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

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(Received 16 April; revised 19 June; accepted 20 June 2019)

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 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. Allelopathic 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 disturbed ecosystems because it initiates pedogenesis and affects the asbestos chemistry.

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

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.\textsuperscript{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.\textsuperscript{2} Prolonged exposure to asbestos fibers can result in development of dangerous diseases such as lung cancer and mesothelioma.\textsuperscript{3,4} Some experts have appealed to countries to cancel asbestos mining and abandon utilization of asbestos containing materials.\textsuperscript{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.\textsuperscript{7,8} Vegetation development on the mine waste prevent wind/water erosion, reduce toxicity of heavy metals and provide aesthetic landscape.\textsuperscript{9} Woody plants with large cover and high biomass may have an important role in the process of revegetation of waste deposits.\textsuperscript{10} Furthermore, organic matter originating from plants could be of great importance in the process of soil humification where phenolic compounds are very significant.\textsuperscript{11,12} Allelopathy presents interactions between plants through the action of allelochemicals.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{16,20,21} According to Inderjit and Weiner,\textsuperscript{22} progress in allelopathy can be reached through connection with soil chemistry rather than in direct plant-plant chemical interactions.

*Radinia pseudoacacia* L. (native in North America) and *Ailanthus altissima* (Mill.) Swingle (native in China) become naturalized in many parts of Europe, and in Serbia are considered as non-indigenous invasive plant species.\textsuperscript{23} High invasion capacity of *R. pseudoacacia* and *A. altissima* is the result of very effective generative and vegetative reproduction,\textsuperscript{24,25} as well as allelopathic activity of plants.\textsuperscript{26-28,25,17} Generally, phenolics that were found in *R. pseudoacacia* and *A. altissima* tissues can act as allelochemicals and possess a high allelopathic activity.\textsuperscript{24,25} Most studies are dealing with allelopathy in natural habitats or in laboratory, but knowledge regarding on 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
Allelopathy of Woody Plants on Asbestos

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 Robinia pseudoacacia L. and Ailanthus 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. S1A,B of the Supplementary material). Therefore, in the Peridotite massif near Stragari there is a large tectonized asbestos deposit formed in contact with cretaceous sediments. Stragaric asbestos (chrysotile type “leather asbestos”, silver-colored, 8MgO·2SiO2·2H2O) is present in the form of lens bodies and asbestos fibers that are intertwined with each other. 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. Although the mine “Stragari” has been closed 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. S2A of the Supplementary material). Populations of R. pseudoacacia are growing in the central part of the asbestos deposit (Fig. S2B of the Supplementary material) whereas on its peripheral parts, populations of A. altissima are developed (Fig. S2C 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 serbicicum 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\text{\textsubscript{ASB}}), whereas the asbestos taken up in the root zone of R. pseudoacacia and A. altissima was marked as rhizospheric asbestos (RP\text{\textsubscript{ASB}} and AA\text{\textsubscript{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**

Flam atomic absorption spectrophotometer (FAAS) model “Perkin Elmer 3300” was used for analyzing heavy metals by choosing 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 = 324.8 nm). 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 the Mn, Pb and Fe. All the sample solutions were analyzed by FAAS using air-acetylene flame (2.0:10.0). The measured values of 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 ºC, 250 × 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: acetonitril). 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 purpose. The standard solution “Acros Organics Standard (USA)”, concentration is 1000 μg mL^{-1}, was used for determinate calibration curve on appropriate heavy metals. EDTA product of “Sigma-Aldrich Company” 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 polyphenolic 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) was 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 (organic solvent) and sodium carbonate were purchased from Zorka Pharma (Šabac, Serbia).

**Determination of chemical characteristics of asbestos**

Asbestos pH was measured in water with pH meter (PHT-026 Multi-function meter). Organic carbon (C) was measured by the method of Tyurin^{29} whereas total nitrogen content (N) was determined by the Benton and Jones.^{32} 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.\textsuperscript{33} Available forms of phosphorus (P$_2$O$_5$) and potassium (K$_2$O) were analysed using the
standard AL-method.\textsuperscript{34}

\textit{Determination of heavy metals in asbestos}

Total concentrations of heavy metals (Fe, Ni, Cu, Zn, Pb, Mn) in asbestos were
determined according to modified method 3051A (EPA SW-846 test methods): \textsuperscript{35} 2-3 g of
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$_4$ for 12 h at 40 °C. Concentrations of available heavy metals in
asbestos were determined according to Zemberyova \textit{et al.}\textsuperscript{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 by magnetic stirring for 1 h at room temperature (20 ± 4) °C. Flamm
atomic absorption spectrophotometer (FAAS) was used for analyzing the concentrations
of chemical elements. Standard solutions were used for the preparation of calibrated diagrams.
The measured values of element content in asbestos are expressed in µg g$^{-1}$ d.w.

\textit{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
another 24 h. Samples of asbestos were centrifuged 20 min at 10000g and supernatants were
filtered through 0.2 μm cellulose filters (Agilent Technologies, Santa Clara, CA, USA) and
stored at 4 °C until use.

\textit{Determination of total phenolic and flavonoid compounds}

The total phenolics were determined using Folin-Ciocalteu’s reagent\textsuperscript{37} and expressed as
µg GAE g$^{-1}$ d.w. The total flavonoid concentration was evaluated using aluminum chloride.\textsuperscript{38}
The concentration of flavonoids was expressed as µg of RUE g$^{-1}$ d.w.

\textit{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). Phenolic acid 3,5–
dihydroxybenzoic (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.

\textit{Growth inhibition test}

Allelopathic activity of control asbestos and plant rhizospheric asbestos was assessed by
modified “rhizosphere soil sandwich method".\textsuperscript{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 \textit{T. pratense} and \textit{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).

\textit{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 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\textsubscript{ASB}) and plant rhizospheric asbestos (RP\textsubscript{ASB} and AA\textsubscript{ASB}) are shown in Table I. The results showed that pH in C\textsubscript{ASB} had higher values than RP\textsubscript{ASB} and AA\textsubscript{ASB} (p<0.05, p<0.001) and AA\textsubscript{ASB} had lower values of pH (H\textsubscript{2}O) than RP\textsubscript{ASB} (p<0.001). Generally, asbestos was characterized by alkaline reaction (7.58-8.13). The higher values of C, N, P\textsubscript{2}O\textsubscript{5} and K\textsubscript{2}O content in asbestos were found in AA\textsubscript{ASB} compared to C\textsubscript{ASB} and RP\textsubscript{ASB} (p<0.05, p<0.01, p<0.001).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C\textsubscript{ASB}</th>
<th>RP\textsubscript{ASB}</th>
<th>AA\textsubscript{ASB}</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (measured in water)</td>
<td>8.13 (±0.070)</td>
<td>7.91 (±0.060)</td>
<td>7.58 (±0.040)</td>
</tr>
<tr>
<td>Carbon content, %</td>
<td>0.38 (±0.012)</td>
<td>0.34 (±0.011)</td>
<td>1.01 (±0.019)</td>
</tr>
<tr>
<td>Nitrogen content, %</td>
<td>0.09 (±0.004)</td>
<td>0.10 (±0.006)</td>
<td>0.22 (±0.009)</td>
</tr>
<tr>
<td>C to N content ratio</td>
<td>4.22 (±0.140)</td>
<td>3.40 (±0.120)</td>
<td>4.59 (±0.130)</td>
</tr>
<tr>
<td>CaCO\textsubscript{3} content, %</td>
<td>1.07 (0.110)</td>
<td>1.25 (0.095)</td>
<td>0.85 (0.071)</td>
</tr>
<tr>
<td>Available content of P\textsubscript{2}O\textsubscript{5}, mg 100 g\textsuperscript{-1}</td>
<td>8.40 (1.400)</td>
<td>0.10 (0.012)</td>
<td>11.60 (3.700)</td>
</tr>
<tr>
<td>Available content of K\textsubscript{2}O, mg 100 g\textsuperscript{-1}</td>
<td>3.60 (0.400)</td>
<td>2.20 (0.250)</td>
<td>26.00 (4.600)</td>
</tr>
</tbody>
</table>

\begin{itemize}
\item a - C\textsubscript{ASB} – RP\textsubscript{ASB};
\item b - C\textsubscript{ASB} – AA\textsubscript{ASB};
\item c - RP\textsubscript{ASB} – AA\textsubscript{ASB};
\end{itemize}

**p<0.05, ***p<0.001, ns = not significant;**

Heavy metal concentrations in asbestos

Total and available concentrations of Fe, Ni, Cu, Zn, Pb and Mn in control (C\textsubscript{ASB}) and plant rhizospheric asbestos (RP\textsubscript{ASB} and AA\textsubscript{ASB}) are shown in Table II. Higher concentrations of Fe\textsubscript{total} were detected in RP\textsubscript{ASB} and AA\textsubscript{ASB} in comparison to C\textsubscript{ASB} (p<0.001). Lower content of Fe\textsubscript{total} was found in AA\textsubscript{ASB} compared to RP\textsubscript{ASB} (p<0.001). Total concentrations of Fe in asbestos were in the range for the serpentine soils.\textsuperscript{40} Higher concentrations of Ni\textsubscript{total} were detected in C\textsubscript{ASB} compared to AA\textsubscript{ASB} (p<0.001) whereas content of Ni\textsubscript{total} was lower in AA\textsubscript{ASB} than in RP\textsubscript{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_ASBS compared to C_ASBS and RP_ASBS (p<0.001) and Zn_total in AA_ASBS compared to C_ASBS (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_ASBS compared to RP_ASBS and AA_ASBS (p<0.001) whereas lower content of Pb_total was in AA_ASBS compared to RP_ASBS (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_ASBS compared to RP_ASBS (p<0.01, p<0.001) while available concentrations of Cu, Zn and Mn were higher in AA_ASBS than in RP_ASBS (p<0.001).

TABLE II. Total and available heavy metal concentrations in control asbestos (C_ASBS) and plant rhizospheric asbestos of R. pseudoacacia (RP_ASBS) and A. altissima (AA_ASBS).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Content, µg g⁻¹ (mean (±SD), n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C_ASBS</td>
</tr>
<tr>
<td>Fe_total</td>
<td>24334.92 (±208.815)</td>
</tr>
<tr>
<td>Ni_total</td>
<td>676.82 (±3.143)</td>
</tr>
<tr>
<td>Cu_total</td>
<td>3.44 (±0.037)</td>
</tr>
<tr>
<td>Zn_total</td>
<td>12.46 (±0.404)</td>
</tr>
<tr>
<td>Pb_total</td>
<td>25.34 (±0.472)</td>
</tr>
<tr>
<td>Mn_total</td>
<td>397.46 (±1.876)</td>
</tr>
<tr>
<td>Fe_available</td>
<td>72.60 (±0.100)</td>
</tr>
<tr>
<td>Ni_available</td>
<td>12.47 (±0.379)</td>
</tr>
<tr>
<td>Cu_available</td>
<td>ND</td>
</tr>
<tr>
<td>Zn_available</td>
<td>1.18 (±0.030)</td>
</tr>
<tr>
<td>Pb_available</td>
<td>5.48 (±0.040)</td>
</tr>
<tr>
<td>Mn_available</td>
<td>15.80 (±0.300)</td>
</tr>
</tbody>
</table>

ND = not detected; a - CASB – RPASB; b - CASB – AASB; c - RPASB – AAASB; *p<0.05, **p<0.01***p<0.001, ns = not significant.

**Phenolics in asbestos and plant allelopathic potential**

Total content of phenolics, phenolic acids and flavonoids in control (C_ASBS) and plant rhizospheric asbestos (RP_ASBS and AA_ASBS) is presented in Table III. Total phenolics, total flavonoids and 3,5 dihydroxybenzoic acid (3,5 DHBA)
were detected only in AAASB. 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.\textsuperscript{42} However, total phenolics, total flavonoids and 3,5 DHBA were significant correlated with C \( (r = +0.969, r = +0.938, r = +0.995, \) respectively), N \( (r = +0.960, r = +0.929, r = +0.995, \) respectively), \( P_2O_5 \) \( (r = +0.720, r = +0.723, \) respectively) and \( K_2O \) content \( (r = +0.933, r = +0.894, r = +0.995, \) respectively). These results are in accordance with Grbović et al.\textsuperscript{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,\textsuperscript{43,19} and can release dissolved organic nitrogen from leaf litter or can increase phosphorous availability due competition for sorption sites on mineral complexes.\textsuperscript{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 (2014)\textsuperscript{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 \( (r = +0.774, r = +0.697, r = +0.889, r = +0.778, \) respectively) and \( M. \) sativa \( (r = +0.958, r = +0.936, r = +0.722, r = +0.963, \) respectively) is more pronounced, which is related to high phenolics content in asbestos.

**TABLE III. Phenolics content in control asbestos (C\textsubscript{ASB}) and plant rhizospheric asbestos (RP\textsubscript{ASB} – Robinia pseudoacacia; AA\textsubscript{ASB} – Ailanthus altissima)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CASB Content, µg g(^{-1}) (mean (±SD), n = 5)</th>
<th>RP\textsubscript{ASB} Content, µg g(^{-1}) (mean (±SD), n = 5)</th>
<th>AA\textsubscript{ASB} Content, µg g(^{-1}) (mean (±SD), n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenolics</td>
<td>ND</td>
<td>ND</td>
<td>5.58 (±1.250)</td>
</tr>
<tr>
<td>3.5 DHBA</td>
<td>ND</td>
<td>ND</td>
<td>0.09 (±0.01)</td>
</tr>
<tr>
<td>Total flavonoids</td>
<td>ND</td>
<td>ND</td>
<td>1.00 (±0.500)</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.21 (±0.040)</td>
<td>0.28 (±0.040)</td>
<td>0.28 (±0.030)</td>
</tr>
</tbody>
</table>

ND = not detected

Total phenolics, total flavonoids and 3,5 DHBA in asbestos was positively correlated with contents of Cu\textsubscript{total} \( (r = +0.908, r = +0.885, r = +0.923, \) respectively), Cu\textsubscript{available} \( (r = +0.788, r = +0.774, r = +0.797, \) respectively), Zn\textsubscript{total} \( (r = +0.969, r = +0.956, r = +0.962, \) respectively), Zn\textsubscript{available} \( (r = +0.895, r = +0.869, r = +0.907, \) respectively), Mn\textsubscript{total} \( (r = +0.954, r = +0.923, r = +0.977, \) respectively), and Mn\textsubscript{available} \( (r = +0.964, r = +0.933, r = +0.986, \) respectively) and negatively correlated with contents of Ni\textsubscript{total} \( (r = -0.929, r = -0.910, r = -0.941, \) respectively), Ni\textsubscript{available} \( (r = -0.833, r = -0.805, r = -0.860, \) respectively), Pb\textsubscript{total} \( (r = -0.950, r = -0.927, r = -0.959, \) respectively), and Pb\textsubscript{available} \( (r = -0.932, r = -0.909, r = -0.948, \) respectively).
According to Pollock et al.\textsuperscript{47} and Li et al.\textsuperscript{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 \textit{M. sativa} was followed by higher values of contents of Cu\textsubscript{total}, Zn\textsubscript{total}, Zn\textsubscript{available}, Mn\textsubscript{total} and Mn\textsubscript{available} ($r = +0.789$, $r = +0.923$, $r = +0.927$, $r = +0.973$, $r = +0.956$, respectively) and lower values of contents of Ni\textsubscript{total}, Ni\textsubscript{available}, Pb\textsubscript{total} and Pb\textsubscript{available} ($r = -0.809$, $r = -0.698$, $r = -0.845$, $r = -0.827$, respectively). Inhibition of radicle growth of \textit{T. pratense} was also followed by higher values of contents of Zn\textsubscript{total}, Zn\textsubscript{available}, Mn\textsubscript{total} and Mn\textsubscript{available} ($r = +0.728$, $r = +0.910$, $r = +0.849$, $r = +0.785$, respectively). Higher inhibition of radicle growth of \textit{T. pratense} and \textit{M. sativa} was noted in AA\textsubscript{ASB} compared to C\textsubscript{ASB} and RP\textsubscript{ASB} ($p<0.05$; $p<0.001$) due to high content of phenolics and flavonoids in asbestos (Fig. 1). Inhibition of \textit{T. pratense} and \textit{M. sativa} growth is increases with a higher content of total phenolics ($r = +0.690$, $r = +0.876$, respectively), total flavonoids ($r = +0.650$, $r = +0.829$, respectively) and 3,5 DHBA ($r = +0.746$, $r = +0.951$, respectively) in asbestos. Similarly, Grbović \textit{et al.}\textsuperscript{17} found that \textit{A. altissima} growing on fly ash deposits had stronger allelopathic potential on growth of \textit{T. pratense} than \textit{R. pseudoaccacia}.

According to Kowarik and Saumel\textsuperscript{25} \textit{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, 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 showed synergistic effects in the combination.

CONCLUSIONS

Results in the present study showed lower values of pH, total and available concentrations of Ni and Pb and higher values of C, N, P2O5, K2O, total and available concentrations of Cu, Zn and Mn in AAASB compared to RPASB and CASB. 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 AAASB than in RPASB, 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, P2O5 and K2O 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 should pay attention to invasion risk due to high allelopathic potential.

SUPPLEMENTARY MATERIAL

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

Acknowledgements: This work was supported by the Ministry of Education, Science and Technological Development, grants III41010 and OI173018. The authors would like to thank the anonymous reviewer, who gave valuable comments for the improvement of this paper.

ИЗВОД

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

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Интезивна експлозија и употреба азбеста у свету представља потенцијални ризик за животну средину и здравље људи. Главни циљ ове студије је детерминација алеопатичког потенцијала багрема (Robinia pseudoacacia L.) и киселог дрвета (Ailanthus altissima (Mill.)
Swingle) чије популације расту на јаловишту напуштеног рудника азбеста „Страгари“ у централној Србији. У контролном азбесту (празне зоне без билног покривача) и ризосферном азбесту испитиваних врста анализирана је киселост (pH), садржај угљеника, азота, калијума, карбоната, доступне форме фосфора и калијума, садржај гвожђа, бакра, манганас, цинка и олова, као и садржај фенолних јединиња. 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 захваћући већем присуством фенолних јединиња. Алелопатска активност фенолних јединиња у ризосферном азбесту била је високо корелисана са pH, садржајем угљеника и азота, доступним облицима фосфора и калијума, али и садржајем бакра, манганаса, цинка и олова, као и садржајем фенолних јединиња. 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 захваћући већем присуством фенолних јединиња. 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 захваћући већем присуством фенолних јединиња. 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 захваћући већем присуством фенолних јединиња. 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 захваћући већем присуством фенолних јединиња. Алелопатска активност испитиваних биљака је утврђена "сендвич методом" ризосферног земљишта, а као индикаторске врсте коришћене су Trifolium pratense L. и Medicago sativa L. Врsta A. altissima је показала већи алелопатски потенцијал у односу на R. pseudoacacia захваћући већем присуством фенолних јединиња.

REFERENCES


SUPPLEMENTARY MATERIAL TO
Complex effect of Robinia pseudoacacia L. and Ailanthus altissima (Mill.) Swingle growing on asbestos deposits: allelopathy and biogeochemistry

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Fig. S1. Study site on map of Serbia (A), and satellite image of asbestos deposits of mine “Stragari” (B)
Fig. S2. Asbestos deposits of mine “Stragari” (A) and woody plant species growing on asbestos deposits: *R. pseudoacacia* (B) and *A. alissima* (C).