

The Effect of Humic Acid on Soil Physicochemical and Biological Properties under Salinity Stress Conditions in Pot Culture of Satureja khuzistanica Jamzad

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ABSTRACT

Aims High salt accumulation has severe adverse effects on soil characteristics. Humic acid can improve the soil structure, soil microbial communities, and absorption and maintenance of mineral nutrients. The aim of the present study was to determine the effects of humic acid on some physicochemical and biological soil properties in soils under salt stress.

Materials & Methods The experiment was conducted as a factorial based on RCBD design with four replications. The first factor included humic acid in five levels (zero, 10, 20, 30, and 40mg kg⁻¹ soil). The second factor was salinity stress in five levels (0, 25, 50, 75, and 100mM NaCl). The sampling was carried out in two stages, before and after harvest.

Findings The results showed that S1H5 treatment had the lowest soil electrical conductivity (EC), soil reaction (pH), bulk density, and population of actinomycetes with average values of 0.26dS m⁻¹, 6.21, 1.12g cm⁻³, and 516cell g⁻¹ of soil and had the highest fungal and bacterial population with an average of 1525000 and 137500000cell g⁻¹ of soil, respectively.

Conclusion Salt stress has a significant effect on physicochemical and biological soil properties except for the population of actinomyces that their activity was better, at a high level of salinity stress, it had adverse effects on other properties. Although using humic acid improved soil properties. According to the results, using humic acid can be a good solution to reduce the adverse effects of salt stress.

Keywords Satureja; Soil Salinity; Humic; Cation; Actinomycetes

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Introduction

Ionic toxicity (soil salinity) is a major environmental factor limiting agricultural and environmental production worldwide. High salt accumulation has severe adverse effects on physical and chemical properties of soil, microbiological processes, plant growth, and in general, on soil fertility [1]. In fact, salinity and sodium content are key factors in limiting access to nutrients and crop production. Physical and chemical properties of the soil, such as soil structure, water content, pH, microorganismś activity, nutrient concentration, soil organic matter, etc., which support plant growth, are significantly affected by the presence of soil ionic salts [2]. In this regard, Tejada and Gonzalez [3] showed that increased electrical conductivity has adverse effects on the stability and stability of soil, density and soil permeability. Increasing salt destroys soil aggregate and microspores and modifies soil porosity. Moreover, the soil bulk density is increased due to increasing microspores, thereby all of these changes instable the soil structure and destroy it [4]. Usually, in soil with a high level of salinity, the amount of soil organic matter is reduced, and thereby microbial activity will be limited [5].

One of the management methods for improving soil properties under adverse conditions, such as salinity and sodium content, is to increase soil organic matter [6]. Organic matter can affect the physical, chemical, and biological properties of soil positively. Humus, a common organic matter in soil, provides a better nutrient (N, P, S, and K) cycling in soil, resulting in soil conservation, environmental aspects, and soil fertility [7].

Various organic compounds such as manure, green manure, compost, etc. have been widely used in various studies to meet the organic material requirements of the soil. Humic acid (HA) is an active ingredient in humus and can play an important role in soil conditioning and plant growth. This combination physically improves the soil structure and increases the water holding capacity. Biologically, development of soil microbial communities is improved and is chemically enriched as an absorption and maintenance complex for mineral nutrients [8]. Humic material (HS) is a major component of soil organic matter. It is used in various agricultural fields such as soil chemistry, fertility, plant physiology, and biotechnology. Humic acid also affects the solubility of many nutrient elements by making complex forms or chemical agents of homocysteine with metal cathode [9].

Humic materials can absorb most of the metals in the soil and thus, make them more accessible to plants. Several studies have shown that plant growth, nutrient concentrations and nutrient uptake by soil have increased significantly by humic materials [10]. using Adding homochemicals to the soil leads to the exchange of cations with H+ with soil colloids, which reduces soil pH. Increasing cationic and anionic interactions reduces aggregation and adhesion of soil particles and increases soil porosity, thereby affecting the flow of water and nutrients[11].

Assumption: Humic acid has not any effect on physicochemical and biological properties in salt stress conditions.

The purpose of the current study was to investigate the effect of humic acid on physicochemical properties and soil biology of pot culture of *Satureja khuzestanica* under salinity stress conditions.

Materials and Methods

The present study was conducted in 2017 at greenhouse of Lorestan University, Iran. The experiment was done as a factorial based on randomized complete block design (RCBD) with four replications. The first factor included humic acid in five levels (0, 10, 20, 30, and 40mg/kg soil) that was applied to the soil before plantation [12]. The second factor was salinity stress in five levels (0, 25, 50, 75, and 100mM NaCl) which was used after sprouting [13] and establishment of the plants during the fourth leaf stage. The levels of the factors are shown in Table 1. The required amounts of humic acid for pots were counted and mixed with the soil before sowing.

Table 1) Different levels of humic acid and salinity used in the present study

Levels	Abbreviation				
Humic acid treatments (mg/kg soil)					
0	H1				
10	H2				
20	Н3				
30	H4				
40	Н5				
Salinity treatments (mM NaCl)					
0	S1				
25	S2				
50	S3				
75	S4				
100	S5				

The used plastic pots were 4kg in volume. The seeds of Satureja khuzistanica Jamzad were sterilized with sodium hypochlorite 10% for three minutes and then, washed by distilled water [14].

To determine the physical, chemical, and biological characteristics of the soil, before planting, soil sampling was carried out and the specimen was transferred to the lab for determination of soil properties (Table 2). This sampling was done in two stages before and after harvest to study the changes and effects of humic acid and salinity stress on the physicochemical and biological properties of soil. The core sampling by auger was used to measure the soil bulk density [15]. The soil specific gravity was determined 2.65g cm⁻³. The soil porosity was calculated by the following equation [16]:

Soil porosity percentage= $1 - \frac{\text{bulk density}}{\text{Particle density}} \times 100$

Soil acidity was measured in saturated flower conditions using a pH meter and electrical conductivity using an electrical conductivity meter in an impregnated extract [17]. The cation exchange capacity (CEC), by sodium acetate [18], soil organic matter by the Wakefield method [19], and the measurement of soil microbial biomass such as bacteria, fungi by the method of Wollum and Kucey [20, 21] and actinomycetes by the method of Alef and Nanipuri [22] were studied. Finally, analysis of variance of data was done using SAS 9.4 software. Also, post hoc analysis was carried out using Duncan's multiple range test at 5% probability level.

Table 2) Soil characteristics before applying the treatments (humic acid and salinity)

Soil characteristics					
Population of actinomycetes	500				
(cell g-1 of soil)					
Bacteria population (cell g-1 of soil)	950000000				
Fungi population (cell g-1 of soil)	1000000				
Cationic exchange capacity (CEC)	11.30				
Porosity (%)	49				
Bulk density (g cm ⁻³)	1.28				
Organic matter (%)	1.78				
Electrical conductivity (ds m ⁻¹)	0.38				
рН	7.02				
Soil texture	Sandy clay loam				

Findings and Discussion Chemical properties Percentage of soil organic matter

ANOVA results on the percentage of soil organic matter (Table 3) and results obtained from comparing the means of interactive effects (Diagram 1) indicated that the highest percentage of soil organic matter was observed in S1H5 treatment with an average of 2.98% and its lowest soil organic matter was seen in S5H1 with an average of 1.58%.

Research has shown that the organic carbon of saline soil is reduced by two ways including reduction of plant growth and, consequently, decreasing the entry of plant residues into the soil and reduced organic matter degradation in the soil [23]. It has been shown that the organic carbon content of the soil is one of the most important chemical indicators of soil quality for soil retrieval and fertility. So, it can make important changes in the biological, chemical, and physical properties of the soil [24]. Humic acid (HA) is a major component of the soil organic matter, which, through its various functions in the soil, affects many soil parameters including the solubility of many nutrients by making complex forms with chemical agents of humic materials [9]. To reduce the salinity stress, new methods such as the use of soil microorganisms, humic acid and fulvic acid, algae and plant extracts can be used. Owing to its specific structure, humic acid has the potential to reduce and neutralize soil salinity [6]. Electrical conductivity (EC) and soil reaction

(pH)

There is a significant interaction between the factors in terms of two traits of electrical conductivity and soil reaction at a 1% level (Table 3). The results showed that the highest electrical conductivity (EC) and soil reaction (pH) were with a mean of 2.6ds m⁻¹ and 7.37 at S5H1 and the lowest of these traits at S1H5 level, 0.26ds m⁻¹ and 6.21 was observed (Table 4). EC values are closely related to soil pH. High amounts of EC soil are affected by the high contents of Na, K, Ca, and Mg of soil. The addition of humic acid to soil leads to the exchange of cationic H+ with soil colloids, where its position is replaced by cationic salts and then decreases the salt concentration in the soil solution to reduce the amount of EC. Humic acid, in addition to EC change caused by cation exchange H+ and replacement with single and double-cationic cations, leads to a decrease in soil pH. The cation exchange capacity of the soil is highly dependent on the organic matter content. A higher organic matter will result in a decrease in pH [11, 25]. It has reported that the humic acid (400mg kg-1 soil) and reduced soil рН cathodic significantly [25].

Table 3) Analysis of variance (mean squares) of physical, chemical, and biological traits of soil under the salinity and humic acid factors

Traits	Rep	Salinity (S)	Humic acid (H)	S×H	CV (%)
Electrical conductivity	0.023	8.59**	1.19**	0.09**	6.69
Organic matter (%)	0.06	0.48*	1.68**	0.06ns	17.75
рН	0.32	0.32**	0.83**	0.17**	3.35
Porosity (%)	413.6	646.19**	65.94**	19.39**	6.5
Bulk density	0.28	0.43**	0.045**	0.013**	5.54
CEC	0.032	3.43**	3.16**	0.12**	1.22
Population of soil fungi	4955666666.7	3.4277465e12**	162151000000**	32575250000**	3.53
Population of soil bacteria	2.5476667e13	4.32039e15**	1.00554e15**	1.151275e14**	3.92
Population of actinomycetes	22.6	125163**	37083**	12350**	1.78

ns, * and ** represent non-significant, significant at 5%, and 1% respectively.

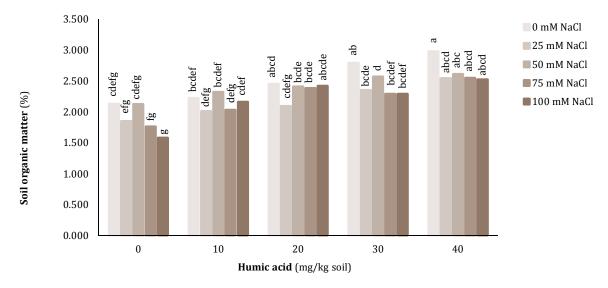


Diagram 1) Percentage of soil organic matter under different combination levels of the salinity and humic acid; Means with at least one common letter have not significant differences based on Duncan's multiple range test (p≤0.05)

Table 4) Mean comparison of soil traits under the combination of salinity and humic acid

Salinity×humic (S×H)	Soil actinomycetes population (cell g ⁻¹ of soil)	Soil bacteria population (cell g ⁻¹ of soil)	Soil fungi population (cell g-1 of soil)	CEC (meq/100g soil)	Bulk density (g cm ⁻³)	Porosity (%)	pН	Electrical conductivity (ds m ⁻¹)
S1H1	518 ⁿ	102500000ef	1025000e	11.32gf	1.31 ^{ijkl}	49.65 ^{cdef}	7.01abc	0.41 ⁿ
S1H2	585jk	107500000cde	1150000 ^d	12.03c	1.27^{jklm}	50.89bcde	6.98bcde	0.33no
S1H3	567 ¹	112500000c	1425000b	12.14bc	1.24^{lm}	52.47bc	6.88bcdefg	0.32no
S1H4	545 ^m	125000000b	1450000b	12.32b	1.18^{nm}	54.61ab	6.68efghij	0.31no
S1H5	516 ⁿ	137500000a	1525000a	12.67a	1.12 ⁿ	56.76a	6.21k	0.26°
S2H1	655 ^f	95750000hij	905000 ^f	10.88ijk	1.37ghij	47.15^{efgh}	6.94bcdef	1.64e
S2H2	610 ^{hi}	97750000fgh	922500 ^f	10.95 ^{ij}	1.34hijkl	48.45 ^{cdefg}	6.88cdefg	1.24i
S2H3	662 ^f	102500000ef	1025000e	11.04hi	1.28^{jklm}	50.55bcde	6.84 ^{defgh}	1.09^{kl}
S2H4	695e	110000000cd	1125000 ^d	11.29gf	1.26 ^{klm}	51.61 ^{bcd}	6.62ghij	1.07^{kl}
S2H5	525 ⁿ	112500000c	1275000c	11.60 ^{de}	1.25 ^{klm}	51.99bcd	6.53hij	0.93 ^m
S3H1	762c	85750000jkl	610000h	10.75klm	1.48cdef	42.81 ^{ijkl}	7.05abcd	1.47gh
S3H2	705e	90750000ij	622500h	11.61 ^{de}	1.46^{defg}	43.85hijk	6.68efghij	1.21 ^{ij}
S3H3	737 ^d	94750000ghi	672500g	11.63 ^{de}	1.42^{efgh}	45.33ghij	6.44 ^{ijk}	1.13 ^{ijk}
S3H4	702e	105000000de	687500g	11.75d	1.35^{hijk}	48.03^{defgh}	6.37^{jk}	1.01 ^{klm}
S3H5	652 ^f	98750000fg	707500g	12.02c	1.34^{hijkl}	48.23 ^{defg}	6.59ghij	0.99lm
S4H1	782 ^b	81000000^{lmno}	425000^{k}	10.59lm	1.54bcd	$40.55^{\rm klm}$	7.1ab	1.99 ^d
S4H2	635g	84750000klm	442500k	10.76 ^{jkl}	1.52bcde	41.34^{jklm}	6.98bcde	1.59ef
S4H3	617h	93250000^{hi}	455000jk	11.53e	1.49cdef	42.53 ^{ijkl}	6.69efghij	1.38h
S4H4	595 ^{ij}	87000000jk	482500 ^{ij}	11.59 ^{de}	1.43^{efgh}	44.95ghij	6.65fghij	1.38h
S4H5	570 ^{kl}	98250000fgh	502500i	11.61 ^{de}	$1.40^{\rm fghi}$	$46.14^{\rm fghi}$	6.6ghij	1.10^{jkl}
S5H1	837a	78750000 ^{nop}	$300000^{\rm m}$	10.57 ^m	1.75a	32.74 ⁿ	7.37a	2.6a
S5H2	822a	74500000p	302500 ^m	11.04 ^{hi}	1.57bc	39.48lm	7.19ab	2.35b
S5H3	767bc	76250000°p	322500lm	11.16gh	1.54bcd	40.54^{klm}	6.85 ^{cdefg}	2.22c
S5H4	652 ^f	79750000mnop	322500^{lm}	11.25g	1.52^{bcde}	41.41^{jklm}	6.81^{defgh}	2.04 ^d
S5H5	620gh	82250000klmn	350000 ¹	11.48ef	1.65 ^b	37.80 ^m	6.78 ^{defgh}	1.51^{fg}

Means with at least one common letter have not significant differences based on Duncan's multiple range test (p≤0.05).

Soil cation exchange capacity (CEC)

The cation exchange capacity of the soil also accepted a significant interaction effect of salinity and humic acid at a 1% level (Table 3). The results showed that the highest amount of exchangeable soil capacity was observed at S1H5 level with 12.67meq/100g and the lowest value of this trait was 10.57% in the S5H1 treatment (Table 4). Narimani and Manafi [26] indicated that the cation exchange capacity of the soils has a very clear relationship with the amount of clay and organic substances. Soils with the highest organic carbon had the highest CEC value and the soils with the least organic carbon had the lowest CEC value.

The higher the dose of humic acid, the higher the CEC value. This was because of the addition of cations will increase them in the mineral surface and between minerals. Colloids do not only adsorbed ions but also absorbed water, so that increased water reserves. HA absorbs more than absorbents used to date [11].

According to Amlinger *et al.* [27], soil organic matter contributes about 20-70% of the CEC for many soils. In absolute terms, CEC of organic matter varies from 300 to 1,400cmol kg-1 soil being much higher than CEC of any inorganic material. These results are in agreement with studies of Agegnehu *et al.* [28], Abdel-Rahman [29] and Mohammad *et al.* [30], who said that compost amendment resulted in an increase of CEC due to input of stabilized OM being rich in functional groups into soil. Similar results were obtained from Dadhich *et al.* [31], who stated that the application of farmyard manure significantly increased the organic carbon and CEC of the soil.

Physical properties

Bulk density and soil porosity

Significant interactions between acidity and salinity levels were observed for bulk density and soil porosity (p≤0.01). Among combination treatments, S1H5 treatment had a higher mean porosity of 56.76%, which was associated with a low soil bulk density with a mean of 1.12g cm⁻³ (Table 4). The lowest soil porosity and hence the highest bulk density were seen under conditions of high salinity and low levels of soil humic acid, S5H1, with a mean of 32.74% and 1.75g cm⁻³ (Table 4). Adding homochemicals to the soil leads to the exchange of cations with H+ with soil colloids, which reduces soil pH. The high cationic and anionic interactions cause the accumulation and condensation of soil particles to decrease and

the porosity of the soil increases significantly. Soil porosity changes affect the flow of water and soil nutrients. Humic acid can increase the stability of aggregates [11].

Mousa [32] showed that concerning the effect of the applied treatments on soil bulk density and hydraulic conductivity values, their values decreased by increasing the application rates of all the applied conditioners. The highest decreases of such values were associated with the treatment (20kg humic acids/fed) as the rate of decreases below the control under recent application reached 24.83 and 42.42%. respectively, while the respective values under residual application reached 12.08 and 50.00%. Such decreases in soil bulk density can be attributed to the low specific gravity of organic materials and the role of organic products in enhancing soil aggregation which increases the apparent soil volume and consequently decrease bulk density.

Tranter *et al.* [33] reported that soil organic carbon affects multiple soil physical properties and increase in soil organic carbon concentration decreases bulk density.

These results are in agreement with those of Vengadaramana *et al.* [34]. Researchers showed that the values of total soil porosity were increased in soil treated with humic acid at any rate compared to control where the highest value was found in the treatment of humic acid high rate of 2400ml/400L water (T4) with El-Salam canal compared to Baher Hados drain [35]. Similar results have been obtained by Oo *et al.* [36] who reported that the use of organic amendments resulted in substantial flocculation and the formation of a large number of soil aggregates.

Also, about bulk density, some researchers showed that the values of soil bulk density of soil profiles treated by humic acid at any rates were relatively lower than those of control, and the maximum decrease exists in case of humic acid high rate of 2400ml/400L water (T4) with El-Salam canal or Baher Hados drain compared to other treatments and control [35]. These results are confirmed with the results of Amlinger et al. [27], who observed that compost application influences soil structure in a beneficial way by lowering soil density as a result for the admixture of low density organic matter into the mineral soil fraction. This positive effect has been detected in most cases and it is typically associated with an increase in porosity because

of the interactions between organic and inorganic fractions. Besides, the organic fraction is much lighter in weight than the mineral fraction in soils. Accordingly, the increase in the organic fraction decreases the total weight and bulk density of the soil [37].

Biological properties

The microbial biomass is the agent for decomposing plant residues and the release of nutrients in the soil, available to uptake by growing plants. Microbial biomass is one of the most important features and indices in the protection of the soil and important ecosystem processes such as carbon cycle, nutrient cycle and bioengineering [38].

The results of the current study showed that microbial communities of the soil were also affected by the interaction of salinity and humic acid at 1% level (Table 3). Soil fungal population in S1H5 treatment with the highest activity was 1525000cells g-1 of soil. This activity was the lowest in S5H1 treatment with an average of 300000cells g-1 of soil, although there was no significant difference with S1H2, S5H3, and S5H4 treatments (Table 4).

The highest soil bacterial population was observed in S1H5 treatment with an average of 137500000cells g-1 of soil and its lowest population was S5H2 with an average of 7450000cells g-1 of soil, although there was no significant difference with S1H1, S5H3, and S5H4 treatments (Table 4). Soil microorganisms play an important role in nutrient cycling and soil fertility by mineralizing, solubilizing, and stabilizing substances. Salinity can cause stress for soil microorganisms or even killing them [39]. Many researchers report the suppression of soil bacterial activity under salinity [40, 41]. Some studies have shown that, under salinity stress, the ratio of bacteria to fungi has increased significantly because of the higher sensitivity of fungi compared to bacteria to the salinity of water and soil [42].

The soil actinomycetes were also affected by acidic and salinity effects (Table 4). The highest number of actinomycetes (837cells g-1 of soil) was observed in S5H1 treatment, although there was no significant difference with S5H2 treatment. The lowest population of these microorganisms was seen in S1H5 treatment with a mean of 516cells g-1 of soil, which was not significantly different with S1H1 treatment.

A study on the flora of soil fungi showed that one of the reasons for the fungal growth restriction

in the samples was high electrical conductivity. The electrical conductivity of the solution is an indicator of the total dissolved solids. As the concentration of anions and cations of a solution increases, its electrical conductivity increases, too [43], and this can be a factor creating the fungal growth restriction. Increased salt concentrations increase the osmolarity outside the microbial cell, lowering the microbial activity. As Na+ accumulates in soils, soil structure would be destabilized and osmotic and specific ion effects would be induced. Thus, the microbial diversity and abundance negatively affected in soils with a pH>7 and high Na+ levels^[44].

Rousk *et al.* [45] found that the short term growth rate of bacteria decreased with increasing EC irrespective of the EC of the soil they were extracted from.

Comparison of four actinomycetes strains under different soil pH and salinity by Ameerah *et al.* [46] has shown that all of the strains had optimum growth at pH= 7 and salinity of 3%. Moreover, in another study on five actinomycetes strains, the highest growth rate was related to pH higher than 7 and salinity of 0.5, 3, and 5% [47]. In the present study, although actinomycetes were able to grow without salt, they generally showed the lowest growth in non-salt conditions, and the reason for this may be the importance of Na⁺ in the membrane transport system to maintain intracellular cell potassium at optimum concentration [48].

The results are in line with those of the researchers, and it was found that fungi and bacteria are increased with increasing organic matter content and are decreased with high salinity without humic acid. Also, humic acid was able to control the effects of high salinity and prevents a large decline in the population of fungi and bacteria in high salinity conditions. But for actinomycetes, the population of this group of microorganisms was much higher in saline conditions, and this is due to the greater need for these microorganisms to salinity, which is consistent with the research reported. When more humic acid is used, it reduces the population of actinomycetes due to improved soil structure and reduced soil salinity.

Conclusion

Salinity, one of the main environmental stress in many parts of the world, has a negative effect on the physical, chemical, and biological properties of the soil. Salinity stress has a negative effect on microbial activity by increasing the electrical conductivity of the soil, so, the microbial activity will have a negative trend. Based on the results of the current study, it can be said that the use of organic matter such as humic acid, can reduce salinity stress due to its properties and its effects on the soil. Also, a higher amount of humic acid will reduce the adverse effects of salinity efficiently.

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author/Methodologist/Original

researcher/Discussion author (35%); Naser Akbari (Second author), Introduction author/Discussion author (15%); Hamid Reza Eisvand (Third author), Introduction author/Methodologist/Discussion author (30%); Ahmad Ismaili (Fourth author), Methodologist/Statistical analyst (10%); Mohamad Feizian (fifth author), Introduction author/Discussion author (10%)

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