# **Response of Soil Biological and Biochemical Activity to Salination**

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## Özet

## Topraktaki Biyolojik ve Biyokimyasal Aktivitenin Tuzluluğa Tepkisi

Bu çalışmanın amacı, tuzluluğun toprak verimliliği ile ilişkili bazı biyolojik ve biyokimyasal özellikler üzerindeki etkisini araştırmaktadır. Toprak örnekleri beş farklı tuz düzeyini içeren (0.65, 2.0, 3.5, 5.0 and 6.5 dS m<sup>-1</sup>) tuzlu sulama suları ile sulanan bir denemeden alınmıştır. Toprağın elektriki geçirgenliğindeki artış toprağın biyolojik ve biyokimyasal verimliliği üzerinde olumsuz bir etkiye sahip olurken, N-mineralizasyonunun tuzluluğa hassasiyeti C-mineralizasyonunu oranla daha fazla olmuştır. En yüksek elektriki geçirgenlikte (6.5 dS m<sup>-1</sup>), N- mineralizasyonu % 47 oranında engellenirken aynı tuz uygulaması C-mineralizasyonunu sadece % 17.5 oranında engellemiştir. Proteaz ve üreaz gibi hidrolaz grubu enzim aktiviteleri bir oksidoredüktaz enzim olan katalaza oranla tuzluluktan daha fazla etkilenmişlerdir. **Anahtar kelimeler:** C-mineralizasyonu, N-mineralizasyonu, enzim aktivitesi, tuzluluk

### Introduction

In lands of low rainfall, irrigation of agricultural fields is closely dependent on underground waters which generally have higher soluble salt contents than surface water since they are permanently in contact with the different minerals which make up the soil (8). Salinity also exits in topographically low lands near the sea where intrusion of seawater to the aquifer is inevitable. Such saline waters are in relatively frequent use on the agricultural soils of Ege Region, İzmir which are proximal to the sea and with high temperature and low rainfall in summer months.

The salt caused both by natural and cultural effects on soil can be of chemical, physical and biological origin. Biological effects are

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the changes in osmotic pressure and alteration of protoplasmic action in plants and the microorganisms (20). A soil's osmotic pressure is a critical factor for microorganism activity and growth, and is closely related with the concentration of salts found therein. The relationships between microbial growth and osmotic pressure is basically a reflection of the inhibition of certain biochemical processes by the high concentrations of endocellular solute (11).

Several microbiological studies of soils affected by salinity are reported. A reduction in the soil respiration and microbial biomass of saline soils was examined by Laura (16) and Carter (5), respectively. Sarig et al (19) studied the influence of the irrigation with saline water on soil microbial activity and soil structure. Garcia and Hernandez (8) stated that the increase of soil electrical conductivity caused by the addition of saline solutions had a negative effect on soil's biological and biochemical fertility, and this effect being more noticeable with NaCl than with Na<sub>2</sub>SO<sub>4</sub>.

Salinity problem has been detected in the soils which covers 1.7 % (1.518.746 ha) of Turkey and 3.8 % (837.405 ha) of agricultural land of Turkey (14) and no data is available regarding the microbial activity in these problematic regions. The objective of the present study was to investigate the effect of salination on biological and biochemical soil characteristics related directly with fertility.

#### **Materials and Methods**

Soil samples were taken from an experimental site which had been established in 1996 to determine the effects of salinity on yield and quality of satsuma mandarins budded onto *Poncirus trifoliata* rootstock at Ege University Campus in İzmir, Turkey. Five different levels of irrigation with saline water  $(0.65 - 2.0 - 3.5 - 5.0 \text{ and } 6.5 \text{ dS} \text{m}^{-1})$  was realized from the beginning of June up to the first half of November (1). Treatment plots were randomly located within each of three replicate blocks and the soil samples under consideration were collected from the upper 20 cm of plots at October 2000.

A part of the sampled soils were sieved (2 mm) and stored at +4 °C for the microbiological analysis and the other part for the determination of physicochemical parameters were air dried and sieved (2 mm) before analysis.

#### Physicochemical analysis

Mechanical analysis of the studied soils were determined by hydrometer method (3) and conductivity and pH measured in a 1:2.5 (w/v) aqueous solution. Calcium carbonate (%) was assessed by measuring the volume of  $CO_2$  from the reaction of the carbonate mineral and excess HCl using the Scheibler calcimeter (6). Organic matter content was calculated from the organic C concentration (21), by multiplying 1.724.

The physicochemical properties of the experimental soil from the Alluvial Great Soil Group are given in Table 1.

Table 1. Soil characteristics						
pH (H <sub>2</sub> 0, 1:2,5w/v)	: 6.83	Sand (%)	: 73.28			
$EC (dS m^{-1})$	: 0.95	Silt (%)	: 16.40			
Total CaCO <sub>3</sub> (%):	0.57	Clay (%)	: 10.32			
Organic matter (%)	: 1.34	Texture	: Sandy loam			

#### **Microbiological analysis**

<u>C- and N-mineralization</u>: C- and N-mineralizations were determined from the quantity of CO<sub>2</sub>-C and NH<sub>4</sub>-N+ NO<sub>3</sub>-N, respectively, mineralized from soil sample during a 10-day incubation at 25 °C (4).

<u>Protease activity</u>: Five ml of tris buffer (pH 8.1) and 5 ml of 2 % casein substrate were added to 1.0 g of soil. The mixture was incubated at 50 °C for 90 minutes. Amino acids released during the incubation period was extracted, and the remaining substrate was precipitated after addition 5 ml of TCA solution. Aromatic amino acids react with Folin Ciocalteu's phenol reagent in an alkaline solution were determined colorimetrically at 700 nm (15).

<u>Urease activity:</u> Twenty ml of pH 10 borate buffer and 2.5 ml of 720 mM urea were added to 5.0 g of soil which was incubated at 37 °C for 90 minutes. Released ammonium was extracted with 2 M KCl solution and determined by a modified Bertholet reaction (12).

<u>Catalase activity:</u> Catalase activity was determined by measuring the amount of  $O_2$  evolved within 3 minutes after the addition of  $H_2O_2$  to the buffered soil suspension (2).

#### Results

Results related to the C and N mineralization rate of the soils irrigated with saline water are given Table 2. Regarding the treatments, two different C-mineralization statistical groups were determined at 5

% significance. The highest rate of C-mineralization was found at the control ( $S_0$ ) and  $S_1$  parcels while the lowest rate at  $S_2$ ,  $S_3$  and  $S_4$  treatments. The amount of mineralizable C decreased after the  $S_2$  treatment (3.5 dS m<sup>-1</sup>) and only 17.5 % was inhibited by an irrigation water with an EC of 6.5 dS m<sup>-1</sup>.

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Treatments	C-mineralization	N-mineralization			
$(dS m^{-1})$	$(mg CO_2$ -C 100 g <sup>-1</sup> soil)	(mg inorganic N 100 kg <sup>-1</sup> soil)			
S <sub>0</sub> (Control)	25.55 a	59.80 a			
$S_1(2.0)$	24.92 a	40.81 b			
$S_2(3.5)$	22.20 ab	35.08 bc			
$S_3(5.0)$	22.15 ab	35.85 bc			
$S_4(6.5)$	21.07 b	31.77 c			
	LSD 0.05 : 3.64	LSD 0.05: 7.67			

Table 2. C-and N-mineralization in soils irrigated with water different EC

In the case of N-mineralization (Table 2), three different statistical groups were assessed at 5 % significance according to the treatments. The highest rate of N-mineralization was determined at the control (S<sub>0</sub>) and the lowest at the S<sub>4</sub> parcel. N-mineralization differing from the C-mineralization began to decrease after the S<sub>1</sub> treatment and the increased electrical conductivity (EC) lead to increased enzyme activity inhibition. At this level (2 dS m<sup>-1</sup>), the rate of N-mineralization was inhibited nearly 32 % and increased to 47 % at S<sub>4</sub> application (6.5 dS m<sup>-1</sup>), compared to the control (S<sub>0</sub>).

The amounts of protease, urease and catalase enzyme activities determined in the research soils are given in Table 3. The effect of enhanced salinity on the studied enzyme activities was found significant at 1 % level. In this respect, protease activity declined sharply after the  $S_1$  application and followed by a decreasing trend that steadily became consistent at the last three salinity levels (S<sub>2</sub>, S<sub>3</sub> and S<sub>4</sub>). Protease activity was inhibited 57 % by a 2 dS  $m^{-1}$  enhancement in salinity (S<sub>1</sub> treatment), and to an average of 82 % at S<sub>2</sub>, S<sub>3</sub> and S<sub>4</sub> treatments. Urease activity had a similar tendency with protease activity however increased salination lead to a constant relative decrease in this regard. Compared to the control  $(S_0)$ , the inhibition rate was 20 % at  $S_1,\; 36$  % at  $S_2$  and an average of 57 % at  $S_3$  and  $S_4$ applications. Results proved that 5.0 and 6.5 dS m<sup>-1</sup> EC had greater inhibitory effects on urease activity. Catalase activity was less sensitive to salination than the other two studied enzyme activities. The highest level was determined at S<sub>0</sub>, S<sub>1</sub> and S<sub>2</sub> treatments and the lowest at S<sub>4</sub>

treatment. This enzyme began to be inhibited at  $S_3$  treatment (5 dS m<sup>-1</sup>) with 57 % which increased to 67 % at  $S_4$  treatment.

Table 3. Protease, urease and catalase activities in soils irrigated with water different EC

Treatments	Protease Act.	Urease Act.	Catalase Act.
$(dS m^{-1})$	( $\mu$ g Tyrosin g <sup>-1</sup> soil/2h)	(µg N g <sup>-1</sup> soil/2h)	(% O <sub>2</sub> )
S <sub>0</sub> (Control)	) 114.02 a	326.80 a	25.12 a
$S_1(2.0)$	49.19 b	262.23 b	26.08 a
$S_2(3.5)$	24.55 c	209.53 c	23.14 a
$S_3(5.0)$	20.21 c	145.55 d	15.96 b
$S_4(6.5)$	17.55 c	136.76 d	8.24 c
	LSD 0.01: 17.10	LSD 0.01: 28.50	LSD 0.01: 5.70

## Discussion

The decomposition of organic carbon provides energy for the growth of heterotrophic microorganisms and supplies carbon for the formation of new cell material. Under aerobic conditions, frequently 60-80 % of the substrate carbon is released as CO<sub>2</sub> or accumulates as waste products. The remaining 20-40 % supplies organic compounds for the synthesis of microbial biomass (10). The conservation of organic nitrogen to the more labile, inorganic state is known as nitrogen mineralization. Microorganisms with different physiological properties (heterotrophs and ototrophs) take part in this process. According to the our results; C and N-mineralizations are differently affected by the salination of the studied soil, meaning that there must be different physiological responses of the microorganisms to salt. Nmineralization was more sensitive than C-mineralization to salinity. At the maximum EC of the irrigation water (6.5 dS m<sup>-1</sup>), N-mineralization was inhibited 47 % while the same treatment lead to an inhibition of only 17.5 % in the case of C-mineralization. Sarig et al (19) reported that irrigation with saline water (EC=5 dS  $m^{-1}$ ) increase the accumulation of C and N in microbial biomass, but decrease the rate of C and N-mineralization. Similarly, Polonenko et al (17) stated an increase in the microbial cell numbers with an increase in salinity of the medium. In studies of microbial ecophysiology (13), the microbial biomass increase when the osmotic potential was lower than -1.0 MPa due to the osmotic adaptation capacity of the microorganisms. Microorganisms prefer a survival strategy under osmotic stress in which they channel the consumed C and N to biomass production or

cell proliferation which naturally results with a decline in the rate of C and N-mineralization.

Valid information on the spectrum of enzymatic activities of a soil is important since it will indicate the potential of the soil to permit the basic biochemical processes necessary for maintaining soil fertility. In the present study, two hydrolases (protease and urease) related with the nitrogen cycle and an oxidoreductase (catalase) related with the number of aerobic microorganisms-soil fertility (18) were determined. The studied enzyme activities were negatively affected from the increased salination in soil. However, the activities of protease and urease were more sensitive to salinity than that of catalase. Similar findings are reported by Garcia and Hernandez (8) who studied enzyme activities under saline conditions.

Many of the enzymes are extracellular, stable and form complexes with the organic and mineral colloids (9). The increase in electrical conductivity disperses the clays resulting in the unprotected stable enzymes which are more susceptible to denaturalization. The cell lysis due to a reduced osmotic potential (7) can also explain this decrease in enzymatic activity.

#### Summary

The objective of the present study was to investigate the effect of salination on the biological and biochemical soil characteristics related with the fertility. Soil samples were taken from an experiment that was established with five different levels of irrigation with saline water (0.65, 2.0, 3.5, 5.0 and 6.5 dS m<sup>-1</sup>). Increase in soil electrical conductivity (EC) had negative effects on soil's biological and biochemical fertility, N- mineralization being more sensitive than C-mineralization. At the highest EC (6.5 dS m<sup>-1</sup>), N-mineralization was inhibited 47 % while the same treatment lead to an inhibition of 17.5 % in C-mineralization. The activity of hydrolases such as protease and urease was more negatively affected by salinity than that of catalase (an oxidoreductase enzyme).

Keywords: C-mineralization, N-mineralization, enzyme activity, salination

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