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The accumulation of metals in *Onobrychis viciifolia* Scop., their distribution in plant parts and the impact on the content of phenols and flavonoids, as well as antioxidant and genotoxic activity

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Abstract: This research investigated the potentially toxic elements (PTE) content and biological activities of *Onobrychis viciifolia* Scop. collected from a tailing site and an uncontaminated locality. Concentrations of Mn, Ni, Ca, Mg, Fe, Zn, Cr, Pb, and Cu were determined in soil and plant parts (roots, aerial parts, and inflorescences) using the wet digestion method, followed by atomic absorption spectrophotometric analysis. Bioaccumulation (*BCF*) and translocation (*TF*) factors were calculated to assess PTE uptake and distribution. Total phenolic and flavonoid contents were determined spectrophotometrically. Antioxidant activity was evaluated using the DPPH radical scavenging assay. DNA damage in human lymphocytes treated with plant extracts was assessed using the comet assay. Results revealed significantly higher concentrations of PTE in soil and plant material from the contaminated site compared to the uncontaminated locality. Plants from the contaminated soil exhibited increased bioaccumulation and translocation of PTE. Moreover, the biological activity of the extracts, including antioxidant capacity and genotoxic effects, was influenced by the exposure of plants to PTE, which affected the synthesis of secondary metabolites. Extracts from plants growing in tailing site showed stronger activity compared to those from the uncontaminated locality. This study highlights the adaptive responses of *O. viciifolia* to PTE-induced stress and provides insights into its potential applications in environmental monitoring and phytopharmacology.

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INTRODUCTION

The genus *Onobrychis* Mill. comprises about 170 species with a cosmopolitan distribution.[1] *Onobrychis viciifolia* is a perennial forage plant which characterised a high nutritional value and good quality of coarse fodder, and it is very adaptable to different climatic and soil conditions. Despite its production-quality properties, its occurrence and use in the agroecological conditions of Serbia is limited and it is mainly known as a honey-bearing species.[2] *O. viciifolia* shows tolerance to high concentrations of potentially toxic elements (PTE) in the soil and can thrive under such conditions.[3] For environmental protection, it is crucial to understand how PTE concentrations in soil can be controlled to remain below the toxicity threshold for plants.[4] Plant tolerance to PTE by exclusion involves two modes of action: preventing the uptake of metals and metalloids by limiting their passage from the soil to the root, or accumulating PTE in the root while preventing their transport to the aerial parts of the plant.[5] The uptake of PTE into the plant parts depends on the movement of PTE from the soil to the plant roots (bioconcentration factor), as well as on the transport of the elements (translocation factor) from the roots, whereby they are transported to the aerial part and inflorescences.[6] According to the literature, *O. viciifolia* has antimicrobial,[7] antibacterial,[8] anti-inflammatory,[9] antioxidant[10] and many other activities.

In the present study, the biological activities (genotoxic and antioxidant) and the total phenolic content (TPC) and total flavonoid content (TFC) were evaluated in methanolic extracts obtained from the root, inflorescence, and aerial parts of *O. viciifolia*. In addition, the concentrations of PTE (Mn, Ni, Ca, Mg, Fe, Zn, Cr, Pb, and Cu) were determined in plant tissues and in the soils collected from two locations: an uncontaminated site (Gornji Milanovac) and a tailing site (Žitkovac, Kosovska Mitrovica former Pb–Zn mining tailings).

EXPERIMENTAL

Chemicals

Gallic acid, rutin hydrate and 2,2-diphenyl-1-picrylhydrazyl (DPPH), Histopaque-1077, Roswell Park Memorial Institute (RPMI) medium, phosphate-buffered saline (PBS), Tris-HCl buffer and ethidium bromide reagent were bought from Sigma Chemicals Co. Folin–Ciocalteu phenol reagent and aluminium chloride hexahydrate were obtained from Fluka Chemie AG, Buchs, Switzerland. Low-melting-point agarose was purchased from AppliChem GmbH (Darmstadt, Germany). Triton X-100 was obtained from Fisher Scientific (Geel, Belgium). Sodium chloride, ethylenediaminetetraacetic acid (EDTA) and dimethyl sulfoxide (DMSO) were purchased from Centrohem (Stara Pazova, Serbia). Electrophoresis equipment (PowerPac Basic) was supplied by Bio-Rad (Hercules, CA, USA).

Plant material

The plants in the flowering phase and soil materials were collected from tailings at Žitkovac (tailing site), 4 km away from Kosovska Mitrovica (42.924052 N; 20.830233 E) in 2018 on 20 June and from an uncontaminated locality (Gornji Milanovac, Brusnica village, 44.002512 N; 20.432371 E) in 2020 on 30 June. Voucher specimen of *O. viciifolia* deposited in the Herbarium of the University of Kragujevac, Faculty of Science, Department of Biology and Ecology, No. 31/018.

Analysis of potentially toxic elements in samples of plant and soil

The content of PTE (manganese, nickel, calcium, magnesium, iron , zinc, chromium, lead and copper) was determined in the soil and in each plant part of *O. viciifolia* collected from both localities. Metals were analysed using the wet digestion method according to Tóth *et al.*[11], in the Laboratory for Analytical Chemistry, Faculty of Science, University of Kragujevac. For each analysis, five replicate samples (soil, roots, aerial part and inflorescence) were prepared, and the average value and standard deviation were calculated. The measurements of PTE content were carried out using an atomic absorption spectrophotometer (Perkin Elmer 3300). After the results were obtained, the bioaccumulation (BCF) and translocation factors (TF) were determined.[12] The BCF was used to determine the PTE accumulation from the soil to the roots, and the TF was used to calculate the ratio of PTE concentrations in the aerial part, inflorescence and roots.[13]

Root, aerial part and inflorescence were air-dried and 50 g of ground material from each plant part was extracted with 250 mL of methanol. After 24 h, the plant material was filtered, made up of 250 mL methanol and the extraction was repeated three times. After the extraction was completed, the extracts were evaporated in a vacuum evaporator (Soxtherm S 306, Gerhardt, Germany). The dry extract was stored at –18 °C until it was used in the tests. For testing purposes, the extract was dissolved in PBS.

Determination of total phenolic content of the extracts

The concentration of TPC in the sample's methanol solutions was determined using the spectrophotometric method (at λmax = 725 nm) by Wootton-Beard *et al.*[14] The reactant blend was made from methanolic dilutions (roots, aerial part and inflorescences), and Folin–Ciocalteu reagent and then incubated. After that, sodium carbonate was added to the mixture. Gallic acid (GA) was used to create the standard curve, which showed a linear regression $r^2 > 0.99$. The TPC is expressed in gallic acid equivalents per gram of plant extract.

Determination of flavonoid content of the extracts

The concentration of flavonoids in the methanol solutions of the samples was determined according to the spectrophotometric method (at λmax = 430 nm) of Quettier-Deleu *et al.*[15] The reagent mixture was prepared from methanolic dilutions (roots, aerial part and inflorescences), and aluminum chloride, after which they were incubated. Rutin (RU) was used to create the standard curve, which showed a linear regression $r^2 > 0.99$. The flavonoid content is expressed in rutin equivalents per gram of plant extract.

Determination of the antioxidant activity of the extracts

The free radical scavenging activity in the sample's methanol solutions was determined using the spectrophotometric method (at λmax = 517 nm) by Takao *et al.*[16] First, a dilution series of methanol solution samples was made, with concentrations of 15.62, 31.25, 62.5, 125, 250 and 500 μg/mL. The reagent mixture was prepared from diluted solutions (roots, aerial part

and inflorescences) and DPPH, which were then incubated. Ascorbic acid was used as a standard. The antioxidant activity is expressed as a concentration that inhibits 50 % of the activity (IC_{50} values in μg/mL).

Measurement of DNA damage

The whole blood was taken by venipuncture from three donors, non-smokers, priorly unexposed to any known genotoxic agents. The study was approved by the Ethics Committee of the Clinical Centre of Kragujevac (01/18/4927), before the start of the study. Written informed consent was obtained from all the patients according to the guidelines of the World Medical Association (Declaration of Helsinki).

To assess the level of DNA damage of peripheral blood lymphocytes in the treatment with different parts *O. viciifolia* plant, single-cell gel electrophoresis (comet assay) was used as described by Singh *et al.*[17] with some modifications given by Collins *et al*. [18] Lymphocytes were isolated from the whole peripheral blood using Histopague-1077 and treated for 30 min at 37 °C in phosphate buffered saline solution with methanolic extract of *O. viciifolia* root, inflorescence or aerial part at concentrations of 125, 250, 500 and 1000 μg/mL. Untreated lymphocytes were used as a negative control, while a positive control was lymphocytes treated with hydrogen peroxide at a final concentration of 10 μg/mL. The cell suspension was mixed with 1 % low-melting-point agarose for slide embedding. The slides were kept on ice for a few seconds to solidify the agarose. The coverslips were then removed and the slides were placed in a lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1 % Triton X-100 and 10 % dimethyl sulfoxide) for 2 h in the dark at 4 °C.

The alkaline denaturation was performed in an electrophoresis buffer solution (10 M NaOH, 200 mM EDTA, pH > 13) in a horizontal position and the slides were electrophoresed for 30 min at 25 V and 300 mA. The slides were washed in neutralizing Tris-HCl buffer (0.4 M Tris, pH 7.5) and stained with 50 μL ethidium bromide (20 μg/mL). One hundred randomly selected cells from each slide (50 cells from each of the two gel replicates) were visually analysed using a Nikon E50i fluorescence microscope at 400× magnification. Each cell was classified into five classes, from 0 (undamaged class cells) to 4 (the most severely damaged one).

The level of DNA damage, expressed as genetic damage index (*GDI*), was calculated for all 100 cells using the Eq. (1) described by Pitarque *et al*.[19] The level of DNA damage, expressed as the *GDI*, is:

$$GDI = \frac{Class1 + 2 \times Class2 + 3 \times Class3 + 4 \times Class4}{Class0 + Class1 + Class2 + Class3 + Class4} \quad (1)$$

Data analysis

The statistical analyses were performed using the SPSS package (IBM SPSS Statistics 21). The results are expressed as mean ± standard deviation (*SD*). *GDI* was evaluated by one-way analysis of variance (ANOVA), with a Tukey post hoc test for comparisons of different treatments v*ersus* negative controls. The relationship between the tested extract concentrations and the *GDI* was determined using the Pearson correlation coefficient. A statistically significant difference in *GDI* between the uncontaminated locality and tailing site was determined by Student's *t*-test. Levels of significance were $p < 0.05$, $p < 0.01$ and $p < 0.001$. To determine the existence of differences between PTE in the soil by localities, PTE, TPC, TFC and antioxidant activity, in plants from different localities, and differences between localities by plants, Tukey's tests were used ($p < 0.05$). In addition, a multivariate statistical method (PCA) was used to investigate the relationship between chemical elements in plant parts and different localities.

RESULTS AND DISCUSSION

Based on the concentrations of PTE in the soil at the tailing site, the analysed PTE (Table I) can be ranked as follows: Fe > Mg > Ca > Mn > Pb > Cr > Ni > Zn > Cu. In contrast, the ranking in uncontaminated soil is as follows: Fe > Ca > Mg > Mn > Ni > Cr > Zn > Pb > Cu.

TABLE I. The content of potentially toxic elements (PTE) in the examined soil samples (mg/kg); different uppercase letter (A, B, C,...) within a column indicates significant differences between PTE at the same locality ($p < 0.05$, ANOVA with Tukey's test). Different lowercase letters (a, b) within a row indicate significant differences in the content of the same PTE between the two localities ($p < 0.05$, ANOVA with Tukey's test)

PTE	Tailing site	Uncontaminated locality
Mn	2685.58±16.0 Aa	676.24±5.8 Ab
Ni	199±0.97 Ba	38.74±0.56 Bb
Ca	3649.76±31.20 Ca	4837.16±32.95 Cb
Mg	4540.54±23.4 Da	2236.86±36.41 Db
Fe	77363.08±682.37 Ea	24332.84±19.58 Eb
Zn	176.24±0.78 Fa	25.48±0.42 Fb
Cr	453.36±1.36 Ga	27.64±0.14 Gb
Pb	873.66±2.057 Ha	11.38±0.07 Hb
Cu	113.88±0.8 Ia	10.44±0.28 Ib

All metals (with the exception of Ca) have significantly higher concentrations at the tailing site. The most abundant PTE at the tailing site is Fe, with a concentration 3.18 times greater than in the uncontaminated soil. Also, an exceptionally high Pb concentration was observed at the tailing site, where it is 76.7 times higher than at an uncontaminated locality. For Ca and Mg, the differences between the two sites are smaller. PTE such as Pb, Cr and Zn exhibit particularly high disparities between the sites.

A comparative analysis of the PTE content in different plant parts from a tailing site and an uncontaminated site is shown in Table II. At the tailing site, the order of PTE concentration in the roots was: Ca > Mg > Fe > Ni > Mn > Zn > Cu > Cr > Pb. In the aerial parts, Ca, Mg, Cr, Fe and Zn were predominant, while in the inflorescences, Ca was dominant, followed by Mg, Zn and Fe. A particularly high Pb concentration was observed at the contaminated site, where the levels in the aerial parts (17.6 mg/kg) and roots (6.16 mg/kg) exceeded the permissible limits, ranging from 2 to 10 mg/kg, according to the regulations of the FAO/ /WHO.[20] Additionally, the Cr content in the aerial parts from the tailing area was significantly above the permissible levels (132.88 mg/kg compared to the allowed range of 5–20 mg/kg). At the uncontaminated site, the most abundant elements in the roots were Ca, Fe and Mg (Ca > Mg >Fe > Mn > Zn > Ni > Cu > Cr > Pb). The aerial parts primarily contained Ca, Mg, and Fe, while the inflorescences followed a similar pattern, with Ca and Mg being dominant. The concentrations of all PTE

at uncontaminated locality were within or below the recommended safety levels, while Pb was below the detection limit. Mn and Cr showed higher concentrations in the roots of the plants from the uncontaminated site compared to the tailing area.

TABLE II. The content of investigated potentially toxic elements (mg/kg) in root, aerial part and inflorescence of *O. viciifolia*; different uppercase letter (A, B, C,…) within a row indicates significant differences between plant parts (root, aerial part, inflorescence) at the same locality ($p < 0.05$, ANOVA with Tukey's test). Different lowercase letters (a, b) within a column indicate significant differences between the two localities (tailing site *vs.* uncontaminated) for the same plant organ ($p < 0.05$, ANOVA with Tukey's test); *n.d. = not detected

PTE	Tailing site			Uncontaminated		
	Root	Aerial part	Inflorescence	Root	Aerial part	Inflorescence
Mn	27.36 ± 1.28^{Ad}	81.92 ± 0.51^{Bc}	77.18 ± 0.29^{Bb}	34.52 ± 0.56^{Aa}	23.94 ± 0.63^{Ba}	23.38 ± 0.49^{b}
Ni	49.4 ± 0.28^{Ad}	12.42 ± 0.33^{Bd}	30.58 ± 0.42^{Cd}	8.67 ± 0.45^{Aa}	9.82 ± 0.14^{Ba}	13.56 ± 0.07^{b}
Ca	11892 ± 62.25^{Aa}	14772 ± 42.99^{Ba}	13856 ± 44.61^{Ba}	7802 ± 21.49^{Aa}	8142 ± 34.08^{Ba}	6258 ± 3.81
Mg	4161 ± 35.21^{Ab}	$6\,087\pm27.77^{Ba}$	7177 ± 37.56^{Ca}	2386 ± 9.26^{Aa}	2229 ± 24.53^{Ba}	1851 ± 38.18^{a}
Fe	447.36 ± 1.44^{Ac}	334.88 ± 38.77^{Bb}	304.82 ± 1.01^{Ca}	1399 ± 30.82^{Aa}	87.98 ± 3.81^{Ba}	424.1 ± 0.28^{a}
Zn	22.22 ± 0.23^{Ad}	41.28 ± 0.41^{Bc}	61.24 ± 0.36^{Cc}	18.56 ± 0.35^{Aa}	17.04 ± 0.56^{Ba}	35.78 ± 0.35^{b}
Cr	7.45 ± 0.04^{Ad}	132.88 ± 0.67^{Bc}	16.54 ± 0.34^{Cd}	9.86 ± 0.09^{Aa}	2.23 ± 0.05^{Ba}	2.28 ± 0.01^{b}
Pb	6.16 ± 0.03^{Ad}	17.6 ± 0.34^{Bd}	5.54 ± 0.03^{Ce}	*n.d.	*n.d.	*n.d.
Cu	9.86 ± 0.03^{Ad}	17.22 ± 0.31^{Bd}	5.30 ± 0.02^{Ce}	7.49 ± 0.15^{Aa}	3.87 ± 0.07^{Ba}	24.54 ± 0.08^{b}

Elevated concentrations of PTE, primarily Cd and Pb, in all plant parts collected from the tailing site compared to the uncontaminated locality, indicate a strong anthropogenic influence and long-term contamination of the soil with toxic metal compounds.[12,21] Furthermore, essential metals such as Ca, Mg and Fe, were present at both sites, they showed significant differences in concentration, which could be linked to alterations in the plant's biochemical regulation under PTE stress.[22,23]

The presentation of the principal component analysis (PCA) in plant parts and localities is presented in Fig. S-1 of the Supplementary material to this paper. The two main components (factor 1 and factor 2) for plant samples are represented with a variation of 69.02 %. The first two factors clearly show the differences in the amount of elements tailing site and the uncontaminated locality around the parts of the plant where PTE accumulate. Elements, such as Mn, Ni, Ca, Mg, Fe, Zn, Cr, Pb and Cu, are included in factor 2. High amounts of the mentioned elements are characteristic of the tailing site, which are grouped on the positive side of the PCA axis and most affect the separation of the plant population. The amounts of Mn, Ni, Ca, Fe, Zn and Cr have negative values around a factor 1, for the uncontaminated locality in the plant samples.

The results of the bioaccumulation and translocation factors (*BCF* and *TF*) for PTE in plant parts of *O. viciifolia* are presented in Fig. 1. The highest *BCF* in individuals of *O. viciifolia* from the tailings site was recorded for Ca, exceeding a

value of 3.5, indicating a high capacity of the plant to accumulate this element in its roots. Moderate accumulation was observed for Mg and Fe, with *BCF* values close to or slightly above 1. Other elements (Mn, Ni, Zn, Cr, Pb, Cu) exhibited low *BCF* values, below 1, suggesting limited accumulation of these metals by the plant. For plants from the uncontaminated site, *BCF* values for all elements were less pronounced compared to the tailings site. However, the highest accumulation was still recorded for Ca, but with a slightly lower value (around 2.5). Significant translocation from the roots to the aerial parts and inflorescences was observed for Cu in the plants collected from the tailing site, with *TF* values above 3. A higher *TF* value was also observed for Zn. In the plants of the uncontaminated site, the highest *TF* was also observed for Cu, albeit with lower values compared to the tailings site. The translocation of Ca and Mg from the roots to the aerial parts and inflorescences was more moderate compared to the tailings site.

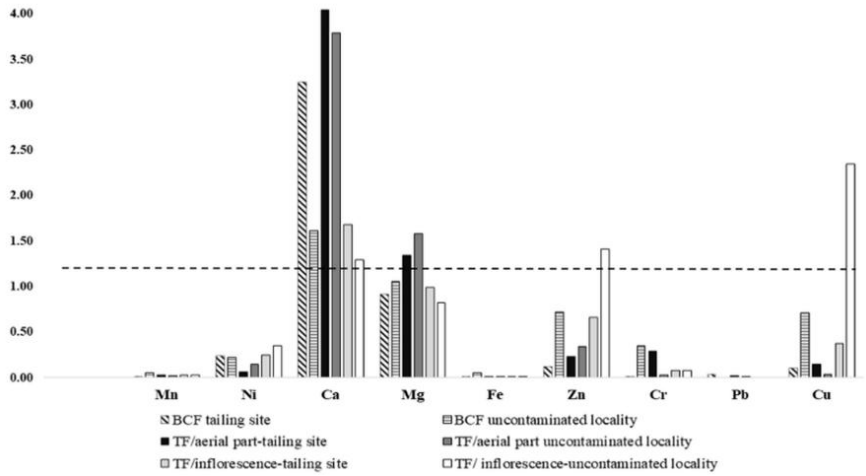

Fig. 1. Bioconcentration factor (B*CF*) and translocation factor (*TF*) of potentially toxic elements in *O. viciifolia*, which grows on the tailing site and an uncontaminated locality.

High *BCF* and *TF* factors at the tailing site indicate effective mechanisms for the uptake and transport of PTE from the roots to the aerial parts, aligning with previous studies suggesting that plants from contaminated sites adaptively enhance metal accumulation.[24] For example, a high *BCF* for calcium could reflect its role in stabilising cell membranes and acting as a signaling molecule under stress conditions, while a high *TF* for copper and zinc could be associated with their roles in antioxidant enzymes and plant metabolism.

The results of *TPC* and *TFC* revealed significant differences in the accumulation of phenolic compounds between plant parts and locations, indicating adaptive responses of the plant to environmental stress conditions (Table III).

TABLE III. Total phenolic and flavonoid content in the different parts of *O. viciifolia*; different capital letters (A, B, C) indicate significant differences among plant parts within the same locality. The same lowercase letters (a, b, c) indicate significant differences between localities for the same plant part, according to ANOVA with Tukey's test ($p < 0.05$)

Locality	Root	Aerial part	Inflorescence
Total phenolic content, mg GAE/g extracts			
Tailing site	80.22 ± 2.77^{Bb}	137.83 ± 1.45^{Aa}	47.72 ± 3.71^{Cc}
Uncontaminated locality	64.88 ± 0.25^{Ba}	86.16 ± 2.96^{Ab}	55.38 ± 0.41^{Ca}
Total flavonoid content, mg RUE/g extracts			
Tailing site	10.11 ± 0.39^{Cc}	66.64 ± 1.87^{Aa}	27.52 ± 1.49^{Bb}
Uncontaminated locality	20.33 ± 0.32^{Cb}	25.41 ± 0.52^{Bc}	28.52 ± 2.18^{Aa}

At the tailing site, the *TPC* in the roots was 80.22±2.77 mg GAE/g, which was significantly higher compared to the uncontaminated locality (64.88±0.25 mg GAE/g). The aerial parts of *O. viciifolia* showed the highest *TPC* value of all plant parts analysed (137.83±1.45 mg GAE/g for the tailing site and 86.16±2.96 mg GAE/g for the uncontaminated locality). The TPC in the inflorescences was lower compared to the roots and aerial parts at both localities.

At the tailing site, the flavonoid content in the roots was 10.11±0.39 mg RUE/g, significantly lower than at the uncontaminated site (20.33±0.32 mg RUE/g). The aerial parts of the plant exhibited the most significant difference in flavonoid content between the two sites. At the tailing site, the flavonoid content in the aerial parts reached 66.64±1.87 mg RUE/g, which is significantly higher than at the uncontaminated site, where it was 25.41±0.52 mg RUE/g. The flavonoid content in the inflorescences was similar at both sites, with slightly higher values at the uncontaminated site (Table III).

The elevated levels of *TPC* and *TFC* in the plants from the tailing site indicate a robust activation response to oxidative stress induced by heavy metals. Phenolic compounds are well-known for their ability to neutralize reactive oxygen species (ROS) and thereby protect cells from oxidative damage.[25] Interestingly, flavonoids were recorded in higher concentrations in the aerial parts of plants from the tailing site, while their content in the roots was reduced. This may indicate a redistribution of secondary metabolites in response to local stress or the inhibition of flavonoid biosynthesis in the roots due to the toxic effect of metals.[26]

A comparative analysis of the flavonoid, phenol and heavy metal content in *O. viciifolia* shows the plant's complex adaptation strategies to environmental stress conditions. At the tailing site, the plant increases the synthesis of flavonoids and phenols to combat oxidative stress caused by PTE contamination. However, the high metal accumulation limits their safety of use. Plants from uncontaminated localities, with a balanced content of secondary metabolites and a low metal content, are more suitable for phytotherapy and the food industry.

Despite the use of *O. viciifolia* in traditional medicine, these results present a dual message. Increased concentrations of phenols and flavonoids may enhance the medicinal properties of the plant, including antioxidant, anti-inflammatory and antimicrobial effects.[25] However, the presence of elevated levels of PTE, especially in the aerial parts used for phytopreparations, poses a serious health risk. Additionally, the ability of *O. viciifolia* to accumulate metals could be beneficial for phytoremediation of polluted areas, but plants growing in such environments must not be used as food or medicine.

The results of antioxidant activity for the root from tailing site was 13.66±0.89 μg/mL, representing significantly better activity compared to the root from the uncontaminated locality (32.75±1.68 μg/mL). The aerial parts of the plant demonstrated moderate antioxidant activity, with significant differences between localities. The inflorescences showed the lowest antioxidant activity compared to the root and aerial parts (Table IV). At the uncontaminated locality, antioxidant activity was more balanced among different plant parts, with inflorescences showing significantly higher activity compared to the tailing site.

TABLE IV. Antioxidant activity of the different parts of *O. viciifolia* (IC_{50} / μg mL^{-1}); different capital letters (A, B, C) indicate significant differences among plant parts within the same locality. The same lowercase letters (a, b, c) indicate significant differences between localities for the same plant part, according to ANOVA with Tukey's test ($p < 0.05$)

Locality	Root 1	Aerial part	Inflorescence
Tailing site	$13.66±0.89^{Cc}$	$74.4±1.57^{Bb}$	$109.6±3.15^{Aa}$
Uncontaminated locality	$32.75±1.68^{Ca}$	$65.4±0.96^{Bb}$	$77.72±1.57^{Ab}$

The genotoxicity of plants from the Fabaceae family has been tested,[27,28] but to our knowledge, similar studies have not yet been conducted on the genus *Onobrychis*, so our results represent a novelty in this field of research. The results of analysis of DNA damage level in treatment with different parts of *O. viciifolia* extracts from uncontaminated locality and tailing site are shown in Fig. 2. All tested concentrations (125–1000 μg/mL) of extracts from root, inflorescence and aerial part of *O. viciifolia* extracts from the uncontaminated locality significantly increased the *GDI* (from 1.16±0.09 to 1.78±0.17 for the root; from 0.91±0.08 to 1.93±0.24 for the inflorescence; from 1.44±0.05 to 2.27±0.07 for the aerial part) in comparison to the negative control (ANOVA, Tukey *post hoc* test, $p < 0.05$). A strong positive correlation between concentrations of the extracts and *GDI* was obtained (Pearson: $r = 0.787$, $p = 0.001$ for the root; $r = 0.931$, $p = 0.000$ for the inflorescence; $r = 0.785$, $p = 0.001$ for the aerial part). All tested extracts of *O. viciifolia* from the tailing site significantly increased the *GDI* in all concentrations compared to the negative control (ANOVA, Tukey *post hoc* test, $p < 0.05$). The highest tested concentration of extracts (1000 μg/mL) increased the *GDI* by 8.2 times for root (2.63±0.19), 8.3 times (2.67±0.10) for inflorescence and 9.9 times

(3.16±0.04) for aerial part, in comparison to the negative control (0.32±0.08). Pearson correlation coefficient revealed a significant association between *GDI* and the tested concentrations of extracts ($r = 0.838$, $p = 0.000$ for root; $r = 0.891$, $p = 0.000$ for inflorescence; $r = 0.756$, $p = 0.001$ for aerial part). Plants from both the uncontaminated locality and tailing site showed a genotoxic effect. All extracts obtained of different plant parts from the tailing site had a significantly higher *GDI*, compared to the same plant parts extracts from the uncontaminated locality ($p < 0.05$). The highest genotoxic effect was observed in the treatment with the extract of the aerial plant part from the tailing site at all tested concentrations. The extract of the inflorescence of the plant from the uncontaminated locality exhibited the lowest *GDI* at all tested concentrations, except at the concentration of 1000 μg/mL, where an insignificantly higher *GDI* was observed for the root extract (1.93±0.24 *vs*. 1.78±0.17).

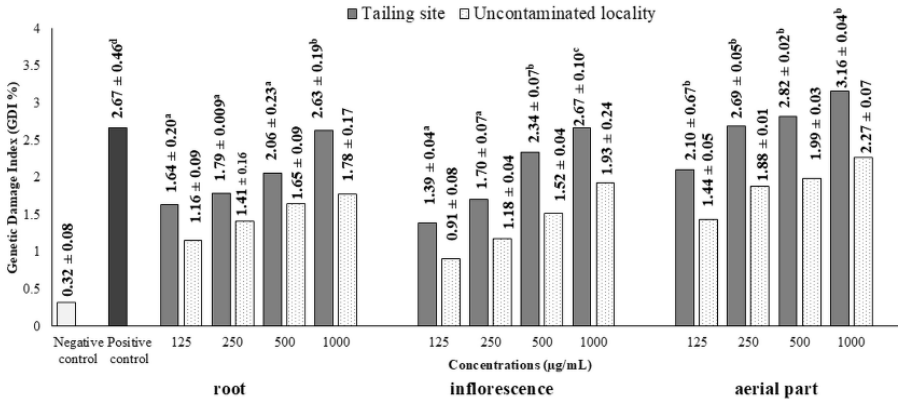

Fig. 2. Comparative results of the level of DNA damage in lymphocytes treated with methanolic *O. viciifolia* extracts from two localities. The results are expressed as mean ± *SD*, $n = 3$; $^{a}p < 0.05$; $^{b}p < 0.01$; $^{c}p < 0.001$, $^{d}p < 0.0005$ statistically significant difference of *GDI* between localities (Student's *t*-test).

The observed genotoxic activity of the methanol extracts of the plants from both localities could be influenced by the exposure of plants to PTE, which affected the synthesis of secondary metabolites. The PTE analysis showed that the plants contained a higher amount of Ni, Zn and Cr compared to the other identified metals, and these amounts were several times higher in the tailings than in the uncontaminated locality. In addition, the high Pb concentration was only detected in various plant parts of the tailings. Previous studies have shown that Ni can induce DNA damage through direct DNA binding and ROS stimulation.[29,30] Low concentrations of Pb also induce genotoxicity.[31] It is also known that Cr has a genotoxic effect and causes chromosomal damage and the formation of oxidized DNA adducts as well as the formation of DNA and protein/amino acid cross-links.

One of the possible mechanisms is the reduction of glutathione, which could generate free radical species such as hydrogen peroxide that produce high levels of oxidative stress and cause DNA damage.[32,33] In addition, Dutta *et al.*[34] showed that high concentrations of heavy metals can trigger genotoxic activities by directly attacking the thiol groups of proteins, leading to disruption of protein structure and function.

CONCLUSION

The results of this study revealed significant differences in phenolic and flavonoid contents, as well as in antioxidant and genotoxic activities, between plants collected from a tailing site and an uncontaminated locality. *O. viciifolia* from the tailing site exhibited pronounced adaptive mechanisms, including increased synthesis of phenolics and flavonoids, which contributed to enhanced antioxidant activity under stressful conditions. These adaptive responses were particularly prominent in the roots and aerial parts of the plants, highlighting their significant role in mitigating oxidative stress induced by potentially toxic elements. Conversely, the plants from the uncontaminated site displayed a more balanced chemical profile, with a lower accumulation of potentially toxic elements and a stable content of bioactive compounds, making them more suitable for medicinal and nutritional applications. Plants from both sites (uncontaminated and tailing site) demonstrated genotoxic effects, with the level of genotoxicity correlating with the concentration of analysed elements in the soil. Further research is needed to isolate bioactive compounds and evaluate their pharmacological safety.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/13515, or from the corresponding author on request.

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И З В О Д

АКУМУЛАЦИЈА МЕТАЛА У БИЉЦИ *Onobrychis viciifolia* SCOP., ЊИХОВА РАСПОДЕЛА У БИЉНИМ ОРГАНИМА И УТИЦАЈ НА САДРЖАЈ ФЕНОЛА И ФЛАВОНОИДА, КАО И НА АНТИОКСИДАТИВНУ И ГЕНОТОКСИЧНУ АКТИВНОСТ

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Ово истраживање је испитивало садржај потенцијално токсичних елемената (ПТЕ) и биолошке активности биљке *Onobrychis viciifolia* Scop. прикупљене са јаловишта и неконтаминираног локалитета. Концентрације Mn, Ni, Ca, Mg, Fe, Zn, Cr, Pb и Cu одређене су у земљишту и биљним деловима (корену, надземним деловима и цвасти) применом методе мокрог спаљивања, а затим атомском апсорпционом спектрофотометријом. Рачунати су фактори биоакумулације (*BCF*) и транслокације (*TF*) ради процене усвајања и расподеле метала. Укупни садржај фенола и флавоноида одређиван је спектрофотометријски. Антиоксидативна активност процењивана је помоћу DPPH теста за неутрализацију слободних радикала. Оштећење ДНК у хуманим лимфоцитима третирано биљним екстрактима испитано је комет тестом. Резултати су показали значајно више концентрације ПТЕ у земљишту и биљним деловима са контаминираног локалитета у поређењу са неконтаминираним. Биљке са контаминираног земљишта показале су повећану биоакумулацију и транслокацију ПТЕ. Поред тога, биолошка активност екстраката, укључујући антиоксидативни капацитет и генотоксичне ефекте, била је под утицајем изложености биљака РТЕ, што је утицало на синтезу секундарних метаболита. Екстракти биљака са јаловишта испољили су јачу активност у поређењу са биљкама са неконтаминираног локалитета. Ово истраживање истиче адаптивне одговоре *O. viciifolia* на стрес изазван РТЕ и пружа увид у њене потенцијалне примене у еколошком мониторингу и фитофармакологији.

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