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New insight into the environmental impact of two imidazolium ionic liquids. Effects on seed germination and soil microbial activity

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HIGHLIGHTS

- Addition effect on seedling emergence and microbial growth of two ILs is analysed.
- High doses of both ILs produced total inhibition of seedling emergence.
- Microcalorimetry provides information about the ILs effect on soil microorganisms.
- Two different patterns of toxic effects on power time curves of soil were observed.
- Plants are more sensitive to the presence of ILs than soil microorganisms.

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ABSTRACT

The next generation of ionic liquids must be synthetized taking into account structures that guarantee the suitable properties for a defined application as well as ecological data. Thus, searching of the right methodologies to know, quickly and efficiently, the ecological effects of these compounds is a preliminary task.

The effects of two imidazolium based ionic liquids with different anions, 1-butyl-3-methylimidazolium tetrafluoroborate, $[C_4C_1Im][BF_4]$, and 1-propyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide, $[C_3C_1Im][NTf_2]$, on seedling emergence of six tree species and on the microbial behaviour of two soils were determined in this work. Results showed that the highest doses of both ionic liquids caused the total inhibition of germination for almost all the species studied and that the seeds are more sensitive to the presence of these compounds than soil microbial activity. Nevertheless, signals of stress and death are observed from the results of heat released by microorganisms after the addition of the highest doses of both ionic liquids.

The novelty of this work resides in the enlargement of knowledge of toxicity of ILs on complex organisms such as arboreal species and microbial activity of soils studied for the first time through a microcalorimetric technique.

1. Introduction

Ionic liquids (ILs) are organic salts made of organic cations and organic or inorganic anions, with melting temperatures lower than 100 °C. The recent increasing interest of these compounds in both industrial and academic communities is mainly due to the knowledge of the dependence of their properties with the structure and, therefore, its possibility of tuning: lots of cations and anions can be combined to create ILs for a particular application. Nowadays it is estimated that more than 10^{12} different ILs could be synthetized with more than 30 000 Imidazolium ILs collected in the CAS database (Amde et al., 2015). In this context, ILs seem to have an important role in 2020 horizon, especially because these compounds have potential use in numerous applications as solvents and battery electrolytes (Plechkova and Seddon, 2008), biosensors (Mundaca et al., 2012), pharmaceutical and antimicrobial ingredients (Smiglak et al., 2014), food industry (Toledo-Hijo et al., 2016), lubricants (Otero et al., 2014), thermal fluids (Holbrey,

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2007), drug recovery from solid pharmaceutical wastes (Silva et al., 2016), herbicides (Cojocaru et al., 2013; Pernak et al., 2015) among other applications.

Because their negligible vapour pressure, atmospheric contamination due to the use of ILs is unlikely, but if water solubility is taken into account, some of these compounds can introduce harmful effects on environmental recipients such as soils, sediments, surface and groundwater, and due to their small biodegradation degree, these effects can be long-lasting (Amde et al., 2015; Cvjetko Bubalo et al., 2014). Recent toxicity studies in bacteria, algae, plants and invertebrates indicated that ILs can be similar in toxicity, even more, than traditional solvents and comparable effects to reference pesticides such as atrazine (Amde et al., 2015; Cvjetko Bubalo et al., 2015; García et al., 2015; Stolte et al., 2007; Tsarpali and Dailianis, 2015; Ventura et al., 2014).

The harmful effects of ILs depend on ion structure (Bado-Nilles et al., 2015; Cvjetko Bubalo et al., 2014; Frade and Afonso, 2010; Oliveira et al., 2016), concentration of IL and time exposition (Biczak et al., 2014); trophic level (Amde et al., 2015; Perales et al., 2016; Santos et al., 2015), kind of bacteria (Gouveia et al., 2014; Yu et al., 2016); plant species (Pawłowska and Biczak, 2016; Studzinska and Buszewski, 2009; Wang et al., 2009); soil organic matter content (Studzinska and Buszewski, 2009), and pH, salinity and temperature (Amde et al., 2015).

Despite the increasing number of papers that try to determine the toxic effects of ILs, the knowledge of their ecotoxicity and biodegradability is still an open question, and even, as pointed out, the bibliographic results present contradictory conclusions.

Another aspect to take into account is that scarce studies about toxicity addressed to know the effects of ILs on organisms with long life, high biomass and coverage and dominant species of ecosystem have been developed. This is very relevant due to the fact that the character additive that the toxic substances can present and the effects of an IL in simple organisms cannot be generalized to the more complex organisms. Peric et al. (2011) provide important information in this field studying the effects of three protic ILs on seedling emergence and seedling growth of onion, grass and radish as well as on the soil microbial functions from the study of carbon and nitrogen mineralization. Thus, the ecological properties are of critical importance for the ongoing utilization of ILs.

In both terrestrial and aquatic ecosystems, the two essential functions for their maintenance are production and decomposition. In terrestrial ecosystems, production or fixing energy is mainly carried out by plants and decomposition by soil microorganisms. Germination is the most sensitive phase of plant life cycle (Reyes et al., 2015a; Reyes and Casal, 1998).

Moreover, a good way to analyse the impact of a substance on soil microorganisms (Núñez-Regueira et al., 2006; She et al., 2013), or even isolated organisms (Wang et al., 2007), is through the variation of their metabolic activity and microcalorimetric techniques with high power sensibility can be used for this purpose; in particular, Isothermal Microcalorimetry measures heat production rate, which accompanies nearly all physical, chemical and biological processes. The role of this technique has been increased during recent years in the microbiological field due to its simplicity, versatility and fast analysis (Wadsö and Gómez Galindo, 2009).

For these reasons and outlining progress in this line opened by Peric et al. (2011), this paper aims to analyse the effect of the addition of five different doses of two imidazolium IL, 1- 3-methylimidazolium tetrafluoroborate, $[C_4C_1Im][BF_4]$ and 1-propyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide, $[C_3C_1Im][NTf_2]$, on two environmental aspects:

- *seed germination* of five species of pine and one species of eucalyptus. The reason for selecting these species was their wide geographical distribution and their great economic, social and ecological interest (Richardson and Rundel, 1998).
- *microbial activity* of two similar soils, one under *Pinus pinaster* Aiton and the other one under *Eucalyptus globulus* Labill., both collected in the same zone, using a new methodology based on the changes of heat released by microorganisms after the addition of a substrate (glucose) and different concentrations of ILs.

The few studies of toxicity of ILs on complex organisms address herbaceous annual plants. One of the most important innovations of this study is the qualitative leap given to having used arboreal species, which are much more complex and persist for a long time, as well as being key species in ecosystems. This fact, together with this being the first time a microcalorimetric technique is used to determine the effect of ILs on microbial activity of soils, are the main novelties of this paper.

The reason to select these ILs is to extend the characterization of the effects of these well-known ILs, enclosed in the most hazardous (Santos et al., 2014; Tsarpali et al., 2015) to more complex systems and verifying the viability of new methodologies (microcalorimetry) on this characterization. In addition, taking in mind that ILs are organic salts, to complete this proposal, the results of this study were compared with that of the effects of a well-known salt, sodium chloride, under the same conditions.

2. Material and methods

2.1. Chemicals

Two imidazolium based ILs were selected for this work. These ILs are $[C_4C_1Im][BF_4]$ and $[C_3C_1Im][NTf_2]$. Both ILs presented high thermal stability and are good candidates to be used in high temperature applications, although special care must be taken with ILs with anion $[BF_4]$ which present tendency to hydrolyse at high temperature in the presence of water.

Table 1 shows the main characteristic of selected ILs, complete and short names, CAS Registry number, chemical structure, molecular mass, purity and supplier.

2.2. Germination response

Seed germination test of six tree species, selected by their wide geographical distributions worldwide (Richardson and Rundel, 1998): *E. globulus*, *Pinus halepensis* Mill. *Pinus nigra* Arnold., *P. pinaster*, *Pinus sylvestris* L. and *Pinus radiata* D. Don were carried out.

For each species, five different concentrations in aqueous solutions for the two selected ILs, namely 0 g/L, 0.1 g/L, 1 g/L, 10 g/L and 100 g/L which correspond to control, $4.39 \cdot 10^{-4}$ mol/L, $4.39 \cdot 10^{-3}$ mol/L, $4.39 \cdot 10^{-2}$ mol/L and $4.39 \cdot 10^{-1}$ mol/L for $[C_4C_1Im][BF_4]$, and, control, $2.44 \cdot 10^{-4}$ mol/L, $2.44 \cdot 10^{-3}$ mol/L, $2.44 \cdot 10^{-2}$ mol/L and $2.44 \cdot 10^{-1}$ mol/L for $[C_3C_1Im][NTf_2]$, respectively were tested. Five replicates with 25 seeds per Petri dish were incubated for each species and treatment (Arán et al., 2013). The effect of both ionic liquids was tested in a total of 7500 seeds. Each replicate was incubated in a Petri dish on a double layer of filter paper in a Phytotron (Climas AGP890) with a photoperiod of 16 h under light at 24 °C and 8 h in darkness at 16 °C. Initially 4 ml of each treatment was added to each replicate. Petri dishes were watered with distilled water when it was necessary to maintain the seed's humidity. Germinated seeds were counted every Monday, Wednesday and Friday. Germination had been completed in all the species after 45 d of incubation.

Additionally, results of this study were compared to those