Effect of Humic Acids Extracted From Different Organic Sources and Inoculation of Bacillus Subtilis or Aspergillus Niger in Alkaline Phosphatase Activity in Calcareous Soil

Israa A.Y. Al-Malaky[1], Meiad M. Al-Jaberi[2], Mohammed A. Abdulkareem[3]

[1]*Department of Soil Sciences & Water Resources, College of Agriculture, University of Basrah, Iraq*

[2]*Department of Soil Sciences & Water Resources, College of Agriculture, University of Basrah, Iraq*

[3]*Department of Soil Sciences & Water Resources, College of Agriculture, University of Basrah, Iraq*

KEYWORDS

Alkaline Phosphatase, Humic Acid , Enzyme, Bacillu Subtilis, Aspergillus Niger.

ABSTRACT

Humic acids have received a large attention of researching and development as amendments for poor fertility conditions and deteriorating physical properties, since the process of extracting is an easy common process to implement, as well as dilution of these acids with water enables to be used at economically acceptable levels over large areas. It is necessary to notice that the properties of these acids depending on the organic source and the concentration added to the soil.A laboratory experiment was conducted at the department of soil Sciences and water resources, collage of Agriculture ,University of Basrah ,Iraq to demonstrate the effect of humic acids extracted from different sources and inoculation with Bacillus subtilis or Aspergillus niger on alkaline phosphatase activity in calcareous soil calcareous, southern Iraq. Humic acids extracted from wheat straw, alfalfa leaves, goat manure, and poultry manure which composted previously . Humic acid levels were 0, 25 and 50 L ha-1 .The soil also inoculated with Bacillus subtilis or Aspergillus niger, then samples were incubated at 28±2°C. After 7 or 30 days of incubation the activity of alkaline phosphatase was estimated . Enzyme phosphatase activity was increased in soils treated with humic acid as compared to control with highest activity at Poultry manure (391.54 µg p-nitrophenol gm-1 soil.1h-1).The results also indicated that increasing the level of humic acid significantly increased enzyme activity. For soil treated with Bacillus subtilis and Aspergillus niger , phosphatase activity increased-as compared to control with a higher activity at Bacilluas subtilis (373.1 µg p-nitrophenol gm-1 soil.1h-1).

1. Introduction

Soil enzymes play a major role in transferring energy through the decomposition of organic matter in the soil and recycling nutrients, and thus play an important role in agriculture, as these enzymes catalyze many biological reactions necessary for the life processes of microorganisms in the soil and also help in stabilizing soil. Although soil microorganisms are the primary source of soil enzymes, plants and animals also contribute production soil enzymes. Soil enzymes respond quickly to any changes in soil management practices and environmental conditions and closely related to the physical, chemical and biological properties of soil. Soil enzymes can used as sensors of soil microbial status and of soil physical and chemical conditions. Understanding the potential roles of different soil enzymes can help in maintaining soil fertility (Rao *et al*.,2017). Alkaline phosphatase (EC 3.1.3.1) is one of the enzymes are capable of catalyzing hydrolysis of organic of phosphorus compounds such as pyrophosphate , metaphosphate and inorganic polyphosphates found in soil. Alkaline phosphatase hydrolysis ester bonds(C – O – P) found in organic phosphate compounds to inorganic phosphatase, so phosphatase activity is an critical factor in continue of phosphorus cycle in nature(Dick and Tabatabai 1993; Chen *et al.*,1996).

$$H_2O \rightarrow 2HPO_4^{-2} - O - \underset{O-}{\overset{O}{\overset{\|}{P}}} - O - \underset{O-}{\overset{O}{\overset{\|}{P}}} - O - +$$

Phosphatase activity depends on the content of organic matter, organic phosphorus, inorganic phosphorus, and nitrogen in the soil . Chen *et al*. (2000) showed that phosphatase activity in soil is mostly associated with the upper surface soil and decreases with soil depth. Humic substances are known as organic substances which are decomposition-resistant nature materials that have high molecular weight with dark color (Anonymous,2010).The resistant materials often are lignin and protein, with bases found in the soil. Humus is characterized by variable characteristics and difficult to determine its chemical formula. The main parts of humic substances are humic acid, fulvic acid, and humin which can be extracted using dissolution with a base and then with acid (Stevenson,1982)

The effect of humic acid on soil properties and plant growth varies depending on the source from which it is extracted, which is mainly due to the concentration of the acid, chemical properties, molecular weight, and the type and quantity of the prevail active groups. This in turn affects the activity of soil microorganisms and the released enzymes due to the interaction of humic acid with clays and changing the nature of their surfaces, and consequently affects enzymes activity and their stabilization. Humic acid is also a source of carbon and some nutrients essential for soil organisms (Li *et al*.,2012). Dubey *et al*.(2022)found an improvement in alkaline phosphatase activity from 48.17 to 70.41 µg p-nitrophenol gm^{-1} soil.$1h^{-1}$ by increasing the dose of humic acid from 0 to 5 mg kg^{-1} soil, indicating that humic acid enhances the nutritional status of soil microbes.Al-Haidrawi (2023) indicated that adding fulvic or humic acid at a concentration of 50 L ha^{-1} led to an increase in the activity of the alkaline phosphatase enzyme, with a higher values at fulvic acid compared to the humic acid.

Microbial inoculation is a promising method to improve soil fertility in an environmentally friendly manner (Zhao *et al*.,2021). The importance role of *Bacillus subtilis* in soil indirectly affects plant growth by influencing the microbial enzymatic activities involved in transformations of phosphorus in soil. Researches have been interested in the role played by phosphate-solubilizing fungi for increasing phosphorus availability through their acids production.Filamentous fungi(A*spergillus niger*)were widely used to produce hydrolysis enzymes in soil wich is a multifunctional fungus capable of dissolving phosphates .Mendes *et. al* .(2015) *found that Aspergillus niger* can dissolved rock phosphate and produce available-p. was added to fermented by 2.4 times more than control due to producing acids as well as enzymes to soil.Al-Haidrawi and Al-Jaberi (2023) obtained an increase in the activity of the alkaline phosphatase enzyme when inoculating the soil with . *Aspergillus niger* or *Bacillus subtilis*, the increase rate were 58.35% and 44.06%, respectively. They indicated that these organisms are among the organisms that produce the phosphatase enzyme. This study aimed to compare the effect of humic acids (humic acid and fulvic acid together) extracing from different soures along with inculation with *Bacillus subtilis* or *Aspergillus niger* on the activity of the alkaine phosphatase at two incubation periods.

2. Materials And Methods

Four organic wastes were collected: wheat , alfalfa Leaves ,goat waste and poultry waste, cleaned, cut into small pieces then composted aerobically in a hole of 2 x 2 x 1.5 dimensions for 3 months .The piles in each hole moisted for 60% and treated with soil suspension and urea solution to accelerate microbial activity , then coverd with plastic. Piles were turning weekly to enhance aeration. After that, the organic waste was removed, air-dried and transported to the laboratory. The humic acids were extracted (humic acid and fulvic acid together) as described in Page *et al*.(1982) by using 0.5 *M* NaOH twice then adjusted pH at 5. The initial samples were analyzed for different properties and listed in table (1).

Table (1) Quantity, ionic analysis and E4/E6 values for humic acids extracted from various organic sources.

Adjective	**Unit**	**Wheat straw**	**Alfalfa leaves**	**Goat waste**	**Poultry waste**
Quantity of humic acids	%	8.6	8.9	3.0	9.6
EC	Decimens M-	15	19.2	13.9	20.2
C	%	60.41	43.26	42.06	45.58
H	%	4.95	4.83	0.96	4.56
N Total	%	1.39	3.88	2.79	4.07
S Total	%	0.85	0.77	1.9	0.71
Please tell me	%	0.042	0.09	0.10	0.12
C/N	----------	43.46	11.14	15.07	12.67
C/P		1438.33	480.66	420.62	379.83
E4/E6	----------	5.0	4.5	1.8	1.4
Total acidity	Millieq gm^{-1}	5.7	5.3	7.3	7.1
Carboxyl groups	Millieq gm^{-1}	3.0	2.9	4.2	3.9
Phenolic aggregates	Millieq gm^{-1}	2.7	2.4	3.1	3.2
Alcoholic aggregates	Millieq gm^{-1}	3.0	2.9	4.2	3.9

Silty clay soil samples(0-30 cm) were collected from agricultural research station, College of Agriculture , University of Basra, air-dried , grinded then passed through a 2 mm sieve. A part of sample was analyzed for initial properties using standard procedures described in Black(1965)and Page *et al.*(1982) (table 2) . The reminded part of soil sample was used for incubation experiments.

Table 2: Initial characteristics of the study soil

The adjective	**Unity**	**Reading**
pH	____________	7.82
EC	dSm^{-1}	4.0
Total solid carbonate	g kg^{-1}	359
CEC	Cmol $+kg^{-1}$	26.73
Organic matter	g kg^{-1}	2.62
Ready phosphorus	mg kg^{-1}	4.9
Ready nitrogen	mg kg^{-1}	19.2
Ready sulfur	mg kg^{-1}	0.62
Bacteria numbersthe college	CFU	106×2.11

Preparation of fungithe college		105×4
Phosphatase activity	µg p-nitro phenol gm-1 soil.1h^{-1}	210
the sand	%	11.2
Clay		54.6
Alluvial		34.2
Histology		Alluvial clay

The bacterial vaccine (*Bacillus subtilis)* growing in tubes containing the nutrient agar (NA) in the form of a slant and pre-insulated in the soil microbiology laboratory of soil department was taken. One ml of sterile distilled water mixed with bacterial colonies and using for inoculation of nutrition broth media and incubated at 30°C for five days. As for the fungal inoculum(*Aspergillus niger)*it was grown in Potato Dextrose Broth (PDB) media by adding 0.5 cm diameter discs of pure colonies growing in PDA medium to the liquid nutrient medium (PDB) and incubating at 28°C for five days. 500 g of soil samples treated with 2%(w\w) of cow waste sterilized at 121C˚ And 15 pounds inch^{-2} and left in the incubator for 14 days for balance. The samples were thoroughly mixed with humic acids at levels of 0, 25 and 50 L ha^{-1}.and inoculated with 20 ml of *Aspergillus niger* at a density of 40 x 103 m^{-1}cfu or with *Bacillus subtilis* at a density of 20.06 x 106 ml^{-1} cfu .Soil moisture was brought to the field capacity and incubated at 28 ± 2 ˚C .Control treatment without inoculation was conducted .After 7 and 30 days of incubation, a set of samples was taken, dried, ground, and sieved, then alkaline phosphatase activity was measured.alkaline phosphatase activity was measured according to method of Bremner and Tabatabai (1969) by incubating 1 g soil with 0.2 ml of toluene and 4 ml of alkaline buffer solution(pH 11) (1.12 g of THAM + 6.11 g of maleic acid + 48 ml of hydroxide sodium + 6.3 g boric acid + 14 ml citric acid). One ml of substrate (di-sodium-4-nitrophenyl phosphate Hexahydrate) with concentration 0.5 *M* was added and samples were incubated at 37°C for one hour. After incubation, 1ml of $CaCl_2$ and 4 ml of NaOH were added . p-nitrophenol released was determined spectro photometrically at 420 nm wave length. Variance analysis of the alkaline phosphatase was performed using GenStat program and the difference between means were obtained by RLSD test (AL-Rawi and Kalaf allah ,1980).

3. Results And Discussion

Figure (1) showed the effect of humic acids on alkaline phosphatase activity after 7 days (A)And 30 days (B) of incubation. At the treatments to which humic acids were added a significantly superior were recorded compared to the control treatment at both incubation periods, the means were 331.77, 345.41, 335.6 and 391.54 at 7 days and 312.93, 310.19, 404.00, and 456.32 µg p-nitrophenol g^{-1} soil1h^{-1} at 30 days of incubation , while it reached 269.4 and 193.15 µg p-nitro phenol g^{-1} soil1h^{-1} for control treatments .This result is consistent with Li *et al.* (2013)who explained that the reason for the increase in enzyme activity in the presence of humic acids is that Humic acids stimulates the production of extracellular enzymes in soil and act as natural activators of enzymes and enhance the biodegradation processes of organic materials. Humic acids improve the soil ability to retain water and creates a better environment for microbes and enzymes, thus enhancing their activity, which provides a suitable environment for enzyme activity. Humic acids also promote the growth and diversity of microbes in the soil, and these microbes produce enzymes help decompose organic matter and improve nutrient cycling and providing the necessary raw materials for enzymes(Lumactud,2022). For comparing the effect of humic acids among themselves, it is observed that the effect of different humic acids on alkaline phosphatase varied according to the following sequence: Poultry < alfalfa <goat <straw, with a mean values of 391.54, 345.41, 335.6, and 331.77 µg p-nitrophenol g^{-1}soil1h^{-1} respectively. This is due to the humic acid contents of total phosphate as a substrate for the enzyme(table 1). Poultry waste gave the highest values than straw waste due to

differences in chemical composition despite its high salinity value. Poultry waste have higher nitrogen and lower C/N and C/P ratios compared to straw(Table 1)this makes it a more suitable environment for enzyme-producing bacteria, due to easily decomposed which increases microbial growth and enzymatic activity. Also, straw waste mainly contains cellulose and lignin, which are difficult compounds to decompose and require a longer time and specialized enzymatic activity (Liu *et al*.,2021) .The acids extracted from poultry waste were superior to the control treatment with an increase of 45.3 and 136.3% for the incubation periods of 7 and 30 days, respectively.

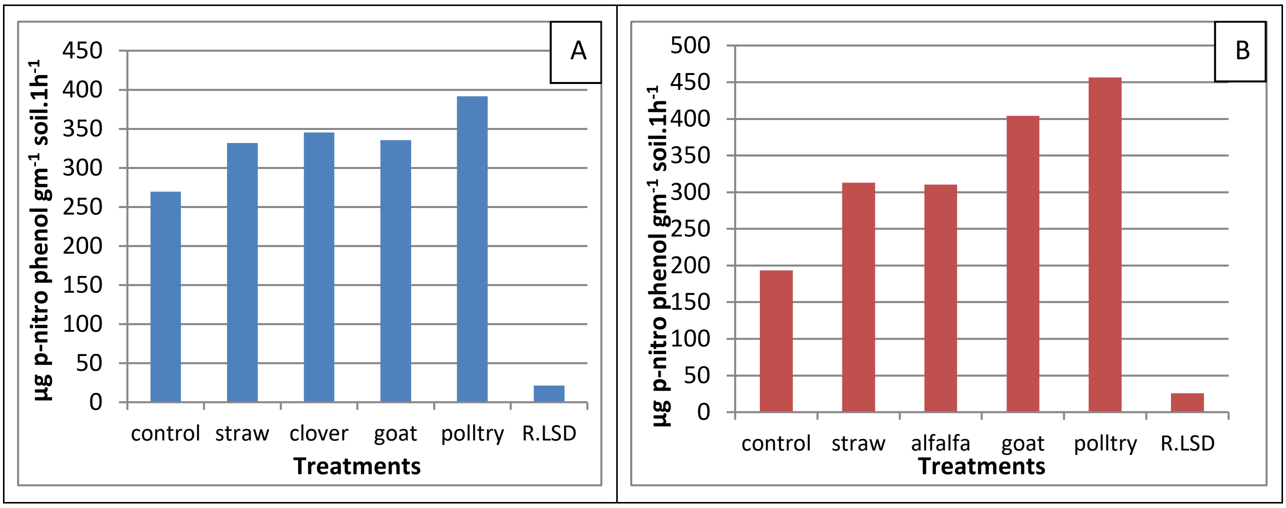
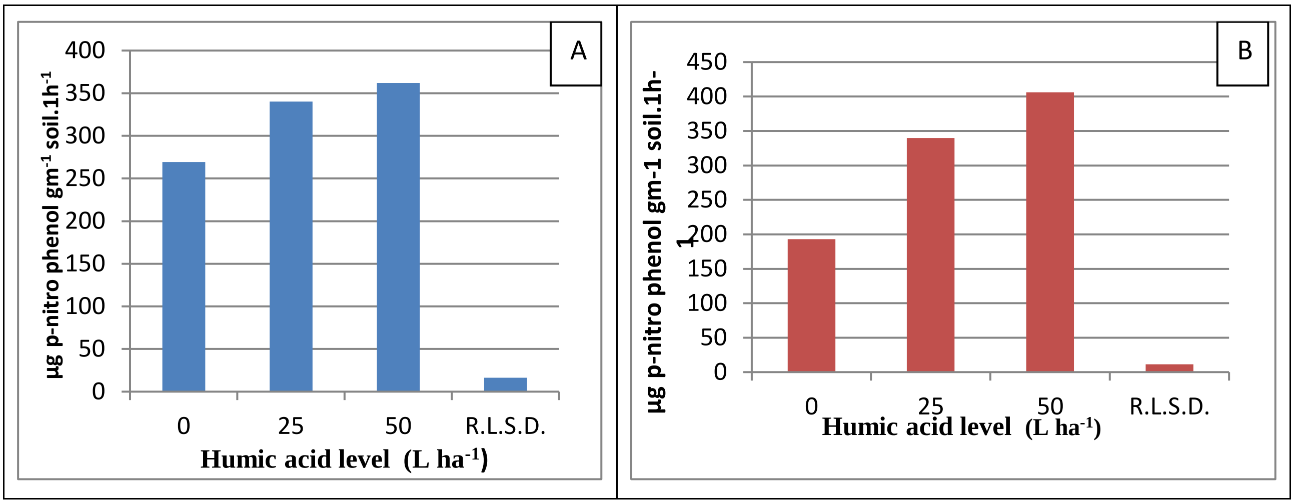


Figure (1) : Effect of a source of humic acid on activity of alkaline phosphatase during 7days(A) and 30 days(B) of incubation.

Increasing the level of humic acid significantly increased alkaline phosphatase activity in soil .The mean values were 269.4, 340.19 and 361.97 µg p-nitro phenol g^{-1} soil1h^{-1} for 7 days of incubation and 193.15, 339.6 and 406.13 µg p-nitro phenol g^{-1} soil1h^{-1} for an incubation period of 30 day, at levels 0,25 and 50L ha^{-1} , respectively (fig.2)This is consistent with the results of Wang *et al.* (2022), who used five concentrations of humic acid as sodium humate and found to an increase in soil pH and alkaline phosphatase activity due to the benefite effect of humic acid to increase soil organic matter content, improve soil strucetur e , create appropriate sizes in the soil, and modify soil pH and increase water-soluble organic carbon.

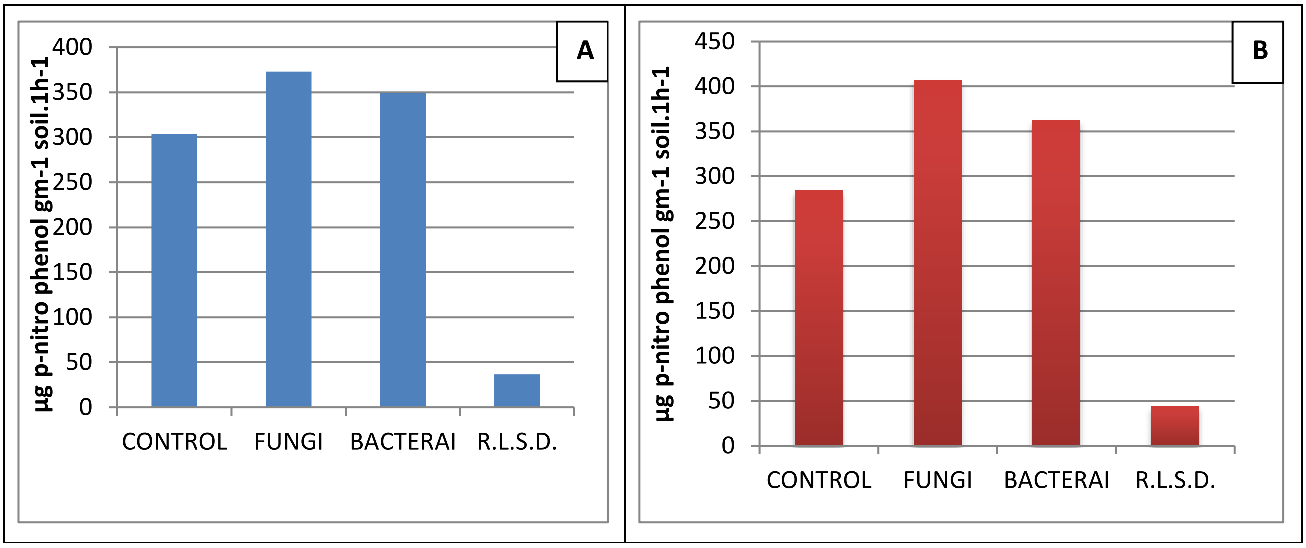
It have been shown from these results that using of mentioned levels of humic acid did not negatively affect the enzyme activity, which confirms the essential role of the extracted humic acids in improving soil properties and preparing the microbial media with carbon and nutrients, including the substrate, resulting in a positive effect on the enzyme activity.



Figure(2): Effect of humic acid level on activity of alkaline phosphatase during 7days(A)and 30 days (B) of incubation.

Figure (3) showed the effect of incubation with *Bacillus subtilis* and *Aspergillus niger* on Alkaline phosphatase activity during incubation periods of 7 and 30 days. Data indicated the superiority of fungal inoculum compared with bacterial inoculum ,as well as the two treatments gave higher value over control treatment with values of 373.1. 349.28 and 303.63 µg p-nitro phenol g^{-1} soil1h^{-1} at incubation period of 7 days, and 406.85, 362.34, and 284.17 µg p-nitro phenol g^{-1} soil1h^{-1} at incubation period of 30 days, respectively. These results are consistent with Saeed and Al-Harbawi (2014) who obtained, an increase in the activity of alkaline phosphatase after treating soil with *Aspergillus niger*, along with sheep waste. *Aspergillus niger* can produce alkaline phosphatase to soil (Tauqeer *et al*.,2021; Wenjie et al.,2020) .Wei et al. (2024).)pointed out the role of fungi in improvement of organic materials decomposition and provides nutrients which enhances soil fertility and growth of microbes and plants .Fungi also secrete organic acids such as citric acid and oxalic acid, which decompose the complex organic phosphates in the soil (Jiao et al.,2022).

Regarding effect of incubation time, the activity of the enzyme decreased with progressing the incubation period from 7 to 30 days in the control treatment due to the depletion of the substance on which the enzyme need and related to the organic matter content. The mean values were 269.4 and 193.15µg p-nitrophenol g^{-1} soil1h^{-1} at incubation periods of 7 and 30 days, respectively .While in humic acid treatments, it is observed that the activity of the alkaline phosphatase increased with increasing incubation period with values of 351.08 and 370.86 µg p-nitrophenol g^{-1} soil1h^{-1} 7 and 30 days, respectively. This increasing confirmed the role of humic acid to supply the soil with the essential materials for microbes activity and enzyme production. Humic acids takes more time to catalyze the enzymatic reaction and over time, the reaction of humic acids with the enzyme will increased.These results are consistent with Al-Taie (2013)who indicated, that there was a significant increase in alkaline phosphatase activity during an increase in the incubation period up to 41 days.



figure(3) : Effect of soil inoculation with *Bacillus subtilis* or *Aspergillus niger* on activity of alkaline phosphatase during 7 days(A) and 30 days (B) of incubation.

Table (3) Effect of source and concentration of humic acids and inoculation with microorganisms on alkaline phosphatase activity (µg p-nitrophenol g^{-1} soil1h^{-1}) at incubation period of 7 days.

Humic acid source	Humic acid level	Inoculation treatment		
		Non	Fungi	Bacteria
Control	0	215.28	307.8	285.12
	25	215.28	307.8	285.12
	50	215.28	307.8	285.12
Straw	0	215.28	307.8	285.12
	25	246.76	348.48	316.8
	50	314.14	382.3	382.15
Alfalfa	0	215.28	307.8	285.12
	25	257.28	385.78	363.38
	50	309.6	387.89	368.54
Goat	0	215.28	307.8	285.12
	25	312.12	326.3	316.8
	50	340.66	374.56	343.2
Poultry	0	215.28	307.8	285.12

	25	392.48	415.36	400.76
	50	344.43	429.44	366.78
RLSD	21.16			

Table (4)

Effect of source and concentration of humic acids and inoculation with microorganisms on alkaline phosphatase activity (µg p-nitrophenol g^{-1} soil1h^{-1}) at incubation period of 30 days.

humic acid source	humic acid level	Inoculation treatment		
		Non	Fungi	Bacteria
Control	0	174.32	207.83	197.3
	25	174.32	207.83	197.3
	50	174.32	207.83	197.3
Straw	0	174.32	207.83	197.3
	25	221.32	275.33	239.71
	50	322.3	480.59	338.35
alfalfa	0	174.32	207.83	197.3
	25	224.0	259.84	268.49
	50	256	454.39	398.44
Goat	0	174.32	207.83	197.3
	25	317.87	481.2	440.45
	50	262.41	478.9	443.28
Poultry	0	174.32	207.83	197.3
	25	329.05	549.07	470.89
	50	450.27	474.5	464.16
RLSD	25.67			

Tables (3) and (4) showed the interaction effect of humic acid treatments and inoculation treatments on alkaline phosphatase activity at 7 and 30 days of incubation. The highest values of enzyme activity obtained in soils treated with acids extracted from poultry, while the lowest values were obtained in humic acid-untreated soils at all levels of acids and inoculation treatments. In the same context, increasing humic acids level significantly increased enzyme activity at all interaction treatments and types. Treatments including fungal inoculum gave the ighest values compared with their counterparts of bacterial inoculum for all interference treatments. These results confirm the superiority of humic acids extracted from various organic sources in increasing enzyme activity at a wide range of levels and different inoculation parameters. The highest interaction value were 429.44 and 549.07 µg p-nitrophenol g^{-1} soil1h^{-1} for 7 and 30 days of incubation , respectively, in soils treated with humic acids extracted from poultry waste at a level of 50 L ha^{-1} or 25 L ha^{-1}, along with fungi inoculant . These values were over the rest treatments by a percentages ranging between 3.38 and 99.47% at 7 days and between 14.10 and 214.97% at 30 days.

It can be concluded that despite the variation in the properties of humic acids and their nutrients content, they did not negatively affect the activity of the alkaline phosphatase under study conditions at all concentrations, and their positive effect increased when added along with *Aspergillus niger* inoculant .This result is considered encouraging for the use of these acids, especially from poultry waste as a soil conditioner for increasing the activity of alkaline phosphatase and consequently controlling the phosphorus cycle in soil .The economics of using humic acids is easy to prepare and inexpensive, and the waste used is present at sufficient quantities in the local areas.

References

[1] Al-Hadrawi ,Worood Bashir Abdul-Kazem and Meiad Mahdi Al-Jaberi (2023)Effect of the Substrate and Inoculation of Phosphate-Dissolving Bacterial and Fungal with Humic Acids in the Activity of the Alkaline Phosphatase Enzyme in Cadmium Contaminated Soil.International Journal of Agriculture and Animal Production.4(1):10-23

[2] Bremner, J. M. and Edwards, A. P. (1965).Determination and Isotoperation analysis of different forms of nitrogen in soils: I. Apparatus and procedure for distillation and determination of ammonium. Soil Sci. Soc. Amer. Proc., 29: 504-507

[3] Burns, R. G. (1986).Interaction of enzymes with soil mineral and organic colloids. In: P. M. Huang, M. Schnitzer, Organics and Microbes. SSSA Spec. Publ. Serv., 17: 429-451.

[4] Burns, R.G.; Deforest, J.L.; Marxsen, J., Sinsabaugh, R.L.; Stromberger, M.E.; Wallenstein, MD; Weintraub, M. N. and Zoppini, A. (2013).. Soil enzymes in a changing environment: current knowledge and future directions. Soil Biol. Biochem. 58:216–234.

[5] Garcia2,AC,Santos,LA.de Soza LGA,Tavares,OCH,Zota,E.,Gomes, ETM, et al.2016.Vermicompost humic acids modulate the accumulation and metabolism of Ros in rice plants. J.Plant Physiol.192(15):56-63.

[6] Gomes MP, Zonta E, Stafanato JB, Pereira AM (2018) Urease activity

[7] Jasim, Ali H., and Kareem A. H. Alghrebawi. (2020). Effect of soil mulch, phosphorous levels and humic acid spray on the growth and green pods yield of broad bean. DYSONA-Applied Science.88-95.

[8] Jiao Wenxiao ,Xin Liu ,Youyuan Li ,Boqiang Li,Yamin Du1,Zhanquan Zhang2,Qingmin Chen3,Maorun Fu(2022) Organic acid, a virulence factor for pathogenic fungi, causing postharvest decay in fruits. Mol Plant Pathol. 23(2):304-312. doi: 10.1111/mpp.13159. Epub

[9] Khil'ko, SL, Efimova, IV, Smirnova, OV 2011. A/ntioxidant properties of humic acids from brown coal. Solid Fuel Chemistry, 45(6), 367-371.

[10] Li, Chunkai; Li, Qisheng; Wang, Zhipeng; Ji, Guanning; Zhao, He; Gao, Fei; Su, Mu; Jiao, Jiaguo; Li, Zhen; Li, Huixin (2019). Environmental fungi and bacteria facilitate lecithin decomposition and the transformation of phosphorus into apatite. Scientific Reports, 9(1), 15291–. doi:10.1038/s41598-019-51804-7 doi: 10.1002/abio.370040210

[11] Li, H.; Shao, H.; Li, W. and Bai, Z. (2012).Improving soil enzyme activities and related quality properties of reclaimed soil by applying weathered coal in opencast mining areas of the Chinese Loess Plateau. Clean Soil Air Water 40:233–238.

[12] Li,Y.; F.Fang; J. Wei ; X.Wu; R. Cui; G.Li; F.Zheng and D. Tan (2019) Humic acid fertilizer improved soil properties and soil microbial diversity of continuous cropping peanut:A three-year experiment.Sci.Rep.9:12014

[13] Lui,Hongdou;Liqiang Zhang;Yu Sun;Guangbo Xu;Weidong Wang;Renzhe Piao;Zongjun CuiandHongyan Zhao(2021)Degradation of lignocelluloses in straw using AC-1, a thermophilic composite microbial system.Peer J.9:e12364.doi:10.7717/peerj.12364.

[14] Lumactud RA; Gorim LY and Thilakarathna MS (2022) Impacts of humic-based products on the microbial community structure and functions toward sustainable agriculture. Front. Sustain. Food System. 6:977121. doi:

10.3389/fsufs.2022.977121

[15] Mazin Faisal Said and Mohammed Ayad Harbawee (2014) Effect of fungi inoculation on alkaline phosphatase enzyme activity insterilized soil.Journal of Tikrit University for the Humanities. 21)2:(142-148

[16] Page, AL; Miller, R. H. and Keeney, D. R. (1982).Methods of soil analysis. Part 2. 2nd. Ed. ASA. Inc. Madison, Wisconsin, USA

[17] Page, AL; RH Miller and DR Kenney (1982). Methods of soil analysis. Part 2 chemical and biological properties. Amer. Soc. Agron. Inc. Pnnblisher, Madison. Wisconsin.

[18] Sun,Q.; J.Liu; L.Huo; YCLi ; X.Li; L.Xia; Z. Zhou; M. Zhang and B. Li (2020) Humic acids derived from leonardite to improve enzymatic activities and bioavailability of nutrients in a calcareous soil Int.J.Ageic. &Eng. 13(3):200-205.

[19] Tabatabai, M. A. and Bremner, J. M. (1970).Arylsulfatase activity of soils. Soil. Sci. Soc. Am. Proc., 34: 225-339.

[20] Tauqeer, Hafiz Muhammad;Zeeshan Basharat;Pia Muhammad Adnan Ramzani,Muniba Farhad d,Karolina Lewińska;Veysel Turan;Anna Karczewska;Shahbaz Ali Khan;Gull-e FaranandMuhammad Iqbal (2021)*Aspergillus niger-mediated release of phosphates from fish bone char reduces Pb phytoavailability in Pb-acid batteries polluted soil, and accumulation in fenugreek.*Environmental Pollution313(15): 569-590.

[21] Wang S, Liang agroecosystems. Soil Sci Soc Am J 76:161–167.https://doi.org/10.2136/sssaj2011.0078

[22] Wenjie Wan; Yi Wang;Jiadan Tan ; Yin Qin ; Wenlong Zuo; Huiqin Wu ; Huangmei He and Donglan He (2020)Alkaline phosphatase-harboring bacterial community and multiple enzyme activity contribute to phosphorus transformation during vegetable waste and chicken manure composting. 297. 1-33.https://doi.org/10.1016/j.biortech.2019.122406

[23] Yang, R.; Tang, J.; Chen, X. and Hu, S. (2007).Effects of coexisting plant species on soil microbes and soil enzymes in metal lead contaminated soils. Appl Soil Ecol 37:240-246.

[24] Zhao, Y., Sun, Y., Pei, M. et al. Enhanced rice yields are related to pronounced shifts in soil resident bacterial community structures in response to Rhodopseudomonas palustris and Bacillus subtilis inoculation. J Soils Sediments 21, 2369–2380 (2021).https://doi.org/10.1007/s11368-021-02929-8