

EXTRACTION OF ACID PHOSPHOMONOESTERASE FROM SOIL: TESTING OF VARIOUS EXTRACTANTS

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Abstract

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The aim of this work was to investigate the suitability of various types of extractants for extraction of acid phosphomonoesterase from soil using various types of extractants. Succinate-borate buffer of pH 4.8 or 0.1 M K_2SO_4 were the most efficient to extract this enzyme compared to 0.1 M glutamic acid or 0.005 M salicylic acid. Extraction using 0.1 M glutamic acid gave significantly ($P < 0.05$) lowest extraction yield. The following extracts were obtained: clear K_2SO_4 and glutamic acid extracts, succinate-borate buffer extracts were of slight coloration, and in some cases, rose-colored extracts of salicylic acid. The results of this work are in accordance with low extraction yields of phosphomonoesterase reported in other studies.

acid phosphomonoesterase, extracts, glutamic acid, K_2SO_4 , salicylic acid, soil

Activity of extracellular enzyme acid phosphomonoesterase (orthophosphoric monoester phosphohydrolase, E.C. 3.1.3.2.) in soil is commonly determined to evaluate release of available phosphorus from soil organic matter. Besides the commonly determined total soil acid phosphomonoesterase activity, several attempts were performed to extract some parts of this activity. For this purpose, various types of extractants were used. For example, extraction using sodium pyrophosphate (0.14 M, pH 7.1) is based on solubilization of soil organic matter when enzyme-humic substances complexes are extracted. In this case, activity of acid phosphomonoesterase may be inhibited by humic substances (Rejšek *et al.*, 2011, *sent to journal*). Sodium pyrophosphate extracts were brown with significant amount of humic substances displaying enzymatic activity mostly below the detection limit, in some cases extraction yield 0.1% of total soil acid phosphomonoesterase activity was reported. Tris-HCl, Tris-HCl with bovine serum albumine (BSA) and Triton X-100 separately or together gave colorless to light yellow extracts indicating very low solubilization of

humic substances. Treatment with Tris-HCl buffer (50 mM, pH 7.5) gave acid phosphomonoesterase extraction yield from 0 to approx. 1% (see Fornasier and Margon, 2007). Tris-HCl buffer plus 1% Triton X-100 extraction may have a loosening effect of the enzymes and soil organic matter complexes and as in case of extraction using Tris-HCl buffer with 4% BSA when protein exchange is supposed, 2–8 times higher extraction yield of enzymes compared to Tris-HCl extraction was reported (Fornasier and Margon, 2007). When both Triton X-100 and BSA were added into Tris-HCl buffer, more than additive extraction yield reaching 2–13% was obtained (Fornasier and Margon, 2007). Other types of extractants used are mentioned in works of Batistic *et al.* (1980), Nannipieri *et al.* (1980), Hayano (1988), Pascual *et al.* (2002) etc. Besides extraction of fraction of acid phosphomonoesterase from soil, other types of treatments were tested. For example, sonication was reported to increase acid phosphomonoesterase activity of soils (De Cesare *et al.*, 2000).

In this work we have attempted to extract acid phosphomonoesterase from soil using various

type of extractants including 0.1M K_2SO_4 , 0.1M glutamic acid, 0.005M salicylic acid and succinate-borate buffer of pH 4.8. The aim of this study was to find the best extractant to extract fraction of acid phosphomonoesterase from soils. The results may be used for various types of ecological studies.

MATERIAL AND METHODS

The soil used for the experiments was obtained from the "Bílý Kříž" ("White Cross") experimental research station, located in the Moravian-Silesian Beskids Mts. in the northeastern part of the Czech Republic. The region has a sub continental climate with a typical mean annual air temperature of 4.9 °C and mean annual precipitation of 1100mm. The number of days with snow cover is 160 per year.

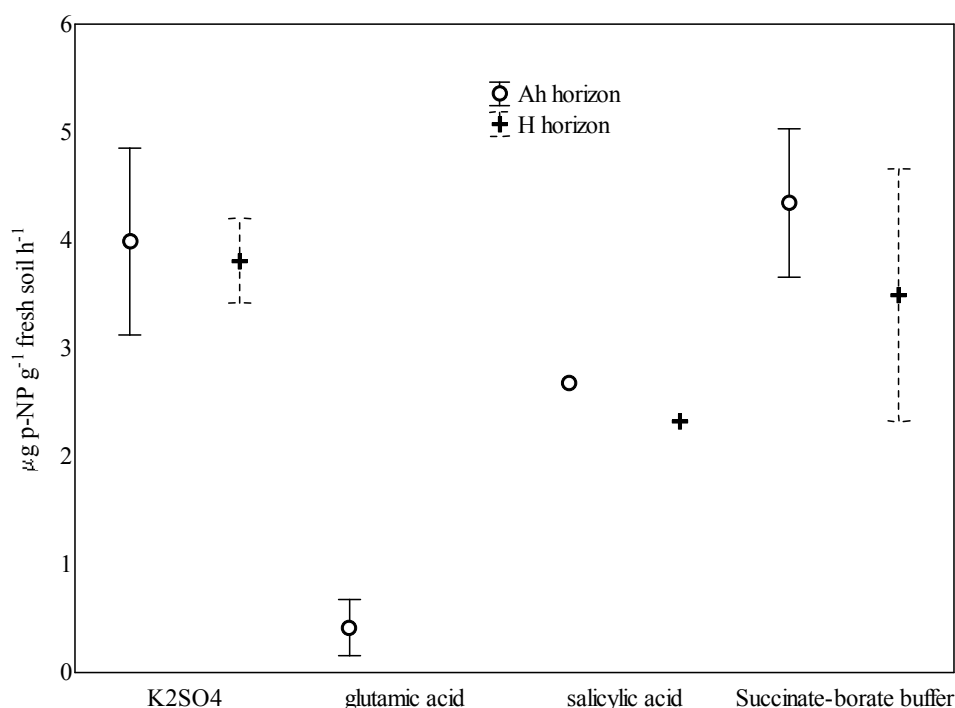
The experimental 23-year-old Norway spruce stand is located at 908m a. s. l. (N 49°30'10", E 18°32'20"). The stand comprises approximately 99% Norway spruce (*Picea abies* (L.) Karsten) and 1% fir (*Abies alba* Mill.). The first mixed sample composed from 5 sub-samples of H horizon was taken there. The second mixed sample was obtained from Ah horizon of the experimental meadow at the same locality. The moderately mown meadow plant association belongs to the *Nardo-Callunetea* class (Formánek *et al.*, 2008).

After sieving through a 5mm sieve, the soil samples were stored at 6 °C until the analyses. Acid phosphomonoesterase was extracted using 0.1M K_2SO_4 , 0.1M glutamic acid, 0.005M salicylic acid or succinate-borate buffer of pH 4.8. In all cases, 5g of wet soil were treated with 25mL of each of the

solution, shaken (20 min., 300 rpm), and filtered. Consequently, 4mL of filtrate were incubated with 6mL p-NPP in succinate-borate buffer of pH 4.8. Determination of acid phosphomonoesterase was performed according to Rejšek (1991). All data were processed using the Statistica 9 software ($\alpha = 0.05$, $n = 3$). One Way Anova plus Fischer's LSD test were used.

RESULTS AND DISCUSSION

The results of this study showed that succinate-borate buffer of pH 4.8 or 0.1M K_2SO_4 were the most efficient agents to extract acid phosphomonoesterase from soil (Fig. 1). While the extracts using K_2SO_4 were colorless, succinate-borate buffer extracts were of slight coloration, but still virtually colorless. Other types of extractants like salicylic acid produced rose-colored extract, when Ah horizon soils were extracted. In case of H horizon of forest soil, colorless extracts were obtained using salicylic acid. Colorless extracts were produced also by glutamic acid treatment giving the lowest extraction yield ($P < 0.05$). The results obtained in this work correspond with low extraction yields when acid phosphomonoesterase or other types of enzymes were extracted from soils within other works. In various studies, extraction of phosphatase was performed using water, Chelex 100- Na^+ active resins and distilled water, 4mM $CaCl_2$, sodium pyrophosphate (e.g. 0.14M, pH 7.1), phosphate buffer (e.g. 0.2M, pH 8) in the presence of EDTA, Tris-HCl buffer (50mM, pH 7.5), Tris-HCl buffer plus 1% (w/v) Triton X-100 (permeabilizing



1: Extraction of acid phosphomonoesterase from soil using various extractants (mean \pm SE)

agent), Tris-HCl buffer plus 4% (w/v) BSA, Tris-HCl buffer plus both 1% Triton X-100 and 4% BSA (Batistic *et al.*, 1980; Nannipieri *et al.*, 1980; Hayano, 1988; Pascual *et al.*, 2002; Fornasier and Margon, 2007). Increased activity of extracted phosphatases appeared after ultrafiltration (Nannipieri *et al.*, 1982). Activity of acid phosphomonoesterase in soil

extracts obtained in this work corresponds with those reported by e.g. Fornasier and Margon (2007) or Margon and Fornasier (2008). Nevertheless, more research is necessary to better evaluate occurrence of easily extractable acid phosphomonoesterase in various soils.

CONCLUSIONS

Various extractants were tested to extract acid phosphomonoesterase fraction from soil. The testing was performed to better understand phosphorus transformation in soils of various types of ecosystems. The results presented in this work show that succinate-borate buffer of pH 4.8 or 0.1 M K_2SO_4 are the most efficient extractants compared to solutions of 5 mM salicylic or 0.1 M glutamic acid. The advantage of treatment with 0.1 M K_2SO_4 is obtaining colorless extracts. Nevertheless, more research is necessary to find the best method how to extract fraction of acid phosphomonoesterase from soil.

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REFERENCES

- BATISTIC, L., SARKAR, J. M., MAYAUDON, J., 1980: Extraction, purification and properties of soil hydrolases. *Soil Biology and Biochemistry*, 12, 1: 59–63. ISSN 0038-0717.
- DECESARE, F., GARZILLO, A. M. V., BUONOCORE, V., BADALUCCO, L., 2000: Use of sonication for measuring acid phosphatase activity in soil. *Soil Biology and Biochemistry*, 32, 6: 825–832. ISSN 0038-0717.
- FORMÁNEK, P., REJŠEK, K., VRANOVÁ, V., MAREK, M., 2008: Bio-available amino acids and mineral nitrogen forms in soil of moderately mown and abandoned mountain meadows. *Amino Acids*, 34, 2: 301–306. ISSN 0939-4451.
- FORNASIER, F., MARGON, A., 2007: Bovine serum albumin and Triton X-100 greatly increase phosphomonoesterases and arylsulphatase extraction yield from soil. *Soil Biology and Biochemistry*, 39, 10: 2682–2684. ISSN 0038-0717.
- HAYANO, K., 1988: Characterization of two phosphodiesterase components in an extract of Larch forest soil. *Soil Science and Plant Nutrition*, 34, 393–403. ISSN 0038-0768.
- MARGON, A., FORNASIER, F., 2008: Determining soil enzymes location and related kinetics using rapid fumigation and high-yield extraction. *Soil Biology and Biochemistry* 40, 9: 2178–2181. ISSN 0038-0717.
- NANNIPIERI, P., CECCANTI, B., CERVELLI, S., MATARESE, E., 1980: Extraction of phosphatase, urease, proteases, organic carbon and nitrogen from soil. *Science Society of America Journal*, 44, 5: 1011–1016. ISSN 0361-5995.
- NANNIPIERI, P., CECCANTI, B., CONTI, C., BIANCHI, D., 1982: Hydrolases extracted from soil: Their properties and activities. *Soil Biology and Biochemistry*, 14, 3: 257–263. ISSN 0038-0717.
- PASCUAL, J. A., MORENO, J. L., HERNÁNDEZ, T., GARCÍA, C., 2002: Persistence of immobilized and total urease and phosphatase activities in a soil amended with organic wastes. *Bioresource Technology*, 82, 1: 73–78. ISSN 0960-8524.
- REJŠEK, K., 1991: Acid phosphomonoesterase activity of ectomycorrhizal roots in Norway spruce pure stands exposed to pollution. *Soil Biology and Biochemistry*, 23, 7: 667–671. ISSN 0038-0717.
- REJŠEK, K., VRANOVÁ, V., HOLÍK, L., PAVELKA, M., FORMÁNEK, P., 2011: Acid phosphomonoesterase (E.C. 3.1.3.2) location in soil. *Journal of Plant Nutrition and Soil Science*. ISSN 1436-8730. (sent to journal).

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