



**Green Agro - Industry
Investment For Our Future**
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**International Conference on Green Agro-Industry
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Preface

Over the past decades, rapid growth of global economic has lifted millions of people out of poverty. In line with rising population, rapid urbanization, and industrialization, it has also led to increase consumption of resources and generate of waste almost beyond the limits of the ecological carrying capacity.

The coming decades will likely witness of the increasing pressures on industries to shift to more resource-efficient and low-carbon production processes as part of global efforts to sustain growth, conserve resources and slow down the pace of climate change. Countries and regions that successfully manage this transition will get a better position to exploit the opportunities created by the shift towards a low-carbon world economy. It is green industry's initiation, a pattern of industrial development that is sustainable in economic, environment and social.

Universitas Pembangunan Nasional "Veteran" Yogyakarta in conjunction with its global partners is proud to announce the International Conference on Green Agro-Industry, to be held on November 11-14, 2013, at Yogyakarta, Indonesia. The basic aim of the conference is to contribute to the development of highly productive methods and technologies for the various segments of the agro-industries. This conference is designed to provide a forum for the presentation, discussion and debate on state-of-the-art and emerging technologies in the field of agro based industry and any issues related to sustain the environment.

Finally, we would like to express our gratitude to the Rector UPN "Veteran", Yogyakarta for the financial support, the Dean of the Faculty of Agriculture for hosting, and the Scientific and Steering Committee. We wish to thank the keynote speaker Director of PT Astra Agro Lestari Tbk and Plenary Speakers: Prof. Sakae Shibusawa (Tokyo University of Agriculture and Technology, Japan), Prof. Raj. Khosla, Ph.D. (Colorado State University, USA), Prof. Dr. Nilda Burgos (University of Arkansas, USA) Ir. Toine Hattink (Director of Department of Horticulture, HAS den Bosch, Netherlands) Prof. Dr. Endang Gumbira Sa'id (Bogor Agricultural University, Indonesia) . Nur Iswanto, PhD. (IKAGI, International Society of Sugar Cane Technologists Councillor), Prof. Wijitapure Wimalaratana. (Department of Economics, University of Colombo), Prof. Hassan M. El Shaer (Desert Research Center, Cairo, Egypt), Dr. Mofit Eko Poerwanto (UPN "Veteran" Yogyakarta, Indonesia) as well as participants for their contribution in making the International Conference on Green Agro-Industry.

We wish to thank PT Astra Agro lestari as the major sponsor and all other sponsors for their contribution in making this Conference possible. As a Chairperson, I highly appreciate the great efforts of the members of the organizing committee whose hard work made this seminar a great success.

Yogyakarta, November 11 , 2013

Sri Wuryani

Chairperson, ICGAI 2013

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ISOLATION AND CHARACTERIZATION OF HUMIC ACID OF VARIOUS WASTE MATERIAL SON SALINE SOIL AND THEIR EFFECTS TO PADDY

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ABSTRACT

Isolation and characterization of humic acid of various waste materials aimed to assess the its quantity and quality. The improvement potency of soil salinity and plant growth is needed to be studied further. Humic acids extracted from sewage plants, livestock and industry with 0.5 N NaOH (1:10) for 24 hours and precipitated approximately 12 hours to separate the humic and humin. Humic material containing humic acid and fulvic acid. Precipitation of humic acid with the addition of 5 N HCl to pH 2, then centrifuged at 200 rpm 30 minutes. Humic acid purification by washing with HF + HCl 1: 3 followed by water up to 3 times. Pure humic acid is dried at a temperature of about 40°C, and then characterized the value of E4/E6, C-organic, EC, pH and humic%. E4/E6 ratio values <5 shows the character of humic acid. Humic acid levels are best obtained from peat followed by coal, compost, filter cake, and manure. Cation exchange capacity in the range of 80-115 g me/100 humic acid at a pH between 6.7-7.5. EC humic acid 0 ms /cm. Humic acid potential in reducing salinity and the growth of rice for 35 DAP was not significantly different between the kinds of waste.

Keyword: C-organic, humid acid, pH, rice, waste matterial

INTRODUCTION

Humic acid is a derivative of the organic matter decomposes (Mikkelsen, 2005); <http://www.ihss.gatech.edu/sources.html>, Eladia, et al. 2005), main obtained in the manure, peat, lignite, coal, leonardite and in soil, water, waste, compost piles, marine and sediments lake. Humic acid is one of the humic substances (SH) which is soluble in alkali but not soluble in acid. A typical humic acid molecule may consist of a polymer structure of six carbon atoms in the aromatic ring of the base-or phenol trihidroksil connected by a bond-O-, -NH-, -N-, and -S-, and contains relationship-OH groups and quinone (-O-C₆H₄-O-). This structure contains a high density of functional groups of humic acid reactive molecules. AH molecular weight greater than AF. Stevenson (1982) in Spark (2003) noted that HS is considered as circular molecules, long chains or cross-linked macromolecular two or three-dimensional negatively charged mainly derived from ionization acid functional groups, eg, carboxyl.

Humic are produced from NaOH extraction in quantitative or qualitative aspects (Pansu,

2003). However, some procedure found from several studies indicating the methods of using NaOH to be reliable within certain limits and suitable for intended purpose (Tan et al., 1994). Isolation of humic acid by extracting dried sediment samples at alkaline conditions (a mixture of 0.1 M NaOH and 0.1 M Na₄P₂O₇ = 1: 1) and then the solution's fraction was added 6 M HCl until pH 2. The most basics of a extraction procedure are to mix a soil with a 0.1 M solution of NaOH at a ratio of soil: solution = 1:5, while the International Humic Substances Society (IHSS) using in 1:10 ratio. The general ratio that is used depends on the organic content of soils (Tan, 2003).

Process of extraction or separation of humic acid with the addition of alkaline NaOH , KOH to a pH of about 9 to form humic dispersed in water and lower the pH to about 2 to precipitate the humic acid. The separation of materials has been carried along by previous researchers. Geoff (2010) resulted in at least 70 percent humic acid through oxidation of the material under alkaline conditions at temperatures between 100 ° C and 200 ° C for at least 1-2 hours at pH 2.9 and precipitate under considerable pressure . Corresponding oxygen pressure in the process is 5-200 psi and alkali source corresponding to the hydroxyl include sodium, potassium, ammonium, lithium or that combination. fulvic acid extract by adding a base solution to humic substances so that the pH of the solution between 9-10. Extraction and fractionation of HA are various in period of about 4 hours to 7 days or from 12-24 hours. Tangible results in the AH isolation of soil is 9 hours (4 hours of extraction, 4 hours and 1 hour fractionation purification) (Nur Hanisah, et al 2008, Chen et al, (2009). Rosliza (2009) There is a linear relationship between the extraction time and the results of humic acid, but there is no relationship between fractionation and results of natural AH C, E4/E6, carboxyl, phenolic and total acidity AH .

Humic acid extraction with NaOH or KOH causes AH saturated with Na or K, so that the negative charge will attract positive charge. Negative charge (CEC) of humus is generally approved for H⁺ dissociation of functional groups, very dependent on pH. Carboxylate of several carboxyl group is quite sour to escape under pH 6 leaving a negative charge on the functional group: R-COOH = R-COO⁻+ H⁺ . as the pH system increases above 6. Other weaker and highly acidic carboxylic groups dissociated with phenolic OH and other weak acids at pH> 8. Dissociation of H⁺ from the acid groups throughout the pH range adds humus total negative charge. Dissociation of H⁺ from the phenolic OH, Amida (= NH), and other groups may also contribute to the negative charge. Protonated groups such as (R-OH₂)⁺ and (R-NH₃)⁺ can produce a positive charge, but overall the humus have negative charge . The charge's side (mainly COO⁻) allow SOM to retain cations can not be washed but the exchange form available to plants (Bohn et al 2001). CEC value of some materials can be used to predict the activity that correlated with humic acid. Humic acid content of various materials that are Leonardite/Humic, black peat, brown coal, Manure, Compost and Soil, respectively 40, 10, 10, 5, and 2%. Fulvic acid content of the same materials, respectively 85, 40, 30, 15, and 5% (<http://humintech.com/001/agriculture/information/general.html>). Chen et al. (1977) believed that the E4:E6 ratio was related to the degree of condensation of the aromatic humic components. Humic acid structure has e4/E6 ratio in between 3.3 - 5.0 (Pansu, 2003) while Orlov (1985) agree by showing a range of 4.1 - 4.8

Role of humic acid on the soil and plants

The use of humic materials in agricultural production continues to grow and evolve. There are a number of reports of both successful and unsuccessful use of this material, due to the wide

variety of materials and methods of manufacturing process (Mikkelsen 2005). Sometimes humic materials can provide a carbon (C) as a source of soil microorganisms, but the mechanism does not seem like, as humic material applications 5-20 gal / A will supply the 3-15 lb C / A if compared with > 4,000 lb C/A that is returned in the form of residues of corn. Humic acid indicates its function as a urease inhibitor and as a nitrification inhibitor in some conditions

Leonardite is beneficial in overhauling clay and compacting soils, assist in the transfer of micronutrients from the soil to the plant, ensuring water retention, accelerate seed germination, penetration, and stimulates the development of microflora populations in soils (Mikkelsen, 2005; Khaled and Fawy, 2011). Filter cake is the result of solid waste from the production process of making sugar. Humic acid (AH) of the filter cake has been beneficial to plant growth, including making lateral roots of maize (*Zea mays* L.) grown, associated with stimulation of plasma membrane ATPase activity. AH ability to support the development of roots showed that the HA filter cake can be used as a plant growth environment stimulator (Busato et al. 2010). Chen et al. (1977) believed that the E4:E6 ratio was related to the degree of condensation of the aromatic humic components. Humic acid structure has e4/E6 ratio in between 3.3 - 5.0. (Pansu, 2003) while Orlov (1985) agree by showing a range of 4.1 - 4.8

Humic substances play an important role in soil fertility and plant nutrition (Tan, 1998; Spark, 2003; Pettit 2011). Humic acid can neutralize soil acidity and alkalinity, improve and optimize the absorption of nutrients and water by plants, increase the buffering properties of soil, acting as a natural chelator for metal ions under alkaline conditions and promote absorption by the roots, has very high cation exchange capacity, promote the conversion of nutrients (N, P, K, Fe, Zn and other elements) into a form available to plants, improving nitrogen uptake by plants, reducing the reaction of phosphorus with Ca, Fe, Mg and Al and released into a form that is available and beneficial to plants (Khaled and Fawy., 2011). Doses of humic substances application is 0, 2 and 4 g.kg⁻¹ to the saline soil one month before planting and humic acid liquid 0, 0.1 and 0.2% in the leaves sprayed twice on days 20 and 40 after emergence of seedlings increase the absorption of nutrients (Khaled and Fawy, 2011).

MATERIAL AND METHODS

A. Site Study and Treatment

Laboratories experiments were conducted at the Faculty of Agriculture UPN "Veteran Jawa Timur on June to September 2012. Humic acids extracted from sewage plants, livestock and industry with 0.5 N NaOH (1:10) for 24 hours and precipitated approximately 12 hours to separate the humic and humin. Compost of sewage plant, manure, filtercake, coal, and peat were sieved to pass a 2-mm sieve, as supposed by Tan (2003). Each of them weighted 50 g entered in to plastic bottle of 1L. and added 500 ml NaOH 0.5 N into bottle (1:10), and then sailed and positioned in shaker. Agitation of this solution were done for 24 hours. After that, the extract (Humic material) displaced from depletion (humin), containing humic acid (gel, brown -black) and fulvic acid (solution, orange). Precipitated of humic acid with the addition of 5 N HCl to pH 2, then centrifuged at 2000 rpm 30 minutes. Humic acid (HA) purification by washing with HF + HCl 1: 3 followed by water up to 3 times. Pure humic acid was dried at a temperature of about 40°C and then characterized the value of E4/E6, C-organic, EC, pH and humic%. E4/E6 ratio values <5 shows the character of humic acid.

Saline Soil samples were taken from Gunuganyar at a depth of 0-20 cm, dried and sieved with a 2 mm sieve. The principle of this soil were pH 7.8, EC 1.5 mS/cm, C-organic 0.8 %, CEC 45 me/ 100g, Exchangeable Ca, Mg, Na, and K were 18, 3, 2, 1 me/100g respectively.

B. Experimental Design

A pot experiment was arranged in a Completely Randomized Factorial Design. It was conducted at Faculty of Agriculture, University of Pembangunan Nasional "Veteran" East Java. The first factor comprises 5 kind organic matter: compost, manure, guano, while the second factor comprises six levels acid humic powder of 0, 0,5, 1,0, 1,5, 2,0, and 2,5 g/kg respectively. Each treatment combination was repeated 3 times.

Soil were weighed 3 kg equivalent to absolute dry weight, mixed with humic acid and than incubated at a field capacity in room condition for 2 weeks. After incubation, the basic NPK fertilizers equivalent to 200 kg ha⁻¹ was added ($3 \times 100 \text{ mg} = 300 \text{ mg pot}^{-1}$). Rice seedlings planted to sink. It taken from a nursery at 15 cm height. Two seedlings of rice were planted per pot and then a light irrigation ($EC_w < 1$) was applied up to saturate. After 2-weeks planting, the plants were thinned to 1 plant per pot and its growth was maintained until harvest. Water was added until the soil is saturated with $EC < 1 \text{ mS.cm}^{-1}$. Plants were maintained in a screen house with the temperatures ranged of 27-30 °C. Pest control was carried out if a pest attack symptom was found and a preventive action was performed by a mechanical handling.

C. Sampling and assessment

Humic acid pH made about pH 6 from pH 2 by adding a few drops of KOH. EC of HA measured in a powder or liquid form. The value of C-organic measured in Walkey and Black method as proposed by Marc Pansudan Jacques Gautheyrou (2003). Organic matter was weighed 0.1 g incorporated into the Erlenmeyer flask and added 10 ml of 1N potassium dichromate and 20 ml of concentrated sulfuric acid, allowed to stand 30 minutes Extractant was added with 200 ml of water, 10 ml of concentrated phosphorus acid, 0.2 g of sodium fluoride and 10 drops of diphenyl amine. Excess dichromate titrated with ferosulfat 1 N. Percent of HA was measured gravimetrically at a temperature of about 100°C. The value of E4 and E6 HA (1 ml HA liquid : 10 ml 0,05 N NaHCO₃) were measured in 465 and 665 nm Spectro Pharo 100 respectively. E4/E6 ratio values was obtained by dividing the value of E4 with E6. CEC of HA was measured with NH₄OAc pH 7 1N extract. Soil chemistry characteristic (pH, EC, C-organic) were measured from a depth of soil sub sample (0-10 cm), 30 DAT (days after treatment) of HA with the same method as before. Growth of rice were evaluated for hay and roots dry weight, plant length, and the number of rice tillers in 35 days after planting (DAP).

D. Statistical analysis

The data of HA characteristic was corrected to kinds of sewage plant, livestock, and industry. The value of characteristic of HA was correlated with the growth parameters, and soil chemical characteristics. The resulting value is correlated with the growth parameters. Data were analyzed by analysis of variance (ANOVA) using Microsoft excel. Means of value were tested by Least Significance Different (LS D) at $P = 0.05$ to determine the soil salinity and

rice growth from effect of level and kinds of humic acid.

RESULTS AND DISCUSION

Characteristic of Humic acids that were extracted from sewage plants, livestock and industry with 0.5 N NaOH (1:10) for 24 hours, precipitated from fulvic acid with the addition of 5 N HCl to pH 2, purification by washing with HF + HCl 1: 3 followed by water up to 3 times, dried at a temperature of about 40oC, is presented in Table 1.

Tabel 1. The value of C-organic, E4/E6, EC, and CEC humic acid

No.	Source	Origin	C-org		HA %	E4/E6	EC mS/cm	CEC Me/100g
			liquid (%)	powder				
1	coal	Kota waringinhilir, Kalimantan	23,07	29.27	4,6.00	2,37	0,00	104,09
2	peat	---“---	46.32	44.96	7,60	2,92	0,00	116,83
3	Compost	GunungAnyar	8.59	16.39	2,60	3,71	0,01	80,72
4	Manure	GunungAnyar	4.21	9.81	1.80	4,40	0,01	59,48
5	Guano	Gresik	15.28	18.07	1.20	4,74	0,03	47,48
6	hay	Gununganyar	5.84	7.72	1.80	4,35	0,01	79,48
7	Filter cake	Sidoarjo	5.34	7.62	1,60	4,58	0,02	69,48

Highest Humic acid levels are obtained from peat followed by coal, compost, filter cake, and manure. Cation exchange capacity in the range of 80-115 g me/100 humic acid at a pH between 6.7-7.5. EC humic acid 0 ms /cm. Peat contains the highest of a C-organic, humic acid, and CEC than other organic materials, respectively by 7.6%, 44, 96%, 116 me/100g followed by coal, compost, rice straw, manure, filter cake, and guano. C-organic content of powder HA is higher than the liquid HA. All of humic acid has a low EC value in the range of 0 to 0.03 mS/cm. C-organic content of organic matter was positively correlated with% humic acid and CEC, but negatively correlated with E4/E6 values. Humic acid was potentially reducing salinity and the growth of rice for 35 DAP. It was not significantly different between the kinds of waste

Table 2. Correlation between humic acid character

	C-org-powder	C-org-liquid	% HA	E4/E6	EC	CEC
C-org-powder	1,00	0,99	0,94	-0,70	-0,57	0,73
C-org-liquid		1,00	0,93	-0,70	-0,40	0,71
% HA			1,00	-0,94	-0,64	0,85
E4/E6				1,00	0,80	-0,78
EC					1,00	-0,69
CEC						1,00

The E4/E6 ratio values of <5 shows the character of humic acid, while value of > 5, is fulvic acid (Tan, 2003). The E4/E6 ratio of humic acid from kinds of these material were < 5, (2, 9- 4,74). This is slightly different from the expressed by Tan (2003), who obtain E4/E6 ratio of humic acid extracted from the ground, which is around 4-5. The higher content of organic material will be high humic acid content, which is characterized by the ratio of the value. The different yield of HA was caused by different extraction and kinds of organic matter that use.

I used of NaOH 0.5N, while others used of NaOH 0.1N, and mixture of NaOH and Na₂PO₄ 0.1 N, or by water. Sodium hydroxide solution is believed as the best extractor than others. The increasing of dose of humic acid did not decrease pH EC yet, but increased number of tiller and hay weight of rice. Purification of humic acid lowering soluble Na content of 25-45%

Humic acid extraction with NaOH causes AH saturated with Na, so that the negative charge will attract positive charge. Surface area and adsorption capacity per unit mass of humus is larger than the layer silicate minerals. Cation Exchange Capacity (CEC) of humus is generally approved for H⁺ dissociation of functional groups. All humus loads are very dependent on pH, with humic acid and fulvic acid behaves as a weak polyelectrolyte. Separation of carboxyl and phenol may produce 85 to 90% of the negative charge of humus. Carboxylate of several carboxyl group is quite source to escape under pH 6 leaving a negative charge on the functional group: R-COOH = R-COO⁻ + H⁺. As the pH of the system increases above 6, unit R-COOH separates on various different pH. The charge's side (mainly COO⁻) allow soil organic matter (SOM) to retain cations can not be washed but the exchange form available to plants (Bohn et al 2001). Application doses of humic acid from a variety of sources up to 2,5 g/kg to saline soil for 30 days after that were not significantly different on soil pH, Ec and C-organic, and the length of the plant, but significantly different on straw weight and number of tillers (Table 4). Although soil salinity indicators (pH and EC) did not affect the application for 30 days, but the number of tillers and straw weight significantly, and the best results on the application of humic acid for about 1,5-2,0 g/kg

Tabel 4. Anova of soil characteristic and plant growth parameter caused humic acid application.

SK	db	F- calculate					F table		
		EC	pH	C-org	Weight of hay	Number of tiller	Lenght	5%	1%
Blok	2	0,014 ns	0,014 ns	0,002 ns	3,04 ns	141,66 **	1,10 ns	4,30	9,92
treatment	17	0,14 ns	0,015 ns	0,104 ns	8,36 **	5,87 **	1,04 ns	2,11	2,90
HA	2	0,16 ns	0,039 ns	0,616 ns	11,61 **	7,03 **	1,90 ns	4,30	9,92
DOSE	5	0,33 ns	0,022 ns	0,018 ns	14,60 **	9,46 **	0,70 ns	2,57	4,03
AH x DOSE	10	0,05 ns	0,007 ns	0,045 ns	4,59 **	3,85 **	1,04 ns	2,23	3,17
Galat	34								
Total	53								

Numbers accompanied by the same letter no significantly different at p = 0.05

Yield experiment above is almost similar with reseach that was doing by Khaled and Fawy (2011), Turan, 2011; Asik, 2011, they find that application dosis of humus 0, 2, 4 g/kg at one month before planting, increased the N of corn uptake. while Barışet al and ÇELİK et al (2010) find that dose of humus 0,1, 2 g /soil kg increase N uptake of wheat and decrease soil salinity from concentration of NaCl of 60 mM or CaCO₃ 40 %. Paksoy, et al. (2010) and Çimrin, et al (2010) find that dose of AH 0- 1500 mg/kg and K 0- 300 mg/kg or in P 0- 150 mg/kg) at salin soil respectively. they can increased N, P, K, Ca, Cu, Fe, Mn or Cu conten and plant growth in p < 0.005. Optimum yield was gained with dose of 69 mg kg⁻¹ P with HA 750 mg kg⁻¹. Value of Na content at shoot and root decreased with the increasing of doses

Table 5. Mean of plant growth parameter caused humic acid application

Treatment	C-Organic	Hay weight	Lenght plant	Number of tillering
AH-Guano	0,90 a	51,23 a	62,78 a	18,35 b
AH-manure	0,85 a	54,14 b	64,81 a	16,87 a
AH-compost	0,73 a	64,66 c	63,97 a	22,61 c
BNT 5 % =	0,15 a	0,47	0,50	0,77

Numbers accompanied by the same letter no significantly different at $p = 0.05$

Treatment	C-Organic	Hay weight	Lenght plant	Number of tillering
0	2,52 a	36,37 a	63,83 a	10,00 a
0,5 g	2,47 a	60,88 d	64,44 a	19,59 b
1,0 g	2,58 a	58,38 c	64,89 b	20,70 b
1,5 g	2,36 a	52,63 b	64,11 a	24,30 c
2,0 g	2,32 a	67,94 f	63,44 a	19,33 b
2,5 g	2,62 a	63,86 e	62,39 a	21,74 b
BNT 5%	2,59 a	0,94	1,01	1,53

of humic. Gulser et al (2010) find that the higher doses of HA 0, - 4 g/kg and calcium nitrate (0 -150mg kg-1) at saline soil (+ 60 mMNaCl) affect germination signficantly. Optimum Doses of HA are 1000 and 2000 mg kg-1 and 50 mg kg-1 CaNO₃ increasing dry weight and fresh leaf, diameter of root and shoot , root lenght and tips. The highest doses of AH (4000 mg kg-1) and CaNO₃ (100 and 150 mg kg-1) decreasing those criteria in salin condition.

CONCLUSION

The value of Humid acid various organic matters, was depended on environmental processes, extraction tecnique, purification and acids solution. Purification is very important proceses for extraction, especially if it used Na in extracxtion, for safety application to soil and plan. Humic acid contain on peat and coal were highest than other matterial. Doses of HA appllication of 2.5 g/kg was not significantly decreaseing soil pH, EC and C-organic matter from Gunungnyar village, but it was increasing plant growth parameter, except plant lenght.

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