Effect of phosphite on soil microbial enzyme activity and the feeding activity of soil mesofauna

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Abstract

The effect of phosphite on the biocoenosis of an arable soil was investigated in a field trial in 2004 – 2005. In particular, the responses of soil micro-organisms and soil mesofauna were objects of the studies. It was shown that no inherent phosphite was detectable in the investigated soil. After the application of phosphite in form of potassium dihydrogenphosphite (KH_2PO_3) to the upper layer of a grass-grown plot, an adaptation of the soil and the metabolic active soil biocoenosis, respectively, caused at least a partly oxidation of phosphite to phosphate.

Microbial enzyme activities demonstrated no significant correlation to soil contents of phosphite and phosphate with exception of dehydrogenase that was correlated to phosphate. The mesofaunal feeding activity was significantly decreased by incorporation of phosphite in combination with mud flat soil originated from the German North Sea.

Keywords: arable soil, fertilisation, phosphite, phosphate, microbial enzyme activity

Zusammenfassung

Wirkung von Phosphit im Boden auf mikrobiologische Enzymaktivitäten und die Fraßaktivität der Bodenmesofauna

Der Einfluss von Phosphit auf die Biozönose von Bodenmikroorganismen und Bodentieren wurde im Feldversuch von 2004 bis 2005 ermittelt. Es konnte gezeigt werden, dass Phosphit im untersuchten Ackerboden per se nicht nachweisbar war. Nach Phosphitapplikation erfolgte nach Adaptation des Bodens bzw. der stoffwechselaktiven Bodenbiozönose zumindest die teilweise Oxidation des applizierten Phosphits zu Phosphat. Die Enzymaktivitäten der Mikroorganismen zeigten bis auf das Enzym Dehydrogenase, welches mit dem Phosphatgehalt korreliert war, keine signifikanten Zusammenhänge mit den Bodengehalten an Phosphit und Phosphat.

Die Fraßaktivität der Bodenmesofauna war nach Applikation von Phosphit und Wattboden signifikant vermindert.

Schlüsselwörter: Ackerboden, Düngung, Phosphit, Phosphat, mikrobielle Enzymaktivitäten

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1 Introduction

The term phosphite refers to an alkaline metal salt of phosphonic acid or hydrogen-phosphonic acid (H_3PO_3), respectively (Rikkard, 2000). Here, potassium (K) is the complexing agent.

Phosphite is mainly used in viticulture and horticulture for pest control purposes but is supposed to have a fertilising effect too (Rickard, 2000; Broschat, 2006; Speiser et al., 1993; McIntire et al., 1950; Angeles-Wedler, 2005; Schroetter et al., 2006). In this study, the effect of potassium dihydrogenphosphite (KH_2PO_3) on soil micro-organisms and mesofaunal organisms was investigated by determining the microbial enzyme activities and the faunal feeding activity of invertebrates. Soil micro-organisms and invertebrate faunal organisms collaboratively decompose soil organic matter. Their metabolic activity can refer to the soil quality (Emmerling et al., 2002). Micro-organisms are key agents in the degrading of soil organic matter (Stevenson and Cole, 1999). The mesofaunal organisms control the microbial growth by feeding (Topp, 1981). The decomposition of organic matter in soil provides the re-cycling of essential nutrients for plant growth. The microbial enzyme activities of dehydrogenase, cellulase, protease and alkaline phosphatase were investigated in this study to get information about the effect of phosphite fertilisation on microbial metabolism. Dehydrogenase is a common measure for the intensity of microbial metabolism (Tabatabai, 1982). Dehydrogenase is an oxidoreductase involved in the intracellular metabolism of all micro-organisms. Cellulose as the most important organic compound in soil (Schinner et al., 1996) will be metabolised by the enzyme cellulase. Cellulase activity is involved in carbon metabolism. Protease belongs to the nitrogen metabolism by microbial degradation of proteins. Proteins are a source of nitrogen in soil mainly derived from dead organisms, plants and animals (Schinner et al., 1996). Alkaline phosphatase is involved in the phosphorus metabolism, its activity represents the microbial ability to release organically bound phosphorus (P) of a soil.

The use of phosphite as P fertiliser presumes its oxidation to plant usable phosphate that is performed by micro-organisms in soil (Malacinski et al., 1966) and in marine sediments (Schink et al., 2005).

2 Materials and methods

The field trial was conducted at the experimental site of the FAL Braunschweig (Northern Germany, Lat/Lon: 10°25‘59 / 52°17‘15). The soil is composed of 46 % sand, 47 % silt and 7 % clay hence it is characterised as a strong silty sand following the German classification system (AG Boden, 1994). The effect of 15 g m^{-2} P, added in form of potassium dihydrogenphosphite, on soil micro-organisms and mesofaunal organisms living within the upper 30 cm soil layer was temporarily monthly investigated between September 2004 and November 2005.

2.1 Soil treatment

On the field, where the sample area was located, a permanent pasture was established. The existing grass sward was removed before the first soil treatment. Afterwards a new grass cover (*Lolium perenne*) was established.

The soil was fertilised twice with 7.5 g P m^{-2} as an aqueous KH_2PO_3 solution*. Additional, at the first phosphite application in September 2004, 2.5 kg m^{-2} mud flat soil (42 % DM) originated from the North Sea shoreline near Husum, Germany, was added to enhance the phosphite oxidation. Because, certain phosphite oxidising micro-organisms are supposed to occur in such substrates (Schink et al., 2005). Corresponding amounts of the phosphite solution and mud flat soil were mixed and replenished with tap water to a volume of 4 L m^{-2}. The finished dispersion was evenly spread on the soil by a watering can, and afterwards superficially raked in. The control plots received 4 L m^{-2} pure tap water in an analogous manner. In April 2005 the application of 7.5 g P m^{-2} and tap water, respectively, was repeated.

2.2 Chemical analysis

The content of phosphite and phosphate in soil was measured by ion chromatography (IC) following the method of Angeles-Wedler (2005). The separation was performed by an analytical column (Metrosep A Supp 5-250) with a guard column (Metrosep A Supp 4/5). The eluent of the column contained 4.0 mM Na_2CO_3 and 3.0 mM NaOH in 10 % acetone.

An aqueous soil extract (1:20 w:v) using moist soil was prepared and passed through a conditioned BOND Elut® C18 SPE (Solid Phase Extraction) cartridge.

2.3 Physical measurement

Soil electrical conductivity (EC) was measured electrometrically at 20 °C. An aqueous extract (1:2.5 w:v) was prepared with distilled water at the same temperature.

* The authors thank KEMIRA GrowHow GmbH Hannover for the friendly provision of the KH_2PO_3 solution.

2.4 Biological properties

The microbial enzyme activities of dehydrogenase, cellulase, protease and alkaline phosphatase were measured in the soil of the upper layer (3 - 15 cm). The faunal feeding activity was also determined within this soil horizon.

The measurement of dehydrogenase activity was conducted following Öhlinger (1996) with triphenyltetrazolium chloride (TTC). TTC was transformed to triphenylformazan (TPF) which was detected by a spectrophotometer at 546 nm.

Cellulase activity was determined by carboxymethyl sodiumsalt (CM - cellulose) as substrate which resulted to glucose equivalents (GE) (von Mersi and Schinner, 1990). The concentration of glucose equivalents was colorimetrically determined as Prussian blue at 690 nm.

Protease activity was performed using casein as substrate (Kandeler, 1996) that was metabolised to amino acids. Amino acids form a blue complex by addition of Folin Ciocalteu´s phenol which concentration was photometrically measured at 700 nm and expressed as tyrosine equivalents (tyr).

Alkaline phosphatase activity was determined with p-nitrophenyl phosphate following Margesin (1996). The substrate p-nitrophenyl phosphate was converted by the enzyme phosphomonoesterase to p-nitrophenol (p-NP) which was photometrically detected at 400 nm.

The feeding activity of soil mesofaunal organisms was determined by the bait lamina test of von Törne (1990). The bait (65 % cellulose, 15 % agar-agar, 10 % bentonite, 10 % wheat bran) was exposed for ten days vertically in the upper soil layer (3 - 12 cm).

2.5 Statistical data analysis

The significance of phosphite and phosphate effects on soil microbial and mesofaunal properties was evaluated using the least significant difference (LSD) test provided by the SPSS 12.0 software (SPSS, Chicago IL).

3 Results and discussion

The application of the KH_2PO_3 solution significantly increased the soil phosphite and phosphate concentrations compared to the control plots without fertilisation (Table 1). Phosphite was not detectable in untreated plots.

Seven days after the first application of 7.5 g m^{-2} P as KH_2PO_3 solution (= 1,905 mg P) in September 2004, phosphite was exclusively observed in aqueous soil extracts of the phosphite treated plots (841 mg kg^{-1} phosphite = 331 mg kg^{-1} P), all other plots read zero (Figure 1). The phosphate content was slightly increased (74 mg kg^{-1}) in phosphite treated plots compared to untreated control plots (52 mg kg^{-1}). The amount of P recovered from phosphite

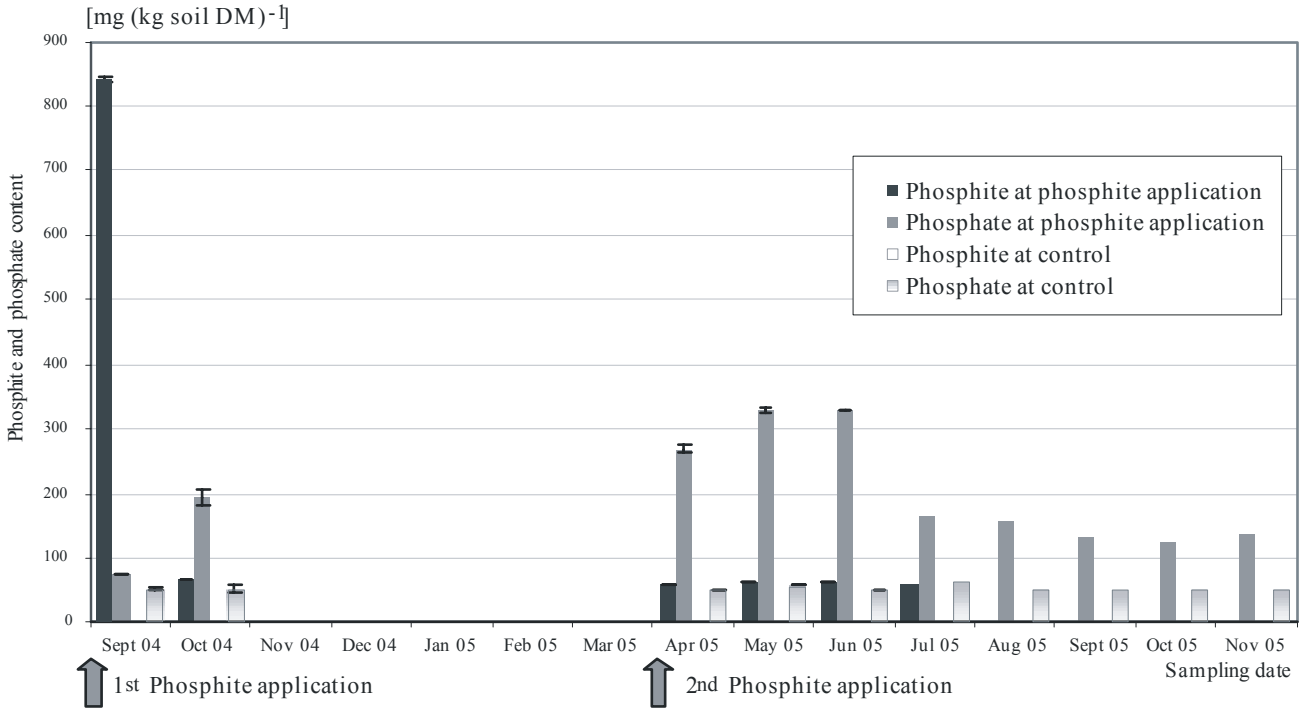

Figure 1:

Phosphite and phosphate contents of a strong silty sand after twice application of 7.5 g m^{-2} P as KH_2PO_3 solution, *Lolium perenne*, model field experiment 2004/2005

application was 18.5 %.

During the following 30 days soil phosphite concentration decreased by 92 % in phosphite treated plots. The phosphate concentration increased in the phosphite treated plots at the same time to 193 mg kg^{-1} phosphate (= 63 mg kg^{-1} P). Rickard (2000) and Malacinski (1966) assumed that phosphite is oxidised by soil micro-organisms to phosphate after a lag phase. After the second phosphite treatment in April 2005 a delay of phosphite oxidation was not observed, oxidation of phosphite re-started immediately. This could be a sign of a well adapted microbial community for phosphite oxidation. The highest phosphate concentration (330 mg kg^{-1}) was found in the soil two month after the second phosphite application. Thereafter, the soil phosphate content dropped slowly to 138 mg kg^{-1} until the experiment was ceased (Figure 1). Phosphite was detected the last time in July 2005. However, a significant correlation between phosphite and phosphate contents was not found (Table 3). Even though, the analytical detected amounts if phosphite and phosphate were accumulated, the computed total amount of applied P (38,100 mg m^{-2} phosphite-P yield 45,900 mg m^{-2} phosphate-P) could not be recovered. The lacking amounts of P (in phosphite or phosphate form) could have been leached and displaced in a deeper soil horizon, or was not extractable fixed to soil particles or integrated in tissues of plants, micro-organisms and soil fauna.

The soil sampling in September 2004 (two weeks after the first treatment) showed that the addition of a phosphite/mud flat soil dispersion resulted in a significantly increased electrical conductivity (EC) of 26 % in the treated soil (control: 8 mS m^{-1}) (Table 1).

Table 1:

Phosphite and phosphate contents and EC of a strong silty sand, two weeks after application of a KH_2PO_4/mud flat soil dispersion, *Lolium perenne*, model field experiment 2004

Treatment	Phosphite [mg kg^{-1}]	Phosphate [mg kg^{-1}]	EC [mS m^{-1}]
Control	0	53	8
Phosphite/ mud flat soil dispersion	114	19,100	30
LSD $_{5\%}$	36	39	12

EC = electrical conductivity
LSD = least significant difference

The microbial enzyme activity of dehydrogenase and alkaline phosphatase (control: 90 µg g^{-1} d^{-1} TPF; 47 µg g^{-1} h^{-1} p-NP) were significantly increased by 21 % and 28 %, respectively, in phosphite treated soils (Table 2). Both enzymes were positively but not significantly correlated to the phosphite content (Table 3).

The cellulase activity (control: 4,142 µg g^{-1} d^{-1} GE) was decreased by 16 % but not significantly by phosphite addition while the protease activity (control: 213 µg $^{-1}$ g^{-1} $2h^{-1}$ tyr) virtually constantly remained in phosphite treated and untreated soils (Table 2). The dehydrogenase as well as the alkaline phosphatase activity were positively correlated to the soil phosphate content, whereas the cellulase and the protease activity were negatively correlated to this parameter (Table 3).

The microbial enzyme activity of dehydrogenase was significant positively correlated to the EC at 0.05 level. No correlations were found to cellulase and protease activity while alkaline phosphatase activity was even significant positively correlated at 0.01 level (Table 3).

Table 2:

Microbial enzyme activities and faunal feeding activity in a strong silty sand after phosphite application, *Lolium perenne*, model field experiment 2004/2005

Treatment	Dehydrogenase [µg g^{-1} d^{-1} TPF]	Cellulase [µg g^{-1} d^{-1} GE]	Protease [µg g^{-1} $2h^{-1}$ tyr]	Alkaline phosphatase [µg g^{-1} h^{-1} p-NP]	Feeding activity [% 10 d^{-1}]
Control	90	4,142	213	47	27
Phosphite application	109	3,479	234	60	23
LSD $_{5\%}$	6	1,341	42	4	2

TPF = triphenyl formazan, DM = dry matter, GE = glucose equivalents, tyr = thyrosine equivalents, p-NP = p nitrophenol, LSD = least significant difference

The combined phosphite/mud flat soil application significantly reduced the feeding activity of soil mesofauna by 15 % (Table 2). The feeding activity was negatively correlated to the microbial enzyme activities of dehydrogenase and cellulase. The feeding activity was significantly negative correlated to the soil phosphite content, the correlation to the phosphate content was also negative but not significant (Table 3).

6 Conclusions

Phosphite did not naturally occur in the investigated soil. However, applied phosphite was oxidised to phosphate after a lag phase. The mud flat soil addition combined with phosphite fertilisation affected the faunal feeding activity. Both, the soil phosphite content as well as the soil salt content displayed as electrical conductivity were significant negatively correlated at the 0.05 level and the 0.01 level, respectively, to the faunal feeding activity. No significant correlations were found between microbial enzyme

Table 3:
Pearson correlation for selected biological, chemical and physical properties of a silty sand, *Lolium perenne*, model field experiment 2004/2005

	Dehydro-genase	Cellulase	Protease	Alkaline phosphatase	Feeding activity	Phosphite	Phosphate	EC
Dehydro-genase	-	0.424**	-0.066	0.138	-0.649**	0.158	0.401*	0.256*
Cellulase		-	-0.385*	-0.195	-0.352*	-0.040	-0.118	0.019
Protease			-	0.257*	0.035	-0.226	-0.159	-0.231
Alkaline phospatase				-	-0.074	0.309	0.248	0.396**
Feeding Activity					-	-0.345*	-0.211	-0.380**
Phosphite						-	0.001	0.976**
Phosphate							-	0.081
EC								-

* = significant at 0.05 level; ** = significant at 0.01 level

activities and soil phosphite content. Phosphite did not harm the biocoenosis of soil micro-organisms and soil mesofauna.

The fate of applied phospite fertiliser in soil remained open. A lysimeter trial should be performed to observe vertical phosphite displacement in soil. Radioactive labelled phosphorus of phosphite may give information about its disposition in chemical structures.

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