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IMPACT OF COMPLEXING COMPOUNDS ON GERMINATION AND EARLY DEVELOPMENT OF MAIZE (*Zea mays* L.)

Summary

The study focused on toxicity evaluation of selected complexing organic compounds (EDTA, rhamnolipids, quaternary pyridylketoximes and pyridineamidoximes) to monocot plant – maize (Zea mays L.) in aquatic and soil systems. Tests evaluating the seed germination and growth inhibition of shoot and root of maize were carried out at different compound concentrations (ranging from 0 to 1000 mg/kg of dry soil). It was observed that the presence of chelating compounds caused different effect on seed germination and early development of plant. The presence of complexing agents in aquatic system was more toxic than in soils and this toxicity depended on the type of chemical compounds as well as on the soil type. The cultivation on soil with chelating substance and polluted with heavy metals resulted in the strong inhibition of the maize seed germination and growth. From all the analyzed compounds synthetic EDTA was less toxic than others, like biosurfactant and new potential ligand of metals.

Key words: *chelating agent, complexing compounds, phytoremediation, phytotoxicity, maize (Zea mays L.)*

WPŁYW ZWIĄZKÓW KOMPLEKSOWYCH NA KIEŁKOWANIE I WCZESNY ROZWÓJ KUKURYDZY (*Zea mays* L.)

Streszczenie

Prezentowane wyniki badań zostały skoncentrowane na ocenie toksyczności wybranych związków organicznych, posiadających właściwości kompleksujące metale (EDTA, rhamnolipidy, czwartorzędowe pirydyloketoksymy oraz pirydynoamidoksymy) w stosunku do kukurydzy (Zea mays L.). Badania określające hamowanie kiełkowania nasion oraz wzrostu pędu i korzenia kukurydzy przeprowadzono w dwóch układach, wodnym i glebowym, przy czym badania w układzie glebowym wykonano dla dwóch gleb różniących się zawartością metali ciężkich. Analizowane związki zastosowano w zakresie stężeń od 0 do 1000 mg. Zaobserwowano, że obecność związków kompleksujących wykazywała różny wpływ na kiełkowanie nasion i wczesny rozwój kukurydzy. Obecność środków kompleksujących w systemie wodnym była bardziej toksyczna niż glebowym. Badania wykazały, iż toksyczność substancji zależy od rodzaju związków chemicznych oraz rodzaju gleby. Z pośród wszystkich analizowanych związków najmniejszą toksyczność wywoływał EDTA.

Słowa kluczowe: *czynniki chelatujące, związki kompleksotwórcze, fitoremediacja, fitotoksyczność, kukurydza (Zea mays L.)*

1. Introduction

The pollution of environment, which includes also pollution with heavy metals and also chemical compounds is today important subject. Remediation of soil and aqueous systems contaminated with heavy metals has traditionally involved the excavation, thermal extraction of volatile metals, electrokinetics, soilidification/stabilization, vitrification, chemical oxidation, soil flushing and bioremediation and phytoremediation [1]. Depending on metal type, disposal history and soil type, metal bioavailability and leachability can differ greatly. Phytoremediation is defined as the use of green plants to remove pollutants from the environment or to render them harmless. Regardless of the used plants, availability of heavy metals to plant roots is considered as the key factor limiting the efficiency of phytoremediation. The degree of availability for uptake i.e., the phytoavailability of metals, is affected by number of soil factors, such as cation exchange capacity, pH, and organic matter content. To improve the metal accumulation capacities, the addition of chelating agents has been proposed [2]. These compounds are also used in other techniques of remediation. It should be noted that although there are a great number of metal chelating agents few of these materials are environmentally benign, while synthetic chemicals such as nitrilotriacetic (NTA), ethylene-diamine-tetraacetic acid (EDTA) and diethyltriamine-pentaacetic acid (DTPA) are extremely effective at metal complexation. Their use in the field for in situ removal is questionable because of their demonstrated toxicity effects. For example, NTA is a Class II carcinogen and DTA is a potential carcinogen [3]. EDTA and NTA were shown as significantly reducing growth and causing leaf abscission in poplars when used to remediate cadmium-contaminated soil [4]. EDTA which has been buried with radioactive wastes through its use in decontamination, has been found in groundwater demonstrating limited biodegradability in the environment [5]. The use of biosurfactants has been recognized as an environmentally friendly way of enhancing the removal of metals and petroleum products from contaminated soil [6]. However, several studies, concerning the phytoextraction of heavy metals, indicate that rhamnolipids may contribute to an inhibition of plant growth. A toxic effect of rhamnolipids was observed at the concentration of 2.5 g/kg of soil [7, 8].

Although EDTA has been shown in several publications to be effective in enhancing phytoextraction, EDTA and EDTA-heavy metal complexes are toxic to soil microorganisms and to the plants by severely decreasing shoot biomass

[2]. Due its low biodegragradability EDTA may remain absorbed on soil particles, even after soil cleaning. Its prolonged presence in the soil dramatically increase the leaching risk of heavy metals and alkaline earth metals such as Ca and Mg. It has been suggested that groundwater pollution by EDTA application could be prevented by proper management of irrigation. In climates displaying a positive water balance difficulties will arise in controlling seepage, practically in sandy substrates, which are representative in many polluted areas. As a consequence EDTA, and also other organic complexing compounds are potentially polluting that occurred at higher concentration in river water [2, 9, 10].

On the basis of the above described possible hazards, this paper focuses on the determination of the effect of selected complexing compounds on germination and growth of maize (*Zea mays L.*).

2. Materials and methods

Effect of complexing agents on germination monocotyledonous (maize (*Zea mays L.*)) was exanimated in hydroponic and soil systems. In the aqueous solutions containing 10, 25, 50, 100, 500 and 1000 mg/L of the analyzed compound were prepared. The control samples were prepared with distilled water.

The investigations of the effect of compounds in the hydroponic systems were carried out in Petri plate. The maize seed were put on a piece of filter paper covered with a thin layer of cotton wool and moistened with 10 ml of water or compounds solution in the appropriate concentration. The examination of germination inhibition in the system containing soil was carried out on plates Phyototoxkit^{TM}. On each plate 90 ml of soil were added containing the appropriate concentration of the test substance (0, 10, 25, 50, 100, 500 and 1000 mg/kg d.w.s.). 10 seeds were placed on each plate. All tests were carried out in 3 replications (3 plates for each concentration of test compounds - analysis for 30 seeds). The prepared samples were left for 7 days at 21°C.

In experiment soil from two places was used. The first composition of the soil was as follows: 81 mg P/kg of soil, 88 mg K/ kg of soil, 69 mg Mg/kg of soil, pH of 5.92, C organic content of 1.01% (10.1 g/kg of soil). This soil did not have heavy metals. The second soil was taken from places near copper smelter. The soil contains Zn 65.7 mg/kg of soil, Fe 1836 mg/kg of soil, Cu 668 mg/kg of soil, Pb 143 mg/kg of soil, Cd 20 mg/kg of soil, Cr 3.3 mg/kg of soil, Sr 13.20 mg/kg of soil, Mg 332 mg/kg of soil, P 95 mg/kg of soil, K 92 mg/kg of soil, pH of 6.35.

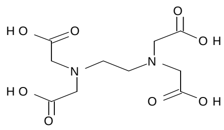
The influence of compounds was investigated using the phytotoxicity test based on the ISO-11269-2:2003. After a certain time, the number of germinated seeds was counted and the root length and shoot height were measured. These values allowed the calculation of the germination index (GI) by the following formula [11]:

$$GI = \left(\left(\frac{Gx}{Gc}\right)\left(\frac{Lx}{Lc}\right)\right) * 100\% \qquad (1)$$

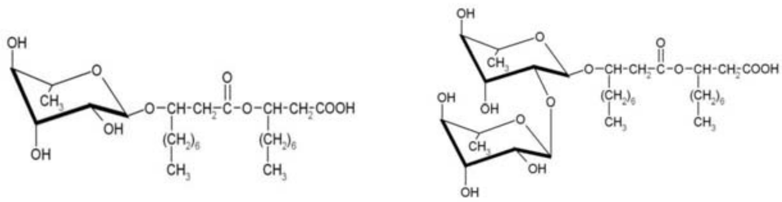
where: Gx and Gc represent the number of germinated seeds, respectively: x – sample, c – control; and Lx and Lc is the length of root respectively: x – sample, c – control.

In these experiments commercial chelating agent such as EDTA and rhamnolipids were analyzed as well as a new potential metal ligand, which was obtained at the Institute of Chemical Technology and Engineering. The structures of these compounds are shown below.

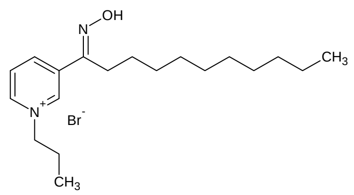
Scheme 1. Structure of complexing compounds:



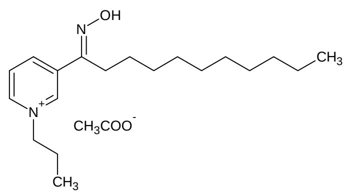
ethylene diamine tetraacetic acid (**EDTA**)



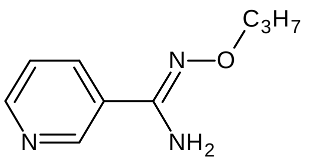
α-L-Rhamnopyranosyl-β-hydroxydecanoyl-β-hydroxydecanoate and
2-O-α-L-Rhamnopyrranosyl-α-L-rhamnopyranosyl-β-hydroxydecanoyl-β-hydroxydecanoate (**Rhamnolipids**)



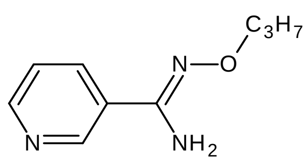
3-[1-(hydroximino)-undecyl]-1-propyl-pyridinium bromide (**3PC10-PrBr**)



3-[1-(hydroximino)- undecyl]-1-propyl-pyridinium acetate (**3PC10-PrCOO**)



3-pyridineamideoxime (**3PAO**)

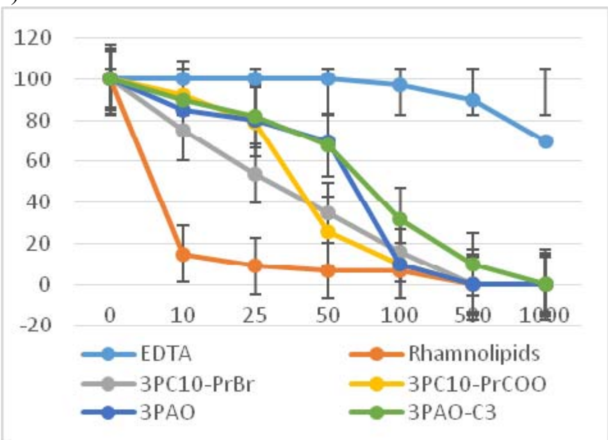


O-propyl-3-pyridineamideoxime (**3PAO-C3**)

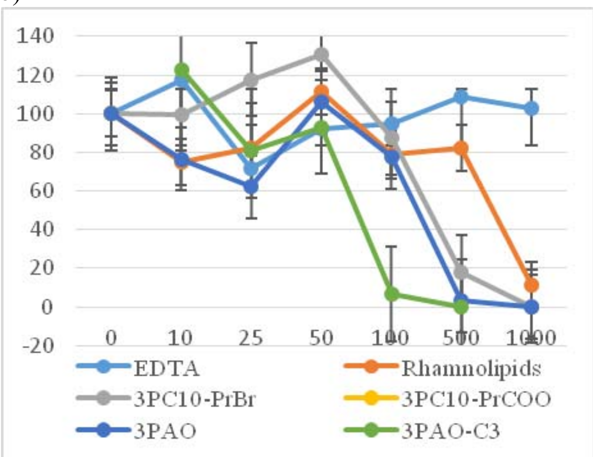
3. Results and discusion

The analysis of EDTA, rhamnolipids, 3-[1-(hydroximino) undecyl]-1-propyl-pyridinium bromide, 3-[1-(hydroximino)undecyl]-1-propyl-pyridinium acetate, 3-pyridineamidoxime and *O*-propyl-3-pyridineamidoxime on germination and development of maize root and shoot was carried out in two variants. The first experiment was carried out in Petri plates containing an aqueous solution of analyzed compounds and the second variant was conducted for systems using a soil test - Phytotoxkit™.

a)



b)



c)

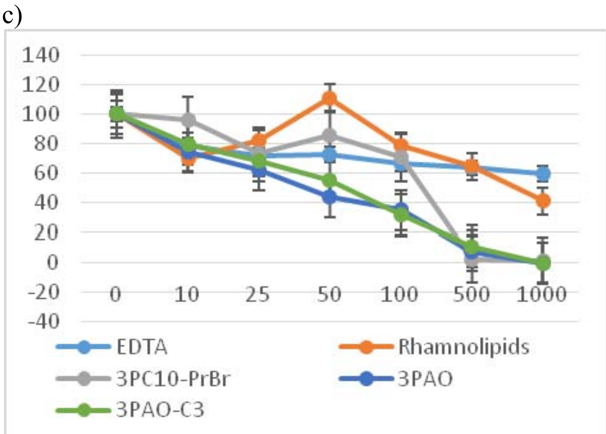


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Fig. 1. Dependence of germination index on analyzed complexing compounds concentration in a) hydroponic systems, b) in soil without heavy metals, c) in soil containing heavy metals

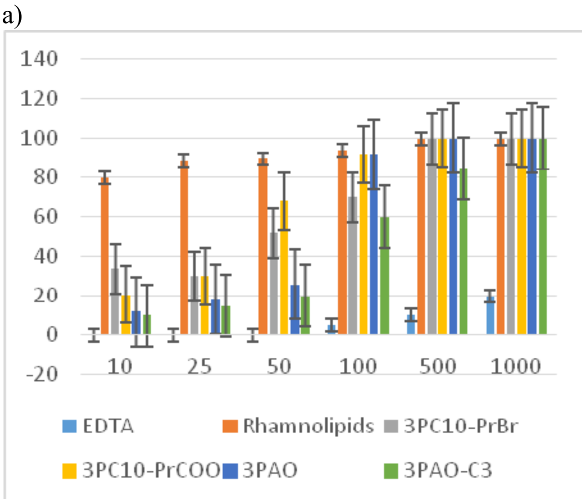
Rys.1. Zależność indeksu kiełkowania od stężenia analizowanych związków kompleksowych a) w układzie hydroponicznym, b) w glebie nie zwierającej metali ciężkich, c) w glebie zawierającej metale ciężkie

On the base of the number of seeds germinated and the root length compared to controls (formula 1) germination index was calculated and then plotted the dependence of the germination index on the concentration of analyzed compounds (Fig. 1). It could be observed that EDTA in all analyzing systems did not inhibit seed germination. In hydrophobic systems rhamnolipids were more toxic than other analyzed compounds, already small concentration (10 mg/L) caused 80% inhibition of seed germination.

Further increase in rhamnoilipids concentration strongly inhibited seed germination and plants development. The pyridine derivatives were less toxic and the concentration above 100 mg/L caused decreasing seed germination. But in the case 3PC10-PrBr and 3PC10-PrCOO at a concentration of 50 mg/L could be observed a high increase in GI values, relative to a concentration of 25 mg/L.

Other situation was observed, when the plant cultivation was carried out in soil containing chemical compounds and other pollutant such as heavy metals. All compounds were less toxic and sometimes, especially in small concentration stimulated shoot and root growth. That confirms the GI value more than 100%. With the increase in the compounds concentration in the soils, germination index decreased. The lowest GI values were observed at a concentration of 100 mg/L. At 500 mg/L and above the germination process did not occur.

a)



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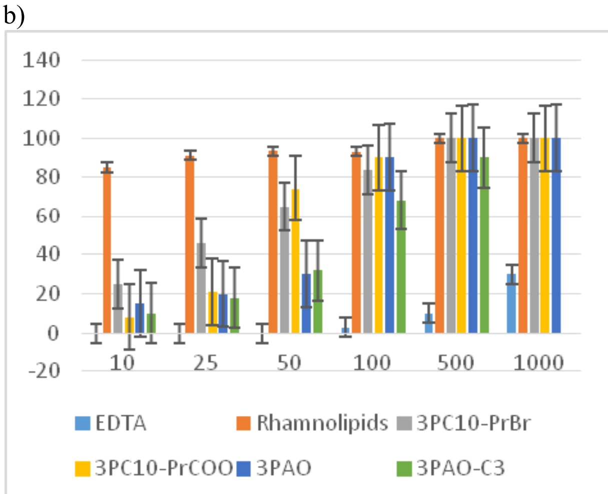

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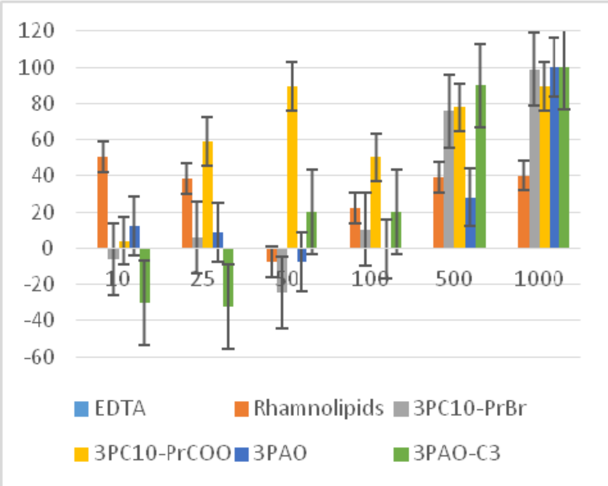
Fig. 2. Effect of complexing compounds on the shoot (a) and root (b) growth of maize in hydroponic system

Rys. 2. Wpływ związków kompleksowych na wzrost pędów i korzeni kukurydzy w układzie hydroponicznym a)



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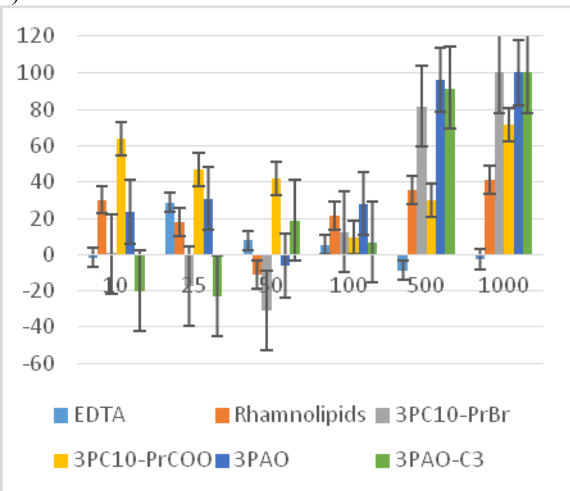

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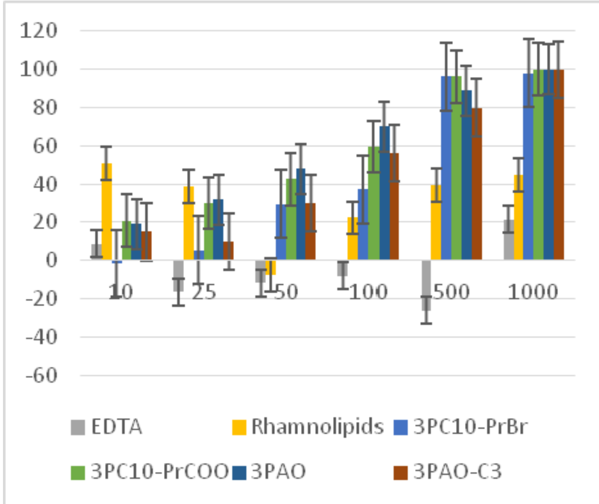
Fig. 3. Effect of complexing compounds on the shoot (a) and root (b) growth of maize in soil without heavy metals

Rys. 3. Wpływ związków kompleksowych na wzrost pędów i korzeni kukurydzy w glebie nie zawierającej metali ciężkich

The length of shoot and root was measured. Root and shoot lengths are the most important parameters for studying xenobiotic stress. This is obvious as roots are in direct contact with contaminated soil and the effects are then translocated and manifested along the shoot On the basis of the measured lengths of the shoots and roots the percent of growth inhibition was calculated for individual plant parts and was shown in the figures 2, 3 and 4. The first analyzed systems was aqueous. The hydroponic system (Fig. 2) was more toxic than soil systems (Fig 2 and 3). In the hydroponic more toxic effect on root development was observed. Most growth inhibition of shoot and root occurred especially in case of rhamnolipids (natural surfactants) and quaternary 3-pyridyloximes. Rhamnolipids already at small concentration provoked a high inhibition of shoot and root growth and at 50 mg/L development of part maize was stopped in 99%. The least growth inhibition was noted for cultivation in medium with EDTA. All investigated pyridine ligands strongly inhibited shoots ad roots of maize development above 50 mg/L. The soil systems were less toxic than aqueous.

The presence of 3PAO-C3 and 3PC10-PrBr in soil without heavy metals at low concentration stimulated shoot and root development, but the increasing in compounds concentration caused decreasing in plant growth. All analyzed compounds strongly inhibited germination and development of plant. The quaternary pyridinium salt with acetate anion at 10 to 50 mg/kg d.w.s. was strongly toxic for maize.

a)



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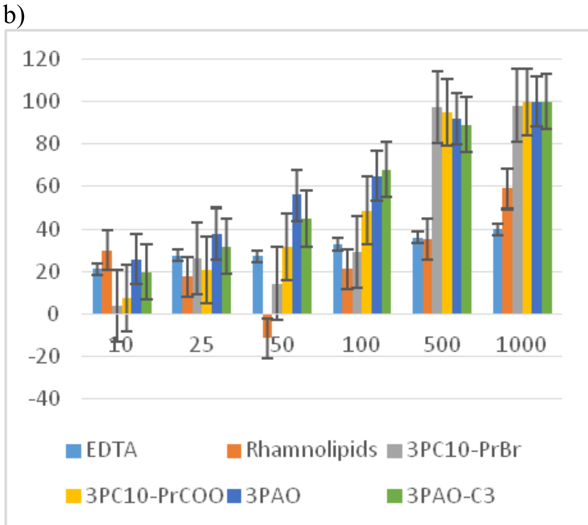

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Fig. 4. Effect of complexing compounds on the shoot (a) and root (b) growth of maize in soil containing heavy metals

Rys. 4. Wpływ związków kompleksowych na wzrost pędów i korzeni kukurydzy w glebie nie zawierającej metali ciężkich

During plant cultivation on soil contaminated by heavy metals, containing also analyzed chelating compounds highest inhibition of growth shoot and root was observed. From these compounds also EDTA was less toxic for plant and only at 1000 mg/kg d.w.s. inhibited root development in 40%, but shoot growth was limited in 20%. The quaternary pyridineoximes and pyridineoximes were more toxic and influenced development of maize. These compounds strongly inhibited growth of shoot and root above concentration of 50 mg/kg d.w.s., and at 500 and 1000 mg/kg d.w.s plant development was inhibited in 90-100%.

Marecik et al. [12] have observed similar effect of rhamnolipids on germination and development of plants. In theirs studies they confirm that the presence of rhamnolipids in soil may potentially be toxic to the natural vegetation. They suggested that phytotoxicity of the chelating agent seems to be species dependent. Also, Millioli et al. [13] demonstrated that the presence of rhamnolipids in soil may influence the GI of lettuce (*Lactuca sativa*). The authors report that in the case of the latter, species, the GI dropped below 50% at a rhamnolipids concentration of 4 g/kg, decreasing futher to 30% with an increase in rhamnolipids content (up to 16 g/kg). Silva et al. [14] indicate that cabbage (*Brassica oleracea*) may tolerate the sole presence of rhamnolipids without any significant drop of its GI. In the literature, the effect of pyridine, pyridine derivatives such as carboxylic acids and their amides on plant growth was described [15, 16]. Those studies concerned the impact of the toxic compounds on the morphology and metabolism of plants (including wheat). They used lower concentrations (up to 10 mg/L). Among other things, we showed a positive effects on plant development of the presence of nicotinic acid amide, but a negative one of dicarboxylic acid amides and other two isomers (i.e., compounds having a functional group at position 2 or 4 of the pyridine ring). The investigators also observed inhibition of plant growth in the presence of pyridine acids and amides concentration of above 0.001M [15, 16]. This contributes to a hypothesis that the phytotoxicity of complexing agents may be related to a type of chemical compounds.

4. Conclusion

The described investigations confirm that complexing agents, such as EDTA, rhamnolipids and also pyridineamidoximes and quaternary pyridylketoximes exert influence on germination and early development of maize. This impact is dependent on the type of systems where plants were cultivated. The all analyzed compounds were more toxic in hydroponic systems than in the soil. This phenomenon is probably caused by the fact that in aqueous systems the access to the pollution is larger even if the compounds are soluble in water. However, in soil the chemical compounds can be adsorbed onto the soil matrix, which can lead to a reduction of access of chemical compounds for plant.

5. References

[1] Wang S. and Mulligan C.N.: Rhamnolipid biosurfactant-enhanced soil flushing for the removal of arsenic and heavy metals form mine tailings. Process Biochemistry, 2009, Vol. 44, 296-301.

[2] Evangelou M.W.H., Ebel M., Schaeffer A.: Chelate assisted phytoextraction of heavy metals form soil. Effect, mechanism, toxicity and fate of chelating agents. Chemosphere. 2007, Vol. 68, 989-1003.

[3] Peters R.W.: Chelant extraction of heavy metals from contaminated soil. J Haz Mat. 1999,Vol 66, 155-210.

[4] Robinson B.H., Mills T.M., Fung L.E., Green S.R., Clothier B.E.: Natural and induce cadmium-accumulation in poplar and willow: Implications for phytoremediation. Plant Soil. 2000, Vol. 227, 301-306.

[5] Baik M.H., Lee K.F.: Transport of radioactive solutes in the presence of chelating agents. Ann Nucl Energy. 1994, Vol 21, 81-96.

[6] Mulligan C.N.: Recent advances in the environmental applications of biosurfactants. Cur. Opin. in Colloid and Interf. Sci., 2009, Vol 14, 372-378.

[7] Wen J., McLaughlin M.J., Stacey S.P., Kirby J.K.: Is rhamnolipids biosurfactant useful in cadmium phytoextraction? J Soils and Sedim., 2010, Vol. 10, 1289-1299.

[8] Gunawardana B., Singhal N., Johnson A.: Amendments and their combined application for enhanced copper, cadmium, lead uptake by *Lolium perenne*. Plant, Soil and Envir. 2010, Vol. 329, 283-294.

[9] Wenezel W.W., Unterbrunner R., Sommer P., Pasquaiina S.: Chelate-assisted phytoremediation using canola (*Brassica napus* L.) in outdoors pot and lysimeter experiments. Plant Soil. 2003, Vol. 249, 83-96.

[10] Barona A., Aranguiz I., Elias A.: Metal associations in soils before and after EDTA extractive decontamination: implications for the effectiveness of further clean-up procedures. Environ. Polut., 2001, Vol. 113, 79-85.

[11] Mosse K.P.M., Patti A.F., Christend E.W., Cavagnaro T.R.: Winery wastewater inhibits seed germination and vegetative growth of common crop species. J Haz Mat. 2010, Vol. 180, 63-70.

[12] Marecik R., Wojtera-Kwiczor J., Ławniczak Łukasz, Cyplik P., Szulc A., Piotrowska-Cyplik A., Chrzanowski Ł.: Rhamnolipids increase the phytotoxicity of diesel oil towards four common plant species in a terrestrial environment. Water Air Soil Pollut, 2012, Vol. 223, 4275-4282.

[13] Millioli V.S., Servulo E.L.C., Sobral L.G.S., De Carvalho D.D.: Bioremediation of crude oil-bearing soil: evaluating the effect of rhamnolipid addition to soil toxicity and to crude oil biodegradation efficiency. Global NEST Journal, 2009, Vol. 11, 181-188.

[14] Silva S.N.R.L., Farias C.B.B., Rufino R.D., Luna J.M., Sarubbo L.A.: Glycerol as substrate for the production of biosurfactant by Pseudomonas aeruginosa UCP0992. Colloids and Surfaces. B. Biointerfaces, 2010, Vol. 79, 174-183.

[15] Berglund T., Ohlsson A.B., Rydström J., Jordan B.R., Strid A.: Effect of nicotinamide on gene expression and glutathione levels in tissue culture of Pisum sativum. Journal of Plant Physiology, 1993, Vol. 142, 676-684.

[16] Taguchi H., Maede M., Nishitari H., Shimabayashi Y., Iwai K.: Effect of cinchomeronic acid and related compounds on the growth of Radish seedlings. Biosci. Biotech. Biochem. 1992, Vol. 56(12): 1921-1923.

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