Ecotoxicology and Environmental Safety 139 (2017) 463–471

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

# Growth inhibition and efficiency of the antioxidant system in spring barley and common radish grown on soil polluted ionic liquids with iodide anions

Robert Biczak[a,*], Martyna Śnioszek[b], Arkadiusz Telesiński[b], Barbara Pawłowska[a]

[a] Jan Długosz University in Częstochowa, The Faculty of Mathematics and Natural Sciences, 13/15 Armii Krajowej Av., 42-200 Częstochowa, Poland
[b] West Pomeranian University of Technology, The Faculty of Environmental Management and Agriculture, Juliusza Słowackiego st. 17, 71-434 Szczecin, Poland

ARTICLE INFO

*Keywords:*
Ionic liquids
Terrestrial plants
Growth inhibition
Photosynthetic pigments
Oxidative stress
Antioxidant enzyme activity

ABSTRACT

Ionic liquids (ILs) constitute a huge group of substances that are increasingly common in the commercial use. This situation may lead to the contamination of the soil environment which being the basic of plants vegetation. This paper presents the effect of four ILs with $I^-$ anion on the growth and development of spring barley (*Hordeum vulgare*) and common radish (*Raphanus sativus* L. subvar. *radicula* Pers) and changes in metabolism of the plants. Seedlings of spring barley and common radish cultivated on soil with increasing ILs concentration exhibited typical phytotoxicity symptoms. A considerable reduction of shoot and root lengths, decrease of fresh weight (FW) and increase of dry weight (DW) occurred in both test plants. Ionic liquids concentration increase in soil was correlated with the decrease of concentrations of all photosynthetic pigments in the plants. The observed increase of malondialdehyde (MDA) concentration and changes in the $H_2O_2$ level indicated presence of oxidative stress in spring barley and common radish, which usually led to the increase of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activity. The most reliable biomarker of oxidative stress was chlorophyll level and changes in POD activity.

## 1. Introduction

According to forecasts of the World Health Organization (WHO) in the next few years the global food production must be constantly increasing, to follow rapidly growing population. The best areas for agricultural production have already been completely utilized, therefore, agricultural lands with increasingly worse quality have to be used, i.e. saline soils, soils characterized by water deficit and lastly, soils polluted or contaminated with certain chemical substances. Such state of affairs poses certain risk for the quality of food, and it is generally known, that soil quality directly influences not only yield size of arable crops, but also its quality. This is related to the fact, that the soil environment constitutes not only the storage of nutrients or reservoir of water for plants, but it also constitutes a "dustbin" for all toxic chemicals produced by humans, or naturally occurring in soil, i.e. heavy metals, dioxins, polycyclic aromatic hydrocarbons, toxic elements such as aluminum or arsenic. Increase of food production of plant origin must occur as a result of conscious activity of man, related to the process of agriculture intensification as a result of the use of mineral fertilizers, pesticides and synthetic products regulating plant growth and development, accelerating and facilitating harvest, regulating plant flowering, leaf fall periods, etc. All these substances, if not entirely used by plants, contaminate soils with these chemical compounds or products of their metabolism (Manzo et al., 2008; Ali et al., 2011; Dragišić Maksimović et al., 2013; Vermue et al., 2013).

Currently, biotests using microorganisms and crustaceans are successfully used for the monitoring of soil environment quality. However, the tests utilizing growth and development of terrestrial plants as the markers of contamination have the greatest practical use and importance for the determination of soil quality. Terrestrial plants are basic biomass and oxygen producers in the terrestrial environment, and their productivity depends on i.a. phytotoxicity of numerous factors naturally occurring in the soil ecosystems and introduced to soils in order to fertilize composts and processed sewage sludge, or other substances of the anthropogenic origin (Manzo et al., 2008; Oleszczuk, 2008).

Due to the above fact, studies on the phytotoxicity of ILs for terrestrial plants are increasingly common in the literature (Matzke et al., 2008, 2009; Sudzińska and Buszewski, 2009; Biczak et al., 2010, 2013a, 2015, 2016a). In these works, the level of phytotoxicity of the studied chemical compounds was determined on the basis of plant growth, the size of fresh and dry weight, or seed germinating capacity, whereas according to Cvjetko Bubalo et al. (2014), the mechanism of toxic effect of these substances has not been entirely understood. As a

* Corresponding author.
*E-mail address:* r.biczak@ajd.czest.pl (R. Biczak).

http://dx.doi.org/10.1016/j.ecoenv.2017.02.016
Received 17 October 2016; Received in revised form 2 February 2017; Accepted 11 February 2017
Available online 23 February 2017

result of scientific studies conducted in this field, the level of toxic effect of ILs for terrestrial plants is increasingly being liked to formation of oxidative stress in these organisms (Wang et al., 2009; Liu et al., 2013, 2015a,b, 2016a,b; Biczak, 2016; Biczak et al., 2016b; Pawłowska and Biczak, 2016). In the cited studies, it was determined beyond all doubt that the used plants had symptoms of oxidative stress identical to the symptoms that had been already observed in plant organisms subjected to other abiotic stress-inducing factors, i.e. soil salinity, drought or presence of heavy metals (Sánchez-Rodríguez et al., 2010; Dragišić Maksimović et al., 2013; Rosalie et al., 2015).

The presented study evaluated the phytotoxicity of four ionic liquids with iodide anion and symmetrical substituents with increasing number of carbon atoms, from 1 to 4, for spring barley and common radish. Iodides were selected for the herein discussed study due to the fact, that the quaternary salts with such anion are very commonly used in the chemical synthesis, electrochemistry, catalysis, and they also have a direct use in the industry and medicine, which is related to their chemical and biological properties, desirable in these fields of human life (Ingalsbe et al., 2009; Smirnova et al., 2009; Obłąk and Gamian, 2010). Such wide spectrum of these ILs use may generate large amounts of postproduction waste, which will be accumulated in i.a. soils, and from there they may influence the growth and development of terrestrial plants. Changes of the level of fresh and dry weight of plants, inhibition of root and stem growth, and the capacity and potential of seed germination served as the basic markers of phytotoxicity of the ILs with iodide anion used in the pot experiment. Moreover, the aim of the discussed study was determination of the level of oxidative stress in both plants used in the experiment on the basis of the content of malondialdehyde (MDA), $H_2O_2$, photosynthetic pigments and efficiency of the plants' detoxification mechanisms of reactive oxygen species (ROS) via the measurement of changes of the activity of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD). The selection of spring barley and common radish stemmed simply from i.a. an attempt to find an answer for the question, whether biologically active ILs cause higher oxidative stress in monocotyledonous or dicotyledonous plants, thus causing in these plants increased symptoms of phytotoxicity. It is essential in the context of selection of such plant species and cultivars, which, cultivated on soils polluted with ILs will provide appropriate yield sizes with the quality required by customers. It should be additionally noted, that spring barley occupies the fourth place among all cereal species in terms of production and cultivated area. Common radish is in turn a very popular vegetable, supplementing the human diet with a selection of microelements and vitamins (Schubert and Jahren, 2011; Dragišić Maksimović et al., 2013; Arias-Baldrich et al., 2015).

## 2. Materials and methods

### 2.1. Chemicals

The ionic liquids: tetramethylammonium iodide [TMA][I] (99% purity), tetraethylammonium iodide [TEA][I] (98% purity), tetrapropylammonium iodide [TPA][I] (≥98% purity) and tetrabutylammonium iodide [TBA][I] (98% purity) used in the study was purchased from Sigma-Aldrich Chemical Co. The structure of the tested ILs is illustrated in Supp. Fig. 1.

### 2.2. Evaluation of ILs phytotoxicity

Determination of phytotoxicity of ionic liquids was performed according to the OECD/OCDE 208, 2006 guidelines. Following the recommendations of this guideline for phytotoxicity study, the following concentrations of ILs were used: 0, 1, 10, 50, 100, 400, 700 and 1000 mg compound per 1 kg of soil dry weight (DW). For each concentration of ILs, 3 independent samples were prepared. Granulometric analysis showed that in this study, loam was used as a soil and contained about 10% of fraction content with a diameter of < 0.02 mm and organic carbon –9.5 g $kg^{-1}$ and pH 5.9. With such prepared medium, plastic pots were filled, which were then seeded with 20 seeds of spring barley (*Hordeum vulgare*) and common radish (*Raphanus sativus* L. *radicula* Pers.) derived from the same source. Seed germination and plant growth (14 days) were carried out under strictly controlled conditions: soil moisture at 70% ppw, temperature of 20 ± 2 °C and constant illumination equal to 170 µmol $m^{-2}$ $s^{-1}$ within the system of 16 h day/8 h night. Such stable growth and development conditions were assured by vegetation hall, which belongs to the Department of Biochemistry and Ecotoxicology at Jan Długosz University in Częstochowa.

Phytotoxicity of the studied ILs for spring barley and common radish was estimated based on, among others, the amount of yield of fresh weight of these plants, dry weight content, length of the aerial parts of plants and their roots. Inhibition factor of fresh weight, length of the plant (shoot length) and root lengths were calculated according to the study published by Wang et al. (2009). Based on the calculated inhibition, using non-linear regression analysis, $EC_{50}$ was estimated using the GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA). Furthermore, germination potential (GP) and germination rate (GR) of spring barley and common radish seeds, were determined. Those seeds for which the germ is more than 2 mm were considered germinated (Liu et al., 2014).

Furthermore, in plant leaves, MDA and $H_2O_2$ content, photosynthetic pigments content and antioxidant enzymes activities were measured and compared.

### 2.3. Pigments assay

Photosynthetic pigments content was determinate according to the method reported by Oren et al. (1993). Fresh weight leaves (0.2*g*) homogenized in 20 ml 80% acetone using mortar and pestle and were put in centrifuge tube. The extraction was carried out the darkness for 24 h and the extract were centrifuged by 10 min and supernatants were used for pigments content determination. The content of chlorophyll a, chlorophyll b and carotenoids by measuring the absorbance at 470 nm, 647 nm and 664 nm. The content photosynthetic pigments were expressed as mg $g^{-1}$ of dry weight (DW).

### 2.4. Determination of lipid peroxidation and hydrogen peroxide

The 500 mg of weighted portion of fresh leaves were homogenized with the addition of 0.1% trichloroacetic acid solution cooled to 4 °C. After centrifugation, MDA and $H_2O_2$ content were determined in the obtained supernatant according to the procedures described by Hodges et al. (1999) and Singh et al. (2007), respectively. As a substrate to determine MDA, thiobarbituric acid was used and the MDA content was determined by measuring the absorbance at 532 nm and 600 nm. In order to determine the content of $H_2O_2$, the absorbance was measured at a wavelength of 390 nm for the reaction mixture consisting of supernatant, phosphate buffer pH – 7.0 and potassium iodide. The content of MDA and $H_2O_2$ was calculated using the extinction coefficient equal to 155 $mM^{-1}$ $cm^{-1}$ and expressed in µmol $g^{-1}$ fresh weight (FW).

### 2.5. Determination of activity of superoxide dismutase, catalase and peroxidase

Fresh leaf samples (0,5*g*) were homogenized in 10 ml of extraction solution containing phosphate buffer (pH 7.4), 1 mM EDTA and 1% polyvinylpyrrolidone (PVP). The mixture was centrifuged for 20 min 4 °C and the supernatant was used for enzyme activity and protein content assays. The total protein content was determined with the method described by Bradford (1976).

Superoxide dismutase (SOD) [EC 1.15.1.1] activity was determined