

Robert BICZAK<sup>1\*</sup>, Piotr BAŁCZEWSKI<sup>1,2</sup>, Barbara PAWŁOWSKA<sup>1</sup>  
 Barbara BACHOWSKA<sup>1</sup> and Piotr RYCHTER<sup>1</sup>

## COMPARISON OF PHYTOTOXICITY OF SELECTED PHOSPHONIUM IONIC LIQUID

### PORÓWNANIE FITOTOKSYCZNOŚCI WYBRANYCH FOSFONIOWYCH CIECZY JONOWYCH

**Abstract:** Ionic liquids have attracted considerable interest in various areas as new, non-volatile and non-flammable organic solvents, catalysts, reaction additives, ligands, drugs and other dedicated materials etc. Their general use, sometimes in bulky quantities, requires determination of their potential ecotoxicity on selected organisms. In the present work, influence of triphenylmethylphosphonium iodide (1) and triphenylhexadecylphosphonium iodide (2), introduced to soil, on germination and early stages of growth and development of superior plants was investigated using the plant growth test based on the OECD/OCDE 208/2006. In this test, the seeds of selected species, i.e. land superior plants - spring barley (*Hordeum vulgare*) and common radish (*Raphanus sativus* L. subvar. *radicula* Pers.) were planted in pots containing soil to which a test chemical compound had been added and in pots with control soil. To evaluate the phytotoxicity of ionic liquids 1 and 2 germination and weight (dry and fresh) of control plant seedlings were determined and compared with the germination and weight (dry and fresh) of the seedlings of plants grown in the soil watered with appropriate amounts of the test chemicals. The visual assessment of any types of damage to the test species, such as growth inhibition, chlorosis and necrosis, was also carried out and documented by digital photographs. Based on the obtained results, magnitudes of the LOEC - the lowest concentration causing observable effects in the form of reduction in growth and germination compared with the control and the NOEC - the highest concentration not causing observable, toxic effects - were also determined.

**Keywords:** ionic liquids, phytotoxicity, land superior plants, spring barley, common radish, yield, dry weight, chlorosis, necrosis

## Introduction

Ionic liquid are chemical compounds that are built solely of ions, an organic cation and most often inorganic anion, with one of both ions having a large volume. Substances of this type may contain an imidazolium, pyridinium, pyrrolidinium, quinolinium, ammonium or

<sup>1</sup> Institute of Chemistry, Environment Protection and Biotechnology, Jan Długosz University, al. Armii Krajowej 13/15, 42-200 Częstochowa, Poland, phone +48 34 361 49 18, fax +48 34 366 53 22, email: r.biczak@ajd.czest.pl; b.pawlowska@ajd.czest.pl; b.bachowska@ajd.czest.pl; p.rychter@ajd.czest.pl

<sup>2</sup> Center of Molecular and Macromolecular Studies, Polish Academy of Sciences, ul. Sienkiewicza 112, 90-363 Łódź, Poland, email: pbalczew@cbmm.lodz.pl

\*Corresponding author: r.biczak@ajd.czest.pl

phosphonium cation, where the positive charge is situated on the nitrogen or phosphorus atom, while the anion can include halides ( $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$ ), nitrates, tetrafluoroborates and hexafluoroborates. The possibilities of anion and cation combinations are virtually unlimited, reaching a value of  $10^{18}$ . The cation of ionic liquids exhibits a low degree of symmetry, which results in a reduction of the lattice energy of such a system, thus lowering the melting point of that compound. Almost all ionic liquids melt at a temperature below  $150^\circ\text{C}$  [1-5].

Although intensive scientific research on ionic liquids have been under way for only a dozen or so years, the history of these compounds dates back to the end of the 19<sup>th</sup> century, when a substance called “red oil” was obtained. The structure of this compound was understood relatively not long ago and found to be characteristic of ionic liquids. In 1911, ethylammonium, dimethylammonium and triethylammonium nitrates(III) were synthesized. In 1914, Walden obtained the first useful salt of this type - ethylammonium nitrate. The name *ionic liquid*, on the other hand, first appeared in specialist literature in 1974. A greater interest was aroused by ionic liquids only in the 90s with the obtaining of salts stable in the presence of air and moisture. First ionic liquids went on sale in 1999, and a dramatic increase in research activity related to the synthesis and practical applicability of these compounds has been seen from that time [2, 6-8].

The attractiveness of ionic liquids as a reaction medium is chiefly due to their numerous properties that are desirable in chemical synthesis, such as very low vapour pressure, incombustibility, thermal (even up to  $350^\circ\text{C}$ ) and electrochemical stability, and good catalytic properties. Ionic liquids are immiscible with many organic substances, and a majority of them exhibits excellent solubility in water; in addition, they are perfect solvents for many substances. All the aforementioned features of ionic liquids make these compounds to be ranked among very attractive solvents that can be used in many processes, both single-phase and multiphase ones. Yet the most important feature of ionic liquids is that they provide the possibility of obtaining substances of optimal properties for a given process through the modification of the cation structure and selection of the appropriate anion; therefore, the term *designer solvents* has been invented for these salts. The unique properties of ionic liquids may bring about a wide range of applications of these substances in all areas of the chemical industry, including catalysis, extraction, synthesis, as well as the pharmaceutical and food industries and electrochemistry and biotechnology [2, 9-16].

The all of the above characteristics and the relatively low production cost may soon lead to the situation, where huge amounts of these chemicals will be brought in to industry, agriculture and commercial trading. Using ionic liquids on a large scale, especially in industry, may pose a potential hazard to the natural environment, where they can get in the form of production wastes, waste water discharges, dump effluents, etc. Hence the need arises for determining the degree of influence of these salts on the natural environment and the potential for their penetration and accumulation in particular living organisms or entire trophic chains. With reference to this problem, along with scientific reports on the synthesis, physical and chemical properties and the potential for practical utilization of ionic liquids, a large number of studies have come up, which are concerned with the examination of the potential toxicity of these substances. Studies described in available literature reveal varied influences of ionic liquids on microorganisms [1, 2, 17-22], plankton [21, 23-26], invertebrates [27-29], vertebrates [27, 30], algae [2, 21, 23, 29, 31-33], fungi [19, 34, 35] or higher land plants [8, 21, 31, 36-39]. The quoted literature shows that a likely toxic

influence of a ionic liquid is determined by a number of factors, of which the primary role is played the cation type and the length of the substituent in the cation (as a rule, the longer alkyl chain, the higher toxicity). The anion type is not without significance, either; but the concentration of the substance used and the habitat conditions, including the presence of other xenobiotics, and the species features of the organism tested are also all-important [1, 4, 8, 9, 15, 17, 18, 21, 36, 37]. The studies of the potential ecotoxicity of ionic liquids has not only a learning and ecological aspect, which eliminates the risk of failure in the process of issuing permits for industrial use and trade in these substances, but are also important for economic reasons, as they prevent any financial expenditures on the rectification of possible damage in the natural environment [29].

The above-mentioned experimental studies belong to ecotoxicology in its broad sense. Contemporary ecotoxicology is an interdisciplinary, intensively growing branch of science encompassing chemistry, ecology and toxicology. The basic objective of this area of toxicology is to assess the state of the environment. Ecotoxicology is therefore concerned with the study of the toxic effects caused by either natural or artificial contaminants in living organisms (both plant and animal, land or aquatic, which make up the biosphere), and also with the interactions of these substances in the physical environment in which those organisms live. Within ecotoxicological studies, classic chemical analysis methods are used, which enable the concentration of chemical compounds to be determined in the samples analyzed. However, bioanalysis and environmental monitoring in ecotoxicological studies more and more often abandon classic chemical analysis, which justified, on the one hand, by very high expenditures on apparatus and operation and, on the other hand, by the fact of introducing additional chemicals to the natural environment, which are pollutants themselves. Moreover, chemical methods are not able to indicate synergic or antagonistic interactions within a population, or organisms. In connection with the above, bioindication methods start gaining increasingly high importance in the examination of the natural environment. Bioindication is a method that uses a living organism as an indicator, whose reaction provides a basis for assessing the degree of toxicity of the medium under examination. Test organisms called bioindicators should be particularly sensitive to chemical compounds harmful to the environment and humans. The basic features of bioindication include accuracy, low costs, the simplicity of use and the objectivity of obtained results. It should be noted, however, that bioindication methods can and should be regarded as a complement to physical and chemical methods; only in that case could the changes occurring in the environment due to a toxic substance showing up be captured in a comprehensive manner [40-46].

Ecotoxicological tests, proven and very fast, can be the base of biomonitoring and provide a basis for examining the history of a foreign substance in the environment. They are recommended for testing the toxicity of water, waste water, sewage sludges, bottoms and soil, and their great popularity resulted in the approval by standardization organizations, such as ISO and OECD. Biotests that are most commonly used in contemporary ecotoxicological studies include Microtox® [1-3, 10, 21, 26, 47-49], Algaltoxkit F and Marine Algaltoxkit [27, 32, 44, 50], Phytotoxkit [38, 50-52], Daphtoxkit F magna and Daphtoxkit F pulex [1, 25], and Ostracodtoxkit F [42, 52-54].

When planning ecotoxicological tests it should be borne in mind that the ultimate "store" of all substance, both nutritive and toxic ones, is the soil. Due to biological transformations, and more often as a result of human activity, soil becomes loaded with

on the germination and growth of superior plants were carried out in a plant house using spring barley (*Hordeum vulgare*) - a monocotyledonous plant - and common radish (*Raphanus sativus* L. subvar. *radicula* Pers.) - a dicotyledonous plant. For this purpose, in

four replicates, pots made of nonporous plastic of a diameter of 90 mm were filled, respectively, with the 250 g control soil and with the soil with the test ionic liquid added at a specific concentration. The grain size analysis of the soil used in the experiment showed that this was light argillaceous sand (las) of floatable particle contents of 10%, an organic carbon content of approx. 9 g/kg of soil dry mass and pH(KCl) equal to 5.8. The salinity of the soil was about 70 mg KCl per 1 dm<sup>3</sup> of soil. 20 identical seeds of each of the selected plant species, originating from the same source, were planted to so prepared pots (Fig. 1). A constant humidity (70% of water hold capacity of soil), light intensity and temperature ( $20 \pm 2^\circ\text{C}$ ) were maintained throughout the tests (14 days).



Fig.1. Digital photographs of pots with planted seeds

The performed phytotoxicity test comprised two testing cycles: a preliminary test and a final test. The preliminary test was carried out to determine the range of concentrations of compounds affecting the soil quality, therefore, in accordance with the PN-ISO 11269-2:2001 standard [63] and OECD/OCDE 208/2006 Guide [64]. The test 1 and 2 ionic liquids were introduced to the soil at the following concentrations: 0 mg (control), and 1 mg, 10 mg, 100 mg and 1000 mg/kg of soil dry weight. In the final test, concentrations were arranged in a geometric progression with a factor of 2, starting from the lowest concentration that reduces the germination and growth of plants. The ionic liquids were introduced to the soil in the form of water solutions.

To evaluate the phytotoxicity of the applied concentrations of the triphenylmethylphosphonium iodide and triphenylhexadecylphosphonium iodide compounds, the germination and (dry and fresh) weight of control plant sprouts were determined and compared with the germination and (dry and fresh) weight of the sprouts of plants grown in the soil with appropriate amounts of the test chemicals added. The visual assessment of any types of damage to the test species, such as growth inhibition, chlorosis and necrosis, was also carried out, which is depicted by digital photographs of the test plants taken. Based on the obtained results, the magnitudes of the LOEC (*the lowest observed effect concentration*) - the lowest concentration causing observable effects in the form of a reduction in growth and germination compared with the control - and the NOEC

(*no observed effect concentration*) - the highest concentration not causing observable, toxic effects - were also determined.

The evaluation of significance of the obtained results was performed using the analysis of variance (Fisher-Snedecor's F-test), while the values of  $NIR_{0.05}$  were calculated using Tukey's test.

## Results and discussion

The results related to the effect of triphenylmethylphosphonium iodide and triphenylhexadecylphosphonium iodide on the emergence and growth of spring barley at his early development stages are presented in Tables 1 and 2 and in Figure 2.

Table 1

Changes in basic parameters of the phytotoxicity test for spring barley following the introduction of triphenylmethylphosphonium iodide compound (in [mg/kg of soil dry mass]) to the soil

Sample	Number of plants	Crop fresh weight [g/pot]	Weight of single plant [g]	Dry weight [mg/g f.m.]
Preliminary test				
0	19	2.911	0.151	0.1120
1	20	2.827	0.144	0.1134
10	20	3.044	0.152	0.1184
100	20	1.451	0.073	0.1409
1000	13	0.313	0.024	-
Final test				
20	20	2.740	0.137	0.1112
40	20	2.076	0.105	0.1144
80	20	1.585	0.079	0.1268
	$LSD_{0.05} - 1$	$LSD_{0.05} - 0.142$	$LSD_{0.05} - 0.007$	$LSD_{0.05} - 0.0052$

Table 2

Changes in basic parameters of the phytotoxicity test for spring barley following the introduction of triphenylhexadecylphosphonium iodide compound (in [mg/kg of soil dry mass]) to the soil

Sample	Number of plants	Crop fresh weight [g/pot]	Weight of single plant [g]	Dry weight [mg/g f.m.]
Preliminary test				
0	20	3.135	0.156	0.0966
1	20	3.029	0.154	0.0999
10	20	3.111	0.155	0.1005
100	19	1.937	0.100	0.0996
1000	20	1.169	0.058	0.1083
Final test				
20	20	3.040	0.152	0.0989
40	20	2.789	0.142	0.0957
80	20	2.423	0.123	0.0959
	$LSD_{0.05} - 0$	$LSD_{0.05} - 0.082$	$LSD_{0.05} - 0.004$	$LSD_{0.05} - 0.0033$

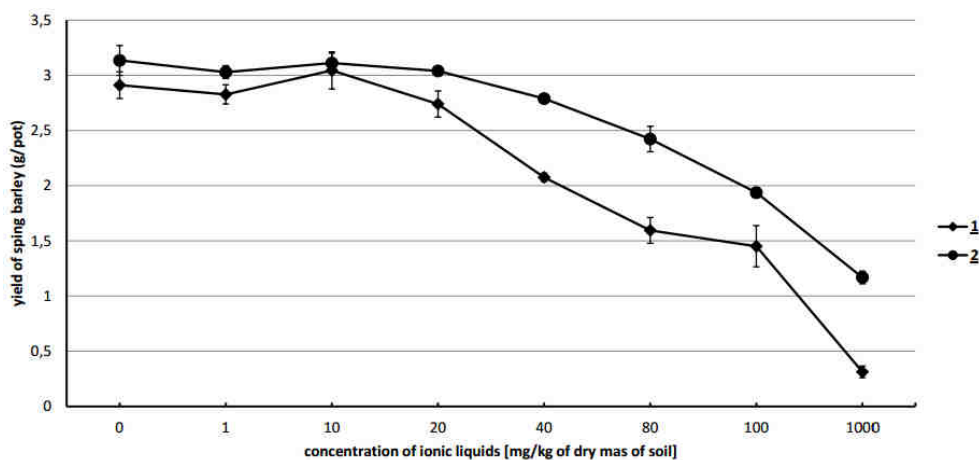


Fig. 2. Comparison of the effects of compounds 1 and 2 on the yield of fresh weight of seedlings of spring barley

The results obtained from the preliminary tests allow one to conclude that the iodides used in the experiment are substances exhibiting relatively high toxicity with respect to spring barley, with triphenylmethylphosphonium iodide turning out to be the most toxic. Only the lowest ionic liquid concentrations, being equal to 1 mg and 10 mg/kg dry soil mass, did not have a significant effect on sprouting and growth of spring barley seedlings. This trend applied to both compounds used in the experiment. This conclusion is based on the fact that, as recognized by standard PN-EN 13432 [65], a substance does not exhibit toxicity if the germination rate of seeds and the overall fresh mass of plants growing on the ground with that substance do not differ by more than  $\pm 10\%$  from those of the control sample. In the case of the visual assessment of the barley plants, no visible differences in the plants' appearance were observed for these concentrations either, for both the control and the test plants; at the same time, no growth inhibition and chlorotic and necrotic changes were found (Figs. 3 and 4). The toxicity of both triphenylmethylphosphonium iodide and triphenylhexadecylphosphonium iodide became apparent after introducing 100 mg of the substance to 1 kg of the soil. The decrease in green barley sprout mass observed in that case was approx. 50% for iodide 1 and 38% for iodide 2. Changes in the appearance of the barley seedlings were also observed; the plants were smaller than the control plants, with visible chlorotic changes; moreover, an increase in dry mass content was found. Upon applying the highest triphenylhexadecylphosphonium iodide concentration - 1000 mg/kg soil, the decrease in fresh barley seedling mass was 63% (Table 2), whereas, after introducing triphenylmethylphosphonium iodide in this concentration, the reduction of the barley seedling crop was as high as 89% (Table 1).

Similar trends can be found by analyzing the obtained results for the effect of triphenylmethylphosphonium iodide and triphenylhexadecylphosphonium iodide on the sprouting and growth of common radish in its early development stages (Tables 3 and 4; Fig. 5).

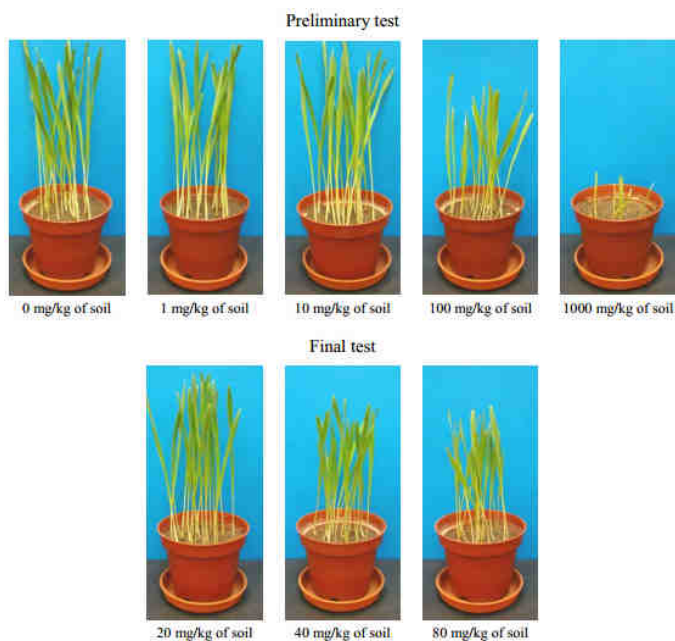


Fig. 3. Digital photographs of spring barley on the 14<sup>th</sup> day after introduction to the soil triphenylmethylphosphonium iodide (in [mg/kg of soil dry weight])

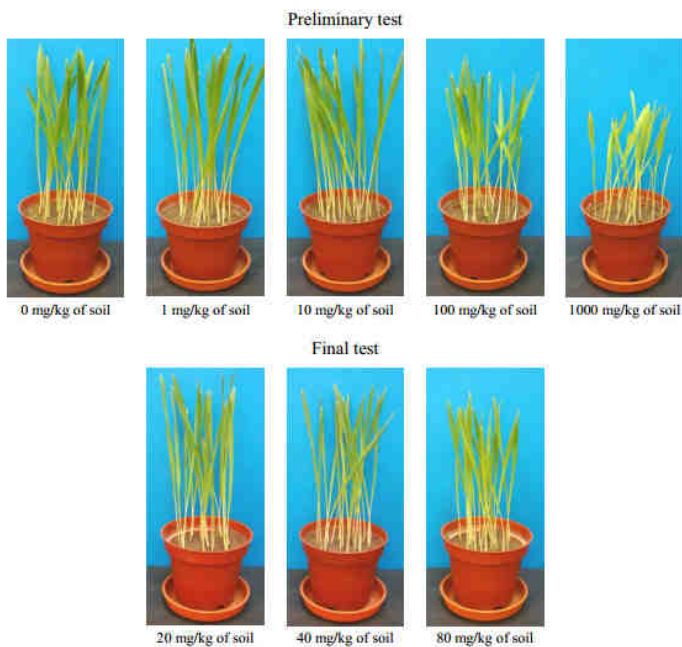


Fig. 4. Digital photographs of spring barley on the 14<sup>th</sup> day after introduction to the soil triphenylhexadecylphosphonium iodide (in [mg/kg of soil dry weight])



Table 3

Changes in basic parameters of the phytotoxicity test for common radish following the introduction of triphenylmethylphosphonium iodide compound (in [mg/kg of soil dry mass]) to the soil

Sample	Number of plants	Crop fresh weight [g/pot]	Weight of single plant [g]	Dry weight [mg/g f.m.]
Preliminary test				
0	20	4.324	0.216	0.1295
1	19	3.999	0.207	0.1335
10	20	4.189	0.210	0.1372
100	20	1.537	0.078	0.1459
1000	17	-	-	-
Final test				
20	20	3.892	0.198	0.1240
40	20	3.407	0.174	0.1099
80	19	1.872	0.098	0.1280
	LSD <sub>0.05</sub> - 1	LSD <sub>0.05</sub> - 0.282	LSD <sub>0.05</sub> - 0.015	LSD <sub>0.05</sub> - 0.0095

Table 4

Changes in basic parameters of the phytotoxicity test for common radish following the introduction of triphenylhexadecylphosphonium iodide compound (in [mg/kg of soil dry mass]) to the soil

Sample	Number of plants	Crop fresh weight [g/pot]	Weight of single plant [g]	Dry weight [mg/g f.m.]
Preliminary test				
0	19	3.432	0.178	0.1039
1	19	3.425	0.177	0.1036
10	19	3.319	0.178	0.0969
100	19	2.603	0.140	0.1075
1000	18	-	-	-
Final test				
20	19	3.517	0.188	0.1032
40	20	3.541	0.177	0.1002
80	19	3.080	0.160	0.0962
	LSD <sub>0.05</sub> - 1	LSD <sub>0.05</sub> - 0.304	LSD <sub>0.05</sub> - 0.013	LSD <sub>0.05</sub> - 0.0061

The iodides used in the experiment are substances exhibiting relatively high toxicity also towards common radish, with triphenylmethylphosphonium iodide turning out to be the most toxic. Only the concentrations of both substances used in the experiment, being equal to 1 mg and 10 mg/kg dry mass, did not have a significant effect on the sprouting and growth of radish. No visible differences in the appearance of plants, both the control and the test ones, were observed either, and not growth inhibition and chlorotic and necrotic changes were found (Figs. 6 and 7). Upon introducing 100 mg of triphenylmethylphosphonium iodide and triphenylhexadecylphosphonium iodide, respectively, to 1 kg of soil, a decrease in green radish sprout mass was found, which amounted to 64% (Table 3) for iodide 1 and 22% (Table 4) for iodide 2, compared to the controls. In addition, distinct changes in the appearance of common radish plants revealed themselves; the plants were dwarf with distinct chlorotic and necrotic changes. After applying the highest concentration of both compounds, being equal to 1000 mg/kg, the common radish seeds only germinated (Figs. 6 and 7).

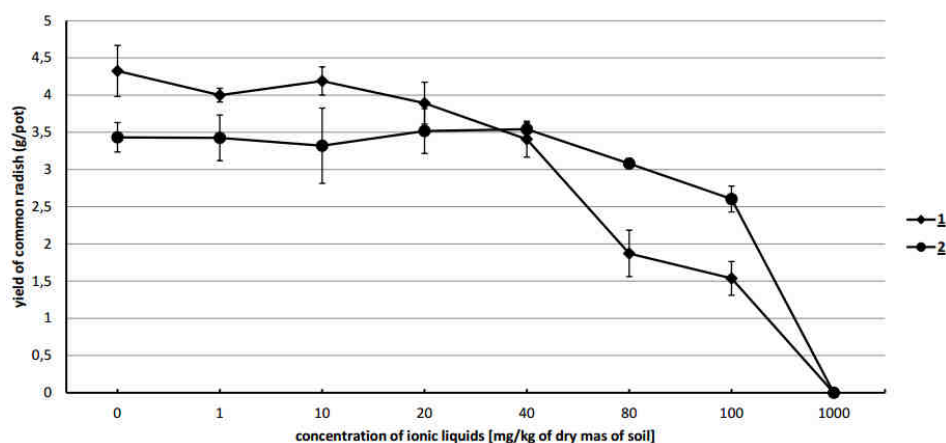


Fig. 5. Comparison of the effects of compounds 1 and 2 on the yield of fresh weight of seedlings of common radish

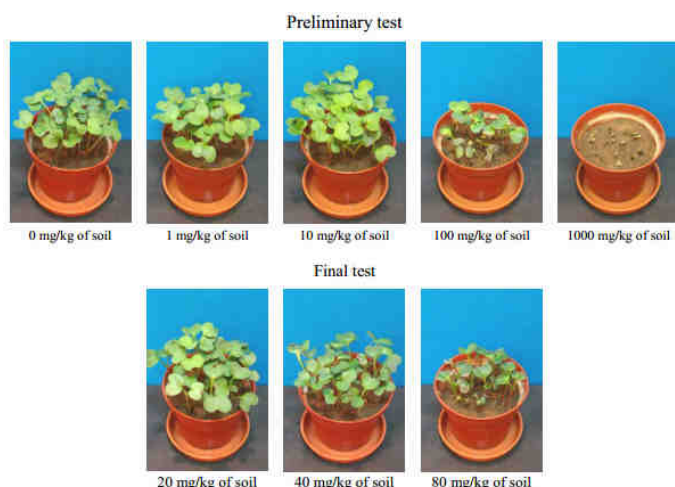


Fig. 6. Digital photographs of common radish on the 14<sup>th</sup> day after introduction to the soil triphenylmethylphosphonium iodide (in [mg/kg of soil dry weight])

The final tests carried out showed that increasing the triphenylmethylphosphonium iodide concentration in soil up to 20 mg of the substance per 1 kg of dry soil mass did not result in any significant changes in the spring barley growth and development parameters being determined; whereas, subsequent ionic liquid concentrations of 40 and 80 mg reduced the fresh plant mass crop by 29 and 46%, respectively. Barley, on the other hand, showed greater tolerance to triphenylhexadecylphosphonium iodide; in this case, only a concentration of 80 mg of the substance per 1 kg of soil caused a 23% reduction in the barley crop, compared to the controls (Tables 1 and 2). For those concentrations, distinct changes in the appearance of spring barley seedlings could be observed (Figs. 3 and 4).

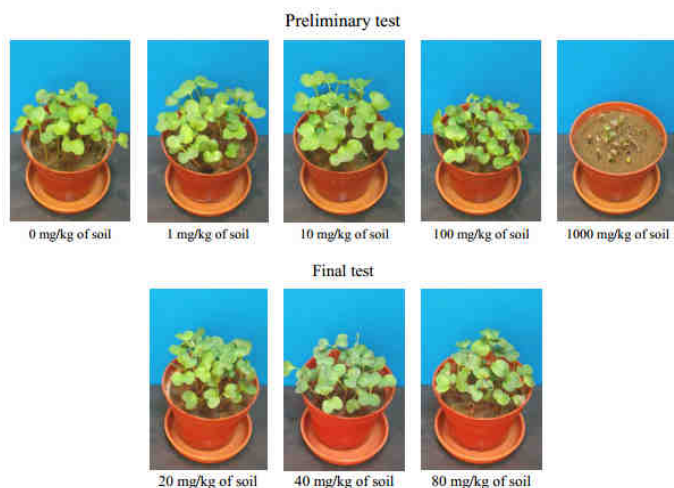


Fig. 7. Digital photographs of common radish on the 14<sup>th</sup> day after introduction to the soil triphenylhexadecylphosphonium iodide (in [mg/kg of soil dry weight])

For common radish, soil triphenylmethylphosphonium iodide concentrations of 40 and 80 mg/kg dry soil mass led to a consistent decrease of the fresh plant mass crop by 21 and 57%, respectively, compared to the controls. Similar changes were observed for the crop per plant. Distinct inhibition of the growth of young plants could also be noticed, whose leaves were covered with spots indicating progressing chlorosis and necrosis. Whereas, the triphenylhexadecylphosphonium iodide concentrations applied in the final tests turned out to be totally nontoxic to common radish (Tables 3 and 4; Figs. 6 and 7).

Triphenylmethylphosphonium iodide adversely influenced the germination capacity of radish seeds and barley grains only at its highest concentration (1000 mg/kg soil).

The results obtained from the experiment under discussion find their confirmation in available literature. A toxic effect of ionic liquids on the growth and development of higher land plants is reported by Bałczewski et al [8], Biczak et al [37] and Matzke et al [36]. The phytotoxicity of ionic liquids observed in the quoted studies was dependent chiefly on the applied concentration of the compound, but the factor determining the toxicity were the genetic features of the species and variety of plants used in the experiment. Matzke et al [31, 38] and Studzińska and Buszewki [39] suggest also that the toxicity of ionic liquids to plants depends also on the structure of the compound and various toxic environmental factors, as well as the organic and mineral colloid contents of the soil. Cybulski et al [15] have also found that the phytotoxicity of ionic liquids is dependent on the type of cation or anion, as well as on the length of the alkyl chain. Some authors report that the highest antimicrobial activity is exhibited by substances whose substituent contains from 10 to 16 carbon atoms [66, 67]. The authors' own studies have not confirmed this observation, because the iodide containing the hexadecyl substituent has turned out to be less toxic to plants than the iodide with the methyl group. Whereas, we found an increase in the toxicity of the ionic liquid group under discussion upon introducing a substituent containing 5 carbon atoms to the cation (the data prepared to be published). These results are in accordance with those obtained by Matzke et al [31]. The phytotoxicity evaluation of

1-ethyl-, 1-buthyl-, 1-oktyl-3-imidazolium ionic liquids on the growth and development of cress and wheat has revealed that the degree of ILs toxicity increased with lengthening of side chain but only up to 4 carbon atoms. They state, that this is due to high mobility of ionic liquids with short side chain when compare to ILs with alkyl chain consisted of more than few carbon atoms.

## Summary

The results obtained from the experiment discussed enable one to claim that both triphenylmethylphosphonium iodide and triphenylhexadecylphosphonium iodide can be regarded as a chemical substance exhibiting quite high toxicity towards higher land plants. The observed toxic action on this ionic liquids was dependent chiefly on the concentration used. The highest concentration of the 1, which did not cause any distinct decrease in the emergence and growth of the plants (NOEC) was 40 mg/kg dry soil mass for spring barley seedlings and radish plants, while the lowest concentration causing a reduction of plants emergence/crops (LOEC) could be established at a level of 80 mg of the substance per 1 kg of dry soil mass for spring barley and common radish, respectively. The same of parameters of toxicity for this study for 2 repectively: NOEC - 80 and LOEC - 100 mg per kg of dry soil mass, both the barley and radish common.

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## PORÓWNANIE FITOTOKSYCZNOŚCI WYBRANYCH FOSFONIOWYCH CIECZY JONOWYCH

<sup>1</sup> Instytut Chemii, Ochrony Środowiska i Biotechnologii, Akademia im. Jana Długosza, Częstochowa

<sup>2</sup> Centrum Badań Molekularnych i Makromolekularnych Polskiej Akademii Nauk, Łódź

**Abstrakt:** Ciecze jonowe wzbudziły duże zainteresowanie w różnych dziedzinach jako nowe nietłone i niepalne rozpuszczalniki organiczne, katalizatory, dodatki do reakcji, ligandy, leki itp. Jednocześnie powszechne stosowanie cieczy jonowych, czasem w dużych ilościach, wymaga określenia ich potencjalnej ekotoksyczności dla wybranych organizmów. W przedstawionej pracy wpływ jodku trifenylometylofosfoniowego (1) i jodku trifenyloteksaedecylofosfoniowego (2), wprowadzonych do gleby w różnych stężeniach, na wschody i wczesne stadia wzrostu i rozwoju roślin wyższych, określono w badaniach fitotoksyczności w oparciu o przewodnik OECD/OCDE 208/2006. W przeprowadzonym eksperymencie nasiona wybranych gatunków lądowych roślin wyższych - jęczmienia jarego (*Hordeum vulgare*) i rzodkiewki zwyczajnej (*Raphanus sativus* L. subvar. *radicula* Pers.) wysiano do wazonów zawierających glebę, do której dodano badany związek chemiczny, i do wazonów zawierających glebę kontrolną. Oceniając fitotoksyczność związków (1) i (2), określono i porównano wschody i masę (suchą i zieloną) pędów roślin kontrolnych ze wschodami i masą (suchą i zieloną) pędów roślin rosnących na glebie, do której wprowadzono odpowiednie ilości związków. Dokonano ponadto oceny wizualnej wszystkich uszkodzeń badanych gatunków roślin, takich jak zahamowanie wzrostu, nekroza i chloroza, czego odzwierciedleniem są wykonane zdjęcia cyfrowe roślin doświadczalnych. Na podstawie otrzymanych wyników określono wielkości LOEC (najniższe stężenie wywołujące zauważalne skutki w postaci obniżki we wschodzie i wschodach w porównaniu z kontrolą) oraz NOEC (najwyższe stężenie niewywołujące zauważalnych, toksycznych skutków).

**Słowa kluczowe:** ciecze jonowe, fitotoksyczność, lądowe rośliny wyższe, jęczmień, rzodkiewka, plon, sucha masa, chloroza, nekroza