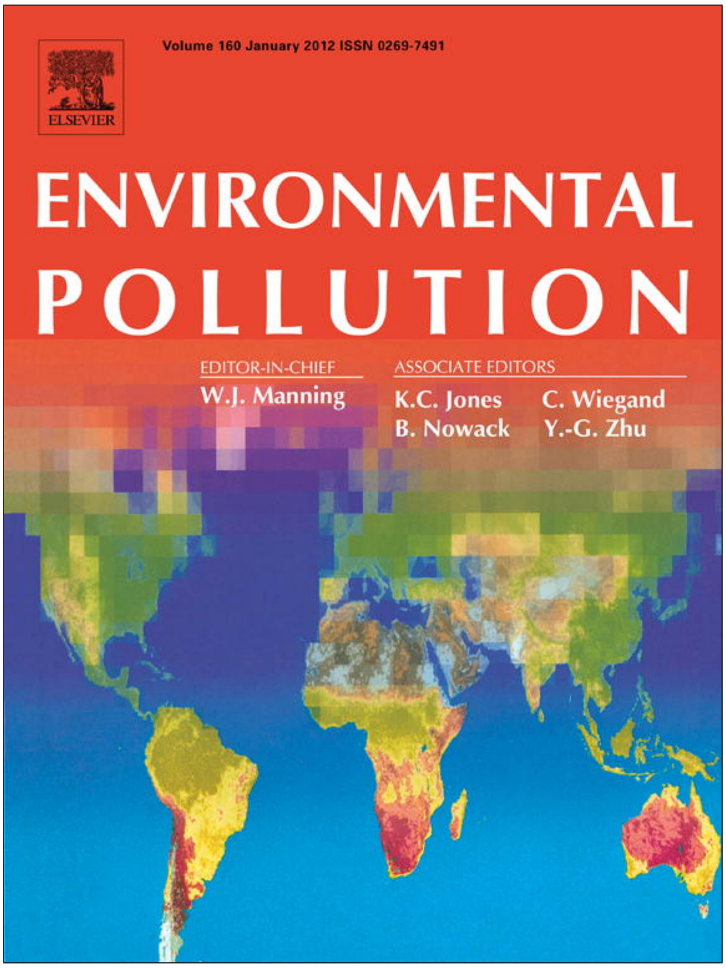
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Potentially toxic contamination of sediments, water and two animal species in Lake Kalimanci, FYR Macedonia: Relevance to human health

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ABSTRACT

The objectives of the research were: (1) to examine the concentrations of metals in *Vimba melanops* and *Rana temporaria* and (2) to evaluate the potential risks of the contaminated organisms to human health in Makedonska Kamenica region. Analyses identified high levels of Cr, Hg, Ni and Pb in studied animals, which also exceeded their permissible levels in food. In sediment and soil samples, levels of Cd, Cu, Cr, Pb, Zn and As were perceived, while Cd, Cu, Ni, Pb, Se and As were increased in water samples. Results of transfer factor revealed that the examined animals had higher bioaccumulation rate from surrounding waters than from sediments or soils. The accomplished Health Risk Index disclosed that studied animals can have considerably high health risks for inhabitants. Conclusively, they could be considered as highly contaminated with metals and can consequently harm human health, especially children in their early development stages.

1. Introduction

Man has used many heavy metals for centuries, but only in the last few decades have the possible effects of heavy metals on the environment and on human health been studied (Yi et al., 2008; Mitra et al., 2012; Mendil et al., 2005; Alkan et al., 2012; Jabeen and Chaudhry, 2010). Heavy metals are introduced into the environment (e.g. aquatic systems) as a result of weathering of rocks and soils (Adeyeye et al., 1996), thus the highest contents of heavy metals are in metal-rich areas, such as ore rich deposits. Other than natural sources, the major causes of heavy metal concentrations in the environment are mining activity and other anthropogenic contributions (industry, traffic, etc.). Mine waters (all natural waters emanating from a mine site, including tailing dams and their leachates) are a part of the water cycle (Riba et al., 2005), and when they are without adequate treatment, aquatic environments can be seriously affected. Once heavy metals enter an aquatic ecosystem, the uptake of metals can occur in all living organisms. Even though some metals are essential for living organisms (e.g. copper (Cu), zinc (Zn), iron (Fe)), they can also be toxic; if the concentrations are too low or too high, it may equal the toxicity of nonessential metals (e.g. tin (Sn), aluminium (Al), cadmium (Cd), mercury (Hg), lead (Pb)) (Kennedy, 2011). Aquatic organisms have a dynamic relationship with the environment and the bioavailability of metals is also influenced by chemical speciation in the surrounding ecosystem. It is suggested that both organisms and their surrounding environment (water, sediments) should be studied together to investigate possible metal toxicity.

It is known that heavy metals are potentially dangerous to aquatic organisms and can accumulate in the food chain, but concern has been raised more recently that the consumption of contaminated fish may lead to adverse health effects in man. In recent decades many studies have dealt with metal toxicity (Phillips and Russo, 1978; Handy, 1994; Awadallah et al., 1985; Dallinger et al., 1987; Chan et al., 1999; Soylak et al., 1999; Rased, 2001; Hiller et al., 2010; Gurkan and Ulusoy, 2010; Alkan et al., 2012; Papadimitriou et al., 2012; Mitra et al., 2012; Mendil et al., 2005), but in area around Sasa mine there is a lack of such studies.

Fish is the main aquatic product of the artificial Lake Kalimanci, located near the small town Makedonska Kamenica, in the eastern part of FYR Macedonia. Heavy metal pollution of lake surficial

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sediments will influence the quality of edible fish, and it is well known that the kidney and liver accumulate higher contents of metals than gill, muscle and bladder (Ay et al., 1999; Liu et al., 2001). Since the aforementioned tissues are usually removed from fish before human consumption, this study focused on measuring potentially toxic metals in the edible parts of selected edible fishes. In areas with a lower economy standard of living, freshwater fish present a major food source. Rased (2001) explained that fish provide a good indicator of heavy metal pollution, thus the study of metal content in edible fish soft tissue were measured to establish the pollution status of Lake Kalimanci, which was affected with Sasa tailings dam material and acid mine drainage. The main objective of this study was to determine the contents of twelve metals in edible fish soft tissue of *Vimba Melanops*, in Lake Kalimanci, and in edible frogs *Rana temporaria* from the nearby Kočani Field, where the water from Lake Kalimanci is used for the irrigation of paddy fields. In addition metal contents in thirty-one surficial sediments from Lake Kalimanci and in twelve lake water samples were determined.

1.1. Study area

Serbo-Macedonian Massif extends from the Republic of Serbia, through eastern part of FYR Macedonia to the southern border with Greece (Fig. 1). Serbo-Macedonian Massif is located between Hellenides, Vardar Zone and Rhodope Massif (Meinhold et al., 2010) and is mainly composed of Precambrian, Riphean-Cambrian and Paleozoik metamorphic rocks (Serafimovski et al., 1997a) (Fig. 2). According to Serafimovski et al. (1997b) and Vrhovnik et al. (2011a), the Sasa ore district contains the most common metaliferious minerals pyrite, sphalerite and galena with some accessory minerals like pyrrothite, chalcopyrite, magnetite and marcasite. Rarely, hematite, Ag- and Bi- bearing minerals also occur there.

The tailings material from the Sasa mine is located between the Sasa mine and the Kamenica River, part of which flows beneath the tailings dam. The Sasa tailings dam mainly consists of quartz, pyrite, galena, sphalerite, gypsum, hornblende, actinolite, albite, anortite, biotite and orthoclase (Vrhovnik et al., 2011a). In year 2003, a major environmental disaster happened, when a part of Sasa tailings dam collapsed and caused an intensive flow of tailings material through the Kamenica River valley. Between 70,000 and 100,000 m^3 of tailings material was discharged into the Lake Kalimanci.

The artificial Lake Kalimanci extends 4 km in length and 0.3 km wide, and the maximum depth is 80 m. Fig. 3 also show a direction of flow inside the lake, which is caused by the inflow of the Kamenica river in the northern part of the Lake Kalimanci. According to our previous research (Vrhovnik et al., 2011b), the surficial sediments from Lake Kalimanci reflects the mineral composition of bedrocks from Osogovo mountains and contains quartz, plagioclases, K-feldspars, clay minerals and occasionally hornblende, dolomite, smithsonite, galena and sphalerite, as well as authigenic minerals like goethite, hematite and pyrite.

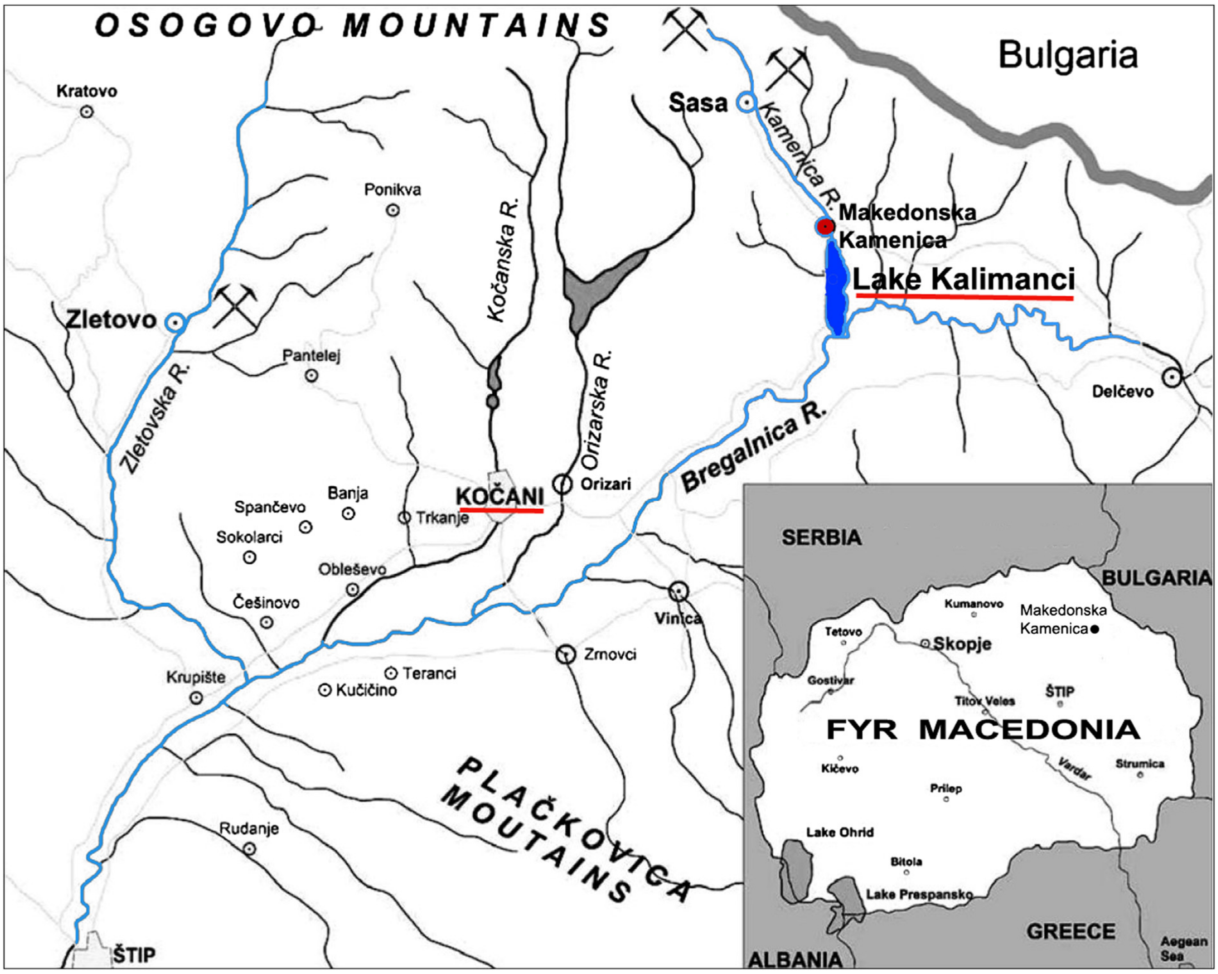

Fig. 1. Study area (blue lines indicates important rivers). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

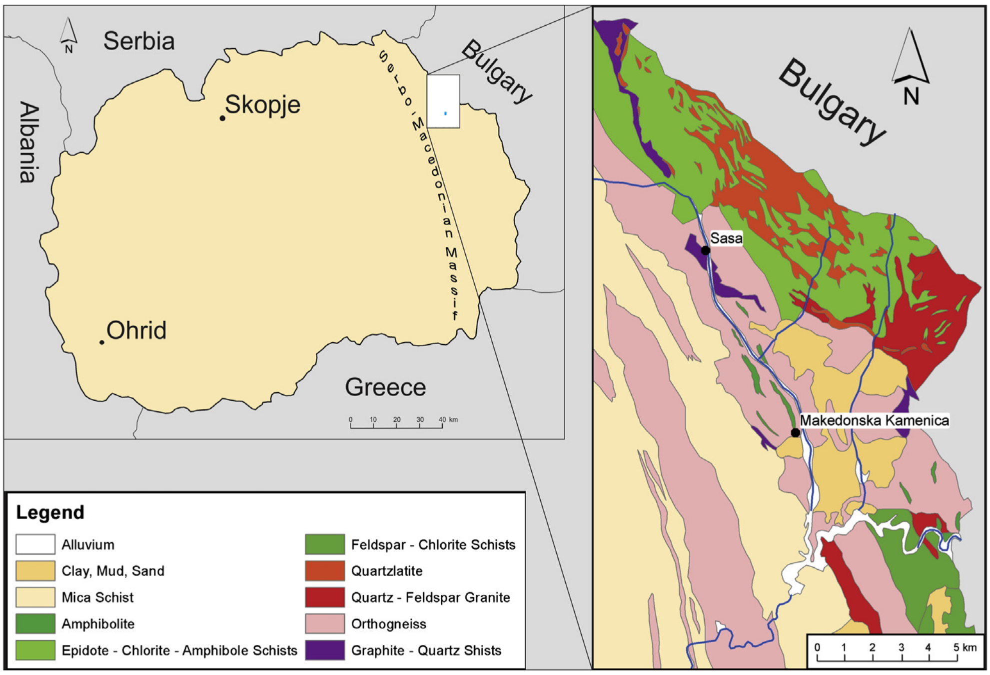

Fig. 2. Geological map of study area.

2. Materials and methods

2.1. Fish, frog, sediment and water sampling

The study was conducted at two different sites in the eastern part of FYR Macedonia (Lake Kalimanci and Kočani Valley). To collect representative fish samples, fishing was performed during late afternoon towards the end of the fishing season in November 2010 with the help of professional local fisherman, as reported by Jabeen and Chaudhry (2009). Fishes were caught in Lake Kalimanci at 16 stations: two samples at each profile (Fig. 3). Afterwards, 4 samples were removed, because they were too small, thus twelve commonly edible fish samples *V. melanops* (Kostov et al., 2010) from Lake Kalimanci were collected and stored in a container, cooled on crushed ice, and brought to the laboratory for further analyses. The length of *V. melanops* ranged from 16 to 20 cm. Adult fish species were collected at sizes which were also commonly captured by local fisherman and used for human consumption. The fish were sectioned, and all non-edible parts (e.g. bones, intestine, gills, fins, etc) were removed so that only soft tissues were freeze-dried (for at least 72 h) until a plateau weight was reached. Dried samples were homogenised and crushed to a fine powder by grinding in an agate mortar.

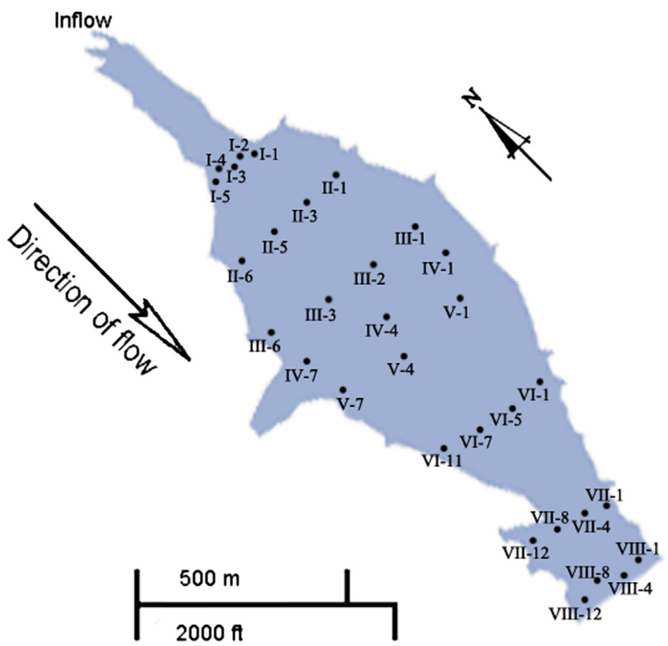

Fig. 3. Lake Kalimanci.

Nine adult frogs (*R. Temporaria*) of both sexes were collected from the Kočani valley, specifically from Zletovska and Bregalnica River and also from maize and paddy fields where the irrigation channels are laid out. The frogs were anaesthetised with ether, individually weighed and measured to vent length. The body was then placed in pre-cleaned plastic bags and deep frozen (at −70° C), before being processed in the same way as fish samples.

Thirty-one surficial sediment samples (marked with roman numbers on the Fig. 3) from Lake Kalimanci were taken in September 2007. The samples were collected using 10 cm long, with 10 cm internal diameter plastic corers. They were than packed into plastic bags and stored in the laboratory at 4 °C. The collected lake sediment samples were dried at 50 °C for 48 h, sieved through a 0.315 mm polyethylene sieve to remove plant debris, and homogenized by mechanical agate grinder to a fine powder (<63 μm) for subsequent geochemical analysis.

Water samples from Lake Kalimanci were collected at all twelve sampling sites at the same time as fish collection. Water samples were filtered using a hand-pump on site through a 0.45 μm membrane filter paper into pre-cleaned sample bottles. After immediate acidification with concentrated HNO_3, the samples were stored in a cooling box (<4 °C) until analysis. The psychochemical properties of the waters, including pH and temperature, were measured in the field.

2.2. Analyses

All samples (fish, frog) were analysed for the following elements: Cd, Co, Cu, Cr, Hg, Mo, Ni, Pb, Sb, Se, Zn and As, at an accredited commercial laboratory, ActLabs (Activation Laboratories Ltd., Ancaster, Ontario, Canada in year 2011), using inductively coupled plasma mass spectrometry (ICP/MS) and microwave digestion. Dry, unashed samples were digested in Aqua Regia solution 3/1 (v/v) (HNO_3 + 3HCl) at 95 °C for two hours. Resultant sample solutions were then diluted and analysed on a Finnegan Mat Element 2 High Resolution ICP/MS (HR-ICP/MS). The quality of the analyses were monitored by comparison to the standard materials NIST 1575a, NIST 1643e and SLRS-5 provided by ActLabs and the measurements of four samples were repeated. Beside ActLab quality control, also our own standard MP-STD-011, and two standards prepared and provided by the International Atomic Energy Agency, Viena (IAEA) – IAEA-407 and by National Research Council Canada – DORM-3 were sent to ActLabs for geochemical analysis as well. Our control standard MP-STD-011 is wild dog fish soft tissue, which was analysed many times by different techniques (ICP/MS, HR-ICP/MS, ICP/AES and XRF). Results of element concentrations in studied samples refer to kg of soft tissue. The results indicated a good agreement between the certified and observed values. For reaching the best control, measurements were repeated on all fish and frog samples and three standards (MP-STD-011, IAEA-407, DORM-3) using X-ray fluorescence (XRF) and inductively coupled plasma atomic emission spectroscopy (ICP-AES). The standard deviations of the means observed for the abovementioned certified materials were between 1 and 6%.

The geochemical analysis for Cd, Co, Cu, Cr, Hg, Mo, Ni, Pb, Sb, Se, Zn and As of Lake Kalimanci surficial sediments were obtained at the commercial ACME Laboratories in Vancouver, Canada. Determination of abundances required 0.5 g of sampled material and were thereupon leached in hot (95 °C) Aqua Regia and afterwards conducted by ICP Mass Spectrometry. The quality of the analyses was monitored by comparison to the standard materials STD SO-18, STD CSC, STD DS7, STD OREAS76A and STD R3A provided by ACME and repeated measurements of five of our samples (I-5, II-1, II-3, V-4, VIII-1). Repeated analyses of different sample aliquots indicated a relative standard deviation ± 5%.

The geochemical analysis for Cd, Co, Cu, Cr, Hg, Mo, Ni, Pb, Sb, Se, Zn and As of Lake Kalimanci water samples was carried out in accredited commercial laboratory Act Labs in year 2011 by ICP-AES for cations. The quality of analyses was checked with standard NIST 1643e and the analytical precision and accuracy were better than ± 5%.

All other used data from Tables 1–3 were collected from our previous researches.

Transfer Factor calculation.

To evaluate the accumulation of heavy metals in fish soft tissue from lake ecosystems the transfer factor (TF) was calculated (Kalfakakour and Akrida-Demertzi, 2000; Rased, 2001). This provided information of metal content in fish soft tissue and was incorporated into fish from water and lake sediment. The TF was given as:

$$\mathrm{TF} = \frac{\text{concentration of metal in fish soft tissue}}{\text{concentration of metal in lake ecosystem(water or sediment/soil)}}$$

According to Kalfakakour and Akrida-Demertzi (2000), a TF greater than 1 indicates bioaccumulation of metals in fish soft tissue.

Estimated Daily Intake (EDI) and Health Risk Index (HRI) were calculated to evaluate the possible health effects on humans via fish and frog consumption. For the calculation of the aforementioned indexes, the wet (fresh) weight of fish and frogs must be provided; therefore, we transformed dry weight to wet weight as Clark and Scheuhammer (2003) described, by multiplying dry weight concentrations by a factor of 3.

The EDI helps us to identify the quantity of metal per inhabitant per day of fish or frog. According to local fisherman and people living nearby Lake Kalimanci, they consume fishes or frogs almost every day, depending on the daily catch. According to questionnaires ($n = 53$ adults), every family member consumes one fish per day (approximately 120 g) and they treat themselves on weekends with the hind legs of a frog.

The EDI of metals from fish and frog species was calculated as described by Oyoo-Okoth et al. (2010), using the formula:

$$\mathrm{EDI} = \left(C_{\mathrm{metal}} \times W_{\mathrm{fish/frog}}\right) \Big/ B_{\mathrm{w}}$$

where C_{metal} (μg kg^{-1}, fresh weight) is the concentration measured in edible parts of fish and frog. $W_{\mathrm{fish/frog}}$ presents the daily average consumption; and B_{w} is body weight of adults (60 kg) and children (30 kg).

HRI was calculated by using EDI and Reference Oral Dose ($\mathrm{R_fD}$) (adopted by US EPA, 2012) using the formula:

$$\mathrm{HRI} = \mathrm{EDI}/\mathrm{R_fD}$$

When calculated values of HRI are less than 1, then the health risk to the population is considered acceptable, but when HRI is equal to or higher than 1, the population will most likely experience health problems (Khan et al., 2009).

2.3. Results

When contaminated particulate or sedimentary material is dispersed through an ecosystem, it equilibrates within water, sediment and living organisms (Di Giulio and Hinton, 2008). Thus, all three media were sampled; the measured concentrations are presented in Tables 1–3.

2.4. Potentially toxic metal contents

The contaminated freshwater ecosystems can provide capability of accumulation of metals in living organisms. According to Forstner and Wittmann (1981), aquatic organisms are capable of accumulating much higher concentrations of metals in their living cells than those in sediments, water and micro flora in their living environment. It is well known that fishes tend to accumulate different amounts of metal in various parts of the body (Jezierska and Witeska, 2001). Many studies researching metal contents in different parts of fishes were published in the last few decades (Mendil et al., 2005; Wang et al., 2005; Castro-Gonzáles and Méndez-Armenta, 2008; De et al., 2010; Jabeen and Chaudhry, 2010; Nwabueze, 2011 Alkan et al., 2012). However, there is a lack of studies dealing only with edible parts of the fish, especially in FYR Macedonia. Table 1 presents concentrations of metals in edible parts of *V. melanops* and *R. temporaria* and allowable contents of

Table 1
Mean, maximum and minimum concentrations of metals in **soft tissue** (edible part) of two different species.

Sample			Unit	Cd	Co	Cu	Cr	Hg	Mo	Ni	Pb	Sb	Se	Zn	As
Detection limit				0.1	0.5	20	10	5	1	0.1	10	0.2	0.2	0.2	5
Lake Kalimanci	*Vimba Melanops*	**Mean**	μg kg^{-1}	7.00	30.9	1653	1090	338.7	58.3	567	1127	3.07	1433	46 500	295
		Min–Max	μg kg^{-1}	4.10–10.4	22.7–41.9	1280–2090	820–1260	201–501	48.0–70.0	400–700	210–2470	0.60–5.40	1300–1600	34 000–68 000	259–328
Kočani Field	*Rana temporaria*	**Mean**	μg kg^{-1}	29.9	112	1520	5290	117	268	5100	435	8.50	650	80 300	183
		Min–Max	μg kg^{-1}	14.8–45.0	96.8–128	1270–1770	3240–9000	51–164	254–282	1900–8300	250–620	6.40–10.6	600–700	36 600–124 000	170–195
[a]FAO//WHO (1984)			μg kg^{-1}	300	/	20 000	1000	500	/	80 000	1500	/	/	5000	1400
[a]FEPA (2003)			μg kg^{-1}	/	/	30 000	150	/	/	/	/	/	/	/	/
[a]FAO (1983)			μg kg^{-1}	300	/	20 000	1000	500	/	/	2000	/	2000	50 000	1400
[a] WHO			μg kg^{-1}	300	/	30 000	50	500	/	600	2000	/	2000	10–75 000	20
US EPA (2012) RfD			μg kg^{-1} day^{-1}	1.00	0.30	40.0	1500	0.50	5.00	50.0	4.00	0.40	5.00	300	0.30

[a] Maximum allowable limit of heavy metals in fish food adopted by different health organisations.

Table 2
Mean, maximum and minimum concentrations of metals in **water samples** from Lake Kalimanci and two rivers from Kočani Field – Bregalnica and Zletovska River.

Sample		Unit	Cd	Co	Cu	Cr	Hg	Mo	Ni	Pb	Sb	Se	Zn	As	pH
Detection limit			0.001	0.002	0.01	0.01	0.001	0.02	0.2	0.01	0.02	1	1	0.01	
Lake Kalimanci	**Mean**	μg L^{-1}	3.27	282	14 035	1.45	0.06	41.1	137	32.7	0.39	90.7	629	2.63	7.4
	Min–Max	μg L^{-1}	0.25–8.25	0.14–1925	1934–96 849	0.69–4.45	0.01–0.18	1.06–147	0.49–915	0.79–98.5	0.12–0.59	6.80–179	24.3–2271	1.77–3.65	7.3–7.5
Kočani Filed Zletovska	**Mean**	μg L^{-1}	2.75	/	8.00	/	/	/	/	17.0	/	/	676	13.3	6.1
River (Rogan et al., 2010)	Min–Max	μg L^{-1}	0.50–5.00	/	6.00–10.0	/	/	/	/	10.0–24.0	/	/	101–1250	1.50–25.0	5.3–6.9
Kočani Field Bregalnica River (Serafimovski et al., 2004)	**Mean**	μg L^{-1}	0.39	/	3.00	/	/	/	/	2.40	/	/	67.0	0.53	/
WHO (2008)		μg L^{-1}	3	/	2000	50	6	70	70	10	20	10	/	10	/

WHO – World Health Organisation (2008). Guidelines for Drinking-water Quality, 3rd Ed., Vol. 1, Recommendations.

Table 3
Mean, maximum and minimum concentrations of metals in **surficial sediments** of Lake Kalimanci and in **soils** from Kočani Field.

Sample		Unit	Cd	Co	Cu	Cr	Hg	Mo	Ni	Pb	Sb	Se	Zn	As	pH
Detection limit			0.1	0.2	0.1	0.002	0.01	0.1	0.1	0.1	0.1	0.5	1	0.5	
Lake Kalimanci	**Mean**	mg kg^{-1}	56.6	19.9	415	59.4	0.05	2.69	46.9	6059	1.77	1.96	8420	78.1	6.5
surficial sediment	Min–max	mg kg^{-1}	16.5–136	11.7–27.7	144–1162	27.4–88.9	0.03–0.10	1.00–4.60	22.0–85.0	1873–16 300	0.60–3.60	0.60–4.60	2944–20 900	27.9–128	5.5–7.5
Kočani Field paddy	**Mean**	mg kg^{-1}	0.90	16.0	33.0	4435	0.08	0.68	21.0	128	0.60	/	206	11.4	5.5
soil (Rogan et al., 2009)	Min–max	mg kg^{-1}	0.10–6.40	8.60–21.3	15.0–99.0	3240–5630	0.02–0.18	0.30–1.80	9.00–37.0	11.0–983	0.10–3.00	/	53.0–1245	3.10–47.6	5.2–6.0
Sediment Chemical Criteria (WAC 172-204-320)	Average	mg kg^{-1}	5.10	/	390	260	0.41	/	/	450	/	/	410	57	/
Upper Continental Crust (Wedepohl, 1995)	Average	mg kg^{-1}	0.102	11.6	14.3	35	0.056	1.4	18.6	17	0.31	0.083	52	2.0	/
Allowable conc. For Agricult. Soil (Kabata-Pendias, 2001)	Average	mg kg^{-1}	1–3	50	30–70	50–80	5	10	30–75	70–150	10	10	100–300	30	/

metal in fish food adopted by different health organisations. Table 2 contains information about metal concentrations in Lake Kalimanci surficial sediments and in Kočani Field paddy soils as also sediment chemical criteria adopted by WAC (172-204-320) and allowable concentrations for agricultural soils (Kabata-Pendias, 2001). Table 3 shows mean, maximum and minimum measured concentrations of metals in water samples from Lake Kalimanci and two rivers from Kočani Field (Bregalnica and Zletovska River). In Table 3, there are added guidelines for drinking water quality (WHO, 2008).

Cadmium (Cd) – In our samples, edible parts (soft tissue) of *V. melanops* and *R. temporaria* have the mean levels of Cd as listed (Table 1): 7.00 μg kg^{-1} and 29.90 μg kg^{-1}. This means that in both cases the mean levels of Cd exceeds the permissible levels of Cd in food by the Join FAO/WHO (Table 1). According to Wetzel (2001), Cd content in natural fresh waters ranges from 0.01 to 0.10 μg Cd L^{-1}. In addition, the recommended Cd value in drinking water (Table 2) is 3 μg Cd L^{-1}. The measured mean levels of Cd in Lake Kalimanci water was 3.27 μg Cd L^{-1} (Table 2), which exceeds all of the abovementioned concentrations. Regarding Table 3, the mean levels of Cd in surficial sediments from Lake Kalimanci and soils from Kočani field also exceed the allowable concentrations.

Cobalt (Co) – The average contents of metals (Table 1) in edible parts (soft tissue) of *V. melanops* and *R. temporaria* are 30.9 μg kg^{-1}, 112 μg kg^{-1}, respectively. No limits for Co content in fish food are available. The WHO (2006) reported that unpolluted sediments and soils contain <20 mg kg^{-1} of cobalt in general. According to Smith and Carson (1981), Co concentrations in polluted lake sediments ranged from 0.16 to 133 mg kg^{-1}, and Kabata-Pendias (2001) also established the upper limit for Co in agricultural soils at 50 mg kg^{-1}. Measured concentrations of Co in studied samples range as follows: in Lake Kalimanci surficial sediments from11.7 to 27.7 mg kg^{-1} and in soils from Kočani Valley from 8.60 to 21.30 mg kg^{-1} (Table 3). The Co content in water samples from Lake Kalimanci ranged from 0.14 to 1925 μg L^{-1} (Table 2). Regarding the aforementioned available regulations Co seems not to be at toxic levels.

Copper (Cu) – Regarding to different health organisations (Table 1), tolerable levels of Cu in fish soft tissue vary from 20 to 30 000 μg kg^{-1}. In studied samples of *V. melanops* and *R. Temporaria*, mean values were: 1653 μg kg^{-1} and 1520 μg kg^{-1}, respectively (Table 1). In both study areas the maximum allowable contents of Cu were not exceeded. The Cu content in sampled water was highly exceeded in Lake Kalimanci water samples where it ranged from 1934 to 96 849 μg L^{-1} with a mean value of 14 035 μg L^{-1}. While there were no lake water criteria available, Cu content was compared to those presented in Table 2, meaning that Cu levels in Lake Kalimanci water are highly above the allowable levels. Meanwhile, the Kočani Field river system had measured Cu contents under the allowable levels. The same picture is shown when levels of Cu in lake sediments and soils are compared, as the only location with excess levels of Cu is Lake Kalimanci, where the mean levels of Cu are 415 mg kg^{-1} and go up to 1162 mg kg^{-1}, which is in accordance with the sediment criteria from Table 3. Kočani Paddy Field sampling locations had lower levels of Cu (Table 3).

Chromium (Cr) – According to Kotaś and Stasicka (2000) the concentrations range in soil is between 1 and 3000 mg kg^{-1}, and in rivers and lakes water is from 26 μg L^{-1}–5.2 mg L^{-1}. The mean value in Paddy soils from the Kočani Valley is 4435 mg kg^{-1} (Table 3) which strongly exceeds the recommended values (Table 3). Regarding the sediment criteria (Table 3), concentrations of Cr in Lake Kalimanci surficial sediments are lower, and range from 27.37 to 88.94 mg kg^{-1} (Table 3). Data for Cr content in studied waters were available only for Lake Kalimanci water and ranged from 0.69 to 4.45 μg L^{-1}, which are below the upper limit for drinking water (Table 2). In studied samples of *V. melanops* and *R. temporaria* were mean values were measured as follows: 1090 μg kg^{-1} and 5290 μg kg^{-1}, respectively (Table 1). According to the measured concentrations and adopted permissible levels, it is shown that the Cr content in the fish species *V. melanops* soft tissue from Lake Kalimanci is slightly increased; meanwhile concentrations of Cr in soft tissue of *R. temporaria* are highly above allowable limit adopted by different health organisations (Table 1).

Mercury (Hg) – In studied samples of *V. melanops* and *R. temporaria* the mean values were measured as follows (Table 1): 338.7 μg kg^{-1}, 117 μg kg^{-1} (Table 1). In samples from Lake Kalimanci and Kočani Field, where higher contents were expected due to mining activity in the vicinity, the Hg concentrations in the soft tissues of fish and frog were below the permissible levels. In addition, only the maximum measured level of *V. melanops* minimally exceeded the adopted level of Hg in fish food. The average Hg content in fresh waters ranges from 500 to 6800 μg L^{-1} (Fitzgerald and Lamborg, 2005). According to Water Quality Guidelines (2001) Hg concentration should never exceed 1.0 μg L^{-1}. All three studied waters (Table 2) have concentrations of Hg below aforementioned recommendations (Table 2). This coincides with Hg contents in surficial sediments of Lake Kalimanci and also soils from Kočani Field, whereas the concentrations were all below the upper maximum levels adopted by different agencies (Table 3).

Molybdenum (Mo) – According to Patty's (1981), soft tissue of fish contain about 1000 μg Mo/kg, and all Mo concentrations in sampled animal soft tissue were below this value. Mean values of Mo of *V. melanops* were 58.3 μg kg^{-1} and in *R. temporaria* were 268 μg kg^{-1} (Table 1). In addition, the measured concentrations of Mo in lake water, lake sediments and river water as well as in paddy field soils, are very low. The waters contain even lower concentrations of Mo per litre than is allowed in drinking water (Table 2). Surficial sediments from Lake Kalimanci contain levels of Mo that are comparable with other lakes (Cook, 2000), and are not very high. In addition, they are close to the upper continental crust average adopted by Wedepohl (1995). Soils from Kočani Paddy field also contain much lower concentrations than Kabata-Pendias (2001) recommends for agricultural soils (Table 3).

Nickel (Ni) – According to the aforementioned limit, in soft tissues of all studied samples, the maximum level of Ni exceeds the allowable level (Table 1), and in *V. melanops* range from 400 to 700 μg kg^{-1} and in *R. temporaria* from 1900 to 8300 μg kg^{-1}. The data of Ni content in waters was only available for Lake Kalimanci (Table 2), where it ranges from 0.49 to 915 μg L^{-1}. The mean value of Ni in Lake Kalimanci also exceeds the recommended concentrations, 70 μg L^{-1}, adopted by the WHO (2008). According to regulations from Table 3, the concentrations of Ni in lake sediments and soils only exceed Lake Kalimanci surficial sediments, whereas it ranges from 22.0 to 85.0 mg kg^{-1} (Table 3) and the toxic effect threshold level of Ni in Sediment Quality Guidelines is 61 mg kg^{-1} (Mulligan et al., 2010).

Lead (Pb) – Measured contents in studied samples ranged from 210 to 2470 μg kg^{-1} in *V. melanops* and from 250 to 620 μg kg^{-1} in *R. temporaria* (Table 1). This means that only one species *V. melanops* has levels that exceed permissible levels in Lake Kalimanci. Concentrations of lead in studied waters from Lake Kalimanci (0.79–98.5 μg L^{-1}) and Zletovska River from Kočani Paddy field (10.0–24.0 μg L^{-1}) also exceeds the allowable upper limit for drinking water (10 μg L^{-1}) presented in Table 2. Among the sampled sediments and soils (Table 3), surficial sediments from Lake Kalimanci (1873–16 300 mg kg^{-1}) and soils from Kočani Paddy field (11.0–983 mg kg^{-1}) exceed allowable contents (450 mg kg^{-1}) for sediments and 70–150 mg kg^{-1} for soils (Table 3).

Antimony (Sb) – According to PHG (1997), even highly contaminated sites show low Sb uptake from soils, and studies on fish and other aquatic organisms also indicate low bioconcentrations of Sb (Callahan et al., 1979). Furthermore, according to ATSDR (2003) there is also little indication that Sb biomagnifies through the food chain. Therefore, scant information about the maximum allowable level of Sb is available. According to Cape Town Permissible Levels of Heavy Metals (2004), the maximum limit of Sb in natural mineral waters is 5.0 μg kg^{-1} and in all other liquid foodstuffs is 150 μg kg^{-1}. The content of Sb in the soft tissue of the studied samples ranged as follows (Table 1): *V. melanops* 0.60–5.40 μg $kg^{-1}$and, *R. temporaria* 6.40–10.6 μg kg^{-1}. Data for Sb content in the studied waters was available only for Lake Kalimanci water (Table 2), where it ranged from 0.12 to 0.59 μg L^{-1}. This can be compared to guidelines for drinking water (Table 2), meaning that levels of Sb in lake water are very low. Low concentrations of Sb were also found in surficial lake sediments from Lake Kalimanci, and also in soils from Kočani paddy field (Table 3), but they do not exceed allowable levels for agricultural soils (Table 3).

Selenium (Se) – All of the studied samples had their mean values below the established limit, and they ranged as follows: *V. melanops* 1300–1600 μg kg^{-1} and *R. temporaria* 600–700 μg kg^{-1} (Table 1). According to the guidelines for Se adopted by Nagpal and Howell (2001) the upper allowable level of Se in freshwater is 2.0 μg L^{-1}, for sediments is 2.0 mg kg^{-1}, and for agricultural soil is 10 mg kg^{-1} (Kabata-Pendias, 2001). Obtained results of Se content in fresh water and soil or sediments in the present study were only available for Lake Kalimanci sampling site and they range from 6.80 to 179 μg L^{-1} in lake water (Table 2) and from 0.60 to 4.60 mg kg^{-1} in lake sediments (Table 3). This means that all of the aforementioned measured concentrations of Se exceed the maximum allowable levels.

Zinc (Zn) – Studied samples had the Zn content measured, and ranged as follows (Table 1): *V. melanops* 34 000–68 000 μg kg^{-1} and *R. temporaria* 36 600–124 000 μg kg^{-1}, and tend to be heavily increased regarding regulations from Table 1. According to Ambient Water Quality Guidelines for Zinc (1999) the recommended value of Zn in drinking water is 5000 μg L^{-1} and concentrations of Zn in all studied samples from two of three sampling sites are below this level (Table 2). On the other hand, the total contents of Zn in lake sediments and soils are above the recommended values (Table 3). The measured values in studied samples ranged as follows: 2944–20 900 mg kg^{-1} in Lake Kalimanci surficial sediments, and 53.0–1245 mg kg^{-1} in agricultural soils from Kočani Valley (Table 3). Results show that Lake Kalimanci surficial sediments and Kočani Paddy Field soils have high Zn contents.

Arsenic (As) – On average, the content of As in food is fairly low, but levels of As in sea food and lake food (e.g. fishes) can be much higher, due to the absorption of As from water, where they live. The content of As in soft tissue of studied samples was measured as follows: *V. melanops* 259–328 μg kg^{-1}, *R. temporaria* 170–195 μg kg^{-1} (Table 1), and is below allowable limits. The mean As content in studied waters are 2.63 μg L^{-1} in Lake Kalimanci water, and at Kočani paddy field sampling sites were 0.53 μg L^{-1} in River Bregalnica and 13.3 μg L^{-1} in Zletovska River (Table 2), which exceeded the upper allowable limit for drinking water. The contents of total As in studied samples are as follows: Lake Kalimanci surficial sediments 27.9–128 mg kg^{-1} and in Kočani Field paddy soils 3.10–47.6 mg kg^{-1} (Table 3). These levels exceed the official recommendations.

2.5. Transfer Factor and estimated daily intake (EDI) and health risk index (HRI)

Table 4 and Fig. 4 show the calculated TFs and the HRIs for Macedonian fishes and frogs, respectively.

Table 4
Mean values of Transfer factor (TF) of heavy metals in *Vimba melanops* from Lake Kalimanci and *Rana temporaria* from Kočani field [water, sediment, soil].

		Cd	Co	Cu	Cr	Hg	Mo	Ni	Pb	Sb	Se	Zn	As
Lake Kalimanci	Sediment/fish	1.24*E-4	1.55* E-3	3.98*E-3	1.84*E-2	6.77	0.02	0.01	1.80*E-4	1.73*E-3	0.73	5.52*E-3	3.78*E-3
	Water/sediment	2.14	0.12	0.12	751.72	564.50	1.42	4.14	34.46	7.87	15.80	73.93	112.17
Kočani Field	Soil/fish	0.03	/	0.05	1.19*E-3	1.46	/	/	3.4*E-3	/	/	0.39	0.02
	Water/fish (Zletovska River)	10.87	/	190	/	/	/	/	25.59	/	/	118.79	13.76
	Water/fish (Bregalnica River)	76.67	/	506.67	/	/	/	/	18.13	/	/	1198.51	345.28

3. Discussion

3.1. Relations between metals in different media

The bioavailability of metals refers to the proportion of element that can be absorbed by the organism, such as fish, frog, etc, in relation to the total amount of element in the surrounding ecosystem. Metals usually accumulate in aquatic animals' tissues and become toxic, when concentrations reach toxicity thresholds. Run-off materials (waters, waste material) from metal mining activities often include a variety of metals, and when there is no proper way of retaining contaminated waters and waste material the risk of pollution in nearby ecosystems is even higher. Aquatic organisms usually receive metal in their bodies through the ingestion of mud (soil, sediment), water and other organisms (fauna and flora). Living organisms usually selectively absorb metals to obtain the essential elements (e.g. Zn, Cu). Even though the selected species in the present research are of different habitats and have different feeding habits, the study of metal content in their soft tissue may provide important information about the metal uptake routes, as well as the accumulation of metal in human beings due to the consumption of fishes and frogs.

According to our previous research (Vrhovnik et al., 2011b), surficial Lake Kalimanci sediments are highly polluted at the northern part of the lake (profile II, III and IV; Fig. 3). Metal levels in fish samples (*V. melanops*) were found to be the same at all sampling stations. Therefore, no matter where the fishes are caught they should not be consumed at any time, because of such high levels of metals (Cr, Hg, Ni, Pb and Zn), which all exceed the maximum allowable levels adopted by different health organisations (Table 1). Much the same story develops when comparing metal content in soils from Kočani valley and metals in *R. temporaria* samples. There is no logical correlation between contaminated soils and sampled frogs. Metals in different media are arranged from high concentrations to lower ones as follows: [*V. Melanops*]: Zn > Cu > Se > Pb > Cr > Ni > Hg > As > Mo > Co > Cd > Sb; [*R. temporaria*]: Zn > Cr > Ni > Cu > Se > Pb > Mo > As > Hg > Co > Cd > Sb; [Lake Kalimanci surficial sediment]: Zn > Pb > Cu > As > Cr > Cd > Ni > Co > Mo > Se > Sb > Hg; [Kočani valley soils]: Cr > Zn > Pb > Cu > Ni > Co > As > Cd > Mo > Sb > Hg; [Lake Kalimanci water]: Cu > Zn > Co > Ni > Se > Mo > Pb > Cd > As > Cr > Sb > Hg; [Zletovska River]: Zn > Pb > As > Cu > Cd; [Bregalnica River]: Zn > Cu > Pb > As > Cd. This shows that Zn, Cu and Pb are elements that occur in all media at the highest concentrations. Even though Zn and Cu are classified as essential elements, at such high levels they can pose several threats to human health, especially to children. Overall, studied metals like Cr, Hg, Ni, Pb and Zn are the most crucial for human health in the present study across Kočani Field and Makedonska Kamenica which surrounds Lake Kalimanci. Even though the Sasa tailings dam failure was a transitory event for the Lake Kalimanci water body, the effects of pollutants may be long-lived due to their tendency to be absorbed in the different particles in bottom sediment, after which they are released in the food chain for longer periods.

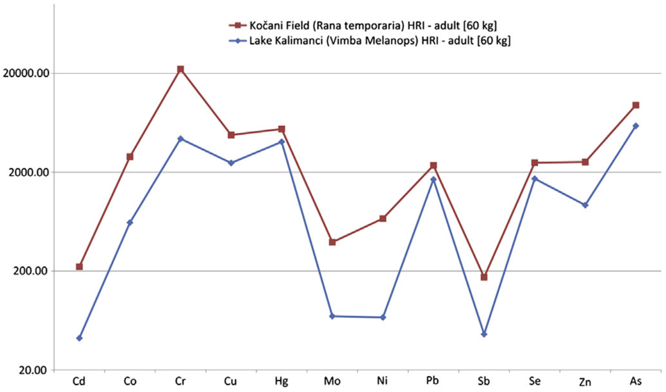

Fig. 4. Calculated values of health risk index (HRI) for adults (logarithmic scale).

3.2. Transfer Factor

The results showed that TF values in relation to water/[fish, frog] were much greater than those among sediments and fauna. In Kalimanci Lake, the mean value of calculated TF between fish soft tissue and water shows that TF was higher than 1 among all studied metals, with the exception of Co and Cu. The same results were found out at Kočani paddy field where the frog soft tissue and water from Zletovska and Bregalnica River were calculated, using the TF equation. Kalfakakour and Akrida-Demertzi (2000) reported that fish undergo the bioaccumulation of metals in cases when TF is higher than 1 in fish from lake water. In addition, Rased (2001) also reported that when the calculated TF is higher than 1 it means that fishes bioaccumulate metals from the environment in all cases (e.g. water, sediment, plant).

Among both locations, the calculated TF to relation fish/sediment showed that the majority of metals have TF values lower than 1, and that only a few of them were greater. Hg in Lake Kalimanci has a TF of 6.77, but the only TF value that exceeds 1 in the Kočani paddy field was calculated for Hg (1.46).

Comparing TF values with other published calculations (Rased, 2001; Abdel-Baki et al., 2011), we can conclude that the examined fishes and frogs from FYR Macedonia have a much higher bioaccumulation rate from surrounding waters than from soils/sediments. This is most likely due to a higher trophic level, as well as their nutrition, but neither of studied the species consumes mud.

3.2.1. Health effects of studied aquatic organisms consumption

Fish consumption has many beneficial effects on human health, but it is important to balance the beneficial effects of fish and detrimental effects of metals. Within areas polluted with metals, where living standards are low (e.g. FYR Macedonia) and the inhabitants eat “home-grown food”, the concern from the toxicological point of view it is not excusable. While fishes are the major food, next to vegetables, it is important to investigate the levels of metals in these organisms to assess whether the concentration is within the permissible level and will not possess any hazards (Krishnamurti and Nair, 1999). According to questionnaires distributed among locals living in or nearby the studied area, they eat fish from Lake Kalimanci and frogs from whole Kočani Valley on a daily basis. That is why the Estimated Daily Intake (EDI) and Health Risk Index (HRI) were calculated.

Results shows extremely high estimated values of HRI for adults, meaning that the consumption of *V. melanops* and *R. temporaria* can have considerable high health risks for inhabitants living across study the area. In addition, the HRI calculated for children (assumed weight 30 kg) is two times higher than for adults; therefore according to Khan et al. (2009), the health of children living in the studied area is at particularly high risk. According to CIA (2001), the unemployment rate in FYR Macedonia in 2011 was 31.4%, among which 55% are between 15 and 24 years old; in addition, in the year 2010, approximately 31% of the population in FYR Macedonia were below the poverty line, which also may be one of the main reasons for equal diets every day. Regarding the abovementioned statement, the living status of inhabitants is presumably low; therefore, their major source of food is captured in nearby waters (e.g. fishes, frogs) or is grown in their gardens, where they use water from Lake Kalimanci for irrigation. Further studies of the detailed study of metal contents in grown vegetables are needed, including a more detailed questionnaire including detailed information of sociodemographic and anthropometric characteristics, as well as health data, reproductive history and lifestyle.

4. Conclusions

The metal contamination of the environment has been occurring for centuries, but in the last decades has increased rapidly due to technological developments, and an increased needfulness of metal-containing objects. The use of metals by itself is not harmful for the environment, but the metal industry always produces wastes which are often released into the ecosystems. When any environmental disasters occur (e.g. tailings dam failure, oil spill, etc.) the ecosystem is "injured" and it takes a long period of time to get back to the primary "un-contaminated" condition. While in the present research we are dealing with an environment that is highly affected by metals, due to the Sasa tailings dam failure in 2003, the results showed that, the presence of released metals is still immense after eight years.

The results of the present study showed that the increased metal content in lake sediments, soils and stream and lake waters of the study area seem to have a marked effect on the bioaccumulation of metal in sampled organisms, resulting in the potentially long-term implication on human health and ecosystems. Among all of the studied metals in the present study, some of them exceeded the maximum allowable concentrations adopted by different organisations. In studied the food (fish, frog), metals like Cr, Hg, Ni and Pb exceeded allowable limits, in surficial lake sediments and soils the elements Cd, Cu, Cr, Pb, Zn and As were found to exceed limits, and in studied water the most crucial elements are Cd, Cu, Ni, Pb, Se and As. If summarised, Pb is the most crucial metal in all of the studied materials and poses the biggest threat to inhabitants, especially to small children, because at such high levels it can affect their brains and central nervous system. Not only do the humans living in the present study area eat contaminated food, but presumably Pb can enter their bodies' through inhalation because of the closeness of the Pb–Zn mine. The third reason which can cause high levels of lead in the human blood is skin contact with materials rich in Pb, which is easy to achieve in such close proximity to a Pb–Zn mine. For some metals, toxic levels can be just above the natural background concentrations normally found in nature. The evaluation of metal concentrations in environmental samples (e.g. water, sediment, food) in this metal-rich zone is extremely important to control its conditions and provide a safe place for all living organisms. This is why it is important to inform the inhabitants about PTM and take protective measures against excessive exposure. In the FYR Macedonia, there is a lack of such information; therefore further investigations into environmental pollution and the possible poisoning of entire ecosystems, including humans, animals, and plants, are urgent.

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