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Fermentative activity and humic acid composition of saline and non-saline soils in Uzbekistan

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Abstract

Soil salinization is one of the critical environmental problems that negatively affects soil microbial activity and enzymatic processes. This study aimed to evaluate the enzymatic activity and humic acid composition of soils from two contrasting regions: non-saline soils of Chinaz district (Tashkent region) and the saline soils of Nishon district (Kashkadarya region). Soil samples (0–30 cm) were analyzed for enzymatic activity (polyphenol oxidase, peroxidase, and phosphatase), while humic acids isolated from the 0–10 cm layer were studied for amino acid and microelement composition. The results showed that saline soils had higher concentrations of soluble salts (Cl^-, SO_4^{2-}, Ca^{2+}, Mg^{2+}), reflecting increased alkalinity and salinity, whereas non-saline soils contained lower salt levels. Enzymatic activity was significantly higher in non-saline soils, with notable seasonal variations, while saline soils showed a pronounced decline in enzyme activity. Amino acid content in humic acids of non-saline soils (531.77 ± 1.75 mg g^{-1} was about 1.5 times higher compared to saline soils (348.80 ± 2.04 mg g^{-1} [1]). In contrast, iron concentration in humic acids was higher in saline soils (853.37 ± 1.15 mg g^{-1}) than in non-saline soils (545.27 ± 1.19 mg g^{-1}). These findings suggest that both enzymatic activity and humic acid composition can serve as reliable biochemical indicators for assessing soil quality and the impact of salinization.

Keywords: Soil, humic acids, polyphenol oxidase, peroxidase, phosphatase, amino acids, microelements, Uzbek soil

Introduction

Soil fertility and the enhancement of its biological activity are of great importance in agriculture. Soil microorganisms and their enzymatic activities play a crucial role in maintaining soil productivity. Their activity serves as a key factor in the decomposition of organic matter, the mobilization of nutrient elements, and the mineral nutrition of plants (Ahmed & Al-Mutairi, 2022; Divya *et al.,* 2023).

However, soil salinization and degradation have emerged as some of the most serious environmental challenges in global agriculture. Salinity reduces both the abundance and activity of soil microorganisms, which in turn negatively affects enzymatic processes. Enzymes such as polyphenol oxidase, peroxidase, and phosphatase participate in the mineralization of organic matter, the cycling of nutrients, and the detoxification of various pollutants in the soil (Kozlov *et al.,* 2017).

Humus and its structural components—humic and fulvic acids—are among the key substances that ensure soil fertility. In particular, humic acids are considered important indicators of the biological state and nutrient balance of soils. The contents of amino acids and microelements within them vary significantly depending on soil conditions (Ivanov *et al.,* 2019).

The agrochemical characteristics of soil encompass parameters that determine its fertility, plant growth, and the potential for efficient crop production (Turaeva *et al.,* 2017; Karimov *et al.,* 2024;Azimova *et al.,* 2024).

Currently, the activity of nearly 60 soil enzymes has been identified. These enzymes participate in matter and energy exchange and contribute to restoring biological

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stability in natural ecosystems disturbed by human activity. Enzymes not only provide essential nutrients for plants but also possess the ability to detoxify various pollutants (Sholihah *et al.,* 2023). Through the analysis of soil enzymatic activity, it is possible to assess soil fertility, monitor contamination by heavy metals and petroleum products, and evaluate technogenic salinization processes and numerous biochemical reactions. Enzyme activity also reflects the formation of humus, the mineralization of organic residues, nitrification, and the biological fixation of molecular nitrogen.

The intensity of enzymatic processes depends on several environmental conditions, including substrate concentration, pH value, temperature, and moisture. Studying the influence of pH on the biological state of soil has significant ecological importance. Under unfavorable soil conditions, negative changes occur within microbial community structures, resulting in a reduction in the number of microorganisms and, consequently, a decline in overall soil enzymatic activity. In studying the alterations in soil processes caused by natural and anthropogenic factors, special attention is paid to oxidation–reduction enzymes (such as polyphenol oxidase and peroxidase) and hydrolytic enzymes (such as invertase and urease).

The transformation of insoluble inorganic phosphorus into plant-available forms is carried out by phosphate-mobilizing microorganisms belonging to the genera *Rahnella*, *Pseudomonas*, *Bacillus*, *Enterobacter*, *Pantoea*, and *Trichoderma* (Saeed *et al.,*2021, Shavkiev *et al.,* 2022;). Another important property of these phosphate-mobilizing microorganisms is their ability to produce the exogenous acid phosphatase enzyme, which hydrolyzes phosphate-containing organic compounds and enriches the soil with available phosphorus (Azene *et al.,* 2023).

Phosphatase enzymes are present in all forms of flora and fauna, although their activity levels vary among different plant and animal tissues, as well as among microorganisms. As a result of phosphatase activity, the cleavage of monoester bonds in phosphorus-containing organic substrates releases phosphate ions and free hydroxyl groups. The production of exogenous, non-specific acid phosphatase by soil microorganisms represents an additional source of available phosphorus for plants (Fitriatin *et al.,* 2024).

Humus is the most essential component of soil and serves as a primary indicator of its fertility. More than 90% of the total nitrogen in the soil is stored within the humus fraction, along with considerable amounts of phosphorus, potassium, calcium, and various microelements, all of which collectively ensure the continuous mineral nutrition of plants (Volkov *et al.,* 2020).

In addition to serving as a nutrient source for plants, humus provides energy for the soil microflora and enhances the mineralization processes of newly formed organic matter (Afanaseva *et al.,* 2021).

The formation of humus in soil results from complex biochemical and chemical transformations involving both organic (proteins, carbohydrates, flavonoids, amino acids, organic acids) and inorganic compounds mediated by microorganisms. These processes include the decomposition and mineralization of organic residues, secondary microbial synthesis, and are strongly influenced by soil moisture and temperature (Canellas *et al.,* 2014).

The residual organic matter in the soil is broken down into simple mobile compounds through microbial activity. A portion of these compounds is assimilated by microorganisms and converted into secondary metabolites, carbon dioxide, and water, while another portion is absorbed by plants. The remaining fraction undergoes further transformation to form humicsubstances — the specific and stable organic component of the soil.

Humus formation in soil is a complex process for which several conceptual theories have been proposed.

-Humification – the polymer condensation theory: according to this concept, humus is formed through the condensation and immobilization of polymeric substances, where organic molecules such as lignin, cellulose, and similar biopolymers undergo adsorption and subsequent transformation into stable, high–molecular–weight compounds.

-The oxidation–carboxylation theory: this model suggests that humification proceeds through several stages, beginning with the oxidation and carboxylation of organic matter (resulting in the formation of organic acids), followed by the fractionation of these compounds into humic and fulvic acid groups, and eventually leading to the complete mineralization of humic acids (Vyalex *et al.,* 2012).

Soil salinization and degradation processes are among the most serious environmental challenges facing global agriculture. Salinity reduces the abundance and activity of soil microorganisms, thereby negatively affecting enzymatic processes and the formation of humic substances. In particular, enzymes such as polyphenol oxidase, peroxidase, and phosphatase are considered reliable indicators for assessing the biological condition and fertility of soils. At the same time, humic acids — as

major components of humus — play a crucial role in maintaining soil fertility through their involvement in the decomposition of organic matter and their association with amino acids and micronutrients.

Although the general characteristics of humic acids and their effects on soil fertility have been widely studied in the literature, the relationship between the composition of humic acids (amino acids and microelements) and enzymatic activity in saline and non-saline soils remains insufficiently understood.

Based on this gap, we hypothesize that soil salinity reduces enzymatic activity and decreases the concentration of amino acids in humic acids.

The novelty of this study lies in the fact that, for the first time, humic acids were isolated in pure form from the soils of Nishon District, Kashkadarya Region (saline), and Chinaz District, Tashkent Region (non-saline). Their amino acid and microelement composition were determined using HPLC and AAS, respectively, and comparatively analyzed in relation to soil enzymatic activity.

Objectives of the study included evaluationof enzymatic activity (polyphenol oxidase, peroxidase, and phosphatase) in saline and non-saline soils; determination of the amino acid and microelement composition of humic acids; and comparative analysis of enzymatic activity and humic acid composition as integrated indicators of soil biological condition.

Materials and Methods

Determination of peroxidase activity

Peroxidase (EC 1.11.1.7) activity in soil samples was determined using a modified colorimetric method. One gram of oven-dried soil (dried at 105 °C for 24 h) was finely ground, sieved, and transferred into a 50 mL conical flask. Subsequently, 10 mL of freshly prepared 1% hydroquinone solution and 1 mL of 0.05% hydrogen peroxide (H_2O_2) solution were added, and the mixture was thoroughly shaken.

The flasks were incubated at 30°C for 30 minutes in a thermostatic chamber. Control samples containing only hydroquinone and H_2O_2 (without soil) were prepared in parallel. After incubation, 10 mL of ethanol was added to each flask, mixed thoroughly, and the resulting mixture was filtered or centrifuged to obtain a clear supernatant.

The yellow-colored filtrate was measured spectrophotometrically at 440 nm, and the amount of oxidized product was quantified using a calibration curve prepared from standard p-benzoquinone solutions ($R^2 \geq 0.99$). Enzyme activity was expressed as mg of product formed per gram of oven-dry soil per hour.

All assays were performed in triplicate (three biological and three technicalreplicates), and the results were expressed as mean ± standard deviation (SD).

Determination of polyphenol oxidase activity

Polyphenol oxidase (EC 1.10.3.1) activity was determined using hydroquinone as a substrate. In the presence of atmospheric oxygen, polyphenol oxidase in soil oxidizes hydroquinone to form a yellow-colored product, 1,4-p-benzoquinone, which was quantified spectrophotometrically at 440 nm (Khaziev, 2005).

The procedure for determining polyphenol oxidase activity was similar to that used for peroxidase, except that hydrogen peroxide (H_2O_2) was not added to the reaction mixture. The amount of 1,4-p-benzoquinone formed was calculated using a calibration curve prepared from standard p-benzoquinone solutions ($R^2 \geq 0.99$).

Enzyme activity was expressed as mg of product formed per gram of oven-dry soil per hour. All analyses were carried out in triplicate (three biological and three technical replicates), and results were reported as mean ± standard deviation (SD).

Determination of acid phosphatase activity

Acid phosphatase (EC 3.1.3.2) activity was determined using p-nitrophenyl phosphate(pNPP) as the substrate. One gram of oven-dry soil sample was moistened with 2% glucose solution to 60% water-holding capacity and incubated at 28 °C for 24 h. After incubation, 1 mL of 50 mM sodium acetate buffer (pH 5.0) was added to the soil sample.

The reaction mixture consisted of the following components:

-3.0 mL sodium acetate buffer (500 mM, pH 5.0),

-30 µL p-nitrophenyl phosphate (65 mM),

-0.1 mL magnesium chloride ($MgCl_2$, 10 mM).

The enzymatic reaction was initiated by adding 0.2 mL of soil extract to the reaction mixture and incubating at 37 °C for 15 min. The reaction was terminated by adding 0.5 mL of 1 M NaOH, and the amount of released p-nitrophenolwas measured spectrophotometrically at 405 nm. Control samples (without enzyme addition) were included for background correction.

The enzyme activity was calculated from a calibration curve prepared using standard p-nitrophenol solutions ($R^2 \geq 0.99$) and expressed as μmol p-nitrophenol g^{-1} oven-dry soil h^{-1}. All measurements were performed in triplicate (three biological and three technical replicates), and the results are presented as mean ± standard deviation (SD).

Extraction of humic acids

Humic acids were extracted following the International Humic Substances Society (IHSS) protocol (Swift, 1996) with minor modifications. Soil samples were collected from 0–10 cm depth in two regions: non-saline cotton monoculture soils from the Chinaz district (Tashkent region) and saline cotton monoculture soils from the Nishon district (Kashkadarya region). Three biological replicates were collected from each site.

For extraction, 100 g of air-dried soil was treated with 400 mL of 0.1 N HCl to release materials bound to calcium ions (Ca^{2+}). The acid-treated soil was then washed twice with distilled water. Subsequently, 400 mL of 0.1 N NaOH was added to each sample and mixed under a nitrogen atmosphere for 24 hours, allowing humic substances to dissolve in the alkaline medium.

The supernatant was centrifuged at 6000 rpm for 30 minutes to separate the soluble humic fraction. The pH of the supernatant was then adjusted to 2.0 using concentrated HCl to precipitate the humic acids. The precipitate was centrifuged again at 12,000 rpm for 20 minutes, washed three times with distilled water, and purified by dialysis (Molecular Weight Cut-Off (MWCO) 3.5 kDa) to remove residual salts.

The purified humic acids were dried at 35 °C in a vacuum oven and stored for subsequent analysis. Results were expressed on a dry-weight basis (μg g^{-1} g oven-dry soil). All extractions were performed in triplicate.

For compositional analysis, the isolated humic acids were hydrolyzed in 6 N HCl at 110 °C for 24 hours. The resulting hydrolysates were used for amino acid profiling and trace element determination.

Determination of amino acids in humic acids

The amino acid composition of humic acid hydrolysates was determined using high-performance liquid chromatography (HPLC) (Agilent 1200 system, equipped with a diode-array detector, DAD). For hydrolysis, humic acid samples were treated with 6 N HCl at 110 °C for 24 h in Teflon-sealed tubes, followed by drying under vacuum.

Derivatization of amino acids was performed as follows: primary amino acids were reacted with ortho-phthaldialdehyde (OPA), while secondary amino acids were derivatized with 9-fluorenylmethyl chloroformate (FMOC). Norvaline (50 μM) was used as an internal standard.

Chromatographic separation was achieved on a Discovery HS C18 column (75 × 4.6 mm, 5 μm). The mobile phases consisted of (A) 10 mM Na_2HPO_4 and 10 mM $Na_2B_4O_7$ (pH ≈ 8.2) and (B) acetonitrile (ACN). The gradient was linearly increased from 10% to 40% ACN within 15–20 min. The flow rate was 1.0 mL min^{-1}, the injection volume 1–5 μL, and the column temperature was maintained at 30 °C.

Detection was carried out at 338 nm for OPA derivatives and 262 nm for FMOC derivatives. Calibration curves were prepared using amino acid standards in the range of 0.5–50 μM ($R^2 \geq 0.99$). The limit of detection (LOD) and limit of quantification (LOQ) were approximately 0.05 μM and 0.1 μM, respectively. The analytical recovery ranged between 92% and 105%.

All analyses were performed in three biological and three technical replicates. The results were expressed as μmol g^{-1} humic acid (on a dry-weight basis). Chromatographic data were processed using ChemStation software (Agilent Technologies, USA).

Determination of Fe, Mn, Cu, and Zn in humic acids

The concentrations of microelements (Fe, Mn, Cu, and Zn) in humic acid hydrolysates were determined using atomic absorption spectroscopy (AAS) (PerkinElmer AAnalyst 400). For the analysis, 0.5 g of humic acid hydrolysate was placed into a porcelain crucible, and 10 mL of concentrated HNO_3 was added. The mixture was digested in a boiling water bath for 2 hours, followed by the addition of 1–2 mL of H_2O_2 to ensure complete mineralization. The obtained solution was filtered and diluted to a final volume of 25 mL with distilled water.

Instrumental parameters were set as follows: Fe – 248.3 nm, Mn – 279.5 nm, Cu – 324.7 nm, and Zn – 213.9 nm. Calibration curves were prepared for each element using standard solutions at 0.5, 1.0, 2.0, and 5.0 mg L^{-1}, showing a linearity of $R^2 \geq 0.995$. The limit of detection (LOD) and limit of quantification (LOQ) were 0.01 mg L^{-1} and 0.05 mg L^{-1}, respectively. The analytical recovery rate ranged between 90% and 105%.

All analyses were performed in triplicate (three biological and three technical replicates), and the results

were expressed as μg g^{-1} humic acid (on a dry-weight basis). Data are presented as mean ± standard deviation (SD).

Statistical analysis of experimental results

All experiments were performed in triplicate. Mean values and standard errors (SE) were calculated using Microsoft Excel (Microsoft Corporation, USA). Statistical significance of differences between the treatment and control groups was determined using one-way ANOVA, with a significance level of $p \leq 0.001$.

Results

Agrochemical analysis of soil samples

To compare the biological activity of two different soil types, samples were collected from the saline soils of the Nishon district (Kashkadarya region) and the non-saline soils of the Chinaz district (Tashkent region) at a depth of 0–30 cm. All soil samples were placed in paper bags, transported to the laboratory, and stored at 4°C until further analysis.

The sampling site in Nishon district, Kashkadarya region, is characterized by a strongly continental climate with considerable temperature fluctuations between seasons. The mean annual temperature is around 14.8°C. In winter, the average temperature can drop to 0.2°C, with the minimum recorded temperature reaching –15°C, while in summer, it rises significantly to an average of 28.8°C, and the maximum can reach up to 45°C.

The region receives low annual precipitation, ranging from 180 to 220 mm, concentrated mainly during the cold months—from late October to early spring. Due to this arid climate, local agriculture heavily depends on irrigation systems such as the Karshi Main Canal and the Tallimarjon Reservoir.

The soils of Nishon district contain a high concentration of soluble salts, predominantly sulfate and chloride salts, which contribute to increased salinity and alkalinity levels. The elevated levels of anions and cations negatively affect soil fertility and nutrient balance, thereby influencing plant growth and productivity (Zakiryaeva *et al.,* 2025; Karimov *et al.,* 2025).

Soil characteristics of Chinaz District (Tashkent Region)

Soil samples were collected from agricultural fields prepared for cotton cultivation in theChinaz district, Tashkent region. The climate of the region is continental, characterized by cold winters and hot summers. The mean annual temperature is 13.4°C; the average temperature in January is –3.1°C, with a minimum recorded temperature of –33°C, while the average temperature in July is 26.5°C,

Table 1: Main physical and chemical characteristics of the studied soils

Parameters	Nishon district (saline soil)	Chinaz district (non-saline soil)	Remarks / Interpretation
pH	8.1–8.3	7.5–7.9	Both soils are alkaline in reaction.
EC (dS m^{-1})	6.5–7.2	1.2–1.5	Salinity index is considerably higher in Nishon.
Na^+ (meq L^{-1})	12.4–15.1	1.8–2.3	High Na^+ concentration observed in Nishon district soils indicates strong salinization and reduced nutrient mobility.
SAR	14–16	2–3	Salinity index is higher in Nishon.
OM (%)	0.85	1.45	Organic matter content is higher in Chinaz soils.
N (%)	0.07	0.12	Chinaz soils have greater biological reserves.
P (mg kg^{-1})	6.2	11.5	Available phosphorus is higher in Chinaz.
CEC (cmol kg^{-1})	18–20	12–14	Nishon soils have a larger adsorption capacity.
HCO_3^- (%)	0.022–0.023	0.028–0.032	Alkalinity is higher in Chinaz.
Cl^- (%)	0.099–0.179	0.001–0.003	Chloride content is higher in Nishon.
SO_4^{2-} (%)	0.856–1.100	0.008–0.013	Overall ion concentration is higher in Nishon.
Ca^{2+} (%)	12.8–14.6	0.08–0.40	Calcium difference is significant — higher in Nishon.
Mg^{2+} (%)	0.028–0.031	0.002	Magnesium ions are more abundant in Target
Anions (meq L^{-1})	28.3–31.1	1.3–1.4	Anion concentrations are considerably higher in Nishon soils.
Cations (meq L^{-1})	15.4–17.6	0.45–0.65	Cation concentrations are also significantly higher in Nishon soils.

and the maximum can reach 42°C. The vegetation period lasts approximately 210 days, and the annual precipitation averages 350 mm.

The Chirchik and Syrdarya rivers flow through the district, providing important water resources. Additionally, irrigation of agricultural crops in the area is carried out using water from the Bozsuv, Kurkuldak, Northern Tashkent, and Jon canals, which cross the district.

Chemical elements present in the soil play a crucial role in supplying essential nutrients for plants and in determining the physical, chemical, and biological properties of the soil. In this study, two contrasting regions were selected for comparison and analysis of saline and non-saline soils.

- Saline soil samples were collected from the Nishon district (Kashkadarya region).
- Non-saline soil samples were collected from theChinaz district (Tashkent region).

All soil samples were analyzed and compared based on their key physical and chemical properties (Table 1).

The conducted analyses demonstrated that the soils of the Nishon district are strongly saline, exhibiting elevated levels of EC, Na^+, SAR, chloride, sulfate, calcium, magnesium, anions, and cations. Such conditions enhance soil alkalinity and salinity, thereby exerting a detrimental effect on soil fertility. In contrast, the soils from the Chinaz district are non-saline, with comparatively higher concentrations of organic matter, nitrogen, and phosphorus, indicating superior agrobiological fertility.

Furthermore, the Chinaz soils showed a higher average moisture content (2.23%), which may be attributed to their higher organic matter content and enhanced water retention capacity. Conversely, the Nishon soils exhibited lower

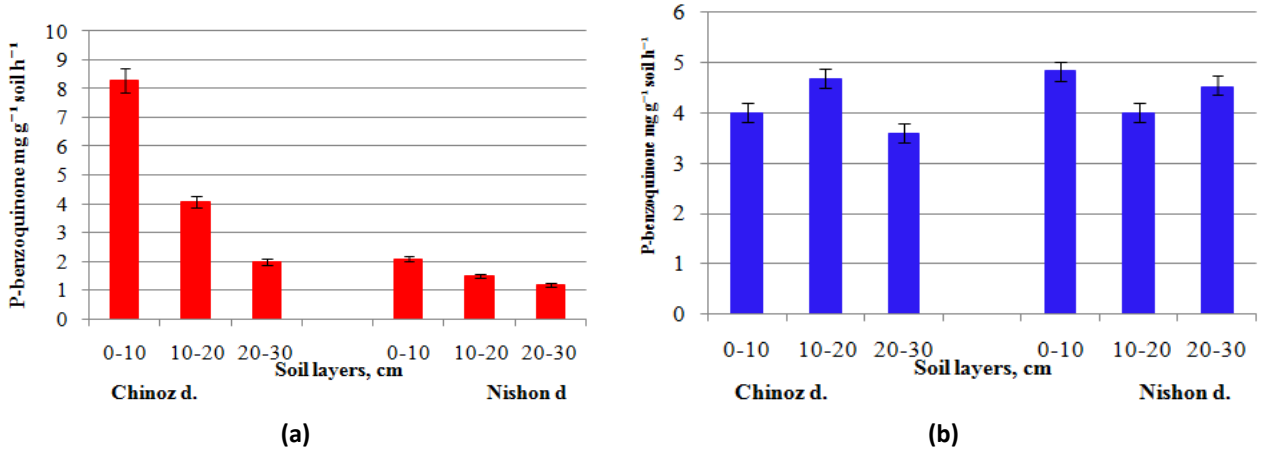


Figure 1: Polyphenol oxidase activity of soil samples (a – spring season, b – autumn season)

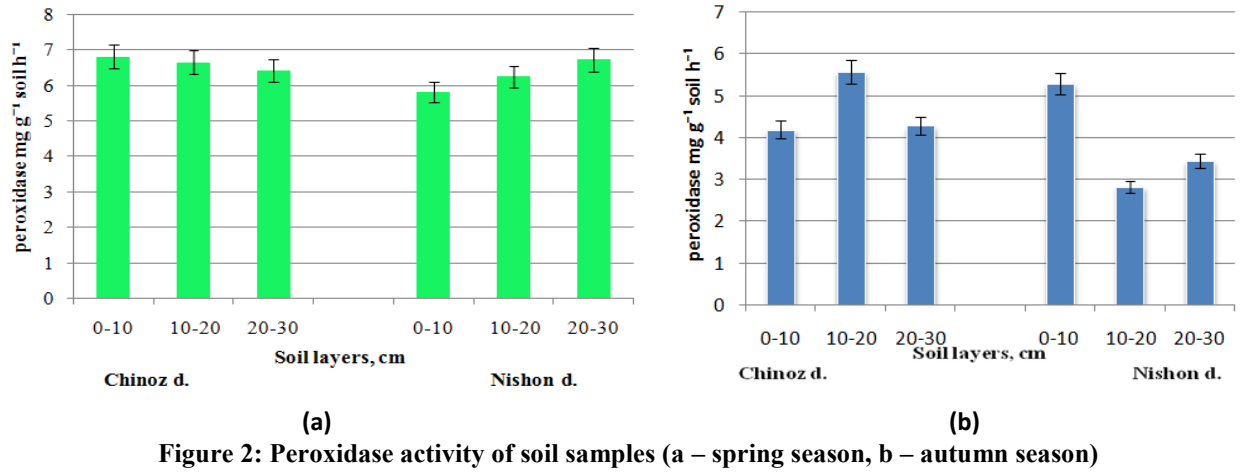


Figure 2: Peroxidase activity of soil samples (a – spring season, b – autumn season)

(1.85%) and more variable (std = 0.91) moisture levels, reflecting reduced water-holding capacity as a consequence of salinization. The lower moisture availability is likely to inhibit microbial activity and suppress enzymatic processes essential for nutrient cycling and organic matter turnover.

Enzymatic activity of soils

To assess the enzymatic activity of soils, samples were collected from three depth layers (0–10 cm, 10–20 cm, and 20–30 cm), and the activities of polyphenol oxidase, peroxidase, and phosphatase enzymes were analyzed.

In soil samples collected during the spring season from agricultural fields of the Tashkent region, the activity of polyphenol oxidase remained relatively stable across different depths, ranging from 3.6 to 4.6 mg p-benzoquinone g^{-1} soil h^{-1} (Figure 1a). Notably, the highest activity was recorded at the 10–20 cm layer, where it reached 4.6 mg p-benzoquinone g^{-1} soil h^{-1}.

In contrast, soil samples collected during the autumn season showed a pronounced increase in polyphenol oxidase activity, particularly in the surface layer (0–10 cm), where the activity was approximately two as high as in spring, reaching 8.28 mg p-benzoquinone g^{-1} soil h^{-1} (Figure 1b). No significant differences were observed at the 10–20 cm depth, whereas the 20–30 cm layer exhibited a 1.8-fold lower enzyme activity compared with the surface layer.

In the saline soils of the Kashkadarya region, the activity of polyphenol oxidase in samples collected during the spring season ranged between 4.0 and 4.82 mg p-benzoquinone g^{-1} soil h^{-1} across the 0–10 cm, 10–20 cm, and 20–30 cm depth intervals (Figure 1a).

A marked decrease in polyphenol oxidase activity was observed across all soil layers in the autumn samples, with activity levels ranging between 1.21 and 2.10 mg p-benzoquinone g^{-1} soil h^{-1} (Figure 1b). This sharp decline in enzymatic activity may be attributed to increased soil salinity, which adversely affects microbial populations and enzyme stability.

In contrast, the relatively higher polyphenol oxidase activity in non-saline soil samples indicates the stable viability of microbial populations within the soil microbial community under favorable biotopic conditions.

In the spring soil samples from the Chinoz district, peroxidase activity remained nearly uniform across all soil depths, ranging from 6.4 to 6.8 mg p-benzoquinone g^{-1} soil h^{-1} (Figure 2a). During the autumn season, a moderate reduction in peroxidase activity was recorded, varying between 4.19 and 5.58 mg p-benzoquinone g^{-1} soil h^{-1} (Figure 2b).

It is noteworthy that, for both spring and autumn samples, peroxidase activity exhibited stable and consistent values across the analyzed soil layers, indicating a relatively uniform enzymatic distribution with depth.

In the saline soils of the Nishon district during the spring season, the activity of the peroxidase enzyme was almost equivalent to that observed in the non-saline soils, ranging between 5.81 and 6.71 mg p-benzoquinone g^{-1} soil h^{-1} (Figure 2a). In the autumn season, however, particularly within the 10–20 cm and 20–30 cm soil layers, peroxidase activity decreased by nearly twofold comparing with spring values (Figure 2b). A similar trend was also evident in the dynamics of polyphenol oxidase activity.

These findings suggest that spring provides relatively optimal conditions for the proliferation and enzymatic activity of soil microbial populations. By autumn, the increased salinity and high temperature likely exert inhibitory effects on microbial metabolism and enzyme synthesis.

To date, studies of soil phosphatase activity have primarily focused on non-specific acid phosphatase. As an extracellular enzyme, acid phosphatase is synthesized by soil microorganisms and can also accumulate in the soil as a result of microbial cell lysis. Free phosphatase enzymes tend to retain their catalytic activity longer in soil than living microorganisms, largely due to immobilization onto soil colloids and mineral particles.

In all soil layers, autumn samples exhibited nearly twice the phosphatase activityobserved in spring, which may be attributed to seasonal factors such as increased soil moisture and higher levels of organic matter.

In the saline soils of the Nishon district, phosphatase activity was slightly lower than in the non-saline soils of the Chinoz district, ranging between 4.87 and 7.12 nM p-nitrophenol min^{-1} g^{-1} soil during the spring season. In contrast, during autumn, phosphatase activity approximately doubled across all soil layers in the same region (Figure 3a, b).

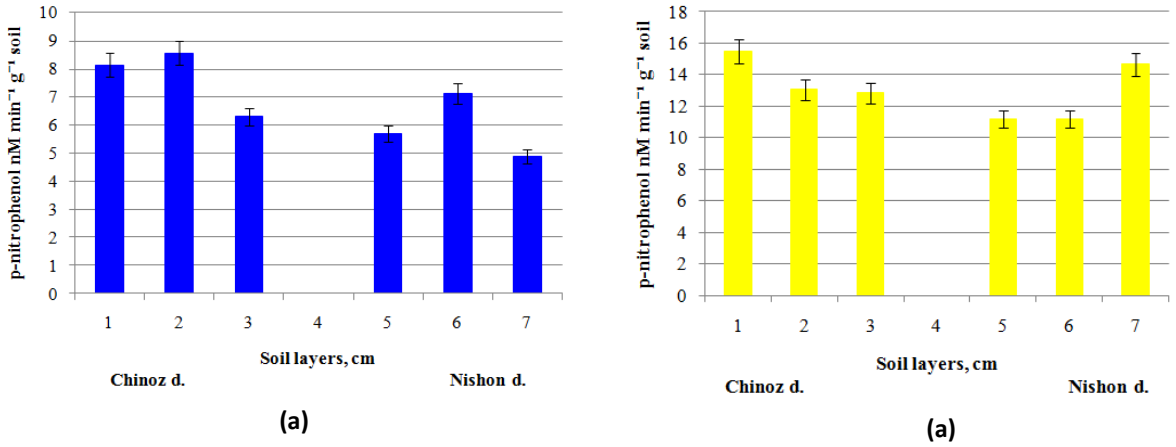


Figure 3: Phosphatase activity of soil samples (a – spring season, b – autumn season)

Based on the obtained results, it can be concluded that the activity of acid phosphatase is highly organism-specific, organism- and environment-dependent, varying with factors suchas pH, temperature, and substrate availability. The activity of phosphatase enzymes in soil serves as a key indicator of soil fertility, since the rate of organic phosphorus mineralization largely determines phosphorus bioavailability for plants. While carbon dioxide and nitrogen are generally available for photosynthesis, phosphorus deficiency often limits plant growth.

In agricultural soils, large quantities of organic phosphorus compounds are present in the form of decomposing root residues, stems, and leaves. However, the release of available phosphorus from these organic compounds primarily depends on phosphatase enzyme activity.

Our findings are consistent with enzymatic activity measurements obtained from the 0–30 cm soil layer, which represents the root zone where both plant roots and soil microorganisms are most active. Additionally, the humic acids extracted from the 0–10 cm surface layer were found to contain higher concentrations of amino acids and microelements, corresponding to the accumulation of surface organic residues and active rhizosphere microflora.

Therefore, combining these two analytical approaches—enzyme activity (0–30 cm) and humic composition (0–10 cm)—provides a complementary assessment of soil biological and chemical status. Enzymatic parameters reflect the overall microbiological activity and nutrient cycling potential, whereas the composition of surface humic acids indicates the source and availability of organic matter and nutrients.

The results demonstrate that soil salinity acts as an inhibitory factor on enzymatic activity, while specific elemental variations in humic composition (e.g., Fe concentration) reveal the influence of salinity on the distribution of trace elements in the soil environment.

Figure 4: Extracted humic acid

Extraction and Composition of humic acids

In the next stage of the study, humic acids were extracted from soils of two different origins. From 100 g of soil, 59 mg of humic acid was obtained from the non-saline soil of the Chinoz District, while 63 mg was extracted from the saline soil of the Nishon District. The extracted humic acids were amorphous, dark brown, and characterized by a distinct glossy surface (Figure 4).

Table 2: Quantity and composition of amino acids in humic acids extracted from soils of Chinaz (non-saline) and Nishon (saline) districts

AminoAcid	Chinaz District (Non-saline soil)	NishonDistrict(Salinesoil)
Asparticacid	36.23 ± 1.18	21.13 ± 1.16
Glutamicacid	39.23 ± 1.18	19.30 ± 1.19
Serine	17.73 ± 0.90	7.07 ± 1.16
Glycine	45.43 ± 1.44	28.27 ± 1.19
Asparagine	44.43 ± 1.79	28.27 ± 1.19
Glutamine	10.00 ± 1.15	4.67 ± 0.88
Cysteine	27.83 ± 0.93	16.33 ± 0.88
Threonine	22.40 ± 1.45	12.03 ± 0.58
Arginine	20.40 ± 1.45	10.60 ± 0.31
Alanine	25.30 ± 1.19	21.77 ± 1.18
Proline	32.77 ± 1.47	26.23 ± 1.18
Tyrosine	7.63 ± 0.88	5.43 ± 0.30
Valine	48.43 ± 0.72	34.23 ± 0.62
Methionine	4.83 ± 0.60	5.40 ± 0.31
Histidine	23.77 ± 2.26	20.00 ± 1.15
Isoleucine	30.13 ± 1.16	21.77 ± 0.91
Leucine	50.90 ± 0.95	30.43 ± 0.87
Tryptophan	16.43 ± 0.81	12.10 ± 1.16
Phenylalanine	23.03 ± 1.16	15.23 ± 0.79
Lysine	2.50 ± 0.29	3.57 ± 0.30
Total	531.77 ± 1.75	348.80 ± 2.04

It should be noted that the slightly higher yield of humic acid obtained from saline soil may be attributed to the fact that plants grown under saline conditions are often unable to fully utilize humic substances. As a result, a portion of these compounds remains accumulated and preserved in the soil matrix.

For the structural characterization of humic acids, 50 mg of sample was placed into a Teflon-sealed ampoule containing 7 mL of 6 N HCl and subjected to hydrolysis at 120°C for 24 hours. After hydrolysis, the digested material was centrifuged at 12,000 rpm for 20 minutes to obtain the supernatant extract.

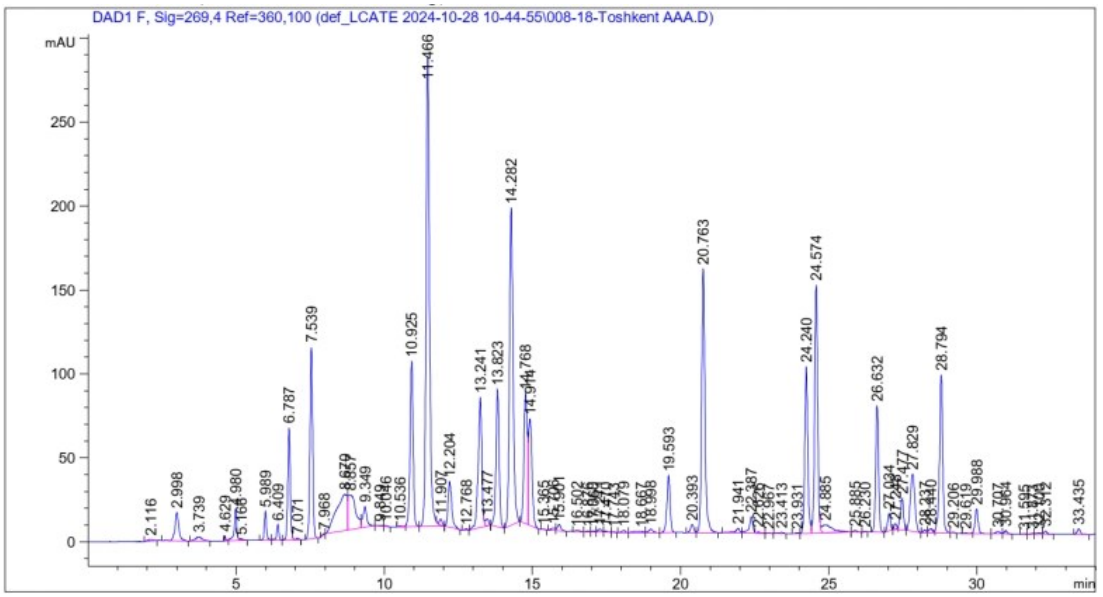
The hydrolysate was transferred into porcelain dishes, and the residual HCl was removed by evaporation on a water bath at 100°C, with intermittent addition of distilled water to ensure complete acid removal. The resulting hydrolysis products were neutralized to pH 7.0 and dried for subsequent analysis.

The amino acid composition of the humic acid hydrolysates was generally similar between samples derived from non-saline (Chinoz) and saline (Nishon) soils; however, there was a considerable quantitative difference. The total amino acid content in humic acids extracted from Chinoz soils was approximately 1.5 times higher than that of the humic acids isolated from Nishon soils (Table 2, Figure 5).

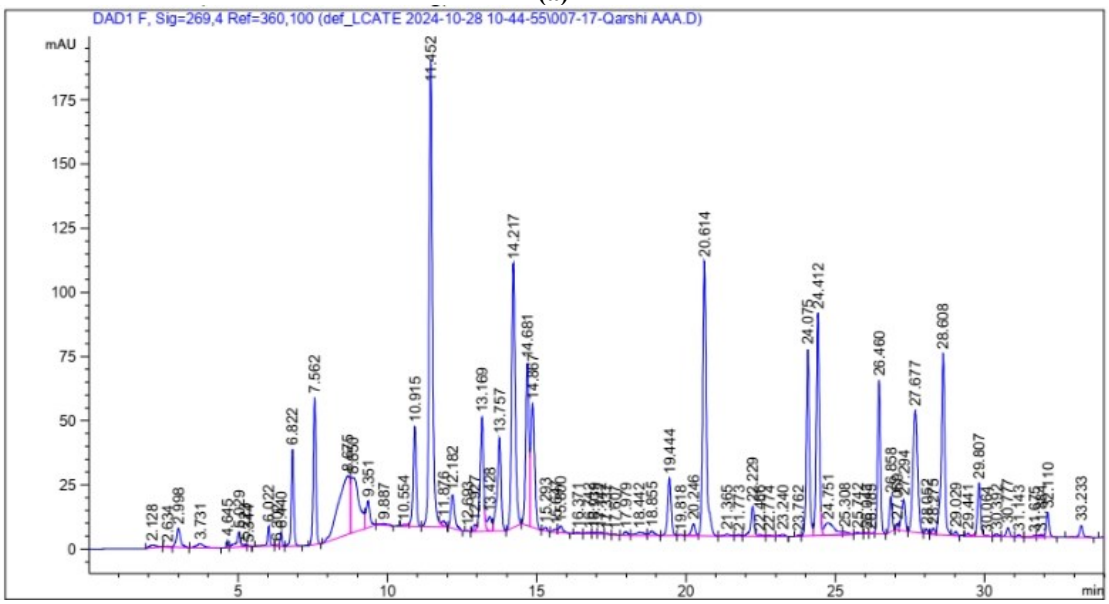
Among the 20 detected amino acids, aspartic acid, glutamic acid, glycine, asparagine, cysteine, arginine, valine, leucine, and phenylalanine were predominant, with total concentrations ranging from 23.1 to 51.7 µg/g of humic acid hydrolysate. In contrast, amino acids such as alanine, tyrosine, methionine, histidine, tryptophan, and lysine were found in nearly equal amounts in both saline and non-saline soil-derived humic acids.

It is noteworthy that the quantitative and compositional variations in amino acids within humic acids are closely related to the agrochemical properties, salinity level, and microbial activity of the soils. These parameters represent important biochemical indicators that reflect the quality and transformation state of humus as well as the fertility potential of the soil ecosystem.

One of the most important and beneficial properties of humic acids is their ability to form complex and chelating compounds (chelates) with polyvalent cations. When humic acids interact with mineral components of the soil, they readily bind elements such as aluminum, iron, copper, and manganese, forming mobile chelated complexes that enhance their bioavailability and transport within the soil matrix.



(a)



(b)

Figure 5: Chromatographic profiles of amino acids in humic acids extracted from soils of Chinoz (a) and Nishon (b) districts

Moreover, humic acids can form insoluble complexes with heavy metals such as mercury, lead, and cadmium, thereby preventing their uptake byplants and reducing their toxicity. This property contributes to the detoxification capacity of humic acids in contaminated environments. When nitrogenous compounds react with humic acids, keto acids are produced, which subsequently participate in the formation of metal–organic chelate complexes.

Humic acids derived particularly from peat have been shown to form chelate complexes with ions of iron, lead, sodium, magnesium, aluminum, zinc, titanium, manganese, and silicon, converting them into bioavailable forms for plant uptake. During this process, the functional groups of humic acids release hydrogen ions, enabling the chelated elements to attain a bioactive state.

In the present study, the concentrations of Fe, Mn, Cu, and Zn in humic acids extracted from saline (Nishon) and non-saline (Chinoz) soils were analyzed. As shown in Table 3, the humic acids obtained from the saline soils contained higher levels of microelements compared to those from the non-saline soils.

Among these, iron (Fe) exhibited the highest accumulation, reaching 853.37 ± 1.15 μg g^{-1} HA in the Nishon samples and 545.27 ± 1.19 μg g^{-1} HA in the Chinoz samples. The concentrations of manganese (Mn), copper (Cu), and zinc (Zn) were also meausured, ranging between 1.4 and 12.4 μg g^{-1} HA, depending on the soil type.

Table 3: Microelement composition of humic acids (HA) extracted from soils of Chinaz and Nishon districts (Flame Photometer: Thermo Scientific)

SoilSource	Element	Concentration (μgg^{-1} HA)
ChinazDistrict	Fe	545.27 ± 1.19
	Mn	11.60 ± 0.31
	Cu	1.47 ± 0.29
	Zn	13.07 ± 0.58
Nishon District	Fe	853.37 ± 1.15
	Mn	12.80 ± 0.58
	Cu	4.43 ± 0.29
	Zn	8.53 ± 0.29

Based on the obtained results, it can be concluded that the higher amino acid content in the humic acids of the Chinoz district soils may result from enhanced microbial decomposition of diverse organic materials, leading to the formation of new, more soluble humic substances.

In contrast, the relatively lower amino acid content in the humic acids of the Nishon district soils may be attributed to long-term stabilization and accumulation of humus compounds over thousands of years. Such humic materials tend to be chemically stable and less soluble, even under alkaline conditions, indicating a higher degree of humification and molecular condensation.

Furthermore, the elevated concentrations of Fe, Mn, Cu, and Zn in the humic acids extracted from Nishon soils are likely associated with the high salinity level and intense ion-exchange activity in these soils. These microelements can form stable chelate complexes with humic substances, promoting their retention and accumulation within the soil matrix.

Discussion

The results of this study revealed significant differences between the soils of the Nishon district (Kashkadarya region) and the Chinoz district (Tashkent region) in terms of their agrochemical and climatic characteristics. The analysis showed that the soils of the Nishon district contained higher concentrations of water-soluble salts, particularly sulfates, chlorides, calcium, and magnesium ions. These factors contribute to an increase in soil alkalinity and overall salinization level, which in turn may negatively affect nutrient availability and plant growth. Moreover, the arid climate, low precipitation, and irrigation practices are likely to play an important role in accelerating these processes and influencing the biogeochemical cycling of nutrients (Yusupov *et al.,* 2019; Khaziev, 2005).

In contrast, the soils of the Chinoz district exhibited favorable agrochemical properties, with a considerably lower content of soluble salts. Although the soil reaction in this area also tends to beslightly alkaline, the low salinity enables more efficient nutrient uptake by plants, creating conditions more conducive to crop productivity (Kadyrov, 2016; Abdurashidov *et al.,* 2020).

The limited rainfall, large temperature fluctuations, and restricted irrigation resources characteristic of the Kashkadarya region intensify the salinization processes. Furthermore, the lower humus content and reduced enzymatic activity observed in saline soils indicate suppressed microbial diversity and metabolic function. This reduction in biological activity is consistent with findings from previous research (Khaziev, 2005; Bashirov, 2013).

Overall, the non-saline soils—particularly those from the Chinoz district—can be regarded as agrobiologically and biochemically more stable, maintaining higher biological activity, enzyme functionality, and humus accumulation. These features promote an active nutrient cycle and support optimal conditions for plant growth and productivity (Tursunov, 2018; Ibragimov *et al.,* 2021; Turaeva *et al.,* 2021).

In our investigation of polyphenol oxidase activity, a marked decline was observed during the autumn season (1.21–2.1 mg p-benzoquinone g^{-1} soil h^{-1}), indicating that saline conditions may adversely affect both microbial population density and enzyme abundance. In contrast, non-saline soils maintained higher polyphenol oxidase activity, which is consistent with microbiological and biogeochemical mechanisms associated with active organic matter turnover. According to literature reports, the combined activities of peroxidase and polyphenol oxidaseare often regarded as a humification index, which tends to increase significantly following the application of

organic amendments (Zinchenko and Zinchenko, 2024; Iqbal *et al.,* 2024).

In the case of peroxidase activity, our findings showed that in the saline soils of the Nishon district, the enzyme activity in autumn samples decreased by up to twofold compared to spring. This reduction is likely related to the degree of salinity and elevated temperature, which can suppress microbial metabolism. Conversely, in the non-saline soils of Chinoz district, peroxidase activity remained relatively stable between spring and autumn, ranging from 6.4 to 6.8 mg p-benzoquinone g^{-1} soil h^{-1}. Such stability indicates that microbial populations are well adapted to the biotope conditions of low-salinity environments, where enzyme systems remain functionally active. Previous studies have reported that variations in peroxidase activity are strongly influenced by agronomic practices, such as the application of organic amendments, microbial inoculants, and different irrigation regimes, all of which highlight the enzyme's sensitivity to soil conditions and its close linkage with microbiological activity within the ecosystem (Fei *et al.,* 2023; Kosimov *et al.,* 2024 (b)).

Studies conducted in the Sangong River Basin near Fukang City, Northern Xinjiang (China) demonstrated that in saline soils under different plant communities, the activities of lignin-degrading enzymes (peroxidase and polyphenol oxidase) and non-lignin-degrading enzymes (β-glucosidase, α-glucosidase, N-acetylglucosaminidase, cellobiohydrolase, and β-xylosidase) varied seasonally (Guan *et al.,* 2014). Specifically, peroxidase activity showed a marked seasonal decline, while the activity of non-ligninolytic enzymes decreased with increasing soil depth. However, such a trend was not observed for peroxidase and polyphenol oxidase, indicating that polyphenol oxidase activity correlated positively with non-ligninolytic enzymes, whereas peroxidase activity was not significantly influenced by soil pH. The combined activities of peroxidase and polyphenol oxidase are thus considered an effective humification index and can serve as a reliable indicator of soil quality.

Our findings similarly revealed that acid phosphatase activity was relatively high in both Chinoz and Nishon district soils, particularly in the 0–10 cm and 10–20 cm soil layers, where the activity ranged from 8.14 to 8.55 nM p-nitrophenol min^{-1} g^{-1}. During the autumn season, these values nearly doubled, likely due to an increase in organic substrates and enhanced microbial activity. In saline soils of the Nishon district, phosphatase activity was somewhat lower (4.87–7.12 nM min^{-1} g^{-1} in spring); however, a subsequent increase was observed in autumn, despite the unfavorable environmental conditions for plants and microbes. This result supports earlier reports suggesting that soil phosphatases can accumulate and remain active for extended periods, even under adverse conditions (Khaziev, 2005).

Fei *et al*. (2023) reported that the application of organic and mineral biohumus amendments (such as NPK combined with compost) significantly enhanced alkaline phosphatase activity in soils. This enzyme, primarily secreted by microorganisms, tends to exhibit greater stability and activity under slightly acidic or saline conditions (Jin *et al.,* 2025). Furthermore, the combined use of biochar and phosphate-solubilizing Bacillus strains has been shown to markedly enhance phosphatase activity and promote plant growth (Iqbal *et al.,* 2024). Likewise, the diversification of organic nutrient inputs and plant species composition has been demonstrated to optimize soil enzymatic activities, including both acidic and alkaline phosphatases (Gao *et al.,* 2023; Khamidova *et al.,* 2024).

Soil pH, moisture content, organic matter, and the structure of microbial populations are among the main factors influencing phosphatase activity. Our findings indicate that salinity and elevated temperature negatively affect bacterial populations; however, exogenous enzymes such as acid phosphatase can retain activity for extended periods in the soil through stabilization by organomineral complexes. This observation is consistent with previously reported theoretical frameworks in the scientific literature.

Our methodological approach—measuring enzymatic activity within the 0–30 cm layer and assessing humic composition in the 0–10 cm layer—allowed simultaneous evaluation of the biological and chemical state of the soil. Enzymatic indicators primarily reflect rhizosphere processes and microbial activity, thereby enabling the assessment of how salinity alters enzymatic dynamics. In contrast, the surface-layer (0–10 cm) humic composition, associated with organic matter sources and microelement interactions, reveals the localized influence of salinity on humic substance formation. Thus, sampling at two different depths provided a comprehensive biochemical and ecological profile of the studied soils (Fierer*et al*. 2003).

This study also focused on comparing the quantitative and compositional characteristics of humic acids extracted from saline (Nishon district) and non-saline (Chinoz district) soils, particularly their amino acid composition and metal-chelating properties. The obtained results demonstrated that both the amount and composition of humic acids varied significantly between the two regions.

Quantitatively, slightly higher yields of humic acids were obtained from the saline soils of Nishon (63 mg per 100 g soil) compared to the non-saline soils of Chinoz (59 mg per 100 g soil). This can be explained by the reduced microbial activity and slower decomposition of organic residues under saline conditions, which may lead to the accumulation of humic substances (Bai *et al.,* 2025). However, such accumulation does not necessarily indicate higher biological activity or soil fertility, as the functional bioavailability of humic matter in saline environments remains limited.

The analysis of amino acids revealed that the total amino acid content in the humic acids of Chinoz district soils (531.4 μg g^{-1}) was considerably higher than that in the Nishon district soils (349.5 μg g^{-1}). In particular, aspartic acid, glutamic acid, glycine, and leucine were more abundant in the non-saline soils. This indicates a higher level of microbial activity and a greater input of organic matter, consistent with the findings of Jin *et al*. (2025).

Furthermore, the elevated amino acid content in the Chinoz humic acids suggests that the humus substances are relatively younger and more biochemically labile. This condition likely results from the enhanced microbial decomposition of organic matter and the formation of highly soluble humic compounds under conditions of active biological turnover (Zavarzina *et al.,* 2021).

Conversely, the lower amino acid content observed in the humic acids of the Nishon saline soils may be attributed to the long-term stabilization of humus substances that have accumulated over extended periods. Such humic materials are more chemically recalcitrant, exhibiting reduced susceptibility to acid hydrolysis and lower enzymatic reactivity (Ding *et al.,* 2001).

Some amino acids—alanine, tyrosine, methionine, histidine, tryptophan, and lysine—showed nearly equal concentrations in both regions, suggesting that these components are relatively stable constituents of humic acids, independent of soil salinity conditions.

Overall, the findings confirm that humic acids act not only as reservoirs of organic nitrogen but also play a critical role in detoxification processes, stabilization of soil composition, and bioactivation of essential elements. Their application, especially in saline and degraded soils, can enhance biological activity, improve soil structural properties, and reduce the toxic effects of heavy metals.

In the present study, the microelemental composition—namely iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn)—of humic acids extracted from the Chinoz (non-saline) and Nishon (saline) soils was also examined. The obtained results demonstrated that the elemental content of humic acids is strongly influenced by the agrochemical properties of the soils, particularly by the degree of salinity.

According to the results of the study, the concentrations of Fe, Mn, and Cu in the humic acids of the saline soils of the Nishon district were higher than those in the non-saline soils of the Chinoz district. In particular, the iron content was significantly elevated in Nishon soils (853.37 ± 1.15 μg g^{-1} HA) compared to Chinoz soils (545.27 ± 1.19 μg g^{-1} HA). This difference can be explained by the increased mobility of cations under saline conditions, although other factors—such as the mineralogical composition and organic matter content of the soils—may also contribute to this variation.

The chelating ability of humic acids, especially their capacity to form stable complexes with polyvalent cations (Fe^{3+}, Al^{3+}, Zn^{2+}, Mn^{2+}), plays a crucial role in nutrient availability and in mitigating the toxic effects of heavy metals (Cd, Pb, Hg). Functional groups such as carboxyl and phenolic hydroxyl groups are primarily responsible for these metal-binding properties.

Moreover, the elevated concentrations of Fe, Mn, Cu, and Zn in humic acids indicate that humic substances act as biologically active components capable of retaining essential micronutrients in chelated forms and serving as a potential source of trace elements for plants. This process strengthens the detoxification and nutrient-retention functions of humic substances, particularly in saline soils (Zanin *et al.,* 2019).

Both our findings and previous studies demonstrate that the bioactivity and chelation potential of humic acids in saline soils can reduce the mobility of heavy metals, thereby contributing to ecological balance and improving soil quality.

Overall, the results suggest that soil salinity, along with microbial activity, exerts a direct influence on the composition and activity of both humic acids and soil enzymes. In the non-saline soils of the Chinoz district, the higher amino acid content in humic acids and the elevated activities of enzymes—particularly polyphenol oxidase, peroxidase, and phosphatase—reflect a greater degree of biological activity and continuous renewal of humus compounds, which together contribute to enhanced soil fertility and ecological resilience.

At the same time, although the saline soils of the Nishon district contained a relatively higher quantity of humic acids, their amino acid content was lower and enzyme activity was also reduced. This pattern indicates a decline in microbial activity, reduced degradability of humic substances, and suppressed enzymatic bioactivity under saline stress.

The enzymatic analyses revealed that the activities of polyphenol oxidase and peroxidase were substantially higher in non-saline soils, whereas in saline soils they decreased sharply due to seasonal variations. Similarly, phosphatase activity was more pronounced in non-saline soils, reflecting a greater availability of phosphorus compounds for plant uptake.

The elevated concentrations of iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn) in the humic acids of saline soils can be attributed to the chelating properties of humic substances and their ability to form stable complexes with micronutrients. This finding supports the notion that humic acids not only play a crucial role in enhancing biological activity but also in reducing the toxic effects of heavy metals by immobilizing them in less bioavailable forms.

Overall, humic acids and soil enzymes represent vital components that sustain the biological integrity, nutrient cycling, and ecological stability of soils. Their concentrations and activities are strongly influenced by salinity, soil structure, and seasonal factors. Therefore, monitoring the levels and activity of humic substances and key soil enzymes can serve as a reliable biochemical indicator for assessing soil fertility and ecosystem health.

Conclusions

In the non-saline soils of the Chinaz district, enzyme activities were generally high: polyphenol oxidase and phosphatase showed notable sensitivity to seasonal variations, while peroxidase exhibited relatively stable activity throughout the sampling periods. In contrast, enzyme activity in the saline soils of the Nishon district was markedly reduced, indicating the negative impact of salinity on microbial dynamics and enzymatic processes.

For the first time, pure humic acids were successfully isolated from both Chinaz (non-saline) and Nishon (saline) soils. The total amino acid content in the humic acids extracted from Chinaz soils was 1.5 times higher than that of Nishon soils, reflecting the greater biological activity and freshness of humus in non-saline conditions.

Among the microelements analyzed, iron (Fe) exhibited particularly high concentrations in both soil types — 853.37 ± 1.15 μg g^{-1} HA in saline soils and 545.27 ± 1.19 μg g^{-1} HA in non-saline soils — whereas the contents of manganese (Mn), copper (Cu), and zinc (Zn) were comparatively low.

Overall, the findings demonstrate that humic acids and soil enzyme activities serve as reliable biochemical indicators for assessing soil quality, biological productivity, and environmental stability, particularly under the stress conditions of salinization.

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