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BIODIVERSITY OF MACROPHYTES AND PHYTOREMEDIATION OF HEAVY METAL ACCUMULATION IN THE AQUATIC ECOSYSTEMS

BIODIVERZITET MAKROFITA I FITOREMEDIJACIJA AKUMULACIJE TEŠKIH METALA U VODENIM EKOSISTEMIMA

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ABSTRACT

Today, pollution of the environment especially water represents the most serious problem in the development and survival of all living organisms. Particular problems are present in water pollution, which also includes drinking water. The goal of this study is to understand the importance of microphytes and macrophytes in accumulation of toxic metals and suggesting the remedial measures for the preservation and restoration of the aquatic ecosystems.

To demonstrate a model for ecological determination and phytoremediation of heavy metal accumulation by using microphytes-macrophytes as bioindicators, structure and dynamics of aquatic ecosystems were studied at more sites of the river basin and sides of the Miljacka River (central Bosnia and Herzegovina). Also, this study again confirmed that, phytoremediation is a cost effective and environmentally friendly technology for the treatment of water contaminated by heavy metals.

Microphytes and macrophytes are the wellknown biological filters and they carry out purification of the water bodies by accumulating dissolved metals and toxins in their tissues. This study showed that the aquatic microphytes and macrophytes play a very significant role in removing the different metals from the aquatic environments. They play an important role in reducing the effect of high concentration of heavy metals.

Therefore, the macrophyte and microphyte communities of the Miljacka River area need to be protected and restored on a priority basis. Accumulation of highly toxic metals like Cu, Zn, Pb and Fe were compared and observed in all locations. Monitoring the diversity, abundance and distribution of microphyte and macrophyte species provides indicative information of environmental impacts upon aquatic ecosystems.

Keywords: *Microphytes*, *Macrophytes*, *Toxic metals*, *Accumulation*, *Phytoremediation*, *Miljacka River*

SAŽETAK

Danas, zagađenje okoliša, posebno voda, predstavlja najozbiljniji problem u razvoju i opstanku svih živih organizama. Poseban problem predstavlja zagađenost vode, uključujući i vodu za piće. Cilj ovog istraživanja je da shvatimo važnost mikrofita i makrofita u akumulaciji toksičnih metala i uputimo na korektivne mjere za očuvanje i obnovu vodenih ekosistema.

Da bismo demonstrirali model za ekološku i fitoremedijaciju akumulacije teških metala pomoću mikrofita-makrofita kao bioindikatora, struktura i dinamika vodenih ekosistema istraživana je na više mjesta u slivu i na obalama rijeke Miljacke (središnja Bosna i Hercegovina). Također, ovo istraživanje ponovno je potvrdilo da je fitoremedijacija jeftina i ekološki prihvatljiva tehnologija za tretman vode zagađene teškim metalima.

Mikrofite i makrofite su dobro poznati biološki filteri koji pročišćavaju vodu iz tijela sakupljanjem otopljenih metala i toksina u svojim tkivima. Ova studija pokazala je da vodene mikrofite i makrofite igraju vrlo značajnu ulogu u uklanjanju različitih metala iz vodenih sredina. Oni igraju važnu ulogu u smanjenju utjecaja visoke koncentracije teških metala.

Dakle, zajednice makrofita i mikrofita na području rijeke Miljacke treba zaštititi i obnoviti na temelju prioriteta. Akumulacija vrlo toksičnih metala poput Cu, Zn, Pb i Fe uspoređena je i promatrana na svim lokacijama. Praćenje različitosti, bogatstva i distribucije mikrofita i makrofita obezbjeđuje indikativne informacije o utjecaju na okoliš nakon vodenih ekosistema.

Ključne riječi: *mikrofita, makrofita, toksični metali, akumulacija, fitoremedijacija, Miljacka*

INTRODUCTION

Miljacka River, flowing through Sarajevo, is crucial for people living in Sarajevo. Because, this river has been an important element for the development of Sarajevo for centuries. The most part of the Miljacka River is being much polluted today. It is still very clean in the upper part and has a high ecological quality, but at the down parts, it is much polluted and very dangerous for people and all living organisms.

The Human Development Report (2006) of UNDP has focused on the global water crisis as one of the most serious problems facing by the humanity today. In many Asian countries and elsewhere the demand for potable water doubles every 10-15 years, not only because of the rising domestic consumption but also due to the increasing needs of industry. The principal sources of water for human use are lakes, rivers, soil moisture and relatively shallow groundwater basins. The usable portion of these sources is less than 1% of all freshwater and only 0.01% of all water on Earth. Although, water quality is an important issue and the subject of much legislation, sometimes the quantity is more important than quality in determining the extent and type of development possible in a given geographic location. Water quality problems can often be as severe as those of water availability but less attention has been paid to them, particularly in developing countries. Many countries do not have sufficient water supplies to meet demand, as a result of which, aquifer depletion due to over exploitation is common. Moreover, the scarcity of water is accompanied by deterioration in the quality of available water due to heavy pollution load and environmental degradation. (Srivastava, Gupta and Chandra, 2008)

Heavy metal contamination in aquatic and soil environments is a serious environmental problem, which threatens aquatic ecosystems, agriculture, and human health (Srivastav et al., 1994; Lasat, 2002; Fediuc and Erdei, 2002; Overesch et al., 2007). Units of metal removal and mobilization include sedimentation, adsorption, complexation, uptaking by plants, and microbially mediated reactions including oxidation and reduction (Dunbabin and Bowmer, 1992).

Many industrial and mining processes cause heavy metal pollution, which can contaminate natural water systems and become a hazard to human health. Therefore, colonization of macrophytes on the sediments polluted with heavy metals and the role of these plants in transportation of metals in shallow areas are very important. This investigation was planned and executed considering the potentials of macrophytes as a biological filter of the aquatic environment.

1. Heavy Metal Accumulation

Heavy metals are metals having a density of 5 g/cc, (Nies et al., 1999). These metals include elements such as copper, cadmium, lead, selenium, arsenic, mercury, chromium and etc. Some sources of heavy metals are industry, municipal wastewater, atmospheric pollution, urban runoff, river dumping, and shore erosion. Heavy metals in surface water systems can be from natural or anthropogenic sources.

Currently, anthropogenic inputs of metals exceed natural inputs. High levels of Cd, Cu, Pb, Fe can act as ecological toxins in aquatic and terrestrial ecosystems (Guilizzoni, 1991; Balsberg-Påhlsson, 1989).

Common Metals and their sources:

- **Lead:** leaded gasoline, tire wear, lubricating oil and grease, bearing wear
- **Zinc:** tire wear, motor oil, grease, brake emissions, corrosion of galvanized parts
- **Iron:** auto body rust, engine parts
- **Copper:** bearing wear, engine parts, brake emissions
- **Cadmium:** tire wear, fuel burning, batteries
- **Chromium:** air conditioning coolants, engine parts, brake emissions
- **Nickel:** diesel fuel and gasoline, lubricating oil, brake emissions
- **Aluminum:** auto body corrosion

Heavy Metal Pollution in Aquatic Ecosystem

Three countries – the United States, Germany, and Russia – with only 8% of the world's population consume about 75% of the world's most widely used metals. The United States, with 4.5% of the world's population, uses about 20% of the worlds metal population and 25% of the fossil fuels produced each year. (http://www.lenntech.com/aquatic/metals.htm#ixzz109YbtAIH)

Toxicity of metals

For the protection of human health, the maximum permissible concentrations for metals in natural waters that are recommended by the Environmental Protection Agency (EPA), are listed below: Maximum Permissible Concentrations (MPC) of Various Metals in Natural Waters For the Protection of Human Health:

Table 1. Relative toxicity of metals
Tabela 1. Relativna toksičnost metala

Metal	Chemical Symbol	$mg\ m^{-3}$
Mercury	Hg	0.144
Lead	Pb	5
Cadmium	Cd	10
Selenium	Se	10
Thallium	Tl	13
Nickel	Ni	13.4
Silver	Ag	50
Manganese	Mn	50
Chromium	Cr	50
Iron	Fe	300
Barium	Ba	1000

Table1; Relative toxicity of metals.
Source: EPA (1987); Federal Register 56 (110): 26460-26564 (1991)

This table gives an idea of the relative toxicity of various metals. Mercury, lead and cadmium are not required even in small amounts by any organism. Because metals are rather insoluble in neutral or basic pH, pHs of 7 or above give a highly misleading picture of the degree of metal pollution. So in some cases it may underestimate significantly the total of metal concentrations in natural waters.

2. The Effects of Heavy Metal Pollution to Human Health;

Heavy metal toxicity can result in damaged or reduced mental and central nervous function, lower energy levels, and damage to blood composition, lungs, kidneys, liver, and other vital organs. Long-term exposure may result in slowly progressing physical, muscular, and neurological degenerative processes that mimic Alzheimer's disease, Parkinson's disease, muscular dystrophy, and multiple sclerosis. (International Occupational Safety and Health Information Centre, 1999)

Heavy metals may enter the human body through food, water, air, or absorption through the skin when they come in contact with humans in agriculture and in manufacturing, pharmaceutical, industrial, or residential settings. Industrial exposure accounts for a common route of exposure for adults. Ingestion is the most common route of exposure in children. (Roberts, 1999)

Exposure to heavy metals has been linked with developmental retardation, various cancers, kidney damage, and even death in some instances of exposure to very high concentrations. Exposure to high levels of mercury, gold, and lead has also been associated with the development of autoimmunity, in which the immune system starts to attack its own cells, mistaking them for foreign invaders. (Glover-Kerkvliet, 1995: 236-237)

3. Microphytes and Macrophytes

Macrophytes and Microphytes are aquatic organisms, living in or near water that are either emergent, submerged or floating. Macrophytes are beneficial to river because they provide food and settler for fish and aquatic invertebrates. They also produce oxygen, which helps in overall river functioning, and provide food for some fish and other wildlife. Also, they are considered as important component of the aquatic ecosystem not only as food source for aquatic invertebrates, but also act as an efficient accumulator of heavy metals (Devlin, 1967; Chung and Jeng, 1974).

Macrophytes are excellent indicators of watershed health because they:

- respond to nutrients, light, toxic contaminants, metals, herbicides, turbidity, water level change, and salt
- are easily sampled through the use of transects or aerial photography
- do not require laboratory analysis
- are easily used for calculating simple abundance metrics
- are integrators of environmental condition (http://www.epa.gov/bioindicators/html/macrophytes.html)

They are unchangeable biological filters and play an important role in the maintenance of aquatic ecosystem. Their characteristics to accumulate metals make them an interesting research objects for testing and modeling ecological theories on plant succession, as well as on nutrient and metal cycling (Forstner and Whittman, 1979). Therefore, it is very important to understand the functions of macrophytes in aquatic ecosystem.

Aquatic macrophytes take up metals from the water, producing an internal concentration several fold greater than their surroundings. Many of the aquatic macrophytes are found to be the potential scavengers of heavy metals from water and wetlands (Gulati et al., 1979).

Some aquatic plants can remove nutrients (Rogers et al., 1991; Moshiri, 1993; Mungur et al., 1997; Miretzky et al., 2004; Maine et al., 2006; Gottschall et al., 2007; Chung et al., 2008) and heavy metals (Rai et al., 1995; Zhulidov, 1996; Miretzky et al., 2004; Maine et al., 2006;) from liquid environments (Iqbal and Tachibana, 2007).

Aquatic macrophytes absorb and accumulate the nutrient ions in the tissues (DeBusk et al., 1995; Mahujchariyawong and Ikeda, 2001). Aquatic macrophyte influence metal retention indirectly by acting as traps for particulate matter, by slowing the water current and favoring sedimentation of suspended particles (Kadlec, 2000). Large aquatic macrophytes possess the ability to breakdown the human and animal derived pollutants in the water (Kadlec and Knight, 1996).

Macrophytes are especially good bioindicators in continuous, long period monitoring. They do not have strong mechanisms regulating the uptake of nutrients and heavy metals. Some species have expressive ability of bioconcentration, and therefore, increased accumulation, of nutrients and heavy metals, (Stankovic et al., 2000). Therefore, macrophytes can be used as bioindicators.

Phytoremediation is emerging as a cost effective and environmentally friendly technology for the treatment of soil and water contaminated by heavy metals and metalloids. (Hoang Ha, Sakakibara, Sano, 2009)

The search for new technologies involving the removal of toxic metals from wastewaters has directed attention to biosorption, based on metal binding capacities of various biological materials. Biosorption can be defined as the ability of biological materials to accumulate heavy metals from wastewater through metabolically mediated or physico-chemical pathways of uptake (Fourest and Roux, 1992). Algae, bacteria and fungi and yeasts have proved to be potential metal biosorbents (Volesky, 1986).

The major advantages of biosorption over conventional treatment methods include (Kratochvil and Volesky, 1998 a):

- Low cost;
- High efficiency;
- Minimisation of chemical and lor biological sludge;
- No additional nutrient requirement;
- Regeneration of biosorbent; and
- Possibility of metal recovery.

MATERIALS AND METHODS

Study areas

The Miljacka River and the source of Bosna River are the rivers in which all investigations were performed. 4 localities were investigated during the investigation; Kozija ćuprija, Bentbaša, Otoka and Vrelo Bosne. (**Figure 1**)

Figure 1. Map of study areas
Slika 1. Karta istraživanog područja

Investigated rivers

All investigated rivers are located in the center of Sarajevo.
Area: Sarajevo
Location Type: Captial Of A Country
Latitude: 43.85
Longitude: 18.38333
Latitude (DMS): 43° 51' 0 N
Longitude (DMS): 18° 22' 60 E

Figure 2. Investigated Localities
Slika 2. Ispitivani lokaliteti

1. Miljacka (river)

The Miljacka is a river in Bosnia and Herzegovina that passes through Sarajevo and East Sarajevo. It is famous for being „Sarajevo's River", and it has come to be identified immediately with the city itself.

The Miljacka river originates from the Paljanska Miljacka river and the Mokranjska Miljacka river confluence, though the Paljanska Miljacka, 13 kilometers long river, spring up around 10 kilometers eastward from the town of Pale, under the slopes of Jahorina near Begovina at 1010 meters a.s.l., and the Mokranjska Miljacka, 21 kilometers long river, spring up from a large cave, yet to be explored, near Kadino Selo village at 1135 meters a.s.l. under the slopes of Romanija mountains.

The Miljacka is a rather small river, only 38 kilometers long with average discharge of 5,7 m^3/s at the City of Sarajevo, and right tributary of the Bosna river. Hence the origin and the estuary, the Miljacka river flows from the east to the west. (http://en.wikipedia.org/)

Figure 3. Miljacka River
Slika 3. Rijeka Miljacka

2. Bosna (river)

The Bosna is the third longest river in Bosnia and Herzegovina, and is considered one of the country's three major internal rivers, along with the Neretva and Vrbas Rivers; the other three major rivers of Bosnia and Herzegovina are the Una, to the northwest, the Sava, to the north, and the Drina, to the east. The Bosna flows for 271 kilometers (168 miles). Its name does not have any foreign variations. In the Roman times the river was called the Bosona, and it is thought that this is probably the Illyrian origin of the name Bosna.

The Bosna river also makes up the Bosna River Valley, the country's industrial center and home to close to a million people, as well as the location of several major cities. The river's biggest tributaries are then Željeznica, Miljacka, Fojnica, Lašva, Gostović, Krivaja, Usora, and Spreča rivers.

Its source is at the spring Vrelo Bosne, at the foothills of the Mount Igman, on the outskirts of Sarajevo, capital of sovereign Bosnia and Herzegovina. The spring is one of Bosnia and Herzegovina's chief natural landmarks and tourist attractions. From there, the Bosna flows northwards, through the heart of Bosnia, eventually becoming a right tributary of the Sava River in Bosanski Šamac.

Although it does not pass through any foreign countries, the Bosna flows through a number of cantons. From its starting point in the Sarajevo Canton, it also flows through Zenica-Doboj Canton, Doboj Region, and Posavina Canton, in that order. On its way north the River Bosna also passes through the cities of Visoko, Zenica, Doboj, Modriča and Bosanski Šamac.

Figure 4: Bosna River
Slika 4. Rijeka Bosna

1. KOZIJA ĆUPRIJA LOCALITY

Picture 4. Locality 1 (Kozija ćuprija)
Slika 4. Lokalitet 1 (Kozija ćuprija)

2. BENTBAŠA LOCALITY

Picture 5. Locality 2 (Bentbasa)
Slika 5. Lokalitet 2 (Bentbaša)

3. OTOKA LOCALITY

Figure 7. Locality 3 (Otoka)
Slika 7. Lokalitet 3 (Otoka)

4. VRELO BOSNE LOCALITY

Figure 8. Locality 4 (Vrelo Bosne)
Slika 8. Lokalitet 4 (Vrelo Bosne)

METHOD

3 main steps were performed during the investigation.

All analysis was done in the center for ecology and natural resources PMF University in Sarajevo and Federal Institute of Agriculture in Sarajevo. Levels of Nitrates, Ammonium, Phosphates and pH were obtained in 4 different localities.

1 – Sample collection and identification: Macrophytes and Microphytes were handpicked from the freshwater habitat from different localities. All samples were labeled according to their localities and scientific nomenclature. In the laboratory, they were sorted by standard taxonomic manuals and one set was kept for preparation of herbarium and confirmation of taxonomic identification.

Table 2: Collected samples from localities
Tabela 2. Sakupljeni uzorci sa lokaliteta

KOZIJA CUPRIJA	BENTBASA	OTOKA	VRELO BOSNE
Menta sp.	*Menta sp.*	*Menta sp.*	*Fontinalis antipyretica*
Willow sp.	*Veronica sp.*	*Willow sp.*	*Elodea sp.*
Equisetum palustre	*Cladophora glomerata*	Rumex sp.	Ranunculus sp.
Cladophora glomerata	*Spirogyra sp.*	*Cladophora glomerata*	*Cladophora glomerata*
	Mougeotia sp.	Chlamydomonas sp.	Scleropodiu purum
		Mougeotia sp.	Spirogyra sp.
			Zgnema sp.
			Ulotrix sp.
			Closterium sp.
			Pediastrum sp.

2 – Sample processing: Then, samples were subjected to air dry at room temperature for 4 days. Dried samples were homogenized and ground to yield fine powder.

Figure 9. Processing of samples
Slika 9. Obrada uzoraka

3 – Sample analysis by ICP-AES: The sample processing and analytical procedures for ICP-AES analysis (Analysis of Major and Trace Elements by Inductively Coupled Plasma–Atomic Emission Spectrometry) was done. Perkin-Elmer plasma-400 ICP-AES operating in sequential mode was used for all the analysis. Since every element has its own characteristic set of energy levels and thus the set of emission wavelengths makes the atomic spectrometer useful for element specific analytical techniques.

All heavy metal analysis was done in the center
Federal Institute of Agriculture in Sarajevo.

Figure 10. Analysis of samples
Slika 10. Analiza uzoraka

RESULTS

According to the measurements, the highest concentration of Zn is detected in *Ranunculus sp.* in locality 4. *Rumex sp.* shows the highest Fe concentration in locality 3 and the highest concentration of Cu was detected in *Menta sp.* in locality 2. In most of the samples, Pb couldn't be detected, probably, because, the measurements were done mg/kg but not µg/g. because lead is supposed to be detected as trace element.

Table 3. Heavy metal concentration of samples obtained by Federal Institute of Agriculture in Sarajevo
Tabela 3. Koncentracija teških metala u uzorcima ostvarena u Federalnom zavodu za poljoprivredu u Sarajevu

Localities	Samples	Pb (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Zn (mg/kg)
Kozija Ćuprija *Locality 1*	*Menta sp.*	32,18	10,69	4.709,43	46,33
	Willow sp.	No Detection	3,92	No Detection	60,68
	Equisetum palustre	No Detection	2,49	324,38	24,96
Bentbaša *Locality 2*	*Veronica sp.*	No Detection	11,17	305,58	36,00
	Menta sp.	29,79	22,37	712,32	83,43
Otoka *Locality 3*	*Willow sp.*	No Detection	11,96	542,10	64,63
	Rumex sp.	No Detection	10,60	6.477,27	39,15
	Mentasp.	No Detection	8,97	5.634,12	38,26
Vrelo Bosne *Locality 4*	*Fontinalis antipyretica*	64,62	13,82	4.213,14	163,04
	Elodea sp.	31,81	13,70	3.780,58	361,13
	Ranunculus sp.	32,17	12,08	1.915,46	430,61

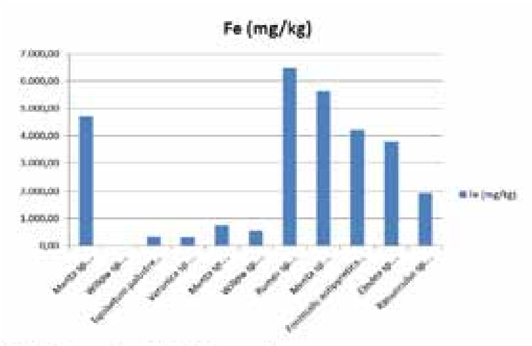

Chart 1: Concentration of Fe in different samples.

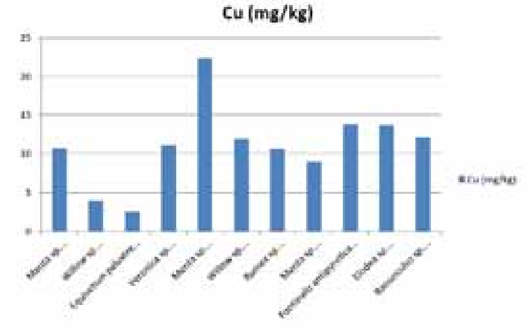

Chart 2: Concentration of Cu in different samples.

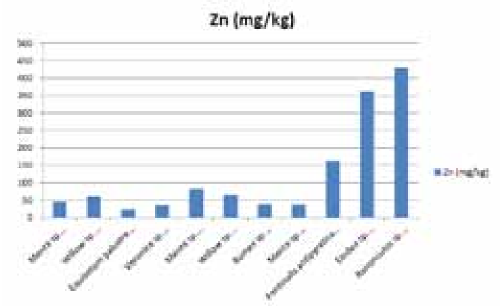

Chart 3: Concentration of Zn in different samples.

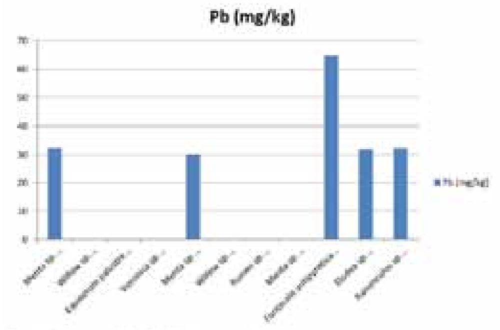

Chart 4: Concentration of Pb in different samples.

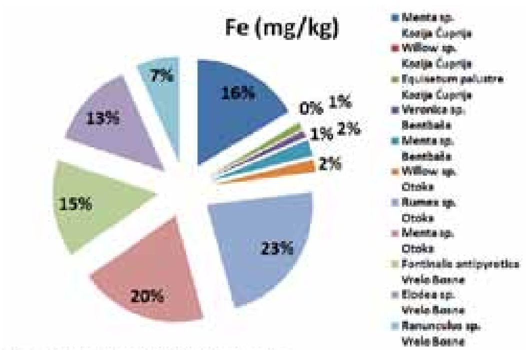

Chart 5: Concentration of Fe in different samples.

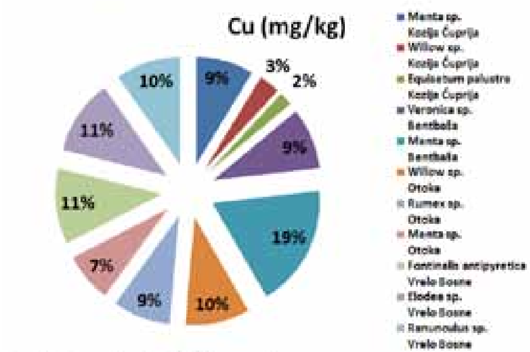

Chart 6: Concentration of Cu in different samples.

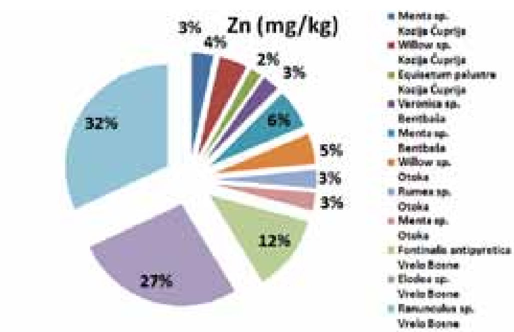

Chart 7: Concentration of Zn in different samples.

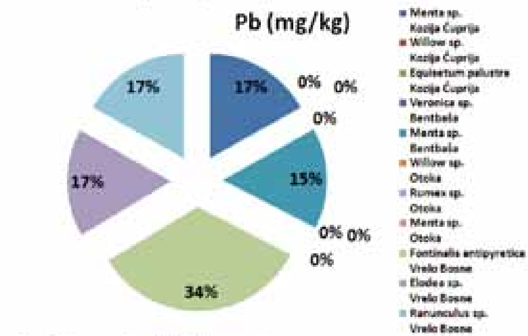

Chart 8: Concentration of Pb in different samples.

As we examine the charts, Cu has been almost absorbed by all organisms with which we have studied. Lead is the one that is absorbed only by 5 species in our concern. The reason behind this result is either because lead is in a trace amount where there is no any Pb detection, or because the species showing 0 Pb level are unable to absorb that element at all at high quantities. In any case, except for *Willow sp.*all other species from every locality more or less absorb the iron.

CONCLUSION

This study concluded that *Ranunculus sp.*, *Menta sp.*, *and Rumex sp.* had promising potential for heavy metal removal. These three aquatic plants could be grown in river, stream, and mining drainage or grown in clean water systems initially and then can be transferred to the polluted water to remove metals from aquatic systems.

In view of the increasing aquatic pollution, the initial survey should be undertaken to acquire an estimation of the range of variability in accumulation of heavy metals in the aquatic plants from the Miljacka River. To determine and evaluate the occurrence, the distribution and their effects of heavy metals, and to prevent them to pass into rivers, lakes and ground water bodies represents an urgent task for applied environmental issues. From the observations and investigations, macrophytes of river systems need protection and municipalities should stimulate researchers to improve the phytoremediation techniques and to demonstrate their reliability to the public. Because of this, some certain macrophytes can be used for the phytoremediation of heavy metal accumulation in the aquatic ecosystems.

Apparently, microphytes and macrophytes could be a remedial solution for heavy metal reduction in aquatic systems. Since, these organisms have potential to absorb the heavy metals. However, ultimately together with all heavy metals that they had been absorbed. Therefore, periodically, microphytes and macrophytes could be eliminated by municipalities. Restoration and elimination of microphytes and macrophytes should be achieved within a proper and good harmony and in accordance. Because, nature is very subtle and sensitive to any external manipulation. By interpreting these data, it could be a better way to figure out which species seems to be the best for which heavy metal remediation.

Acknowledgement

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