

**Research Article**

**Effects of humic acid-based buffer + cation on chemical characteristics of saline soils and maize growth**

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**Abstract:** Humic acid is believed to maintain the stability of the soil reaction, adsorption / fixation / chelate of cation, thereby increasing the availability of water and plant nutrients. On the other hand, the dynamics of saline soil cation is strongly influenced by the change of seasons that disrupt water and plant nutrients uptake. This experiment was aimed to examine the characteristics of the humic acid from compost, coal, and peat and its function in the adsorption of  $K^+$  and  $NH_4^+$  cations, thus increasing the availability of nutrients and of maize growth. Eighteen treatments consisted of three humic acid sources (compost, peat and coal), two cation additives ( $K^+$  and  $NH_4^+$ ), and three doses of humic acid-based buffer (10, 20, and 30 g / 3kg), were arranged in a factorial completely randomized with three replicates. The treatments were evaluated against changes in pH, electric conductivity (EC), cation exchange capacity (CEC), chlorophyll content, plant dry weight and plant height. The results showed that the addition of  $K^+$  and  $NH_4^+$  affected pH, CEC,  $K^+$ ,  $NH_4^+$ , and water content of the buffer. Application of humic acid-based buffer significantly decreased soil pH from  $> 7$  to about 6.3, decreased soil EC to 0.9 mS / cm, and increased exchangeable Na from 0.40 to 0.56 me / 100g soil, Ca from 15.57 to 20.21 me/100 g soil, Mg from 1.76 to 6.52 me/100 g soil, and K from 0.05-0.51 me / 100g soil. Plant growth (plant height, chlorophyll content, leaf area, and stem weight) at 35 days after planting increased with increasing dose of humic acid. The dose of 2.0g peat humic acid +  $NH_4^+$  / 3 kg of soil or 30g peat humic acid +  $K^+$  / 3 kg of oil gave the best results of maize growth.

**Keywords:** *compost, cations, humic acids, maize, saline soil*

**Introduction**

Humic substances play an important role in soil fertility and plant nutrition (Tan, 1998; Spark, 2003; Pettit, 2011). Humic acid is a derivative product of decomposed organic material that is soluble in alkali but insoluble in acid (Mikkelsen, 2005; Pena-Méndez et al., 2005). A typical humic acid molecule polymer structure may consist of six carbon aromatic ring of the basis of di- or tri-hydroxyl phenols linked by -O-, -NH-, -N-, -S-, and contain group-OH and quinone (O-  $C_6H_4$ -O-) (Tan, 1998). Humic acid is a cyclic organic compound having high molecular weight, long-chain, and active carboxyl group (-COOH) and

phenolic (-OH), which are ampoter, binding of cations/anions at certain pH conditions (pH dependent charge) (Stevenson, 1994; Bohn et al., 2001; Pena-Méndez et al., 2005; Khaled and Fawy, 2011). Carboxylate of some carboxyl group is released below pH 6 leaving a negative charge on the functional group:  $R-COOH = R-COO^- + H^+$  (Pettit, 2011). Dissociation of  $H^+$  from amide (= NH) also can increase the negative charge. Protonated groups such as  $R-OH_2^+$  and  $R-NH_3^+$  can produce a positive charge, but the overall humus is negatively charged.

Extraction of humic acid with NaOH or KOH causes the negative charge of humic acid is saturated with Na or K, so that the ions are easily

exchanged. NaOH or KOH saturation increases the pH up to 11, encouraging all acids at the level of maximum solubility and stabilizing hydrocolloid in suspension. Humic acid levels in compost, manure, straw, and other are relatively low (<1%), while that in coal deposits "Leonardite" is relatively very high (~ 15%) (Humintech, 2012). Mindari et al. (2013) obtained 2.6%, 4.6% and 7.6% humic acid extracted from compost, coal and peat with 0.5 N NaOH and deposition to pH 2, respectively. Goff (1982), Lebo et al. (1997), Anaya-Onala (2009), and Chen et al. (2009) obtained humic acid at least 60-80% and has a high solubility. They extracted humic acid in alkaline conditions (pH 9-12), and precipitated at pH 0.5-2.9, temperature of 100°C - 200°C, pressure of 5-200 psi, and time of 0.5 - 2 hours.

Nur Hasinah et al. (2008) reported that reduction of the duration of the extraction time from 24 hours to 12 hours gave a similar character humic acid. Humic acids can be characterized from the ratio of the value of E4 / E6, which is the value of absorbance at 465 nm (E4) and at 665 nm (E6). Kononova (1966) and Chen et al. (1977) believed that the ratio of E4/ E6 is associated with the degree of condensation of aromatic carbon lattice. Weak ratio values indicate weak condensation of high levels of humic aromatic components, while strong ratio indicates the proportion of higher aliphatic structure. The ratio value of E4 / E6 is primarily governed by the size of the molecule or molecular weight or particle, which is correlated with the concentration of free radicals with O, C, CO<sub>2</sub>H and total acidity (Chen et al., 1977), but it does not depend on the concentration of humic acid or fulvic. The structure of humic acids have E4 / E6 ratio that ranges from 4.1 to 4.8 (Orlov et al., 1975) and from 3.3 to 5.0 (Pansu and Gautheyrou, 2006).

The ability humic acid to adsorb cations follows the lyotropic sequence, i.e., Al<sup>3+</sup> = (H<sup>+</sup>) > Fe<sup>3+</sup> > Fe<sup>2+</sup> > Ca<sup>2+</sup> > Mg<sup>2+</sup> > K<sup>+</sup> = NH<sub>4</sub><sup>+</sup> > Na<sup>+</sup> (Tan, 1998). Sorption of NH<sub>4</sub><sup>+</sup> is similar to Na<sup>+</sup> (Nursyamsi et al., 2009). Sorption and maximum buffering capacity of the NH<sub>4</sub><sup>+</sup> and Na<sup>+</sup> are relatively different. Application of NH<sub>4</sub><sup>+</sup> significantly increased exchangeable K in Cromic Endoaquert and Typic Endoaquert. Cation adsorption by humic acid occurs through the exchange of cations in solution or that adsorbed by clay-humic. The cations are easily absorbed by the roots, increasing the transfer of micronutrients to the plant circulatory system and then change the balance of K:Na:Ca:Mg (Sharma and Kappler, 2011). Adsorption of cations or metals by humic acid can be through (a) direct adsorption (Ca<sup>2+</sup> that release PO<sub>4</sub><sup>3-</sup>), (b) complexation of Cu<sup>2+</sup> or

outer-sphere interactions for hydrated Mg<sup>2+</sup> (c) serving as a cation bridge (outer sphere complex) through direct or indirect chelation, and (d) interaction with Ca<sup>2+</sup>- humic acid aggregates or with amine groups (Sharma and Kappler, 2011). Clay or humic materials have a strong affinity to weak acids containing phenolic hydroxyl, carboxyl group, or aminosulfonyl. Alkaline cations (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>) are primary detained by simple cation exchange with COOH groups (RCOONa, RCOOK) (Zhang et al., 2013).

Food and Agriculture Organization (2005) reported that salt affects plant growth mainly through: (a) poisoning due to absorption of excessive salt constituent elements (Na), (b) decreased uptake of water, and (c) decreased uptake of essential elements (K). Maize (*Zea mays* L.) is the second staple food after rice, but the growth of maize is very susceptible to salinity, with tolerance levels of 1.5 - 2 mS / cm (Ayers and Wescot, 1976; Goudarzi and Pakniyat, 2008; Mindari et al., 2011). Maize plant is more tolerant to water shortages, lack of nitrogen and high temperature than the soil salinity. Turan et al. (2011) found that concentrations of 45 and 60 mM NaCl had a negative impact on the dry weight and uptake of N, P, K, Ca, Mg, Fe, Cu, Zn and Mn by maize.

Application of humic acid has been identified to reduce the soil salinity and improve nutrient uptake by plant (Çelik et al., 2010; Paksoy et al., 2010; Khaled and Fawy, 2011; Turan et al., 2011), increase plant physiology and biochemistry, and crop productivity (Canellas and Olivares, 2014). Variation of humic acid dose amongst researchers is determined by the source of organic matter, humic acid extraction techniques, and cationic additives, as well as the nutrients studied. Dose of humic acid and N of 1.5 to 2 g/kg, 0- 150 mg P/kg, and 0-300 mg K/kg have been reported to reduce soil salinity (60 mM NaCl or 40% CaCO<sub>3</sub>) and increase N uptake by wheat (Çelik et al., 2010).

Application of 4 g humic acid / kg to saline soil also improved N uptake by maize (Khaled and Fawy, 2011). Mindari et al. (2013) have reported application of 1.5-2.0 g humic acid / kg and 150 mg NPK / kg to saline soil that increased rice straw weight and number rice seedlings at 35 days after planting. Turan et al. (2011) reported that uptake of N and P do not need additional humic acid, but the uptake of Mg and Mn need application of 1 g humic acid /kg, and Cu uptake need application of 2 g humic acid /kg for maize grown in saline soils. Value of K<sup>+</sup> / Na<sup>+</sup> ratio in leaves can be used to determine indicators of plant susceptibility (Goudarzi and Pakniyat, 2008).

Based on the above findings, this study was aimed to elucidate the characteristics of the humic-based buffer of various organic materials (humic acid +  $K^+$  or  $NH_4^+$ ) on the chemical properties of saline soil and maize growth. The characteristics of humic acids were used to determine their potential in the cation exchange, to control cation equilibrium in saline soil dominated by Na or Ca. Addition of  $K^+$  or  $NH_4^+$  is expected to decrease exchangeable Na so it does not toxic to plant and increase nutrient uptake and plant growth.

**Materials and Methods**

The experiment was conducted from April 2013 to July 2013 in the glasshouse and laboratory of Land Resources, Faculty of Agriculture, Universitas Pembangunan Nasional “Veteran”, East Java, and in the laboratory of Soil Science Department, Brawijaya University.

**Experiment 1: Formulation and characterization of humic acid-based buffer**

Humic acid-based buffer was prepared from a mixture of humic acid with  $K^+$  or  $NH_4^+$  cation. Humic acids were extracted from compost, coal and peat with 0.5 N NaOH solution (1:10) through 24-hour agitation and precipitation with 6N HCl up to pH 2 in accordance with the method of Stevenson (1994). The extracted humic acid was purified by adding a mixture of HCl and HF (2:1 by volume) which were then forwarded with

water up to 3 times by centrifugation or settling in the separator tube. Humic acid precipitate was heated at 40°C to obtain concentrated humic acid.

Organic-C content of the humic acid was determined using the method of Walkey and Black. The percentage of humic acid was calculated by gravimetric method at a temperature of 100°C. The value of E4 and E6 were analyzed by diluting humic acid with 0.05 N  $NaHCO_3$  (1 mL of humic acid: 10 mL), and then each of which was analyzed at a wavelength of 465 and 665 nm using a Spectro Pharo 100. Ratio of E4 / E6 was obtained by dividing the value of E4 with E6 (Kononova, 1966; Chen et al., 1977). The value E4 / E6 ratio of less than 5 shows the character of humic acid, while that of more than >5 is fulvic acid (Tan, 2003). CEC value of the humic acid was analyzed by saturation 1N  $NH_4OAc$  at pH 7. Results of the analysis of the three different humic acid materials are presented in Table 1.

Buffer was formulated from a mixture of humic acid, 30-40% compost, 40-50%, 10-20% clay and 10-20% cations. The mixture was processed to become grains or granules in a pan granulator. At first, compost and clay were evenly mixed and then fed to the pan granulator before the granulation machine was started. Once the materials were evenly mixed, the concentrated humic acid was diluted and sprayed into the mixture until the materials formed a uniform grain with diameter of about 2 mm.

Table 1. Characteristics of humic acid materials

Sources of Humic Acid	Origin	Organic-C (%)	Humic Acid-C (%)	Humic Acid (%)	Ratio E4/E6	CEC (me/100g)
Compost	Gunung Anyar, Surabaya	25.15	16.39	2.60	3.71	80.72
Coal	Bukit Asam	34.80	23.87	4.60	2.37	104.09
Peat (0-10 cm)	Kotawaringin Hilir Village, Central Kalimantan	46.32	22.61	7.60	2.92	116.83

The granules were then removed from the pan granulator, air-dried, sieved to pass through a 1-2 mm sieve, and water content of the granule was made around 10-20%. Large granules was returned to the pan granulator and smoothed again for granulation process. Addition of  $KH_2PO_4$  and  $NH_4SO_4$  to the buffer was directed to increase the CEC to meet the standard of soil amendment according to Permentan (2011). Humic acid-based buffer was analyzed for C-organic content, CEC, pH,  $K^+$ ,  $NH_4^+$ , and water content. The C organic content and CEC were analyzed according to the

previous methods in organic materials that have been oven dried at 70°C as proposed by Pansu and Gautheyrou (2006). CEC was analyzed by saturation 1N Ammonium Acetate. pH and EC values were analyzed in pasta humic acid 1:1.

**Experiment 2: Effect of buffer on soil chemical characteristics and growth of maize**

The experiment was arranged according to a factorial completely randomized design where factor 1 was three types of humic acid source

(compost, peat, and coal), factor 2 was two types of cation ( $K^+$  and  $NH_4^+$ ), and factor 3 was dose of buffer (10, 20, and 30g / 3kg soil). Each treatment was repeated three times. Soil used for this experiment was top soils (0-20 cm depth) collected from Gununganyar Village of Surabaya to represent saline soils. Soil samples were air dried and sieved to pass through a 2 mm sieve for chemical analyses. The soil has the following characteristic: slow to moderate permeability (2.2-25.5 mL / h), clay texture (60-62% clay), pH 7.5-7.55, EC 1.77-1.83 mS /cm, exchangeable Ca 19.4-20.4 me/100g, exchangeable Mg 4.3-3.8 me/100g, exchangeable K 2.6-2.73, exchangeable Na 0.9-0.8 me/100g, CEC48.22-52.95 me/100g, and 1.58-2.46% organic C.

Three kilograms of dried soil were mixed with the appropriate treatment and placed in a plastic pot. Ten grams of NPK fertilizer were evenly mixed into the soil. Water with salinity of less than 1 mS / cm was added to the soil to approximate field capacity and incubated for two weeks at room temperature. After incubation of two weeks, 50 g of soil subsample was collected from each pot, air-dried and sieved to pass through 0.5 mm sieve for analyses of pH, EC, and exchangeable bases. Two pre-germinated maize seeds were planted in each pot and thinned to one seedling after one week. Maize was grown for 35 days.

**Data analysis**

The data obtained were subjected to analysis variance followed by 5% and 1% last significance different test. The statistical analysis was performed using Excel. Regression and

correlation treatment of the results were used to assess the buffer dose optimization

**Results and Discussion**

**Buffer characteristics**

There was a positive correlation between humic acid content and organic-C content, and CEC, but negative correlation to E4/E6 ratio. The values of E4 / E6 ratio of humic acids from various types of materials were less than 5 (Table 1). This was slightly different from that previously expressed by Tan (2003), who obtained the ratio E4 / E6 of soil humic acid extracts of about 4-5. Humic acid-based buffer from the mixture of humic acids, clay and cations ( $K^+$  and  $NH_4^+$ ), was intended as a soil conditioner for saline soil to stabilize pH and to balance cations in order to improve soil nutrient availability, plant growth and production. The characteristics of humic acid-based buffer from a variety of organic materials enriched with cations are presented in Table 2. Organomineral buffer complexes were various with different substrate. Association of humic acid and clay mineral form colloidal complexes of humic acid-clay and humic acid-silt aggregate (Petit, 2011). The addition of  $K^+$  decreased the amount of  $NH_4^+$  and conversely the addition of  $NH_4^+$  decreased soil  $K^+$  exchange. The addition of  $NH_4^+$  increased buffer CEC higher than the addition of  $K^+$ , because the colloid prefers to absorb  $NH_4^+$  than  $K^+$  (Nursyamsi et al., 2009). Peat humic acid-based buffer had slightly higher CEC than others. This is consistent with the characteristics of peat containing higher organic-C, humic acid, CEC, and smaller E4 / E6 ratio than others.

Table 2. Chemical characteristics of buffer after addition of cations in mixture of humic acid, compost, clay and mineral

<b>Humic acid + cations</b>	<b>pH 1:2.5</b>	<b>Organic-C (%)</b>	<b>CEC (me/100g)</b>	<b>Exch <math>K^+</math> (me/100g)</b>	<b><math>NH_4^+</math> (mg/kg)</b>	<b>Water content (%)</b>
Humic acid <sub>peat</sub>	6.3	8.17	42.80	4.50	2443.92	12
Humic acid <sub>peat</sub> + $K^+$	4.9	9.44	67.53	6.33	1105.32	59
Humic acid <sub>peat</sub> + $NH_4^+$	8.0	10.17	65.40	4.13	14791.45	62
Humic acid <sub>compost</sub>	6.8	7.43	59.58	3.19	367.20	64
Humic acid <sub>compost</sub> + $K^+$	5.9	3.93	58.43	9.42	160.71	61
Humic acid <sub>compost</sub> + $NH_4^+$	8.1	10.35	156.48	2.75	10129.52	65
Humic acid <sub>coal</sub>	6.5	9.65	52.58	4.15	150.40	63
Humic acid <sub>coal</sub> + $K^+$	5.8	8.92	61.32	6.98	48.24	60
Humic acid <sub>coal</sub> + $NH_4^+$	8.0	10.30	56.31	4.51	13017.70	64

The addition of cations did not significantly change water content and organic C content of the materials. Overall, the addition of humic acid

changed pH from > 7 to about 6.3, EC soil was about 0.9 mS / cm, exchangeable Ca, Mg, Na and K decreased from 15.57 to 20,21, from 1.76 to

6.52, from 0.40 to 0.56 and from 0.05 to 0.51 me / 100g soil, respectively.

#### **Effects of buffer on chemical characteristics of saline soil**

Application of humic acid + cations significantly affected soil cation exchange (Ca, Mg, K, Na) and soil pH, but not for EC and organic-C (Table 3). The three types of humic acid material affected the value of exchangeable Na and K but did not significantly affect exchangeable Ca and Mg. Although the dose and type of humic acid only partially affected soil cations, there was a strong interaction between them on all the cations evaluated.

Dose of buffer + cations up to 30 g / 3kg (10 g / kg, equivalent to 3 g of humic acid / kg soil) applied at 2 weeks after incubation increased the average C-organic content of more than 20% and CEC of 80 -156 me /100g. Dose of treatment of 20g humic acid with  $K^+$  or  $NH_4^+$  / 3kg increased the exchangeable K, Ca and Mg, as well as decreased the exchangeable Na better than the other treatments (Table 5). This condition is similar to that found by Çelik et al. (2010), Paksoy et al. (2010), Khaled and Fawy (2011) and Turan et al. (2011), where application of humic acid reduced the soil salinity that was detected from the decrease of Na.

Humic acids extracted from compost, peat and coal were abundant sources of organic materials having variations of humic acids content, organic-C content, and CEC. Highly oxidized organic matter such as coal, in which parts of its chemical structure are oxidized, will create site adsorption to bind micronutrients, microflora, and the molecules. According to Tan (2003) and Miklesen (2005), positive ions bound to oxidized site adsorption provide space for the entry of negatively charged molecules which causes them to absorb micronutrients.

Decomposed organic matters (peat and compost) that were saturated with NaOH or KOH caused the site was oxidized to molecules saturated with Na or K, which was ready to be exchanged with all ions in the soil. Saturation NaOH on decomposed or oxidized organic material increased the pH up to 11, leading to maximum acid solubilization and stabilizing of hydrocolloid in suspension. Formulation of humic acid-based buffer was designed to meet the quality requirements of organic soil amendment according to Permentan (2011), i.e. minimum organic-C content is 15%, pH is 4-9, and water content is 15-25%. The characteristics of inorganic soil amendment are as follows:

minimum CEC is 60 cmol/kg, maximum moisture content is 10%, and minimum fineness of 50-60 mesh is 90%. The results obtained from buffer formulation showed that the characteristics approached the quality requirements of organic soil amendment with the addition of  $K^+$  and  $NH_4^+$ . The addition of  $K^+$  ( $KH_2PO_4$ ) in a mixture of humic acid, compost and clay increased CEC, exchangeable  $K^+$ , but reduced exchangeable  $NH_4^+$ , and exchangeable  $Na^+$ . This reduction was because of  $K^+$  replaced them on the surface of colloid adsorption so the proportion of K increased. Because the three ions have similar valence, their exchange ability are determined by the affinity of the cations (Tan, 2003). In line with the increasing charge, addition of cations also increased CEC. The ability of  $K^+$  to exchange  $H^+$  potential of missel was greater than  $NH_4^+$ , this has made solution to become more acid. The pH values of 4.9-5.9 were lower than the original pH buffer of 6.3-6.5.

The content of buffer- $NH_4^+$  increased 3-8 times after addition of  $NH_4^+$  and decreased 2-4 times after addition of  $K^+$ . As adsorption of  $K^+$  and  $NH_4^+$  follows a similar liotropic (Tan, 1998), the ability to remove both cations is also similar. The difference in the results obtained was probably because of other ions in the  $KH_2PO_4$  and  $NH_4SO_4$  used. The addition of  $NH_4^+$  increased CEC higher than that of  $K^+$ . This condition occurred because of  $NH_4^+$  is easier or more preferable adsorbed to the colloid surface than  $K^+$  (Nursyamsi et al., 2009).

The high amount of  $NH_4^+$  or  $K^+$  adsorbed by colloid determines the amount of nutrient supply to the plant. Because of the ability of colloid to absorb  $NH_4^+$  or  $K^+$  is similar, addition of one of the ions will exchange the others in the same amount. The higher the dose of humic acid increased the CEC value. This was because of the increase of cations at the mineral surface and between minerals. Colloids do not only adsorbed ions, but also absorbed water, so that increase water reserves. Humic acid absorbs more than absorbents used to date (Pena-Méndez et al., 2005). This was evident that the absorption ability of the humic acid-based buffer to water was high, around 50-60%, yet during buffer formulation process the water added was only 20%. Soil reaction greatly affects the availability of nutrients to plants.

Under neutral soil pH, nutrients are available in considerable amounts. However, if the soil pH is more than 8.0, nitrogen, iron, manganese, boron, copper, and zinc will be less available to plants (Tan, 1998).

Table 3. ANOVA of humic acid –based buffer on chemical characteristics of saline soil

Source of Variation	F Calculated					F table			
	exch. Ca	exch.Mg	exch.Na	exch.K	pH	EC	Organic-C	0.05	0.01
Replicate	1.33ns	0.94ns	1.56ns	1.14ns	0.52ns	0.69ns	2.85ns	2.92	6.96
Factor A (dose)	0.15ns	0.51ns	3.59*	3.49ns	12.31*	1.58ns	0.10ns	2.92	6.96
Factor B (humic acid type)	1.11ns	0.61ns	2.62**	7.26**	1.04ns	0.85ns	2.66ns	2.92	6.96
A x B	1.09ns	1.61ns	0.79ns	1.48*	0.46ns	1.32ns	3.35*	2.13	3.75
Additive (K <sup>+</sup> , NH <sub>4</sub> <sup>+</sup> )	23.23**	30.72*	3.15ns	23.31**	3.33ns	0.004ns	10.04*	6.31	31.82
Type x Additive	1353.96**	40.19**	187.68**	29.10**	23.90**	0.60ns	1.21ns	1.8595	2.8965

Remarks: ns not significance, \* significance at 5% level, \*\* significance at 1% level

Saline intrusion on soil causes (a) fixation or absorption of other nutrients in the soil by the compounds and silica carbonate or oxide Fe, Ca, and Mg, and (b) disturbance in the balance of Ca<sup>2+</sup>, Mg<sup>2+</sup>, Na<sup>+</sup>, and K<sup>+</sup> in the soil, and further strengthen the aggregate stability (Mikklesen, 2005; Khaled and Wafy, 2011). Humic acid can increase aggregate stability (Pena-Méndez et al., 2005) which leads to improvement of physical properties of saline soils. The addition of buffer reduced the soil pH from > 7 to about 6.3. This was presumably because of the release of H<sup>+</sup> from humic acid because the cation exchange with Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> or Mg<sup>2+</sup> which made the pH of the solution slightly acid. The ability of K<sup>+</sup> to exchange with potential-H of missel was greater than NH<sub>4</sub><sup>+</sup>. This has made solution become more acid. The decline of soil pH was thought to affect the solubility of Fe<sup>3+</sup>, H<sub>2</sub>PO<sub>4</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> fixed by minerals into plant available forms (Mikkelsen, 2005).

**Effects of buffer on plant growth**

Application of humic acid-based buffer and K<sup>+</sup> or NH<sub>4</sub><sup>+</sup> significantly affected chlorophyll content, biomass dry weight, plant height and leaf area of maize at 35 days after planting (Table 4). The different types of humic acid significantly affected the chlorophyll content and plant height,

but did not affect biomass dry weight and leaf area. Application of humic acid-based buffer and NH<sub>4</sub><sup>+</sup> at 20g/3 kg increased chlorophyll content, biomass dry weight, plant height and leaf area greater than the other treatments (Table 5). In order to obtain a better yield, humic acid-based and K<sup>+</sup> should be applied at a dose of up to 30g /3kg, that is equivalent to 3g humic acid/kg as a buffer was made of 30% humic acid, although the results were lower when compared with the addition of NH<sub>4</sub><sup>+</sup>. This value was in the range of dose from 1-4 g/kg (Khaled and Wafy, 2011), but higher than 2 g/kg (Turan et al., 2011; Celik et al., 2010). Differences in the results of this study with previous studies reported by other researchers are determined by the sources of humic acid, soil texture and nutrients added. Previous researcher added Zn in humic acid-clay colloid to improve plant growth.

Petrus et al. (2010) reported that addition of humin to humic acid-NK increased maize dry matter and nutrient efficiency. Application of humic substance to soil makes the soil becomes more susceptible to interact with bioinoculants, as humic substances can modify the structure / activity of the microbial community in the rhizosphere compartment, and increase plant physiology, biochemistry, and productivity (Canellas and Olivares, 2014)

Table 4 ANOVA of humic acid –based buffer on the growth of maize growth at 35 days

Source of Variation	F Calculated				F table	
	Chlorophyll	Dry Weight	Plant Height	Leaf area	0.05	0.01
Replicate	0.01ns	0.08ns	0.57ns	0.33ns	2.92	6.96
Factor A (dose)	51.32**	48.51**	22.91**	14.88**	2.92	6.96
Factor B (humic acid type)	95.97**	0.98ns	4.33*	0.04ns	2.92	6.96
A x B	6.88**	0.77ns	1.07ns	1.97ns	2.13	3.75
Additive (K <sup>+</sup> , NH <sub>4</sub> <sup>+</sup> )	17.61**	24.61**	6.97*	1.46ns	6.31	31.82
Type x Additive	156.61**	305.02**	1.016,64**	323.54**	1.8595	2.8965

Remarks: ns not significance, \* significance at 5% level, \*\* significance at 1% level

Table 5. Effects of humic acid-based buffer and cations on soil chemical properties and maize growth.

Additive Cations	Dose (g/3kg)	exch.Na	exch.K	exch.Ca (me/100g)	exch.M	pH	Dry Weight (g/plant)	Chlorophyll	Plant Height (cm)	Leaf area (cm <sup>2</sup> )
K <sup>+</sup>	10	0.56c	0.48c	15.57a	5.16c	7.39a	86.11b	0.56a	150.82a	403.02a
	20	0.54c	0.34b	17.41b	6.52e	7.58b	93.67c	0.80a	154.06b	403.98a
	30	0.40a	0.41b	15.76a	5.82d	7.67c	112.44e	1.14b	158.92c	471.09c
NH <sub>4</sub> <sup>+</sup>	10	0.45a	0.05a	20.21c	1.82a	7.41a	47.78a	0.34a	163.02d	408.38b
	20	0.46ab	0.51c	19.80c	2.54b	7.38a	487.22f	2.23c	179.24f	474.12d
	30	0.42a	0.08a	20.13c	1.76a	7.41a	105.44d	0.69a	177.96e	530.53e
LSD 5%		0.04	0.06	0.59	0.59	0.09	0.59	0.59	0.59	0.59

Remarks: numbers in one column followed by same letter are not significantly different at 5% level

Plant will grow optimally if all nutrients needed are available. The need of nitrogen is greater than phosphorus and potassium. Addition of NH<sub>4</sub><sup>+</sup> is expected to increase maize growth better than the addition of K<sup>+</sup>, as N plays a role in the formation of chlorophyll. Chlorophyll is crucial to plant photosynthetic. The high release of humic acid-H<sup>+</sup> affected the adsorption of cation added. The addition of K<sup>+</sup> or NH<sub>4</sub><sup>+</sup> improved soil exchangeable of Na and Ca, as well as increased their availability in the soil for plants.

The higher concentrations of humic acid-based buffer was added, the greater amount availability of K<sup>+</sup> or NH<sub>4</sub><sup>+</sup> for plant uptake. These conditions increased the process of photosynthesis that promoted growth of plant organs, i.e. weight of biomass (Figure 1), the amount of chlorophyll (Figure 2), and the plant height (Figure 3). The improvement of those plant organs was determined by the interaction of different sources of humic acid, various cations, and doses applied. Addition of K<sup>+</sup> in the humic acid will increase the proportion of K in the sorption colloid so that the equilibrium of cations shifted toward increasing

ratios of K/Na and K/Mg (Goudarzi and Pakniyat, 2008). The increase of K/Na value will reduce constraints of Na uptake by plants. Provision of K will increase ion transport in the process of assimilation of sugars and carbohydrates which further increases the plant height, leaf area, and biomass.

Although the addition of K increased crop yields, the biomass dry weight of the plant was lower than that with the addition of NH<sub>4</sub><sup>+</sup>. Ammonium (NH<sub>4</sub><sup>+</sup>) is one of mayor nutrients for plant uptake and it is required more than K<sup>+</sup>, so that the availability of N determines the development of plant organs. The formation of plant leaf chlorophyll will help the process of photosynthesis with the availability of CO<sub>2</sub>, water and sunlight into carbohydrates. These nutrients are available in abundance in the air and in the soil. The addition of humic acid also indirectly increase the reserves of H<sub>2</sub>O in the soil because of its ability to absorb water becomes high (Pena-Méndez et al., 2005).

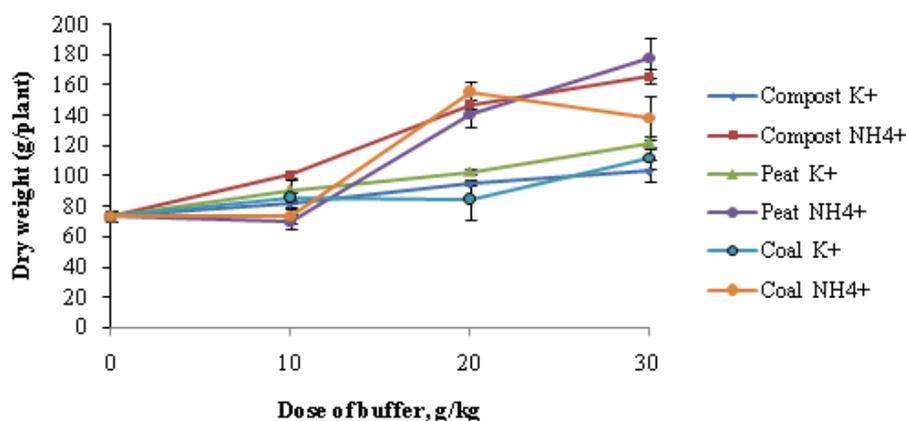


Figure 1. Dry weight of maize at 35 days after planting under various doses of humic acid and cations

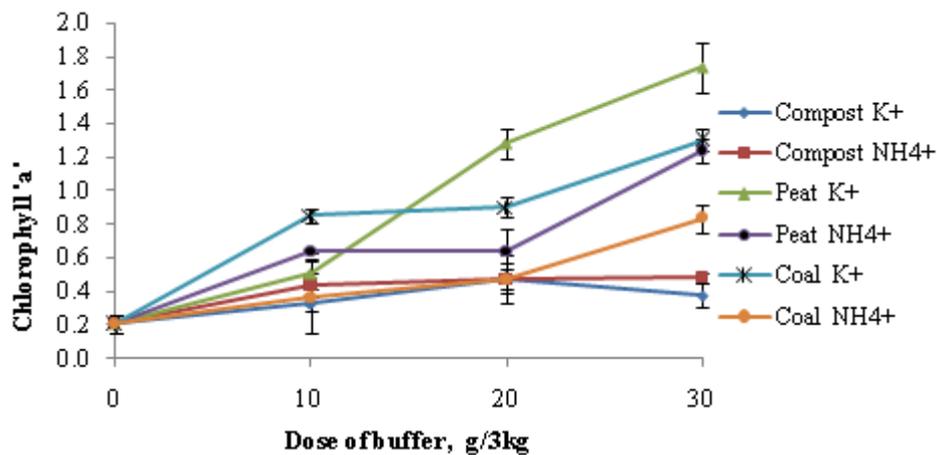


Figure 2. Chlorophyll 'a' of maize at 35 days after planting under various doses of humic acid and cations

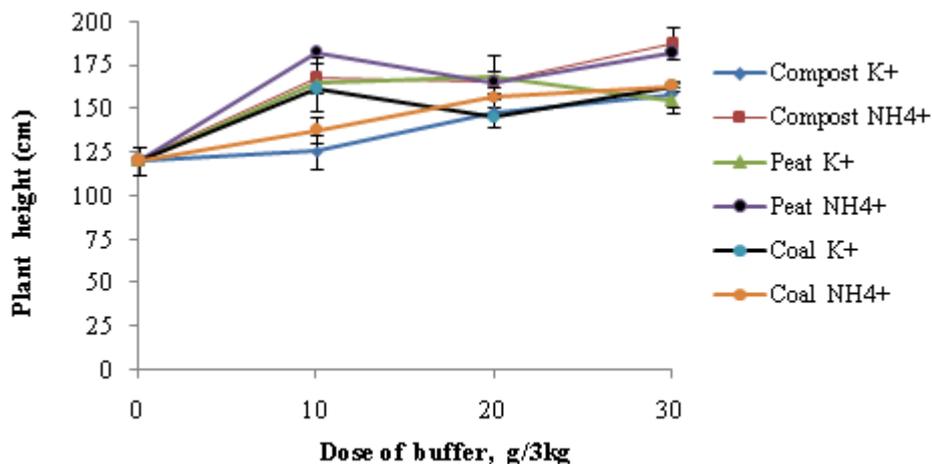


Figure 3. Plant height of maize of maize at 35 days after planting under various doses of humic acid and cations

In line with the increasing dose of buffers, the chlorophyll content of leaves increased. Total leaf chlorophyll in the treatment of peat +  $\text{NH}_4^+$  was higher than the other buffer additions. This was thought to be the high capability of peat humic acid to exchange cations that led to maximum nutrient uptake by the plant (Figure 1). As shown in Figure 1, application of buffer up to 30g/3kg linearly increased maize growth. This indicates that additional dose is needed to achieve optimum results. The addition of  $\text{NH}_4^+$  increased biomass dry weight and plant height, but did not increase chlorophyll. This condition occurred because of the part—of  $\text{NH}_4^+$  exchanged to  $\text{NH}_3$  form in alkaline soil, so that the availability of N reduced. The decrease of N resulted in the reduction of

chlorophyll formation. On the contrary, the addition of  $\text{K}^+$  increased available Mg (Table 5) and inhibited other cations, which led to the increase of chlorophyll content. There was a strong correlation of biomass dry weight at 35 days after planting with leaf chlorophyll and exchangeable Ca and inversely correlated to soil pH and soil EC (Table 6). This indicates that the maize growth was limited by soil reaction, soil salinity, and nutrient availability. The strongest correlation between chlorophyll content and biomass dry weight 35 days after planting was probably due to the important role of chlorophyll in photosynthesis that generate energy for plant organ development.

Table 6. Correlation of the biomass dry weight with soil chemical characteristics of and plant growth

	Chlorophyll (%)	Exch.Ca (me/100g)	Soil pH	Soil EC (mS/cm)	Organic-C (%)
<b>Biomass dry weight (g/plant)</b>	0.876700216	0.64612	-0.62737751	-0.58332144	0.243036103

## Conclusion

Humic acid-based buffer made of a mixture of humic acid extract, compost, clay and cations of  $K^+$  or  $NH_4^+$  with a proportion of 30-40%, 40-50%, and 10-20%, 10%, respectively, had CEC of 60-156 me/100g, organic C-organic content of 20-30%, pH of about 6.0, black in colour, and slow water soluble. Application of buffer up to 30g/3kg of soil significantly increased the exchange cations, biomass dry weight, plant height, chlorophyll, and leaf area. There was interaction between the dose and the type of humic acid in affecting soil cation exchange and plant growth. The best treatment combination was 20 g peat humic acid +  $NH_4^+$  or 30 g peat humic acid +  $K^+$  per 3 kg of saline soil.

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## References

Anaya-Olvera, A. 2009. Method of Obtaining A Concentrate Humic Extract from Organic and Inorganic Sources. Patent No: Us 7,510, 590, B2.

Ayers, R.S. and Westcot, D.W. 1976. Water Quality for Agriculture. Food and Agriculture Organization of the United Nations, Rome.

Bohn, H., McNeal, B.L. and O'Connor, G.A. 2001. *Soil Chemistry*, Third Edition. John Wiley & Sons, Inc. 307p.

Canellas, L.P. and Olivares, F.L. 2014. Physiological responses to humic substances as plant growth promoter. *Chemical and Biological Technologies in Agriculture* 1:3 [http:// www. ChemBioagro .com/content /pdf/2196-5641-1-3](http://www.ChemBioagro.com/content/pdf/2196-5641-1-3)

Çelik, H., Katkat, A.V., Aşık, B.B. and Turan, M.A. 2010. Effect of humus on growth and nutrient uptake of maize under saline and calcareous conditions. *Agriculture* 97(4): 15-22.

Chen, C.H.H., Ahmed, O.H., Majid, N.M.Ab. and Jalloh, M.B. 2009. Reduction of isolation period of coal humic acids. *American Journal of Applied Sciences* 6 (7): 1327-1330.

Chen, Y., Senesi, N. and Schnitzer, M. 1977. Information provided on humic substances by E4:E6 ratios. *Soil Science Society of America Journal* 41: 352-358

Food and Agriculture Organization. 2005. Management of Irrigation Induced Salt Affected Soils.

Goff, D.W. 1982. Method of Producing Humic Acid. Us Patent C 4,319,041

Goudarzi M. and Pakniyat, H. 2008. Comparison between salt tolerance of various cultivars of wheat and maize. *Journal of Applied Sciences* 8 (12): 2300-2305

Humintech. 2012. <http://humintech.com/001/agriculture/information/general.html>

Khaled, H. and Fawy, H.A. 2011. Effect of different levels of humic acids on the nutrient content, plant growth, and soil properties under conditions of salinity. *Soil and Water Research* 6 (1): 21-29.

Kononova, M.M. 1966. *Soil Organic Matter, Its Nature, Its Role in Soil Formation and in Soil Fertility*, 2nd English ed. Pergamon Oxford, 544 p

Lebo Jr. S.E., Kevin, W.R., Stephen, W. and Dickman, L. 1997. Production of oxidized humic acids by an oxygen-alkali. *Process* 5:688-999.

Mikkelsen, R.L. 2005. Humic materials for agriculture. *Better Crops* 89 (3): 6-10.

Mindari, W., Kusuma, Z. and Syekhfani. 2013. Isolation and Characterization of Humic Acid of Various Waste Materials on Saline Soil and Their Effects to Paddy. *Proceeding of International Conference on Green-Agro Industry*, Ina GarudaHotel, Yogyakarta 12-14 November, p 255-233

Mindari, W., Maroeto. and Syekhfani. 2011. Maize tolerance to water salinity. *Journal of Tropical Soil* 16 (3): 211-218

Nur Hanisah, M. R., Ahmed, O.H., Susilawati, K., Ab.Majid, N.M. and Jalloh, M.B. 2008. Determination of minimal duration essential for isolation of humic acids from soils in forest restoration programmes. *American Journal of Agricultural and Biological Sciences* 3 (3): 597-601.

Nursyamsi, D., Idris, K. Sabiham S., Rachim D. A., dan Sofyan, A. 2009. Jerapan dan pengaruh  $Na^+$ ,  $NH_4^+$ , dan  $Fe^{3+}$  terhadap ketersediaan K pada tanah-tanah yang didominasi mineral liat smektit. *Jurnal Tanah Tropika* 14 (1): 33-40.

Orlov, D.S., Ammosova, Y.M. and Glebova, G.I. 1975. Molecular parameters of humic acids. *Geoderma*, 13: 211-229

Paksoy, M., Türkmen, Ö. and Dursun, A. 2010. Effects of potassium and humic acid on emergence, growth and nutrient contents of okra (*Abelmoschus esculentus*) seedling under saline soil

- conditions. *African Journal of Biotechnology* 9 (33): 5343-5346.
- Pansu, M. and Gautheyrou, J. 2006. *Handbook of Soil Analysis Mineralogical, Organic and Inorganic Methods*. Springer-Verlag Berlin Heidelberg. 993p
- Pena-Méndez, E.M., Havel, J. and Patocka, J. 2005. Humic substances, compounds of still unknown structure: applications in agriculture, industry, environment, and biomedicine. *Journal of Applied Biomedicine* 3:13-24.
- Permentan No. 70-11. 2011. Tentang Pupuk Organik, Pupuk Hayati dan Pembenh Tanah. <http://perundangan.pertanian.go.id/admin/file/permentan-70-11.pdf>
- Petrus, A.C., Ahmed, O.H., Nik Muhamad, A.M., Nasir, H.M. and Jiwan, M. 2010. Effect of K-N-Humates on dry matter production and nutrient use efficiency of maize in Sarawak, Malaysia. *The Scientific World Journal*10:1282–1292
- Pettit, R.E. 2011. Organic Matter, Humus, Humate, Humic Acid, Fulvic Acid, and Humin: [http://www.calciumproducts.com/articles/dr\\_pettit\\_humate.pdf](http://www.calciumproducts.com/articles/dr_pettit_humate.pdf)
- Sharma, P. and Kappler, A. 2011. Desorption of arsenic from clay and humic acid-coated clay by dissolved phosphate and silicate. *Journal of Contaminant Hydrology* 126 (2011) 216–225
- Sparks, D. L. 2003. *Environmental Soil Chemistry*. Second Edition. University of Delaware. Academic Press. 345 P.
- Stevenson, F.J. 1994. *Humus Chemistry: Genesis, Composition, Reaction*. 2nd Ed. John Wiley & Sons, Inc. New York. p. 36.
- Tan, K.H. 2003. *Humic Matter in Soil and the Environment. Principles and Controversies*. University of Georgia. Athens, Georgia, U.S.A.
- Tan, K.H. 1998. *Principles of Soil Chemistry*. 3rd Ed. 521 P.
- Turan, M.A., Asik, B.B., Katkat, A.V. and Celik, H. 2011. The effects of soil-applied humic substances to the dry weight and mineral nutrient uptake of maize plants under soil-salinity conditions. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 39(1):171-177
- Zhang, W.Z., Chen, X.Q., Zhou, J.M., Liu, D.H., Wang, H.Y. and Du, C.W. 2013. Influence of humic acid on interaction of ammonium and potassium ions on clay minerals. *Pedosphere* 23(4): 493–502.