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Accumulation of Heavy Metals and Metabolites in Taraxacum officinale (L.) Weber ex F.H.Wigg. on Polymetallic Contaminated Area

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Abstract. Technogenic terrains are one of the most unfavorable terrains for occupying of plant species. However, there are species that are distributed in such places and their reaction to such conditions is interesting. Some of them can be used for remediation of such terrains. The purpose of this study is to establish relationships among the heavy metals in the roots and aboveground part of T. officinale on the one hand and the metabolites synthesized under conditions of polymetallic contamination in Osogovo Mine, West Bulgaria. The metabolites were identified by GC/MS. There were determined 19 soil characteristics, content of 15 elements in aboveground and in the root system of *Taraxacum officinale*, as well as 20 metabolites. The concentrations of the studied elements in the aboveground part were significantly higher than the ones in the root system. There were found 40 significant correlations among the studied soil indicators and the studied metabolites in the roots. The number of statistically significant correlations (106) among the studied soil indicators and the studied metabolites in the aboveground parts were determined. There are also statistically significant correlations (38) among the studied chemical elements in the roots, the metabolites in the roots, and the metabolites in the aboveground part as well as 32 statistically significant correlations among the studied elements in the aboveground part of *T. officinale* and the studied metabolites in the aboveground and underground parts.

Key words: technogenic soils, heavy metals, *Taraxacum officinale*, metabolites.

Introduction

Terrain and soil resources formed or affected by mining activities are characterised by degrading properties. Depending on the mined resources, the newly formed and/or affected soils display different and specific characteristics. Sustainable management of post-mining areas is strongly linked to having good knowledge of soil and plant resources, and of the changes in the synthesis of important metabolites caused by unfavourable soil characteristics.

As a result of ore mineral extraction and processing, post-mining areas form specific habitats (Donov et al., 1978), and the formed tailings ponds, especially after mining and processing activities have ceased, can be a significant point source of pollution. Due to the high levels of heavy metals and metalloids, these sites can pose a serious environmental problem, but also a significant threat to people's health due to the degree of persistence and accumulation (Conesa et al., 2006; Martínez-Carlos et al., 2022). The environmental consequences of the presence of heavy metals and metalloids in tailings ponds can also be serious for the surrounding areas which have not been affected by ore mining and processing (García-Lorenzo et al., 2015; Tsolova et al., 2016; Khademi et al., 2018; Martínez-Carlos et al., 2022).

The substrates formed can be characterized by a high content of the sand fraction and a low content of the clay fraction, high particle density and bulk density which result in degraded hydro-physical properties (Donov et al., 1978; Martínez-Pagán et al., 2011; Chrastný et al., 2011). The availability of plant nutrients (N, P, K) and Soil Organic Matter (SOM) is usually low. Soil acidity can be extremely high and can vary considerably (Hossner & Hons, 1992; Martínez-Pagán et al., 2011). Tailings ponds formed as a result of ore mining activities can have very high, even toxic levels of heavy metals and metalloids, which can significantly affect the formation of phytocoenosis (Fernandez et al., 2017; Kovačević et al., 2020).

Many authors have studied the stages of vegetation succession on such terrains, for example Charzyński et al. (2013) present a scheme for the replacement of plant communities of technogenic substrates in parallel with soil development. Denisenko et al. (1996) investigate primary successions that occur in areas disturbed by mining and open pit mining. Titova & Vershinina (2014) comment on the changes in the floristic structure of the phytocoenosis of mechanically disturbed soil in the conditions of natural ecosystem and against the background of partial biological reclamation. The possibility for self-restoration of the phytocoenosis after technogenic impact has been assessed and the importance of reclamation for the regenerative capacity of the natural ecosystem has been established. The main component of the newly formed phytocoenosis after the disturbance is the weed-ruderal vegetation. The greatest development of weeds of the family Asteraceae is observed - more than 44%. Partial recultivation (liming) contributes to increasing the total number of plants, intensive development of cereals and the emergence of legumes.

The macro- and microelements play an extremely important role in normal plant development (Gorbanov et al., 2005). Different plant species need certain species-specific levels of macro- and micro-elements for their normal development, and both deficiencies and high concentrations of them can have an inhibiting effect on plant development (Gorbanov et al., 2005; Verbruggen et al., 2009). According to a number of authors, the optimal levels for plant development are not only species-specific but also genotype-dependent (Yang et al., 2006; Zalesny & Bauer, 2007). Elements such as Co, Cu, Fe, Mn, Mo, Ni and Zn are essential elements required for the normal growth and photosynthesis, respiration, and metabolic processes, whereas elements such as As, Cd, Hg, Pb are not considered to be essential elements (Gorbanov et al., 2005; Rascio & Navari-Izzo 2011; Martínez-Carlos et al., 2022. A number of angiosperm species can be classified as hyperaccumulators of elements such as As, Cd, Co, Cu, Mn, Ni, Pb, Sb, Se, Tl, Zn (Rascio & Navari-Izzo, 2011). Plant species accumulate different heavy metals and metalloids in different concentrations in different plant organs (Gorbanov et al., 2005).

Heavy metals can affect the synthesis of metabolites and metabolic processes to varying degrees (Lajayer et al., 2017; Zheljazkov et al., 2006). A number of authors report that stress from heavy metal contamination can increase the content of some metabolites (Tirillini et al., 2006; Lajayer, et al., 2016), while other authors report a reduction of some metabolites under heavy metal stress (Murch et al., 2003). On the other hand, differences in the synthesis of secondary metabolites can be observed in different populations of the same species, which may be due to abiotic factors (Doncheva et. al., 2014; Semerdjieva et. al., 2020). One of the species found in areas subject to anthropogenic pressure associated to heavy metal contamination is *Taraxacum officinale* (L.) Weber ex FHWigg (Maleci et al., 2013; Fazekašová et al., 2015), which has been the subject of extensive research related to the synthesized metabolites (Hook, 1994; Huber et al., 2015).

The aim of this study was to determine the influence of heavy metals in soil, roots and aerial parts of *T. officinale* and the content of metabolites in response to stress caused by polymetallic pollution. The formulated hypothesis, which we tested is that the stress caused by heavy metals in the soil lead to a change in the content of metabolites in plants.

Materials and Methods

The object of study were technogenic soils (Technosols (IUSS, 2014) formed in a tailings pond created as a result of ore mining and lead-zinc ore processing in Osogovo Mine complex, situated near the village of Gyueshevo, Kyustendil district, West Bulgaria, 950 m above sea level. They were found in the Middle forest vegetation zone (700-1200 m above sea level) of the Thracian forest vegetation area (Zahariev, 1979).

The taxonomy of the plant species is presented according to Delipavlov et al. (2003). The quantitative participation of species in phytocoenoses has been assessed by the 7 degree abundant scales of abundance and coverage (Braun-Blanquet, 1964). Five sample plots, 2x2 m have been set.

Five soil samples and plant (roots and aboveground parts) materials were collected in 2021 during peak flowering (May) of *T. officinale* and by means of applying a systematic sampling method (Petersen & Calvin, 1996), where the studied soils were taken from a depth of 0-20 cm, as close to the root system of *T. officinale* as possible. Soil sampling was done in accordance with the recommendations of Donov et al. (1974), and with the procedures described in ISO 10381-1: 2002. Preliminary sample preparation was performed according to ISO 11464: 2006.

Soil samples were analyzed for: pH (H_2O) - ISO 10390:2005; specific Electrical Conductivity EC (μS.cm^{-1}) was determined according to ISO 11265:1994; $CaCO_3$ was determined using the volumetric method in accordance with ISO 10693:1995; Total Kjeldahl Nitrogen TKN, mg.kg^{-1} - according to FAO.2021; plant available P_2O_5, mg.100g^{-1} and K_2O, mg.100g^{-1} were determined using an Acetate-Lactate extraction solution (Ivanov, 1984); Soil Organic Matter – SOM, % was determined according to Donov et al. (1984); Al, Ba, Ca, Cd, Cu, Fe, Mg, Mn, Na, Ni, Pb, Zn, mg.kg^{-1} were determined by extraction with NH_4NO_3 and subsequent determination by ICP-OES (ISO 19730:2008; Borge, 1997; Schöning & Brümmer, 2008); the results were converted to absolute dry weight in accordance with ISO 11465:1993.

Plant samples were cleaned to remove deposits, dried and ground with an agate mill, and homogenized in a metal-free homogenizer (Kowalenko, 1984). Procedures set out in ISO 10381-1:2002 were applied; microwave digestion system (M6 PreeKem) was used in accordance with Method 3052 (USEPA, 1996) for digestion of samples and subsequent determination by ICP-OES (Al, Ba, Ca, Cd, Cu, Fe, Mg, Mn, Na, Ni, Pb, Zn, mg.kg^{-1}); Total Kjeldahl Nitrogen was determined in accordance with the AOAC method 2001.11; the results were converted to absolute dry weight in accordance with Rautio et al. (2010).

Preparation of extracts and GC/MS analysis: crude extracts were prepared from 100 mg powdered plant material macerated with 1 mL methanol in 2 mL Eppendorf tubes. Fifty µL of 3,5 dichloro-4-hidroxybenzoic acid (1 mg/mL) were placed in the beginning of the extraction procedure as internal standard. After 24 h of extraction at room temperature aliquot of 500 µL of each simple was transferred to glass vial and was dried. 100 µL pyridine and 100 µL of N,O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA) were added to the dried samples and heated at 70 °C for 2 h. After cooling, 300 mL of chloroform were added and the samples were analyzed by GC/MS. The GC-MS spectra were recorded on a Thermo Scientific Focus GC coupled with Thermo Scientific DSQ mass detector operating in EI mode at 70 eV. A DB-5MS column (30 m x 0.25 mm x 0.25 µm) was used. The conditions of the analysis were described by Berkov et al. (2021). The GC-MS spectra of the compounds in the extracts were deconvoluted by AMDIS 2.64 software (NIST, National Institute of Standardization and Technology, Gaithersburg, MD) before their comparison with those of standard compounds and NIST spectra library. The response ratios were calculated for each metabolite relative to the internal standard using the calculated areas for both components.

Descriptive statistics and t-test was applied for the purposes of data analysis using Excel in Mac. Pearson's product-moment correlation and LSD Post-Hoc (SPSS 26 for Mac) were used to measure the associations that exist among the soil characteristics, elements and metabolites in the plants studied. The selected level of significance was α=0.05.

Results and Discussion

Most of the plant species that are distributed in the territory are pioneering and represent a stage of primary succession of vegetation (Table 1).

Table 1. Floristic composition of the tailings pond of the Osogovo Mine.

Plant species	Cover/Abundance of species	Family	Biological type
Tussilago farfara L.	1	Asteraceae	perennial
Taraxacum officinale (L.) Weber ex F.H.Wigg.	2	Asteraceae	perennial
Senecio vulgaris L.	1	Asteraceae	annual
Centaurea stoebe L.	1	Asteraceae	perennial
Cirsium arvense L. (Scop.)	1	Asteraceae	perennial
Hieracium pilosella L.	1	Asteraceae	perennial
Echium vulgare L.	2	Boraginaceae	biennial
Equisetum palustre L.	1	Equisetaceae	perennial
Euphorbia cyparissias L.	1	Euphorbiaceae	perennial
Medicago lupulina L.	1	Fabaceae	annual
Pinus sylvestris L.	1	Pinaceae	tree
Festuca valesiaca Schleich. ex Gaudin	1	Poaceae	perennial
Poa pratensis L.	1	Poaceae	perennial
Clematis recta L.	1	Ranunculaceae	perennial
Populus nigra L.	2	Salicaceae	tree

Fifteen plant species have been identified, of which 2 are trees (*Pinus sylvestris* L. and *Populus nigra* L.), 1 biennial herbaceous, 2 annual herbaceous and 10 perennial herbaceous plants. *Pinus sylvestris* is represented by single plants up to 40 cm high, and from *Populus nigra* single plants are 1.5 m high. Prevailing species are of Asteraceae (40% of all species) and Poaceae (13%) families.

Taraxacum officinale was chosen for sampling for chemical analysis because it has higher average cover and occurrence than other species in research site.

The studied soils (Table 2) according to Penkov's classification (1996) are characterised by moderately alkaline reaction in water, low carbonate content, low SOM levels, very low TKN levels, low reserve of plant available K_2O (Nikolova et al., 2014), and very low reserve of plant available P_2O_5 (Nikolova et al., 2014). The studied elements in the soil (Al, Ba, Ca, Cd, Cu, Fe, Mg, Mn, Na, Ni, Pb, Zn) are arranged in descending order by mean values as follows: Ca>Mg>Mn>Zn>Na>Fe>Pb>Al>Ba>Cu>Cd>Ni. Although there were significant variations, SOM, Ca, K_2O, $CaCO_3$, EC, TKN had the lowest levels of variation (under 20%), whereas Pb, Al, Cu, Cd, Ni, Zn, Fe had the highest levels of variation (over 50%). SOM's lowest level of variation is most likely due to the nature of formation and accumulation of soil organic matter and is related to SOM being considered a stable soil indicator (Bogdanov, 2018). Essential and non-essential elements are present in the soil in different forms (Doichinova et al., 2013), which determines their availability to plants. A number of soil indicators such as pH, SOM, soil texture can affect the availability of essential and non-essential elements to plants. Heavy metal immobilization by forming organo-mineral complexes has been discussed by various authors (Doichinova et al., 2013; Gorbanov et al., 2005).

The element with the most significant difference between its content in the root system and in the aboveground part was ~~is~~ nickel, which is the element with the lowest concentration in the studied soils (Table 3). Significantly greater variations in the concentrations of most elements in the root system were found, while only N, Ca, Cu, Na and Ni were characterized by a greater variation in the aboveground part.

Table 2. Arithmetic means and coefficients of variation of studied soil indicators.

	mean	CV, %
$pH(H_2O)$	8,33	1,28
$CaCO_3$, %	1,5	16,79
EC, $\mu S.cm^{-1}$	156,92	17,77
K_2O, $mg.100g^{-1}$	12,08	12,84
P_2O_5, $mg.100g^{-1}$	0,74	33,26
TKN, $mg.kg^{-1}$	162,9	18,49
SOM, %	0,56	10,56
Al, $mg.kg^{-1}$	1,34	59,02
Ba, $mg.kg^{-1}$	1,11	20,13
Ca, $mg.kg^{-1}$	1037,49	11,47
Cd, $mg.kg^{-1}$	0,09	62,63
Cu, $mg.kg^{-1}$	0,68	59,62
Fe, $mg.kg^{-1}$	2,3	75,99
Mg, $mg.kg^{-1}$	33,68	36,36
Mn, $mg.kg^{-1}$	10,72	41,03
Na, $mg.kg^{-1}$	2,64	33,67
Ni, $mg.kg^{-1}$	0,005	67,82
Pb, $mg.kg^{-1}$	1,49	55,51
Zn, $mg.kg^{-1}$	9,96	69,23

CV – coefficient of variation

The elements were accumulated in the root system of *T. officinale* in the following order (arranged by mean values): Ca>N>K>Fe>Al>Mg>Mn>P>Zn>Na>Pb>Cu>Ni>Ba>Cd. The accumulation of the elements in the aboveground part of the plant was in the following order (arranged by mean values): K>Ca>N>Fe>Al>Mg>Mn>P>Zn>Pb>Na>Ni>Cu>Ba>Cd. Significant differences were found between the content of K and Ca in the roots and in the leaves, where the higher values of K and Ca in the aboveground part compared to the roots have been discussed by other authors (Hook et al., 1993). Various studies (Kabata-Pendias & Dudka, 1991; Krolak, 2003; Lisiak-Zielińska et al., 2020) have reported higher concentrations of the elements Mn, Ni, Pb, Zn, Fe in the leaves compared to their levels in the roots, which has also been confirmed by the data obtained from this study. In a study, Krolak (2003) reported higher concentrations of Cd and Pb in the leaves compared to the concentrations of Cd and Pb in the roots, but these data have not been confirmed by the present study. Although the accumulation of toxic elements in the roots and their immobilization is considered a good strategy for dealing with toxic concentrations of elements (Bini et al., 2012) *T. officinale* does not accumulate toxic elements in larger quantities in the roots, and successfully translocates essential and non-essential elements from the roots to the aboveground part.

Twenty one metabolites were identified in the aboveground part and root system of *T. officinale* including phenolic, organic and fatty acids, mono- and disaccharides, triterpene and triterpene acids, sugars alcohols (Table 4). The content of the most metabolites, except malic acid, fructose 2, myo-inositol, sucrose, β-amyrin, β-sitosterol and triterpene 2, were higher in the aboveground part than in the root system.

Table 3. Arithmetic means and coefficients of variation of the studied elements in the roots and aboveground part of *T. officinale*. Legend: CV – coefficient of variation; The values in **Bold** indicate statistically significant difference at p≤0,05.

	Roots		Aboveground part	
	Mean	CV, %	mean	CV, %
N, %	0,96	13,5	1,18	32,82
P, mg.kg^{-1}	**498,1**	26,99	**822**	10,44
K, mg.kg^{-1}	**8452**	13,43	**15846**	8,85
Al, mg.kg^{-1}	**3808,8**	18,33	**6452**	11,73
Ba, mg.kg^{-1}	**26,69**	18,88	**39,34**	13,38
Ca, mg.kg^{-1}	11333	17,51	12468	17,96
Cd, mg.kg^{-1}	2,78	24,5	2,97	17,92
Cu, mg.kg^{-1}	45,09	16,38	54,56	16,69
Fe, mg.kg^{-1}	**6486**	18,14	**11295**	12,17
Mg, mg.kg^{-1}	**1766**	12,67	**2787**	11,4
Mn, mg.kg^{-1}	**1582**	20,73	**2682**	13,25
Na, mg.kg^{-1}	274,4	10,51	277,8	19,87
Ni, mg.kg^{-1}	**32,85**	26,93	**67,46**	35,51
Pb, mg.kg^{-1}	**202,41**	19,32	**341,51**	16,29
Zn, mg.kg^{-1}	389,92	23,8	458,54	19,69

Table 4. Arithmetic means and coefficients of variation of the studied metabolites in the roots and aboveground part of *T. officinale*. Legend: CV – coefficient of variation; The values in **Bold** indicate statistically significant difference at p≤0,05.

Metabolites in the roots			Metabolites in the aboveground part		
	mean	CV, %		mean	CV, %
Protocatechuic acid	0,43	80,87	Protocatechuic acid	2,06	97,35
Quinic acid	21,56	76,74	Quinic acid	68,55	142,59
Caffeic acid	26,78	77,18	Caffeic acid	560,59	204,19

Chlorogenic acid	12,08	195,18	Chlorogenic acid	37,58	178,67
Malic acid	365,15	41,14	Malic acid	204,72	126,11
Meso-erythritrol	8,39	95,15	Meso-erythritrol	15,39	59,2
Octanoic acid	594,39	88,03	Octanoic acid	717,14	137,88
Fructose 1	556,53	78,85	Fructose 1	718,51	114,88
Fructose 2	1924,63	63,89	Fructose 2	73,77	101,82
Fructose 3	44,12	138,77	Fructose 3	210,12	134,05
Glucose	97,99	173	Glucose	550,56	176,68
Myo-Inositol	**626,23**	**75,75**	**Myo-Inositol**	**1779,8**	**69,24**
Sucrose	2471,99	61,9	Sucrose	1778,71	109
β-Amyrin	77,88	44,3	β-Amyrin	61,13	115,63
Tririterpe acid	188,11	51,58	Tririterpe acid	119,86	142,25
Succinic acid	11,94	79,86	Glyceric acid	105,64	54,67
Palmitic acid	44,91	82,78	Palmitic acid	301,6	131,28
β-Sitosterol	80,09	55,59	β-Sitosterol	44,6	102,32
Triterpene 1	62,41	56,08	Triterpene 1	96,63	123,33
Triterpene 2	187,25	60,54	Triterpene 2	126,98	125

There were found 40 significant correlations among the studied soil indicators and the studied metabolites in the roots (Table 5). Twenty of the studied metabolites (in the roots) correlated with 13 out of 18 soil indicators. The following considerable numbers of significant correlations have been established – 9 for protocatechuic acid, 6 for palmitic acid, triterpene 1, triterpene 2, 5 for sucrose. Much fewer significant correlations have been found for the other metabolites. The significant correlations between fructose 1 - Na and glucose – Ca are negative, whereas all other significant correlations are positive. As regards the studied chemical elements, there were no significant correlations only for plant available P and K, and Mg, as well as for $CaCO_3$, EC and SOM. Ba and Fe had 6 significant correlations each, lead – 5; TKN, Al, Cd have 4 each, and Zn – 3. A large number of statistically significant correlations (106) among the studied soil indicators and the studied metabolites in the aboveground parts, all of which are positive (Table 6) were determined. All metabolites showed statistically significant correlations with the studied soil parameters. Three to eight were the significant correlations with quinic acid and fructose, 7 significant correlations were observed with octanoic acid, myo-inositol, sucrose, β-amyrin, palmitic acid. Six correlations were observed with chlorogenic acid, malic acid, tririterpe acid, triterpene 1; five significant correlations were observed with protocatechuic acid, caffeic acid, glucose and triterpene 2. The other metabolites had fewer significant correlations with the studied soil parameters. Of the studied soil parameters, only pH (H_2O), $CaCO_3$ K_2O, P_2O_5, TKN, Ca and Mg didn't show any significant correlations. Copper correlated with 17 metabolites, lead and zinc with 15 metabolites each, aluminum and barium with 14 metabolites each, and cadmium with 8. The other elements and EC had fewer correlations.

A number of studies (Hook et al., 1993; Rai et al., 2004; Pandey & Tripathi, 2011) discuss the relationships between the concentrations of essential and non-essential elements in the growth medium and the synthesis of metabolites in different plants. The significant correlations were found among a number of elements (Cd, Cu, Fe, Mn, Mg, Ni, Pb, Zn) and the metabolites synthesized in the organs of *T. officinale*. They confirmed the results obtained by other studies, which showed that those elements affected the synthesis of metabolites (Lajayer et al., 2017). Despite the important role of essential nutrients (N, P, K) in the synthesis of metabolites and in the regulation of the metabolic regime (Gorbanov et al., 2005), the present study did not find any significant correlations among P_2O_5, K_2O, TKN (with the exception of TKN which correlated with the metabolites in the roots) and the metabolites in the roots and the aboveground part.

Table 5. Table of the Pearson correlation coefficients among the pH (H_2O), EC, SOM and the studied chemical elements in the soil and the metabolites in the root system of *T. officinale*. Legend: * Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed). Due to the large amount of data, the correlation coefficients have been presented without 0 before the decimal point.

	Ca, $mg.kg^{-1}$	Ba, $mg.kg^{-1}$	Al, $mg.kg^{-1}$	SOM, %	TKN, $mg.kg^{-1}$	P_2O_5 $mg.100^{-1}$	K_2O $mg.100^{-1}$	EC, $\mu S.cm^{-1}$	$CaCO_3$, %	pH (H_2O)
Protocatechuic acid	,61	,993**	,933*	,27	,882*	-,49	-,03	,02	-,31	-,01
Quinic acid	,37	-,02	-,23	-,58	-,03	,56	,32	-,28	,34	,22
Caffeic acid	,73	,62	,62	,51	,61	,16	,61	,15	-,84	,73
Chloro-genic acid	,41	,27	,26	,69	,47	,44	,60	,45	-,85	,880*
Succinic acid	,29	,59	,56	,85	,83	-,01	,15	,61	-,69	,50
Malic acid	,63	,84	,71	-,27	,63	-,39	-,07	-,32	,14	-,22
Meso-erythritrol	,45	-,03	-,15	,11	,14	,84	,73	,16	-,42	,879*
Octanoic acid	,65	,64	,61	,59	,70	,14	,52	,27	-,81	,69
Fructose 1	-,24	-,44	-,65	,00	-,04	,84	,12	,44	,24	,41
Fructose 2	,74	,57	,51	,40	,61	,31	,63	,15	-,72	,76
Fructose 3	,39	,29	,29	,73	,48	,39	,57	,47	-,87	,86
Glucose	-,944*	-,39	-,43	,43	,06	-,10	-,78	,75	,41	-,40
Myo-Inositol	,58	,83	,69	,37	,912*	-,07	,13	,25	-,35	,30
Sucrose	,52	,926*	,79	,09	,87	-,41	-,15	,02	-,02	-,13
β-Amyrin	,53	,883*	,78	,47	,955*	-,21	,06	,29	-,40	,24
Tririterpe acid	-,17	-,47	-,66	-,65	-,37	,56	-,02	-,16	,69	-,07
Palmitic acid	,47	,905*	,918*	,59	,87	-,50	-,01	,23	-,55	,11
β-Sitosterol	-,85	-,23	-,29	,56	,27	-,10	-,75	,86	,32	-,31
Triterpene 1	,54	,960**	,883*	,40	,943*	-,41	-,04	,18	-,34	,06
Triterpene 2	,62	,980**	,889*	,17	,87	-,45	-,05	-,02	-,20	-,05

Table 6. Table of the Pearson correlation coefficients among the pH (H_2O), EC, SOM and the studied chemical elements in the soil and the metabolites in the aboveground part of *T. officinale*. Legend: * Correlation is significant at the 0,05 level (2-tailed). ** Correlation is significant at the 0,01 level (2-tailed). Due to the large amount of data, the correlation coefficients have been presented without 0 before the decimal point.

	K_2O mg.100^{-1}	EC, µS.cm^{-1}	$CaCO_3$, %	pH (H_2O)	Zn, mg.kg^{-1}	Pb, mg.kg^{-1}	Ni, mg.kg^{-1}	Na, mg.kg^{-1}	Mn, mg.kg^{-1}	Mg, mg.kg^{-1}	Fe, mg.kg^{-1}	Cu, mg.kg^{-1}	Cd, mg.kg^{-1}
Protocatechuic acid	-,091	-,494	-,123	-,386	,915*	,970**	,896*	,49	,88	,70	,988**	,908*	,905*
Quinic acid	-,137	-,251	-,141	-,288	-,15	-,14	-,36	-,29	-,43	-,34	-,10	-,20	-,11
Caffeic acid	-,287	-,266	,07	-,448	,33	,55	,59	,23	,64	,00	,73	,49	,30
Chlorogenic acid	-,224	-,217	,015	-,351	-,03	,19	,26	-,15	,36	-,25	,44	,10	-,01
Malic acid	-,123	-,392	-,089	-,354	,41	,55	,58	-,04	,67	,28	,75	,42	,46
Meso-erythritrol	-,142	,951*	-,364	,377	,81	,80	,61	,44	,53	,60	,74	,75	,80
Octanoic acid	-,157	-,168	-,089	-,254	-,34	-,16	-,23	-,41	-,19	-,61	,06	-,27	-,31
Fructose 1	-,039	-,529	-,18	-,356	,36	,57	,59	,15	,65	,07	,76	,48	,36
Fructose 2	,164	-,649	-,329	-,224	-,56	-,55	-,70	-,898*	-,67	-,53	-,38	-,69	-,43
Fructose 3	-,139	-,06	-,154	-,173	,27	,47	,45	,09	,49	-,08	,67	,38	,26
Glucose	-,287	-,303	,118	-,468	,00	,22	,30	-,13	,40	-,22	,46	,12	,01
Myo-Inositol	,177	-,39	-,447	-,048	-,18	-,34	-,34	-,63	-,29	,23	-,32	-,42	-,04
Sucrose	-,083	-,334	-,16	-,28	,66	,75	,63	,10	,64	,42	,88	,62	,70
β-Amyrin	-,228	-,351	-,014	-,443	,881*	,885*	,73	,32	,68	,71	,887*	,80	,907*
Tririterpe acid	-,252	-,336	,044	-,454	,74	,83	,74	,18	,76	,53	,939*	,71	,77
Glyceric acid	,319	,077	-,481	,393	-,46	-,55	-,75	-,56	-,81	-,42	-,56	-,59	-,38
Palmitic acid	-,227	-,352	-,044	-,444	,82	,908*	,930*	,46	,958*	,66	,967**	,85	,81
β-Sitosterol	-,244	-,408	-,004	-,496	-,06	-,19	-,22	-,65	-,15	,31	-,13	-,31	,10
Triterpene 1	-,22	-,387	-,001	-,454	,87	,928*	,85	,35	,85	,67	,984**	,84	,880*
Triterpene 2	-,303	-,298	,08	-,487	,907*	,947*	,84	,45	,80	,69	,955*	,88	,906*

Zn, mg.kg^{-1}	Pb, mg.kg^{-1}	Ni, mg.kg^{-1}	Na, mg.kg^{-1}	Mn, mg.kg^{-1}	Mg, mg.kg^{-1}	Fe, mg.kg^{-1}	Cu, mg.kg^{-1}	Cd, mg.kg^{-1}	Ca, mg.kg^{-1}	Ba, mg.kg^{-1}	Al, mg.kg^{-1}	SOM, %	TKN, mg.kg^{-1}	P_2O_5 mg.100^{-1}
,882*	,878	,880*	,905*	,81	,715	,729	,952*	,777	,569	,828	,896*	-,14	,41	-,808
,976**	,982**	,925*	,72	,87	,798	,898*	,984**	,923*	,583	,964**	,956*	,032	,691	-,722
,977**	,933*	,837	,655	,764	,852	,818	,932*	,943*	,474	,913*	,875	-,084	,654	-,757
,974**	,950*	,841	,605	,774	,819	,865	,930*	,948*	,528	,946*	,888*	-,039	,72	-,678
,946*	,941*	,895*	,804	,825	,768	,821	,975**	,871	,593	,913*	,927*	-,096	,564	-,758
-,225	-,174	-,098	-,614	,031	-,058	,031	-,302	-,103	-,474	-,164	-,175	,905*	,347	,262
,972**	,974**	,877	,597	,822	,793	,916*	,945*	,947*	,573	,977**	,921*	,045	,77	-,637
,832	,84	,872	,945*	,807	,662	,687	,932*	,711	,572	,782	,878*	-,136	,338	-,803
,517	,561	,695	,984**	,649	,355	,404	,716	,35	,512	,481	,673	-,17	-,027	-,675
,961**	,978**	,882*	,527	,842	,78	,952*	,928*	,950*	,563	,989**	,924*	,152	,841	-,584
,962**	,911*	,802	,647	,722	,836	,788	,912*	,927*	,477	,893*	,847	-,144	,624	-,74
,827	,910*	,949*	,879*	,913*	,568	,844	,963**	,715	,739	,886*	,964**	,068	,51	-,628
,954*	,967**	,923*	,78	,863	,758	,871	,988**	,884*	,624	,946*	,955*	-,023	,625	-,723
,962**	,932*	,881*	,776	,81	,827	,799	,963**	,898*	,504	,895*	,906*	-,088	,569	-,812
,967**	,928*	,85	,725	,774	,832	,798	,946*	,914*	,499	,899*	,884*	-,117	,595	-,783
,644	,778	,692	,264	,693	,335	,893*	,682	,645	,746	,856	,756	,297	,823	-,057
,956*	,929*	,894*	,801	,827	,827	,793	,968**	,886*	,492	,884*	,912*	-,065	,551	-,835
,929*	,891*	,865	,831	,793	,815	,734	,947*	,85	,463	,837	,879*	-,121	,474	-,862
,951*	,919*	,868	,792	,794	,813	,778	,957*	,881*	,509	,880*	,894*	-,123	,536	-,814
,973**	,923*	,838	,691	,763	,859	,793	,934*	,930*	,454	,893*	,871	-,103	,612	-,795

There were statistically significant correlations (38) among the studied chemical elements in the roots, the metabolites in the roots, and the metabolites in the aboveground part, which have been presented in Table 7. Only one statistically significant correlation was positive and all the rest were negative. There were found 21 statistically significant correlations among the studied elements in the roots and the identified metabolites in the roots. Mallic acid had ten significant correlations with the studied elements in the roots, and only one significant correlation with the studied chemical elements (with sodium) in the aboveground part.A large number of significant correlations were observed among sucrose in the roots and the studied elements in the roots (5), where, as in the case with malic acid, sucrose also had only one significant correlation with the studied elements and it was with Na.

A number of the identified metabolites in the aboveground part (quinic acid, caffeic acid, chlorogenic acid, meso-Erythritrol, octanoic acid, glucose, triterpene acid) correlated significantly with the studied elements in the roots, but on the other hand these same metabolites (in the roots) did not correlate with the studied elements in the roots.

Table 7. Table of the Pearson correlation coefficients among the studied chemical elements in the root system and the metabolites in the root system and the aboveground part of *T. officinale*. Legend: * Correlation is significant at the 0,05 level (2-tailed). ** Correlation is significant at the 0,01 level (2-tailed). ± the compound has been found only in the roots. No statistically significant correlations were found among the studied elements in the roots and Protocatechuic acid, Fructose 1, Fructose 2, Fructose 3, Palmitic acid, β-Sitosterol, Triterpene 1, due to which they have not been presented in the table. The correlations marked in brown are among the studied elements in the roots and the metabolites in the roots. The correlations marked in green are among the studied elements in the roots and the metabolites in the aboveground part. Due to the large amount of data, the correlation coefficients have been presented without 0 before the decimal point.

Malic acid		Succinic acid ±	Chlorogenic acid		Caffeic acid		Quinic acid		
,249	-,250	-,407	-,02	-,393	,082	-,255	,124	-,614	N, %
-,745	-,925*	,132	-,829	,413	-,832	,043	-,724	-,472	P, $mg.kg^{-1}$
-,736	-,799	,211	-,8	,595	-,836	,253	-,704	-,220	K, $mg.kg^{-1}$
-,411	-,755	-,616	-,625	-,428	-,528	-,572	-,525	-,515	Al, $mg.kg^{-1}$
-,402	-,729	-,662	-,615	-,469	-,516	-,601	-,522	-,471	Ba, $mg.kg^{-1}$
-,763	-,980**	-,248	-,899*	,058	-,861	-,278	-,801	-,457	Ca, $mg.kg^{-1}$
-,613	-,907*	-,225	-,706	-,184	-,637	-,503	-,636	-,699	Cd, $mg.kg^{-1}$
-,684	-,924*	-,035	-,737	,020	-,694	-,350	-,671	-,668	Cu, $mg.kg^{-1}$
-,647	-,949*	-,289	-,777	-,151	-,71	-,462	-,689	-,65	Fe, $mg.kg^{-1}$
-,806	-,950*	-,061	-,909*	,295	-,904*	-,068	-,813	-,357	Mg, $mg.kg^{-1}$
-,586	-,934*	-,247	-,744	-,091	-,678	-,375	-,632	-,691	Mn, $mg.kg^{-1}$
-,884*	-,982**	-,165	-,922*	,052	-,898*	-,367	-,881*	-,397	Na, $mg.kg^{-1}$
-,664	-,696	,479	-,594	,544	-,632	,148	-,56	-,404	Ni, $mg.kg^{-1}$
-,545	-,902*	-,098	-,665	-,044	-,603	-,343	-,562	-,783	Pb, $mg.kg^{-1}$
-,815	-,974**	-,268	-,876	-,105	-,83	-,492	-,831	-,483	Zn, $mg.kg^{-1}$

Triterpene 2		Tririterpe acid		β-Amyrin		Sucrose		Myo-Inositol		Glucose		Octanoic acid		Meso-erythritrol	
,149	-,252	,174	-,508	,252	-,459	,198	-,375	,361	-,573	,072	-,218	-,05	-,333	-,41	-,658
-,820	-,700	-,812	-,263	-,759	-,414	-,715	-,779	-,475	-,457	-,865	,31	-,774	,045	,654	,18
-,841	-,599	-,822	-,151	-,781	-,287	-,691	-,682	-,427	-,284	-,864	,071	-,728	,236	,588	,477
-,466	-,807	-,455	-,135	-,392	-,875	-,466	-,867	-,306	-,939*	-,529	,162	-,661	-,628	-,109	-,493
-,455	-,808	-,443	-,089	-,383	-,894*	-,461	-,861	-,311	-,951*	-,514	,139	-,657	-,66	-,162	-,496
-,829	-,889*	-,82	-,121	-,764	-,723	-,77	-,952*	-,551	-,761	-,878	,294	-,883*	-,303	,377	-,047
-,596	-,779	-,612	-,234	-,562	-,66	-,634	-,805	-,534	-,755	-,661	,671	-,716	-,476	,483	-,442
-,667	-,732	-,685	-,249	-,64	-,528	-,683	-,758	-,574	-,615	-,725	,71	-,725	-,306	,667	-,27
-,666	-,846	-,671	-,216	-,614	-,734	-,669	-,891*	-,518	-,815	-,731	,517	-,783	-,461	,394	-,361
-,887*	-,812	-,875	-,115	-,825	-,569	-,79	-,880*	-,548	-,586	-,925*	,227	-,868	-,088	,498	,193
-,631	-,807	-,63	-,308	-,563	-,699	-,605	-,878	-,418	-,789	-,702	,419	-,742	-,385	,366	-,348
-,880*	-,879*	-,891*	,02	-,86	-,652	-,884*	-,884*	-,758	-,679	-,914*	,591	-,911*	-,344	,593	-,024
-,649	-,388	-,668	-,227	-,65	-,021	-,608	-,406	-,509	-,064	-,674	,623	-,53	,225	,965**	,244
-,562	-,711	-,57	-,393	-,507	-,57	-,554	-,773	-,403	-,682	-,636	,576	-,658	-,323	,521	-,400
-,800	-,895*	-,814	-,014	-,778	-,725	-,83	-,897*	-,723	-,77	-,844	,633	-,881*	-,471	,509	-,204

There were 32 statistically significant correlations among the studied elements in the aboveground part of *T. officinale* and the studied metabolites in the aboveground and underground part (Table 8). All 22 significant correlations among the studied elements in the aboveground part and the metabolites in the roots were positive. All ten correlations between the studied elements and the metabolites in the aboveground part were negative. The only metabolites found both in the roots and in the aboveground part that correlated with some of the studied chemical elements were fructose 1, fructose 2 and β-sitosterol. No significant correlations were found between the studied elements in the aboveground part and malic acid (both in the roots and in the aboveground part), although malic acid (in the roots) correlated in many of the cases with the studied elements in the roots.

The toxic levels of essential and non-essential elements can cause oxidative stress and a number of other changes in physiology, including different localization of metal ions (in roots and leaves), accumulation and storage as non-toxic forms, formation of complexes with organic acids or peptides, etc., and unlock various mechanisms in the plant organism for adaptation to stress (Clijsters et al., 1999; Bretzel et al., 2013), which can result in a change in the amount of secondary metabolites (Lajayer et al., 2017). The increase or decrease in the content of various metabolites in plants subjected to metal stress has been discussed by other authors (Misra & Sharma, 1991;

Zheljazkov & Nielsen, 1996). Our data confirmed the increase in secondary metabolites caused by heavy metal stress reported by other authors (Lajayer et al., 2017), but there was also a decrease in the synthesis of secondary metabolites, which has been discussed by other authors (Murch et al., 2003). The significant correlations among Cu, Pb, Zn and the secondary metabolites in the leaves, and the lower concentrations of these same metals in the roots compared to those in the leaves confirmed data obtained from a study conducted by other authors on the impact of heavy metals on metabolites in *T. officinale* (Bretzel et al., 2013).

Table 8. Table of the Pearson correlation coefficients among the studied chemical elements in the aboveground part and the metabolites in the root system and the aboveground part of *T. officinale*. Legend: * Correlation is significant at the 0,05 level (2-tailed). ** Correlation is significant at the 0,01 level (2-tailed). No statistically significant correlations were found among the studied elements in the aboveground part and Quinic acid, Succinic acid, Malic acid, Meso-erythritol, Sucrose, β-Amyrin, Triterpene 1, Triterpene 2, Glyceric acid, due to which they have not been presented in the table. The correlations marked in brown are among the studied elements in the aboveground part and the metabolites in the roots. The correlations marked in green are among the studied elements in the aboveground part and the metabolites in the aboveground part. Due to the large amount of data, the correlation coefficients have been presented without 0 before the decimal point.

	Fructose 2	Fructose 1		Octanoic acid		Chlorogenic acid		Caffeic acid		Protocatechuic acid	
N, %	-,176	-,969**	,920*	-,714	-,305	-,706	,014	-,746	-,348	-,941*	-,65
P, mg.kg^{-1}	-,058	-,894*	,879*	-,497	-,111	-,505	,158	-,558	-,203	-,843	-,386
K, mg.kg^{-1}	-,292	-,862	,859	-,686	-,477	-,651	-,21	-,674	-,488	-,835	-,699
Al, mg.kg^{-1}	,995**	,107	,165	,383	,977**	,282	,919*	,173	,979**	,121	,605
Ba, mg.kg^{-1}	,986**	,197	,052	,403	,978**	,302	,903*	,201	,994**	,201	,614
Ca, mg.kg^{-1}	,051	-,644	,902*	-,263	-,176	-,239	-,067	-,301	-,195	-,575	-,264
Cd, mg.kg^{-1}	-,307	-,684	,628	-,317	-,286	-,298	-,108	-,313	-,404	-,622	-,277
Cu, mg.kg^{-1}	,321	-,54	,697	,018	,325	-,02	,422	-,103	,208	-,461	,178
Fe, mg.kg^{-1}	,404	-,509	,703	,064	,396	,02	,477	-,071	,286	-,428	,233
Mg, mg.kg^{-1}	,024	-,693	,913*	-,204	-,128	-,187	-,011	-,25	-,197	-,607	-,171
Mn, mg.kg^{-1}	,362	-,356	,526	,195	,409	,153	,438	,076	,286	-,275	,352
Na, mg.kg^{-1}	-,298	-,62	,525	-,276	-,234	-,264	-,063	-,275	-,359	-,565	-,222
Ni, mg.kg^{-1}	,519	-,772	,859	-,388	,431	-,457	,69	-,563	,388	-,746	-,161
Pb, mg.kg^{-1}	,577	-,203	,392	,314	,647	,247	,642	,157	,539	-,136	,513
Zn, mg.kg^{-1}	-,334	-,679	,628	-,316	-,32	-,292	-,147	-,305	-,436	-,616	-,288

β-Sitosterol		Palmitic acid		Tririterpe acid		Myo-Inositol		Glucose		Fructose 3		
-,891*	,44	-,879*	-,678	-,799	,733	-,943*	-,265	-,722	,474	-,671	-,022	-,942*
-,755	,706	-,718	-,362	-,635	,523	-,825	-,008	-,553	,671	-,422	,136	-,986**
-,796	,222	-,804	-,828	-,712	,895*	-,903*	-,371	-,632	,297	-,681	-,256	-,822
,052	-,292	,132	,65	,147	-,295	,437	,807	,145	-,483	,468	,907*	-,005
,116	-,378	,189	,671	,188	-,372	,518	,769	,172	-,557	,478	,895*	,12
-,526	,077	-,501	-,518	-,362	,935*	-,613	,092	-,254	,062	-,247	-,123	-,793
-,485	,907*	-,462	-,251	-,389	,383	-,716	-,049	-,311	,885*	-,261	-,111	-,845
-,36	,696	-,286	,197	-,197	,212	-,377	,509	-,115	,547	,12	,411	-,808
-,337	,627	-,258	,24	-,165	,208	-,323	,575	-,083	,466	,168	,464	-,786
-,511	,429	-,472	-,353	-,333	,794	-,652	,191	-,219	,377	-,16	-,053	-,918*
-,17	,705	-,092	,389	-,016	,035	-,193	,612	,055	,535	,297	,438	-,662
-,427	,962**	-,402	-,146	-,348	,231	-,645	-,031	-,283	,931*	-,214	-,057	-,774
-,751	,254	-,686	-,097	-,624	,319	-,516	,298	-,57	,151	-,284	,659	-,799
-,065	,552	,022	,593	,076	-,175	,025	,75	,124	,351	,426	,647	-,489
-,477	,897*	-,456	-,276	-,381	,412	-,723	-,066	-,3	,880*	-,265	-,151	-,842

Conclusions

Significant concentrations of available heavy metals have been found in technogenic soils on which phytocoenoses with the participation of *T. officinale* have been formed. There was a greater accumulation of elements (P, K, Al, Ba, Fe, Mg, Mn, Ni, Pb) in the aboveground part compared to the roots, which has confirmed previous data reported by other authors on the greater accumulation of heavy metals in the aboveground part. There were significantly more (38) correlations between the investigated chemical elements in the roots, metabolites in the roots and metabolites in the aerial part, compared to the correlations (32) between the investigated elements in the aerial part of *T. officinale* and the investigated metabolites in the aerial and underground parts. It was established that the investigated elements can influence (positively and/or negatively) the content of metabolites in the different parts of the plants. Also, affecting the content of a given metabolite in one plant part does not necessarily mean that the same metabolite will be affected in the same way in other plant parts. The obtained results confirm the formulated hypothesis - that the stress caused by heavy metals in the soil causes a change in the content of metabolites in plants.

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