

The influence of compost humic acid quality and its ability to alleviate soil salinity stress

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Abstract

Purpose Soil salinization is one of the most severe abiotic stresses which threaten sustainable agriculture in arid and semi-arid regions including Morocco. The application of organic amendments like composts to saline soils has been confirmed to alleviate soil salinity stress. The present work aims to study the effect of humic acids (HA), as one of the products resulting from organic matter decomposition, on soil salinity reduction.

Methods In this perspective, four composts made from mixtures corresponding to initial C/N ratios of 25, 30, 35 and 40 were tested. The four composts were made from mixtures of different proportions of greenhouse wastes, olive mill waste and sheep manure in Souss-Massa region. Representative samples underwent physicochemical analysis, extraction, purification and analysis of HA. The maturity of compost was assessed through some maturation index, prior to the main trial of the interaction between extracted HA and sodium carbonates salt (Na_2CO_3), largely encountered in saline soils.

Results The results showed that the compost with initial C/N ratio of 35 is the most recommended for application as an amendment to alleviate soil salinity.

Conclusion The salt stress alleviating effect of compost may be advocated to compost humic acids and their ability to chelate sodium on their carboxylic sites.

Keywords Alkalinity, C/N ratio, Compost, Humic acid, Soil salinity

Introduction

Soil salinity is a rising agricultural problem throughout the world, and concerning about 100 million hectares in 1994 (Heuer 1999). The severity has been continuously increasing to reach more than 800 million hectares in 2008, which represents 6 % of the world's agricultural land surface with a share of 3.8 % in Africa only (Manchanda and Garg 2008). In Morocco, soil salinity constitutes the second threat

after soil erosion and concerns nearly 160,000 ha of agricultural land surface (Montanarella et al. 2016; Kahime et al. 2018).

The salinization is a type of soil chemical degradation, restricting its environmental quality and agricultural production (Liang et al. 2005). The soil salinity has been expanded at a broader scale and caused an adverse impact on soil structural stability, permeability and bulk density (Tejada and Gonzalez 2005). Salt accretion is generally sprung from seawater and irrigation water holding few quantities of sodium chloride (NaCl) (Flowers and Yeo 1995; Tester and Davenport 2003). Such ions, especially sodium, have a critical effect on plants growth by affecting the metabolism and reducing its photosynthetic efficiency (Flowers and Yeo 1995; Mäser et al. 2002). To overcome this abiotic stress, the organic amendments (OA), like composts, have proved their effectiveness as a good solution to soil salinity issue. Compost can improve soil fertility and increase the crop accessibility to nutrients, leading to

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good plant growth as well as reducing the damaging effects of salt stress (Çimrin et al. 2010). The OA efficiency consists of its ability to decrease the exchangeable sodium (Na⁺) percentage, accelerate its leaching and increase simultaneously the water-holding capacity while maintaining adequate electrical conductivity (El-Shakweer et al. 1998). Additionally, an appropriate amount of organic matter (OM) in saline soils increases soil bulk density, soil structural stability, and as a result, soil microbial biomass (Tejada and Gonzalez 2005).

The fate of the OM within the soil, takes place generally through two stages, degradation and formation/development. The degradation step is known by the reduction of the size of the particles after fragmentation and decomposition of the initial products as plant and animal residues by the microorganisms. In the phase of development, the OM is reorganized in the form of recalcitrant macromolecules called humic substances (non-mineralized organic constituents) (Lashermes 2010).

Humic substances are heterogeneous macromolecules that can be divided into three fractions: humins, humic acids (HA) and fulvic acids (FA) (Mosley and Mosley 1998). Regarding solubility, HA and FA are extractable in alkaline conditions, and separable from the humins which represents the non-soluble fraction. The separation of HA from FA occurs after, by changing alkaline to acidic conditions, where the HA precipitate, leaving the FA in solution as supernatant (MacCarthy et al. 1990; Andreux and Munier-Lamy 1994). About 65 to 70 % of humic substances are constituted only from fulvic and humic acids (Hatami et al. 2018). In comparison with FA, HA are characterized by more dark color and higher molecular weight (103-105 Da) (Badr et al. 2012; Zingaretti et al. 2018), and are characterized by higher carbon content and lower oxygen content (Zingaretti et al. 2018). Among these build products from OM breakdown, special interest has been attributed many decades to HA that have shown a particular potential in reducing soil salinity and improving soil fertility. There is no unanimous defined structure of these elements. Several models of humic acid structures have been established over time by: Fuchs, Dragunov, Flaig, Steelink, Stevenson, and Schulten models (cited in: Al-Faiyz 2017). Nevertheless, these macromolecules are known to contain a large amount of ionizable functional groups such as phenols, carboxylic acids, alcohols, thiols, and ammonium, in which the major binding sites are attributed to the presence of the oxygen (Badr et al.

2012). Several studies had proved the role of HA as crops anti-stress agent under saline conditions and for improving soil properties, favoring plant nutrient uptake, and then increasing crop productivity (Bacilio et al. 2016; Baris et al. 2009; Javanshah and Nasab 2016; Kulikova et al. 2005; Mosa 2012; Ouni et al. 2014; Xudan 1986). However, their negative effect was reported in some cases for certain plants by decreasing nutrient intake even when alleviating soil salinity (Aydin et al. 2012; Liu and Cooper 2002).

The properties of humic substances in composts may not be well studied alone as organic fraction unless stripped to its inorganic components. The extraction of humic substances, from soils or organic wastes, was widely performed by alkaline extraction according to several methods, e.g. with Na₄P₂O₇ (Ayuso et al. 1997), NaOH (Piccolo et al. 1992) and KOH (Sortino et al. 2014).

Regarding origins, it has been demonstrated recently that green waste compost constitutes an excellent source of humic substances (Zhang et al. 2019). However, Smidt et al. (2004) reported that pure green wastes show lower humification than mixtures from different input materials (biowaste from households + green wastes + others), especially when using a mixture of yard and kitchen waste with 50 % of organic matter at the commencement of composting process.

Compost maturity is not described by a single property, but best tested by different physical (odor, color, temperature), biological (microbial activity indicators, phytotoxicity, etc.), and physical/chemical (C/N, CEC, pH, humification indices, etc.) properties. The degree of humification of OM is generally accepted as an indicator of maturity, since the maturation is known by the formation of some humic-like substances. But it had been proved that humification parameters are not feasible to indicate maturity for all kinds of compost (Bernal et al. 1998; Paredes et al. 2000). However, in the case of city refuse compost, Iglesias-Jimenez and Perez-Garcia (1992a) had established the validity of humification level as a criterion of compost stability.

Compost initial C/N ratio is another parameter commonly known to influence the maturity of the final compost. By comparing three initial C/N ratios (15, 18, and 21) of pig feces and corn stalks co-composting, Guo et al. (2012) reported that compost with the lowest initial C/N had significantly the lowest germination index. On the other hand, the co-composting of pig manure and sawdust revealed that lower initial C/N would require 63 days as

composting period, but it can reduce the quantity of sawdust used (Huang et al. 2004).

The objectives of this work is twofold: first to evaluate compost maturity by investigating some humification quality parameters, and second to study the ability of HA to alleviate salinity induced by sodium carbonate (Na_2CO_3) through electrical conductivity (EC) and pH assessment in the resulted solution after adding increasing quantities of the Na_2CO_3 solution.

Material and methods

Composts properties

The composts preparation was made based on the waste availability and the use of their additional carbon and nitrogen content. Four types of wastes were selected: end-cycle tomato plants, melon wastes, olive mill wastes and sheep manure. Raw materials

were ground using silage brewer, and representative triplicate samples were taken for carbon, nitrogen, and moisture analysis (Table 1) prior to mixture using formula below given by Soudi (2005):

$$\frac{C}{N}(\text{mixture}) = \frac{\sum_{n=1}^{\infty} (Q_n [C_n (100 - M_n)])}{\sum_{n=1}^{\infty} (Q_n [N_n (100 - M_n)])}$$

Where:

C/N (mixture): C/N ratio of the resulting materials to compost

Qn: Quantity of the fresh material (n) in tons WM

Cn: Total carbon content of the dry material (n) in % DM

Mn: moisture content of the fresh material (n) in % WM

Nn: Total nitrogen content of the dry material (n) in % DM

Table 1 Analyses of carbon, nitrogen, and moisture content of raw materials

Organic wastes	Total organic Carbon (%) DM	Total Nitrogen (%) DM	C/N ratio	Moisture content (%) WM
End-cycle tomato plants	39.2 ± 0.7	0.78 ± 0.19	50 ± 1	26.0 ± 2.0
Melon wastes	30.5 ± 0.5	1.47 ± 0.23	21 ± 1	17.0 ± 1.6
Sheep manure	38.0 ± 0.8	2.00 ± 0.27	19 ± 1	32.0 ± 1.8
Olive mill waste	34.6 ± 0.5	0.87 ± 0.14	40 ± 1	36.0 ± 2.2

The precedent formula was used to calculate the needed shares of the different materials to get the wanted (defined) C/N-ratios of 25 to 40, and has

resulted in the composition (fresh weight / fresh weight) of the following four mixtures (Table 2).

Table 2 Proportion of raw material used to constitute the 4 treatments

Treatments (Initial C/N ratio)	Proportion (% w/w of fresh matter)			
	Melon waste	Sheep manure	Olive mill waste	Tomato waste
CN 25	10	55	5	30
CN 30	10	30	20	40
CN 35	5	20	20	55
CN 40	5	10	15	70

The standard pile composting was with open windrows of 3 m width and 1,6 m height. Turning was performed using backhoe loader every 10-15

days when the temperature fell below 45 °C. Until reaching the optimal value, the moisture content of the mixture was adjusted within the optimal range

(50-65 %) using an onward sprinkler. Composting process has lasted for 172 days, and resulted in four composts: CN25, CN30, CN35 and CN40 (Table 3). Subsamples of each mixture were collected from the produced compost, air dried, and characterized. pH and EC were measured by the extraction method with distilled water 1:5 soil/water (w/w). Total nitrogen was determined by Kjeldahl's method. The analysis

of nitrates and ammonium was based on the extraction, followed by titration.

The total organic matter content was determined by the mass loss of ignition of dry sample at 550 °C for 24 hours. Total organic carbon (TOC) was estimated from the OM based on the following formula of Iglesias Jiménez and Pérez Gracia (1992b). $TOC = 1,703 + 0,520 OM$.

Table 3 Chemical properties of the studied composts

Treatments	C/N 25	C/N 30	C/N 35	C/N 40
Initial C/N ratio of feedstock mixture	25	30	35	40
Prof final composts	-	-	-	-
pH	7.9	7.9	7.8	7.9
EC (mS/cm)	23.4	21.6	19	22
OM (% DM)	45,2	68,6	57,3	73,5
TOC (% DM)	25,2	37,4	31,5	39,9
N tot (% DM)	2.1	2.2	2.1	2.1
C/N ratio	12	17	15	19
NO ₃ -N (mg/kg DM)	39	42	50	37
NH ₄ -N (mg/kg DM)	13	14	14	14
NO ₃ -N / NH ₄ -N	2.9	3.1	3.7	2.7

Alkaline extraction

The extraction of humic substances was performed according to De Nobili and Petrusi (1988). It was carried out with 0.1 M Na₄P₂O₇ added to 0.1 M NaOH solution with a ratio of sample/extractant (1/10) and shaking for 1 hour under nitrogen flux. After centrifugation at 36.000 g for 30 min, supernatant was filtered through 0.2 µm membrane filters and stored at 4 °C. One part of the solution was used to separate organic and inorganic fractions, and the rest was kept for isolation and purification of humic acids.

Solid-phase extraction (SPE)

An aliquot of 25 ml of the supernatant was carefully removed and adjusted to pH 1.5 by adding concentrated H₂SO₄ (96 %). Humic acids were separated as a precipitant by centrifugation at 18.000 g for 30 min and stored. The separation of non-humified organic matter from fulvic phase and the following isolation of the fulvic-like acids were made according to Sequi et al. (1986) by using a column packed with polyvinylpyrrolidone (Polyclar). The

non-retained (non-phenolic) fractions were eluted from the columns with H₂SO₄ 0.01 N. The eluate was collected in 50 ml volumetric flasks. The adsorbed fractions were eluted with NaOH 0.5 N and collected in the centrifuge tubes which contained the precipitated humic acids. The contents of the tubes (phenolic fractions) were quantitatively transferred into calibrated 25 ml flasks and brought to a volume of 25 ml with NaOH 0.5 N. Solutions in 25- and 50-ml flasks contained phenolic and non-phenolic fractions, respectively, in addition to the non-separated solutions which were analyzed separately to determine the amount of carbon. The organic carbon of the extracts was determined after appropriate dilution by high temperature catalytic oxidation and subsequent non-dispersive infrared spectroscopy and chemoluminescence detection (TOC-VCPN Shimadzu) after eventually neutralizing samples with concentrated sulphuric acid. The total organic carbon was determined with the Elemental Analyzer Costech Instruments Elemental Combustion System.

Since the humic substances constitute the final compounds in the pathway of the organic matter decomposition, the evaluation of humification indices constitutes one of the issues in defining compost

maturity (De Nobili and Petrusi 1988; Iglesias-Jimenez and Perez-Garcia 1992a; Tittarelli et al. 2002). In this work, C_{ext} (extractable carbon), C_{phen} (phenolic carbon) and C_{n-phen} (non-phenolic carbon) (Fig. 1) were used to evaluate the humification index (HI) defined by De Nobili and Petrusi (1988) as the

carbon ratio of non-phenolic fraction to the carbon in humified fraction (phenolic fraction). The percentage of humified C in compost is commonly expressed by the degree of humification (DH) calculated as the ratio of humified carbon (phenolic fraction) to the total extractable carbon (Ciavatta 1988).

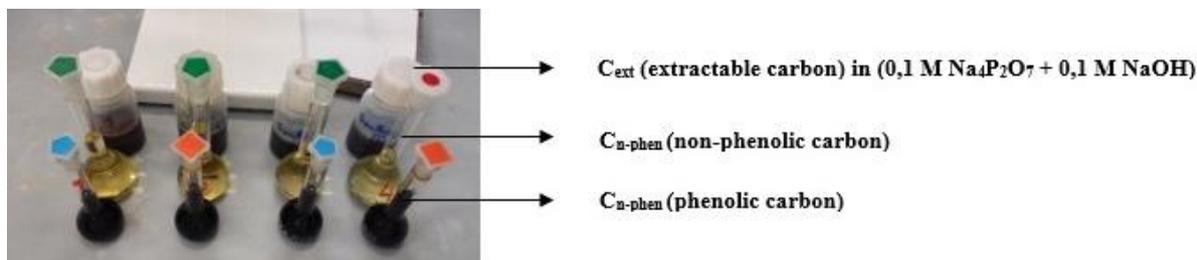


Fig. 1 The different fractions of carbon

Purification of humic acid

In order to isolate humic acids, the solution was separated by centrifugation from the supernatant (fulvic fraction). The precipitate obtained (humic acids) was washed with acidified distilled water, coagulated for 24 h at 4 °C and underwent a lyophilization to get its powder form.

Interaction between humic acids and sodium carbonate (Na_2CO_3)

During the whole process pH-value and EC were monitored (results see fig 2 and 3) to assess alkalinity and salinity. The idea is that humic acids from compost react with dissolved Na_2CO_3 which is dissolved, releasing carbon dioxide and thereby decreasing the salinity of the soil solution: $2HA + Na_2CO_3 = 2NaA + H_2CO_3 = 2NaA$.

To perform a reaction with Na_2CO_3 (0.2M) in solution, the humic acids must be prepared in an aqueous state. For this reason, 20 mg of dry humic acid was transferred to a beaker of 50 ml and a sufficient quantity of diluted NaOH was added to get a solution with pH of 7. The interaction was carried out using a Mettler Memo 40 automatic titrator containing a solution of Na_2CO_3 . A volume of 0.1 ml of this solution was added every 3 minutes or longer if required to stabilize the electrode reading. A blank experiment was performed simultaneously with an aliquot part of distilled water into which the same amount of Na_2CO_3 was introduced.

Statistical analysis

The four C/N compost ratios plus the water, as a control, were considered as a factor in the analysis of variance (ANOVA) to evaluate if there are differences for pH and EC for each level of this factor. Also, since Na_2CO_3 is a quantitative factor, it was included in the ANOVA that leading to an analysis of covariance (ANCOVA). In case the compost factor has a significant effect, Duncan's multiple range test was used to identify homogeneous groups of composts. The SAS software (SAS) was used for statistical analysis.

Results and discussion

Compost stability

Table 4 shows the obtained values of carbon content of different fractions, humification index (HI) and degree of humification (DH) for each of the four composts. The values of HI for the different mixtures taken simultaneously at the end of composting process indicate that C/N 35 had relatively the lowest HI due to the lowest quantities of non-humified compounds and then a high quantity of humified carbon as confirmed by the values of DH. According to Ciavatta (1988), treatments C/N 30 and C/N 35 are then classified as stable composts since their HI are not exceeding 0.35. The high HI values of C/N 25 and C/N 40 may be largely explained by a simultaneous release of other degradable substances soluble in the extracting solution. On the light of these data, compost of C/N 35 seems to constitute the mixture apt to reach rapidly the maturation phase.

This was proved equally by Azim et al. (2014) who studied these four feedstock-mixtures in the perspective to assess the development of the C/N ratio and the temperature during the composting

process. Azim et al. (2014) reported that the third mixture (corresponding to the initial C/N=35) had the longest thermophilic phase, and had produced the most mature compost.

Table 4 Values of carbon content in different fractions and the humification indices

Initial C/N	Fraction	Carbon content (mg/g DM)	HI	DH
25	C _{ext}	3,3	0.43	0.78
	C _{n-phen}	1,1		
	C _{phen}	2,6		
30	C _{ext}	2,7	0.33	0.77
	C _{n-phen}	0,7		
	C _{phen}	2,1		
35	C _{ext}	2,9	0.27	0.81
	C _{n-phen}	0,6		
	C _{phen}	2,4		
40	C _{ext}	2,9	0.39	0.81
	C _{n-phen}	0,9		
	C _{phen}	2,4		

C_{ext}: total alkaline extractable carbon

C_{n-phen}: Carbon content in the non-phenolic fraction

C_{phen}: Carbon content in the phenolic fraction

Reaction of HA with Na₂CO₃

As previously mentioned, the titration was done with Na₂CO₃ 0.2 M at high conductivity (23 mS.cm⁻¹) and alkaline pH (11) which represents the specific features of the salts in saline soils. Humic acids from different mixtures behave differently in presence of the salt. The curves in Fig. 2 and 3 illustrate the variation in EC and pH, respectively, in the humic acid solution with the increasing amount of Na₂CO₃.

The ANCOVA results showed that the treatment (4 composts and water as a control) has a very highly significant effect on EC (F value=97, p-value<0.0001), that EC and Na₂CO₃ are significantly related (F value=2932, p-value<0.0001), and that the interaction between the treatment and Na₂CO₃ is very highly significant (F value=244, p-value<0.0001). This means that the mean EC is different for the 5 treatments (4 composts and water as a control), that there is a strong relationship between EC and Na₂CO₃, and that this relationship differs from one treatment to another; the latter is clearly shown on Fig 2.

The Duncan posthoc test indicates that there are 4 homogeneous groups of treatments:

- Group 1: C/N 40 compost with the highest mean EC (508 μS/cm),
- Group 2: water (380 μS/cm)
- Group 3: C/N 25 compost (313 μS/cm)
- Group 4: C/N 30 and 35 composts which have the lowest mean EC (151 and 157 μS/cm, respectively).

The four EC curves underwent an immediate decrease directly after the addition of the first salt drops in solution. This decrease may be largely explained by the reaction that can be happening between the salt and the functional groups of humic acids. However, the major functional groups that could be involved with salt reaction in this present experiment are carboxylic groups. This hypothesis is supported by the fact that carboxylic groups are subject to dissociation at pH>4 while the dissociation of phenolic groups occurs only after pH>8 (Motta et al. 2016; Randelović et al. 2016). This is also demonstrated by the formation of electrical double layer resulting from a dissociation of acidic functional groups in aqueous media (Tombácz 1999).

However, other authors have considered that proton dissociation is higher for phenolic sites due to the high relative level of proton occupation and the higher energy required to remove protons (Rey-Castro et al. 2009; Xu et al. 2016). Besides, functional groups with S and N should be also considered whatever the responsible function. Humic acids have proved their ability to perform complex formation, ionic exchange, and oxidation-reduction reactions (Davies et al. 1997). These substances had shown also their aptitude as a sorption agent of some pollutants present in solution, and their chelating potential (Zingaretti et al. 2018). The complexation property toward the metal ions, illustrated by Stevenson (1982) has been considered in several studies. In fact, the spectrometric analysis had allowed estimating the ability of humic acids, issued from compost, to chelate 0.04 mg Fe^{2+} /mg HA (Zingaretti et al. 2018). Also, SEM-EDX image and FTIR of HA fractions, before and after adsorption, disclosed the responsibility of phenolic and carboxylic groups for the Cu^{2+} sorption (He et al. 2016). Humic acid, extracted from compost, had shown its capacity to adsorb heavy metals such as Cd and Ni from contaminated sediments with regard to commercial HA (Zhang et al. 2019). HA has proved overall its tendency to coagulate many metallic ions such Mn^{2+} , Co^{2+} , Ni^{+} , Zn^{2+} , Cu^{2+} , Fe^{3+} , and Al^{+} (Khan 1969). Consequently, and considering the low relative molar weight of sodium cation (Na^{2+}), it is possible to suggest a complexation of sodium on the carboxylic sites of humic acid fractions in the earlier drop of Na_2CO_3 corresponding to lower pH values. The following EC increase may be attributed to the saturation of sites and the association of all protons. This last was released as soon as adding almost 3 mg of Na_2CO_3 as shown by pH curves that drift slightly downward. EC curves took new ascending directions after adding 3 mg of Na_2CO_3 , indicating proton binding and then decrease of net negative charge.

The ANCOVA results indicated very highly significant effect of compost on pH (F value=207, p-value<0.0001), showing that the mean pH differs between the 5 treatments. Indeed, there were two groups of treatments following the Duncan's multiple range test:

- Group 1: the control treatment (water) with the highest mean pH (9.7)

- Group 2: all the 4 composts with much lower mean pH ranging from 5.6 for C/N=35 to 5.8 for C/N=25.

Also, there is a very highly significant effect of Na_2CO_3 on pH (F value=999, p-value<0.0001). It is clear that there is a strong relationship between pH and Na_2CO_3 . Thus, the covariate (Na_2CO_3) can predict pH or the pH is influenced by Na_2CO_3 . Finally, there exists a highly significant interaction between compost and Na_2CO_3 . This implies that the relationship between pH and Na_2CO_3 is not the same for the 5 treatments (the regression slopes are different). Indeed, Fig. 3 shows this clearly.

The increase of pH curves occurred differently depending on the treatments. For example, at the same increment of the added salt, the alkalinity of the solutions increased for all composts but with respect to the following order: pH (C/N 35) < pH (C/N 30) < pH (C/N 25) < pH (C/N 40). After reaching the maximum decrease, EC curves have taken upward trends, but the difference recorded consists of the quantity of added salt to reach minimum EC values for each of the 4 composts. In fact, minimum EC value was reached after adding the minimum amount of Na_2CO_3 (2.54 mg) to C/N 25, intermediate amount (3.82 mg) to C/N 30 and C/N 40, but the maximum amount (5.08 mg) to C/N 35. This result reflects the high relative tolerance of C/N 35 to higher salt addition. The properties of the studied humic acids therefore varied depending on the matrix of the mixture from which they were produced. It has been shown that the sensitivity of humic solutions to salt depends mainly on their acidic group density and their size (Tombácz 1999). Likewise, the strength and the dissociation of the functional groups contained in humic acids as well as the composition of humic leachates depend upon the equilibrium between dissolved fraction and recalcitrant solid humic particles (Klučáková 2018). Furthermore, HA fractions, extracted after shorter duration of composting in some previous projects, contained more oxygen functional groups (He et al. 2016).

The effectiveness of humic acids in reducing sodium concentration is rarely investigated in the soil alone, but in presence of vegetation. Humic acids contribute effectively in the salt tolerance by accumulation of sodium in the root reducing, as a result, its translocation to shoot part of the plant (Cimrin et al. 2010).

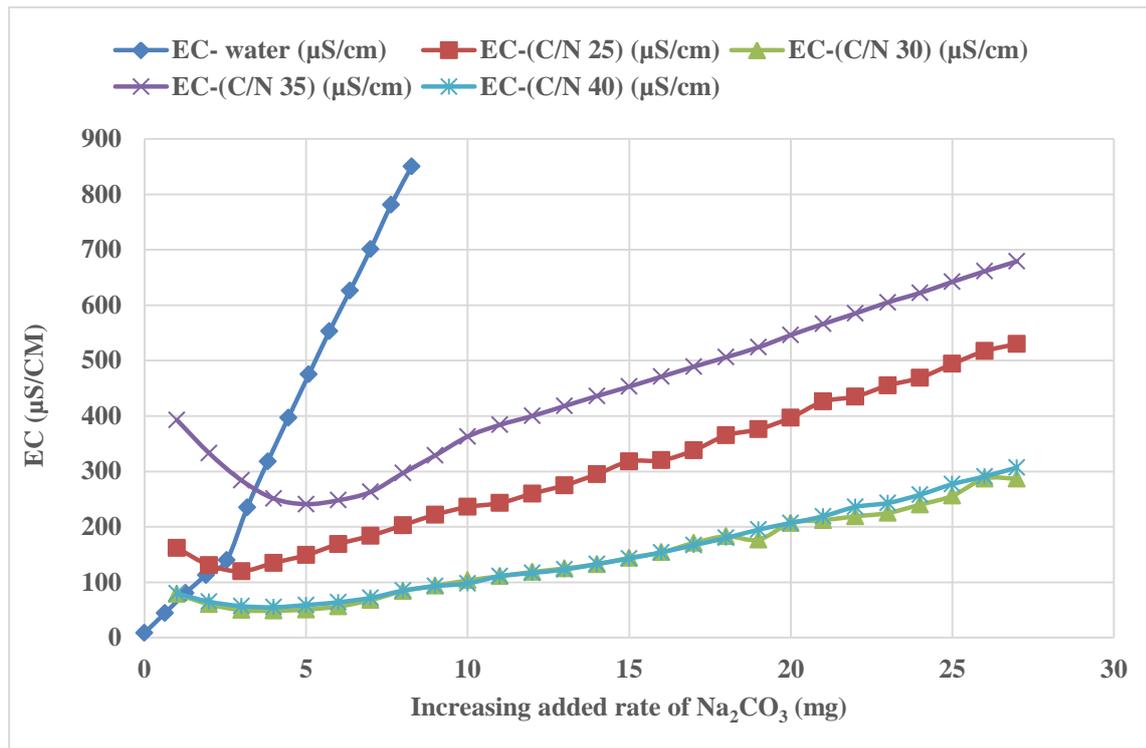


Fig. 2 Variation of electrical conductivity (EC) of humic acids solutions extracted from different composts under the effect of increasing added rate of Na_2CO_3

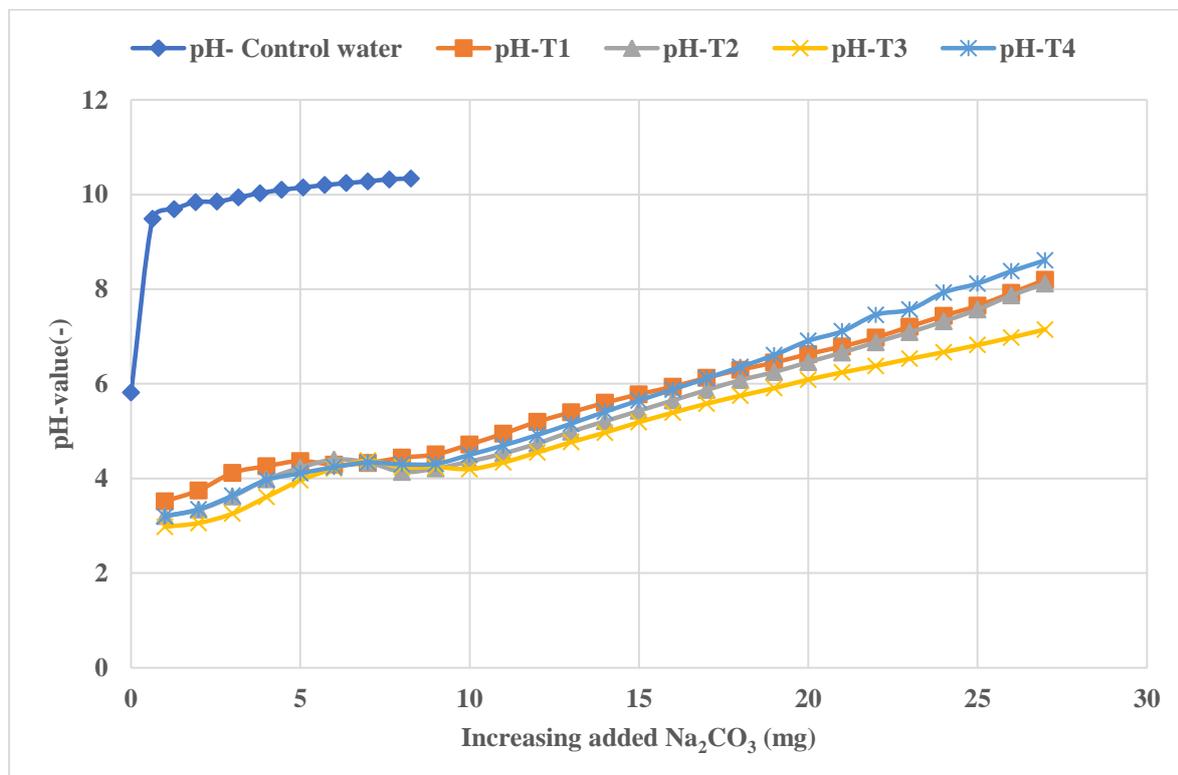


Fig. 3 Variation of pH of humic acids solutions extracted from different composts under the effect of increasing added rate of Na_2CO_3

Conclusion

In the light of the previous results, the consistent combination that seems to produce a compost, which is rich in humic acid with the highest relative resistance against salinity and alkalinity, is the one made from tomato waste (55 %), melon waste (5 %), olive mill waste (20 %), and sheep manure (20 %), which corresponds to the compost with C/N = 35. This effect may be advocated to the uneven ability of compost humic acids to chelate sodium on their carboxylic sites. The compost, with the above-mentioned quality, could be recommended for amendment by farmers on their saline soils. Further studies are needed to elucidate the reaction of different soil types to such organic amendment under different soil and/or irrigation water salinity.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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