International Journal of Plant Production 8 (3), July 2014
ISSN: 1735-6814 (Print), 1735-8043 (Online)
www.ijpp.info

GUASNR

The role of humic substances in mitigating the harmful effects of soil salinity and improve plant productivity

Y. Ouni[a], T. Ghnaya[a], F. Montemurro[b], Ch. Abdelly[a], A. Lakhdar[a,*]

[a]*Laboratoire des Plantes Extremphiles, Centre de Biotechnologies de la Technopole de Borj Cedria, BP 901, Hammam Lif 2050, Tunisia.*
[b]*Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Unità di Ricerca per lo Studio dei Sistemi Colturali, 75010 Metaponto, Italy.*
*Corresponding author. E-mail: abdelbassetlak@yahoo.fr

Received 30 October 2013; Accepted after revision 26 March 2014; Published online 22 May 2014

Abstract

The environmental, social and economical reasons indicate that the conventional agriculture constitute a continuous pressure contributing to the progressive worsening of environmental conditions, especially by an increasing apply high level of inputs. In this matter, the increasing use of saline irrigation water is one of the main factors resulting in salt accumulation in the plant rhizosphere influencing both physical, chemical and biological soil properties and agroecosystem productivity. In this situation, soil degradation emphasized the need to develop strategies of salt effected soil reclamation. One of the possible solutions is to use humic substances (HS), since there is an increasing need to their utilization in agriculture. They are the major components of soil organic matter, have multiple roles in plant growth and are the subject of study in various areas of agriculture, such as soil chemistry, soil fertility and plant physiology. Thus, our hypothesis account for a beneficial effect of HS in salt affected rhizosphere likely due to a 'direct' action on the plant together with an 'indirect action' on the metabolism of soil microorganisms, the dynamics of uptake of soil nutrients and soil physical conditions. In this paper we review the HS formation and components and their influences on improving saline soil properties as both direct and indirect effects.

Keywords: Humic substance; Salt accumulation; Soil degradation; Plant productivity; Hormone-like activity.

Introduction

Soil salinity is one of the most important problems in arid and semi-arid regions of the world reducing the yield of wide variety of crops (Tester and Davenport, 2003; Ashraf and Foolad, 2007; Gulser et al., 2010). The processes of salinization and alkalinisation induce upon soils certain chemical and physical characters, which might have a profound effect on the agro-ecosystem. The excess exchangeable sodium (Na) and the high soil pH, as a result of salt accumulation, cause deformation of soil structure and decrease in hydraulic conductivity and infiltration rate of soils (Lauchli and Epstein, 1990). These processes, which affect plant growth, are related to the increase in the concentration of salt in the root zone, as water is removed from the soil profile due evapotranspiration. According to Wong (2007), slaking occurs upon wetting, causing larger aggregates to break into smaller ones as result of swelling and air entrapment. Further wetting induces dispersion causing clay particles to diffuse out of the aggregates. The accumulation of Na^+ causes the interparticle distance to continuously increase and the individual clay particles to disperse (Rengasamy and Summner, 1998) inducing likely root elongation thwart. The mechanisms of growth inhibition include disturbance of plant water retention, due to the high osmotic potential of the external medium and also adverse effects on gas exchange, photosynthesis and protein synthesis (Romero-Aranda et al., 2001).

The reclamation of salt affected soil requires an improvement of physical, chemical biological properties. Soil humic substances (HS) such as humic acid (HA) and fulvic acid (FA), are mainly derived from the (bio) chemical degradation of plant and animal residues and from microbial synthetic activity and they constitute a significant fraction of the soil organic matter (65-70%) (Gulser et al., 2010). There is increasing interest in the potential use of HS as plant growth promoter (Nardi et al., 2002, Pizzeghello et al., 2013). Their applications also provide many benefits to agricultural soil, including increased ability to retain moisture, a better nutrient-holding capacity, a better soil structure and a higher levels of microbial activity. The humic acids can significantly reduce water evaporation and increase its use by plants in non-clay, arid and sandy soils. In addition, HS promote the conversion of a number of mineral elements into forms available to plants. The increased availability of P_2O_5, in the presence of humic acids has been well documented (Delgado et al., 2002; Guppy et al., 2005; Hua et al., 2008). The HS presence in soil may exert several effects on plant functions and some of these may result, directly or indirectly, in a modulation of ion uptake (Nardi et al., 2002). The HS also enhance plant growth significantly due to the increasing cell membrane permeability, respiration, photosynthesis, oxygen and phosphorus uptake and supplying root cell growth (Russo and Berlyn, 1990; Gulser et al., 2010, Pizzeghello et al., 2013). Accordingly, under moderate salinity conditions, Çimrin et al. (2010) found that humic acid application improved the growth of both shoots and roots fresh and dry weight, shoot and root lengths, shoot width, cotyledon length and width and hypocotyls length of pepper plant.

On the other hand, some studies reported the hormone-like role of HS. In particular, humic acid can be used as a growth regulator to control hormone level, improve plant growth and enhance stress tolerance (Çimrin et al., 2010). The complex biological activity of humic matter depends on its concentration, chemical characteristics and molecular size and weight (Muscolo et al., 2007). These authors also found a similar effect produced by indole-3-acetic acid (IAA). The low molecular fraction of HS caused an increase in carrot cell growth similar to that induced by 2,4-dichlorophenoxyacetic acid (2,4-D) and promoted morphological changes similar to those induced by IAA (Muscolo et al., 2007). In addition, Muscolo and Nardi (1999) reported the possible binding site of low molecular weight HS on the IAA cell membrane receptors. The HS seem to influence positively the metabolic and signaling pathways involved in the plant development, by acting directly the specific physiological targets (Trevisan et al., 2010; Pizzeghello et al., 2013). At the molecular level, the effects of HS have been demonstrated also on the expression of specific genes, such as the two H^+-ATPase isoforms Mha1 and Mha2 (Quaggiotti et al., 2004).

This review presents an overview on the HS formation according to 4 theories: (*i*) theory of lignin, (*ii* and *iii*) theory of polyphenols and (*iv*) theory of amino-saccharidic condensation. It aimed to investigate the role of these substances on improving salt-affected soil physic-chemical properties, in order to mitigate soil salinity. In addition, it focuses also the direct role of humic substances on plant growth, hormone like activities and effect on heavy metals.

Humic substances

Humic substances formation

Rosa et al. (2005) indicated that the investigations of the structure of HS are important to govern the properties and reactions of these materials in the environment. For decades, to gain a better understanding of the structure of humic matter, the chemical structure of HS has been investigated using several techniques, particularly infrared and UV-visible spectroscopy, nuclear magnetic resonance, spin electron resonance, fluorescence and PY-CG-MS (Milori et al., 2002; Rosa et al., 2005). Moreda-Piñeiro et al. (2004) suggested that the HS are a series of naturally high molecular weight compounds, which are the main component of organic matter in soils and waters. The bacterial and chemical degradation of lignins (substances deposited in cell membranes that help to give the plant support and rigidity) and of other structural carbohydrates in plants are responsible to build the intermediate product of HS, such as amino acids, aromatics, fatty acid and organic acids (Wersahw, 1989; Gramss et al., 1999; Williams and Jackson, 2002). These intermediate products are then polymerised in presence of polyphenols. These metabolites can be oxidised to quinones either spontaneously in presence of molecular oxygen or enzymatically, mediated by a wide variety of microorganisms. Since polyphenols from plant degradation are involved in the formation of HS, the phenolic acids (pyrocatechic acid, vanillic acid, vanillin, resorcinol, ferulic acid and benzoic acid) would then contribute an important part to the structure of HS. These phenolic acids have at least one carboxyl group (-COOH) and one phenolic hydroxyl (-OH) group. These are the functional groups responsible of mineral chelating capacity of HA and FA. The phenolic compounds, quinones and proteins condensate by the action of soil microorganisms on soil carbohydrates. This helps to form the structure and composition of humic and fulvic acids and thus HS (Aiken et al., 1985; Gramss et al., 1999) (Figure 1).

In Figure 1, the mechanism of humic substances formation is reported. Several investigations have demonstrated that there are four ways of HS formation that are described below.

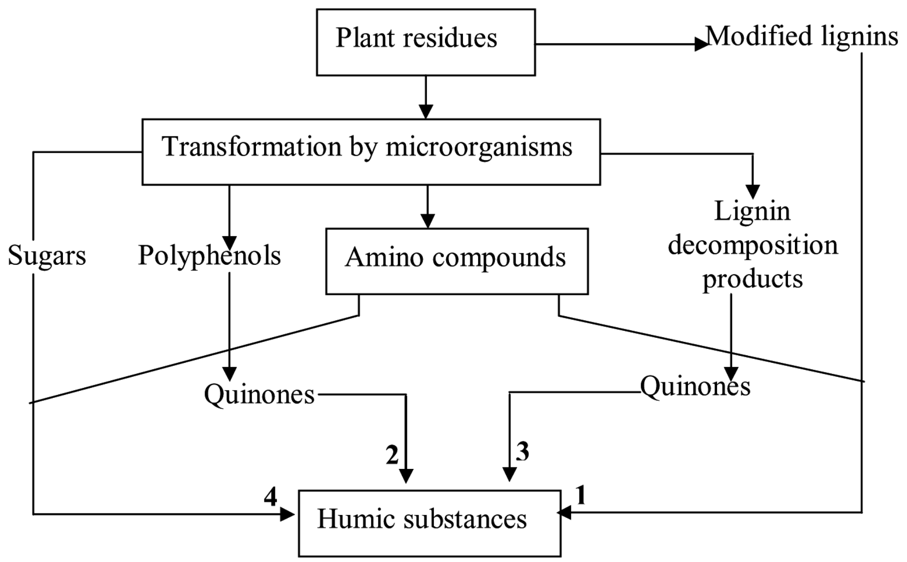

Figure 1. Mechanism of humic substances formation (Stevenson 1982). (1) theory of Lignin, (2, 3) theory of polyphenols and (4) theory of amino-saccharidic condensation.

Theory of Lignin (way formation 1)

It is the classical theory defined by Waksman (1932). It hypothesized that HS formation starts from the modified lignin (Figure 1). The latter component is an easily biodegradable bio-polymer made up of an assembly of three types of units phenylpropanoïds connected between them by various types of connections C-C and ethyl oxide. The process consists of a combination of modified lignin molecules with nitrogenized components of proteinic type (amino compounds) synthesized by the micro-organisms (Stenvenson, 1994), according to the following reaction:

$$\text{(Modified lignin)-CHO} + RNH_2 \rightarrow \text{(modified lignin)-CH=NR} + H_2O$$

Accordingly, the lignin is incompletely used by the micro-organisms, because of the solidity of its structure (stable connections C-C) and, as a consequence, its residue becomes the constituent part of the soil humus (Saiz-Jimenez et al., 1989).

Theory of polyphenols (ways formation 2 and 3)

According to the theory of polyphenols, the HS can be considered as a combination of biopolymers resulting from the degradation of the plants and other organic components (way number 2). Also in this case, the HS formation occurred as a result of the transformation by microorganism (Peña-Méndez et al., 2005). According to way 3 (Figure 1), the lignin released of its bond with cellulose, during the decomposition of the vegetable residues, is subjected to a degradation and is broken up into its primary structural units (units phenylpropenes). These latter components are subsequently demethylated and oxidized out of polyphenols. The acids and the phenolic aldehydes, resulting from lignin during the microbial attack, are converted into quinones by specific enzymes, in particular the polyphenoloxydases, which remove a hydrogen atom with the phenolic OH. The quinones are then polymerized or recombined with compounds nitrogenized to form humic macromolecules (Huang, 2000).

The way 2 follows similar principle of way 3 of the HS formation. However, the only difference is that the polyphenols are synthesized by micro-organisms starting from the no woody sources of carbon (for example the cellulose, but also of the no vegetable sources). The oxidation of these bacterial polyphenols in quinones also leads to the HS formation.

Theory of amino-saccharidic condensation (way formation 4)

Mriaillard (1913) proposed the combination of reducing sugars and amines to explain the formation of HS. It is known as complex reactions implementing compounds presenting of the reducing groupings (aldehydic or cetonic) and of the amino compounds which react each others to produce aromatic and coloured substances. The compounds with grouping carbonyl (C=O) can be carbohydrates (sugars) or products of oxidation of the lipids and the function amine come from the amino acids themselves, proteins or natural or exogenic amines. The sugars and the amino acids resulting from the microbial metabolism and degradation from the organic matter are polymerized by abiotic condensation (Anderson et al., 1989; Jokic et al., 2001).

Component of humic substances

The chemical structure of HS is not simple and well known (Yang et al., 2004). However, Cheshire et al. (1967) indicated that the main structure of HS is a polycyclic aromatic core linked with side chain structures, such as carbohydrates phenolics, proteins, peptides and metal ions. The term HS is used as a generic name to describe the colored material or its fractions obtained on the basis of solubility characteristics (Moreda-Piñeiro et al., 2004). In any case, according with these properties the HS are divided into three fractions humic acids, fulvics acids and humin, which are described as follow.

Humic acids

The humic acids are the brown to black polymeric constituent of soils. They consisted of lignite and peat, containing aromatic and heterocyclic structures, carboxyl groups and nitrogen (Stevenson, 1982; Mosley and Mosley, 1998). Their elemental composition is reported in the Figure 2.A. This fraction of HS is naturally formed from the decomposition of cellular substances and acts to decompose cell walls and gluing materials (hydrocarbons) in decaying plant life. The humic acids constitute the fraction of HS that is not soluble in water under acidic conditions ($pH<2$) but they are soluble at higher pH values (Chung et al., 2005). They can be extracted from soil by various reagents and they are insoluble in diluted acid. The same authors also indicated that the humic acids are the major extractable component of soil HS.

Fulvics acids

The fulvics acids are the light orange to brown constituent of soil humus. These natural materials are formed from the decomposition of cellular material and they act as a natural chelator of minerals and metals in soils (Mosley and Mosley, 1998) (Figure 2.B). The material enters the food chain and is soluble in acid solutions. The fulvic acids are the fraction of HS that is soluble in water under all pH conditions. They remain in solution after removal of humic acid by acidification (Eyheraguibel et al., 2008).

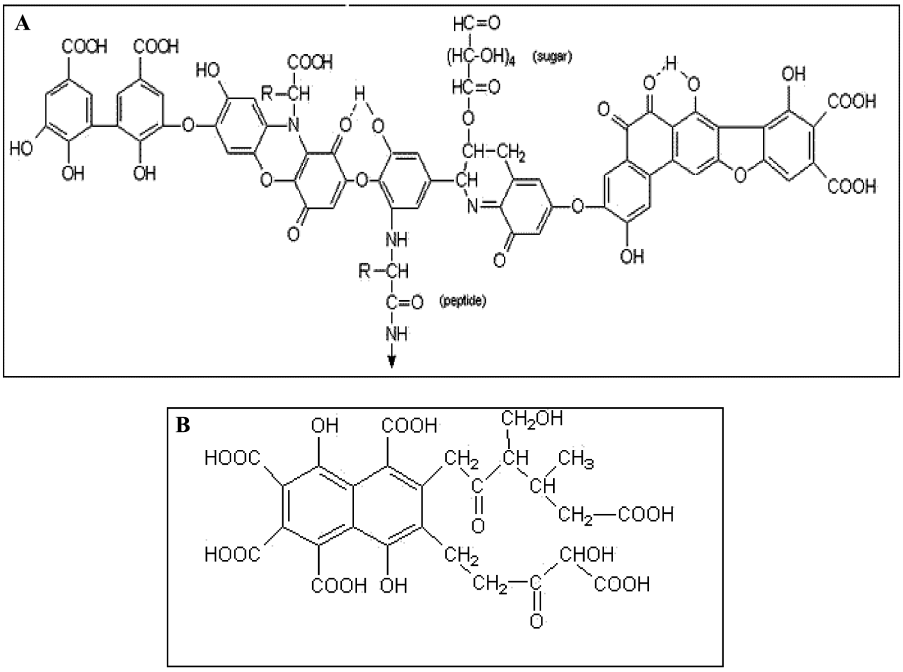

Figure 2. Elemental composition of humic substance (A: humic acid, B: fulvic acid) (Stevenson, 1982).

Humin

The humin is the fraction of HS that is not soluble in water at any pH value (Kulikova et al., 2005). It is not even soluble in the alkaline media. It breaks down slowly by soil microbial activity and affects the soil by regulating water holding capacity ion exchange rates, electrical conductivity and pH (Mosley and Mosley, 1998). The color of humin substances are black in soil humus.

Effects of humic substances on plant in saline media

The effects of HS on plant growth stimulation have been observed and well documented in a large number of researches (Chen and Avid, 1990; Nardi et al., 1996; Cesco et al., 2002; Yang et al., 2004; Rady, 2012). These authors have confirmed that HS can indirectly and directly affect the physiological processes of plant growth.

Indirect effects: improving saline soil properties

Organic matter is an important soil component in improving soil chemical and physical properties (Montemurro et al., 2007; Diacono and Montemurro, 2010). Gulser et al. (2010) indicated that they constitute 65-70% of the soil organic matter. Due to their structural characteristics, the HS control several physical and chemical properties of soil. In particular, they influence the stability of aggregates, buffering capacity, sorption of hydrophobic organic compounds and transport, bioavailability and complexation of metals present in the environment (Rosa et al., 2005). The HS supply provide, throughout the organic matter distribution, the energy for the beneficial organisms within the soil, influencing the soil water holding capacity, the soil structure, the release of plant nutrients from soil minerals, the increase availability of trace minerals, so improving salt-affected soil fertility. These beneficial effects are particularly important to sustain the productivity of soils, especially in arid regions where there are large zones of low input of organic matter. The role and the importance of the HS in soils and in particular of the humus, are proven since a long time (Thomann, 1964). Their multiple properties, especially their "sequestering" capacity (adsorbent, chelating) with organic compounds and mineral (pesticides and metals) confer to them an essential role in the solubilization, the biodisponibility, the degradability, the transport and the exchanges of these compounds in water and soil (Busnot et al., 1995).

In salt affected soil, the sodium percentage in water generally increases. In this situation, there is a tendency for great adsorption of sodium ions on the humus complex leading to the salinization of the soil and the toxicity of plants (Tchiadje, 2007), which may be represented by:

$$2Na^{+} + Ca(humus) \rightarrow (humus)Na + Ca^{2+} \qquad \text{(Figure 3.A)}$$

The most effective amelioration methods are based on the removal of exchange and soluble sodium and changing the ionic composition of soils with, at the same time, leaching the sodium salts out of the soil profile (Chhabra, 1994). Raychev et al. (2001) reported that the result of such procedures is to reduce pH and osmotic pressure of soil solution, thus promoting good conditions for the decrease of the dispersion of soil colloidal fractions. The organic matter application and, as a consequence, the HS distribution decreased soil Na, EC and pH likely due to high supplies of Ca, Mg and K. These mineral elements kept the cation-exchange sites on soil particles, minimizing adsorption of Na, so enhancing Na leaching losses during precipitation events (Figure 3.B) (Lakhdar et al., 2009). In fact, the reduction of salinity means to reduce the monovalent Na^+ and this is particularly evident when the replace of the monovalent K^+ to the humate (salt) of the humic complex occurred. Thus, by electrostatic repulsion the high concentration of K^+ the Na^+ will be replaced on the humic complex and the previous equation becomes:

$$2\,K^+ + Na(humus) \rightarrow (humus)K + Na^+$$

Then, by the water dilution of the cation H^+, the K^+ will replace and, at last, it will be available to plants. The effective removal of Na^+ by leaching depends on the amendment-soil contact, soil permeability and the availability of drainage practices. The addition of Ca-based compounds improves near-surface soil physical quality and reduces surface sealing and crusting apart from replacing/neutralizing the Na^+. Different authors (Garcia et al., 1994; Albiach et al., 2001; Madejon et al., 2001; Arancon et al., 2004) indicate that, due to this particular effect, the HS are used as additives in fertilizers. Furthermore, Buckau et al. (2000) suggested that different salts of HS, such as calcium humate, were used to increase soil fertility.

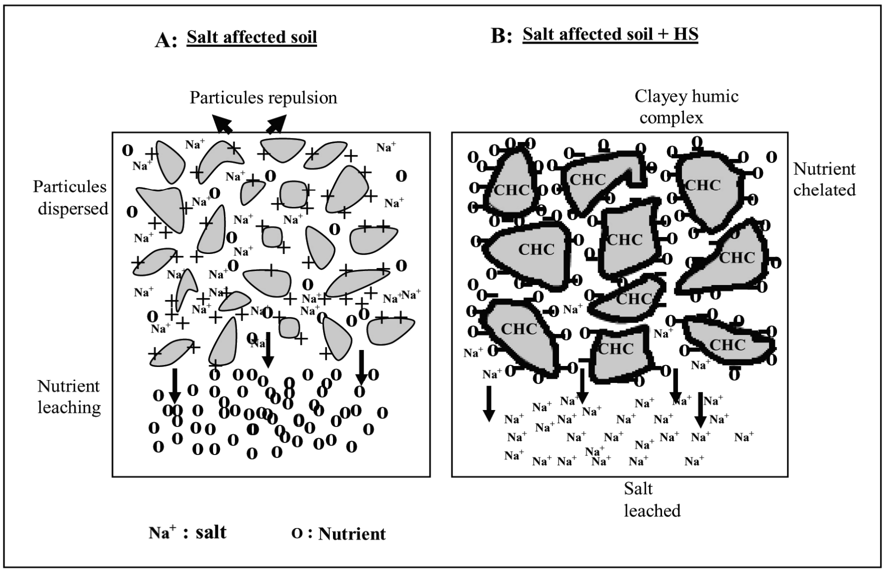

Figure 3. Schematic representation of the indirect effect of humic substances added to salt-affected soil.

Direct effects on plants

Several investigations have demonstrated the direct effects of HS on some crops and the main researches are summarized in the Table 1. The direct effects of HS on plants include those changes in plant metabolism that occur following the uptake of organic macromolecules, such as HA and FA. Once these compounds enter in plant cells, several biochemical changes occur in both membranes and cytoplasmic components of plant cells. The HS influence several metabolic processes, such as photosynthesis, respiration, nucleic acid synthesis and ion uptake (Nardi et al., 2002). In particular, the humic acid influences the production of RNA, which is essential for many biochemical processes in the cell (Trevisan et al., 2010).

Table 1. Direct effects of humic substances on plant.

HS effect	References
Stimulation of germination	Masciandaro et al. 2002; Orlova and Arkhipchenko, 2009
Increase of plant growth	Chen and Aviad, 1990; Brunetti et al., 2007; Paksoy et al., 2010
Stimulation of nutrient uptake	Varanini and Pinton, 1995; Turkmen et al., 2004
Stimulation of protein synthesis and activities	Canellas et al., 2002; Carletti et al., 2008; Dantas et al., 2007
Hormone like-activities	Piccolo et al., 1992; Trevisan et al., 2010

Germination

The HS play an important role in seed germination which can be considered as the earlier stimulation induced by the humic molecules (Eyheraguibel et al., 2008). As the HS enter into the seed cells carrying both micronutrients and water, the respiration rate increases and the cell division processes are accelerated improving the growth of the root. Under saline media, Masciandaro et al. (2002) found that the addition of HS to the solution of NaCl (NaCl+HS) at high electrical conduttivity (4 mS cm^{-1}) increased the germination index (GI) from 68.5 to 118%, suggesting that HS could reduce the inhibitor effects induced by salinity on *Lepidium sativum* and *Zea mays*. According to these authors, the reduction of germination inhibition could be due to the formation of the Na-humus complexes, so that the Na is not available to be accumulated in plant cells, thus avoiding osmotic effects. Furthermore, Orlova and Arkhipchenko (2009) found a direct relationship between the concentration of HS in soil solution and their stimulating effect on cress germination. The enhanced stimulation of seed germination using humic acid solution can compensate the cool or rainy conditions and give a wider margin of safety. The addition of HS on seed (seed treatment) or within the seed furrow significantly improve seed germination and seedling development (Piccolo et al., 1993). However, the excessive concentrations of HA and/or FA can inhibit seed germination at high concentrations and can reduce the growth of young seedlings.

Plant growth

Root

Chen and Aviad (1990) and Varanini and Pinton (1995) showed a stimulator effect of HS on the root growth and development and on the uptake of macro and microelements. In particular, Turkmen et al. (2004) reported a significant increase in mineral nutrient uptake in tomato cultivated in saline medium added with humic acid. This behavior is probably due to the increasing permeability of membranes of root cells. The HS substance may also act through a stimulation of both root growth and proliferation. These effects are particularly important for the adaptation of plants to adverse soil conditions, such as salinity and could be useful for the definition of rhizosphere management practices (Romheld and Neumann, 2006). According to these authors, the improvement of nutrient uptake, particularly of micronutrients, might be important to increasing plant resistance to biotic and abiotic stresses. In addition, in a pot experiment of olive trees, Tattini et al. (1991) reported that nitrogen uptake rate by the roots increased after application of humic acid, when it was applied in the range of 30-120 mg pot^{-1}. Accordingly, Orlova and Arkhipchenko (2009) observed a maximum stimulating effect of HS treatment on roots, comparatively to shoots. In particular, the HS increase the lateral root emergence and induce the production of smaller, but more ramified, secondary roots. Eyheraguibel et al. (2008) found that the root development induces an increase in total radicular length and enhances root surface, resulting in a better mineral nutrition. The HS maintain an additional enrichment of the rhizosphere with Ca^{2+}, in salt-affected soil, which tends to replace exchangeable Na^+ on the root adsorption sites (Garcia et al., 2000). These effects on ion uptake may depend on modifications of membrane fluidity and permeability, as a result of the interaction between the humic molecules and the lipidic matrix of the plasma membrane (Samson and Visser, 1989) and/or on the interference with specific ion carriers (Vaughan and McDonald, 1971; Guminski et al., 1983). Pinton et al. (1992) found that the HS enhanced the KCl-stimulated ATPase activity of oat root when the concentration was increased up to 0.5 µg org C mL^{-1}. This result confirms previous findings found by Maggioni et al. (1987), which suggested a regulatory capacity of humic molecules on membrane activities connected with ion uptake and plant nutrition. Furthermore, in a more recent research, Canellas et al., (2002) found that the HS affect the enzymes activities associated with the plasma membrane of roots cell. In fact, Carletti et al. (2008) the plasma membrane represents the site for the exchange of information and substances between the cell and its environment. Thus, the proteins associated with root cell plasma membrane can be reasonably be involved in HS action. The changes in their expression present likely the first reaction leading to the biological responses (mainly in salt-affected soil) (Carletti et al., 2008).

Shoot

The humic acid application positively affected the yield parameters of plant grown in salinity condition (Türkmen et al., 2005; Paksoy et al., 2010). According to Chen and Aviad (1990), the effects of HS on plant growth consistently show positive effects on plant biomass. The HS interact with the organic component of soil and the root apparatus of plants in the soil matrix. Nardi et al. (2002) indicate that the HS can have a fundamental influence not only on overall soil fertility and conservation, but also on plant physiology. It enhances plant growth, as measured in terms of an increase in length or in the fresh and dry weights of shoots and roots (Carletti et al., 2008). Furthermore, Brunetti et al. (2007) found a positive correlation between wheat grain yield and the components of HS, such as HA and FA. These substances affect the solubility of many nutrient elements by building complex forms or chelating agents of humic matter with metallic cations (Lobartini et al., 1997). Many studies of nutrient solution have been conducted to determine the direct effects of HA and FA on plant growth (Schnitzer and Poapst, 1967; Linehan, 1976; Rauthan and Schnitzer, 1981).

These researches indicated that the uptake of major plant nutrients is mediated by HS and in particular they enhanced the uptake of the major plant nutrients such as nitrogen, phosphorus and potassium. Under saline medium and to ensure a good level of growth, the plants have to maintain a high K^+ and a low Na^+ content in the cytosol. Therefore, a high K^+/Na^+ ratio is important for salt tolerance and this situation could be achieved by a high presence of HS, which stimulated the K uptake. Maggioni et al. (1987) indicated that, among the components of HS, the HA and FA can influence the nutrient absorption, due to their effect on the K^+ and Mg^{2+} dependent ATPase. The findings of Pinton et al. (1992) demonstrated that the HS affect the activity of microsomal and tonoplast. Finally, in a more recent research, Dantas et al. (2007) indicated that, when the HS is associated with the enzymes, it can lead to the enhancement of many enzymes activity (phosphorilase, phosphatase and cytochrome oxidase), to the inhibition of others (IAA oxidase, fitase and peroxidase) and to the synthesis of some (invertase).

Hormone like activity

The hormone-like activity of HS has received increasing attention and, as a consequence, there is an enhancing utilization of HS as nutrient supplements in agriculture and horticulture (Zhang and Schmidt, 1999; Selim et al., 2009). According to Trevisan et al. (2010), the stimulatory effect on Arabidopsis lateral root development observed in response to the HS application was mainly evident in the first growing stages, when cells start to divide. These findings suggest that the HS response may involve mechanisms of the stimulation of cell division and differentiation, which it is known to be under the auxin control. Piccolo et al. (1993) also found that the HS application regulate hormone level, improve tomato and lettuce growth and enhance stress tolerance. In addition, cytokinins- and auxin- like properties have been identified in HS by Clapp et al. (1998). These natural substances have been shown to enhance tolerance to abiotic stress delaying plant senescence and improving quality (Zhang and Ervin, 2004), which may mitigate deleterious effect of salt. Furthermore, the auxin causes root and shoot elongation in plants and allows them to produce tropic responses, such as shoots bending toward a light source (phototropism) and roots growing downward (geotropism). It is known that the cytokinins exhibit antisenescence properties that are related to their antioxidant activity maintaining the integrity of the tonoplast membrane (Thimann, 1987; Musgrave, 1994). Moreover this latter hormone is responsible of the formation of both roots and buds, it promotes cell division and tends to counteract the effects of aging and stress in plants. Accordingly, the application of organic matter rich in HS on sunflower cultivated in 0.3% sea salt solution (electrical conductivity equal to 4.8 dS/m) induced an improvement of plant yield (Ahmad and Jabeen, 2009). The higher vigor found after HS application has not only overcome sodium induced toxicity of substrate but also helped in restoring growth. The activation of related biochemical processes results in an increase in enzyme synthesis and protein contents (Carletti et al., 2008; Trevisan et al., 2010). During these metabolic changes an increase in the concentration of several important enzymes is detected. Experiments performed on rice cells in suspension culture seem to suggest that HS may be also used as carbon skeletons to synthesize proteins and DNA (Wang et al., 1999). Furthermore, in a recent research (Trevisan et al., 2010), the auxinic activity of the HS was investigated on the initial and lateral roots in the model plant Arabidopsis thaliana, by utilizing a combination of genetic and molecular approaches. The finding indicates an increase in root mass, photochemical efficiency, antioxidant levels and resistance to senescence during abiotic stress. Pizzeghello et al. (2013), found also a positive effect of HS on plant via isopentenyladenosine activity. In addition treatment of plants with the organo-mineral fertilizer based on HS enhanced the level of proline under salt stress (Rady, 2012). According to this researcher, the acceleration of increased pool of proline resulted in an increase in the capacity of tolerance to salinity. These results may be associated with an increased endogenous cytokinin and auxin levels because of the exogenous applications of humic acid (Zhang and Ervin, 2004), which may offer a promising opportunity to improve crop production in salt-affected soil.

Effect of HS on heavy metals

The HS are natural organic compounds, that contain structures, produced by biological and geochemical processes that contain structures. They can complex metals, sequester anthropogenic organic compounds, oxidize and reduce elements to and from toxic forms, photosensitize chemical reactions, enhance and retard the uptake of toxic compounds or micronutrients to plants and microbial organisms (Pandey et al., 2003). The HS bear a large number of functional moieties, such as carboxylic, phenolic and alcoholic groups which allow a free-cations chelation in the soil solution. Several divalent heavy metals cations would be fixed reversely via S-containing ligands. As a consequence, these acids play an important role in the homeostasis of metals in soil solution especially in the modulation of metals bioavailability to root uptake (Kalis et al., 2006).

Depending on the experimental design and the type of metal, the increased or decreased uptake of Cd, Cu, Pb by organic matter addition to soil have been observed (Garcia-Mina et al., 2004; Inaba and Takenaka, 2005; Kungolos et al., 2006; Montemurro et al., 2008; Montemurro et al., 2010). In fact Evangelou et al. (2004) demonstrated that the addition of humic acids to soil increased the cadmium uptake of tobacco plants from an artificially contaminated soil, thus confirming the statement of Halim et al. (2003), which found an enhancement of the bioavailability and mobility of other heavy metals in soil as a result of humic acids application. These organic acids could also form an enhance through their functional groups, which is not resorbed by plants and delivers the heavy metals in a more available form to the exudates of the plants. However, these effects depend essentially to the pH values of soil. When soil pH increases, H^+ dissociates from functional groups, such as carboxyl, phenolic, hydroxyl and carbonyl functional groups. As a consequence there in an increasing affinity of the metal cations with HS. On the other hand, it is known that in saline soils, pH is usually alkaline (Qadir et al., 2004; Rabhi et al., 2010). The presence of humic acids could ameliorate significantly the bioavailability of bivalent cations to the root absorption. Thus the amendment of saline soils with HS reduce the harmful effects of salinity, due to the increase of both the bioavailability of metals and the absorption by the roots of halophytes. This practice is particularly important in the sites of urban and industrial releases which are characterized by saline depression and are usually contaminated with toxic substances, especially toxic metals. These no biodegradable elements are very toxic for human health and their implication in several diseases was demonstrated (Morgano et al., 2011). Although saline soils are not agricultural productive areas, the heavy metals must be removed because of the possible risk of their migration to ground and surface waters. The use of salt sensitive plants for the phytoextraction of metals from saline soils is limited by the low phytoavailability of metals cations to root absorption. The addition of organic matter to these soils could enhance the capability of halophytes to extract metals by the increase of the concentration of free metals in soil solution (Rabhi et al., 2010).

Conclusion

Only few published studies focused the role of HS in reducing the harmful effects of salinity. Therefore, our manuscript attempted to address this issue by reviewing the effects of HS in salt affected soils. First, we reported the formation way of HS, indicating the four theories that are proposed till now (lignin, amino compounds, polyphenols and amino-saccharidic condensation). Then, the indirect and direct action of HS on plant-soil system to mitigate salinity was emphasized. The indirect effects of HS are linked to improve the physical, chemical and microbiological properties of soils. The direct actions on plant are due to their effects on germination, plant growth (root and shoot) and hormone like activity. The HS can ameliorate the deleterious effects of salt stress by increasing root growth, altering mineral uptake and decreasing membrane damage, thus inducing salt tolerance. The HS also increase the bioavailability of metals to the absorption by hyperaccumulator halophytes species which are normally used in phytoextraction.

References

Ahmad, R., Jabeen, N., 2009. Demonstration of growth improvement in sunflower (*Helianthus annuus* L.) by the use of organic fertilizers under saline conditions. Pak. J. Bot. 41, 1373-1384.

Aiken, G.R., McKnight, D.M., Wershaw, R.L., MacCarthy, P., 1985. Introduction to humic substances in soil, sediment and water. p. 1-9. *In:* Humic substances in soil, sediment and water: geochemistry, isolation and characterization. Wiley-Interscience, New York.

Albiach, R., Canet, R., Pomares, F., Ingelmo, F., 2001. Organic matter components, aggregate stability and biological activity in a horticultural soil fertilized with different rates of two sewage sludges during ten years. Biores. Technol. 77, 109-114.

Anderson, H.A., Bick, W., Hepburn, A., Stewart, M., 1989. Nitrogen in Humic Substances. Humic Substances II: In Search of Structure, John Wiley & Sons, Inc.

Arancon, N.Q., Lee, S., Edwards, C.A., Atiyeh, R., 2004. Effects of humic acids derived from cattle, food and paper-waste vermicomposts on growth of greenhouse plants. Pedobiologia. 47, 741-744.

Ashraf, M., Foolad, M.A., 2007. Improving plant abiotic-stress resistance by exogenous application of osmoprotectants glycine betaine and proline. Environ. Exp. Bot. 59, 206-216.

Brunetti, G., Senesi, N., Plaza, C., 2007. Effects of amendment with treated and untreated olive oil mill wastewaters on soil properties, soil humic substances and wheat yield. Geoderma. 138, 144-152.

Buckau, G., Artinger, R., Fritz, P., Geyer, S., Kim, J.I., Wolf, M., 2000. Origin and mobility of humic colloids in the Gorleben aquifer system. Appl. Geochem. 15, 171-179.

Busnot, A., Busnot, F., Le Querler, J.F., Yazbeck, J., 1995. Caractérisation de materiel humique, d'origine terrestre de la région Bas-Normande. Thermochim. Acta 254, 319-330.
Canellas, L.P., Olivares, F.L., Okorokova-Façanha, A.L., Façanha, A.R., 2002. Humic acids isolated from earthworm compost enhance root elongation, lateral root emergence and plasma membrane H^+-ATPase activity in maize roots. Plant Physiol. 130, 1951-1957.
Carletti, P., Masi, A., Spolaore, B., De Laureto, P.P., De Zorzi, M., Turetta, L., Ferretti, M., Nardi, S., 2008. Protein expression changes in maize roots in response to humic substances. J. Chem. Ecol. 34, 804-18.
Cesco, S., Nikolic, M., Römheld, V., Varanini, Z., Pinton, R., 2002. Uptake of 59Fe from soluble Fe-humate complexes by cucumber and barley plants. Plant Soil. 241, 121-128.
Chen, Y., Aviad, T., 1990. Effects of humic substances on plant growth. In: humic substances in soil and crop science; Selected Readinds, American Society of Agronomy and Soil Science Society of America. Madison, pp. 161-186.
Cheshire, M.V., Cranwell, P.A., Falshaw, C.P., Floyd, A.J., Hartworth, R.O., 1967. Humic acid II. Structure of humic acids. Tetrahedron. 23, 1669-1682.
Chhabra, R., 1994. Soil salinity and water quality. Oxford and IBH Publ. Co. New Delhi.
Chung, K.H., Choi, G.S., Shin, H.S., Lee, C.W., 2005. Vertical distribution and characteristics of soil humic substances affecting radionuclide distribution. J. Environ. Radioactiv. 79, 369-379.
Çimrin, K.M., Türkmen, Ö., Turan, M., Tuncer, B., 2010. Phosphorus and humic acid application alleviate salinity stress of pepper seedling. Afr. J. Biotechnol. 9, 5845-5851.
Clapp, C.E., Liu, R., Cline, V.W., Chen, Y., Hayes, M.H.B., 1998. Humic substances for enhancing turfgrass growth. p. 227-234. *In:* G. Davies and E.A. Ghabbour (ed.) Humic substances: Structures, properties and uses. Royal Soc. Chem. Publ. Cambridge, UK.
Dantas, B.F., Pereira, M.S., Ribeiro, L.S., Maia, J.L.T., Bassoi, L.H., 2007. Effect of humic substances and weather conditions on leaf biochemical changes of fertigated guava tree, during orchard establishment. Rev. Bras. Frutic. Jaboticabal. 29, 632-638.
Delgado, A., Madrid, A., Kassem, S., Andreu, L., del Campillo, M.C., 2002. Phosphorus fertilizer recovery from calcareous soils amended with humic and fulvic acids. Plant Soil. 245, 277-286.
Diacono, M., Montemurro, F., 2010. Long-term effects of organic amendments on soil fertility. A review. Agron. Sustain. Dev. 30, 401-422.
Kalis, E.J., Temminghoff, E.J., Weng, L., van Riemsdijk, W.H., 2006. Effects of humic acid and competing cations on metal uptake by *Lolium perenne*. Environ. Toxicol. Chem. 25, 702-711.
Evangelou, M.W.H., Daghan, H., Schaeffer, A., 2004. The influence of humic acids on the phytoextraction of cadmium from soil. Chemosphere. 57, 207-213.
Eyheraguibel, B., Silvestre, J., Morard, P., 2008. Effects of humic substances derived from organic waste enhancement on the growth and mineral nutrition of maize. Biores. Technol. 99, 4206-4212.
Garcia, C., Hernandez, T., Costa, F., Ceccanti, B., 1994. Biochemical parameters in soils regenerated by the addition of organic wastes. Waste Manage. Res. 12, 457-466.
Garcia, C., Hernandez, T., Pascual, J.A., Moreno, J.L., Ros, M., 2000. Microbial activity in soils of SE Spain exposed to degradation and desertification processes. Strategies for their rehabilitation. p. 93-143. In C. Garcia and M.T. Hernandez (ed.) Research and perspectives of soil enzymology in Spain. CEBAS-CSIC, Spain.

Garcia-Mina, J.M., Antolin, M.C., Sanchez-Diaz, M., 2004. Metal-humic complexes and plant micronutrient uptake: a study based on different plant species cultivated in diverse soil types. Plant Soil. 258, 57-68.
Gramss, G., Ziegenhagen, D., Sorge, S., 1999. Degradation of soil humic extract by wood- and soilassociated fungi, bacteria and commercial enzymes. Microbial Ecol. 37, 140-151.
Gulser, F., Sonmez, F., Boysan, S., 2010. Effects of calcium nitrate and humic acid on pepper seedling growth under saline condition. J. Environ. Biol. 31, 873-876.
Guminski, S., Sulej, J., Glabiszewski, J.J., 1983. Influence of sodium humate on the uptake of some ions by tomato seedlings. Acta Soc. Bot. Poloniae. 52, 149-164.
Guppy, C.N., Menzies, N.W., Moody, P.W., Blamey, F.P.C., 2005. Competitive sorption reactions between phosphorus and organic matter in soil: A review. Aust. J. Soil Res. 43, 189-202.
Halim, M., Conte, P., Piccolo, A., 2003. Potential availability of heavy metals to phytoextraction from contaminated soils induced by exogenous humic substances. Chemosphere. 52, 265-275.
Hua, Q.X., Li, J.Y., Zhou, J.M., Wang, H.Y., Du, C.W., Chen, X.Q., 2008. Enhancement of Phosphorus Solubility by Humic Substances in Ferrosols. Pedosphere. 18, 533-538.
Huang, P.M., 2000. Abiotic catalysis. In: Sumner, M.E. (Ed.), Handbook of Soil Science. CRC Press, Boca Raton, pp. 303-332.
Inaba, S., Takenaka C., 2005. Effects of dissolved organic matter on toxicity and bioavailability of copper for lettuce sprouts. Environ. Int. 31, 603-608.
Jokic, A., Frenkel, A.I., Vairavamurthy, M., Huang, P.M., 2001. Birnessite catalysis of the Maillard reaction: its significance in natural humification. Geophys. Res. Lett. 28, 3899-3902.
Kulikova, N.A., Stepanova, E.V., Koroleva, O.V., 2005. Mitigating activity of humic substances: direct influence on biota. Use of humic substances to remediate polluted environments: from theory to practice. I.V. Perminova et al. (eds.).
Kungolos, A., Samaras, P., Tsiridis, V., Petala, M., Sakellaropoulos, G., 2006. Bioavailability and toxicity of heavy metals in the presence of natural organic matter. J. Environ. Sci. Heal. A. 41, 1509-1517.
Lakhdar, A., Rabhi, M., Ghnaya, T., Montemurro, F., Jedidi, N., Abdelly, C., 2009. Effectiveness of compost use in salt-affected soil. J. Hazard. Mater. 171, 29-37.
Lauchli, A., Epstein, E., 1990. Plant response to salinity and sodic conditions. In: Agricultural Salinity Assesment and Management (Ed. K.K. Tanji). American Society of Civil Engineers, Manual and Report Engineering Practice. 71, 113-137.
Linehan, D.J., 1976. Some effects of a fulvic acid component of soil organic matter on the growth of cultured excised tomato roots. S. Biol. Biochem. 8, 511-517.
Lobartini, J.C., Orioli, G.A., Tan, K.H., 1997. Characteristics of soil humic acid fractions separated by ultrafiltration, Commun. Soil Sci. and Plant Anal. 28, 787-796.
Madejon, E., López, R., Murillo, J.M., Cabrera, F., 2001. Agricultural use of three (sugar-beet) vinasse composts: effect on crops and chemical properties of a Cambisol soil in the Guadalquivir river valley (SW Spain). Agric. Ecosyst. Environm. 84, 55-65.
Maggioni, A., Varanini, Z., Nardi, S., Pinton, R., 1987. Action of soil humic matter on plant roots: Stimulation of ion uptake and effects on (Mg^{2+} K^{+}) ATPase activity. Sci. Tot. Environ. 62, 355-363.

Masciandaro, G., Ceccanti, B., Ronchi, V., Benedicto, S., Howard, L., 2002. Humic substances to reduce salt effect on plant germination and growth. Commun. Soil Sci. Plant Anal. 33, 365-378.

Milori, D.M.B.P., Martin-Neto, L., Bayer, C., Mielniczuk, J., Bagnato, V., 2002. Humification degree of soil humic acids determined by fluorescence spectroscopy. Soil Sci. 167, 739-749.

Montemurro, F., Maiorana, M., Convertini, G., Ferri, D., 2007. Alternative sugar beet production using shallow tillage and municipal solid waste fertiliser. Agron. Sustain. Dev. 27, 129-137.

Montemurro, F., Canali, S., Convertini, G., Ferri, D., Tittarelli, F., Vitti, C., 2008. Anaerobic digestates application on fodder crops: effects on plant and soil. Agrochimica. 52, 297-312.

Montemurro, F., Charfeddine, M., Maiorana, M., Convertini, G., 2010. Compost use in agriculture: the fate of heavy metals in soil and fodder crop plants. Compost Sci. Util. 18, 47-54.

Moreda-Piñeiro, A., Bermejo-Barrera, A., Bermejo-Barrera, P., 2004. New trends involving the use of ultrasound energy for the extraction of humic substances from marine sediments. Anal. Chim. Acta. 524, 97-107.

Morgano, M.A., Rabonato, L.C., Milani, R.F., Miyagusku, L., Balian, S.C., 2011. Assessment of trace elements in fishes of Japanese foods marketed in São Paulo (Brazil). Food Control. 22, 778-785.

Mosley, R., Mosley, M., 1998. The effects of humates on remediation of hydrocarbon and salt contaminated soils 5th International Petroleum Environmental Conference, Albuquerque, New Mexico, October 20th-23rd.

Mriaillard, L.C., 1913. Formation de matieres humiques par action de polypeptides sur sucres. CR Acard Sci. 156, 148-149.

Muscolo, A., Nardi, S., 1999. Effetti di due frazioni umiche sul metabolismo azotato di cellule di *Daucus carota*. IV Convegno Nazionale dell' IHSS, Le ricerche di base e le applicazioni delle sostanze umiche alle soglie del 2000. Alghero, Italy, pp. 103-106.

Muscolo, A., Sidari, M., Francioso, O., Tugnoli, V., Nardi, S., 2007. The auxin-like activity of humic substances is related to membrane interactions in carrot cell cultures. J. Chem. Ecol. 33, 115-129.

Musgrave, M.E., 1994. Cytokinins and oxidative processes. p. 167-178. *In:* D.W.S. Mokand M.C. Mok (ed.) Cytokinins: Chemistry, activity and function. CRC Press, Inc., Boca Raton, FL.

Nardi, S., Pizzeghello, D., Muscolo, A., Vianello, A., 2002. Physiological effects of humic substances on higher plants. Soil Biol. Biochem. 34, 1527-1536.

Nardi, S., Concheri, G., Dell'Agnola, G., 1996. Biological activity of humus. In: Piccolo A, ed. Humic Substances in Terrestrial Ecosystems. The Netherlands: Elsevier. pp. 361-406.

Orlova, O.V., Arkhipchenko, I.A., 2009. Humic substances of composts from municipal Solid wastes as a promising plant growth stimulator. Russ. Agr. Sci. 35, 175-178.

Paksoy, M., Türkmen, Ö., Dursun, A., 2010. Effects of potassium and humic acid on emergence, growth and nutrient contents of okra (*Abelmoschus esculentus* L.) seedling under saline soil conditions. Afr. J. Biotechnol. 9, 5343-5346.

Pandey, K., Pandey, S.D., Virendra, M., Santha, D., 2003. Role of humic acid entrapped calcium alginate beads in removal of heavy metals. J. Hazard. Mater. 98, 177-181.

Peña-Méndez, E.M., Havel, J., Jiří, P., 2005. Humic substances . compounds of still unknown structure: applications in agriculture, industry, environment and biomedicine. J. Appl. Biomed. 3, 13-24.

Pizzeghello, D., Francioso, O., Ertani, A., Muscolo, A., Nardi, S., 2013. Isopentenyladenosine and cytokinin-like activity of different humic substances. J. Geochem. Ex. 129, 70-75.

Piccolo, A., Celano, G., Pietramellara, G., 1993. Effects of fractions of coal-derived humic substances on seed germination and growth of seedlings (*Lactuga sativa* and *Lycopersicum esculentum*). Biol. Fertil. Soil. 16, 11-5.

Pinton, R., Varanini, Z., Vizotto, G., Maggioni, A., 1992. Soil humic substances affect transport properties of tonoplast vesicles isolated from oak roots. Plant Soil. 142, 203-210.

Qadir, M., Oster, J.D., 2004. Crop and irrigation management strategies for saline-sodic soils and waters aimed at environmentally sustainable agriculture. Sci. Total Environ. 323, 1-19.

Quaggiotti, S., Ruperti, B., Pizzeghello, D., Francioso, O., Tugnoli, V., Nardi, S., 2004. Effect of low molecular size humic substances on nitrate uptake and expression of genes involved in nitrate transport in maize (*Zea mays* L.). J. Exp. Bot. 55, 803-813.

Rabhi, M., Ferchichi, S., Jouini, J., Hamrouni, M.H., Koyro, H.W., Ranieri, A., Abdelly, C., Smaoui, A., 2010. Phytodesalination of a salt-affected soil with the halophyte *Sesuvium portulacastrum* L. to arrange in advance the requirements for the successful growth of a glycophytic crop. Biores. Technol. 101, 6822-6828.

Rady, A.A., 2012. A novel organo-mineral fertilizer can mitigate salinity stress effects for tomato production on reclaimed saline soil. South Afr. J. Bot. 81, 8-14.

Rauthan, B.S., Schnitzer, M., 1981. Effects of soil fulvic acid on the growth and nutrient content of cucumber (*Cucumus sativus*) plants. Plant Soil. 63, 491-495.

Raychev, T., Popandova, S., Józefaciuk, G., Hajnos, M., Sokoowska, Z., 2001. Physicochemical reclamation of saline soils using coal powder. Int. Agrophysics. 15, 51-54.

Rengasamy, P., Summner, ME., 1998. Processes involved in sodic behaviour. In ‘Sodic soils: Distrubition, Properties, Management and Environmental Consequences’. (Eds ME Sumner, R Naidu). (Oxford University Press: New York).

Romero-Aranda, R., Soria, T., Cuartero, J., 2001. Tomato plant water uptake and plant-water relationships under saline growth conditions. Plant Science. 160, 265-272.

Römheld, V., Neumann, G., 2006. The Rhizosphere: Contributions of the soil-Root Interface to Sustainable Soil Systems. In: Uphoff, N. et al. (ed.) Biological Approaches to Sustainable Soil Systems. CRC Press, Boca Raton, FL. pp. 91-107.

Rosa, A.H., Simoes, M.L., Oliveira, L.C., Rocha, J.C., Neto, L.M., Milori, D.M.B.P., 2005. Multimethod study of the degree of humification of humic substances extracted from different tropical soil profiles in Brazil’s Amazonian region. Geoderma. 127, 1-10.

Russo, R.O., Berlyn, G.P., 1990. The use of organic biostimulants to help low input sustainable agriculture. J. Sust. Agric. 2, 19-42.

Saiz-Jimenez, C., Senesi, N., De Leeum, J.W., 1989. Evidence of lignin residues in Humic acids isolated from vermicomposts. J. Anal. Appl. Pyrol. 15, 121-128.

Samson, G., Visser, S.A., 1989. Surface-active effects of humic acids on potato cell membrane properties, Soil Biol. Biochem. 21, 343-347.

Schnitzer, M., Poapst, P.A., 1967. Effects of a soil humic compound on root initiation. Nature. 213, 598-599.

Selim, E.M., Mosa, A.A., El-Ghamry, A.M., 2009. Evaluation of humic substances fertigation through surface and subsurface drip irrigation systems on potato grown under Egyptian sandy soil conditions. Agr. Water Manage. 96, 1218-1222.
Stevenson, F.J., 1982. Extraction, fraction and general chemical composition of soil organic matter. *In* Stevenson FJ, editor. Humus Chemistry, Genesis, Composition, Reactions. John Wiley and Sons, New York.
Stevenson, F.J., 1994. Humus Chemistry. Genesis, composition, reactions, 2nd Ed. New York: John Wiley & Sons, 1994. 496p.
Tattini, M., Bertoni, P., Landi A., Traversi, M.L., 1991. Effect of humic acids on growth and biomass partition of container grown olive plant. Acta Hortic. 294, 75-80.
Tchiadje, N.F.T., 2007. Strategies to reduce the impact of salt on crops (rice, cotton and chilli) production: A case study of the tsunami-affected area on India. Desalination. 206, 524-530.
Tester, M., Davenport, R., 2003. Na^+ tolerance and Na^+ transport in higher plants. Ann. Bot. 91, 503-527.
Thimann, K.V., 1987. Plant senescence: A proposed integration of the constituent processes. p. 1-19. *In:* W.W. Thomson and E.A. Nothnagel (ed.) Plant senescence: Its biochemistry and physiology. Amer. Soc. Plant Physiol. Rockville, MD.
Thomann, C., 1964. Les différentes fractions humiques de quelques sols tropicaux de l'Ouest africain. Cahiers de Pédologie O.R.S.T.O.M., 2 (3), 48-79.
Trevisan, S., Francioso, O., Quaggiotti, S., Nardi, S., 2010. Humic substances biological activity at the plant-soil interface. Plant Signal. Behav. 5, 1-9.
Türkmen, Ö., Dursun, A., Turan, M., Erdinç, C., 2004. Calcium and Humic Acid Affect Seed Germination, Growth and Nutrient Content of Tomato (*Lycopersicon esculentum* L.) Seedlings Under Saline Soil Conditions. Acta Agr. Scan B-S-P. 54, 168-174.
Türkmen, O., Demir, S., Sensoy, S., Dursun, A., 2005. Effects of arbuscular mycorrhizal fungus and humic acid on seedling development and nutrient content of pepper grown under saline soil conditions. J. Biol. Sci. 5, 568-574.
Varanini, Z., Pinton, R., 1995. Humic substances and plant nutrition, In: U. Lüttge (ed.), *Progress in Botany, Vol. 56*, Springer-Verlag, Berlin, Heidelberg, Germany, pp. 97-117.
Vaughan, D., McDonald, I.R., 1971. Effects of humic acid on protein synthesis and ion uptake in beet discs. J. Exp. Bot. 22, 400-410.
Waksman, S.A., 1932. Principles of Soil Microbiology. Baltimore, Williams & Wilkins Co.
Wang, W.H., Bray, C.M., Jones, M.N., 1999. The fate of 14C-labelled humic substances in rice cells in cultures. J. Plant Physiol. 154, 203-211.
Wersahw, R.L., 1989. Application of a membrane model to the sorptive interactions of humic substances. Environ. Helath Perspect. 83, 191-203.
Williams, R., Jackson, P.D., 2002. From humic substances in Academia to the needs of international commerce. Environmental Health Founadation, Martinez, California 94553-4010, USA. 32p.
Wong, V.N.L., 2007. The effect of salinity and sodicity on soil organic carbon stocks and fluxes. Thesis of Philosphy at the Australian National University, 245p.
Yang, H.L., Hseu, Y.C., Hseu, Y.T., Lu, F.J., Lin, E., Lai, J.S., 2004. Humic acid induces apoptosis in human premyelocytic leukemia HL-60 cells. Life Sci. 75, 1817-1831.
Zhang, X., Ervin, E.H., 2004. Cytokinin-containing seaweed and humic acid extracts associated with creeping bentgrass leaf cytokinins and drought resistance. Crop Sci. 44, 1737-1745.
Zhang, X., Schmidt, R.E., 1999. Antioxidant response to hormone-containing product in Kentucky bluegrass subjected to drought. Crop Sci. 39, 545-551.