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Bioaccumulation of heavy metals by the leaves of *Robinia pseudoacacia* as a bioindicator tree in industrial zones

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Abstract

The study is a part of research project on using short rotation plantations in biomass production. The aim of this investigation was to determine the accumulation characteristics of *Robinia pseudoacacia* leaves as a biomonitor of heavy-metal pollution in the industrial regions in Bulgaria. The study was carried out in 25-year-old plantations located in close vicinity of industrial area Devnia, Eastern Bulgaria. Devnia is a zone, highly contaminated by cement factories, nitrogen fertilizers and polyvinylchloride factories. Controls were similarly aged plantations on a background area 15 km away from the emission sources. The concentration of nutrients and heavy metals in the leaves of damaged and control plants were investigated. Decreased levels of total nitrogen (6 %), total phosphorous (11 %), potassium (36 %) and magnesium (3 %) were detected in September for polluted trees as compared to control trees. Lead content (30.7 mg kg^{-1}) was 1.38 times higher as compared to control, whereas accumulation of zinc (19.0 mg kg^{-1}) about 1.37 times more than control. An excessive accumulation of copper in the leaves collected from the polluted area (17.2 mg kg^{-1}) was 2.15 times higher than control. The concentrations of Pb, Zn and Cu in the contaminated soil under black locust plantation were 38.2, 77.4 and 101.3 mg kg^{-1}, respectively. Our results showed that the leaves of *R. pseudoacacia* accumulated Pb, Zn and Cu in parallelity with their increase in the contaminated soil. *R. pseudoacacia* may be considered as a good biomonitor of soil pollution especially with Cu, and at lower extent for Pb and Zn in the industrial region of Devnia.

Key words

Biomonitoring, Heavy metal pollution, Remediation, *Robinia pseudoacacia*

Introduction

Industrial sources make a significant contribution to plant contamination with the emitted heavy metals. Apart from the weather conditions, heavy metals are know to be one of the primary causes of tree damage. Spreading the residuals by wind erosion to the environment causes pollution in the soil and plants equally (Ozturk, 1989; Aksoy and Ozturk, 1996, 1997; Aksoy *et al.*, 2000; Gucel *et al.*, 2009 a, b; Uysal *et al.*, 2012).

Large areas of natural tree stands and plantations in Eastern Bulgaria are showing sign of serious damage due to industrial emissions during last few decades (Yorova *et al.*, 1998). Most heavy metals accumulate at the top soil and in long term their contamination increases in the soil, as a result the absorption and accumulation in plants increases (Filipović-Trajković *et al.*, 2012). Plant contamination in most cases arises from atmospheric particle accumulation through their leaves, and the degree of contamination depends on the smoothness of leaves, wind speed and amount of rainfall. Plants can withstand heavy-metal accumulation till the metal reaches the toxicity threshold in level tissues (Sawidis *et al.*, 1995; Ozturk *et al.*, 2008; Ashraf *et al.*, 2010).

Remediation of toxic metals in polluted areas is an actual ecological approach. A successful reforestation is possible by growing plants strongly tolerant to the pollutants with high capacity of bioaccumulation (Gu *et al.*, 2007). Bioaccumulation of heavy metals is dependent on the species of tree used as bioindicator. Deciduous trees have the potential advantage that they are able to monitor short-term or annual changes in pollution (Aksoy *et al.,* 2000; Yilmaz *et al*., 2006; Celik *et al*., 2010; Ozturk *et al*., 2012, 2013).

Many researchers have demonstrated the role of black locust in the phytoremediation processes and in the absorbtion of heavy metals from the soil (Kim and Lee, 2005; Baycu *et al.,* 2006). Robinia *pseudoacacia* improves the quality of soil as well as its physico-chemical features as a nitrogen-fixing species. The leaves of this plant seem to act as an effective biomonitor of environmental quality in areas subjected to industrial and traffic pollution. It can be used as a pioneer species to reforest the degraded land with high content of heavy metals, where other species fail to do so (Çelik *et al*., 2005; Corneanu *et al*., 2009). In addition to high metal accumulation by its leaves, this plant flourishes in the industrial areas because of its adaptability to dryness, fast growth and ability to fix nitrogen (Converse and Betters, 1995). It can survive under a wide temperature range and grows in almost any type of soil (Aksoy *et al*., 2000). Recently, interest in this species has increased as it is used for forestation of abandoned lands based on an E.U. regulation, as well as for phytoremediation (Corneanu *et al*., 2009). In Bulgaria, at present the area occupied by this species is about 150 000 ha, amounting to 4 % of the total forest area. *R. pseudoacacia* is commonly applied for establishment of plantations with short rotation (Gyuleva *et al*., 2013).

In view of the above, the aim of this study was to examine alterations in the nutrient content of damaged *R. pseudoacacia* plants, from a big industrial source, in order to estimate the capacity of leaves to accumulate heavy metals, together with an evaluation of this species as possible bioindicator of heavy-metal pollution in Devnia region.

Materials and Methods

The present study was carried out in close vicinity of industrial region of Devnia in Eastern Bulgaria, a highly polluted zone due to the wastes generated from cement factories, nitrogen fertilizers and polyvinylchloride factories.

The material consisted of 25-year-old *Robinia pseudoacacia* plantation, growing at 500 m from the industrial complex on the prevailing direction of the local winds. The plantation of same age, in unpolluted region at 15 km away from the main transfer of the polluted air masses, was used as control site. The average stem height of the trees was between 8 to 15 m and the average stem diameter at breast height varied between10.5 to 13 cm (Table 1). Site index was determined on the table for the black locust plantations (Poryazov *et al.,* 2004).

Climate of the region was continental, under the influence of sea breezes. The mean air temperature within the vegetation period was about 22°C. The prevailing wind directions were characterized by east and west components. The soil under the sample plantations was Haplic kastanozem (FAO), sandy-clay type, with light mechanical composition and 7.0 - 7.5 pH. The humus content varied between 2.00 to 3.56 % and total nitrogen was about 0.344 % (Yorova *et al*., 1998).

The main atmospheric pollutants of the region, originated from the chemical plants and nearby highroad with dense traffic, were NOx, SO_2, O_3, aerosols of HCl, nitric and sulfuric acids, CO, HF, NH_3, Cl_2, CaO, $CaCO_3$, solid and liquid aerosols, organic compounds, particulate matter of dust and soot, silicon, Al and heavy metals (Tzvetkova and Petkova, 2006). Dust originated from both soil and industrial sources, containing N, K, Mg and P and other elements which get deposited on leaf surfaces, providing an alternative source of nutrients for the plants (Yorova *et al.,* 1998).

Almost all of the 24-hour means for O_3 concentrations were above the critical level of 30 ppb during the growing season. There were many short time events of high SO_2 concentrations (Tzvetkova and Petkova, 2007).

Leaf samples were collected from the middle part of the crown of three trees collected from polluted and control sites during June and September, 2009. Sampled leaves were cleaned mechanically, dried at 60 °C and stored in plastic containers. Total nitrogen concentration was measured by micro-Kjeldahl procedure. Total phosphorous content was estimated colorimetrically by molybdophospforic blue reaction. Potassium, calcium, magnesium, iron, manganese, cadmium, copper and zinc content in the samples were estimated by atomic spectroscopy, after wet digestion with HNO_3 and $HClO_4$. A Perkin-Elmer M-5000 atomic absorption spectrometer was used to detect these elements. Coefficients of total contamination were presented as a ratio between heavy metal content in the leaves of trees, collected from polluted and control sites.

Statistical analysis : The analysis was performed in three replicated. Differences between the mean concentration of

Table 1 : Site description and characteristics of *R. pseudoacacia* plantations

Characteristics	Polluted	Control
Latitude	43.235	43.180
Longitude	24.600	24.873
Altitude (m)	100	100
Exposition	South	East
Mean annual temperature (°C)	12.5	12.5
Annual precipitation (mm)	550	550
Age (years)	25	25
Planting density (number/ha)	333	500
Planting scheme	2 x 1.5 m	2 x 1 m
Average height (m)	8	15
Average DBH (cm)	10.5	13
Site index	V	IV

elements in the damaged and control trees were estimated by t-test at $p<0.05$ (Systat, 7.0).

Results and Discussion

The total nitrogen, phosphorus and potassium content were higher, in June than in September both in polluted and control leaves (Table 2). The calcium level increased from June to September in the leaves of both tree groups. Similar trend was shown by magnesium but only in the polluted leaves. Damaged leaves had lower phosphorus, potassium and magnesium content both in June and September. Low potassium level in damaged leaves was found in June as well as September (53 % and 38 %, respectively). In June, the phosphorus and magnesium content decreased by 16 and 23 % respectively. A slight decrease in total nitrogen (6 %) was observed in damaged leaves only in September, while total phosphorous and magnesium decreased by 11 and 3%, respectively.

Most of the metal content increased towards the end of the growth season in damaged leaves as well as in control. However, foliar content of copper and zinc in control trees was higher in June than in September. Manganese in polluted leaves exceeded that in control mainly in June, whereas iron and lead had higher concentrations in damaged leaves as compared to control, both during spring and autumn season. The ratio between nutrients and metal concentrations in the polluted and control leaves is shown in Table 3.

The foliar analysis revealed changes in the levels of macronutrients and some micronutrients. Lower nutrient levels in damaged leaves indicated alteration in the growth potential and physiological status of afflicted plants (Table 1). P, K and Mg had higher levels in younger leaves. Retranslocation of highly mobile macronutriens could support the younger leaves in polluted site to maintain a good physiological activity. During leaf senescence, the mobile nutrients decrease extremely. The decreased concentration of nitrogen, phosphorus and potassium in September was a result of their export to the stem, low uptake and remobilization (Tzvetkova and Kolarov, 1996). According to Nieminen and Helmisaari (1996), retranslocation of P and K was less efficient in the polluted stands. Higher amount of calcium at the end of growth period was due to its low mobility and presence of calcium compounds in the pollutants (Yorova *et al*., 1998).

Pb, Zn and Cu were found at higher levels in plant samples, collected from industrial area. In autumn, copper was taken up most intensively by damaged leaves of *R. pseudoacacia* as compared to other metals examined. Higher metal content of older leaves could be attributed to longer exposure period in the polluted environment, although young leaves showed increased ability for nutrient uptake (Sawidis *et al*., 1995). At polluted region, soil was contaminated with metals and uptake from the soil *via* roots and translocation to the leaves contributed to leaf contamination. Pb, Zn and Cu concentrations in contaminated soil under black locust plantation were 38.2, 77.4 and 101.3 mg kg^{-1}, respectively (Yorova *et al*., 1998). The trend of heavy metal accumulation indicated similarity with the results reported previously by several workers (Cicek and Koparal, 2004; Baycu *et al*., 2006; Filipović-Trajković *et al*., 2012). In response to air pollution, resistant plants accumulated high amount of toxic elements. The intensity of copper, zinc and lead contamination as well as its accumulation in plant leaves declined with distance from the industrial sources. Despite accumulation in foliage, metals like Cu, Zn and Pb in polluted areas were below toxicity levels which can decrease physiological activity. Root uptake was influenced by decrease in the transpiration rate, leading to reduced water and nutrients flow and alterations in nutrient balance (Suwannapinunt and Kozlowski, 1980). Leaf surface has an important morphological mechanism for survival that allows this species to maintain good photosynthetic efficiency during stress (Azmat *et al*., 2009). Despite significant accumulation of lead and copper young black locust trees not only succeeded in surviving in the

Table 2 : Nutrient and metal contents in the leaves of *R. pseudoacacia* from the polluted and control site

Elements	Damaged leaves		Control	
	June	September	June	September
N %	2.17 ± 0.02	1.63 ± 0.01	2.10 ± 0.02	1.74 ± 0.07
P (mg g^{-1} d. wt.)	2.13 ± 0.18*	1.68 ± 0.17	2.53 ± 0.23	1.88 ± 0.17
K (mg g^{-1} d. wt.)	19.75 ± 0.50*	14.53±0.24*	42.47 ± 1.10	23.55 ± 0.47
Ca (mg g^{-1} d. wt.)	15.40 ± 0.57	38.92 ± 0.75	15.33 ± 0.50	37.12 ± 0.66
Mg (mg g^{-1} d. wt.)	1.87 ± 0.20*	2.25 ± 0.22	2.42 ± 0.25	2.32 ± 0.17
Cu (mg 100 g^{-1} d. wt.)	1.33 ± 0.15*	1.72 ± 0.17*	1.78 ± 0.19	0.80 ± 0.09
Fe (mg 100g^{-1} d. wt.)	6.83 ± 0.36	14.35 ± 0.96	6.46 ± 0.28	13.62 ± 0.98
Mn (mg 100g^{-1} d. wt.)	4.33 ± 0.20*	10.07 ± 0.40	2.89 ± 0.14	9.30 ± 0.47
Zn (mg 100 g^{-1} d. wt.)	3.00 ± 0.43*	1.90 ± 0.31*	2.32 ± 0.30	1.38 ± 0.24
Pb (mg 100 g^{-1} d. wt.)	2.85 ± 0.42*	3.07 ± 0.99*	1.49 ± 0.23	2.22 ± 0. 34

Significant versus control at $p<0.05$

Table 3 : Ratio between the nutrients and metal concentrations in damaged and control leaves.

Elements	June	September
N	1.038	0.937
P	0.842	0.894
K	0.465	0.617
Ca	1.004	1.048
Mg	0.770	0.971
Cu	0.478	2.146
Fe	1.057	1.054
Mn	1.498	1.083
Zn	1.293	1.377
Pb	1.913	1.383

polluted area, but also maintained a good physiological status. Previous study on *R. pseudoacacia* showed that reduction in the rate of photosynthesis was only 22 % at the polluted site (Tzvetkova and Petkova, 2005). Brake *et al*. (2010) established the ability of black locust to survive in any significant numbers after reclamation at a coal mine. Filipović-Trajković *et al.* (2012) suggested that leaves from plants in the polluted area were better indicator of Pb pollution than other plant parts. Atiq-Ur-Rehman and Iqbal (2008) reported excessive copper in foliage of plants collected in the vicinity of industrial areas of Karachi.

Thus, it can be concluded that in the present study, *R. pseudoacacia* accumulated Pb, Zn and Cu in increased rates during season. The most pronounced metal accumulated by damaged leaves was copper. Consequently, trees growing near industrial sites in Eastern Bulgaria can be used as bioindicator of trace metals zones.

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