

Influence of Varying Degree of Salinity-Sodicity Stress on Enzyme Activities and Bacterial Populations of Coastal Soils of Yellow Sea, South Korea

Siddikee, Md. Ashaduzzaman¹, Sherlyn C. Tipayno¹, Kiyoon Kim¹, Jongbae Chung², and Tongmin Sa^{1*}

¹Department of Agricultural Chemistry, Chungbuk National University, Cheongju, Chungbuk 361-763, Korea

²Division of Life and Environmental Sciences, Daegu University, Gyeongsan 712-714, Korea

Received: December 13, 2010 / Revised: January 3, 2011 / Accepted: January 17, 2011

To study the effects of salinity-sodicity on bacterial population and enzyme activities, soil samples were collected from the Bay of Yellow Sea, Incheon, South Korea. In the soils nearest to the coastline, pH, electrical conductivity (EC_e), sodium adsorption ratio (SAR), and exchangeable sodium percentage (ESP) were greater than the criteria of saline-sodic soil, and soils collected from sites 1.5–2 km away from the coastline were not substantially affected by the intrusion and spray of seawater. Halotolerant bacteria showed similar trends, whereas non-tolerant bacteria and enzymatic activities had opposite trends. Significant positive correlations were found between EC, exchangeable Na⁺, and pH with SAR and ESP. In contrast, EC_e, SAR, ESP, and exchangeable Na⁺ exhibited significant negative correlations with bacterial populations and enzyme activities. The results of this study indicate that the soil chemical variables related with salinity-sodicity are significantly related with the sampling distance from the coastline and are the key stress factors, which greatly affect microbial and biochemical properties.

Keywords: Coastal soil, salinity, sodicity, soil enzyme activity, bacterial population

In coastal regions, soils are rich in salts, and the characteristics are developed as a result of the presence of saline ground water-table at a shallow depth and due to periodical inundation with tidal water. The salt-laden sands and seawater spray with sea winds are also accountable for the formation of coastal saline soils. The soil salinity thus shows high temporal and spatial variations depending on the climate, elevation, drainage, soil texture, and other related factors [1, 2, 15, 30, 36, 37]. There are three soil classifications used to describe salt accumulation in soils. Saline soils

contain a high amount of soluble salts, primarily Ca²⁺, Mg²⁺, K⁺, and Na⁺ salt of Cl⁻, NO₃⁻, SO₄⁻² and CO₃⁻³, etc, whereas sodic soils are dominated by Na⁺ salt. Saline-sodic soils have high salt of Ca²⁺, Mg²⁺, and K⁺ as well as Na⁺.

Salts in soil have a negative impact on soil physical, chemical, and biological properties and can ultimately deteriorate soil quality in both ecological and agricultural aspects. A lot of really useful and practical information is available on the subject of salinity effects on the physical and chemical properties of soil [16, 18, 23, 24, 29, 34]. However, the soil microbial properties of natural saline environments are rarely studied [36, 37]. Salinity detrimentally affects microbial communities and their activities in irrigation-induced saline soils, which are important in nutrients recycling within the soil, increasing fertility, and maintaining ecological function [27].

Although saline environments harbor taxonomically diverse microbial groups exhibiting modified physiological and structural characteristics [40], microbial biomass and soil enzyme activities are detrimentally affected under the prevailing saline conditions [2, 3, 7, 10, 12, 13, 14, 27, 31, 36, 37]. Such reduction in microorganism growth and activity in saline environments appears to be associated with a change in osmotic potential of the soil–water phase and possibly with specific ion effects [12, 17]. In fact, coastal ecosystems are severely constrained systems subjected to high stresses of salinity, drought, and nutrient limitation.

Since biological processes in soils are fundamental to their ecological function and to maintain biodiversity, it is important to manage the sensitive coastal soil to maintain the microbial and biochemical activities of the constrained saline environment. However, to manage the salt-affected soil, particularly soils of a coastal region, a prerequisite is to have knowledge on the variables related with salinity-sodicity and biological processes. Little is known regarding the effects of salinity and/or sodicity gradients on the microbial population and enzyme activities of naturally salt-affected soil, particularly in coastal regions [36, 37].

*Corresponding author

Phone: +82-43-261-2561; Fax: +82-43-271-5921;
E-mail: tomsa@chungbuk.ac.kr

Therefore, in the present study, we attempted to investigate the bacterial population and enzyme activities in soils of the coastal saline region of the Bay of Incheon, South Korea, with the aim to understand how a gradient in salinity/sodicity affects the soil microbial variables.

The sampling sites were located within 15 km² of the coastal region of the Bay of Incheon, South Korea. A total of seven soil samples were collected (designated as site 1, site 2, and site 3 nearest to the coastline; site 4 and site 5, and site 6 and site 7 about 500 m and 1.5–2 km away from the coastline, respectively) during the Spring of 2009. The site 4 was a barren land and the other sites were natural habitats of halophytic plants. Triplicate soil samples were collected from the 0–15-cm depth in each site, and soils were sieved through 2-mm mesh screens to sort out plant debris and visible fauna and then stored at 4°C until use.

Soil pH was measured in 1:2.5 soil–water suspensions. Organic matter content was measured following Nelson and Sommers [25]. The electrical conductivity (EC_e) in saturation paste extracts was measured following the method described by the United States Department of Agriculture [38]. Concentrations of Ca²⁺, Mg²⁺, and Na⁺ from the same extracts were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES, Varian Liberty Series II; Mulgrave, Australia). The sodium adsorption ratio (SAR) was calculated as follows: $SAR = Na^+ / (\{[Ca^{2+}] + [Mg^{2+}]\} / 2)^{1/2}$ [38]. The exchangeable cations Ca²⁺, Mg²⁺, K⁺, and Na⁺ were extracted with 1 M ammonium acetate (pH 7.0) after the soil was prewashed with glycol-ethanol, and analyzed using the ICP-OES (Varian Liberty Series II; Mulgrave, Australia). Exchangeable sodium percentage (ESP) was calculated as follows: $ESP = [Exch. Na^+ / Exch. (Ca^{2+} + Mg^{2+} + K^+ + Na^+)] \times 100$ [38].

For counting bacteria, collected soil samples of each site were composited to yield one sample. Ten-fold serial dilutions of the soil samples were made by mixing 5 g of soil with sterile 0.85% saline water and shaken for 15 min at 150 rpm. The aliquots of soil dilution were plated in tryptic soy agar (TSA) media (Pepton 15 g/l, Trypton 5 g/l, Dextrose

2.5 g/l; pH 7.2) for counting the total number of bacteria or in TSA medium containing 10% NaCl as final concentration (pH 8.5) for halophilic bacteria. Plates were incubated at 30°C for 3–4 days and the bacterial populations were determined by counting the visible colonies. Since, non-halophilic/non-halotolerant bacteria cannot tolerate salt or can grow in the presence of <1% NaCl, whereas halophilic bacterial populations require salt to grow and halotolerant/facultative halophilic bacteria can tolerate a wide range of salt concentration (*i.e.*, adapted to saline-sodic soil condition) [19, 21], thus TSA media without NaCl amendment were used to count the culturable non-tolerant bacterial population, and 10% NaCl and pH 8.5 amended media were used to count high salt and pH enduring halophilic/halotolerant bacteria [5, 39].

Dehydrogenase activity was estimated as reported by Casida *et al.* [6]. Cellulase activity was determined as described by Schinner *et al.* [32]. Protease activity was measured according to Ladd and Butler [20]. Alkaline and acid phosphatases and β-glucosidase activities were assayed following the methods of Tabatabai and Bremner [35]. Results were calculated on the basis of air dry weight of soil and each assay was repeated three times. The corresponding controls were carried out for each soil and enzyme activity by proceeding with the same analytical protocol described without the addition of the substrate at the moment of initiating the enzymatic reaction.

Pearson's correlation coefficients (*r*) were calculated between some selected variables measured. All the analyses were conducted using the Statistical Analysis System (SAS) software (version 9.1; Cary, NC, USA). Mean separation was accomplished by Duncan's new multiple range test (DMRT). Significance was tested at the 1% and 5% levels.

Physicochemical properties of soils collected from the different sites are shown in Table 1. Soil pH values were in the range of 6.3–8.7. Soils from sites 1, 2, 3, and 4 were alkaline, and sites 5 and 6 soils were slightly alkaline, whereas site 7 soil was slightly acidic. Soil organic matter content ranged from 0.40% to 1.07%, and the lowest

Table 1. Physicochemical properties of soils collected from the various sampling locations in the coastal region of the Bay of Incheon.

Sampling Sites	pH (1:5 H ₂ O)	Organic matter (g/kg soil)	EC _e (dS/m) ^a	SAR ^a	Exchangeable cations ^b (cmol ⁺ /kg soil)				ESP ^b
					Na	K	Ca	Mg	
Site 1	8.7a	6.26d	44.47a	58.87a	2.52a	1.7a	8.9a	1.69b	17.19b
Site 2	8.4ab	7.10c	29.15c	46.92b	1.82b	1.5a	6.0abc	1.84ab	16.90b
Site 3	8.4ab	9.90b	11.92d	14.54d	1.93b	1.3a	7.6ab	1.72ab	15.40b
Site 4	8.7a	4.00e	37.30b	38.15c	2.05b	1.7a	3.4c	1.99a	22.43a
Site 5	7.4c	9.30b	1.17c	2.14d	0.39c	1.2a	5.2bc	2.00a	4.93c
Site 6	7.4c	9.70b	2.32c	1.82d	0.17c	1.7a	6.1abc	1.96ab	1.87c
Site 7	6.3d	10.63a	1.05c	4.05d	0.10c	1.5a	6.2abc	1.24c	1.01c

^aElectrical conductivity (EC) and sodium adsorption ratio (SAR) were measured in saturation paste extracts.

^bExchangeable cations were extracted using 1 N ammonium acetate (pH 7.0) after removing free ions.

*Values (mean ± SD, n=3) with the same letters are not significantly different at 0.05% (LSD).

Table 2. Bacterial population in different soil samples measured in TSA and TSA+10% NaCl media.

Sampling site	Population in TSA ×10 ⁶ CFU/g soil	Population in TSA (10% NaCl) ×10 ⁶ CFU/g soil
Site 1	14.9±0.9 ^c	18.3±0.8 ^a
Site 2	15.8±0.4 ^c	15.3±0.8 ^b
Site 3	15.4±0.2 ^c	16.3±0.8 ^{ab}
Site 4	1.1±0.06 ^d	1.7±0.2 ^d
Site 5	34.7±1.7 ^a	5.3±0.5 ^c
Site 6	27.3±1.8 ^b	4.6±0.4 ^c
Site 7	36.3±1.7 ^a	3.3±0.2 ^{cd}

Values (mean ± SD, n=3) with the same letters are not significantly different at 0.05% (DMRT).

organic matter content was recorded in the soil collected from barren land. The range of EC_e was 1.0–44.4 dS/m. The concentrations of cations Ca²⁺, Mg²⁺, K⁺, and Na⁺ were significantly higher in soils of sites 1, 2, 3, and 4 than that found in soils of sites 5, 6, and 7, and the Na⁺ ion was the predominant cation in saturated paste extracts of soils of sites 1, 2, 3, and 4 (data not shown). Soils of sites 1, 2, 3, and 4 were highly salt affected, and soils collected from sites 5, 6, and 7 were not in saline condition. Since the percentage of Na⁺ in the composition of solution and exchangeable cations was very high in the soils of sites 1, 2, 3, and 4, SAR and ESP were very high, with the higher EC_e values in soils at sites 1, 2, 3, and 4 than sites 5, 6, and 7. Considering the EC_e, SAR, and ESP values, soils of sites 1, 2, 3, and 4 were predominantly saline-sodic (EC_e>4, SAR>13 and ESP>15) and soils of sites 5, 6, and 7 with an EC_e value less than 4 dS/m could not be considered as salt-affected based on the classification of the USDA [38].

The number of colony forming units of bacteria were counted from different sampling sites and are shown in Table 2. The bacterial population measured in TSA medium was found to be higher in non-saline soils of sites 5, 6, and 7 (27.3–36.3× 10⁶ CFU/g dry soil) compared with those found in soils of sites with high salinity and sodicity (14.8–

15.7×10⁶ CFU/g dry soil). Higher numbers of halophilic/halotolerant bacteria were obtained from sites 1, 2, and 3 (ranging from 15.3–18.3×10⁶ CFU/g dry soil) whereas sites 5, 6, and 7 had far lower counts (ranging from 3.3–5.3×10⁶ CFU/g air dry soil). The lowest number of bacteria was observed in the soil collected from barren land (site 4) where salinity and sodicity were relatively higher but the lowest organic matter content was observed.

Maximum dehydrogenase activities were observed at site 5, glucosidase and alkaline phosphatase at site 6, and cellulase, acid phosphatase, and protease at site 7, and the lowest for all the enzymes was at site 4 (Table 3). The ranges of activity were from 3–15 mg TPF (kg air dry soil)⁻¹ h⁻¹ for dehydrogenase, 26.4–94 mg glucose (kg air dry soil)⁻¹ h⁻¹ for cellulase, 22.4–73.9 mg tyrosine (kg air dry soil)⁻¹ h⁻¹ for protease, and 5.2–41.7, 130.2–376.2, and 182.5–523.8 mg pNP (kg air dry soil)⁻¹ h⁻¹ for β-glucosidase, alkaline, and acid phosphatase, respectively (Table 3). Overall, enzyme activities were found to be much lower in salt-affected soils compared with non-saline soil. The activities of all enzymes studied were much lower in soils collected from barren land (site 4), compared with the soils collected from the sites where halophytic vegetation was found (Table 3).

Correlation analyses among soil chemical properties and microbial activities indicated several significant trends (Table 4). Soil chemical properties of pH, EC_e, exchangeable Na⁺, SAR, and ESP tended to co-vary (*i.e.*, one property causes changes of another). Briefly, the content of exchangeable Na⁺ revealed significant positive correlations with pH, EC_e, SAR, and ESP, and this result suggests that the content of Na⁺ is responsible for the saline-sodic characteristics of the investigated soils. Soil pH, EC_e, SAR, or ESP and exchangeable Na⁺ content showed significant negative correlations with soil enzyme activities. However, bacterial population showed significant positive correlation with the activities of all enzymes. Similarly, soil organic matter contents significantly and positively correlated with enzymes activity, whereas significant negative correlations were found with pH, EC, SAR, and ESP. Highly significant

Table 3. Enzyme activity of soil samples collected from different sites of the coastal region of the Bay of Incheon, South Korea.

Sampling sites	Dehydrogenase ^a	Cellulase ^b	Glucosidase ^c	Protease ^d	Alkaline phosphatase ^e	Acid phosphatase ^e
Site 1	8.5±0.2 ^b	26.3±1.2 ^d	21.9±0.3 ^c	46.6±2.9 ^{bc}	155.5±9.1 ^{de}	214.3±10.5 ^d
Site 2	8.8±2.3 ^b	32.9±1.6 ^d	19.9±0.9 ^c	41.6±3.3 ^c	206.1±7.2 ^c	257.9±9.9 ^{cd}
Site 3	10.0±0.1 ^{ab}	50.9±1.4 ^c	22.8±0.5 ^c	50.3±2.5 ^{bc}	188.0±7.5 ^{cd}	305.5±12.1 ^c
Site 4	3.0±0.1 ^c	20.3±1.5 ^d	5.1±1.4 ^d	22.3±0.6 ^d	130.2±3.6 ^e	182.5±8.2 ^d
Site 5	15.0±0.5 ^a	70.7±3.9 ^b	36.3±0.8 ^b	55.3±5.1 ^{bc}	354.4±5.5 ^a	476.2±20.6 ^{ab}
Site 6	14.6±0.4 ^a	83.3±4.5 ^{ab}	41.7±0.5 ^a	60.9±0.9 ^{ab}	376.1±7.5 ^a	436.5±9.9 ^b
Site 7	14.4±0.2 ^a	94.0±3.4 ^a	37.8±0.5 ^{ab}	73.9±1.9 ^a	292.9±14.5 ^b	523.8±24.7 ^a

^aDehydrogenase=mg TPF (kg air dry soil)⁻¹ h⁻¹.

^bCellulase=mg glucose (kg air dry soil)⁻¹ h⁻¹.

^cβ-Glucosidase and phosphatase (acid and alkaline)=mg pNP (kg air dry soil)⁻¹ h⁻¹.

^dProtease=mg tyrosine (kg air dry soil)⁻¹ h⁻¹.

*Values (mean±SD, n=3) with the same letters are not significantly different at 0.05% (DMRT).

Table 4. Pearson's correlation coefficients among physicochemical and biological properties of coastal soil.

	Cellulase	Protease	Alkaline phosphatase	Acid phosphatase	Glucosidase	Organic matter	pH	Electrical conductivity	Exchangeable Na ⁺	log CFU (g soil) ^{-1a}	Sodium adsorption ratio	Exchangeable sodium percentage
Dehydrogenase	0.91**	0.92**	0.91**	0.93**	0.98**	0.90**	-0.81**	-0.86**	-0.84**	0.92**	-0.76**	-0.96**
Cellulase		0.91**	0.87**	0.97**	0.92**	0.89**	-0.94**	-0.93**	-0.94**	0.73*	-0.89**	-0.92**
Protease			0.73*	0.89**	0.92**	0.92**	-0.86**	-0.77**	-0.75*	0.89**	-0.67*	-0.96**
Alkaline phosphatase				0.89**	0.92**	0.73*	-0.77**	-0.86**	-0.93**	0.72*	-0.81**	-0.83**
Acid phosphatase					0.91**	0.86**	-0.95**	-0.93**	-0.95**	0.76**	-0.88**	-0.90**
Glucosidase						0.87**	-0.81**	-0.83**	-0.85**	0.89**	-0.74*	-0.97**
Organic matter							-0.75**	-0.89**	-0.72*	0.86**	-0.79**	-0.83**
pH								0.83**	0.92**	-0.63	0.78**	0.81**
Electrical conductivity									0.90**	-0.67	0.97**	0.82**
Exchangeable Na ⁺										-0.60	0.88**	0.79**
log CFU (g soil) ⁻¹											-0.02	-0.51
Sodium adsorption ratio												0.76*

^aBacterial population used in the correlation study was counted in tryptic soy agar (TSA) media.

*and ** indicate significant at the 5% and 1% level of probability, respectively.

positive correlation coefficients were obtained in the correlation matrix among all enzymes activities studied.

Soils of the coastal region of the Bay of Yellow Sea exhibited large variations in pH (site wise) and in EC_e (location wise) (Table 1). Such wide variations in soil pH and EC have also been found among the sampling sites and even between the seasons in coastal regions [2, 36, 37], but the variation found in this study could be due to the distance of soil sampling sites from the coastline. Soil pH and EC_e were significantly higher at the sampling sites 1, 2, and 3 nearest to the coastline and at site 4 about 500 m away from the coastline, respectively. Soils collected from sites 5, 6, and 7 about 1.5–2 km away from the coastline are considered to be not substantially affected by the intrusion and spray of seawater. Soil organic matter content in the coastal region was relatively lower compared with those found in saline-sodic cultivated fields [27, 36, 37]. The lowest organic matter content found in the soil from barren land could be due to fact that soil organic matter is created mostly by the cycling of organic compounds of plants (Table 1).

Soils of sites 1, 2, 3, and 4 revealed EC_e values much higher than the saline soil criteria of 4 dS/m, and SAR and ESP were also greater than the criteria of sodic soil (*i.e.*, SAR, >13; ESP, >15) [38], whereas soils of sites 5, 6, and 7 showed normal soil properties. In salt-affected coastal soils, Na⁺ is present in the highest amount among cations

and Cl⁻ is the dominant one among the anions [4, 36]. The saline-sodic condition of the soils of sites 1, 2, 3, and 4 are quite extreme compared with the conditions where detrimental influences of salinity/sodicity on biological and biochemical activities are commonly found, such as in irrigation-induced saline soil [27] and natural saline soil under rice cultivation nearby the coastal region of the Bay of Bengal [36, 37].

Bacterial population (mesophilic) was higher in soils of non-saline sites compared with sites with high salinity and sodicity (Table 2). Increased salinity and sodicity becomes increasingly detrimental to the microbial community, as demonstrated by the decline in bacterial population [9, 27, 28, 36]. On the other hand, the halophilic/halotolerant bacterial population measured in a TSA medium containing 10% NaCl was higher in saline than in non-saline soils (Table 2). This result indicates that there are bacterial populations that require salt to grow (halophilic) and are adapted to saline-sodic soil condition or can tolerate (halotolerant/facultative halophilic) the extreme condition. Obligate halophiles only develop in media with higher concentrations of Na⁺, whereas the facultative halophiles/halotolerant bacteria usually develop in media containing a wide range of NaCl as well as in its absence too [19, 21]. In barren land soil (site 4), the fewest number of bacterial population was measured in media of both TSA and TSA + 10% NaCl, and such lower bacterial population could be due to the low organic matter content in the soil.

The presence of soil organic matter usually leads to an increase in the number and types of microorganisms. Hence, in addition to salinity, organic matter content is also an important factor that determines the biological characteristics of saline soils [22, 26, 33, 41].

Enzyme activities were significantly lower for soils collected from sites 1, 2, and 3 nearest to the coastline than sites 5, 6, and 7 that were 1.5–2 km away from coastline. Similar to earlier studies, in the coastal region [36, 37, 40, 41], where saline-sodic characteristics of soils were induced mostly by the seawater intrusion and aerosol spray, the high concentration of Na^+ and Cl^- in soil-solution phase could be one of the most important factors in the inhibition of enzyme activities [12]. Comparing the salt-affected soils, barren land (site 4) soil showed much lower enzyme activities than soils of natural habitats of halophytic plants (Table 3). Considering the ameliorative influence of organic matter on biological activity in salt-affected soils [28], the further inhibition of enzyme activities in the barren land soil might be due to the lower bacterial population and activity under the limited carbonaceous substrates and Na^+ toxicity.

Correlation matrix analyses showed significant positive correlations between the activities of different enzymes, number of bacterial population, and organic matter content, indicating that an increase in soil enzymatic activity is a consequence of increased OM content, and microbial population and activity. Although the halotolerant bacterial population size was higher in saline-sodic soils compared with non-saline soils, even the extracellular hydrolytic enzyme activity was lower. Significant negative correlations were also found between enzyme activities and EC, SAR, or ESP (Table 4). In addition, our result disagrees with earlier reports that alkaline phosphatase activity was predominant in neutral or alkaline soils [8, 11]. Such reduced enzyme activities in salt-affected soils may be due to the osmotic desiccation of microbial cells, modification of the ionic conformation of the active site of enzyme–protein, specific ion toxicities causing nutritional imbalances for microbial growth and subsequent enzyme synthesis, and limitation of carbonaceous substrates [12, 40].

In conclusion, the results of this study revealed that both the salinity and sodicity properties of coastal soil vary with distance from coastline and determine the degrees of inhibition of microbial activity and biochemical processes that are fundamental in maintaining ecological quality and productivity in soils of coastal regions.

Acknowledgments

This study was carried out with the support of the Mid-career Researcher Program through NRF grant funded by the MEST (No. 2010-0000418), Republic of Korea. M. A.

Siddikee acknowledges the Brain Korea21 (BK21) for awarding a PhD fellowship.

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