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Influence of physical-chemical properties of soil on bioavailability of copper in the roots of Calendula officinalis L.

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Abstract: Determination of total and singular content of copper in soil and determination of physical-chemical characteristics of soil was performed, as well as influence of copper on growth, bioavailability and translocation of marigold (*Calendula officinalis L.)*. Total and singular copper content was determined in natural soil conditions on locations Petrovo and Banovici, total content of copper in marigold roots was determined by use of AAS method and sequential extraction. BCF factor was used for assessment of dynamics and intensity of metal adoption from the soil. In our experiment, all samples had the BCF <1 value from both locations. It was concluded that marigold is not hyperaccumulator of copper.

Keywords*:* Copper, soil, roots, marigold, extraction, AAS

INTRODUCTION

Metal traces are always present in the nature, and natural processes regulate their concentration as disintegration of rocks and soil due to influence of weather. Very important fact considering the metal traces is that they are not biodegradable (unlike organic substance), but they do go from one form to another.

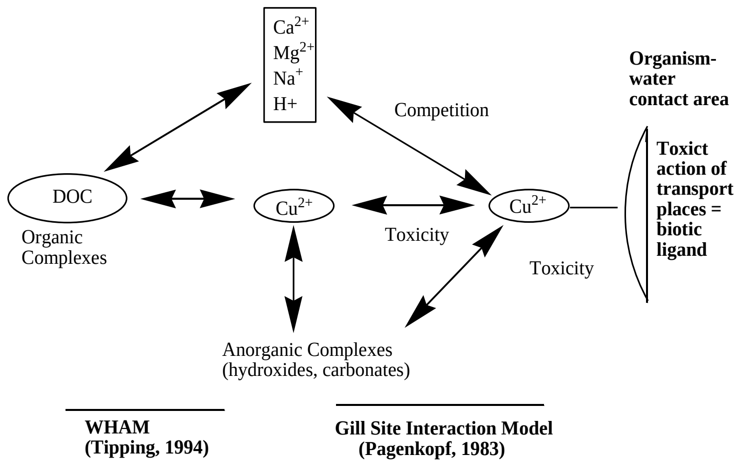
Copper is an essential element for important cellular functions such as photosynthesis, pigmentsynthesis and plasma membrane permeability.[1]

One of the more important mechanisms for plant tolerance towards copper is immobilization of this metal in cell walls, but its efficiency is limited by capacity of cell wall.

Other mechanisms responsible for plant tolerance towards copper are deposition in vacuoles and binding in complexes with phytochelatines (proteins with small molecule mass) in cytoplasm.[2]

Copper is included in function of different enzymes, mostly those that participate in redox reactions.[3] Examples of enzymes are: cytochrome c oxidase, (key enzyme in electron transport chain), superoxide dismutase (has a role of catching the free radicals), lysyl oxidase (crucial for networking of collagen fibers), ferroxidase (important in transport of iron), dopamine β-monooxigenase (needed for metabolism of dopamine and catecholamine), tyrosinase (needed for melanin production) and many other.

Enzymes that contain copper mainly participate in phenol metabolism. Cu-enzymes that participate in creation and transformation of phenols are different phenoloxidases. With the help of Cu valency change ($Cu^{2+} \leftrightarrow Cu^{+}$), they catalyze oxygenation of phenols to quinone. Many outside factors influence the intensity of ion adoption by roots (ion concentration in external environment, selectivity, temperature, pH value of environment and other) and inside factors (species, genotype, health condition, ontogenetic development and other).[4] At the start of growth and development of roots, adoption of mineral matter is very intensive. During that period, mineral matter accumulates faster than organic matter.



Fugure I: Reduction of activity of free copper ions by forming of copper complexes with organic and inorganic ligands, and competition of copper, calcium, magnesium, sodium and hydrogen cations, which leads to reduction of ability to bind copper and biotic ligands, which leads to reduction of toxicity. [5]

Essential elements are usually adopted selectively in the form of ions. When they bind into complexes, selective binding is disabled, and metal concentration with chelators in solution of soil must be high enough to favor apoplastic transport of metals, and so to compensate reduced adoption of metals through symplastic transport.[6] Individual species of plants differ by their ability to adopt and accumulate individual elements, efficiency of use, their distribution inside the plant and their sensitivity to the excess or lack of certain elements.

EXPERIMENTAL

Materials and methods

In order to determine content of absorbable copper in soil in *Calendula officinalis L.* (marigold), experiment in semi-controlled conditions was set in soil in Petrovo, as well as an open field experiment in Banovici, during the February-August period.

For physical-chemical analysis, part of the sample was air-dried, and then homogenized (size of granules < 2 mm). pH-value was determined in soil suspension with water (active acidity) and in suspension with 1 M KCl (substitutional acidity), by using potentiometer following ISO 10390:1994 method. Content of $CaCO_3$ in soil was determined gas volumetrically with Scheibler's calcimeter following ISO 10693:1995 method. Content of organic matter in soil was determined by annealing of samples in an oven on 500-600°C. Preparation of soil samples for AAS analysis was performed by weighing of 1 g of air-dry soil sample with 0.001 g accuracy in reaction flask and dissolving it in aqua regia (33.33 % HNO_3 + 66.66 % HCl). After dissolution and cooling, solution was carried over to 100 mL measuring flask and supplemented to label with the distilled water. Prepared sample is then filtered, and the concentracion of copper in filtrate was measured by AAS. Preparation of marigold roots for AAS analysis was performed by weighing of 0.5 g of sample in platinum pot, and then burning the sample on burner. To improve the mineralization, pot with the sample is put on the heated furnace on 900^{o}C and kept there for 2-3 minutes. After cooling, 2 mL of concentrated HNO_3 and 5mL of distilled water were added. Then, the pot is put on the mild temperature, so the content could be properly dissolved. After cooling, content is moved to 10 mL flask, filled up to the label with distilled water and then the concentracion of copper was measured by AAS analysis.

After determination of total copper content in the examined samples, specific forms of copper binding in soil was determined by the sequential and fractional extraction method, in 4 phases.[7] Fifth phase is residue, which includes silicates that contain significant copper content. *Tessier* method was chosen because, unlike the other methods of sequential extraction, it enables separation of exchangeably copper bound to carbonates, which was important for this research.

Dissolved and changeable Cu: extraction from the soil with CH_3COONa (1 M, pH 8.2 with NaOH), 1 hour.

Cu bound to carbonate: extraction from the residue of previous phase with CH_3COONa (1 M, pH 5.0 with CH_3COOH), on room temperature, 4 hours.

Copper occluded on iron and manganese oxides: extraction from the residue of previous phase with 0.04 M NH_2OH x HCl in 25% (v/v) CH_3COOH, 96 ± 3 °C, 5.5 hours.

Copper bound to organic matter: extraction form residue of previous phase with 0.02 M HNO_3 i and 10 mL 30% H_2O_2 (pH 2 with HNO_3), on 85 ± 2 °C, 2 hours. After that, 6 mL of 30 % H_2O_2 (pH 2 with HNO_3) was added, and extraction continued for 3 hours more on 85±2 °C. After this, 10 mL more of 3.2 M CH_3COONH_4 in 20% (v/v) HNO_3 was added, and cuvette was filled up with 40 mL of distilled water for 30 minutes on room temperature.

Residual Cu: is what is left. Blank probe for each of extraction phases was prepared along with the samples. For each fraction, series of multi elementary standards were made, and so called „matrix matching" was performed, in each standard series appropriate extraction medium was added in the same volume as in the sample. Content of total copper and specifically bound forms of copper in the prepared samples was measured by AAS, Perkin Elmer, 3110 spectrometer.

RESULTS AND DISCUSSION

Results of $CaCO_3$ content presented in Table 1. shaws that soil samples from Banovici are non-carbonate soils, and soil from Petrovo is medium carbonated soil. Total content of organic matter in Banovici soil makes it as a soil with a very high content of organic matter, while samples from Petrovo are soils with high content of organic matter. pH value of soil is a parameter that influences the amount of available ions for plants in soil. The more acidity is in the soil, the higher is metal solubility in it. Banovici soil is considered as neutral, soil from Petrovo is base soil. Desirable pH value for arable layer is 6.5 and more, because it provides good immobilization of heavy metals and their minimal bioavailability, and also optimal availability of essential micro and macroelements.[8]

Table 1. Soil parameters

Parameters	Locations	
	Banovici	Petrovo
pH-H_2O	6.72	7.84
pH-KCl	6.27	7.38
$CaCO_3$ (%)	0	13.96
Organic Matter (%)???	13.69	7.55

The results show that in the untreated Banovici soil samples obtained in the first phase of extraction, the content of exchangeable copper ions is 1.63 %, and in Petrovo soil 1.66 %, which is in corelation to total copper content in the soil. These values are low in second fraction, and they are 0.20 % for Banovici, and 0.16 % for Petrovo. Cu-fraction bound to Mn and Fe oxides has same value as fraction bound to carbonates in both samples. Solubility of copper in soil increases with the acidity of soil, and decreases with the rise of pH value. Decrease of mobility of copper is achieved by the increase of organic matter in the soil. Most of copper content is presented in the fourth fraction, and it is in corelation to the total content 21.96 % for Banovici and 11 % for Petrovo). That means that copper is mostly bound to the organic matter forming organometallic compounds which turn copper into the form that is hardly accessible for the plant, and because of that there is no negative influence of copper on the plant. Finally, copper bound to clay minerals remainde from previous phases and it is highly present phase in samples with the values of: 76.40 % Cu in Banovici and 87.33 % Cu in Petrovo.

Table 2: Content of single forms of copper bonds

Forms of copper / mg kg^{-1}	Location	
	Banovici	Petrovo
changeable copper	0.4	0.5
copper bound to carbonate	0.05	0.05
copper bound to Mn and Fe	0.05	0.05
copper bound to organic matter	5.38	3.3
copper bound to mineral clays	18.72	26.2

Natural content of copper in soil is around 1-20 mg kg^{-1} of air-dry soil sample, and the average value of noncontaminated copper in soil is around 6-60 mg kg^{-1}, or 2-100 mg kg^{-1} .[9, 10,11] Copper content in the examined soil exceeds the border value of 100 mg kg^{-1} for Petrovo area ($Cu_{(600)}$ =117.00 mg kg^{-1}). For samples from the other areas, values do not exceed the border value.

Average content of copper in noncontaminated soil is 60 mg kg^{-1}. This value is higher only in samples from Banovici ($Cu_{(200)}$ = 64.5 mg kg^{-1}), while in other samples those values were lower.

Table 3: Concentracion of Cu in soil /mg kg^{-1}

Soil/ mg kg^{-1}	Banovici	Soil/ mg kg^{-1}	Petrovo	Soil/ mg kg^{-1}	Petrovo
(control)	24.5	(control)	30	(control)	30
Cu $_{(0*)}$	25	Cu $_{(0*)}$	27	Cu $_{(0*)}$	25
Cu $_{(3*)}$	32.5	Cu $_{(3*)}$	26	Cu $_{(9*)}$	30
Cu $_{(50*)}$	33	Cu $_{(50*)}$	26	Cu $_{(150*)}$	40
Cu $_{(100*)}$	41	Cu $_{(100*)}$	27	Cu $_{(300*)}$	59
Cu $_{(200*)}$	64.5	Cu $_{(200*)}$	32	Cu $_{(600*)}$	117

*The concentration of copper added to the soil expressed in mg kg^{-1}

Cu concentration in roots of cultivated plants is within the allowed limits (Banovici 8.2 mg kg^{-1}, Petrovo 4.8 mg kg^{-1}). Critical values for copper are 15 mg kg^{-1} or more, and toxic values are 20 mg kg^{-1} or more.[4] By monitoring copper concentration in roots, it was determined that the highest Cu-concentration value was recorded in treated soil of Banovici and Petrovo, in the sample $Cu_{(150)}$ with the value of 16 mg kg^{-1}. In the samples of roots cultivated in the soil with alkali reaction, more significant accumulation of copper in the roots was determined. Accumulation in the roots is probably a consequence of copper immobilization in cell walls, because that is one of the basic mechanisms of defense of excessive copper adoption.[2]

Root system of *Calendula officinalis L.* analyzed in this paper has a high capacity for copper bonding, and thus protecting the plant from toxic activity. Obtained results show that copper adoption in the roots is relatively low in all plant samples, regardless of the soil where they were growing, which can be explained by good immobilization of copper in the soil.

Reduction of biomass of roots from Banovici and Petrovo soil 3·c /mg kg^{-1} (on soil with three times more concentration) occurred. It is considered that biomass reduction of roots is a common response of the plant on complex interactions of copper in large number of physiologic and biochemical processes. It was determined that after the treatment of soil with copper salt, the concentration of this metal significantly increased, but it did not increase proportionally with the increase of added salt, which implies that copper was tied to firmly in some fractions of soil.

Table 4: Concentration of Cu in roots, / mg kg^{-1}

Roots/ mg kg^{-1}	Banovici	Roots/ mg kg^{-1}	Petrovo	Roots/ mg kg^{-1}	Petrovo
(control)	8.2	(control)	4.8	(control)	4.8
Cu $_{(0*)}$	10	Cu $_{(0*)}$	8.2	Cu $_{(0*)}$	6.6
Cu $_{(3*)}$	9.8	Cu $_{(3*)}$	8.6	Cu $_{(9*)}$	5
Cu $_{(50*)}$	10.4	Cu $_{(50*)}$	7	Cu $_{(150*)}$	16
Cu $_{(100*)}$	13.6	Cu $_{(100*)}$	9.6	Cu $_{(300*)}$	10
Cu $_{(200*)}$	14	Cu $_{(200*)}$	8.4	Cu $_{(600*)}$	9.2

* The concentration of copper added to the soil expressed in mg kg^{-1}

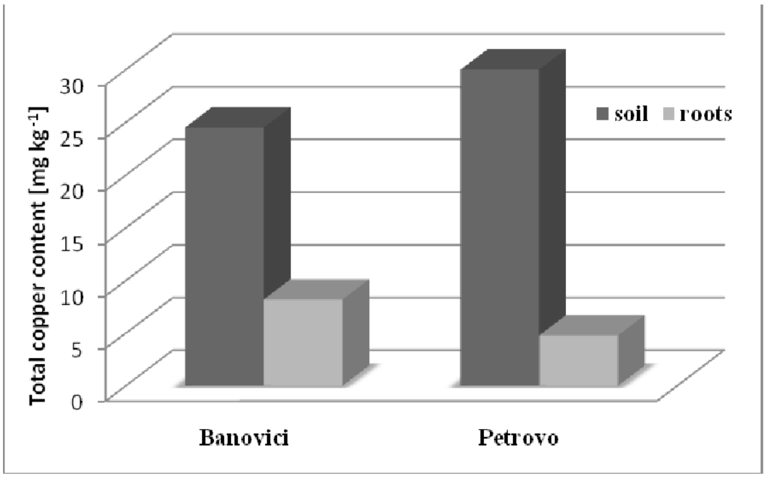


Figure 2: Total concentration of copper in soil and roots of nontreated samples

Soil that was kept in pots during the experiment, over the time has settled down which probably resulted in lowered permeability, lowered drainage, and worsed water-air regime.

Bioconcentration factor (BCF) was calculated as ratio of metal concentration in the plant (used part) and metal concentration in soil in which the plant grew.[12]

$$BCF = [metal]_{roots}/[metal]_{soil}$$

Plant species whose BCF factor value is >1 can be used as potential species for phytoextraction and phytostabilization.[13] In order to become a hyperaccumulator, plant must have high rate of growth and high biomass level, and to be adaptable on the environmental stress. In our experiment, all samples had BCF value <1 from both locations. It is concluded that marigold is not hyperaccumulator.

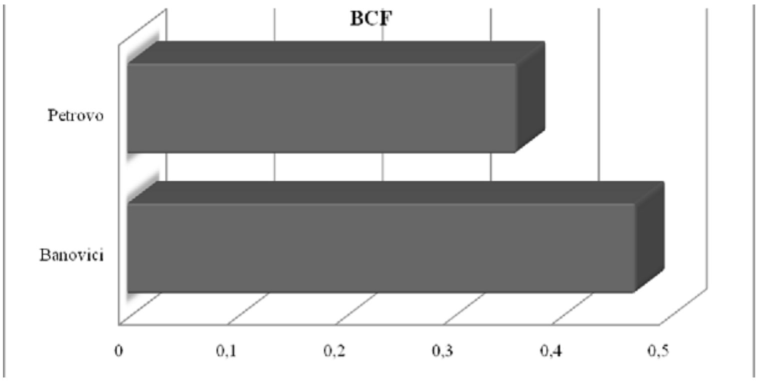


Figure 3: BCF values of Cu in the examined samples

CONCLUSIONS

Acidity of the examined soil is not in optimal range (pH_{KCl} from 5 to 6.5) for cultivation of medicinal plants. Bioavailability and absorption of copper in marigold roots is optimal on the areas with pH <7, and its bioavailability decreases with the increase of soil pH value. These findings were proved by experimental examination. Copper mostly bonds with organic matter and clay minerals in all soil samples.

Concentrations of copper in the examined area do not exceed the border value of 100 mg/kg, and concentrations of copper in roots of cultivated plants are within the regular limits. In the soil samples with alkali reaction, we found a more significant copper accumulation in the roots. Plants growing on the soil with high heavy metal content created a physiological mechanism that enables them to survive higher heavy metal concentrations. This mechanism is based on the slower adoption, or on the certain system of internal detoxication.

From everything mentioned, it can be concluded that the cultivation of marigold in semi controlled conditions is difficult to compare with real natural conditions (open field), and that cultivation of marigold in outdoor conditions is one complex and open system which is difficult to control. Further investigations should be targeted at the improvement of physical and chemical characteristics of soil, and on mechanisms of survival of marigold in polluted soil.

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