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## EVALUATION THE EXTRACTION METHODS OF HUMUS ACIDS FROM VARIOUS ORGANIC SOURCES AND THEIR EFFECT ON THE CHEMICAL BEHAVIOR OF PHOSPHOROUS IN THE SOIL

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### ABSTRACT

This study was carried out in order to evaluate the efficiency of some compounds in extracting humus acids (humic and fulvic) from various organic sources and to evaluate the efficiency of the extracted acids in phosphorous availability in the soil. Three chemical compounds were selected for the extraction, which were 0.1 standard of sodium pyrophosphate  $\text{Na}_4\text{P}_2\text{O}_7$ , 0.5 standard of sodium hydroxide NaOH, and a 0.1 standard mixture solution of  $\text{Na}_4\text{P}_2\text{O}_7$  and 0.5 standard of NaOH and symbolized as  $M_1$ ,  $M_2$ ,  $M_3$  and four organic residues (corn molasses  $O_1$ , licorice  $O_2$ ,  $O_3$  poultry, and  $O_4$  sheep Residues). The humus acids were extracted by adding extraction solutions to the organic residues in a ratio of 1:10 solution: organic matter and the purification and separation process was carried out using a centrifuge. The chemical composition of the extracts was analyzed and the organic acids concentrations were determined. The efficiency of these extracts was tested in the laboratory in terms of phosphorus adsorption and release by conducting an adsorption and release experiment. The results revealed that the effect of the extraction method and the type of organic residue on the concentrations of the extracted humic and fulvic acids, as the concentration of humic acid was significantly higher than the fulvic acid in all treatments by 80%. The concentration of humic acid extracted from animal Residues increased by 283% compared to plant Residues. While for the fulvic acid, the increment was by 539%. As for the extraction method, the  $M_3$  treatment was significantly higher than the treatments  $M_1$  and  $M_2$  by 87% and 33% for humic acid, 324% and 11% for fulvic acid, respectively. The adsorption and release experiment also showed a high efficiency of extracted acids in reducing the values of binding energy and maximum adsorption, and the humic acid was significantly higher than fulvic acid in reducing the values of binding energy and maximum adsorption by 81% and 17% respectively. The results also revealed the significant increment of humic acids extracted from animal residues compared to plant residues in a ratio of 43% and 7% for each of the binding energy and the maximum adsorption. Humic acid was significantly higher in reducing binding energy and the maximum adsorption by 57% and 10% compared to fulvic acid. As for the extraction methods, the treatment of  $M_3$  was significantly higher than the treatments  $M_1$  and  $M_2$ . Moreover, in the effect of organic residue, the treatment of  $O_3$  was significantly higher than  $O_1$ ,  $O_2$  and  $O_4$ .

**Keywords :** Extraction methods, humus acids, soil

### Introduction

Humus acids are a component of the basic organic matter in the soil after decomposition which is responsible for many chemical activities in the soil; it is involved in complex reaction and can be used as soil conditioners in addition to its great role in influencing many chemical properties of soil and fertility, such as reducing the degree of soil interaction and increasing the availability of some nutrients that suffer from lack of adsorption and sedimentation especially in calcareous soils (Al-Alwani, 2019). This group of acids includes fulvic acid, humic acid, and Hymatomelanic acid, in addition other types in small quantities, and that the recognition of these different types of acids can be based on their solubility in acids, Basics and Alcohols (Flaig *et al.*, 1975). The International Humic Substances Society (IHSS) has been interested in determining the fragmentation of humus materials from soils, sediments, organic matter and water; for the importance of that topic, this organization developed some typical characteristics that must be provided in the fractionation

solutions of humic substances, which were not to change the composition of these materials (humus acids) upon the extraction, and that the extracted humus materials are free of inorganic contaminants and polyvalent ions. And that the extraction solution is suitable to apply in all soils. Depending on these foundations, many methods have emerged in which extraction solutions for these humus substances are used, including the method proposed by Haynes (1985), which used organic solutions such as formic acid and Formamidic acid, while Achard (1786) used an extraction solution of (0.5N) of NaOH, this method is described in (pege *et al.*, 1982). A solution (0.1N) of  $\text{Na}_4\text{P}_2\text{O}_7$  sodium pyrophosphate was used, which is a method proposed by Lee and Bremner (1949) and described in (Pege *et al.*, 1982). Whereas Schnitzere (1982) proposed a method in which an extraction solution (N 0.5 NaOH and 0.1 N  $\text{Na}_4\text{p}_2\text{O}_7$ ). After all given above, it is clear that there is no specific extraction compound that can be used and considered the best compound; Therefore, this study was conducted in order to evaluation the extraction methods of humus acids from

various organic sources and their effect on the chemical behavior of phosphorous in the soil.

**Materials and Methods**

**Preparation of the organic Residues:** Organic residues were brought from the Organic Agriculture Center - the

Ministry of Agriculture which was (corn molasses O<sub>1</sub>, licorice O<sub>2</sub>, O<sub>3</sub> poultry, and O<sub>4</sub> sheep residues). Air-dried, milled and sifted in a diameter of 0.5 mm to obtain regular particles in terms of size and to get rid of the impurities; the chemical analysis was conducted as shown in Table (1) that revealed some chemical properties of the organic residues.

**Table 1:** Some chemical properties of the organic residues used in the experiment

Compost				Unit	Adjective
Sheep residues O <sub>4</sub>	poultry O <sub>3</sub>	Licorice O <sub>2</sub>	corn molasses O <sub>1</sub>		
5.48	4.31	6.20	6.71	-	PH 1:10
6.31	9.21	5.3	4.2	ds.m <sup>-1</sup>	EC 1:10
653	785.5	567.2	521.9	gm.kg <sup>-1</sup>	Organic matter
378.7	455.59	328.9	302.7	gm.kg <sup>-1</sup>	Organic carbon
25.2	34.2	21.3	18.7	gm.kg <sup>-1</sup>	Nitrogen
15.02	13.32	15.44	16.1	-	C / N ratio
0.65	0.75	0.49	0.51	%	Phosphorous
1.73	1.78	1.52	1.03	%	Potassium
27	33	21	18	%	Moisture content

**Humus acids extraction**

Humus acids (fulvic and humic) were extracted from different organic sources using three types of solutions, which were (0.1N) of Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> (M<sub>1</sub>) sodium pyrophosphate. The method proposed by Bremner and Lee, which mentioned in Page *et al.* (1982). (0.5N) of NaOH suggested by Achard (M<sub>2</sub>) mention in Page *et al.* (1982). A mixture solution of 0.1N (Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> and 0.5N) of NaOH which is the method suggested by Schintzer *et al.* (1982) (M<sub>3</sub>). 1 kg of the organic residue has taken and put into plastic containers; then, 10 liters of the aforementioned extraction solution was added and left for 48 hours, and continuously shaken from time to time. Then the solutions were separated from the solids using a piece of cloth. The process was repeated 3 times to ensure the purification from the solids. The fumigants were collected, which represent the organic acids. The humic was separated from fulvic using the hydrochloric acid HCl solution at a concentration of 6 N for 24 hours for the purpose of coagulation. Then, the fulvic was Isolated from the humic using a centrifuge at a rate of 6000 round / min. The materials were kept in glass containers and putted into the refrigerator. The humus acids concentration were measured using HPLC (High Performance Liquid Chromatography) device the acid concentrations were calculated according to the following equation

$$C_{sample} = \frac{A_{sample}}{A_{stander}} \times C_{stander} \times 10$$

C Sample Represent Sample concentration / A sample Represent Sample Area

C Stander Represent Focus measurement model / A stander Area of the measurement model

**Isothermic adsorption experiment:**

To study the isothermic adsorption of phosphorus, 5 grams of soil was sifted through a diameter of (2 mm), Table (2) revealed the characteristics of the soil used in the study. The sample was placed in a tube of (100) ml capacity; then, added 0.125 ml of humus acids (humic and fulvic) extracted from various organic sources and various methods, mixed with 5 ml of distilled water and left for 24 hours for the purpose of reaction and equilibrium; then, the concentrations of phosphorus (0,25, 50, 75, 100) micrograms. ml<sup>1</sup> were added in the form of a solution of mono potassium phosphate (KH<sub>2</sub>PO<sub>4</sub>), volume of 45 ml and kept for 24 hours after shaking for two hours and then placed in a centrifuge to separate the pure solution from the soil at a speed of 2500 round / minute for 10 minutes, then the samples were filtered. The amount of adsorbed phosphorus was calculated. Then the relationship between the adsorbed phosphorus and the phosphorous in the equilibrium solution was described using Lancemire's equation with a single surface in which the values of binding energy K and the maximum adsorption capacity Xm were calculated.

**Table 2 :** Some chemical and physical characteristics of the study soil

Unit	the value	Adjective
-	7.37	pH 1:1
DCI Siemens M <sup>-1</sup>	1.48	EC 1:1
Centimol of the charge kg <sup>-1</sup> soil	19.18	CEC
	7.71	Soil organic matter
	0.15	Gypsum
	180.00	Carbonate minerals
	4.60	<sup>2+</sup> Ca
	2.10	<sup>2+</sup> Mg

D i s s o l v e d p o

Mmol liter <sup>-1</sup>	0.85	Na <sup>+</sup>	Dissolved negative ions
	0.58	K <sup>+</sup>	
	Nil	<sup>2-</sup> CO <sub>3</sub>	
	1.40	HCO <sub>3</sub> <sup>-</sup>	
	1.01	<sup>2-</sup> SO <sub>4</sub>	
	11.40	Cl <sup>-</sup>	
Mg / kg <sup>-1</sup> soil	31.3	Ready nitrogen	
	11.6	Ready phosphorous	
	102.5	Ready Potassium	
GM kg <sup>-1</sup>		the sand	Minute analysis
	400.00	Silt	
	208.00	Clay	
		Loam	

### Collective emancipation

The same soil was taken on which adsorption treatments were performed and the combined release of available phosphorus was studied by taking the highest concentration only, i.e. (100) micrograms ml<sup>-1</sup>, as 100 ml of sodium bicarbonate solution at a concentration of 0.5 N was added, and the sample was shaken for 2 hours, then placed in The centrifuge at 2500 r / min for 10 minutes after which the soil is separated from the solution. The process was repeated 5 times for each sample, and the available phosphorus was estimated for different treatments.

### Results and Discussions

#### The effect of the extraction method and the source of the organic residue on the concentration of humic and fulvic acids in the extracts

Results in Table 3 showing the effect of the extraction method and the source of the organic residue on the concentration of humic and fulvic acids . Results showed the significant effect of the M<sub>3</sub> method over the M<sub>1</sub> and M<sub>2</sub> method, with an increment percent of 87% and 33% for humic acid, and the increment percent of fulvic acid reached 324% and 11%, respectively. As for the effect of the organic residue source, the results showed the significant effect of the O<sub>3</sub> treatment compared to the rest of the treatments O<sub>1</sub>, O<sub>2</sub> and O<sub>4</sub>, with an increment of 324%, 278% and 9% for humic acid, and an increment of 754%, 586% and 47% for fulvic acid, respectively. The increment in the concentration of humic and fulvic acid extracted from the O<sub>3</sub> treatment (poultry) compared to other residues is due to the nature of the chemical composition of those residues as a result of the combination of the urine and fecal channels and the increase in the percent of carboxylic and phenolic groups (Chen 1996, Al-Balkhi *et al.*, 2006). The effect of the interaction between the extraction method and the type of residue with regard to organic humic acid, the results showed that the highest value was 61.60 micrograms ml<sup>-1</sup> in the treatment of M<sub>3</sub>O<sub>3</sub>, while the lowest value was 8 micrograms. ml<sup>-1</sup> in the treatment of M<sub>1</sub>O<sub>1</sub> in humic acid, and the highest value was 49.60 micrograms ml<sup>-1</sup> in the treatment of M<sub>3</sub>O<sub>3</sub>, while the lowest value was 2.30 micrograms. ml<sup>-1</sup> in M<sub>1</sub>O<sub>1</sub> treatment for the fulvic acid. The effect of the extraction method may be attributed to the chemical compound's layer containing sodium, which contributes effectively to extracting a largest amount of organic acids, in addition to the properties of the compound, such as the degree of basic reaction compared to the other two compounds, the highest value was 12.53 in the M<sub>3</sub> method and the lowest value was 8.65 in the M<sub>1</sub> method.

These results were confirmed by (Al-Tamimi, 1997 and Yaqoub, 1995). Also an increment noticed in the concentration of humic and fulvic acid in animal residues compared to plant residues, with an increase of 283% and 539%, respectively, due to the nature of the chemical composition of those residues and the increase in the percentage of carboxylic COOH and phenolic OH groups.

#### The effect of humus acids (humic and fulvic) extracted by different methods and different organic sources on the values of binding energy K and maximum adsorption X<sub>m</sub>.

Results in Table 4 and 5 revealed the values of binding energy for soil after the application of humic and fulvic acid compared to non-treated soil with humus acids. The results showed that the humic acid gave a significant effect among the fulvic acid in reducing the average values of the K binding energy by 81%. The results also showed the significant effect of the humic acids extracted from animal sources over the humic acids extracted from plant sources in reducing the values of phosphorous binding energy in soil by 43%. As for the effect of the extraction method on the average values of the binding energy of phosphorus; the results indicated the significant effect of the M<sub>3</sub> method in reducing the binding energy values compared to M<sub>1</sub> and M<sub>2</sub>, and by 36% and 26% for humic acid, respectively; this might be due to the high extractive capacity of this compound and the basic characteristics that make it more efficient to extract these compounds. As for the effect of the type of organic residue on the K values, it was found that the humic acid extracted from The treatment of O<sub>3</sub> (poultry) gave the lowest value of K compared to the other treatments O<sub>1</sub>, O<sub>2</sub> and O<sub>4</sub>, with a decrease of 48%, 43% and 8%, respectively, the reason for this is due to the nature of the chemical composition of these residues and its high ability to form organometallic complexes with phosphorous (Hemati *et al.*, 2012). As for the fulvic acid extracted from different organic sources and by different methods, the results showed an increase in the average values of the binding energy K compared to the addition of humic acid with a general rate of all treatments reached to 433%. M<sub>3</sub>, based on the parameters M<sub>1</sub> and M<sub>2</sub>, reduced the binding energy values by 53% and 26%, respectively. As for the effect of the organic residue types, the results showed the significant effect of the O<sub>3</sub> treatment (poultry) in reducing the binding energy compared to the other treatments, as the average binding energy was 0.373 while the binding energy values were 1.09, 0.916 and 0.482 for the treatments O<sub>1</sub>, O<sub>2</sub> and O<sub>4</sub>, respectively. The average values of the binding

energy under the application of fulvic acid compared to humic acid indicates a decrease in the efficiency of this acid in releasing phosphorous from the soil compared to humic acid; this is due to the nature of their chemical composition as humic acid is characterized by the high molecular weight and thus its concentration was increased compared to fulvic acid (Al-Alwani, 2019). While the effect of adding humus acids (humic and fulvic) on the values of the greatest adsorption of soil; the results showed in general that the application of humus acids has decreased the values of the greatest adsorption by 39% and 26% for humic and fulvic acids respectively. The results showed the significant effect of humic acid over fulvic acid in reducing the values of maximum adsorption  $X_m$ , by a rate of 17%. Also, the humic acids extracted from animal source were significantly higher than the same acids extracted from plant sources by a percent 7%, as the maximum adsorption values under the application of humic acid were 640 and 652 micrograms  $gm^{-1}$  for treatment of  $O_3$  and  $O_4$ , while the ratio was 698 micrograms.  $gm^{-1}$  for each of  $O_1$  and  $O_2$ . While the application of fulvic acid has reached 769 micrograms.  $gm^{-1}$  And fulvic as the values were 636, 690 and 690 for each of  $M_3$ ,  $M_2$  and  $M_1$  respectively. As for the fulvic acid, the values were 801, 801 and 839 for the treatments of  $M_3$ ,  $M_2$  and  $M_1$ , respectively. The high level of humic acid reduces the effect of carbonate minerals in the soil, which is considered one of the main factors that negatively affect the availability of phosphorous in the soil by reducing the degree of reaction and dissolving those compounds containing phosphorous by regulating the availability of phosphorous in the equilibrium and reducing its adsorption or sedimentation on Carbonate minerals surfaces (Hemati *et al.*, 2013).

#### The effect of humus acids (humic and fulvic) extracted by different methods and different organic sources on the collective release of phosphorous:

Results in Table 6 revealed the effect of the humus acids application (humic and fulvic) extracted by different methods and different organic sources on the amount of released phosphorous collectively. The results showed that the amount of released phosphorous increased under the application of humus acids by 266% and 102% to humic and fulvic acids respectively. It also revealed that the released amount was higher under the application of humic compared to fulvic acid for all treatments. It was also found that, at the level of one acid type, the results also showed the significant effect of the humic acid extracted from animal sources gave high efficiency in the released phosphorous compared to the same acid extracted from plant sources, at a rate of 43.39. The reason for this is due to the increase in the concentration

of the acid extracted from those residues. As for the effect of the extraction method on the amount of phosphorous collectively under the application of humic acid; the results showed the significant effect of the  $M_3$  method over  $M_1$  and  $M_2$ , by 27% and 13%, respectively. The results showed the significant effect of the humic acid extracted from the  $O_3$  treatment (poultry) and showed high efficiency in the release of the largest amount of phosphorous compared to the same acid extracted from the treatments  $O_1$ ,  $O_2$  and  $O_4$ , by 54%, 41% and 6% respectively, and the reason for this is due to the increase in the acid concentration extracted from those residues compared to other residues. As for the effect of fulvic acid extracted by different methods and from different organic sources; the results in Table 2 showed a decrease in the average values of the released phosphorous, It contains humic acid at a rate of 44%, which is due to the nature of the chemical composition and its low molecular weight (Al-Alwani, 2019). As for the effect of the extraction method of fulvic acid on the accumulative release of phosphorous; the results showed the significant effect of the  $M_3$  method over  $M_1$  and  $M_2$ , as the rate of the released phosphorous for the treatments reached 31.83, 29.31 and 27.03  $mlg Kg^{-1}$  for the treatments  $M_1$ ,  $M_2$  and  $M_3$  respectively, due to the high capacity of this The compound in extracting the largest amount of acid from the residues, in addition to the high basicity compared to the other two compounds (Omid *et al.*, 2015). As for the effect of the type of organic residue on the efficiency of the extracted fulvic acid on the release of the combined phosphorous, the results in Table 2 showed the significant effect of the extracted fulvic acid under the treatment of  $O_3$ , over the treatments of  $O_1$ ,  $O_2$ , and  $O_4$ , as the rate of released phosphorous reached 24.26, 25.64 and 32.83  $mlg Kg^{-1}$ , respectively, while the rate of free phosphorous in the treatment of  $O_3$  was 34.58  $mlg. kg^{-1}$ . The different nature of the chemical composition of the treatment  $O_3$  compared to the rest of the treatments and the high concentration of humus acids, which have the ability to form organic complexes with phosphorous and increase their availability (Huey *et al.*, 2010). according to Table 12 and 13, the increase in the average values of binding and saturating energy can be noticed. The greatest increase in the two treatments  $M_1$  and  $M_2$  compared to the treatment  $M_3$ , but on the basis of the type of organic residue, we also notice the high values of binding energy and greatest adsorption in the treatments  $O_1$ ,  $O_2$  and  $O_4$  compared to the treatment  $O_3$ . This indicates that the extraction method and the type of organic residue are among the most important factors affecting the acid efficiency. The extracted humus and its concentration, which is reflected in its behavior in the equilibrium. These are in agreement with Ghosh and Dassanyal (2012).

**Table 3 :** The effect of the extraction method and the source of the organic residue on the concentration of humic and fulvic acids ( $micrograms.ml^{-1}$ )

Medians of extraction methods	Residues ( O )				Sour type	Method (M)
	$O_4$	$O_3$	$O_2$	$O_1$		
20.92	30.00	36.90	8.80	8.00	H	$M_1$
5.85	7.30	10.40	3.40	2.30	F	
29.37	44.70	49.60	12.30	10.90	H	$M_2$
22.36	20.80	38.00	4.80	3.50	F	
39.17	61.10	61.60	18.00	16.00	H	$M_3$
24.82	38.10	49.60	6.00	5.60	F	
	45.26	49.36	13.03	11.63	H	Residue averages
	22.06	32.46	4.73	3.80	F	

**Table 4 :** The effect of addition of humic and fulvic acids extracted by different methods and from different organic sources on the values of binding energy K (micrograms P. ml<sup>-1</sup>)

Medians of extraction methods	Residues ( O )				Binding energy	Method (M)	Sour type
	O <sub>1</sub>	O <sub>2</sub>	O <sub>3</sub>	O <sub>4</sub>			
0.161	0.206	0.203	0.112	0.123	H	M <sub>1</sub>	K
1.025	1.570	1.375	0.502	0.653	F		
0.140	0.191	0.168	0.095	0.107	H	M <sub>2</sub>	
0.646	1.00	0.709	0.395	0.483	F		
0.103	0.141	0.124	0.074	0.076	H	M <sub>3</sub>	
0.476	0.704	0.666	0.224	0.310	F		
	0.179	0.165	0.093	0.102	H	Residue averages	
	1.09	0.916	0.373	0.482	F		
	2.20				control		

**Table 5 :** Effect of addition of humic and fulvic acids extracted by different methods and from different organic sources on the values of maximum adsorption X<sub>m</sub> (micrograms P. ml<sup>-1</sup>)

Medians of extraction methods	Residues ( O )				Binding energy	method (M)	Sour type
	O <sub>1</sub>	O <sub>2</sub>	O <sub>3</sub>	O <sub>4</sub>			
690	714	714	666	666	H	M <sub>1</sub>	X <sub>m</sub>
839	909	909	769	769	F		
690	714	714	666	666	H	M <sub>2</sub>	
801	833	833	769	769	F		
636	666	666	588	625	H	M <sub>3</sub>	
801	833	833	769	769	F		
	698	698	640	652	H	Residue averages	
	858	858	769	769	F		
	909				control		

**Table 6:** Effect of addition of humic and fulvic acids extracted by different methods and from different organic sources on the cumulatively released phosphorous (mg. P kg<sup>-1</sup>).

Medians of extraction methods	Residues ( O )				Binding energy	method (M)	Sour type
	O <sub>1</sub>	O <sub>2</sub>	O <sub>3</sub>	O <sub>4</sub>			
47.06	35.01	39.65	59.51	54.07	H	M <sub>1</sub>	X <sub>m</sub>
27.03	22.77	22.98	32.02	30.35	F		
52.77	42.20	45.14	62.12	61.65	H	M <sub>2</sub>	
29.31	24.93	25.01	33.75	33.58	F		
29.77	48.32	52.00	71.95	66.82	H	M <sub>3</sub>	
31.83	25.83	28.95	37.98	34.58	F		
	41.84	45.59	64.52	60.84	H	Residue averages	
	24.26	25.64	34.58	32.83	F		
	14.50				control		

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