Pak. J. Chem. 4(1): 19-25, 2014 Full Paper
ISSN (Print): 2220-2625
ISSN (Online): 2222-307X DOI: 10.15228/2014.v04.i01.p04

# Chemical Composition of Lignitic Humic Acid and Evaluating its Positive impacts on Nutrient Uptake, Growth and Yield of Maize

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## ABSTRACT
Lignite derived humic acid (HA), obtained from Thar coal mines were extracted by 0.5 N KOH; characterized and used to evaluate its impact on maize yield and soil health. Having highest HA recovery (20%) black coal derived HA were applied in different level [(@ no HA ($HA_0$), 25 ($HA_1$) and 50 ($HA_2$) mg $kg^{-1}$ soil)] in conjoint with different nitrogen (N) levels [no N application ($N_0$) (150 ($N_1$) and 300 ($N_2$) mg $kg^{-1}$]. Results showed that plant fresh biomass increased by 23% and 44% with application of HA at $HA_1$ and $HA_2$ respectively, about 23% increase was observed in dry plant biomass at both the HA levels. Cob weight and grain weight increased significantly (29% and 40%) with HA at 25 and 50 mg kg $^{-1}$ respectively vis as vis control (no HA applied), with N at 150 and 300 mg $kg^{-1}$ the increase was 51% and 103%. The grain weight increased by 12% and 41% with $HA_1$ and $HA_2$ whereas, 31 % and 43 % with N application ($N_1$ and $N_2$ over the control). The HA application increased plant N contents by 20% and 26 %, P by 14% and 20% and K by 15% and 10% in $HA_1$ and in $HA_2$, respectively. Nutrient uptake also enhanced with both N and HA application. From the results it can be concluded that HA application along with N help improved growth, yield and nutrient uptake by maize.

**Keywords**: Lignite, humic acid, fulvic acid, maize yield, organic fertilizer

## 1. INTRODUCTION
Most of agriculture soils in Pakistan are alkaline calcareous in nature and dominantly low in nutrients particularly micronutrients and organic matter (< 1 %). As to compensate for nutrients deficiency, inorganic fertilizers such as urea, diammonium phosphate, muriate or sulphate of potassium, zinc sulphate and boric acid for N, P K Zn and B respectively. However, having high intensity and cropping around the year, the soils are being continuously depleted of organic matter (OM). The low soil OM on one hand and growing needs for more food on the other end emphasized the need for improving soil fertility status either alone or in combination with inorganic fertilizers to enhance productivity. The HA as substitute and synonym for organic matter[1,2] and so considered as an appropriate candidate for enhancing crop productivity through improving organic matter status of soil.

Earlier research[3] underlined the significance of HA in enhancing crop productivity. Increase in cropped production could be ascribed HA potential role of complexing micronutrients (Fe and Al) and making it available for plant uptake[4]. Hence, the dual goals of improving soil organic acid along with increasing micronutrients availability could be achieved.

As source of plant nutrients (54% C, 5% N, and 0.6% P), HA can serve as slow release fertilizer particularly for N[3] where ground water pollution of $NO_3$ is getting at alarming rate. The significance of HA in increasing crop productivity has been extensively reported[5, 6, 7, 8, 9]. Recently, Ahmad and Khan et al. (2013)[10, 11] reported the positive impact of plant derived HA both by foliar and soil application in vegetables (pea and pepper). Findings of first study[10] recommended that soil application of HA @ 15-30 kg/ha and foliar application @ 45 kg/ha could improve plant growth and increase pea yield in addition to increase in foliar nutrients (P, K and Fe) and chlorophyll contents.
The HA can be derived both from coal and waste plant materials. As the positive impact of plant derived HA has been reported earlier[10, 11]. Coal derived HA can also acts as potential source of HA and having long retention in soil and slow decomposition[12] hence it can possibly play role in C sequestration.

## 2. RESULTS AND DISCUSSION

### 2.1 *Chemical Composition of HA*
Analysis of three lignitic coals (black, brown and half white) showed the existence of essential plant nutrients, HA and FA content (Table 1-2). Highest HA recovery (42%) was obtained in black coal followed by 15.3 % in brown and < 1 % (0.02 %) in half white coal. Based on HA recovery and micronutrients availability, black coal can be classified as of superior quality. However unlike black coal, the P and K contents were relatively high in brown white coal HA. These results simply imply that the possible combination of all three coal type could provide macro (P and K) as well as micronutrients (Cu, Zn and Fe) in addition to potential source of HA.

### 2.2 *Agronomic Measurements*
#### 2.2.1 *Cob weight and grain weight per cob*

*Corresponding Author Received 24th November 2013, Accepted 18th February 2014

Cob weight and grain weight per cob were significantly increased with application of N and HA alone as well as in combinations. An increase of 29% and 42% and 11.7% and 29% increase in cob weight and grain weight per cob were obtained with the HA applied as $HA_1$ and $HA_2$ respectively over control ($HA_0$) treatment (Fig 1 a and Fig 1 b). Nitrogen application alone increased cob weight by 52 and 93 % and grain per cob by 31 % to 44 % with $N_1$ and $N_2$ respectively vis a vis control. The interaction effect (HA × N) was also significant for both yield parameters (Fig 1 a).

**Table-1:** Chemical composition of lignite coal

| Characteristic | Unit | Type of lignite | | |
|---|---|---|---|---|
| | | Black | Brown | Half white |
| LOI | (%) | 85.00 | 77.00 | 4.00 |
| N | | 0.70 | 0.60 | 0.02 |
| K | | 0.75 | 0.95 | 0.40 |
| P | | 7.25 | 7.25 | 2.90 |
| Zn | mg $kg^{-1}$ | 11.95 | 4.25 | 4.85 |
| Cu | | 5.35 | 5.85 | 1.20 |
| Fe | | 606.9 | 390.0 | 51.65 |
| Mn | | 71.25 | 96.40 | 2.80 |

LOI = Loss on Ignition

**Table-2:** Humic acid recovery and its nutrients composition using various coal sources

| Coal type | HA recovery | N | K | Na | P | Cu | Zn | Fe |
|---|---|---|---|---|---|---|---|---|
| | -------------------(%)---------------- | | | | ------------------mg $kg^{-1}$----------------- | | | |
| Black coal | 49.2 | 1.23 | 0.04 | 7.9 | 11.38 | 22.0 | 20.6 | 3201 |
| Brown coal | 15.3 | 0.54 | 0.06 | 10.6 | 15.74 | 7.2 | 6.4 | 629 |
| Half white coal | 0.2 | 0.02 | 0.05 | 11.2 | 15.03 | 7.0 | 5.6 | 64 |

### 2.2.2 *Chlorophyll content and fresh weight*

Chlorophyll content was also positively affected with applied HA alone as well in combination with different N levels. Results showed increase of 7% and 13% with $HA_1$ and $HA_2$ respectively. Whereas, N application also improved chlorophyll content by 9% and 31% at $N_1$ and $N_2$ respectively. Interaction effect (HA × N) effect was also significant ($p \leq 0.05$) and the maximum chlorophyll contents were recorded in $HA_1$ x $N_2$ and the minimum in control [$HA_0$ × $N_0$] (Fig 1 c). Plant biomass as fresh weight also significantly increased with HA application. The increase of 27% and 44% were obtained with $HA_1$ and $HA_2$ respectively. Application of N increased plant biomass yield by 19% and 33 % at $N_1$ and $N_2$, respectively over the control ($N_0$). Interactive effect of (HA × N) also showed positive significantly increase fresh weight (Fig 1 d).

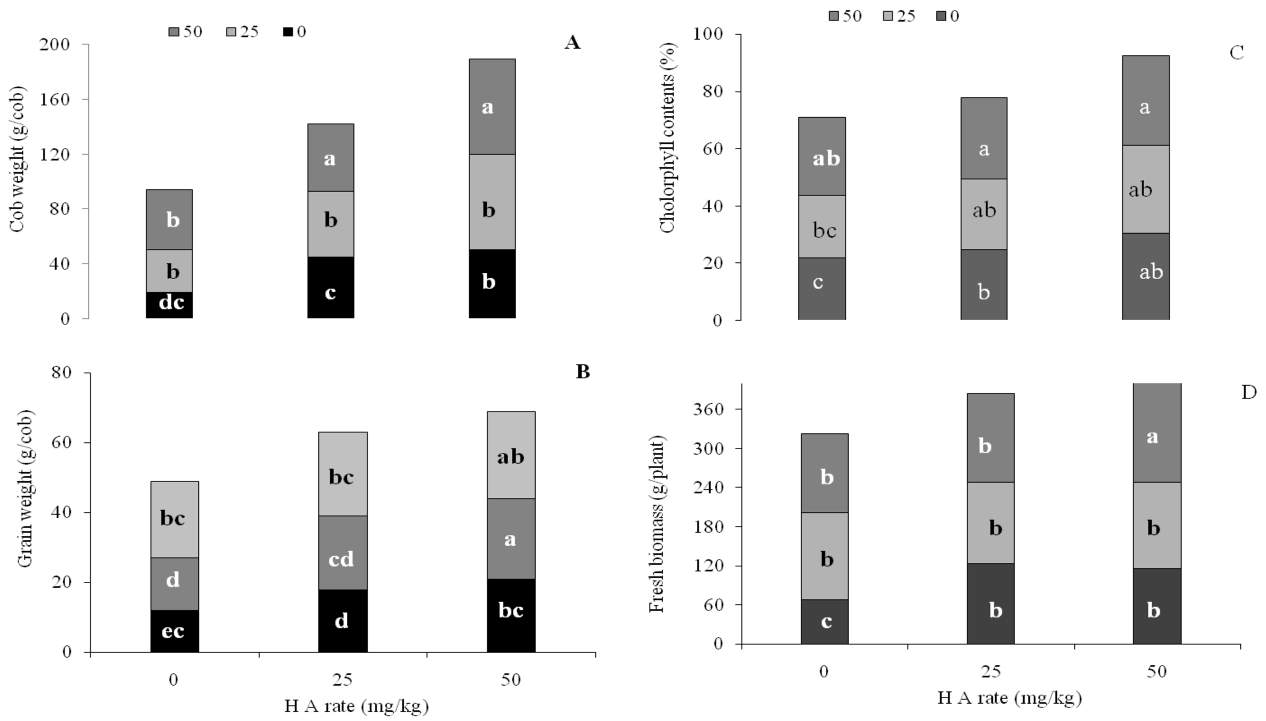


**Fig-1:** Response of (A) Cob weight (B) Gram weight (C) Chlorophyll and (D) Fresh biomass of Maize to HA and Nitrogen

### 2.2.3 *Effect of application on N, P, K and micronutrients*

Both HA application alone and in combination with HA significantly affected (<*P* 0.05), macronutrients and micronutrients in soil and its uptake by plant. N contents of maize plant significantly (Fig 2 a-f and Fig 3 a, b). The

HA application not only individual but interaction of both N and HA increased N, P and K concentration. Similarly, it also differed significantly with N levels as compared to control treatment (Fig 2 a). The combined effect of N and HA on plant N concentration was also significant and the maximum N was recorded in $HA_2 \times N_2$ treatment and the minimum in $HA_0 \times N_0$ combination.

The P and K contents of plants were positively affected with applied HA levels showing the increase of 14 % and 22% and 15 and 10 % with $HA_1$ and $HA_2$ respectively. Results showed that N application in absence of HA decreases P contents at $N_1$ (Fig 2 b). The interactions (HA × N) also showed significant differences ($p \leq 0.05$) on P content of maize (Fig 2 b). Increase in plant K with increasing application rate of N can be ascribed to synergistic effect of N on plant K content and increased it by 6 and 8 % with applied nitrogen as $N_1$ and $N_2$, respectively over the control. The combined effect of (HA × N) on potassium content of maize was also significant ($p \leq 0.05$). Similar to macronutrients, HA application increased plant available Zn, B, Cu and Fe and Mn under different HA and N application (Fig 2 d, e-f; Fig 3 a, b). In overall, plant available Zn content increased by 31% with $HA_2$ over $HA_0$ treatment and conversely, plant B contents decreased as HA application increased. Application of N increased Zn and B contents in plants variably. Non-significant difference was obtained in interactive effect (HA × N) on Zn and B contents. Plant B concentration in maize plants was less affected with different N and HA levels showing antagonistic response of HA on B plant uptake. However, it was noticed that in comparison

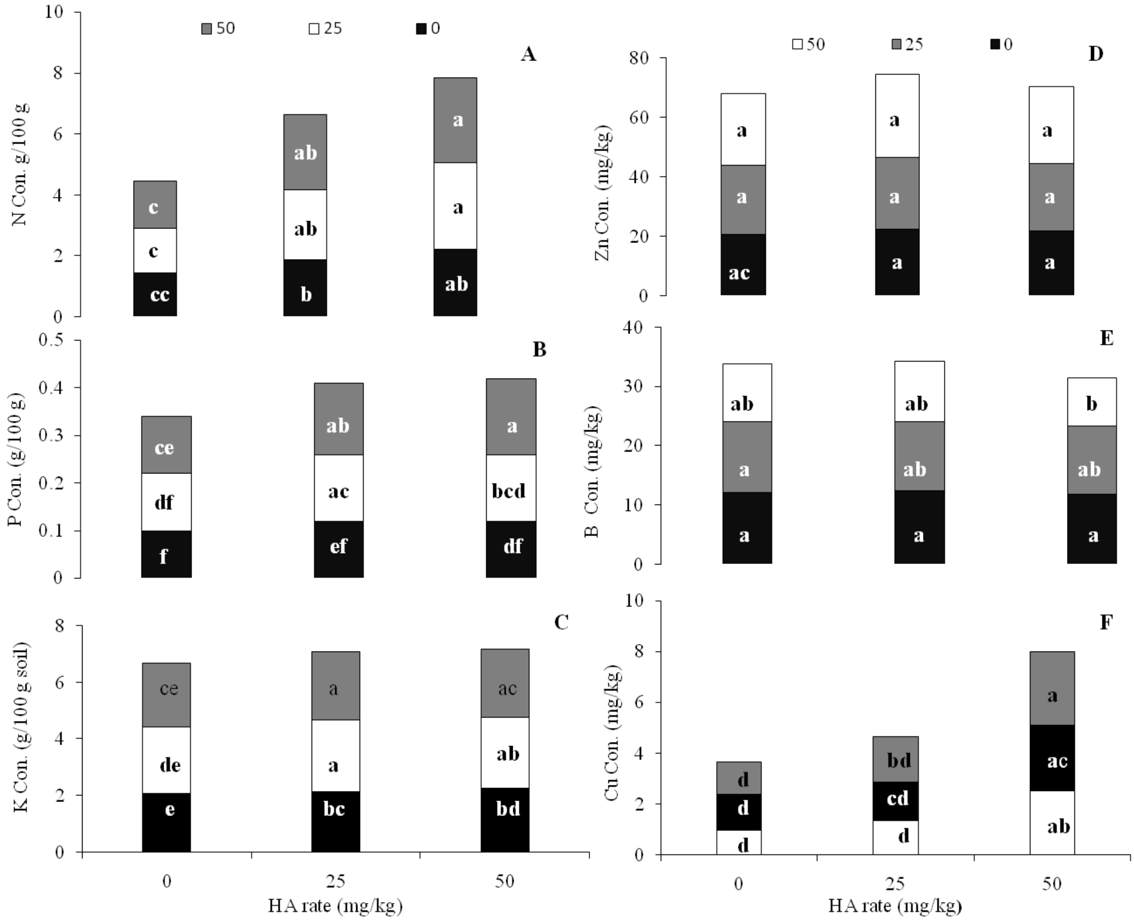


**Fig-2:** Response of plant (A) N conc. (B) P conc. (C) K conc. (D) Zn conc. (E) B conc. (F) Cu conc. In maize to HA and N application rates

to control (where no HA applied) HA application @ 25 mg/kg resulted better B uptake. Concentration of Fe was also significantly increased with HA application to maize crop (Fig 4 a) and Cu showed significant differences in plants grown on pots applied with different HA levels ($p \leq 0.05$) and an increase of 29% and 28% for Fe and 11% and 22% for Cu was recorded with $HA_1$ and $HA_2$ respectively over $HA_0$ treatment. The B contents decreased linearly with increasing application rates of HA (Fig 3 b). Application of nitrogen (N) also had synergistic effect on micronutrient uptake by plants. Iron concentration in maize plants increased by 31% and 25 %, Cu by 29% and 120% and Mn by 8 and 43% with applied $N_1$ and $N_2$, respectively over the control ($N_0$). The interactions HA×N also showed significant effect ($p \leq 0.05$) on micronutrient concentration of maize plants. Results showed that maximum Fe concentration (168 mg $kg^{-1}$) obtained by $HA_2$ x $N_2$ treatments whereas minimum Fe concentration (62 mg $kg^{-1}$) was obtained where both HA and N were controlled ($HA_0$ x $N_0$). The Mn concentration increased linearly with increasing rates of HA and N. Manganese (Mn) contents were increased by 7% and 14% at $HA_1$ and $HA_2$ respectively over $HA_0$. Applied $N_1$ increased Mn by 8% and $N_2$ by 48% over control ($N_0$). The interactions HA × N regarding maize Mn contents significantly differed ($p \leq 0.05$) and the maximum Mn concentration was recorded in plants grown in $HA_2 \times N_2$ treatments (66.38 mg $kg^{-1}$) and the minimum in $HA_0$ x $N_0$ treatment (40.95 mg $kg^{-1}$). It showed that HA application at different rate help solubilised micronutrients and hence increased their bioavailability. Earlier studies reported that HA application increased micronutrients availability such as Fe, Zn and particularly Mn.

These results are of particular interest as having low micronutrients bio available in most soil of Pakistan, HA application either alone or in combination with N could improve availability of micronutrient in soil and hence uptake by plant. The classical work[18] (Schnitzer and Khan (1972) reported that by complexing micronutrients HA improve plant root access to soil micronutrients and acts as carrier but also increase their availability to plant.

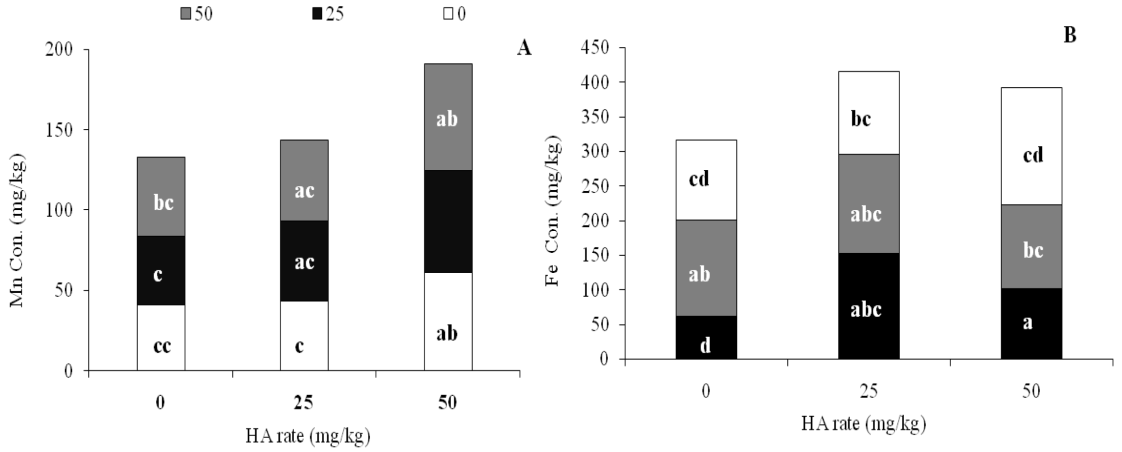


**Fig-3:** Response of plant (A) Mn conc. And (B) Fe conc. in maize to HA and N application rates

### 2.2.4 *Effect of coal derived HA on soil pH, N, P K and micronutrients*

#### 2.2.4.1 *Soil pH, N, P and K*

Results showed that soil pH ranged from 7.5-8.02 across all treatments (Table 3). Application of N also tended to decrease soil pH and a slight decrease (7.94 to 7.92 and 7.83) with $N_1$ and $N_2$ over the control was recorded. The interactions (HA× N) also showed non-significant effect ($p \leq 0.05$) on soil pH. The soil pH decreased with applied HA as $HA_1$ and $HA_2$ gradually under $N_1$ and $N_2$ as well as $N_0$.

**Table-3:** Interactive effect of N and humic acid levels on soil pH after crop harvest

| HA Level | pH | | |
|---|---|---|---|
| | N Levels | | |
| | $N_0$ | $N_1$ | $N_2$ |
| $HA_0$ | 8.02 | 8.00 | 8.01 |
| $HA_1$ | 7.97 | 7.93 | 8.00 |
| $HA_2$ | 7.84 | 7.84 | 7.50 b |

Though there has been shift in soil $NO_3$-N however it was found to be statistically non-significant (Fig 4 a). As a whole an increase of 11-17% was found by HA application at the rate of $HA_1$with $HA_2$ vis a vis control treatment where no HA was applied ($HA_0$). As expected soil $NO_3$-N responded to N application in various levels. An increase of about 6 and 12% was recorded with $N_1$ and $N_2$ over $N_0$. The interactions effect (HA × N) showed a significant increase ($p \leq 0.05$) in soil $NO_3$ status. Adani et al. 1998[19] reported that peas and leonardite derived HA increased plant uptake of N. Like N soil P also responded to HA application alone and in combination with N. It is reported that $PO_4$ usually rendered unavailable owing its complexing potential with Fe/Al and Ca in acidic and alkaline soils respectively. As chelating agent HA help $PO_4$ releasing out of this complexing and make it available for plant uptake. Results from this study showed significant differences (54% and 21%) at $HA_1$ and $HA_2$ over $HA_0$ treatment (Fig 4 b). Application of N increased the P status by 14% at $N_1$ only over the control ($N_0$). The interactions (HA × N) also showed significant change ($p \leq 0.05$) in soil P status after crop harvest. Earlier work of Ahmad and Tan, (1998)[20] and Cimrina and Yilmaz, (2005)[21] also reported the HA effects phosphate availability and ultimate uptake by plant.

The K contents increased with N as well as HA application. Results showed significant differences among HA levels ($p \leq 0.05$) and increased soil K status by 14% and 34% with the applied $HA_1$ and $HA_2$ over control treatment $HA_0$. The results regarding effect of application of N also showed the similar trend and K increased linearly with increasing N rates. An increase of 14% and 30% was recorded at $N_1$ and $N_2$ over that of $N_0$. The interactions (HA × N) also showed significant decrease ($p \leq 0.05$) in soil K status (Fig 4 c). The maximum K was recorded in $HA_2$ and $N_2$ treatment and the minimum in $HA_0$ and $N_0$ treatment.

#### 2.2.4.2 *Effect of HA on Micronutrients availability in soil*

The AB-DTPA extractable micronutrients were affected with the application of not only HA but N as well. Results showed significant differences for micronutrients with HA treatment at $p \leq 0.05$. Iron contents increased by 29% and 27% and Mn by 82% and 73% at $HA_1$ and $HA_2$, respectively over $HA_0$ while Cu content decreased with application of

HA in soil (Fig 4 a-f). In case of N application Fe contents also increased by 60 %, Cu by 14% and Mn by 25% with $N_1$ over $N_0$ while, in case of $N_2$ the increase was non-significant. The interactions (HA×N) also showed variable effect ($p \leq 0.05$) on micronutrient concentration in soil. Regarding zinc (Zn) contents there was a non significant difference in soil after crop harvest with HA and N levels. The interactive effect of HA×N on soil was significant ($p \leq 0.05$) for Zn content. The maximum Zn was recorded in $HA_2 \times N_2$ treatment and the minimum in $HA_0$ and $N_0$ treatment.

By complexing micronutrients HA application improved soil micronutrients. Having alkaline calcarious soils, these results have special implication for Pakistani soil where micronutrients availability is generally low in soil.

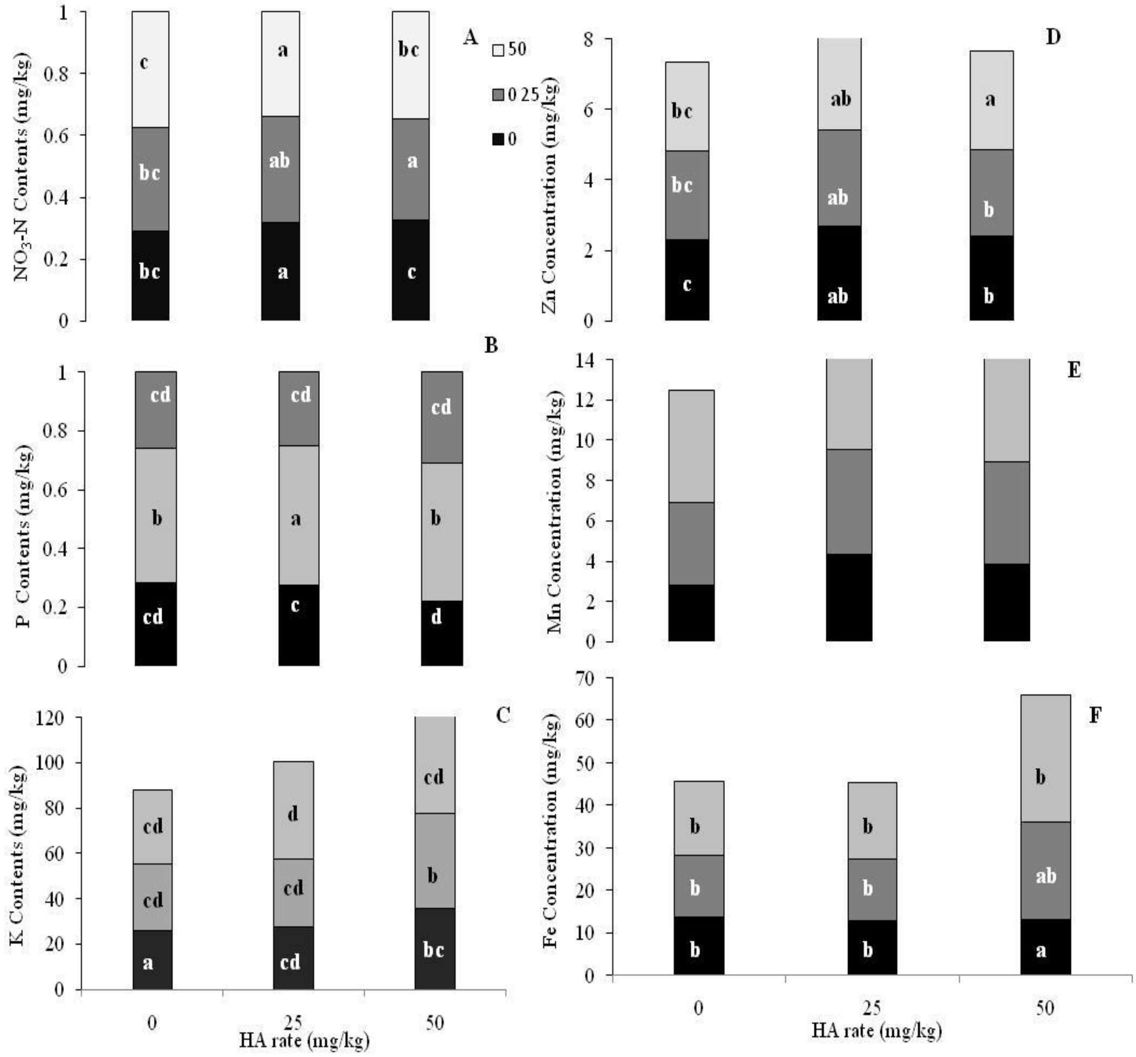


**Fig-4:** Response of (A) N conc. (B) P conc. (C) K conc. (D) Zn conc. € Mn conc. and (F) Fe conc. in soil to HA and N application rates

## 3. EXPERIMENTAL

### 3.1 *Extraction of HA and FA from coal*

Three types of coal viz. black, brown and half white (color based) were used for HA extraction. Coal samples were ground; sieved (2 mm), treated with KOH (0.5N) and NaOH (0.5N) separately; filtered, centrifuged and dried to HA (Fig 5), following grinding and sieving, extraction was acidified (10% $HNO_3$) as (1:4; coal: acid); filtered and dried (65 $^0$C) to fulvic acid (FA), a yellow color substances (Fig 5). Purity of FA and HA was determined by spectrophotometer. The chemical composition of coal derived HA was determined as described[12].

### 3.2 *Trial setup*

#### 3.2.1 *Soil sampling, preparation and analysis*

Pot experiment was carried out at National Agricultural Research Centre (NARC) Islamabad using Nabipur soil series with chemical properties listed in Table 4. The soil was collected from top 15 cm field layer; air dried, sieved (2 mm) and filled into 7 kg plastic container. Treatments consisting three HA levels ($HA_0$ = no HA, $HA_1$ = HA applied at 25 mg $kg^{-1}$ soil and $HA_2$ = HA applied at 50 mg $kg^{-1}$ soil) with or without three N levels [($N_0$ = no N, $N_1$ = N @ 150 mg $kg^{-1}$ soil and $N_2$ = N @ 300 mg $kg^{-1}$)] and replicated thrice using completely randomized design (CRD). The N applied as urea solution (in three splits at planting, at plants attained one foot height and before tussling) frequency. Earlier grown 6 seeds per pot were reduced to 3. Soil pH, EC, organic matter, macronutrients ($NO_3$-N, P, and K) and micronutrients (Zn, Fe, Cu, B and Mn) were determined following standard procedures as described[13].

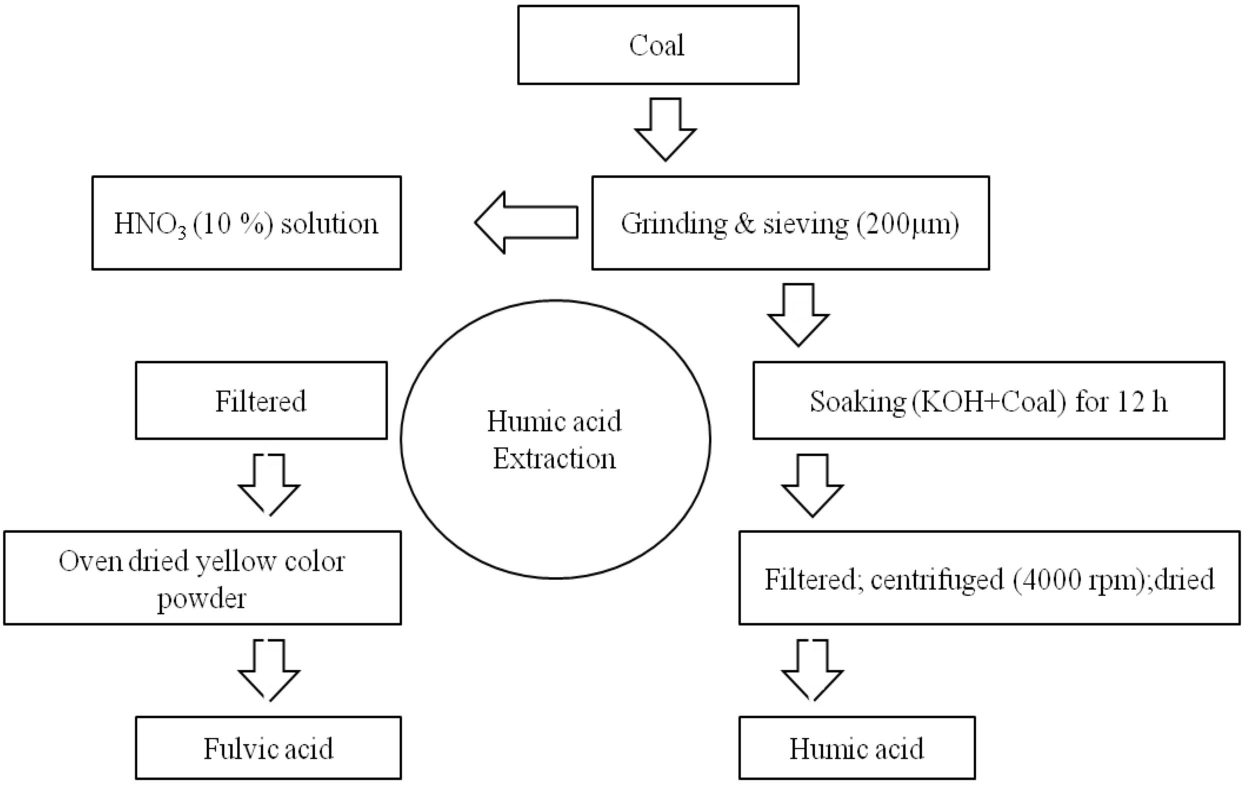


**Fig-5:** Humic and fulvic acid extraction from coal

**Table-4:** Chemical properties of soil science

| Property | unit | value | property | unit | value |
|---|---|---|---|---|---|
| **pH** | dS $m^{-1}$ | 8.10 | K | mg $kg^{-1}$ | 48.0 |
| **$EC_{1:1}$** | | 0.16 | Zn | | 0. 4 |
| **$CaCO_3$** | % | 4.00 | Cu | | 0.5 |
| **OM** | | 1. 02 | Fe | | 6.6 |
| **$NO_3$** | | 1.34 | Mn | | 6.2 |
| **$NaHCO_3$-P** | mg $kg^{-1}$ | 4.90 | | | |

### 3.3 *Measurement and chemical analysis*

#### 3.3.1 *Plants analysis*

The plant samples previously rinsed, washed; oven dried (65 $^0$C), ground and analyzed for total N (Kjeldahl method[14], tissue B by dry ashing[15]. Nutrients (P, K, Zn, Fe, Cu and Mn) were determined in plant materials following mixed acid ($HNO_3$: $HClO_4$ in 2:1) digestion as described[16]. Leaf chlorophyll content was measured using chlorophyll meter (SPAD 502, Konica Inc. Japan).

#### 3.3.2 *Statistical analysis*

The data obtained were analyzed using the MSTATC 5.4.2[17]. Difference in means was compared using least significant difference (LSD) test.

## 4. CONCLUSION

Combine use of HA and N helped in booting biomass production and enhancing grain yield. This was attributed to enhance uptake of nutrients by plants with applied HA and N in different concentrations. The application of HA improved soil characteristic by playing its role in chelating nutrients that became available to plant. It laid a positive effect on soil indicated the improvements in soil health which is a key to sustain crop productivity and on over all sustainable agriculture.

## 5. ACKNOWLEDGEMENTS

Authors are thankful to Pakistan Agricultural Research Council for sponsoring the study under the Agricultural Linkage funding Program (ALP) *NR010* on production of humic substances based fertilizer plant nutrients products for increasing crop productivity.

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