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Acid phosphatase activity in Saudi Arabian soils

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ABSTRACT

Since little is known about the activity of acid phosphatase in Saudi Arabian soils and no studies have been previously published comparing the activity of acid phosphatase enzyme at different regions of the Kingdom of Saudi Arabia, a laboratory study was conducted to determine the acid phosphatase activity in soil from eight localities of Saudi Arabia. The effect of phosphorus fertilizer application on phosphatase activity and related properties of calcareous soils in Saudi Arabia was also investigated. Phosphorus fertilizer (100 mg P/kg soil) was solubilized to available phosphate in all soils tested. The soils from the eight localities differed in their acid phosphatase activity according to varying soil characteristics. The maximum activity of phosphatase enzyme was recorded in Alkharj (125 μg *p*-nitrophenol/g/h) and Riyadh (107 μg *p*-nitrophenol/g/h) soils. These sites also exhibited the largest concentration of available phosphorus. In contrast, the Hail and Qassium soils showed a slight phosphatase activity (30 and 33 μg *p*-nitrophenol/g/h, respectively). The addition of phosphorus fertilizer to soils led to a tenfold increase in the concentration of available phosphorus and microbial numbers. Also an insgnificant decline in soil pH was observed after supplementation of soils with phosphorus fertilizer.

INTRODUCTION

Soil phosphatases mediate biochemical transformations involving organic residue decomposition and phosphorus cycling in soil. Phosphorus is an essential element in all living systems and is often a limiting mineral nutrient in plant nutrition and production. The presence of the soil acid phosphatase enzyme can greatly influence the utilization of phosphate fertilizers in agricultural applications (Burns 1978).

Soil enzyme activity results from a combination of the activity of accumulated enzymes and those produced by proliferating microorganisms (Martens *et al.* 1992). Soil phosphatases primarily originate from the microbial biomass, although they can also originate from plant and animal residues (Burns 1978).

The activity of phosphatase is influenced by various soil properties, vegetation cover and the presence of inhibitors and activators. Wainwright (1980) found that the activity of soil phosphatase decreased after its exposure to atmospheric deposition. In contrast, Al-Falih & Wainwright (1996) found that the activity of this enzyme increased when a carbon source was added to the soil.

Phosphatase activity in soils has been studied by many workers (Halstead 1964, Ramirez-Martinez & McLaren 1966, Wainwright 1981, Martens *et al.* 1992). Many of these studies have been concerned with the activity of phosphomonoesterases, especially acid phosphatase. Information about phosphatase activity in soils has been reviewed by Ramirez-Martinez (1968), Kiss *et al.* (1975), Skujins (1976), Westermann (1992), Atlas & Bartha (1993) and Killham (1994). Aliev & Gadzhiev (1973) reported that phosphatase activity increased with increasing numbers of microorganisms.

Little is known about the activity of acid phosphatase in Saudi Arabian soils and no studies appear to have reported a comparison of acid phosphatase and phosphate availability in soil from diferent regions of the Kingdom of Saudi Arabia.

The aim of this present investigation is to measure the activity of acid phosphatase in the soils of various regions of Saudi Arabia before and after supplementation with phosphate fertilizer. In order to learn more about how phosphatase activity in these soils correlates with P nutrition and P availability, calcium carbonate content, salt content, pH and organic matter were also quantitated.

MATERIALS AND METHODS

Collection and analysis of soil samples

Soil samples were collected from eight different localities in Saudi Arabia. Five surface (0–15 cm depth) samples from each site were combined into a sterile polyethylene bag. Mechanical analysis of the soil was made by the sieve method and soil texture was determined using the soil texture triangle. The method described by Jackson (1962) was used for determination of total soluble salts and calcium carbonates. Soil pH was determined with a glass electrode (10:1, water to soil ratio). The soil organic matter was determined colorimetrically using the method described by Walinga *et al.* (1992).

Determination ofphosphate availability

Soil samples were amended with triple superphosphate ($Ca(H_2PO_4)_2$) fertilizer (100 mg P/kg soil) in order to determine the effect of phosphorus availability on phosphatase activity in soil. The soil samples were incubated in triplicate in polythene bags closed with a small hole to allow for gas exchange. The soils were moistened to a water potential of −0.9 MPa and incubated at 25°C for one month.

Phosphate was extracted from soil with 0.5N $NaHCO_3$. The soil to extractant ratio was 1:10, and the slurry was shaken for 15 mins (100 throws min^{-1}). After being shaken, the soil slurries were filtered through Whatman No. 1 filter paper and the concentration of phosphate was determined colorimetrically according to Hesse (1971).

Determination of phosphatase enzyme

Phosphatase activity was assayed using the method of Tabatabai & Bremner (1969). Triplicate samples of soil (1 g) were placed in 50-ml Erlenmeyer flasks and 0.25 ml of toluene was added to wet the soil, followed by the addition of 4 ml of 0.5 M sodium acetate buffer (pH 6.5) and 1 ml of the substrate (*p*-nitrophenyl phosphate) solution. The contents were shaken for a few seconds to ensure adequate mixing and the flasks were then closed and placed in an incubator at 37°C. After one hour, 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH was added and the slurry was thoroughly mixed by hand. The soil suspensions were then filtered through Whatman No. 1 filter paper.

The *p*-nitrophenol concentration in the filtrate was determined by absorbance at 400 nm and by comparison with a *p*-nitrophenol standard curve (0–50 μg). Standards were included in each series of analysis.

Determination of soil microbial numbers

For the quantitative estimation of bacteria, fungi and actinomycetes, the dilution plate method of Waksman as described by Johnson & Curl (1972) was used. Soil (1 g, sieved to <2 mm) was shaken in sterile Ringer's solution (10 ml) for 15 min. Samples of the resulting suspension were then serially diluted in Ringer's solution (Al-Gounaim *et al.* 1995). The final dilution was then spread on the surface of the medium. Incubation periods were 2 days at 37°C for total bacteria and 3–5 days at 30°C for total fungi and actinomycetes.

Statistical analysis

Analysis of variance was carried out and statistical significance between groups was evaluated with the Duncan test ($P = 0.05$). Statistical analysis was carried out with SPSS software (SPSS for Windows 5.01, 1992).

RESUILTS AND DISCUSSION

The characteristics of soil samples collected from different sites of Saudi Arabia are given in Table 1. The soil texture class is sandy in most cases, with the exception of sandy loam and loamy sand soils in Qassium and Wadi dawaser, respectively. All soil samples tested contained low percentages of organic matter ranging between 0.02 and 0. 80%.

Table 1. Soil characteristics of different regions in Saudi Arabia (All values are means of triplicates).

Soil locality	Mechanical fraction %			Texture class	OM %	pH	T.S.S. %	$CaCO_3$ %
	Sand	Silt	Clay					
Alhassa	89.0	5.8	5.2	Sand	0.80	7.4	0.23	7.02
Aljouf	98.3	0.9	0.8	Sand	0.13	7.8	0.08	12.30
Alkharj	92.6	1.2	6.2	Sand	0.07	7.2	0.13	1.87
Hail	91.3	3.3	5.4	Sand	0.09	8.3	0.61	20.13
Qassium	56.4	6.1	37.5	Sandy loam	0.68	8.1	0.54	15.22
Riyadh	90.6	4.2	5.2	Sand	0.62	7.5	0.19	9.62
Tabouk	89.0	3.3	7.7	Sand	0.74	7.5	0.16	5.43
Wadi dawaser	82.0	5.5	12.5	Loamy sand	0.02	7.3	0.34	11.92

OM = Organic Matter T.S.S. = Total Soluble Salts

The pH of the soils was slightly alkaline and ranging between 7.2 in the Alkharj soil and 8.3 in the soil from the Hail region. In general, the soil samples had a low content of total soluble salts. Aljouf soil recorded the lowest percentage (0.08%) and the soil from Hail had the highest amount of total soluble salts (0.61%), followed by the Qassium soil with 0.54%. The Hail and Qassium soils had the highest concentrations of calcium carbonate (20.13% and 15.22%, respectively) and the lowest calcium carbonate concentration was recorded in the Alkharj soil (1.87%). The Alkharj soil had a high activity of acid phosphatase which may be due to the low concentration of calcium carbonate.

The types and activities of the soil microflora are very important factors in soil development because of their role in soil mineral cycling and in the flow of energy (Ehrlich 1981). Table 2 contains the counts of soil microorganisms in different regions of Saudi Arabia. Changes in counts of soil bacteria, actinomycetes and fungi after addition of phosphate fertilizer are presented in Table 3. In this study the lowest bacterial count was 11.0×10^5/g soil in the Hail soil. The total count of bacteria in the Alhassa and Tabouk soils were 52.7 and 43.1×10^5, respectively. These sites

Table 2. Counts of soil microorganisms per gram soil at different regions in Saudi Arabia (All values are means of six replicates ± S.D; $P < 0.05$).

Soil locality	Total bacteria $\times 10^5$	Actinomycetes $\times 10^3$	Total fungi $\times 10^3$
Alhassa	52.7 ± 0.6	50 ± 2.4	43 ± 1.1
Aljouf	13.5 ± 0.4	33 ± 4.5	28 ± 3.0
Alkharj	39.7 ± 1.3	67 ± 1.7	75 ± 2.4
Hail	11.0 ± 0.9	28 ± 1.6	39 ± 1.0
Qassium	12.3 ± 1.4	45 ± 3.2	55 ± 0.4
Riyadh	32.1 ± 0.6	70 ± 1.4	68 ± 1.9
Tabouk	43.1 ± 4.0	17 ± 2.5	22 ± 1.5
Wadi dawaser	14.5 ± 0.8	10 ± 1.3	38 ± 0.6

Table 3. Changes in counts of soil microorganisms at different regions in Saudi Arabia per gram soil after amendment of 100 mg P/kg soil of triple superphosphate fertilizer. (All values are means of six replicates ± S.D.; $P < 0.05$).

Soil locality	Total bacteria $\times 10^7$	Actinomycetes $\times 10^3$	Total fungi $\times 10^5$
Alhassa	14.5 ± 1.0**	28.4 ± 0.3	20.0 ± 0.6**
Aljouf	3.6 ± 1.2*	12.5 ± 0.3	17.1 ± 0.4**
Alkharj	92.7 ± 2.7**	85.0 ± 0.7	81.2 ± 0.9**
Hail	1.7 ± 0.4*	4.0 ± 0.3	9.3 ± 1.7*
Qassium	2.1 ± 0.8*	5.0 ± 1.9	5.5 ± 0.2*
Riyadh	62.1 ± 0.3**	60.2 ± 0.6	53.0 ± 0.6**
Tabouk	35.2 ± 1.8**	29.1 ± 0.4	19.1 ± 0.9**
Wadi dawaser	27.1 ± 1.4**	25.7 ± 2.1	21.3 ± 1.8**

*: Significant.
**: Highly significant at 0.05.

exhibited the largest content of organic matter. The addition of phosphate fertilizer caused an increase in the number of soil microorganisms in all Saudi Arabian soils studied. The highest microorganism numbers were observed in the Alkharej and Riyadh soils following amendment of the soil with triple superphosphate fertilizer. Overall, the addition of phosphate fertilizer to soil led to a highly significant increase in the bacterial and fungal content ranging from 1.7 to 92.7 $\times$ 10^7/g soil and 5.5 to 81.2 $\times$ 10^5/g soil, respectively. There was no significant increase in the total count of soil actinomycetes following the addition of phosphate fertilizer in all Saudi Arabian soils tested.

Acid phosphatase activities of different Saudi Arabian soils are given in Fig. 1. The acid phosphatase activities varied between 17–125 μg *p*-nitrophenol released per gram of soil per hour. Alkharj and Riyadh soils exhibited the highest phosphatase activity (125 and 107 μg *p*-nitrophenol per gram of soil per hour, respectively). The primary reason for the high acid phosphatase activity in the Alkharj soil appeared to be the low calcium carbonate concentration. Moderate phosphatase activity was exhibited by the Alhassa, Aljouf, Tabouk and Wadi dawaser soils (78, 75, 80 and 73 μg *p*-nitrophenol per gram of soil per hour, respectively). Low acid phosphatase activity was shown by the Hail (30 μg *p*-nitrophenol/g/h) and Qassium soils (33 μg *p*-nitrophenol/g/h). Addition of phosphate fertilizer caused a marked increase in the activity of soil phosphatase in all samples studied. The presence of the soil phosphatase enzyme is of paramount importance to the utilization of phosphate fertilizers in agricultural applications (Ramirez-Martinez & McLaren 1966, Burns 1978). Soil enzyme activity results from a combination of the activity of accumulated enzymes and those produced by proliferating microorganisms (Martens *et al.* 1992).

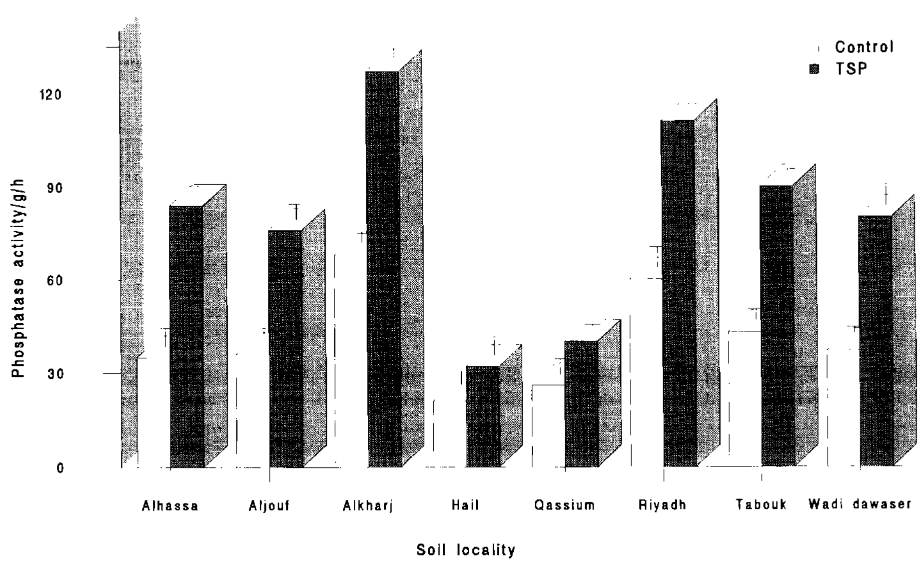

Fig. 1 Phosphatase activity (μg *p*-nitrophenol/g/h) in soils collected from different regions in Saudi Arabia after amendment with 100 mg P/kg soil. All values are means of triplicates $\pm$S.D; $P<0.05$.

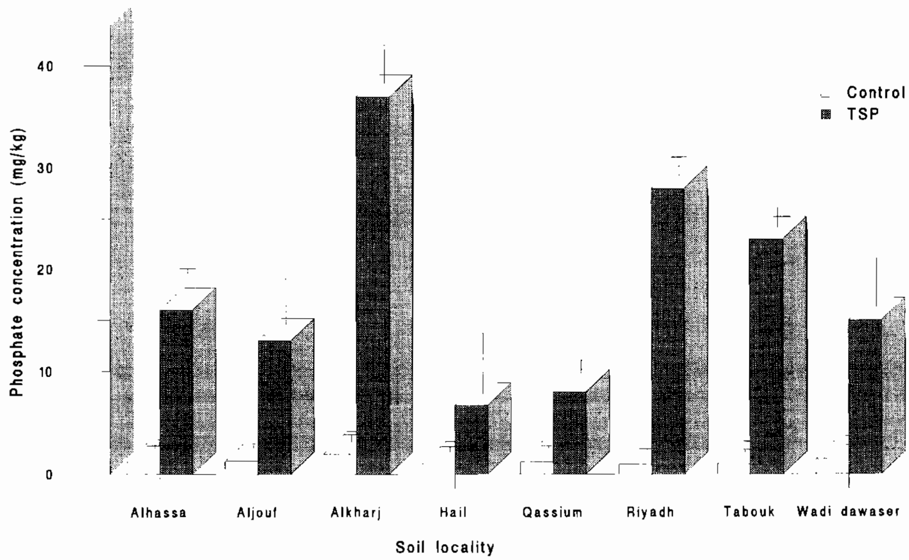

Fig. 2 Phosphate solubilization (100 mg P/kg soil) in Saudi Arabian soils. All values are means of triplicates ±S.D; $P<0.05$.

Changes in phosphate concentration of soils from different regions in Saudi Arabia after additions of triple superphosphate fertilizer (100 mg P/kg) are shown in Fig. 2. In all control soils, the phosphate concentration ranged between 0.7 and 3.0 mg/kg. In the present study, the soil samples supplemented with triple superphosphate fertilizer had high concentrations of available phosphate that varied from one soil to another.

All of the Saudi Arabian soils solubilized triple superphosphate fertilizer forming available orthophosphate (Fig. 2). Alkharj soil was particularly active in this process forming 38 mg/kg of phosphate at the end of the incubation period followed by Riyadh soil with 27 mg/kg of phosphate. In contrast, the Hail and Qassium soils had the lowest amount of phosphate with 6.7 and 8.1 mg/kg of phosphate, respectively, at the end of the incubation period.

The various factors that are believed to influence soil phosphorus availability include calcium carbonate content, soil pH, clay content, salinity, phosphatase enzyme activity and microorganism population (Bashour *et al.* 1985). Robertson & Alexander (1992) found that pH, and Ca and Fe concentrations alter the availability of phosphate.

The highest levels of phosphate, microbial population and phosphatase activity were observed in the Alkharj soil. In addition, the Alkharj soil exhibited the lowest concentration of calcium carbonate and total soluble salts. In contrast, the Hail soil that had the lowest microbial population, phosphatase activity, and a high percentage of calcium carbonate and total soluble salts, also had the lowest available phosphate concentration. Thus, the soil phosphate availability in Saudi Arabian soils appears to be correlated with acid phosphatase activity, microbial population, calcium carbonate and total soluble salts, consistent with the findings of previous studies (Halstead 1964, Bashour *et al.* 1985, Al-Falih & Wainwright 1996, Al-Sewailem 1997). A similar inverse relationship between calcium carbonate content and phosphate availability in soil was reported earlier by Sharpley *et al.* (1984) and Westermann (1992).

Addition of phosphate fertilizer to Saudi Arabian soils did not significantly change soil pH and this could be attributed to the availability of calcium carbonate in these soils. In calcareous soils (high $CaCO_3$), such as the Saudi Arabian soils, calcium carbonate works as a buffer (Al-Falih & Wainwright 1996). The low concentrations of calcium carbonate in the Alhassa, Riyadh and Tabouk soils led to a reduction in the pH of the soil upon the addition of phosphate fertilizer.

CONCLUSION

Soils from the eight localities of Saudi Arabia differ in their acid phosphatase activity according to soil characteristics. The maximum activity of acid phosphatase enzyme was recorded in Alkharj soil (125 μg *p*-nitrophenol/g/h) primarily due to the low calcium carbonate concentration.. The Hail soil showed a slight phosphatase activity (30 μg *p*-nitrophenol/g/h) and a high calcium carbonate concentration.

Addition of phosphorus fertilizer to soils led to a highly significant increase (tenfold) of available phosphorus and microbial numbers. The available phosphorus in the Alkharj soil increased from 3 to 38 mg/kg, while the available phosphorus in the Hail soil changed from 1.2 to 6 mg/kg after supplementation with fertilizer

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نشاط إنزيم الفوسفتيز في عدد من الترب الزراعية بالمملكة العربية السعودية

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خلاصة

أجريت دراسات معملية لتقدير نشاط إنزيم الفوسفتيز في عينات التربة التي جمعت من ثمان مواقع في المملكة العربية السعودية . وقد درس في هذا البحث تأثير إضافة السماد الفوسفاتي على خصائص التربة ، ونشاط إنزيم الفوسفتيز فيها.

وجد في هذه الدراسة أن أعلى نشاط لإنزيم الفوسفتيز سجل في كل من تربة الخرج (125) ، وتربة الرياض (107) ، وهذان الموقعان يحتويان على أعلى معدل من الفوسفور الذائب. بينما ظهر أقل تركيز للفوسفور في تربة منطقة حائل ، وتربة القصيم .

إضافة السماد الفوسفاتي (100 ملجم P/كجم تربة) أدت إلى زيادة كبيرة في تركيز فوسفور التربة الذائب ، وفي الأعداد الكلية لميكروبات التربة. كما أدت إلى انخفاض بسيط في درجة حموضة التربة.