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Toxic effects of [BMIM][BF4] on early growth of Eucalyptus globulus Labill.

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Abstract: Atmospheric contamination from ionic liquids (ILs) is very improbable due to the low vapour pressure of these compounds. Nevertheless many ILs are water soluble and they can generate harmful effects on aquatic organisms, soils and plants.

Inhibition of bioluminescence of *Vibrio fischeri* bacteria is one of the most used tests to determine the toxicity of ILs, however these results cannot be extrapolated to other trophic levels.

This work presents the main conclusions of a study on the effect of the IL 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF$_4$]) on early growth of *Eucalyptus globulus* Labill. plants on two soils with different pH and treated with five different concentrations of aqueous solutions of the IL (0 (control), 0.01, 0.1, 1, 10, 25 %) with the rate of 0.1 ml of solution per gram of soil. The stem and root lengths, the leaf number and the dry weight were determined in every case.

[BMIM][BF$_4$] has important effects on the survival and early growth of plants characterized by a decrease with the increasing IL concentration, and for the dose higher than 10% the total mortality was observed for both soils. Soil influence has been detected for the lowest concentrations, observing that highest survival and early growth correspond to the highest pH soil.

Keywords: Ionic liquids, [BMIM][BF$_4$], toxicity, early growth, *Eucalyptus globulus* Labill

1. Introduction

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

Atmospheric contamination from ionic liquids (ILs) is very improbable due to the low vapour pressure of these compounds. Nevertheless many ILs are water soluble and they can generate harmful effects on aquatic organisms, soils and plants. Inhibition of bioluminescence of *Vibrio fischeri* bacteria is one of the most used tests to determine the toxicity of ILs, however these results cannot be extrapolated to other trophic levels.

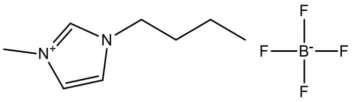
On the other hand, the soil, according to its characteristics, can modulate the response of the microorganisms to the ILs (Salgado et al., 2017), however it is not known how the soil can interfere in the response of the plants to the ILs. There are still very few studies of ILs effects on plants and generally focused on studying herbaceous species (Peric et al. 2014). The knowledge about the early growth of plants living in soils contaminated with ILs is scarce. For these reasons, and for contributing to the understanding of ILs effects, the early growth of the arboreal species *Eucalyptus globulus* Labill in two soils with different pH and contaminated with different doses of [BMIM][BF$_4$] was analysed in this work.

2. Materials and Methods

2.1. Chemicals

The ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF$_4$]), was purchased from IOLITEC (Heilbronn, Germany) with the highest available purity (99%). The corresponding chemical structural and main characteristics of this IL are shown in Table 1.

Table 1. Main characteristics, CAS Identification number, structure, molecular mass and purity of [BMIM][BF$_4$].

Ionic liquid CAS Number	Short Name	Structure	Mm (g mol^{-1})	Purity
1-Butyl-3-methylimidazolium tetrafluoroborate [174501-65-6]	[BMIM][BF$_4$]		226.02	>0.99

2.2. Plant and soils

The plant species chosen to analyse changes on its early growth as consequence of IL addition was *Eucalyptus globulus* Labill. The reason to select this species is because its abundance, distribution and high biomass, as well as for its high rate of germination and rapid early development.

Two soils of contrasting pH and different organic matter content were selected for the study: a soil developed under *Pinus pinaster* Aiton (Calcareous soil), located at Rubiá (Lugo, Spain) and a soil developed under *Quercus robur* L. (Acid soil), located at Negreira (A Coruña, Spain). The main characteristics of both soils are shown in Table 2. The surface horizon (0-10 cm) was collected after removing the litter layer and the soils were immediately transported to the laboratory in isothermal bags and maintained at 4 ºC until the beginning of the experiment.

2.3. Analysis of soils

The soils were analysed for pH in water and in 1 M KCl, total carbon and nitrogen contents and particle size distribution following the methods described in Guitián and Carballas (1976).

2.4. Experimental set-up

The *E. globulus* seeds were sown in a Petri dish with two filter paper layers and distilled water and maintained at 25 ºC for germination (one week approximately).

Table 2. Main characteristics of the soils used in the study.

Soil	**Total C** %	**Total N** %	**C/N**	**pH$_{H2O}$**	**pH$_{KCl}$**	**Moist** %	**Sand** %	**Silt** %	**Clay** %
Acid soil	12.65±0.81	0.61±0.02	21	4.47±0.05	3.48±0.01	55.8	62	26	12
Calcareous soil	5.31±0.02	0.28±0.01	19	7.74±0.01	7.18±0.01	31.7	56	36	9

Different solutions of [BMIM][BF$_4$] were prepared by dilution of this compound in water, to obtain final concentrations of 0 (control), 1, 2.5, 10 and 25%. Thereafter, the soils were spiked with 0.1 ml of each of the solutions per gram of soil, and the soil-[BMIM][BF$_4$] mixture was maintained at 20 ºC for three days, before the start of the planting experiment. A soil sample without [BMIM][BF$_4$], but with identical quantity of water than for the soil samples contaminated, was maintained as a control. This water was the amount needed to reach the 80% of Water Holding Capacity (Moist %) for each soil. Three days after contamination, quadruplicate samples of 55 g each were prepared for each dose of [BMIM][BF$_4$] in plastic containers.

The early growth of *E. globulus* was tested by percentage survival, the stem and root lengths, the leaves number and the dry weight of eucalyptus seedlings. For this, seedlings were transplanted to these containers (6 seedlings per container, 24 seedlings per treatment). The plants were maintained in a chamber with photoperiod (25 ºC, ambient humidity of 60% and light/dark cycles of 16 h/8 h) for 2 weeks and watered three times a week with distilled water to keep the initial moisture content (80% of Water Holding Capacity). After the two weeks the IL effects were clear and the experiment was finished. The seedlings were removed from the container; the number of leaves was counted and the length of the stems and roots were measured. Then the plants were dried in an oven at 40 ºC for three days. Thereafter, the plants were weighted to determine their dry weight.

3. Results and Discussion

The survival of *E. globulus* seedlings varied according to the amount of [BMIM][BF_4] applied. The highest doses, 10% and 25%, resulted in the death of all seedlings in both soils and for this reason there are no emergency data for these doses.

3.1. Stems length

The length of the stems was measured and the average values were calculated (Fig. 1). Seedlings reached up to 2.8 cm and 2.4 cm of height in acid in calcareous control soils, respectively, whereas the corresponding values for soils treated with the dose of 1% were lower: 1.6 and 1.9 cm in the acid and the calcareous soils, respectively. Additionally, the stem only reached 1.1 cm and 1.2 cm in acid and calcareous soils, respectively, spiked with 2.5% of [BMIM][BF_4]. In summary, a clear reduction in seedlings growth of *E. globulus* caused by [BMIM][BF_4] was observed and, since the reduction is similar in both soils, the characteristics of the soil do not appear to have any influence on this reduction.

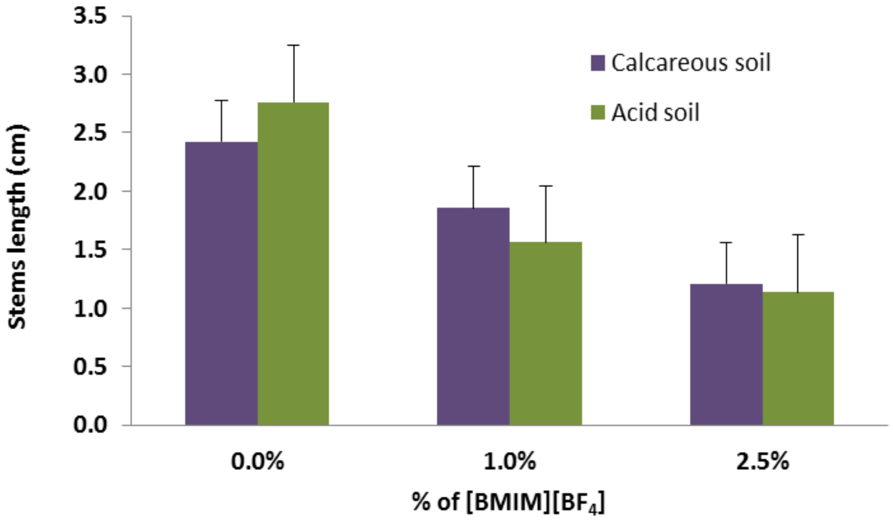


Figure 1. Average stems length and standard deviation (SD) reached by *E. globulus* seedlings after 14 days of growth with each treatment.

3.2. Roots length

Increasing amounts of [BMIM][BF_4] caused a decrease in the length of the roots (Fig 2). Thus, in the acid soil the roots length changed from 5.4±1.3 cm in the control to 1.9±1.1 cm in the 2.5% treatment. In the calcareous soil, the roots in the control soil reached a length of 5.6±1.6 cm, while in the 2.5% treatment the roots only reached 3.4±2.3 cm.

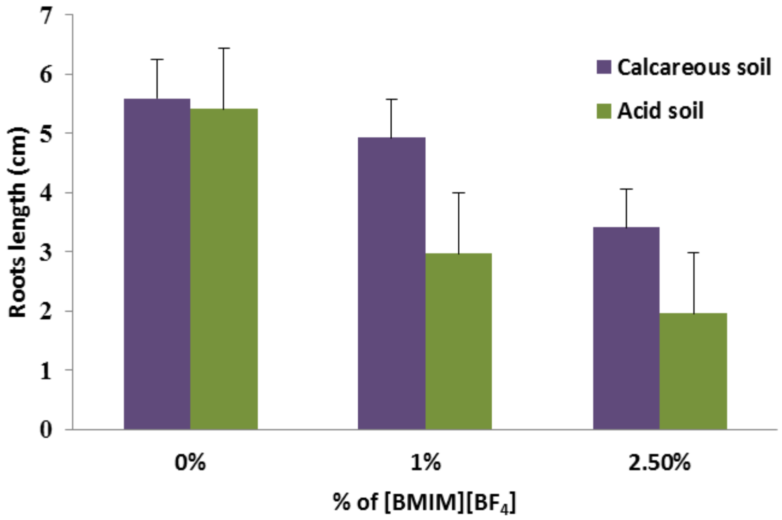


Figure 2. Average roots length and SD reached by *E. globulus* seedlings after 14 days of growth with each treatment.

The decrease in the length of the roots is, therefore, stronger in the acid than in the calcareous soil, showing that not only the characteristics of the compound, but also those of the soil are relevant for the effect of [BMIM][BF_4] on the growth of the roots.

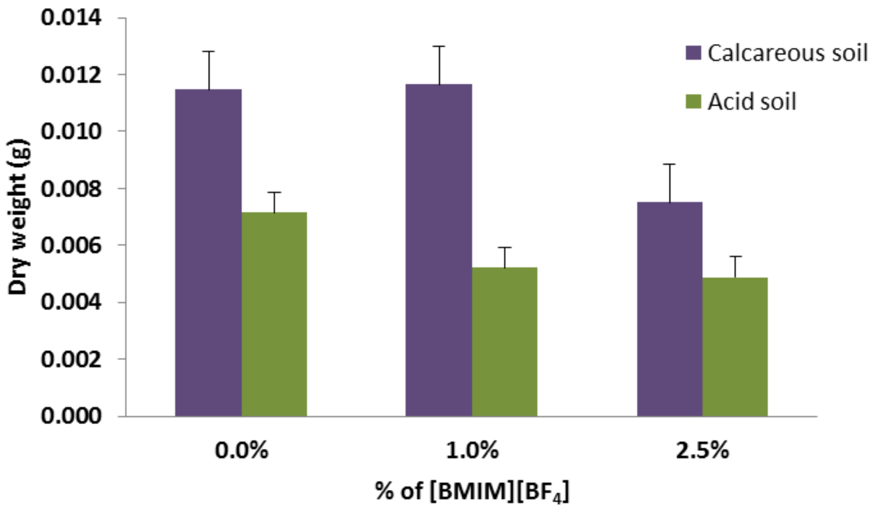


Figure 3. Average dry weight and SD of *E. globulus* seedlings after 14 days of growth with each treatment.

These results are in relatively good agreement with the significant inhibitions of seed germination of several plant species (Salgado et al., 2015) and the reduction of early growth of two herbaceous species (Reyes and Salgado, 2016) as a consequence of the addition of different ILs, obtained in previous studies by the same authors. However, in previous studies the effect of the IL on root growth was stronger than in the current study. This difference can be partly attributed to the fact that in previous studies the effect of the IL was investigated by testing their effect directly on the seeds (different amounts of each of the ILs), while in the present study the presence of soil could modulate the effect of the [BMIM][BF_4] .

3.3. *Dry weight and leaves number*

The dry weight of the seedling growing in the two soils varied depending on both the soil and the amount of [BMIM][BF_4] added to the soil. Values of 0.007±0.003 g and 0.011±0.005 g were observed in calcareous and in acid control soils, respectively. Differences between the two soils were also detected in the soils added with [BMIM][BF_4]. Between treatments, the main differences were observed for the acid soil, among the 1% and 2.5% treatments (0.011±0.002 g and 0.007±0.003 g, respectively).

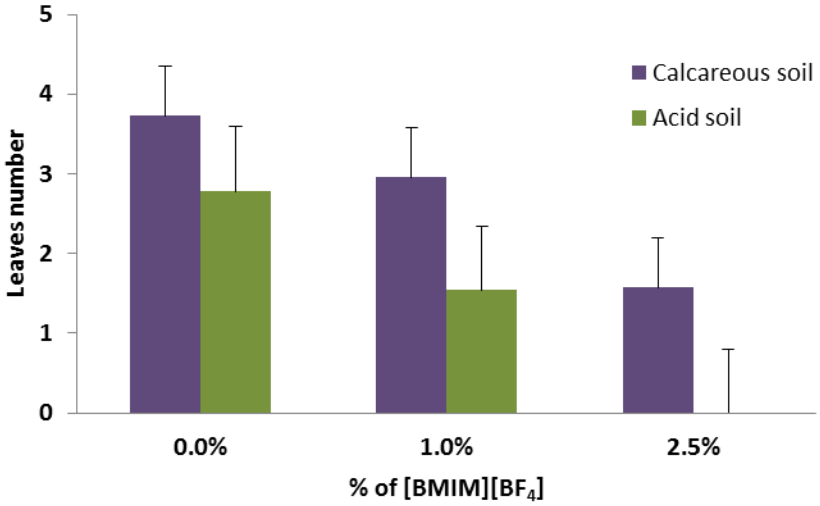


Figure 4. Average number of leaves and SD of *E. globulus* seedlings after 14 days of growth with each treatment.

The last factor determined was the number of leaves after the two weeks of seedlings growth. It was observed a decrease in the number of leaves with the addition of [BMIM][BF_4]. The seedlings of the acid soil lost all their leaves with the 2.5% [BMIM][BF_4] treatment, while the seedling of calcareous soil kept some leaves for the same dose of [BMIM][BF_4]. Thus, the seedlings of the acid soil had 2.8±0.9, 1.5±0.9 and 0 leaves in the control, 1% and 2.5% treatments, respectively, and the seedlings of the calcareous soil had 3.7±0.9 leaves in the control, 2.9±1.2 in the 1% and 1.6±1.4 in the 2.5% treatment of [BMIM][BF_4].

Similar behaviour was also found in previous studies for the growth of seedlings of *Avena sulacata* (Gay ex Boiss.) Dumort. and *Daucus carota* L. after the addition of the same ionic liquid (unpublished data).

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