The influence of long-term sewage sludge application on the activity of phosphatases in the rhizosphere of plants

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ABSTRACT

Model experiments using rhizoboxes were carried out in order to evaluate the influence of different plants (wheat, rape) on the changes in water extractable contents of P, the pH/H_2O value and the activity of acidic and alkaline phosphatase in soil of plant rhizosphere. For this experiment, a Cambisol with different long-term fertilizing systems was used: (i) control (with no fertilizer application), (ii) sewage sludge, and (iii) manure. A lower content of water-soluble P was observed in close vicinities of root surfaces (up to 2 mm) at all the studied variants. The control (non-treated) variant reflected a significantly lower content of water-soluble P in the rhizosphere compared to the fertilized ones. The activities of the acidic and alkaline phosphatases were significantly higher in the rhizosphere compared to the bulk soil (soil outside the rhizosphere). The long-term application of organic fertilizers significantly increased phosphatase activity; the activity of the acidic phosphatase was significantly higher in the rhizosphere of rape plants compared to wheat. The variant treated with manure exhibited an increased activity of both the acidic and alkaline phosphatases compared to the variant treated with sewage sludge. In the case of the variant treated long-term with sewage sludge, the portion of inorganic P to total soil P content proportionally increased compared to the manure-treated variant. Soil of the rape rhizosphere showed a trend of lower pH/H_2O value of all variants, whereas the wheat rhizosphere showed an opposite pH tendency.

Keywords: sewage sludge; phosphorus; acidic and alkaline phosphatase; wheat; rape; rhizosphere

Total phosphorus concentration in soils varies from 0.01–0.15% according to the soil type, organic matter content and fertilizing intensity. Organic P contents (P_{org}) account generally for 20–85% of the total P content with the largest fraction of organic P in the form of phytin (Dalal 1977). Tarafdar and Claassen (1988) mention that organic compounds (such as phytin, lecithin and glycerol-phosphate) are very important in plant nutrition. Phosphorus release from organic compounds is governed by the activity of different enzymes. Phosphatases catalyze the release of P from organically bound P in the form of esters: C-O-P (Eivazi and Tabatabai 1977). Acidic phosphatases are present in the rhizosphere of most plant species. In plants, this enzyme is mainly present in the cell nucleus, cell walls and intracellular spaces, and to a lower extent in amyloplasts, mitochondria and the endoplasmatic reticulum (Chen et al. 1992). Acidic phosphatase from roots is an ectoenzyme released mostly in apical root zones.

The activity of the acidic phosphatase is not only plant-specific (Helal 1990, Yadav and Tarafdar 2001) but it is also dependent on the level of P deficit in plant nutrition (Tarafdar and Jungh 1987, Tadano and Sakai 1991, Mudge et al. 2003, Wasaki et al. 2003). Phosphatase activity produced by roots of rice without sufficient nutrition was twice higher compared to the control (Hirata et al. 1982). A similar effect was observed by Goldstein et al. (1988) in an experiment with tomatoes. Nevertheless, Li et al. (2004) found that the selected plant species play an important role as well. The activity of the acidic phosphatase was 2–3-fold higher in the case of Chinese cabbage compared to corn; corn and Chinese cabbage produced more acidic phos-

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phatase when phytin was added to the soil, compared to the addition of KH_2PO_4 or $Ca(H_2PO_4)_2$. Marschner et al. (2007) showed that the P uptake by plants (*Brassicae* and *Poaceae*) significantly correlated with the activity of acidic phosphatase only at low P levels in soils. Interestingly, the activity of the acidic phosphatase and P uptake by plants correlated well at early stages of plant growth (phase of 6 leaves, blooming phase), but not at later stages of mature plants. Therefore, the authors assume that the mobilization of organic P is more emphasized at earlier growth stages. Similarly, the alkaline phosphatase hydrolyzes P from organic compounds. It is produced by soil fungi and bacteria (Dick and Kandeler 2005). Bhadraray et al. (2002) found a high correlation between the activity of the alkaline and acidic phosphatases in the rhizosphere of plants and the degree of evolution of the rice panicle.

Nannipieri et al. (1978) and Spiers and McGill (1979) state, that organic fertilizers stimulate phosphatase activity in the rhizosphere of cereals due to the increased microbial activity in the soils. At the same time, the activity was inhibited by inorganic soil P. Furthermore, in the experiment of Kizilkaya and Bayrakli (2005) the addition of sewage sludge to soils increased the activity of alkaline phosphatase. On the other hand, Speir et al. (2007) showed that the activity of the acidic phosphatase was partly inhibited at high dosages of applied sewage sludge. A negative correlation was found between the activities of the acidic phosphatase and zinc concentrations in soil. Crecchio et al. (2004) found a positive correlation between compost addition to soil and phosphatase activity. The work of Landi et al. (2000), based on balance procedures, confirmed that the addition of only inorganic P or combined with stable manure, decreases phosphatase activity.

Sewage sludge presents an important source of nutrients and organic matter; however, it can contain increased concentrations of risk elements, organic pollutants and pathogenic microorganisms (Balík et al. 2002). It is thus necessary to evaluate the changes of soil characteristics during intensive long-term sewage sludge application. Studies dealing with the rhizosphere are important to determine the dynamics of nutrient changes in soils (Hinsinger 2001).

The aim of this work was to evaluate the changes in water-soluble P and the activity of acidic and alkaline phosphatase in the rhizosphere of plants in relation to different sources of organic fertilization.

MATERIAL AND METHODS

Rhizobox experiments were carried out in order to evaluate the changes in P contents in the rhizosphere of plants. The type of rhizobox used was described in detail in Wenzel et al. (2001) and Tlustoš et al. (2006). Cambisols from selected long-term field experiments were chosen to represent non-treated variant and variants treated with sewage sludge and manure. The soil was air-dried and the fraction of < 2 mm was used for rhizobox experiments.

Wheat and oilseed rape were selected as experimental plants; they were grown under controlled conditions in a growth chamber (day: 24°C, humidity 95%; night: 16°C, humidity 60%) and were watered using only demineralized water. After the harvest, the rhizosphere was precisely sliced into 1mm layers.

Water-extractable P in the soil was determined using the H_2O extraction with the w/v ratio of 1:10. After 16 hours of shaking, the suspension was filtered and was analyzed for P using optical emission spectrometry with inductively coupled plasma (ICP-OES; Varian VistaPro, Australia).

The pH value was determined using the extraction with demineralized H_2O with the w/v ratio of 1:10. The suspension was shaken for 16 hours and left to settle for 1 hour. Prior to analysis, the sample was mechanically mixed and the pH/H_2O value was determined potentiometrically using a glass electrode.

Phosphatase activity in the soil was determined as follows (Tabatabai and Bremner 1969): the soil was incubated for 2 hours at 25°C in the solution of *p*-nitrophenylphosphate (Fluka) (1:1 w/v) with added buffer [0.1M TRIS = hydroxymethylaminomethan solution (Merck)]. In order to determine the acidic and alkaline phosphatase, the pH of the buffer was adjusted using concentrated HCl and NaOH, respectively. The formed *p*-nitrophenol was determined spectrophotometrically at λ = 400 nm.

Total concentrations of P in plant samples were determined after dry ashing dissolution using ICP-OES.

RESULTS AND DISCUSSION

Table 1 summarizes the contents of different P forms at tested variants prior to the rhizobox experiment. It is apparent that the control variant exhibited a lower total P content (aqua re-

Table 1. Phosphorus concentration in the Cambisol before the rhizobox experiment (mg P/kg)

Treatment	pH/$CaCl_2$	Mehlich 3	H_2O	AEM-PES	Ammonium oxalate			DL-NaOH			P_{ar}
					P_{ox}	Fe_{ox}*	Al_{ox}**	P_{an}	P_{tot}	P_{org}	
Control	5.0	105	12.0	51	505	3077	1305	252	855	603	678
Sewage sludge	5.1	216	16.2	79	774	3907	1593	350	1046	696	878
Manure	5.1	174	16.2	60	726	3293	1408	279	907	628	758

*mg Fe/kg, **mg Al/kg; Mehlich 3 – extraction according to Mehlich (1984); H_2O – water extraction
AEM-PES – determination of P using anion exchange membranes (Tiessen and Moir 1993)
P_{ox}, Fe_{ox}, Al_{ox} – P, Fe, Al determined by ammonium oxalate extraction (Schwertmann 1964)
P_{an}, P_{tot}, P_{org} – mineral, total and organic P determined by DL-NaOH extraction: [$(CH_3CHOHHCOO)_2Ca$ (c = 0.01 mol/l) + HCl (c = 0.01 mol/l); pH = 3.6] + subsequent extraction with NaOH (c = 1 mol/l) (Marks 1977)
P_{ar} – total P determined by aqua regia extraction (EN 13346)

gia/DL-NaOH extraction) and a lower content of more mobile P forms. The content of water-extractable P accounted here for 12 mg/kg, while at the variants treated with sewage sludge and manure, the concentration reached 16.2 mg/kg for both variants. Similarly, the values determined using AEM-PES membranes and by the method Mehlich 3 confirmed significantly higher P contents at fertilized variants. It is clear, however, that variants treated with sewage sludge reflected higher total and mobile P concentrations compared to the variants treated with manure, which corresponds well to long-term P application to separate variants. When comparing the values of P_{org} and P_{tot} determined by the DL-NaOH extraction (Marks 1977) it is evident that the variant with manure exhibited a slightly higher organic P fraction (69.2%) compared to the variant treated with sewage sludge (66.5%). This is in accordance with different sorption characteristics of P from different fertilizers. Data obtained from the ammonium oxalate extraction suggest that in the case of sewage sludge variant a larger portion of P is bound to Fe and Al oxides compared to the variant with manure.

Dry biomass yields of the aboveground and root biomass are presented in Table 2, with the lowest values found for the control variant and the highest for the variant with sewage sludge. This is due to the fact that the sludge has higher macro- and micro-nutrients contents (with the exception of N, K) compared to the manure. Phosphorus contents in plants are summarized in Table 3, with the lowest value for the control variant (with the only exception of rape roots). The yield factor and P content in plants corresponds well with total P uptake (Table 4). It is possible to assume that these results reflect well the total and mobile P contents at the control variant as well as at fertilized variants. Furthermore, the aboveground biomass accumulated significantly higher P concentrations compared to roots.

Phosphorus contents in the rhizosphere of plants are summarized in Figure 1. It is important to note that the presented values are not the real concentrations in the soil solution but P concentrations obtained from the water extraction (w/v 1:10). It is widely accepted that the concentrations of salts in the soil solution in the field are 2- to 4-times higher than in the soil extract obtained from the

Table 2. Dry yields of aboveground and root biomass (g/rhizobox)

Treatment	Wheat		Rape	
	aboveground biomass	roots	aboveground biomass	roots
Control	1.6	0.3	3.7	1.1
Sewage sludge	2.2	0.5	4.0	1.1
Manure	1.8	0.2	3.8	1.0
F-test	11.89	10.03	0.89	1.18
d_{min} (α = 0.05)	0.28	0.13	ns	ns

Table 3. Phosphorus concentrations in plants (mg/kg dw)

Treatment	Wheat		Rape	
	aboveground biomass	roots	aboveground biomass	roots
Control	2297	1181	3118	3462
Sewage sludge	2810	2372	3539	3155
Manure	4223	2355	3127	3786
F-test	3.03	7.63	5.47	4.32
d_{min} (α = 0.05)	ns	323	329	486

Table 4. Phosphorus uptake by plants (mg/rhizobox)

Treatment	Wheat			Rape		
	aboveground biomass	roots	total uptake	aboveground biomass	roots	total uptake
Control	3.62	0.52	4.14	11.46	3.72	15.18
Sewage sludge	6.11	1.19	7.30	14.16	3.32	17.48
Manure	7.50	0.47	7.97	11.73	3.71	15.44
F-test	5.18	12.25	45.43	4.74	2.20	2.70
d_{min} (α = 0.05)	2.65	0.38	2.73	2.17	ns	ns

"saturated soil paste" (Marschner 2003). For both wheat and rape, the lowest content was found in the close vicinities of plant roots, which is in accordance with the results of Fusseder and Kraus (1986). Results from the control variant also suggest that low P concentrations were found at 3–6 mm from root surfaces. Interestingly, the sewage sludge variant exhibited lower concentrations compared to the variant with manure even though before the start of the experiment, soil analyses showed that water-extractable P was similar for both variants; still, the contents of available P (obtained from Mehlich 3, AEM-PES) were higher at the variant with sewage sludge. Higher plant uptake can explain only the experiments with rape, not with wheat; it is related to an increased P sorption to Fe and Al oxides and/or a lower mineralization rate of P from organic compounds at the variants with sewage sludge. Furthermore, higher P concentrations were found in the rhizosphere of rape compared to wheat, which confirms a higher ability of rape to mobilize P from less available forms. This ability can be further confirmed by the pH/H_2O values (Figure 2). The results reflect the acidifying effect of rape on the rhizosphere environment. Hypothesis that this acidification was caused by the lack of P

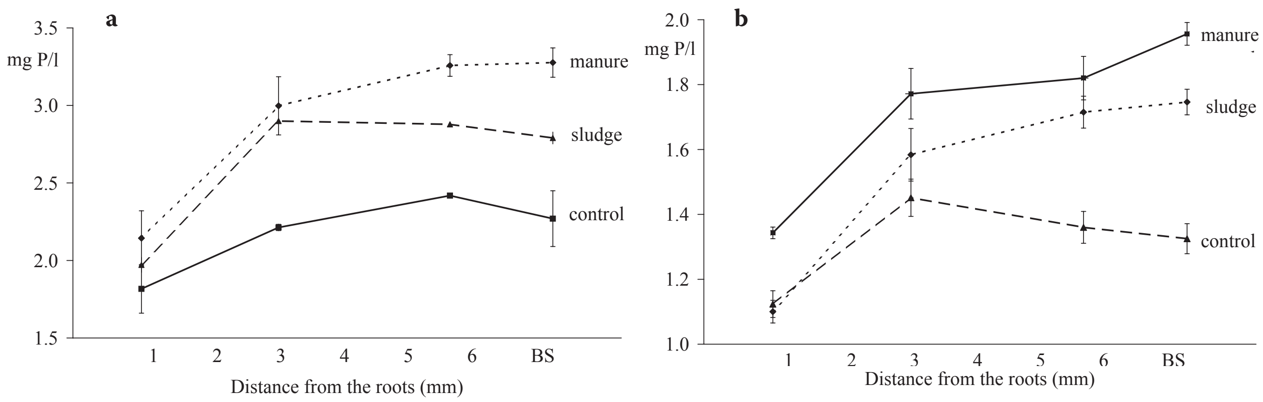

Figure 1. Changes of water extractable P (mg/l) in (a) oilseed rape and (b) wheat rhizosphere

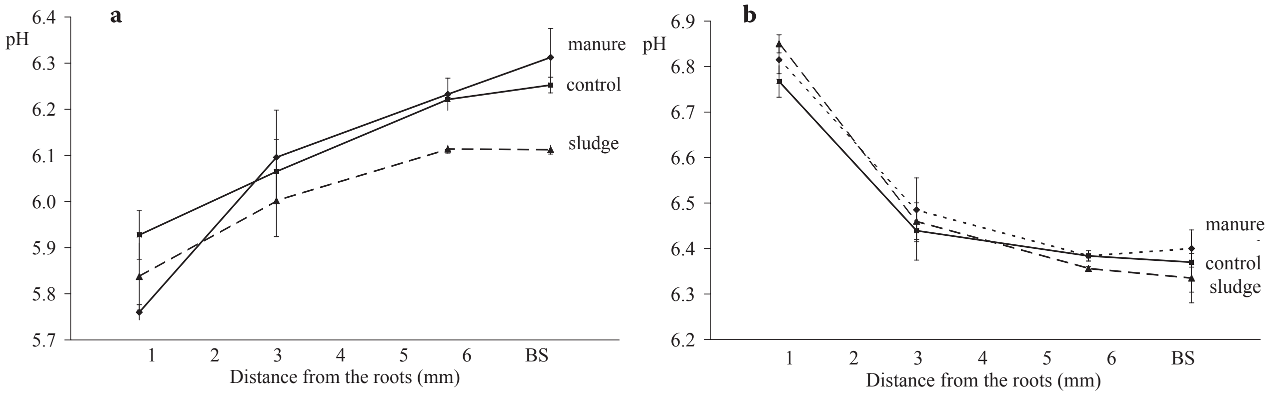

Figure 2. Changes of pH/H_2O in (a) oilseed rape and (b) wheat rhizosphere

cannot be approved because the acidification occurred even at variants fertilized with P. According to the Czech regulations using the Mehlich 3 procedure, very high concentrations of available P are present in the soils. A more probable reason for the pH change is the unbalanced ratio between the cations and anions taken up by the rape plants (Marschner 2003, Balík et al. 2007).

Contrary to the rape, an increase of pH was observed in the close vicinities of wheat roots. This can be caused by the presence of increased concentrations of cations (Ca, Mg) (data not shown). A comparable increase of accumulation of Ca and Mg in the rhizosphere of barley was found by Youssef and Chino (1987).

Due to the fact that direct N fertilizing was not applied, the influence of N forms is not so important as for the acid-base equilibrium and the pH of the rhizosphere. Analyses of N_{min} in soils at all variants showed that the prevailing form was nitrate. Its influence was more emphasized at the control variant compared to the fertilized ones.

The results of phosphatase activity analyses are presented in Figure 3. Significant differences in the acidic phosphatase activity were found in the rhizosphere and in the bulk soil. In our case, it was an average sample of the rhizosphere at 0–4 mm from root surfaces. The chosen distance is in accordance with the work of Nuruzzaman et al. (2006) who found an increased activity at as far as 3–4 mm from root surfaces. Contrary to that, Dick and Kandeler (2005) highlight that the activity of enzymes is the highest at root surfaces and their closest vicinities (0–1.3 mm). This is mostly caused by the accumulation of fast degradable exudates, mass flow and limited diffusion of dissolved organic compounds, which are used by soil microorganisms. Dick and Kandeler (2005) summarize information about positive correlations between enzymatic activities and amounts of exudates; both these factors decrease with increasing distance from root surface. It is thus possible to assume that the values in the close vicinities of roots would be significantly higher. The influence

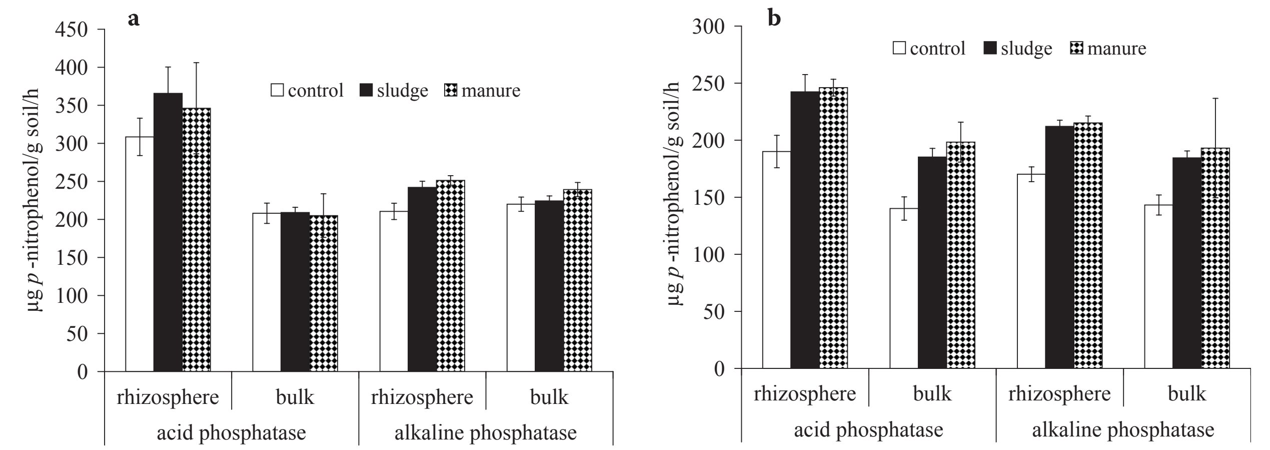

Figure 3. The phosphatase activity in (a) oilseed rape and (b) wheat rhizosphere (μg *p*-nitrophenol per 1 g of soil per hour)

of the plant species on the activity of the acidic phosphatase was also proved, which is in compliance with the works of other authors (Helal 1990, Yadav and Tarafdar 2001, Li et al. 2004). Based on the results obtained from plant analyses and P contents in the rhizosphere it is possible to anticipate that the increased activity of acidic phosphatase at variants with sewage sludge and manure were not caused by insufficient P nutrition. The increased phosphatase activity is probably related to the overall higher microbial activity caused by fertilization (Nannipieri et al. 1978, Spiers and McGill 1979) and/or by an increased production of root exudates as a consequence of more intensive plant growth. Marschner et al. (2007) found that P uptake by plants (*Brassicae* and *Poaceae*) significantly correlated with the activity of the acidic phosphatase only at low P levels in soils. Furthermore, it is important to note that in our experiments, the sampling along the root axis was not performed, but a whole volume of soil was sampled at 0–4 mm distance from the roots. It is possible to expect more significant differences between variants at root tips. As it was stated in the work of Eltrop (1993) hydrolytic enzymes (acidic phosphatase, phytase) are characterized by low mobility in soils and are mostly connected to cell walls and mucilage at the root tips. Similar results were obtained by Dinkelaker and Marschner (1992).

Alkaline phosphatase hydrolyzes P from organic compounds. It is produced by fungi and bacteria in the soil (Dick and Kandeler 2005). Phosphatase activity was higher in the rhizosphere than in the bulk soil, which confirms a higher microbial activity in this zone caused by a higher content of easily degradable root exudates (Figure 3). An increased activity was found at the variant with sewage sludge compared to the control, which is in good agreement with the work of Kizilkaya and Bayrakli (2005). From the figures mentioned above, it is clear that the increased activity was found at the variant with manure compared to the variant with sewage sludge. This is probably caused by the addition of a higher concentration of higher quality organic matter, together with an increase of microbial activity at the variant with manure.

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