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Original scientific paper

Complex effect of *Robinia pseudoacacia* L. and *Ailanthus altissima* (Mill.) Swingle growing on asbestos deposits: Allelopathy and biogeochemistry

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Abstract: Asbestos is widely mined and used around the globe posing a great risk to environment and human health. The main objective of this study was to determine allelopathic potential of *Robinia pseudoacacia* L. and *Ailanthus altissima* (Mill.) Swingle growing on the asbestos deposits at abandoned mine "Stragari" in central Serbia. The pH, content of carbon, nitrogen, calcium carbonate, available phosphorous and potassium, content of Fe, Ni, Cu, Zn, Pb, Mn, and phenolics were analyzed in the control asbestos (zones without vegetation cover) and plant rhizospheric asbestos. Allelopathic activity of plant species was assessed by "rhizosphere soil method", and *Trifolium pratense* L. and *Medicago sativa* L. were used as the indicator species. *A. altissima* showed higher allelopathic potential compared to *R. pseudoacacia* for *T. pratense* and *M. sativa* due to greater content of phenolics. Alleopathic activity of phenolics in rhizospheric asbestos was highly correlated with pH, content of carbon and nitrogen, available phosphate and potassium, and content of Ni, Cu, Zn, Pb and Mn. *A. altissima* increased phenolics content in rhizospheric asbestos inhibiting the plant growth. This woody plant in spite of high allelopathic potential is suitable for revegetation of distrurbed ecosystems because it initiates pedogenesis and affects the asbestos chemistry.

Keywords: woody species; allelochemicals; degraded habitats; phenolic acids; flavonoids; radicle growth inhibition.

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INTRODUCTION

Asbestos minerals are naturally-occurring fibrous silicates (chrysotile, amosite, crocidolite, tremolite, anthophyllite, and actinolite) which have been widely mined and used due to low thermal conductivity, high mechanical strength, resistance to chemical and biological attacks, and low cost.[1] Asbestos and asbestos--containing materials have been used for over a millennium, and evidence for respiratory diseases is associated with human exposure to asbestos fibers.[2] Prolonged exposure to asbestos fibers can result in development of dangerous diseases such as lung cancer and mesothelioma.[3,4] Some experts have appealed to countries to cancel asbestos mining and abandon utilization of asbestos-containing materials.[5,6] The abandoned asbestos mines leave deposits which pose a potential risk to environment and human health because they are very close to settlements, rivers, agricultural fields and pastures.[7,8]

Vegetation development on the mine waste prevents wind/water erosion, reduces toxicity of heavy metals and provides aesthetic landscape.[9] Woody plants with large cover and high biomass may have an important role in the process of revegetation of waste deposits.[10] Furthermore, organic matter originating from plants could be of great importance in the process of soil humification where phenolic compounds are very significant.[11,12]

Allelopathy presents interactions between plants through the action of allelochemicals.[12,13] Phenolic compounds as the most important group of allelochemicals in ecosystems have important role in the dynamics of mineral and organic compounds in the soil due to their effect on the soil chemical properties, availability of heavy metals and the microorganism community.[12,14–17] Phenolics enrich the soil through leachates from plant parts and plant litter, and can be transformed and metabolized by soil microbes, or bound to the soil organic matter.[18,19] High content of phenolics in the soil leads to the inhibition of seed germination and plant growth reducing the number of herbaceous plant species.[16,20,21] According to Inderjit and Weiner,[22] progress in allelopathy can be reached through connection with soil chemistry rather than in direct plant–plant chemical interactions.

Robinia pseudoacacia L. (native in North America) and *Ailanthus altissima* (Mill.) Swingle (native in China) have become naturalized in many parts of Europe, and in Serbia are considered as non-indigenous invasive plant species.[23] High invasion capacity of *R. pseudoacacia* and *A. altissima* is the result of very effective generative and vegetative reproduction,[24,25] as well as allelopathic activity of plants.[17,25–28] Generally, phenolics that were found in *R. pseudoacacia* and *A. altissima* tissues can act as allelochemicals and possess a high allelopathic activity.[24,25] Most studies are dealing with allelopathy in natural habitats or in laboratory, but knowledge regarding allelopathic activity of woody plants from anthropogenically disturbed sites is still missing.

Woody plants are important for understanding the mechanisms by which some plant species can alter plant community structure and ecosystem processes on contaminated sites. No comprehensive study of revegetation of asbestos mine deposits in Serbia and allelopathic interactions between non-native woody and herbaceous native plant species has been performed. In addition, abandoned asbestos mine deposits present biologically empty space suitable for plant colonization and revegetation. Therefore, the objectives of this study were: a) analysis of chemical characteristics and heavy metal concentrations in control asbestos and rhizospheric asbestos of *R. pseudoacacia* L. and *A. altissima* (Mill.) Swingle; b) evaluation of the phenolics content in control asbestos and rhizospheric asbestos; c) determination of the allelopathic potential of woody plant species through radicle growth inhibition of indicator species *Trifolium pratense* L. and *Medicago sativa* L., whose populations grow on asbestos deposits, but they are sparse and suppressed. This research also explore the potential of *R. pseudoacacia* and *A. altissima* for transformation of asbestos to more fertile substrate and their capability for successful revegetation of asbestos deposits.

EXPERIMENTAL

Study area

The locality Kotraža (N 44°30', E 20°67'), situated near the rural settlement Stragari in the central part of Serbia (Kragujevac municipality), is the locality where the serpentine asbestos was formed by the metamorphosis process (Fig. S-1A and B of the Supplementary material to this paper). Therefore, in the peridotite massif near Stragari there is a large tectonized asbestos deposit formed in contact with cretaceous sediments.[29] Stragaric asbestos (chrysotile type "leather asbestos", silver-colored, $8MgO{\times}2SiO_2{\times}2H_2O$) is present in the form of lens bodies and asbestos fibers that are intertwined with each other.[30] The intensive mining and exploitation of asbestos began in the 1950s and lasted almost forty years, when production stopped and the mine closed. Asbestos tailing was formed near the mine at sites where large quantities of materials were deposited after the asbestos processing.[30] Although the mine "Stragari" has been closed for more than two decades, the process of spontaneous revegetation on the asbestos deposits is running very slowly, and the main part of the deposits is biologically empty space (Fig. S-2A of the Supplementary material). Populations of *R. pseudoacacia* are growing in the central part of the asbestos deposit (Fig. S-2B of the Supplementary material) whereas on it peripheral parts, populations of *A. altissima* are developed (Fig. S-2C of the Supplementary material). Plant species that spontaneously grow on the asbestos deposits are: *Alyssum murale* Waldst. et Kit., *Artemisia absinthium* L., *Chrysopogon gryllus* (L.) Trin., *Eryngium serbicum* Pančić, *Euphorbia cyparissias* L., *Helleborus odorus* Waldst et Kit. in Willd., *Medicago sativa* L., *Melica ciliata* L., *Potentilla cinerea* Chaix ex Vill., *Sanguisorba minor* Scop., *Saponaria officinalis* L., and *Trifolium pratense* L.

Collection of asbestos

The field research on asbestos deposits was taken in abandoned asbestos mine „Stragari" at Kotraža locality during August of 2016. The asbestos that was collected at a depth of 0–30 cm on bare zones without vegetation cover represented the control asbestos (C_{ASB}), whereas

the asbestos taken up in the root zone of *R. pseudoacacia* and *A. altissima* was marked as rhizospheric asbestos (RP_{ASB} and AA_{ASB}, respectively). Asbestos samples were packed into plastic bags and brought to the laboratory for analysis. After the removal of visible plant remains samples were dried at room temperature (25 °C) and sifted through a sieve (0.5 mm mesh). For chemical, elemental and biochemical analysis five composite samples of asbestos were used ($n = 5$).

Instrument and apparatures

Flame atomic absorption spectrophotometer (FAAS) model "Perkin Elmer 3300" was used for heavy metal analysis with D_2-lamp as a background corrector; manganese (λ = 279.8 nm), nickel (λ = 232.0 nm), iron (λ = 248.3 nm), zinc (λ = 213.9 nm), lead (λ = 283.3 nm), copper (λ = 324.8nm). For the preparation of calibrated diagrams standard solutions of the corresponding concentrations were used. A range of concentrations of test elements of the standard solutions was 0.5–2.0 mg L^{-1} for Cu, Zn and Ni, or 1.0–5.0 mg L^{-1} for Mn, Pb and Fe. All the sample solutions were analyzed by FAAS using air–acetylene flame (2.0:10.0). The measured values of the element content in asbestos are expressed in micrograms per gram of the dry asbestos weight (μg g^{-1} d.w.).

HPLC system (Shimadzu, Kyoto, Japan), which consisted of degasser DGU-20A3, analytical pumps LC-20AT, 7125 injectors and SPD-M20A diode array detector and CBM-20A system controller, was used for determination of phenolic acids and flavonoids. Separation was achieved on Luna C18 column at 30 °C, 250 mm×4.6 mm I.D., 5 μm (Phenomenex, Torrance, CA, USA) with a flow rate of 1.0 mL min^{-1}. Injection volume was 20 μL. The chromatographic data were processed using LC Solution computer software (Shimadzu). Gradient elution was used (5 % B 0–5 min, gradient 5–60 % B during 5–30 min, 60 % B held for 5 min, then ramped from 60 to 90 % B for 2–3 min and equilibrated for further 5 min; mobile phases – A: water acidified with formic acid, pH 3, B: acetonitrile). The identity of compounds was determined by comparing the retention times and absorption maxima of known peaks with pure standards (Sigma) at 290 and 245 nm.

UV–Vis spectrophotometer (Shimadzu UV-160) was used for determination of total phenolics (λ = 725 nm) and total flavonoids (λ = 430 nm).

Chemicals and reagents

For determination of heavy metal concentrations in asbestos samples, analytical grade chemicals were purchased from Sigma–Aldrich Company: 65 % nitric acid (HNO_3), and 70 % perchloric acid ($HClO_4$) were used for digestion procedure. The standard solution "Acros Organics Standard (USA)", of concentration 1000 μg mL^{-1}, was used to determine calibration curve of appropriate heavy metals. EDTA product of Sigma–Aldrich Company was used for extractions of mobile heavy metals in asbestos.

For HPLC analysis, acetonitrile was obtained from J.T. Baker (Deventer, The Netherlands) while formic acid was product of Merck (Darmstadt, Germany). Quantification was based on external calibration of purified standard of flavonoids (quercetin) and phenolic acids (3,5-dihydroxybenzoic acid, Sigma–Aldrich Company, St. Louis, MO, USA). All reagents were HPLC reagent grade purity unless stated otherwise.

Gallic acid and rutin (Sigma–Aldrich, St. Louis, MO, USA) were used as a standards for determination of total phenolics and total flavonoids, respectively. Folin–Ciocalteu's reagent and aluminium chloride were purchased from Sigma–Aldrich, St. Louis, MO, USA. Methanol and sodium carbonate were purchased from Zorka Pharma (Šabac, Serbia).

Determination of chemical characteristics of asbestos

Asbestos pH was measured in water with PHT-026 multi-function meter. Organic carbon (C) was measured by the method of Tyurin[31] whereas total nitrogen content (N) was determined by the method of Benton Jones[32] and the C/N ratio was calculated. The content of free carbonates ($CaCO_3$) was determined by volumetric method, by the action of the hydrochloric acid solution on the soil and by measuring the volume of released carbon dioxide.[33] Available forms of phosphorus (P_2O_5) and potassium (K_2O) were analysed using the standard ammonium lactate/acetic acid (AL)-method.[34]

Determination of heavy metals in asbestos

Total concentrations of heavy metals (Fe, Ni, Cu, Zn, Pb, Mn) in asbestos were determined according to the modified method 3051A (EPA SW-846 test methods): [35] 2–3 g of the asbestos sample was oven dried for 1 h at 105 °C. The sample was dissolved in a mixture of 25.0 mL of HNO_3 and $HClO_4$ in ratio of 3:1 for 12 h at 40 °C. Concentrations of available heavy metals in asbestos were determined according to Žemberyová *et al*.[36] The extraction was performed with 0.05 mol L^{-1} EDTA (pH 7.00). The sample of dried asbestos (2–3 g) was added in 25 mL of 0.05 M EDTA and mixed with magnetic stirring for 1 h at room temperature 20±4 °C. Flame atomic absorption spectrophotometer (FAAS) was used for analyzing the concentrations of chemical elements. Standard solutions were used for the preparation of calibration diagrams. The measured values of element content in asbestos are expressed in μg g^{-1} d.w.

Extraction of phenolics from asbestos

Phenolic acids and flavonoids were extracted by dissolving 10 g of asbestos (d.w.) in 30 mL of pure methanol (99.8 %) in an ultrasonic bath for 15 min and then left to dissolve for another 24 h. Samples of asbestos were centrifuged for 20 min at 10000 g and supernatants were filtered through 0.2 μm cellulose filters (Agilent Technologies, Santa Clara, CA, USA) and stored at 4 °C until use.

Determination of total phenolic and flavonoid compounds

The total phenolics were determined using Folin-Ciocalteu's reagent[37] and expressed as μg of gallic acid equivalent (GAE) g^{-1} d.w. The total flavonoid concentration was evaluated using aluminum chloride.[38] The concentration of flavonoids was expressed as μg of rutin equivalent (RUE) g^{-1} d.w.

Determination of phenolic acids and flavonoids by HPLC

For qualification and quantification of phenolic acids, methanolic extracts of asbestos were analysed by HPLC system (Shimadzu, Kyoto, Japan). 3,5-Dihydroxybenzoic acid (3,5-DHBA) was used as phenolic acid standard whereas quercetin was used for identification of flavonoids. Concentrations of phenolic acids and flavonoids are expressed in μg g^{-1} d.w.

Growth inhibition test

Allelopathic activity of control asbestos and plant rhizospheric asbestos was assessed by modified "rhizosphere soil sandwich method".[39] In the experiment, 5 mL of agar (0.5 %) cooled at 42 °C was added into a multi-dish plate (6 dishes) containing 3 g of dried asbestos. After solidification, 3.2 mL of agar (0.5 %) was added on asbestos–agar layer. After 1 h, 5 seeds of *T. pratense* and *M. sativa* were added on the gelled agar culture medium in one dish (30 seeds per multi-dish plate). Control plates contained only agar medium. The multi-dishes were incubated at 25 °C in the dark. After 7 days, the length of the radicle was measured and

the percentage of growth inhibition was calculated (compared to control). The bioassays were done in 5 replications (30 seeds per replications, $n = 150$).

Statistical analyses

Statistical analyses included determination of the mean (*M*) and standard deviation (*SD*) for each of the analyzed parameters. Differences between groups in terms of the chemical properties of asbestos, total and available concentrations of heavy metals, and content of phenolics in asbestos, as well as the inhibition of radicle growth of indicator species were determined by analysis of variance (ANOVA) and Scheffé's post-hoc test. Correlations between analyzed parameters in asbestos were determined by Pearson correlation coefficients (*r*). Statistical analysis was performed by using the package Statistica 10.0.

RESULTS AND DISCUSSION

Chemical properties of asbestos

Chemical properties of control (C_{ASB}) and plant rhizospheric asbestos (RP_{ASB} and AA_{ASB}) are shown in Table I. The results show that pH in C_{ASB} had higher values than RP_{ASB} and AA_{ASB} ($p < 0.05$, $p < 0.001$) and AA_{ASB} had lower values of pH (H_2O) than RP_{ASB} ($p < 0.001$). Generally, asbestos was characterized by alkaline reaction (7.58–8.13). The higher values of C, N, P_2O_5 and K_2O content in asbestos were found in AA_{ASB} compared to C_{ASB} and RP_{ASB}.

TABLE I. Chemical properties (mean (±*SD*), $n = 5$) of control asbestos (C_{ASB}) and plant rhizospheric asbestos (RP_{ASB} – *Robinia pseudoacacia*; AA_{ASB} – *Ailanthus altissima*); a – C_{ASB}–RP_{ASB}; b – C_{ASB}–AA_{ASB}; c – RP_{ASB}–AA_{ASB}; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns = not significant

Parameter	C_{ASB}	RP_{ASB}	AA_{ASB}
pH (measured in water)	8.13 (±0.070) a* b***	7.91 (±0.060) c***	7.58 (±0.040)
Carbon content, %	0.38 (±0.012) a*	0.34 (±0.011)	1.01 (±0.019) b*** c***
Nitrogen content, %	0.09 (±0.004)	0.10 (±0.006) a^{ns}	0.22 (±0.009) b*** c***
C to N content ratio	4.22 (±0.140) a**	3.40 (±0.120)	4.59 (±0.130) b* c***
$CaCO_3$ content, %	1.07 (0.110) b*	1.25 (0.095) a^{ns} c**	0.85 (0.071)
Available content of P_2O_5, mg 100 g^{-1}	8.40 (1.400) a***	0.10 (0.012)	11.60 (3.700) b^{ns} c**
Available content of K_2O, mg 100 g^{-1}	3.60 (0.400) a**	2.20 (0.250)	26.00 (4.600) b** c***

Heavy metal concentrations in asbestos

Total and available concentrations of Fe, Ni, Cu, Zn, Pb and Mn in control (C_{ASB}) and plant rhizospheric asbestos (RP_{ASB} and AA_{ASB}) are shown in Table II. Higher concentrations of Fe_{total} were detected in RP_{ASB} and AA_{ASB} in

comparison to C_{ASB} ($p < 0.001$). Lower content of Fe_{total} was found in AA_{ASB} compared to RP_{ASB} ($p < 0.001$). Total concentrations of Fe in asbestos were in the range for the serpentine soils.[40] Higher concentrations of Ni_{total} were detected in C_{ASB} compared to AA_{ASB} ($p < 0.001$) whereas content of Ni_{total} was lower in AA_{ASB} than in RP_{ASB} ($p < 0.001$). In this study, total concentrations of Ni in asbestos were at toxic levels (13–34 μg g^{-1}).[41] The results showed higher concentrations of Cu_{total} and Mn_{total} in AA_{ASB} compared to C_{ASB} and RP_{ASB} ($p < 0.001$) and Zn_{total} in AA_{ASB} compared to C_{ASB} ($p < 0.01$). Total concentrations of Cu and Zn in asbestos were below the normal range whereas Mn concentrations were in normal range (13–24 μg g^{-1}, 45–100 μg g^{-1}, 270–525 μg g^{-1}, respectively).[41] Higher content of Pb_{total} was detected in C_{ASB} compared to RP_{ASB} and AA_{ASB} ($p < 0.001$) whereas lower content of Pb_{total} was in AA_{ASB} compared to RP_{ASB} ($p < 0.001$). Pb concentrations in asbestos were in normal range (22–28 μg g^{-1}).[41] Furthermore, available concentrations of Fe, Ni and Pb were lower in AA_{ASB} compared to RP_{ASB} while available concentrations of Cu, Zn and Mn were higher in AA_{ASB} than in RP_{ASB} ($p < 0.001$).

TABLE II. Total and available heavy metal concentrations (mean (±*SD*), μg g^{-1}, $n = 5$) in control asbestos (C_{ASB}) and plant rhizospheric asbestos of *R. pseudoacacia* (RP_{ASB}) and *A. altissima* (AA_{ASB}); ND = not detected; a – C_{ASB}–RP_{ASB}; b – C_{ASB}–AA_{ASB}; c – RP_{ASB}––AA_{ASB}; *$p < 0.05$, **$p < 0.01$***$p < 0.001$, ns = not significant

Parameter	C_{ASB}	RP_{ASB}	AA_{ASB}
Fe_{total}	24334.92 (±208.815)	28516.30 (±131.155) a*** c***	25527.94 (±76.622) b***
Ni_{total}	676.82 (±3.143) a*** b***	647.24 (±2.423) c***	587 (±1.015)
Cu_{total}	3.44 (±0.037)	4.87 (±0.048) a***	7.35 (±0.043) b*** c***
Zn_{total}	12.46 (±0.404)	12.47 (±0.403) a^{ns}	15.54 (±0.329) b** c***
Pb_{total}	25.34 (±0.472) a*** b***	21.76 (±0.541) c***	13.58 (±0.356)
Mn_{total}	397.46 (±1.876) a***	377.35 (±3.137)	471.08 (±0.934) b*** c***
$Fe_{available}$	72.60(±0.100)	93.20 (±0.265) a*** c***	83.50 (±0.394) b***
$Ni_{available}$	12.47 (±0.379) a* b***	11.46 (0.351) c**	10.26 (±0.207)
$Cu_{available}$	ND	0.82 (±0.015) a***	1.31 (±0.007) b*** c***
$Zn_{available}$	1.18 (±0.030) a**	1.07 (±0.020)	1.36 (±0.024) b** c***
$Pb_{available}$	5.48 (±0.040) a*** b***	4.45 (±0.025) c***	2.11 (±0.016)
$Mn_{available}$	15.80 (±0.300) a^{ns}	15.40 (±0.100)	19.60 (±0.158) b*** c***

Phenolics in asbestos and plant allelopathic potential

Total content of phenolics, phenolic acids and flavonoids in control (C_{ASB}) and plant rhizospheric asbestos (RP_{ASB} and AA_{ASB}) is presented in Table III. Total phenolics, total flavonoids and 3,5-dihydroxybenzoic acid (3,5-DHBA) were detected only in AA_{ASB}. In this study, the negative correlation between pH and total phenolics (r = –0.888), total flavonoids (r = –0.873) and 3,5-DHBA (r = –0.884) indicates that in alkaline conditions phytotoxic activity of allelochemicals released from woody plant species can be reduced. This can be explained by the rapid mineralization of phenolic compounds in conditions of alkaline reaction of the substrate.[42] However, total phenolics, total flavonoids and 3,5-DHBA were significantly correlated with C (r = +0.969, +0.938 and +0.995, respectively), N (r = +0.960, +0.929 and +0.995, respectively), P_2O_5 (r = +0.720 0.723 and 0.645, respectively) and K_2O content (r = +0.933, +0.894 and +0.995, respectively). These results are in accordance with Grbović *et al.*,[17] who found positive correlations between phenolics and C, N and P_2O_5 in fly ash. Phenolics as carbon rich compounds released from plants may contribute to the C-stock in soils, [19,43] and can release dissolved organic nitrogen from leaf litter or can increase phosphorus availability due to competition for sorption sites on mineral complexes.[44,45] Results also showed that as pH in asbestos increases, inhibition of radicle growth of *M. sativa* is less pronounced (r = –0.719) which is in agreement with Makoi and Ndakemi[46] statement that in neutral or slightly alkaline conditions phytotoxic activity of allelochemicals is limited due to its sorption to organic matter. However, as the content of C, N, P_2O_5 and K_2O increases, inhibition of radicle growth of *T. pratense* (r = +0.774, +0.697, +0.889 and +0.778, respectively) and *M. sativa* (r = +0.958 +0.936, +0.722 and +0.963, respectively) is more pronounced, which is related to high phenolics content in asbestos.

TABLE III. Phenolics content (mean (±*SD*), μg g^{-1}, n = 5) in control asbestos (C_{ASB}) and plant rhizospheric asbestos (RP_{ASB} – *Robinia pseudoacacia*; AA_{ASB} – *Ailanthus altissima*); ND = not detected

Parameter	C_{ASB}	RP_{ASB}	AA_{ASB}
Total phenolics	ND	ND	5.58 (±1.250)
3,5-DHBA	ND	ND	0.09 (±0.01)
Total flavonoids	ND	ND	1.00 (±0.500)
Quercetin	0.21 (±0.040)	0.28 (±0.040)	0.28 (±0.030)

Total phenolics, total flavonoids and 3,5 DHBA in asbestos were positively correlated with contents of Cu_{total} (r = +0.908, +0.885 and +0.923, respectively), $Cu_{available}$ (r = +0.788, +0.774 and +0.797, respectively), Zn_{total} (r = +0.969, +0.956 and +0.962, respectively), $Zn_{available}$ (r = +0.895, +0.869 and +0.907, respectively), Mn_{total} (r = +0.954, +0.923 and +0.977, respectively) and $Mn_{available}$ (r = +0.964, +0.933 and +0.986, respectively) and negatively cor-

related with contents of Ni_{total} (r = –0.929, –0.910 and –0.941, respectively), $Ni_{available}$ (r = –0.833, –0.805 and –0.860, respectively), Pb_{total} (r = –0.950, –0.927 and –0.959, respectively), and $Pb_{available}$ (r = –0.932, –0.909 and –0.948, respectively). According to Pollock *et al.*[47] and Li *et al.*,[48] heavy metals in soil can affect the activity of allelochemicals, *i.e.*, phenolics can increase their availability or can fix them in the form of chelates. In this study, inhibition of radicle growth of *M. sativa* was followed by higher values of contents of Cu_{total}, Zn_{total}, $Zn_{available}$, Mn_{total} and $Mn_{available}$ (r = +0.789, +0.923, +0.927, +0.973, and +0.956, respectively) and lower values of contents of Ni_{total}, $Ni_{available}$, Pb_{total} and $Pb_{available}$ (r = –0.809, –0.698, –0.845 and –0.827, respectively). Inhibition of radicle growth of *T. pratense* was also followed by higher values of contents of Zn_{total}, $Zn_{available}$, Mn_{total} and $Mn_{available}$ (r = +0.728, +0.910, +0.849 and +0.785, respectively).

Higher inhibition of radicle growth of *T. pratense* and *M. sativa* was noted in AA_{ASB} compared to C_{ASB} and RP_{ASB} ($p < 0.05$; $p < 0.001$) due to high content of phenolics and flavonoids in asbestos (Fig. 1). Inhibition of *T. pratense* and *M. sativa* growth increases with a higher content of total phenolics (r = +0.690 and +0.876, respectively), total flavonoids (r = +0.650 and +0.829, respectively) and 3,5-DHBA (r = +0.746 and +0.951, respectively) in asbestos. Similarly, Grbović *et al.*[17] found that *A. altissima* growing on fly ash deposits had stronger allelopathic potential on growth of *T. pratense* than *R. pseudoaccacia*.

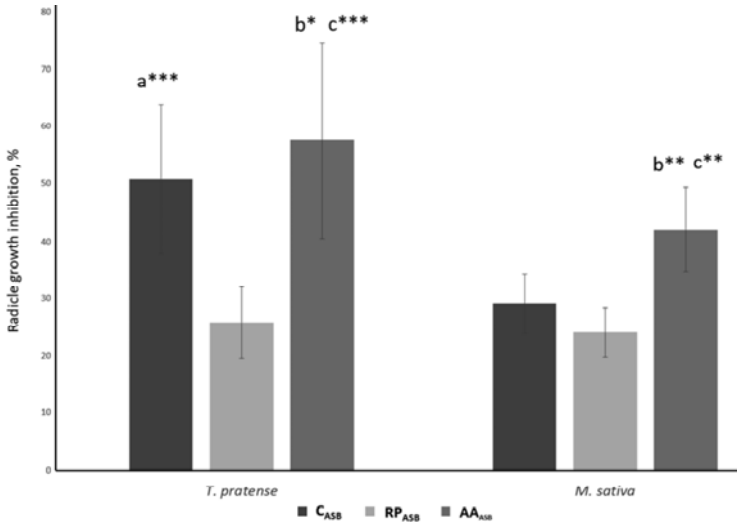

Fig. 1. Radicle growth inhibition of *Trifolium pratense* and *Medicago sativa* in control asbestos (C_{ASB}) and plant rhizospheric asbestos (RP_{ASB} – *Robinia pseudoacacia*; AA_{ASB} – *Ailanthus altissima*); ANOVA, data represents means (± *SD*) (n = 150), ND = not detected; a – C_{ASB}–RP_{ASB}; b – C_{ASB}–AA_{ASB}; c – RP_{ASB}–AA_{ASB}; *$p < 0.05$, **$p < 0.01$ ***$p < 0.001$, ns = not significant.

According to Kowarik and Säumel,[25] *A. altissima* can decrease pH and increase organic carbon and total nitrogen, and due to high rate of litter decomposition it can increase the nutrient and heavy metal availability,[48,49] which is in agreement with our results. In our study, in the condition of lower pH, total and available content of Ni and Pb decreased whereas total and available content of Cu, Zn and Mn increased which is associated with high phenolics content in asbestos and high allelopathic potential of *A. altissima*. In this study, some phenolics had high allelopathic effect, probably due to reduced sensitivity to microbial activity or different phenolics showing synergistic effects in the combination.[15,19]

CONCLUSIONS

Results in the present study show lower values of pH, total and available concentrations of Ni and Pb and higher values of C, N, P_2O_5, K_2O, total and available concentrations of Cu, Zn and Mn in AA_{ASB} compared to RP_{ASB} and C_{ASB}. Total phenolics, phenolic acids and flavonoids in rhizospheric asbestos of *A. altissima* indicate changes in soil chemistry, humus formation and initiation of pedogenesis. Furthermore, higher inhibition of radicle growth of *T. pratense* and *M. sativa* was in AA_{ASB} rather than in $RP_{ASB,}$ indicating that *A. altissima* has strong allelopathic potential due to high content of phenolic compounds which have the allelochemical properties. Alleopathic activity of phenolic compounds is correlated with pH, C, N, P_2O_5 and K_2O content, as well as with concentration of Ni, Cu, Zn, Pb and Mn in asbestos. Results in this study indicate that *A. altissima* is suitable for revegetation of disturbed sites because it improves asbestos chemical properties and affects the biogeochemistry of anthropogenic ecosystems, but attention must be paid to invasion risk due to high allelopathic potential.

SUPPLEMENTARY MATERIAL

Additional data are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

КОМПЛЕКСНИ ЕФЕКАТ *Robinia pseudoacacia* L. И *Ailanthus altissima* (Mill.) Swingle КОЈЕ РАСТУ НА ДЕПОЗИТУ АЗБЕСТА: АЛЕЛОПАТИЈА И БИОГЕОХЕМИЈА

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Интензивна експлоатација и употреба азбеста у свету представља потенцијални ризик за животну средину и здравље људи. Главни циљ ове студије је одређивање алелопатског

потенцијала багрема (*Robinia pseudoacacia* L.) и киселог дрвета (*Ailanthus altissima* (Mill.) Swingle) чије популације расту на јаловишту напуштеног рудника азбеста „Стрaгари“ у централној Србији. У контролном азбесту (празне зоне без биљног покривача) и ризосферном азбесту испитиваних врста анализирана је киселост (pH), садржај угљеника, азота, калцијум-карбоната, доступне форме фосфора и калијума, садржај гвожђа, бакра, мангана, никла, цинка и олова, као и садржај фенолних једињења. Алелопатска активност испитиваних биљака је утврђена „сендвич методом ризосферног земљишта“, а као индикаторске врсте коришћене су *Trifolium pratense* L. и *Medicago sativa* L. Врста *A. altissima* је показала већи алелопатски потенцијал у односу на *R. pseudoacacia* захваљујући већем присуству фенолних једињења. Алелопатска активност фенолних једињења у ризосферном азбесту је била високо корелисана са pH, садржајем угљеника и азота, доступним облицима фосфора и калијума, као и садржајем бакра, мангана и цинка. Резултати су показали да висок садржај фенола у ризосферном азбесту *A. altissima* може да инхибира раст биљака. Ова дрвенаста биљка упркос високом алелопатском потенцијалу је погодна за обнову вегетације нарушених станишта, јер иницира процес педогенезе и утиче на хемизам азбеста.

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