Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 2010, 22(12) 1974–1979
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Toxic effects of 1-methyl-3-octylimidazolium bromide on the wheat seedlings

Ping Liu[1], Yifeng Ding[1], Haiying Liu[1], Liping Sun[1], Xiaoyu Li[1,*], Jianji Wang[2,3,*]

1. College of Life Sciences, Henan Normal University, Xinxiang 453007, China. E-mail: liuping5812@sina.com
2. Key Laboratory for Environmental Pollution Control, Xinxiang 453007, China
3. College of Chemistry and Environmental Science, Henan Normal University, Xinxiang 453007, China

Received 01 December 2009; revised 30 March 2010; accepted 06 May 2010

Abstract

The toxic effects of 1-methyl-3-octylimidazolium bromide ([C_8mim]Br) on wheat seedlings were evaluated. Wheat seedlings were cultivated in aqueous solution with [C_8mim]Br at different concentrations (0, 1, 2, 4, 6, 8 mg/L). The contents of photosynthetic pigment and proline, peroxidation of membrane lipid, and activities of antioxidation enzymes (superoxide dismutase, catalase, peroxidase and ascorbate peroxidase) in leaves were measured on day 7 after treatment with [C_8mim]Br. The results showed that [C_8mim]Br significantly decreased the contents of photosynthetic pigments, activities of antioxidant enzymes in the wheat leaves and in dry weight of seedlings, while increased the proline content and membrane lipid peroxidation. The results suggested that [C_8mim]Br can inhibit photosynthesis and lead to oxidative stress to wheat seedlings.

Key words: [C_8mim]Br; wheat seedlings; photosynthetic pigments; antioxidant enzymes; membrane lipid peroxidation
DOI: 10.1016/S1001-0742(09)60348-X

Introduction

Ionic liquid solutions (ILs), defined as pure ionic compounds with melting points below 100°C, represent a dramatic success for their applications in industry and engineering chemistry at the beginning of the 21st century (Pernak et al., 2004). They have drawn a great deal of attention due to their unique properties ranging from excellent solution ability for lots of compounds, high thermal stability to immeasurably low vapour pressure (Couling et al., 2006). Now, ILs were applied in many aspects, including many reactions, solvent in liquid-liquid extractions, and as electrolytes in batteries. In 2003, Badische Anilin- and Soda- Fabrik (BASF) company developed the industrial preparation of ionic liquid for the first time, and predicted that ILs would be put into use extensively in industry as one of most promising green solvent (Rogers and Seddon, 2003). However, with the development of research and application continue rapidly, the people began to pay more and more attention to the toxicity of ionic liquid and the environment problems caused by ionic liquid (Hu et al., 2005). Several recent studies have proved that ILs have toxic effects on algae, microorganisms, and animals (Ranke et al., 2004; Skladanowski et al., 2005; Stepnowski et al., 2004; Stock et al., 2004; Couling et al., 2006). Once applied on large-scale, it will not be long before ILs become a permanent component of industrial effluents. It is their stability that ILs could go through traditional water treatment systems to become persistent pollutants of natural aquatic environment and, in turn, enter soil by irrigation or subsurface groundwater. Therefore, it is critical and pressing to determine the toxicity of existing ILs before they are released into environment.

Wheat is one of main crops in the world. It is most frequently consumed cereals and its cultivation area covers about 22% of the total arable lands in China. Therefore, wheat seedlings were used as the testing material and the toxicity of [C_8mim]Br was estimated.

1 Materials and methods

1.1 Materials

[C_8mim]Br, a kind of the ILs, was prepared according to the method by Bonhôte et al., (1996). ^{1}H-NMR spectra data for this IL are in good agreement with those reported in literature (Chun et al., 2001). All other reagents were of analytical grade. The wheat variety used for evaluation is the Zhengmai 9023 (*Triticum aestivum*).

1.2 Experiment design and toxicity determination

Prior to germination, all seeds were sterilized in 0.1% $HgCl_2$ for 15 min and then thoroughly washed with distilled water. Two pieces of filter paper were placed in Petri plate (15 cm diameter) and moistened with 15 mL of treatment solution. Controls were maintained with 15 mL deionized water. Each plate with fifty seeds in was covered by lid and was incubated in the dark at 23°C. Seeds were

* Corresponding author.
E-mail: lixiaoyu65@263.net; jwang@henannu.edu.cn

considered germinated when both the plumule and radicle extended to more than 3 mm from their junction.

The treatment concentration of [C_8mim]Br for wheat seedlings was set to 0, 1, 2, 4, 6 and 8 mg/L according to the preliminary tests on the inhibitory rate of wheat germination by [C_8mim]Br. After being kept in the dark for two days, the germinated seeds were transferred and arrayed onto fixed nylon net adjacent to the solution with different [C_8mim]Br concentrations above in culture jar and with the 0 mg/L as the control. Wheat was grown in a greenhouse under controlled conditions with a shift cycle of 14 hr/25°C on day and 10 hr/20°C at night, and photo flux intensity of 70–80 μmol/(m^2·sec). The solutions were daily renewed to keep [C_8mim]Br concentration stable.

All the treatments were replicated three times. After 7 days of exposure, the content of pigments and proline, activities of antioxidant enzymes, the degree of membrane peroxidation in leaves and dry weight of seedlings were determined.

1.3 Pigment assay

The content of photosynthetic pigments (chlorophyll *a* and *b*, total chlorophyll, and carotenoids) was determined with UV-Vis spectrophotometer (model UV–9600, Rayleigh, Beijing, China). The pigments of seedling leaves were extracted with 15 mL of 80% acetone according to the method reported by Arnon et al. (1949). The contents of chlorophyll *a* and *b*, total chlorophyll, and carotenoids could be calculated by

$$C_{\text{Chl-}a} = 15(12.7A_{663\text{ nm}} - 2.69A_{645\text{ nm}})/0.2 \quad (1)$$

$$C_{\text{Chl-}b} = 15(22.9A_{645\text{ nm}} - 4.68A_{663\text{ nm}})/0.2 \quad (2)$$

$$C_{\text{Chl-tot}} = C_{\text{Chl-}a} + C_{\text{Chl-}b} = 15(20.2A_{645\text{ nm}} + 8.02A_{663\text{ nm}})/0.2 \quad (3)$$

$$C_{\text{car}} = 15(4.70A_{440\text{ nm}})/0.2 - 0.27C_{\text{Chl-tot}} \quad (4)$$

where, $C_{\text{Chl-}a}$, $C_{\text{Chl-}b}$, $C_{\text{Chl-tot}}$ and C_{Car} are the contents of chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids, and $A_{663\text{ nm}}$, $A_{645\text{ nm}}$, $A_{440\text{ nm}}$ are the absorbances at 663, 645, 440 nm, respectively.

1.4 Proline content

Free proline content was determined according to Bates et al. (1973). Leave samples (0.2 g) from each group were first homogenized in 5 mL of 3% (*W/V*) sulphosalycylic acid and then filtered through filter paper. After addition of 2 mL acid ninhydrin and 2 mL glacial acetic acid, the mixture was heated at 100°C for 1 hr in water bath. Reaction was then terminated in ice bath. The mixture was extracted with toluene, and red toluene solution was obtained. The absorbance of red solution was read at 520 nm. Proline concentration was determined from calibration curve and expressed as mg proline/g fresh weight (fw).

1.5 Lipid peroxidation and electrolyte leakage

Malondialdehyde (MDA) is an end product of lipid peroxidation. Following the method of He et al. (2003), it can be quantitative reacted with thiobarbituric acid (TBA) (Yuanfan, Shanghai, China). Fresh plant sample (0.1 g) was homogenized in 2 mL of 0.2 mol/L citrate-phosphate buffer (pH 6.5) containing 0.5% Triton X-100, with mortar and pestle. The homogenate was filtered through two layers of filter paper and centrifuged for 15 min at 6×10^3 r/min. One milliliter of the supernatant fraction was added to an equal volume of 20% (*W/V*) trichloroacetic acid (TCA) containing 0.5% (*W/V*) TBA. The mixture was heated at 95°C for 30 min and then quickly cooled in ice bath. Afterwards, the mixture was centrifuged at 6×10^3 r/min for 15 min; the absorbance of the supernatant was measured at 450, 532 and 600 nm. The lipid peroxides were expressed as nmol MDA/g fw.

Electrolyte leakage was measured according to Sairam et al. (1997). The leaves were washed with deionized water and placed in test tubes containing 30 mL deionized water at 30°C for 4 hr in darkness. Each sample was centrifuged and the initial electrical conductivity (EC_1) of the supernatant was measured by conductometer (DDS-11A, Shanghai, China). Then, the sample was put in the 100°C water bath for 15 min to release all electrolytes. The sample was cooled, centrifuged and the final electrical conductivity (EC_2) of supernatant was measured. Electrolyte leakage, expressed as a percentage of total electrolytes, was calculated with the formula: $EC_1/EC_2 \times 100\%$.

1.6 Enzyme activity assay

For enzyme extractions, 0.5 g leaves were homogenized in ice bath with 2 mL of 50 mmol/L sodium phosphate buffer (pH 7.0) containing 1 mmol/L EDTA and 2% (*W/V*) polyvinylpolypyrrolidone (PVP) (Xilong, Guangdong, China). The extraction procedure was carried out at 4°C. Homogenates were then centrifuged at 4°C for 20 min at 1.3×10^4 r/min, and supernatants were used for enzyme activity determination.

Superoxide dismutase (SOD) activity was measured with UV-Vis spectrophotometer as described by Beyer and Fridovich (1987). The assay mixture (3.0 mL) contained 1.5 mL of 50 mmol/L sodium phosphate buffer (pH 7.0), 0.3 mL of 130 mmol/L L-methionine, 0.3 mL of 750 μmol/L nitrobluetetrazolium (NBT) (Sinopharm, Shanghai, China), 0.3 mL of 100 μmol/L EDTA-Na_2, 0.3 mL of 20 μmol/L riboflavin, 0.25 mL distilled water and 50 μL enzyme extract. The reaction was initiated by addition of riboflavin and exposed under photon flux of 55 μmol/(m^2·sec) at 25°C for 5 min. In this assay, 1 unit (U) of SOD is defined as the amount required to inhibit the photoreduction of NBT by 50%.

The activity of catalase (CAT) was assayed according to the method of Chance and Maehly (1955) with some modifications. A reaction mixture contained 50 μL of 750 mmol/L H_2O_2, 2.9 mL of 50 mmol/L sodium phosphate buffer (pH 7.0) and 50 μL enzyme extract. This mixture was incubated at 25°C for 5 min and catalase activity was determined by monitoring the disappearance of H_2O_2 by measuring the decrease in absorbance at 240 nm. Activity was expressed as $\Delta A_{240\text{ nm}}$/(min·g fw).

Peroxidase (POD) activity was measured by the method of Chance and Maehly (1955) with some modification. The reaction mixture containing 50 μL enzyme extract, 100

μL of 40 mmol/L H_2O_2, 100 μL of 30 mmol/L guaiacol, and 2.75 mL of 50 mmol/L sodium phosphate buffer (pH 7.0). The increase in absorbance was recorded at 470 nm. One unit of POD activity was expressed as $\Delta A_{470\,nm}$ (increasing) 0.01/(min·g fw).

Ascorbate peroxidase (APX) activity was assayed following the method of Zhu et al. (2004). The total reaction mixture volume of 3 mL, contained 2.9 mL of 50 mmol/L sodium phosphate buffer (pH 7.0), 0.1 mmol/L EDTA, 50 μL of 15 mmol/L ascorbate, and 50 μL enzyme extract. Activity was expressed as $\Delta A_{290\,nm}$/(min·g fw).

1.7 Dry weight of seedlings determination

Seedlings on day 7 after treatment (DAT) were oven-dried at 105°C for 8 hr and at 80°C for 40 hr, and then the dry weights were weighed.

1.8 Statistical analysis

With SPSS11.5 software, data were statistically analyzed to calculate average, standard deviations, correlation and regression. Differences among means were detected using one way ANOVA.

2 Results

2.1 Effect of [C_8mim]Br on pigment content in leaves

The pigment levels of seedling leaves for different [C_8mim]Br treatments are listed in Table 1. At the highest levels of [C_8mim]Br (8 mg/L), chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid content were 1.81, 0.31, 2.12 and 1.18 μg/g fw, which were 56.59%, 59.21%, 56.99% and 60.40% lower than those of the controls on the 7th DAT, respectively. Decreases in the chlorophyll and carotenoid content indicated that [C_8mim]Br exhibited toxic effect on the leaves of wheat seedlings.

2.2 Effect of [C_8mim]Br on dry weight of wheat seedlings

Table 1 indicates that [C_8mim]Br decreased dry weights markedly ($p < 0.01$) in all the treatment. The highest concentration (8 mg/L) resulted in dry weight 47.82% lower than that of control on the 7th DAT.

2.3 Effect of [C_8mim]Br on proline content

The content of proline in wheat leaves markedly increased on 7th DAT, compared with the control ($p < 0.01$), the levels of proline in the leaves of wheat seedlings treated with 4, 6 and 8 mg/L of [C_8mim]Br increased by 3, 7 and 11-fold of control, respectively (Fig. 1).

2.4 Effect of [C_8mim]Br on lipid peroxidation and membrane permeability

Elevated lipid peroxidation was observed in the [C_8mim]Br-treated seedlings. MDA contents in leaves significantly increased with [C_8mim]Br concentration (Fig. 2a).

Except for 1 mg/L treatment, [C_8mim]Br also promoted leaf membrane leakage expressed as relative electrical conductivity in all treated wheat seedlings (Fig. 2b, $p < 0.01$), indicating that some concentration of [C_8mim]Br (over 1 mg/L) could strongly induce oxidative damage or cell membrane disruption to wheat seedlings.

2.5 Effect of [C_8mim]Br on activities of antioxidant enzymes

As compared with control, after 7 day IL-treatment, the activities of SOD, CAT, POD and APX in the leaves of wheat seedlings decreased (Fig. 3, $p < 0.05$). The SOD activity decreased gradually in seedlings treated with 1 and 2 mg/L [C_8mim]Br, whereas significantly decreased with 6 and 8 mg/L [C_8mim]Br. The similar trend happened to CAT and POD. SOD was 15.2% and 20.6% lower (Fig. 3a) for samples treated with 6 and 8 mg/L [C_8mim]Br, and CAT activity in leaves was 59.3% and 63.8% (Fig. 3b), and POD was 61.9% and 64.0% lower than that of control, respectively (Fig. 3c). The activity of APX in the leaves of wheat seedlings treated with 6 and 8 mg/L [C_8mim]Br decreased markedly on 7th DAT (Fig. 3d, *p*

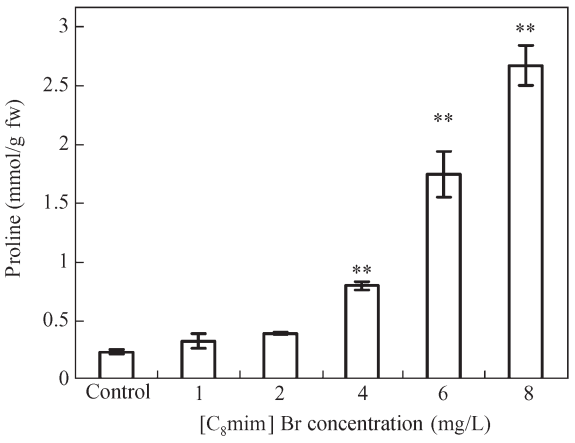

Fig. 1 Effect of [C_8mim]Br on the proline content in wheat seedlings. Values are means of three replicates ± SD. ** significant ($p < 0.01$), * significant ($p < 0.05$) compared to control.

Table 1 Effects of [C_8mim]Br on leaf pigment content and dry weight of wheat seedlings

Treatment (mg/L)	$C_{Chl\text{-}a}$ (μg/g fw)	$C_{Chl\text{-}b}$ (μg/g fw)	$C_{Chl\text{-}tot}$ (μg/g fw)	C_{Car} (μg/g fw)	Dry weight (mg)
Control	4.17 ± 0.37	0.76 ± 0.09	4.93 ± 0.51	2.98 ± 0.46	14.43 ± 0.47
1	3.48 ± 0.11**	0.58 ± 0.01*	4.06 ± 0.11**	2.31 ± 0.15**	13.10 ± 0.80**
2	2.66 ± 0.10**	0.39 ± 0.04**	3.04 ± 0.11**	1.65 ± 0.12**	9.63 ± 0.50**
4	2.43 ± 0.06**	0.36 ± 0.03**	2.79 ± 0.07**	1.60 ± 0.13**	8.63 ± 0.31**
6	2.09 ± 0.04**	0.35 ± 0.04**	2.45 ± 0.03**	1.34 ± 0.01**	8.03 ± 0.21**
8	1.81 ± 0.09**	0.31 ± 0.04**	2.12 ± 0.08**	1.18 ± 0.25**	7.53 ± 0.21**

Values are means of three replicates ± SD. ** significant ($p < 0.01$), * significant ($p < 0.05$) compared to control.
fw: fresh weight.

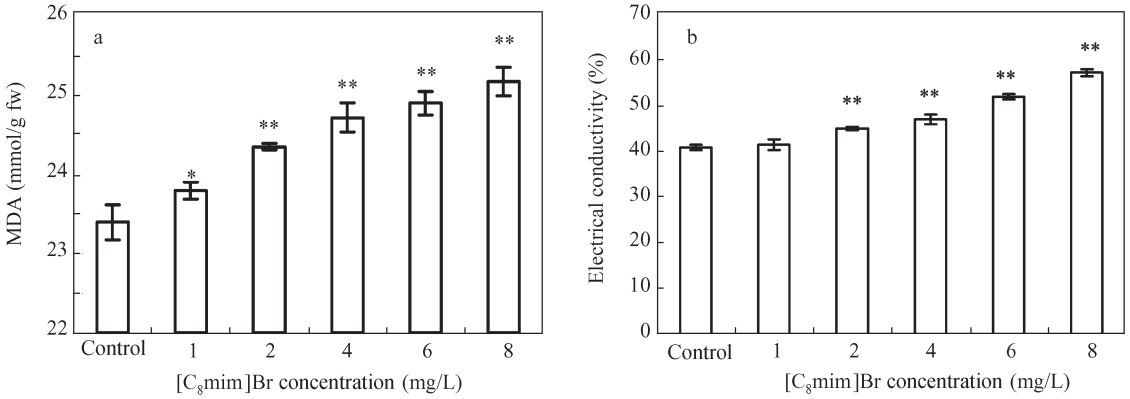

Fig. 2 Effect of [C_8mim]Br on MDA content (a) and electrical conductivity (b). Values are means of three replicates ± SD. ** significant ($p < 0.01$), * significant ($p < 0.05$) compared to control.

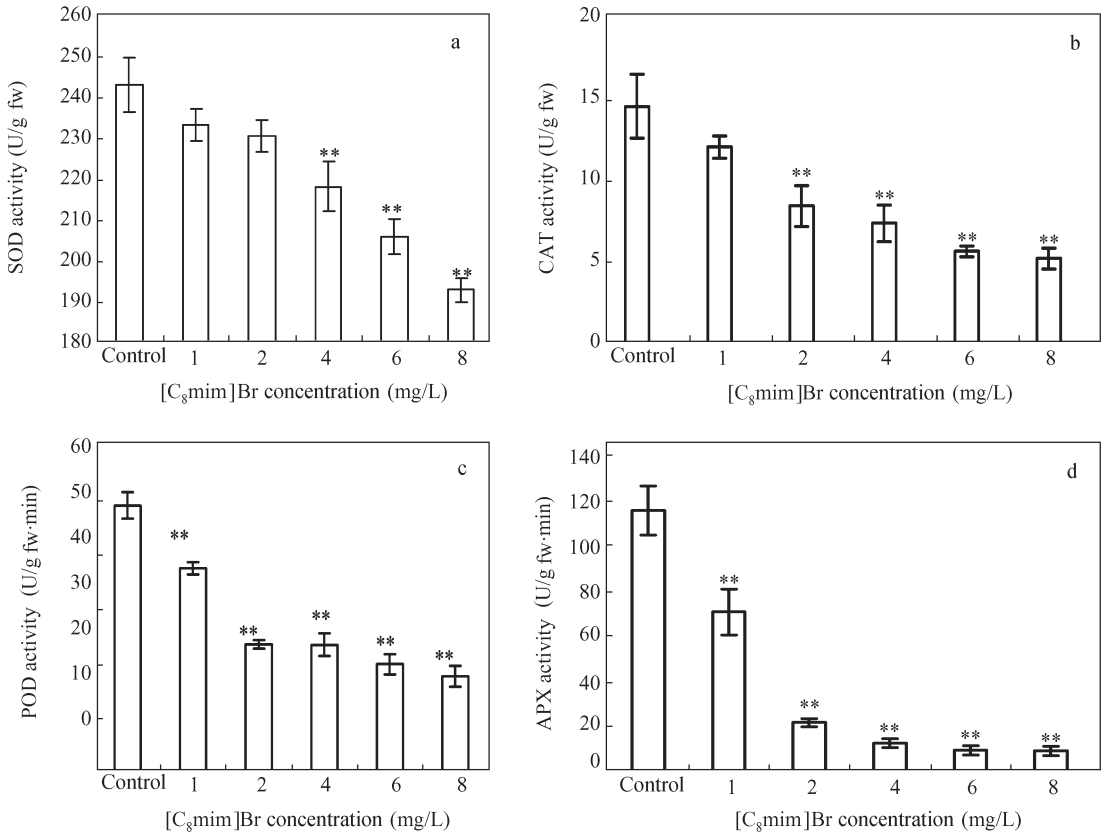

Fig. 3 Effects of [C_8mim]Br on SOD (a), CAT (b), POD (c) and APX (d) in the leaves of wheat seedling. Values are means of three replicates ± SD. ** significant ($p < 0.01$), * significant ($p < 0.05$) compared to control.

< 0.01) and was 91.7% and 91.9% lower than those of control respectively.

3 Discussion

The contents of total chlorophyll, chlorophyll *a*, and chlorophyll *b* in wheat seedlings decreased after [C_8mim]Br treatment and which might be due to the toxicity of [C_8mim]Br to chlorophyll biosynthesis. [C_8mim]Br can significantly reduce the activity of antioxidant enzymes, and result in massive accumulation of reactive oxygen species, give rise to quick degradation of photosynthetic pigments (Rai et al., 2004). In addition, carotenoids can serve as nonenzymatic oxygen radical scavengers (Qin et al., 2006) and whose decrease, in turn, increase the toxicity of [C_8mim]Br to lipid membrane.

As we know, proline accumulates in plant tissues when they are under a broad range of stress conditions such as water shortage, salinity and high-radiation intensity. The increase of proline content in the leaves of wheat seedlings under [C_8mim]Br (> 4 mg/L) stress might be protective reaction to cope with [C_8mim]Br toxicity. Proline has multiple functions, e.g., it can be osmotic regulator, scavenger of free radicals, protector of cytoplasmic enzymes, source

of nitrogen and carbon for growth, stabilizer of membranes and a sink for energy to regulate redox potential (Rout and Shaw, 1998). Its accumulation enhances the abilities of plants to regulate cytoplasmic osmotic pressure, to protect enzymes against stress, and to stabilize protein synthesis.

Lipid peroxidation induced by free radicals is important characteristics in membrane deterioration of plant cell (McCord, 2000). MDA is a cytotoxic product of lipid peroxidation (Rai et al., 2004) and a sensitive diagnostic indicator of oxidative injury (Janero, 1990). [C_8mim]Br treatment may cause loss of differential permeability of cellular membranes and in turn lead to increase in ion leakage and cell membrane disruption in the leaves of wheat seedlings. The increase in electrical conductivity of ion leakage suggested that [C_8mim]Br can bring some damages to the cell membrane structure of leaves.

The direct and rapid response of plant to toxicants is reduction in antioxidant enzyme activity at cellular level. There are many defense enzymes which make up antioxidant systems, such as SOD (E.C.1.15.1.1), POD (E.C.1.11.1.7), CAT (E.C.1.11.1.6), and APX (E.C.1.11.1.11). The protective systems can scavenge active oxygen and removal of toxic compounds, to prevent cell from damage (Cho and Park, 2000). They also play an important role for plants in protecting cellular membranes and organelles from various environmental stresses and damages.

Activity declines of SOD, CAT, POD and APX in [C_8mim]Br-treated plants strongly suggested that antioxidant enzymes become incompetent in protection, and H_2O_2 and toxic peroxides could not promptly be removed or changed. And it is natural for the cell membrane to be destroyed or disrupted. This point can also be confirmed by correlation between decrease in protection enzyme activity and increase in MDA content.

Seedling weight can reflect the situation of plant growth quite well. [C_8mim]Br reduced significantly seedling weight with the increase in concentration, indicating that [C_8mim]Br can affect the metabolic activity and substance the accumulation of seedlings.

4 Conclusions

In conclusion, [C_8mim]Br treatment can cause the decrease in chlorophyll content and the decline in activities of antioxidant enzymes in leaves and reduce dry weight of seedlings at last. These results indicated that [C_8mim]Br has obviously toxic effects on wheat seedling growth and development. It is important for agricultural production and environmental protection departments and relevant departments pay enough attention to its impact on agro-ecological system safety.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 20273019, 20573034), the Innovation Project for University Prominent Research Talents of Henan, China (No. 2006KYCX021), and the Project-sponsored by SRF for ROCS, SEM (No. [2006] 331).

References

Arnon D I, 1949. Copper enzyme in isolated chloroplasts: polyphenol oxidase in *Beta vulgaris*. *Plant Physiology*, 24(1): 1–15.

Bates L S, Waldren R P, Teare I D, 1973. Rapid determination of free proline for water stress studies. *Plant Soil*, 39(1): 205–207.

Beyer W F, Fridovich I, 1987. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. *Analytical Biochemistry*, 161(2): 559–566.

Bonhôte P, Dias A P, Papageorgiou N, Kalyanasundaram K, Grätzel M, 1996. Hydrophobic, highly conductive ambient-temperature molten salts. *Inorganic Chemistry*, 35(5): 1168–1178.

Chance B, Maehly C, 1955. Assay of catalase and peroxidases. *Methods in Enzymology*, 2(11): 764–775.

Chun S, Dzyuba S V, Bartsch R A, 2001. Influence of structural variation in room-temperature ionic liquids on the selectivity and efficiency of competitive alkali metal salt extraction by a crown ether. *Analytical Chemistry*, 73(15): 3737–3741.

Cho U H, Park J O, 2000. Mercury-induced oxidative stress in tomato seedlings. *Plant Science*, 156(1): 1–9.

Couling D J, Bernot R J, Docherty K M, Dixona J K, Maginn E J, 2006. Assessing the factors responsible for ionic liquid toxicity to aquatic organisms via quantitative structure-property relationship modeling. *Green Chemistry*, 8(1): 82–90.

He B, Ye H B, Yang X E, 2003. Effects of Pb on chlorophyll contents and antioxidant enzyme activity in leaf for Pb accumulating and non-accumulating ecotypes of *Sedum Alfredii* (Hance). *Journal of Agro-Environment Science*, 22(3): 274–278.

Hu X S, Yu J, Xia H S, Liu Q F, Liu H Z, 2005. Green synthesis and the environmental properties of ionic liquids. *Chemistry*, 68(12): 906–910.

Janero D R, 1990. Malondialdehyde and thiobabituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Journal of Free Radicals in Biology and Medicine*, 9(6): 515–540.

McCord J M, 2000. The evolution of free radicals and oxidative stress. *American Journal of Medicine*, 108(8): 652–659.

Pernak J, Goc I, Mirska I, 2004. Anti-microbial activities of protic liquids with lactate anion. *Green Chemistry*, 6(7): 323–329.

Qin T T, Fu J F, Zhang N H, Du L F, 2006. Comparative studies of senescence-related enzymes in the cotyledon of chlorophyll b-deficient mutant and its wild type oilseed rape during senescence. *Plant Science*, 171(3): 293–299.

Rai V, Vajpayee P, Singh S N, Mehrotra S, 2004. Effect of chromium accumulation on photosynthetic pigments, oxidative stress defense system, nitrate reduction, proline level and eugenol content of *Ocimum tenuiflorum* L. *Plant Science*, 167(5): 1159–1169.

Ranke J, Mölter K, Stock F, Bottin-Weber U, Poczobutt J, Hoffmann J et al., 2004. Biological effects of imidazolium ionic liquids with varying chain lengths in acute *Vibrio fischeri* and WST-1 cell viability assays. *Ecotoxicology and Environmental Safety*, 58(3): 396–404.

Rogers R D, Seddon K R, 2003. Ionic liquids – solvents of the future? *Science*, 302(5646): 792–793.

Rout N P, Shaw B P, 1998. Salinity tolerance in aquatic macrophytes, robable role of proline, the enzymes involved in its

synthesis and C_4 type of metabolism. *Plant Science*, 136(2): 121–130.

Sairam R K, Deshmukh P S, Shukla D S, 1997. Tolerance of drought and temperature stress in relation to increased antioxidant enzyme activity in wheat. *Journal of Agronomy and Crop Science*, 178(3): 171–178.

Skladanowski A C, Stepnowski P, Kleszczyński K, Dmochowska B, 2005. AMP deaminase *in vitro* inhibition by xenobiotics. A potential molecular method for risk assessment of synthetic nitro- and polycyclic musks, imidazolium ionic liquids and N-glucopyranosyl ammonium salts. *Environmental Toxicology and Pharmacology*, 19(2): 291–296.

Stepnowski P, Skladanowski A C, Ludwiczak A, Laczyńska E, 2004. Evaluating the cytotoxicity of ionic liquids using human cell line Hela. *Human & Experimental Toxicology*, 23(11): 513–517.

Stock F, Hoffman J, Ranke J, Störmann R, Ondruschka B, Jastorff B, 2004. Effects of ionic liquids on the acetylcholinesterase – a structure-activity relationship consideration. *Green Chemistry*, 6(6): 286–290.

Zhu Z J, Wei G Q, Li J, Qian Q Q, Yu J Q, 2004. Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber (*Cucumis sativus* L.). *Plant Science*, 167(3): 527–533.