COPPER, MANGANESE AND ZINC BIOACCUMULATION IN THREE COMMON WOODY SPECIES FROM BLACK SEA COASTAL AREA

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Cu, Zn and Mn phytoaccumulation potential was assessed in three common woody species growing in the Black Sea coastal area, Constanţa County, Romania, Elaeagnus angustifolia L., Robinia pseudoacacia L. and Salix alba L.

Tissular heavy metal concentrations were determined through AAS in aboveground plant organs and compared to that in soils. Biological Accumulation Coefficients (BAC) were determined.

The highest average concentrations were found in Salix alba L. species: 8.89 mg/kg Cu, 43.94 mg/kg Mn and 94.10 mg/kg Zn, values that are not consistent with hyperaccumulation. Salix alba L. was the only species with BAC > 1 for Cu (constant in all samples) and Zn (only as average value).

Keywords: bioaccumulation, heavy metals, *Elaeagnus angustifolia L.*, *Robinia pseudoacacia L.*, *Salix alba L.*

1. Introduction

Heavy metals pose a growing problem as soil pollutants, due to their increasing usage in industry and agriculture (in pesticides etc.). In turn, soil pollution affects wildlife, agriculture and livestock and, not least, human health.

High concentrations of heavy metals can also occur naturally, in certain soils. Plants that are constantly exposed to high amounts metal ions have developed various mechanisms to avoid toxicity.

Root exclusion and root sequestration (i.e. limiting uptake or avoiding metal transfer to sensitive photosynthetic organs) are two common strategies. However, other species react by distributing metals throughout their tissues, including aboveground organs and/or storing them in secure areas. This is called phytoaccumulation, if the organisms concentrate more metal in its tissues than in surrounding soil. The extreme version is hyperaccumulation, when tissular metal concentration surpasses that in regular vegetation 100-1,000 times [1, 2]. Phytoaccumulation is important, because it relates to food safety, but also due to

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its potential applications in bioprospection, bioremediation of polluted soils and biomining of subeconomic ores [1, 2].

This research is part of a wider effort to screen local vegetation for possibly valuable metal-accumulating plants. Copper, manganese and zinc are three metals widely used in industry and also, common pollutants.

Elaeagnus angustifolia L., Fig. 1, (known as Russian/Persian olive, Trebizond date, silver berry, oleaster) is member of Elaeagnaceae family, a thorny shrub or small tree (up to 8 m height), with reddish bark and deciduous, lanceolate leaves, covered with silver scales. It blossoms in spring, producing small, yellow flowers. Fruits are small, oval, with a single seed each. Reproduction occurs mainly by seeds. It is a native of Mediterranean Europe and Central Asia, but found (often as invasive) in many other regions. It prefers wetlands (coasts, riverbanks, marshes) and tolerates a large variety of soils (including sandy or salty ones) and temperatures, temporary flooding and drought. It is grown mostly for ornamental purposes, but also for nitrogen enrichment of soils and, in some regions, for its edible fruits, which also have medicinal applications [3, 4].

Robinia pseudoacacia L., Fig. 2, (known as black/honey locust, false acacia, Chinese scholar tree), a member of the Fabaceae family, is a medium-sized tree (up to 30 m), with gray-brown to dark brown bark, long, composed, pinnate leaves with spiny stipules, white flowers grouped in pendulous racemes and dark brown legumes as fruits. A native of Southeastern USA, it is now grown for its valuable wood and ornamental and apicultural purposes throughout temperate areas, including most of Central Europe. It is tolerant to many types of soils, but less tolerant to shade and frost. It is often planted in non-native regions, for decorative and apicultural purposes. It is considered as invasive in some areas. Its root system also helps soil enrichment with nitrogen [5, 6].

Salix alba L. (known as white willow), Fig. 3, from Salicaceae family, is a medium tree, sometimes shrub, growing throughout Northern Eurasia, as a native species, and in other temperate areas. It has a dark grey bark, yellow to reddish-orange on thin branches. Branches are flexible, with deciduous, lanceolate, silver-grey leaves. It is dioecious, with both male and female flowers grouped in catkins. Reproduction is done by seeds and twigs. It grows in wetland areas, on various types of soils, but necessarily needs moisture. It is sometimes grown as an ornamental plant, sometimes used for wood and for medicinal purposes (for salycin) [7, 8].

Fig. 1. *Elaeagnus angustifolia* L. (from ref. [4])

Fig. 2. *Robinia pseudoacacia* L. (from ref. [6])

Fig. 3. *Salix alba* L. (from ref. [8])

2. Material and methods

Samples consisting of branch fragments were taken from the *Trei Papuci-Modern* beaches, Black Sea coastal area, Constanţa County, Romania, from three individuals for each of the species studied. Soil samples were taken from the rhizospheric area of each tree. Branches were cut in smaller pieces, oven dried at 80ºC for several days. Soil samples underwent the same procedure.

Quantity of 0.25 g of each sample was left to digest in 5 mL concentrated HNO_3 and boiled one hour at 150ºC. 2 mL H_2O_2 were added and the mixture was boiled again, for two hours at 150ºC. Resulting solutions were diluted to 50 mL, with NH_4Cl (2% final concentration) and $CaCl_2$ (0.5%) [9-12].

Concentrations of selected metals were determined through Atomic Absorption Spectrometry, using a HR-CS Spectrophotometer ContrAA 700, Analytik Jena AG, Germany, with acetylene flame, at specific wavelengths for copper (324 nm), manganese (279 nm) and zinc (213 nm). Metal concentration values were expressed as mg/kg [10-15]. The device has, as minimal detection limits, 0.46 mg/kg Cu, 0.16 mg/kg Mn and 1.44 mg/kg Zn.

The biological accumulation coefficient (BAC) was calculated for each plant, as a ratio of metal concentration in shoots to the concentration found in soil. BAC (eq. 1) is important for showing accumulation and translocation of metal ions to aboveground organs [16, 17].

Eq. 1: $BAC = [Metal]_{Shoot} / [Metal]_{Soil}$

3. Results and discussion

Average concentration levels of each heavy metal in the selected plants are shown in Fig. 4. Biological Accumulation Coefficient (BAC) values are shown in Fig. 5. There are several factors that determine whether a plant species can be considered a valuable metal accumulator or not.

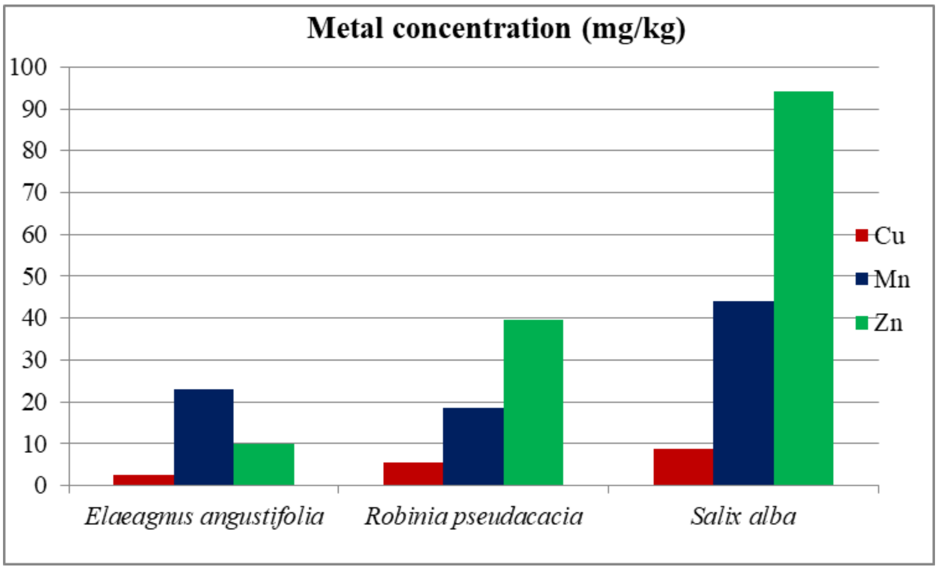

Fig. 4. Cu, Mn and Zn content in branch tissues of selected species (average values; mg/kg)

Every living organism contains various heavy metals in low concentrations. An average plant, for instance (the so-called „standard reference plant") contains around 10 mg/kg Cu, 200 mg/kg Mn and 50 mg/kg Zn [18]. Average values for the three selected metals and plant species fall below these standard values with one exception: 94.10 mg/kg Zn in *Salix alba* L. However, there was a high degree of variation among individuals, from 16.70 to 246.60 mg/kg.

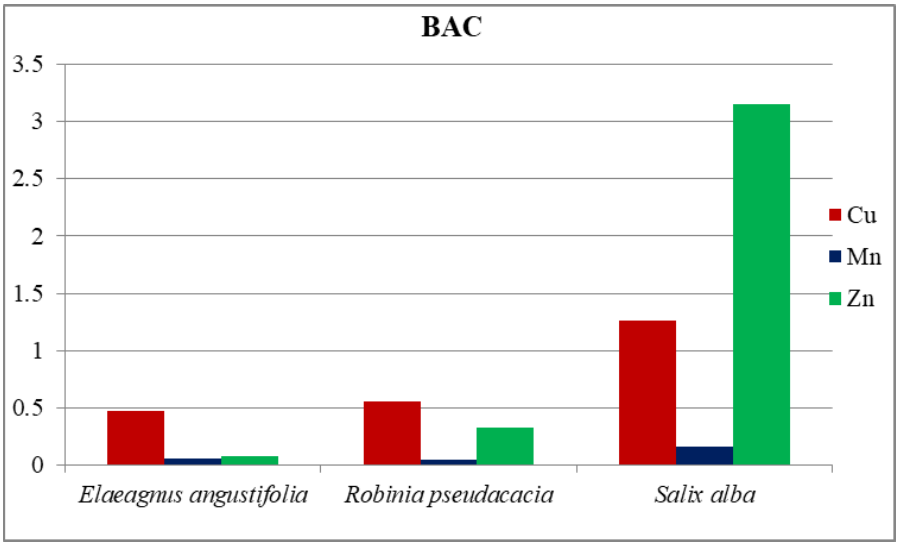

Fig. 5. Biological Accumulation Coefficients for selected plant species and heavy metals (average values)

A much higher threshold, is the one defining hyperaccumulation. Most authors consider 1,000 mg/kg to be the minimal threshold for Cu hyperaccumulators (while some consider 300 mg/kg), while 10,000 (or 3,000) mg/kg is the threshold for Mn and Zn [1, 18]. None of the three woody species in this study is a Cu, Mn or Zn hyperaccumulator under this definition.

The BAC, while it might not be constant under all types of ecological conditions and by itself does not indicate the actual amount of metal accumulated, it is an extremely valuable index, coupled with other quantitative data.

A BAC constantly above 1, means that the plant concentrates metals in its tissues and, even more importantly, it translocates them in aboveground organs, where they can be harvested. A plant with BAC > 1 could be valuable as a phytostabilizer or phytoextractor [18].

According to general classification, plants with a BAC below 0.01 are considered as non-accumulating, at BAC between 0.01-0.1, low-accumulating, at 0.1-1, moderately-accumulating and, at 1-10, high/hyper-accumulating [19].

According to the values determined, the three species should be considered as manganese excluders or low accumulators. Moderate to high copper accumulation occurred in *Robinia pseudoacacia* L. and *Salix alba* L., but starting from low copper concentration in local soils. While most soils in Romania contain 0-120 mg/kg Cu [20, 21], samples analyzed in this experiment had 13.01 mg/kg as the highest value.

Zinc was found in normal concentrations in soil samples: over 100 mg/kg for most soils, 28.27-89.65 mg/kg in soils around selected *Salix alba* L.

individuals (compared to 0-275 mg/kg range in Romanian soils and 100 mg/kg legal reference threshold for sensitive soils [20, 21]).

Moderate zinc accumulation occured in *Robinia pseudoacacia* L. (BAC 0.29-0.37). In *Salix alba* L., results were very different among individuals: from 0.21 (at the highest concentration in soil – 89.65 mg/kg), to 8.72, indicating high accumulation (but at the lowest soil concentration – 28.27 mg/kg), with an average BAC of 3.15.

Salix species are known as tolerant to high concentrations of several heavy metals, including zinc and often exhibit high metal accumulation and translocation to upper organs [22, 23].

6. Conclusions

According to these results, only for *Salix alba* L. encouraging data for metal accumulation for metal accumulation and translocation (BAC > 1) were found, for copper and zinc. In the case of copper, the values were constantly high, but at a low soil concentration. For zinc, BAC was highly variable, with an extremely high value only at low soil levels.

In both cases, *Salix alba* L. seems suitable for further experiments, regarding the potential for chemically-enhanced bioaccumulation at higher metal ion levels in soil.

Robinia pseudoacacia L. had BAC values below 1, but still can be considered as a moderate Cu and Zn accumulator, with a possible potential for phytostabilization.

No significant accumulation was observed in *Elaeagnus angustifolia* L. samples, and all three species are manganese excluders.

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