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RESPONSE OF SOIL PEROXIDASES TO 1-ALKYL-3-METHYLIMIDAZOLIUM IONIC LIQUIDS WITH TETRAFLUOROBORATE ANION

REAKCJA PEROKSYDAZ GLEBOWYCH NA 1-ALKILO-3-METYLOIMIDAZOLIOWE CIECZE JONOWE Z ANIONEM TETRAFLUOROBORANOWYM

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Streszczenie. Celem przeprowadzonych badań było określenie oddziaływania 1-alkilo-3--metyloimidazoliowych soli amoniowych (ILs) z anionem tetrafluoroboranowycm na aktywność peroksydaz w glebie. Doświadczenie laboratoryjne przeprowadzono na próbkach piasku gliniastego (C_{org} 8,71 g · kg^{-1}, pH_{KCl} 6,36), gliny lekkiej (C_{org} 10,92 g · kg^{-1}, pH_{KCl} 6,81) oraz gliny piaszczysto--ilastej (C_{org} 33.81 g · kg^{-1}, pH_{KCl} 7,13). Próbki gleby przesiano przez sito o średnicy oczek 2 mm i wprowadzono do nich trzy ciecze jonowe: tetrafluoroboran 1-butylo-3-metyloimidazoliowy [BMIM][BF_4], tetrafluoroboran 1-heksylo-3-metyloimidazoliowy [HMIM][BF_4] oraz tetrafluoroboran 1-oktylo-3-metyloimidazoliowy [OMIM][BF_4] w ilości: 0, 10, 100 i 1000 mg · kg^{-1}. Aktywność peroksydaz oznaczono spektrofotometrycznie w 1., 3., 7., 14., 28., 56. i 112. dniu doświadczenia. Wprowadzenie 1-alkilo-3-metyloimidazoliowych soli amoniowych spowodowało w większości istotne zmiany aktywności peroksydaz glebowych, które zależały od rodzaju soli, jej dawki, czasu inkubacji oraz właściwości gleby. Zaobserwowane zmiany zwiększały się wraz ze wzrostem dawki oraz z wydłużaniem podstawnika alkilowego w kationie. Największy wpływ ILs na aktywność peroksydaz wykazano w piasku gliniastym.

Key words: ionic liquids, peroxidase, resistance index, soil.
Słowa kluczowe: ciecze jonowe, gleba, index oporności, peroksydazy.

INTRODUCTION

In recent years, ionic liquids (ILs) are included in the category of abiotic factors the cause ecotoxicological changes. These compounds, with revealing biological activity, are often used as pesticides, as disinfectant in medicine, in the production of surfactants, and also in organic synthesis and electrochemical and biocatalysis reactions (Biczak et al. 2016; Sun et al. 2017). ILs become air pollutants because of their low vapor pressure. Moreover, they are inevitably released into the water or the soil through accidental spills, effluents or irrigation (Liwarska-Bizukojc 2011). Likewise, ILs with anions [PF_6] and [BF] as well as being sensitive to hydrolytic processes are poorly biodegradable, while the acetate and sulfate anion appear to be more environmentally friendly (Peric et al. 2013).

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Soil, as a major part of terrestrial ecosystems, performs important functions in sustainable agricultural production (Sun et al. 2017). One of the markers of changes occurring in the soil environment with the effect of natural and anthropogenic factors, especially organic xenobiotics is the activity of enzymes (Bielińska and Mocek-Płóciniak 2010). The changes in soil enzymatic activities are among the earliest signals indicating the changes in the intensity of environmental life processes because multiple chemical compounds assume a toxic or mutagenic character following the metabolic transformations occurring in the living organisms (Filipek-Mazur et al. 2014). Extracellular enzymes are the proximate agents of organic matter decomposition, and thus the activities of hydrolytic enzymes (e.g. cellulases, phosphatases, chitinases, proteases) are widely measured in ecosystem studies (German et al. 2011). In contrast, activity of peroxidases have been measured in a smaller number of soil enzyme studies. Fungi and bacteria express oxidases for a variety of functions, including cellular processes and defense, as well as carbon (C) and nitrogen (N) acquisition (Sinsabaugh 2010). Once in the soil environment, these enzymes mediate the biogeochemical processes of lignin degradation, carbon mineralization and sequestration, and dissolved organic C export (Bach et al. 2013). Peroxidases such as manganese peroxidase and lignin peroxidase use H_2O_2 as an electron acceptor (Wiedermann et al. 2017). These enzymes have Fe-containing heme prosthetic groups with redox potentials up to 1490 mV, giving them the capacity to break aryl and alkyl bonds within lignin either directly or through redox intermediates such as Mn^{3+} (Rabinovich et al. 2004).

In this paper, we characterize the impact of three 1-alkyl-3-methylimidazolium ionic liquids with tetrafluoroborate anion on the activity of peroxidases in three different soils: loamy sand, sandy loam and sandy clay loam.

MATERIAL AND METHODS

The tests were performed on three soil materials collected from the topsoil in Agricultural Experimental Station in Lipnik, the Gumieniecka Plain and the Pyrzycka Plain, in the West Pomeranian District, Poland. According to the classification of the United States Department of Agriculture, it was soil with a granulometric composition of loamy sand, sandy loam and sandy clay loam, respectively. Some of the soil properties are presented in Table 1.

Table 1. Properties of soils used in experiment
Tabela 1. Właściwości gleb użytych w doświadczeniu

Properties Właściwości	Loamy sand Piasek gliniasty	Sandy loam Glina lekka	Sandy clay loam Glina piaszczysto--ilasta
Organic carbon – Węgiel organiczny [$g \cdot kg^{-1}$]	8.71	10.92	33.81
Total nitrogen – Azot ogółem [$g \cdot kg^{-1}$]	0.83	1.03	3.12
Hydrolytic acidity – Kwasowość hydrolityczna [mmol(+) kg^{-1}]	13.02	11.81	14.20
Cation exchangeable bases – Suma zasad wymiennych [mmol(+) kg^{-1}]	10.50	13.39	11.84
pH in 1 M KCl – pH w 1 M KCl	6.36	6.81	7.13

The experiments were carried out in triplicate under laboratory conditions, with the three different imidazolium ionic liquids: 1-butyl-3-methylimidazolium tetrafluoroborate – [BMIM][BF_4], 1-hexyl-3-methylimidazolium tetrafluoroborate – [HMIM][BF_4] and 1-octyl-3-methylimidazolium tetrafluoroborate – [OMIM][BF_4]. These ionic liquids (ILs) were added into soil ant the dosages of 0, 10, 100 and 1000 mg · kg^{-1} d.m. soil. The 1-kg soil samples with ILs were adjusted to 60% maximum water holding capacity, and they were incubated in tightly closed glass containers at a temperature of 20°C. The activity of peroxidases (EC 1.1.3.x) was determined on days: 1, 3, 7, 14, 28, 56 and 112 using Bartha and Bordeleau (1969) method. The analyses were carried out using spectrophotometer UV-1800 produced by Shimadzu.

The results were processed with one-way ANOVA using Statistica 12.5 software (StatSoft, Inc.). The homogeneous groups were identified using Tukey's test at a significance level of $P < 0.05$.

Additionally, mean activities of peroxidases were used to calculate the index of these enzymes resistance (RS) to effect of ILs according to the formula developed by Orwin and Wardle (2004):

$$RS = 1 - \frac{2|D|}{C + |D|}$$

where:

C − the soil resistance under natural conditions (not exposed to ILs);

P − the resistance of soil exposed to ILs;

D = C−P.

RESULTS

In loamy sand, application of [BMIM][BF_4] at all dosages caused significant increase in activity of peroxidases during 14 days of experiment, and compared to control, for dosage of 10 mg · kg^{-1} it was ranged from 10.80 to 18.32%, for dosage of 100 mg · kg^{-1} from 15.15 to 29.39% and for dosage of 1000 mg · kg^{-1} from 15.35 to 46.18%. Moreover, significant stimulation was reported on day 112 in loamy sand treated with dosage of 1000 mg · kg^{-1} (19.89%, compared to control). In sandy loam significant increase in peroxidases was observed after treatment with [BMIM][BF_4] at dosage of 100 mg·kg^{-1} on day 28 (16.14%, compared to control) and dosage of 1000 mg · kg^{-1} on days 1, 14 and 28 (14.67, 15.66 and 18.52%, compared to control, respectively). In sandy clay loam the activity of peroxidases was significantly higher than in soil control on day 1 for dosage 1000 mg · kg^{-1} (26.92%, compared to control), and on day 3 for all [BMIM][BF_4] dosages (19.88–33.04%, compared to control). However, decrease in peroxidase activities occurred in loamy sand treated with [BMIM][BF_4] at all dosages on day 56 (29.12–66.30%, compared to control) and the dosage of 10 mg · kg^{-1} on day 112 (36.92%, compared to control). In sandy loam inhibition of peroxidase activities was noted on day 14 after treatment with [BMIM][BF_4] at the dosage of 10 mg · kg^{-1} (17.37%, compared to control) and on day 56 after treatment with all [BMIM][BF_4] dosages (28.24––61.66%, compared to control). In sandy clay loam the inhibitory effect, compared to other soil samples, was observed most frequently. The highest decrease in activity of peroxidases was reported on day 56, and for the dosages of 10, 100 and 1000 mg · kg^{-1} it was 37.63, 47.24, and 56.85%, respectively (Fig. 1).

Application of [HMIM][BF_4] at the dosage of 10 mg · kg^{-1} caused significant increase in the activity of peroxidases in loamy sand on day 3 (40.46%, compared to control), in sandy loam on days 14 and 28 (19.47% and 23.54%, compared to control, respectively), and in sandy clay loam on days 1 and 28 (19.89% and 17.32%, compared to control, respectively). Significant decrease in activity of peroxidases was more often observed.

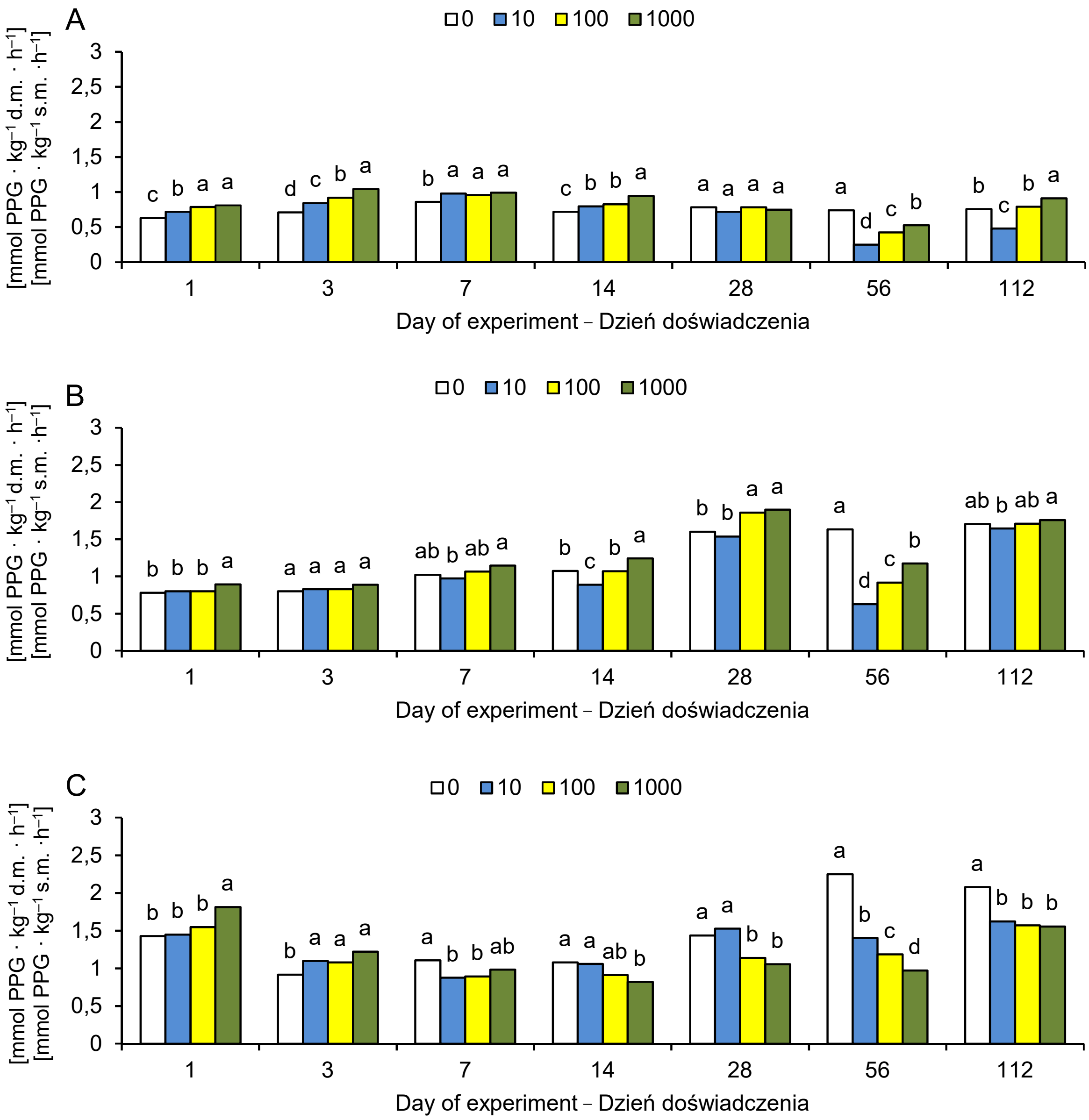

Fig. 1. Activity of peroxidases in loamy sand (A), sandy loam (B) and sandy clay loam (C) treated with [BMIM][BF_4]; the same letter means a homogenous group in every day ($P < 0.05$); values of 0, 10, 100 and 1000 are dosages of [BMIM][BF_4] in mg · kg^{-1}

Ryc. 1. Aktywność peroksydaz w piasku gliniastym (A), glinie lekkiej (B) i glinie piaszczysto-ilastej (C) z dodatkiem [BMIM][BF_4]; te same litery oznaczają grupy jednorodne w danym dniu doświadczenia ($P < 0,05$); wartości 0, 10, 100 i 1000 są dawkami [BMIM][BF_4] wyrażonymi w mg · kg^{-1}

The inhibition of activity was noted in loamy sand on days 56, and 112 (48.90% and 42.28, compared to control, respectively), in sandy loam on days 7 and 56 (17.40% and 41.80%, compared to control, respectively), and in sandy clay loam on days 7, 14 and 112 (47.57%, 12.04% and 18.34%%, compared to control, respectively). After treatment with [HMIM][BF_4] at the dosage of 100 mg · kg^{-1} significant increase in activity of peroxidases in loamy sand on days 3, 7 and 14, in sandy loam on days 3, 14 and 28, and in sandy clay loam on days 1, 7 and 56. The highest stimulation was noted in sandy loam on day 28 (46.56%, compared to control). However application of [HMIM][BF_4] at the dosage of 100 mg · kg^{-1} resulted in inhibition of activity on day 56 in all soil samples (for loamy sand, sandy loam and sandy clay loam 40.29%, 42.75% and 10.43%, compared to control, respectively), and on day 112 in loamy sand and sandy clay loam (20.07% and 22.83%, compared to control, respectively). In soil samples contained [HMIM][BF_4] at the dosage of 1000 mg · kg^{-1} significant increase in activity of peroxidases was reported. This effect was observed in loamy sand in the whole of experiment (10.45–71.37%, compared to control), in sandy loam from day 1 to day 28 (14.09–44.18%, compared to control), and in sandy clay loam on days 1, 3, 14 and 28 (25.44–47.83%, compared to control). The significance inhibition of activity occurred only in sandy loam on day 56 (42.75%, compared to control), and in sandy clay loam on days 7 and 112 (36.65% and 19.65%, compared to control, respectively) – Fig. 2.

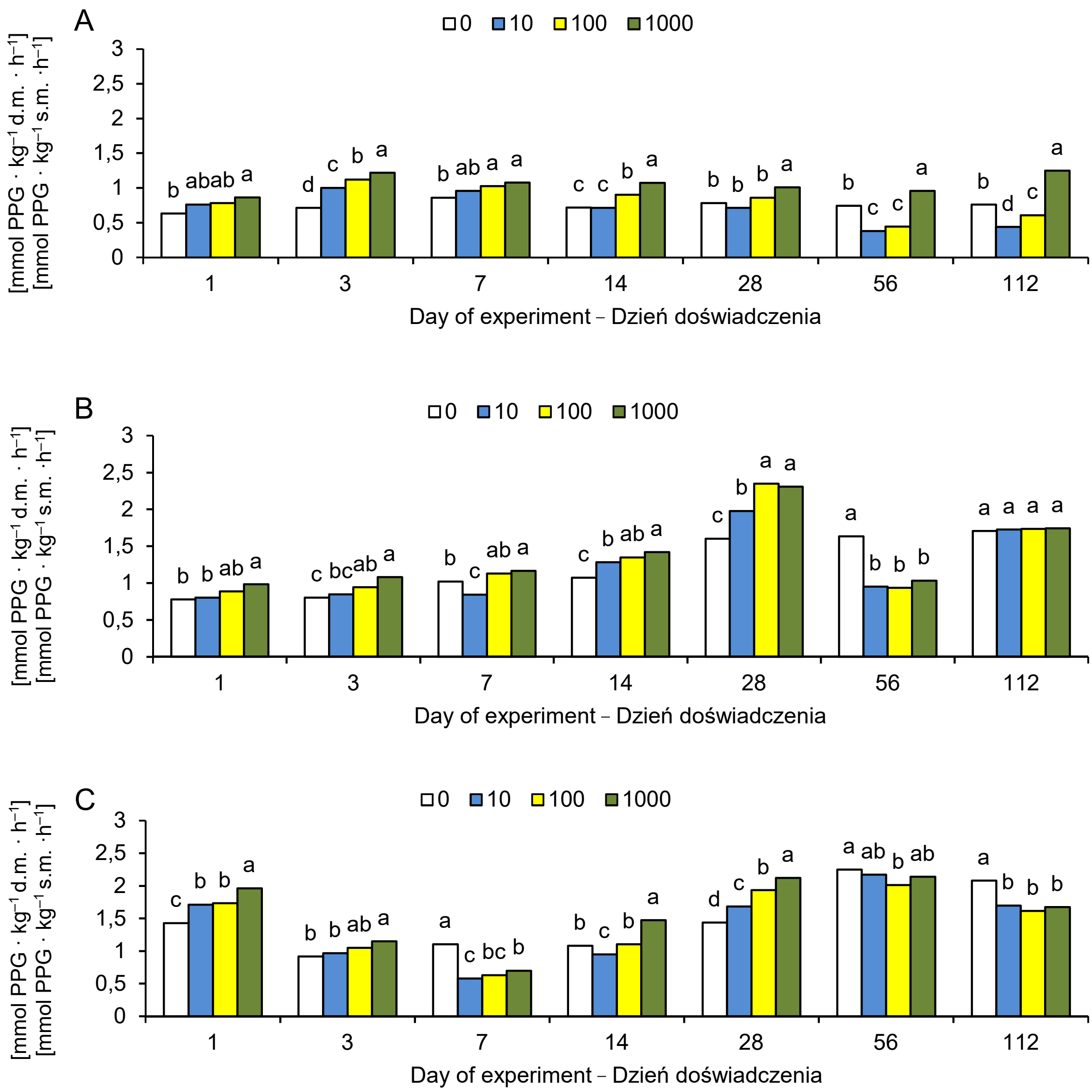

Fig. 2. Activity of peroxidases in loamy sand (A), sandy loam (B) and sandy clay loam (C) treated with [HMIM][BF_4]; the same letter means a homogenous group in every day ($P < 0.05$); values of 0, 10, 100 and 1000 are dosages of [HMIM][BF_4] in mg · kg^{-1}

Ryc. 2. Aktywność peroksydaz w piasku gliniastym (A), glinie lekkiej (B) i glinie piaszczysto-ilastej (C) z dodatkiem [HMIM][BF_4]; te same litery oznaczają grupy jednorodne w danym dniu doświadczenia ($P < 0,05$); wartości 0, 10, 100 i 1000 są dawkami [HMIM][BF_4] wyrażonymi w mg · kg^{-1}

In soil samples treated with [BMIM][BF_4] significant stimulation in activity of peroxidases was mainly reported. This effect increased with increase of ionic liquid dosage. In loamy sand the highest stimulation for dosages of 10, 100, and 1000 mg · kg^{-1} was observed on day 3, and it was 37.79%, 64.88%, and 80.53%, compared to control, respectively. In sandy loam application of [BMIM][BF_4] at the dosages of 10 mg · kg^{-1} caused significant increase in activity of peroxidases only on days 1, 28 and 113, whereas dosages of 100 and 1000 mg · kg^{-1} resulted in activation of peroxidases (except day 56 for dosage of 100 mg · kg^{-1}) on the level of 14.64–44.80%, and 9.58–53,20%, respectively. In sandy clay loam with added [BMIM][BF_4] at the dosages of 10, 100 and 1000 mg · kg^{-1}, there was significant increase in activity of peroxidases of 5.30–24.41%; 10.30–34.21%, and 16.85–49.89%, compared to control, respectively. Only on day 7 for dosages of 10 and 100 mg · kg^{-1}, and on day 14 for dosage 10 mg · kg^{-1} significant changes in activity of peroxidases were not noted (Fig. 3).

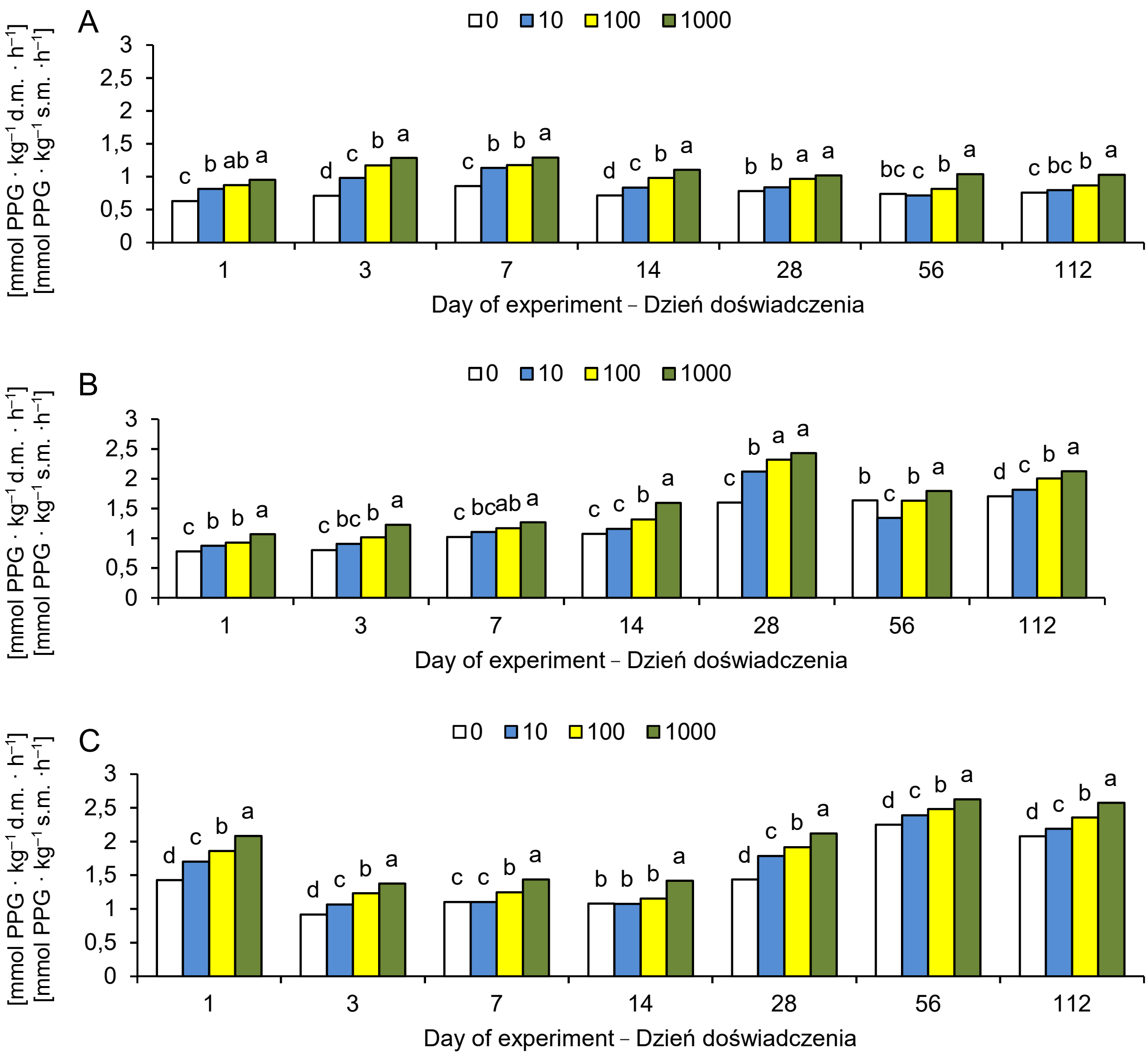

Fig. 3. Activity of peroxidases in loamy sand (A), sandy loam (B) and sandy clay loam treated with [OMIM][BF_4]; the same letter means a homogenous group in every day ($P < 0.05$); values of 0, 10, 100 and 1000 are dosages of [OMIM][BF_4] in mg · kg^{-1}

Ryc. 3. Aktywność peroksydaz w piasku gliniastym (A), glinie lekkiej (B) i glinie piaszczysto-ilastej (C) z dodatkiem [OMIM][BF_4]; te same litery oznaczają grupy jednorodne w danym dniu doświadczenia ($P < 0.05$); wartości 0, 10, 100 and 1000 są dawkami [OMIM][BF_4] wyrażonymi w mg · kg^{-1}

An interpretation of the effect of different ILs and any other pollutant on soil enzyme activities is difficult because the enzyme assays currently used have some limitations and because of the existence of direct and indirect effects on the measured enzyme (Nannipieri et al. 2003). According to Orwin and Wardle (2004), soil resistance values can be used to determine the analyzed ecosystem's sensitivity to various stressors. Soil resistance indicators provide information about the status of soil environments contaminated with organic compounds, including ionic liquids (Kaczyńska et al. 2015; Telesiński et al. 2017).

It was found based on the calculated mean values of resistance index (RS) that the most toxic to soil peroxidases was [OMIM][BF_4] (Table 2). In loamy sand with all dosages, the lowest values of RS were noted for [OMIM][BF_4] (for dosages of 10, 100 and 1000 mg · kg^{-1} they were 0.699, 0.515, and 0.346 respectively). However, in sandy loam and sandy clay loam treated with ILs at the dosage of 10 mg · kg^{-1} the largest decrease in RS values was reported for [BMIM][BF_4] (0.733 and 0.782, respectively). Similar to loamy sand, in sandy loam, and in sandy clay loam treated with higher dosages of ILs the lowest RS values were noted for [OMIM][BF_4] (for dosages of 100 and 1000 mg · kg^{-1}, in sandy loam – 0.659 and 0.498, in sandy clay loam – 0.681 and 0.510, respectively).

Table 2. Mean values of resistance peroxidases indices (RS) in soil treated with 1-alkyl-3--methylimidazolium ionic liquids with tetrafluoroborate anion
Tabela 2. Średnie wartości współczynników oporności (RS) peroksydaz w glebie z dodatkiem 1-alkilo--3-metyloimidazoliowych cieczy jonowych z anionem tetrafluoroboranowym

Soil Gleba	ILs dosages [mg · kg^{-1} d.m.] Dawki cieczy jonowych [mg · kg^{-1} s.m.]	Kind of ionic liquid Rodzaj cieczy jonowej		
		[BMIM][BF_4]	[HMIM][BF_4]	[OMIM][BF_4]
Loamy sand Piasek gliniasty	10	0.850	0.912	0.699
	100	0.895	0.814	0.516
	1000	0.742	0.397	0.346
Sandy loam Glina lekka	10	0.733	0.957	0.849
	100	0.919	0.848	0.659
	1000	0.916	0.771	0.498
Sandy clay loam Glina piaszczysto--ilasta	10	0.782	0.901	0.819
	100	0.682	0.959	0.681
	1000	0.692	0.835	0.510

Obtained results showed that the effect on activity of peroxidases depended on the dosage of ionic liquids, incubation time, and soil properties. This is similar with previous research regarding impact of 1-alkyl-3-methylimidazolium ionic liquids on *o*-diphenol oxidase (Telesiński and Sułkowska 2016), dehydrogenases (Liwarska-Bizukojc 2011) and respiration rate (Azimova et al. 2009). Markiewicz et al. (2009) estimated that at 1-methyl-3-octylimidazolium chloride of concentration higher than 0.2 mM, the dehydrogenase activities of the cells dropped markedly.

Li et al. (2013) reported that imidazolium ionic liquids may improve enzyme activities, because they participated in the biocatalytic processes. But, results obtained by Telesiński et al. (2017) showed that the application of three ionic liquids with hexafluorophosphate anion caused mainly non-significant changes in the activity of phosphatases.

CONCLUSIONS

In the present study, the effects of three 1-alkyl-3-methylimidazolium ionic liquids with tetrafluorophosphate on activity of soil peroxidases were examined. The application of different dosages of all ILs caused mainly significant changes in activity of peroxidases. Changes in activity of peroxidases depended on the dosage of ionic liquids, incubation time, and soil properties. The effect of ILs on activity of peroxidases increased not only with an increasing dosage, but also with elongation of alkyl substituents in the cation. The values of resistance indices showed that the highest impact of ILs on activity of peroxidase was in loamy sand. The result of the presented study is beneficial for future research about ILs toxicity.

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Abstract. The aim of study was to determine effect of 1-alkyl-3-methylimidazolium ionic liquids with tetradluoroborate anion on activity of soil peroxidases. The experiment was carried out in laboratory conditions with samples of loamy sand (C_{org} 8.71 g · kg^{-1}, pH_{KCl} 6.36), sandy loam (C_{org} 10.92 g · kg^{-1}, pH_{KCl} 6.81) and sandy clay loam (C_{org} 33.81 g · kg^{-1}, pH_{KCl} 7.13). Samples were sieved through a 2-mm mesh, and then three ionic liquids: 1-butyl-3-methylimidazolium tetrafluoroborate [BMIM][BF_4], 1-bhexyl-3-methylimidazolium tetrafluoroborate [HMIM][BF_4], and 1-octyl-3-methylimidazolium tetrafluoroborate [OMIM][BF_4] at the dosages of 0, 10, 100, and 1000 mg · kg^{-1} were added to soil. Activity of peroxidases was measured spectrophotometrically on days 1, 3, 7, 14, 28, 56 and 112. Application of different dosages of all ILs caused mainly significant changes in activity of peroxidases. Changes in activity of peroxidases depended on the dosage of ionic liquids, incubation time, and soil properties. The effect of ILs on activity of peroxidases increased not only with an increasing dosage, but also with elongation of alkyl substituents in the cation. The highest impact of ILs on activity of peroxidases was observed in loamy sand.