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Assessing the influence of 1-dodecyl-3-methylimidazolium chloride on soil characteristics and *Vicia faba* seedlings

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ABSTRACT

Imidazolium-based ionic liquids (ILs) have attracted increasing attention in recent years. The IL 1-dodecyl-3-methylimidazolium chloride ([C_{12}mim]Cl) has been widely used in the chemical industry. In this study, the influence of [C_{12}mim]Cl on *Vicia faba* seedlings, soil physicochemical properties and soil enzyme activities was investigated for the first time. Meanwhile, the variation of [C_{12}mim]Cl concentrations in soil was monitored during the exposure period. The present results showed that the concentration of [C_{12}mim]Cl remained stable in the tested soil with a change rate of no more than 10% during the exposure period. The 50% effective concentration (EC_{50}) values for shoot length, root length and dry weight were 188, 69 and 132 mg kg^{-1}, respectively. At 200 mg kg^{-1} and 400 mg kg^{-1}, [C_{12}mim]Cl had significant influence on soil organic matter content, pH value and conductivity value. At 40 mg kg^{-1}, the reactive oxygen species (ROS) levels were obviously enhanced, resulting in oxidative stress effects in *Vicia faba* seedling leaves. Additionally, the soil enzyme activities changed significantly at 40 mg kg^{-1}.

1. Introduction

In recent years, people have paid increasing attention to ionic liquids (ILs) as part of the search for a kind of environmentally friendly solvent. ILs are a broad class of novel semi-organic salts with a melting point below 100 °C and are completely composed of cations and anions (Gouveia et al., 2014). ILs are considered an alternative to conventional volatile organic solvents due to their unique properties, such as non-volatility, high thermal stability and high solvent capacity (Yan et al., 2015). Therefore, ILs have a broad range of applications in the fields of catalysis, organic synthesis, electrochemistry and separation processes (Ma et al., 2015).

Although ILs have been widely used in all walks of life, relatively little is known regarding their toxicological information (Hezave and Dorostkar, 2013; Made et al., 2015; Zhou et al., 2015). As a kind of promising solvent, ILs will eventually be released into the environment due to extensive use. Moreover, the exposure concentrations of ILs in the environment may be very high due to accidental spills or effluents. ILs may be a water contaminant due to their strong solubility and stability in water. It has been widely reported that ILs have toxic effects on aquatic organisms even at low concentrations (Du et al., 2012; Dong et al., 2013). Nonetheless, ILs will eventually get into the soil in various ways. Therefore, ILs may also be a kind of soil contaminant, and we must consider the influence of ILs on soil environments.

Plants are an important component of the soil ecosystem, and the growth condition for plants is related to soil quality. Although some studies have reported the phytotoxicity of ILs, few of them have studied this topic from the molecular level (Matzke et al., 2008; Biczak et al., 2014). In addition, there are few studies focusing on the influence of ILs on soil physicochemical properties and soil enzyme activities. Therefore, studying the influences of ILs on both plant growth and soil characteristics can be a better way to investigate the toxicity of ILs.

The imidazolium-based IL 1-dodecyl-3-methylimidazolium chloride ([C_{12}mim]Cl) has been widely used in the chemical industry in recent years (Hezave and Dorostkar, 2013). However, few studies focus on the influence of [C_{12}mim]Cl in the environment, especially in the soil environment. *Vicia faba* is one of the main crops in the world and has been widely used in toxicological testing (Song et al., 2009).

Therefore, the ecotoxicity of [C_{12}mim]Cl on *Vicia faba* seedlings was studied in the present study from the molecular level. Meanwhile, the influence of [C_{12}mim]Cl on the tested soil was evaluated. The main purpose of the present study was to evaluate the risk of [C_{12}mim]Cl in

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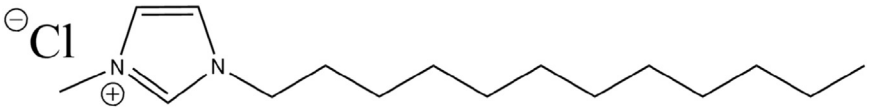

Fig. 1. The chemical structural formula of 1-dodecyl-3-methylimidazolium chloride. The image was completed using the ChemBioDraw software (version 11.0).

soil environments.

2. Materials and methods

2.1. Materials

[C_{12}mim]Cl with 99% purity was provided by Chengjie Chemical Co. Ltd. (Shanghai, China) (Fig. 1).

Vicia faba seeds were provided by the College of Life Science, Shandong Agricultural University (Taian, China).

The tested soil was collected from the test field of Shandong Agricultural University (Taian, China).

2.2. Experimental design

[C_{12}mim]Cl is a new type of compound, and the database of background environmental values is limited. The concentrations used in the present study were selected according to the toxicology experiment method. First, range-finding tests were carried out to assess the toxicity of [C_{12}mim]Cl. The concentrations were set to 0, 10, 50, 100, 200 and 400 mg kg^{-1} to investigate the influence of [C_{12}mim]Cl on the growth of *Vicia faba* seedlings after 10 d exposure, and then the 50% effective concentration (EC_{50}) values were calculated. Meanwhile, the physicochemical characteristics of the tested soil were assayed before and after exposure to [C_{12}mim]Cl, according to Li et al. (2009). The pH and the conductivity of the tested soil were determined using a pH-meter (CG842, Schott, Germany) and a conductivity meter (FE30, Mettler-Toledo, China), respectively. Subsequently, the concentrations were set to 0, 1, 10, 20 and 40 mg kg^{-1} according to the EC_{50} values to investigate the influence of [C_{12}mim]Cl on the physicochemical properties of *Vicia faba* seedlings and the activity of soil enzymes after 10 d exposure.

The *Vicia faba* seed was sterilized using a 0.1% sodium hypochlorite solution and dormancy broken by soaking in water for 12 h. The appropriate amount of [C_{12}mim]Cl solution was sprayed onto the soil (1000 g). Subsequently, the *Vicia faba* seeds were planted in the soil and cultured at 22 °C for 14 h in light and 18 °C for 10 h in dark with a light intensity of 200 μmol m^{-2} s^{-1}.

2.3. Determination of [C_{12}mim]Cl concentrations

The [C_{12}mim]Cl in the tested soil was extracted using extracting solution, which contained methanol (90%), saturated ammonium chloride (10%) and ethylenediamine tetraacetic acid disodium salt (1 g L^{-1}). After sonicating for 1 h, the samples were shaken for 2 h at 160 rpm and centrifuged for 5 min at 4000 rpm. Finally, the samples were filtered using a 0.22-μm syringe filter.

The [C_{12}mim]Cl concentrations were determined using a high performance liquid chromatography (HPLC, Agilent 1100, USA) method as stated by Zhou et al. (2015). A C_{18} column (Eclipse XDB-C_{18}, 4.6 × 250 mm, 5 μm) was used in the present study to separate samples. The column temperature, injection volume and flow rate were 30 °C, 10 μL, and 0.8 mL min^{-1}, respectively. The mobile phase was acetonitrile (50%) and 25 mM of phosphate buffer in 0.5% triethylamine (50%, pH 3.0).

2.4. Determination of Hill reaction activity and proline content

The activity of the Hill reaction was measured using the method of Liu et al. (2013). Chloroplasts were extracted using extracting buffer (pH 7.6) containing 0.01 M NaCl, 0.05 M Tris and 0.4 M sucrose. The chloroplast solution was added to a tube which contained 1 mM of $K_3Fe(CN)_6$, 5 mM of $MgCl_2$, 10 mM of NaCl, 50 mM of Tris and distilled water. The reaction was stopped by adding 0.2 mL of 10% trichloroacetic acid (TCA) after 1 min of light exposure. Subsequently, 0.7 mL of reacting solution was added to another tube containing 0.01 M $FeCl_3$, 0.05 M phenanthroline, 0.2 M sodium citrate and distilled water. The tube was placed in the dark for 15 min, and then the absorbance of the sample was measured at 520 nm.

The proline content was determined using the method of Bates et al. (1973). The proline in leaves was extracted with 3% sulfosalicylic acid. The extracting solution reacted with 2.5% acid ninhydrin and glacial acetic acid for 30 min. Subsequently, 4 mL of toluene was added, and the absorbance of the toluene fraction was read at 520 nm.

2.5. Determination of ROS levels

The ROS levels in *Vicia faba* seedling leaves were determined according to the method of Liu et al. (2016). *Vicia faba* seedling leaves were randomly selected from each treatment. The petioles of *Vicia faba* seedling leaves were immersed in a 6-mM nitroblue tetrazolium solution and 1 mg mL^{-1} 3,3-diaminobenzidine solution. Subsequently, the leaves were placed under light for 8 h and then discolored using ethanol. The results were recorded using a digital camera (Canon, 760D).

2.6. Determination of oxidative damage degree

The malondialdehyde (MDA) content was assayed using the method of Song et al. (2007). The leaves were homogenized in 1% TCA solution and centrifuged at 10,000 g for 10 min. Subsequently, the supernatant was reacted with 0.5% TBA solution for 30 min, and the absorbance was read at 532 nm and 600 nm.

The protein carbonyl (PCO) content was assayed using the method of Levine et al. (1990). The protein was denatured using 20% TCA and centrifuged at 10,000 rpm for 3 min. The precipitate was dissolved in 10 mM of 2,4-dinitrophenylhydrazine (DNPH) buffer. Subsequently, the pellet was washed three times using ethanol and ethyl acetate (1:1, v/v), and re-dissolved in 6 M guanidine hydrochloride. The absorbance of the mixture was recorded at 366 nm.

The DNA damage degree was determined using the single cell gel electrophoresis method (Song et al., 2009). A slide was prepared using the three layers of glue method. After electrophoresing at 25 V (300 mA) for 15 min, the slide was washed using Tris buffer (0.4 M, pH 7.5). Finally, 13 μg mL^{-1} of ethidium bromide (EB) solution was added onto the slide, and the slide was analyzed using a fluorescence microscope (Olympus, BX71).

2.7. Antioxidant enzyme gene expression and antioxidant compound content

The expression profiles of one housekeeping and two target genes were quantified according to Sta et al. (2014). The RNA in *Vicia faba* seedling leaves was extracted and purified using an RNApure plant kit (Cwbio, China). The concentration of RNA was measured using a nucleic acid concentration meter (Nanodrop 2000, USA). Subsequently, the purified RNA was reversely transcribed using a 1st strand cDNA synthesis kit (Cwbio, China). PCR reactions were prepared using a 2 × Sybr Green qPCR Mix kit (Aidlab, China) and performed on a Step One Real-Time PCR System (ABI, USA). The gene expression level was