



Properties of humic acids from copper tailings 20 years after reclamation

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Abstract: Part of Cu post flotation tailings of Serbia Ziljin Bor Copper, Serbia, was reclaimed by restoration of top soil with arable soils, and revegetation in 1991. Humic acids isolated from these Technosols were investigated to find out if their properties underwent any changes since reclamation. Two groups of control samples were used. Elemental composition (CHNS analysis) falls within the range of average soil humic acids. Humic acids belong to the type B pointing out to its lower humification degree (UV–Vis). Relative abundances of functional groups are ranged as follows: polysaccharide C ≥ aromatic C > carboxyl C > OH group > aliphatic C. Aromaticity indexes are low, 1.88–3.25 (ATR-FTIR). Basic units at pH 10 are in the 11.7–26.8 nm range. Pronounced reaggregation (1462–5218 nm) at pH 3 points out to less expressed humic acid sol stability, as well as to increase in aromatic condensation degree (dynamic light scattering). No significant changes have occurred in technosol humic acids since the recultivation, confirming stability of their properties over time (PCA). Nevertheless, humic acids from very strongly acidic Technosols show higher humification degree possibly originating from arable soils used in reclamation, but more likely from low soil pH and low litter input, results of unsuccessful reclamation.

Keywords: Technosols; top soil restoration; revegetation; humification degree.

INTRODUCTION

Numerous mines exploit ores and, after extraction of economic elements, discharge residual wastes in the environment.¹ Study of post-mining soils focuses on two important aspects: soil formation processes and soil restoration as a basic precondition of ecosystem recovery. Development of these soils is influenced by various environmental conditions and types of land use. Rate of pedogenesis and

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degree of soil evolution particularly depend on residence time and nature of parent material. Technogenic materials may control functioning and evolution of soils, their potential impact on the surrounding environment and soil suitability for plants growth. Since organic matter addition and humification are the key processes in the first steps of soil development, vegetation plays the key role as initial soil forming factor. Dominant problems in revegetation are physical, nutritional and/or toxicological properties of mine wastes.²⁻⁴

Copper mining and processing have a profound impact on the environment producing a huge amount of waste worldwide.⁵ One of the largest mines in Serbia is Serbia Zijin Bor Copper. Beside other mine wastes, post-flotation tailings (PFT) dumps as a result of copper ore processing were formed. The PFT dump "Polje 2" was definitively abandoned in 1987.⁶ In addition to degradation of large land areas these mine wastes are sources of pollution to the surrounding environment, especially for the nearby city of Bor. PFT formed are of very pure soil conditions, predominantly high As and Cu concentration, which disable plant growth, and associated soil organic matter (SOM) accumulation.^{1,6} Therefore recultivation is the only way to recover these degraded areas. An area of PFT (about 16 ha) was reclaimed by reconstructing the topsoil with natural arable soil material, an average depth of 40 cm, in 1991. The soil was taken from the southern part of the city of Bor where the residential area expanded. One part of the reclaimed area was planted with grass and the other with trees, without any other management practice applied.

Our previous investigations on pedological characteristics of PFT⁶ showed that there were no spontaneous revegetation and SOM genesis on non-reclaimed areas due to pure soil characteristics, predominantly high As and Cu content, as already mentioned. On reclaimed area of PFT formed A horizon (about 45 cm deep) is a heterogeneous mixture of arable soil materials and tailings in various ratios, characterized by degraded structure, low humus content, mostly acid, high As and Cu concentrations and low soil microbial activity. According to Lilić *et al.*,⁶ these soils are classified as Spolic Technosols (Phytotoxic). Part of the A horizon is covered with wind-deposited tailings (originating from a non-reclaimed part of PFT), soils belonging to the Spolic Technosols (Phytotoxic, Areninovic). According to unfavourable soil characteristics and poor vegetation, it is obvious that this soil reclamation attempt was unsuccessful, as already noticed.⁷

Humic acids (HAs) from Technosols on Cu PFT about 20 years after reclamation were investigated in this study. It is well known that the main processes in soil formation are the SOM accumulation and transformation. Humic substances, including HAs, are the most abundant fractions of SOM. Their composition, structure and properties predetermined by conditions of soil formation⁸ can be used as indicators of pedogenetic processes.⁹ HAs are the most reactive compounds in soil, *i.e.*, have the capacity for diverse chemical and physical interact-

ions in the environment. These interactions are well known to depend on the HA chemical structure and composition.¹⁰ According to Chaudhuri *et al.*,¹¹ molecular properties of soil organic carbon (SOC) were useful indicators of both SOC dynamics and soil quality in mine soil chronosequence.

As the A horizon of investigated Technosols is a mixture of arable soils and tailings, it can be assumed that the investigated HAs originate from the arable soils. HA properties (elemental composition, fractions of carbon of aliphatic and aromatic groups and their ratio, optical and other properties) are related to soil genesis environmental conditions and are persistent over time,¹² retaining characteristics typical for the given soil type.^{8,13} Therefore, there is a possibility for technosol HAs investigated in this study to retain main properties of HAs from the arable soils used. However, these HAs existed under exchanged soil environmental conditions and plant residues entrance during the last 20 years. Hence, technosol HAs were investigated to find out if they underwent any changes in elemental composition, optical properties and reaggregation ability 20 years from recultivation.

EXPERIMENTAL

The study area (Fig. S-1 of the Supplementary material to this paper) is located in the immediate vicinity of the city of Bor, East Serbia, at about 400 m above sea level (44°05' N, 22°06' E). The climate in the region is of a temperate continental type characterized by short hot summers and a long, cold winters with average annual air temperature of 11 °C, and precipitation of 550 mm. The winds blowing from the northwest are the most frequent and the strongest. On the reclaimed area of PFT, birch (*Betula pendula* L.), shrubs (*Rosa canina* L. and *Rubus caesus* L.), and several grass species among which dominant *Nardus stricta* L. and *Agropyrum repens* L. are present, but very sporadically. Here and there *Verbascum phlomoides* L. and *Bryopsida* spp sedge appear too.

In our previous investigations of pedological characteristics of PTF,⁶ 30 soil samples were taken at equal intervals in the direction of the letter L. To investigate soil HA properties, 6 soil samples (TC1-6) were selected due to the differences in pH (4.17–7.24), C (0.39–1.24 %) and clay content (18.46–27.34 %), Table S-I of the Supplementary material. Five soil samples (TC1–TC5) were taken from the A horizon at a 0–25 cm depth. Sixth soil sample (TC6) was collected at a 15–50 cm depth, from the A horizon covered with a wind-deposited 15 cm thin layer originating from non-reclaimed area of post-flotation tailings.

To the best of our knowledge, there is no appropriate HA sample which could be used as control (there are no data on TC HA properties immediately after the reclamation as well as of HA properties of arable soils used in the reclamation process). Therefore, two groups of HA control samples were chosen for this study. The first group consisted of three HAs (CB7–CB9) isolated from natural arable soils used in crop production (0–25 cm depth), originating from the location nearby a new residential area of Bor, which surface soil layers were used for reclamation of PFT (Fig. S-1). The second group of control samples includes two HAs isolated from soils formed under different environmental conditions. The first HA (CCH) was isolated from Chernozem (0–25 cm depth), originating from Novi Banovci (44°57' N, 20°16' E), Serbia. The second HA control sample (CES) was IHSS standard HA obtained from Elliott soil.^{14,15} Detailed description of control samples is given in the Supplementary material. Soil

texture, aggregate stability (mean weight diameter - MWD), organic C and pH were determined by common methods.¹⁶

HA sample was isolated from approx. 5 kg TC and 2 kg control soil material from each sampling location and used for all measurements in three replicates. HA samples were isolated using a modified IHSS method (HA gel was dried at 35 °C, powdered, and sieved using a 0.05 mm sieve).¹⁴

The C, H and N contents of HA samples were determined using elemental analyzer (CHNS 628, LECO Corporation, USA) after drying the samples over P₂O₅ under vacuum. Their percentages were calculated on an ash-free basis. The oxygen was calculated by the difference. Ash content was determined by a dry combustion method (50 mg HA at 750 °C for 8 h). The C/N, O/C, H/C and O/H atomic ratios were calculated by determining the ratio of C to N, O to C, H to C, and C to H contents, respectively. Internal oxidation degree (ω) was calculated according to the formula: $\omega = (2O+3N-H)/C$,¹³ where *O*, *N*, *H* and *C* are contents (in at. %) of oxygen, nitrogen, hydrogen and carbon, respectively.

UV-Vis absorption spectra of HA sol (prepared in 0.05 M NaHCO₃ solution to contain 0.1 g dm⁻³ C, pH 8.3–8.4), in the 200–800 nm range were recorded using a UV-Vis spectrophotometer (Evolution 60s, Thermo Fisher Scientific, USA) with 1 nm resolution. Optical indexes E_{280} (absorbance at 280 nm/mg C),¹¹ E_4/E_6 (absorbance ratio A_{465}/A_{665}),⁸ E_3/E_5 (A_{350}/A_{550}),¹⁷ E_2/E_3 (A_{225}/A_{350}),¹⁸ then $\Delta\log K$ ($\log A_{400} - \log A_{600}$) and RF ($15A_{600}/c$, *c* is concentration in mg of C in 1 ml of HA solution)¹⁹ were used as indicators of humification degree, most widely used for comparison of HA samples.

ATR-FTIR spectra of HAs in the 4000–400 cm⁻¹ range were recorded by an Alpha spectrometer (Bruker, Germany, 4 cm⁻¹ resolution, 64 scans). Air spectrum was used as background. Peak intensities were determined relative to the baseline dependant on the spectral region. Baselines in the 3700–1800 and 3000–2800 cm⁻¹ range were used for 3283 and 2920 cm⁻¹ bands, respectively. Intensities of 1705, 1620, 1520, 1080 and 1030 cm⁻¹ bands were determined using the baseline between 1830 and 400 cm⁻¹. Relative peak intensities of 3283, 2920, 1705, 1620, 1080 and 1030 cm⁻¹ bands were calculated by dividing peak intensity values by that for the 1520 cm⁻¹ band. Aromaticity index (I_{1620}/I_{2920}) was calculated by dividing the peak height for the 1620 cm⁻¹ band (determined by using the baseline between 1696 and 1530 cm⁻¹) by previously determined peak height for the 2920 cm⁻¹ band.²⁰ Each peak height was calculated as a mean of two replicates.

To perform the dynamic light scattering (DLS) and zeta potential (*ZP*) measurements, the HA sols (0.02 g dm⁻³) were prepared using deionized water and their pHs were adjusted by adding 0.1 and 1.0 M HCl or NaOH solutions. NaCl was added to maintain ionic strength constant (0.1 M) within $\pm 10\%$, even though the maximum amount of HCl or NaOH solution was used. The prepared HA sols were equilibrated for 24 h at 25 \pm 2 °C and their pHs determined before the measurement. The measurements were performed using a Zeta-sizer Nano ZS with 633 nm He-Ne laser (Malvern, UK), and the data were analyzed by the Zetasizer software version 6.20 (Malvern, UK). Measurement details are given by Jovanović *et al.*²¹ Absorbances of alkaline and acid sols at 465 and 665 nm were recorded by UV-Vis spectroscopy to calculate the E_4/E_6 index.

The HA characteristics were organized in three datasets as follows: the first, Technosols and all control soils; the second, Technosols and control Bor soils; and the third, Technosols. The correlation of soils and HAs data, as well as the PCA of HAs data (auto scaled prior to analysis) were performed using an IBM SPSS Statistics 19 software package.

RESULTS AND DISCUSSION

Elemental composition of investigated HA samples (Table I) reveals the contents obtained to fall within the range of average values reported for soil HA.²² Atomic ratios have been used as indicators of variations in HA properties as a function of HA origin.^{22,23} Generally, high C/N and O/H and low O/C and H/C reflect high degree of aromatic condensation, maturity and stability of HAs, *i.e.*, the degree of HA humification. Besides, ω has been used as an indicator of the progress in plant residue humification.¹³ Positive values of ω indicate well-drained soils with prevailing oxidizing conditions favorable for HA oxidation, while negative values reflect anaerobic soil conditions.²⁴ The atomic ratios appear to be qualitatively useful parameters to compare compositional differences between various humic materials.²² Differentiation in the degree of oxidation between the second control HAs, CCH and CES and other HAs is clearly indicated in Table I. At the same time, there is no clear difference between investigated TC HAs and the first control CB HAs. Elemental composition of TC and CB HAs point out to their similar origin. H/C atomic ratios were higher than 1, indicating prevalence of aliphatic structures in both TC and CB HAs,²³ while lower O/H ratios reveal lower degree of oxidation and low content of oxygen-containing functional groups.²⁵ According to negative ω values, it can be assumed that TC3 and CB9 HAs were formed under soil anaerobic conditions.¹³

TABLE I. Elemental composition, ash content, atomic ratios and internal oxidation degree of humic acids (HA) isolated from Technosols (TC1–TC6) and control natural soils: arable soils near the city of Bor (CB7–CB9), Chernozem (CCH) and standard Elliot soil (CES)

HA	C	H	O	N	Content of ash, %	C/N	O/C	H/C	O/H	ω
	Content ^a , wt. %					Atomic ratio				
TC1	52.38	5.36	37.23	5.03	0.63	12.15	0.605	1.2281	0.492	0.228
TC2	52.11	5.28	37.83	4.78	0.32	12.70	0.574	1.2160	0.475	0.186
TC3	53.68	5.46	35.81	4.91	0	12.76	0.490	1.2206	0.401	-0.006
TC4	57.97	5.03	32.00	5.00	0.27	13.54	0.467	1.0413	0.448	0.113
TC5	57.48	5.17	32.09	5.26	0.44	12.76	0.447	1.0794	0.414	0.049
TC6	52.86	4.87	37.12	5.15	0	11.97	0.642	1.1053	0.581	0.430
CB7	56.95	5.03	32.62	5.40	0.20	12.31	0.487	1.0599	0.459	0.158
CB8	56.23	5.29	32.84	5.64	0.07	11.63	0.447	1.1288	0.396	0.023
CB9	50.76	5.79	39.01	4.44	0.19	13.35	0.505	1.3624	0.369	-0.134
CCH	52.29	4.31	39.57	3.83	0.23	15.95	0.810	0.9888	0.820	0.820
CES	58.13	3.60	34.13	4.14	0.88	16.39	0.770	0.7430	1.037	0.981

^aAsh and moisture-free basis

According to the literature data,^{11,17,18,26} the lower E_2/E_3 , E_3/E_5 , E_4/E_6 and $\Delta\log K$ and higher E_{280} and RF indexes, the higher the HA optical density. The obtained E_{280} , E_2/E_3 , E_3/E_5 , E_4/E_6 , $\Delta\log K$ and RF values (<43.31, >1.44, >4.67, 4.89–5.36, > 0.6399 and < 66.6, respectively, Table II) indicate low humification

degree of TC HAs. The E_4/E_6 ratio slightly higher than 5 suggests a relatively low degree of aromatic polycondensation and/or relatively small particle size and molecular weight of TC HAs.²⁷ Control CB HAs have very different optical indexes, but lower humification degree than TC HAs. CCH and CES HAs reveals higher E_{280} and RF and lower E_2/E_3 , E_3/E_5 , E_4/E_6 and $\Delta\log K$ indicating higher humification degree in comparison with that of TC and CB HAs. According to Kumada,¹⁹ CCH and CES belong to type A, TC1–TC6 and CB9 are of type B, while CB7 and CB8 are of type P soil HAs, meaning that their optical density decreases in the order: CCH \approx CES > TC1–TC6 \approx CB9 > CB7 \approx CB8.

TABLE II. Optical indexes of humic acids (HA) isolated from Technosols (TC1–TC6) and control natural soils: arable soils near the city of Bor (CB7–CB9), Chernozem (CCH) and standard Elliot soil (CES), obtained by UV–Vis spectroscopy

HA	E_{280}	E_2/E_3	E_3/E_5	E_4/E_6	$\Delta\log K$	RF
TC1	40.76±0.27	1.74±0.07	5.21±0.01	5.12±0.01	0.67401±0.0035	48.1±0.4
TC2	42.34±0.51	1.50±0.03	4.71±0.01	4.89±0.01	0.6399±0.0029	61.6±0.4
TC3	43.31±1.58	1.46±0.04	5.21±0.04	5.36±0.01	0.6794±0.0036	57.9±0.4
TC4	41.26±0.28	1.65±0.03	5.05±0.01	5.06±0.01	0.6650±0.0028	52.2±0.3
TC5	40.69±0.95	1.73±0.04	5.20±0.01	5.19±0.07	0.6776±0.0022	48.3±0.2
TC6	43.24±0.59	1.44±0.03	4.671±0.004	5.12±0.01	0.6442±0.0016	66.6±0.2
CB7	38.94±0.04	1.94±0.05	4.99±0.01	5.15±0.02	0.6572±0.0039	45.6±0.4
CB8	42.21±0.15	1.70±0.07	5.22±0.01	5.33±0.01	0.6844±0.0037	49.1±0.4
CB9	37.32±0.05	2.11±0.09	5.52±0.01	5.79±0.01	0.6969±0.0043	36.5±0.3
CCH	47.24±3.58	1.14±0.11	3.94±0.05	4.390±0.003	0.6065±0.0006	105.5±0.1
CES	45.53±2.47	1.24±0.04	4.05±0.08	3.900±0.002	0.5736±0.0006	94.5±0.1

Relative band intensities (ATR-FTIR spectra) have been used to estimate relative abundances of broad classes of functional groups (OH, N–H, I_{3300} ; alkyl, I_{2923} ; carboxyl, I_{1702} ; aromatic C, I_{1620} ; carbohydrate-like structures/polysaccharides, I_{1080} and I_{1030}).¹¹ These relative band intensities, as well as aromaticity index (I_{1620}/I_{2920}) values^{20,21} have been used to compare HA molecular characteristics. Relative band intensities and aromaticity index values obtained in this study are shown in Table III. Relative abundances of functional groups present are ranged as follows: polysaccharide C = aromatic C > carboxyl C > OH group > aliphatic C for TC1–TC3 and TC6 HAs, and polysaccharide C > aromatic C > carboxyl C > OH group > aliphatic C for TC4–5 HAs. Aromaticity index values are low (1.88–3.25). It is obvious from Table III that relative band intensities, as well as aromaticity index values, for CCH and CES are different from those of TC and CB HAs. Additionally, TC and CB HAs are similar, as confirmed by elemental and UV–Vis analysis. Lower abundances of oxygen-containing groups (I_{3293} , I_{1705} , I_{1080} and I_{1030}) in TC and CB HAs are in agreement with lower ω , O/C and O/H. Less aromatic and more aliphatic component abundance, as well as lower aromaticity indexes, are consistent with higher H/C ratio and lower optical

density obtained by UV–Vis indexes, pointing out to their lower humification degree.^{19,29}

TABLE III. Some relative band intensities and aromaticity index values of humic acids (HA) from TTechnosols (TC1–TC6) and control natural soils: arable soils near the city of Bor (CB7–CB9), Chernozem (CCH) and standard Elliot soil (CES), obtained by ATR-FTIR (measurement uncertainty < 5 %)

HA	I_{3273}^a	I_{2923}^a	I_{1705}^a	I_{1620}^a	I_{1080}^a	I_{1030}^a	I_{1620}/I_{2920}^b
TC1	0.71	0.18	0.85	1.31	0.59	0.58	2.50
TC2	0.71	0.21	1.04	1.41	0.70	0.79	2.02
TC3	0.65	0.18	1.00	1.40	0.67	0.66	2.42
TC4	0.76	0.24	1.05	1.44	0.73	0.76	1.88
TC5	0.75	0.23	1.00	1.41	0.72	0.75	1.91
TC6	0.59	0.14	1.08	1.45	0.66	0.63	3.25
CB7	0.76	0.22	0.93	1.36	0.68	0.70	1.98
CB8	0.77	0.20	1.02	1.41	0.69	0.70	2.09
CB9	0.70	0.17	1.00	1.48	0.86	0.89	3.16
CCH	0.88	0.12	1.73	1.52	0.75	0.69	4.29
CES	0.90	0.11	1.35	1.74	0.86	0.91	4.24

^aRelative band intensities (to intensity of C=C aromatic band at 1520 cm⁻¹); ^baromaticity index

Humic substances are able to rearrange and restructure themselves in response to environmental changes such as pH, ionic strength, moisture as well as HS concentration.^{21,30} Aggregation properties of HAs significantly influence their interactions in nature. For example metal complexation³¹ is the reason why is important to study their colloidal character. In this study, PSD and ZP were measured in highly alkaline solutions (pH 10), low HA concentration (0.02 g dm⁻³) and constant ionic strength (0.1 M NaCl solution) to obtain HA basic unit size.²¹ Alkaline solutions were acidified by HCl to pH 3 to examine the HA reaggregation ability. Since *PS* is closely related to colloidal stability of humic particles,^{21,32} *ZP* at both pHs was measured.

It is obvious (Table IV) that *ZPs* are negative for all HAs at both pHs (–22.7 to –31.9 mV at pH 10 and –16.3 to –19.4 mV at pH 3). Basic units of TC HAs at pH 10 are in the range 11.7–26.8 nm, except for TC1 (151.4 nm). After acidification, pronounced TC HA reaggregation (1462–5218 nm) is evident, pointing out to less negative *ZP* values, *i.e.*, less expressed HA sol stability. By inspecting *PS* and *ZP* values in Table IV, difference between all control HAs and TC HAs is obvious at both pHs, but more pronounced at pH 10. Pronounced aggregation of CCH and CES HA control samples can be related to higher relative ATR-FTIR band intensities (I_{1705} and I_{3272}) typical of carboxyl and phenolic (OH) functional groups. On the contrary, aggregation of other control samples (CB7 and CB8, as well as TC5) could not be explained by already mentioned relative intensities likely due to the presence of two competitive intramolecular contraction and intermolecular aggregation processes influencing HA particle size.³⁰ Com-

paring E_4/E_6 indexes under alkaline and acid conditions, an increase in aromatic condensation degree for TC HAs can be noticed, opposite to its decrease for BC HAs and almost no change for CCH and CES HAs.

TABLE IV. Particle size (PS), zeta potential (ZP) and E_4/E_6 index of humic acids (HA) isolated from Technosols (TC1–TC6) and control natural soils: arable soils near the city of Bor (CB7–CB9), Chernozem (CCH) and Elliot soil (CES)

HA	pH 10			pH 3		
	PS^a / nm	ZP / mV	E_4/E_6^b	PS^a / nm	ZP / mV	E_4/E_6
TC1	151.4	-27.5±1.4	4.77	2282	-16.3±1.9	3.86
TC2	26.6	-23.0±2.7	2.59	1977	-18.5±1.2	3.75
TC3	15.0	-31.9±1.4	2.54	3284	-19.4±1.3	3.29
TC4	18.0	-22.7±4.4	2.65	1462	-18.9±1.2	4.52
TC5	26.8	-27.5±2.4	2.49	5218	-19.1±1.2	2.78
TC6	11.7	-29.4±1.9	2.69	3337	-17.4±1.1	3.03
CB7	17.0	-19.1±2.9	5.06	5031	-17.2±1.0	3.70
CB8	14.7	-14.0±2.2	4.69	5363	-17.6±0.7	3.60
CB9	14.3	-22.4±3.0	4.65	491	-13.7±0.7	3.73
CCH	10.9	-23.1±2.0	5.07	5359	-16.8±1.1	3.65
CES	10.2	-18.5±4.7	4.85	4658	-16.9±2.0	3.67

^aMeasurements uncertainty <6 %; ^bmeasurement uncertainty <2 %

According to the literature,³³ HA chemical structure and properties significantly influence the efficiency of HA-metal binding. The results obtained in this study have shown changes in TC HA structure and properties with pH, which likely affect their interaction with metals. HA-metal binding is of great importance at Cu post-flotation tailings due to extremely high As and Cu concentrations⁶ and this problem should be emphasized in future investigations.

PCA score plots obtained for HA characteristics for three datasets: TCs, CBs, CCH and CES (Fig. S-2 of the Supplementary material), TCs and CBs (Fig. S-3 of the Supplementary material) and TCs (Fig. S-4 of the Supplementary material). The first three principal components (Fig. S-2) describe more than 84 % of the total variance (56.6 and 17.0 % for the first (PC1) and the second (PC2) principal component, respectively) enabling very good insight into data structure. Similarly, the first three principal components describe nearly 79 % in the second dataset (Fig. S-3, PC1 36.0 % and PC2 26.1 %), and nearly 84 % of the total variance in the third dataset (Fig. S-4, PC1 40.9 % and PC2 27.2 %).

The score plot (Fig. S-2) shows that two groups of the first dataset can be considered as separated along the PC2 axis in terms of linear separability and classification. Clear separation of CCH and CES from TC and CB HAs can be noticed, as well as from each other, which is expected regarding their origins. Obviously, RF , O/H , C/N , ω , O/C , I_{1620}/I_{2923} (along positive PC1 axis) and E_3/E_5 , $\Delta\log K$, E_4/E_6 and H content (along negative PC1 axis) are informative for

clear difference between CCH and CES HAs and TC and CB HAs. Also, C and O contents carry the most of variance along the PC2 axis causing separation inside the TC and CB group. TC and CB HAs had similar properties since 79 % of the total variance has been preserved (Fig. S-3), while the content of H, I_{1080} and I_{1030} cause separation of CB9 HAs. Considerable heterogeneity noticed in Fig. S-4 confirms differences in TC HAs. TC1 (characterized by PS at pH 10), TC2 and TC6 (RF , E_{280} , carboxyl and aromatic C), TC3 (ZP at pH 10, PS at pH 3, E_4/E_6 and N) as well as TC4 and TC5 HAs (aliphatic C and polysaccharides), can be considered as separated. TC1–TC3 and TC6 HAs originate from very strongly to strongly acid soils with low SOC content, while TC4 and TC5 HAs come from slightly acid and neutral soils with slightly higher SOC and clay content, as well as higher MWD. Dependence of HAs properties on some basic characteristics of Technosols was confirmed by correlation analysis, indicating to dependence of humification process on soil conditions.¹⁹

Higher TC soil clay content is related to higher abundance of COOH acidic groups, aromatic C, polysaccharides and degree of aromatic condensation (H/C ratio, 0.880*, 0.912*, 0.899*, 0.812* and -0.835^* , respectively; * $p < 0.05$, ** $p < 0.01$). It is well known that clay minerals absorb humic substances, which makes them less available to microorganisms,^{8,34} affects the stability of some components such as polysaccharides¹¹ and contributes to the formation of more stable forms.² TC soil MWD is highly correlated with HA C and O content, as well as O/C and H/C ratio (0.979**, -0.991^{**} , -0.857^* and -0.757 , respectively) suggesting increase in soil aggregate stability with increasing HA humification degree.

The C content, C/N ratio, I_{3273} , I_{2923} , and I_{1030} of HAs are in high positive correlation with TC soil pH, while it is highly negatively correlated with O content, O/C and O/H ratio, ω and I_{1620}/I_{2923} (0.928**, 0.701, 0.722, 0.805, 0.762, -0.950^{**} , -0.936^{**} , -0.732 , -0.715 and -0.730 , respectively). According to Kumada,¹⁹ higher HA humification degree in acid to strongly acid soils can be a consequence of autoxidative changes in organic materials accelerated by Fe and Mn oxides. Also, E_2/E_3 , I_{3273} and I_{2923} are significantly higher and E_{280} , RF and I_{1620}/I_{2923} lower with increasing soil organic C (0.891*, 0.932**, 0.844*, -0.887^* , -0.933^{**} , and -0.812^* , respectively). HAs have more aliphatic and less aromatic constituents, *i.e.*, lower humification degree with increase in SOM. Higher soil pH influences better vegetation cover and hence higher plant litter input, likely causing lower HAs humification degree. The highest aromaticity index, oxidation and aromatic condensation degree were obtained for TC6 HA extracted from a 15–50 cm layer covered with wind deposited Cu post-flotation tailings and no vegetation. These TC6 HA characteristics might originate from arable soils used in reclamation, but the contribution of very low soil pH and the absence of vegetation (fresh litter input) to the highest HA humification degree in

the last 20 years is more likely. Decrease in HA humification degree with increasing fresh plant litter input has already been reported.^{11,13,25}

Comparison of the results obtained in this study with those for other mine soils is difficult due to different reclamation methods or mine wastes. According to our knowledge, comparable investigations of HAs from soils formed on Cu post-flotation tailings recultivated by top layer reconstruction with arable soils are missing. Humified organic C was increased in depleted copper mine soil using organic amendments, on the contrary to tree vegetation.³⁵ Although soils on coal-mine spoils are quite different from those on Cu post-flotation tailings, results obtained indicate an increase in HS humification degree on reclaimed areas.¹¹ According to Abakumov *et al.*² calorificity of HA tends to increase with increasing site age in a reclaimed mine soil chronosequence (maximum 7–10 years), while on unreclaimed site no trend was evident, explained by ecosystem heterogeneity. In this study, increase of humification degree is not noticed for all TC HAs likely due to heterogeneous soil properties. Humification degree is probably increased only for HAs from very strongly acid soils due to influence of autoxidative changes accelerated by Fe and Mn oxides and absence of fresh litter input. These results confirm that quantity of total organic matter entering the soil, as well as soil conditions^{11,13,19,25} influence soil humification process. Pure soil conditions of Technosols studied are likely results of ineffective PFT reclamation. Soil top reconstruction process was performed by irregular mixing of arable soils and tailing, the consequence of which are strong acid conditions. Also, acid tailing was wind-transferred from non-reclaimed to reclaimed PFT areas. As already mentioned,⁷ Cu PFT remediation is difficult to accomplish, expensive and often unsuccessful. Further, residence time is an important factor influencing HA humification.^{2,11} Results of this study have shown that in the 20 years period humification changes occur only in extremely different soil conditions, while HAs in similar soil conditions were not significantly changed.

CONCLUSION

Results of elemental analysis, UV–Vis and ATR-FTIR spectroscopy point out to lower humification degree of TC HAs. Pronounced TC HAs reaggregation at pH 3 points out to less negative *ZP* values, as well as to increase in aromatic condensation degree.

According to PCA, TC and CB HAs are clearly differentiated from the second HA control group (CCH and CES) indicating that TC and CB HAs are likely of the same origin. TC HAs are not separated from the first HA control group (CB – arable soils near Bor), which probably means that no significant changes have occurred in TC HAs over the past 20 years since the recultivation, confirming stability of HAs properties over time.

Nevertheless, it is obvious that HAs extracted from very strongly acid TC soils show higher aromaticity index, oxidation and aromatic condensation degree compared to HAs from slightly acid and neutral TC, as well as CB soils. These TC HA characteristics could originate from arable soils used in reclamation, but the contribution of very strongly acid soil conditions and low fresh litter input (bare or absent vegetation) to high humification degree is more likely. Unsuccessful reclamation resulted in both very low soil pH and fresh litter input probably causing higher humification degree of HAs originated from very strongly acid TC soils. Also, their aggregation is less pronounced in comparison to other TC and control HAs studied, which could influence efficiency of HA–metal binding. It was shown that 20 years period is long enough to change TC HAs humification degree only under extremely changed soil conditions.

SUPPLEMENTARY MATERIAL

Additional data are available electronically at the pages of journal website: <http://www.shd.org.rs/JSCS/>, or from the corresponding author on request.

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ИЗВОД

ОСОБИНЕ ХУМИНСКИХ КИСЕЛИНА СА ЈАЛОВИШТА БАКРА 20 ГОДИНА НАКОН РЕКУЛТИВАЦИЈЕ

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Део постфлотационог јаловишта Serbia Zijin Bor Copper, Бор, је 1991. године рекултивисан реконструкцијом површинског слоја обрадивим земљиштем и ревегетацијом. Из формираних техносола су изоловане хуминске киселине које су испитиване са циљем да се утврди да ли је дошло до промене њихових особина 20 година после рекултивације. Коришћене су две групе контролних узорака. Елементални састав (CHNS анализа) се налазио унутар опсега карактеристичког за земљишне хуминске киселине. Хуминске киселине припадају Б типу што указује на њихов нижи степен хумифицираности (UV–Vis). Редослед релативне заступљености функционалних група је следећи: полисахаридни С ≥ ароматични С > карбоксилни С > ОН група > алифатични С. Вредности ароматичног индекса су ниске (1,88–3,25, ATR-FTIR). Основне јединице на рН 10 су у опсегу 11,7–26,8 nm. Изражена реагрегација (1462–5218 nm) на рН 3 указује на нижу стабилност сола хуминских киселина, као и на повећање степена ароматичности (DLS). Резултати показују да није дошло до значајних промена особина хуминских киселина техносола 20 година после рекултивације (РСА), што указује на њихову стабилност. Ипак, хуминске киселине из веома киселих техносола имају већи степен хумифицираности који је можда наслеђен из обрадивог земљишта коришћеног у рекултивацији, али је вероватније последица ниског рН и нижег прилива органских остатака, а који су резултат неуспешне рекултивације.

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