



Characterization and metal loading capacity of humic acids derived from composted rice straw and olive pomace affected by the humification degree

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ARTICLE INFO

Keywords:

Rice Straw (RS)
Olive pomace
Humic acids
Metal loading capacity
Humification degree

Article history

Submitted: 2020-10-07

Accepted: 2021-02-20

Available online: 2021-06-25

Published regularly: June 2021

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ABSTRACT

The humification degree is a valuable characteristic of humic acids that indicates the maturity and intensity of aromatic rings, important not only for the maturation of composted materials but also for the ability to interact with metal ions. This investigation was conducted to extract humic acids from composted rice straw (RS) and olive pomace (OP) and determine their characteristics (total acidity, carboxylic groups and phenolic groups, elemental analysis and IR absorption). As well as, to assess the impact of humification degree on the metal loading capacity of isolated humic acids *i.e.* HA-a (60% RS+10% OP), HA-b (50% RS+20% OP), HA-c (40% RS+30% OP) and HA-d (cotton stalks and rice straw, farmyard manure). For this purpose, a laboratory study was performed to assess the metal loading capacity of extracted humic acids with Fe⁺², Zn⁺², and Mn⁺² at different pH values (4, 5, and 6). The results revealed that the highest values of total acidity, carboxyl, and phenolic-OH groups were achieved by HA-c. HA-c realized more pronounced values of total carbon and nitrogen content. Adversely, HA-c achieved less pronounced values of oxygen and hydrogen as well as H/C, O/C, and C/N atomic ratios. The loading capacity of Fe⁺² reached optimum values at pH 5 for all studied humic acids. While the maximum loading capacity of Zn⁺² or Mn⁺² was achieved at pH 6. In this respect, the maximum loading capacity of Fe⁺², Zn⁺², and Mn⁺² was obtained for HA-c followed by HA-d. These results seem to confirm that the humification degree governs the loading capacity of humic acids for Fe⁺², Zn⁺², and Mn⁺² which plays an important role in regulating their bioavailability in soil.

How to Cite: Mostafa, M. A. M., Hegazy, A. S. S. I., El-Sedfy, O. M. F., & Abd El-Rhaman, Z. M. (2021). Characterization and metal loading capacity of humic acids derived from composted rice straw and olive pomace affected by the humification degree [Research]. *Sains Tanah Journal of Soil Science and Agroclimatology*, 18(1): 1-6. <https://dx.doi.org/10.20961/stjssa.v18i1.44741>

1. Introduction

Humic acids (HAs) constitute a principal fraction of humic substances and they are commonly described as heterogeneous compounds mixtures of polydispersed dark brown to black colored substances. HAs are formed by humification as a result of the biochemical transformation of decay plants and animals remain (Kosobucki & Buszewski, 2011).

HAs are a complex class of biogenic poly-electrolytes that have various functional groups such as COOH, phenolic-OH, alcoholic-OH, and carbonyl groups responsible for the

formation of complexes with metals. The formation of a covalent link between metal ions and the HAs, the formation of H-bonding, as well as, the electrostatic interface can promote the interaction between humic acid and metal ions. In the micellar aggregates, the hydrophilic part of the molecule including carboxyl groups and phenolic hydroxyl groups apprehends the aromatic structure. Complexation with the hydrophilic part of HAs has been formed due to the interaction with metal ions, also increasing the ratio of aromatic structure (Dudare & Klavins, 2013).

Table 1. HAs samples used and their Humification degree.

Sample	Source	Humification degree (%)
HA-a	Compost A (60% RS + 10% OP + 10% tomato hallum + 10% banana residues + 10% farmyard manure).	52.12
HA-b	Compost B (50% RS + 20% OP + 10% tomato hallum + 10% banana residues + 10% farmyard manure).	71.87
HA-c	Compost C (40% RS + 30% OP + 10% tomato hallum + 10% banana residues + 10% farmyard manure).	77.80
HA-d	Compost D* (cotton stalks, rice straw and farmyard manure) as a comparative sample.	76.15

*Obtained from Soil, Water and Environ. Res. Inst., ARC, Giza, Egypt (Mohamed et al., 2010)

Humic substances complexing capacity varies as a function of pH. Two binding patterns are possibly presented at acid-neutral pH (carboxylates) and alkaline pH (carboxylate and phenolic groups). The binding patterns, binding strength, stability and solubility depend on the complex metal. Complex solubility depends on the concentration of free ionized functional groups and the molecular weight (Garcia-Mina, 2006).

Further, the maximum binding capacity of substances is often equal to the content of acidic functional groups. The binding is influenced by several factors such as pH, ionic strength, kind and amount of acidic functional groups, and molecular weight. According to Cao et al. (1995), changing pH affects metal-ligand binding due to: a) competition between H^+ and M^+ for binding sites, b) competition between HA and inorganic ligands (OH^-) for the metals, c) potential formation of mixed ligand species such as hydrolyzed metal-ligand complexes.

Humification is a natural process of changing organic matter (OM) into humic substances by geo-microbiological mechanisms. The concept of humification refers to the quality of compost. So evaluation of the humification degree of the OM during composting is an agronomic criterion for compost quality (Mayhew, 2004). The humification of OM during composting is demonstrated by humic acid formation with increasing molecular weight, aromatic features, concentrations of oxygen and nitrogen and functional groups in line with the generally accepted humification process Soil OM Theories (Bernal et al., 2009). Dudare and Klavins (2013) focused on the interaction of metal ions with humic substances using different methods and comparing humic substances from different sources. But the impact of humification degree on the formation of humic-metal complexes has not received much attention until now. This investigation was conducted to characterize the humic acids derived from three compost piles made from mainly rice straw (RS) and different ratios of olive pomace (OP). Additionally, to assess the impact of the humification degree of these piles on the loading capacity of extracted humic acids with Fe^{2+} , Mn^{2+} , and Zn^{2+} at different pH values (4, 5, and 6).

2. Materials and Method

2.1 Extraction and purification of humic acids

HAs samples (Table 1) were isolated from mature compost piles (90 days old) conducted in the previous study (Abd El-Rhman et al., 2018). HAs were extracted from compost samples according to Syahren and Wong (2008) as follows:

ten grams of each compost sample was shaken with 100 mL KOH 0.25M (1:10 w/v) in an orbital shaker under N_2 gas atmosphere in sealed bottles for 24 h. The extracts were separated by centrifugation at 10,000 rpm for 20 min. The supernatant was acidified with HCl 6 M to pH 1.0 to precipitate humic acids and left to coagulate for 24 h. Then, humic acids were separated from soluble fulvic acids by centrifugation at 6000 rpm for 15 min.

Humic acid samples were purified by treating with a 5% HF-HCl (5 ml HCl and 5 ml HF per liter) for 36 h (Sánchez-Monedero et al., 2002). After that, they were washed twice by 0.1 M HCl and once with deionized water until no trace of Cl^- was detected. The purified HAs were dried at 40°C under vacuum.

2.2 Characterization of humic acids

The total acidity of humic acids was determined based on the barium chloride ($BaCl_2$) method (Dragunova, 1958). Carboxylic groups ($COOH$) was estimated based on the calcium acetate [$Ca(CH_3COO)_2$] method according to the method described by Schnitzer and Gupta (1965). While phenolic OH groups were calculated by subtracting carboxylic groups content from total acidity.

Elemental analysis for HAs was determined using a combustion-gas chromatography technique Hewlett-Packard 185 (C, H, N, S automatic) microanalysis (VarioElementar/C, H, N, S Germany). Elemental analysis was carried out using the Micro Analytical Center, Faculty of Science, Cairo University. The oxygen content was calculated based on the difference between the sum of the C, H, and N percentages from 100 (Goh & Stevenson, 1971).

Fourier transformed infrared spectroscopy (FT-IR) spectra of humic acids were recorded on KBr pellets in the 4,000–400 cm^{-1} wavenumber using a Jasco FT/IR-4100-A spectrophotometer at Micro Analytical Centre, Faculty of Science, Cairo University.

2.3 Loading capacity of the studied humic acids with metal ions (Fe^{2+} , Zn^{2+} , and Mn^{2+})

Stock solutions for the studied metal ions were prepared using metal sulphate salts, $ZnSO_4$, $MnSO_4$ or $FeSO_4$ at 5×10^{-3} M. Fifty milliliters of metal ion solutions were added to 0.1 g of HA sample and adjusted at different pH values (4, 5, and 6) using NaOH or HCl (0.1N) before shaking for 2 h at room temperature (30°C). After shaking, the suspension was filtered with Whatman paper No.42. The final concentration

of a metal ion in solution was measured using ICP-AAS Model Ultima 2 JY Plasma- JobinYvon. The metal ion concentration uptake from solution was calculated as the amount loaded (q , mg g^{-1}) on HA by the Equation [1].

$$q (\text{mg g}^{-1}) = \frac{C_i(\text{mg g}^{-1}) - C_f(\text{mg g}^{-1})}{\text{wt (g)}} \times \text{Total vol (L)} \dots [1]$$

Where C_i is the initial metal concentration for metal ion before adding to HA, C_f is the final concentration after shaking and filtration. Wt (g) is the weight of HA and Total vol (L) is the metal solution volume.

3. Results

3.1 Total acidity and functional groups of humic acids

The total acidity of the studied humic acids presented in Table 2 ranged from 275 to 377 $\text{mmol } 100\text{g}^{-1}$. Whereas HA-c extracted from compost C contained more pronounced quantities of total acidity followed by HA-d derived from compost D (comparative sample).

The COOH groups content of the studied humic acids ranged from 141 to 200 $\text{mmol } 100\text{g}^{-1}$. Whereas, the maximum values of COOH groups were obtained for HA-d (200 $\text{mmol } 100\text{g}^{-1}$) followed by HA-c (185 $\text{mmol } 100\text{g}^{-1}$) that drove from compost D and C respectively. Meanwhile, the minimum value (141 $\text{mmol } 100\text{g}^{-1}$) was found for HA-a. In the case of the phenolic-OH groups, the maximum value of phenolic-OH was 192 $\text{mmol}/100 \text{ g}$ for HA-c while the minimum value (134 $\text{mmol } 100\text{g}^{-1}$) was found for HA-a isolated from compost A.

3.2 Elementary analysis

The elemental composition and atomic ratios of humic acids extracted from different composts are shown in Table 3. The carbon content of humic acids is ranged from 43.3 to 49.7%. Whereas, the maximum values of carbon (49.7 %) occurred with HA-c followed by HA-d (47.6 %). The nitrogen and sulfur contents were higher in HA-c. In contrast, HA-c contained lower amounts of hydrogen and oxygen. Data are given in Table 2 clear that the maximum sulfur content is 5.1% for HA-b. Whereas, the lowest content of sulfur (2.1 and 4.1%) obtained by HA-a and HA-d, respectively.

Regarding atomic ratios, The HA-c isolated from HA-c can be characterized by the relatively low H/C ratio (0.07). The HA-c achieved the least values (12.43) of the C/N ratio. However, the highest values (14.0) were recorded on HA-d. Concerning the O/C ratio, the most significant values of the O/C ratio were obtained for HA-a.

3.3 Infrared Spectroscopy (IR)

Fourier-Transform Infrared (FT-IR) spectra of the humic acids isolated from the compost samples (Figure 1) exhibited similar bands with different intensity distribution as the following:

- The strong broadband at 3432 cm^{-1} assigned to the H bonded OH group of carboxylates, phenol, alcohols, and NH groups was observed in all humic acids spectra.
- Another band at $2930\text{-}2928 \text{ cm}^{-1}$ is attributed to C–H stretching of aliphatic carbon (CH_2 groups), which is more intense in HA-d and HA-c than in HA-b.
- The spectrum of HA-c and HA-d has a peak at 1714 cm^{-1} corresponding to C=O stretching of COOH. Although, this band doesn't appear at the spectra of HA-d.
- The band at $1660\text{-}1630 \text{ cm}^{-1}$ characterizing the C=O stretching of amide groups (amide I band), quinone C=O were more intense in the HA-d and HA-c.
- Absorption peaks appeared near 1510 cm^{-1} assigned to aromatic–C=C stretch and C=N stretch from amide II.
- Absorption bands in $1460\text{-}1440 \text{ cm}^{-1}$ are due to C-H bending of CH_2 or CH_3 groups. Whereas, the bands of HA-a and HA-c demonstrated a high proportion of aliphatic structures. But this band doesn't appear at the spectra of HA-d.
- The absorption at range $1428\text{-}1423 \text{ cm}^{-1}$ due to C-H bending showed an increase in intensity peaks for HA-b and HA-d.
- In the region of $1265\text{-}1200 \text{ cm}^{-1}$, the absorption band at 1216 cm^{-1} corresponding to C-O stretch and OH deformation of phenols and the OH deformation of carboxylic acids is only found in the spectrum of HA-a and HA-d.
- The peak corresponding to the C-O stretch and OH bending from stretch and OH deformation of phenols and/or COOH appeared in the range from 1126 cm^{-1} for HA-a, HA-b and HA-c.
- A peak at $1050\text{-}1034 \text{ cm}^{-1}$ (C–O stretching of polysaccharide) was found in HA's of HA-a and HA-d.

3.4 Loading capacity of extracted humic acids with Fe, Mn, and Zn

The obtained results in Figure 2 revealed that the amounts of Fe^{2+} loaded onto humic acids at pH values (4, 5 and 6). The loading capacity of Fe^{2+} reached optimum values at pH 5 for all humic acids. Whereas, the highest amount of Fe^{2+} (79.2 mg g^{-1}) was loaded by HA-c followed by HA-d (36.3 mg g^{-1}). However, the amount of Fe^{2+} loaded onto humic acids at pH 6 was still higher than that obtained at pH 4.

Table 2. Total acidity and functional groups of humic acids isolated from different compost samples.

Samples	Total acidity	Carboxyl group	Phenolic group
		(mmol 100g^{-1})	
HA-a	275	141	134
HA-b	301	153	148
HA-c	377	185	192
HA-d	350	200	150

Remarks: HA-a = Humic acid (60% rice straw+ 10% olive pomace); HA-b = Humic acid (50% rice straw+ 20% olive pomace); HA-c = Humic acid (40% rice straw+ 30% olive pomace); HA-d = Humic acid (cotton stalks and rice straw, farmyard manure)

Table 3. Elemental composition and atomic ratios of humic acids isolated from compost samples

Samples	Elemental composition					Atomic ratios		
	C %	H %	N %	S %	O %	H/C	O/C	C/N
HA-a	43.3	7.6	3.4	2.1	43.6	0.18	1.01	12.74
HA-b	44.7	6.5	3.3	5.1	40.4	0.15	0.90	13.55
HA-c	49.7	3.5	4.0	4.3	38.5	0.07	0.77	12.43
HA-d	47.6	4.2	3.4	4.1	40.7	0.09	0.86	14.00

Remarks: HA-a = Humic acid (60% rice straw+ 10% olive pomace); HA-b = Humic acid (50% rice straw+ 20% olive pomace); HA-c = Humic acid (40% rice straw+ 30% olive pomace); HA-d = Humic acid (cotton stalks and rice straw, farmyard manure)

In the case of Zn^{2+} , the loaded Zn^{2+} ($mg\ g^{-1}$) gradually increased when increasing pH from 4 to 6 for all humic acid samples. Whereas, the highest loaded Zn^{2+} was achieved by HA-c ($174.01\ mg\ g^{-1}$) and followed by HA-d ($161.1\ mg\ g^{-1}$) > HA-b ($151.0\ mg\ g^{-1}$), shown in Figure 2.

In the case of Mn^{2+} , the obtained results in Figure 2 cleared that the lowest values of loaded Mn^{2+} on the studied humic acids were achieved at pH 4 and 5. However, loaded Mn^{2+} on the humic acids were maximized at pH 6. Hence, it could be arranged as the following descending order: HA-c ($98.8\ mg\ g^{-1}$) > HA-d ($77.7\ mg\ g^{-1}$) > HA-b ($53.6\ mg\ g^{-1}$) > HA-a ($45.7\ mg\ g^{-1}$).

4. Discussion

Humic acids extracted from composted materials which contain a higher ratio of olive pomace (Table 1) were able to produce more acidic functional groups of humic substances. These results seem to confirm that the higher humification degree of these materials leads to increases in the formation of oxygen-containing functional groups such as carboxyl and phenol (Baddi et al., 2004; Senesi & Plaza, 2007).

Regarding the elementary analysis of the studied humic acids, the maximum values of carbon and nitrogen occurred

with HA-c (40% RS+30% OP). These values depending on the type of composted materials and the humification degree that occurred which is supported by Barje et al. (2012) 's studies who reported that humic acids extracted during composting of olive mill waste mixed with municipal solid waste showed a high level of carbon (49.9%-54.4%). Conversely, the minimum values of oxygen and hydrogen as well as H/C, O/C and C/N atomic ratios were realized for HA-c. Therefore, the lower values of H/C (Table 2) indicate the higher aromaticity and condensation of aromatic unite. However, the highest values of H/C are indicative of humic substances with less condensed or open aromatic structures and a relatively high content of aliphatic compounds (Amir et al., 2010; Boguta & Sokołowska, 2016). The decrease in C/N (Table 2) was attributed to an increase of N content of the structure of humic substances arising from organic material decomposition and may indicate a greater humification level (Ahmadou et al., 2013). However, the lowest one was realized for HA-c. Whereas, the O/C ratio is related to the amount of the functional groups containing oxygen of humic substances. The decrease in the O/C value refers to carbohydrate and functional groups containing oxygen of humic substances (Canellas & Façanha, 2004).

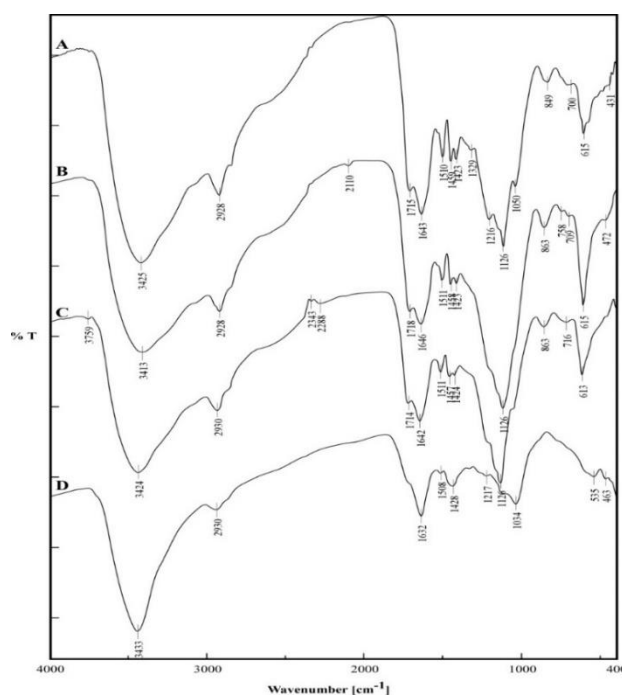


Figure 1. Fourier-Transform Infrared (FT-IR) spectra of humic acids extracted from different compost samples. (Remarks: A = HA-a (60% rice straw+ 10% olive pomace); B = HA-b (50% rice straw+ 20% olive pomace); C = HA-c (40% rice straw+ 30% olive pomace); D = HA-d (cotton stalks and rice straw, farmyard manure)

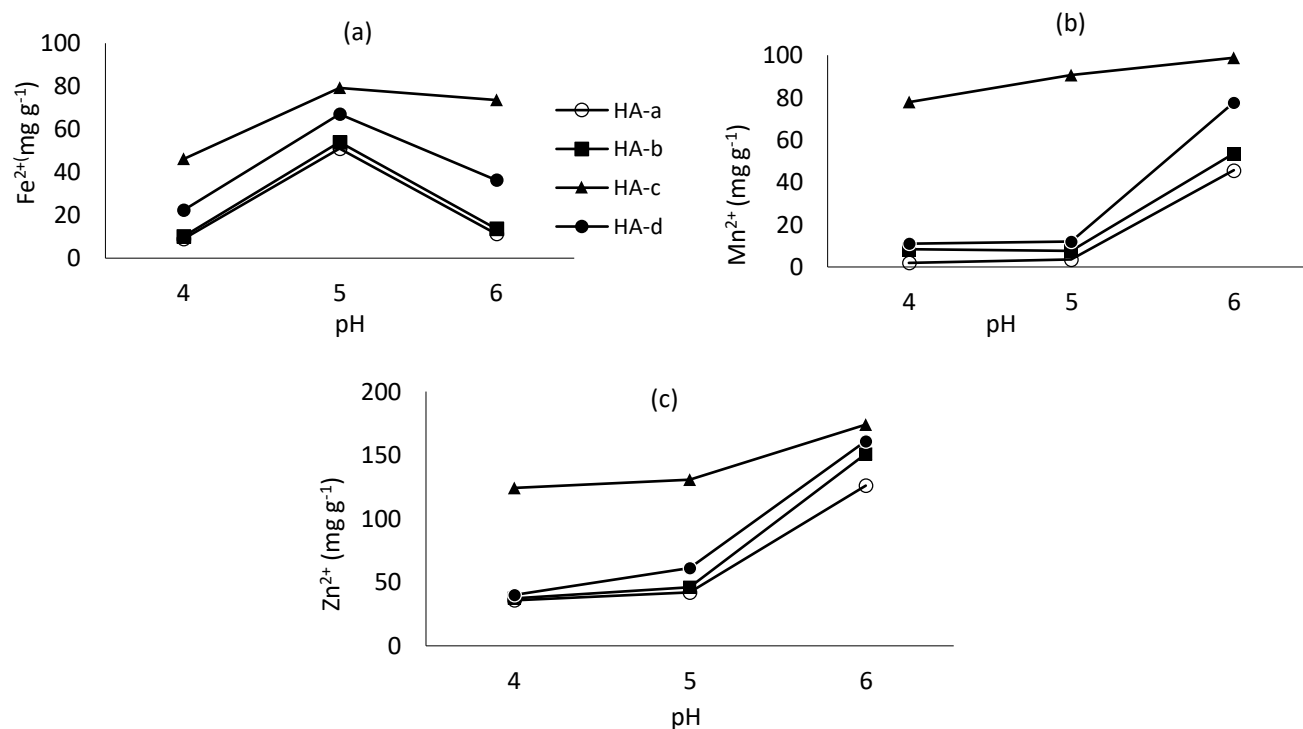


Figure 2. Metals ((a) Fe²⁺, (b) Mn²⁺, (c) Zn²⁺) loading capacity of humic acids isolated from different compost samples.

Moreover, the FTIR spectrum of the extracted HA's showed a predominance of aromatic, aliphatic, carbonyl, and amid groups that contained all major characteristic absorption peaks of humic soil materials as described by [Stevenson \(1994\)](#). A typical HA molecule contains aromatic rings and aliphatic chains that host numerous carboxylic, phenolic, hydroxyl, and other functional groups. The oxygen-containing groups (-O- or O=) are the most numerous; and there is a small percentage of N and S binding sites exist. This phenomenon is related to the humification of organic matter during composting increased resulting in the formation of humic acids with high molecular weight, aromatic characteristics, and functional groups. Humic substances are produced and humic acid-like organic increases, while fulvic acid-like organic and water-extractable organic decrease due to microbial degradation ([Bernal et al., 2009](#)). Also, the humification degree governs the adsorptive capacity of HA molecules for various organic and inorganic soil constituents to exert their influence on the formation, conditioning, and fertility of the soil.

Regarding the effect of pH on the metal loading capacity of humic acids, the amounts of Fe²⁺ ([Figure 2](#)) are complexed with humic acids depends on the pH and oxygen-containing the functional group which correlated with the origin of humic acids (formation and humification process). ([Catrouillet et al., 2014](#)) reported that Fe²⁺ forms mainly bidentate complexes, some tridentate, and only a few monodentate complexes with HA. It is mainly adsorbed on carboxylic groups at acidic and neutral pH. Carboxy-phenolic and phenolic groups play a major role at basic pH. Also, they mentioned that the major adsorbed species are Fe²⁺ at acidic and neutral pH (2.95, 4.95, and 6.93), Fe (OH)⁺ appears at alkaline pH (8.13-9.9).

The highest values of the loading capacity of humic acids by Zn²⁺ ([Figure 2](#)) were achieved at pH 6. This phenomenon refers to the competition of H⁺ with Zn²⁺ on ion-exchange sites of adsorbent at acidic pH values. At alkaline pH, the negative charge on humic particle surfaces increased. The metal adsorbed onto these negatively charged surfaces more easily as a result of strong electrostatic attractive force between organic ligands and metal ions ([Li et al., 2010](#)).

The results confirmed that the humification degree of the final compost product reflects on the extracted humic acid content of carboxylic and phenolic groups. Consequently, the loading capacity of the extracted humic acids by Fe²⁺, Mn²⁺, and Zn²⁺ markedly enhanced. Therefore, increasing the loading capacity of the studied humic acids could be enhanced soil nutrient availability which reflected on nutrient content and their uptake by the plant.

5. Conclusion

The increasing olive pomace ratio up to 30% of pile composition could be maximized the humification degree of the final compost product which reflects on raising the loading capacity of the extracted humic acids by Fe²⁺, Mn²⁺, and Zn²⁺. In this respect, HA-c realized the maximum value of loading capacity by Fe²⁺ (79.2 mg g⁻¹), Mn²⁺ (98.8 mg g⁻¹), and Zn²⁺ (174.0 mg g⁻¹). Consequently, the maximized loading capacity of the studied humic acids could be enhanced soil nutrient availability and nutrient uptake.

Declaration of Competing Interest

The authors declare no competing financial or personal interests that may appear and influence the work reported in this paper.

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