

Original Research

HLs vs. MCPA: Which is Better for the Uptake of Ca, Mg, and Fe by *Hordeum vulgare* L.

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Abstract

The aim of this study is to examine the effect of spraying two commercially available MCPA (4-chloro-2-methylphenoxyacetic acid) formulations as a salt (Chwastox Extra 300 SL), as an ester (Chwastox AS 600 EC), and as two herbicidal ionic liquid (HIL) forms of MCPA – namely Ethoquad O-12 (ETQ-O12) and didecyldimethylammonium (DDA, a cation), and MCPA (an anion) – on the uptake of Ca, Mg, and Fe by spring barley (*Hordeum vulgare* L.). The total elemental contents of the aboveground parts of the spring barley were measured using FAAS (Varian AA240FS) 24 h and 72 h after fields were sprayed and compared with untreated plants. The field studies revealed lower contents of Ca, Mg, and Fe in the material after use of the commercial formulation compared to HILs. The greatest reduction in the uptake of macroelements was observed after using MCPA as a salt; the reduction amounted to 28% for Fe and 14% Mg, while for Ca it was 10%, but for Mg the differences between treatments were not significant in any case. The greatest drops in the uptake of the reference macroelements were observed for Chwastox 300 SL < Chwastox AS 600 EC < [ETQ-O12][MCPA] < [DDA][MCPA] in comparison to the control sample.

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

Introduction

The earliest references to weed control products date back to ancient times. According to the literature, salt and a mixture of animal waste with salt, ash, and liquid from olive oil extraction was used to control weeds. Until the 19th century, iron sulphate, copper nitrate, and ammonium and potassium salts were used for selective weed removal in agriculture. The first organic herbicides were produced in 1932 and the application of nitrophenols, i.e., 2,4-D [1] and MCPA (4-chloro-2-methylphenoxyacetic acid) [2], initiated a new weed-removal method.

Derivatives of phenoxy acids, MCPA in particular, are among the most common in agriculture due to their low manufacturing cost and high efficiency [3]. Common market preparations contain salts or esters as the active substance. It has been demonstrated, however, that excessive use of the formulations poses a serious hazard for the environment, as their residues remain in the soil long after their application [4-5]. The compounds can affect non-target organisms and can contribute to the compensation of weeds and selection of biotypes resistant to the herbicides previously used. They can also negatively affect the soil environment binding micro- and macroelements, forming their complexes and limiting their availability for arable plants. Micro- and macroelements in the soil are necessary for the correct

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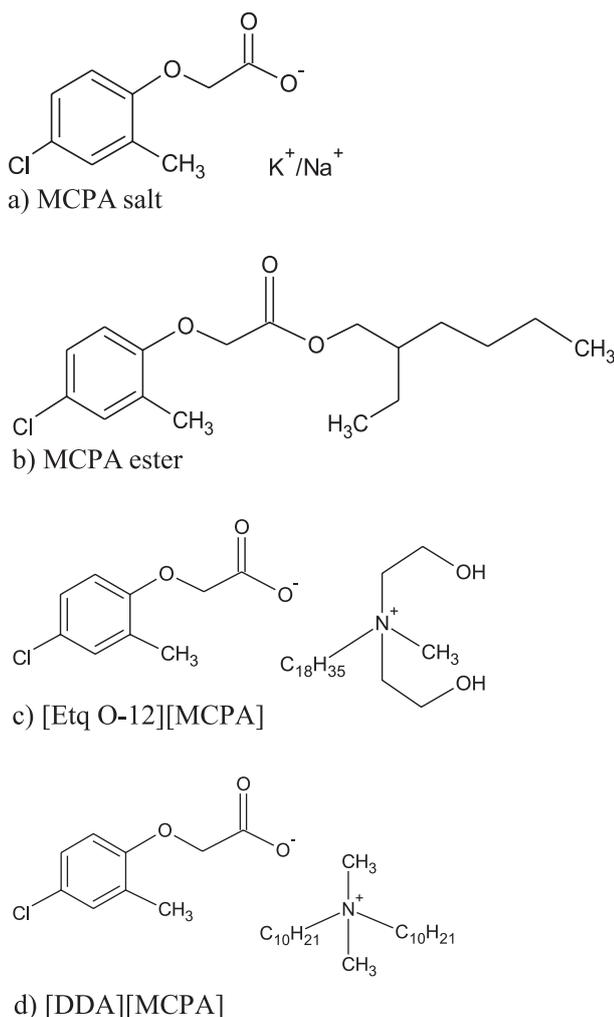


Fig. 1. Structural formulas of applied herbicides and HILs.

growth and development of plants. Common pesticides containing active substances (i.e., glifosat 2,4-D or MCPA) tend to create durable complexes with metals in the soil, hence blocking nutrients for plants [6-12]. Reduced availability of micro- and macroelements for plants causes metabolic dysfunctions as a result of the poor function of proteins in cells, which are responsible for the metabolism of nitrogen, photosynthesis, or respiration [13-14]. Therefore, it is important to limit to the minimum the negative impact of herbicides on the environment.

Despite the fact that there is plenty of literature data concerning the formation of durable bonds between MCPA and metals in the soil, the studies on metal bonding by HILs are rarely available. There is information in

literature on HILs characterized by their lower mobility in the soil and groundwater and their surface activity, efficacy, biodegradation, and their impact on non-target organisms compared to the currently used herbicides. However, there is no information on HILs potential ability to bond macroelements (as in the case of MCPA). Therefore, this study can be identified as important and new.

With regard to the above, it is more important to improve and modify the existing herbicides or to introduce new ones in order to improve their effectiveness with a simultaneous reduction of negative environmental impact (EFSA 2009, Directive EC 2009, Regulation EC 2009) [15].

The transformation of herbicides into an ionic liquid form is an innovative possibility for reducing their negative impact [16-18]. A new generation of ionic liquids (ILs), whose structure contains a herbicidal ion, is called herbicidal ionic liquids (HILs). These preserve oriented biological properties with selected chemical and physical properties. The literature describes such combinations with common herbicides: 2,4-D [19-20], MCPA [21-22], dicamba [23], fomesafen [24], and glifosat [25]. HILs are characterised by lower mobility in the soil and groundwater and their surface activity is high, which eliminates the need for adjuvants or surfactants; they are safer to use and their residue in the environment is much lower because the dose of the active system can be greatly reduced compared to the currently used herbicides [17, 19-20, 26-27].

The aim of this study is to identify the changes in the uptake of macro-elements (Ca, Mg, Fe) by spring barley, depending on the applied MCPA-based herbicides in the forms of salts and esters, and selected HILs. These are among the essential macroelements for plant development. The studies were carried out under field conditions.

Material 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 C₉H₉ClO₃Na/K; Z. Ch. Organika-Sarzyna S.A. Nowa Sarzyna, Poland; Ch300) and MCPA as an ester (Chwastox AS 600 EC 2-ethylhexyl ester containing 56.44% of the active substance C₁₆H₂₅ClO₃; Z. Ch. Organika-Sarzyna S.A. Nowa Sarzyna, Poland; Ch600). The HILs were: [Etq O-12][MCPA] Ethoquad

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

Table 2. Optimal instrumental parameters for FAAS determination of metals.

Element	Wavelength (nm)	Spectra width slit (nm)	Background correction	Flame	C ₂ H ₂ flow rate (L min ⁻¹)
Ca	422.7	0.5	Off	C ₂ H ₂ /N ₂ O	6.35
Mg	285.2	0.5	On	C ₂ H ₂ /Air	2.00
Fe	248.3	0.2	On	C ₂ H ₂ /Air	2.00

Table 3. Overview of the results obtained in the validation study.

Element	Slope coefficient	Correlation coefficient r	LOD (mg L ⁻¹)	LOQ (mg L ⁻¹)	Repeatability (SD, %)	Linear range (mg L ⁻¹)	Accuracy (Recovery) (%)
Ca	0.2543	0.9997	0.0267	0.0801	2.28	0.1-1.0	101.59
Mg	1.3370	0.9995	0.0260	0.0780	4.98	0.025-0.4	95.67
Fe	0.0766	0.9999	0.0285	0.0856	3.06	0.25-2.0	103.20

O-12 (4-chloro-2-methylphenoxy)acetate (containing 35.56% of the active substance; ETQ) and [DDA][MCPA] (4-chloro- 2-methylphenoxy)acetate (containing 37.93% of the active substance; DDA). HILs were synthesized by the metathesis reaction in water or in organic solvents with a high yield and characterized by ¹H and ¹³C NMR spectroscopy and elemental analysis in the Faculty of Chemical Technology of the Poznań University of Technology, and described by J. Pernak et al. [17] (Fig. 1).

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 on experimental fields in a block arrangement on 16.5 m² plots, in 4 repetitions, according to the local agricultural practice. The treated plants were compared to an untreated control. The commercially available herbicides Chwastox Extra 300 SL (Ch300) and Chwastox AS 600 EC (Ch600) were used as reference substances. The discussed herbicides and HILs were applied in such a dose so that the active substance concentration was the same and equaled 900 g h⁻¹. The prepared solutions of herbicides and HILs were applied at the developmental stage of BBCH14 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.

Identifying Metal Content in Soil and Plant Material

The soil used for the experiment was classified as sandy-clay (Table 1) with an approximate content of

organic matter of 1.04% and pH value of 4.55, determined according to PN-ISO 10390:1997 "Soil Quality – Determination of pH," in a soil suspension of one mol/L KCl using a Teleko N5893 pH meter.

An analysis of the total content of Ca, Mg, and Fe in the soil was conducted after the experimental fields were selected. The experimental material was analyzed 24 and 72 hours after the application of herbicides using flame atomic spectroscopy FAAS (VARIAN AA240FS). The timing of sampling was coordinated with DT50 MCPA [28] and to allow observing the changes occurring during macroelement uptake by barley.

Plant samples (0.5 g) consisting of leaves were mineralized in a closed microwave system (CEM MARS 5) in 10 ml of 65% HNO₃ (Merck Suprapur) according to methodologies developed in an accredited laboratory of environmental analysis [7]. The total content of tested metals was determined by the FAAS method (Varian AA240FS; Tables 2-3).

The accuracy of the completed mineralization and FAAS determination was confirmed in the analysis of the certified material INCT-MPH2 (mixture of Polish herbs; Table 4).

Statistical Analysis

R (R Core Team) [29] and lme4 [30] were used to perform a linear mixed effects analysis of the relationship

Table 4. Results of selected metals analyses of the certified material.

Metal	Value identified in laboratory (wt. % d.m.)	Reference value (wt. % d.m.)	Recovery (%)
Ca	1.049	1.08	97.13
Mg	0.297	0.292	101.71
Fe	0.0484	0.046	105.22

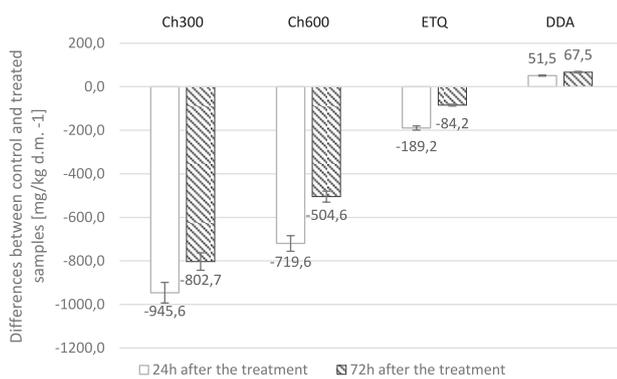


Fig. 2. Decrease in Ca uptake by spring barley after the treatment, as compared with the control sample.

between metal concentrations, treatment, and time. As fixed effects, we entered treatment and time into the model (with interaction). As random effects, we had intercepts for repeated measurements on fields. Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. Then, p-values (the probability of type I error) were obtained by likelihood ratio tests of the full model (with time and treatment as fixed effects) against the simplified model with only time or treatment as fixed effect. As the next step, we performed post-hoc tests using the pairwise test with Tukey's adjustment from the ls means package [31].

Results and Discussion

Calcium

The identified total Ca content in the soil of 0.123% is below the mean content of the element for arable soil in Poland, i.e., below 0.3% [32].

In the experiment, the total mean Ca content in the control sample after 24 h was 8,536 ppm, while after 72 h it was 8,162 ppm. For samples collected from fields where Ch300 (salt) was used as a plant protection product, lower Ca values were observed. After spraying, the content of Ca in the analysed material was lower by 11.1% (24 h) and by 9.8% (72 h). For fields where ETQ was used as an equivalent to Ch300, the percentage drop in the Ca content was lower, i.e., 2.2% (24 h) and 1.0% (72 h), respectively. The percent content of Ca after MCPA application in an ester form (Ch600) was lower compared to the control sample by 8.4% (24 h) and 6.1% (72 h). For HIL DDA, 24 h and 72 h after the treatment, the Ca content was the same as in the control sample (Fig. 2). In the case of post-hoc comparisons between particular time points, significant differences were found for the following pairs of treatments: control vs. Ch300, control vs. Ch600, Ch300 vs. DDA, Ch300 vs. ETQ, Ch600 vs. DDA in the first time point, control vs. Ch300, and Ch300 vs. DDA in the second time point (detailed statistics are presented in Table 5).

Magnesium

The total identified content of Mg in the soil amounted to 0.08%, and it was lower than the average content of this element in arable soil in Poland, which is 0.14% [32].

Differences in Mg uptake by spring barley after the application of herbicides and ionic liquids are similar to the differences for Ca uptake. After the application of Ch300, the decrease, compared to the control sample, was 12.2% 24 h after the treatment and 14.1% after 72 h. Following the application of Ch600, the drop was 7.9% after 24 h and 10.2% after 72 h. The use of herbicidal ionic liquids also causes drops in Mg uptake, but at a lower level. For ETQ the drop in the Mg content was

Table 5. Pairwise test for differences between means of treatments for Ca uptake with Tukey's adjustment.

Contrast	Time 1-24 h after treatment				Time 2-72 h after treatment			
	Dif.	SE	t ratio	p	Dif.	SE	t ratio	p
Control vs. Ch300	945.6	223.8	4.225	0.001	802.7	241.7	3.321	0.017
Control vs. Ch600	719.6	223.8	3.215	0.022	504.6	241.7	2.088	0.248
Control vs. DDA	-51.5	241.7	-0.213	1.000	-67.5	223.8	-0.302	0.998
Control vs. ETQ	189.2	241.7	0.783	0.934	84.20	241.7	0.348	0.997
Ch300 vs. Ch600	-226.0	223.8	-1.010	0.849	-298.1	258.3	-1.154	0.777
Ch300 vs. DDA	-997.1	241.7	-4.126	0.002	-870.2	241.7	-3.600	0.008
Ch300 vs. ETQ	-756.4	241.7	-3.129	0.027	-718.5	258.4	-2.781	0.062
Ch600 vs. DDA	-771.1	241.7	-3.190	0.023	-572.1	241.7	-2.367	0.149
Ch600 vs. ETQ	-530.4	241.7	-2.194	0.206	-420.4	258.4	-1.627	0.491
DDA vs. ETQ	240.7	258.4	0.932	0.883	151.7	241.7	0.627	0.970

Dif. – differences between means [ppm], SE – standard error, t ratio – ratio of the departure of mean from standard error, p – the probability of type I error

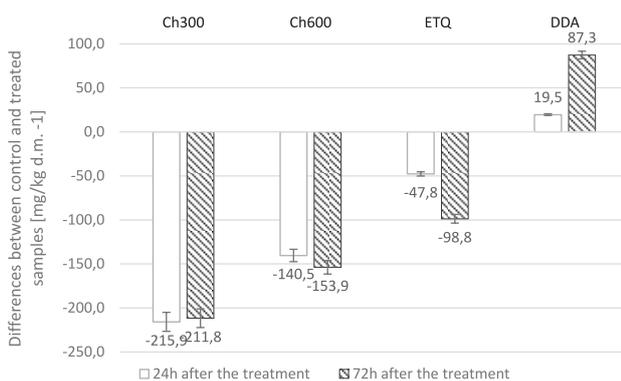


Fig. 3. Decrease in Mg uptake by spring barley after the treatments, against the control sample.

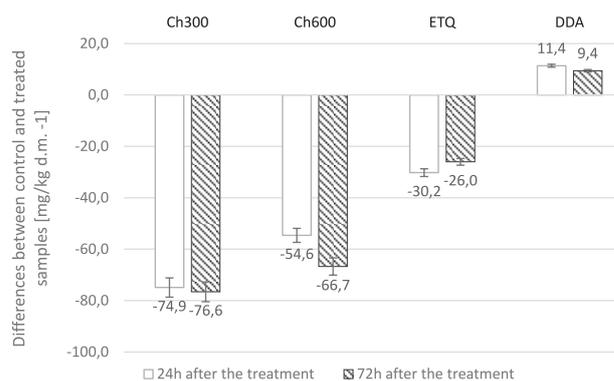


Fig. 4. Decrease in Fe uptake by spring barley after the treatments, as compared with the control sample.

2.7% after 24 h and 6.6% after 72 h. Following the application of DDA, the Mg content in the collected plant material slightly exceeded its values in the control sample (Fig. 3). In the case of post-hoc comparisons at a particular time point, no significant differences occurred between pairs of treatments and control vs. treatments (detailed statistics are presented in Table 6).

Iron

Iron occurs in soil in large quantities and its distribution is quite diversified. Surface soil levels in Poland usually contain between 0.8 and 1.8% Fe [33], and the mean content of the element for arable soil amounts to ca. 1% [32]. In the soil collected for the experiment, the total Fe content was lower than the mean value and amounted to 0.56%.

The tests revealed that the level of Fe content in the analyzed plant material differed significantly. The greatest decrease in iron uptake was observed after the application

of commercial plant protection products. The application of Ch300 caused a drop in Fe uptake by as much as 27.6% (24 h) and 27.9% (72 h). For fields where ETQ had been applied, the drop in Fe content was lower and amounted to 11.1% (24 h) and 9.4% (72 h), respectively. The content of Fe after the application of Ch600 was lower by 20.1% (24 h) and 24.3% (72 h) compared to the control sample. For HIL DDA, 24 h and 72 h after the treatment, the Fe content was much higher than the control sample value (Fig. 4). In the case of post-hoc comparisons in particular time points, significant differences were observed between the following pairs: control vs. Ch300, control vs. Ch600, Ch300 vs. DDA, Ch300 vs. ETQ, Ch600 vs. DDA, and DDA vs. ETQ at the first time point, and in all pairs of treatments besides control vs. DDA, control vs. ETQ, and Ch300 vs. Ch600 at the second time point (detailed statistics are presented in Table 7).

Due to their structure, phenoxycarboxylic acid derivatives have a tendency to bind bi- and tri-valent metals (Mn, Zn, Cu, Ca, Mg, Al, etc.), which form

Table 6. Pairwise test for differences between means of treatments for Mg uptake with Tukey’s adjustment.

Contrast	Time 1-24 h after treatment				Time 2-72 h after treatment			
	Dif.	SE	t ratio	p	Dif.	SE	t ratio	p
Control vs. Ch300	215.9	116.8	1.848	0.363	211.8	108.1	1.959	0.307
Control vs. Ch600	140.5	116.8	1.202	0.750	153.9	108.1	1.423	0.618
Control vs. DDA	-19.50	108.1	-0.180	1.000	-87.23	116.8	-0.747	0.944
Control vs. ETQ	47.83	116.8	0.409	0.994	98.78	116.8	0.846	0.914
Ch300 vs. Ch600	-75.43	124.9	-0.604	0.974	-57.95	108.1	-0.536	0.983
Ch300 vs. DDA	-235.4	116.8	-2.015	0.280	-299.1	116.8	-2.560	0.100
Ch300 vs. ETQ	-168.1	124.9	-1.346	0.665	-113.1	116.8	-0.968	0.868
Ch600 vs. DDA	-159.9	116.8	-1.369	0.651	-241.1	116.8	-2.064	0.258
Ch600 vs. ETQ	-92.63	124.9	-0.742	0.945	-55.10	116.8	-0.472	0.989
DDA vs. ETQ	67.33	116.8	0.576	0.978	186.0	124.9	1.490	0.576

Dif. – differences between means [ppm], SE – standard error, t ratio – ratio of the departure of mean from standard error, p – the probability of type I error

Table 7. Pairwise test for differences between means of treatments for Fe uptake with Tukey's adjustment.

Contrast	Time 1-24 h after treatment				Time 2-72 h after treatment			
	Dif.	SE	t ratio	p	Dif.	SE	t ratio	p
Control vs. Ch300	74.9	12.7	5.89	<0.0001	76.6	12.7	6.03	<0.0001
Control vs. Ch600	54.6	12.7	4.30	0.002	66.7	12.7	5.25	0.000
Control vs. DDA	-11.4	12.7	-0.90	0.894	-9.4	12.7	-0.74	0.944
Control vs. ETQ	30.2	11.8	2.57	0.105	26.0	12.5	2.09	0.252
Ch300 vs. Ch600	-20.3	13.6	-1.49	0.576	-9.9	13.6	-0.73	0.948
Ch300 vs. DDA	-86.3	13.6	-6.36	<0.0001	-86.0	13.6	-6.34	<0.0001
Ch300 vs. ETQ	-44.7	12.7	-3.52	0.013	-50.6	13.3	-3.79	0.006
Ch600 vs. DDA	-66.1	13.9	-4.87	0.000	-76.2	13.6	-5.61	0.000
Ch600 vs. ETQ	-24.4	12.7	-1.92	0.331	-40.7	13.3	-3.05	0.036
DDA vs. ETQ	41.7	12.7	3.28	0.022	35.5	13.3	2.66	0.087

Dif. – differences between means [ppm], SE – standard error, t ratio – ratio of the departure of mean from standard error, p – the probability of type I error

complexes of different and lower solubilities [7-8, 34-35]. Another factor that fosters the binding of micro- and macroelements is the formulation used in Ch300, where MCPA comes in the form of a sodium and potassium salt easily soluble in water.

The difference between a traditional plant protection product (i.e., MCPA salt) and ETQ 24 h after application in the parts of spring barley aboveground was 18.5% for Fe and 20.4% after 72 h. Lower differences could be observed for Ca, where the drop was 9.1% after 24 h and 8.9% after 72 h, while the differences for Mg amounted to 9.7% and 8.0%, respectively. The application of the Ch600 compared to DDA also caused a decrease in the uptake of the reference macro-elements. The differences were the highest for Fe and amounted to 23.3% after 24 h and 26.8% after 72 h, but were much lower for Ca and amounted to 8.9% after 24 h and 6.9% after 72 h. The drop was diversified for Mg and reached 8.9% after 24 h and 15.1% after 72 h.

Following the application of Ch600, the quantity of macroelements consumed by spring barley is higher compared to Ch300, but the level of elements taken up differs from the value for the control sample. An application of MCPA as HIL ETQ or DDA revealed that the quantity of macroelements consumed by spring barley is similar to the quantity of the ingredients identified in the control sample. Their molecular structure can be the cause of the reduced blocking of nutrients by HILs (Fig.1). The inclusion of Ethoquad O-12 or a didecyldimethylammonium cation into the structure of a phenoxy acid derivative makes it almost impossible to insert or replace other metal cations, and that is why the total content of the studied macro-elements in the analysed plant material was at the same level as the control sample.

One should remember that soil factors such as granulometric composition, content of organic matter,

cation forms, sorption volume, redox potential, activity of microorganisms, and pH value have the greatest impact on metal uptake from the soil by plants.

Conclusions

In the experiment, the greatest impact on the uptake of the analysed macroelements was observed after the application of Chwastox 300 SL. The greatest differences, compared with the control sample, were observed for the MCPA salt 72 h after treatment (Fe ~28%, Mg ~14, and Ca ~10%).

The most statistically significant differences of Fe content in barley leaves after 24 and 72 h were observed after spraying with commercially available pesticides in comparison to HILs. In the case of Ca the differences were mostly noted after 24 h, while after 72 h these differences were only significant for control/Ch300 and Ch300/DDA pairs. Both formulations were not statistically significant for Mg uptake.

Calcium, iron, and magnesium uptake were least affected by the ester form of HIL DDA. Following its application, the content of the reference macroelements was at the same level as in the control sample.

The studies showed that the application of herbicidal ionic liquids, (4-chloro-2-methylphenoxy)acetate Ethoquad O-12 and ammonia didecyldimethyl(4-chloro-2-methylphenoxy)acetate, in plant protection fosters improved uptake of major macroelements (Ca, Mg, Fe) by spring barley, compared to common plant protection products in the form of 4-chloro-2-methylphenoxyacetic acid sodium salt or 2-ethylhexyl ester of 4-chloro-2-methylphenoxyacetic acid.

Using HILs in plant protection seems like a perfect way to reduce the negative impact of herbicides on the

environment. They have desirable characteristics such as higher effectiveness, lower volatility, and solubility in soil. Compared to the traditional MCPA-based plant protection products, they greatly contribute to the proper growth of plants (spring barley). This is why appropriately prepared HILs can eliminate all significant drawbacks of MCPA, and constitute a good candidate for future use.

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