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The effect of imidazolium based ionic liquids on wheat and barley germination and growth: Influence of length and oxygen functionalization of alkyl side chain

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ABSTRACT

In this work five different imidazolium based ionic liquids, namely: 1-(2-oxybutyl)-3-methylimidazolium chloride, $[C_2OC_2mIm][Cl]$; 1-(2-oxypropyl)-3-methylimidazolium chloride, $[C_1OC_2mIm][Cl]$; 1-(3-hydroxypropyl)-3-ethylimidazolium chloride, $[OHC_3eIm][Cl]$; 1-(3-hydroxypropyl)-3-methylimidazolium chloride, $[OHC_3mIm][Cl]$; 1-(2-hydroxyethyl)-3-methylimidazolium chloride, $[OHC_2mIm][Cl]$, together with commercial 1-butyl-3-methylimidazolium chloride, [bmim][Cl] and synthesized protic imidazolium chloride, [Im][Cl], were prepared and their toxicity examined towards wheat and barley germination and growth. Introduction of the polar groups (in the form of hydroxyde and/or ether group) in the alkyl side chain of the imidazolium cation and their influence on the reduction of the ionic liquid's toxicity is demonstrated. The results indicate that toxicity of oxygen functionalized ILs is significantly lower against wheat comparing to non-functionalized analogues. In the case of barley, influence on germination follow the same trend as in the case of wheat, but for seedlings growth different trend is observed with more pronounced toxicity of ether functionalized ILs. From these results it was also shown that alkylation in the position N-3 atom of the imidazole significantly reduces toxicity of cation.

1. Introduction

An intensive research in the field of green chemistry is devoted to design environmentally friendly solvents. One of the most popular strategies is synthesis of new classes of ionic liquids (ILs) due to their extraordinary (but not universal) physicochemical properties. Scientific community consider ILs as an innovative solvents of the 21st century with a broad range of potential applications (Earle and Seddon, 2000; Plechkova and Seddon, 2008). However, ILs comprise a very heterogeneous group of liquids that cannot be considered a priori benign, especially because of huge gap of fundamental data and knowledge (Biczak at al, 2015; Matzke et al., 2007; Zhao et al., 2007). Also, due to their high stability which is desirable in many industrial processes, ILs can be turned into persistent pollutants (Docherty and Kulpa, 2005; Gathergood et al., 2004, 2006). Therefore, prior to their industrial scale applications, it is crucial to evaluate their potential environmental hazards by determining their persistence in water and soil, their biodegradation, migration in groundwater, bioaccumulation in aquatic or terrestrial organisms and their overall ecotoxicity (Ranke et al., 2004; Bernot et al., 2005; Cornmell et al., 2008; Radošević et al., 2013; Stolte et al., 2007; Kumar et al., 2011; Liu et al., 2015; Zhang et al., 2017). Numerous studies have implied that ILs could become persistent and toxic contaminants in the environment due to their low biodegradability towards model organisms, which puts the greenness of many ILs in question (Gathergood et al., 2004, 2006; Petkovic et al., 2011). Toxicity studies of ILs have been focused on the ILs toxicity to aquatic ecosystems, various bacteria, yeast, algae, multicellular organisms and different cell lines (Thuy Pham et al., 2010; Amde et al., 2015; Costa et al., 2015), while knowledge regarding their toxicity toward terrestrial ecosystems, especially plants, are rather scarce (Matzke et al., 2008).

So far, the impact of two different structural elements of ILs (alkyl substituted imidazolium cation and anion) on terrestrial plants was investigated, indicating numerous toxic effects of both structural elements with more obvious cationic toxicity (Biczak et al., 2015; Peric et al., 2011; Matzke et al., 2008, 2009; Liu et al., 2013). In the work of Cho et al. (2016) it was suggested that a cation needs more descriptors to predict its toxicity comparing to an anion, concluding that origin of cation toxicity is complex and complicate to be predicted. Hence, detailed and comprehensive investigation of different ionic liquids must be conducted in order to obtain reliable information about features that defined their potential toxicity. Some authors found that

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imidazolium based ILs are toxic in the early stage of barley growth and wheat seedlings, and that the most toxic ionic liquid was one that contains the longest alkyl substituent on the imidazolium ring (Cvjetko Bubalo et al., 2014; Chen et al., 2016). These studies confirmed the tendency that the longer alkyl chain of the ionic liquid, the higher toxicity it has. In the work of Stolte et al. (2007) it was described that toxicity of imidazolium based ionic liquids may be reduced by introduction of polar groups in the cation alkyl substituent. In our previous work (Vraneš et al., 2016a, 2016b), it was shown that introduction of hydroxyl and/or ether group in the alkyl side chain of imidazolium cation significantly reduced toxicity of ionic liquids to *Artemia salina* and MRC-5 cell lines.

From the above said, it is clear that further investigation about potential toxic effects of ILs on the living organisms is extremely important for the risk assessment as well as for their living environment. Thus, in this work we investigated the effect of the alkyl chain length and different functionalization of the imidazolium cation on the early development of barley and wheat as two extremely important feed and food crops for human and animal nutrition.

2. Experimental section

2.1. Synthesis

All chemicals for ILs synthesis were used without purification and their provenance and purity are given in Table S1 in Supporting information of Manuscript.

Five different chloride based ionic liquids and imidazolium chloride are synthesized, while 1-butyl-3-methylimidazolium chlorid was purchased. The structures of investigated ILs are presented in Fig. 1. Synthesis of 1-(2-hydroxyethyl)-3-methylimidazolium chloride ionic liquid, 1-(3-hydroxypropyl)-3-methylimidazolium chloride, 1-(2-oxybutyl)-3-methylimidazolium chloride and imidazolium chloride are described in our previous papers (Vraneš et al., 2016a, 2016b).

In order to prepare 1-(2-oxypropyl)-3-methylimidazolium chloride and 1-(3-hydroxypropyl)-3-ethylimidazolium chloride, 3-chloro-1-propanol in 10% excess (or 2-chloroethyl methyl ether in 10% excess) and 1-methylimidazole (or 1-ethylimidazole) were mixed in a round-bottom flask together with ethyl acetate as a solvent. The mixture was kept under the reflux for 48 h at 70 °C with stirring. The top phase, containing unreacted starting material was removed. The bottom phase was washed four times with new portions of ethyl acetate. The products were obtained in the liquid state, and additionally stored under vacuum with P_2O_5 for the next 72 h. Water content was determined by the Karl-Fischer titration (Stark et al., 2008). It was found that water content was less than 200 ppm in all synthesized ionic liquids.

For additional characterization the IR and NMR spectra (Figs. S1–S12 in Supporting information) of the synthesized ionic liquids were performed. NMR spectra were recorded in D_2O at 25 °C on a Bruker Advance III 400 MHz spectrometer. Tetramethylsilane was used as accepted internal standard for calibrating chemical shift for 1H and 13C. 1H homodecoupling and 2D COSY method were used routinely for the assignation of the obtained NMR spectra. 13C spectra were assigned by selective decoupling technique. Infrared spectra were recorded as neat samples from (4000-650) cm^{-1} on a Thermo-Nicolet Nexus 670 spectrometer fitted with a Universal ATR Sampling Accessory.

2.2. Experiments with wheat and barley culture

Seeds of wheat (cultivar NS 40S) and barley (cultivar NONIUS) were sown in glass Petri-dishes (R = 90 mm) on a filter paper, to which was added 7.5 ml of either deionized water (control) or 10, 100 or 1000 mg L^{-1} of selected ionic liquids. In each Petri dish 20 seeds were sown, in two replications. The experiment was repeated three times. Germination was done at 26 °C, in the dark. Number of germinated seeds was counted after 24, 48 and 72 h, since we observed the differences between treatments already after 24 h and no differences were recorded after 72 h (germination was observed until 120 h from sowing). Eight seedlings from each Petri dish were transferred to pots V = 750 ml, filled with ½ strength Hoagland nutrient solution (Hoagland and Arnon, 1959). Plants were grown in a growth chamber (RK-340 CH) under controlled conditions: 12 h day/night period, 23 °C/19 °C temperature regime, 45% humidity, 80% ventilation, light provided by FLUORA 18W/77 lamps. After eight days, plants were harvested. Root and shoot lengths were recorded and dry weight was assessed after drying plant material to constant mass.

All experimental data were used to calculate percentage of respective controls, to allow comparison of effects of different ionic liquids on different plant species and on a variety of measured parameters. The presented endpoint is [%] growth or germination inhibition, calculated in relation to the untreated controls. Experimental data were analyzed with the Origin 8.1 statistical software, by the one-way ANOVA (analysis of variance) technique. The data processing methods were compared using the Least Significant Difference (LSD) test at the 0.05 probability level.

In order to investigate the impact of imidazolium based ILs on growth parameters of barley and wheat, plants were treated with the following compounds: [Im][Cl], [Bmim][Cl], [C_2OC_2mIm][Cl], [C_1OC_2mIm][Cl], [OHC_3eIm][Cl], [OHC_3mIm][Cl], [OHC_2mIm][Cl]. The melting point of [Im][Cl] is higher than 100 °C, so generally it can not be consider as an ionic liquid, but [Im][Cl] was included in this study as a representative of protic imidazolium salt without any alkyl substituents. [Bmim][Cl] is an ionic liquid with hydrocarbon side chains and melting point of 70 °C. Other investigated compounds are room temperature ionic liquids with one side chain functionalized with oxygen in the form of hydroxyl or ether group. The observed growth parameters were germination, growth and dry mass of root and shoot of wheat and barley.

3. Results and discussion

3.1. Effect on germination

The effect of investigated ionic liquids and imidazolium chloride

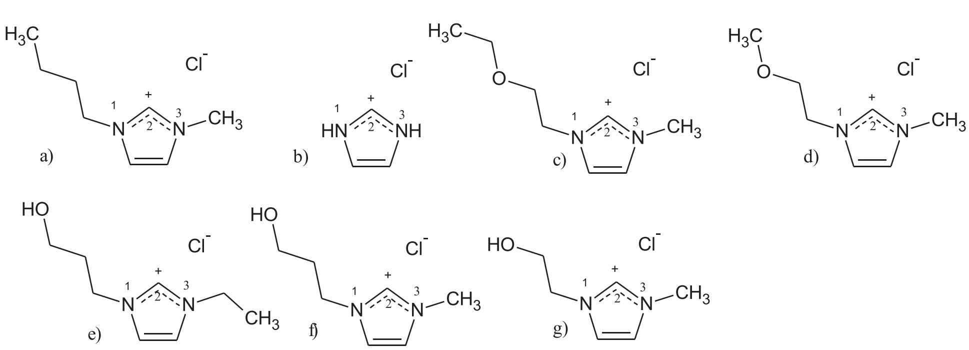

Fig. 1. Structure of investigated compounds: a)1-butyl-3-methylimidazolium chloride, [Bmim][Cl]; b) imidazolium chloride, [Im][Cl]; c) 1-(2-oxybutyl)-3-methylimidazolium chloride, [C_2OC_2mIm][Cl]; d) 1-(2-oxypropyl)-3-methylimidazolium chloride, [C_1OC_2mIm][Cl]; e) 1-(3-hydroxypropyl)-3-ethylimidazolium chloride, [OHC_3eIm][Cl]; f) 1-(3-hydroxypropyl)-3-methylimidazolium chloride, [OHC_3mIm][Cl]; g) 1-(2-hydroxyethyl)-3-methylimidazolium chloride, [OHC_2mIm][Cl].