

Phenolic Acids as Bioindicators of Fly Ash Deposit Revegetation

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Abstract. The floristic composition, the abundance, and the cover of pioneer plant species of spontaneously formed plant communities and the content of total phenolics and phenolic acids, as humus constituents, of an ash deposit after 7 years of recultivation were studied. The restoration of both the soil and the vegetation on the ash deposits of the “Nikola Tesla-A” thermoelectric power plant in Obrenovac (Serbia) is an extremely slow process. Unfavorable physical and chemical characteristics, the toxicity of fly ash, and extreme microclimatic conditions prevented the development of compact plant cover. The abundance and cover of plants increased from the central part of the deposit towards its edges (ranging from 1–80%). *Festuca rubra* L., *Crepis setosa* Hall., *Erigeron canadensis* L., *Cirsium arvense* (L.) Scop., *Calamagrostis epigeios* (L.) Roth., and *Tamarix gallica* L. were the most abundant species, thus giving the highest cover. Humus generated during the decomposition process of plant remains represents a completely new product absent in the ash as the starting material. The amount of total phenolics and phenolic acids (38.07–185.16 µg/g of total phenolics and 4.12–27.28 µg/g of phenolic acids) in fly ash increased from the center of the deposit towards its edges in correlation with the increase in plant abundance and cover. Ash samples contained high amounts of ferulic, vanillic, and *p*-coumaric acid, while the content of both *p*-hydroxybenzoic and syringic acid was relatively low. The presence of phenolic acids indicates the ongoing process of humus formation in the ash, in which the most abundant pioneer plants of spontaneously formed plant communities play the main role. Phenolic compounds can serve as reliable bioindicators in an assessment of the success of the recultivation process of thermoelectric power plants’ ash deposits.

plant in Obrenovac (Serbia) are being deposited at designated ash depots that are subject to wind erosion. Restoration of the vegetation on ash deposits is an extremely slow process. The unfavorable physical and chemical characteristics of the fly ash and extreme microclimatic conditions prevent the development of compact plant cover, and also the development of microorganisms and collembolans and their activity as decomposers of organic matter and thus humus formation processes. According to Bogdanović (1990), the number of microorganisms in recultivated ash deposits is rather low in relation to natural soils. Bååth *et al.* (1995) found a decrease in the number of microorganisms in forest ecosystems after an application of wood ash. Also, Liiri *et al.* (2002) reported a decrease in the number of collembolans in the field, and the number of mesostigmatic mites in the laboratory after wood ash treatment. The major factors that limit the establishment of vegetation on ash disposal sites are: a lack of essential nutrients, usually N and P, toxicity caused by a high pH and/or high soluble salt concentrations, a high boron content and high concentrations of other potentially toxic trace elements, an accumulation of As, Mo, and Se in plants in quantities considered hazardous to animals, and the presence of compacted and/or cemented layers in the ash (Adriano *et al.* 1980; Haering and Daniels 1991; Dosskey and Adriano 1993; Carlson and Adriano 1993; Pavlović *et al.* 2004). Fly ash contains particles ranging in size from 0.01 to 100 µm (Adriano *et al.* 1980) whereas bottom ash contains particles greater than 2 mm in size (Carlson and Adriano 1991). In order to prevent the dispersion of fly ash, revitalization measures at the “Nikola Tesla-A” thermoelectric power plant are usually applied through the formation of a continual plant cover comprising herbaceous and woody plant species (Bogdanović 1990; Dželetović and Filipović 1995). The organic matter of plants growing at the fly ash deposits are of great importance for the formation of soil humus, which represents the product of biological soil activity and contains mixtures of different organic compounds. In addition to humic acids, humus also contains different peptides, carbohydrates, metals, and phenolic compounds (Felbeck 1971; Haworth 1971). Phenolic compounds are an important component in the humus layer of different soil types (Guenzi and McCalla 1966; Hennequin and Juste 1967; Katase 1981a,b, Whitehead *et al.* 1983; Kögel 1986; Kuiters and Denneman 1987; Djurdjević *et al.* 2000, 2003, 2004). Phenolics as secondary metabolic products of plants represent a widely distributed group of compounds in

As well as the disturbance and deterioration of large areas of arable land, the destruction of plant cover, and the disorder of ground and surface water, the effects of surface coal exploitation lead inevitably to technogenic deserts. Each year enormous quantities of ash (fly + bottom ash) resulting from coal combustion at the “Nikola Tesla-A” thermoelectric power

plant tissues. They are located in all plant organs, both as free forms and in association with other compounds such as lignin (Bate-Smith 1969; Whittaker and Feeny 1971; Harborne 1980; Kögel and Bochter 1985; Kögel 1986; Kögel-Knabner 2002) and polysaccharides of cell walls (Whitmore 1976).

Taking into account the great significance of phenolic compounds in the process of humus formation and, thus, soil fertility, the aims of this study were: (1) an analysis of the composition, abundance, and cover of pioneer plant communities seven years after the initiation of ash deposit recultivation; and (2) an estimation of the influence of vegetation abundance and cover on the content and composition of phenolic compounds in the surface layer of the ash deposit. The ash as the starting substrate contains no humus. In line with this, we hypothesized that phenolic acids as the products of the excretion of living plants, the decomposition of plant remains, and the humification process could serve as bioindicators of humus presence.

Materials and Methods

Experimental Site

The study was performed on an ash deposit from the “Nikola Tesla-A” thermoelectric power plant in Obrenovac (a town 30 km southwest of the city of Belgrade). At this thermoelectric power plant, with a total installed generator capacity of 2892 MW, 12×10^6 tons of low-caloric lignite coal are burnt per year and approximately 2.4×10^6 tons of ash are produced over the same period (Knežević *et al.* 1991). Because of this, the ash deposit area, which has been formed on fertile cultivated lands (fluvisol), at present covers an area of 400 ha and is located in the immediate vicinity of the power plant on the right bank of the Sava River. Deposition is performed by introducing the ash into three lagoons, two of which are inactive at any time. Ash deposit super structuring, carried out by erecting side dikes, is done by means of the hydrocycling of the ash, which is piped in the form of a hydraulically transported pulp (ash:water = 1:10). Coarser fractions (bottom ash) are separated by hydrocycling to form the side dikes. In terms of its chemical characteristics, the ash produced by lignite combustion is alumo-silicate (approximately 80%) with a significant share of Fe, Ca, Mg, K, and Ti oxides (approximately 18%). The ash is of alkaline reaction (pH of 7.9) and the input of fly ash contributes 80–85%, bottom ash 15–20%, and unburned coal 0.2–2.0%. Organic matter, even if present in the ash, only includes unburned coal particles. Chemical analyses demonstrated a very low concentration of total N (<0.05%, Dželetović and Filipović 1995), available P₂O₅ and K₂O (0.05–0.2%, and 0.5–0.7%, Simonović 2003), a high concentration of B, and a lack of Cu and Mn (Pavlović *et al.* 2004). Organic carbon content originating from unburned coal varied between 0.7–1%, (Filipović *et al.* 1993).

The experimental area was located at lagoon 3 (an area of approximately 75 ha). In order to stabilize the fly ash deposit, recultivation was initiated with the formation of plant cover consisting of 12 land-reclamation herbaceous plants (*Secale cereale* L., *Arrhenatherum elatius* L., *Lolium italicum* L., *Poa pratensis* L., *Festuca rubra* L., *Festuca pratensis* Huds., *Phleum pratense* L., *Dactylis glomerata* L., *Lotus corniculatus* L., *Trifolium pratense* L., *Medicago sativa* L., and *Brassica napus* L.) and 7 woody species (*Tamarix gallica* L., *Salix alba* L., *Salix viminalis* L., *Populus euramericana* (Dode) Guier, *Spiraea van-houttei* (Briot) Zbl., *Forsitia europaea* Deg. et Bald, and *Elaeagnus angustifolia* L.).

Research was undertaken at lagoon 3, seven years after the initiation of the recultivation process. Five 20-m-wide radial representative transects were established, from the overflow channel (in the center of the lagoon) towards its edges. The transects were of different lengths (Fig. 1) and were established on the basis of an estimation of the vegetation cover at the lagoon as a whole. For each transect, an estimation of the abundance and cover of the vegetation (from the central part of the lagoon with the lowest plant cover towards the peripheral parts of the deposit with the highest plant cover) was made. Each of the transects was divided into six zones in accordance with the level of cover: 1 (1%); 2 (5%); 3 (50%); 4 (60%); 5 (70%); and 6 (80%). The number and cover of plant species were estimated using the scale of Westhoff and van der Marrel (1973). In each zone, an experimental plot, 20 m \times 20 m in size, was established. There was an overall total of 30 experimental plots with each being divided into rectangles, 5 m \times 5 m in size. Fly ash samples were collected from the center of each rectangle, at a depth of 0–10 cm, for analysis, giving a collective sample from each experimental plot, made up of 16 individual samples. In this way, five collective samples were obtained for each of the cover categories.

Sampling of Fly Ash

Fly ash samples for analysis were collected, under plants, at a depth of 0–10 cm. After the removal of visible plant remains, the ash samples were air dried (25°C) at room temperature (Kögel and Bochter 1985), and sifted through a sieve (0.5-mm mesh). Native ash collected from the active lagoon where there is no vegetation served as a control. Control samples are labelled as 0 in all figures.

Extraction of Phenolics from Ash Samples

Total phenolics and phenolic acids were extracted by boiling 30 g of dry ash in 30 ml of 2N NaOH for 24 hours. After that, the aqueous phase was separated, the pH adjusted to 2.0 with concentrated HCl, and the mixture was transferred to ethyl acetate and evaporated. The dry residues were dissolved in 4.0 ml of 80% (v/v) methanol and maintained at –20°C until analysis (Katase 1981a, b; Hennequin and Juste 1967). Each sample was taken in five replicates.

Determination of Total Phenolics

Total phenolics were measured spectrophotometrically (a Shimadzu UV 160 spectrophotometer) using the Folin-Ciocalteu reagent (Feldman and Hanks 1968). A standard curve was constructed with different concentrations of ferulic acid. Units of total phenolics were expressed in μ g of ferulic acid equivalent per gram of fly ash dry weight.

Determination of Phenolic Acids by HPLC

Phenolic acids were detected between 210 and 360 nm using a Hewlett Packard diode array detector (HP 1100 HPLC system). The separation was achieved with a Nucleosil 100–5 C₁₈ column; 5 μ m; 4.0 \times 250 mm (Agilent Technologies). A step-gradient of acetonitrile in water was used: 15% acetonitrile (5 min, gradient), 30% acetonitrile (20 min, gradient), 40% acetonitrile (25 min, gradient), 60% acetonitrile (30 min, gradient), 60% acetonitrile (35 min, gradient), and 100% acetonitrile (45 min, isocratic) at a flow rate of 1.0 ml/min,

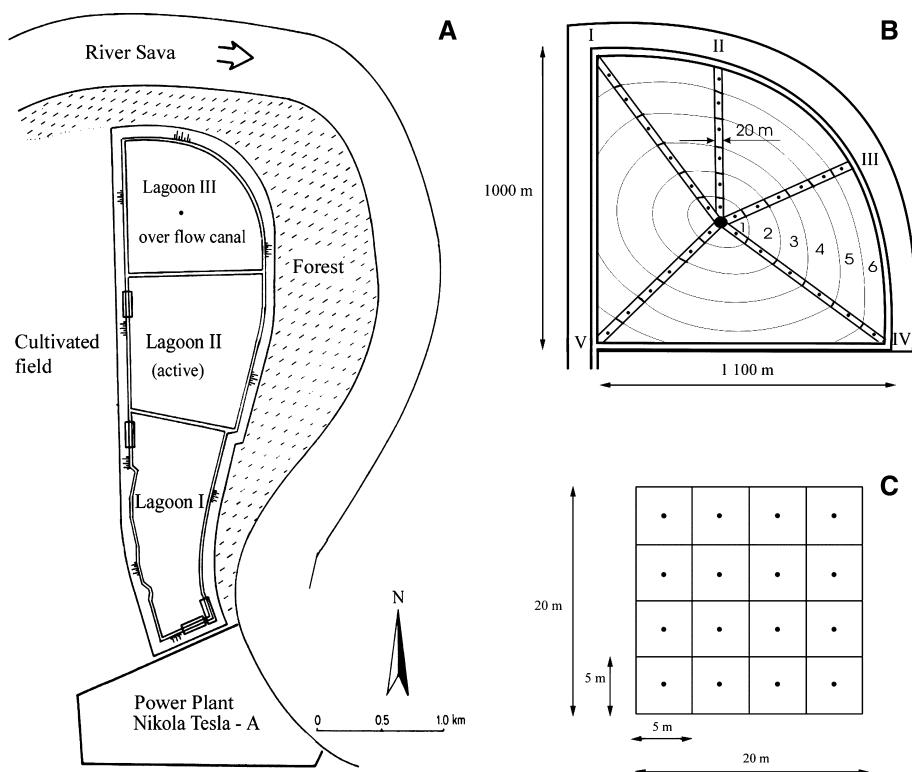


Fig. 1. Plan of the Nikola Tesla-A ash deposit in Obrenovac. Research was undertaken at lagoon 3 (A); transects (I–V) where the dots represent a total of 30 experimental plots, 6 on each transect: (1) a zone with 1% vegetation cover, (2) with 5% cover, (3) with 50% cover, (4) 60%, (5) 70% and (6) 80% (B); a diagram of the experimental plots from which 16 samples of ash were taken from a depth of 0–10 cm and a collective sample obtained (C).

the injection volume being 5.0 μl . In order to avoid the tailing of the phenolic acids, 0.05% *o*-phosphoric acid was added to the solvents. Individual phenolic acids were identified by comparing the retention times and absorption maximum with those of *p*-hydroxybenzoic and syringic acid (Acros Organics), ferulic, vanillic, and *p*-coumaric acid (Serva, Germany), serving as phenolic standards. The content of phenolic acids was expressed in $\mu\text{g/g}$ of fly ash dry weight.

The statistical evaluation of the differences in the total content of phenolics and the composition of phenolic acids in ash samples was performed with two-sided ANOVA tests. Data were processed using the statistical package Statistica 6.0 for Windows.

Results

Analysis of Pioneer Plant Communities

In the ash deposit of the “Nikola Tesla-A” thermoelectric power plant, only fragments of spontaneously formed plant communities were developed during the 7-year period. Out of 19 plant species at the start of the recultivation process, only 5 species (*Tamarix gallica* L., *Festuca rubra* L., *Spiraea van-houttei* (Briot) Zbl., *Lotus corniculatus* L., and *Medicago sativa* L.) remained. In the meantime, 31 plant species originating from the surrounding area had appeared, making a total of 36 plant species in the ash deposit itself. The highest number of plant species was recorded at the periphery and the lowest in the center of the ash deposit (Table 1). The abundance and cover of pioneer plant species was found to increase from the center of the ash deposit towards its edges from 1% to 80%. The most numerous were *Festuca rubra* L., *Crepis setosa* Hall., *Calamagrostis epigeios* (L.) Roth., *Erigeron canadensis* L., *Cirsium arvense* (L.) Scop., *Xanthium italicum* Mor., *Tamarix*

gallica L., *Verbascum phlomoides* L., and *Eupatorium cannabinum* L. Herbaceous plants accounted for 89% of the total number of plant species present in the ash deposit. In terms of the soil acidity, 63% of plant species were neutrophil growing in neutral to weakly acidic soils. In addition, mesotrophic plants occurring in soil that was moderately rich in mineral matters accounted for 61% of the total. Most of the recorded plants inhabit not only extremely arid, but also mesophyllic habitats (according to ecological groups by Ellenberg 1956; Landolt 1977; Soo 1980).

Total Phenolics and Phenolic Acids in Ash Samples

The results of the amount of total phenolics in ash samples are depicted in Figure 2. The presence of phenolics was not detected in native ash (control). The content of total phenolics increased from the center of the deposit towards its edges, parallel to the increase in plant abundance and cover. The difference in the content of total phenolics was especially pronounced in the experimental zones along the transects with the highest plant cover (70% and 80%), 4 to 5 times more than in the zone with 1% cover.

In ash samples collected from zones with differing vegetation cover, five phenolic acids (*p*-coumaric, ferulic, *p*-hydroxybenzoic, vanillic, and syringic) were detected. Ferulic acid (4-hydroxy-3-methoxycinnamic) and *p*-coumaric acid (*trans*-4-hydroxycinnamic acid) represent cinnamic acid derivatives, and *p*-hydroxybenzoic acid, vanillic acid (4-hydroxy-3-methoxy-benzoic acid), and syringic (3,5-dimethoxy-4-hydroxybenzoic acid) acid are benzoic acid derivatives. The amount of phenolic acids increased with distance

Table 1. Abundance and cover of plants at transects at the ash deposit

Plant species	1. 1%	2. 5%	3. 50%	4. 60%	5. 70%	6. 80%
<i>Tamarix gallica</i> L.	—	5	—	3	—	—
<i>Festuca rubra</i> L.	1	3	7	5	7	3
<i>Crepis setosa</i> Hall.	—	3	5	3	5	5
<i>Epilobium collinum</i> Gmell.	—	2	—	—	2	2
<i>Erigeron canadensis</i> L.	2	2	3	3	3	5
<i>Verbascum phlomoides</i> L.	1	2	—	2	—	3
<i>Cirsium arvense</i> (L.) Scop.	1	2	2	—	3	5
<i>Calamagrostis epigeios</i> L.	—	—	7	—	3	2
<i>Dianthus armeria</i> L.	—	—	3	—	—	—
<i>Eupatorium canabinum</i> L.	—	—	2	—	2	3
<i>Spiraea van-houttei</i> Br. Zal.	—	—	—	3	—	—
<i>Linaria vulgaris</i> Mill.	—	—	—	2	—	—
<i>Echium vulgare</i> L.	—	—	—	2	—	3
<i>Bromus arvensis</i> L.	—	—	—	2	3	—
<i>Xanthium italicum</i> Mor.	—	—	—	2	2	3
<i>Geranium dissectum</i> Jusl.	—	—	—	2	—	—
<i>Sorghum halepense</i> L.	—	—	—	2	—	3
<i>Silene vulgaris</i> Garcke	—	—	—	2	—	—
<i>Melilotus albus</i> Med.	—	—	—	—	2	2
<i>Medicago lupulina</i> L.	—	—	—	—	2	2
<i>Rubus caesius</i> L.	—	—	—	—	2	—
<i>Centaurium umbellatum</i> Gil.	—	—	—	—	2	—
<i>Cynodon dactylon</i> L.	—	—	—	—	—	3
<i>Ambrosia artemisifolia</i> L.	—	—	—	—	—	3
<i>Amorpha fruticosa</i> L.	—	—	—	—	—	3
<i>Plantago lanceolata</i> L.	—	—	—	—	—	3
<i>Cichorium intybus</i> L.	—	—	—	—	—	3
<i>Agrostis capillaris</i> L.	—	—	—	—	—	2
<i>Lotus corniculatus</i> L.	—	—	—	—	—	2
<i>Cardus acanthoides</i> L.	—	—	—	—	—	2
<i>Setaria viridis</i> B.P.	—	—	—	—	—	2
<i>Dipsacus laciniatus</i> L.	—	—	—	—	—	2
<i>Echium italicum</i> L.	—	—	—	—	—	2
<i>Plantago major</i> L.	—	—	—	—	—	2
<i>Medicago sativa</i> L.	—	—	—	—	—	2
<i>Daucus carota</i> L.	—	—	—	—	—	2
Number of plant species	4	7	7	13	13	27

Each transect was divided into 6 zones, from the central part of the lagoon with the lowest plant cover, towards the peripheral parts of the deposit with the highest plant cover: 1: 1%; 2: 5%; 3: 50%; 4: 60%; 5: 70%; and 6: 80%. For each plant species, the numbers represent an estimation of its abundance and cover in the examined transects, expressed in numbers on a scale of 1–9, equivalent to abundance of 1–100%, according to Westhoff and van der Marrel (1973).

from the center of the deposit, parallel to the increase in plant abundance and cover (Figs. 3 and 4). In all the ash samples collected from the different zones (except the native ash), ferulic, vanillic, and *p*-coumaric acid prevailed, while the lowest content found was of *p*-hydroxybenzoic acid. Syringic acid was identified only in those samples taken from the zone with 70% vegetation cover. The share of cinnamic acid derivatives in total phenolics ranged from 6.75% to 9.60% and that of benzoic acid derivatives from 6.05% to 6.50%. A significant increase in the content of benzoic acid derivatives,

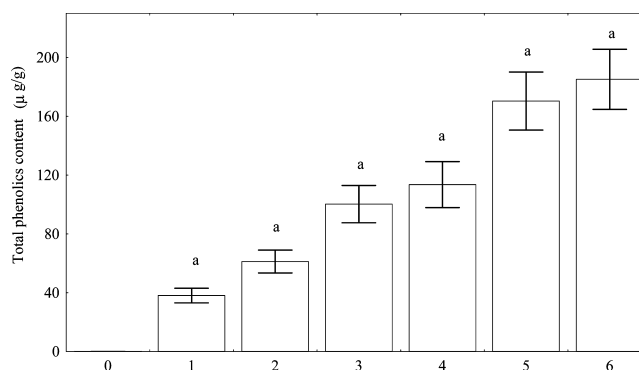


Fig. 2. Content of total phenolics ($\mu\text{g/g}$) in the ash samples taken from ash deposit transects differing with regard to plant cover (0: control, 1: zone with 1% vegetation cover, 2: 5%, 3: 50%, 4: 60%, 5: 70%, and 6: 80%, ANOVA; $^a p < 0.001$; $n = 5$)

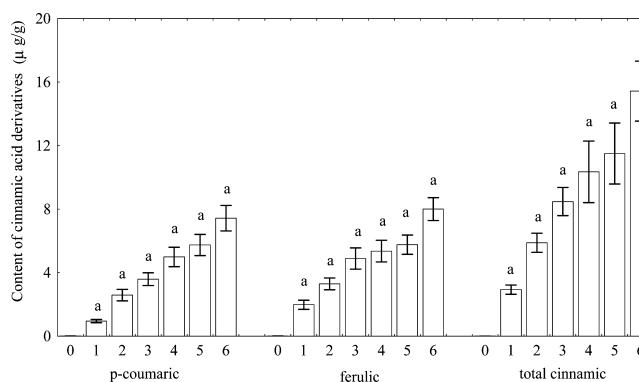


Fig. 3. Content of cinnamic acid derivatives ($\mu\text{g/g}$) in the ash samples taken from ash deposit transects differing with regard to plant cover (0: control, 1: zone with 1% vegetation cover, 2: 5%, 3: 50%, 4: 60%, 5: 70%, and 6: 80%, ANOVA; $^a p < 0.001$; $n = 5$)

above all of vanillic acid, corresponded closely to the increase in cover and was much more prominent than that of cinnamic acid derivatives (Fig. 5). This is also obvious from the cinnamic acid/benzoic acid ratio that decreased from 3.97 in the center (1% cover) to 1.30 that was found in the periphery (80% cover). A statistically significant difference between the content of cinnamic acid derivatives and benzoic acid derivatives was recorded in those samples collected from zones with vegetation cover ranging from 1% to 50% ($p = 0.05$ – 0.01). However, the difference between the content of these two groups of phenolic acids in the samples taken from zones with the highest plant cover (60%–80%) was not statistically significant. An approximately uniform content of cinnamic and benzoic acid derivatives in the zones characterized by 60–80% plant cover resulted from an abrupt increase in the content of vanillic acid. Phenolic acids contributed as much as 12.22–15.61% of total phenolics in the ash samples. A positive correlation between the content of total phenolics, total phenolic acids, cinnamic, and benzoic acid derivatives on the one hand and the plant cover of zones along the transects on the other hand was observed (Fig. 5). The greatest difference ($p < 0.001$) in the content of total phenolics and phenolic acids was found between native ash and zones with different vegetation cover (Figs. 2–4).

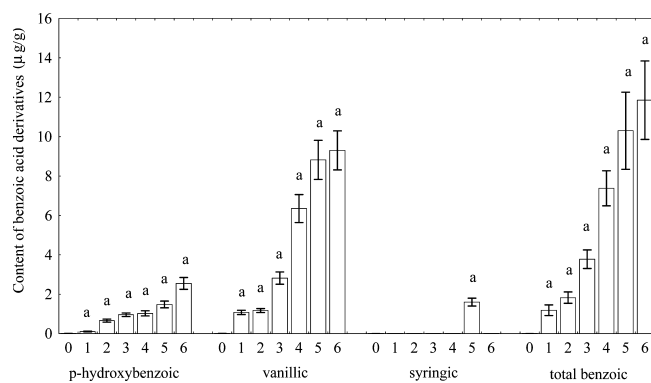


Fig. 4. Content of benzoic acid derivatives ($\mu\text{g/g}$) in the ash samples taken from ash deposit transects differing with regard to plant cover (0: control, 1: zone with 1% vegetation cover, 2: 5%, 3: 50%, 4: 60%, 5: 70%, and 6: 80%, ANOVA; ^a $p < 0.001$; $n = 5$)

Discussion

Vegetation establishment on ash deposit sites is limited by the lack of essential nutrients (mostly N and P), toxicity caused by a high pH and/or high concentrations of soluble salts, a high boron content, high concentrations of some potentially toxic heavy metals, and the presence of compact and/or cemented layers (Haering and Daniels 1991; Carlson and Adriano 1993). Because of this, soil and vegetation restoration on ash deposits is an extremely slow process. In the fly ash deposit of the Nikola Tesla-A thermoelectric power plant in Obrenovac, only fragments of spontaneous plant communities had been developed after 7 years after recultivation. Our recent study showed that species that are tolerant to drought and toxic B concentrations, with moderate demands in terms of mineral elements, have the ecological potential to overgrow an ash deposit site (Pavlović *et al.* 2004). According to Hodgson and Townsend (1973) and Bradshaw and Chadwick (1980), plants that are N_2 fixers or are tolerant to high salinity and boron are the most successful early colonizers. Results of this study confirmed the findings mentioned above since only 5 of the 19 plant species used for recultivation remained after the 7-year period, and 31 species appeared at the deposit through the natural invasion of vegetation from surrounding areas. The natural invasion of herbaceous plants, which plays an important role in protecting the terrain from wind erosion, could provide one course for recultivation activities, which, with a minimum of investment, would enable the binding of a mobile substrate such as ash.

The highest abundance and cover of plants was recorded on the periphery of the lagoon, which indicates less stressful ecological conditions for plants in such zones. The abundance and cover of vegetation decreased towards the central parts of the lagoon as ecological conditions became more stressful. It can be concluded that the 7 years of succession of vegetation is a very short period to allow it to overgrow the whole area. However, the existing composition of plant species as a factor of utmost importance for soil formation corresponded closely to the extreme ecological conditions for plant growth in the ash deposit examined.

All the plant species growing in the ash deposit over the 7 years greatly influenced the revegetation process and humus formation. The dominant plant species, *i.e.*, the most abundant

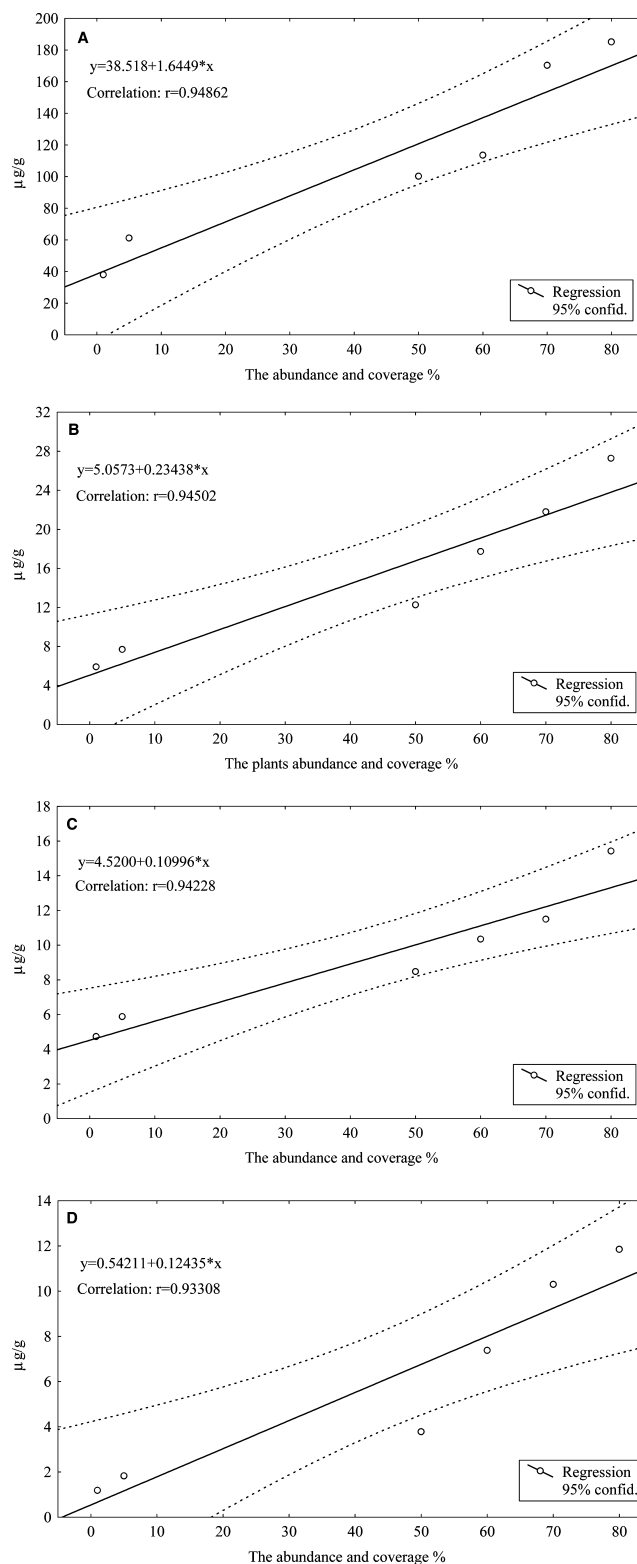


Fig. 5. Correlation between the content of total phenolics (A), total phenolic acids (B), cinnamic acid derivatives (C), and benzoic acid derivatives (D) and plant cover of the ash deposit

plants with the largest above-ground and below-ground mass, were the main source of phenolic compounds at the ash deposit site. Our results proved an absence of phenolic compounds in

native ash. Phenolic compounds detected in ash 7 years later are the result of the establishment of a vegetative canopy at the lagoon, with herbaceous plants dominant. Also, the gradient of increase in phenolic compound content was established in relation to the increase in vegetation cover. Namely, the content of total phenolics detected in the experimental zones along the transects with the highest plant cover (70% and 80%) was approximately five times higher than in the zones with the lowest cover (1%). Our results are in accordance with Gallet *et al.* (1999) and Séné *et al.* (2001), who found a higher content of water-soluble phenolic compounds in soils where *Empetrum hermaphroditum* and *Sorgum bicolor* dominate than in areas without these plant species, indicating the influence of vegetation on the content of total phenolics and phenolic acids in soils.

In natural ecosystems, phenolics from plants transfer into soil by foliage and stem leaching, leaf falling, as well as by root system exudates (Rice 1974, 1979; Tang and Young 1982). Similar processes are occurring at the fly ash deposit where a spontaneous pioneer community is being developed. Our results demonstrate that the increase in the amount of total phenolics and phenolic acids in fly ash with distance from the center of the ash deposit was closely correlated to changes in plant abundance and cover, *i.e.*, with an increase in above-ground and below-ground plant biomass. The presence of phenolic acids was detected in all ash samples collected from the different zones, except in native ash. Ferulic, vanillic, and *p*-coumaric acid prevailed, while the lowest content found was of *p*-hydroxybenzoic acid. Derivatives of cinnamic acid dominated in comparison to derivatives of benzoic acid in all ash samples. One possible explanation for this could be the domination of herbaceous plants, mainly grasses, over woody species at the ash deposit site. Namely, the results of Tsutsuki *et al.* (1994) indicated a high percentage of cinnamic acids mainly derived from the non-woody tissues of angiosperm plants (*e.g.*, mainly grasses), suggesting that grasses were thriving.

Our results showed that the significant increase in vanillic acid content in fly ash corresponded closely to the increase in vegetation cover. This is in accordance with the findings of Gallet *et al.* (1999), Djurdjević *et al.* (2000), and Séné *et al.* (2001), who found a higher content of vanillic acids than other phenolic acids in soil samples although the content in vegetative plant parts is rather low. This could be connected to the microbial metabolism of lignin, *i.e.*, the degradation of lignin results in ferulic acid, giving vanillin that is converting to vanillic acid. Also, some bacteria, especially *Pseudomonas fluorescens*, can convert the vanillin biologically into vanillic acid. Lignin or lignin-derived molecules are of significance in the formation of humus (Martin and Haider 1969, 1971; Alexander 1977; Crawford and Crawford 1980; Bare *et al.* 1994). Microorganisms themselves synthesize some phenolic compounds that contribute to the content of phenolics found in soil samples. Microorganisms are involved primarily in the synthesis of monomer phenolic acids, *e.g.*, derivatives of benzoic or cinnamic acid, but also of some polyphenol compounds (Martin and Haider 1969, 1971). An increase in the content of vanillic acid as distance from the center increased was detected in relation to plant cover. Therefore, zones with the highest vegetation cover were the source of the highest content of lignin, resulting in vanillin as a deg-

radation product, and it was converted to vanillic acid by microbial activity.

The content of total phenolics in the fly ash was several times lower in comparison to the natural soils. Thus, the content of total phenolics in different soils is as follows: in forest-peat bog 6561.3 µg/g (Djurdjević *et al.* 2003), in mixed Austrian and Scots pine forest 2678.5 (Djurdjević *et al.* 1998a), in beech-fir forest 1770 µg/g (Djurdjević *et al.* 2004), in inundate pedunculate oak forest 1589.6 µg/g (Djurdjević *et al.* 1999), in virgilius' oak forest 708.9 µg/g (Djurdjević *et al.* 1998b), and in forest-steppe sandy soil 598.8 µg/g (Djurdjević *et al.* 2000). After 7 years, fly ash contained only 185.16 µg/g of total phenolics in the zone with the highest vegetation cover (80%), *i.e.*, 35 times less than in peat soil and approximately 3 times less than in sandy soil. In native ash samples, there were no phenolics. Their presence in zones with different cover, although less than in natural soils, indicated the initiation of pedogenetic processes, processes of organic matter transformation, and humus formation. The usage of phenolics as indicators of initial pedogenetic processes at fly ash deposits can be a more reliable approach than a simple measurement of organic matter. Organic matter content in fly ash ranging from 0.2 to 2% as a result of unburned coal can cause a deviation in estimates of organic matter alone, and in the influence of plants in restoring such sites. On the other hand, the presence of phenolics as secondary plant metabolites is a reliable sign of the influence of plants in the overbuilding of biologically sterile substrate such as fly ash. Also, they show the level of improvement of substrate quality for plant colonization in comparison to native ash. An analysis of phenolics in combination with an estimation of the abundance, cover, and adaptive potential of plants can provide a clearly defined course for the restoration (revegetation) not only of such sites but of other anthropogenically disturbed landscapes as well.

Conclusions

In the ash deposit of the Nikola Tesla-A thermoelectric power plant in Obrenovac (Serbia), only fragments of spontaneously formed plant communities had been developed in the 7 years since the start of the recultivation process. Out of the 19 plant species at the start of this process, only 5 species remained. Those plant species from the surrounding areas with the highest adaptive potential were the most abundant in the ash deposit. The dominant, *i.e.*, the most abundant, plant species with the largest above-ground and below-ground mass such as *Festuca rubra* L., *Crepis setosa* Hall., *Erigeron canadensis* L., *Cirsium arvense* (L.) Scop., *Calamagrostis epigeios* (L.) Roth., and *Tamarix gallica* L., played the most important role in humus formation. The abundance and cover of pioneer plant species increased gradually with distance from the center of the ash deposit from 1% to 80%. In native ash, no phenolics were detected. The amount of total phenolics and phenolic acids in fly ash samples also increased with distance from the center of the deposit, corresponding closely to the increase that was observed in plant abundance and cover. Phenolic acids constituted 12.22–15.61% of total phenolics. Ferulic, vanillic, and *p*-coumaric acid prevailed in all samples, while the content of *p*-hydroxybenzoic and syringic acid was the lowest. An increase in the share of benzoic acid derivatives was in clear

correlation with the increase in plant cover, primarily due to the elevated levels of vanillic acid. The presence of phenolic compounds in biologically empty spaces such as ash deposit sites, after their recultivation and spontaneous recolonization, is a reliable indicator of the continuance of initial pedogenetic and humus formation processes. Through the detection and analysis of phenolics, it is possible to estimate the level of success of different recultivation activities for creating more favourable sites for plant colonization and increasing the biodiversity of disturbed landscapes.

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References

- Adriano DC, Page AL, Elseewi AA, Chang AC, Straughan I (1980) Utilization and disposal of fly ash and other coal residues in terrestrial ecosystems: A review. *J Environ Qual* 9:333–334
- Alexander M (1977) Lignin decomposition. In: *Introduction to soil microbiology*, 2nd ed. John Wiley and Sons, New York, pp 174–187
- Bååth E, Frostegård Å, Pennanen T, Fritze H (1995) Microbial community structure and pH response in relation to soil organic matter quality in wood-ash fertilized, clear-cut or burned coniferous forest soils. *Soil Biol Biochem* 27:229–240
- Bare G, Delaunoy V, Rikir R, Thonart P (1994) Bioconversion of vanillin into vanillic acid by *Pseudomonas fluorescens* strain Btp9-reactor design and parameters optimization. *Appl Biochem Biotechnol* 45:599–610
- Bate-Smith EC (1969) Flavonoid patterns in the Monocotyledons. In: Harborne JB, Swain T (eds) *Perspectives in phytochemistry*. Academic Press, New York, pp 167–177
- Bogdanović V (1990) The number of some microorganisms in ash deposit under *Robinia pseudoacacia* of the "Lazarevac" thermoelectric power plant. *Soil Plant* 39:139–145 (in Serbian)
- Bradshaw AD, Chadwick MJ (1980) The restoration of land. University of California Press, Berkeley, California
- Carlson CL, Adriano DC (1991) Growth and elemental content of two tree species growing on abandoned coal fly ash basins. *J Environ Qual* 20:581–587
- Carlson CL, Adriano DC (1993) Environmental impacts of coal combustion residues. *J Environ Qual* 22:227–247
- Crawford DL, Crawford RL (1980) Microbial degradation of lignin. *Enzyme Microb Technol* 2:11–22
- Djurđević L, Dinić A, Mitrović M, Pavlović P (1998a) Allelopathy of *Erica carnea*, a dominant species in the mixed pine forest at Maljen mountain (Serbia). In: Tsekos J, Moustakas M (eds) *Progress in botanical research*. Kluwer Academic Publishers, Dordrecht, pp 289–293
- Djurđević L, Dinić A, Kuzmanović A, Kalinić M (1998b) Phenolic acids (PA) and total phenols in soil, litter and dominating plant species in community *Orno-Quercetum virgilianae* Gajić 1952. *Arch Biol Sci (Belgrade)* 50:21–28
- Djurđević L, Milenković M, Pavlović P, Kostić O (1999) Allelopathic investigations in the *Fraxino angustifoliae-Quercetum roboris* (Jov. et Tomić 1979) forest community with the autumnal truffle (*Tuber macrosporum* Vitt.). *Arch Biol Sci (Belgrade)* 51:27–33
- Djurđević L, Dinić A, Stojčić V, Mitrović M, Pavlović P, Oldja M (2000) Allelopathy of *Paeonia officinalis* L. 1753 ssp. banatica (Rochel) Soo 1945, a Pannonian endemic and relict species. *Arch Biol Sci (Belgrade)* 52:195–201
- Djurđević L, Dinić A, Mitrović M, Pavlović P, Tešević V (2003) Phenolic acids distribution in a peat of the relict community with Serbian spruce in the Tara Mt. forest reserve (Serbia). *Eur J Soil Biol* 39:97–103
- Djurđević L, Dinić A, Pavlović P, Mitrović M, Karadžić B, Tešević V (2004) Allelopathic potential of *Allium ursinum* L. *Biochem System Ecol* 32:533–544
- Dosskey MG, Adriano DC (1993) Trace element toxicity in VA mycorrhizal cucumber grown on weathered coal fly ash. *Soil Biol Biochem* 25:1547–1552
- Dželetović S, Filipović R (1995) Grain characteristics of crops grown on power plant ash and bottom slag deposit. *Resourc Conserv Recycl* 13:105–113
- Ellenberg H (1956) Basis of vegetation degradation. Part 1: Tasks and Methods in Vegetation Science. In: Walter H (ed) *Introduction to Phytology*, Vol. 4. Ulmer, Stuttgart
- Felbeck GTJR (1971) Structural hypothesis of soil humic acids. *Soil Sci* 111:42–48
- Feldman AW, Hanks RW (1968) Phenolic content in the roots and leaves of tolerant and susceptible citrus cultivars attacked by *Rodopholus similis*. *Phytochemistry* 7:5–12
- Filipović R, Simić S, Stojanović D, Dražić G, Dželetović Ž, Lazarević M (1993) Power plants as potential sources of pollution to ecosystems, with special attention to ash deposits. *Energetics of Belgrade, status and perspectives*, Society of Engineers and Technicians of Belgrade, Proceedings, pp 371–379
- Gallet C, Nilsson MC, Zackrisson O (1999) Phenolic metabolites of ecological significance in *Empetrum hermaphroditum* leaves and associated humus. *Plant Soil* 210:1–9
- Guenzi WD, McCalla TM (1966) Phytotoxic substances extracted from soil. *Soil Sci Soc Am Proc* 30:214–216
- Haering KC, Daniels LW (1991) Fly ash: Characteristics and use in mined land reclamation: a literature review. *Virginia Coal Energy Res J* 3:33–46
- Harborne JB (1980) Plant phenolics. Secondary plant products. In: Bell EA, Charlwood BV (eds) *Encyclopedia of Plant Physiology*, New series. Springer-Verlag, New York, Vol 8, pp 329–402
- Haworth RD (1971) The chemical nature of humic acid. *Soil Sci* 111:71–79
- Hennequin JR, Juste C (1967) Présence d'acides phénols libre dans le sol: Etude de leur influence sur la germination et la croissance des végétaux. *Ann Agron* 18:545–569
- Hodgson DR, Townsend WN (1973) The amelioration and revegetation of pulverized fuel ash. In: Hutnik RJ, Davis G (eds) *Ecology and reclamation of devastated land*, Vol. 2. Gordon and Breach, London, pp 247–270
- Katase T (1981a) The different forms in which *p*-coumaric acid exists in a peat soil. *Soil Sci* 131:271–275
- Katase T (1981b) The different forms in which *p*-hydroxybenzoic, vanillic, and ferulic acids exist in a peat soil. *Soil Sci* 132:436–443
- Knežević D, Grbović B, Jovičić Ž, Djuknić P, Stupar S, Dražević D (1991) Ash deposits as air pollutions and measures of protection. In: *Proceedings of Influence of Power Plants in Belgrade Zone in Air Quality Symposium*, 17–18 October 1991, Belgrade, Yugoslavia (in Serbian), pp 77–86
- Kögel I (1986) Estimation and decomposition pattern of the lignin component in forest humus layers. *Soil Biol Biochem* 18:589–594
- Kögel I, Bochter R (1985) Characterization of lignin in forest humus layers by high-performance liquid chromatography of cupric oxide oxidation products. *Soil Biol Biochem* 17:637–640
- Kögel-Knabner I (2002) The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil Biol Biochem* 34:139–162

- Kuiters AT, Denneman CAJ (1987) Water-soluble phenolic substances in soils under several coniferous and deciduous tree species. *Soil Biol Biochem* 19:765–769
- Landolt E (1977) Ecological Survey of Switzerland Flora. Geobot Inst ETH, Zurich
- Liiri M, Haimi J, Setälä H (2002) Community composition of soil microarthropods of acid forest soils as affected by wood ash application. *Pedobiologia* 46:108–124
- Martin JP, Haider K (1969) Phenolic polymers of *Stachybotrys atra*, *Stachybotrys chartarum* and *Epicoccum nigrum* in relation to humic acid formation. *Soil Sci* 107:260–270
- Martin JP, Haider K (1971) Microbial activity in relation to soil humus formation. *Soil Sci* 111:54–70
- Pavlović P, Mitrović M, Djurdjević L (2004) An ecophysiological study of plants growing on the fly ash deposits from the “Nikola Tesla-A” thermal power station in Serbia. *Environ Manage* 33:654–663
- Rice EL (1974) Allelopathy. Academic Press, New York, San Francisco, London
- Rice EL (1979) Allelopathy—an update. *Botan Rev* 45:15–109
- Séne M, Gallet T, Doré T (2001) Phenolic compounds in a Sahelian sorghum (*Sorghum bicolor*) genotype (CE145-66) and associated soils. *J Chem Ecol* 27:81–92
- Simonović B (2003) Report on waste, surface and ground waters monitoring in the “Nikola Tesla-A” thermal power station at Obrenovac. Holding Institute of General and Physical Chemistry, Belgrade, p 36
- Soo R (1980) Flora and Vegetation of Hungary. Akademia Kiado, Budapest
- Tang CS, Young CC (1982) Collection and identification of allelopathic compounds from the undisturbed root system of Bigalta Limpograss (*Hemarthria altissima*). *Plant Physiol* 69:55–160
- Tsutsuki I, Esaki K, Kuwatsuka S (1994) CuO-oxidation products of peat as a key to the analysis of the paleo-environmental changes in a wetland. *Soil Sci Plant Nutr* 40:107–116
- Westhoff V, van der Marrel E (1973) The Braun-Blanquet approach. In: Whittaker RH (ed) *Handbook of Vegetation Science V. Ordination and Classification of Communities*. Junk, The Hague, pp 617–726
- Whitehead DC, Dibb H, Hartley RD (1983) Bound phenolic compounds in water extracts of soils, plant roots and leaf litter. *Soil Biol Biochem* 15:133–136
- Whitmore FW (1976) Binding of ferulic acid to cell walls by peroxidases of *Pinus elliotii*. *Phytochemistry* 15:375–378
- Whittaker RH, Feeny PP (1971) Allelochemicals: Chemical interaction between species. *Science* 171:757–770