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Original Research Article

Influence of Ionic Liquid 1-butyl-3-Methylmidazolium Chloride on the Soil Micro-Ecological System

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A B S T R A C T

Ionic liquids (ILs) are a group of new organic salts that exist as liquids at a relatively low temperature (<100^{0}C). In order to evaluate the influence of ionic liquid 1- butyl-3-methylimidazolium chloride ([Bmim]Cl) on the soil micro-ecological system, the toxicity of [Bmim]Cl to soil microorganisms and its impact on soil physico-chemical properties were investigated. Three soil samples, which were taken from the agricultural land, nursery land and the industrial land were used for this study. The toxicity test results showed that the [Bmim]Cl inhibited the growth of soil microorganisms including bacteria and *Actinomycetes*. The [Bmim]Cl also caused the pH change in the soil micro-ecological system. It suggests that the ionic liquid [Bmim]Cl would influence the soil micro- ecological system by inhibiting the growth of soil microorganisms and altering the soil physico-chemical properties when it contaminated the soil system.

Keywords

Ionic liquid, [Bmim]Cl, soil micro-ecological system, microorganism, physico-chemical property.

Introduction

Ionic liquids (I.L.s) are defined as low melting salts with a melting point below 100°C. These substances are variously called liquid electrolytes, ionic melts, ionic fluids, fused salts, liquid salts, or ionic glasses. Mostly they consist of organic cations and inorganic anions. Owing to their physicochemical properties, I.L.s is substitutes for conventional solvents. The structural heterogeneity within the pool of ionic liquid cations and anions provides an almost unlimited number of theoretically available substances complicating their hazard assessment (MacFarlane *et al*., 2001).

The discovery date of the "first" ionic liquid is disputed, along with the identity of its discoverer. Ethanolamminium nitrate (m.p. 52–55^{0}C) was reported in 1888 by Gabriel and Weiner (Walden, 1914). One of the earliest truly room temperature ionic liquids was ethylammonium nitrate (m.p. 12^{0}C), synthesized in 1914 by Walden (Chum *et al*., 1975). In the 1970s and 1980s ionic liquids based on alkyl-substituted imidazolium and pyridinium cations, with halide or tetrahalogenoaluminate anions, were initially developed for use as electrolytes in battery applications (Wilkes *et al*., 1982; Adam *et al*., 2004). Because of their distinctive properties, ionic liquids are attracting increasing attention in many fields, including organic chemistry, electrochemistry, catalysis, physical chemistry, and engineering.

The soil is home to a large proportion of the world's biodiversity. The links between soil organisms and soil functions are observed to be incredibly complex. We know that soil organisms break down organic matter, making nutrients available for uptake by plants and other organisms. The nutrients stored in the bodies of soil organisms prevent nutrient loss by leaching. Microbial exudates act to maintain soil structure, and earthworms are important in bioturbation. In balanced soil, plants grow in an active and steady environment. The mineral content of the soil and its heartiful structure are important for their well-being, but it is the life in the earth that powers its cycles and provides its fertility. Without the activities of soil organisms, organic materials would accumulate and litter the soil surface, and there would be no food for plants, bacteria and fungi which play key roles in maintaining a healthy soil (Tong *et al*., 2012).

Due to unique properties of Ionic Liquids, now-a-days they are used in almost all industries like Chemical industry, Pharmaceuticals, Cellulose processing, Fundamental Research, Algae processing, Dispersants, Gas handling, Nuclear fuel reprocessing, Solar thermal energy, Food and bioproducts, Waste recycling and Batteries. Besides this it was reported that from industrial waste Ionic Liquid gets spread all over in the environment like water and soil ecosystem. It is very hazardous to living organisms like bacteria and *Actinomycetes*. It disturbs the balance of soil micro-ecology (Plechkova and Seddon, 2008). Therefore aim of the present project was to study the influence of ionic liquid 1-butyl-3-methylimidazolium Chloride ([Bmim]Cl) on the Soil Micro-Ecological System.

Materials and Methods

Collection of Soil samples

The soil samples were collected from different sites, Agricultural soil, Nursery soil and Industrial soil in air tight bags. Sampling was carried out at 5 randomly chosen points from each site. Samples were collected at a depth of 1~15 cm, after removing the top layer. All samples from one site were mixed, then were air-dried, homogenized by sieving to less than 2 mm to separate roots and large objects, and stored in a polythene bag at room temperature for further use (Zheng *et al*., 2007).

Examination of physicochemical properties of soil samples (pH)

The soil suspensions of Agricultural soil, Industrial soil and Nursery soil were prepared in sterilized distilled water. The pH of the soil suspensions of all the three samples was measured by pH meter. All the three soil samples were incubated with IL BmimCl for 15 days at room temperature. The pH of these treated soil samples was measured by using pH meter after treatment with I.L. BmimCl (Tong *et al*., 2012).

Preparation of IL BmimCl solution

The solution of distilled water and Ionic Liquid 1-butyl-3-methylimidazolium chloride was prepared by taking 1g of I.L. in 1000ml sterile distilled water (Tong *et al*., 2010).

Enumeration of Bacteria by Serial Dilution and Spread Plate Method

The soil suspension was made in sterile distilled water and stirred it for 30 minutes. A series of 10-fold dilution for the sample was prepared starting with 10 ml of sterilized distilled water added to 1.0 g soil sample. The 9ml of distilled water was taken in 7 test tubes and autoclaved it at 15lbs pressure for 20 minutes. The test tubes were allowed to cool at room temperature. Later on 1ml of the soil suspension was transferred in first test tube by using micropipette near burner (in sterile environment). Now from the first test tube 1ml of the solution was taken by using micropipette and transferred to second test tube. Above transferring was repeated upto test tube No. 7. All the three samples were diluted. From these diluted samples 0.1 ml each of sample was taken on Nutrient Agar plates and Actinomycetes agar plates from the test tube having concentration 10^{-5}, 10^{-6} and 10^{-7} each. This 0.1 ml solution was spreaded uniformly by using spreader to get isolated colonies. All the plates were incubated for two days at 28^{0}C until colonies appeared. By counting CFU, the bacterial and *Actinomycetes* count of the soil was noted after performing serial dilution and spread plate technique (Zheng *et al*., 2007).

Bacterial and *Actinomycetes* Count after treatment with I.L. [Bmim]Cl

The 1ml of I.L. was added in 1g of soil from each of the three soil samples. This soil with I.L. was incubated at room temperature for 15 days. It was mixed with 9 ml sterile distilled water after treatment with I.L. Microbial count of this mixture of I.L. with soil samples were also taken in the same way by performing serial dilution and spread plate technique (Zheng *et al*., 2007).

Examination of Morphology of Wheat crop (*Triticum aestivum*)

The 6 pots were taken for the study having small hole for aeration purpose which were filled up with the soil samples (by dividing each sample in 2 pots). The 100ml solution of IL in distilled water was added in three pots with agricultural, nursery and industrial soil. In this way 3 pots without I.L. and 3 pots with I.L. were prepared. The Wheat seeds (around 40 seeds in each pot) were sown in it. All the pots were allowed to grow naturally by providing daily sufficient amount of water for 15 days. The two pots of each sample were compared for growth and change in morphology (with reference to the height) of wheat crops i.e. untreated and treated crops with Ionic Liquid (Pretti *et al*., 2006).

Results and Discussion

The soil microorganisms are an important element in soil micro-ecological system and play a vital role in it. The toxicity of [Bmim]Cl to the soil microorganisms is an important part of its influence on the soil micro-ecological system. In the present study three soil samples were taken for influence of Ionic Liquid [Bmim]Cl on soil microecological system. For this, isolation of micro-organisms including bacteria and *Actinomycetes* was carried out. The bacterial and *Actinomycetes* count was taken on the nutrient agar and *Actinomycetes* isolation agar respectively.

Enumeration of bacterial count by spread plate technique showed that bacterial count was decreased after the treatment of the same soil by I.L. In this the bacterial count of soil without I.L., for Agricultural soil (89 CFU/ml g^{-1}) followed by Nursery soil (48 CFU/ml g^{-1}) and Industrial soil (33 CFU/ml g^{-1}) was obtained. After treatment of same soil with Ionic Liquid [Bmim]Cl for 15 days, the bacterial count for Agricultural soil (52 CFU/ml g^{-1}) followed by Nursery soil (9 CFU/ml g^{-1}) and Industrial soil (5 CFU/ml g^{-1}) was obtained (Table 1).

The *Actinomycetes* count on different soil samples revealed that the count for Agricultural soil was (22 CFU/ml g^{-1}) followed by Nursery soil (20 CFU/ml g^{-1}) and Industrial soil (15 CFU/ml g^{-1}). Soil samples with I.L. showed decrease in *Actinomycetes* count i.e. Agricultural soil (8 CFU/ml g^{-1}) followed by Nursery soil (6 CFU/ml g^{-1}) and Industrial soil (5 CFU/ml g^{-1}) (Table 2).

The effect of Ionic Liquid [Bmim]Cl was also studied *invivo*. In the *invivo* study, the field test was performed by taking 6 pots, in which wheat grains were grown. Morphological analysis of the Wheat crop reported that there was excellent growth in Agricultural soil compared with that of the Nursery soil and Industrial soil. On the other hand the wheat crops treated with Ionic Liquid [Bmim]Cl has comparatively stunted growth and less height with respect to that of non-treated crops (Table 3) (Figure 1-3).

One of the important physico-chemical properties of soil is its pH. Change in pH affects the soil microecological system. The [Bmim]Cl itself is an organic compound and it has strong solubility to salts in the soil samples. The [Bmim]Cl also caused the pH change in the soil micro-ecological system. The pH of Agricultural soil, Industrial soil and Nursery soil was 7.4, 6.6 and 7.0 respectively. This pH gets changed when these soil samples were treated with Ionic Liquid [Bmim]Cl for 15 days and it becomes changed to 7.9, 8.1 and 7.8 respectively. The variations in the pH of the soil samples after treatment with I.L. have demonstrated the alkalinity in the treated soil samples (Table 4) (Graph 1).

The soil microorganisms are an important element in soil micro-ecological system and play a vital role in it. The toxicity of [Bmim]Cl to the soil microorganisms is an important part of its influence on the soil micro-ecological system. The present project was selected for these studies because Ionic Liquids are very interesting class of substances at present. I.L.s are used in almost all industries, hence are present in industrial wastes. Industrial wastes are released in water bodies and I.L. ultimately comes into the soil either in traces or in bulk that's why soil was selected as a sample. Soil is very important component of environment. If soil is affected in any aspect then this will also affects all the living beings directly or indirectly. From the present study I.L.s are found to be toxic to soil micro-ecological system.

In the present study three soil samples were taken for influence of Ionic Liquid [Bmim]Cl on soil microecological system. For this, isolation of micro-organisms including bacteria and *Actinomycetes* was carried out. The bacterial and *Actinomycetes* count was taken on the nutrient agar and *Actinomycetes* isolation agar respectively. It was found that bacterial count was decreased in all the soil samples containing I.L. which was correlated with the work of Docherty and Kulpa (2005) in which they reported that bacterial count decreases after treatment with I.L. The *Actinomycetes* count was also decreased in soil containing I.L. According to the work of Zheng *et al.*, (2007) *Actinomycetes* in the soil were highly affected by I.L.

The effect of Ionic Liquid [Bmim]Cl was also studied *invivo*. In the *invivo* study, the field test was performed by taking 6 pots, in which wheat grains were grown.

Morphological analysis of wheat crop states that the wheat crops treated with Ionic Liquid [Bmim]Cl has comparatively stunted growth and less height with respect to that of non-treated crops. Similar work was also carried out by Pretti *et al.,* (2006) they reported that morphology of the wheat plant was highly affected and quantity as well as quality of the grain was immensely decreased.

One of the important physico-chemical properties of soil was its pH. Change in pH affects the soil microecological system. The pH gets changed when these soil samples were treated with Ionic Liquid [Bmim]Cl for 15 days and get increased. The variations in the pH of the soil samples after treatment with I.L. have demonstrated the alkalinity in the soil samples. The similar type of research work was carried out by Tong *et al*., (2010). They reported that Ionic Liquid [Bmim]Cl is hazardous to soil microecological system. The study on Ionic Liquids was also carried out by Grades *et al*., (2003). They worked on aquatic ecosystem. *Invivo* study was also done and they showed that Ionic Liquids is harmful to living world and it disturbs the balance of environment.

Table.1 Bacterial Count on Nutrient Agar Plates

Sr.No.	Type of Soil	Bacterial Count CFU/ml g^{-1} (Before treatment with I.L.)	Bacterial Count CFU/ml g^{-1} (After treatment with I.L.)
1.	Agricultural Soil	89	52
2.	Nursery Soil	48	9
3.	Industrial Soil	33	5

Table.2 *Actinomycetes* Count on Actinomycetes Isolation Agar

Sr.No.	Type of Soil	*Actinomycetes* Count CFU/ml g^{-1} (Before treatment with I.L.)	*Actinomycetes* Count CFU/ml g^{-1} (After treatment with I.L.)
1.	Agricultural Soil	22	8
2.	Nursery Soil	20	6
3.	Industrial Soil	15	5

Table.3 Effect of IL [Bmim]Cl on Growth of Wheat Crop (*Triticum aestivum*)

Sr.No.	Type of Soil	Growth (Without I.L.)	Growth (With I.L.)
1.	Agricultural Soil	++++	+++
2.	Nursery Soil	+++	++
3.	Industrial Soil	++	+

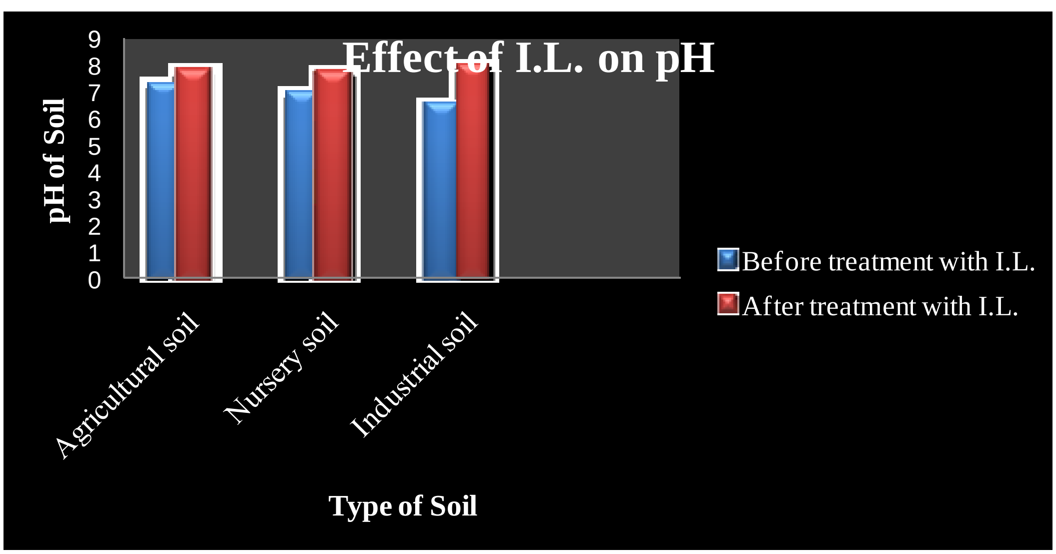
Where,

+ ⟶ Less Growth
++ ⟶ Good Growth
+++ ⟶ Moderate Growth
++++ ⟶ Excellent Growth

Table.4 Variation in pH of Soil

Sr.No.	Type of Soil	pH (Before treatment with I.L.)	pH (After treatment with I.L.)
1.	Agricultural Soil	7.4	7.9
2.	Nursery Soil	7.0	7.8
3.	Industrial Soil	6.6	8.1

Graph.1 Effect of Ionic Liquid on soil pH



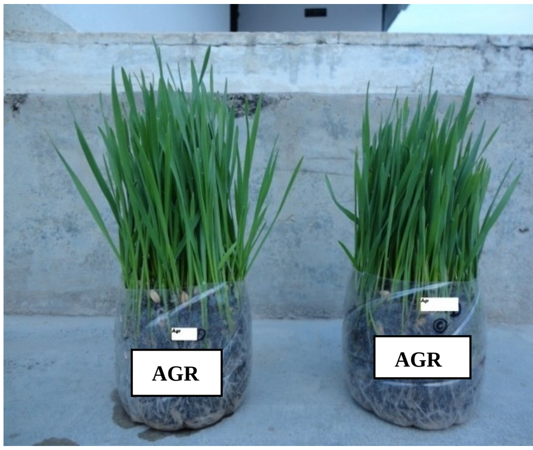


Figure 1: Wheat Crop in Agricultural Soil without Treatment of I.L. (Left) with Treatment of I.L. (Right)

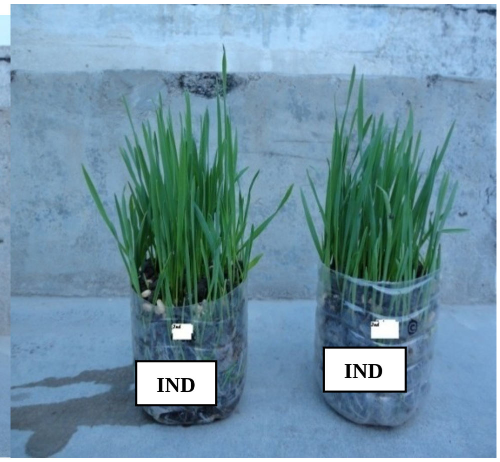


Figure 2: Wheat Crop in Industrial Soil without Treatment of I.L. (Left) with Treatment of I.L. (Right)

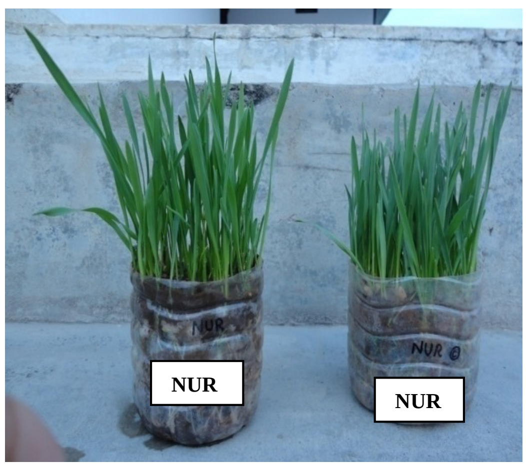


Figure 3: Wheat Crop in Nursery Soil without Treatment of I.L. (Left) with Treatment of I.L. (Right)

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