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The Potential Role of Humic Substances in the Amelioration of Saline Soils and Its Affecting Factors

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Abstract

The application of organic amendments and humic acids (HA) often ameliorates saline soils, but the mechanisms responsible for their positive action have never been fully clarified. HA from four different origins (Elliott soil—EHA, peat—PHA, leonardite—LHA and compost—CHA) and polyacrylic acid (PAA) were characterized by acid–base titrations and ^{1}H-NMR spectroscopy and tested in laboratory experiments by measuring changes in electric conductivity (EC) and pH following micro-additions of Na_2CO_3 or NaCl. The effective salinity amelioration potential (SAP_{eff}) of HA, which expresses the amount of Na_2CO_3 neutralized per unit weight of HA at a given pH, was calculated. PAA had the highest capacity of mitigation, corresponding to 49.9 mg Na_2CO_3 g^{-1}, followed by LHA, EHA and PHA, whose SAP_{eff} values were similar and only slightly lower, and with CHA having the lowest value (25.1 mg Na_2CO_3 g^{-1} HA). All substances failed to display any effect at constant pH when NaCl was the only salt present. The dissociation of acid groups, when HA become exposed to a more alkaline pH, produces an excess of negative charges that attracts more cations within the diffuse double layer. Because of the slower diffusion of HA and their tendency to aggregate at high ionic strengths, this action reduces the osmolarity of the soil solution and therefore mitigates salinity stress.

Keywords: humic acids; soil salinity; soil electrical conductivity; alkalinity; salinity mitigation

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1. Introduction

Soil salinization is a major driver of agricultural land degradation worldwide [1–3]: it is estimated that salt-affected soils occupy more than 20% of global irrigated land, with an estimated annual economic loss of USD 27.3 billion [4]. Salinization, in fact, reduces crop yields, causing osmotic stress and specific ion toxicity [5]. In salt-affected soil, plant growth is highly affected by reduced water potential, which induces osmotic stress and ion imbalance in plants [6–8]. Future projections forecast an increase of up to 1.5 million ha of soil experiencing worsening salt accumulation within a few decades [9].

Coastal and sub-arid regions are both susceptible to salt accumulation but differ both in the causes of salinization and in the composition of soluble salts. In arid and semi-arid regions, excess soluble salts predominantly arise from rock weathering and paucity of precipitation combined with high evapotranspiration rates [4,10]. Here, carbonates predominate and, because of the low solubility of calcium carbonate, which precipitates, sodium carbonate often prevails in the soil, along with other salts such as calcium and magnesium chlorides and sulphates [11]. Saline-alkali soils [12] are typically strongly affected by the high solubility of Na_2CO_3 and high levels of exchangeable sodium [13], so that their scarce buffering capacity causes a dramatic increase in soil pH, which can easily reach pH values above 10 [14].

Conversely, in coastal soils, salinization is primarily driven by marine aerosol and saline water intrusion [15,16]. The soluble salts present in these soils are predominantly chlorides, with NaCl and $MgCl_2$ making up about 83% of total dissolved salts. In this case, as in sodic saline soils, a high exchangeable sodium level (>15% Cation Exchange Capacity) results in unbalanced nutrient uptake for soil microorganisms and plants [17].

A wide variety of approaches is currently being applied to alleviate the negative effects of high salinity levels: in addition to the removal of salts by leaching with good quality irrigation water [3,18] combined with the correction of soil pH, the use of organic amendments such as compost or manure, which seem to efficiently mitigate the alkalinity of sodic soils [19] and the adverse effects on crop yields, is a valuable and sustainable strategy. The application of organic amendments remediates nutrient imbalance [19,20] and improves soil water retention and cation exchange capacity, sustaining plant growth and soil microbial activity [21], and can also have positive effects on salt stress.

The application of organic amendments was often reported to successfully ameliorate saline soils [22]. Although debates persist on possible increases in Na^+ and Cl^- concentrations after the application of certain salt-rich organic amendments [23], multiple studies have highlighted the benefits of organic amendments on salinity tolerance in plants [24–26]. Addition of organic materials to soil, especially more humified sources including animal manure and compost, but also biochar, straw, and other amendments, has been proven to be beneficial against salt stress and the consequent reduction of salt stress in crop yields. For instance, Shukry et al. [27] reported that rice growing in saline soil treated with 40 mg L^{-1} of humic acids (HA) exhibited increased plant height and dry weight. Atero-Calvo et al. [28] documented a consistent reduction in the oxidative stress of lettuce plants after treatment with a commercial leonardite-based product, while Vitti et al. [29] observed a strong increase in the germination capacity and root growth of *Lepidium sativum* after application of humic substances (HS) extracted from compost. These results were imputed to a possible enhanced ionic balance caused by a lower Na uptake and to an improved potassium level, together with the activation of plant antioxidant defenses or hormone-like activity of HS [28,30,31].

The complex positive impact of organic matter addition on saline soil productivity and, in particular, its mechanisms of action has been so far scarcely investigated. Apart from the well-known positive effects on soil physical characteristics and nutrient availability, the influencing factors involved in the amelioration of saline soils by organic amendments are still largely unknown. Among the components contained in organic amendments or formed within the soil as a consequence of their application, humic substances have been proven to enhance resistance to osmotic stress in plants and to increase the availability of nutrients [32,33]. Many studies have shown that HA, which also stimulate germination and growth by increasing membrane permeability and facilitating nutrient uptake [34,35], can be used as biostimulants and alleviate stress to seeds and seedlings [36–39]. However, it is reasonable to presume that there are other ways by which humic substances can reduce the impact of large concentrations of dissolved salts.

This study aims at elucidating the mechanism by which humic substances, by acting directly on the osmolarity of a soil solution, can alleviate its effects on plants and microorganisms. We hypothesized that this mechanism cannot be only ascribed to the biostimulant action of HS on plant metabolism, which alleviates salinity stress symptoms, but that it also resides in an abiotic direct effect on the osmolarity of the soil solution. In fact, the ionisable functional groups on the HS molecules can potentially interact with the dissolved osmolytes, reducing their concentration and thereby their mobility, exerting a direct mitigatory effect on the osmotic potential experienced by roots. Specifically, humic acids (HA), which are composed of less polar and less soluble molecules [40] but have their ionizable moieties solvated and in dynamic equilibrium with the soil solution, may be particularly effective. Upon dissociation, these moieties release protons that are neutralized by the alkalinity of the soil solution. The negative charges, thus left uncompensated, are able to attract cationic osmolytes within the electrostatic double layer, thereby virtually subtracting them from the soil solution and preventing their contact with plant roots. Our hypothesis is therefore that HA have a stronger effect on Na_2CO_3 than on NaCl because a more alkaline pH supports further dissociation of HA acidic functional groups, and the released protons may shift the equilibrium of carbonate ions towards the formation of carbon dioxide.

In this study, we performed a series of laboratory experiments in which micro-additions of the two salts, namely Na_2CO_3 and NaCl, that typically predominate in the soil solution of saline soils were added to de-aerated solutions containing or not different types of HA, monitoring pH and electrical conductivity (EC) to quantify the effects of HA on the osmolarity of solutions.

2. Materials and Methods

2.1. HA Sources

Four HA and an anionic synthetic polyelectrolyte, polyacrylic acid (PAA), were used to investigate mechanisms of potential mitigation effects on salt stress. PAA was chosen because it has a well characterized chemical structure and properties and, as a potassium salt, it is used in agriculture to improve soil structure [41,42]. PAA and Aldrich HA were purchased from Sigma Aldrich (St. Louis, MO, USA), whereas soil (EHA) and peat humic acids (PHA) were extracted following the procedure suggested by the IHSS from, respectively, the Elliott silt loam soil and the Pahokee (Florida) Peat materials provided by the IHSS. Compost humic acids (CHA) were obtained from a well matured sample of compost obtained from an anaerobically digested separately collected organic fraction of municipal solid waste provided by Bioman SpA (Maniago, Italy). The compost was shaken for one hour in 0.1 M NaOH plus 0.1 M $Na_4P_2O_7$ at a 1:10 ratio (*w*:*v*) under N_2; to obtain the HA, the extract was acidified to pH 1 using concentrated H_2SO_4. Leonardite humic acids (LHA) purchased from Sigma Aldrich (H16752, Sigma Aldrich) were purified by dissolution in 0.1 M KOH and re-precipitation by adding 6 M HCl. All HA were purified by re-suspension in milliQ water followed by dialysis and freeze-drying in a Scanvac CoolSafe 55–4 (LaboGene, Allerød, Denmark).

2.2. Mitigation Capacity

The capacity of humic acids to mitigate salinity was tested by means of a DL50 automatic Graphix Titrator (Mettler Toledo, Columbus, OH, USA), adding stepwise microvolumes (30 µL) of 0.167 M NaCl and 0.213 M Na_2CO_3 solutions to a cell equipped with a pH electrode connected to the titrator and a conductivity cell connected to a Cond 7 Vio portable conductimeter (XS Instruments, Modena, Italy). At the beginning of the experiments, the cell was filled with de-aerated milliQ water or solutions containing HA or PAA.

Sodium carbonate (anhydrous, ≥99.5%, Carlo Erba Reagents, Cornaredo, Italy) was oven-dried at 105 °C for 24 h and stored in a desiccator before being used to prepare solutions. Solutions containing HA were prepared by dissolving 50 mg of freeze-dried preparation in a minimum amount of NaOH solution, then adding de-aerated MilliQ water to reach a final concentration of 2.5 mg mL^{-1}. All solutions were then acidified with

Amberlite IR-120 H^+ resin (39389-20-3, Sigma Aldrich) under a nitrogen atmosphere to prevent CO_2 interference until the lowest possible pH value, which differed for each HA solution, was reached. Before the experiment, solutions were filtered through a 0.2 µm cellulose syringe filter. The final concentrations of dissolved C in the resin-treated solutions were quantified with a Shimadzu TOC-V CPN analyzer (Shimadzu, Kyoto, Japan). In the case of NaCl, pH was afterwards corrected to pH~6 to simulate pH levels commonly found in saline coastal soils (e.g., [43,44]).

The micro-additions were performed every 5 min to allow the pH electrode to reach equilibrium. For sodium carbonate, the endpoint was set to pH~10, which is commonly adopted as the endpoint of ionization for all acidic groups of HA [45]. All experiments were performed in triplicate.

From the obtained graphs, the slopes of EC for solutions without or with HA were retrieved for comparison. In addition, the capability of the HA and PAA to reduce the concentration of free dissolved ions in a solution of a given soil was calculated by subtracting the difference between the EC of a solution containing Na_2CO_3 only and that of a Na_2CO_3 + HA solution measured at pH 7.5 from the difference between the conductivity of a Na_2CO_3 solution and that of a Na_2CO_3 + HA solution measured at pH 8.5.

2.3. Acid-Base Titrations

Solutions were prepared by dissolving each humic acid and the PAA in a minimum volume of 0.1 M NaOH solution, followed by the addition of de-aerated MilliQ water to achieve a final concentration of 2.5 mg mL^{-1}. The solution was then acidified to pH 3 using 1 M HCl and immediately titrated. From titration curves, the negative charges from strong (Q1) and weak (Q2) acidic functional groups were calculated following the procedure proposed by Ritchie and Perdue [46]. They represent, respectively, the total number of negative charge sites developed by an organic polyelectrolyte at pH 8 (Q1) and twice the difference between the number of total negative charge sites at pH 11 and pH 8 (Q2). In addition, the total amount of ionized groups at pH 7, pH 8.5 and 10 were graphically retrieved.

2.4. H-NMR Spectroscopy

^{1}H-NMR spectra were acquired using a Bruker Advance III 400 spectrometer equipped with a 5 mm Iprobe operating at 400.13 MHz. The samples were prepared by dissolving 2 mg of each humic or humic-like substance in 0.6 mL of deuterium oxide (D_2O) and were then placed in NMR tubes for analysis.

Spectra were recorded using the zgesgp sequence for selective saturation of the HDO resonance. Experimental parameters were selected as follows: relaxation time 2 s, acquisition time 1.39 s and number of scans 1280, for a total acquisition time of 75 min. The spectra were divided into the following five regions: 0–1.7 ppm, corresponding to terminal methyl and methylene groups of methylene chains, methylene of alicyclic groups and CH_2 and CH groups at least two carbons away from aromatic rings or polar functional groups; 1.7–3.0 ppm, corresponding to protons of methyl and methylene groups α to aromatic or carboxylic acid groups; 3.0–5.0 ppm, corresponding to protons α to carbon attached to oxygen groups (polysaccharides or carbohydrates); 5.0–6.5 ppm, corresponding to protons of olefins and 6.5–9.0 ppm, corresponding to aromatic protons [47,48]. The relative intensity of each region was calculated by integrating the areas under the corresponding peaks and expressing them as percentages of the total spectral area.

2.5. Statistical Analysis

All statistical analyses were conducted using R studio v4.4.3 [49]. One-way ANOVA was performed to evaluate significant differences in EC trend slopes between control and experimental treatments. Data normality (Shapiro–Wilk test) and homogeneity of variance (Bartlett's test) were tested across all experimental groups. ANOVA was conducted to determine the effects of HA from different origins and PAA on EC in solutions titrated with Na_2CO_3 or NaCl. Post hoc comparisons using Tukey's HSD test were conducted to identify pairwise differences between treatment groups. Data analyses were conducted using the 'aov' and 'TukeyHSD' functions of the 'multcomp' package [50].

3. Results

3.1. The Effect of the Type of Salt

In Figure 1 we report changes in the EC and pH of water and water-plus-HA solutions during stepwise micro-additions of Na_2CO_3. The data obtained from these graphs allowed us to compare the behavior of HA from different origins and to calculate, by means of specifically devised equations, the salinity neutralized by a unit addition of each humic acid at any given change in pH. A polyelectrolyte of known structure, PAA, which is also sometimes used in agriculture as a salt (potassium polyacrylate) to improve soil structure, was also considered to better understand the mechanisms involved.

Addition of a weak electrolyte such as Na_2CO_3 to water (Figure 1) determines a steep immediate increase in pH from about 6 to ~9, already after the addition of 6.4 µg of the salt, because of the hydrolysis of the carbonate ions, which tend to associate with protons, thereby increasing the equilibrium concentration of OH^- ions. Afterwards in the absence of PAA and HA, the pH of the solution increases only slightly, whereas as expected its EC increases linearly (a = 0.072, $p < 0.001$) with salt additions.

All HA and, even more so, the PAA buffered the solution pH and strongly counteracted the pH increase imposed by Na_2CO_3 additions (Figure 1). Polyacrylic acid (PAA) exhibited the strongest buffering effect, with pH values increased by only 1.85 units at the end of the experiment (Figure 1a). In the presence of HA, after a brief initial decrease in conductivity caused by the neutralization of protons derived from the dissociation of the stronger acidic groups, the EC increases steadily, displaying again a linear positive trend. However, the increase rates measured per unit salt addition were, in all cases, significantly lower than those of the control solution (Figure 2). Therefore, in the case of salinity caused by Na_2CO_3, all tested HA and the synthetic polyelectrolyte PAA resulted in effective countering of the increase in conductivity of the solutions at increasing salt levels, as confirmed by the significant statistical differences among EC slopes calculated from experimental data (Figure 2).

Among the tested HA, CHA and EHA were the most effective at mitigating the Na_2CO_3-induced increase in EC, yielding final EC values nearly 50% lower than those of the control (Figure 2a). LHA and PAA also reduced the EC increase rate, whereas PHA was the least effective (Figure 2a).

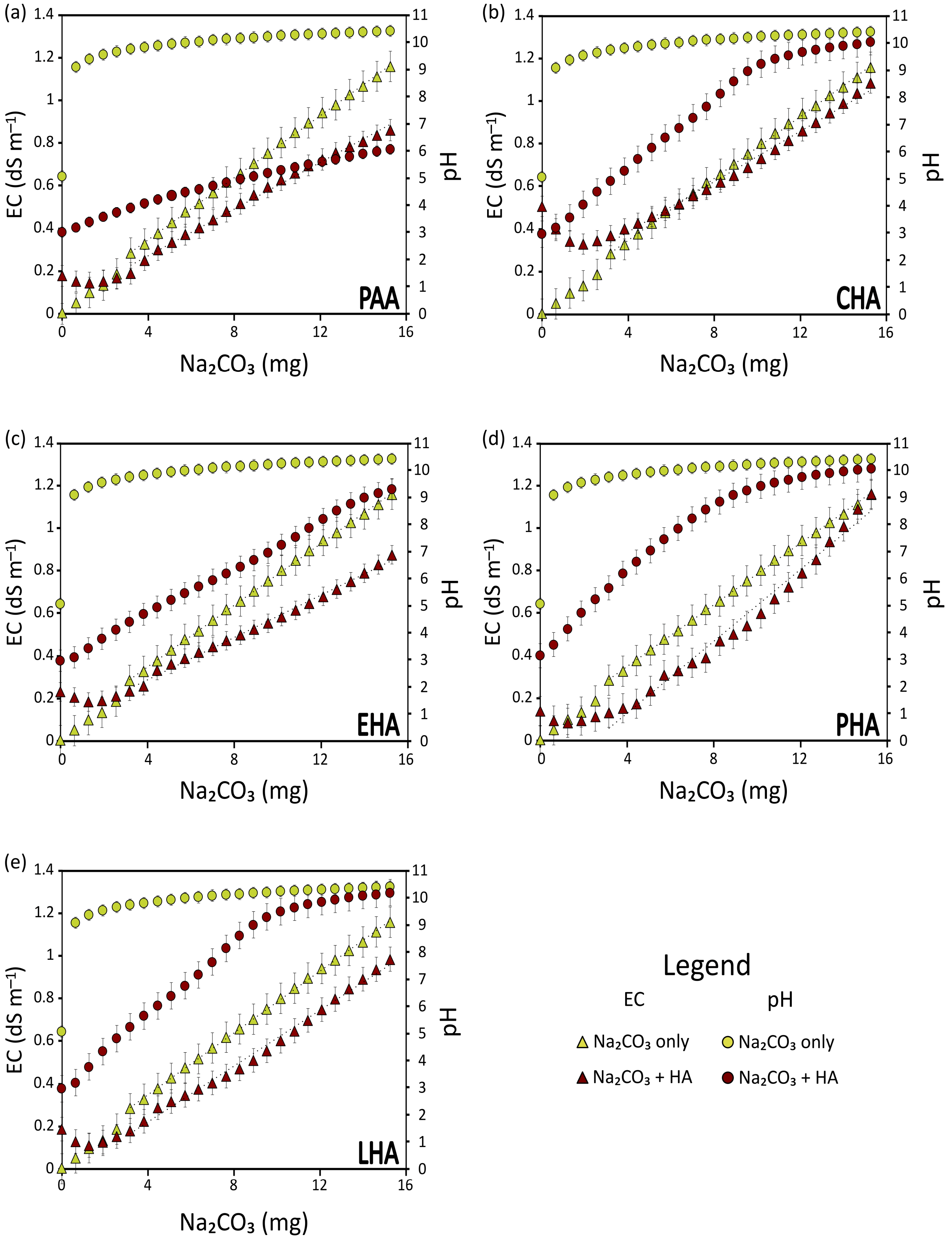

Figure 1. Changes in pH and EC registered following micro-additions of 0.2 M Na_2CO_3 to de-aerated water and to solutions containing polyacrylic acid PAA (**a**) or HA of different origins, i.e., compost CHA (**b**), soil EHA (**c**), peat PHA (**d**) and leonardite LHA (**e**). Data points represent the mean of three independent replicates and bars represent the standard deviation.

Conversely, when the same experiment was performed with NaCl, which is a strong electrolyte and does not cause hydrolysis of water, additions did not have any significant effect on the pH of the solutions either in the absence or in the presence of HA or PAA (Figure 3). The presence of the latter, however, strongly acidified the solution and the pH remained around 3 throughout the experiment. In this situation the EC of the solution containing PAA remained always well above that which was measured in the NaCl-only control (Figure 3).

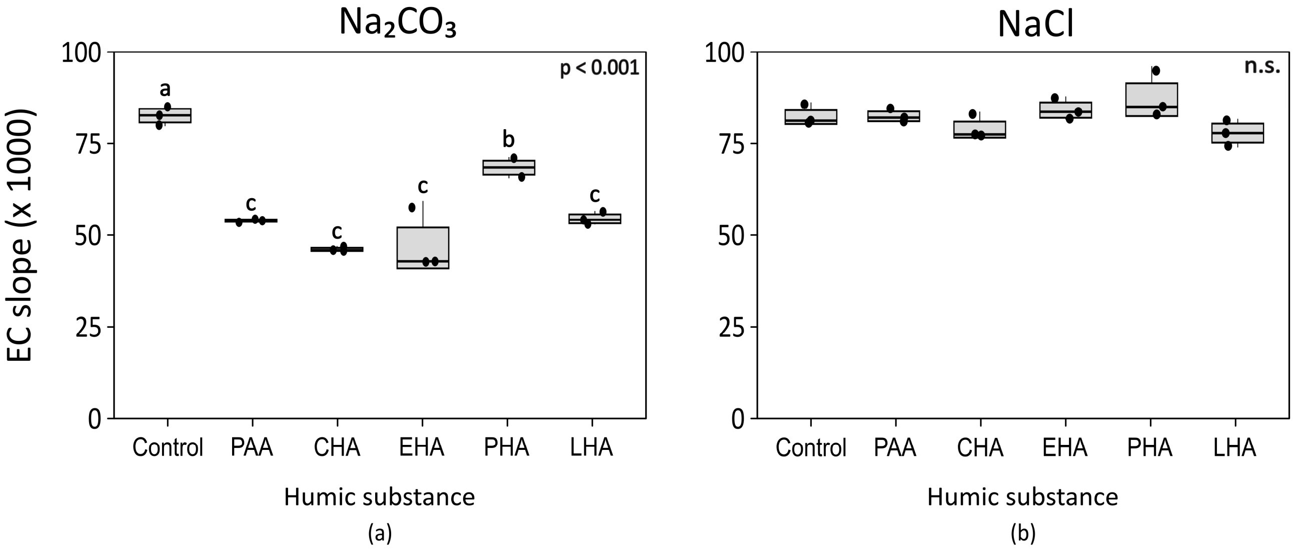

Figure 2. Effect of HA on EC increments (dEC/dmg) measured per unit addition of Na_2CO_3 (**a**) and NaCl (**b**) up to pH values of 7. ANOVA analysis highlights the statistical differences in EC slopes between the control (no PAA or HA) and the PAA and HA treatments. Different letters refer to statistically significant differences based on Tukey's post hoc test (Na_2CO_3 and NaCl: $p < 0.001$ and n.s., respectively).

Considering the fact that HA cannot be expected to possess any dissociated, negatively charged acid group to attract free sodium ions at the equilibrium pH attained after treatment with the cation exchange resin, which differed slightly for each type of HA (see Figure 1), the solutions were adjusted to pH 6.0 before we started adding NaCl (Figure 3).

This allowed us to better compare the eventual effects of their presence because, in the NaCl-only control, the pH remains around pH 6 throughout the experiment. Under these conditions (initial pH raised to 6.0) and in the presence of HA, the pH slightly decreases after the addition of 6 mg of the salt due to a minor cation exchange reaction driven by the increase in ionic strength and concentration of sodium ions. However, in this case, ANOVA did not detect statistical differences among the slopes of the control, of the PAA or of any of the HA (Figure 2b).

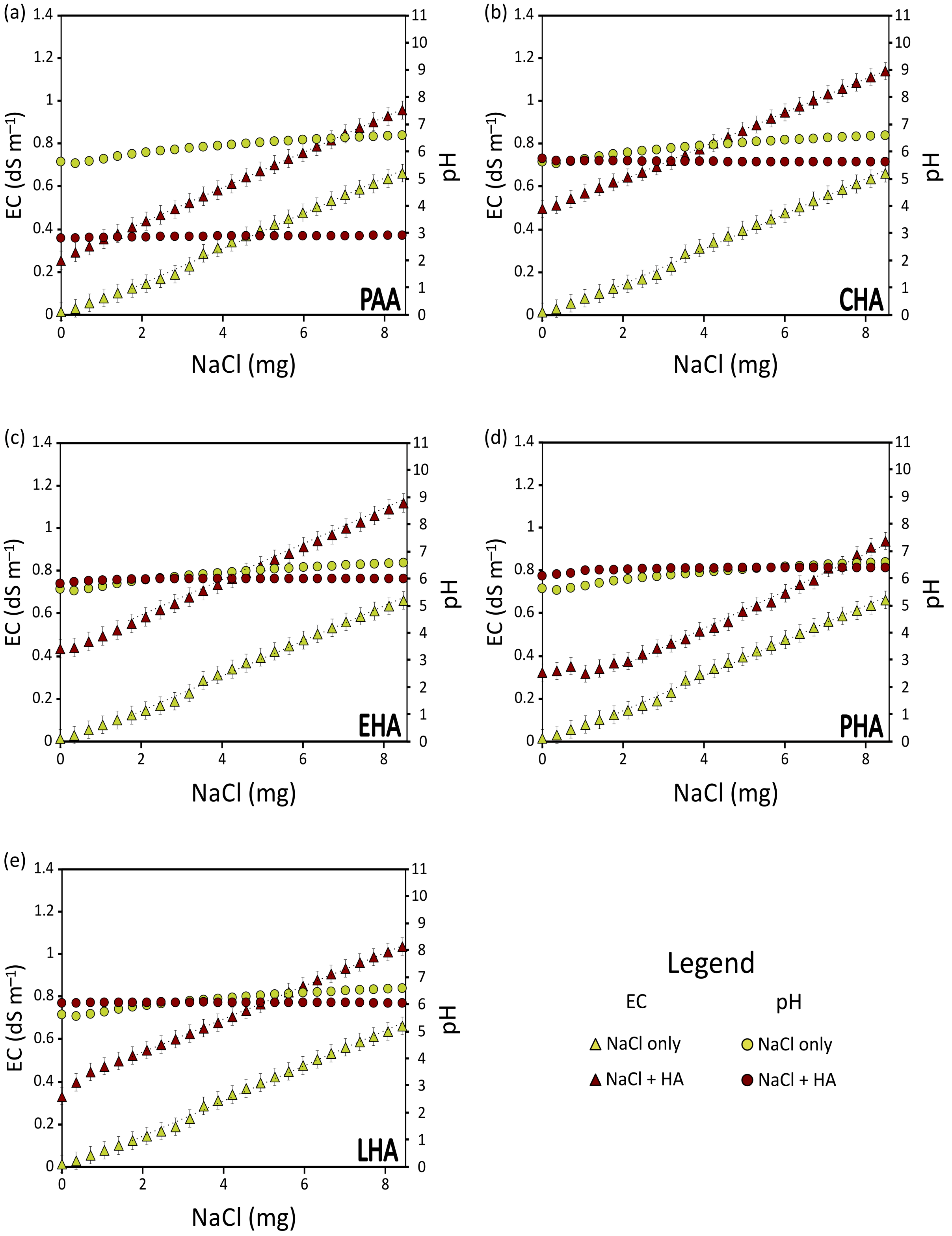

Figure 3. Changes in pH and EC following micro-additions of 0.2 M NaCl to de-aerated water and solutions containing polyacrylic acid PAA (**a**) or HA of different origins, i.e., compost CHA (**b**), soil EHA (**c**), peat PHA (**d**) and leonardite LHA (**e**). Data points represent the mean of three independent replicates and bars represent the standard deviation.

3.2. The Effect of the Type of HA

The EC data measured during the experiments allow us to evaluate and compare the ability of each HA to mitigate salinity caused by dissolved Na_2CO_3. The overall salinity amelioration potential (SAP_{HA}) of each HA at a given pH can then expressed as the amount of Na_2CO_3 neutralized per unit of weight of HA and can be calculated from the values in the graphs of Figure 1 using the following equation:

$$SAP_{HA} = \frac{(\mathrm{A} - \mathrm{A}')}{W_{HA}} \bullet 1000 \quad (1)$$

where

- A is the amount of Na_2CO_3 that, in the water + HA solution, yields the pH experienced by the HA in the soil;
- A′ is the amount of Na_2CO_3 that, if added to water, would produce a solution with the same conductivity as A;
- W_{HA} is the weight of HA expressed in mg.

Considering that HA are generally not applied in their fully protonated form, the effective SAP ($SAP_{HA\ eff}$) of the HA contained in an amendment added to a certain soil can still be calculated (Equation (2)) from the graph by subtracting the amount of Na_2CO_3 added to HA to bring the acid-saturated HA to the pH at which they are actually applied:

$$SAP_{HA\ eff} = \frac{(\mathrm{A} - \mathrm{A}') - (\mathrm{B} - \mathrm{B}')}{W_{HA}} \bullet 1000 \quad (2)$$

where

- A is the amount of Na_2CO_3 that, in the water + HA solution, yields the pH experienced by the HA in the soil;
- A′ is the amount of Na_2CO_3 that, if added to water, would produce a solution with the same conductivity as A;
- B is the quantity of Na_2CO_3 added to HA to bring the acid-saturated HA to the pH of the soil at which they are applied;
- B′ is the quantity of Na_2CO_3 added to water to obtain a solution that, at the original pH of the HA, has the same conductivity as B.

As an example, and to underline the dependence of SAP on the pH variation experienced by HA, we calculated the SAP of each of the examined HA (Table 1) and their capability to neutralize the increase in EC corresponding to the maximum change in pH (i.e., from 7 to 8.5) that can likely occur to the HA contained in an organic amendment, such as compost, when incorporated in a typical saline soil.

Table 1. Salinity amelioration potential (SAP) of HA and PAA at pH 7 towards osmolarity caused by Na_2CO_3. The table shows the effectiveness of the different HA in mitigating EC rise in terms of mg of Na_2CO_3 neutralized per gram of HA following a change in pH from the fully protonated form to pH 7 (SAP_{HA}) and from pH 7.0 to 8.5 ($SAP_{HA\ eff}$).

Humic Acid	**Origin**	**SAP_{HA} (mg Na_2CO_3 g^{-1} HA)**	**$SAP_{HA\ eff}$ (mg Na_2CO_3 g^{-1} HA)**
PAA	Polyacrylic Acid	142.59	49.86
CHA	Compost	28.56	25.10
PHA	Peat Soil	155.65	43.60
EHA	Elliott Soil	164.55	44.35
LHA	Leonardite	142.62	46.44

When results were normalized per g of HA, PAA showed the highest potential capacity of mitigation, corresponding to 49.9 mg Na_2CO_3 g^{-1}, followed by LHA, EHA and PHA, whose calculated SAP_{eff} values were only slightly lower and were very close to each other (i.e., ~45 mg Na_2CO_3 g HA^{-1}), and with CHA having the lowest potential, i.e., 25.1 mg Na_2CO_3 g HA^{-1} (Table 1).

The mitigation effect of PAA is somewhat lower that would be expected by its high content of carboxyl groups, which significantly influences its surface charges at increasing pH levels (Table 2).

Table 2. Relative abundance of protons in functional groups of PAA and HA determined by 1H-NMR analysis. The table displays the percentage distribution of protons bound to different structural units across the following chemical shift regions: aromatic-H (9.0–9.5 ppm), α to double bonds H (6.5–5.0 ppm), H in α to carbons bound to oxygen (5.0–3.0 ppm), methyl and methylene H associated with carboxyl groups (3.0–1.7 ppm) and methyl and methylene H in terminal aliphatic groups (1.7–0 ppm). Data are normalized to a total area of 100% for each substance.

Humic Acid	Aromatic-H	H in α to Double Bonds	H in α to Carbons Bound to Oxygen	Methylene-H Associated with Carboxyl Groups	H in Terminal Methyl and Methylene Groups
PAA *	0.31	0.00	61.58	36.92	1.19
CHA	10.43	0.16	21.86	30.64	37.19
PHA	11.82	2.79	29.25	19.50	36.62
EHA	14.70	1.64	25.53	23.82	34.30
LHA	19.15	3.54	5.38	33.56	38.35

* Deduced from Chollakup et al. [51].

3.3. HA Characterization

The characterization of the selected HA, which were chosen in order to examine the behavior of HA of widely different origins and humification degrees, was performed in order to understand which structural factors determine the different capabilities displayed. To highlight the range of structural variations in the different HA employed, the percent distribution of proton types was obtained from ^{1}H-NMR spectra and the quantitation of the negative charge density development following pH changes was calculated based on acid–base titrations.

According to the respective ^{1}H-NMR spectra (Supplementary Materials Figure S1), LHA have the highest percentage of aromatic protons (Table 2), about twice that of CHA, indicating, in the latter, a lower degree of aromaticity. The amount of aromatic protons in EHA was lower than in LHA, but 50% higher than in CHA. The percentage of olefinic protons (protons directly bound to double bonds) was low in all compounds, never exceeding 4%. Protons bound to oxygen in polysaccharides and carbohydrates were abundant in PHA and EHA (~29% and ~25%, respectively), while they were poorly represented in LHA (5.4%).

On the contrary, as expected from the polymer's composition, PAA shows, in the ^{1}H-NMR spectrum and proton type distribution (retrieved from the literature [51]), the highest percentages of methyl and methylene protons associated with terminal aliphatic groups and with carboxylic and aromatic groups, at least 1.6 times higher than those of the other HA considered, which on the contrary showed similar values as each other. Regarding aliphatic structures, EHA had the lowest amount of alkyl protons.

In order to obtain information on the charge density and charge pH dependence, we calculated the Q1 and Q2 parameters (Table 3) of the examined HA from their acid–base titration curves (see Supplementary Materials Figure S2). According to Ritchie and Purdue [46] these parameters provide a quantitative estimation of carboxyl and phenolic groups. The model substance, PAA, has the highest concentration of carboxyls, i.e., Q1, (17.0 meq g^{-1} C). The HA that have the largest quantity of ionizable groups that can be assimilated to carboxyls, being dissociated at pH 8, are PHA and EHA (~10 meq g^{-1} C) followed by LHA and CHA (~9 meq g^{-1} C). As for Q2, which has been considered to correspond to phenolic groups, the largest values were recorded for CHA and PHA (5.50 meq g^{-1} C) followed by EHA (4.0 meq g^{-1} C), while the lowest was recorded for LHA (2.45 meq g^{-1} C). The limits of this assumption are highlighted by the Q2 value of PAA (3.50 meq g^{-1} C): PAA does not actually contain any phenolic groups, only carboxyls. These groups were made progressively weaker by the build-up of the negative charge density on the polymer molecules during titration.

Table 3. Calculated maximum charge densities for the first (Q1) and second (Q2) types of negatively charged binding sites at pH 8 and 10, respectively, total ionized groups at pH 7, 8.5 and 10 and ionization increases from pH 7 to pH 8.5 and from pH 8.5 to pH 10.

Humic Acid	Q1 (mmol g^{-1} C)	Q2 (mmol g^{-1} C)	Total Ionized Groups at pH 7 (mmol g^{-1} C)	Total Ionized Groups at pH 8.5 (mmol g^{-1} C)	Total Ionized Groups at pH 10 (mmol g^{-1} C)	Charge Increments from pH 7 to 8.5 (mmol g^{-1} C)	Charge Increments from pH 8.5 to 10 (mmol g^{-1} C)
PAA	17.00	3.50	15.25	17.50	17.75	2.50	0.25
CHA	8.80	5.50	9.20	10.10	11.55	2.35	1.45
EHA	10.20	4.00	9.60	10.50	12.25	2.67	1.75
PHA	10.13	5.50	9.63	10.50	12.88	3.25	2.38
LHA	9.03	2.45	8.55	9.33	10.25	1.70	0.92

The numbers of total ionized groups at pH 7, which represents the average pH of good quality composts, and at pH 8.5, which is considered the upper limit for saline soils according to the U.S. Salinity Laboratory [12], are reported in Table 3. The numbers of total ionized groups at pH 10, a representative pH for alkaline saline soils [14], was also calculated. In this way, the negative charge increment corresponding to the lower (7 to 8.5) and upper extremes (7 to 10, in the case of alkaline saline soils) of the pH change that may be experienced by HA contained in an organic amendment when added to soil was also calculated and reported (Table 3) to evaluate the effect of soil pH on the amelioration potential of HA. Total ionized groups were always highest in PAA, but, although the synthetic polymer between pH 7 and 8.5 had a negative charge increment similar to those of the HA, only a negligible further increment occurred from pH 8.5 to 10. This is reasonable, considering that PAA does not possess acidic groups weaker than carboxyls. On the contrary the contribution of phenolic groups is sizeable in the case of HA. At pH 8.5, EHA PHA and CHA displayed about the same amount of total ionized groups (10.50 ÷ 10.10 meq g^{-1} C). However, differences were displayed at pH 10, with EHA and PHA showing the highest number of ionized groups, around 30% more than CHA. At all the examined pH values, the lowest amount of ionized groups was that of LHA (8.55, 9.33 and 10.25 meq g^{-1} C at pH 7, 8.5 and 10, respectively) which is consistent with the strong aromatic nature revealed by ^{1}H-NMR spectra (Table 2).

4. Discussion

Among the different strategies that have shown some potential for enhancing the productivity of saline soils, the use of organic amendments has been often reported to improve soil conditions [52]. This work does not aim to recommend any specific strategy, but only to explain how humic substances can act to alleviate the osmolarity stress caused by dissolved salts. Addition of HA to soil may occur not only through the use of commercial humate products but, more frequently, through addition of compost, sewage sludge or crop residues, which either contain HA or will generate them during their decomposition in soil. Several studies assessed a reduction in EC after the application of organic amendments in saline soils where pH values exceeded 8.0 [53–55]. Based on our hypothesis, which was confirmed by the results of salt addition experiments, this action is driven by the increase in pH experienced by HA that causes ionisable weak acid groups to dissociate [56]. The increased negative charge on the humic molecules causes more cations to become attracted within the diffuse double layer [57,58]. At the same time, the release of protons shifts the equilibrium among carbonate, bicarbonate ions and dissolved CO_2 in the solution. Because CO_2 is a gas, in a non-equilibrium condition, it may be lost by diffusion into the gas phase. This means that, in the field, the amelioration that can be achieved depends on the actual pH difference between the original pH of the HA contained in a commercial product or in an organic amendment such as compost and the pH of the soil. The larger the difference, the stronger the decrease in osmolarity (EC) of the soil solution produced by a given amendment. Our work also shows that, as an undesirable side effect, the addition of organic amendments to ameliorate saline soils may trigger the release of CO2 from soluble carbonates and therefore mobilize CO2 trapped in mineral soil components. However, this effect is not long lasting and is limited to a short pulse.

The fact that HA and PAA fail to display any SAP when NaCl is the only salt present and at constant pH is actually not surprising. It is well known, in fact, that sodium ions are very weakly retained within the diffuse double layer and would never be able to cause the displacement of H^+ from acid groups which has been observed in the presence of divalent metals [59].

Strongly alkaline saline soils are therefore likely to profit more, and in particular saline-sodic soils, whose pH is bound to increase when excess salts are amended [60], may particularly benefit from addition of organic materials containing or producing HA through their humification in soil. The addition of non-humified substrates may be potentially more beneficial. In fact, in this case HS would be produced in the soil in their acid form and not added together with the stoichiometric amount of positive counter ions, as when compost is added to soil.

The decrease in osmolarity, which is demonstrated by the decrease in EC, also strongly depends on the anionic composition of the soil solution. In fact, no amelioration was observed in our experiments in the presence of NaCl. In fact, in this case, besides the lack of change in pH, that does not allow the formation of novel negative charges, chloride ions, contrary to carbonate ions, which may be re-moved from the solution as gaseous carbon dioxide by a shift in their acid-base equilibrium, are never lost from the solution.

4.1. Dependence of SAP on the Structural Traits of HA

Nevertheless, the density of acid groups on HS molecules and their strength (see Table 3) seems to only partially explain the documented reduction in EC. In fact, PAA, in spite of the much larger number of carboxyls, displayed a similar SAP_{eff} as the rest of the HA, with the only exception of CHA (Table 1).

It is likely that other structural factors may contribute in determining the amelioration potential of HA. Other structural differences, such as molecular size and tendency to aggregation, may in fact contribute to the observed decrease in osmolarity. Molecular size, in fact, promotes the aggregation of humic molecules, occluding attracted counter-ions within a supramolecular structure and further decreasing the number of free molecules in the soil solution [61]. Flocculation of HA, although impaired by Na, is at the same time driven by the shielding of the negative charge at increasing ionic strength and is a phenomenon normally observed in saline-sodic soils [62].

Total ionized groups were calculated at pH 7 and 10 which, respectively, represent the pH generally exhibited by compost and the pH frequently measured in salt-affected soils of high sodicity. Total ionized group density was highest in PAA at both pH levels, which is consistent with the high amount of calculated strong acidic groups. While this specific aspect of HS performance in salt-affected soils with different initial pH levels has not been extensively investigated, numerous studies have confirmed the effectiveness of HS in efficiently reducing soil pH in saline-sodic and sodic soils [38,63,64].

Despite LHA exhibiting the lowest number of dissociated groups at both pH 8.5 and 10, our salt addition experiment showed that leonardite has potentially an ability to mitigate the increase in EC following Na_2CO_3 additions which is comparable to those of the other HA. This could be explained considering that phenolic groups become increasingly relevant in retaining cations at alkaline pH (pKa~10) [65].

4.2. Potential Practical Applications

These findings can have practical applications. In fact, knowing the content of HA, or the amount of HA potentially produced in the soil, of a given compost or amendment and the salinity amelioration potential ($SAP_{HA\ eff}$) of its HA allows for the quantity which should be applied in the field to be easily calculated for the specific soil considered by applying Equation (2). As an example, knowing that, for instance, from the SAP_{HAeff} of CHA, approximately 25 mg of Na_2CO_3 can be neutralized per gram of compost HA when the pH increases from 7.0 to 8.5 (Table 1), we can assess the amount of compost that should be applied in the field in order to alleviate salinity. If we consider, for instance, a soil with a EC of 4 dS m^{-1}, a moisture content of 25% and a bulk density of 1.3 g cm^{-3} [66], and the aim to reduce EC to 2 dS m^{-1} in the top 5 cm layer, the amount of good quality mature compost to be applied will be about 40 t per hectare. This quantity is quite for arable crops also considering the content of plant nutrients (see Supplementary Materials S3 for details on calculations). It must be noted that the amount required may be much larger in the case of compost that is not well humified: the HA content of the compost and their SAP_{HAeff} must be both known in order to obtain reliable predictions on short term effects of the application. From $SAP_{HA\ eff}$ data reported in Table 1, it is also evident that PAA may represent a possible alternative to compost in the amelioration of salinity.

5. Conclusions

Our results revealed the plausible mechanism behind salt stress relief driven by application of organic amendments to salt-affected soils and showed that it depends on (i) the nature of the salts, (ii) the pH of the soil and (iii) the quantity and properties of HA in the organic amendment. The mechanism is based on, but not only limited to, the electrostatic interaction of the excess negative charges that are generated by the dissociation of acidic ionisable groups on HA molecules with the cations dissolved in the soil solution of saline soils. The dissociation of acid groups that occurs when HA are eventually exposed to a more alkaline soil pH will produce an excess of negative charges that will attract and hold more cations in the diffuse double layer of HA. Because of the very slow diffusion of HA and their tendency to aggregate in saline solutions, it is expected that the concentration of free ions in the bulk soil solution will be lowered. This will reduce the osmolarity of the soil solution and potentially mitigate salinity stress to plants and microorganisms.

However, an essential part of the mechanism is the concomitant neutralization of carbonate ions and the consequent non-equilibrium release of CO_2. In fact, when the amendment has a pH lower than the soil, the weak acid functional groups of the humic substances contained in it will dissociate and release H^+ in solution. These will then react with carbonate or bicarbonate ions, shifting the equilibrium towards gaseous CO_2. This situation is highly likely to occur, as good quality composts generally have a pH around 7, whereas the pH of many soils where salts have been accumulated are alkaline or even, in some cases (saline-sodic soils), extremely alkaline.

The calculated $SAP_{HA\ eff}$ that can be obtained in the laboratory from easy EC and pH measurements also provides a mean to obtain preliminary information the minimum amount of amendment to be applied under field conditions to a given soil. However, extrapolation of outcomes from laboratory experiments to field conditions is never straightforward and needs to be validated with field research. Further research is also needed to advance our insight into concomitant factors, such as aggregation and precipitation, which may also contribute to the amelioration potential of humic substances towards soil salinity.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/su17198621/s1: Figure S1: Acid–base titration curves of PAA (polyacrylic acid) and HA of different origins (compost CHA, soil EHA, peat PHA and leonardite LHA); Figure S2: Comparative ^{1}H-NMR spectra of HA of different origin (compost CHA, soil EHA, peat PHA and leonardite LHA); S3: Example of calculations of quantity of compost required to reduce salinity in one hectare of land, based on $\mathrm{SAP_{HA\,eff}}$.

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