POLISH JOURNAL OF SOIL SCIENCE
VOL. L/2 2017 PL ISSN 0079-2985

DOI: 10.17951/pjss/2017.50.2.189

ARKADIUSZ TELESIŃSKI*, BARBARA PAWŁOWSKA**,
ROBERT BICZAK**, JACEK PATER*

ACTIVITY OF DEHYDROGENASES IN CLAY SOIL EXPOSED TO QUATERNARY AMMONIUM SALTS WITH THE IODINE ANION

Received: 21.08.2017
Accepted: 30.10.2017

Abstract. The aim of the research was to compare the effects of four quaternary ammonium salts (QAS) with the iodine anion – tetramethylammonium iodine [TMA][I], tetraethylammonium iodine [TEA][I], tetrapropylammonium iodine [TEA][I], tetrabutylammonium iodine [TBA][I] – on the activity of dehydrogenases in soil. The experiment was performed on sandy clay loam samples with organic carbon content of 33.82 $g \cdot kg^{-1}$, and pH 7.13 in 1 M KCl. QAS were added to soil at the dosages: 0, 10, 100, and 1,000 $mg \cdot kg^{-1}$. Activity of dehydrogenases was assayed on days: 1, 14, 35, and 70. The obtained results have shown that the treatment of soil with quaternary ammonium salts along with the iodine anion caused decrease in the activity of dehydrogenases. This inhibition increased with the increase of QAS dosages and increase with alkyl chains in cations. Analysis of variance η^2 indicatedthat the type of QAS had the biggest impact on formation of the activity of dehydrogenases in soil.

Keywords: quaternary ammonium salts, iodine, dehydrogenases, relative changes

* Department of Plant Physiology and Biochemistry, West Pomeranian University of Technology in Szczecin, Poland, 71-434 Szczecin, Słowackiego 17.

** Department of Biochemistry and Ecotoxicology, Jan Długosz University in Częstochowa, Poland, 42-200 Częstochowa, ArmiiKrajowej Av. 13/15. Corresponding author's e-mail: arkadiusz.telesinski@zut.edu.pl

INTRODUCTION

Due to many useful properties, quaternary ammonium salts (QAS) are increasingly used in industries (Telesiński *et al*. 2016). In terms of chemical structure, these substances belong to ionic compounds that contain four organic groups linked to the nitrogen atoms in the molecule (Pernak *et al.* 2006). Naturally, there are more than 100 known metabolites that are quaternary ammonium salts. These metabolites occur in a wide range of species, from bacteria through algae, fungi, plants, and invertebrates to vertebrates, and they perform various functions in the organisms. The organisms synthesize these substances for a better adaptation to the environmental conditions, such as salinity or sudden temperature changes (Obłąk and Gamian 2010). However, synthetic quaternary ammonium salts have been known for a long time; QAS are recognized for their supremely desirable froth like, moisturizing, emulsifying, surfactant, anti-electrostatic, preserving, algicide, antifungal, and bactericidal properties, which resulted in the worldwide QAS production of one million tons at the end of the 20th century (Biczak *et al.* 2016). The variety of applications of quaternary ammonium salts leads to a high possibility of their transfer into the soil environment either directly or indirectly together with domestic and industrial waste water or rainwater (Telesiński *et al*. 2016).

Preservation of the biological equilibrium in the soil depends on numerous factors, which may be divided into chemical, physical, and biological. The last group of parameters is particularly susceptible to modifications caused by any disturbance occurring in the soil and water environment. Enzymatic activity of the soil and proliferation of soil microorganisms are the best indicators of the stability and fertility of soil ecosystems (Kaczyńska *et al*. 2015). This is due to the immediate response of the biochemical activity of the soil to any disturbance of the environment. The soil environment is a source of an immense pool of enzymes. It includes representatives of every enzyme class, i.e. oxidoreductases, hydrolases, isomerases, ligases, lyases, and transferases (Baldrian 2009). All these enzymes are involved in the process of conversion of organic substances and energy (Gu *et al*. 2009). Soil dehydrogenases are the main representatives of the oxidoreductase class (Kaczyńska *et al*. 2015).

Dehydrogenases play a significant role in the biological oxidation of soil organic matter by transferring hydrogen from organic substrates to inorganic acceptors (Zhang *et al.* 2010). Many specific dehydrogenases transfer hydrogen to either nicotinamide adenine dinucleotide or nicotinamide adenine dinucleotide phosphate (Kuziemska *et al*. 2012). Kaczyńska *et al*. (2015) reported that dehydrogenases are very good indicators of the microbiological redox-systems and could be considered as a good and adequate measure of microbial oxidative activities in the soil. Brzezińska *et al*. (2001) found that active dehydrogenases can utilize both O_2 and other compounds as terminal electron acceptors,

although anaerobic microorganisms produce most dehydrogenases. Therefore, dehydrogenases reflect metabolic ability of the soil and its activity is considered to be proportional to the biomass of the microorganisms in the soil (Stręk and Telesiński 2016). But, the relationship between an individual biochemical property of soil dehydrogenases activity and the total microbial activity is not always obvious, especially for complex systems like soils where the microorganisms and processes involved in the degradation of the organic compounds are highly diverse (Salazar *et al*. 2011).

This paper describes the effects of different quaternary ammonium salts (varying in the length of the alkyl substituents), with the iodine anion, on the activity of dehydrogenases in sandy clay loam.

MATERIALS AND METHODS

The testing was performed on a soil material taken from the topsoil of Pyrzycka Plain (53°15′N, 14°90′E), located in the West Pomeranian Voivodeship, Poland. According to the classification of the United States Department of Agriculture, it was a soil with granulometric composition of sandy clay loam. The content of particular fractions, expressed in $g \cdot kg^{-1}$, was as follows: sand (0.05–2 mm) – 531.28; silt (0.002–0.05 mm) – 192.31; and clay (<0.002 mm) – 276.41. The soil contained, in $g \cdot kg^{-1}$: C_{org} – 33.82; N_{tot} – 2.92. Its hydrolytic acidity was 14.22 $mmol(+) \cdot kg^{-1}$, and the pH was 7.13 in1 MKCl. The soil was air-dried and sieved through a 2-mm mesh.

The experiments were performed in triplicate under laboratory conditions, with the following variable factors: (a) type of QAS: tetramethylammonium iodine [TMA][I], tetraethylammonium iodine [TEA][I], tetrapropylammonium iodine [TPA][I], tetrabutylammonium iodine [TBA][I]; (b) QAS dosages: 0, 10, 100, and 1,000 $mg \cdot kg^{-1}$. The 1-kg soil samples were adjusted to 60% maximum water holding capacity, and then were incubated in tightly closed glass containers at a temperature of 20°C.

During the experiment, the activity of dehydrogenases was measured four times (on day 1, 14, 35, and 70) in the soil samples from each repetition in 3 subsequent replications. Soil samples are suspended in a triphenyltetrazolium chloride (TTC) solution and incubated for 16h at 25°C. The triphenylformazan (TPF) produced is extracted with acetone and measured photometrically at 546 nm (Thalmann 1968).

Values of the activity of dehydrogenases were also used to calculate the median relative changes according to the formula defined by Chaer *et al.* (2009):

$$RCh = \left(\frac{T}{C} - 1 \right) \cdot 100\%$$

Where: T – the activity of dehydrogenases in the soil treated with QAS; C – the activity of dehydrogenases in control soil.

The results of the studies were determined statistically using a statistical software package Statistica v. 13.1 (Statsoft, Inc.). Based on the analysis of the effect measure η^2 by variance analysis – ANOVA, the percentage shares of all the variable factors affecting the activity of dehydrogenases were defined. Homogeneous groups were calculated using the Tukey's test with $p<0.05$.

RESULTS AND DISCUSSION

The application of quaternary ammonium salts into the soil caused the inhibition of activity of soil dehydrogenases activity. However, the demonstrated changes depended on the type of quaternary ammonium salts, their dosage, and the day of the experiment. A statistically significant ($p<0.05$) decrease in activity of dehydrogenases was noted in the soil containing [TMA][I] on day 1 for doses of 100 and 1,000 mg·kg^{-1}, by 31.19% and 40.30%, respectively (Table 1). At two consecutive measurement dates, all [TMA][I] dosages decreased statistically significantly ($p<0.05$) the activity of the examined group of enzymes and the observed effect increased with an increasing dosage of this quaternary ammonium salt (up to 35.77% on day 14 and up to 32.38% on day 35). But, on day 50, only the dose of 1,000 mg·kg^{-1} inhibited the activity of dehydrogenases in the soil (22.37%, compared to the control), statistically significantly ($p<0.05$).

An application of [TEA][I] to the soil at all dosages resulted in a statistically significant ($p<0.05$) decrease in the activity of dehydrogenases in the first three measurements, and it was higher with increasing salt doses. The demonstrated inhibition for subsequent dosages of [TEA][I] was 17.52–20.34%, 23.63–47.14%, and 38.86–58.17%, respectively. In turn, on day 70, a statistically significant decrease in the activity of dehydrogenases was recorded only for the dosage of 1,000 mg·kg^{-1} (26.09%).

TABLE 1. ACTIVITY OF DEHYDROGENASES (mg TPF·kg^{-1} dm·h^{-1}) IN SOIL TREATED WITH QUATERNARY AMMONIUM SALTS WITH THE IODINE ANION

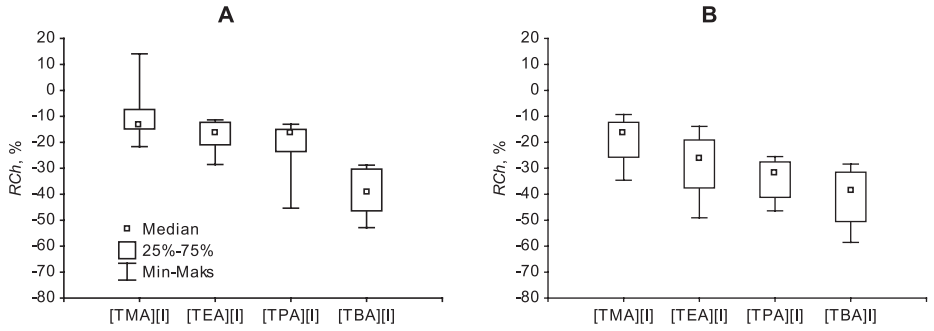
Type of QAS	QAS dosage (mg·kg^{-1} DM)	Day of experiment				
		1	14	35	70	70
Control		54.22 a	54.22 a	52.00 a	54.12 a	49.79 a
[TMA][I]	10	53.91 a	53.91 a	44.76 b	46.28 b	44.32 ab
	100	37.31 c	43.42 b	45.35 b	43.50 ab	43.50 ab
	1,000	32.37 cd	34.04 c	36.59 c	38.65 b	38.65 b

Type of QAS	QAS dosage (mg·kg^{-1} DM)	Day of experiment				
		1	14	35	70	70
[TEA][I]	10	43.19 b	43.19 b	42.89 b	44.01 b	43.60 ab
	100	28.66 d	36.99 c	41.33 bc	42.57 ab	42.57 ab
	1,000	22.68 e	26.01 d	36.08 c	36.80 b	36.80 b
[TPA][I]	10	45.97 b	45.97 b	32.97 cd	44.43 b	42.57 ab
	100	31.34 cd	31.09 cd	38.55 c	36.70 bc	36.70 bc
	1,000	23.50 de	30.29 cd	33.09 cd	33.91 cd	33.91 cd
[TBA][I]	10	27.32 d	27.32 d	28.41 d	36.39 c	35.36 bc
	100	23.81 de	27.34 d	35.97 c	35.13 bc	35.13 bc
	1,000	16.80 f	25.20 d	27.93 d	22.37 d	22.37 d

The same letter means a homogenous group in the columns for a day ($p<0.05$).

In contrast, the introduction of [TPA][I] and [TBA][I] at all doses inhibited the activity of dehydrogenases throughout the experiment. As for [TEA][I], the observed effect increased with increasing salt doses. The greatest degree of activity inhibition was observed on day 1, and the demonstrated inhibition for the highest dose of [TPA][I] and [TBA][I] was 56.65% and 69.01%, respectively.

Based on the calculated values of median relative changes (*RCh*), the inhibition of activity of dehydrogenases increased not only with an increasing dosage, but also with elongation of alkyl substituents in the cation (Fig. 1). Liwarska-Bizukojc (2011) also reported the inhibitory effect of 1-alkyl-3-methylimidazolium bromides on the activity of dehydrogenases in activated sludge biomass, which increased with the increase in the chain length of the alkyl substituent, whereas Markiewicz *et al.* (2009) estimated that at 1-methyl-3-octylimidazolium chloride of concentration higher than 0.2 mM, the activity of dehydrogenases in the cells dropped markedly. Also Azimova *et al.* (2009) measured the effect of imidazolium-derived ionic liquids on the bacterial respiration rate.



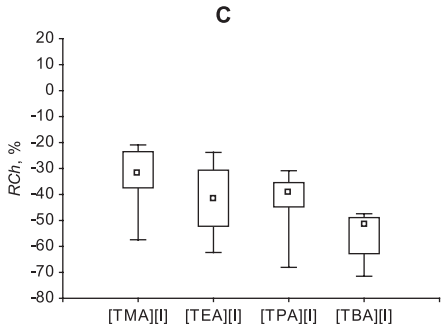


Fig. 1. Median relative changes in activity of dehydrogenases in soil treated with quaternary ammonium salts with the iodine anion at dosages of: 10 mg·kg^{-1} (A), 100 mg·kg^{-1} (B), 1.000 mg·kg^{-1} (C)

However, the results obtained by Sun *et al*. (2017) revealed that dehydrogenases, as well as acid phosphatase, were not sensitive to 1-methyl-3-octylimidazolium tetrafluoroborate, while the urease activity was first stimulated and then inhibited by this ionic liquid (observed after 30-day incubation). Telesiński and Sułkowska (2016) reported that two imidazolium ionic liquids: 3-butyl-1-methylimidazolium tetrafluoroborate and 3-hexyl-1-methylimidazolium tetrafluoroborate, decreased the activity of o-diphenol oxidase, but the size of this effect depended on the dosage of ionic liquids, incubation time, and soil properties. Li *et al.* (2013) showed that imidazolium ionic liquids may improve enzyme activities, because they participated in the biocatalytic processes.Telesiński *et al.* (2017) have shown that the application of three ionic liquids with a hexafluorophosphate anion caused mainly non-significant changes in the activity of phosphatases. These ionic liquids were often not dependent on their doses.

Our previous studies have also showed the phytotoxicity of quaternary ammonium salts with the iodine anion in soil. The increase of QAS concentration in the soil was correlated with the decrease of concentrations of all photosynthetic pigments in the plants. The observed increase of malondialdehyde (MDA) concentration and the changes in the H_2O_2 level has indicated the presence of oxidative stress in plants, which usually led to the increase of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) activity (Biczak *et al*. 2017).

The data presented in Table 2 unequivocally indicates that the activity of dehydrogenases varied over time, and that it depended on the QAS dosage. The share of this factor in the formation of activity of dehydrogenases was 76.80%. The type of QAS also affected dehydrogenases, significantly, and the share of this factor in the formation of the activity was 13.74%.

TABLE 2. PARTICIPATION OF VARIABLE FACTORS IN THE FORMATION OF DEHYDROGENASES ACTIVITIES (%)

Factor	Percentage participation
Dosage of QAS (A)	76.80
Type of QAS (B)	13.74
Day of experiment (C)	4.40
A × B	1.82
A × C	2.16
B × C	0.54
A × B× C	0.34
Error	0.20

CONCLUSIONS

The quaternary ammonium salts with the iodine anion caused decrease in the activity of dehydrogenases in the soil. This effect increased with an increasing dose and also with the elongation of alkyl substituents in the cation. The highest inhibition was observed in the soil with [TBA][I] at a dose of 1,000 $mg \cdot kg^{-1}$, and it was nearly 70% compared to the control. The analysis of variance η^2 showed that the activity of dehydrogenases was affected, to the highest degree, by the type of QAS.

REFERENCES

[1] Azimova, M., Morton, S.A. III, Frymier, P.,2009.*Comparison of three bacterial toxicity assays for imidazolium-derived ionic liquids*. Journal of Environmental Engineering, 135(12): 1388–1392, DOI: 10.1061/(ASCE)EE.1943-7870.0000092.

[2] Baldrian, P., 2009. *Microbial enzyme-catalyzed processes in soils and their analysis*. Plant Soil and Environment, 55(9): 370–378.

[3] Biczak, R., Telesiński, A., Pawłowska, B., 2016. *Oxidative stress in spring barley and common radish exposed to quaternary ammonium salts with hexafluorophosphate anion*. Plant Physiology and Biochemistry, 107: 248–256, DOI: 10.1016/j.plaphy.2016.05.016.

[4] Biczak, R., Śnioszek, M., Telesiński, A., Pawłowska, B., 2017. *Growth inhibition and efficiency of the antioxidant system in spring barley and common radish grown on soil polluted ionic liquids with iodide anions*. Ecotoxicology and Environmental Safety, 139: 463–471, DOI: 10.1016/j.ecoenv.2017.02.016.

[5] Brzezińska, M., Stępniewski, W., Stępniewska, Z., Przywara, G., 2001. *Effect of oxygen deficiency on soil dehydrogenase activity in a pot experiment with Triticale cv. Jago vegetation*. International Agrophysics, 15: 145–149.

[6] Chaer, G., Fernandes, M., Myrold, D., Bottomley, P., 2009.*Comparative resistance and resilience of soil microbial communities and enzyme activities in adjacent native forest and agricultural soils*. Microbial Ecology, 58: 414–424, DOI: 10.1007/s00248-009-9508-x.

[7] Gu, Y., Wag, P., Kong, C., 2009. *Urease, invertase, dehydrogenase and polyphenoloxidase activities in paddy soil influenced by allelophatic rice variety*. European Journal of Soil Biology, 45(4–5): 436–441, DOI: 10.1016/j.ejsobi.2009.06.003.

[8] Kaczyńska, G., Borowik, A., Wyszkowska, J., 2015. *Soil dehydrogenases as an indicator of contamination of the environment with petroleum products*. Water Air Soil Pollution, 226: 372, DOI: 10.1007/s11270-015-2642-9.

[9] Kuziemska, B., Kalembasa, S., Wysokiński, A., Jaremko, D., 2012. *Assessment of impact of organic waste materials and liming on dehydrogenase activity, content of organic carbon, and pH value in soil contaminated with nickel*. Polish Journal of Soil Science, 45(2): 177–184.

[10] Li, N., Du, W., Huang, Z., Zhao, W., Wang, S., 2013. *Effect of imidazolium ionic liquids on the hydrolytic activity of lipase*. Chinese Journal of Catalysis, 34: 769–780, DOI: 10.1016/S1872-2067(11)60521-4.

[11] Liwarska-Bizukojc, E., 2011. *Influence of imidazolium ionic liquids on dehydrogenase activity of activated sludge microorganisms*. Water, Air and Soil Pollution, 221: 327–335, DOI: 10.1007/s11270-011-0793-x.

[12] Markiewicz, M., Jungnickel, C., Markowska, A., Szczepaniak, U., Paszkiewicz, M., Hupka, J., 2009. *1-Methyl-3-octylimidazolium chloride: sorption and primary biodegradation analysis in activated sewage sludge*. Molecules, 14: 4396–4405, DOI: 10.3390/molecules14114396.

[13] Obłąk, E., Gamian, A., 2010. *The biological activity of quaternary ammonium salts (QASs)* (in Polish). Postępy Higieny i Medycyny Doświadczalnej, 64: 201–211.

[14] Pernak, J., Śmiglak, M., Griffin, S.T., Hough, W.L., Wilson, T.B., Pernak, A., Zabielska-Matejuk, J., Fojutowski, A., Kitad, K., Rogers, R.D., 2006. *Long alkyl chain quaternary ammonium-based ionic liquids and potential applications*. Green Chemistry, 8: 798–806, DOI: 10.1039/B604353D.

[15] Salazar, S., Sanchez, L., Alvarez, J., Valverde, A., Galindo, P., Igual, J., Peix, A., Santa Regina, I. 2011.*Correlation among soil enzyme activities under different forest system management practices*. Ecological Engineering, 37: 1123–1131, DOI: 10.1016/j.ecoleng.2011.02.007.

[16] Stręk, M., Telesiński, A., 2016. *Comparison of selenite (IV) and selenate (VI) effect on some oxidoreductive enzymes in soil contaminated with spent engine oil*. Plant Soil and Environment, 62(4): 157–163, DOI: 10.17221/740/2015-PSE.

[17] Sun, X., Zhu, L., Wang, J., Wang, J., Su, J., Liu, T., Zhang, C., Gao, C., Shao, Y., 2017. *Toxic effects of ionic liquid 1-octyl-3-methylimidazolium tetrafluoroborate on soil enzyme activity and soil microbial community diversity*. Ecotoxicology and Environmental Safety, 135: 201–208, DOI: 10.1016/j.ecoenv.2016.09.026.

[18] Telesiński, A., Sułkowska, N., 2016. *Dissipation dynamic of 1-alkyl-3-methylimidazolium ionic liquids and their effect on o-diphenol oxidase activity in soil* (in Polish). Chemistry. Environment. Biotechnology, 19: 87–92, DOI: 10.16926/cebj.2016.19.11.

[19] Telesiński, A., Śnioszek, M., Biczak, R., Pawłowska, B., 2016. *Environmental and toxicological risk of using quaternary ammonium salts* (in Polish). Kosmos, 65(4): 495–502.

[20] Telesiński, A., Śnioszek, M., Biczak, R., Pawłowska, B., 2017. *Response of soil phosphatases to three different ionic liquids with hexafluorophosphate anion*. Journal of Ecological Engineering, 18(2): 86–91, DOI: 10.12911/22998993/68140.

[21] Thalmann, A., 1968. *Zur methodik der Bestimmung der Dehydrogenase aktivität im Boden Mittels Triphenyltetrazoliumchlorid (TTC)*. Landwirtschaftliche Forschung, 21: 249–258.

[22] Zhang, N., He, X., Gao, Y., Li, Y., Wang, H., Ma, D., Zhang, R., Yang, S., 2010. *Pedogenic carbonate and soil dehydrogenase activity in response to soil organic matter in Artemisia ordosica community*. Pedosphere, 20: 229–235, DOI: 10.1016/S1002-0160(10)60010-0.