Parus, A., Zdebelak, O., Ciesielski, T., Szumski, R., Woźniak-Karczewska, M., Framski, G., Baranowski, D., Niemczak, M., Zembrzuska, J., Cajthaml, T., **Heipieper, H.J.**, **Chrzanowski, Ł.** (2023):
Can ionic liquids exist in the soil environment? Effect of quaternary ammonium cations on glyphosate sorption, mobility and toxicity in the selected herbicidal ionic liquids
J. Mol. Liq. **370** , art. 120981

http://dx.doi.org/10.1016/j.molliq.2022.120981

Can ionic liquids exist in the soil environment? Effect of quaternary ammonium cations on glyphosate sorption, mobility and toxicity in the selected herbicidal ionic liquids.

Anna Parus[1*], Oskar Zdebelak[1], Tomasz Ciesielski[1], Radosław Szumski[1], Marta Woźniak-Karczewska[1,4], Grzegorz Framski[2], Daniel Baranowski[2], Michał Niemczak[1], Joanna Zembrzuska[1], Tomáš Cajthaml[3], Hermann J. Heipieper[4], Łukasz Chrzanowski[1,4]

[1] Faculty of Chemical Technology, Poznan University of Technology, Berdychowo 4, 60-965 Poznan, Poland

[2] Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznań, Poland

[3] Institute for Environmental Studies, Faculty of Science, Charles University, Benátská 2, Prague 2, Czech Republic

[4] Department of Environmental Biotechnology, Helmholtz Centre for Environmental Research – UFZ, Permoserstraße 15, 04318 Leipzig, Germany

Corresponding author: anna.parus@put.poznan.pl

Electronic supplementary information (ESI) available.

Keywords: ionic bond energy, ion integrity; phytotoxicity; sorption isotherm; synthesis; toxicity

ABSTRACT

Ionic liquids (ILs) are described as low-impact green chemicals in which the cation can influence the properties of the anion through ion pair formation. We decided to see how stable ILs are in the soil environment. Herbicidal ILs consisting of hydrophilic glyphosate [Glyph] paired with hydrophilic choline [Chol], hydrophobic C_{12} alkyl modified choline [C_{12}Chol] and hydrophobic quaternary ammonium cation [C_{16}TMA] were synthesized. The calculated interaction energies between ions (75.9-88.9 kcal/mol) were typical for ILs. The results showed independent sorption of cations and anions and no statistical effect of cations on anion sorption. Hydrophobic [C_{16}TMA], [C_{12}Chol] cations followed 99% adsorption, and hydrophilic [Chol] revealed 42-45% adsorption. The Freundlich constants were [C_{16}TMA] K_f≈4000 > [C_{12}Chol] K_f≈3000 > [Chol] K_f≈3 ($mg^{1-1/n}$ $L^{1/n\ g-1}$). The preferential sorption of hydrophobic cations was also visible during leaching experiments and resembles the behavior of cationic surfactants. [Glyph] sorption was higher in organic-rich agricultural soil (approx. 45%) compared to reference OECD soil (30%), and the K_f values were constant, regardless of the presence of cations. The lack of ionic pair integrity was also visualized by the toxicity and phytotoxicity results. In the soil environment, ILs are a mixture of independent cations and anions rather than a novel emerging contaminant.

1. INTRODUCTION

Ionic liquids (ILs) are, next to graphene, graphene oxide, nanoparticles, etc., among the most addressed scientific topics of the last 1-2 decades (Randviir et al., 2014; Talapin and Shevchenko, 2016). However, only recently have issues related to the toxicity and potential environmental impact of ionic liquids begun to be raised (Docherty and Kulpa, Jr., 2005; Markiewicz et al., 2013; Peric et al., 2013). Despite the vast amount of research, the behavior of the cation and anion that enter the environment still remains unknown. By definition, the interaction of the cation with the anion, which is due to the ion size and resulting low lattice energy, will define the properties of the resulting ionic liquid (Krossing et al., 2006). Many researchers state that the cation and anion form an ionic pair to explain the specific properties of ionic liquids (Köddermann et al., 2006; Tubbs and Hoffmann, 2004; Zhang and Maginn, 2015). Additionally, the advantage of the high designability of ionic liquids is highlighted. This includes the possibility of modifying, e.g., the hydrophilicity/hydrophobicity of the anion, by an appropriate choice of cation (Choudhary et al., 2017). Consequently, without proper consideration, ILs are often described as green chemicals with low environmental impact, which unfortunately is repeated as a mantra by many researchers.

Even if we reject the obvious cases of scientific blundering, the problem that is apparent at first glance is that almost all of these studies are conducted for artificial aqueous systems without the presence of any other compounds or for systems in which the IL is the solvent and other compounds are present in small amounts, e.g., as substrates. To date, virtually no one has analyzed how stable the cation–anion interaction is in a real environment. It is true that sorption of ILs in soil has been analyzed, but the selection concerned mainly ILs in which the organic cation was linked with a simple inorganic anion (Mrozik et al., 2013, 2012, 2009). Consequently, none of these reports showed a real effect of the potential interaction between cation and anion on the behavior of the ILs in soil. Currently, toxicity studies of ILs are also becoming very popular, but they still do not take into account the role and importance of the postulated cation–anion interactions on the described toxicity.

Therefore, analyzing the current state of knowledge, we observed the need to conduct research addressing the issue of cation and anion integrity in an IL upon release to environmental conditions by using a selected herbicidal ionic liquid as an example. Such a choice makes it easy to trace the influence of organic cations differing in hydrophobicity on the sorption of the glyphosate anion in the soil environment. In addition, changes in toxicity can be easily traced. Glyphosate was chosen because it is the most important herbicide in terms of frequency and amount of use worldwide (Benbrook, 2016; Myers et al., 2016). As a total herbicide, it is a very popular active ingredient in many nonselective preparations (Bento et al., 2016; Botero-Coy et al., 2013; Meftaul et al., 2021) used to control both monocotyledonous and dicotyledonous weeds and primarily to desiccate crops before harvest (e.g., oilseed rape, lupine) (Fernández-Moreno et al., 2016). Based on literature data, it is known that glyphosate is characterized by considerable sorption in soil. This property is responsible for a reduced availability of glyphosate for soil microorganisms and translates to a prolonged degradation time (up to 200 days) compared to 5-7 days when no sorption occurs (Singh et al., 2019). The combination of a cationic surfactant with glyphosate in the form of an herbicidal ionic liquid is thus an ideal test system for analyzing the possible increase in anion sorption under the influence of different cations. The sorption of cationic surfactants is a widely studied topic and has been explained in great detail for the soil environment. Therefore, we chose a series of cations with increasing hydrophobicity, which is related to the presence of a long alkyl chain.

It should be emphasized here again that, as previously reported by many researchers, it is possible to increase the hydrophobicity of the entire IL by appropriate selection of the hydrophobic cation (Kowalska et al., 2021; Stepnowski et al., 2007).

Such designability of the IL could only be visible if both ions in the IL were forming a tight ionic pair. In theory, our study provides important verification for postulated changes in anion properties by the selection of an appropriate cation. In a broader context, such studies will provide an answer to the question of whether adjuvants affect the sorption of glyphosate in soil. Will glyphosate be more mobile or, on the contrary, will it be more adsorbed in the soil? Additionally, it will be possible to understand the effect of surfactant compounds on the observed toxicity of glyphosate. In addition, sorption of herbicides is important for proper biodegradation processes; otherwise, herbicides, not being sorbed, will easily leach into groundwater and then drinking water. First, we will investigate how an IL behaves in soil. Whether as a characteristic ionic pair with specific properties or as an independent collection of cations and anions with behavior specific to each individual.

2. MATERIALS AND Methods

2.1. Synthesis of compounds with glyphosate anions

The herbicidal ionic liquids $[Chol]_2[Glyph]_{1.8}$ and $[C_{16}TMA]_2[Glyph]_{0.8}$ used in this study were synthesized and characterized using previously described patents WO2010123871 A1 (M. Li, H. Tank, L. Liu, K. Quin, S. Wilson, 2010) and EP124351 A1 (M.P. Prisbylla, n.d.), respectively. The potassium salt of glyphosate and $[C_{12}Chol]_2[Glyph]_{1.3}$ was synthesized in this study according to the procedure presented in the abovementioned references. A detailed description of the reagents and synthesis is presented in the ESI (Section 1.1. Synthesis of herbicidal ionic liquids).

2.1.1. Spectral analysis

1D and 2D ^1H, ^{13}C and ^{15}N NMR spectra were recorded on a Bruker Avance II 400 MHz (Billerica, Massachusetts, US) spectrometer equipped with a 5 mm broad-band multinuclear (BBI) probe in D_2O at 298 K. Chemical shifts (δ) for ^1H and ^{13}C NMR were reported in ppm relative to the tetramethylsilane (TMS) peak, and for ^{15}N NMR, δ was reported in ppm relative to liquid NH_3. ^1H, ^{13}C and ^{15}N signals were assigned by means of ^1H–^1H COSY, ^1H–^{13}C/ ^{15}N HSQC, and ^1H –^{13}C/ ^{15}N HMBC experiments. The 2-D homo or heteronuclear correlation experiments were carried out using a standard set of parameters. NOESY experiments utilized standard gradient selected sequences with 100 scans per increments, a spectral width of 10 ppm in both dimensions, and mixing times of 150 and 450 ms. Spectra were processed and prepared with Top-Spin 3.1 Bruker Software.

2.2. Properties of the obtained herbicidal ionic liquids

2.2.1. Melting point

The melting point of the obtained compounds was analyzed using an MP 90 Melting Point System apparatus (Mettler Toledo, Switzerland). The precision of the measurements was ensured by calibrating the apparatus using certified reference substances.

2.2.2. Water content

The water content of all products obtained was measured using a trace TitroLine 7500 KF apparatus (SI Analytics, Germany) by Karl Fischer titration. First, each compound was dissolved in dehydrated methanol. After determining the water content of the pure methanol used as well as the resulting methanol solutions, the water content of the products was calculated.

2.2.3. Density

Density was determined using a DDM2911 automatic densitometer (Rudolph Research Analytical, USA) by the mechanical oscillator method. The density of ILs (approximately 1.0 cm^{-3}) was measured at 25 °C, and the temperature was controlled using a Peltier module. The apparatus was calibrated using distilled water as a standard. After each series of measurements, the densimeter was washed with water and organic solvents (methanol and acetone) and dried with a stream of air.

2.2.4. Refractive index

The refractive index was determined using a J357 automatic refractometer (Rudolph Research Analytical, USA) with electronic temperature control. Approximately 1 cm^3 of IL was analyzed at 25 °C. The uncertainty of measurement was less than 0.00005.

2.2.5. Solubility

Water and nine commonly used organic solvents, arranged in order of decreasing Snyder's polarity index, were selected for solubility testing: water, 9.0; methanol, 6.6; DMSO, 6.5; acetonitrile, 6.2; acetone, 5.1; isopropanol, 4.3; ethyl acetate, 4.3; chloroform, 4.1; toluene, 2.3 and hexane, 0.0. (See Table S1. in ESI for details). The solubility of the obtained ILs in organic solvents was determined according to the methodology described in Vogel's Textbook of Practical Organic Chemistry (A.I. Vogel, A.R. Tatchell, B.S. Furnis, A.J. Hannaford, 1996). A sample of ILs (0.1 ± 0.0001 g) was introduced into a defined volume of solvent. Measurements were carried out at 25 °C. Depending on the volume of solvent used, three results were recorded: "good solubility" refers to ILs that dissolved in 1 cm^3 of solvent (>10.0% m/v), "medium solubility" refers to compounds that dissolved in 3 cm^3 of solvent (between 3.33-10.0% m/v), and "low solubility" refers to ILs that did not dissolve in 3 cm^3 of solvent (<3.33% m/v).

2.3. Soil characteristics

Two soil types were used in this study. The first was the OECD standard soil, which provides a baseline for comparison by a wide range of researchers. The second was an actual agricultural soil.

2.3.1. OECD soil

The OECD soil used in this study consisted of 70% air-dried quartz sand, 20% kaolin clay, and 10% peat. Calcium carbonate was used to obtain an initial pH of 6±0.5 (in KCl) and 6.5 (in water). The water holding capacity was 40%, the total carbon was 5%, and the cation exchange capacity was 8.76 cmol/kg. Data represent the mean of triplicate soil analysis (standard error of mean < ±5%, n=3).

2.3.2. Agricultural soil

The agricultural soil used in this study was collected from the field in Rzgów (Poznan, Poland N 52.151102, E 18.050041). The soil samples were taken from a depth of 10–20 cm and sieved through a 2.0 mm sieve. According to the United Soil Classification System, the soil was characterized as fine-grained sandy loam type OL. The detailed composition of the soil was as follows: clay 1.9%, silt 27.0%, and sand 71.0%. The pH 5.9 (in KCl), 6.9 (in water), bulk density 1.32±0.06 Mg/m^3, porosity 0.39±0.03 m^3/m^3, field water capacity 0.22±0.04 m^3/m^3, relative field capacity 0,564±0.04, moisture during sampling 18%±1%, and cation exchange capacity 24.3±0.5 cmol/kg. The symbol '±' represents the standard deviation from three independent replicates.

2.4. Sorption of herbicidal ionic liquids in soil

Adsorption experiments were performed according to OECD guidelines, 2000 (OECD/OCDE 106, 2000). The soil used for studies was sterilized in an autoclave for 20 minutes at 121 °C. Sterilization was carried out for three consecutive days. The soil between sterilizations was dried at 60 °C according to the procedure described in Shen et al. (Shen et al., 2018). The experiments were conducted for four concentrations of [K][Glyph] and herbicidal ionic liquids containing the anion [Glyph], in which the concentration of the active ingredient (glyphosate) was 1, 10, 20, 50 mg/L. Herbicidal ionic liquid solution (25 mL) and dried sterile soil (5 g) were added to the PP centrifuge tubes and shaken in an orbital shaker at 240 rpm at 20±1 °C in the dark (to avoid photodegradation) for 0-24 h. Samples were analyzed after 1, 6, 12, and 24 h. After reaching equilibrium, the tubes were centrifuged at 10,000 rpm for 10 min. The obtained supernatant was filtered through a 0.22 µm PTFE syringe filter (Whatman® Puradisc, Sigma Aldrich). HPLC–MS/MS was used to determine the cation and anion concentrations. Possible sorption on the surface of PP centrifuge tubes was carried out. Blank tests were performed by shaking 25 mL of the solution of the analyzed compounds without substrate. Additionally, a control was performed by shaking 5 g of soil with 25 mL of water (without the analyzed compounds). All experiments were performed in triplicate.

The adsorption efficiency of herbicides on soil is calculated using the following formula:

$$removal\ \% = \frac{c_0 - c_e}{c_0} \cdot 100\ \%\ (1)$$

where c_o and c_e are the initial and equilibrium concentrations of herbicide in solution, respectively (mg/L).

2.5. Adsorption isotherms

Langmuir and Freundlich isotherm models were applied to calculate the adsorption parameters.

The Langmuir model (Meftaul et al., 2020):

$$\frac{c_e}{q_e} = \frac{c_e}{q_{max}} + \frac{1}{bq_{max}}\ (2)$$

where q_e – is the concentration of cations or anions adsorbed on the soil per unit mass calculated from the following formula:

$$q_e = \frac{V \cdot (c_0 - c_e)}{m}\ (3)$$

where c_o is the initial concentration of cation or anion (mg/L), c_e is the equilibrium concentration of cation or anion (mg/L) in the solution, V is the volume of the solution (L), m is the mass of the sorbent (kg), and q_{max} (mg/kg) and b (L/mg) are the maximum amounts of cation or anion per unit mass of sorbent to form a complete monolayer on the surface at c_e. q_{max} and b can be determined from the slope and intercept of the linear plot of c_e / q_e versus c_e. The Freundlich model describes c_e (mg/L) and q_e (mg/kg) as the equilibrium concentration of cations or anions that remain unadsorbed and the concentration of cations or anions adsorbed on soil per unit mass, respectively. The Freundlich constants K_f ($\mathrm{mg^{1-1/n}\ L^{1/n}\ g^{-1}}$) and n indicate the adsorption capacity of the sorbent and the favorability of the adsorption process, respectively. The magnitude of the intercept and slope of the plot of $log\ q_e$ relative to $logc_e$.

$$\log q_e = \frac{1}{n}\ logc_e + logK_f \quad (4)$$

2.6. Leaching of herbicidal ionic liquids from OECD and agricultural soils

The tests were adapted from OECD Guideline No. 312 "Leaching in soil columns" (OECD 2004) (“OECD/OCDE 312 - Leaching in Soil Columns,” n.d.). Briefly, a glass wool plug was packed into glass chromatography columns (17 cm long, 4.0 cm wide) with an outlet control valve to avoid particle leaching, followed by 15 cm of appropriate soil (previously described OECD or agricultural soil from Rzgów). The soil was autoclaved and added using small portions under gentle vibration. The columns were then saturated from bottom to top with water and allowed to percolate freely for 12 h as described by Khan et al. (Khan et al., 2017). After this time, 5 mL of 50 mg/L (calculated for active ingredient – glyphosate) aqueous solution of herbicidal ionic liquid was applied to the top of the column. The surface of the soil columns was covered with circular filter paper for uniform distribution of artificial rain, which was prepared from 0.01 M $CaCl_2$ in Milli-Q water. The columns were mounted on a universal laboratory tripod and connected to a multichannel peristaltic pump with a flow rate of artificial rain set at 0.2 mL/min. The total amount of artificial rain was 250 mL, which corresponds to a rainfall of 200 mm as recommended by OECD, 2004 (“OECD/OCDE 312 - Leaching in Soil Columns,” n.d.). The columns were stored at room temperature away from light. At specified intervals, leachate samples were collected into Erlenmeyer flasks and stored at 4 °C before analysis. Finally, 5 mL of each sample was passed through a 0.22 µm syringe filter for HPLC–MS/MS cation and anion analysis.

After leaching, the soil was removed from the columns, homogenized and preserved at 4 °C before extraction. Subsequently, 2 g of soil in triplicate was taken for the extraction. The anion extraction was carried out using 5 ml distilled water, while 5 ml of methanol supplemented with 0.5 ml of HCl was used to extract the cations. The whole sample was placed in an ultrasonic bath, and the sonication process was carried out for 30 minutes. Then, the samples were centrifuged for 10 minutes at 10,000 rpm. The obtained supernatant was decanted and filtered through a syringe filter (PTFE) and then analyzed by HPLC–MS/MS.

2.7. HPLC–MS/MS analysis

HPLC–MS/MS analysis was performed on an UltiMate 3000 RSLC chromatographic system from Dionex (Sunnyvale, CA) connected to an API 4000 QTRAP triple quadrupole mass spectrometer from Biosystems, MDS Sciex (Foster City, CA) via an electrospray ionization source operating in positive ion mode for the determination of cations and in negative ion mode for the determination of anions. Five microliters of prepared samples with ionic liquids were injected into a Luna C18 column (100 mm × 2.0 mm ID; 3 µm) from Phenomenex (Torrance, CA, USA) with a security guard cartridge (4 mm × 2.0 mm ID) of the same type thermostated at 35 °C. The mobile phase employed in the analysis consisted of 5 mmol/L ammonium acetate in water (phase A) and methanol (phase B) at a flow rate of 0.2 mL/min. The analysis was performed using gradient elution (Table S2). The dwell time for each mass transition detected in the multiple reaction monitoring mode was set to 200 ms. The following mass spectrometer settings were used for the analysis: curtain gas 10 psi, GS1/GS2 40 psi, temperature 400 °C, and collision gas medium. The ion spray voltage was 5500 V for cations and −4500 V for anions. The parameters characteristic for the particular ions are summarized in Table S3.

2.8. Antimicrobial activity test

Stock solutions of glyphosate-based HILs as well as glyphosate potassium salt were diluted in distilled water, and 5000, 3750, 2500, 1250, 500, 250, 50, and 5 mg/L concentrations were prepared for the assessment of antimicrobial activity. All solutions were stored at 4 °C until use but no longer than 48 h. The toxicity was tested against the following microorganisms: Gram-negative *Escherichia coli* (isolated from the soil sample and identified by the 16S ribosomal RNA gene), Gram-positive *Staphylococcus aureus* (ATCC 6538) and fungal species *Candida albicans* (ATCC 10231). All strains belong to the microbial collection of the Faculty of Chemical Technology (Poznan University of Technology). Strains were cultivated in rich media according to their individual species preferences. Microbial cells were transferred from the agar plates to appropriate rich media – *E. coli* to 50% TSB (BTL, Poland), S. *aureus* and *C. albicans* to 50% nutrient broth (BTL, Poland). The initial optical density (OD_{600}) was equal to 0.100±0.015. Subsequently, the microbial suspensions (200 μL) were transferred to sterile 96-well plates and incubated at 30 °C with continuous shaking in a Synergy™ HTX Multi-Mode Microplate Reader until the cell suspension reached an exponential phase. Then, 50 μL of previously prepared solutions of HILs was added to each well to obtain final tested concentrations of 1000, 500, 250, 100, 50, 10, 5, and 1 mg/L. The microbial cells were cultivated for 10 h under the above-described conditions. Inoculated media without tested compounds were used as biotic controls; additionally, media without toxicants and microorganisms (abiotic controls) as well as media without microorganisms but with toxicants were also included in the experiment. Three replicates of all samples and controls were prepared and treated identically. After the strains reached stationary phase, growth curves were plotted, and the degree of inhibition of microbial growth was calculated. The concentration of the half maximum effective concentration (EC_{50}) was determined according to the procedure and formulas described in Syguda et al. (Syguda et al., 2020).

Additionally, growth inhibition caused by glyphosate-based HILs was determined according to the procedure described by Piotrowska et al. (2016) using *Pseudomonas putida* KT2440 as a model microorganism for toxicity and adaptation assessment. The growth experiments were performed in the Department of Environmental Biotechnology, UFZ (Leipzig, Germany).

2.9. Determination of the impact of herbicidal ionic liquids on the early development of plants

The effect of HILs on germination and early development of plants was examined on wheat (*Triticum*

L.) and cornflower (*Centaurea cyanus*) using the phytotoxicity test based on the ISO-11269-2:2003 International Standard (ISO-11269-2, 2003) ("ISO-11269-2:2003 International Standard (ISO-11269-2, 2003)," n.d.). Both model plants belong to different taxonomic units. Experiments were conducted in Phytotoxkit plastic containers (Phytotoxkit, Tigret, Belgium). The plates were filled with 100 g of soil, and then 20 ml of a solution of HILs was added at a concentration corresponding to 1, 5, 10, 25, 50, 100 [mg/kg soil dry weight (DM)] of glyphosate as an active ingredient for wheat and 0.1, 0.5, 1, 5, 10, 25 [mg/kg soil dry weight (DM)] of glyphosate as an active ingredient for cornflower. To soil samples without analyzed HILs, 20 mL of water was added. Potassium salts of glyphosate were used as the herbicide reference sample. Ten plant seeds were placed in the soil in one plastic container, and three plastic containers were prepared for each concentration. They were then placed in the dark and kept at a constant temperature (25±1 °C). At the end of the experiment, the number of germinated seeds was determined, and root length and shoot height were measured. The germination index (GI) was calculated according to the equation provided by Parus et al. (Parus et al., 2020). All phytotoxicity tests were carried out in three replicates. Average values were evaluated with their standard deviations (SD). The data were compared by Student's t test, and statistical significance was set as $p < 0.05$.

2.10. Structure Modeling

Structural modeling was carried out in HyperChem 8.0.6 software. Individual models were built using a graphical interface and then optimized by the semiempirical PM3 method using the Fletcher-Reeves algorithm (conjugate gradient) on isolated chemical individuals (i.e., in a vacuum). Modeling was performed for HILs with a cation and anion molar ratio of 1:1

Calculations were carried out until the total energy gradient was less than or equal to 0.01 $\mathrm{kcal \cdot mol^{-1}}$. The interaction energy between the components of the complex was calculated as a number opposite to the enthalpy of the complex formation reaction, which is the difference between the heats of formation of the complex and its components:

$$E_b = -\Delta H_r = -(HoF_{complex} - \sum HoF_{individuals}) \quad (6)$$

where E_b is the interaction energy [kcal·mol^{-1}], ΔH_r is the enthalpy of the complex formation reaction [kcal·mol^{-1}], $HoF_{complex}$ is the heat of formation of the complex [kcal·mol^{-1}], and $HoF_{individuals}$ is the heat of formation of components [kcal·mol^{-1}].

2.11. Statistical analysis

One-way ANOVA with $p < 0.05$ was used for statistical comparisons. This approach was also employed for statistical analysis of metagenomic data to establish the significance of differences for all systems.

3. RESULTS AND DISCUSSION

3.1. Synthesis of herbicidal ionic liquids

To investigate the effect of cations on glyphosate sorption, cations differing in hydrophobicity were synthesized and combined with the herbicide anion in the form of herbicidal ionic liquids. The resulting salts meet the definition of ionic liquids and therefore are liquids below 100 °C. The exact physicochemical parameters are shown in Table 1. From a practical point of view, the most important feature of these compounds is their very good solubility in water, which enables their wide use in agriculture. Solubility in other solvents is presented in Table S1 in ESI.

Table 1. Synthesized HILs containing glyphosate as the anion

Salt	R^1	R^2	Water content [%]	Appearance at 25 °C	Refractive index at 25 °C	Density at 25 °C [g cm^{-3}]
$[Chol]_2[Glyph]_{1.8}$	CH_3	CH_2CH_2OH	0.89	Liquid	1.5002	1.305
$[C_{12}Chol]_2[Glyph]_{1.3}$	$C_{12}H_{25}$	CH_2CH_2OH	1.36	Wax	1.4793	---
$[C_{16}TMA]_2[Glyph]_{0.8}$	$C_{16}H_{33}$	CH_3	0.89	Solid*	---	---

*m.p. = 79-84 °C Where: [a] WO2010123871 A1 [40] ; [b] EP124351 A1 [41]

3.2. NMR analysis

^{1}H, ^{13}C, ^{15}N NMR experiments revealed that the hydrophobicity of the cation evidently affects the structure of the analyzed HILs, which was confirmed by unequivocal assignment of ^{1}H, ^{13}C and ^{15}N resonances by thorough analysis of 2D spectra (^{1}H–^{1}H COSY, ^{1}H–^{13}C gHSQC/gHMBC, ^{1}H–^{15}N gHSQC/gHMBC) (for more information, see Figure S3-S50 in ESI). Comparison of the ^{1}H and ^{13}C resonances for glyphosate as well as the amine part of the analyzed pairs with ^{1}H and ^{13}C resonances for each individual compound showed no significant differences, which may suggest a lack of any interaction between the amine and acidic moieties (Table S4 and S5). Based on ^{1}H spectra, it can be observed that the reduction of the ratio between [Glyph] and [Chol], [C_{12}Chol], [C_{16}TMA] depending on hydrophobicity associated with the presence of long alkyl chains occurred. The determined ratio is $[Chol]_2[Glyph]_{1.8}$, $[C_{12}Chol]_2[Glyph]_{1.3}$, and $[C_{16}TMA]_2[Glyph]_{0.8}$. Additionally, NOESY spectra showed no inter-NOE correlations between the protons of the cation part and glyphosate protons. These findings indicated that both cation and anion molecules are spatially far from each other; thus, the formation of ionic pairs should be excluded in D_2O.

3.3. Sorption of herbicidal ionic liquids in soil

The only potential explanation of cation–anion interactions that could support the possibility of modeling the properties of a cation–anion system is the formation of ionic pairs. Only in the case of a stable ionic pair can strong interactions between the cation and anion lead to a decrease in the total charge of the molecule, as well as an increase in the hydrophobicity of the ionic pair. All this should be observable in sorption experiments. To increase the chances of observing interactions of anions, which are known to sorb poorly in soil, we selected glyphosate. It is often paired with cations in commercial formulations, and it also shows sorption in soil. Therefore, we decided to evaluate the behavior of herbicidal ionic liquids based on choline-based cations and glyphosate as an anion in two soil types. The first soil was selected as a typical OECD soil with a low organic fraction content. The second soil is a typical agricultural soil with medium fertility but a higher organic fraction content compared to the OECD soil.

Analysis according to the OECD procedure clearly revealed that cation sorption in both soils correlated with increasing hydrophobicity of the cation. [Chol], as the most hydrophilic cation, underwent the weakest sorption, while hydrophobic [C_{12}Chol] and [C_{16}TMA] were almost quantitatively sorbed in both soils. No differences in cation sorption efficiency are observed between OECD soil and agricultural soil. Differences between soils were observed for the glyphosate anion. This herbicide bound more to the real soil matrix than to the matrix present in the OECD model soil. The sorption values for cations and anions are summarized in Table 2.

Table 2. Sorption of cations and anions of the analyzed herbicidal ionic liquids in OECD and agricultural soils. Mean values for experiments at a concentration of 50 mg/L of active ingredient (glyphosate) in HILs.

Herbicidal ionic liquid	Cation sorption OECD soil [%]	Cation sorption Agricultural soil [%]	Anion sorption OECD soil [%]	Anion sorption Agricultural soil [%]
[K][Glyph]	-	-	36.1±2.4	49.9±2.5
[Chol]$_2$[Glyph]$_{1.8}$	42.1±1.8	44.4±1.2	36.2±0.9	50.4±2.2
[C_{12}Chol]$_2$[Glyph]$_{1.3}$	99.6±0.4	99.7±0.3	37.6±1.4	48.4±1.7
[C_{16}TMA]$_2$[Glyph]$_{0.8}$	99.7±0.3	99.8±0.2	37.5±0.8	49.7±2.3

The sorption of cations and anions was fit to the Freundlich adsorption isotherm. We applied this model following the reports of leading researchers of ionic liquid sorption (Kowalska et al., 2021) and OECD recommendations (Figure S1 and Figure S2 in ESI0. Freundlich demonstrated the essence of the separate functioning of cations and anions in soils.

Figure 1.

The grouping of isotherms for the hydrophilic cation [Chol] and the hydrophobic cations [C_{12}Chol] and [C_{16}TMA] is clearly visible in Figure 1. As mentioned above, both cations [C_{12}Chol] and [C_{16}TMA] showed almost quantitative sorption in OECD soil and agricultural soil. This was confirmed by the very high Freundlich K_f values, which for [C_{12}Chol] and [C_{16}TMA] are approximately 2 000 and above 4 000 $mg^{1-1/n}$ $L^{1/n}$ g^{-1}, respectively (Table 3). The [Chol] cation was also sorbed in both soils, although its hydrophilic nature would suggest a lower sorption efficiency. Some reports indicate that since choline hydroxide is a quaternary ammonium compound, adsorption will depend on the cation exchange capacity of the sorbent and many other parameters. Not surprisingly, choline is often not included in software used to estimate physical/chemical properties and environmental fate, such as KOCWIN developed by EPA and Syracuse Research Corp. ("[https://echa.europa.eu/registration-dossier/-/registered-dossier/5289/5/5/2] [access 18.11.2021]," n.d.). Interestingly, we observed no difference between the sorption isotherms for cations in either OECD or agricultural soil.

Table 3. Comparison of K_f ($mg^{1-1/n}$ $L^{1/n}$ g^{-1}) values calculated for [Chol], [C_{12}Chol], [C_{16}TMA] cations and [Glyph] anions in the appropriate salts

Herbicidal ionic liquid	**K_f ($mg^{1-1/n}$ $L^{1/n}$ g^{-1}) values**			
	Cation in OECD soil	**Cation in Agricultural soil**	**Anion in OECD soil**	**Anion in Agricultural soil**
[K][Glyph]	-	-	3.3±0.3	4.4±0.3
$[Chol]_2[Glyph]_{1.8}$	3.4±0.3	4.1±0.5	2.9±0.2	4.3±0.4
$[C_{12}Chol]_2[Glyph]_{1.3}$	2208.6±109.3	2516.1±142.7	3.9±0.2	5.2±0.2
$[C_{16}TMA]_2[Glyph]_{0.8}$	4616.5±67.1	4883.4±130.3	3.3±0.1	5.4±0.5

In the case of isotherms for [Glyph] anions, no significant differences are observed between HILs in OECD or agricultural soil (see Figure 2). However, significant differences are visible between OECD and agricultural soil. The sorption isotherm for glyphosate is lower than that for agricultural soil. These differences can be explained very easily. The sorption of glyphosate depends on the following factors: cation exchange capacity, clay and organic carbon content and pH (Dollinger et al., 2015). In addition, according to the Freundlich isotherm model, glyphosate undergoes partitioning on heterogeneous surfaces of soil organic matter (OM) and clay minerals, followed by diffusion into soil micropores (Meftaul et al., 2021). Comparing the OECD model soil and the real agricultural soil, it is easy to see that the agricultural soil has twice as much organic carbon and a much higher CEC, so that the analysis of soil physicochemical parameters alone can easily explain why glyphosate sorbs more strongly in agricultural soil (see Figure 3).

Figure 2.

Figure 3.

3.4. Leaching of herbicidal ionic liquids from OECD and agricultural soils

The purpose of the OECD "Leaching in soil columns" test is to mimic the leaching of xenobiotics under precipitation conditions. The hydrophobic cations [C_{12}Chol] and [C_{16}TMA] with surfactant properties are strongly adsorbed in both soils and are subjected to little leaching (Table 4). However, [Chol], as the most hydrophilic cation, is highly mobile in soils (75-88%). Interestingly, this quaternary ammonium compound was partially sorbed, most likely due to the high cation exchange capacity of the agricultural soil used in this study. However, the sorption trend was also evident in the OECD soil.

Table 4. Leaching and sorption of herbicidal ionic liquids in OECD and agricultural soils at the initial concentration corresponding to 50 mg of the active substance – glyphosate.

HILs	Sorbed [%]		Leached [%]	
	Cation	Anion [Glyph]	Cation	Anion [Glyph]
	OECD soil			
[K][Glyph]	nd	59.2 ± 4.1	nd	44.8 ± 3.0
[Chol]$_2$[Glyph]$_{1.8}$	23.2 ± 1.5	58.4 ± 2.7	88.4 ± 1.6	46.6 ± 1.9
[C_{12}Chol]$_2$[Glyph]$_{1.3}$	92.3 ± 5.8	56.1 ± 2.9	10.5 ± 1.3	45.6 ± 3.3
[C_{16}TMA]$_2$[Glyph]$_{0.8}$	100.2 ± 4.3	59.4 ± 3.4	2.1 ± 0.1	42.9 ± 3.4
	Agricultural soil			
[K][Glyph]	nd	71.8 ± 4.0	nd	30.5 ± 2.7
[Chol]$_2$[Glyph]$_{1.8}$	26.7 ± 2.6	70.7 ± 4.5	75.3 ± 1.6	29.7 ± 2.8
[C_{12}Chol]$_2$[Glyph]$_{1.3}$	92.3 ± 5.8	69.4 ± 3.3	12.0 ± 2.0	32.7 ± 3.1
[C_{16}TMA]$_2$[Glyph]$_{0.8}$	100.6 ± 4.9	72.5 ± 3.0	1.7 ± 0.1	29.3 ± 2.6

nd – not determined

In contrast, the glyphosate content of the column leachates (Table 4) was the same and independent of the type of herbicidal ionic liquid applied (approximately 40% in OECD soil and 30% in agricultural soil). These results confirmed the independent action of cations and anions of the herbicidal ionic liquids in the soil environment, confirming the same trends as described above in the sorption of herbicidal ionic liquids in the soil section.

The sorption of glyphosate to both soils was found to be relatively high, approximately 60 and 70% for OECD and agricultural soils, respectively. This is a very typical trend described by many researchers who have proven the role of organic matter in glyphosate sorption. The more organic matter there is, the greater the sorption of glyphosate in the soil (Meftaul et al., 2021).

From an environmental point of view, xenobiotics adsorbed in soil tend to be poorly accessible to microorganisms and therefore degrade slowly, extending the time the xenobiotic remains in the environment. In the case of glyphosate, the half-life in soil has been determined to range from several up to more than one hundred days depending on the soil type (Bento et al., 2016). The cationic surfactants used to synthesize HILs can undergo virtually quantitative sorption, as was the case for [C_{16}TMA]. However, under aerobic conditions, sorption might be beneficial because it will reduce the migration of the xenobiotic, allowing it to be slowly biodegraded before entering drinking water. Deeper in soil, under anaerobic conditions, all biological processes are much slower, which may increase the migration of xenobiotics into drinking water. It is worth mentioning that extensive agriculture causes a decrease in soil carbon content, which results in the rapid release of pesticides into ground and surface water. The best example of this is the more frequent detection of pesticides in drinking water despite the same consumption in the Czech Republic. Therefore, if we consider the fact that only a small portion of the pesticide will reach the target plants and a large portion will end up in the soil after spraying (Haberey et al., 2021), then it is worth further analyzing the balance of advantages and disadvantages resulting from the use of cationic surfactants in agricultural applications.

3.5. Antimicrobial activity test

The data presented in Table 5 clearly indicate the known fact that glyphosate is not toxic to microorganisms. The toxicity of glyphosate to higher organisms was obviously different, but as far as bacteria and fungi are concerned, the results obtained were consistent with those presented by others (Amorós et al., 2007; Busse et al., 2001). According to the classification by Passino and Smith (Passino and Smith, 1987), if the toxicity is higher than 1000 mg/L, then the compound is defined as harmless.

However, choline is a biomolecule incorporated in some phospholipids, especially lecithin and sphingolipids (Zeisel and Canty, 1993). The fact that it is nontoxic has attracted much interest in the chemical community (Gadilohar and Shankarling, 2017; Glier et al., 2014; Zeisel and da Costa, 2009). Based on this molecule of natural origin, a number of modifications have been proposed that show great potential to use this compound as a base for the synthesis of ILs (Weaver et al., 2010) and deep eutectic solvents (Radošević et al., 2015) or lead to novel compounds with new properties, e.g., advanced cationic surfactants. Choline itself is a hydrophilic compound, and due to its positive charge on the nitrogen atom, it can interact with electron-dense molecular fragments present in the environment. Therefore, an important issue is to increase hydrophobicity by introducing long alkyl chains into the choline molecule. In this way, compounds with a positively charged center on the nitrogen and characterized by high hydrophobicity are formed. As expected, the obtained cations [C$_{12}$Chol] and [C$_{16}$TMA] already showed bactericidal properties and thus behave as typical cationic surfactants. [C$_{12}$Chol] exhibits toxicity in the range of 5 to 200 mg/L, while [C$_{16}$TMA] exhibits toxicity in the range of 1 to 30 mg/L, which, according to the Passino and Smith classification, translates into slightly to moderately toxic compounds. The [C$_{16}$TMA] cation is generally more toxic than [C$_{12}$Chol], which would confirm the benefits of modifying molecules of natural origin rather than synthesis based on strictly synthetic compounds. The toxicity data correlated with the values for this group of compounds, as shown by Costa et al. (Costa et al., 2015). The most interesting conclusion from the analysis of the results presented in Table 5 is that the toxicity determined for HILs perfectly matches the toxicity of the cations used. This is even more evident in the figure (Figure 4.)

Table 5. Antimicrobial activity of glyphosate-based HILs towards model microorganisms.

Microorganisms	**Antimicrobial activity [mg/L]**						
	[K] [Glyph]	**[Chol] [Cl]**	**[Chol]$_2$ [Glyph]$_{1.8}$**	**[C$_{12}$Chol] [Cl]**	**[C$_{12}$Chol]$_2$ [Glyph]$_{1.3}$**	**[C$_{16}$TMA] [Cl]**	**[C$_{16}$TMA]$_2$ [Glyph]$_{0.8}$**
Escherichia coli	>1000 (H)	>1000 (H)	>1000 (H)	70.3±0.8 (ST)	72.2±0.7 (ST)	25.2±0.6 (ST)	28.1±0.7 (ST)
Staphylococcus aureus	>1000 (H)	>1000 (H)	>1000 (H)	5.8±0.3 (MT)	7.5±0.2 (MT)	3.2±0.3 (MT)	1.1±0.1 (MT)
Candida albicans	>1000 (H)	>1000 (H)	>1000 (H)	195.6±8.2 (PH)	207.7±9.5 (PH)	2.7±0.2 (MT)	3.8±0.1 (MT)

± standard error of the mean of three independent experiments, Toxicity classification according to Passino and Smith (1987) [49]; <1 mg/L toxic (T), 1-10 mg/L moderately toxic (MT); 10-100 mg/L slightly toxic (ST); 100-1000 mg/L practically harmless (PH); >1000 mg/L harmless (H)

Figure 4.

If HILs formed ionic pairs, then the toxicity of the cation–anion pair would in no case be a simple sum of the toxicity of the cation and anion. If the anion is nontoxic and only the cation is toxic, then the interactions between the combined cation and anion should manifest at least in a reduction of formal charge, and consequently, the ion pair should exhibit lower toxicity. However, we observed that the toxicity of the obtained HILs was not reduced in comparison with the toxicity of the cations themselves, which may suggest that only the cation is responsible for the final toxicity. This observation was consistent with reports of many authors that have suggested that the cation determines the properties of the resulting ILs (Gathergood et al., 2004).

Cationic surfactants are known for their toxic effects, and the main factors responsible for this are the presence of a positive charge and a hydrophobic chain. In our opinion, the same toxicity results for cations and HILs provide indirect evidence that there is no permanent interaction between cations and anions. Consequently, a solvated cation with toxic properties acts independently of the anion. It can incorporate into the cell membrane, leading to cell lysis, etc., which is a typical action of cationic surfactants (Timmer and Droge, 2017). If there is an interaction between the cation and the anion, one would expect a reduction in the toxic effects of the cations. A question that arises when analyzing toxicity results concerns the integrity of ionic liquids in an aqueous environment. The analyzed HILs are just one of many ILs but still provide a good justification for more advanced analysis of the role of the interaction between cation and anion when researching, e.g., degradation of ILs in the natural environment.

3.6. Structure Modeling

Glyphosate is usually referred to as a zwitterion, which is not entirely true, as it exists in different ionic states depending on pH, and not all of the forms have equal numbers of positive and negative charges. The amine group can be protonated, but both the phosphonic and carboxylic moieties ensure an overall negative charge under environmental conditions. Sheals et al. (Borggaard and Gimsing, 2008; Sheals et al., 2002) presented the following acid dissociation constants: $pK_{a1} = 2.22$, $pK_{a2} = 5.44$ and $pK_{a3} = 10.13$.

In the first step, the cation–anion interactions in pure ionic liquids were modeled using the HyperChem 8.0.6 program. The interaction energies of cations ([Chol], [C_{12}Chol], [C_{16}TMA]) with glyphosate anion ([Glyph]) were in the range of 75.9-88.9 kcal/mol (Table S6).

Both phosphonic and carboxylic moieties of glyphosate were taken into consideration when setting up the initial positions of ions (i.e., indicating which moieties are more plausible to interact with cation). No significant effect was observed. The obtained energy values indicate that the interactions between cations and anions are possibly enough to support the existence of herbicidal ionic liquids as the energies for bonds present in ionic liquids. Those have been reported to be 85.8-95.2 kcal/mol and 83.4-91.0 kcal/mol in dimethylpyrrolidinium chloride and methanesulfonate, respectively; 67.7-78.0 kcal/mol in the combination of dimethylpyrrolidinium cation with bis[(trifluoromethyl)sulfonyl]imide anion (Izgorodina et al., 2009); 100.7 kcal/mol in trimethylammonium methanesulfonate, or 72.4 kcal/mol in 1-ethyl-3-methylimidazolium methanesulfonate per ion pair (Fumino et al., 2013). Notably, when the ammonium group of the cation was positioned to the phosphonic moiety of the glyphosate ion, the latter rotated to embrace the ammonium center with both phosphonic and carboxylic moieties.

To explain why the cations were preferentially sorbed in soils to an extent that exceeded the sorption of glyphosate, additional modeling was needed. Due to the pH of the analyzed soils, which was in the range of 5.9-6.8, glyphosate will mainly exist in fully ionized form, i.e., All three acidic moieties will have a negative charge, and the amine group will be protonated; as a result, the net charge will be minus 2. Therefore, the second step involved modeling interactions between the cations, anions and representative moieties present in soil: silanol and aluminol in both neutral and dissociated forms (Thompson A., 2012), as well as carboxyl, carboxylate and sulfonate.

The glyphosate ion with both OH groups dissociated was found to interact more strongly with ammonium cations with an energy of 164.6-170.1 kcal/mol. This higher interaction energy between cations and anions may explain the behavior of the analyzed HILs in OECD soil, where increased cation sorption translated into increased anion sorption. However, this does not explain why a similar effect was not observed for the agricultural soil, where cation sorption ([C_{12}Chol], [C_{16}TMA]) was almost quantitative but in no way translated into increased glyphosate sorption. This was interesting because even some publications indicate that the presence of surfactants in soil promotes sorption of herbicides (Rodríguez-Cruz et al., 2006).

3.7. Determination of the impact of herbicidal ionic liquids on the early development of plants

To test the effect of cations on the toxicity of glyphosate to plants, we determined the germination index [GI], and based on this, we calculated EC_{50} values expressed as the concentration of the active substance glyphosate (Table 6), which provides an easier way to see significant trends. Additionally, the effects of salt concentration on both plant shoot and root growth are presented in Figures S51-S54 in ESI. The effect of glyphosate alone was identical to the effect observed for the $[C_{12}Chol]_2[Glyph]_{1.3}$ and $[C_{16}TMA]_2[Glyph]_{0.8}$ salts. The same trend was observed in both monocotyledonous and dicotyledonous plants.

Table 6. EC_{50} values of the tested salts against cornflower and wheat expressed as concentration of active substance – glyphosate.

	EC_{50} [mg/kg d.w.s.]	
	Cornflower	**Wheat**
[K][Glyph]	19 ± 2	70 ± 7
$[Chol]_2[Glyph]_{1.8}$	9 ± 1	92 ± 7
$[C_{12}Chol]_2[Glyph]_{1.3}$	21 ± 2	73 ± 6
$[C_{16}TMA]_2[Glyph]_{0.8}$	17 ± 2	79 ± 5

No effect of the cation is seen here in any way, which should be explained by its sorption. This is due to the germination index determination methodology, in which the analyzed compound is introduced into the soil, to which seeds are then added. Since the cations $[C_{12}Chol]$ and $[C_{16}TMA]$ undergo almost quantitative sorption, they are not bioavailable and have no effect on plant growth. Therefore, in each case, the effectiveness of glyphosate is the same, regardless of whether it was introduced as a potassium salt or accompanied by the abovementioned cations in the form of HILs.

In the case of choline, the [Chol] cation undergoes only partial sorption in soil and therefore, due to its mobility, may have an effect on germinating plants. Many researchers have described both positive and negative effects of this biomolecule on plant growth. In plants, choline and glycine are involved in the synthesis of glycine betaine (GB), which is a major and effective osmoprotectant. It is widely believed that GB can protect plants from exposure to harsh environmental conditions such as drought, high temperature, and salinity without causing cell toxicity (Ashraf and Foolad, 2007). According to Pinheiro et al., low doses of 2,4-D herbicide containing choline salt can increase the shoots and roots of soybean (Pinheiro et al., 2021). However, in some cases, choline addition was proven to also have a negative effect on plant growth (Briggs, 1968).

Our results clearly indicated that the selected salts in the soil environment do not function as ionic liquids, so there is no interaction between cations and anions, the existence of which would undoubtedly translate into EC_{50} values.

4. CONCLUSION

In our work, the ability of an ionic pair to exist in the soil environment was studied in the course of sorption, leaching, toxicity, and structure modeling experiments.

Conversion of the choline cation into the hydrophobic derivative [C_{12}Chol] contributed to an increase in microbial toxicity comparable to that of a typical hydrophobic quaternary ammonium cation such as [C_{16}TMA]. In fact, the glyphosate anion is not toxic to the test microorganisms, and the toxicity of the analyzed HILs corresponded to the toxicity of the sole cations. The same trend also occurred during phytotoxicity testing. The sorption of HILs on OECD and agricultural soils revealed the following trend: cation [C_{16}TMA] > [C_{12}Chol] > [Chol]. An important aspect is that the calculated values of the Freundlich constant K_f for anions [Glyph] are almost constant, regardless of the presence of cations. The differences in K_f results between OECD soils and agricultural soils are because soils differ in factors such as cation exchange capacity, clay and organic carbon content, and pH. This showed that cations and anions sorb in soil independently of each other and that cations have no statistical effect on anion sorption. The leaching results indicated that combining herbicides with hydrophobic cations with surfactant properties does not make sense because such compounds are preferentially sorbed in soil and the mobility of the herbicide does not change at all. The interaction energies between cations ([Chol], [C_{12}Chol], [C_{16}TMA]) and zwitterion [Glyph] were within the range of interactions between ions forming ionic liquids reported previously in the literature. If we consider the pH of the analyzed soils, glyphosate should exist in a fully ionized form and interact strongly with ammonium cations, but increased sorption of hydrophobic cations was not observed. Nor did this translate into increased sorption of glyphosate. In our opinion, a much more thorough analysis of the interactions of individual cations and anions with the soil matrix should be conducted in the future to fully understand the nature of interactions between cations and anions in the soil matrix.

The results obtained indicate one important fact. The unique interactions between cations and anions, which are important for ionic liquids, are no longer an important factor in sorption on negatively charged soil components. This is strong evidence that in soil environments, choline-based ionic liquids and the herbicide glyphosate do not form any ionic pairs and undergo physicochemical processes as independent cations and anions. We have demonstrated the need for increasingly sophisticated studies from which the environmental fate of cations as well as anions can be more accurately analyzed, as it currently appears that the use of the term ionic liquids to refer to such substances introduced into the soil environment is highly debatable.

5. ACKNOWLEDGMENTS

This study was conducted in the frame of OPUS 15 funded by the National Science Centre in Poland, conferred on the basis of decision DEC-2018/29/B/NZ9/01136. Grant title “Bioaugmentation with herbicide-degrading bacteria as a potential factor in spreading resistance to herbicides among plants”.

6. REFERENCES

[https://echa.europa.eu/registration-dossier/-/registered-dossier/5289/5/5/2] [access 18.11.2021], n.d.

A.I. Vogel, A.R. Tatchell, B.S. Furnis, A.J. Hannaford, P.W.G.S., 1996. Vogel’s Textbook of Practical Organic Chemistry.

Amorós, I., Alonso, J.L., Romaguera, S., Carrasco, J.M., 2007. Assessment of toxicity of a glyphosate-based formulation using bacterial systems in lake water. Chemosphere 67, 2221–2228. https://doi.org/10.1016/j.chemosphere.2006.12.020

Ashraf, M., Foolad, M.R., 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ. Exp. Bot. 59, 206–216. https://doi.org/10.1016/j.envexpbot.2005.12.006

Benbrook, C.M., 2016. Trends in glyphosate herbicide use in the United States and globally. Environ. Sci. Eur. 28, 3. https://doi.org/10.1186/s12302-016-0070-0

Bento, C.P.M., Yang, X., Gort, G., Xue, S., van Dam, R., Zomer, P., Mol, H.G.J., Ritsema, C.J., Geissen, V., 2016. Persistence of glyphosate and aminomethylphosphonic acid in loess soil under different combinations of temperature, soil moisture and light/darkness. Sci. Total Environ. 572, 301–311. https://doi.org/10.1016/j.scitotenv.2016.07.215

Borggaard, O.K., Gimsing, A.L., 2008. Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review. Pest Manag. Sci. 64, 441–456. https://doi.org/10.1002/ps.1512

Botero-Coy, A.M., Ibáñez, M., Sancho, J.V., Hernández, F., 2013. Improvements in the analytical methodology for the residue determination of the herbicide glyphosate in soils by liquid chromatography coupled to mass spectrometry. J. Chromatogr. A 1292, 132–141. https://doi.org/10.1016/j.chroma.2012.12.007

Briggs, D.E., 1968. α-amylase in germinating, decorticated barley—III. Phytochemistry 7, 539–554. https://doi.org/10.1016/S0031-9422(00)88225-4

Busse, M.D., Ratcliff, A.W., Shestak, C.J., Powers, R.F., 2001. Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities. Soil Biol. Biochem. 33, 1777–1789. https://doi.org/10.1016/S0038-0717(01)00103-1

Choudhary, H., Pernak, J., Shamshina, J.L., Niemczak, M., Giszter, R., Chrzanowski, Ł., Praczyk, T., Marcinkowska, K., Cojocaru, O.A., Rogers, R.D., 2017. Two Herbicides in a Single Compound: Double Salt Herbicidal Ionic Liquids Exemplified with Glyphosate, Dicamba, and MCPA. ACS Sustain. Chem. Eng. 5, 6261–6273. https://doi.org/10.1021/acssuschemeng.7b01224

Costa, S.P.F., Pinto, P.C.A.G., Lapa, R.A.S., Saraiva, M.L.M.F.S., 2015. Toxicity assessment of ionic liquids with Vibrio fischeri: An alternative fully automated methodology. J. Hazard. Mater. 284, 136–142. https://doi.org/10.1016/j.jhazmat.2014.10.049

Docherty, K.M., Kulpa, Jr., C.F., 2005. Toxicity and antimicrobial activity of imidazolium and pyridinium ionic liquids. Green Chem. 7, 185. https://doi.org/10.1039/b419172b

Dollinger, J., Dagès, C., Voltz, M., 2015. Glyphosate sorption to soils and sediments predicted by pedotransfer functions. Environ. Chem. Lett. 13, 293–307. https://doi.org/10.1007/s10311-015-0515-5

Fernández-Moreno, P.T., Alcantara-de la Cruz, R., Cruz-Hipólito, H.E., Rojano-Delgado, A.M., Travlos, I., De Prado, R., 2016. Non-target Site Tolerance Mechanisms Describe Tolerance to Glyphosate in Avena sterilis. Front. Plant Sci. 7. https://doi.org/10.3389/fpls.2016.01220

Fumino, K., Fossog, V., Wittler, K., Hempelmann, R., Ludwig, R., 2013. Dissecting Anion-Cation Interaction Energies in Protic Ionic Liquids. Angew. Chemie Int. Ed. 52, 2368–2372. https://doi.org/10.1002/anie.201207065

Gadilohar, B.L., Shankarling, G.S., 2017. Choline based ionic liquids and their applications in organic transformation. J. Mol. Liq. 227, 234–261. https://doi.org/10.1016/j.molliq.2016.11.136

Gathergood, N., Garcia, M.T., Scammells, P.J., 2004. Biodegradable ionic liquids: Part I. Concept, preliminary targets and evaluation. Green Chem. 6, 166. https://doi.org/10.1039/b315270g

Glier, M.B., Green, T.J., Devlin, A.M., 2014. Methyl nutrients, DNA methylation, and cardiovascular disease. Mol. Nutr. Food Res. 58, 172–182. https://doi.org/10.1002/mnfr.201200636

Haberey, P., Hodel, D., Collet, L., Bucher, C., Anken, T., Total, R., Keller, M., 2021. 13. Efficiency evaluation of automated insecticide spot spraying in lettuce and bok choy fields, in: Precision Agriculture '21. Wageningen Academic Publishers, The Netherlands, pp. 121–128. https://doi.org/10.3920/978-90-8686-916-9_13

ISO-11269-2:2003 International Standard (ISO-11269-2, 2003), n.d.

Izgorodina, E.I., Bernard, U.L., MacFarlane, D.R., 2009. Ion-Pair Binding Energies of Ionic Liquids: Can DFT Compete with Ab Initio-Based Methods? J. Phys. Chem. A 113, 7064–7072. https://doi.org/10.1021/jp8107649

Khan, S., Shah, A., Nawaz, M., Khan, M., 2017. Impact of different tillage practices on soil physical properties, nitrate leaching and yield attributes of maize (Zea mays L.). J. soil Sci. plant Nutr. 0–0. https://doi.org/10.4067/S0718-95162017005000019

Köddermann, T., Wertz, C., Heintz, A., Ludwig, R., 2006. Ion-Pair Formation in the Ionic Liquid 1-Ethyl-3-methylimidazolium Bis(triflyl)imide as a Function of Temperature and Concentration.

ChemPhysChem 7, 1944–1949. https://doi.org/10.1002/cphc.200600034

Kowalska, D., Maculewicz, J., Stepnowski, P., Dołżonek, J., 2021. Ionic liquids as environmental hazards – Crucial data in view of future PBT and PMT assessment. J. Hazard. Mater. 403, 123896. https://doi.org/10.1016/j.jhazmat.2020.123896

Krossing, I., Slattery, J.M., Daguenet, C., Dyson, P.J., Oleinikova, A., Weingärtner, H., 2006. Why Are Ionic Liquids Liquid? A Simple Explanation Based on Lattice and Solvation Energies. J. Am. Chem. Soc. 128, 13427–13434. https://doi.org/10.1021/ja0619612

M. Li, H. Tank, L. Liu, K. Quin, S. Wilson, D.O., 2010. High-strength, herbicidal compositions of glyphosate and 2,4-d salts.

M.P. Prisbylla, n.d. EP124351 Mixed long-chain alkylammonium salts of glyphosate.

Markiewicz, M., Piszora, M., Caicedo, N., Jungnickel, C., Stolte, S., 2013. Toxicity of ionic liquid cations and anions towards activated sewage sludge organisms from different sources – Consequences for biodegradation testing and wastewater treatment plant operation. Water Res. 47, 2921–2928. https://doi.org/10.1016/j.watres.2013.02.055

Meftaul, I.M., Venkateswarlu, K., Annamalai, P., Parven, A., Megharaj, M., 2021. Glyphosate use in urban landscape soils: Fate, distribution, and potential human and environmental health risks. J. Environ. Manage. 292, 112786. https://doi.org/10.1016/j.jenvman.2021.112786

Meftaul, I.M., Venkateswarlu, K., Dharmarajan, R., Annamalai, P., Megharaj, M., 2020. Movement and Fate of 2,4-D in Urban Soils: A Potential Environmental Health Concern. ACS Omega 5, 13287–13295. https://doi.org/10.1021/acsomega.0c01330

Mrozik, W., Jungnickel, C., Ciborowski, T., Pitner, W.R., Kumirska, J., Kaczyński, Z., Stepnowski, P., 2009. Predicting mobility of alkylimidazolium ionic liquids in soils. J. Soils Sediments 9, 237–245. https://doi.org/10.1007/s11368-009-0057-1

Mrozik, W., Jungnickel, C., Paszkiewicz, M., Stepnowski, P., 2013. Interaction of Novel Ionic Liquids with Soils. Water, Air, Soil Pollut. 224, 1759. https://doi.org/10.1007/s11270-013-1759-y

Mrozik, W., Kotłowska, A., Kamysz, W., Stepnowski, P., 2012. Sorption of ionic liquids onto soils: Experimental and chemometric studies. Chemosphere 88, 1202–1207. https://doi.org/10.1016/j.chemosphere.2012.03.070

Myers, J.P., Antoniou, M.N., Blumberg, B., Carroll, L., Colborn, T., Everett, L.G., Hansen, M., Landrigan, P.J., Lanphear, B.P., Mesnage, R., Vandenberg, L.N., vom Saal, F.S., Welshons, W. V., Benbrook, C.M., 2016. Concerns over use of glyphosate-based herbicides and risks associated with exposures: a consensus statement. Environ. Heal. 15, 19. https://doi.org/10.1186/s12940-016-0117-0

OECD/OCDE 106, 2000. Adsorption - Desorption Using a Batch Equilibrium Method.

OECD/OCDE 312 - Leaching in Soil Columns, n.d.

Parus, A., Wilms, W., Verkhovetska, V., Framski, G., Woźniak-Karczewska, M., Syguda, A., Strzemiecka, B., Borkowski, A., Ławniczak, Ł., Chrzanowski, Ł., 2020. Transformation of herbicides into dual function quaternary tropinium salts. New J. Chem. 44, 8869–8877. https://doi.org/10.1039/D0NJ01597K

Passino, D.R.M., Smith, S.B., 1987. Acute bioassays and hazard evaluation of representative contaminants detected in great lakes fish. Environ. Toxicol. Chem. 6, 901–907. https://doi.org/10.1002/etc.5620061111

Peric, B., Sierra, J., Martí, E., Cruañas, R., Garau, M.A., Arning, J., Bottin-Weber, U., Stolte, S., 2013. (Eco)toxicity and biodegradability of selected protic and aprotic ionic liquids. J. Hazard. Mater. 261, 99–105. https://doi.org/10.1016/j.jhazmat.2013.06.070

Pinheiro, G.H.R., Marques, R.F., Araújo, P.P.S., Martins, D., Marchi, S.R., 2021. Hormesis effect of 2,4-D choline salt on soybean biometric variables. Chil. J. Agric. Res. 81, 536–545. https://doi.org/10.4067/S0718-58392021000400536

Radošević, K., Cvjetko Bubalo, M., Gaurina Srček, V., Grgas, D., Landeka Dragičević, T., Radojčić Redovniković, I., 2015. Evaluation of toxicity and biodegradability of choline chloride based deep eutectic solvents. Ecotoxicol. Environ. Saf. 112, 46–53. https://doi.org/10.1016/j.ecoenv.2014.09.034

Randviir, E.P., Brownson, D.A.C., Banks, C.E., 2014. A decade of graphene research: production, applications and outlook. Mater. Today 17, 426–432. https://doi.org/10.1016/j.mattod.2014.06.001

Rodríguez-Cruz, M.S., Sánchez-Martín, M.J., Andrades, M.S., Sánchez-Camazano, M., 2006.

Comparison of Pesticide Sorption by Physicochemically Modified Soils with Natural Soils as a Function of Soil Properties and Pesticide Hydrophobicity. Soil Sediment Contam. An Int. J. 15, 401–415. https://doi.org/10.1080/15320380600751769

Sheals, J., Sjöberg, S., Persson, P., 2002. Adsorption of Glyphosate on Goethite: Molecular Characterization of Surface Complexes. Environ. Sci. Technol. 36, 3090–3095. https://doi.org/10.1021/es010295w

Shen, G., Zhang, Y., Hu, S., Zhang, H., Yuan, Z., Zhang, W., 2018. Adsorption and degradation of sulfadiazine and sulfamethoxazole in an agricultural soil system under an anaerobic condition: Kinetics and environmental risks. Chemosphere 194, 266–274. https://doi.org/10.1016/j.chemosphere.2017.11.175

Singh, S., Kumar, V., Singh, J., 2019. Kinetic study of the biodegradation of glyphosate by indigenous soil bacterial isolates in presence of humic acid, Fe(III) and Cu(II) ions. J. Environ. Chem. Eng. 7, 103098. https://doi.org/10.1016/j.jece.2019.103098

Stepnowski, P., Mrozik, W., Nichthauser, J., 2007. Adsorption of Alkylimidazolium and Alkylpyridinium Ionic Liquids onto Natural Soils. Environ. Sci. Technol. 41, 511–516. https://doi.org/10.1021/es062014w

Syguda, A., Wojcieszak, M., Materna, K., Woźniak-Karczewska, M., Parus, A., Ławniczak, Ł., Chrzanowski, Ł., 2020. Double-Action Herbicidal Ionic Liquids Based on Dicamba Esterquats with 4-CPA, 2,4-D, MCPA, MCPP, and Clopyralid Anions. ACS Sustain. Chem. Eng. 8, 14584–14594. https://doi.org/10.1021/acssuschemeng.0c05603

Talapin, D. V., Shevchenko, E. V., 2016. Introduction: Nanoparticle Chemistry. Chem. Rev. 116, 10343–10345. https://doi.org/10.1021/acs.chemrev.6b00566

Thompson A., G.K.W., 2012. Introduction to the Sorption of Chemical Constituents in Soils. Nat. Educ. Knowl. 4, 7.

Timmer, N., Droge, S.T.J., 2017. Sorption of Cationic Surfactants to Artificial Cell Membranes: Comparing Phospholipid Bilayers with Monolayer Coatings and Molecular Simulations. Environ. Sci. Technol. 51, 2890–2898. https://doi.org/10.1021/acs.est.6b05662

Tubbs, J.D., Hoffmann, M.M., 2004. Ion-Pair Formation of the Ionic Liquid 1-Ethyl-3-

methylimidazolium bis(trifyl)imide in Low Dielectric Media. J. Solution Chem. 33, 381–394. https://doi.org/10.1023/B:JOSL.0000036308.36052.01

Weaver, K.D., Kim, H.J., Sun, J., MacFarlane, D.R., Elliott, G.D., 2010. Cyto-toxicity and biocompatibility of a family of choline phosphate ionic liquids designed for pharmaceutical applications. Green Chem. 12, 507. https://doi.org/10.1039/b918726j

Zeisel, S.H., Canty, D.J., 1993. Choline phospholipids: molecular mechanisms for human diseases: A meeting report. J. Nutr. Biochem. 4, 258–263. https://doi.org/10.1016/0955-2863(93)90094-D

Zeisel, S.H., da Costa, K.-A., 2009. Choline: an essential nutrient for public health. Nutr. Rev. 67, 615–623. https://doi.org/10.1111/j.1753-4887.2009.00246.x

Zhang, Y., Maginn, E.J., 2015. Direct Correlation between Ionic Liquid Transport Properties and Ion Pair Lifetimes: A Molecular Dynamics Study. J. Phys. Chem. Lett. 6, 700–705. https://doi.org/10.1021/acs.jpclett.5b00003

Figure captions

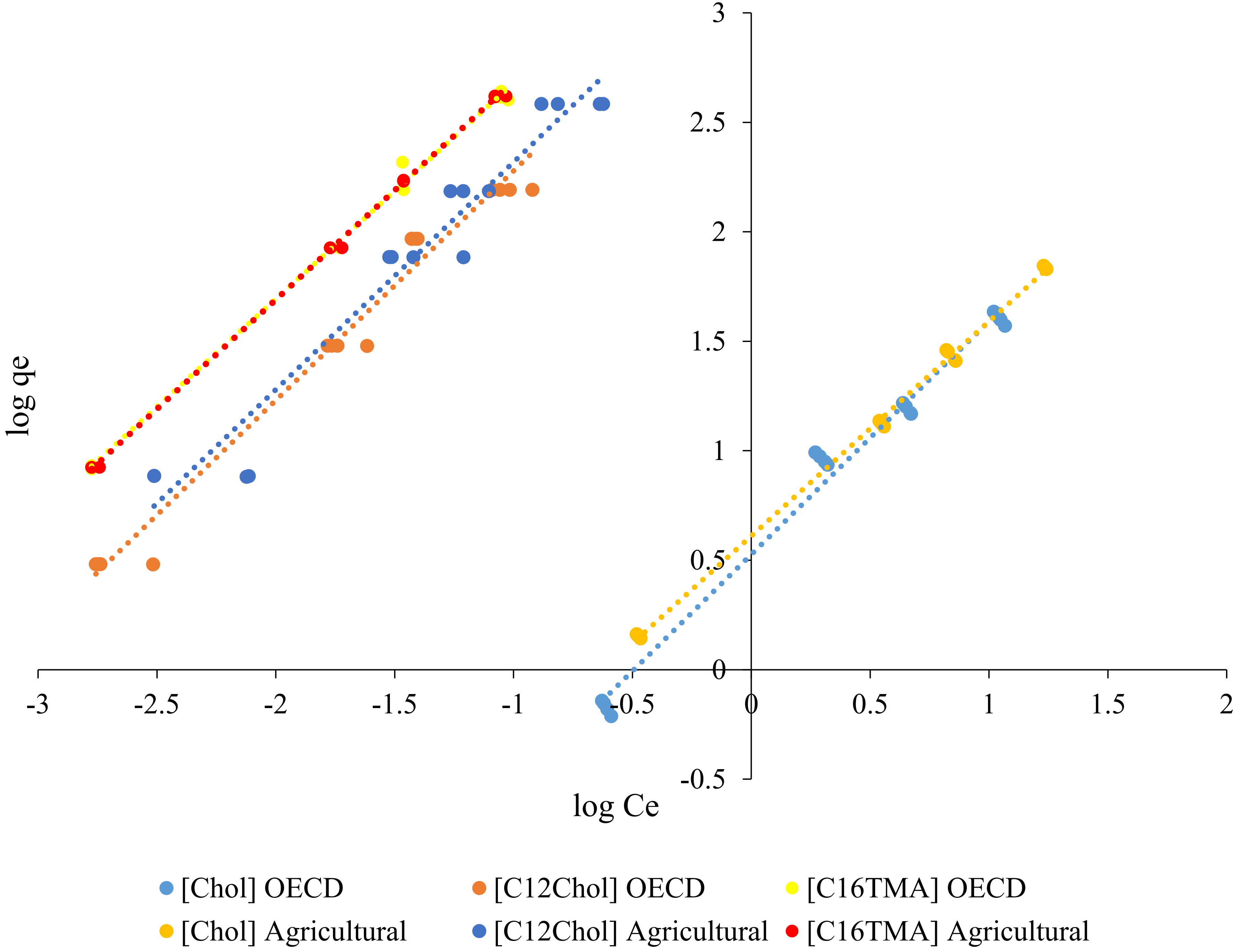
Figure. 1. Freundlich isotherms for [Chol], [C_{12}Chol], [C_{16}TMA] cations (a) and [Glyph] anions in the appropriate salts (b).

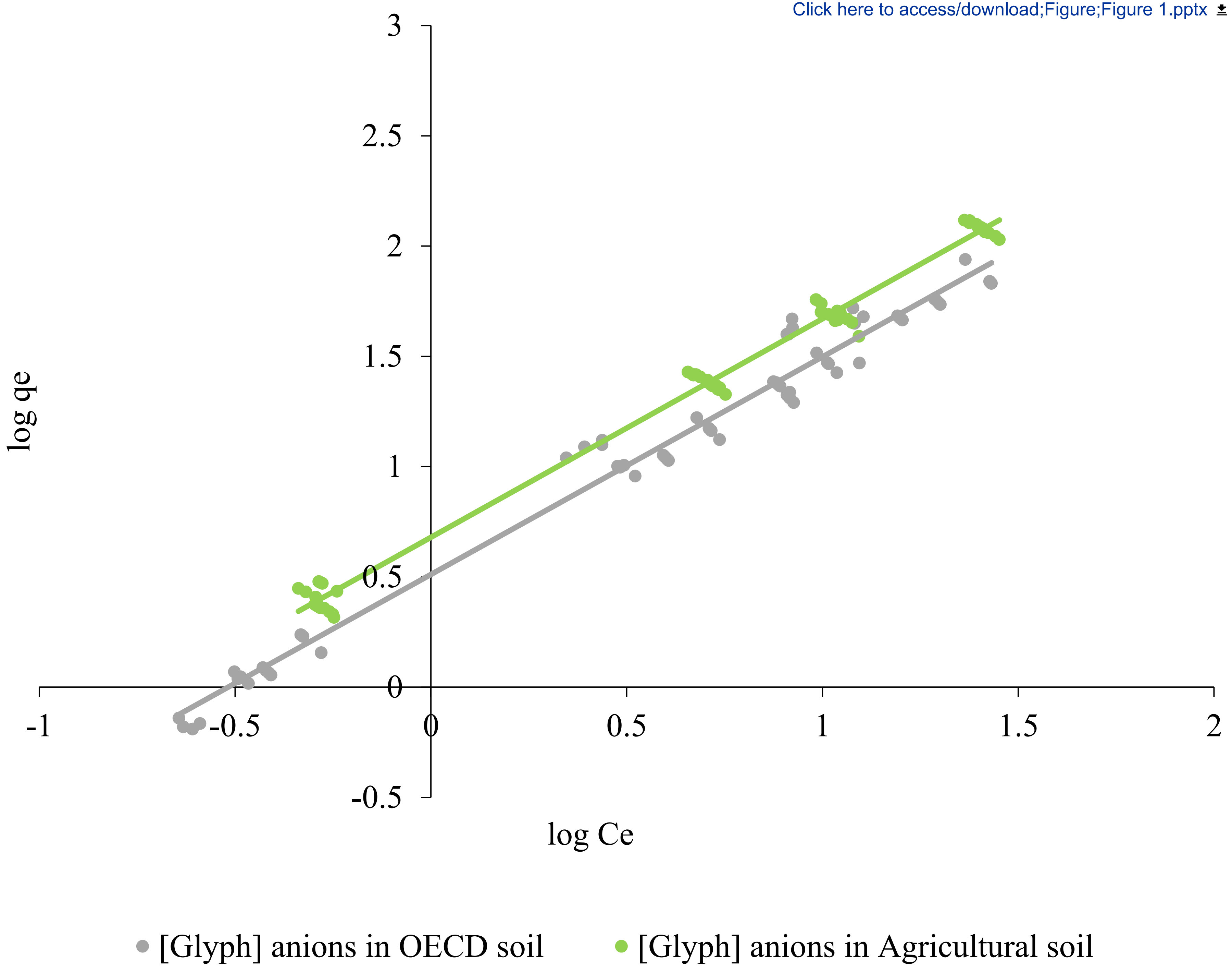
Figure. 2. Sorption of glyphosate [K][Glyph] versus sorption of glyphosate incorporated into appropriate ionic liquids ($[Chol]_2[Glyph]_{1.8}$, $[C_{12}Chol]_2[Glyph]_{1.3}$, $[C_{16}TMA]_2[Glyph]_{0.8}$) for both OECD and agricultural soils.

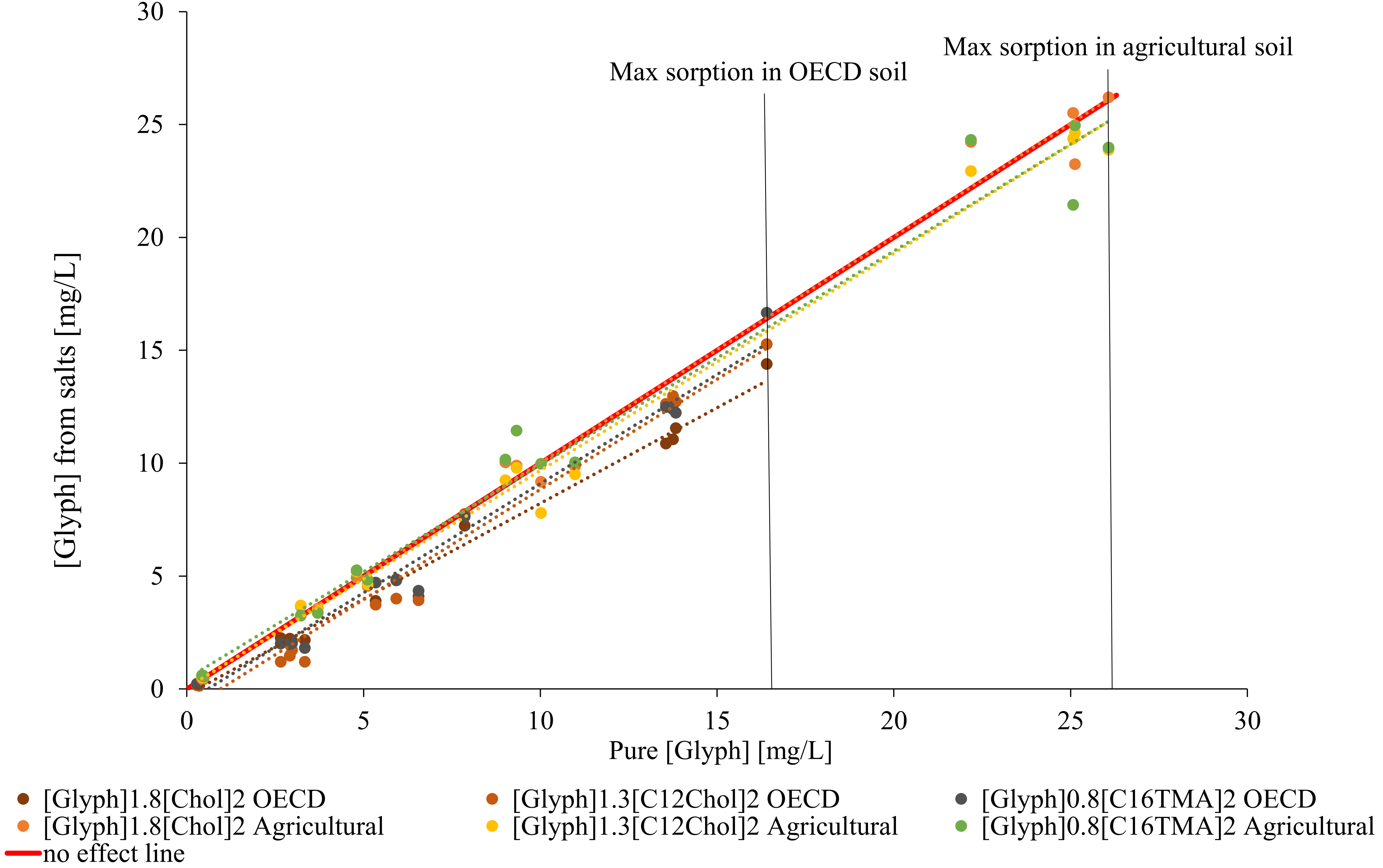
Figure 3. Adsorption percentage in OECD and agricultural soils for [Chol], $[C_{12}Chol]$, $[C_{16}TMA]$ cations (a) and [Glyph] anions in the appropriate salts (b). The [Glyph] anion is listed first, and the cation is listed second to make the figure easier to read. The graphs shown are for experiments with a concentration of 50 mg/L of active ingredient (glyphosate) and an experimental time of 24 hours.

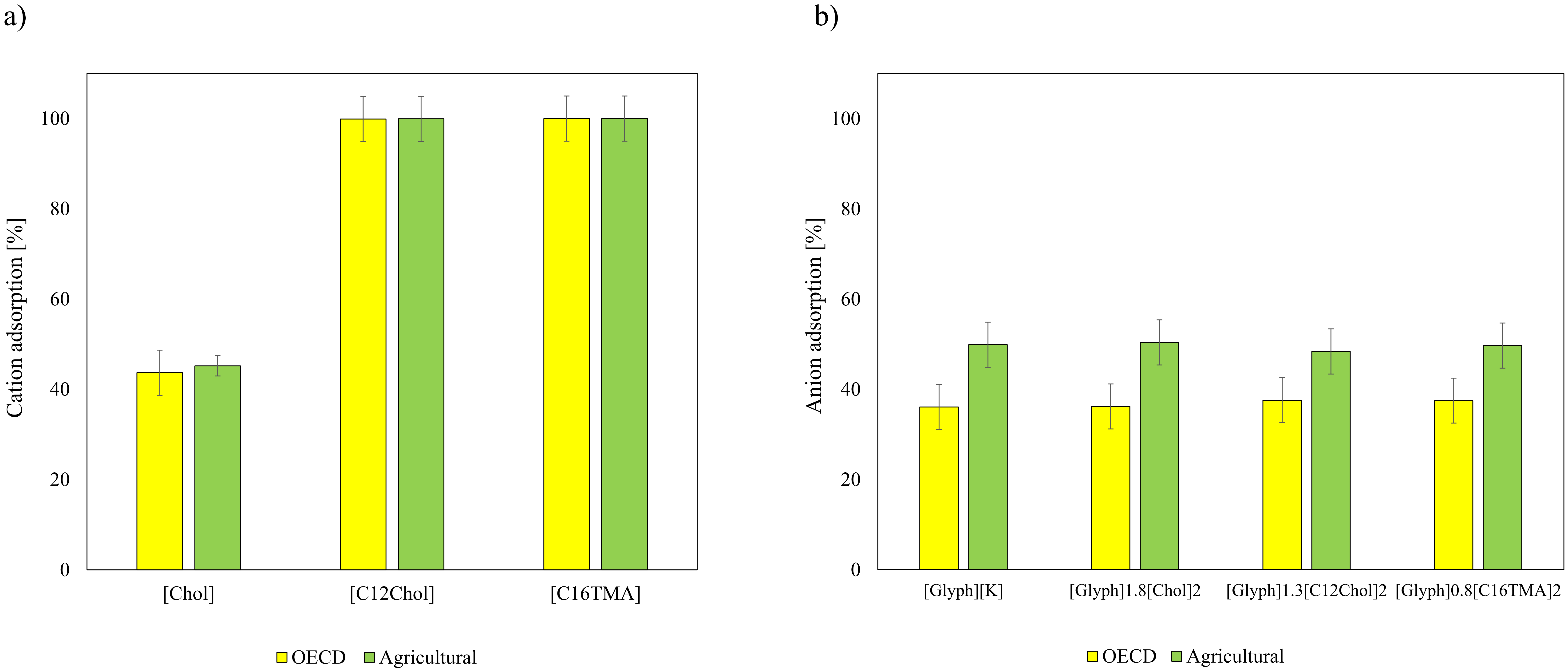
Figure 4. Relationship between toxicity of cations present in chloride salts [Chol][Cl] (□), $[C_{12}Chol][Cl]$ (○), $[C_{16}TMA][Cl]$ (Δ) and toxicity of HILs containing the same cations paired with glyphosate anion $[Chol]_2[Glyph]_{1.8}$ (■), $[C_{12}Chol]_2[Glyph]_{1.3}$ (●), $[C_{16}TMA]_2[Glyph]_{0.8}$ (▲) during growth of *Pseudomonas putida* KT2440.

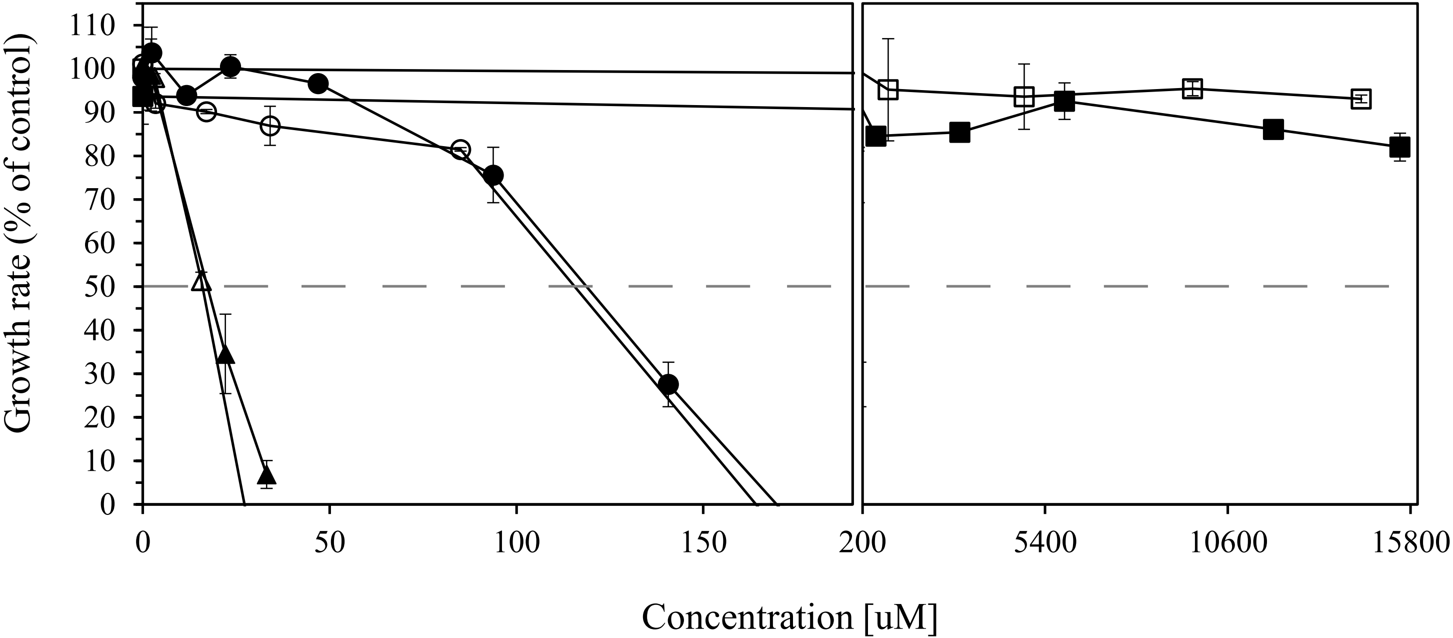
Figure


log qe
log Ce
3
2.5
2
1.5
1
0.5
0
-0.5
-3
-2.5
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2
● [Chol] OECD
● [C12Chol] OECD
● [C16TMA] OECD
● [Chol] Agricultural
● [C12Chol] Agricultural
● [C16TMA] Agricultural


log qe
log Ce
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-0.5
0
0.5
1
1.5
2
● [Glyph] anions in OECD soil
● [Glyph] anions in Agricultural soil


30
25
20
15
10
5
0
[Glyph] from salts [mg/L]
0
5
10
15
20
25
30
Pure [Glyph] [mg/L]
Max sorption in OECD soil
Max sorption in agricultural soil
● [Glyph]1.8[Chol]2 OECD
● [Glyph]1.8[Chol]2 Agricultural
no effect line
● [Glyph]1.3[C12Chol]2 OECD
● [Glyph]1.3[C12Chol]2 Agricultural
● [Glyph]0.8[C16TMA]2 OECD
● [Glyph]0.8[C16TMA]2 Agricultural


a)
Cation adsorption [%]
0
20
40
60
80
100
[Chol]
[C12Chol]
[C16TMA]
🟨 OECD
🟩 Agricultural
b)
Anion adsorption [%]
0
20
40
60
80
100
[Glyph][K]
[Glyph]1.8[Chol]2
[Glyph]1.3[C12Chol]2
[Glyph]0.8[C16TMA]2
🟨 OECD
🟩 Agricultural


Growth rate (% of control)
110
100
90
80
70
60
50
40
30
20
10
0
0
50
100
150
200
5400
10600
15800
Concentration [uM]

Author Contributions Statement

Anna Parus: Conceptualization, Methodology, Investigation, Resources, Writing - Review & Editing;

Oskar Zdebelak: Investigation;

Tomasz Ciesielski: Writing - Original Draft, Visualization;

Radosła Szumski: Formal analysis

Marta Woźniak-Karczewska: Investigation, Resources, Review & Editing;

Grzegorz Framski: Investigation;

Daniel Baranowski: Investigation, Data Curation;

Michał Niemczak: Investigation;

Joanna Zembrzuska: Investigation, Data Curation;

Tomáš Cajthaml: Review & Editing

Hermann J. Heipieper: Review & Editing

Łukasz Chrzanowski: Supervision, Conceptualization, Writing - Original Draft, Funding acquisition, Project administration