Effect of the ionic liquid [BMIM] [OTf] on germination and early growth of *D. carota* and *A. sulcata* and on soil microbial activity of an oakland.

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Abstract

Although the ionic liquids (ILs) are considered as green solvents, mainly due to their negligible vapor pressure, the effects on the environment are scarcely studied.

In this work, effects of the IL 1-butyl-3-methylimidazolium triflate, [BMIM] [OTf], on germination and on growth during the seedling stage of two herbaceous forest species with wide geographical distribution: *Daucus car*ota L. and *Avena sulcata* (Gay ex Boiss.) was determined. Changes on soil microbial activity of *Quercus robur* L. oakland as consequence of the addition of different concentrations of this IL were also studied.

Different effects of this IL on plants and soil have been found. Thus, in terms of germination, the dose of 10% (of IL in distillated water) presented total inhibition in the germination of both species; whereas this dose presented a stimulant effect on soil microbial activity. The effect of IL on plant growth is similar to that obtained in the germination test. Concentration of 50% of IL provoked the total inhibition of the soil microbial activity.

Keywords: ionic liquids toxicity**,** [BMIM] [OTf], germination, plant growth, soil microbial activity.

Introduction

Ionic liquids (ILs) are organic salts made of organic cations and organic or inorganic anions, with melting temperatures lower than 100ºC. These compounds have been cited as important elements of green chemistry because they have been shown similar properties than organic solvents, which are often toxic, flammable and volatile

releasing to the atmosphere hazardous substances. Nevertheless, ILs have negligible vapor pressure reducing the likely risk of atmospheric contamination, which is an important property for improving safety with regard to conventional organic solvents. Because of their non-volatility, it is unlikely an atmospheric contamination due to the use of these compounds, however if water solubility is taking into account, some of these compounds can introduce handful effect on environmental recipients as soils, sediments, surface and groundwater, and due to their resistance to photo-degradation and small biodegradation degree, these effects can be long-lasting (Amde et al. 2015). In spite this fact, literature about the effects of ionic liquids on environment is scarce. There are some investigations in aquatic organisms (Pham *et al*. 2010) and very few in terrestrial organisms (Peric *et al*. 2014) that study the possible toxicity of different ILs. It is therefore necessary to carry out studies on the effects of ionic liquids on organisms, starting with those in the first trophic level.

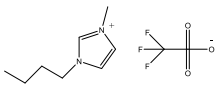
In this paper we have focused on an ionic liquid, [BMIM][OTF], with the main aim to determine their effect on germination and early growth of *A. sulcata* and *D. carota* and on microbial activity of an oakland soil.

Material and methods

Chemical

The ionic liquid selected for this work, which chemical structural and main characteristic are presented in table 1 was purchased to Iolitec.

Table 1. Main characteristic of the [BMIM][OTF] ionic liquid, CAS Identification number, structure, molecular mass and purity

Ionic liquid CAS Number	Name	Structure	Mm (g/mol)	Purity
1-Butyl-3-methylimidazolium triflate [174899-66-2]	[BMIM][OTf]		288.29	>0.99

Germination and growth

Germination test with 600 seeds of each species other were performed to determine the effect of ionic liquid [BMIM] [OTf] on germination of the two selected species,. Treatments of 0.01%, 0.1%, 1% and 10% and control (0%) of IL dissolved in distilled water were applied to seeds. Five replicates of each treatment and species were performed with each dose. Each replicate consisted of a Petri dish with a double filter paper layer on which 25 seeds of a species were sowed. 4 ml of the corresponding dose

was applied initially and afterwards samples were watered with distilled water. After application of IL seeds were incubated in Phytotron chamber with 16h photoperiod at 24 ° C light and 8h dark at 16 ° C (Reyes et al. 2015).

Additionally, a second test was performed to determine the effect of IL on early growth of the two species. This test was done with concentrations that showed some germination in the previous test: control, 0.1% and 0.01%. 200 seeds were sown per replicate to ensure having sufficient emerged seedlings of the same age with the same treatment. Emerged seedlings were transplanted to plastic recipients with perlite and incubated, in the same conditions as seeds, for 21 days. 5 replicates of each treatment and species were performed. Each replicate consisted of a recipient with perlite and 8 seedlings of the same age. At the end of this period the length of the stems of each seedling was measured.

Soil calorimetry

An organic soil under *Quercus robur* L, located in Ons de Abaixo, Brión, A Coruña, Spain (42º53´36.82´´ 8º43´41.59´´) was seleted for this work.

Six grids of 1m2 were selected randomly in a surface of 100 m2 in the sampling zone. After removal of the litter layers, which consisted mainly of undecomposed leaves, samples were collected at a depth of up to 10 cm. Soil sample was dried in the air naturally up to residual moisture. Before the analysis, visible plants and other large particles were removed from the soil by hand and sample was sieved at 2 mm. Fraction less than 2 mm was homogenised and used for this study. Samples were kept to 4 ºC in hermetic bags during three months to field capacity to assure a minimum microbial activity before start the calorimetric experiments and to guarantee a suitable reproducibility in calorimetric determinations.

The effect of the addition of five different concentrations of aqueous solutions (1, 10, 25, 50 y 75% in weight in distilled water, as well as the corresponding control) of the IL on microbial activity of this soils was studied by calorimetry. Experiments were performed using an isothermal microcalorimeter 2277 Thermal Activity Monitor (TAM) Thermometric AB. Measurements were carried out at 25 ºC in hermetically sealed 5 ml stainless steel ampoules closed with a Teflon coated septa and an steel cap. Soil samples of 1 g size at water-holding capacity, treated with 0.2 ml of a glucose solution in water with a concentration of 6.25 g / l to activate the metabolism of soil microorganisms, were used as control. The heat released by the microorganisms was recorded until the total consumption of glucose (at least three days).

Results and discussion

Results showed that the germination of both species are in different way by de addition of the IL [BMIM] [OTf]. The most affected species was *A. sulcata*, which only germinated in the same proportion as in natural conditions with the lowest dose, 0.01%. *D. carota* was able to germinate at doses of 0.01% and 0.1% and in the same amount as the control (Fig1).

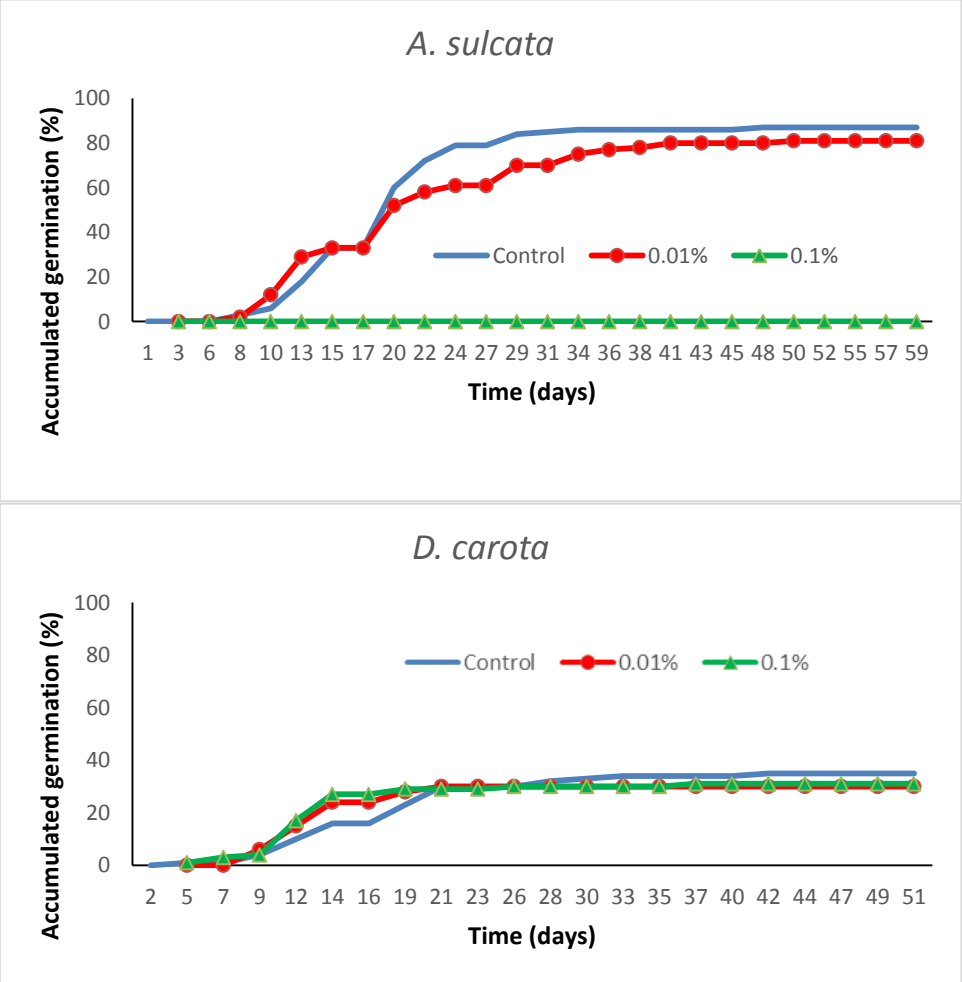


Figure 1. Accumulated germination reached by *A. sulcata* (up) and *D. carota* (down) with each applied dose of [BMIM][OTf].

Values of stem length reached by *A. sulcata* seedlings were always lower than those of *D. carota*, but no significant differences between control and IL tested dose were found (Fig. 2).

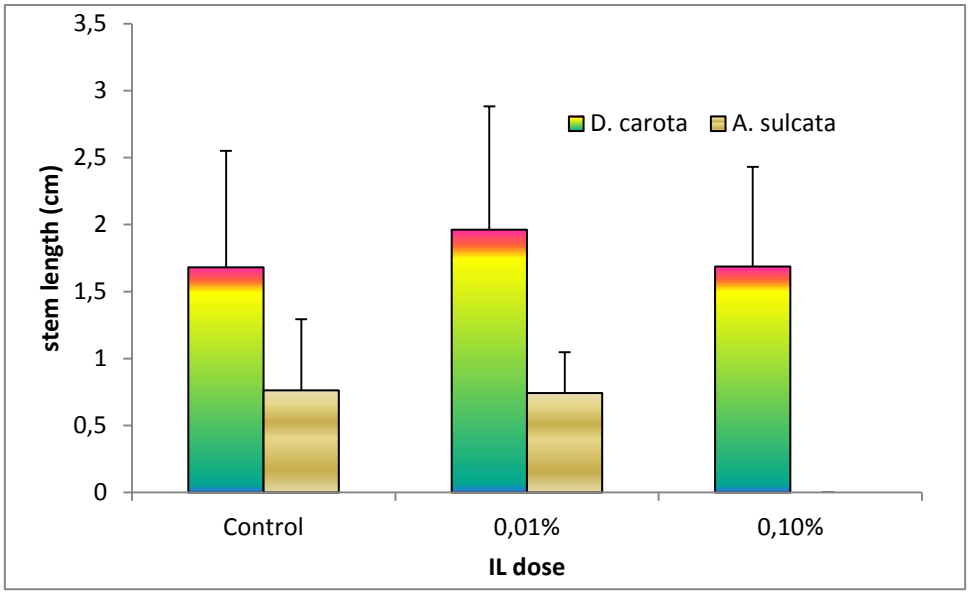


Figure 2. Average stem length and SD, in cm, reached by *A. sulcata* and *D. carota* after the application of [BMIM][OTf].

Figure 3 shows the power-time curves associated to the microbial activity of the soil after the addition of the different doses of the selected IL. The main observation is that the two lowest doses provoked an stimulation of the activity whereas the doses of 50 and 75% provoked the total inhibition of the microbial growth.

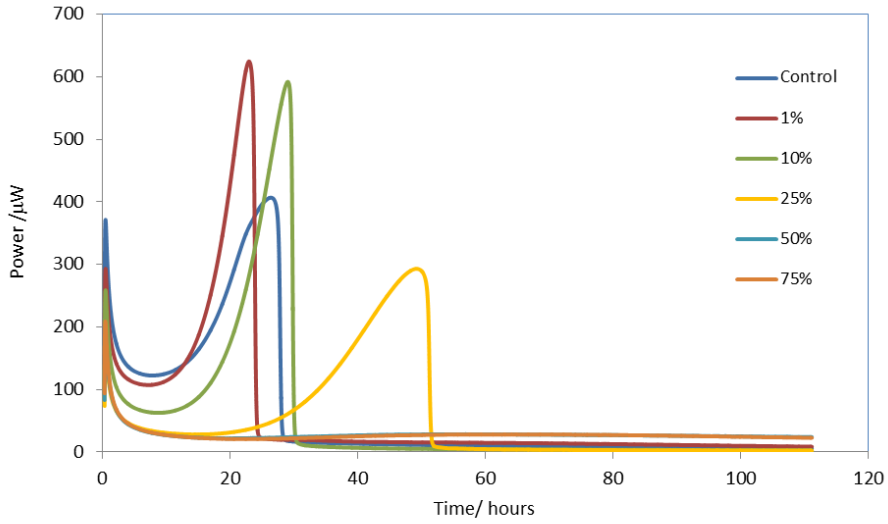


Figure 3: Power time curves of microbial activity of the soil after the addition of the different concentrations of [BMIM][OTF].

In conclusion we can say that [BMIM] [OTf] negatively affects germination of both species, inhibiting it completely with concentrations of 1% or higher. This negative effect is more noticeable in *A. sulcata* than in *D. carota* becaouse of dose of 0.1% IL *provokes a total inhibition of germination of A. sulcata.* Similar results had already been

obtained on the germination of other species using other based immidazolium ILs (Delatorre-Herrera and Pinto 2009, 2015 Cruz, Salgado et al. 2016).

On the other hand, the lowest doses do not appear to affect early seedling growth, but taking into account that the seeds were only in contact with the IL from sowing until germination. The result could change if the seedlings were in contact with the IL during growth. This is an aspect that deserves to be studied in future research.

Nevertheless the effect of the IL on soil is very different that on germination, the inhibition of soil microbial growth takes place for doses higher of 50% of IL.

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