## Using Microphyte-Macrophyte Species As Bioindicators For The Determination And Phytoremediation Of Heavy Metal Accumulation In The Aquatic Ecosystems

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#### Abstract

Heavy metal accumulation and contamination have become a serious problem in recent years. Therefore, it is crucial to take a closer look at the microphytes and macrophytes species. These two elements are recommended for the determination and phytoremediation of the heavy metals in the contaminated aquatic ecosystems. It is clear that, the excess amount of heavy metals can have negative impacts on the environment and these influents can be weak, strong, long lasting and short lasting, and they can also be present in different levels such as global, regional or local. Moreover, heavy metal contamination in aquatic environments is a serious environmental problem, which threatens aquatic ecosystems, agriculture, and human health. The goal of this study is to understand the importance of microphytes and macrophytes in accumulation of toxic metals and suggest some effective measurements for the preservation and restoration of the aquatic ecosystems. Thus, in order to protect our living ecosystems, it is necessary to study the sources, level and quantity of contamination of heavy metals. Different treatment methods have been developed for the elimination of these metals from water including coagulation, adsorption, ion exchange and other chemical and biological processes. However, these methods are expensive and require major investments in equipment and facilities and they also introduce various chemicals which can have a harmful impact on our environment. In contrast, this study verifies that phytoremediation is an efficient and cheap technology for the treatment of water polluted by heavy metals. Furthermore, it is suggested a new model called wetland construction, which is an expansion on a river bed and it serves as a water purification system as well, in order to enable easy collection and replacement. After the collection, the biomass obtained would be processed to obtain biogas, biofertilizers, animal food and recycled heavy metals. As a result, the macrophyte and microphyte communities of the Miljacka River area need to be protected and restored on a priority basis. 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 and sides of the Miljacka River (central Bosnia and Herzegovina). All in all, this study shows that the aquatic microphytes and macrophytes play a very significant role in removing the different metals from the aquatic environments and they can both be used as bioindicators of heavy metals and other toxic substances in a given area.

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

## **1.INTRODUCTION**

Water is one of the essentials that support all forms of plant and animal life (Vanloon and Duffy, 2005) and it is generally obtained from two principal natural sources. Surface water such as fresh water lakes, rivers, streams, etc. and ground water such as borehole water and well water (McMurry and Fay, 2004; Mendie, 2005).

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 usable portion of water convenient for human use is less than 1% of all freshwater and only 0.01% of all water on Earth. 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 environmental load and degradation (Srivastava, Gupta and Chandra 2008).

Nowadays, at the beginning of the third millennium, everyone should be aware of the fact that we have reached the ecological limit of endangering the life and survival beyond which we cannot go anymore. Lately, great importance has been given to studying of the concentration and impact of heavy metal effects to flora and fauna. The researches in this area show 350 that observing of residues of these metals and their chemical compounds in different systems is crucial, first of all from the aspect of human health protection, as well as from the aspect of protection of entire flora and fauna and human environment. For a successful implementation of protection of humans, animals and plants from any harmful heavy metals, it is necessary to study the source, degree and extent of the pollution.

# 2.MATERIALS & METHODS

As a first step in the field investigation part we had to determine the investigation areas. These are as follows: Kozija Cuprija locality, Bentbasa locality, Otoka locality and Vrelo Bosne locality. All these are found in the area of Sarajevo which is the capital of Bosnia and Herzegovina. First three investigated rivers are located on the Miljacka River and the last one ids located on the Bosnia river. From these areas we have observed microphytes such as Cladophora glomerata, and macrophytes such as Rumex sp., Ranunculus sp., Veronica sp., Willow Salix, Equisetum palustre and Mentha sp.After the collection process these plants went through various treatments in which we have used: hand mortar and pestle, nitric acid, perchloric acid, flasks , electrical plates, filters and, Ultra-pure water. The research was conducted in order to obtain the concentrations of Fe, Zn, Cu and Pb which are all heavy metals found in observed microphytes and macrophytes.

### **3.STUDY AREAS**

Bosnia and Herzegovina, often referred to in the West as simply Bosnia, is a country in Southern Europe, on the Balkan Peninsula. Bordered by Croatia to the north. west and south, Serbia to the east. and Montenegro to the southeast, Bosnia and Herzegovina is almost landlocked, except for 26 kilometres (16 miles) of Adriatic Sea coastline, centered on the town of Neum. According to its geographical position if is found on the intersection of various biogeographical influents and roads. The interior of the country is mountainous centrally and to the south, hilly in the northwest, and flatland in the northeast. Out of its total area 5% makes up valleys, 24% hills, 42% mountains and 29% makes up karsts. Medium altitude is about 500 m and the highest peak is Maglic, 2386m. Around 41% of area is covered by woods. Inland is the larger geographic region with a moderate continental climate, marked by hot summers and cold, snowy winters.

# 4.RESULTS

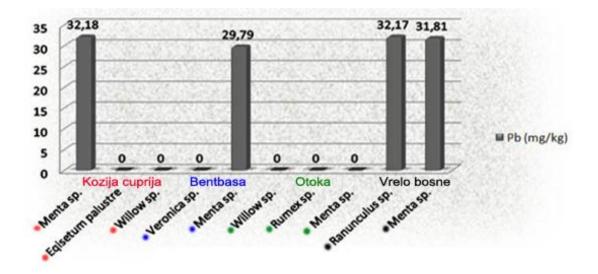
## 4.1.Macrophyte Analysis

Results show the concetrations of Lead, Copper, Iron and Zinc in the localities where the investigation was conducted on the macrophyte species which are Mentha sp., Equisetum palustre, Veronica sp., Ranunculus sp., Rumex sp. and Willow salix. The obtained valuesshow that heavy metal contamination is present in a very high level. The results are given below;

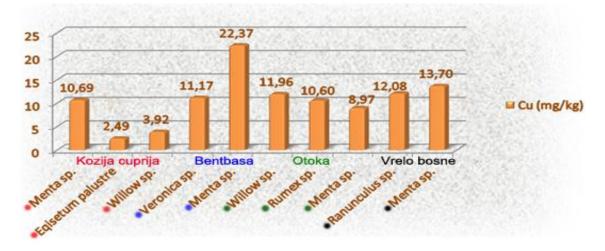
Localities	Samples	Pb (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Zn (mg/kg)
Kozija Ćuprija	Menta sp.	32,18	10,69	4.709,43	46,33
	Eqisetum palustre	No detection	2,49	324,38	24,96
	Willow salix	No detection	3,92	No detection	60,68
Bentbaša	Veronica sp.	No detection	11,17	305,58	36,00
	Menta sp.	29,79	22,37	712,32	83,43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

Table 3.1: Results obtained from macrophyte analysis

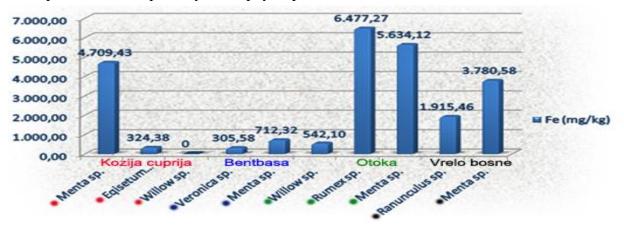
Graph 3.1: Pb absorption by macrophyte species



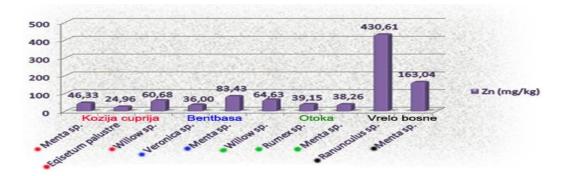
Graph 3.2: Cu absorption by macrophyte species



Graph 3.3: Fe absorption by macrophyte species



Graph 3.4: Zn absorption by macrophyte species 352



According to the measurements, the highest concentration of Zn, 430.61mg/kg, is detected in Ranunculus sp. in locality 4 (Vrelo Bosne) and the lowest concentration of Zn was obtained from the locality 1 (Kozija Cuprija), by Equisetum palustre, where the value was 24.96 mg/kg. The medium value is 98.709 mg/kg.Rumex sp. shows the highest Fe concentration, 6,477.27 mg/kg, in locality 3 (Otoka) and Equisetum palustre shows the lowest value of 324.38 mg/kg in locality 1 (Kozija Cuprija) if we don't take in consideration Willow salix found in the same locality where iron wasn't detected at all. The medium absorption level is 3790.58 highest result mg/kg.The for Cu. 22.37mg/kg, is detected in Menta sp. in locality 2 (Bentbaša) and the lowest, 2.49 mg/kg, in Equisetum palustre in locality 1 The average value (Kozija Cuprija). obtained for copper is 10.795 mg/kg.Menta sp. also shows the highest concentration of Pb, 32.18 mg/kg, in locality 1 (Kozija ćuprija). The lowest value detected is 29.79 mg/kg in the locality 2 (Bentbasa) by Mentha sp. The values of Pb that could be detected had very heterogeneous values on

each locality and the average of those values is 31.49 mg/kg. In most of the samples Pb couldn't be detected probably because the measurements were done in mg/kg but not  $\mu$ g/g and lead is supposed to be detected as trace element. The higest Pb concentration measured by Chlorophyta sp., 52.72 mg/kg, is detected in locality 3 (Otoka) and the lowest one, 24.72 mg/kg, in locality 1 (Kozija Cuprija). The average value is 40.73 mg/kg.The higest value of Cu, 45.70 mg/kg, was obtained in locality (Otoka) and the lowest 3 value. 11.94mg/kg, in the locality 1 (Kozija Cuprija). Cu average value is 24.18 mg/kg. Fe is the element with highest detection, 9,001.42 mg/kg,by Chlorophyta sp. in locality 3 (Otoka). On the other hand the lowest detection of iron, 3078.68 mg/kg, was obtained from locality 4 (Vrelo Bosne). The average value obtained for Fe is 6310.04 mg/kg. As our last listed metal, Zn highest concentration, 256.21 mg/kg, in Chlorophyta sp. was detected in locality 4 (Vrelo Bosne). Zn lowest concentration was obtained from locality 1 (Kozija Cuprija) which is 53.40 mg/kg. Zn average concentration level was 126.74mg/kg.

	Localities		Pb (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Zn (mg/kg)
1	Kozija L.1.1.1.	Ćuprija	27.27	13.02	7,588.28	50.67
2	Kozija L.1.1.2.	Ćuprija	30.56	13.38	4,499.53	39.29
3	Kozija L.1.1.3.	Ćuprija	18.29	12.09	2,922.73	48.26
4	Kozija L.1.1.4.	Ćuprija	25.30	10.11	5,715.24	40.22
5	Bentbaša L.2.1.1.		25.40	20.13	7,736.33	106.42
6	Bentbaša L.2.1.2.		31.84	17.15	9,487.16	71.51
7	Bentbaša L.2.1.3.		49.76	20.11	3,900.61	60.97
8	Bentbaša L.2.1.4.		26.59	7.14	4,745.73	132.8
9	Otoka L.3.1.1.		50.33	44.37	8,920.73	105.31
10	Otoka L.3.1.2.		35.56	38.97	7,619.46	78.81
11	Otoka L.3.1.3.		43.23	42.96	7,490.07	112.04
12	Otoka L.3.1.4.		81.74	56.48	11,975.41	121.68
13	Vrelo Bosr L.4.1.1.	ne	40.20	30.88	286.12	74.18

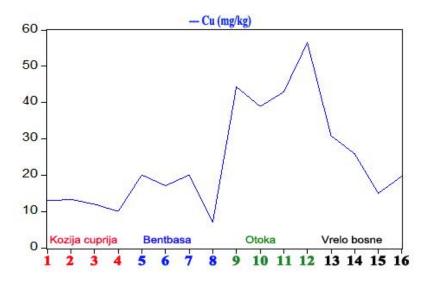
Table 4.1: Results obtained from microphyte analysis

14	Vrelo Bosne L.4.1.2.	101.74	25.96	9,406.15	638.45
15	Vrelo Bosne L.4.1.3.	27.8	15.10	1,689.63	179.62
16	Vrelo Bosne L.4.1.4.	38.51	19.81	932.8	132.60

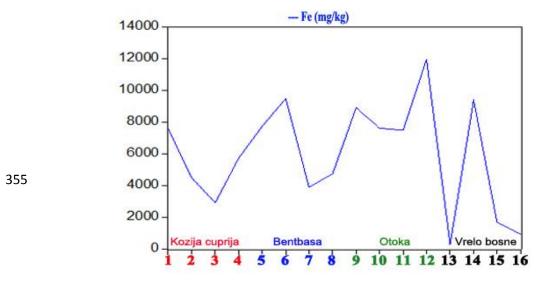
## 4.2.Descriptive analysis

In the first step, descriptive analysis was performed and the results are reported in Graphs 1,2,3,4.

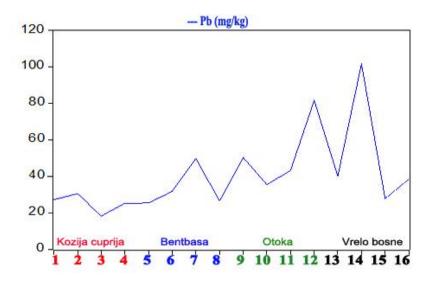
Graph 4.1:Empirical analysis of Cu

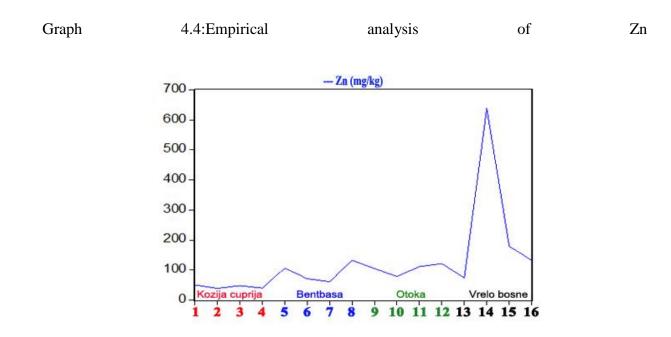


Graph 4.2:Empirical analysis of Fe



Graph 4.3:Empirical analysis of Pb





## **5. CONCLUSION**

Results indicate significant variation of element concentrations in different macrophyte and microphyte species. Since concentration of a substance in a plant is the result of the physical and chemical properties of the compound, environmental characteristics and plant characteristics these results are reasonable. Some species turned to be more successful bioaccumulators for certain elements therefore showing high potential in possible use as environment phytoremediators. As the most successful from the macrophytes Ranunculus sp., Menta sp., and Rumex sp. had promising potential for heavy metal removal according to our studies. Also, the high bioaccumulation abilities of Chlorophyta species for selected metals were confirmed by obtained results. They turned to be very successful in iron absorption which is very useful since too high values are rather lethal. Cladophora glomerata turned to be a microphyte with enormous potential for heavy metal absorption and in this way a phytoremediator. This plant 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. The goal of this study was to prove that macrophytes and microphytes can have a great impact in heavy metals accumulation in aquatic ecosystems. Results show that the hypothesis was correct because both macrophytes and microphytes turned to be very efficient as heavy metals accumulators and bioindicators. In this way microphytes and macrophytes work as an excellent team in the maintenance of natural homeostasis. Considering the scientific articles we can also say that they are very cheap phytoremediators with high efficiency. At the same time this research offers a complete solution for heavy metal removal from aquatic ecosystems by introducing wetlands that handling and replacement of macrophytes and microphytes.While easy enable phytoremediation can occur naturally, it is more effective when good design, planting, and site management processes are carried out properly. Successful phytoremediation requires and integrated approach for each specific site, which must consider plant selection, genetic engineering, and soil and water management. Also, the empirical analysis has showed that there is no significant relationship between Fe and other metals. Moreover, no significant relationship is seen between Fe and Cu and a significant positive relationship between Pb and Zn are found; however, there exists a significant negative relationship between Zn and Cu as well. Taking these results into consideration, one can obviously see that these metals affect each other in a way. Therefore, phytoremediation types need to be identified and applied, and from time to time the microphytes and macrophytes used for phytoremediation should be change. Another way for increasing the efficiency of heavy metal absorption is genetic modification. Genetic engineering in macrophytes and microphytes for enhanced heavy metal accumulation is still in embryonic stage and needs more attention in this area. A multidisciplinary research effort that integrates the work of plant biologists, soil chemists, microbiologists, and environmental engineers is essential for greater success of phytoremediation as a viable water cleanup technique. Therefore, it is clear that the utilization of the remarkable potential of green plants to accumulate environmental pollutants and to perform biochemical transformation is becoming a new frontier in environmental science and technology. 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. By interpreting these data, it could be a better way to figure out which species seem to be the best for which heavy metal remediation. To determine and evaluate the occurrence, the distribution and the 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. Obviously, macrophytes and microphytes could be a remedial solution for heavy metal reduction in aquatic systems. However, together with all heavy metals that they had absorbed, macrophytes and microphytes, could be periodically eliminated by municipalities. Restoration and elimination of macrophytes and microphytes should be achieved within a proper and good harmony and in accordance because nature is very subtle and sensitive to any external manipulation. As mentioned before, wetlands are an excellent solution that enables easy replacement and further processing and metal recycling.

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