SPRUCE BARK AQUEOUS EXTRACTS AS MODULATOR IN COPPER INDUCED STRESS

Alina STINGU[1], Ioana IGNAT[1], Irina VOLF[2], V.I. POPA[1]

[1] "Gheorghe Asachi" Technical University, Department of Natural and Synthetic Polymers

[2] "Gheorghe Asachi" Technical University, Environmental Engineering and Management Department, *e-mail vipopa@chtuiasi.ro*

Plants manifest a certain reaction against increasing of heavy metals toxic concentration in the cultivated soils. The differences depends on plant sensitivity, species, time exposure intensity (concentration of heavy metals, cultivation length), or amendments applied on cultivated site. The challenge of this work is to investigate the influences of different amendments such as biologic active compounds, on the seedling stage and growth responses, copper uptake and accumulated at different evolution stage of plants. Thus, physiological responses of maize (Zea mays) under copper ions stress and polyphenolic amendments were evaluated in pot cultivation experiments through roots and stem length measurements and quantitative determination of wet biomass. The distribution of copper ions concentration in different parts of the plants was estimated by flame atomic absorption spectrometry. In some cases stimulating effects were identified for different concentrations of spruce bark extracts applied on maize seed under copper stress. Other extracts seems to have inhibiting proprieties on maize plant growth. In conclusion spruce bark extracts can have stimulator as well as inhibitor effect depending on the concentrations of biological active compounds and the presence of copper ions.

***Key words**: phytextraction, polyphenols, heavy metal*

Agricultural soil pollution with toxic-metals is of increasing concern due to food safety issues and potential health risks.Traditional methods of dealing with metal pollution are either the extremely costly process of removal and burial or simply isolation of the contaminated sites. Thus, new methods based on environmentally friendly and low-cost technology are needed. Phytoextraction by using hyperaccumulator plants has been proposed for decreasing the toxic-metal concentrations of contaminated soils. To maximize the efficiency of phytoextraction, it is important to select a plant with a strong metal-accumulating ability that is also compatible with mechanized cultivation techniques and local weather conditions. Selection of such plants may yield more immediately practical results than selection-based solely on high tolerance to toxic-metals [3].

On the other hand, polyphenols extracted from different sources of plant biomass can have stimulating or inhibitory effects regarding plant growth and development. Also, polyphenols could interact with different pollutants agents with important contributions to their sequestration, regulation their transfer in different organs of the plants or metabolisation by different mechanisms including enzymatic systems.

Studies carried out in the case of spruce bark extracts have proved stimulating effects in appropriate concentration on the different crops such as maize, bean, tomatoes, oats, barley and rice [5, 6, 8]. On the other hand, stimulating or inhibitory effects on growth and development of plant could also be influenced by the concentration of polyphenolic extracts. Different activities of polyphenolic products can be similar to those of growth hormones (auxines, cytokines) effects [6,7].

Taking into account this information, the purpose of this study was to analyze the interaction of copper ions and polyphenolic compounds from the spruce bark extracts on maize plants growth and developments.

MATERIAL AND METHOD

Heating extraction

Spruce bark was grinded in a mill to reduce it to a fine powder with size of 0.5 mm. Different amounts of dried ground material were extracted for three times with 125 mL water in water bath at 80-90 C for 45 min.The aqueous extracts were collected and the volume was completed to 500 mL with distilled water, and characterized from dry matter and polyphenols contents point of view.

Determination of total phenolic compounds by Folin– Ciocalteu method

The total phenolics were assayed colorimetrically by the Folin–Ciocalteu method.[1]

Pot experiments

Pot tests were carried out using sandy soil. Maize (*Zea mays*) seeds were directly sown into pots after applying 10mL of tested solution. To contaminate sandy soil with copper ions it was used a stock solution of copper at 50mg/L, obtained by dissolution of copper sulfate $CuSO_4 \cdot 5H_2O$. Three different concentration of copper sulfate were tested (10, 25, 50mg/L CuSO4- C1, C2, C3) in different condition, in the absence or presence of spruce bark extracts. It has been used spruce bark extracts obtained from three different quantity of raw material (5, 10, 20 g spruce bark-S1, S2,S3) and combination between copper solution and polyphenolic extracts (C1S1, C2S2. C3S3). Each Cu ions treatment was replicated in five pots, and three uniform plants were allowed to grow in each pot, at a uniform spacing.

For one week the cultivated soils were wetted daily with 15mL tap water until germination. After that, every two days maize plants were wetted with 15 mL tested solution. The experiments were carried out for 45 days.

Evaluation of plant growth and metal accumulation

Maize plants were harvested after 45 days and the root, stem and leaf lengths were measured. The plants were then washed with running tap water and rinsed with deionized water to remove any sand and perlite particles attached to the plant surfaces. Root, stem and leaf were separated and weighed. Plants samples were oven dried at 70 $^{\circ}C$ until constant mass. About 0.5g plant material were digested with concentrated HNO_3 and H_2O_2 on a hot plate for at least 6 hours [2]. The digested solutions were

filtered and then analyzed. Copper concentrations were determined in air acetylene using a flame atomic absorption spectrophotometer (GBC Avanta 2003) and expressed on the basis of dry mass.

RESULTS AND DISCUSSIONS

Dry matter and total phenolic compounds

Dry matter content determination shows that the concentration values increase proportionally with the raw material quantity used in extraction process (*tab1*). At the same time total polyhenols content (TPC) is correelated with the quantity of spruce bark used in experiments.

Table 1

Dry matter content in spruce bark extracts

Sample	Dry matter content (g/L)	Organic matter content (g/L)	TPC (mg/L)
S1	0.47	0.44	130
S2	0.81	0.76	191
S3	1.56	1.47	190

Pot experiments

The results obatined by applying different treatments with solutions of copper ions and spruce bark extracts to maize plant are presented in (*fig.1*). A stimulative effect of copper solutions C1(10mg/L $CuSO_4$), C2 (25mg/L $CuSO_4$), all spruce bark extracts (S1, S2 S3 - see *tab.1*), and combination between coper ions and polyphenols extracts (C1S1, C1S2 and C1S3 , C3S3) on maize root length was observed. When a combination (C2S3) was used inhibitory effects on maize root length were evidenced, while the samples C3, C3S1,C3S2 and C2S2 seems to have similar effect as in the case of Control sample (tap water).

Stem length of maize plant is stimulated in the presence of C3, C2S1, C3S2, C3S3 tested solutions. An inhibitory effect is evidenced when the solution of C1S3was applied. The other samples used in this pot experiments seems to have similar effects on maize stem length as in the situation of reference samples (*fig.2).*

The accumulation of biomass in plant roots was stimulated in all variants of treatment excepting the sample treated with C3S1 which has inhibitory effects, as one can observe in *fig.3*. The obviously stimulating effects are provided when the plants were treated with C1, S1, S2, C1S1, C1S2, C1S3, C2S1, C2S2 samples.

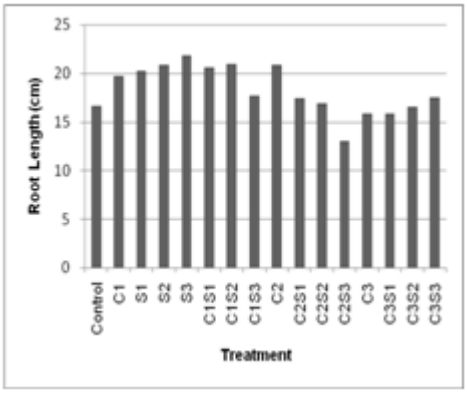

Figure 1 **Influence of copper ions and spruce bark extracts on maize root length**

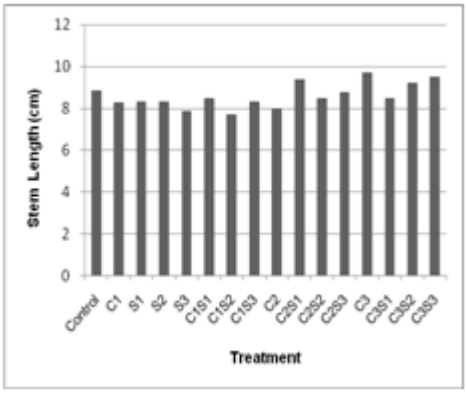

Figure 2 **Influence of copper ions and spruce bark extracts on maize stem length**

All studied treatments applied to maize plants evidenced inhibitory effects (*fig.4*) on the stem green biomass. The strongest inhibitory effects was observed when the samples C1S2, C1S3, C2 were applied to water the maize plants cultivated in vegetation pots.

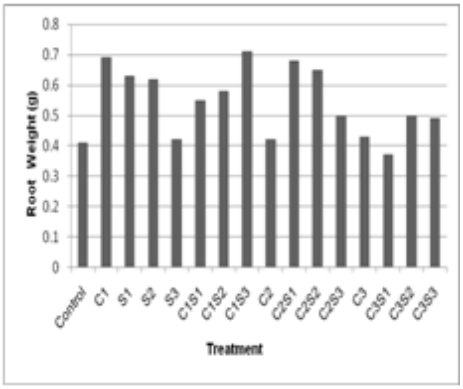

Figure 3 **Influence of copper and spruce bark extracts on maize root mass**

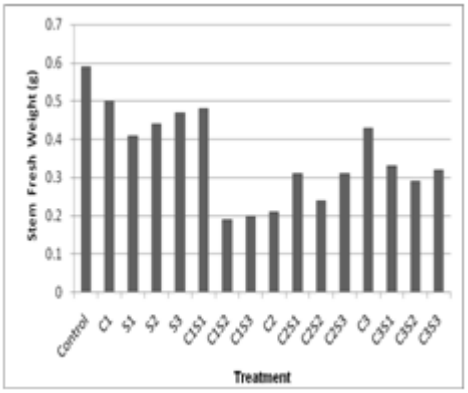

Figure 4 **Influence of copper and spruce bark extracts on maize stem green biomass**

The quantification of copper ions distributed in different morphological part of maize plant shown that in the presence of spruce bark polyphenolic amendments, retention of copper is more efficiently. Copper ions are predominantly retained in the roots of maize plants (*fig.5*), when these are cultivated in the presence of a small concentration of copper. Then a higher concentration of metal ions solution are used, copper ions are transferred from roots to stems. By increasing the metal ions concentration and in the absence of spruce bark extracts, *Zea mays* is capable to absorb less copper in aerial parts of the plant.

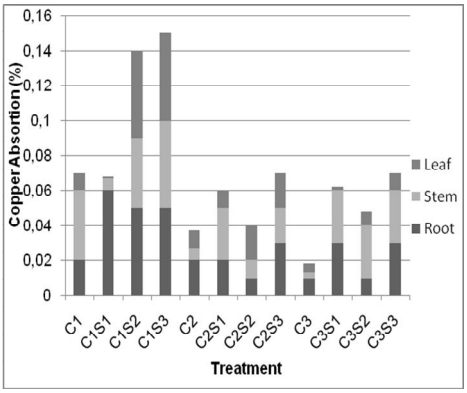

Figure 5 **Copper absorption in different parts of maize plant**

CONCLUSIONS

The polyphenols compounds were extracted from spruce bark using water as extraction agent. The obtained extracts were characterized from dry matter and polyphenols contents point of view. The extracts of different concentration along with solutions of copper ions, individually or in combinations were tested to establish their influence in the case of maize cultivation in vegetation pots using sandy soil. As a function of applied treatment, the stimulation or inhibition effects were evidenced following biometric characteristics and distribution of biomass in different morphological parts of the plants.

Thus, spruce bark extracts used as amendments stimulate roots elongation and roots biomass mass even in the presence of heavy metals ions. The most evident stimulating effects was observed in the case of the treatments with the samples S1, S2, C1S1, C1S3,C2S1, C2S2 from roots biomass point of view. Root elongation is obvious strongly stimulated in the presence of S1, S2, S3, C1S2. The utilization of C2S1, C3S2, C3S3 combinations stimulates stem length. Stimulating effect on root elongation is notice also in contaminated sandy soil with C1, C2, C3 samples. Copper sulphate solution C1, C2 stimulates also root biomass accumulation. Sample C3 characterized by a highest concentration in copper ions determines inhibitory effects on stem biomass and root elongation, but stimulating effects on stem length. Stem biomass accumulation is inhibiting by all the treatments applied. The strongest inhibitory effects are observed in the presence of C2, C1S2, C1S3.

Polyphenolic amendments could be used to increase the absorption of copper ions in different parts of maize plant as compared with the sample cultivated in the absence of natural aromatic compounds.

The higher copper ions retention is achieved in the less contaminated sandy soil and in the presence of spruce bark extraction with increased concentration (S2 and S3), what proves the important role of polyphenols in the metabolism of heavy metals.

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