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Influence of Lead Nitrate and Acetate Applied to Sod-Podzolic Soil on its Bioindicative Parameters

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Abstract—The influence of two lead salts on the soil enzymatic activity dynamics (urease, dehydrogenase, the total hydrolase activity, acidic phosphatase, and peroxidase), with lead ion concentrations of 10, 100, 300, 500, and 1000 mg/kg, was assessed in a 21-day-long model experiment. A reliable inhibitory effect of lead nitrate applied at doses of 500 and 1 000 mg (Pb^{2+})/kg on the total activity of hydrolases, dehydrogenase, and peroxidase has been identified, while lead acetate mostly caused a stimulating effect. Based on the data obtained, the diagnostic indicators have been ranked by the reduction of their sensitivity to lead nitrate pollution in the following order: total activity of hydrolases > peroxidase > dehydrogenase > urease ~ acidic phosphatase.

Keywords: heavy metals, soil assessment, soil enzymes, significance of bioindication parameters, regulation

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INTRODUCTION

Amid the global pollution of the soil cover, the identification of sensitive and informative biodiagnostics parameters making it possible to assess the soil biota condition becomes increasingly important. Biodiagnostics parameters based on direct assessment of the biota activity or obtained through applicative methods using test soil organisms are considered most promising, while studies on soil–water extracts under conditions of chemical pollution raise numerous questions (Terekhova, 2011; Olkova et al., 2016; Vestberg et al., 2001).

As pollutants, heavy metals, including lead, are able to accumulate in the soil cover and exercise long-term effects on the pace and direction of biochemical reactions ongoing in the soil and toxic impacts on living organisms inhabiting it (Zvyagintsev, 1987; Fokina, 2008). To assess the lead impact on soils, methods involving measurements of activity of such enzymes as urease (Hemida et al., 1997), dehydrogenase (Aoyama and Nagumo, 1996), phosphatase (Doelman and Haanstra, 1989), and total hydrolase activity method (Yang et al., 2014) are commonly used. However, the published data on the sensitivity of the above enzymes to lead are very inconsistent and often make it impossible to select the most sensitive parameter (Table 1).

The purpose of this study was to assess the influence of lead nitrate and acetate on the dynamics of the biological parameters in a sod-podzolic soil in a model experiment and rank the diagnostic indicators by their sensitivity to lead pollution.

MATERIALS AND METHODS

Soil characteristics. Sod-podzolic and previously cultivated middle loamy soils collected in September 2015 in Chashnikovo Training and Experimental Soil Ecological Center of Moscow State University located in the Solnechnogorsk district of Moscow oblast were used in the experiment. The soil samples were collected using the "envelope" method on a site 10 × 10 m in size in the upper ploughing horizon (0–20 cm); the volume was reduced and averaged by quartering. The averaged samples were air dried; plant roots were removed and then pushed through a sieve with a mesh size of 1 mm. Prior to the experiment, the soil had the following characteristics: physical clay (fraction < 0.01 mm) content, 35%; pH_{KCl}, 6.00 ± 0.05; pH_{H_2O}, 7.58 ± 0.05; organic carbon content, 2.55%; humus content, 4.39%; content of potassium (K_2O) and phosphorus (P_2O_5) inactive form, 10 and 20 mg/100 g respectively; and background lead content (the total form), 5 mg/kg.

Model experiment. The following lead (Pb^{2+}) concentrations were studied: 0 (control), 10, 100, 300, 500, and 1000 mg/kg, which is equivalent to 0, 0.3, 3.1, 9.4, 15.6, and 31.3 maximum permissible lead concentrations according to the Hygienic Standards 2.1.7.2041-06 (2006). Lead application forms (nitrate and acetate) have been chosen due to their good water

Table 1. Effective lead concentrations affecting the soil enzymatic activity (EC_{50}) based on literature data

Parameter	EC_{50}, mg/kg	Soil characteristics	Application form	Source
1	2	3	4	5
Urease	>400	Sod-podzolic heavy loamy, pH 4.8; humus content, 1.5%	$Pb(NO_3)_2$	Faiza, 1993
	1727–2459	Melanic brunisol, C_{org}, 5.5%; pH 5.7	$PbCl_2$	Chaperon and Sauvé, 2008
	n/a (stimulating effect)	Krasnozem, C_{org}, 1.2–2.6%	$Pb(NO_3)_2$	Yang et al., 2014
	n/a (stimulating effect)	Chernozem leached low-humic middle loamy	$Pb(CH_3COO)_2$	Ananyeva and Shpis, 2010
	320	Typical chernozem	$Pb(CH_3COO)_2$	Semenova et al., 2011
	n/a (stimulating effect)	Sod-podzolic previously cultivated	$Pb(CH_3COO)_2$	Fokina, 2008
Dehydrogenase	1266–1895	Brown forest, C_{org}, 5.5%; pH 5.7	$PbCl_2$	Chaperon, Sauvé, 2008
	n/a (stimulating effect)	Krasnozem, C_{org}, 1.2–2.6%	$Pb(NO_3)_2$	Yang et al., 2014
	>2000	Brown forest, pH 5.4; humus content, 4.4%	PbO	Mazanko et al., 2013
	32–320	Brown carbonate	PbO	Kolesnikov et al., 2015
	500	Brown, C_{org}, 1.78%	$Pb(NO_3)_2$	Pan and Yu, 2011
Hydrolases (by fluorescein diacetate (FDA) hydrolysis)	>1600	Krasnozem, C_{org}, 1.2–2.6%	$Pb(NO_3)_2$	Yang et al., 2014
Phosphatase	>800	Krasnozem, C_{org}, 1.2–2.6%	$Pb(NO_3)_2$	Yang et al., 2014
	>500	Sod-podzolic, humus content, 1.8–2.1%	$Pb(CH_3COO)_2$	Arzamazova, 2004
	36.7– 381 – n/a	Sand–loam–clays	$Pb(NO_3)_2$	Doelman and Haanstra, 1989
Phosphatase	n/a in the range of 10–100 mg/kg	Chernozem carbonate, pH, 7.6; C_{org}, 3.1%	$Pb(CH_3COO)_2$	Belyaeva et al., 2005

n/a, no inhibitory effect identified.

solubility and large number of studies dedicated to lead mobility in soils and its impact on bioindicative parameters in these forms (Table 1).

Soil lots with a mass of 200 g were placed in jars. Lead nitrate or acetate water solutions were added to reach 60% of the maximum field water capacity of the substrate and the required lead ion concentrations. The soil was stirred thoroughly with a metal palette knife. The jars were incubated for 21 days at a temperature of 22 ± 2°C. The moisture level was maintained by regular moistening with distilled water. The moisture level was controlled using the weight method: weight loss in each jar due to water evaporation should not exceed 5%. Each experiment was replicated three times. On the 3rd, 7th, and 21st day, series of specimen were taken for measurements of the enzymatic activity.

Specimens taken for enzymatic activity measurements were stored in leakless polyethylene bags in a fridge at a temperature of 4°C; the storage period did not exceed ten days after specimen collection.

Methods used to assess the soil enzymatic activity. The impact of the lead salts on the condition of soil as a habitat of living organisms was assessed by changes in the soil enzymatic activity.

The dehydrogenase activity was assessed using the methodology proposed by Lenhard (1962) with modifications. To soil lots 0.1 g $CaCO_3$ and 1% glucose solution were added with a mass of 1 g to reach 90% of the maximum soil water holding capacity and incubated for 24 h in a thermostat at 30°C. Then 3 mL of 1% triphenyltetrazolium chloride and 1% glucose solution were added to the flask and incubated again for 3 hours at 30°C. The resultant triphenylformazan (TPF), a degradation product of triphenyltetrazolium chloride, was extracted by 25 mL of ethanol, then centrifuged, and the optic density was measured in the supernatant at 456 nm. The device readings were recalculated, using a calibration curve, into μg TPF g^{-1} 24 h^{-1}.

The activity of acidic phosphomonesterases (phosphatase) was assessed using the methodology proposed by M. Tabatabai and J. Bremner (1969). One-gram lots of soil were put into test tubes, then 4 mL of modified universal buffer (MUB) (pH 6.5) and 1 mL of 5 mM solution of para-nitrophenyl sodium phosphate were added. The test tubes were then sealed and incubated for 2 h in a thermostat at 37°C. The resultant para-nitrophenol (p-NP) was extracted by 1 mL of 0.5 M solution of $CaCl_2$ and 4 mL of 0.5 M NaOH solution. The optical density of the resultant colored solutions was measured after filtering the samples through a white ribbon filter at 405 nm. The device readings were recalculated, using a calibration curve, into μg p-NP g^{-1} h^{-1}.

The total activity of hydrolases was assessed through the fluorescein diacetate (FDA) hydrolysis reaction modified by A.V. Yakushev and B.A. Byzova (2009). Soil lots with a mass of 1 g were put into test tubes; then 0.1 mL of fluorescein diacetate solution (2 g/L) and 10 mL of 0.1 M potassium phosphate buffer (pH 7.6) were added. The specimens were incubated for 1 hour at 30°C; the optical density of the solutions was measured after centrifuging for 3 min at 2000 rotations per minute at 490 nm. The device readings were recalculated, using a calibration curve, into μg of fluorescein g^{-1} h^{-1}.

The urease activity was assessed using the methodology proposed by L. Kong et al. (2009). Two milliliters of potassium phosphate buffer (pH 7.6) and 2 mL of 10% urea solution were added to soil lots with a mass of 1 g. The specimens were incubated for 48 h at 37°C; then 4 mL of 1 M KCl solution were added to the test tubes and shaken using a rotation shaker for 30 min at 180 rotations per minute. After filtering through the white ribbon filter, 1 mL of aliquot was put into a 25-mL measuring flask. Distilled water, 4 mL of 1 M NaOH solution, 1 mL of 50% Siegnette salt water solution, and 0.4 mL of Nessler's reagent were added; the volume was made up to the mark. The optical density of the resultant colored solutions was measured using a color filter with a wave length of 460 nm. The results were recalculated, using a calibration curve, into μg NH_3 g^{-1} 24 h^{-1}.

The peroxidase activity was assessed using the methodology proposed by L.A. Karyagina and N.A. Mikhailovskaya (1986). Ten milliliters of 1% hydroquinone solution and 1 mL of 0.05% hydrogen peroxide solution were added to soil lots with a mass of 1 g and incubated for 30 min in a thermostat at 30°C. Then 10 mL of ethanol were added to the flask, the mix was filtered and centrifuged, and the optical density was measured in the supernatant at 405 nm. The device readings were recalculated, using a calibration curve, into μg of benzoquinone (BQ) g^{-1} h^{-1}.

The activity measurements for all the enzymes were replicated four times for each soil specimen with a blank test correction (the soil calcined at 120°C for 3 h).

The *statistical data processing* was performed using the Statistica 10 software package. The significance and reliability of identified differences were assessed through single-factor variance analysis and pairwise comparison of the mean values using Fisher's Least Significant Difference (LSD) test for each parameter. The curves were constructed using the SigmaPlot 11.0 software (United States).

Toxicometric parameters (i.e., effective lead concentrations $NOEC_{10}$, EC_{20}, and EC_{50}) in the enzymatic activity measurement experiments have been computed using a complementary log–log model $\varphi(x) = 1 - \exp[-\exp(\beta X)]$ from the XLSTAT-Ecology (AddinSoft) software package.

RESULTS AND DISCUSSION

A series of experiments have been performed to assess the impact of lead nitrate and acetate applied in lead ion concentrations of 10, 100, 300, 500, and 1000 mg/kg on the activity of certain enzymes in a previously cultivated sod-podzolic soil.

Figure 1 shows the dehydrogenase activity assessment results depending on the lead concentration and form. With an increase in the lead concentration in the soil, a trend toward inhibition of dehydrogenase activity has been identified in specimens taken on the 7th and 21st day of the experiment; with the maximum application dose (1000 mg/kg), the deviation from the control had reached 42.5 and 26.1%, respectively. The values of enzyme activity in specimens containing less than 300 mg/kg of lead did not differ reliably from the control specimens. A reliable negative correlation ($r = -0.85$, $p < 0.05$) between the dehydrogenase activity on the 21st day of the experiment and the Pb^{2+} concentration in the nitrate form has been identified; the results obtained are consistent with the literature data (Khan et al., 2007; Pan and Yu, 2011).

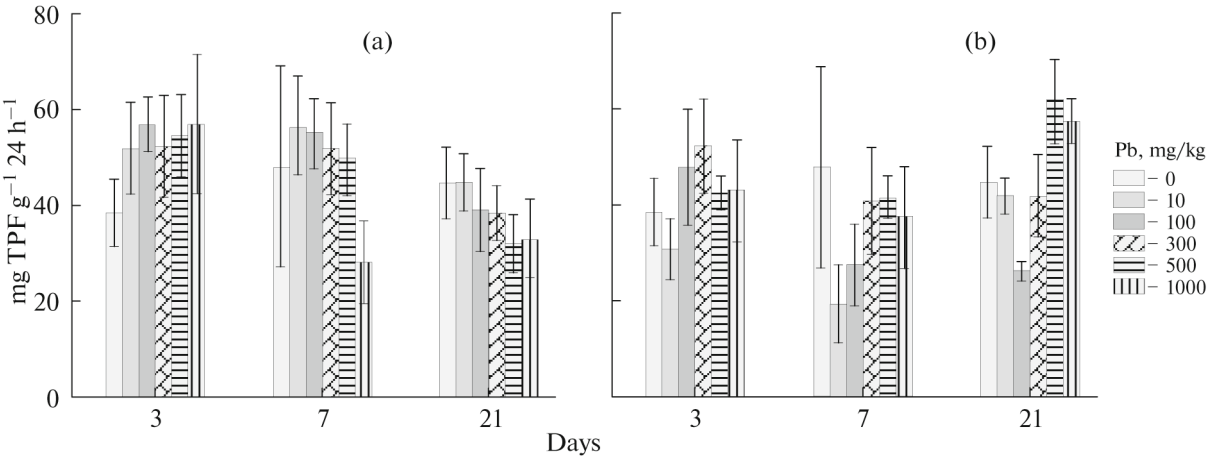

Fig. 1. Effect of various lead concentrations on the dehydrogenase activity of a sod-podzolic soil in a model experiment: (a) $Pb(NO_3)_2$; (b) $Pb(CH_3COO)_2$. Hereinafter, the charts show mean values of the observed parameters and error bars reflecting standard deviations ($N = 12$).

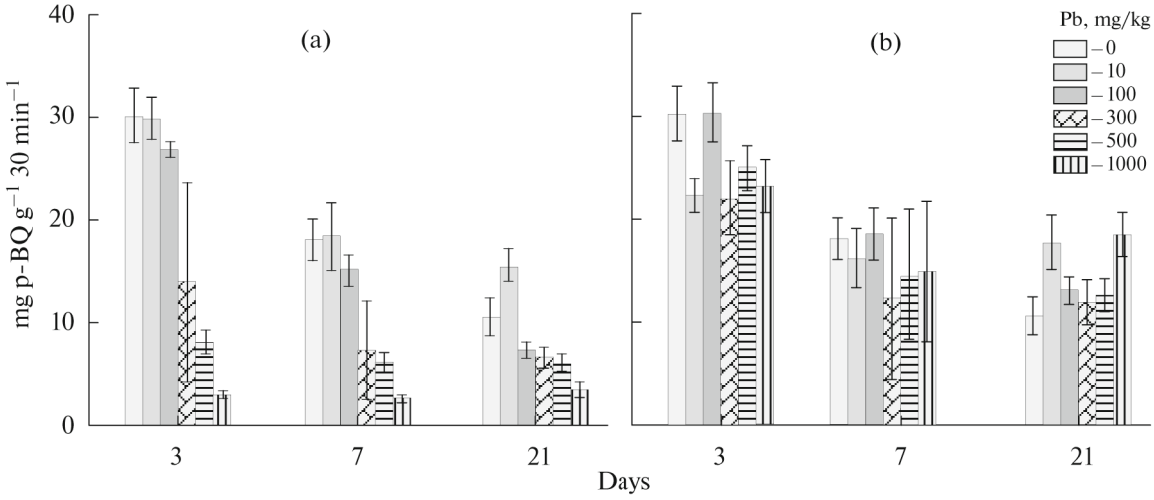

Fig. 2. Effect of various lead concentrations on the peroxidase activity of a sod-podzolic soil in a model experiment: (a) $Pb(NO_3)_2$; (b) $Pb(CH_3COO)_2$.

The application of lead acetate caused mixed effects. A significant variability of the responses in the lead concentration gradient in comparison with the control has been identified, while the maximum application doses (500 and 1000 mg/kg) caused a discernable stimulating effect on the 21st day of the experiment; the activity in these specimens was higher than the control values by 66.7 and 55.1%, respectively. Similar data indicating the potential stimulating effect of lead on the dehydrogenase activity were obtained by T. Stuczynski et al. (2003) for lead concentrations of 500 and 700 mg/kg applied in the chloride form. The authors link the effect with possible transformations of the soil microbial community: death of species sensitive to lead chloride contamination and biological growth of other bacteria resistant to that contamination and using the dead cell walls as a nutrition source. In the course of such processes, the concentration of dehydrogenase, as an enzyme involved in oxidation–reduction processes of the soil, had increased. It is possible to assume that the stimulating effect of lead acetate identified in our experiment has a similar explanation.

Figure 2 presents the results of the assessment of lead nitrate and acetate impacts on the peroxidase activity. The experiment has shown that, similarly to dehydrogenase, lead nitrate causes a reliable inhibitory effect on the peroxidase activity, while certain concentrations of lead acetate cause a stimulating effect.

The total activity of hydrolases assessed using the FDA hydrolysis method in the specimens with lead

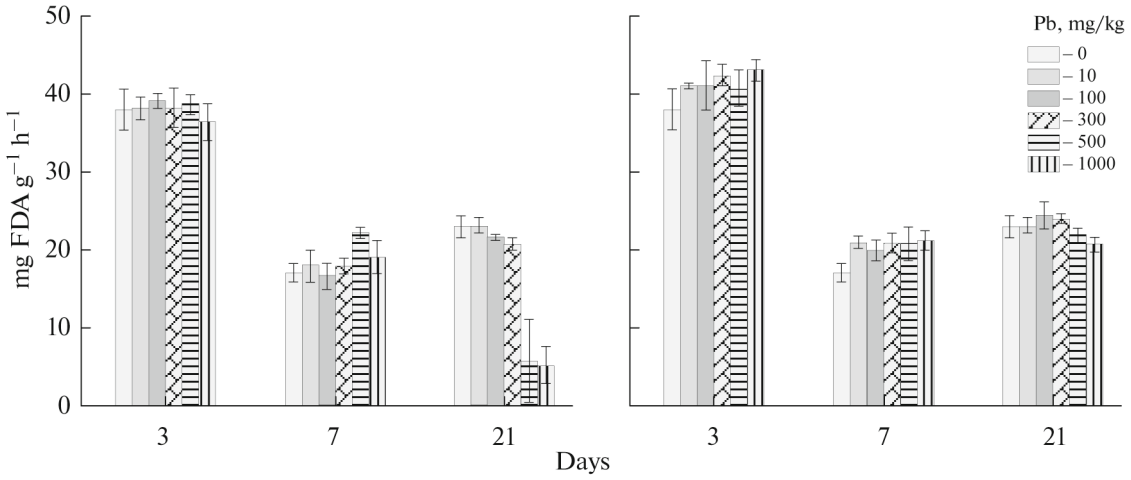

Fig. 3. Effect of various lead concentrations on the total hydrolase activity (FDA) of a sod-podzolic soil in a model experiment: (a) $Pb(NO_3)_2$; (b) $Pb(CH_3COO)_2$.

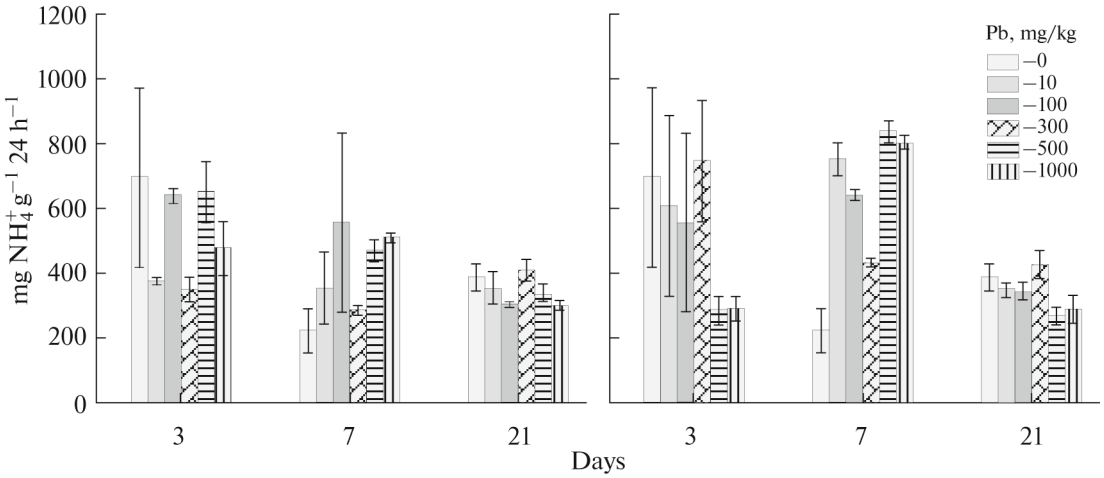

Fig. 4. Effect of various lead concentrations on the urease activity of a sod-podzolic soil in a model experiment: (a) $Pb(NO_3)_2$; (b) $Pb(CH_3COO)_2$.

acetate and nitrate was not different from the control in the first seven days of exposition. However, on the 21st day, a differentiation in the FDA hydrolysis activity was identified in specimens with different lead nitrate concentrations (Fig. 3a). In specimens with lead ion concentrations of 500 and 1000 mg/kg, the activity was reduced by more than 76% in comparison with the control samples containing no lead. All the lead acetate concentrations studied were not different from the control values (Fig. 3b). The FDA hydrolysis activity in soils determines the general course of hydrolytic processes, i.e., the total activity of lipases, proteases, and esterases (Guilbault and Kramer, 1964; Schnürer and Rosswall, 1982); a number of works have shown a close correlation relationship between the FDA activity and the length of the fungal mycelium (Söderström, 1977; Ingham and Klein, 1984).

The application of lead nitrate and acetate did not affect significantly the activity of urease and phosphatase (Figs. 4, 5).

A significant variability in the urease activity values was observed within the variants, both in the control specimens and with application of lead nitrate and acetate, on the 3rd and 7th days of the experiment; this may indicate the presence of additional factors affecting the urease activity at the initial stages of succession of the microbial community. The average enzyme activity in control specimens had reliably decreased by the end of the experiment; this may have been caused by gradual depletion of easily accessible organic substrates in the model experiment (Moreno et al., 2003).

The lack of discernable effects from lead application on the acidic phosphatase activity was, most likely, caused by the heavy granulometric composition

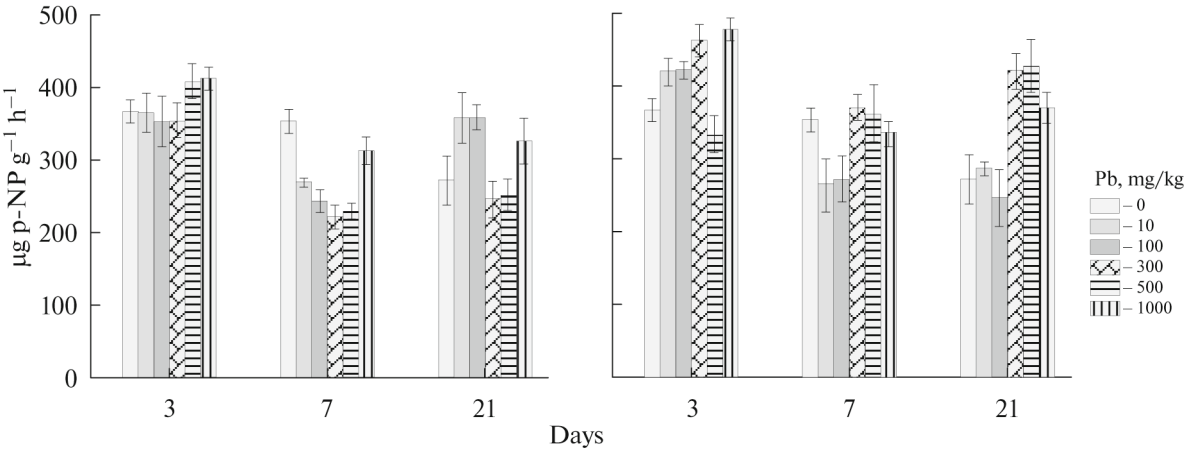

Fig. 5. Effect of various lead concentrations on the acidic phosphatase activity of a sod-podzolic soil in a model experiment: (a) $Pb(NO_3)_2$; (b) $Pb(CH_3COO)_2$.

of the soil, including a high content of the clay fraction able to adsorb the enzymes in clay interplanar spaces, thus, protecting those against inhibiting factors (Zvyagintsev, 1979; Doelman and Haanstra, 1989; Zhang et al., 2015).

Differences between the impacts on the soil enzymatic activity caused by lead nitrate and acetate can be clearly illustrated by a comparison of the median parameter deviation coefficients in specimens containing lead expressed in percentage points of the control values (Fig. 6).

The results presented indicate that lead applied in the nitrate form causes a more discernable inhibiting effect in comparison with lead acetate. When lead nitrate is applied, the median values of deviation coefficients for hydrolases (FDA), peroxidase, and dehydrogenase have been identified in the inhibition range. When lead acetate is applied, the median values of the deviation coefficients for the above enzymes are identified in the enzymatic activity stimulation range. The phosphatase and urease activities basically were not affected by the lead form and concentration; this may indicate a low sensitivity and informative value of these parameters in the assessment of lead pollution of sod-podzolic soils. The obtained data clearly demonstrate the influence of the selected form of heavy metal application on registered responses in model experiments.

Using an equation describing the logistic curve of the enzymatic activity response to the lead concentration gradient, the effective impact levels of lead nitrate on sod-podzolic soils have been computed for the data registering reliable deviations from the control values at least for the maximum lead application doses on the 21st day of the experiment. Therefore, changes in the dehydrogenase, peroxidase, and total hydrolase activities have been taken into account, while the activities of urease and acidic phosphatase were disregarded. The results of the computations performed are shown in Table 2.

The method used by the authors to calculate the correlation between the application dose and its effect and to assess lead concentrations sufficient to identify the value of the negative effect on the activity of cer-

Table 2. Effective (reacting) lead concentrations affecting the soil enzymatic activity

Parameter	Effective (reacting) lead concentrations, Pb mg/kg			R (McFadden)*
	NOEC (EC_{10})	EC_{20}	EC_{50}	
Dehydrogenase	49.5 (15.2–136.2)	450.4 (160.1–3122.7)	8985.4 (1646.2–516750.4)	0.131
Peroxidase	0.04 (0.0–0.90)	0.60 (0.0–7.06)	377.65 (37.33–98785.5)	0.138
Hydrolases	128.4 (99.7–162.4)	191.3 (151.1–244.1)	488.2 (371.3–688.2)	0.391

* The calculated McFadden's "pseudo" correlation index shows how adequately the complementary log-log model describes the empiric data. Values in the 0.2–0.4 range can be interpreted as a "very close" correlation relationship (Behavioral Travel Modelling, 1979).

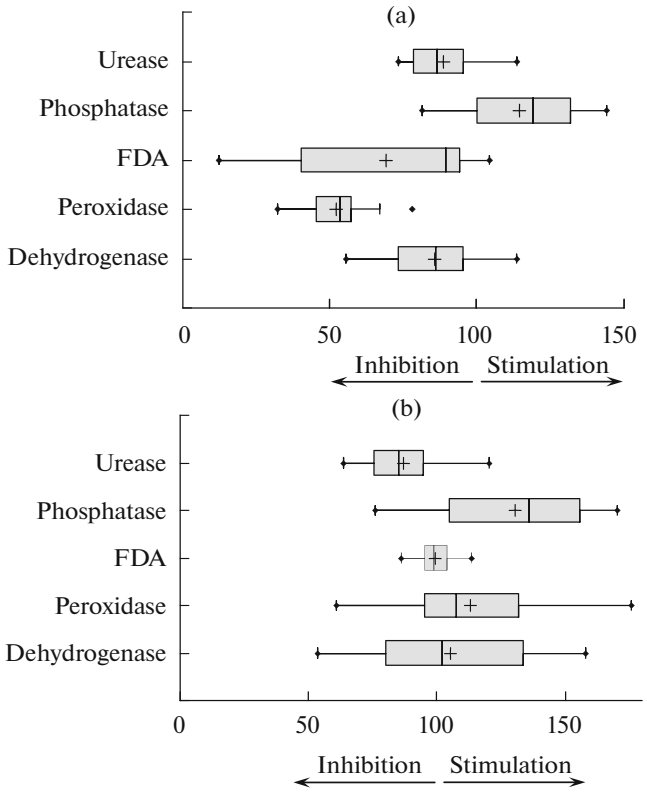

Fig. 6. Diagrams showing the range of enzymatic activity deviation coefficients for a sod-podzolic soil under the influence of (a) lead nitrate ($Pb(NO_3)_2$); and (b) lead acetate ($Pb(CH_3COO)_2$) in comparison with the control (100% control) for all lead application doses on the 21st day of the experiment. The charts provide the median, quartile, and range of the observed values.

tain soil enzymes has shown that the logistical model describes optimally the response of the total activity of hydrolases (the FDA-based method) to the application of lead nitrate. With regards to dehydrogenase and peroxidase, the computed correlation index values were located in the "weak correlation" range, which has resulted in high standard error values for the calculated concentrations. Based on the data obtained, the activity of soil enzymes can be ranked by the reduction of their sensitivity to lead nitrate pollution in the following order: FDA > peroxidase > dehydrogenase > urease ~ acidic phosphatase.

CONCLUSIONS

The experimental results show that lead salts of different origin (organic and inorganic ones) mostly cause oppositely directed effects on the enzymatic activity of sod-podzolic soils. A mostly stimulating effect of lead acetate manifested through increased activities of dehydrogenase, peroxidase, and acidic phosphatase has been noted. Lead nitrate caused a more significant inhibitory effect. Based on the dehydrogenase, peroxidase, and total hydrolase (the FDA-based method) activity values, the effective (reacting) lead concentrations have been calculated for the 21st day of the experiment. The EC_{50} effective lead concentration is mostly located in the range over 500 mg/kg of Pb^{2+} in the form of lead nitrate.

ACKNOWLEDGMENTS

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