

1 **Amelioration effect of humic acid extracted from solubilized excess**  
2 **sludge on saline-alkali soil**

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16

17 **Abstract** In this study, the main characteristics and soil amendment  
18 effect on the saline-alkali soil of humic acid extracted from solubilized  
19 excess sludge (SS-HA) were investigated. The excess sludge was  
20 solubilized prior to extraction to improve the humic acid recovery rate.  
21 The structural features of SS-HA were characterized by an elemental  
22 analysis, Fourier transform infrared spectroscopy, and <sup>1</sup>H-nuclear

1 magnetic resonance spectroscopy, and compared with those of HA  
2 extracted from non-solubilized excess sludge (ES-HA). The results  
3 showed that extraction efficiency of humic acid was enhanced by using  
4 solubilization, although structural properties of humic acid extracted  
5 from solubilized excess sludge were almost same as those of ES-HA.  
6 To study a utilization method of SS-HA, the soil amendment effect on  
7 saline-alkali soil by mixture of SS-HA were investigated with model  
8 soil-column experiment. SS-HA reduced the pH of the saline-alkali  
9 soil, and the effect was faster or immediate observed compared with the  
10 case which is only peat added. Moreover, the cation exchangeable  
11 capacity of the saline-alkali soil was enhanced by addition of SS-HA.

12

13 **Key words** humic acid, solubilized excess sludge, pH, exchangeable  
14 cation, saline-alkali soil

15

## 16 **Introduction**

17 Activated sludge process has been widely employed for wastewater  
18 treatment in many countries. Generation of excess sludge (ES) is one  
19 of the drawbacks of this process. Recently, adjustment to sustainable  
20 developing society is policy of the Japanese Government in waste  
21 management. Organic waste (biomass) is ranked as part of the  
22 untouched natural resources, and proactive utilization of those came to  
23 be strongly demanded. One possible way for ES to be utilized is to

1 extract some of the beneficial compounds that ES contains for use. It  
2 is well known that excess sludge is degraded and humified by  
3 microorganisms to become compost etc, and it contains some bioactive  
4 compounds such as humic substances (HS).<sup>1</sup> HS is broken down  
5 organic components formed by plant decay and other biological  
6 activities and is found in almost all terrestrial and aquatic  
7 environments. HS have various useful effects due to their functional  
8 groups. There are three types of HS, according to their solubility in  
9 water: fulvic acid (FA), humic acid (HA) and humin. The fraction that  
10 is precipitated by acid is referred to as HA and those that are not  
11 precipitated by acid are FA and humin is not soluble in water.<sup>2</sup> HA  
12 is a very important component of soil that affects its physical and  
13 chemical properties and improves the fertility of agricultural fields.  
14 HA can form complexes with various metal ions and thereby increase  
15 the cation exchange capacity (CEC) of the soil.<sup>3,4</sup> HA also promotes  
16 very complex biological activity. For example, HA has many different  
17 effects on plant metabolism in the diverse systems that have been  
18 tested.<sup>5-7</sup> One practical example of soil improvement in response to  
19 the addition of HA is the positive effect on the pH of saline-alkali soil  
20 after addition of Canadian peat reported by Kawakami *et al.*<sup>8,9</sup> In the  
21 environmental field, FA is known to supply iron nutrients to the sea  
22 because of its characteristically strongly bonding to iron.<sup>10</sup> FA also  
23 has a role in environmental cleanup operations where its ability to form

1 complexes with metals makes it a useful coagulant for harmful heavy  
2 metals and assists in detoxification of carcinogenic hexavalent  
3 chromium.<sup>11</sup> In healthcare field, physiological action on the human  
4 body of HS has been studied in recent years.<sup>12,13</sup>

5 In this study, to examine whether ES could be a new source of HS,  
6 we extracted and purified HS from ES. HS are abundant in peat,  
7 weathered coal, and other humified substances.<sup>14</sup> However, the cost of  
8 transporting peat from wetlands to arid lands where it might be utilized  
9 is very large. Similarly, weathered coal is available only near coal  
10 mines. In contrast, ES is an industrial waste that is widely available  
11 and continually produced. It thus has the potential to become an  
12 environmentally sustainable source of HS. Excess sludge is often  
13 chemically reduced by solubilization. Previously, we compared the  
14 characteristics of solubilized excess sludge (SS) obtained by using  
15 alkalization, microwave irradiation, boiling, and the bead-mill  
16 technique, is higher than that of raw excess sludge, or of sludge  
17 solubilized by other techniques.<sup>15</sup> We found that the dissolved total  
18 organic carbon (TOC) content of SS, particularly sludge solubilized by  
19 the bead-mill technique, is higher than that of raw excess sludge, or of  
20 sludge solubilized by other techniques. HS constitute an important  
21 reservoir of organic carbon in aquatic and terrestrial environmental  
22 compartments and account for as much as 50% of the dissolved organic  
23 carbon in surface waters.<sup>16</sup> 11% to 66% of TOC was HS in

1 groundwater.<sup>17</sup> It is expected the TOC-rich fraction of SS contains  
2 high concentration of HS. Therefore, solubilization using the  
3 beads-mill technique is an effective means for their extraction.  
4 Compared with the application of raw sewage sludge, the application of  
5 extracted and purified HS to agricultural fields is likely to result in  
6 fewer adverse effects on the environment such as the addition of heavy  
7 metals.

8 Previously, the inhibitory effect of FA extracted from ES or SS on  
9 type I allergy using rat basophilic leukemia cells was investigated.<sup>18,19</sup>  
10 In this study, structure of the HA obtained by solubilization (SS-HA)  
11 was compared with the structure of HA from non-solubilized excess  
12 (ES-HA) with elemental analysis, Fourier transform infrared (FT-IR)  
13 spectroscopy and <sup>1</sup>H-nuclear magnetic resonance (<sup>1</sup>H-NMR)  
14 spectroscopy. As case study for utilization of SS-HA, the application  
15 effect of SS-HA on the saline-alkali soil was investigated with modeled  
16 column experiment.

17

## 18 **Materials and methods**

19

### 20 **Collection of sludge samples and solubilization conditions**

21

22 The ES used as a source for extraction of HA and FA in this research  
23 was collected from the return line of an aeration tank at a wastewater

1 treatment plant in Gunma, Japan. The mixed liquor suspended solid  
2 (MLSS) concentration of the sludge was 4200 mg/l. A total of 8 liters  
3 of ES was divided into two 4 liters samples. One of these was  
4 solubilized to verify improved HS extraction efficiency after  
5 solubilization, and HS were extracted from the other sample without  
6 solubilization.

7 A rotary drum cylindrical bead mill was used for solubilization of  
8 ES. The cylindrical container (139.8 mm × 400.0 mm) (Fig. 1) had  
9 four mixing blades, each 2 cm high, on the inside wall of the container.  
10 The conditions under which solubilization was carried out were as in  
11 the previous study of Motojima *et al.*<sup>15</sup> The procedure for the  
12 features analysis of solubilized sludge was described in our previous  
13 study.<sup>18</sup>

#### 14 15 Extraction of humic acid

16  
17 To investigate the improvement of extraction rate and changes to the  
18 structure of HA after solubilization, it was extracted from unsolubilized  
19 ES as a control. Extraction and isolation procedures were carried out  
20 following the protocol of the International Humic Substances Society.

#### 21 22 Analyses of extracted humic acids

23

1 The structural features of ES-HA and SS-HA were characterized by  
2 using an elemental analysis, Fourier transform infrared spectroscopy  
3 and  $^1\text{H}$ -nuclear magnetic resonance spectroscopy. The procedure for  
4 the structural features analysis of SS-HA was described in the previous  
5 report.<sup>18</sup> Carboxyl group content of ES-HA and SS-HA was  
6 determined by the calcium acetate method of Ikeya *et al.*<sup>20</sup> Dried  
7 ES-HA and SS-HA were placed in 50 ml of 0.5 M aqueous calcium  
8 acetate solution. After mixing for 16 h, filtrate was treated with 0.1 M  
9 NaOH, with phenolphthalein used as an indicator. The carboxyl group  
10 content of HA was calculated by subtracting from the result of the  
11 blank test. Measurement of cation exchange capacity (CEC) was by  
12 barium acetate saturation according to the standard method of the  
13 National Institute for Agro-Environmental Science Japan.<sup>21</sup>

14

#### 15 Modeling of saline-alkali soil

16

17 An artificial saline-alkali soil was prepared by adding a saline solution  
18 to sandy material. The saline solution was a mixture of NaCl, KCl,  
19  $\text{Ca}(\text{OH})_2$ ,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $(\text{NH}_4)_2\text{SO}_4$ , and  $\text{KH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ . The sandy  
20 material was a mixture of coarse sand (0.2–2.0 mm), fine sand  
21 (0.02–0.2 mm) and clay (<0.002 mm) in ratios of 85.5, 13.5, and 1.0  
22 wt %, respectively, to form a texture similar to that of Sahara desert  
23 soil studied by Yazawa *et al.*<sup>22</sup> The particle size distribution of the

1 model soil was determined according to Japanese Industrial Standard  
2 (JIS) A 1204 which is an internationally recognized standard. The pH  
3 and electrical conductivity (EC) of the model soil were adjusted by  
4 addition of saline solution to 8.8 and 3.7 mS/cm, respectively. The pH  
5 of the model soil was measured in suspension (soil:pure water = 1:2.5)  
6 with a UC-23 pH meter (Central Kagaku Co.) according to the glass  
7 electrode method of the Japanese Geotechnical Society (JGS  
8 0211-2000), and EC of the soil solution (soil:pure water = 1:5) with a  
9 YK-22CT EC meter (Sato-Shoji Co.) following the EC electrode method  
10 (JGS 0212-2000). The physical and chemical properties of the model  
11 soil are provided in Table 1.

12

### 13 Batch testing

14

15 The pH buffering ability and pH of the model soil after application of  
16 SS-HA were measured by a batch test before the soil-column  
17 experiment (described in the next section). The initial pH buffering  
18 capacity of SS-HA was determined by the amount (moles) of strong acid  
19 or base needed to produce a unit change of pH in the model soil. HCl  
20 and NaOH solutions of the required concentrations (0.5, 1.0, 2.0, 5.0,  
21 8.0, and 10.0 cmol/kg) were prepared; pure water was used for the  
22 neutral point. Freeze-dried SS-HA (0.05, 0.1, and 0.5% of the weight  
23 of the base or acid solution) was added to the model soil in NaOH and

1 HCl solution. After shaking for 24 h, the pH of soil solution was  
2 measured. The pH change of the model soil in response to SS-HA  
3 application was measured as follows. A 1000 mg/l solution of SS-HA  
4 was prepared and added to the model soil (0, 0.005, 0.05, and 0.5% of  
5 the dry weight of the soil). The mixtures of soil and SS-HA were  
6 allowed to stand for three days, after which they were dried at 40 °C for  
7 24 h. Each dried soil sample was then shaken in pure water for 2 h  
8 (soil:pure water = 1:2.5 for pH, soil:pure water = 1:5 for EC), and pH  
9 and EC were measured after filtering through a 1- $\mu$ m paper filter.

10

11 Soil-column experiment and parameters measured

12

13 To investigate the ameliorative effect of application of SS-HA to  
14 saline-alkali soil, a soil-column experiment was performed at Tsukuba  
15 University over 157 days from 30 March to 26 August 2009. The  
16 experiment was performed in a cylindrical column of 5.0 cm diameter.  
17 Two soil sampling shoes (steel columns; diameter 5.5 cm, length 5.0  
18 cm) were connected to the top of a plastic column (length 31.0 cm) (Fig.  
19 2). To minimize soil movement, the bottom of the column was covered  
20 with glass wool. Experiments were performed for three cases:

21 1. Control 1: Saline-alkali soil only

22 2. Control 2: Saline-alkali soil + 2 wt % peat in the top 10 cm of the  
23 column

1       3. Experimental treatment: Saline-alkali soil + 2 wt % peat + 1 wt %  
2       SS-HA in the top 10 cm of the column.

3       For the second and third cases, freeze-dried SS-HA was re-dissolved  
4       and evenly mixed into the saline-alkali soil with pure water. We  
5       assumed that the liquid in the water-SS-HA mixture would have  
6       immediately permeated through the top soil layer if the SS-HA had been  
7       applied directly to the soil surface. Peat was also added in the second  
8       and third cases in order to retain the SS-HA in the top 10 cm of the soil  
9       column. Each of the three cases was replicated three times. Peat  
10       contains HA, so the second treatment was undertaken as a control to  
11       clearly identify the effect of SS-HA. The properties of the peat used  
12       are provided in Table 2. The surface of the column was lit with a lamp  
13       (57 W) for 8 h each day to accelerate evaporation during the experiment.  
14       After 21 days, pure water was poured into the apparatus to cause  
15       leaching. The upper surface of the soil column was then dried until  
16       day 90, and then held at room temperature until day 157.

17       Holes at 10-cm intervals along the length of the soil column were used  
18       to obtain samples for measurement of the variations of pH and EC with  
19       depth (Fig.2). On days 1, 14, 21, and 157, pH and EC were measured  
20       using a direct-reading electrode soil pH Tester 30  
21       (Takemuradenkiseisakusho Co.) and a soil EC meter HI98331-S  
22       (Takemuradenkiseisakusho Co.), respectively. These direct methods  
23       were used so that the soil layer in the column would not be disturbed by

1 repeated collection of soil samples for pH and EC measurements.

2 To measure the exchangeable cation concentration ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  
3 and  $\text{Ca}^{2+}$ ) of the soil, 1.5-g soil samples were collected from the holes  
4 in the side of the column (depths of 0, 10, 20, and 30 cm) on day 90,  
5 air-dried and passed through a 2-mm sieve and preserved for analysis.  
6 A 1-g subsample was extracted from the air-dried soil and shaken for 2  
7 h in 10 ml of 0.5 M  $\text{BaCl}_2$ , as described by the method of Kamewada *et*  
8 *al.*.<sup>23</sup> Exchangeable cation concentrations were determined by ion  
9 chromatograph DX-100 (DIONEX) after passing samples through a  
10 0.45- $\mu\text{m}$  filter paper.

11

12 Statistical analysis

13

14 The Student's *t*-test was used for comparison between two groups. To  
15 compare three groups, statistical significance ( $P < 0.05$ ) was evaluated  
16 by one-way ANOVA, and if a significant difference was found, group  
17 means were compared using Bonferroni's post hoc test.

18

19 **Results**

20

21 Extraction rate of humic acid

22

23 A comparison of the main properties of the ES and SS which are

1 resource of HAs are shown in Table 3. The results of our MLSS  
2 measurement and water-quality analysis show the typical  
3 characteristics of SS. The MLSS of SS was lower than that of ES and  
4 the soluble fraction was higher (measured as TOC, TN, and carboxylic  
5 acid). The HA extraction efficiency was enhanced by an increase of  
6 the soluble fraction in response to solubilization (Table 4). The HA  
7 recovery rates of ES and SS were 3.7% and 9.1% respectively. As  
8 reference, previously reported HA and FA extraction rates from  
9 Canadian *Sphagnum* peat (CP), from weathered coals of China, and  
10 from soils are presented.<sup>24,25</sup> The extraction rate obtained for SS-HA  
11 approaches that of CP-HA.

12

### 13 Characterizations of ES-HA and SS-HA

14

15 Knowledge of the elemental composition of HS allows evaluation of the  
16 role of those elements in building macromolecules of humus and  
17 analysis (in simple terms) of the influence of the origin of humus on its  
18 composition and structure. The elemental compositions of ES-HA and  
19 SS-HA were similar and showed higher H and N contents than other HA  
20 materials such as CP-HA (Table 5).

21 FT-IR spectra can be used to identify the presence of most of the  
22 important functional groups in HS, to compare HS of different origins,  
23 and to estimate the concentrations of functional groups in HS, and are

1 thus important for the development of structural models. The IR  
2 spectra of HS from ES and SS are shown in Fig. 3 with that of CP shown  
3 for comparison. These IR spectra were identified based on the study  
4 by Stevenson and Goh.<sup>26</sup> The broad band at 3400–3200  $\text{cm}^{-1}$  is  
5 attributed to intermolecular OH stretching of carboxylic and alcoholic  
6 groups in different electrostatic environments,<sup>27</sup> and the band at  
7 2920–2850 $\text{cm}^{-1}$  reflects aliphatic C–H stretching vibration, indicating  
8 the presence of methyl and methylene groups. The shoulder at 1720  
9  $\text{cm}^{-1}$ , which is commonly attributed to C=O stretching vibration of  
10 COOH groups. The band near 1620  $\text{cm}^{-1}$  is attributed to structural  
11 vibration of aromatic C=C, H-bonded C=O of quinones, or H-bonded  
12 and conjugated ketones. The lack of absorption near 1540  $\text{cm}^{-1}$  is  
13 attributed to amide groups in the peptide linkages of protein. The  
14 absorption band at 1400  $\text{cm}^{-1}$  reflects C–H deformation of  $\text{CH}_3$  and  $\text{CH}_2$   
15 groups. The broad adsorption band near 1230–1220 $\text{cm}^{-1}$  represents  
16 C–O stretching and OH deformation of carboxylic acids, alcohols, and  
17 esters. Comparing the spectra of our samples to that of CP-HA, we  
18 found some differences in the component ratios of functional groups:  
19 the absorption at 2920  $\text{cm}^{-1}$  was relatively stronger in ES-HA and  
20 SS-HA. ES-HA and SS-HA showed stronger aliphatic characteristics  
21 due to the different intensities of oxidation and humification processes  
22 they had undergone. Absorption near 1540  $\text{cm}^{-1}$  is attributed to amide  
23 groups in the peptide linkages of protein. The spectra of ES-HA and

1 SS-HA show absorption of COOH at  $1230\text{ cm}^{-1}$ .

2 NMR techniques provide an efficient tool to determine the  
3 properties of humus. The  $^1\text{H}$ -NMR spectra of HA and FA from ES and  
4 SS are shown in Fig. 4. They showed peaks in the region 7.0–8.1 ppm  
5 (aromatic H, including quinone phenol), stronger peaks at 3.3–4.6 ppm  
6 (carbohydrate H), and weaker peaks in the region 0.8–3.3 ppm  
7 (methylene and aromatic  $\text{CH}_2$ ,  $\text{CH}_3$ ). For a more effective comparison,  
8 the proton chemical shift has been quantitatively classified into four  
9 regions (I to IV) representing protons in different chemical  
10 environments (Table 6).<sup>28,29</sup> The structure of SS-HA was shown to be  
11 similar to that of ES-HA. The results of  $^1\text{H}$ -NMR are consistent with  
12 the results obtained from elemental analyses and FT-IR.

13

14 Batch experiment

15

16 Before the soil-column experiment, the pH buffering ability of SS-HA  
17 was confirmed by batch test. SS-HA showed pH buffering ability in  
18 an alkaline environment (Fig. 5), which suggested that application of  
19 SS-HA would lower the pH of the saline-alkali soil. This was  
20 confirmed, as the pH of the saline-alkali soil decreased as the amount  
21 of SS-HA applied increased (Fig. 6). On the other hand, the EC  
22 increased rapidly as the pH of the saline-alkali soil decreased.

23

## 1 Effect of humic substances on pH and EC in soil-column experiment

2

3 The high pH of alkali soil causes phosphate and metal ions to  
4 precipitate and thus lowers their availability, causing a serious  
5 imbalance in the supply of mineral nutrient ions in the root zone. This  
6 may destroy the structure of roots, reducing their activity, and finally  
7 causing a reduction or total loss of root function. Moreover, high pH  
8 reduces the number of protons outside the roots, which impedes  
9 establishment of a transmembrane proton gradient.<sup>30</sup> Because more  
10 than half of the total root length of maize is within 20 cm of the soil  
11 surface, and the maximum root densities of peanut are within the top 30  
12 cm soil depth,<sup>31</sup> and the column length in the soil-column experiment  
13 was selected to be 31 cm.

14 The effect of SS-HA on the pH of the soil profile during the  
15 soil-column experiments are shown in Fig. 7. For the cases where  
16 peat+SS-HA and peat alone (control 2) were added to the saline-alkali  
17 soil, the pH in the top 10 cm of the column was lower than that of  
18 control 1 on days 1 and 14, and that for peat+SS-HA was lower than  
19 that for peat alone. After leaching on day 21, the pH profile of the  
20 column in which peat+SS-HA had been added was almost identical to  
21 that of control 2 (peat alone), and remained so until day 157.

22 High salt levels, which are indicated by high EC, can reduce  
23 water uptake by plants, restrict root growth, cause marginal burning of

1 foliage, inhibit flowering, limit seed germination, and reduce fruit and  
2 vegetable yields.<sup>32</sup> The changes of soil EC during the column  
3 experiments are shown in Fig. 8. Although the batch tests showed that  
4 as SS-HA concentrations increased, EC increased and pH decreased, EC  
5 during the column experiment showed no significant difference among  
6 the three experimental cases.

7

8 Effect of humic substances on exchangeable cations in soil-column  
9 experiment

10

11 CEC provides a measure of the capacity of a soil for exchange of  
12 cations between soils and soil solutions and gives an indication of the  
13 amount and ratios of available plant nutrients in soil. Base saturation,  
14 which is the fraction of exchangeable cations that are base cations ( $K^+$ ,  
15  $NH_4^+$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ ), is closely related to CEC. Therefore,  
16 exchangeable cation ( $NH_4^+$ ,  $K^+$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ , and  $Na^+$ ) concentrations on  
17 day 90 of the soil-column experiments were conformed (Fig. 9).  
18 Application of peat+SS-HA showed significantly greater  $NH_4^+$   
19 concentrations at the top and bottom of the soil column than was the  
20 case for peat alone. The  $K^+$  concentration was also greater in the top  
21 layer after application of peat+SS-HA. Application of peat+SS-HA  
22 had a significant affect on  $Na^+$  and  $Ca^{2+}$  concentrations at the bottom of  
23 the soil column. There were no notable differences of  $Mg^{2+}$

1 concentrations after application of peat and peat+SS-HA. Moreover,  
2 compared to a single treatment of peat, concentrations of trace element  
3  $\text{Fe}^{3+}$  were increased by addition of peat+SS-HA.  $\text{Fe}^{3+}$  concentration  
4 after addition of peat alone was 7.5 mmol/kg, whereas addition of peat  
5 + SS-HA gave a  $\text{Fe}^{3+}$  concentration of 9.7 mmol/kg (data not shown).

6

## 7 **Discussion**

8

9 ES solubilization techniques have been developed, including chemical  
10 processes such as ozone oxidation and alkali treatment, physical  
11 processes such as supercritical water oxidation and treatment with  
12 ultrasonic waves, and aerobic biotreatments using specific  
13 microorganisms.<sup>33,34</sup> From the available solubilization techniques, we  
14 chose to use a bead mill. Previously, we showed that sludge  
15 solubilized by using a beads mill had a higher TOC concentration than  
16 sludge solubilized by alkalization, microwaves, or boiling.<sup>15</sup>  
17 Moreover, as a mechanical treatment, a beads-mill provides stable  
18 solubilization results, and its operation and maintenance is simple.

19 The extraction rate of HA from SS was higher than that of ES-HA  
20 (Table 4). The use of solubilization as a pretreatment for extraction  
21 of HS increased the extraction efficiency for HA. The extraction rate  
22 we achieved for SS-HA approached that of CP-HA.

23 HA can have a wide range of molecular weights and sizes,

1 ranging from a few hundred to several hundred thousand atomic mass  
2 units, depending on its elemental composition, structure, and the  
3 number and position of functional groups, which differ according to the  
4 origin, method of extraction, and natural conditions prevailing during  
5 the formation of the HA.<sup>35,36</sup> The similarities among different HAs,  
6 however, are greater than the differences. The physicochemical and  
7 biological activities of HA also differ depending on the origin and  
8 method of extraction of the HA. For example, HA extracted from peat  
9 and that extracted from weathered coal have different physiochemical  
10 effects on rice cultivated in a saline-alkali soil.<sup>37</sup> For this reason, we  
11 investigated the structure of HA extracted from ES after solubilization  
12 by using elemental analysis, FT-IR spectroscopy, and <sup>1</sup>H-NMR  
13 spectroscopy, and compared it to the structure of HA extracted from ES  
14 without solubilization. Then, for reference, we compared the  
15 characteristics of SS-HA and ES-HA with those of CP-HA. The results  
16 of elemental analysis, FT-IR, and <sup>1</sup>H-NMR showed that the extraction  
17 efficiency of HA from ES was enhanced by solubilization, although  
18 solubilization had little influence on the structural properties of the HA.  
19 The elemental compositions of ES-HA and SS-HA showed higher H and  
20 N contents than other HA materials such as CP-HA. The higher H  
21 content of ES-HA and SS-HA was probably related to the stronger  
22 aliphatic characteristics of these HAs (shown by the FT-IR results), and  
23 their higher N content to less humification.

1        HA can form various complexes with metal ions and thus increase  
2 the CEC of soils.<sup>3,4</sup> CEC is the capacity of a soil to exchange cations  
3 between the soil and the soil solution, and it is used as a measure of the  
4 soil's fertility and nutrient retention capacity. Carboxylate and  
5 phenolic groups are primarily responsible for the binding of metals  
6 under most natural conditions.<sup>38</sup> Our structural analysis showed that  
7 SS-HA contains a number of acid functional groups such as the  
8 carboxylate and phenolic groups. We measured carboxylate groups'  
9 content and CEC of SS-HA for conformation, and those results were  
10 compared with that of previous research results.<sup>39,40</sup> It became clear  
11 that carboxyl group exist in SS-HA, and they have cation exchange  
12 capacity, although those concentrations were not high compared to  
13 CP-HA (Table 7).

14        Prior to column experiment, pH buffering ability of SS-HA was  
15 confirmed by batch test. As shown in Fig. 5, SS-HA had pH buffering  
16 ability on alkali area. From this result, it was expected that the pH of  
17 the saline-alkali soil decreased by SS-HA application. Then, effects  
18 of SS-HA application on the pH of the saline-alkali soil were confirmed.  
19 As the results, the pH of the saline-alkali soil decreased  
20 dose-dependently by SS-HA application (Fig. 6).

21        Our soil-column experiment, we confirmed the amelioration  
22 effects of SS-HA (Fig. 7). The water potential gradient is affected by  
23 gravitational potential, capillary potential, and osmotic potential. In

1 control 1 (soil alone), the vapor phase gradually reached equilibrium  
2 with the liquid phase of soil water. While, soil additives peat+SS-HA  
3 reduced the pH of the saline-alkali soil, and the effect of additional  
4 SS-HA on the pH reduction was faster or immediate observed compared  
5 with control 2 (peat alone). After leaching (from 21 to 157 days), the  
6 pH of the experimental treatment was not different from that of control  
7 2. Thus, application of SS-HA to saline-alkali soil enhanced the pH  
8 reduction achieved by the application of peat. The more immediate  
9 pH reduction achieved with SS-HA treatment may improve crop  
10 germination. It was considered that the mechanism by which the  
11 saline-alkali soil was ameliorated by addition of SS-HA was as follows.  
12 Carboxyl groups in SS-HA released  $H^+$ , which neutralized the  $OH^-$  in  
13 the soil solution, thus decreasing soil pH (Fig. 10A). Negatively  
14 charged carboxyl groups adsorbed cations from the soil solution,  
15 causing the EC of the soil solution to decrease. However, the results  
16 of the batch and soil-column experiments were unexpected.  
17 Kawakami *et al.*<sup>8</sup> reported that the EC of saline-alkali soils tends to  
18 increase in response to the application of peat. They showed that  
19 calcium and magnesium ions, which are poorly soluble in alkaline  
20 solution, were released from the soil and dissolved under low pH  
21 conditions. However, application of peat+SS-HA in our experiments  
22 produced a significant increase in exchangeable cations in the  
23 saline-alkali soil by their CEC (Fig. 9). An important property of

1 cations is their attraction to surfaces carrying a negative charge, upon  
2 which they adsorb strongly (Fig. 10B). Compared with the results  
3 for addition of peat alone, the added SS-HA produced a significant  
4 increase in  $\text{NH}_4^+$  concentrations at the top and bottom of the soil  
5 column. This occurred because the high-N component of SS-HA was  
6 broken down to form  $\text{NH}_4^+$ . The  $\text{K}^+$  concentration also increased in the  
7 top layer of the column after application of SS-HA.  $\text{NH}_4^+$ ,  $\text{K}^+$ , and  
8  $\text{Ca}^{2+}$  are especially important nutrients for plant growth. There was no  
9 noticeable change in the  $\text{Mg}^{2+}$  concentration at the top or bottom of the  
10 column after application of either peat alone or peat+SS-HA.  
11 Application of peat+SS-HA significantly affected the  $\text{Ca}^{2+}$   
12 concentration at the bottom of the column. Fe availability in  
13 calcareous soils is very low because of the high soil pH of such soils,  
14 which reduces the solubility of ferric iron. Our results also show that  
15 application of SS-HA to the saline-alkali soil increased Fe content,  
16 which is a trace element for plant growth.

17

## 18 **Conclusion**

19

20 The application of SS-HA was clearly shown to have a beneficial effect  
21 by improving the physicochemical properties of the saline-alkali soil.  
22 The SS-HA reduced the soil pH, and increased the concentrations of  
23 exchangeable cations in the soil. Naturally, higher concentration of

1 exchangeable base cations allow, more acidity to be neutralized and in  
2 a shorter time. HS are abundant in peat, weathered coal, and other  
3 humified substances. However, exploitation of wetlands as a source  
4 of peat has recently been severely restricted by the Ramsar  
5 Convention.<sup>41</sup> By comparison, ES is industrial waste that is widely  
6 and continually produced, and thus has the potential to be an  
7 environmentally sustainable source of HS. We hope that this research  
8 will contribute to the growth of the recycling society in the future to  
9 minimize raw material consumption and the amount of waste disposal in  
10 the environment.

11

12 **Acknowledgments** This research was partially supported by the  
13 JST-JAICA Science and Technology Research Partnership for  
14 Sustainable Development program (SATREPS).

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1 **Figure legend**

2

3 **Fig. 1.** Laboratory equipment used for solubilization of excess sludge

4

5 **Fig. 2.** Apparatus used for the saline-alkali soil-column experiment

6 Soil additives were mixed in the top 10-cm of the soil. The surface of  
7 the column was exposed to heat from a 57-W lamp for 8 h each day to  
8 promote evaporation.

9

10 **Fig. 3.** FT-IR spectra of humic acid extracted from non-solubilized

11 excess sludge (ES-HA) and solubilized excess sludge (SS-HA)

12 CP-HA, humic acid extracted from Canadian *sphagnum* peat

13

14 **Fig. 4.**  $^1\text{H-NMR}$  spectra of humic acid extracted from excess sludge

15 without solubilization (ES-HA) and humic acid extracted from

16 solubilized excess sludge (SS-HA)

17

18 **Fig. 5.** pH buffer curves of SS-HA obtained by batch tests

19 The pH buffering capacity of the model soil after application of  
20 different amounts of SS-HA was determined by as the number of moles  
21 of strong acid or base needed to produce a unit change of pH of the  
22 model soil.

23

1 **Fig. 6.** Effects of SS-HA on pH and EC in a saline-alkali soil  
 2 determined by batch tests (3 days after addition of SS-HA)

3

4 **Fig. 7.** Effects of humic substances on pH of the saline-alkali soil in  
 5 the column experiments

6 (■) Peat (2 wt %) added in the top 10-cm of soil; (□) peat (2 wt%) +  
 7 SS-HA (1 wt %) added in the top 10-cm of soil (peat 2%/soil plus  
 8 SS-HA 1%/peat); (○) control (without humic substances added).

9 Statistical significance ( $P < 0.05$ ) was evaluated by one-way ANOVA,  
 10 and then, group means that were significantly different were compared  
 11 using Bonferroni's post hoc test.  $*P < 0.05$ , peat alone and  
 12 peat+SS-HA versus control 1;  $**P < 0.01$ , peat alone and peat+SS-HA  
 13 versus control 1; <sup>a</sup>Significant difference between SS-HA+peat and peat  
 14 alone (control 2).

15

16 **Fig. 8.** Effect of humic substances on EC of the saline-alkali soil in  
 17 the column experiments

18 (■) Peat (2 wt %) added in the top 10-cm of soil; (□) peat (2 wt %) +  
 19 SS-HA (1 wt %) added in the 10-cm of soil; (○) control (without humic  
 20 substances added). Statistical significance ( $P < 0.05$ ) was evaluated  
 21 by one-way ANOVA and then, group means that were significantly  
 22 different compared using Bonferroni's post hoc test.  $*P < 0.05$ , peat  
 23 alone and peat+SS-HA versus control 1;  $**P < 0.01$ , peat alone and

1 peat+SS-HA versus control 1.

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3 **Fig. 9.** Effects of humic substances on exchangeable cation  
4 concentrations on day 90 of the column experiments

5  $P < 0.05$ , peat+SS-HA versus peat alone.

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7 **Fig. 10.** Mechanism of amelioration of saline-alkali soil by addition  
8 of humic substances extracted from solubilized excess sludge

9 (A) pH reduction. (B) Increase of exchangeable cation concentration.

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**Table 1.** Properties of the experimental soil (model saline-alkali soil)

## (1) Physical properties

$\rho_b$	Particle size distribution				
	Clay ~0.002mm	Silt 0.002~0.02	Fine sand 0.02~0.2	Coarse sand 0.2~2.0	Gravel 2.0~
g/cm	%				
1.54	1.00	0.00	13.47	85.53	0.00

## (2) Chemical properties

pH <sup>1)</sup>	Ash	EC <sup>2)</sup>	CEC	ESP <sup>3)</sup>	Exchangeable bases			
					Na <sup>+</sup>	K <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>
	%	mS/cm	cmol/kg	%	cmol/kg			
8.81	99.35	3.69	12.02	54.10	6.50	0.99	2.97	4.28

1) Soil-water suspension (2:5) 2) Soil-water suspension (1:5)

3) ESP, exchangeable\_sodium\_percentage (Na<sup>+</sup> / CEC)

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**Table 2.** Properties of the peat

Property	Value
Moisture content (% dry w/w)	64.0
Ash	1.5
Elemental composition (% d.a.f.)	
C	48.0
H	5.8
N	0.5
O	45.8
Extraction rate (% d.a.f.)	
Humic acid	3.6
Fulvic acid	5.2

d.a.f., dry ash free

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**Table 3.** Main Properties of non-solubilized excess sludge (ES) and solubilized excess sludge (SS)

Sample	Sludge concentration	TOC	IC	TN	Acetic acid	Propionic acid
		(mg/l)			(mg-soluble fraction/g-dry sludge)	
ES	4200	5.2	13.6	8.8	ND	ND
SS	3108	547.5	38.0	144.5	11.4	3.5

Each value of represents the mean of two measurements.

TOC, total organic carbon; IC, inorganic carbon; TN, total nitrogen; ND, not detected

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**Table 4.** Extraction rates of humic acid from excess sludge (ES) and solubilized excess sludge (SS) compared with rates reported in the literature

	HA %	Reference
ES	3.7	
SS	9.1	
<i>Sphagnum</i> peat (Canada)	12.8	
Weathered coal (Menggu, China)	26.4	Yamada <i>et al.</i> , 2002 <sup>24</sup>
Weathered coal (Urumqi, China)	7.2	
Inokashira soil (Japan)	2.1	
Dando-soil (Japan)	1.5	Watanabe <i>et al.</i> , 2007 <sup>25</sup>

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**Table 5.** Composition of humic acid extracted from excess sludge (ES) and solubilized excess sludge (SS) compared with values from the literature

Sample	Ash (% dry w/w)	Elemental composition (% d.a.f.)						
		C	H	N	S	O	H/C	O/C
Humic acid								
ES	1.9	47.2	7.0	9.4	1.4	35.1	1.8	0.6
SS	2.1	50.6	7.4	8.2	1.9	31.9	1.8	0.5
<i>Sphagnum</i> peat (Canada)	1.0	56.5	5.1	1.9	—	36.6 (O+S)	1.1	0.5
Weathered coal (Menggu China)	11.0	59.8	3.7	1.1	—	35.4 (O+S)	0.7	0.4
Weathered coal (Urumqi China)	2.1	62.5	4.7	2.9	—	29.9	0.9	0.4
Inokashira soil (Japan)	—	54.8	4.3	4.0	0.3	36.6 (O+S)	0.9	0.5
Dando-soil (Japan)	—	53.0	5.3	4.5	0.3	37.0	1.2	0.5

—, Not decided

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**Table 6.** Hydrogen distribution calculated from  $^1\text{H-NMR}$  results for humic acid extracted from excess sludge (ES) and solubilized excess sludge (SS)

Chemical shift region $\delta$ (ppm)	Assignment	Relative contribution (%)	
		ES-HA	SS-HA
I. (0.4-1.7)	Terminal $\text{CH}_3$ , $\text{CH}_2$ , and $\text{CH}$ of methylene chains, etc	28.55	30.93
II. (1.7-3.3)	$\text{CH}_3$ , $\text{CH}_2$ , and $\text{CH}$ proton $\alpha$ to aromatic or carboxyl groups, etc	25.40	23.34
III. (3.3-4.6)	Protons on carbon $\alpha$ to oxygen, carbohydrate, etc	36.06	32.85
IV. (6.5-8.1)	Aromatic protons (including quinone, phenol, etc)	9.99	12.88

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**Table 7.** Carboxyl group content and cation exchange capacity of humic substances extracted from solubilized excess sludge (SS) compared with values from the literature

		CEC	Carboxyl group (cmol/kg)	Reference
HA	SS	140	98	
	Sphagnum peat (Canada)	—	192	
	Reed-sedge peat (Belarus)	—	186	Yamaguchi <i>et al.</i> , 1998 <sup>39</sup>
	Reed peat (Japan)	—	68	
	Woody peat (Indonesia)	—	25	
FA	SS	175	159	
	Sphagnum peat (Canada)	—	398	Yamada <i>et al.</i> , 2007 <sup>40</sup>

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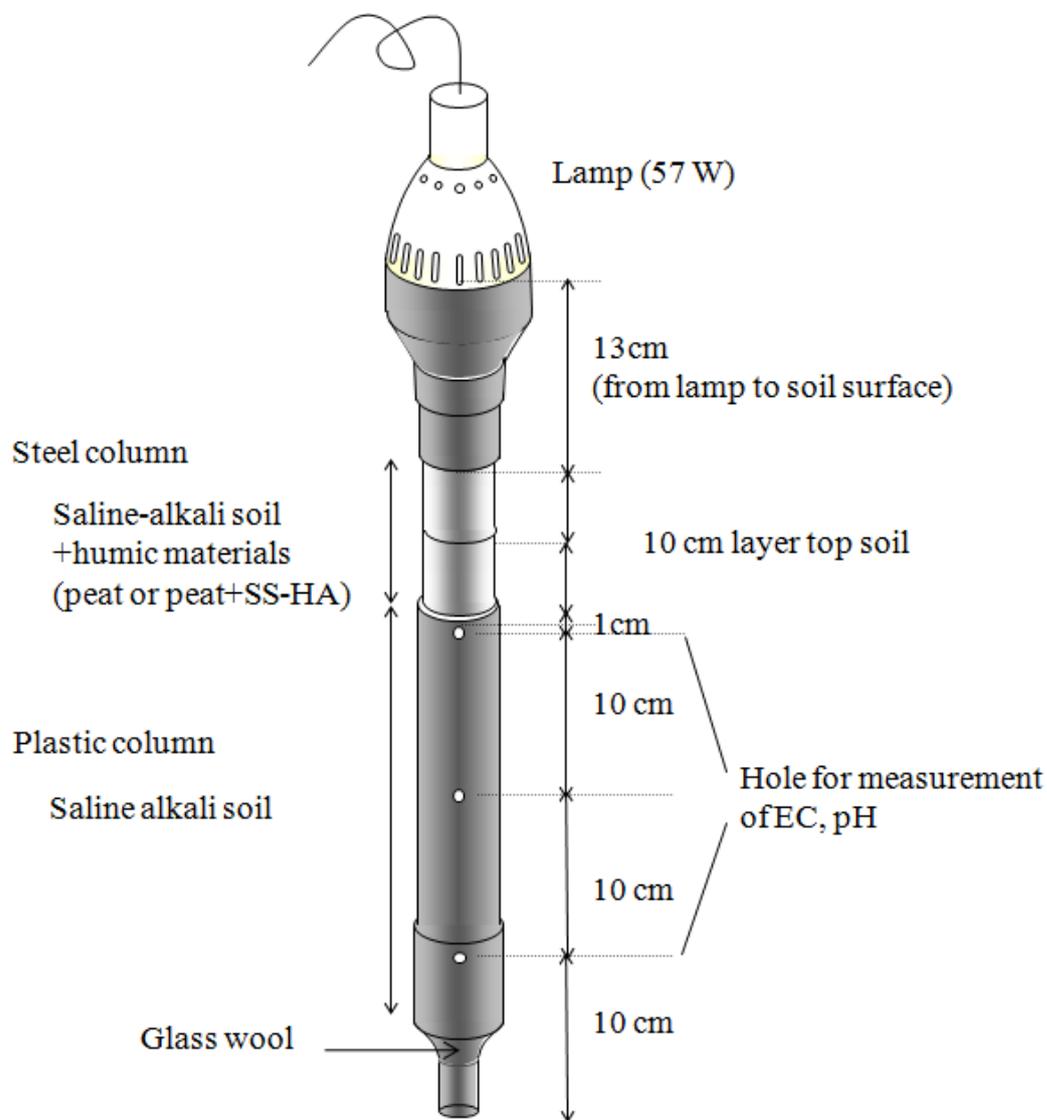
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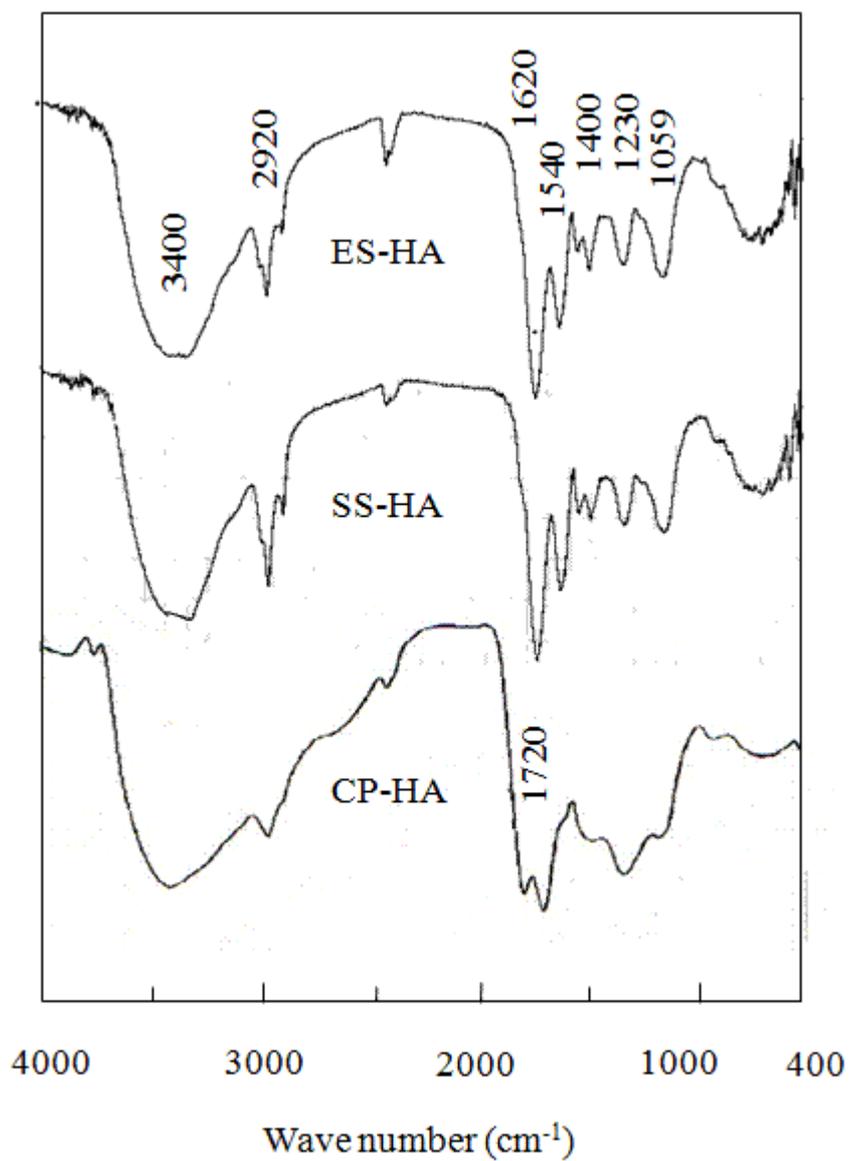


Fig 3

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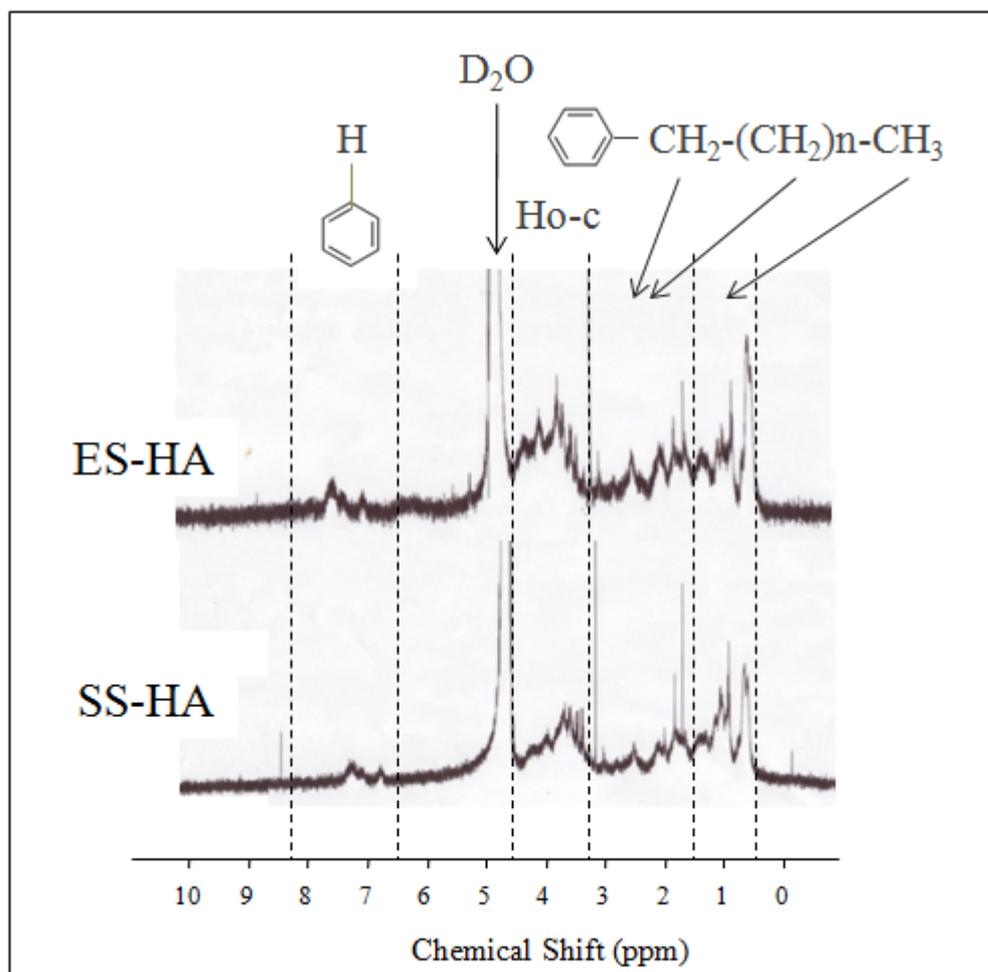


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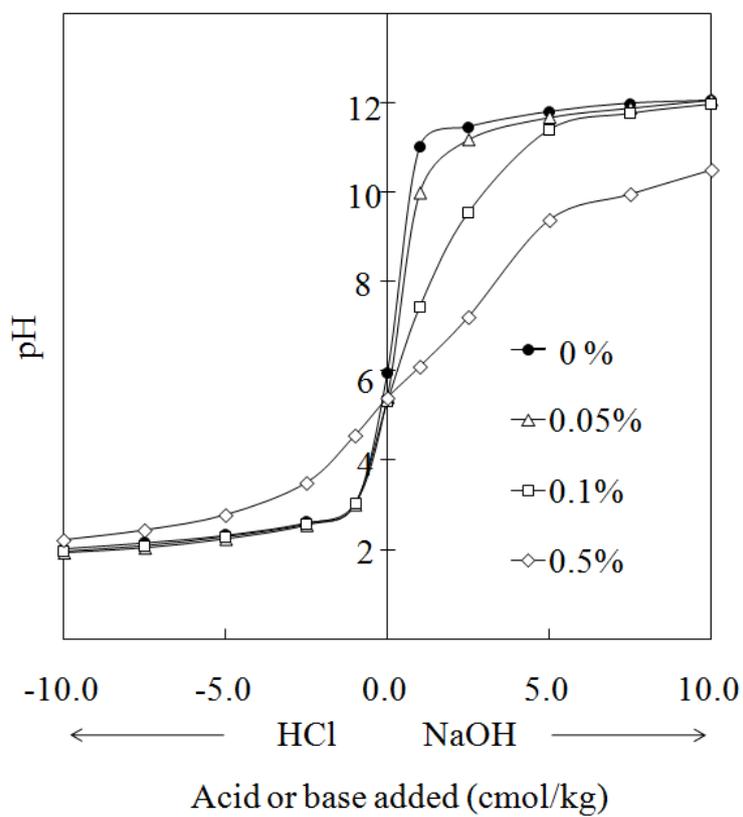


Fig 5

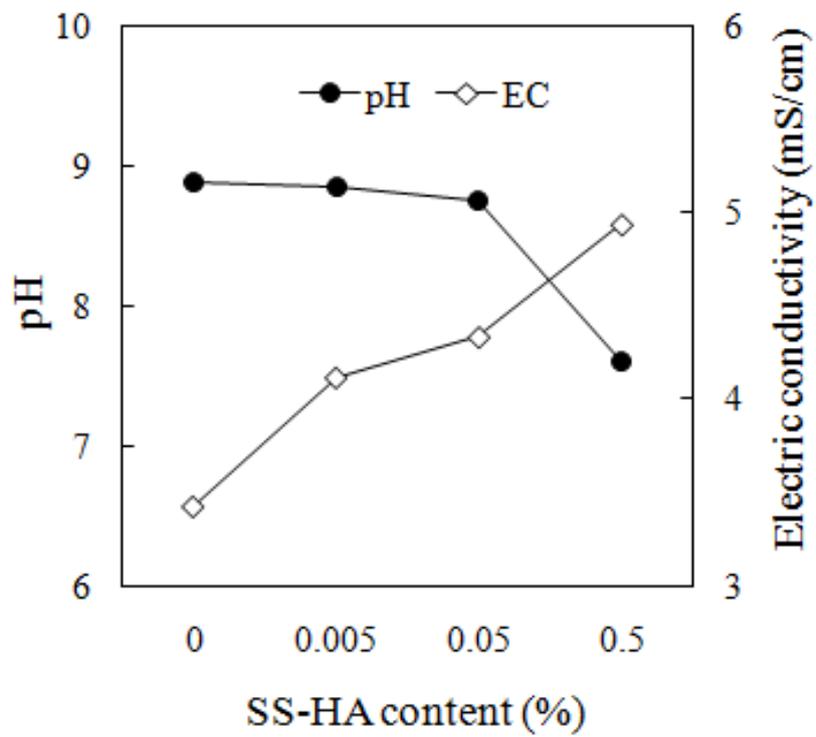
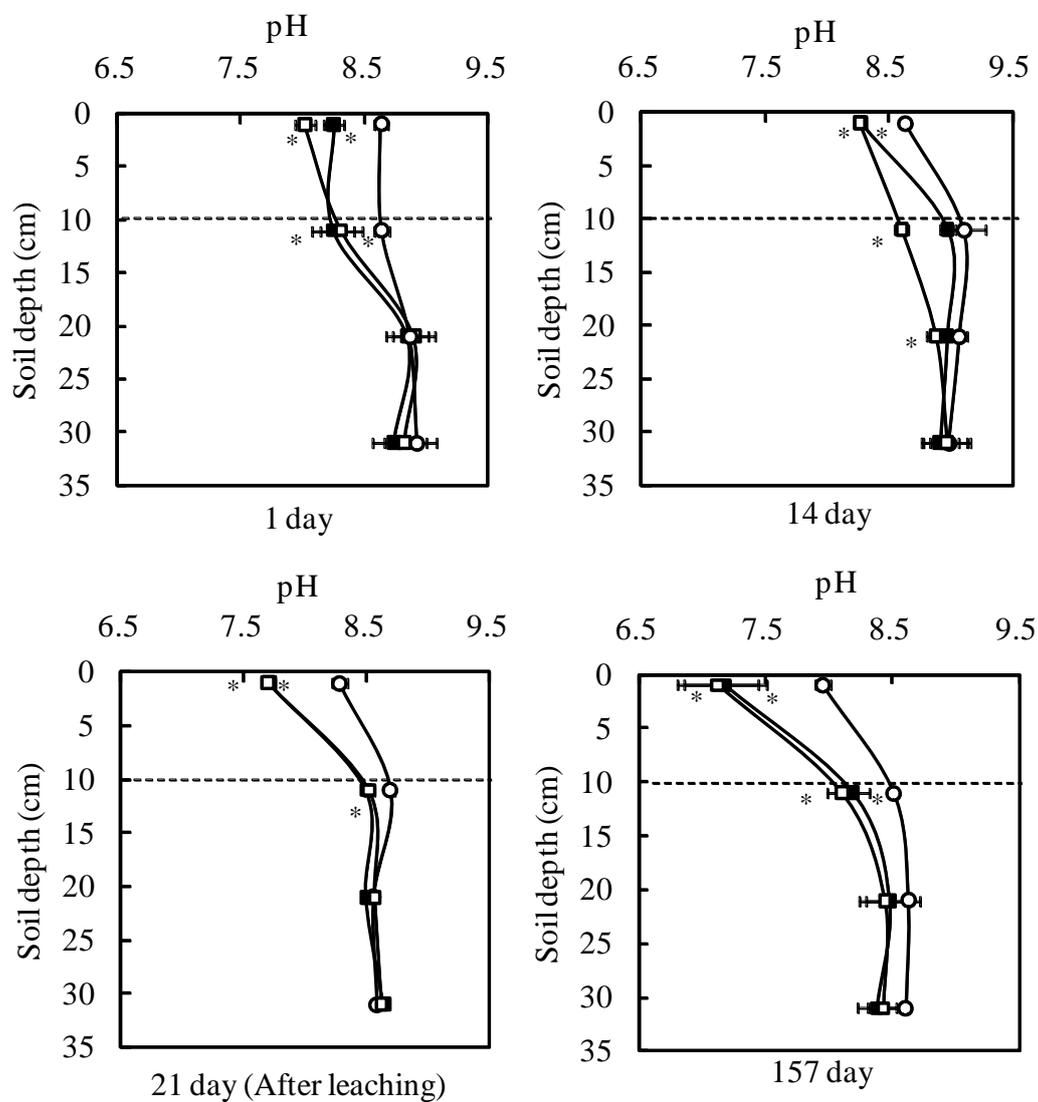


Fig 6

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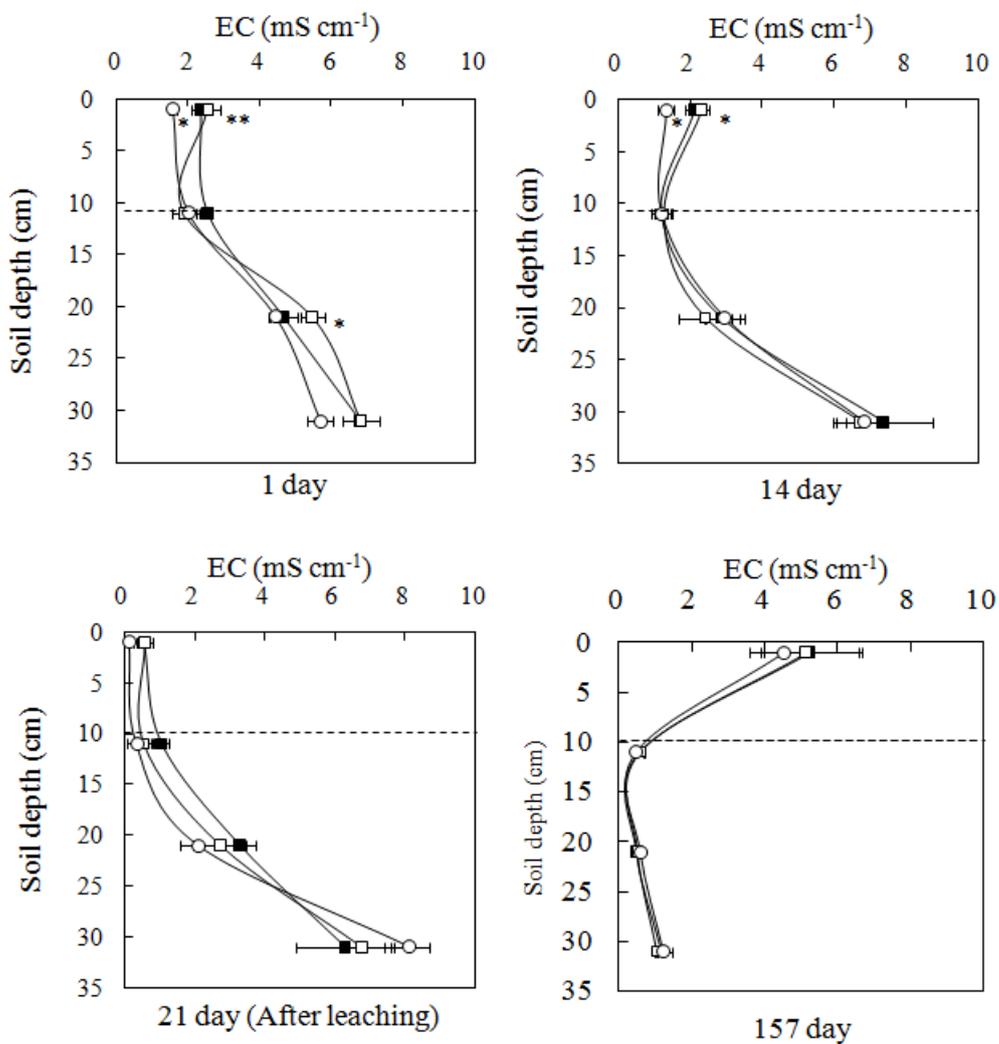
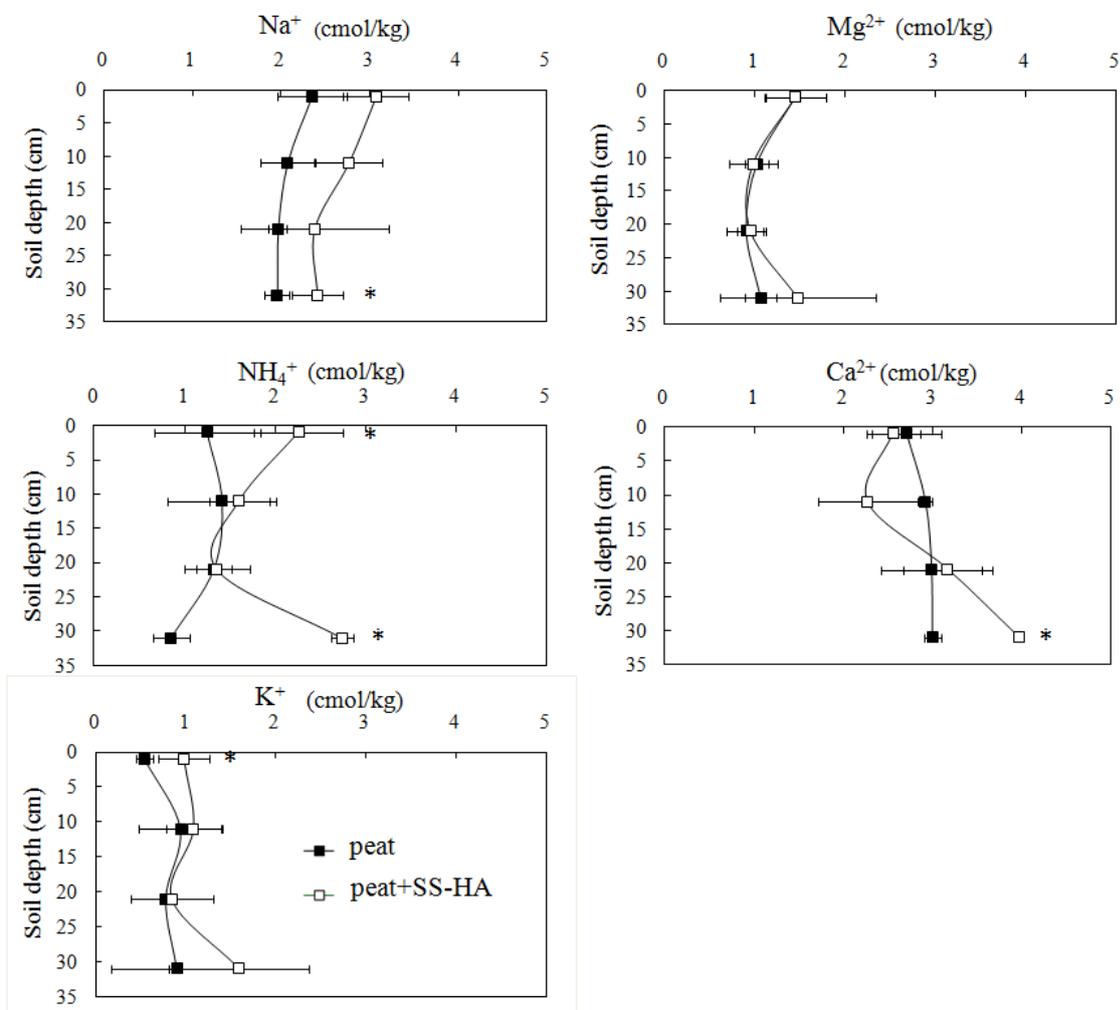


Fig 8

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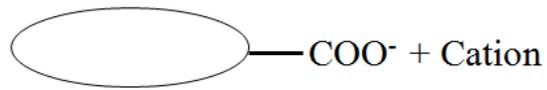
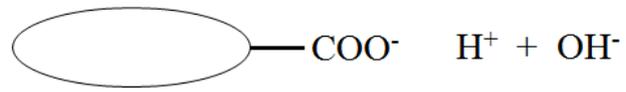
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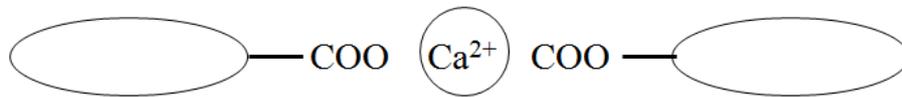
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(B)



25 Fig 10