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BIOACCUMULATION OF METALS, TOTAL PHENOLIC AND FLAVONOID CONTENTS AND ANTIOXIDANT ACTIVITY OF *Rumex acetosella* L. FROM TAILINGS IN ŽITKOVAC (KOSOVO & METOHIJA)

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ABSTRACT. The aim of this study was to determine the bioaccumulation of the metals in *Rumex acetosella* L. collected from the tailings of abandoned mine in Žitkovac (near Kosovska Mitrovica, Kosovo and Metohija), and to establish the possibility of using those plants for medicinal purposes. Concentrations of heavy metals (Mn, Fe, Pb, Ni, Cr, Cu, Cd, Zn, Ca and Mg) in soil, roots and aboveground parts of *R. acetosella* were determined by wet mineralization with nitric and perchloric acid. The results of the research indicate that *R. acetosella* accumulates large amounts of Ni, Cu, Pb, Zn, Cr, and Mn. The total phenol content, the total amount of flavonoids and the antioxidant activity of three different extracts of the root and aboveground parts of *R. acetosella* were determined by spectrophotometric methods. The acetone extracts contain the highest concentration of phenols and flavonoids. Ethyl acetate has proven to be a solvent that releases the least amount of phenols and flavonoids. The methanol extract of aboveground parts has the highest, while acetone root extract has the lowest antioxidant activity. *Rumex acetosella* can be used for medicinal purposes, but only collected from uncontaminated soil.

Keywords: *Rumex acetosella*, heavy metals, secondary metabolites, antioxidant activity.

INTRODUCTION

For centuries, plants with aromatic and medicinal properties have been in the daily use of many people around the world. In a certain way, this is the oldest form of medicine. Plants contain compounds, plant secondary metabolites, that have antibacterial, anti-inflammatory, antihypertensive, anticancer, antioxidant, antiscorbutic, hypocholesterolemic, detoxification, anti-refrigerant, astringent, aperient and many other activities (RAO *et al*., 2011; ASGARPANAH and HAGHIGHAT, 2012; AL DISI *et al*., 2015; REZAIE *et al*., 2015). Today, a

large number of extraction techniques and solvents are used to isolate a various classis of biologically active compounds from plant material (terpenes, vitamins, carotenoids, fatty acids, etc.) (SELVAMUTHUKUMARAN and SHI, 2017). The final extraction products depend not only on the extraction method, but also on the solvent used for extraction (BHANDARY *et al*., 2012; NGO *et al*., 2017, VUKOVIĆ, *et al*., 2018). When choosing the appropriate extraction technique we should take into account the toxicity of the solvent, the selectivity of the process in relation to the desired group of compounds, as well as the possibility of degradation of active components in the extract (SELVAMUTHUKUMARAN and SHI, 2017). Water and organic solvents (ethanol, acetone, ethyl acetate) should be used more often for the extraction of plant material, because they are less toxic than methanol, hexane and chloform (DAILEY and VUONG, 2015).

The genus *Rumex* comprises about 250 different species (KHAN and BHAT, 2018). VASAS *et al*. (2015) showed that species of the genus *Rumex* are rich in anthraquinones, naphthalenes, flavonoids, stilbenoids, triterpenes, carotenoids and phenolic acids from which various pharmacological activities originate. *Rumex acetosella* L. (Polygonaceae) is a perennial weed herbaceous plant that grow along roadsides, on pastures and meadows (STOPPS *et al*., 2011). This species has a great tolerance for the geological substrate. It grows on different substrates such as serpentine, andesite and dolomite limestone (TOPUZOVIĆ, 1989).

Several studies have confirmed the medicinal benefits of this plant as mildly laxative and potentially useful in a long-term treatment for chronic disease in the gastrointestinal tract (ALKUSHI, 2017). It is used in traditional medicine and as a food source in Europe and North America. Leaves contain vitamin A, vitamin B, complex E, D, K, P, C, U and a large number of metals, such as Fe and Ca (SVIRČEV, 2014). This plant is used as a remedy for diseases of the stomach and respiratory tract, as a diuretic (the fresh juice of the leaves having a pronounced diuretic effect) and in stopping vomiting blood (STOPPS *et al*., 2011). A leaf poultice applied to tumors, cysts is a folk treatment for cancer (LEONARD, 2006). In the study by RAO *et al*. (2011) *R. acetosella* as a natural source of antioxidants was studied in vitro, and its' antioxidant capacity tested because of widespread using of this plant in traditional medicine as an anticancer, antiscorbutic, antirefrigerant and diuretic.

Plants, including medicinal can be accumulators of heavy metals (FRITSCH *et al.*, 2010), since they can accumulate metals essential to their growth (OKAREH *et al*., 2018). Large amounts of heavy (toxic) metals affect the parameters of plant metabolism, particularly photosynthesis and the antioxidant defense system (KISA, 2016). The mechanisms of plant tolerance to metals and metalloids are based on strategies of exclusion or accumulation of metals. It is believed that these traits are a consequence of the evolutionary development of plants due to the modification of their physiological functions in the process of adapting to the environments rich in metals (SINGH *et al*., 2016). Plant tolerance to metals through exclusion encompasses two forms of behaviors: avoiding the uptake of metals and metalloids by limiting their transfer from soil to root, or exclusion of metal through accumulation in root along at the same time preventing the transport of metals to the aboveground parts of the plant (SMITH *et al*., 2014).

Exposure of plants to heavy metals increases the production of phenolic compounds (KISA, 2016). To protect the environment, it is important to know how to control the quantity of heavy metals in the soil, so that their amount does not exceed the toxicity limit for the plants (BRIFFA *et al*., 2020). Therefore, determining the heavy metals accumulation in plants is of high importance.

Bioconcentration and translocation factor values have a role in identifying plant suitability for phytoextraction and phytostabilization. These factors explain the characteristics of accumulation and translocation of metals in plants also (RADZIEMSKA, 2018). The uptake of metals from the soil into plants and their transfer to the aboveground parts shows the ability

of certain plant species to accumulate metals. If plants are accumulators, then they can be classified as indicators of pollutants useful phytoremediation (PIETRZYKOWSKI *et al*., 2018). It is considered that the solubility of certain trace elements in the soil and the degree of their transport through the plant, according to KABATA-PENDIAS (2011) is as follows: Cr slightly dissolved in the soil and plants do not absorb it easily; Cu is mobile in the soil, from where it is easily taken up by plants; Zn is very mobile in the soil and plants very easily accumulate it in their biomass. pH reduces the mobility of trace metals in the soil, which affects the reduction of biological absorption by plants (PIETRZYKOWSKI *et al*., 2018).

Tailings as a waste product of ore processing are alkaline. On the basis of the claim ZARINKAMAR (2013) accumulators are considered to be plants that actively absorb metals and transport them in its aboveground parts. *Rumex acetosella* is an accumulator for Ni, Ca, Mg, Pb and Cu.

Our study had a few aims. The first – analysis of content of Mn, Fe, Pb, Ni, Cr, Cu, Cd, Zn, Ca and Mg in soil, roots and aboveground parts of *R. acetosella* from the tailings of abandoned mine in Žitkovac (near Kosovska Mitrovica, Kosovo and Metohija) was performed to determine the bioaccumulation potential of *R. acetosella*. The second aim was to determine total content of phenols and flavonoids, as well as the antioxidant effect of methanol-, ethyl acetate- and acetone extracts of the roots and aboveground parts of *R. acetosella*. Based on that, the possibility of its medicinal use was assessed.

MATERIALS AND METHODS

Plant material

Plant material, *R. acetosella* (the aboveground parts and roots i.e. *herba et radix*), and soil were collected from tailings at Žitkovac, 4 km away from Kosovska Mitrovica (42.924052 N; 20.830233 E). The experimental plant was harvested in the flowering phase. Žitkovac is an abandoned mine of lead and zinc, where tailings were formed. Even though it is partially covered by vegetation, this area still looks like desert, represents a huge source of dust and water pollution, which mainly occurs through erosion. Plants were sampled in 2018 on 20 June. Plant material was identified and the voucher specimen of *R. acetosella* deposited in the Herbarium at the University of Kragujevac, Faculty of Science, Department of Biology and Ecology. When collected, plant material (underground and aboveground parts) was air-dried and prepared for analysis. From each sample of dried plant material *R. acetosella*, part of the material was finely ground with a grinder, and 50 g was extracted with 250 ml of one of the organic solvents (methanol, acetone, and ethyl acetate) and after 24 hours filtrated, and then 250 ml of adequate solvents were added to the remaining plant material. After next 24 hours, the procedure was repeated. After extraction, the material was evaporated to dryness using a rotary evaporator at 40°C. The obtained extracts (nine samples) were stored in sterile tubes at 4°C till the chemical analysis.

For the determination of total phenols and flavonoids content immediately before analysis 1 mg of dry extract was solved by adding 2 ml of methanolic 1 mg/ml solution, which is in the future text named samples methanol solutions.

Analysis of heavy metals in samples of plant and soil

The concentration of heavy metals such as manganese (Mn), iron (Fe), lead (Pb), nickel (Ni), chromium (Cr), copper (Cu), cadmium (Cd), zinc (Zn), calcium (Ca), magnesium (Mg) was measured in the soil, root, and herb part of the plants. The plant material is washed and then dried in the shade. Analysis of heavy metals was performed in the Laboratory for

Analytical Chemistry, at the University of Kragujevac, Faculty of Science. For the determination of heavy metals in the soil a method according to MICKOVSKI-STEFANOVIĆ (2012) was performed. Plant material was subjected to wet digestion. Five copies of samples (soil, roots and herba separately) were prepared for each analysis and the average value and standard deviation were calculated. The results were read on an atomic spectrophotometer (Perkin Elmer 3300), after which the bioaccumulation and translocation factor were determined (KABATA-PENDIAS, 2001). Bioaccumulation factor was used for determining the accumulation of metal from the soil to the root is calculated as a ratio of metal concentrations in the root and in the soil. For metal translocation analysis from the root to the aboveground parts of the plant, a translocation factor was calculated as the ratio of the concentrations of metal in the aboveground parts and in the root (GUPTA *et al*., 2008).

Determination of total phenols content of the extracts

The total phenols content of the samples methanol solutions was determined by the Folin-Ciocalteu method as described by PETER *et al*. (2011). The 0.2 ml of each of six methanolic dilutions and 1.5 ml of Folin-Ciocalteu's reagent (1:10 water solution) were incubated for 5 minutes at 25°C in dark. After incubation, 1.5 ml of sodium carbonate (6%) was added, and then the test samples were vortexed for 15 seconds. The mixture was incubated for 90 minutes at 25°C in dark. The test samples were prepared in triplicate for each analysis and the average absorbance values were obtained at different concentrations of gallic acid. The absorbance was determined using a spectrophotometer at λ max = 725 nm. Gallic acid (GA) was used to prepare the standard curve, which showed the linear regression of $r^2 > 0.99$, and the level of phenolics was expressed in terms of gallic acid equivalent per gram of plant extract. The values obtained for the concentration of total phenols are expressed as GA/g of extract.

Determination of total flavonoid content of the extracts

The concentration of flavonoids in plant extracts was determined by method of QUETTIER-DELEU *et al*. (2000). The 2 ml of each of six samples methanol solutions and 2 ml of $AlCl_3$ (2%) dissolved in methanol. The test samples were incubated for 10 minutes at 25°C in dark. The test samples were prepared in triplicate for each analysis and the average absorbance values were obtained at different concentrations of rutin. The absorbance was determined using a spectrophotometer at λ max = 430 nm. Rutin (RU) was used to prepare the standard curve, which showed the linear regression of $r^2 > 0.99$, and the level of flavonoid was expressed in terms of rutin equivalent per gram of plant extract. The values obtained for the concentration of total flavonoids are expressed as RU/g of extract.

Determination of the antioxidant activity of the extracts

The free radical scavenging activity of samples methanol solutions the same as for total flavonoid contents analysis, were analyzed using 2,2-diphenyl-1-picrylhydroxyl (DPPH) according to the TAKAO *et al*. (1994). Dilutions were made to obtain concentrations of 500, 250, 125, 62.5, 31.25, 15.62 mg/mL. Diluted solutions were mixed with prepared DPPH reagent, and then the test samples were incubated for 30 minutes at 25°C, in dark. The test samples were prepared in triplicate for each analysis and the average absorbance values were obtained at different concentrations of ascorbic acid. Ascorbic acid was used as the standard. The absorbance was measured at λ max =517 nm. Antioxidant activity was expressed as the 50% inhibitory concentration (IC_{50} values in mg/ml).

Data analysis

All measurements (the content of phenols, flavonoids and antioxidant activity in roots and aboveground parts) were carried out in triplicate and expressed as the average value of three independent measurements ± standard deviation. Statistical analyses of data was made by using IBM SPSS Statistics 21.0 (2012). The values of the correlation coefficient (r) between the concentrations of heavy metals in the root and aboveground parts were determined using the Pearson correlation coefficient. Statistically significant difference was defined as $p < 0.05$.

RESULTS AND DISCUSSION

Concentration of metals in soil, root and aboveground part

Based on the mean values of the metal concentration measured in the soil, from the highest to the lowest, they can be arranged in the following order: Fe> Mg> Ca> Mn> Pb> Cr> Zn> Ni> Cu. SORIANO *et al*. (2010) and KABATA-PENDIAS (2011) described the classification of contaminated soil according to heavy metal content (Tab. 1). The values obtained in our study show that the concentration of heavy metals (Mn, Ni, Ca, Mg, Fe, Zn, Cr, and Pb) in the soil were very high, except Cu (113.88 mg/kg), whose concentration is within the limits for the unpolluted soil.

Table 1. Classification of contaminated soil according to heavy metal content (mg/kg) (SORIANO *et al*., 2010; KABATA-PENDIAS, 2011).

Chemical element	Unpolluted	Low polluted	Moderately polluted	Highly polluted	Extremely polluted
Lead (Pb)	0-500	500-1000	1000-2000	2000-1%	>1%
Magnesium (Mg)	0-500	500-1000	1000-2000	2000-1%	>1%
Nickel (Ni)	0-20	20-50	50-200	200-1000	>1000
Chromium (Cr)	0-100	100-200	200-500	500-2500	>2500
Copper (Cu)	0-100	100-200	200-500	500-2500	>2500
Cadmium (Cd)	0-1	1-3	3-10	10-50	> 50
Zinc (Zn)	0-20	20-50	50-200	200-1000	>1000
Arsenic (As)	0-30	30-50	50-100	100-500	>500
Iron (Fe)	0-50 000	/	/	/	/
Manganese (Mn)	0- 2 000	/	/	/	/
Calcium (Ca)	600-1000	/	/	/	/

Based on concentrations of Mg (4,540.54 mg/kg), Pb (873.66 mg/kg), Cr (453.36 mg/kg), Zn (276.24 mg/kg) and Ni (199 mg/kg) in the tested soil, it can be concluded that the tested soil is moderately polluted, according to the classification by SORIANO *et al*., (2010). ČIAMPOROVÁ *et al*. (2021) reported that *R. acetosella* grows on soil with high concentrations of heavy metals such as Zn, Pb and Cu, which is in accordance with our results.

The results presented in Table 2 show that the analyzed soil had a high content of iron (Fe 77,363.08 mg/kg), higher than the maximum allowed concentration. KABATA-PENDIAS (2011) points out that the mean Fe concentration in the soil is 20,000-50,000 mg/kg (. The iron content in plants may vary. In general, a sufficient concentration of iron for most plants is from 50–100 mg/kg of dry matter. The iron accumulates in plants without any obvious harmful effects, but its deficiency in the plant is manifested by a loss of green color (MARIĆ, 2014). Our results show that iron, as an essential element, is present in large quantities it the tested *R. acetosella* samples (Tab. 2).

Table 2. The content of investigated metals (mg/kg) in soil, root and aboveground parts of *Rumex acetosella*.

Metal	Soil	Root	Aboveground part
Mn	2,685.58 ± 16.06	619.6 ± 2.15	189.76 ± 1.4
Ni	199 ± 0.97	25.32 ± 0.36	37.28 ± 0.4
Ca	3,649.76 ± 31.20	2,460.6 ± 28.76	8,716.38 ± 37.91
Mg	4,540.54 ± 23.4	2,703.4 ± 36.81	3,653.42 ± 52.88
Fe	77,363.08 ± 682.37	3,867.8 ± 36.73	2,514.04 ± 25.56
Zn	276.24 ± 0.78	146.46 ± 0.35	103.32 ± 0.54
Cr	453.36 ± 1.36	39.24 ± 0.31	26.32 ± 0.38
Pb	873.66 ± 2.057	36.12 ± 0.32	77.16 ± 0.48
Cu	113.88 ± 0.8	12.46 ± 0.19	19.16 ± 0.21

Mean value (n = 5) ± standard deviation (mg/kg).

As shown in Table 2, tested soil had a high content of Mg (4,540.54 mg/kg), what is higher than the maximum allowed concentration. KABATA-PENDIAS (2011) points out that the mean Mg concentration in the soil is 0–500 mg/kg. Magnesium plays a specific role in phosphorus metabolism, and in plants it has been recognized as a very important ion in signaling processes for activating and maintaining biochemical reactions (FIORENTINI *et al*., 2021). Symptoms of Mg deficiencies are even more noticeable in older plant tissues (e.g. jaundice and necrosis, lower leaves, so-called chlorosis) (MARIĆ, 2014). Our results show that Mg, as an essential element, is present in large quantities in tested soil (Tab. 2).

The content of investigated metals (mg/kg) in soil and *R. acetosella* root and aboveground parts are shown in Table 2. The higher amounts of Mn, Fe, Cr and Zn are in the root than in the aboveground part of plants.

Calcium is a macronutrient needed for plant growth and development, and above all, for fruit quality (BAI *et al*., 2021). It is found in large quantities in the green parts of the plant, and by aging this amount increases (WEBB, 1999). The results (Tab. 3) show that Ca is present in large quantities (8,716.38 mg/kg). According to study conducted by WEBB (1999), the optimal range for Ca in leaf is 600–1,000 mg/kg.

The average amount of Mn in plants is 20–300 mg/kg, while the toxic value is estimated at 300–500 mg/kg of dry matter (KASTORI, 1993). The amount of Mn we measured in the root (619.6 mg/kg) is significantly above the toxic values, which is a strong argument that *R. acetosella* from the analyzed locality must not be used for the human feeding or medicine.

The examined soil is very polluted in terms of Zn content (276.24 mg/kg) compared to the study by SORIANO *et al*., 2010. According to KABATA-PENDIAS (2011), the optimal content of Zn in the aboveground part is 27–150 mg/kg, and toxic 200–400 mg/kg, and our results are within (103.32 mg/kg).

Excessive amounts of Cu affect the growth of the plant, and the symptoms are similar to iron deficiency (WATERS and ARMBRUST 2013). Our results show that Cu (19.16 mg/kg in the aboveground part) content is lower than phytotoxic concentrations, but is significantly above the optimal content: critical Cu concentration for plants is 15 mg/kg, and this metal is considered high phytotoxic if exceeded 30 mg/kg. Optimal Cu concentrations in plants range from 2 to 15 mg/kg (KABATA-PENDIAS, 2011).

In our study, Ni in aboveground part is in the toxicity range (37.28 mg/kg), suggested by different scientific studies. For instance, KABATA-PENDIAS (2011) states that early signs of

Ni toxicity for plants were in the range of 10–100 mg/kg, and the optimal range Ni is 0.1–5 mg/kg.

The measured Cr content in the root of *R. acetosella* (39.24 mg/kg) is significantly above the toxic concentration range 5–30 mg/kg of dry matter given by NAGAJYOTI *et al.* (2010). The optimal content of Cr in plants is 0.2–1 mg/kg.

The content of Pb in the root (36.12 mg/kg) and aboveground part (77.16 mg/kg) is far higher than the average content of Pb in plants stated by KABATA-PENDIAS (2011). The optimal content of Pb in plants is 5–10 mg/kg, while the toxic value of Pb for plant tissues is estimated at 30–300 mg/kg (KABATA-PENDIAS, 2011). The toxicity of Pb to plants is reflected in the inhibition of plant growth and photosynthesis, loss of chlorophyll and a large amount can lead to plant cell death (HADI *et al.*, 2015).

As presented in Table 3, a significantly negative correlation (r = -0.896*) is registered between Cr and Mn for the root, but a strong positive correlation (r = 0.926*) between Ca and Mn for the root.

The Tab. 4 shows a significantly negative correlation (r = -0.95*) between root in Fe content and aboveground part in Ni content. Strong negative correlation (r = -0.87*) between root in Zn content and aboveground part in Ni content. Significantly strong positive correlation (r = 0.88*) between root in the Mn content and aboveground part in Cr content.

The Tab. 5 shows a significantly negative correlation (r = -0.938*) between Ca and Cu in the aboveground part. Significantly strong positive correlation (r = 0.917*) is registered between Fe and Zn in the aboveground part, and strong positive correlation (r = 0.936*) between Cr and Mn in the aboveground part.

Table 3. Pearson's correlation between heavy metals at the root of *Rumex acetosella.*

		Root								
		Mn	**Ni**	**Ca**	**Mg**	**Fe**	**Zn**	**Cr**	**Pb**	**Cu**
Root	**Mn**	1								
	Ni	-0.502	1							
	Ca	**0.926***	-0.468	1						
	Mg	-0.024	-0.416	-0.322	1					
	Fe	0.077	0.680	-0.076	-0.137	1				
	Zn	0.360	0.491	0.220	-0.015	0.833	1			
	Cr	**-0.896***	0.772	-0.828	-0.087	0.504	0.068	1		
	Pb	0.177	-0.397	0.039	0.728	0.293	0.249	-0.086	1	
	Cu	-0.350	0.04	-0.07	-0.22	0.338	-0.16	0.416	0.327	1

* is significant at $0.05 < p \leq 0.1$ levels, ** is significant at 0.01.

Table 4. Pearson's Correlation of heavy metals among root and aboveground part of *Rumex acetosella.*

		Root								
		Mn	**Ni**	**Ca**	**Mg**	**Fe**	**Zn**	**Cr**	**Pb**	**Cu**
Aboveground part	**Mn**	0.802	0.108	0.766	-0.38	0.346	0.709	-0.509	-0.103	-0.362
	Ni	-0.219	-0.522	-0.24	0.237	**-0.95***	**-0.87***	-0.232	-0.289	0.586
	Ca	-0.435	0.193	-0.317	-0.134	-0.56	-0.454	-0.737	-0.150	0.135
	Mg	-0.141	0.868	0.001	-0.757	0.658	0.537	0.46	-0.518	0.105
	Fe	0.564	-0.694	0.762	-0.365	-0.56	-0.454	-0.737	-0.15	0.135
	Zn	0.584	-0.376	0.812	-0.664	-0.365	-0.231	-0.643	-0.417	0.045
	Cr	**0.889***	-0.188	0.868	-0.232	0.361	0.675	-0.60	0.172	-0.148
	Pb	0.506	0.16	0.753	-0.683	0.368	0.316	-0.266	-0.046	0.447
	Cu	0.499	-0.48	-.443	-0.116	-0.783	-0.36	-0.746	-0.503	-0.701

* is significant at $0.05 < p \leq 0.1$ levels, ** is significant at 0.01.

Table 5. Pearson's correlation between heavy metals at the aboveground part of *Rumex acetosella*.

		Aboveground part								
		Mn	**Ni**	**Ca**	**Mg**	**Fe**	**Zn**	**Cr**	**Pb**	**Cu**
Aboveground part	**Mn**	1								
	Ni	-0.586	1							
	Ca	-0.392	-0.461	1						
	Mg	0.457	-0.647	0.071	1					
	Fe	0.224	0.312	-0.297	-0.27	1				
	Zn	0.475	0.101	-0.406	0.105	**0.917***	1			
	Cr	**0.936***	-0.628	-0.177	0.256	0.344	0.488	1		
	Pb	0.627	-0.593	0.179	0.473	0.550	0.716	0.723	1	
	Cu	0.279	0.614	**-0.938***	-0.29	0.573	0.582	0.154	-0.092	1

* is significant at $0.05 < p \leq 0.1$ levels, ** is significant at 0.01.

Bioconcentration (BCF) and translocation factors (TF)

Bioconcentration factor (Fig. 1) was used to calculate the accumulation of heavy metals from soil to aboveground plant parts. The obtained results show that BCF < 1, which means that *R. acetosella* heavy metal excluders. This indicates that the plant roots can solubilize and take up the metals from very low levels in the soil, even from nearly insoluble precipitates (TANGAHU *et al.*, 2011). Efficient translocation (TF > 1) was found for Ni, Ca, Mg, Pb, Cu. The BF and TF for Zn were both less than 1 (0.62, 0.96, respectively), which is in accordance with the research by KOTHE and VARMA (2012).

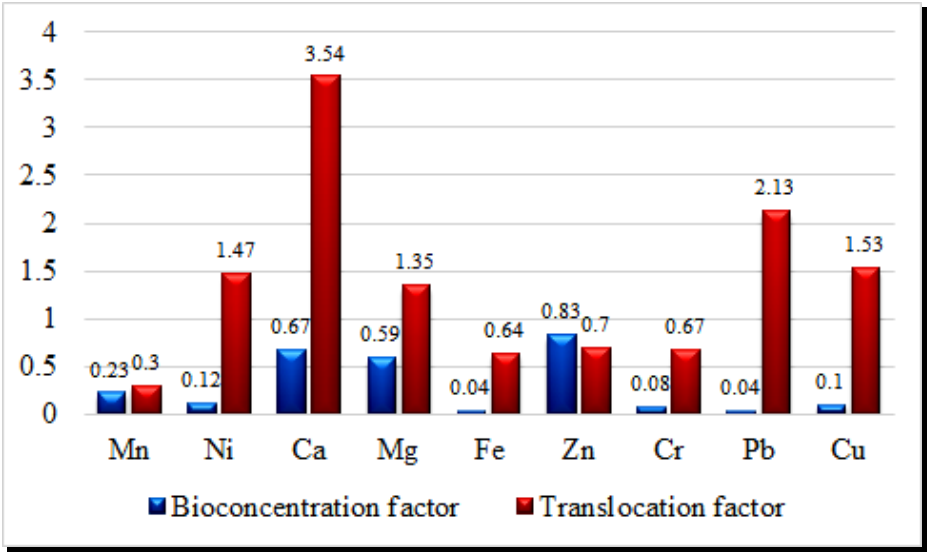

Figure 1. Bioconcentration factor (blue) and translocation factor (red) for *Rumex acetosella*.

Total contents phenol of the extracts

Results of the total amount of phenols in different plant parts (root and aboveground part) of species *R. acetosella* from the locality Žitkovac are shown in Tab. 6, as an average value and standard deviation based on measurements in three samples. The values obtained for the concentration of total phenols are expressed as GA/g of extract. The results of the total phenol content in the tested plant extracts are expressed as gallic acid equivalent (the standard curve equation: $y= 76.735x+0.069$, $r^2= 0.994$).

The highest concentration of phenols was extracted from the root with polar solvents - acetone (248.88 ± 3.57 mg GA/g) and methanol (211.33 ± 1.73 mg GA/g), and the lowest in

aboveground part extracts obtained with non-polar solvent (ethyl acetate) (115.22 ± 3.43 mg GA/g). Differences between concentration of phenols gained by different extraction solvents were significant ($p < 0.05$).

Our study shows a higher phenol content in methanol extracts of aboveground part in comparison to previous studies, such as PEREIRA *et al.* 2011. They reported the phenol concentration of 141.5 ± 3.67 mg GA/g from aboveground part. FEDURAEV *et al*. (2022) reported that 70% ethanol aboveground part extracts contained the 117 ± 7 mg GA/g of phenol, while 96% ethanol aboveground part extract (ISBILIR and SAGIROGLU, 2013) 69.21 ± 8.5 mg GA/g. Mentioned results are a much lower compared to our results (polar solvents (acetone and methanol)). SARIKURKCU *et al.* (2017) reported that *R. acetosella* ethanolic extract contains phenols in the concentration of 34.14 ± 1.05 mg GA/g, which is much lower than the amount we extracted with polar solvents (acetone and methanol). Compared to ÖZEN (2010) aboveground part water extracts contained 76.6 ± 1.5 mg GA/g of phenols, compared to the concentrations in the polar (acetone and methanol) solvents in our study. AHMED *et al*., (2013) reported that *R. acetosella* n-butanolic fraction showed the highest total phenolic content (252.19 mg GA/g) from root, while the chloroform fraction showed the lowest (34.44 mg GA/g) from root. Second highest phenolic content was in ethyl acetate fraction (230.71 mg GA/g) from root followed by residual aqueous (94.07 mg GA/g) from root. The results of the research coincide with the scientific claim that the content of phenolic compounds in polar solvents is higher, because phenols contain polar OH groups (MILIAUSKAS *et al*., 2004). The successfulness of the used solvents varies depending form the plant part. From the obtained results we infer that the acetone proved effective when it comes to the extracts of root, whereas the methanol was found as the most adequate solvent of the extracts of aboveground part.

Table 6. The total amount of phenols determined in different *Rumex acetosella* extracts presented as equivalents of gallic acid, mg of GA/g extract.

Extract	Methanol	Acetone	Ethyl acetate
Root	211.33 ± 1.73^{a}	248.88 ± 3.57^{b}	132.88 ± 4.41^{c}
Aboveground part	178.22 ± 1.27^{a}	165.83 ± 5.78^{a}	115.22 ± 3.43^{b}

Values are mean ± standard deviation of triplicate analyses. Values within the row followed by the same letter (a, b, c), are not significantly different according to Tukey's test ($p<0.05$).

Total contents flavonoids of the extracts

Results of the total amount of flavonoids in different plant parts (root and abovegro-und parts) of the analysed *R. acetosella* are shown in Tab. 7, as an average value and standard deviation calculated, based on measurements in three samples. The values obtained are expressed as rutin equivalent (the standard curve equation: $y= 14.78x+0.027$, $r^2= 0.995$), mg of RU/g of extract. The highest concentration of flavonoids was extracted from the herb, with polar solvents - acetone (140.83 ± 0.04 mg RU/g) and methanol (118.23 ± 2.68 mg RU/g), while the lowest values were measured in the root extract obtained with non-polar solvent (ethyl acetate) (16.61 ± 0.39 mg RU/g). Differences in flavonoid content among extracts made by different solvent were significant ($p < 0.05$). FEDURAEV *et al.* (2022) reported that *R. acetosella* herb extract made with 70% ethanol contains phenols in the concentration of 106 ± 4 mg RU/g, which is lower than the amount we extracted with polar solvents (acetone and methanol). SVIRČEV (2014) reported the flavonoid concentration in the herb extract made with polar solvent (80% ethanol) 98.77 mg RU/g, which is, again, lower concentration compared to the polar solvents (acetone and methanol) in our study. SARIKURKCU *et al*. (2017) reported the following results: herb ethanol extracts had the flavonoids concentration 70.48 ± 1.79 mg

RU/g, again, a lower concentration compared to the polar solvents (acetone and methanol) in our study. Similar in the study by ÖZEN (2010), the flavonoids content in the polar solvent (water) is much lower (51.6 ± 1.2 mg RU/g) compared to the concentrations in the polar solvents (acetone and methanol) in our study. The successfulness of the used solvents varies in comparison to different plant parts. From the obtained results we infer that the acetone proved effective when it comes to the extracts of root and aboveground part.

Table 7. The total amount of flavonoids determined in *Rumex acetosella* extracts presented as equivalents of rutin, mg of RU/g extract.

Extract	Methanol	Acetone	Ethyl acetate
Root	60.85 ± 0.61^{a}	81.28 ± 0.37^{a}	16.61 ± 0.39^{b}
Aboveground part	118.23 ± 2.68^{a}	140.83 ± 0.04^{b}	87.19 ± 0.54^{c}

Values are mean ± standard deviation of triplicate analyses. Values within the row followed by the same letter (a, b, c), are not significantly different according to Tukey's test ($p<0.05$).

The antioxidant activity of the extracts

The antioxidant activity is expressed in terms of IC_{50} (µg/mL) values (Tab. 8). The lower IC_{50} value reflects greater activity. The highest antioxidant activity was measured in methanol and acetone extract from aboveground part (7.27 ± 0.57; 9.77 ± 0.15 µg/mL, respectively), while the lowest capacity to neutralized DPPH radicals was measured in the root acetone extract (67.5 ± 1.14 µg/mL). Differences between antioxidant activity of extracts gained by different extraction solvents were significant ($p<0.05$). Our results show much lower IC_{50} values in methanol herb extract compared to results reported by PEREIRA *et al*. (2011). In mentioned study, IC_{50} values of methanol aboveground part extract of *R. acetosella* were 30 ± 0.0 µg/mL. In another study (ISBILIR and SAGIROGLU, 2013), the herb extracts were gained with 96% ethanol and the reported IC_{50} value was 3.67 ± 1.05 µg/mL, which is a much higer value in comparision to the results gathered by using polar solvents (acetone (7.27 ± 0.57 µg/mL) and methanol (9.77 ± 0.15 µg/mL)) in our study. Also, FEDURAEV *et al.* (2022) report that the IC_{50} (31 ± 1 µg/mL) for the aboveground part extracts made by using 70% ethanol as solvent, which is, however, lower concentration compared to the polar solvents (acetone (7.27 ± 0.57 µg/mL) and methanol (9.77 ± 0.15 µg/mL)) in our study. AHMED *et al*., (2013) reported that *R. acetosella* aqueous, methanolic, n-butanolic, chloroform extract from root had the IC_{50} value 0.85, 1.29, 1.31, 1.38 µg/mL, respectively, which is a higher concentration compared to the polar solvents (acetone (67.5 ± 1.14 µg/mL) and methanol (24.11 ± 0.01 µg/mL)) in our study. The methanol extracts from aboveground part exhibited potent scavenging capacity against the free radical DPPH, besides commonly used solvents such as acetone and ethanol. Moreover, found lowe IC_{50} value in ethyl acetate extract from root (17.42 ± 0.53 µg/mL), primarily due to the low polarity of ethyl acetate.

Table 8. Antioxidant activity of investigated *Rumex acetosella* extracts, IC_{50} values (µg/mL).

Extract	Methanol	Acetone	Ethyl acetate
Root	24.11 ± 0.01^{a}	67.5 ± 1.14^{b}	17.42 ± 0.53^{c}
Aboveground part	7.27 ± 0.57^{a}	9.77 ± 0.15^{a}	58.3 ± 1.05^{b}

Values are mean ± standard deviation of triplicate analyses. Values within the row followed by the same letter (a, b, c), are not significantly different according to Tukey's test ($p<0.05$).

Based on these results, *R. acetosella* could be a potential medicinal plant, because of high content of secondary metabolites and antioxidant activity, but content of Cu, Cr, Pb and

Ni, which are present in the concentrations higher than phytotoxic, strongly disable this possibility.

CONCLUSION

Analysis of the content of heavy metals in plants is obvious if we plan to made any plant products for human or animal consumption. Based on the obtained results, we can conclude thar *R. acetosella* grows successfully on tailings soil at Žitkovac contaminated by heavy metals (Mn, Fe, Pb, Ni, Cr, Cu, Cd, Zn, Ca, and Mg). Soil is moderately polluted in terms of Mn, Fe, Pb, Ni, Cr, Cu, Cd, Zn, Ca, and Mg. *Rumex acetosella* accumulates Cu, Cr, Pb, Ni in higher than phytotoxic concentrations. We can conclude that individuals of the species *R. acetosella* can absorb and accumulate large amounts of Ni and Cr in the aboveground parts, Cr and Mn in the roots, and Pb in the whole plant.

Based on the obtained results, it can be concluded that analyzed *Rumex acetosella* contains a large total amount of flavonoids and phenols, which is also responsible for the registered high antioxidant activity. This study indicates that acetone is the most efficient medium for the extraction of phenols and flavonoids. The obtained results show that *R. acetosella* can be used more for medical purposes, but due to its ability to accumulate metal, only from unpolluted soil.

Further research of this plant species should be aimed to comparative studies of biological activities of phenolic and flavonoid compounds of plants from contaminated and non-contaminated sites.

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