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GERMINATION AND DEVELOPMENT OF *LEPIDIUM SATIVUM* L. AND *EUCALYPTUS GLOBULUS* LABILL IN TWO DIFFERENT SOILS SPIKED WITH THE IONIC LIQUID [C_1C_1Im][DMP]

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INTRODUCTION

Ionic liquids (ILs) have many potential applications in diverse fields because of their distinctive properties. The increasing interest in ILs in industrial and academic sectors is mainly due to recent demonstration of the close relationship between the compound structure and their properties, which could be modified for different purposes. ILs contain an inorganic anion and an organic cation, and different cations and anions could potentially be combined to create ILs for specific applications as, for example, bioseparation agents of active compounds (with special importance in petroleum industry), drug delivery systems, lithium ion batteries, paint additives, lubricants for high temperatures and low pressures and absorbents for heat pump devices [1-5]. More than 30000 imidazolium-based ILs have already been included in the CAS database, and it has been estimated that more than 10^{12} different ILs could be synthesized [4]. The vapour pressure of ILs is very low [4], and in some contexts ILs are considered innocuous because they are not harmful to the atmosphere. They are often referred to as "green fluids". However, their inocuousness has still to be proven, as the fact that they do not act as atmospheric toxins does not mean they are also harmless to aquatic and terrestrial environments. Furthermore, in case of accidental spillage, water-soluble ILs would quickly reach the soil surface in the surrounding area, as well as the deeper layers of soil and surface and subsurface waters. Moreover, given the large number of combinations of anions and cations and the lack of knowledge about the effects of IL structure on toxicity, it is not possible to generalize about the potential impact of ILs on the environment.

The ionic liquid 1,3-dimethylimidazolium dimethylphosphate ([C_1C_1Im][DMP]) has many potential applications and is already used as, e.g., a lubricant-hydraulic fluid, an absorbent in heat pumps, a heat transfer fluid (in heaters or freezers) and a surfactant [3,5-6]. However, the toxicity of this IL to the soil remains to be investigated. This is especially important as soils with different characteristics, particularly in relation to organic matter (OM) and pH, may react differently to the presence of any exogenous compound [7].

Ecotoxicology testing is often carried out with agricultural plants because these are sensitive to environmental stress and pollution [8-9]. Seed germination and seedling development are crucial and particularly sensitive stages of plant development. If plants are grown in contaminated soil, the seeds and roots will be in direct contact with the pollutants, thus potentially affecting germination and/or plant development [10-11]. Moreover, toxicity tests based on seed germination and elongation can be carried out with a wide variety of plant species that are radily available and also germinate and grow rapidly [8-9].

The aim of this study was to investigate the toxicity of [C_1C_1Im][DMP] in soil by analyzing the germination and early development of seeds of one forest and one agricultural plant species in two different types of soil spiked in different compound concentrations.

KEYWORDS:
Ionic liquids, 1,3-dimethylimidazolium dimethylphosphate, ecotoxicity, soils, seed germination, seedling development

MATERIALS AND METHODS

The ionic liquid 1,3-dimethylimidazolium dimethylphosphate, [C_1C_1Im][DMP], (99% pure, analytical grade) was purchased from IOLITEC (Heilbronn, Germany). The main chemical and structural characteristics of this IL are summarized in Table 1.

The plant species selected for the study were garden cress (*Lepidium sativum* L.) and eucalyptus (*Eucalyptus globulus* Labill.). Both plants are suitable for use in ecotoxicity studies because of their abundance, high rate of germination and rapid, early development [8, 12-13].

TABLE 1
Main characteristics of the ionic liquid [C_1C_1Im][DMP: CAS identification number, structure, molecular mass and purity

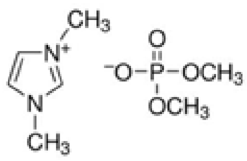
Ionic liquid	Short Name [CAS Number Id.]	Structure	Mm (g mol^{-1})	Purity
1,3-dimethylimidazolium dimethylphosphate	[C_1C_1Im][DMP] [654058-04-5]		222.18	> 0.99

TABLE 2
Main characteristics of the soils used in the study

Soil	pH H_2O	pH KCl	Ct (%)	Nt (%)	C/N	Fe_2O_3 (%)	Al_2O_3 (%)	Texture
Agricultural	5.44±0.05	4.23±0.06	2.22±0.03	0.20±0.00	11	0.89±0.02	0.46±0.01	Loamy
Forest	4.45±0.02	3.51±0.03	11.91±0.08	0.58±0.00	21	0.95±0.01	1.08±0.01	Sandy-loam

Two soils destined for different use (agricultural and forest), containing different amounts of OM of different quality, were selected for the study. At each site, 10-15 subsamples of the A horizon (0-10 cm) were obtained at random points and pooled in the field to produce a composite sample. The samples were transported in isothermal bags to the laboratory where they were sieved (< 4 mm). A sub-sample of each soil was air-dried for determination of general soil properties, and the remainder was stored at 4 ºC until required for the germination and early seed development tests.

Spiked soil samples were prepared from different solutions of [C_1C_1Im][DMP] by diluting the compound in water, to yield final concentrations of 0, 1, 2.5, 5, 10, 25, 50, 75 and 100%. The soils were spiked with 0.1 ml of each of these solutions per gram of soil (equivalent to doses of 0, 0.47, 1.2, 2.4, 4.7, 11.7, 23.3, 35 and 46.7 g of [C_1C_1Im][DMP] kg^{-1} dry soil), and water was added to reach 80% of the water holding capacity of the soil. The spiked soils were maintained at 20 ºC for three days before the start of the planting experiment to maximize the contact between the soil and the IL. Quadruplicate samples were prepared in plastic pots for each treatment and plant species. Nine seeds of each plant species were placed on the surface of each replicate spiked soil. The pots containing soil were placed for 16 days in a growth chamber at 25 °C, ambient humidity of 60% and light/dark cycles of 16/8 h. Water was added daily to replace the water lost by evaporation. Seed germination (%) was determined during the incubation period. Diverse plant growth parameters (length, number of leaves and dry weight) were measured 16 days after the seeds were sown.

An air-dried sub-sample of each soil was analyzed to determine the following physical and chemical soil properties, by previously described methods [14]: pH in water (1:2.5 w:v, soil:water ratio), pH in 1 M KCl (1:2.5 w:v, soil:solution ratio), total carbon (dichromate oxidation in acid medium), total nitrogen content (Kjeldahl procedure), amorphous Al_2O_3 and Fe_2O_3 [15], particle size distribution (Robinson pipette, with Calgon® as dispersant) and texture. The mean values and standard deviations of these properties in the agricultural and forest soils are shown in Table 2.

RESULTS AND DISCUSSION

General soil data. Both the agricultural and the forest soil were strongly acidic, with pHs in KCl of respectively 4.23 and 3.51. As expected, the total organic carbon content was lower in the agricultural soil (2.22% Ct) than in the forest soil (11.91% Ct). The total nitrogen content was also different in the agricultural soil (0.20% Nt) and in the forest soil (0.58% Nt). The amorphous Fe_2O_3 and Al_2O_3 contents of the agricultural soil were respectively 0.89 and 0.46, and the corresponding values in the forest soil were 0.95 and 1.08%. The texture of the agricultural soil was loamy while the forest soil was sandy-loam (Table 2).

Plant parameters. [C_1C_1Im][DMP] had a negative effect on the germination of garden cress and eucalyptus seeds. Thus, for doses of 11.7 (11.7 g of [C_1C_1Im][DMP] kg^{-1} dry soil) and higher the germination percentage for seeds of both species in both soils was zero, except for garden cress in the forest soil (20% germination) (Figs. 1 and 2).

In general, for doses lower than 11.7 g of [C_1C_1Im][DMP] kg^{-1} soil the germination percentage (%) for both garden cress and eucalyptus was higher in the forest soil than in the agricultural soil (Fig. 1 and 2).

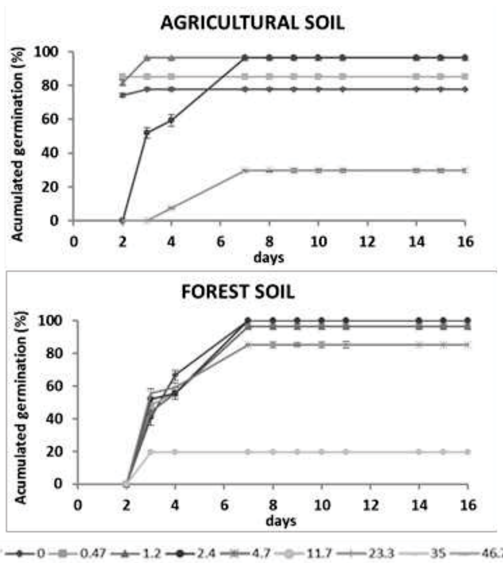


FIGURE 1
Accumulated germination (%, mean±standard deviation) of garden cress during 16 days in the agricultural and forest soils spiked with different doses of [C_1C_1Im][DMP]

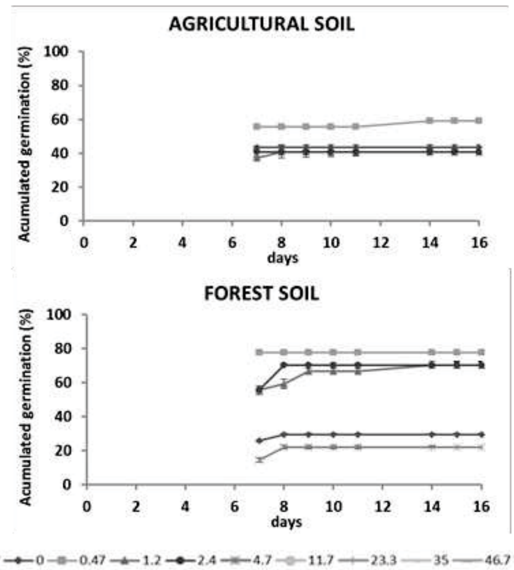


FIGURE 2
Accumulated germination (%, mean±standard deviation) of eucalyptus during 16 days in the agricultural and forest soils spiked with different doses of [C_1C_1Im][DMP]

Germination of both species was very variable, depending on the type of soil and dose of IL, and was the same, higher or lower than in the control (non-spiked) soil. Thus, in some samples spiked with doses of up to 2.4 g of [C_1C_1Im][DMP] kg^{-1}, germination was higher than in the control samples (Figs. 1 and 2); this was observed for the garden cress in the agricultural soil (10% higher) and eucalyptus in the forest soil (40% higher) spiked with 0.47, 1.2 and 2.4 g of [C_1C_1Im][DMP] kg^{-1} or eucalyptus in the agricultural soil spiked with 0.47 g of [C_1C_1Im][DMP] kg^{-1} (almost 15% higher). On the other hand, the response of the samples of garden cress in the forest soil spiked with doses of 0.47, 1.2 and 2.4 g of [C_1C_1Im][DMP] kg^{-1} was very similar to that of the control sample, and germination was only lower in the soil spiked with 4.7 and 11.7 g of [C_1C_1Im][DMP] kg^{-1}. In previous studies, large reductions in germination were also observed in response to the presence of other imidazolium-based ILs such as [C_4C_1Im][BF_4], [C_3C_1Im][NTf_2] and [C_4C_1Im][OTf] [16-17], although in these cases the seeds were in direct contact with the IL solutions. Germination of the cress seeds also tended to take longer as the dose of [C_1C_1Im][DMP] increased. This effect was not observed for eucalyptus seeds, probably because germination is slower (Figs. 1 and 2).

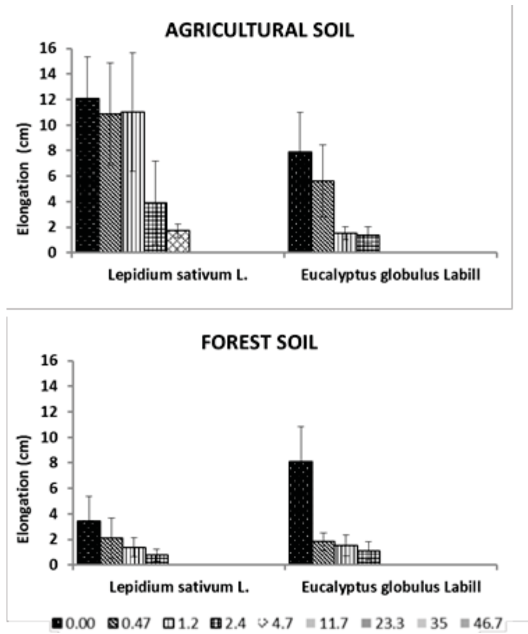


FIGURE 3
Elongation (mean±standard deviation) of garden cress and eucalyptus seedlings grown for 16 days in the agricultural and forest soils spiked with different doses of [C_1C_1Im][DMP]

As germination of both species was inhibited in soils spiked with doses of 11.7 g of [C_1C_1Im][DMP] kg^{-1} soil and higher, seedling elongation at these doses was also zero. For doses of 4.7 g of [C_1C_1Im][DMP] kg^{-1} or lower, and although the germination was stimulated in some samples, there were no differences in the pattern of elongation between the two soils or the two species. Thus, for doses of between 0 and 4.7 g [C_1C_1Im][DMP] kg^{-1} soil, the elongation of garden cress and eucalyptus seedlings decreased as the dose of the IL increased (Fig. 3). The [C_1C_1Im][DMP] had a stronger effect on the elongation of eucalyptus compared to that of garden cress, especially in the forest soil; elongation of both plant species, especially garden cress, was higher in the agricultural than in the forest soil (Fig. 3).

Studzinska and Buszewski [12] also observed that the development of garden cress in soils spiked with three different 1-alkyl-3-methylimidazolium chloride ILs was hindered above a certain concentration of IL, and the effect was dose-dependent. The highest doses of IL killed the plants. The authors attributed the effect to the uptake of IL by plants via the primary cell wall, which is semipermeable and thus allows the passage of small molecules. They attributed the toxic effect to the salts that formed inside the plants. A similar process may have occurred in the present study, as $[C_1C_1Im][DMP]$ should be able to pass through the cell wall pores in both garden cress and eucalyptus (pores of diameter between 5 and 10 nm) [18]. However, it is difficult to explain the stimulatory effect of doses of IL up to 2.4 g kg^{-1} soil on germination of both garden cress and eucalyptus. The addition of nutrients such as N or P with non toxic doses of the IL might be expected to have a stimulatory effect; however, this should affect elongation rather than germination and is unlikely to explain the enhanced germination.

Other researchers have also observed that the effects of ILs vary depending on the type of soil, attributing this to the OM content and suggesting that the OM will adsorb the IL and thus prevent it appearing in the soil solution and thereby reducing its toxicity [12]. A similar relationship between OM content and toxicity has also been observed in Galician soils contaminated with different organic compounds, such as chlorophenols [19]. However, the pH and OM also interact, exerting an effect that is regulated by the pK of the chlorophenol [7]. Likewise, in the present study, the OM content did not appear to be the only factor regulating the negative effects on plant development. If this was the case, the elongation would be greater in the forest soil than in the agricultural soil, which is the opposite to what was observed. It is impossible to clarify which factors determine the toxicity with the data available, and further studies should be carried out with different types of soil in order to clarify this point.

The presence of $[C_1C_1Im][DMP]$ reduced the number of leaves in the garden cress and eucalyptus plants in both soils. This effect increased with the dose of $[C_1C_1Im][DMP]$ (Fig. 4). The garden cress grown in the agricultural soil had statistically significant higher number of leaves than the garden cress grown in the forest soil, whereas the eucalyptus plants grown in both types of soil had very similar numbers of leaves.

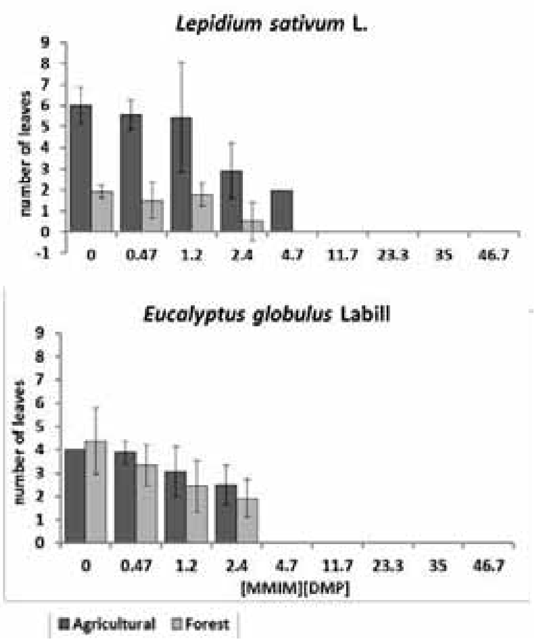


FIGURE 4
Number of leaves (mean±standard deviation) in garden cress and eucalyptus plants grown in both types of soils spiked with $[C_1C_1Im][DMP]$ 16 days after sowing

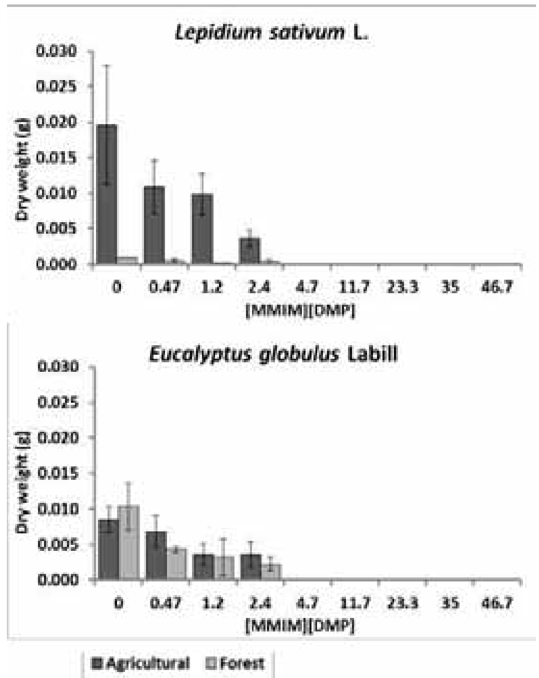


FIGURE 5
Dry weight (mean±standard deviation) of garden cress and eucalyptus grown in both types of spiked with of $[C_1C_1Im][DMP]$, measured 16 days after sowing

Taking into account that the presence of $[C_1C_1Im][DMP]$ reduced the elongation and the number of leaves of both plants species, the different doses of $[C_1C_1Im][DMP]$ were also expected to cause a decrease in the dry weight of the plants, as was observed. Thus, the decrease in dry weight increased with the dose of $[C_1C_1Im][DMP]$ (Fig. 5). The decrease in the dry weight of garden cress was greater in the forest soil, while the effect on eucalyptus was similar in both types of soil (Fig. 5).

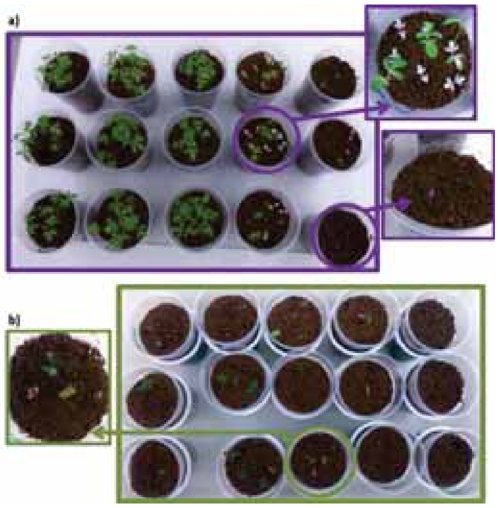


IMAGE 1
a) Garden cress and b) eucalyptus plants 16 days after sowing

The $[C_1C_1Im][DMP]$ also caused chromatosis, i.e. the leaves and stems of the garden cress and eucalyptus plants grown in both agricultural and forest soils spiked with doses above 1.2 g of $[C_1C_1Im][DMP]$ kg^{-1} soil underwent a colour change. The colour of some of the leaves of the garden cress gradually changed to violet, while the colour of the eucalyptus plants changed to light green or dark yellow (Image 1). This phenomenon, like the reduction in seedling size at the highest doses of IL, has been related to chlorosis [12], a plant disease caused by a lack of chlorophyll and/or macronutrient deficiency. It was suggested that the ILs present in the soil solution may block nutrient transport to the plant, or that ILs are preferentially absorbed by the plants, thus causing a deficit of macroelements in the plants [12].

The results of the present study demonstrate that the presence of $[C_1C_1Im][DMP]$ has harmful effects on the germination of *L. sativum* and *E. globulus* seeds and on the early development of these plants.

Although ILs are often considered to be less toxic than other chemical substances and even non toxic to the atmosphere, the findings of this study show that the IL $[C_1C_1Im][DMP]$ strongly affects the early development of plants and that the effect depends on both the dose of IL and on the soil characteristics. Therefore, before this compound can be used as lubricant-hydraulic fluid, absorbent in heat pumps, heat transfer fluid, surfactant, and/or other purposes, further studies should be carried out to investigate its toxicity to soils and plants and, if necessary, its presence in the environment should be regulated.

CONCLUSIONS

Doses of 4.7 g of $[C_1C_1Im][DMP]$ kg^{-1} soil and higher had a negative effect on the germination and development of both garden cress and eucalyptus, regardless of the soil type.

Doses up to 2.4 g of $[C_1C_1Im][DMP]$ kg^{-1} soil stimulated the germination of *L. sativum* and *E. globulus* seeds.

The reduction in elongation, dry weight and number of leaves of *L. sativum* and *E. globulus* plants increased with the dose of $[C_1C_1Im][DMP]$ added to the soil.

Doses of $[C_1C_1Im][DMP]$ above 1.2 g kg^{-1} caused chromatosis in garden cress and eucalyptus plants, a phenomenon resembling chlorosis and probably caused by blockage of nutrient transport or the preferential absorption of ILs by plants, and the consequent reduction of macroelements in the plant tissues.

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REFERENCES

[1] Plechcova, N.V., Seddon, K.R. (2008) Applications of ionic liquids in the chemical industry. Chemical Society Reviews. 37, 123-150.

[2] Toledo-Hijo, A.A.C., Maximo, G.J., Costa, M.C., Batista, E.A.C., Meirelles, A.J.A. (2016) Applications of ionic liquids in the food and bioproducts industries, ACS Sustainable Chemistry and Engineering. 4, 5347–5369.

[3] Gaciño, F.M., Regueira, T., Bolotov, A.V., Sharipov, A., Lugo, L., Comunas, M.J.P., Fernández, J. (2016) Volumetric behavior of six ionic liquids from T = (278 to 398) K and up to 120 MPa. Journal of Chemical Thermodynamics. 93, 24-33.

[4] Made, M., Liu, J-F., Pang, L. (2015) Environmental Application, Fate, Effects, and Concerns of Ionic Liquids: A Review. Environmental Science and Technology. 49, 12611−12627.

[5] He, Z., Zhao, Z., Zhang, X., Feng, H. (2010) Thermodynamic properties of new heat pump working pairs: 1,3-Dimethylimidazolium dimethylphosphate and water, ethanol and methanol. Fluid Phase Equilibria. 298, 83-91.
[6] Ren, N.N., Gong, Y.H., Lu, Y.Z., Meng, H., Li C.X. (2014) Surface Tension Measurements for Seven Imidazolium-Based Dialkylphosphate Ionic Liquids and Their Binary Mixtures with Water (Methanol or Ethanol) at 298.15 K and 1 atm. Journal of Chemical and Engineering Data. 59, 189-196.
[7] Bello, D., Trasar-Cepeda, C., Leirós, M.C., Gil-Sotres, F (2013) Modification of enzymatic activity in soils of contrasting pH contaminated with 2,4-dichlorophenol and 2,4,5-trichlorophenol. Soil Biology and Biochemistry. 56, 80-86.
[8] Zucconi, F., Pera, A., Forte, M., De Bertolli, M. (1981) Evaluating Toxicity of Immature Compost. Biocycle. 22, 54-57.
[9] OECD/OCDE (2003) OECD guidelines for the testing of chemicals. Test 208. Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test.
[10] Rey, P.J., Alcántara, J.M. (2000) Recruitment dynamics of a fleshy-fruited plant (*Olea europaea*): connecting patterns of seed dispersal to seedling establishment. Journal of Ecology. 88, 622-633.
[11] Reyes, O., García-Duro, J., Salgado, J. (2015) Fire affects soil organic matter and the emergence of *Pinus radiata* seedlings. Annals of Forest Science. 72, 267-275.
[12] Studzińska Buszewski, B. (2009) Study of toxicity of imidazolium ionic liquids to watercress (*Lepidium sativum* L.) Analytical and Bioanalytical Chemistry. 393, 983–990.
[13] Arán, D., García-Duro, J., Reyes, O., Casal, M. (2013) Fire and invasive species: Modifications in the germination potential of *Acacia melanoxylon, Conyza canadensis* and *Eucalyptus globulus.* Forest Ecology and Management. 302, 7–13.
[14] Guitián-Ojea, F., Carballas, T. (1976) Técnicas de análisis de suelos. Pico Sacro Editorial, Santiago de Compostela.
[15] McKeague, J.A., Day, J.H. (1966) Dithionite and oxalate extractable Fe and Al as an aid in differentiating various classes of soils. Canadian Journal of Soil Science. 46, 13–22.
[16] Reyes, O., Salgado, J. (2016) 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. The 20th International Electronic Conference on Synthetic Organic Chemistry (http://www.usc.es/congresos/ecsoc/20/START.htm).
[17] Salgado, J., Parajó, J.J., Teijeira, T., Cruz, O., Proupín, J, Villanueva, M, Rodríguez-Añón, J.A., Verdes, P.V., Reyes, O. (2017) New insight into the environmental impact of two imidazolium ionic liquids. Effects on seed germination and soil microbial activity. Chemosphere. 185, 665-672.
[18] Rose, J.K.C. (2003) The Plant Cell Wall. CRC Press, Oxford.
[19] Bello, D., Trasar-Cepeda, C., Leirós, M.C., Gil-Sotres, F. (2008) Evaluation of various tests for the diagnosis of soil contamination by 2,4,5-trichlorophenol (2,4,5-TCP). Environmental Pollution. 156, 611-617.

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