Original Research

Differences in the Uptake of Mn, Zn, and Cu by *Hordeum Vulgare* L. Following Applications of MCPA-based Herbicides and their Ionic Liquid Forms

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Abstract

The aim of this study was to examine the effect of spraying two herbicidal ionic liquid (HIL) forms of MCPA – namely Ethoquad O-12 and didecyldimethylammonium (a cation) and MCPA (an anion) – on the uptake of Mn, Zn, and Cu by spring barley (*Hordeum vulgare* L.). The total elemental contents of the aboveground parts of the spring barley were measured using flame atomic absorption spectroscopy 24 and 72h after fields were sprayed. The data was compared to controls that consisted of the corresponding treatments using a commercial formulation of MCPA as a salt or ester. The field studies revealed lower contents of Mn, Zn, and Cu in the material after use of commercial formulation compared to HILs. This clean technology provides the means to reduce toxic waste from an industrial chemical process and increase its efficiency in terms of weed control by increasing the absorption rate of macro- and microelements.

Keywords: herbicidal ionic liquids, MCPA, uptake of microelements, spring barley

Introduction

Herbicides are used in agriculture for selective and non-selective weed control in agricultural crops. Besides being efficient for the control of undesired plants, the compounds can boost the binding of macro- and microelements, forming complexes with lower solubility and, hence, reducing their availability for crops [1]. Herbicides containing a phenoxy-acid derivative, in particular MCPA (4-chloro-2-methylphenoxyacetic acid), are the most popular and commonly used in agriculture, due to low manufacturing costs and high efficiency

rates. The active substance in the commercially available preparations comes as salts or esters. Excessive use of these preparations poses a serious hazard to the environment.

For many years the literature has provided information on the formation of complexes of active pesticides such as glyphosate [2-6], or phenoxy-acid derivatives with metals [1, 7-11], which can result in a reduced uptake of macro- and microelements (e.g., Cu, Zn, Mg, Ca, and Fe) that are necessary for proper crop development. This is why it seems increasingly important to improve or modify the current herbicides, or introduce their forms, in order to reduce the negative impact on the environment [12]. One of the possible solutions to the problem is using ionic liquids (ILs) in agriculture. An anion with a herbicidal action was

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introduced into the structure of the ILs. The new group of the formed compounds was named herbicidal ionic liquids (HILs), with directed biological properties and selected chemical and physical characteristics [13].

The literature describes combinations of ammonia and phosphonium cations containing anions of phenoxyacetate [14-16], dicamba [17], and fomesafen [18], confirming that they maintain their biological activity, which tends to be higher than that of the currently popular commercial preparations. Herbicidal ionic liquids are also characterized by lower mobility in the soil and groundwater. The long alkyl chains in the cation ensure their high surface activity, which eliminates the need to use adjuvants or surfactants, and its type determines their toxicity. New forms of herbicides are non-volatile and that is why, contrary to the ester form, they do not pose a risk of displacement or damage to plants in the area adjacent to where they were used [13-14, 16].

The goal of the study was to identify the changes in the uptake of microelements (Mn, Zn, Cu) by spring barley, depending on the applied MCPA-based herbicides in the forms of salts and esters, and selected herbicidal ionic liquids. These are one of the essential microelements for plant development. The studies were carried out under field conditions.

Materials and Methods

MCPA Formulations

Two commercially available MCPA formulations were used for the studies: MCPA as a salt (Chwastox Extra 300 SL potassium-sodium salt, containing 26.5% of the active substance $C_9H_9ClO_3Na/K$; Z. Ch. Organika-Sarzyna S.A. Nowa Sarzyna, Poland) and MCPA as an ester (Chwastox AS 600 EC 2-ethylhexyl ester, containing 56.44% of the active substance $C_{16}H_{25}ClO_3$; Z. Ch. Organika-Sarzyna S.A. Nowa Sarzyna, Poland), and the ionic herbicidal liquids were: [Etq O-12][MCPA] Ethoquad O-12 (4-chloro-2-methylphenoxy)acetate (containing 35.56% of the active substance) and [DDA][MCPA] (4-chloro-2-methylphenoxy)acetate (containing 37.93% of the active substance); Fig. 1. Herbicidal ionic liquids were synthesized in the Faculty of Chemical Technology of the Poznań University of Technology, and described by J. Pernak et al. [13, 19].

The maximum dose of phenoxy-acetic acid-based herbicides recommended in agriculture is 2 kg/ha. Single doses of the mentioned herbicides and HILs were prepared for the tests in such a way that the doses contained the same quantity of the active substance.

Field Experiments

Field tests were conducted in 2014 at the Experimental Station in Winna Góra (E: 17°26', N: 52°12'). Spring barley (*Hordeum vulgare L.*) of the KWS Olof variety (entered into the National Register in 2010) was cultivated
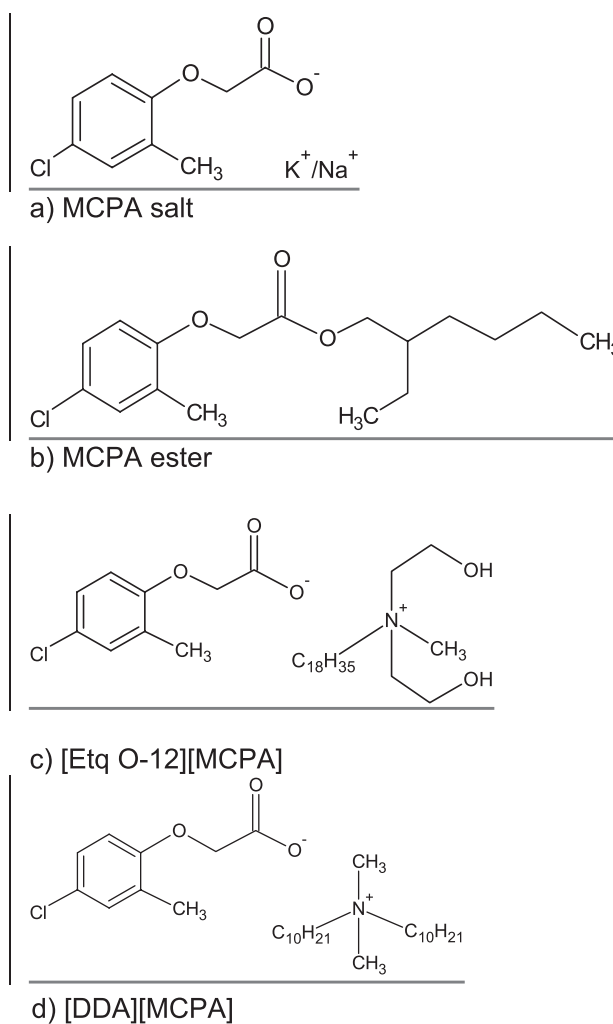

a) MCPA salt

b) MCPA ester

c) [Etq O-12][MCPA]

d) [DDA][MCPA]

Fig. 1. Fig. 1. Structural formulas of applied herbicides and HILs.

on experimental fields in a block arrangement and over an area of 16.5 m², in four repetitions, according to the local agricultural practice. The commercially available herbicides Chwastox Extra 300 SL (300 g of sodium-potassium salt MCPA in 1 litre) and Chwastox AS 600 EC (600 g of MCPA ester in 1 litre) were used as reference substances. The appropriate quantity of HILs as an active substance, single-dose equivalent was dissolved in deionized water.

The prepared solutions of herbicides and HILs were applied at the developmental stage of BBCH14 (developmental stage of leaf 4-6) using a portable pressure sprayer (Aporo, Poznań, Poland) and with a TeeJet DG110/02 flat-jet sprayer (TeeJet Technologies, Wheaton, IL, USA) that has a 200 L ha⁻¹ capacity and a 0.3 MPa working pressure.

The analysis of total Mn, Zn, and Cu content in the plant material was made after applying the herbicides at 24 and 72 h intervals using flame atomic-absorption spectroscopy (FAAS; VARIAN AA240FS). The total content of selected metals in the soil was also measured before and after spray application.

Table 1. Soil characteristics.

Soil reaction		Organic matter content [%]	Grain size [%]		
pH-H$_2$O	pH-KCl		2.0-0.05 mm	0.05-0.002 mm	<0.002 mm
5.55	4.55	1.04	74.48	22.87	2.64

Analysis of Metal Content of Soils and Plant Tissue

The soil for the tests was collected from organic fields in Winna Góra, where the studied plants were subsequently cultivated. The collected soil was dried in the open air and sieved through 2 mm-thick screens. The grain size in the soil prepared this way was measured using a laser particle meter: ANALYSETTE 22 MicroTec plus. Additionally, the soil pH was identified according to PN-ISO 10390:1997P "Soil quality – pH identification" in the soil suspension in 1 mol/L KCl solution using a Teleko N5893 pH-meter (Table 1).

Metal contents of samples were analyzed in an accredited laboratory No. AB 1281, using certified reference materials INCT-OBTL5 (tobacco leaves) and INCT-MPH2 (mixed Polish herbs; Table 2).

The aboveground parts of the plant material were collected for tests after applying herbicides at 24 and 72 h intervals.

Ground samples of the plants (0.5 g) were digested in a closed microwave system (CEM MARS 5) in 10 ml of 65% HNO$_3$ (Merck suprapur). The samples were heated during digestion in two cycles by extending the time, increasing the temperature, and using the microwave oven at a fixed power level (1600W, 100% for five minutes, temperature increased to 135ºC, then five minutes maintained at 135ºC; 1600W, 100% for five minutes, temperature increased to 165ºC, then 20 minutes maintained at 165ºC). After cooling, the samples were filtrated into 50 ml volumetric flasks. The total content of the selected metals were analyzed using the FAAS (Varian AA240FS) method (Table 3). Nitric acid (Merck suprapur), prepared in the same manner as was the plant material, was used as a blind sample.

Water with 0.05µS/cm conductivity, obtained from a Polwater CDRX-200 deionizer, was used in all analyses.

A solution of 65% HNO$_3$ (Merck suprapur) was used to digest the soil and the plant material.

Table 2. Results of selected metals analyses.

Metal	Value identified in laboratory [mg/kg d.m.]	Reference value [mg/kg d.m.]	Recovery [%]
Mn	182	180	101
Zn	35.2*	33.5	105
Cu	11.1	10.1	110

*INCT-MPH2

All laboratory vessels used in the experiment were immersed in 10% HNO$_3$ for 24 h and then rinsed with deionized water.

Statistical Analysis

Compliance of the empirical distribution with the normal distribution was tested by means of the Shapiro-Wilk test. Mean values and standard deviations were assessed. A monocomponent analysis of variance (testing at the significance level α = 0.05) was carried out in order to verify the zero hypothesis regarding the treatment's lack of impact on the variance of Mn, Zn, and Cu.

Results and Discussion

Individual granulometric fractions of the identified soil demonstrated that it belonged to the sand and clay soil grade. The soil collected for the experiment has been classified as grade IIIA – good arable land (according to Polish Soil Classification), with 1.04% organic matter content (according to the *Tiurin method*). The pH value (4.55) identified in the soil fosters the plants' uptake of the studied metals, although their absorption capacity varies [20]. Consequently, higher contents of Mn and Zn than that of Cu can be observed in the spring-barley leaves. The conducted tests revealed a significant drop in the content of the selected metals after spraying with commercial plant-protection chemicals, as compared with equivalent spraying using herbicidal ionic liquids (Table 3).

Manganese

The Mn level the soil used in this study was at 111 mg/kg. This was 240 mg/kg, which was the found to be the mean level of the element in the surface levels of sandy soils in Poland. Mn uptake by plants is metabolically regulated; however, at high concentrations of the element, the dominant mechanism of uptake is by passive absorption [20].

The mean total content of Mn in the control sample collected after 24 h was 117.3 ppm, while after 72 h it amounted to 122.2 ppm. Much lower contents of Mn were observed for samples collected from the fields where Chwastox Super 300 SL was used (in the form of a salt). After spraying, the content of Mn in the analyzed material was lower by 16.3% (24 h) and 19.4% (72 h). The percentage drop of the Mn content was lower for the fields where [Etq-O12][MCPA] was used as a Chwastox Super 300 SL equivalent, and amounted to drops of 2.7%

Table 3. Mean total contents of Mn, Zn, and Cu in spring barley, depending on the sample's collection time. The same letter after the result indicate the lack of a significant difference ($p<0.05$) – the Shapiro-Wilk test.

Treatment	Total metal content [mg/kg d.m.$^{-1}$]					
	24 h after treatment					
	Mn	SD	Zn	SD	Cu	SD
Control	117.3 a	15.47	29.27 a	2.632	7.88 a	0.1646
Chwastox Extra 300 SL	98.2 c	7.01	24.37 b	0.802	7.65 a	0.1089
Chwastox AS 600 SL	101.8 bc	0.92	25.15 b	4.165	7.743 a	0.215
[Etq-O12][MCPA]	114.1 ab	16.65	26.8 ab	3.934	7.872 a	0.0723
[DDA][MCPA]	118.6 a	32.43	29.35 a	4.296	8.00 a	0.3643
NIR 0.05	15.2		4.46		0.231	
p-ANOVA	0.03		0.037		0.224	
Treatment	Total metal content [mg/kg d.m.$^{-1}$]					
	72 h after treatment					
	Mn	SD	Zn	SD	Cu	SD
Control	122.2 ab	20.3	31.27 a	4.36	7.423 ab	0.1966
Chwastox Extra 300 SL	98.5 c	14.95	26.83 b	2.255	6.71 d	0.1552
Chwastox AS 600 SL	107.8 bc	7.19	29 ab	1.735	7.063 cd	0.2977
[Etq-O12][MCPA]	120.5 a	14.13	30.57 ab	4.291	7.12 bc	0.2687
[DDA][MCPA]	119.2 ab	12.96	33.03 a	2.06	7.69 a	0.1697
NIR 0.05	12.5		6.1		0.317	
p-ANOVA	0.025		0.044		0.002	

(24 h) and 1.4% (72 h), respectively. The percentage content of Mn after using MCPA in an ester form (Chwastox AS 600 EC) was lower than that of the control sample by 13.3% (24 h) and 11.8% (72 h). For HIL [DDA][MCPA], the content of Mn was at the level of the control sample 24 h after the treatment, and after 72 h it was lower by 2.5%.

Fig. 2 presents differences in the mg/kg d.m. of Mn absorbed by spring barley 24 and 72 h after the treatment with herbicides and HILs, as compared with the control sample.
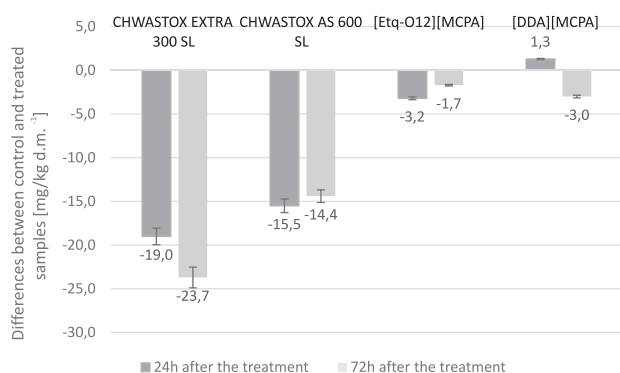

Fig. 2. Decrease in Mn uptake by spring barley after treatment as compared with the control sample.

Zinc

All forms of easily soluble zinc are easily absorbed by plants, but their uptake rates vary depending on the kind of plant, or even its species or variety. Zinc is more easily assimilated by plants when it comes from anthropogenic sources than in the natural form occurring in the soil [20].

The differences in Zn uptake by spring barley after using herbicides and ionic liquids are similar to the differences occurring in Mn uptake; the lowest content of Zn was observed after spraying the soil with Chwastox Super 300 SL. Using the herbicide resulted in drops in Zn uptake of 16.7% (24 h) and 14.4% (72 h). In the case of fields where [Etq-O12][MCPA] was used, the percentage drop for Zn was lower, amounting to 8.5% (24 h) and 2.2% (72 h). The percentage content of Zn after using MCPA in an ester form (Chwastox AS 600 EC) was lower when compared with that of the control sample, by 14.0% (24 h) and 7.4% (72h). For HIL [DDA][MCPA] 24 and 72 h after the treatment, the Zn content was at the level of the control sample Fig. 3.

Zn uptake by plants is greatly determined by soil conditions, mainly by the Ca:Zn ratio. It is not easy to identify the total Zn content in the analyzed soil or plant material, because it mainly depends on the correct Fe: Zn ratio and on the content of other antagonistic elements, i.e., Cd, Al, Se, Mn, and Fe.
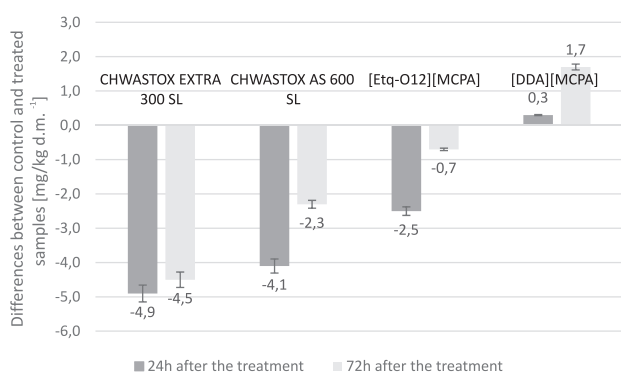
Fig. 3. Decrease in Zn uptake by spring barley after treatments as compared with the control sample.

Copper

The intensity of copper uptake by plants depends on the form of its occurrence in the soil. It is more easily absorbed from anthropogenic sources than from its natural source, which is present in soil, as in the case of zinc. As compared with other trace metals, the plant roots block significant quantities of copper, both deficiencies and surpluses, which is why it demonstrates low mobility [20].

It was observed in the study that copper content in the analyzed plant material ranged from 6.7 to 8.0 mg/kg d.m. The greatest drops in the copper uptake were observed after using commercial herbicides. After using Chwastox Extra 300 SL, the decrease was 2.9% 24 h after treatment and 9.6% 72 h after treatment, as compared with the control sample. After using MCPA in an ester form (Chwastox AS 600 EC), the drop amounted to 1.8% after 24 h and to 4.9% after 72 h. The use of herbicidal ionic liquids does not cause a reduction in copper uptake. In the case of [Etq-O12][MCPA], the total copper content was comparable to that of the control sample. However, after using [DDA][MCPA], the copper content in the collected plant material slightly exceeded its values in the control sample.

Fig. 4 presents differences in the mg/kg d.m. of Cu absorbed by spring barley 24 and 72 h after treatment with the studied herbicides and HILs, against the control sample.
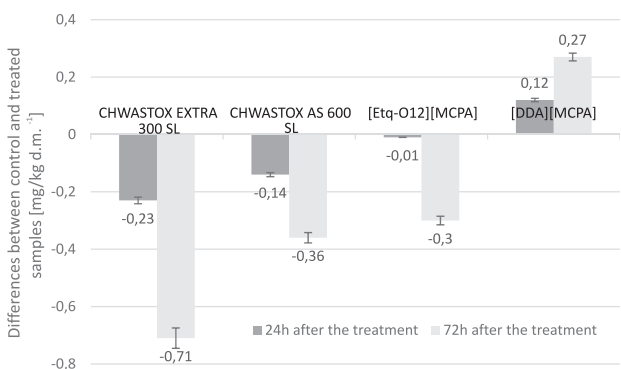

Fig. 4. Decrease in Cu uptake by spring barley after treatments as compared with the control sample.

According to the conducted studies, using popular commercial MCPA-based plant-protection chemicals in agriculture can block the availability of microelements for crops (cereals). The greatest impact on the Mn and Zn uptake was observed after using Chwastox Extra 300 SL, while less significant differences were observed for Cu, which results from lower mobility and an accumulation of the element in the plants' roots. The difference between traditional plant-protection chemicals and [Etq O-12][MCPA] 24 h after treatment of the aboveground parts of the spring barley was 13.8% for Mn, and 18.3% after 72 h. Slightly smaller differences can be observed for Zn, where the decrease amounted to 9.0% after 24 h and 12.4% after 72 h. The differences were minor for Cu, amounting to 2.8% and 5.8%, respectively. Using an MCPA in an ester form of Chwastox AS 600 EC commercially available herbicide, as compared with [DDA][MCPA], also caused a decrease in the uptake of the reference microelements. For Mn, the differences amounted to 14.2% after 24 h and 9.6% after 72 h, while for Zn they were slightly higher, amounting to 14.3% after 24 h and 12.1% after 72 h. In the case of copper, the decrease was lower: 3.3% after 24 h and 8.2% after 72 h.

As compared with the results obtained for the control sample, the greatest drops in the uptake of the reference microelements were observed after using Chwastox Extra 300 SL, while smaller ones were observed for Chwastox AS 600 EC and [Etq O-12][MCPA] (this was only a comparison of the ester and salt forms), while the smallest or no differences were discovered after applying the herbicidal ionic liquid [DDA][MCPA].

The reason why metals are so easily bound by a phenoxycarboxylic-acid derivative is the presence of a non-covered carboxyl group in its molecule. The formulation used in the case of Chwastox Extra 300 SL, where MCPA occurs in the form of a sodium-potassium salt that is easily soluble in water, fosters the binding of other di- and trivalent metals (Mn, Zn, Cu, Ca, Mg, Al, etc.) that form complexes of different, but lower, solubilities [1, 8]. Chwastox AS 600 EC, where an ester-based formulation is used, has a lower capacity for metal binding. The study revealed that using MCPA in an ester form and HIL [Etq O-12][MCPA] results in the reduced uptake of metals by spring barley at a similar level; however, the level is lower for HIL. The structure of an HIL [DDA][MCPA] molecule makes it practically impossible to include a metal cation into its structure, which is why the total content of the reference metals in the analyzed plant material was at the control sample level. One should also remember that the main factors determining the uptake of metals by plants from soil include the soil's granulometric composition, content of organic matter, form of cations, sorption capacity, oxidation-reduction potential, the action of microorganisms, and, mainly, its pH value.

Conclusions

The innovation of the conducted experiments is based on the fact that they demonstrated for the first

time that using the herbicidal ionic liquids (4-chloro-2-methylphenoxy)acetate of Ethoquad O-12 and (4-chloro-2-methylphenoxy)actetate in the protection of plants does not cause a significant decrease in *Hordeum vulgare* L's uptake of manganese, zinc, and copper that accompanies the use of commercial herbicides in the form of the sodium-potassium salt of 4-chloro-2-methylphenoxyacetic acid or 2-ethylohexyl acid 4-chloro-2-methylphenoxy acid ester.

The lowest impact on the uptake of manganese, zinc, and copper was observed for HIL [DDA][MCPA] in an ester form, the use of which resulted in the reference microelements' contents being at the control sample's level. The greatest drops were observed for MCPA salt 72 h after the treatment (Mn ~20%, Zn ~15%, Cu ~10%).

As compared with traditional MCPA-based plant-protection chemicals, the use of HILs significantly (in terms of statistical analysis) contributed to an increased uptake of manganese and zinc by *Hordeum vulgare* L*.,* and those elements are the main microelements necessary for correct plant growth.

This study revealed that substituting traditional plant-protection chemicals with herbicidal ionic liquids can improve the efficiency of new formulations [16], but also reduce their adverse impact on the changes occurring in the natural environment (e.g., the uptake of macro- and microelements by plants). This clean technology provides the means to reduce toxic waste from an industrial chemical process and increase its efficiency in terms of weed control by increasing the absorption rate of macro- and microelements.

References

1. KOBYŁECKA J., SKIBA E. The Effect of Phenoxyacetic Herbicides on the Uptake of Copper, Zinc and Manganese by *Triticum Aestivum* L. Polish J. of Environment. Stud. **17**, 895, **2008**.
2. EKER S., OZTURK L., YAZICI A., ERENOGLU B., ROMHELD V., CAKMAK I. Foliar-applied glyphosate substantially reduced uptake and transport of iron and manganese in sunflower (*Helianthus annuus* L.) plants. Agric. Food Chem. **54**, 10019, **2006**.
3. LANE M., LORENZ N., SAXENA J., RAMSIER C., DICK R.P. The effect of glyphosate on soil microbial activity, microbial community structure, and soil potassium. Pedobiologia **55**, 335, **2012**.
4. BOTT S., TESFAMARIAM T., CANDAN H., CAKMAK I., ROMHELD V., NEUMANN G. Glyphosate-induced impairment of plant growth and micronutrient status in glyphosate-resistant soyabean (*Glycine max* L.). Plant and Soil **312**, 185, **2008**.
5. CAKMAK I., YAZICI A., TUTUS Y., OZTURK L. Glyphosate reduced seed and leaf concentrations of calcium, manganese, magnesium, and iron in non-glyphosate resistant soybean. Europ. J. Agronomy **31**, 114, **2009**.
6. CAETANO M.S., RAMALHO T.C., BORTEL D.F., CUNHA E., MELLO W.C. Understanding the inactivation process of organophosphorus herbicides: a DFT study of glyphosate metallic complexes with Zn^{2+}, Ca^{2+}, Mg^{2+}, Cu^{2+}, Co^{3+}, Fe^{3+}, Cr^{3+} and Al^{3+}. Int. Journal of Quant. Chem. **112**, 2752, **2012**.
7. PRAMANIK A., ABBINA S., DAS G. Molecular, supra-molecular structure and catalytic activity of transition metal complexes of phenoxy acetic acid derivatives. Polyhedron **18**, 5225, **2007**.
8. KOBYŁECKA J., PTASZYNSKI B., ROGACZEWSKI R., TUREK A. Phenoxyalkanoic acid complexes. Part I. Complexes of lead(II), cadmium(II) and copper(II) with 4-chloro-2-methylphenoxyacetic acid (MCPA). Thermochimica Acta **407**, 25, **2003**.
9. KOBYŁECKA J., TUREK A., SIEROŃ L. Phenoxyalkanoic acid complexes. Part II. Complexes of selected bivalent metals with 2,4-dichlorophenoxyacetic acid (2,4D) and 2-(2,4-dichlorophenoxy)propionic acid (2,4DP). Thermochimica Acta **482**, 49, **2009**.
10. GRZESIAK P., MOTAŁA R., GROBELA M., SCHROEDER G. Synergizm metali ciężkich ze środkami ochrony roślin. Prog. Plant Prot. **47**, 4, **2007**.
11. TUREK A., KOBYŁECKA J., PTASZYŃSKI B. Complexes of dicamba with cadmium(II), copper(II), mercury(II), lead(II) and zinc(II). Synthesis and properties. J. Thermal Anal. Calorimetry **78**, 513, **2004**.
12. [EFSA http://www.efsa.europa.eu/en/topics/topic/pesticides; Directive 2009/128/EC http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:309:0071:0086:en:PDF; Regulation (EC) No 1107/2009 http://eur-lex.europa.eu/LexUriServ/ LexUriServ.do?uri_=OJ:L:2009:309:0001:0050:EN:PDF;
13. PERNAK J., SYGUDA A., JANISZEWSKA D., MATERNA K., PRACZYK T. Ionic liquids with herbicidal anions. Tetrahedron **67**, 4838, **2011**.
14. PERNAK J., SYGUDA A., MATERNA K., JANUS E., KARDASZ P., PRACZYK T. 2,4-D based herbicidal ionic liquids. Tetrahedron **68**, 4267, **2012**.
15. KORDALA-MARKIEWICZ R., RODAK H., MARKIEWICZ B., WALKIEWICZ F., SZNAJDROWSKA A., MATERNA K., MARCINKOWSKA K., PRACZYK T., PERNAK J. Phenoxy herbicidal ammonium ionic liquids. Tetrahedron **70**, 4784,**2014**.
16. ERNAK J., NIEMCZYK M., ZAKROCKA K., PRACZYK T. Herbicidal ionic liquid with dual-function. Tetrahedron **69**, 8132, **2013**.
17. COJOCARU O.A., SHAMSHINA J.L., GURAU G., SYGUDA A., PRACZYK T., PERNAK J., ROGERS R.D. Ionic liquid forms of the herbicide dicamba with increased efficacy and reduced volatility. Green Chem. **15**, 2110, **2013**.
18. DING G., LIU Y., WANG B., PUNYAPITAK D., GUO M., DUAN Y., LI J., CAO Y. Preparation and characterization of fomesafen ionic liquids for reducing the risk to the aquatic environment. New J. Chem. **38**, 5590, **2014**.
19. POLIT J.T., PRACZYK T., PERNAK J., SOBIECH Ł., JAKUBIAK E., SKRZYPCZAK G. Inhibition of germination and early growth of rape seed (*Brassica napus* L.) by MCPA in anionic and ester form. Acta Physiol. Plant **36**, 699, **2014**.
20. KABATA-PENDIAS A., PENDIAS H. Biogeochemistry of Trace Elements, 2nd ed; PWN: Warsaw, 1, **1999** [in Polish].