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PHYTOREMEDIATION POTENTIAL OF WILD PLANTS

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ABSTRACT

Phytoremediation with wild plant species could be an environmentally friendly and economical solution. Selected wild plants like mint, oregano, chamomile, nettle, and St. John's wort, from rural areas in Bosnia and Herzegovina, were collected, lyophilized, and acid-digested for heavy metals analysis. Nickel (Ni), copper (Cu), chromium (Cr), cadmium (Cd), iron (Fe), zinc (Zn), manganese (Mn), and lead (Pb) were determined by using an Atomic Absorption Spectrometry-flame technique. Results showed that some of the investigated wild plants have a high content of multiple heavy metals. This work showed that collected wild plants can accumulate heavy metals and could be used for the phytoremediation of multi-metal-contaminated soil.

Keywords: wild plants; heavy metals; bioremediation

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1. INTRODUCTION

Due to the rapid growth of the world population, the industrialization of society, and the excessive use of protective agens in agriculture, the amount of available unpolluted agricultural soil is rapidly decreasing. Accumulation of toxic pollutants, such as heavy metals, radionuclides, and organic pollutants, in soil and water burdens the ecosystem's production capacity. Also, pollution affects human health and quality of life. The remediation (from lat. *remediare* or *remedium*; to treat or restore balance) of soils and waters from the above-mentioned pollutants is, and remains, one of the major challenges facing our society today. Many physical, chemical, and biological approaches have been used to remediate environmental pollution. Biological methods, including bioremediation, bioventilation, and phytoremediation of soil remediation use microorganisms or plants to block or remove pollutants from the soil [1-4].

Selected chemical methods of soil remediation are soil electroremediation, soil flooding with various solutions (water or water with additives (acid, base, surfactants)), soil leaching (water and various chemical additives), and soil solidification and stabilization. However, their applications are limited due to cost and labor requirements, safety hazards, and risks to ecosystems and people. As a biological method, phytoremediation (greek: *φυτό* – plant, latin: *remedio* –to cure, to heal), can be an effective and alternative technique and is gaining popularity, acceptance, and application. Plants, both wild or cultivated, have been used for hundreds of years to prevent soil erosion and waste processing, and preservation mostly of water quality. Recently, a recognition of phytoremediation as a potential solution for soil purification and prevention has directed the focus of the scientific community on research into these processes [5].

Phytoremediation is considered an environmentally friendly soil remediation, often referred to as *green remediation*. The main mechanism of phytoremediation is based on the use of fast-growing plants to remove toxic pollutants in soil or water sources. During the phytoremediation process, green plants concentrate certain individual elements and then excrete them into the ecosystem. This is a relatively new method of soil cleaning. The approach of phytoremediation is based on plants, is economically acceptable, and has attracted a lot of attention in the last few years. Main advances have been made in understanding the mechanisms and types of phytoremediation application possibilities, which have resulted in many implementations of an innovative method. According to the mechanism of action and effects on pollutants, phytoremediation techniques are divided into: phytoextraction, phytofiltration, phytostabilization, phytovolatization, phytodegradation, and rhizodegradation. Phytoextraction (also known as phytoaccumulation and phytoabsorption), refers to the uptake of pollutants from soil or water by the root system, transfer to vascular tissues, and accumulation in plant shoots. This is how soils contaminated mainly with heavy metals are remediated. Phytofiltration is the process by which pollutants are removed from an aqueous medium, either surface water or wastewater. Depending on the part of the plant responsible for the removal, phytofiltration is divided into rhizofiltration (root), blastofiltration (seedlings), and caulofiltration (separated plant shoots). In phytofiltration, pollutants are absorbed or adsorbed onto plant tissue, thereby minimizing their movement. In addition, phytofiltration in an aqueous medium is equivalent to phytostabilization in soil. Phytostabilization is based on the placement of plant cover on the surface of contaminated soil with the aim of limiting the movement and bioavailability of pollutants by accumulating them within the roots or immobilizing them in the rhizosphere by precipitation, complexation, or reduction. This process reduces the migration of pollutants due to wind and water erosion. Phytoremediation is applied when remediation of the contaminated area is not a priority. Pollutants are contained *in situ* to prevent migration until remediation [6,7].

The phytoremediation plant species must be a non-invasive species that animals do not like to eat. Also, plant is chosen according to its ability to extract toxins from the environment, adaptability to local climatic conditions, large production of green mass, the depth to which the root penetrates, compatibility with the type of soil to be rehabilitated, speed of growth, ease of planting and maintenance, and the ability to absorb large amounts of water. Since the remediation reach is equal to the root depth, fast-growing plant species are ideal for phytoextraction due to their extensive root system, rapid growth, biomass, and ability to sprout after harvest. The advantage of using these plant species is a lower risk of heavy metals entering the environment and thus the food chain. For example, zinc and cadmium have been found to accumulate in the leaves of such plants, so maintenance is possible by harvesting the leaves, while the disposal of other heavy metals requires digging up and disposal of the roots at the end of the process. It has also been found that aromatic plants such as *Mentha piperita* (peppermint) accumulate heavy metals in their aboveground biomass, and during the processing of plant material, heavy metals are separated and are not found in the aromatic oils as the final products of the process. For soils with a high degree of metal contamination, such as those in mining and post-industrial areas, phytostabilization is specifically interesting and is applied to prevent further spread of contamination. When choosing a plant species, it is desirable to choose one that has a widespread and dense root system. Since immobilization takes place in the root zone, a dense vegetative cover is needed to stop wind dispersion and washing out by precipitation. Phytostabilization is rarely limited to plant species, but is mainly a synergy with microorganisms from the root zone. Soil microorganisms release organic acids, extracellular polymers of polysaccharides and proteins, and chelating agents that help immobilize and convert heavy metals into less toxic forms. Thus, heavy metals remain in the soil, their bioavailability and toxicity are reduced, and the possibility of contaminating waterways or the food chain is drastically reduced [8,9].

Advantages of applying phytoremediation methods for removing heavy metals from soils are:

- the cost of energy, thus the total financial cost of phytoremediation is significantly lower
- than conventional decontamination and purification processes,
- by observing plants (wild or cultivated), changes in the concentration of toxic substances
- can also be monitored,
- there is a possibility of "recycling" valuable metals from the ashes of used plants,
- it is the least harmful method because it uses living organisms and not chemicals, so it has
- the least impact on the environment and humans,
- plant material that has absorbed toxins can be processed by drying and burning,
- the final amount of toxic waste created after treatment is less than the amount of toxic waste produced by using conventional methods.

This work aims to use selected wild plants of mint, oregano, chamomile, nettle, and St. John’s wort, from rural areas in Bosnia and Herzegovina, as an indicator of pollution of soil, and possible applications of these plants for phytoremediation of soil contaminated with heavy metals.

2. EXPERIMENTAL

2.1 Plant mterials

Selected wild plant species of mint, oregano, chamomile, nettle, and St. John's wort were collected from rural areas in Bosnia and Herzegovina. The collection was carried out in completely dry conditions, as wet soil, rain, or high humidity are not desirable. The collection of plant material and harvesting were carried out only in clean, unpolluted habitats and in clean paper bags. The harvesting site was at an appropriate distance of 200 m, and the minimum distance from roads, industrial facilities, landfills of various types of waste, garbage, or agricultural areas with crops treated with pesticides.

2.2 Preparation of plant samples

Previous extraction of heavy metals from plant material was performed using nitric and sulfuric acid. Briefly, a mass of 0.5 g of dry plant material is weighed into a 100 mL flask, and 5 mL of HNO_3 and 2 mL of H_2SO_4 are carefully added. The prepared solution is left for 5-6 hours at room temperature, and then heated on a hot plate until the plant material is completely decomposed (30 minutes). After cooling, the solution is filtered through filter paper (blue tape) into a 50 mL volumetric flask and made up to the mark with distilled deionized water [10].

2.3 Determination of heavy metal content in samples by flame atomic absorption spectrophotometry, FAAS (ISO 11047)

The content of heavy metals in plant material was determined by flame atomic absorption spectrophotometry on a Shimadzu 7000 AA apparatus, according to the instructions contained in the ISO 11047 method. Preparation of standard working solutions for tested heavy metals: working standard solutions (series of standards) for the tested elements are prepared from original certified working solutions (MERCK) with a concentration of 1000 ppm, and further purification was not required. Standard stock solutions containing 1.000 mg/mL of nitrate salts of Pb(II), Cr(III), Zn(II), Ni(II); 1.000 mg/mL of chloride salts of Mn(II), Fe(III), and 1.000 mg/mL of sulfate of Cd(II), Cu(II) were prepared in 1 L volumetric flasks and made up to the mark with distilled deionized water (Milli-Q purification system). The selection of concentrations in the series of standards for each element is done in accordance with the expected average values of the content of the tested element in the zeolite, i.e., plant material. All of the volumetric glassware was of A grade, and soaked in nitric acid for 12 h and rinsed with Milli-Q water before use [11].

2.4 Measurement of heavy metal content by FAAS

The concentrations of heavy metals in the prepared solutions of plant material samples are then read on an atomic absorption spectrophotometer (Shimadzu 7000 AA device). The sample and blank measurements were performed in triplicate. This device has a software program that directly reads the results based on the equation of the calibration curve obtained for each tested element.

3. RESULTS AND DISCUSSION

In selected wild plant species of mint, oregano, chamomile, nettle and St. John's wort, from rural areas in Bosnia and Herzegovina, the content of heavy metals: nickel (Ni), copper (Cu), chromium (Cr), cadmium (Cd), iron (Fe), zinc (Zn), manganese (Mn) and lead (Pb) were analyzed using Flame Atomic Absorption Spectrometry (FAAS). Tables 1, 3, 5, 7, and 9 show the measured content of heavy metals (mg/kg) in wild plant species, while Tables 2, 4, 6, 8, and 10 present the correlations of the total content of elements in the used plants.

Interestingly, cadmium was not detected in any of the samples of wild plant species used, while chromium was not detected or determined in mint (all three samples) and oregano (sample I). High concentrations (mean value, mg/kg) for heavy metals were: Cu 11.06 in nettle, Cr 1.73 in nettle, Mn 33.13 in mint, Ni 3.67 in mint, Fe 339.33 in nettle, Pb 0.53 in mint, and Zn 34.19 in oregano. These measured values indicate that the highest accumulation of heavy metals, i.e., Cu, Cr, and Fe in nettle; Mn, Pb in mint; and Zn in oregano, was demonstrated, while other plant species, St. John's wort and chamomile, bind and accumulate the tested heavy metals moderately.

Previous studies have shown that most heavy metals have been found at elevated concentrations in plants as a consequence of contamination and pollution of soil and sediments, as well as water resources [12-14]. It is well known that some heavy metals, depending on their chemical affinity, affect microbial changes and enzymatic activities in plants, and also have consequences for human health. For example, it has been found that Pb affects catalases, ureases, invertase, and acid phosphatase; Cu restricts β-glucosidase; high Cr concentration in soil exhibits distinct toxicity, causing negative effects in soil microbial cell metabolism. Mainly heavy metal such as Cd, Cu, Zn, Ni, Pb, and Cr, accumulation and higher concentrations cause various severe diseases and metabolic conditions including Itai-itai, hepatic and central nervous system damage, kidney dysfunction and anemia, skin irritation and nervous complications, cardiovascular problems and nervous damage, cancer, respectively; or other hard disturbances [15-19].

Table 1. Heavy metal content in wild Oregano

Element, mg/kg	Sample I	Sample II	Sample III
Cu	6.96	4.63	4.87
Cr	n.d.	0.036	0.023
Mn	26.28	17.48	27.3
Ni	0.39	1.46	2.37
Fe	108.7	68.79	152.6
Pb	n.d.	0.0204	0.002
Zn	51.28	34.48	16.81
Cd	n.d.	n.d.	n.d.

Table 2. The correlation coefficients between the elements in wild Oregano; statistically significant values are in bold type (α<0.05)

	Cu	Cr	Mn	Ni	Fe	Pb	Zn
Cu	1	**-0.96**	0.50	**-0.84**	0.07	-0.65	**0.81**
Cr		1	-0.71	0.67	-0.33	**0.83**	-0.62
Mn			1	0.05	**0.90**	**-0.98**	-0.11
Ni				1	0.48	0.14	**-1.00**
Fe					1	**-0.80**	-0.54
Pb						1	-0.07
Zn							1

Table 3. Heavy metal content in wild Mint

Element, mg/kg	Sample I	Sample II	Sample III
Cu	7	7,3	0,01
Cr	n.d.	0	0
Mn	40,3	21,3	37,8
Ni	0,7	3,7	6,6
Fe	64,5	75,5	65,9
Pb	0,1	0,6	0,9
Zn	13,9	14,5	29,9
Cd	n.d.	n.d.	n.d.

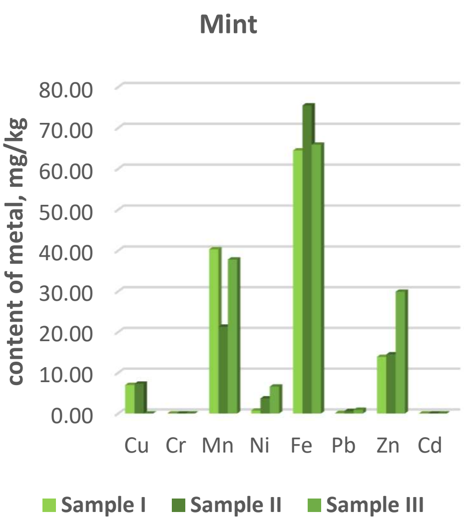

Figure 1. Content of metal in Mint

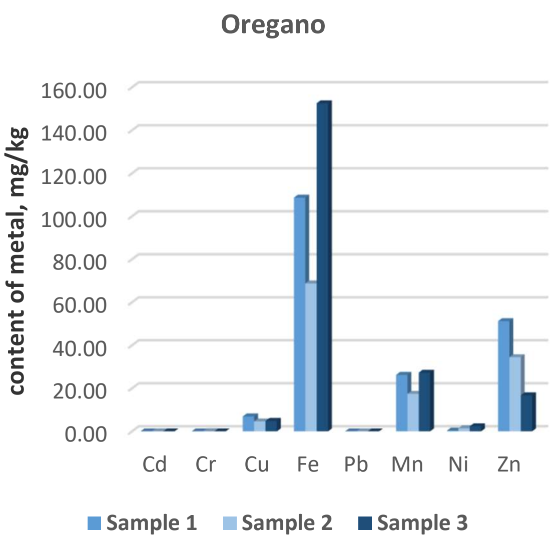

Figure 2. Content of metal in Oregano

Table 4. The correlation coefficients for total element content in wild Mint; statistically significant values are in bold type (α<0.05)

	Cu	Mn	Ni	Fe	Pb	Zn
Cu	1	-0.42	**-0.84**	0.43	-0.76	**-1.00**
Mn		1	-0.13	**-1.00**	-0.26	0.36
Ni			1	0.13	**0.99**	**0.88**
Fe				1	0.26	-0.36
Pb					1	**0.81**
Zn						1

Table 5. Heavy metal content in wild St John's wort

Element, mg/kg	Sample I	Sample II	Sample III
Cu	6.23	7.98	8.05
Cr	0.87	1.23	1.73
Mn	36.17	9.1	11.85
Ni	0.14	0.36	0.87
Fe	7.59	26.06	32.91
Pb	0.19	0.24	0.36
Zn	5.23	4.87	3.17
Cd	n.d.	n.d.	n.d.

Table 6. The correlation coefficients between the elements in wild St John's wort; statistically significant values are in bold type ($\alpha<0.05$)

	Cu	Cr	Mn	Ni	Fe	Pb	Zn
Cu	1	**0.83**	**-0.99**	0.76	**0.97**	0.75	-0.66
Cr		1	-0.76	**0.99**	**0.94**	**0.99**	**-0.96**
Mn			1	-0.67	**-0.94**	-0.66	0.56
Ni				1	**0.88**	**1.00**	**-0.99**
Fe					**1**	**0.88**	**-0.81**
Pb						1	**-0.99**
Zn							1

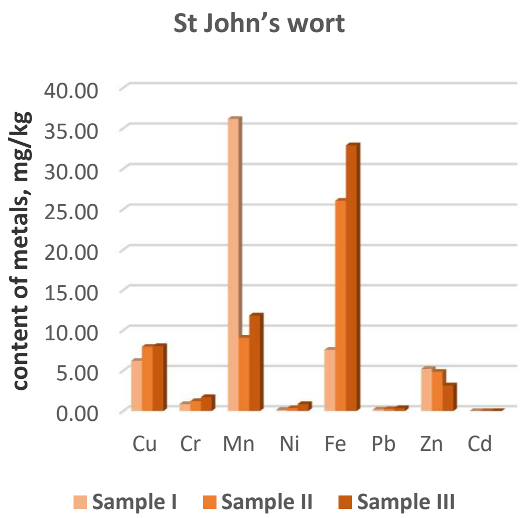

Figure 3. Content of metal in St John's wort

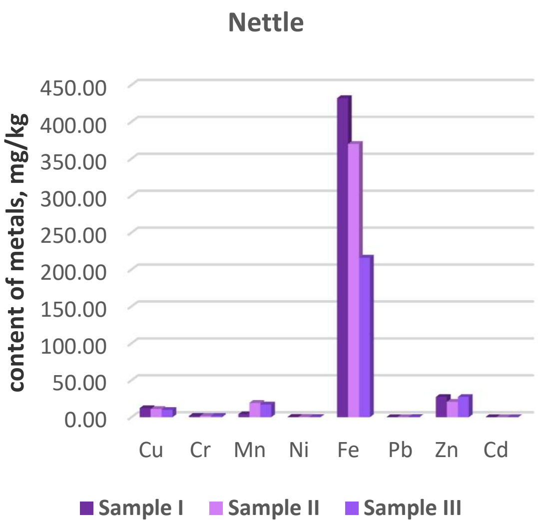

Figure 4. Content of metal in Nettle

Table 7. Heavy metal content in wild Nettle

Element, mg/kg	Sample I	Sample II	Sample III
Cu	12.26	11.07	9.85
Cr	1.73	1.66	1.81
Mn	4.03	19.35	17.17
Ni	0.46	0.47	0.31
Fe	432	370	216
Pb	0.12	0.13	0.28
Zn	27.44	20.87	27.43
Cd	n.d.	n.d.	n.d.

Table 8. The correlation coefficients between the elements in wild Mint; statistically significant values are in bold type (α<0.05)

	Cu	Cr	Mn	Ni	Fe	Pb	Zn
Cu	1	-0.54	-0.79	**0.84**	**0.97**	**-0.90**	-0.01
Cr		1	-0.09	**-0.91**	-0.72	**0.86**	**0.85**
Mn			1	-0.33	-0.62	0.43	-0.61
Ni				1	**0.94**	**-0.99**	-0.55
Fe					1	**-0.97**	-0.24
Pb						1	0.45
Zn							1

Table 9. Heavy metal content in wild Chammomile

Elemen, mg/kg	**Sample I**	**Sample II**	**Sample III**
Cu	6.3	7.3	6.7
Cr	1.14	1.27	1.22
Mn	9.3	26.9	33.3
Ni	0.1	0.21	0.12
Fe	115.5	175.85	116.51
Pb	0.5	0.3	0.2
Zn	15.1	17.3	12.7
Cd	n.d.	n.d.	n.d.

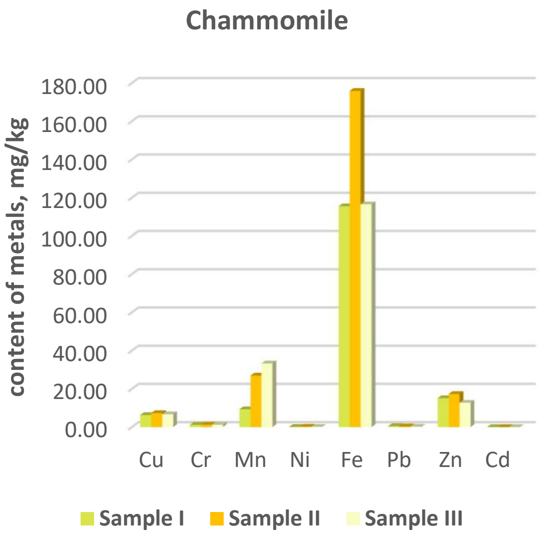

Figure 5. Content of metal in Chammomile

Table 10. The correlation coefficients between the elements in wild Chammomile; statistically significant values are in bold type (α<0.05)

	Cu	Cr	Mn	Ni	Fe	Pb	Zn
Cu	1	**0.97**	0.62	**0.97**	**0.92**	-0.56	0.58
Cr		1	**0.80**	**0.88**	**0.80**	-0.75	0.36
Mn			1	0.42	0.27	**-1.00**	-0.28
Ni				1	**0.99**	-0.35	0.75
Fe					1	-0.20	**0.85**
Pb						1	0.35
Zn							1

Also, eight elements (Cr, Cu, Mn, Fe, Ni, Cd, Pb, and Zn) were compared in all five plant samples by correlation analysis using Pearson correlation (r). Complete. strong. and moderate or weak correlations, and absence of correlation with r values of $0.9 \le r < 1$, $0.5 \le r < 0.8$, $0.2 \le r < 0.5$, and $r \le 0$ respectively. were found between the analyzed heavy metals in the tested plant species.

The element correlations in the studied wild plant species samples from various sampling sites are reported in Tables 2, 4, 6, 8, and 10. Since the content of Cd (in samples of all wild plant species) and Cr (sample I. oregano) was not analyzed in plant samples, because the values were below the limit of detection by FAAS. Pearson's correlation was not performed. The correlations are the result of biological, chemical, and metabolic reactions and the different signaling pathways that these elements have in plants. A strong and complete correlation. positive or negative. between two elements indicating the same source of these elements in the plant as in the soil (e.g., Cu and Fe, Cu and Zn, Cu and Mn, as well as Cr and Zn, Mn, Zn, and Fe, Cu, and Cr, etc.) was observed [20-23]. The strong correlations between Pb and Ni. Cr and Ni, as well as Ni and Fe. primarily indicate the influence of various environmental factors on the chemical/metal composition and concentration of wild plant species. An important factor is the soil pH since the adsorption capacity of the soil for certain heavy metals (e.g., Cd) triples for each pH unit in the interval 4-7. Then there are other factors, such as the solubility of some heavy metals. the organic matter content. the cation exchange capacity and the concentration of other cations. For instance. organic matter binds heavy toxic metals (Cd, Pb, and Ni) and converts them into organically bound fractions, reducing their bioavailability. Replacement of the structural cation of magnesium (Mg) with heavy metals is an important mechanism in cation absorption that affects the cation exchange capacity. The measured concentrations of Ni and Pb were lower. and cadmium was not detected in any sample of the examined plant species [7.8.23].

In the samples of mint. chamomile. nettle and St. John's wort. correlation. especially for Cu. Cr. Fe and Ni, and for Pb and Ni in nettle and St. John's wort, were observed. Also. our results showed the potential application of the investigated plant species. nettle and mint. and in some cases also St. John's wort, as plants for phytoremediation by using various mechanisms that can increase the efficiency of phytoremediation processes. Furthermore. the application of these plant species is due to the fact that they are cheap. available and renewable resources compared to other plant species or conventional. mainly chemical technologies [23-25].

4. CONCLUSION

In this work. the heavy metal content of wild plant species of mint. oregano. chamomile. nettle and St. John's wort, originating from various areas of Bosnia and Herzegovina, were determined. This work showed that collected wild plants can accumulate heavy metals and could be used for the phytoremediation of multi-metal-contaminated soil. Also. the obtained results could serve as a basis for future research on the possible use of these plant species for soil phytoremediation. and to contribute to a more comprehensive use of this plant species. both ecologically and naturally.

Conflicts of Interest

The authors declare no conflict of interest.

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