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Phytoremediation potential of the grapevine in regard to lithium

ABSTRACT

In this paper, the phytoremediation potential of the grapevine (Vitis vinifera) cv Tamjanika in regard to lithium (Li) was investigated using chemical and statistical analysis, as well as the calculation of bioaccumulation and enrichment factors. Plant and soil material was collected from the Bor region. Based on the obtained results it can be concluded that plants of the grapevine cv Tamjanika acted as exluders of Li which may candidate this plant species as a suitable choice for phytostabilization purposes. Its application in phytoextraction could not be estimated due to the fact that detected Li concentrations in the aboveground parts could not be considered as a real bioaccumulation. However, based on Li contents in unwashed aboveground parts (especially in leaves) it was possible to detect that particular part of Li load which came from the atmosphere, i.e. from the industrial facilities of the Copper Mining and Smelting Complex Bor which may point to their biomonitoring potential.

Keywords: grapevine; ICP-OES; lithium; phytoremediation; soil.

1. INTRODUCTION

Phytoremediation exemplifies a set of innovative technologies (phytotechnologies) that uses the specific extractive and metabolic capabilities of plants in order to remediate, control or stabilize the contamination in the environment [1-5]. For instance, many specific adaptations of some plant species, which naturally grow in soils enriched by heavy metals allowed these plants to survive in hostile environments but at the same time, very conveniently, they had been used for application in phytoremediation of contaminated soils [1, 6-8]. Two main plants' strategies that were developed against toxic metal effects are exclusion and hyperaccumulation. Plants excluders are capable to maintain the metal concentrations in the aboveground parts up to a critical value, at a low level across a wide range of soil concentration. They usually prevent the uptake of toxic metals into root tissues keeping them outside the roots or take care

of rapid efflux in the case when toxic metals have entered root cells, so that excluders can be used for restriction of spreading of heavy metals in soil (phytostabilization purposes). In the oppositet to the described tactic, plants hyperaccumulators have a capability to extract metals from the soil in a large extent and translocate them into the aboveground parts where they are usually stored in specific cell organelles, vacuoles [1, 9-11]. The most successful plants may accumulate 100-1000 times the levels normally accumulated in plants, with no adverse effects to their growth [1-3, 12]. These plants represent an ideal choice for phytoremediation in and particular for phytoextraction purposes, because they provide a sufficient clean-up of polluted soils [3, 5, 13].

Many plant species have been investigated for possible use in phytoremediation [3, 12-17]. In this work, a plant species of *Vitaceae* family, the grapevine (*Vitis vinifera*), cultivar Tamjanika was examined with the aim of ascertaining its potential for lithium (Li) extraction from the soil, and eventual further translocation into the aerial parts. The extraction of this metal by plants was not so extensively investigated in the past. Kabata-

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Pendias (2011) [8] cited several studies, which aimed to detect the content of Li and its accumulation rates in plants from different families such as: Solanaceae, Leguminosae, Rosaceae, Ranunculaceae, Violaceae, Polygonaceae, Compositae, Cruciferae, Chenopodiaceae, Urticaceae, Gramineae, and Lichenes. They reported that the content of Li in mentioned families ranged from 0.02 mg/kg in Lichenes (Worlwide) and Urticaceae from New Zealand to 2.9 mg/kg in Rosaceae from Russia and 143 mg/kg in Leguminosae from New Zealand. However, some members of Solanaceae family when grown in an aridic climatic zone can accumulate more than 1000 mg/kg Li (USA) [8]. For plants of the Rosaceae family the calculated value for Li accumulation rate was 0.6, while for plants of the Polygonaceae family, it was 0.04. The highest value, 0.8, was calculated for plants of the Solanaceae family, for which is known to have the highest tolerance to Li [8]. Some members of this family may be applied in phytoremediation of polluted sites, since industrial and consumer use of Li in batteries, drugs, and alloys has increased dramatically over the past decade [18].

The experiment of the present work was conducted as a field study, at the territory of the Bor's municipality, which is known as one of the most polluted areas in Serbia. More than 100 years of the mining-smelting activities of RTB Bor (The Copper Mining and Smelting Complex Bor) has had a strong negative impact on the ecosystem of Bor and its surroundings. Waste gases emitted from the copper smelter as the main source of pollution contain high concentrations of SO₂ and particulate matter with heavy metal(oid)s such as: Cu, As, Zn, Cd, and Pb [2, 19, 20]. However, little is known about possible presence of Li in these gases. High concentrations of mentioned heavy metals have been already detected in the grapevine (Vitis vinifera), cultivar Tamjanika from the investigated region [19] but in parallel with their analysis, the evaluation of Li concentrations was also performed. From this point of view, this study will also represent a very first insight into the status of the environment of the Bor region regarding metal such as Li.

2. EXPERIMENTAL PART

2.1. Description of the Sampling Area

Geographic coordinates of the town of Bor are 44°25'N latitude and 22°06'E longitude. The town of Bor is surrounded by mountains: Crni Vrh, Stol, and Veliki Krs. It is under the influence of

moderately continental climate with the annual average temperature of 10.2°C, and the annual average rainfall of 688 mm/m². The air humidity is 76%. The dominant winds in this region are: the northwest (NW), the west-northwest (WNW) and the west (W). They distribute contaminants from the industrial facilities to the town of Bor and its surrounding areas.

In this work, the concentrations of Li were determined in spatial soils and plant parts of the grapevine *Vitis vinifera* cv Tamjanika from the most contaminated zones as well as from a clean zone (control zone, C) of the rural settlement Gornjane - G which is located 19 km away from the Bor town and which is naturally protected from any pollution by mountain Veliki Krš (Table 1, Fig. 1). The urban-industrial (UI) zone included 4 sampling sites: FJ - Flotacijsko jalovište, an old abandoned flotation tailings pond, BN - Bolni ko naselje, a part of the town near the city hospital, and the two suburbs: SN - Slatinsko naselje and NS - Naselje Sunce. The rural zone (R) included 3 rural settlements: O - Oštrelj, S - Slatina and D - Dubašnica.

 Table 1 - Positions of the sampling sites in regard to the copper smelter

Sampling site	Zone	Distance (km)	Wind direction
Flotacijsko Jalovište (FJ)	UI	0.7	WNW, NW
Bolni ko naselje (BN)	UI	1.7	E, ESE
Slatinsko naselje (SN)	UI	2.3	WNW, NW
Naselje Sunce (NS)	UI	2.5	E, ENE
Oštrelj (O)	R	4	W, WNW
Slatina (S)	R	7	NW, WNW
Dubašnica (D)	R	17	E, ESE
Gornjane (G)	С	19	S

The sites where soil- and plant-material was collected are selected according to the following: the presence of investigated plant species, the position of the industrial facilities, type of the settlement, and the meteorological and topographic parameters.

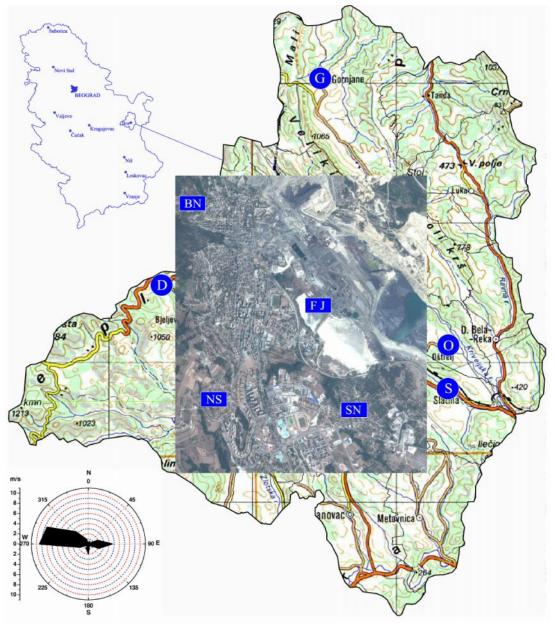


Figure 1 - Map of the study area with labels of measuring sites and wind rose diagram (%) for the year of sampling

2.2. Sample Collection, Preparation and Analysis

Plant material and spatial soil samples of *Vitis vinifera* cv Tamjanika were collected in autumn 2012 and analyzed as it was described in Alagi et al. (2015) [19]: Collected root samples were washed with tap water followed by distilled water, whereas aboveground plant parts remained unwashed with the aim of assessing the level of possible atmospheric pollution. The samples of soil were taken from the topsoil, from which root samples were taken, too. All samples were airdried to a constant weight during a period of several weeks. The dried plant samples were milled in a laboratory mill, whereas soil samples were sieve.

Prepared samples were mineralized with the nitric acid (65% HNO₃, Merck, Darmstadt) in combination with hydrogen peroxide (10% H₂O₂, Merck, Darmstadt), according to a microwave assisted strong acid digestion method for complex matrices recommended by United States Environmental Protection Agency known as USEPA method 3052. The digestion was performed in a microwave digestion system ETHOS 1 (Milestone, Bergamo, Italy).

iCAP 6000 inductively coupled plasma optical emission spectrometer (Thermo Scientific, Cambridge, United Kingdom) was used for determination of Li content. The chosen wavelength for Li, based upon tables of known interferences, baseline shifts and the background correction which was manually selected for the quantitative measurements was 670.784 nm. The limit of detection (LOD), limit of quantification (LOQ) and correlation coefficient (r) were as follows: 0.000064 mg/L, 0.000213 mg/L and 0.99706, respectively. The multi-element standard solution (Ultra scientific, USA) of about 20.00±0.10 mg/L was used for calibration.

Several soil parameters that may influence metal uptake by plants such as soil pH, electrical conductivity (EC) and organic matter (OM) (Alloway 2013; Kabata-Pendias 2011) were measured using a pH meter (3510 Jenway, UK) and an EC meter (4510 Jenway, UK), respectively; OM was determined by LOI (loss-on-ignition) method at 550°C.

2.3. Data Analysis

Li accumulation rates in the roots of the investigated plant as well as its translocation were estimated using two biological factors as follows:

- Biological Concentration Factor (BCF), is calculated as the ratio of metal concentration in plant roots to its concentration in soil: BCF=Croot/Csoil. The values of BCF>1 point to a good accumulation of metal in roots, whereas BCF<1 characterize exclusion of metals (plant excluder).
- Translocation Factor (TF) is calculated as a ratio of heavy metal in the aboveground plant part (stem or leaves) to that in plant root: TF=Cabove ground plant part/Croot; the values of TF>1 point to a good translocation from the root to shoot [2, 19].

For processing grapevine samples from contaminated zones, i.e. to detect the degree of

eventual anthropogenic influence in regard to Li, the enrichment factor (EF) is used. The element enrichment factor is calculated as: EF=Cpolluted/ Ccontrol, where Cpolluted and Ccontrol are the metal concentrations in grapevine organs (root, stem, or leaves) from the contaminated sampling site and the control site, respectively. Element enrichment factors are usually evaluated using local background values. Values of EF>2, point to the enriched samples [2, 19]. As the EF values increase, the contribution of the anthropogenic origin also increases.

Additionally, a ratio of concentrations between aboveground plant parts (R) was estimated using: R=Cleaves/Cstem. Values of R>1 indicate pollution via atmosphere [20].

To investigate the relationships between parameters that were measured in this work such as: metal contents in soil and plant samples, soil parameters (pH, EC, and OM), and distance from the copper smelter, the Pearson's correlation study was performed [21], using IBM SPSS statistic 20 software (USA).

3. RESULTS AND DISCUSSION

ICP-OES analysis of soil samples revealed that Li concentrations ranged from 12.6 mg/kg at the site SN to 51.1 mg/kg at the site G from the control zone, which is in the range of common abundance of Li in different surface soils, except histosols (Table 2). This result indicated that, in the Bor region, there are no elevated concentrations of Li, which was additionally supported by very low values of EFs calculated for soils from polluted locations (Table 3).

Sampling site	Li soil	Li root	Li stem	Li leaves	рН	EC (µS/cm)	OM (%)
FJ	22.9±0.3	0.395±0.005	0.245±0.002	1.380±0.005	8.02	166	7.6
BN	15.21±0.09	4.305±0.004	0.655±0.005	1.99±0.02	7.35	170.6	10.4
NS	23.03±0.05	0.275±0.005	0.31±0.01	1.08±0.02	7.23	152.2	12.2
SN	12.6±0.2	1.390±0.005	1.58±0.02	1.12±0.02	6.76	115.6	9.8
0	22.7±0.2	1.68±0.03	1.6±0.2	3.110±0.005	7.45	146.9	9.2
S	50.7±0.7	1.76±0.01	1.08±0.02	1.38±0.01	7.46	151.7	10.4
D	15.4±0.2	0.48±0.02	0.345±0.005	0.345±0.005	6.79	90.3	10.4
G	51.1±0.9	14.8±0.3	0.64±0.01	0.54±0.01	7.25	100.8	5.8
Literature data for different plant tissues ^a		0.2 ^b		6.6 ^c ; 6.2 ^d ; 0.5 ^e ; 0.3 ^f			
Soil literature data (common abundance in topsoils) ^a	$\begin{array}{c} 5-70 \ (22)^{9**} \\ 2-130 \ (46)^h \\ 9-175 \ (53)^i \\ 6-105 \ (56)^j \\ 0.01-3 \ (1.3)^k \end{array}$						

Table 2 - Concentrations of the element (mg/kg DW) in soil and plant samples* and soil pH, EC and OM

* - Data are presented as the mean ± standard deviation (SD) for triplicate determinations

** - Within parentheses: arithmetic means

a - Kabata-Pendias (2011) [8], b - Carrot; c - Celery; d - Chard; e - Cabbage; f - Lettuce; g - Arenosols (sandy);

h – Podzols (medium loamy); i – Cambisols (heavy loamy); j – Calcisols (calcareous); k - Histosols (organic)

Table 3 - Element enrichm	ent factor	(EF) and ratio
of concentrations	between	aboveground
plant parts (R)		

Sampling		R			
site	Soil	Root	Stem	Leaves	n
FJ	0.45	0.03	0.38	2.58	5.63
BN	0.30	0.29	1.02	3.72	3.04
NS	0.45	0.02	0.48	2.01	3.47
SN	0.25	0.09	2.46	2.08	0.70
0	0.44	0.11	2.48	5.81	1.94
S	0.99	0.12	1.68	2.59	1.28
D	0.30	0.03	0.53	0.64	1.00
G					0.83

EF=Cpolluted/Control; R=Cleaves/Cstem

The results obtained from chemical analysis of plant parts (Table 2) showed that Li concentrations were in the range of concentrations normally found in plants and much lower than concentrations in plants from the Solanaceae family (1000 mg/kg) that have the highest tolerance to Li [8]. The highest Li concentration in grapevine from the Bor region was found in root samples from the site G (C zone): 14.8 mg/kg, while the lowest one was present in stems from the site FJ (UI zone): 0.245 mg/kg. Calculated EF values for plant parts from polluted sites (Table 3) was not in accordance with EF values obtained for corresponding soils, except in the case of root EFs. Namely, soil and root EFs didn't point to any enrichment by Li, whereas some EF values for stem and leaves pointed to elevated presence of Li at investigated locations such as: FJ (EFleaves=2.58),ΒN (EFleaves=3.72),NS (Efleaves = 2.01), SN (EFleaves=2.08; Efstem = 2.46), O (EFleaves=5.81 EFstem=2.48) and S (Efleaves = 2.59). It is possible that aboveground parts of the grapevine cv Tamjanika were capable to "record" even a slight pollution that came from the atmosphere, i.e. the copper smelter which may recommend these plant parts as suitable biomonitors in regard to Li. In addition, the calculated ratio, R: Cleaves/Cstem (Table 3), for different locations confirmed presence of atmospheric pollution. However, the recorded pollution has a limitation effect on further steps in planed estimation of grapevine potential to Li extraction from the soil and additional translocation into the aboveground parts. More precisely, it can be said that detected concentrations in unwashed stems and leaves cannot represent a real bioaccumulation and also that calculated values for stem and leaf TF (Table 4) do not represent a true reflection of grapevine capability to transport assimilated Li from roots to the shoot. However, since root samples were washed before the analysis by ICP-OES, the calculated BCF values can be considered as a good tool in the estimation of the potential of the grapevine to Li uptake from soil to the root. Based on very low, almost insignificant values of BCFs for all investigated locations (Table 4), it can be concluded that the grapevine cv Tamjanika has a very low affinity to soil Li in the circumstances where the soil Li concentrations are within the common ranges. Remain unknown the behavior under severe Li contamination.

Table 4 - Biological Concentration Factor (BCF) and Translocation Factor (TF)

Sampling site	BCF TF (stem)		TF (leaves)		
FJ	0.017	0.620	3.494		
BN	0.283	0.152	0.462		
NS	0.012	1.127	3.909		
SN	0.110	1.140	0.802		
0	0.074	0.952	1.851		
S	0.035	0.615	0.785		
D	0.031	0.726	0.726		
G	0.290	0.044	0.036		

BCF=Croot/Csoil; TF=Caboveground part (stem or leaves)/Croot

The results obtained from the Pearson's correlation study (Table 5) revealed that the best uptake of Li was realized at the sites with the lowest OM content, as it can be seen from very strong negative correlation between root Li concentrations and soil OM: -0.717 (significant at the 0.05 level). Soil pH and EC were in very low correlations with Li contents in roots. Root contents were in a good positive correlation with soil contents (0.604) but the confidence of this correlation is not at the significant level. The results from the Pearson's correlation study confirmed that BCF values correlated positively and very significantly withroot Li contents (0.809). The obtained results also showed a negative correlation between Li contents in leaves and distance from the copper smelter (-0.599); although not significant at the level of high confidence, this correlation may support the findings obtained from calculated EFs and Rs, i.e. it supports the conclusion that slightly elevated concentrations of Li in the unwashed grapevine leaves may originate from the present industrial activities.

	Pearson's correlation coefficients								
	Distance	рН	EC	ОМ	Lisoil	Liroot	Listem	Lileaves	BCF
Lisoil	0.458	0.284	-0.089	-0.445	1				
Liroot	0.609	-0.032	-0.364	-0.717 [*]	0.604	1			
Listem	-0.207	-0.251	-0.053	0.002	-0.008	-0.041	1		
Lileaves	-0.599	0.419	0.635	0.110	-0.190	-0.254	0.546	1	
BCF	0.274	-0.116	-0.107	-0.455	0.187	0.809 [*]	0.073	0.004	1

Table 5 - Correlation	coefficient	matrix for the	e investigated	variables

*- Correlation is significant at the 0.05 level (2-tailed) and is given in bold

4. CONCLUSIONS

Based on the results obtained from chemical and Pearson's correlation analysis, as well as from calculated BCFs, it can be concluded that plants of the grapevine cv Tamjanika from the Bor region acted as exluders of Li (BCF<1) which may candidate this plant species as suitable for phytostabilization purposes. Its application in phytoextraction could not be estimated due to the fact that detected Li concentrations in the aboveground parts could not be considered as a real bioaccumulation. However, based on Li contents in unwashed aboveground parts (especially in leaves) it was possible to detect a specific part of Li load, which came from the atmosphere, i.e. from the industrial facilities of RTB Bor, which may point to possible utilization of these parts in biomonitoring procedures.

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IZVOD

FITOREMEDIJACIONI POTENCIJAL VINOVE LOZE U ODNOSU NA LITIJUM

U ovom radu, ispitivan je fitoremedijacioni potencijal vinove loze (Vitis vinifera) cv Tamjanika u odnosu na litijum (Li), koriste i hemijsku i statisti ku analizu, kao i izra unavanje faktora bioakumulacije i oboga enja. Biljni i zemljišni materijal bio je sakupljen u regionu Bora. Bazirano na dobijenim rezultatima, može se zaklju iti da su se biljke vinove loze ponašale kao ekskluderi litijuma, što može kandidovati ovu biljnu vrstu kao pogodnu za svrhe fitostabilizacije. Njena primena u fitoekstrakciji nije se mogla proceniti, jer se detektovane koncentracije Li u nadzemnim delovima nisu mogle smatrati pravom bioakumulacijom. Me utim, na bazi sadržaja Li u neopranim nadzemnim delovima (a posebno u liš u), bilo je mogu e detektovati upravo onaj sadržaj Li koji je došao iz atmosfere, tj. iz industrijskih postrojenja rudarsko-topioni arskog kompleksa Bor, što može da ukaže na njihov biomonitoring-potencijal.

Klju ne re i: vinova loza; ICP-OES; litijum; fitoremedijacija; zemljište.

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