



Research Article

INVESTIGATION OF SOME SOIL MICROBIOLOGICAL PROPERTIES OF  
RHIZOSPHERE SOIL OF HALOPHYTIC FORAGE PLANTS

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ABSTRACT

The purpose of this study was to determine the halophytic forage crops and some microbiological properties i.e. catalase activity, dehydrogenase activity, microbial biomass carbon and basal soil respiration of rhizosphere soils. Halophytic forage crops like *Salsola crassa*, *Salsola dendroides*, *Cressa cretica*, *Salsola soda*, *Alopecurus myosuroides*, *Prosopis farcta*, *Alhagi pseudalhagi*, *Tamarix smyrnensis*, *Chenopodium album*, *Chenopodium* sp. were widely determined in Harran Plain, Turkey. Rhizosphere soil samples of these crops were collected from rhizosphere of each plant. The rhizosphere soils were analyzed by measuring microbiological properties i.e. basal soil respiration, microorganism population, microbial biomass carbon, catalase and dehydrogenase activities. Basal soil respiration and microbial biomass carbon content of samples were between 10.67-62.3 µg CO<sub>2</sub> /g dry soil and 104.47-216.59 µg C/ g dry soil, respectively. Soil basal respiration was obtained highest in the rhizosphere of *Salsola soda*. Enzyme activities were affected by the rhizosphere soils, depending on the plant species. Catalase activity and dehydrogenase activity were highest in the rhizosphere of *Alopecurus myosuroides*, 12.3 ml O<sub>2</sub>/5g soil and 348.36 µg TPF/ g dry soil, respectively.

**Keywords:** Soil respiration, salt affected soil, catalase, dehydrogenase, halophytic forage plants.

1. INTRODUCTION

The Southeastern Anatolian Project (GAP) was initiated by the Turkish Government after 1970s. One of the most important aims of this project is to irrigate at least 1.7 million ha agricultural area [1]. Harran Plain, which is the most important agricultural regions of Turkey and has the potential of the major components of the GAP. Harran Plain has 225 000 ha area and currently 132 000 ha area is now irrigated. 15 000 ha area have salinity and sodicity problems due to excessive irrigation and poor drainage [1]. The most important problem is salt affected in irrigated areas. Therefore, saline soils in these area has been using as pasture. More or less amount of salt, accumulated in the soil with irrigation. All these negative factors, available in the Harran Plain [1].

The majority of plants growing in salt affected areas was found to belong to the family Chenopodiaceae [2, 3]. Although there are many halophytes belonging to the family Chenopodiaceae, *Atriplex* species can grow in areas [4]. *A.halimus*, *Salsola vermiculata* can grow in salt affected soils and also forage plants such as *Festuca rubra*, *Agrostis capillaris*,

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*A.stolonifera*, *Holcus lanatus*, *Leptochloa fusca*, *Puccinellia distans* (barren grass) have been determined by the same investigators [3, 5].

Organisms in the soil, soil structure and structural formation of the organic residues, atmospheric nitrogen fixation and nutrient cycles of plants and symbiotic relationships provide the necessary conditions for soil fertility [6]. Enzymes secreted by microorganisms play an important role in the realization of these events. Enzymes such as dehydrogenase, catalase, urease were adsorbed on the soil colloids [7, 8]. In the soil, more than fifty the enzyme activity was determined [6]. The enzymes such as catalase, dehydrogenase are responsible for the decomposition of plant litter [9].

In this study, halophytic forage crops were collected from the salt affected areas of Harran Plain and identified, rhizosphere soil samples of halophytic plants were taken and microbial population, basal soil respiration (carbon dioxide production), catalase and dehydrogenase enzyme activities were determined. This research is the first study of the research area. For later research, it is important for the comparison of microbiological activity in salt affected soils.

## 2. MATERIAL AND METHODS

### 2.1. Study sites

The study area is located in the Harran Plain, one of Turkey's major plains. The Harran Plain lies between 38° 41' and 39° 15' east longitudes and 36° 40' and 37° 21' north latitudes in southeastern part of Turkey (Figure 1). The climate is semi-arid. The average maximum and minimum temperature are 46.8 °C (July) and -9.3 °C (February), respectively. Yearly total amount of rainfall is 363.1 mm [10].

### 2.2. Soil Sampling procedures

Soil samples were collected from plants rhizospheres in salt affected areas of Harran Plain (Figure 1) in 2012 year. The soil samples were collected at 0-4 mm from the root surface. Rhizosphere soil samples of each halophytic plants were sieved through a 2 mm grid and were kept at 4 °C in a plastic box and then analyzed. Plant identifications were made according to Davis [11], Donner [12] and Güner [13].

### 2.3. Analysis of soil

The organic matter content of soil was determined to Walkey-Black method, total N was determined by the Kjeldahl method and available P was extracted with sodium bicarbonate extraction method of Olsen [14]. The soil texture was determined by the hydrometer method, pH was measured using pH-meter, available K was measured by flame photometry, electrical conductivity was measured in a aqueous extract and CaCO<sub>3</sub> was determined by Scheibler calcimeter [14]. The some chemical and physical properties of the soil samples used in this study were shown in Table 1.

### 2.4. Basal soil microbial respiration

Basal soil microbial respiration was measured according to Aşkın and Kızılkaya [15] by absorption of the CO<sub>2</sub> produced during 24 hours incubation period. A 20 g soil samples were placed in a jar with 10 ml of BaOH. Soil respiration was trapped inside the alkaline solution and titrated using a diluted HCl solution. Data were expressed as µg CO<sub>2</sub>-C /g dry soil.

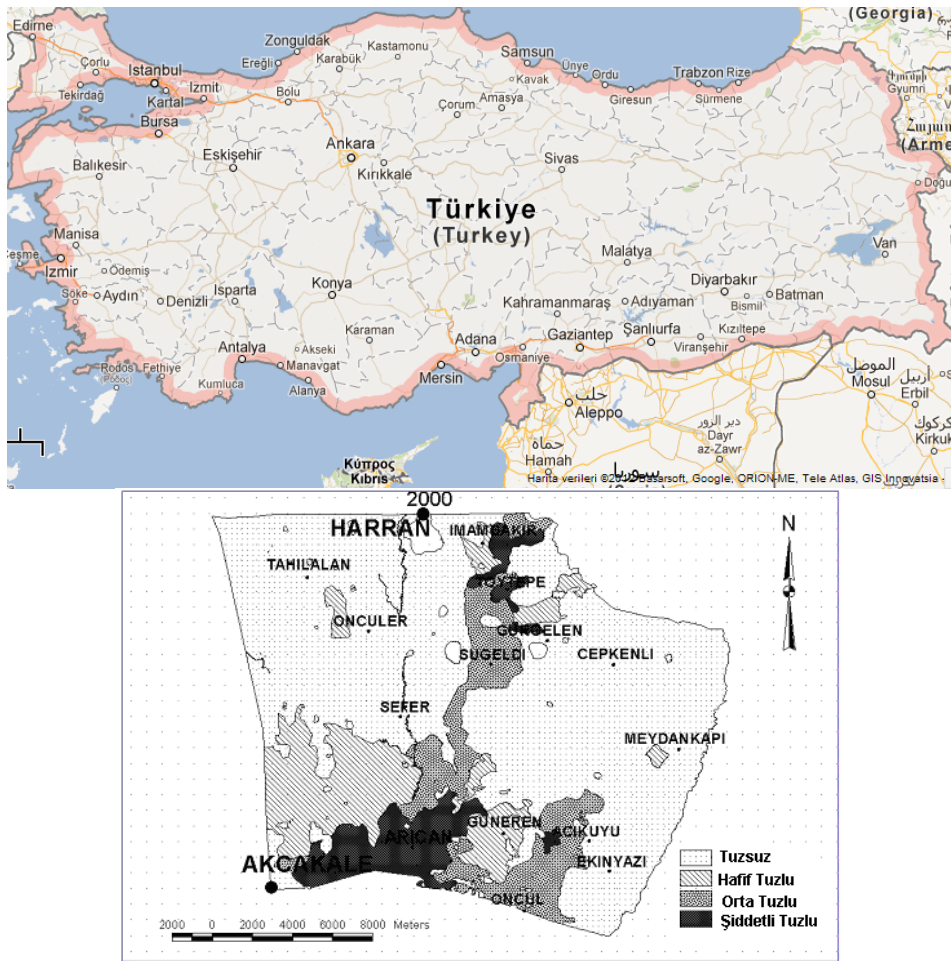


Figure 1. Map of sampling area

Table 1. The physico-chemical properties of soil samples

Halophytic plants	Electrical conductivity (dS/m)	% CaCO <sub>3</sub>	pH	Organic matter (%)	K <sub>2</sub> O (kg/ da)	% N	Texture
<i>Chenopodium sp.</i>	40.1	9.1	8.83	1.58	254.4	0.17	Silty loam
<i>Prosopis farcta</i>	1.73	13.6	8.10	1.64	394	0.15	Clay
<i>Cressa cretica</i>	40.1	9.1	8.83	1.58	254.4	0.17	Silty loam
<i>Alopecurus myosuroides</i>	1.73	13.6	8.10	1.64	394	0.15	Clay
<i>Salsola soda</i>	40.1	9.1	8.83	1.58	254.4	0.17	Silty loam
<i>Chenopodium album</i>	40.1	9.1	8.83	1.58	254.4	0.17	Silty loam
<i>Salsola dendroides</i>	40.1	9.1	8.83	1.58	254.4	0.17	Silty loam
<i>Salsola cressa</i>	40.1	9.1	8.83	1.58	254.4	0.17	Silty loam
<i>Tamarix smyrnensis</i>	40.1	9.1	8.83	1.58	254.4	0.17	Silty loam
<i>Alhagi pseudalhagi</i>	40.1	9.1	8.83	1.58	254.4	0.17	Silty loam
Control	1.73	13.6	8.10	1.64	394	0.15	Clay

## 2.5. Microbial biomass carbon

Glucose (200 mg) was added to the soil samples and incubated for 4 hours at 25 °C [16]. Microbial biomass carbon contents from the amount of CO<sub>2</sub> production at the end of the incubation period were calculated using the equation  $40.04 \text{ mg CO}_2/\text{g} + 0.37$  [16].

## 2.6. Soil enzyme activity

### 2.6.1. Catalase activity

Catalase was evaluated following Liu et al. [8]. 10 ml of phosphate buffer solution was added to the soil sample (5 g) and incubated for 10 minutes. 3 ml of 30 % H<sub>2</sub>O<sub>2</sub> was placed in small glass tubes and placed in jars so as not to spill onto the soil. The jars are covered with rubber stoppers and connected to Scheibler calcimeters. Then, H<sub>2</sub>O<sub>2</sub> was poured into the soil, the oxygen output was determined as ml by shaking for 3 minutes.

Controls were tested in the same way, but with the addition of 2 ml of 6.5 % (w/v) NaN<sub>3</sub>. Data were calculated on the basis of oven dry weight of soil.

### 2.6.2. Dehydrogenase activity

Dehydrogenase activity was evaluated following Liu et al. [17]. 3% TTC (2,3,5-triphenyl tetrazolium chlorid) solution, 2.5 ml distille water, glucose were added to by of soil. The content has been incubated for 24 hr at 25 °C. The formation of TPF (1,3,5 triphenylformazon) was found spectrophotometrically at 485 nm.

## 2.7. Microbial counts

Microbial number were quantified by the spread plate technique. Total bacterial number, actinomycetes numbers and a fungi number were counted by plating dilutions of soil a Plate Count Agar (PCA, MERCK), Glycerol-asparagine agar (1g asparagine, 10 g glycerol, 1g K<sub>2</sub>HPO<sub>4</sub>, 1ml trace salt solution, 10 g agar, 1000 ml distilled water) and Potato Dextrose Agar (PDA, MERCK), respectively. The number of colonies was determined from three replicate plate after 3-5 days incubation at 28 °C in incubator [17-19]. Dilution blanks were prepared with sterile distilled water. The plate counts of microorganisms were reported as the number of colony forming units (c.f.u).

The tests were performed in triplicate. The results were evaluated using the TARIST statistical program. The Students-t test was used the criterion in checking the importance of the applications and comparison of the average.

## 3. RESULTS AND DISCUSSION

The physico-chemical characteristics and some microbiological activity of the salt affected rhizosphere soil samples were given Table 1 and Table 2. Soils have a high pH values, pH of samples was between 8.1 and 8.83. CaCO<sub>3</sub> content of soil samples was determined between 13.6 - 9.1% (Table 1). These features are a direct effect on the efficiency of the microbiological activity in the soil [20, 21]. This area are using as a pasture, because it is affected by salt. *Salsola* species, high-quality fodder such as *Cressa cretica*, the seeds of these plants and the growth of salt-resistant plants can growth at by sprinkling salt fields unusable for agriculture and pasture areas. These plants may be economically important. In the studied area, halophytic plants such as *Salsola crassa*, *Salsola dendroides*, *Cressa cretica*, *Salsola soda*, *Alopecurus myosuroides*, *Prosopis farcta*, *Alhagi pseudalhagi*, *Tamarix smyrnensis*, *Chenopodium album*, *Chenopodium*

sp. were determined (Table 2). The rhizosphere soil of *Alopecurus myosuroides* showed the highest values of microbial biomass (Table 2).

**Table 2.** List of the halophytic plants at the sampling sites and some microbiological properties in the rhizosphere soils of halophytes plants

Halophytic plants	Basal soil respiration ( $\mu\text{g/g}$ dry soil)	Soil microbial biomass ( $C_{\text{mic}}$ ) ( $\mu\text{g/g}$ dry soil)	DHA ( $\mu\text{g}$ TPF/ g dry soil)	CA ( $\text{ml O}_2/5$ g dry soil)
<i>Chenopodium sp.</i>	57.67 $\pm$ 1.3 b	176.55 $\pm$ 0.02 b	91.10 $\pm$ 0.1 h	2.43 $\pm$ 0.05 ef
<i>Prosopis farcta</i>	40.1 $\pm$ 2.2 e	128.50 $\pm$ 0.02 g	123.16 $\pm$ 0.2 g	3.13 $\pm$ 0.04 e
<i>Cressa cretica</i>	45.3 $\pm$ 0.05 d	114.47 $\pm$ 0.01 h	181.36 $\pm$ 0.7 c	6.4 $\pm$ 0.07 d
<i>Alopecurus myosuroides</i>	54.67 $\pm$ 0.1 bc	216.59 $\pm$ 0.03 a	348.36 $\pm$ 1.0 a	12.3 $\pm$ 0.05 a
<i>Salsola soda</i>	62.3 $\pm$ 0.7 a	140.15 $\pm$ 0.01 d	133.63 $\pm$ 0.5 f	8.93 $\pm$ 0.01 c
<i>Chenopodium album</i>	21.3 $\pm$ 0.1 h	136.51 $\pm$ 0.05 e	312.76 $\pm$ 0.1 f	1.87 $\pm$ 0.01 f
<i>Salsola dendroides</i>	31.3 $\pm$ 0.3 g	144.51 $\pm$ 0.02 c	162.76 $\pm$ 0.3 d	8.73 $\pm$ 0.05 c
<i>S.cressa</i>	35.3 $\pm$ 0.2 f	124.63 $\pm$ 0.03 f	147.73 $\pm$ 0.1 e	10.07 $\pm$ 0.03 b
<i>Tamarix smyrnensis</i>	51.7 $\pm$ 0.1 c	131.43 $\pm$ 0.04 f	92.73 $\pm$ 0.2 h	6.83 $\pm$ 0.02 d
<i>Alhagi pseudalhagi</i>	48.3 $\pm$ 0.1 d	128.50 $\pm$ 0.01 g	132.50 $\pm$ 0.1 f	10.33 $\pm$ 0.02 b
Control	10.67 $\pm$ 0.2 k	104.47 $\pm$ 0.01 k	86.26 $\pm$ 0.1 k	3.67 $\pm$ 0.05 e
LSD	3.072 <sup>***</sup>	1.28 <sup>***</sup>	2.075 <sup>***</sup>	0.794 <sup>***</sup>

<sup>\*\*\*</sup> significant at the %0.1 probability level.

$C_{\text{mic}}$ :Microbial biomass carbon; DHA:Dehydrogenase activity; CA:Catalase activity; Control: non-rhizospheric soil

In our study, we determined the basal soil respiration was changed from 10.67  $\mu\text{g}$  to 62.3  $\mu\text{g}$   $\text{CO}_2\text{-C}$  /g soil (Table 2). Dehydrogenase activities of soil samples were changed from 86.26 to 348.36  $\mu\text{g}$  TPF/g soil, catalase activities were changed from 1.87 to 12.3  $\text{ml O}_2$  /5 g soil, microbial biomass carbon contents were changed from 104.47 to 216.59  $\mu\text{g/g}$  soil. Microbial population in these different rhizosphere soils were shown in Table 3.

**Table 3.** Microbial population on rhizosphere soils of the halophytic plants

Halophytic plants	Total bacteria ( $10^7$ c.f.u $\text{g}^{-1}$ soil )	Actinomycetes ( $10^7$ c.f.u $\text{g}^{-1}$ soil )	Fungi ( $10^6$ c.f.u $\text{g}^{-1}$ soil )
<i>Chenopodium sp.</i>	47.3 de	2.53 b	5.70 b
<i>Prosopis farcta</i>	82.3 a	2.30 b	7.17 a
<i>Cressa cretica</i>	75.0 b	5.67 a	5.40 bc
<i>Alopecurus myosuroides</i>	44.3 e	5.00 a	4.40 bcd
<i>Salsola soda</i>	50.0 d	2.50 b	3.33 d
<i>Chenopodium album</i>	26.0 f	1.73 b	5.37 bc
<i>Salsola dendroides</i>	44.0 e	1.67 b	4.13 cd
<i>S.cressa</i>	54.3 c	2.01 b	3.37 d
<i>Tamarix smyrnensis</i>	82.0 a	1.57 b	3.93 d
<i>Alhagi pseudalhagi</i>	50.3 d	1.27 b	3.33 d
Control	17.7 g	2.00 b	1.63 e
LSD	3.690 <sup>***</sup>	1.745 <sup>***</sup>	1.319 <sup>***</sup>

<sup>\*\*\*</sup> significant at the %0.1 probability level, respectively

Microbial biomass and soil respiration are the most commonly used microbial indicators for soil health monitoring by Kızılkaya and Dengiz [14], Gill-Sotres et al. [21]. The lowest microbial biomass value were recorded in rhizosphere soil of *Cressa cretica* (Table 2). The Students-t test was done to determine the differences among rhizosphere soils. According to results, some microbiological properties were statistically significant differences determined in different soil samples (Table 2).

It has been described that microbial growth in the rhizosphere is closely related to root exudates and plant debris [16,22,24]. Studied soils are salt affected soils, so, basal soil respiration values were low (Table 2). The basal respiration from rhizosphere soil is a good indicator of overall biological activity of the soil [6]. Low rates of soil respiration reflected the environmental stress as a result of salinity conditions on the soil microbial community. The basal soil respiration rate was decreased tested samples. This corresponds well with other studies, which showed decreased soil respiration in salt affected soils [9, 22]. It is possible that the result as osmotic stresses may play a role in restricting respiration in the salt affected soils. High soil respiration content indicates of high biological activity [7, 21,24].

Decreasing rates of microbial biomass are an indication of soil degradation, pollution, salinity and clay. Salinity can directly kill many microorganism therefore, microbial biomass is a decrease [20]. Soil micro-climatic conditions are play a role in determining microbial biomass [24].

The lowest dehydrogenase activity was determined in *Chenopodium* sp. rhizosphere soil and the highest dehydrogenase activity was determined in *Alopecurus myosuroides* rhizosphere soil (Table 2). In this study, the rhizosphere of *Alopecurus myosuroides* showed the highest dehydrogenase and catalase activity, the lowest dehydrogenase activity was found in the rhizosphere *Tamarix smyrnensis*. Various enzymes are adsorbed on clay minerals [22]. Studied soils have clay texture. Dehydrogenase is an intracellular enzyme and it is very sensitive to natural factors. In soil microbiological activity, dehydrogenase activity is an important indicator for monitoring the oxidation of organic matter reported by Ouni et al. [22].

The rhizosphere of *Chenopodium album* showed the lowest catalase activity as 1.87 mg O<sub>2</sub>/5 g soil (Table 2). The different enzyme activities may differ in the rhizosphere of each plant species. Catalase activity in soils is affected by clay content, soil moisture, salinity, temperature, organic matter. Microbial population play a key role among them. Increasing salinity in soils might be affect the activity of the enzymes. Garcia et al. [7] shown that, population of microorganisms in soil content were increase or decrease depending on the plant type and age.

The fodders have to be grown with other cultivated plants that resistant to salt. In semi-arid regions, at the beginning and of the summer, evaporation is very high. Seasons of growing and development of halophytics are spring and end of the summer. The plant growth and microbial activity in rhizosphere might be affecting from increasing salinity. In this study, bacterial communities are higher than fungal communities (Table 3). On average, the populations of bacteria in soil samples of *Prosopis farcta* and *Tamarix symrnensis* were higher than those in the other rhizosphere soil samples (Table 3). Similar observations have been reported by Liu et al. [17] and Ouni et al. [22], where the high concentration of salt into resulted in decreasing soil microbial activity. Tripathi et al. [9] examined that the number of microorganisms decreased when salt was added to soil. Yuan et al. [24] studied that the application of salt concentrations to soil decreased the incidence of bacteria in the plants rhizosphere soil. Bacterial communities have dominate in salt affected soils according to Saviozzi et al. [20], Yuan et al. [24]. Low organic matter content and high salinity were affected in growth of fungi and actinomycetes and microbial biomass. In this study, fungi and actinomycetes numbers low than bacterial numbers (Table 3).

In salt affected soils, bacterial dominance may inhibit the decomposition of complex organic material [6, 23]. Because fungi ise especially important for the breakdown of lignin and cellulose in decaying plant residues [7]. Root biomass and levels of soluble carbohydrates in soil water were related in rhizosphere soil of plants. According to Garcia et al. [7] the binding effect of

polysaccharides and carbohydrates is attributable to the increases in microbial activity. Soil dehydrogenase activity reflects the total range of oxidative activity of soil microflora [22]. Garcia et al. [7] found that dehydrogenase activity is a good index of the status of soil microbial activity in semi-arid area.

Arid and semi-arid lands of the region shows a very high evaporation. Halophytes the end of summer and early fall coincides with periods of growth and development of plants for this region. Halophytic plants will consist largely of high evaporation and thus prevent the salt of the soil to reach the lower parts of the plant root zone will be blocked. In addition, largely for livestock feed supplies will be provided. Akinshina et al. [25], Kumar et al. [26] and Öztürk et al. [5] have been reported that *Salsola crassa*, *Salsola dendroides*, *Chenopodium album* and *Cressa cretica* collected from salt affected soil. In addition forage crops has been reported to increase the positive effects on soil structural structures, soil organic matter in saline soil [5].

#### 4. CONCLUSIONS

In this study, amount of tested enzyme is lower in rhizosphere soil samples because organic matter levels is low. As a result, in the region, the problem of salt depends on the climatic conditions. Salinization of soil and water management has resulted in the wrong territory. Improper use of irrigation systems has increased and the excess water has become an environmental problem of salinity. Forage crops were identified in salt affected conditions and microbiological properties in rhizosphere soils of halophytic forage crops were determined. In rhizosphere soil samples, microbial activity were quite low. In addition, due to stress caused by the salt, the number of microorganisms and enzymatic activity of rhizosphere soils is low.

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