# Effect of the addition of $[C_3C_1im][NTf_2]$ in pine seed germination and in soil microbial activity


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Ionic liquids (ILs) are chemicals with great design potential. At present there are many details of physical properties of ILs and the dependence of these with the structure [1]. Lots of cations and anions can be combined to create ILs for a particular application. Most of these compounds have negligible vapor pressure and low flammability, important properties for improved safety compared to conventional organic solvents. In this context ILs seem to have an important role in 2020 horizon and ILs will be used for the production of substances of low toxicity and low persistence in the environment (environmental compatibility). These conditions seem feasible to consider the enormous potential of design LIs.

Despite the increasing number of papers about the study of the properties of the ionic liquids (ILs), the knowledge of their ecotoxicity and biodegradability are not still well established. The few studies that deal with it [2] provides that derived imidazolium ILs are those with lower biodegradability while pyridinium have greater potential for degradation. Likewise, ILs with anions $[PF_6]$ and $[BF_4]$ as well as being sensitive to hydrolytic processes are poorly biodegradable, while the acetate and sulfate anion appear to be more environmentally friendly. These studies do not address in any way the effects of these substances on organisms with long life, high biomass and dominant species coverage or ecosystem [3].

This paper aims to analyze the effect of addition of five different doses of the IL $[C_3C_1im][NTf_2]$ on the seed germination of five species of pine , and on the microbial activity of a soil under *P. pinaster* Aiton using microcalorimetry. The reason to select these species was their wide geographical distribution and their great economic, social and ecological interest [4]. The IL was selected taking into account that its thermophysical properties [5, 6] make it a good candidate for to be used as lubricant or lubricant additive and / or thermal fluid, with important applications in renewable energy. Additionally we compare the results of this study with the effects of a salt, sodium chloride, under the same conditions.

## Material and methods

### Germination response

Seed germination test of species of *Pinus halepensis*, *Pinus nigra*, *Pinus pinaster, Pinus sylvestris* and *Pinus radiata* were carried out. In each, five different concentrations of aqueous solutions (10%, 1%, 0.1%, 0.01% and 0% (control) in weight) of the ILs were tested. Five replies with 25 seeds per Petri dish were incubated for every species and treatments. The seeds were incubated in a Phytotron (Climas AGP890) and were maintained for 16 h under light at 24 ºC and in the dark for 8 h at 16 ºC. Germinated seeds were counting every Monday, Wednesday and Friday. Germination had been completed in all the species after 45 days of incubation.

### Soil calorimetry

The effect of the addition of the same doses of the previous IL on organic matter and microbial activity of a Galician soil, under *P. pinaster* Aiton was studied by calorimetry. Calorimetric experiments were performed using a microcalorimeter 2277 Thermal Activity Monitor (TAM) Thermometric AB. Measurements were carried out in hermetically sealed 5 ml stainless steel ampoules. 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 [7]. The heat released by the microorganisms was recorded until the total consumption of glucose (at least three days). The same experiment was performed by adding IL doses indicated above. Three replicates were performed for each case. Additionally, a comparison between the effects of this IL in the soil microbial activity and the corresponding to a well-known salt, sodium chloride, in the same conditions, was stablished.

## Results

### Germination response

The germination percentages obtained show significant differences between specific response of each species. *P radiata* reaches a higher percentage of germination in the control 62% followed in a second group *P. nigra* with 52%; in a third group *P. pinaster* stands with 33%, with 26% *P. sylvestris* and *P. halepensis* with 18% (Fig. 1). Overall, the two higher doses of the IL produce total inhibition of germination except *P. radiata* presenting for the treatment of 1% IL low germination rate, but nonzero. Germination increases as IL content decreases reaching, in less severe treatments, similar values to the control. No germination was obtained for the highest dose of ClNa, althought results for 1% of ClNa are higher than the corresponding to the same dose of IL.

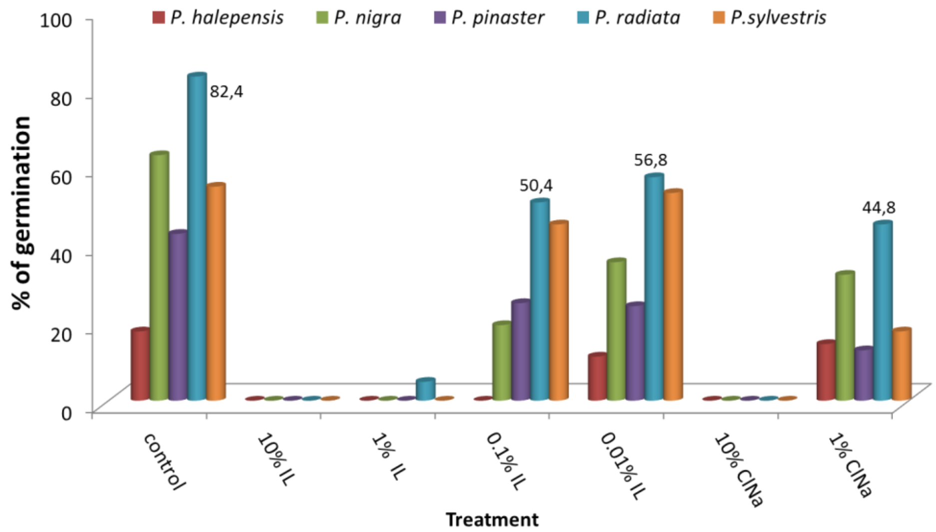


Fig 1. Germination percentages obtained for each species with four doses of $[C_3C_1Im][NTf_2]$ and two doses of ClNa.

**Soil calorimetry**

In Figure 2 the thermal power (μW) curves against time (hours) corresponding to the under *P. pinaster* soil samples to which different doses of $[C_3C_1Im][NTf_2]$ have been added, and that corresponding to the control curve (soil activated glucose), are shown. Integration of these curves provides values of the total heat evolved during the process. As it can be seen, control curve is characterized by an exothermic peak from the beginning of the experiment until elapsed 30 hours, reaching its maximum after 15 hours of reaction. From the comparison between the curves corresponding to this control and the two highest concentrations (1% and 10%), the peak is narrower and higher, in both cases, with regard to control curve. This behavior could be explained with the assumption that the high concentrations of the IL have a selective toxic effect, by inhibiting the growth of some type of microorganisms and strengthening the growth of others that can support the presence of the IL in the soil. Even more, the death of those microorganisms could improve the proliferation of the more resistant giving rise to a higher peak when comparing to the control curve. Nevertheless the differences between the curves of the two lowest doses and the corresponding control are small, only a broader time interval of growth can be observed in samples of soil treated with the IL. It could be even said that the lower doses of IL could strengthen the growth of the microorganisms present in the soil, this fact reflected in a higher peak height and in a broader time interval of growth when compare with control curve. Thus, it can be concluded that the addition of the selected IL in low concentrations does not induce toxic effects on the microbial growth of this soil.

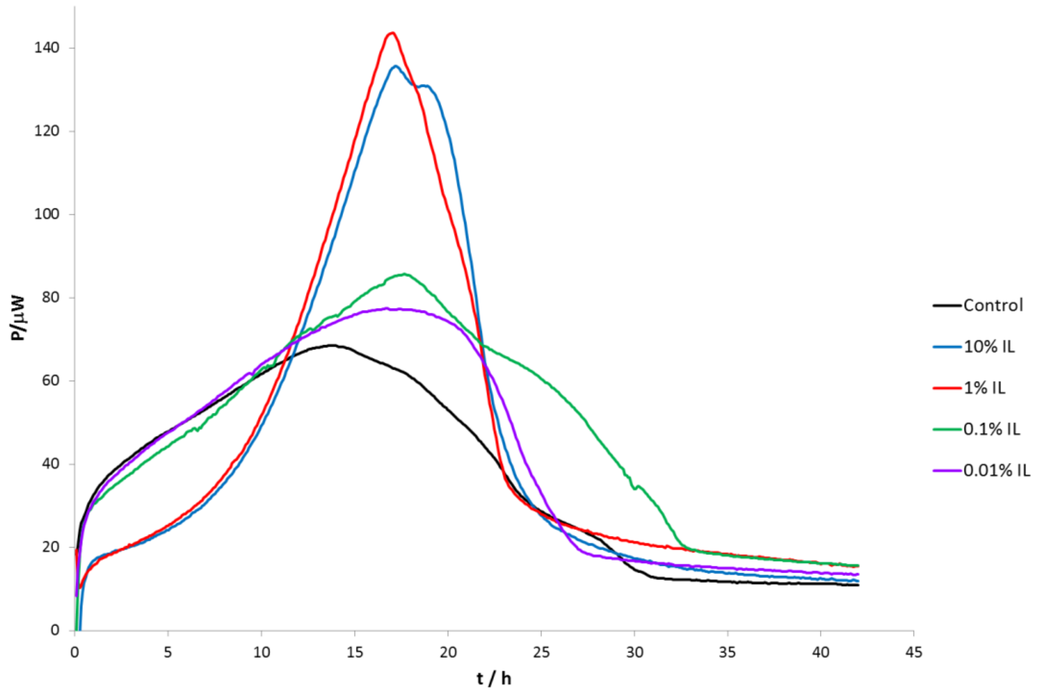


Fig 2. Power –time curves of soil samples with the different treatments of $[C_3C_1Im][NTf_2]$.

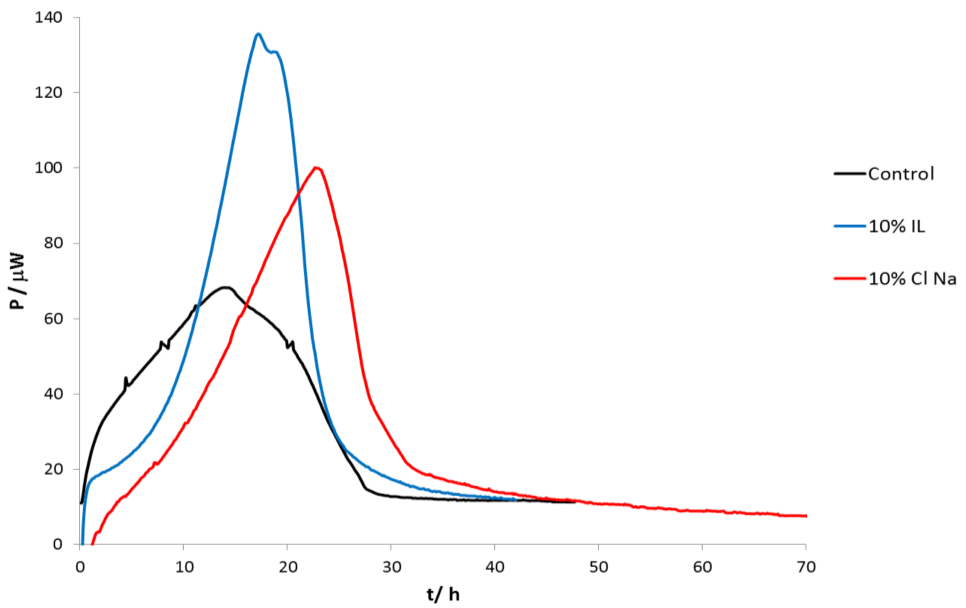


Fig 3. Comparison of the power –time curves of soil samples corresponding to the treatment with aqueous solution at 10% of $[C_3C_1Im][NTf_2]$, with aqueous solution at 10% Cl Na and control.

Figure 3 shows a comparison between the effects of this IL on the soil microbial activity and the corresponding to a well-known salt, sodium chloride, in the same conditions

(10%). From these curves it can be said that, qualitatively, the effects of the IL are similar to that detected with the sodium chloride; a slight retard on the growth of microbial population producing an increase on the peak time and also a higher value of the peak height. A difference between salt and IL curves can be observed at the beginning of the reaction; for ClNa, an immediate and continuous growth is observed whereas for the IL curve a slight retard on the initial growth step was detected.

## Conclusion

In spite of the fact that ILs are considered low-toxic compounds due their negligible vapor pressure and low flammability, they must be treated with caution because ILs can have a negative effect on the environment, especially at high doses.

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