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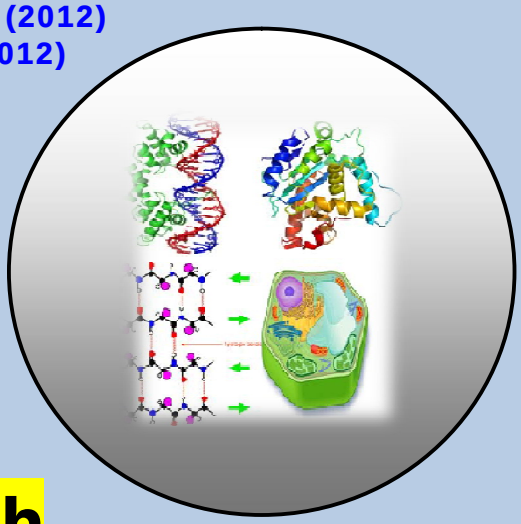

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Impact of Salinity on Enzyme Activities in Calcareous Soils of Uzbekistan

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ABSTRACT

Progressive increases in salinilization of irrigated soil is a major problem in agriculture. To evalaute the effects of secondary salinity on soil enzyme activities under long-term irrigated cotton (Gossypium hirsutum L.) monoculture, a study was conducted in Syrhound district of the Syr-Darya region of north-west Uzbekistan. Composite soil samples were randomly collected at 0 - 30 cm depth from rhizosphere soil of weakly saline (2.3 dS m^{-1}), moderately saline (5.6 dS m^{-1}), and strongly saline (7.1 dS m^{-1}) fields, 2-mm sieved, and analyzed for acid and alkaline phosphoesterase, β-galactosidase, β-glucosidase, urease, proteasase, and fluorescein diacetate (FDA) hydrolase activities. Results showed that increasing salinity accounted for significant decrease in all enzyme activities, and the adverse effect was more pronounced on FDA activities. We conclude that salinization of soils due to prolonged irrigation and continous use of high amounts of fertilizers in cotton monoculture reduced several enzyme activities, indicating a progressive decline of soil microbiological activities in agricultural lands of Uzbekistan.
Key words: Soil Salinity, Enzyme Activity, Cotton and Uzbekistan.

INTRODUCTION

Salinity is a major concern for irrigated agriculture in arid and semi-arid regions of the world (Shirokova et al. 200; Egamberdiyeva et al. 2007). Several studies on the effect of salinity on plant growth development and soil properties were reported (Egamberdieva et al. 2010; 2011). The negative effect of salinity on soil enzyme activities were also reported by Rietz and Haynes (2003), Sardinha et al. (2003) and Tripathi et al. (2006). Hydrolase activities in the soil can be quantitatively important in processes related to plant nutrition; for example the hydrolysis of organic phosphate monoesters by phosphomonoesterases may account up to 80% of P assimilated by plants in agricultural soils (Gilbert at al. 1999).

Measures of enzyme activities in saline soils can be also used for evaluating the degree of degradation of salinated soils and the possibilities of soil restoration. For example soil enzymes such dehydrogenase, phosphatase, sulfatase, amylase, and b-glucosidase activity was severely inhibited in salinized soils and their variation in soils seemed to be related to the physicochemical microbial properties of soils (Zahir et al. 2001). In arid or semiarid climates, salinity is usually combined with alkaline soil pH, because of $CaCO_3$ enrichment in the uppermost soil layers (saline soils) or hydrolysis of sodium carbonate (sodic soils) (Pascual et al. 2000). The soil enzymes activities such as ureases, proteases, phosphatases, and glucosidases are considered as sensitive indicators to detect changes occurring in soils under field conditions (Nannipieri et al. 2002). In spite of the large information on the effects of soil secondary salinization on soil enzyme activity, very few studies have focused on the effects of salinization on the enzyme activity in the rhizosphere. In this paper we report the effects of increasing soil salinity on enzyme activities of soils under cotton crop located in Syrdarya province.

MATERIAL AND METHODS

Description of the study area

Twelve conventionally tilled (0 - 40 cm depth) irrigated cotton fields affected by various degress of salinity were selected to collect soil samples at Sayhunobod district ($41^0$00'N, $64^0$00'E) of the Syr-Darya province of north-east Uzbekistan. According to the the WRB-FAO (2006) classification, the soils of all the selected fields were identified as Calcisol (silt loam seirozem) which formed from loess, eluvial and proluvial parent materials. The soils have been cropped with cotton monoculture for the last 50 to 60 years under flood irrigation without proper drainage facilities using natural flow system. On average, the soil contained 43±9 g sand kg^{-1}, 708±12 g silt kg^{-1}, and 250±13 g clay kg^{-1} and had a cation exchange capacity of 23.6±1 cmol kg^{-1} with an exchangeable Na percentage of 4.41, and a Na absorption ratio of 0.32. The climate of the area is continental with a yearly average rainfall of 200±36 mm and more than 90 percent of the total rain falling between October to May. The average minimum monthly air temperature is 0^oC in January and the maximum is 37^oC in July.

Soil collection, processing and analysis

For soil sampling, the selected fields (3.5 ha each) were categorized into three different salinilty levels based on electrical conductivity (E_C): (1) weakly saline (2.3±0.3 dS m^{-1}), (2) moderately saline (5.6±0.6 dS m^{-1}), and (3) strongly saline (7.1±0.6 dS m^{-1}) soils. In each soil salinity category, four relatively uniform fields were selected as replicates followed by random demarcation of three subplots (1-m x 1-m) in each replicated field. From each subplot three soil cores (3.5 cm internal diameter) were sampled at 0 - 30 cm soil depth before harvesting cotton in September 2005 between plant rows (80 cm apart), and another three core samples between plants (40 cm apart) within rows. The soil cores were pooled and mixed to obtain six composite samples for each replicated field, and 24 replicated composite samples for each salinity level. The soil samples were gently sieved through a 2-mm mesh (visible pieces of crop residues and roots were removed), and a portion of the field-moist soil was analyzed for enzyme activities.

Measurement of soil enzyme activities

Fluorescein diacetate (FDA) hydrolase was determined according to Schnürer and Rosswall (1982), adapted to micro plates by using 50µl sodium acetate-acetic acid buffer (0.2 m, pH 5.0), 50µl of FDA as substrate (100µg ml^{-1}) and 100µl of soil extract, and determined spectrophotometrically at 492 nm (Wirth, 1992). The β-glucosidase activity was determined using 0.1M maleate buffer, pH 6.5 at 37ºC for 90min. The reaction was stopped with tris-hydroxymethl aminomethane (THAM) according to Tabatabai (1982). The β-galactosidase activity was assayed using MUB buffer at pH 6 according to Tabatabai (1982). Acid and alkaline phosphomonoesterase activities were assayed according to Tabatabai and Bremner (1969) and phosphodiesterase activity as reported by Browman and Tabatabai (1978). Urease activity was measured using 0.1 phosphate buffer at pH 7, as reported by Nannipieri et al. (1974). Protease activity was determined by hydrolysis of *N*-benzoylargininamide (*N*-BAA) according to Ladd and Butler (1972). All enzyme activities were assayed at 37°C for 1 h, after centrifugation of soil mixtures at 6000*g* at 4°C. Concentrations of *p*-nitrophenol (*p*-NP) produced in the assays of acid and alkaline phosphomonoesterase, phosphodiesterase, β-glucosidase and β-galactosidase activities were calculated from a *p*-NP calibration curve after subtracting the absorbance of the control at 400nm wavelength using a UV-VIS spectrophotometer (Lambda 2, Perkin Elmer). The NH_4^+ produced by urease and *N*-BAA-hydrolysing activities was extracted using 2M KCl and quantified by a flow injection analyzer (FIAStar, Tecator, Sweden). To account for the NH_4^+ fixation by soils, NH_4^+ solutions with concentrations in the range of those released by urease and protease activities were incubated with these soils. The recovery of NH_4^+ ranged between 93 and 96%. All results were the means of 3 replicates. The LSD values (Tukey-Kramer test, $P < 0.05$) were calculated to assess the significance of differences of the means (n = 3).

RESULTS AND DICUSSION

In Table 1 we report rhizosphere soil enzyme activities in cotton grown at various levels of soil salinity. We observed that salinity inhibited urease, protease, alkaline phosphomonoesterases, acidic phosphomonoesterases, phosphodiesterase, glucosidase and fluorescein diacetate (FDA) hydrolase activities of rhizosphere soil, whreas non-saline soil showed highest enzyme activities.

Table 1. Acid (AcP), alkaline phosphomonoesterases (AlP), phosphodiesterase (PD), galactosidase (GA), glucosidase (GL), urease (UR), protease (PR) and fluorescein diacetate (FDA) hydrolase activities in non saline (NS), mid saline (MS) and strong saline (SS) soils.

Treatments	AcP	AlP	PD	GA	GL	UR	PR	FDA
EC	(mg *p*-nitrophenol kg^{-1} soil*h^{-1})					(mg NH_4^+-N kg^{-1}soil*h^{-1})		
NS (1.3 dS m^{-1})	821.3	2811.5	914.8	442.7	256.9	7.9	23.9	23.9
MS (5.6 dS m^{-1})	952.2	2367.8	761.2*	482.0	200.1	6.5*	22.2	12.0*
SS (7.1 dS m^{-1})	456.1*	1884.0*	796.2	491.6	127.6*	5.8*	17.0*	6.1*

Garcia et al. (1994) and Egamberdieva et al. (2010) reported that the decrease of enzyme activities can be due to lower contents of microbial biomass releasing less amounts of enzymes, but also by the fact that in semi-arid soils the enzyme activity is mainly extracellular, stabilized by forming complexes with organic and mineral colloids. The increase of salinity as measured by increased conductivity disperses clay minerals and stable enzymes thus remain unprotected and more susceptible to denaturation (Frankenberger and Bingham 1982). Moreover, the reduction of organic matter content accumulated in salinized soils could also be a cause of reduced soil enzyme activity as the soil organic matter plays an important role in providing substrates, and also protecting enzymes through immobilization in organo-mineral complexes (Tabatabai 1994). Alkaline phosphatase was higher in all soils than other hydrolase enzymes (Table 1). Generally, alkaline phosphatase predominated in soils with neutral or slightly alkaline pH (Tripathi et al. 2006). Since, higher plants are devoid of alkaline phosphatase, the alkaline phosphatase of soils originates totally from microorganisms (Juma and Tabatabai 1988) and thus provides a sensitive indicator for microbial activity.

Acid phosphomonoestrase, phosphodiestrase, and galactosidase were not affected by an increase of soil salinity, and did not correlate with soil electrical conductivity, organic matter content, Cl, and Na (Table 1). Other enzymes such proteases, glucosidases, ureases, and fluorescein diacetate hydrolase were inhibited in saline soils, in relation to the soil organic C and microbial biomass (data not shown). Urease and protease activities appear to be more sensitive to salinity than phosphatases. In saline soils, salt tolerant bacteria produce enzymes, whose activity has a greater salt requirement than that of corresponding enzymes produced by non- salt tolerant bacteria (Zahran 1997; Egamberdieva 2005; 2007; 2012). Garzia and Hernandez (1996) in their work reported that salinity negatively affects biological and biochemical fertility of the soils and is more pronounced with NaCl than Na_2SO_4 which can be attributed to the toxic effect of a particular ion in saline soils on microbial growth.

In conclusion, our study showed that salinization of soils due to prolonged irrigation and continous use of high amount of fertilizers in cotton monoculture reduced several hydrolase and esterase activities, indicating a progressive degradation of the agricultural lands of Uzbekistan and a need of urgent remediation actions to be taken.

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