millions displaced, with the enormous destruction of civilian targets and infrastructure<sup>1</sup>.

A systematic research of the metal and metalloid concentrations in the environment of Croatia or in the wider region was never made, not even in war affected areas. Some published papers only partially covered certain

segments, such as the metals in water of a limited geographical area, for example arsenic in drinking water from the local water supply in eastern Croatia or arable and forest soils<sup>2,3</sup>. Only a few studies that include the presence of some metals in food products or soil and the presence of heavy metals in air, sea water and sediment<sup>4</sup> in central Croatia have been published<sup>5–8</sup>. More has been written on the wartime use of depleted uranium in the wider Balkans region but nothing about Croatia<sup>9,10</sup>. The Republic of Croatia was affected by the longest combat

activities in the region, however only one paper was published investigating the presence of heavy metals in the blood, urine and hair of soldiers exposed to heavy combat operations in Croatia<sup>11</sup>. The results obtained in that study were a pretext for this research.

The aims of this paper were to determine if 15 years after the war, selected metals and metalloids can be found in the soil, water, vegetation, hair, blood and urine at sampling sites and among population exposed to heavy combat activities; to determine whether there is any regularity in the grouping of selected metals and metalloids, and to see if this can be linked to the intensity of war, type of activity and chemical composition of the means used.

## Materials and Methods

### Study area

The eastern part of Croatia, the regions of Slavonia and Baranja, is an area bordered by three rivers: the Danube in the east (border with Serbia), the Sava River in the south (border with Bosnia and Herzegovina) and the Drava River to the north (border with Hungary) and represents about a quarter of Croatian territory and is inhabited by nearly one million people.

Osijek-Baranja County represents a significant part of eastern Croatia, covering 4.152 km<sup>2</sup> with 330,506 inhabitants. The area is predominantly lowland; the population is mainly engaged in agriculture and animal farming with a highly developed food industry.

### Sampling sites

Soil and vegetation sampling was performed at 28 sites in 11 settlements (two towns and nine villages). Nine of the settlements: Vladislavci, Dopsin, Hrastin, Čepin, Čelije, Erdut-Bridge, Dalj, Ernestinovo and Osijek were in war areas of higher intensity combat activity (HICA), a total of 22 collected soil and plant samples, and three of the settlements: Potnjani, Draž and Našice in the areas of low intensity combat activity (LICA), a total

of six collected soil and plant samples. Out of the eight HICA settlements affected by war, three villages were occupied during the war and destroyed (Čelije, Ernestinovo, Dalj), and four villages were on the front line of defense held by the Croatian forces (Čepin, Vladislavci, Dopsin, Hrastin) as well as the city of Osijek (Figure 1).

The principle of soil sampling was as follows: one sample was taken from the scene immediately subjected to infantry, artillery projectile or, from a current or former minefield; one sample from the center of a settlement (usually around a church or school) and a sample from the agricultural ground on the edge of the settlement. In the control sites i.e. LICA sites: one soil sample was taken from the center of the village and another one from agricultural soil on the edge of the village. From each of these locations, a 200 g of dandelion leaves (*Taraxacum officinale*) were collected, at close proximity of a soil sampling site.

Dandelion has been chosen since, according to literature, among hovel plants it is an excellent bio-indicator for heavy metal contamination in environments with various degrees of metal contamination, also fulfilling criteria for phytoindicators in general<sup>12,13</sup>. Dandelion is re-emerging as a food source not only for animals but also for humans<sup>14</sup>. Dandelion is known for its content of beta-carotene, beta-sitosterol, caffeic acid, cryptoxanthin, lutein, stigmasterol, saponin and p-coumaric acid. Their potential benefit lies in increasing bile production, repairing liver functions, reduction in serum cholesterol, relieving of menopausal symptoms and in helping prevention of breast cancer<sup>15</sup>. It also has an antioxidant and antiradical activity that is being investigated<sup>16</sup>. Dandelions in this case, were also chosen because they were common to all of the sampling sites.

Sampling included the following settlements: Čelije, the first destroyed village during the war in Croatia; Dalj, a village on the border with Serbia; the vicinity of Erdut-Bogojevo Bridge at the border crossing on the Danube, which was destroyed by NATO aircrafts in 1999. Other war sites (Vladislavci, Hrastin, Čepin, Dopsin,) were villages on the front line, except the city of



Fig. 1. Map of sampling sites.

Osijek, where soil samples were also taken from army barracks. For the control group three sampling sites: Potnjani, Draž, Našice (LICA) were used (without registered war activities), which according to the criteria of population, agricultural production and soil composition, correspond with the study group. Apart from the city of Osijek, all other places have no metal industry. Agriculture and animal farming are the main activities, and hunting areas have been avoided in sampling.

Drinking water has been taken from local water supplies (68 samples), all of which are being used for human consumption.

Blood, hair and urine samples have been taken from 391 subjects, based upon strict criteria and according to the Guidelines of the Osijek Medical faculty's Ethical Committee and with the signed informed consent by each of the patients. Subjects were previously triaged to exclude subject with potential professional exposure, smokers, minors and residents from other parts of the country. For this purpose a questionnaire was made, covering all aspects of possible professional or wartime exposure.

#### *Sampling procedure and measurement technique*

Soil samples were taken from predefined points (according to Gauss-Krueger coordinates). After removing the top layer of grass with a shovel, probes were used to extract deeper parts of the soil for analysis, which was then packed into containers resistant to the ingredients of the soil. The measured soil interval was 5–50 cm. Dandelion (*Taraxacum officinale*) leaves were collected at the same places and packed in containers for further analysis. Dandelions were washed with distilled water, oven dried at 80 °C for 48 h and ground to fine powder before analysis. Soil samples were air dried and passed through a 2 mm sieve. Plant and soil samples were digested with HNO<sub>3</sub>. Water was collected from 68 spots (taps) all from local water supplies, according to map. Hair samples were taken from the back of the head (cca. 1 cm wide and 3 cm long) using stainless steel scissors and collected in a polyethylene bag. For each of 0.1 g of sample 1 mL 65% HNO<sub>3</sub> was added. After two hours of soaking sample was placed in a microwave oven, heated and cooled and diluted to a volume of 12 mL then followed by ICP-MS. Urine sampling was done in a way that each of the patients gave a first morning urine sample, which was then collected in polyethylene urine containers (urine set 100 mL, Greiner Bio-One, Frickenhausen, Germany). Each of the subjects gave one full test tube of blood, which was then centrifuged for serum. Blood was drawn by a laboratory technician using needles (Vacuette Blood Collection Needle, 38x0.9 mm, Greiner Bio-One, Frickenhausen, Germany) and test tubes (Vacuette Serum Gel Tube 3.5 mL, Greiner Bio-One, Frickenhausen, Germany). Samples were then centrifuged after which supernatant was transferred to cryotubes (Cryotube, 3.8 mL, TPP, Switzerland) and stored at -30 °C. After defrosting, to each cuvette a 10 mL 1% HNO<sub>3</sub> was added and 0.5 mL of sample (serum or urine) was added. After

determining the dry residue, the samples were analysed using inductively coupled plasma mass spectrometry (ICP-MS, ELAN DRC-e, Perkin Elmer, Waltham, MA, USA).

Although by using ICP-MS we determined the values of all 66 elements; for further data processing we used only the ones that are used in military production, and that have already been investigated in this particular region: Mg, Cr, Cu, Zn, Ni, Fe, Pb, Cd, Al, Li, B, Si, P, V, Co, As, Sr, Sb, Ba, Hg and U<sup>11,17</sup>.

#### *Data analysis*

Besides the standard descriptive statistics, the principal component analysis (PCA) was carried out to discover the structure of data and to observe the sources of variation in the data set. The interpretation of the results of PCA is usually carried out by visualization of the component scores and loadings. The plot can be examined or outlined for influential observations, or it shows if the observations can be visually clustered. The data were calculated statistically using Statistica 7.0 software package.

## **Results**

#### *Soil analysis*

A total of 11 (40%) soil samples (all from HICA) recorded values above the maximum allowed values for organic production according to the national regulation, however, they did not exceed the maximum allowed concentrations (MAC) for general agricultural use<sup>18</sup>.

According to the sampling locations: eight samples with increased As and two with higher Hg recorded in soil samples were located in southern parts of the investigated region while in one sample increased Pb was recorded in the eastern part of the region. Compared with soil samples from areas exposed to low intensity combat activity soil samples from areas exposed to heavy fighting had higher concentrations of As, Hg and Pb than allowed by national legislation for ecologic farming as well as even higher concentrations of Hg than the maximum allowed values for agriculture in general. Location Dalj has recorded Sb value of 3.10 mg/kg, which is the highest among those measured. The sites with the highest concentrations of Mg, Sb and Pb in soil samples were Osijek-army barracks, Erdut-Bogojevo Bridge, Dalj-village and Dalj-agricultural soil sample (all HICA). Compared with the other members of the cluster, sampling site Erdut-Bogojevo Bridge is characterized by the highest concentrations of Pb, Mg and Sb.

#### *Water analysis*

Regarding investigated elements it is founded that, drinking water with the exceptions of the concentrations of Fe (Vladislavci: mean 842.68 µg/L, Čepin: mean 226.39 µg/L) MAC=200 µg/L, concentrations of As (Vladislavci: mean 16.32 µg/L, Čepin: mean 184.34 µg/L, Osijek: mean

23.60 µg/L), MAC=10 µg/L and concentrations of Mn (Vladislavci: mean 66.71 µg/L, Dalj mean 70.167 µg/L), MAC=50 µg/L; is in accordance with National Croatian limits for drinking water<sup>19</sup>.

*Plant sample analysis*

Dandelion samples results were in accordance to national legislation with only minor exceptions. Among HICA locations two samples (Osijek, Hrastin) had Pb concentration higher than maximum allowed (0.30 mg/kg) and one had As (Hrastin) concentration above MAC (0.30 mg/kg). Among LICA locations one (Potnjani) had As concentration above MAC (0.30 mg/kg), one (Draž) had Hg concentration above MAC (0.05 mg/kg) and one (Potnjani) had a Pb concentration above MAC (0.30 mg/kg)<sup>20</sup>.

*Biological samples analysis*

Results of the data regarding serum, urine and hair samples among soldiers and civilians have been previously published by Jergović<sup>11,21</sup>. Concentrations were determined in serum, hair and urine of 391 examinees and compared to the results of research from other countries, determining higher concentrations of Al, As, Ba, Cd, Cr, Cu, Li, Mg, Mn, Ni, Pb, U, V and Zn – all of which were connected with military production. Bio monitoring has determined differences among population coming from HICA then from LICA. In population from HICA significantly higher concentrations of: Al, B, Hg in serum; As, Cu, Si in urine; Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, Hg, Li, Mg, Ni, P, Sb, Sn, Sr and Zn were observed. Among former soldiers higher concentrations of metals connected to military production were observed, regardless of location.

*Principal component analysis (PCA)*

Due to the complexity of the numerous relationships, it was difficult to draw clear conclusions directly. However, principal component analysis can extract the hidden information and explain the structure of the complex data sets. PCA revealed two significant principal components (eigenvalue > 1 criterion) that explained 65–88% of the total variance of the data set. The loadings and score plots (i.e. biplot) of the first two PC's are presented in Figures 2–6. The biplots showed grouping as well as a relationship between analyzed samples (water, serum, urine, hair, soil, plant) and concentrations of 22 monitored elements: Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, Hg, Li, Mg, Ni, P, Pb, Sb, Si, Sn, Sr, U, V and Zn. The closer together the two types of samples were, the stronger was the mutual correlation. In Figure 3, the highest positive correlation was observed between hair and plant samples but only in the sampling site Dalj (located in area of high intensity combat activity). The same figure showed that variation in Fe, Al, Ni and Mg concentrations were important for the hair and plant samples. By contrast, the most of remaining elements (Hg, Sb, Cd, Co, U, Sn, As, P, Ba, Cr, Pb, Li, V) associated with more intensive war operations

behave in a different way as they were not strongly correlated either with Fe, Al, Ni and Mg, or with the rest of the samples (i.e. urine, serum, water, soil). Input of elements from combat activities can be ruled out since it is similar in all other sampling sites (Figures 2, 3, 5, 6) regardless of the level of combat activity.

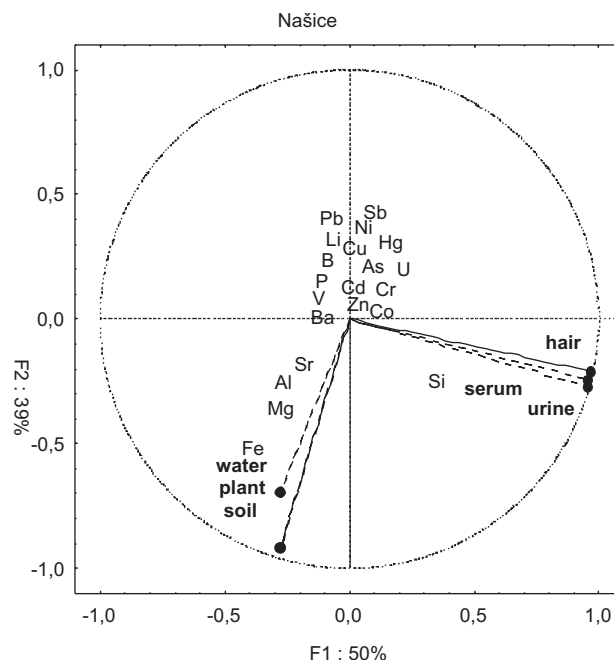


Fig. 2. Principal component analysis of the samples obtained from location Našice.

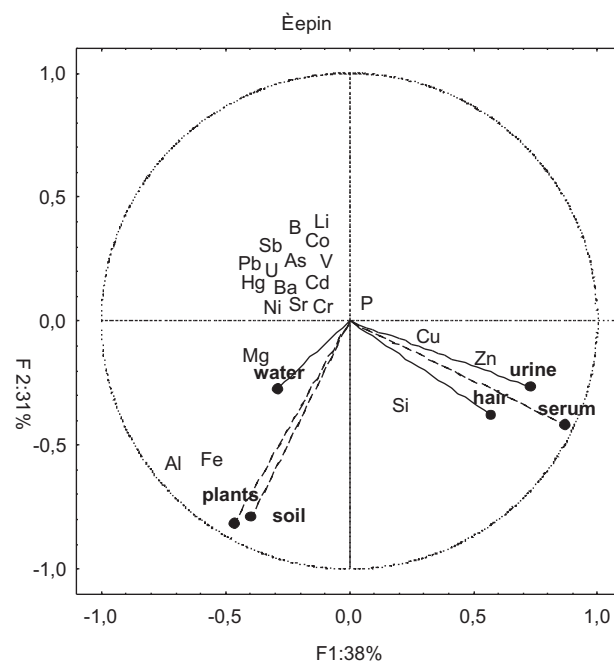


Fig. 3. Principal component analysis of the samples obtained from location Čepin.



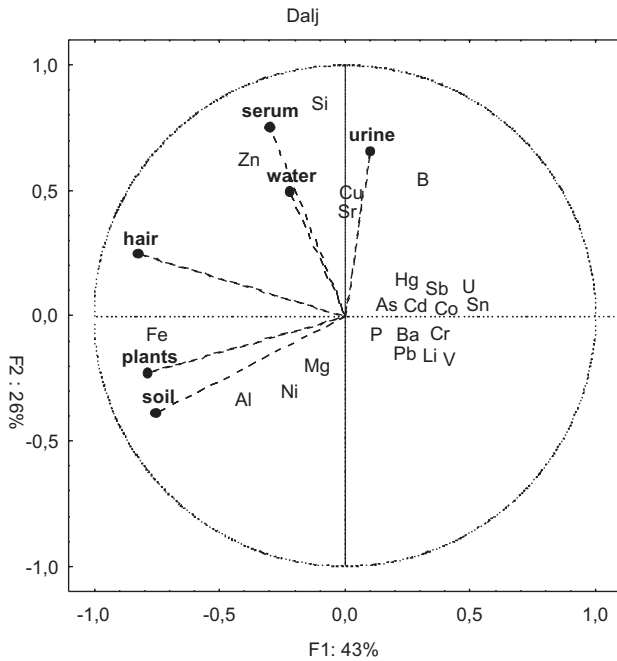


Fig. 4. Principal component analysis of the samples obtained from location Dalj.

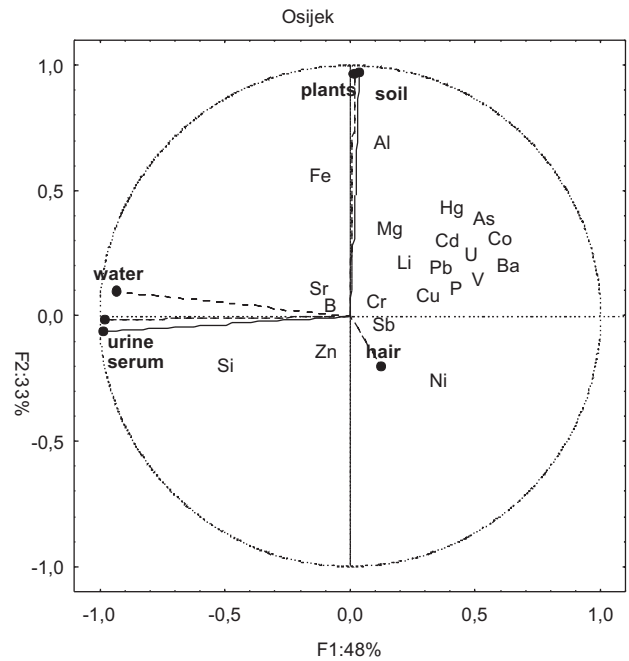


Fig. 6. Principal component analysis of the samples obtained from location Osijek.

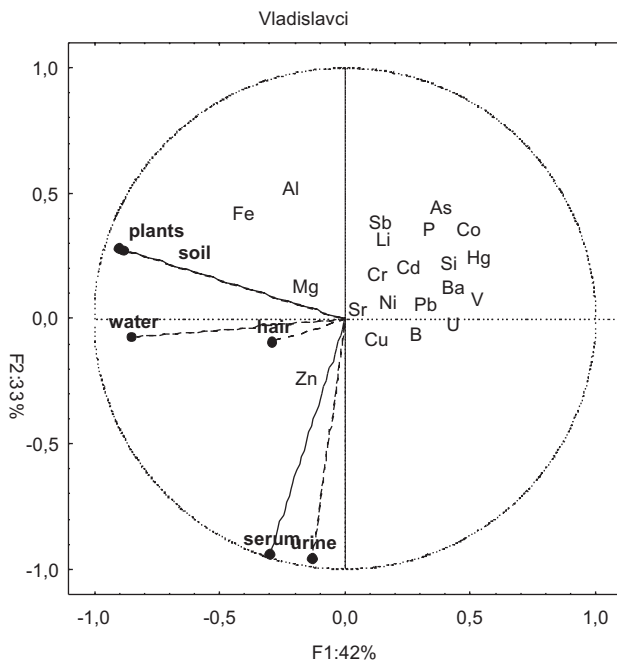


Fig. 5. Principal component analysis of the samples obtained from location Vladislavci.

## Discussion

In our study, the measured concentrations of selected metals and metalloids associated with war activities in all the sampling sites, except one, did not exceed the maximum allowed concentration (MAC) for soil accord-

ing to national and EU regulations<sup>18</sup>. However, referral values are available for only a few metals (Cd, As, Hg, Pb) for agricultural soils in general and for soil use in organic production. It was found that a total of 11 (40%) sampling sites (all from HICA) have higher values of As, Hg and Pb than allowed by national legislation for organic farming, and one sample had a level of Hg higher than the maximum allowed values for soil in general, however that sample was not taken from arable soil.

When water analysis is concerned than drinking water with exceptions of the concentrations of Fe and As for locations of Vladislavci, Čepin, Osijek and Dalj is in accordance with National limits for drinking water. Problems with As, Fe and Mn presence have been previously described by Čavar et al. and Santo et al.<sup>2,22</sup> and represents a biogeochemical problem which has been known for at least a decade and is being resolved by introducing regional water supply instead of a local one.

Results of the dandelion leaves sampling can be compared with similar published papers by Polish, Canadian and Macedonian authors<sup>23–26</sup>. Wilkomirski et al. have published their research on railway transportation as source of pollution, comparing presence of Cd, Fe, Zn, Hg and Cu in soil and *Taraxacum officinale* at major railway junction in Poland, ending with similar results<sup>23</sup>. Czar-nowska and Milewska did a similar analysis on dandelion leaves in Warsaw’s main streets as well as Marr et al. with the dandelions in the streets of Montreal, all with similar results<sup>24,25</sup>. However when comparing the highest measured values of Pb (19.80 mg/kg), although it is higher than ones measured in the streets of Warsaw (5.3–8.9 mg/kg) or Montreal (5.8–6.83 mg/kg) it is still below the measured values in the railway junction in Upper

Silesia, Poland (29.98 mg/kg). Higher concentrations of As were observed in plants taken from the wider Čepin area.

When biological samples are concerned, significantly higher concentrations of Al, As, Ba, P and V in the blood, significantly higher concentrations of As and Cd in urine and Al, Fe, Cd, Pb and V in hair samples of subjects exposed to more intensive war operations (soldiers and civilians) from these locations were found<sup>11,21</sup>. Some of these results could be expected: Čepin and nearby Vladislavci have higher concentrations of As in drinking water and our study revealed higher concentrations of As in soil and plants, therefore elevated values of As in biological samples of residents could be expected. Nevertheless, this requires further research since prolonged arsenic exposure can cause damage to the central nervous system, lungs, digestive tract, circulatory system and kidneys leading to a series of chronic health effects ranging from reproductive and developmental abnormalities to cancers<sup>27</sup>.

Principal component analysis revealed Dalj and Danube Bridge as the most interesting spots: the highest positive correlation was observed between hair and plant samples on location Dalj (HICA), showing correlation between presence of trace metals in the environment and long-time exposure in humans. Also, at the same location were the highest measured concentrations of Sb in soil (3.10 mg/kg). Examinees from Dalj had mean hair Sb level 10–19x higher (0.38 mg/kg) than examinees from any other location (0.020, 0.034 and 0.036 mg/kg, respectively) suggesting potential long term exposure. Antimony, along with lead, is considered a very important compound of ammunition of all types. Olive, in her study of Swiss army shooting ranges determined higher values of Sb than these measured in Dalj, but on the target location and in the topsoil depth (0–5 cm); while in our case this location is of civilian use, and sampling depth interval was up to 50 cm<sup>28</sup>. These measured values of antimony (3.10 mg/kg) can be seen as a result of combat action of high intensity over a small area. However, if the U.S. Army Corps of Engineers proposed criteria are to be followed (>2 mg/kg), then a decontamination of the Dalj Bridge area should be done<sup>29</sup>.

In 1991, this was the location of anti-aircraft cannons and machine guns, as well as in 1999 when on the other side of the bridge (Serbian side) anti-aircraft weapons were located (cannons, machine guns, rockets) fighting against NATO aircrafts bombing the bridge.

Although represented in a statistically significant amount compared to other samples this value is still far from the toxic value (MAC=6 mg/kg) prescribed by WHO<sup>30</sup>. Antimony itself has a limited effect on humans, with mainly dermatological consequences and with unconfirmed reports on a higher frequency of stillbirths<sup>31</sup>. Higher values of Pb were also found at the army barracks in the town of Osijek, but it can be also attributed to city traffic.

Some measured values can be correlated with the geological composition of soil, for instance, the content of

Co, Cr, Fe, Mn, Ni and Zn in soil samples that are associated with the lithological component of rock or soil from which they originate. Cd, Cu, P and Mg can also be found in fertilizers. Certain trace elements, such as Mn, V, Co, Mo are also called »heavy metals associated with storm water« suggesting that the storm runoff can strain the debris<sup>32</sup>.

Opposite to this are the elements Cd, Cu and Pb, whose presence is associated only with anthropogenic (wartime and peacetime) activities<sup>33,34</sup>. Published studies on the presence of Pb as a result of the use of munitions in shooting facilities<sup>35,36</sup> or on the influence of weapons on the environment are available<sup>37</sup> so elevated concentrations of Pb in soil, plants or biological samples of exposed population could be expected all over the sampling area. This cannot be regarded only as a consequence of wartime activities, but can also be due to increased road traffic. The measured value of uranium in any of the materials does not differ from values in other samples, so we can determine that there was no use of missiles with depleted uranium. Uranium values in all of the samples were in approximately equal amounts and it is most likely of geological origin<sup>38</sup>. The high value of Hg in the soil taken from location of Hrastin (HICA) cannot be directly explained by war activities but it represents major concern. The measured value above the MAC gives a warning signal, not only because of possible health effects but also because of its possible effect on agriculture – the main source of income for inhabitants. Exposure to Hg can cause central nervous system damage, lung and kidney damage and impaired intellectual functions in children<sup>39</sup>.

Environmental toxicity is becoming a highly emerging topic in modern science, especially due to industrial progress creating more and more waste and destroying more and more eco systems. The fate of heavy metals and metalloids in the environment as well as their toxicity through the food chain are always at the top of discussions. The interest in studying the uptake and deposition of heavy metals in ecosystems is focusing on soil and plants, but also on the intake via the food chain in animal tissues and products, and ultimately in humans. Thus, elevated values of Pb, As and Hg in soil (making organic production impossible) are a matter of great concern, and it should be an alarm for further research and action – possibly through phytoremediation.

In assessing these data an additional problem was the fact that no similar papers have been published in this region, and the fact that the data linked to the military industry in the former Yugoslavia is very scarce and difficult to gain access to.

## Conclusions

To our knowledge this is the first study relating to the presence of metals and metalloids in soil, water, plants and biological samples and its correlation with previous war actions in the region. Although in some locations abnormalities in the presence of certain trace metals (Sb in

location Dalj) have been without a doubt proven, however, in general, there is no serious contamination of the environment. Despite the fact that elevated concentrations of some elements such as Pb, As, Hg in samples may not be the direct consequence of the war, and still they are a matter of great public concern and require further extended research.

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D. Vidosavljević

General Hospital Vukovar, Bolnička 5, 32100 Vinkovci, Croatia  
e-mail: d-vidosavljevic@inet.hr

## PRISUTNOST METALA U OKOLIŠU I POPULACIJI KAO MOGUĆA POSLJEDICA DUGOTRAJNIH RATNIH DJELOVANJA U OSJEČKO-BARANJSKOJ ŽUPANIJI

### SAŽETAK

Rat u Hrvatskoj je rezultirao sa značajnom kontaminacijom okoliša kao posljedicom uporabe borbenih sredstava, uglavnom konvencionalnih i usmjerenih na civilne ciljeve. Cilj istraživanja bio je ispitati koncentraciju metala i metaloida u tlu, vodi, biljkama (maslačak-Taraxacum officinale), serumu, urinu i kosi stanovnika Osječko-baranjske županije. Ukupni rezultati pokazuju manje abnormalnosti u prisutstvu nekih metala u tlu (As, Hg, Pb, Sb), vode (As, Fe) i listovima maslačaka (As) u nekim mjestima. U usporedbi s uzorcima tla iz područja izloženih borbenim aktivnostima niskog intenziteta, uzorci tla iz područja izloženih teškim borbama su imali više koncentracije As, Hg i Pb od dopuštene

nacionalnim zakonodavstvom za ekološku proizvodnju. Pitka voda, s izuzetkom po jednog uzorka s povišenim koncentracijama Fe, As i Mn je u skladu s propisima. Ispitanici iz Dalja su imali prosječnu vrijednost Sb u kosi 10–19x veću u odnosu na ispitanike s drugih ispitivanih lokacija. Međutim, kada se ovi podaci koreliraju metodom analize glavnih komponentata (PCA), prisutnost metala u nekim ratom pogođenim područjima može se pratiti od tla kroz biljke do populacije što dokazuje da intenzivne borbene aktivnosti na malom prostoru mogu ostaviti tragove u vidu prisutnosti određenih koncentracija metala koje se mogu detektirati i više od 15 godina nakon rata.