

ARKADIUSZ TELESIŃSKI\*, KORNEL CURYŁO\*,  
MACIEJ PŁATKOWSKI\*, TERESA KRZYŚKO-ŁUPICKA\*\*,  
BARBARA PAWŁOWSKA\*\*\*, KRYSZYNA CYBULSKA\*\*\*\*

## COMBINED EFFECT OF LOW-MOLECULAR-WEIGHT ORGANIC ACIDS AND CREOSOTE ON PHOSPHATASE ACTIVITIES IN SANDY SOIL

*Received: 07.08.2017*

*Accepted: 30.10.2017*

*Abstract.* This paper assesses the impact of creosote and low-molecular-weight organic acids (LMWOAs) on the activity of acid phosphomonoesterase, alkaline phosphomonoesterase, phosphotriesterase, and inorganic pyrophosphatase in soil. The experiment was carried out on loamy sand samples with organic carbon content of  $8.71 \text{ g}\cdot\text{kg}^{-1}$ , with the following variable factors: dosages of creosote: 0, 0.5%, and 2.5%; type of LMWOAs: oxalic acid, tartaric acid, and citric acid in the amount of  $50 \text{ mmol}\cdot\text{kg}^{-1}$  of soil; days of experiment: 1, 7, 14, 28, 56, 112. Obtained results showed that contamination with creosote caused decrease in the activity of soil phosphatases. The observed effect did not always increase with increase in the dosage of the pollutant. Among the assayed phosphatases, the biggest changes were noted in the activity of phosphomonoesterases. Application of LMWOAs to contaminated soil mainly effected the inhibition of phosphatases.

---

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

\*\* Independent Department of Biotechnology and Molecular Biology, University of Opole, Poland, 45-032 Opole, Kardynała Kominka 6.

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

\*\*\*\* Department of Chemistry, Microbiology and Environmental Biotechnology, West Pomeranian University of Technology in Szczecin, Poland, 71-434 Szczecin, Słowackiego 17.

tase, especially the activity of acid phosphomonoesterase. Comparison of the effects of LMWOAs showed that the citric acid was the least toxic to soil phosphatases.

**Keywords:** sandy loam, phosphatases, oxalic acid, tartaric acid, citric acid, creosote

## INTRODUCTION

Creosote is a wood-preserving product obtained by fractional distillation of crude coal tar and produced by high-temperature carbonization of bituminous coal (Gallego *et al.* 2008). It is composed of approximately 85% polycyclic aromatic hydrocarbons (PAH), 10% phenolic compounds, and 5% N-, S-, and O-heterocyclics (Simarro *et al.* 2013). Following the Regulation of the European Parliament and of the Council no. 528/2012 of 22 May 2012, beginning from 1 May 2013, creosote, used for impregnation of railway track ties, has been considered a non-threshold carcinogen and it has been classified as a 1B carcinogen, and some of the PAHs have been considered as persistent, bioaccumulative, and toxic (Kukulska-Zajac *et al.* 2014).

According to the estimations of the World Health Organization, at the beginning of the 21<sup>st</sup> century, the annual production of creosote in the EU Member States was from 60 to 100 thousand tons (Ikarashi *et al.* 2005). Thus, large amounts of the substance may penetrate into the soil. Polycyclic aromatic hydrocarbons as well as other creosote components may cause negative impacts on the biochemical processes of the soil, including transformations of the phosphorus compounds. The amount of organic phosphorus in the upper soil layers remains in a wide range: from 20% to over 80% of the total phosphorus content (Turner and Haygarth 2005). The hydrolysis of esters and orthophosphoric acid anhydrides is catalyzed by a large group of enzymes, referred to as phosphatases (Nannipieri *et al.* 2011). The most commonly studied phosphatases include phosphomonoesterases, which catalyze the hydrolysis of organic phosphoric monoesters (Wang *et al.* 2011). Apart from these enzymes, soil contains phosphodiesterases and phosphotriesterases, which catalyze the hydrolysis of phosphate diesters and triesters, respectively, as well as an inorganic pyrophosphatase that catalyzes the decomposition of pyrophosphate to orthophosphates (Reitzel and Turner 2014).

Low-molecular-weight organic acids (LMWOAs) occur widely in soils and primarily originate from root exudation (Gao *et al.* 2003, White *et al.* 2003, Zhao *et al.* 2006, Lu *et al.* 2007). The most common LMWOAs identified in soils include oxalic, succinic, tartaric, fumaric, malic, and citric acids (Kpombekou-A and Tabatabai 2003). LMWOAs have been known to disrupt the sequestering of soil matrix, and thereby enhancing the desorption of organic pollutants in soil (White *et al.* 2003). Consequently, based on the theory, it has been assumed that LMWOAs influence the PAH availability in the soil environ-

ment. However, to date, little research has been conducted in this area, and there is limited information on the availability and sorption–desorption behavior of PAHs from natural soils by organic acids.

The aim of the study was to assess the effect of creosote and three low-molecular-weight organic acids, namely oxalic, tartaric, and citric acids on the activity of phosphatases (acid phosphomonoesterase, alkaline phosphomonoesterase, phosphotriesterase, and inorganic pyrophosphatase) in loamy sand.

## MATERIALS AND METHODS

The testing was performed on soil samples taken from the topsoil at the Agricultural Experimental Station in Lipnik (53°24'N, 14°28'E), located in the West Pomeranian Voivodeship, Poland. According to the classification of the United States Department of Agriculture, it is a soil with granulometric composition of loamy sand. The content of particular fractions, expressed in  $\text{g}\cdot\text{kg}^{-1}$ , was as follows: sand (0.05–2 mm) – 748.6; silt (0.002–0.05 mm) – 231.3; and clay (<0.002 mm) – 20.1. The soil contained, in  $\text{g}\cdot\text{kg}^{-1}$ :  $C_{\text{org}}$  – 8.71 and  $N_{\text{tot}}$  – 0.97. Its hydrolytic acidity was  $9.9 \text{ mmol}(+)\cdot\text{kg}^{-1}$ , and the pH value in  $1 \text{ mol KCl}\cdot\text{dm}^{-3}$  was 6.4. The soil was air-dried and sieved through a 2-mm mesh.

The experiments were carried out in triplicate under laboratory conditions, with the following variable factors: (a) creosote dosages: 0, 0.5%, and 2.5%; (b) type of LMWOAs: oxalic acid, tartaric acid, and citric acid; (c) days of experiment: 1, 7, 14, 28, 56, and 112. The content of LMWOAs added to soil was  $50 \text{ mmol}\cdot\text{kg}^{-1}$ . The 1-kg soil samples were adjusted to 60% maximum water holding capacity, and they were incubated in tightly closed glass containers at a temperature of 20°C.

The acid phosphomonoesterase (Pac – EC 3.1.3.2) and alkaline phosphomonoesterase (Pal – EC 3.1.3.1) activities were determined, as described by Tabatabai and Bremner (1969), with disodium p-nitrophenyl phosphate hexahydrate as a substrate. The phosphotriesterase (PT – EC 3.1.8.1) activity was determined, according to Eivazi and Tabatabai (1977), with tris(p-nitrophenyl) phosphate as a substrate. The yellow band of absorbance of the filtrate, due to p-nitrophenol, was measured at 400 nm. Inorganic pyrophosphatase activity (IPP – EC 3.1.6.1) was determined, as described by Dick and Tabatabai (1978), with sodium pyrophosphate decahydrate as a substrate. Orthophosphate released by inorganic pyrophosphatase activity was extracted with sulfuric acid, and determined photometrically at 700 nm after colorization with ammonium molybdate. Enzyme activities were calculated using a calibration curve.

Based on the mean activity of assayed soil phosphatases, indices on effects of creosote and LMWOAs were calculated using the following formulas (Kaczyńska *et al.* 2015):

$$F_C = \frac{A_C}{A_0}$$

$$F_{LMWOAs} = \frac{A_{LMWOAs}}{A_C}$$

Where:  $IF_C$  – index of the creosote effect;  $IF_{LMWOAs}$  – index of low-molecular-weight organic acids effect;  $A_0$  – activity of phosphatases in non-contaminated soil with the creosote;  $A_C$  – activity of phosphatases in soil contaminated with the creosote;  $A_{LMWOAs}$  – activity of phosphatases in the soil treated with low-molecular-weight organic acids.

If  $IF=1$ , there is no influence of the tested factor on oxidoreductases. If  $IF<1$ , there is inhibition of the oxidoreductases activity by the tested factor and if  $IF>1$ , there is stimulation of the oxidoreductases activity by the tested factor (Stręk and Telesiński 2016a).

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  $\eta^2$  by variance analysis – ANOVA, the percentage shares of all variable factors affecting the activity of phosphatases were defined. Homogeneous groups were calculated using the Tukey's test with  $p<0.05$ .

## RESULTS AND DISCUSSION

The activities of phosphatases in non-contaminated soil with creosote were 111.24–288.81 mg p-NP·kg<sup>-1</sup> dm·h<sup>-1</sup>, 15.83–35.51 mg p-NP·kg<sup>-1</sup> dm·h<sup>-1</sup>, 18.25–22.55 mg p-NP·kg<sup>-1</sup> dm·h<sup>-1</sup> 200.78–259.44 mg p-PO<sub>4</sub><sup>3-</sup>·kg<sup>-1</sup> dm·h<sup>-1</sup> for Pac, Pal, PT, and IPP, respectively (Table 1). Application of all LMWOAs caused a significant decrease in Pac and Pal in the whole experiment. The highest inhibition was observed for Pac and Pal activity in soil treated with oxalic acid on day 1 (96.54%, compared to control) and on day 7 (85.08%, compared to control), respectively. IPP activity was significantly decreased during all experiment in soil with the addition of oxalic acid and tartaric acid, whereas application of citric acid inhibited IPP activity only from day 1 to day 28. On almost all test days, the activity of PT in soil treated with LMWOAs was significantly lower than or similar to control. Moreover, on day 1, increase in PT activity was reported in soil with the addition of tartaric acid. Renella *et al.* (2007) have shown that the activity of phosphatase in the rhizosphere soil layer was generally stimulated by LMWOAs. The alkaline phosphomonoesterase activity was significantly stimulated by citric acid in both clay and sandy soils, whereas the acid phosphomonoesterase activity was significantly stimulated by citric acid in the clay soil and by citric acid and oxalic acid in the sandy soil. Phosphodiesterase activity was

significantly increased by citric acid in the sandy and clay soil. Furthermore, Huang *et al.* (2003) reported that LMWOAs may have an effect on the sorption/desorption activity of acid phosphomonoesterase in soil.

In the whole experiment, creosote at both doses caused a decrease in alkaline phosphomonoesterase activity in soil. Generally, creosote dosage of 2.5% induced higher inhibitory effect on Pac activity than the dosage of 0.5%. Activity of Pal, PT, and IPP in soil containing creosote at the dosage of 0.5% was mainly lower than the control. However, soil contamination with creosote at the dosage of 2.5% caused also an increase in activity of Pal, PT and IPP. This stimulation was the highest for Pal on day 14 (Table 2).

Summarizing the evaluation of the influence of creosote on soil phosphatases, one may ascertain that creosote at the dosage of 0.5% inhibited the activity of Pac, Pal, PT, and IPP (Fig. 1). The mean index of the effect of creosote ( $IF_c$ ) on the activity of Pac and PT decreased, together with an increase in the dosage of creosote. Inhibition of the activity of different phosphatases in soil contaminated with polyaromatic hydrocarbons (PAHs) and other petroleum products was reported by many researchers (Bielńska *et al.* 2014, Ma *et al.* 2014, Bastida *et al.* 2016, Markowicz *et al.* 2016, del Carmen Cuevas-Díaz *et al.* 2017).

The effect of LMWOAs on the activity of phosphatases in soil contaminated with creosote proved to be diversified, and depended on the enzyme, incubation time, creosote dosage and the form of LMWOAs. In soil with creosote at the dosage of 0.5%, on the most days of experiment, application of all LMWOAs affected Pac, Pal, and IPP negatively, but PT – favorably (Table 3). Furthermore, on days 56 and 112, the activity of IPP was stimulated. However, in soil contaminated with creosote at the dosage of 2.5% after treatment with LMWOAs, a decrease in the activities of Pac and IPP and an increase in PT activities were mainly observed. For Pal, stimulation of the activity was reported on days 1, 56, and 112, whereas at other measurement dates, application of LMWOAs caused inhibition (Table 4).

TABLE 1. ACTIVITIES OF PHOSPHATASES IN SOIL NON-CONTAMINATED WITH CREOSOTE

Enzyme	LMWOAs	Incubation time (days)					
		1	7	14	28	56	112
Pac mg p-NP·kg <sup>-1</sup> dm·h <sup>-1</sup>	0	111.24 <sup>a</sup>	172.86 <sup>a</sup>	202.81 <sup>a</sup>	176.71 <sup>a</sup>	288.81 <sup>a</sup>	274.26 <sup>a</sup>
	C <sub>2</sub> H <sub>2</sub> O <sub>4</sub>	3.85 <sup>c</sup>	26.95 <sup>b</sup>	12.84 <sup>d</sup>	180.13 <sup>a</sup>	18.83 <sup>d</sup>	77.44 <sup>d</sup>
	C <sub>4</sub> H <sub>6</sub> O <sub>6</sub>	11.12 <sup>b</sup>	15.40 <sup>c</sup>	67.17 <sup>c</sup>	25.54 <sup>c</sup>	39.36 <sup>c</sup>	92.42 <sup>c</sup>
	C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>	13.26 <sup>b</sup>	13.69 <sup>c</sup>	85.57 <sup>b</sup>	56.91 <sup>b</sup>	59.04 <sup>b</sup>	100.55 <sup>b</sup>
Pal mg p-NP·kg <sup>-1</sup> dm·h <sup>-1</sup>	0	35.51 <sup>a</sup>	28.69 <sup>a</sup>	15.83 <sup>a</sup>	22.68 <sup>b</sup>	27.81 <sup>a</sup>	29.95 <sup>a</sup>
	C <sub>2</sub> H <sub>2</sub> O <sub>4</sub>	12.84 <sup>c</sup>	4.28 <sup>c</sup>	6.42 <sup>c</sup>	12.84 <sup>d</sup>	16.69 <sup>c</sup>	23.53 <sup>b</sup>
	C <sub>4</sub> H <sub>6</sub> O <sub>6</sub>	16.69 <sup>b</sup>	4.71 <sup>c</sup>	13.69 <sup>b</sup>	27.81 <sup>a</sup>	27.38 <sup>a</sup>	30.81 <sup>a</sup>
	C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>	17.11 <sup>b</sup>	15.40 <sup>b</sup>	14.55 <sup>ab</sup>	16.69 <sup>c</sup>	21.39 <sup>b</sup>	30.38 <sup>a</sup>

Enzyme	LMWOAs	Incubation time (days)					
		1	7	14	28	56	112
PT mg p-NP·kg <sup>-1</sup> dm·h <sup>-1</sup>	0	18.43 <sup>b</sup>	20.31 <sup>a</sup>	18.25 <sup>a</sup>	26.66 <sup>a</sup>	21.56 <sup>a</sup>	22.55 <sup>a</sup>
	C <sub>2</sub> H <sub>2</sub> O <sub>4</sub>	20.58 <sup>ab</sup>	17.83 <sup>b</sup>	16.61 <sup>b</sup>	21.95 <sup>b</sup>	19.72 <sup>b</sup>	22.25 <sup>a</sup>
	C <sub>4</sub> H <sub>6</sub> O <sub>6</sub>	21.94 <sup>a</sup>	18.47 <sup>b</sup>	16.39 <sup>b</sup>	23.79 <sup>ab</sup>	19.60 <sup>b</sup>	21.22 <sup>a</sup>
	C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>	20.58 <sup>ab</sup>	19.11 <sup>ab</sup>	16.52 <sup>b</sup>	21.26 <sup>b</sup>	20.62 <sup>ab</sup>	21.95 <sup>a</sup>
IPP mg p-PO <sub>4</sub> <sup>3-</sup> ·kg <sup>-1</sup> dm·h <sup>-1</sup>	0	259.44 <sup>a</sup>	233.12 <sup>a</sup>	200.78 <sup>a</sup>	222.59 <sup>a</sup>	221.09 <sup>a</sup>	221.84 <sup>a</sup>
	C <sub>2</sub> H <sub>2</sub> O <sub>4</sub>	115.06 <sup>d</sup>	91.74 <sup>d</sup>	115.06 <sup>c</sup>	140.62 <sup>c</sup>	147.39 <sup>b</sup>	160.93 <sup>b</sup>
	C <sub>4</sub> H <sub>6</sub> O <sub>6</sub>	135.36 <sup>c</sup>	151.90 <sup>c</sup>	112.05 <sup>c</sup>	124.83 <sup>d</sup>	145.89 <sup>b</sup>	176.72 <sup>b</sup>
	C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>	177.47 <sup>b</sup>	184.24 <sup>b</sup>	127.84 <sup>b</sup>	187.25 <sup>b</sup>	223.34 <sup>a</sup>	215.07 <sup>a</sup>

The same letter means a homogenous group in the columns for an enzyme ( $p < 0.05$ ); Pac – acid phosphomonoesterase, Pal – alkaline phosphomonoesterase, PT – phosphotriesterase, IPP – inorganic pyrophosphatase

TABLE 2. INDICES OF THE CREOSOTE EFFECT ( $IF_c$ ) ON THE ACTIVITY OF SOIL PHOSPHATASES

Enzyme	LMWOAs	Incubation time (days)					
		1	7	14	28	56	112
Pac	0.5%	0.95 <sup>a</sup>	0.84 <sup>a</sup>	0.71 <sup>a</sup>	0.67 <sup>a</sup>	0.68 <sup>a</sup>	0.78 <sup>a</sup>
	2.5%	0.89 <sup>b</sup>	0.77 <sup>b</sup>	0.73 <sup>a</sup>	0.52 <sup>b</sup>	0.58 <sup>b</sup>	0.60 <sup>b</sup>
Pal	0.5%	0.72 <sup>a</sup>	0.50 <sup>b</sup>	0.83 <sup>b</sup>	0.87 <sup>a</sup>	0.72 <sup>b</sup>	0.62 <sup>b</sup>
	2.5%	0.79 <sup>a</sup>	1.20 <sup>a</sup>	1.60 <sup>a</sup>	0.90 <sup>a</sup>	1.26 <sup>a</sup>	0.92 <sup>a</sup>
PT	0.5%	0.97 <sup>b</sup>	0.94 <sup>a</sup>	0.83 <sup>a</sup>	0.68 <sup>b</sup>	1.02 <sup>a</sup>	0.95 <sup>a</sup>
	2.5%	1.13 <sup>a</sup>	0.75 <sup>b</sup>	0.77 <sup>b</sup>	0.91 <sup>a</sup>	0.75 <sup>b</sup>	0.54 <sup>b</sup>
IPP	0.5%	0.78 <sup>b</sup>	0.62 <sup>b</sup>	0.91 <sup>a</sup>	0.61 <sup>b</sup>	0.45 <sup>b</sup>	0.53 <sup>b</sup>
	2.5%	1.10 <sup>a</sup>	1.47 <sup>a</sup>	0.94 <sup>a</sup>	0.90 <sup>a</sup>	0.80 <sup>a</sup>	0.77 <sup>a</sup>

The same letter means a homogenous group in the columns for an enzyme ( $p < 0.05$ ); abbreviations as in Table 1

From the index of LMWOAs effect ( $IF_{LMWOAs}$ ) in soil contaminated with creosote, mean values of below 1 were observed in the activity of Pac for both 0.5% and 2.5% creosote dosages, for Pal at creosote dosage of 0.5%, and for IPP at creosote dosage of 2.5%. Moreover, in soil with creosote at the dosage of 0.5%, mean  $IF_{LMWOAs}$  was lower than 1 after the application of citric acid. However, mean values of  $IF_{LMWOAs}$  above 1 were reported for PT in soil with both creosote dosages treated with all LMWOAs, for Pal in soil with creosote at the dosage of 2.5% treated with all LMWOAs, and for IPP in soil with creosote at the dosage of 0.5% treated with oxalic acid and tartaric acid (Fig. 2).

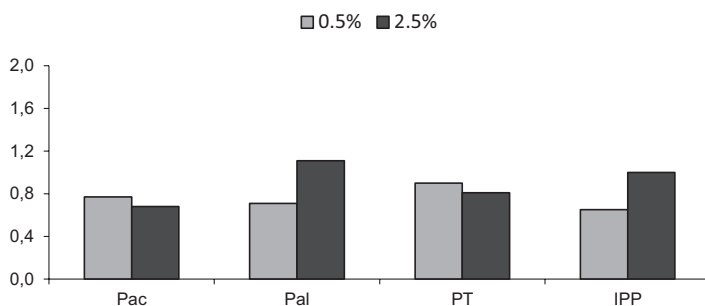


Fig. 1. Mean index of the creosote effect ( $IF_c$ ) on the activity of acid phosphomonoesterase (Pac), alkaline phosphomonoesterase (Pal), phosphotriesterase (PT), and inorganic pyrophosphatase (IPP) activities in soil

TABLE 3. INDICES OF THE LMWOAS EFFECT ( $IF_{LMWOAS}$ ) ON THE ACTIVITY OF PHOSPHATASES IN SOIL CONTAMINATED WITH CREOSOTE AT THE DOSAGE OF 0.5%

Enzyme	LMWOAs	Incubation time (days)					
		1	7	14	28	56	112
Pac	$C_2H_2O_4$	0.07 <sup>a</sup>	0.09 <sup>a</sup>	0.04 <sup>b</sup>	0.03 <sup>a</sup>	0.08 <sup>ab</sup>	0.13 <sup>b</sup>
	$C_4H_6O_6$	0.09 <sup>a</sup>	0.06 <sup>a</sup>	0.06 <sup>b</sup>	0.07 <sup>a</sup>	0.07 <sup>b</sup>	0.15 <sup>b</sup>
	$C_6H_8O_7$	0.07 <sup>a</sup>	0.05 <sup>a</sup>	0.12 <sup>a</sup>	0.06 <sup>a</sup>	0.12 <sup>a</sup>	0.32 <sup>a</sup>
Pal	$C_2H_2O_4$	0.41 <sup>c</sup>	0.09 <sup>c</sup>	0.35 <sup>c</sup>	0.41 <sup>b</sup>	0.37 <sup>b</sup>	0.41 <sup>b</sup>
	$C_4H_6O_6$	0.84 <sup>b</sup>	0.33 <sup>b</sup>	0.72 <sup>a</sup>	0.51 <sup>a</sup>	0.64 <sup>a</sup>	0.66 <sup>a</sup>
	$C_6H_8O_7$	1.04 <sup>a</sup>	0.56 <sup>a</sup>	0.58 <sup>b</sup>	0.51 <sup>a</sup>	0.63 <sup>a</sup>	0.61 <sup>a</sup>
PT	$C_2H_2O_4$	1.08 <sup>b</sup>	1.02 <sup>b</sup>	1.03 <sup>a</sup>	1.15 <sup>b</sup>	0.96 <sup>a</sup>	1.05 <sup>a</sup>
	$C_4H_6O_6$	1.15 <sup>a</sup>	1.12 <sup>a</sup>	1.02 <sup>a</sup>	1.18 <sup>ab</sup>	0.89 <sup>a</sup>	0.93 <sup>b</sup>
	$C_6H_8O_7$	1.10 <sup>ab</sup>	1.16 <sup>a</sup>	1.07 <sup>a</sup>	1.24 <sup>a</sup>	0.93 <sup>a</sup>	0.97 <sup>ab</sup>
IPP	$C_2H_2O_4$	0.49 <sup>a</sup>	0.79 <sup>a</sup>	0.30 <sup>b</sup>	0.81 <sup>a</sup>	1.98 <sup>a</sup>	1.78 <sup>a</sup>
	$C_4H_6O_6$	0.53 <sup>a</sup>	0.76 <sup>a</sup>	0.61 <sup>a</sup>	0.90 <sup>a</sup>	1.93 <sup>a</sup>	1.66 <sup>b</sup>
	$C_6H_8O_7$	0.54 <sup>a</sup>	0.83 <sup>a</sup>	0.39 <sup>b</sup>	0.78 <sup>a</sup>	1.75 <sup>b</sup>	1.39 <sup>c</sup>

The same letter means a homogenous group in the columns for an enzyme ( $p < 0.05$ ); abbreviations as in Table 1

TABLE 4. INDICES OF THE LMWOAS EFFECT ( $IF_{LMWOAS}$ ) ON THE ACTIVITY OF PHOSPHATASES IN SOIL CONTAMINATED WITH CREOSOTE AT THE DOSAGE OF 2.5%

Enzyme	LMWOAs	Incubation time (days)					
		1	7	14	28	56	112
Pac	C <sub>2</sub> H <sub>2</sub> O <sub>4</sub>	0.09 <sup>b</sup>	0.05 <sup>b</sup>	0.06 <sup>a</sup>	0.09 <sup>b</sup>	0.12 <sup>b</sup>	0.18 <sup>b</sup>
	C <sub>4</sub> H <sub>6</sub> O <sub>6</sub>	0.17 <sup>a</sup>	0.06 <sup>b</sup>	0.06 <sup>a</sup>	0.09 <sup>b</sup>	0.21 <sup>a</sup>	0.18 <sup>b</sup>
	C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>	0.20 <sup>a</sup>	0.11 <sup>a</sup>	0.10 <sup>a</sup>	0.20 <sup>a</sup>	0.13 <sup>b</sup>	0.43 <sup>a</sup>
Pal	C <sub>2</sub> H <sub>2</sub> O <sub>4</sub>	1.64 <sup>c</sup>	0.29 <sup>c</sup>	0.44 <sup>a</sup>	0.53 <sup>c</sup>	1.81 <sup>a</sup>	2.20 <sup>b</sup>
	C <sub>4</sub> H <sub>6</sub> O <sub>6</sub>	3.50 <sup>b</sup>	0.61 <sup>b</sup>	0.40 <sup>a</sup>	0.69 <sup>b</sup>	1.37 <sup>b</sup>	2.30 <sup>b</sup>
	C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>	4.68 <sup>a</sup>	0.90 <sup>a</sup>	0.29 <sup>b</sup>	0.83 <sup>a</sup>	1.85 <sup>a</sup>	2.70 <sup>a</sup>
PT	C <sub>2</sub> H <sub>2</sub> O <sub>4</sub>	0.92 <sup>a</sup>	1.20 <sup>b</sup>	1.03 <sup>b</sup>	0.79 <sup>b</sup>	1.15 <sup>b</sup>	1.51 <sup>b</sup>
	C <sub>4</sub> H <sub>6</sub> O <sub>6</sub>	1.01 <sup>a</sup>	1.24 <sup>b</sup>	1.06 <sup>b</sup>	0.97 <sup>a</sup>	1.44 <sup>a</sup>	1.74 <sup>a</sup>
	C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>	0.96 <sup>a</sup>	1.41 <sup>a</sup>	1.14 <sup>a</sup>	0.85 <sup>ab</sup>	1.35 <sup>a</sup>	1.76 <sup>a</sup>
IPP	C <sub>2</sub> H <sub>2</sub> O <sub>4</sub>	0.99 <sup>a</sup>	0.89 <sup>a</sup>	0.38 <sup>c</sup>	0.70 <sup>b</sup>	0.91 <sup>a</sup>	0.91 <sup>b</sup>
	C <sub>4</sub> H <sub>6</sub> O <sub>6</sub>	0.77 <sup>b</sup>	0.77 <sup>b</sup>	0.74 <sup>a</sup>	0.88 <sup>a</sup>	0.82 <sup>b</sup>	1.03 <sup>a</sup>
	C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>	0.57 <sup>c</sup>	0.57 <sup>c</sup>	0.52 <sup>b</sup>	0.50 <sup>c</sup>	0.66 <sup>c</sup>	0.66 <sup>c</sup>

The same letter means a homogenous group in the columns for an enzyme ( $p < 0.05$ ); abbreviations as in Table 1

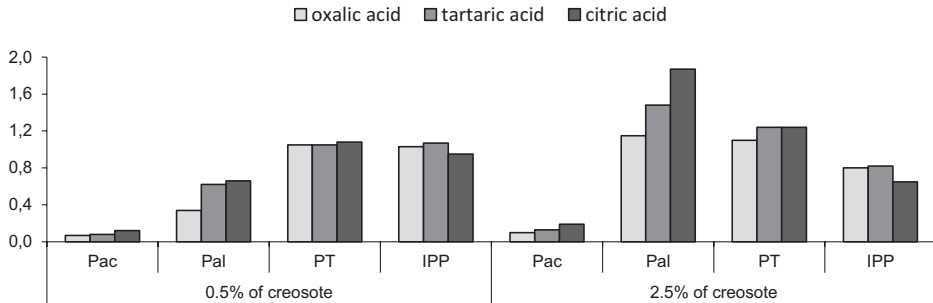


Fig. 2. Mean index of the LMWOAs effect ( $IF_{LMWOAS}$ ) of acid phosphomonoesterase (Pac), alkaline phosphomonoesterase (Pal), phosphotriesterase (PT), and inorganic pyrophosphatase (IPP) activities in soil contaminated with creosote

$IF_{LMWOAS} > 1$  indicated the stimulation of phosphatase activities by the LMWOAs: oxalic acid, tartaric acid, or citric acid. Previous study showed that the application of calcium peroxide improved the activity of dehydrogenases and catalase in soil contaminated with creosote (Stręk and Telesiński 2016b).

The information about the impact of LMWOAs on the enzyme activity in soil contaminated with petroleum hydrocarbons is limited. However, some studies have shown that LMWOAs could accelerate the degradation and desorption of polycyclic aromatic hydrocarbons (PAHs) in soils (LeFevre *et al.* 2013, Ling



*et al.* 2015). Binet *et al.* (2000) have found that rhizosphere of ryegrass potentially enhanced the dissipation or biotransformation of a range of PAHs including 5-and 6-ring PAHs. Ling *et al.* (2015) have reported that the availability of phenanthrene and pyrene in soils increased with the increasing concentration of LMWOAs. Further, several studies suggested that desorption of PAHs in soils could be significantly enhanced with the presence of LMWOAs, thus improving the bioavailability and biodegradation of the residual pollutants (Gao *et al.* 2015, Sun *et al.* 2016).

The data presented in Table 5 indicates, unequivocally, that the activity of phosphatases varied over time. Also, it depended on the creosote dosage. The share of this factor in the formation of dehydrogenase activity ranged from 10.81% (Pal) to 34.49% (IPP). The application of the LMWOAs affected all phosphatases, significantly. The share of this factor in the initiation of the activity ranged from 6.67% (PT) to 78.89% (Pac). Also, the incubation time of the soil affected these enzymes, significantly. This independent variable determined the activity of phosphatases in the range from 5.68% (Pac) to 60.18% (PT).

TABLE 5. PARTICIPATION OF VARIABLE FACTORS IN THE FORMATION OF PHOSPHATASE ACTIVITIES (%)

Factor	Pac	Pal	PT	IPP
Creosote dosage (A)	11.87	10.81	13.35	34.49
Type of LMWOAs (B)	78.89	41.24	6.67	22.85
Day of experiment (C)	5.68	31.01	60.18	15.78
A × B	0.64	9.16	8.35	8.92
A × C	0.99	1.31	4.87	12.62
B × C	1.44	3.02	2.38	3.56
A × B × C	0.47	3.33	3.56	1.67
Error	0.01	0.13	0.63	0.09

## CONCLUSIONS

Soil contaminated with creosote oil resulted in the inhibition of the soil phosphatase activities. The observed effect was not always increased with the increase of the contamination dosage. The greatest changes among the determined enzymes, caused by creosote oil, occurred for the activity of phosphomonoesterases. The introduction of low-molecular-weight organic acids, both to non-contaminated soil and soil contaminated with creosote, resulted primarily in the reduction of phosphatase activity. The biggest visible effect was in the case of acid phosphomonoesterase. A comparison of the effect of different organic acids such as oxalic, tartaric, and citric allows concluding that citric acid was characterized with the lowest toxicity to soil phosphatases.

## REFERENCES

- [1] Bastida, F., Jehmlich, N., Lima, K., Morris, B.E.L., Richnow, H.H., Fernandez T., von Berger, M., Garcia, C., 2016. *The ecological and physiological responses of the microbiological community from a semiarid soil to hydrocarbon contamination and its bioremediation using compost amendment*. Journal of Proteomics, 135: 162–169, DOI: 10.1016/j.jprot.2015.07.023.
- [2] Bielińska, E.J., Mocek-Płóćiniak, A., Futa, B., Kawecka-Radomska, M., 2014. *The relationship between the activity of dehydrogenases and phosphatases and the content of polycyclic aromatic hydrocarbons in urban soils*. Journal of Research and Applications in Agricultural Engineering, 59(3): 8–11.
- [3] Binet, P., Portal, J.M., Leyval, C., 2000. *Dissipation of 3-6-ring polycyclic aromatic hydrocarbons in the rhizosphere of ryegrass*. Soil Biology and Biochemistry, 32: 2011–2017, DOI: 10.1016/S0038-0717(00)00100-0.
- [4] del Carmen Cuevas-Díaz, M., Martínez-Toledo, A., Guzmán-López, O., Torres-López, C.P., del C. Ortega-Martínez, A., Hermida-Mendoza, L.J., 2017. *Catalase and phosphatase activities during hydrocarbon removal from oil-contaminated soil amended with agro-industrial by-products and macronutrients*. Water, Air and Soil Pollution, 228: 159, DOI: 10.1007/s11270-017-3336-2.
- [5] Dick, W.A., Tabatabai, M.A., 1978. *Inorganic pyrophosphatase activity of soils*. Soil Biology and Biochemistry, 10, 59–65.
- [6] Eivazi, F., Tabatabai, M.A., 1977. *Phosphatases in soils*. Soil Biology and Biochemistry, 9: 167–172.
- [7] Huang, Q., Zhao, Z., Chen, W., 2003. *Effects of several low-molecular weight organic acids and phosphate on the adsorption of acid phosphatase by soil colloids and minerals*. Chemosphere, 52: 571–579, DOI: 10.1016/S0045-6535(03)00238-8.
- [8] Gallego, E., Roca, F.J., Perales, J.F., Guardino, X., Berenguer, M.J., 2008. *VOCs and PAHs emissions from creosote-treated wood in a field storage area*. Science of the Total Environment, 402: 130–138, DOI: 10.1016/j.scitotenv.2008.04.008.
- [9] Gao, Y.Z., He, J.Z., Ling, W.T., Hu, H.Q., Liu, F., 2003. *Effects of organic acids on copper and cadmium desorption from contaminated soils*. Environment International, 29: 613–618, DOI: 10.1016/S0160-4120(03)00048-5.
- [10] Gao, Y., Yuan, X., Lin, X., Sun, B., Zhao, Z., 2015. *Low-molecular-weight organic acids enhance the release of bound PAH residues in soils*. Soil and Tillage Research, 145: 103–110, DOI: 10.1016/j.still.2014.09.008.
- [11] Ikarashi, Y., Kaniwa, M., Tsuchiya, T., 2005. *Monitoring of polycyclic aromatic hydrocarbons and water-extractable phenols in creosotes and creosote-treated woods made and procurable in Japan*. Chemosphere, 60: 1279–1287, DOI: 10.1016/j.chemosphere.2005.01.054.
- [12] Kaczyńska, G., Borowik, A., Wyszowska, J., 2015. *Soil dehydrogenases as an indicator of contamination of the environment with petroleum products*. Water, Air and Soil Pollution, 226: 372, DOI: 10.1007/s11270-015-2642-9.
- [13] Kpombekou-A, K., Tabatabai, M.A., 2003. *Effect of low-molecular weight organic acids on phosphorus release and phytoavailability of phosphorus in phosphate rocks added to soils*. Agriculture Ecosystems and Environment, 100(2–3): 275–284.
- [14] Kukulska-Zajac, E., Król, A., Krasińska, A., 2014. *Legal aspects of the management of waste railway sleepers* (in Polish). Chemik, 68(11): 979–982.
- [15] LeFevre, G.H., Hozalski, R.M., Novak, P.J., 2013. *Root exudate enhanced contaminant desorption: an abiotic contribution to the rhizosphere effect*. Environmental Science and Technology 47: 11545–11553, DOI: 10.1021/es402446v.
- [16] Ling, W., Sun, R., Gao, X., Xu, R., Li H., 2015. *Low-molecular-weight organic acids enhance desorption of polycyclic aromatic hydrocarbons from soil*. European Journal of Soil Science 66: 339–347, DOI: 10.1111/ejss.12227.

- [17] Lu, H.L., Yan, C.L., Liu, J.C., 2007. *Low-molecular-weight organic acids exuded by Mangrove (Kandeliacandel (L.) Druce) roots and their effect on cadmium species change in the rhizosphere*. Environmental and Experimental Botany 61: 159–166, DOI: 10.1016/j.envexpbot.2007.05.007.
- [18] Ma, J., Shen, J., Liu, Q., Fang, F., Cai, H., Guo, C., 2014. *Risk assessment of petroleum-contaminated soil using soil enzyme activities and genotoxicity to Vicia faba*. Ecotoxicology, 23(4): 665–673, DOI: 10.1007/s10646-014-1196-8.
- [19] Markowicz, A., Plaza, G., Piotrowska-Seget, Z., 2016. *Activity and functional diversity of microbial communities in long-term hydrocarbon and heavy metal contaminated soils*. Archives of Environmental Protection, 42(4): 3–11, DOI: 10.1515/aep-2016-0041.
- [20] Nannipieri, P., Giagnoni, L., Landi, L., 2011. *Role of phosphatase enzymes in soil*. In: E.K. Bunemann, A. Obreson, E. Frossard (eds.), Phosphorus in action. Berlin, Springer, 215–243.
- [21] Renella, G., Landi, L., Valori, F., Nannipieri, P., 2007. *Microbial and hydrolase activity after release of low molecular weight organic compounds by a model root surface in a clayey and a sandy soil*. Applied Soil Ecology, 36: 124–129, DOI: 10.1016/j.apsoil.2007.01.001.
- [22] Reitzel, K., Turner, B.L., 2014. *Quantification of pyrophosphate in soil solution by pyrophosphatase hydrolysis*. Soil Biology and Biochemistry, 74: 95–97, DOI: 10.1016/j.soilbio.2014.03.001.
- [23] Simarro, R., González, N., Bautista, L.F., Molina, M.C., 2013. *Assessment of the efficiency of in situ bioremediation techniques in a creosote polluted soil: Change in bacterial community*. Journal of Hazardous Materials, 301: 17–26, DOI: 10.1016/j.jhazmat.2013.08.025.
- [24] Stręk, M., Telesiński, A., 2016a. *Comparison of selenite (IV) and selenate (VI) effect on some oxidoreductive enzymes in soil contaminated with spent engine oil*. Plant Soil Environment, 62(4): 157–163, DOI: 10.17221/740/2015-PSE.
- [25] Stręk, M., Telesiński, A., 2016b. *The effect of calcium peroxide on the activity of some oxidoreductases in soil contaminated with creosote* (in Polish). Chemistry. Environment. Biotechnology, 19: 7–11, DOI: 10.16926/cebj.2016.19.01.
- [26] Sun, B., Lian, F., Bao, O., Liu, Z., Song, Z., Zhu, L., 2016. *Impact of low molecular weight organic acids (LMWOAs) on biochar micropores and sorption properties for sulfamethoxazole*. Environmental Pollution, 214: 142–148, DOI: 10.1016/j.envpol.2016.04.017.
- [27] Tabatabai, M.A., Bremner, J.M., 1969. *Use of p-nitrophenyl phosphate for assay of soil phosphatase activity*. Soil Biology and Biochemistry, 1: 301–307.
- [28] Turner, B.L., Haygarth, P.M., 2005. *Phosphatase activity in temperate pasture soils: potential regulation of labile organic phosphorus turnover by phosphodiesterase activity*. Science of the Total Environment, 344: 27–36, DOI: 10.1016/j.scitotenv.2005.02.003.
- [29] Wang, F., Sims, J.T., Ma, L., Ma, W., Dou, Z., Zhang, F., 2011. *The phosphorus footprint of China's food chain: implications for food security, natural resource management, and environmental quality*. Journal of Environmental Quality, 40(4): 1081–1089, DOI: 10.2134/jeq2010.0444.
- [30] White, J.C., Mattina, M.I., Lee, W.Y., Eitzer, B.D., Iannucci-Berger, W., 2003. *Role of organic acids in enhancing the desorption and uptake of weathered p,p0-DDE by Cucurbita pepo*. Environmental Pollution, 124: 71–80.
- [31] Zhao, Z.H., Wang, L.G., Jiang, X., Wang, F., 2006. *Influence of three low-molecular-weight organic acids on the release behavior of HCHs from red soil*. China Environmental Science 26: 324–327.