Pak. J. Bot., 47(6): 2231-2238, 2015.

EFFECTS OF LIGNITE-DERIVED HUMIC ACID ON SOME SELECTED SOIL PROPERTIES, GROWTH AND NUTRIENT UPTAKE OF WHEAT (*TRITICUM AESTIVUM* L.) GROWN UNDER GREENHOUSE CONDITIONS

TUBA ARJUMEND[1], M. KALEEM ABBASI[1]* AND EJAZ RAFIQUE[2]

[1]*Department of Soil & Environmental Sciences, Faculty of Agriculture, The University of Poonch, Rawalakot, Azad Jammu and Kashmir, Pakistan*

[2]*Land Resources Research Institute, National Agriculture Research Center, Islamabad, Pakistan*

[*]*Corresponding author e-mail: mkaleemabbasi@gmail.com; Tel.: +92 (0) 5824 960041, Fax: +92 (0) 5824 960004*

Abstract

Humic acid (HA) has been reported a promising natural resource showing persistent effects on plant growth promotion, nutrient uptake and soil nutrient status. A greenhouse experiment was conducted to determine the effects of soil and foliar applied HA on the changes in selected soil properties and growth/nutrient accumulation of wheat (*Triticum aestivum* L.). The experiment comprised of 05 levels of soil applied HA *viz*., 0, 50, 100, 150 and 200 mg kg^{-1}; 02 levels of soil+foliar applied HA i.e., 0 and 100 mg kg^{-1} tested on two different soils i.e. loam and silt loam. Results indicated that application of HA increased plant growth in terms of shoot length (18%), root length (29%), shoot dry weight (76%), root dry weight (100%) and chlorophyll content (96%). Response of yield and yield components displayed a significant increase in 1000-grain weight (8–16%), biological yield (18–36%), dry matter yield (15–25%) and grain yield (19–58%). The relative increase in NPK uptake in plants grown under HA was 57, 96 and 62%, respectively over the control. HA improved soil nutrient status by increasing organic matter (9%), total N (30%), available P (166%) and available K (52%), indicating a substantial increase in soil nutrient status. The improvement in soil fertility and wheat productivity in response to humic acid observed in this study is critical in the degraded and eroded soils generally exist in the State of Azad Jammu and Kashmir and other parts of the world. The quality and productivity of these degraded and eroded soils may be upgraded by including HA in our agricultural cropping pattern/system.

Key words: Humic acid, lignite coal, nutrient accumulation, NPK uptake, soil properties

Introduction

Humic acid is a naturally occurring polymeric-heterocyclic organic compound with carboxylic ($COOH^-$), phenolic (OH^-), alcoholic and carbonyl fractions extracted from various sources such as lignite, peat, coal, farmyard manure, coirpith besides natural persistence in soil (Sharif *et al*., 2002). Humic acids is not only found in soil, plants, peat, natural water, rivers, sea sediments, and other chemically and biologically transformed materials but also extracted from lignite, oxidized bituminous coal, leonardite and gyttja (Karaca *et al*., 2006). Pakistan is rich in coal and the total coal reserves are estimated to 548 million metric tons (Hai & Mir, 1998). Studies have shown that these coal reserves have reasonable concentrations of HA that can be utilized efficiently and effectively as organic input to boost-up agricultural production. Hai & Mir (1998) conducted various experiments to determine the physio-chemical characteristics of HA derived from lignitic coal of Pakistan and reported that this HA contains 57% C, 7% N, 4% H, 30% O and 1% S.

It has been reported that HA affects plant growth both by direct and indirect action (Sharif *et al*., 2002; Saruhan *et al*., 2011). Indirect effects comprise improvement/modification of soil physiochemical and biological environment such as aggregation, aeration, permeability, water holding capacity, hormonal activity, microbial growth, organic matter mineralization, transport and availability of micro (Fe, Zn and Mn) (Saruhan *et al*., 2011) and some macro nutrients (P, K and Ca) (Sharif *et al*., 2002; Daur *et al*., 2013). Directly, humic compounds may have various biochemical effects either at cell wall, membrane level or in the cytoplasm, including increased photosynthesis and respiration rates in plants, enhanced protein synthesis and plant hormone like activity (Nardi *et al*., 2002). In general, the effect of HA on plant physiology is recognized with regard to enhancement of root growth (Eyheraguibel *et al*., 2008) and nutrient uptake (Pinton *et al*., 2007).

Addition of HA improve yield and quality of a variety of plants by acting on mechanisms involved in cell respiration, photosynthesis, protein synthesis, water and nutrient uptake and enzyme activities (Chen *et al.,* 2004). The stimulatory effects of humic substances have been directly correlated with enhanced uptake of macronutrients, such as nitrogen, phosphorus and sulfur (Sharif *et al*., 2002; Jones *et al*., 2007), and micronutrients lke Fe, Zn, Cu and Mn (Sharif *et al*., 2002; Eyheraguibel *et al.,* 2008).

Humic acid plays a key role in soil fertilization via contributing to various soil properties including chelation, buffering, clay mineral–organic interaction and cation-exchange capacity which are essential for soil quality (Selim & Mosa, 2012). Mikkelsen (2005) reported that HA are able to act as a sink for polyvalent cations and form complex various cations in the soil. Amending soils with HA tend to improve soil biochemical quality through increasing activities of several enzymes (Bastida *et al*., 2008) and possibly enhance the uptake of minerals through the stimulation of microbiological activity. Turgay *et al*. (2011) reported in detailed that the positive effect of humic substances on enhancing plant growth is attributed to their promoting effects on soil physical, chemical, and biological-biochemical characteristics and increasing soil quality in general and hence providing better plant growth. When compared the efficiency of HA with chemical fertilizers, Kirn *et al.* (2010) reported that

HA, no doubt has its role in plant growth stimulation yet it was not able to sustain the yield when reducing the fertilizer from recommended levels and HA can be a supplement but not a substitute of fertilizers.

The soils of arid region of Pakistan and eroded soils of mountainous regions of Northern part of the country including the state of Azad Jammu and Kashmir are generally deficient in organic matter ranged between 0.3–1.0% only. Exploitation and proper utilization of natural resources especially coal/lignite will be a major step towards economic viability and agriculture sustainability of the country. Keeping in view, present study was designated on two soils collected from the mountainous and hilly region of Rawalakot Azad Jammu and Kashmir to examine the effects of lignite-derived HA on growth, yield and nutrient uptake of wheat and evaluate the changes in the nutrient status of soil after crop harvest.

Materials and Methods

Soil collection-sampling: The bulk soil samples were collected from two different locations i.e., Tolipir and Rawalakot located at about 8800 ft and 5374 ft, respectively from the sea level. The soil samples were taken at 0–15 cm depth from five sub sampling points marked in a uniform field and mixed to make composite soil samples. Soil was then air dried and crushed to pass through a 4-mm mesh screen. A sub sample of about half kg of each location was taken, sieved through 2-mm mesh screen and analyzed for physical and chemical characteristics of the soils used in the study (Table 1).

Table 1. Physical and chemical properties of soils before actual experiment.

Soil parameters	Units	Locations	
		Tolipir	Rawalakot
Total N	(g kg^{-1})	3.8	1.9
Available P	(mg kg^{-1})	1.18	1.97
Available K	(mg kg^{-1})	108	98
Organic matter	(g kg^{-1})	59	17.8
Soil pH	----	5.50	7.63
ECe	(dSm^{-1})	0.34	0.21
Sand	(%)	44	22
Silt	(%)	47	64
Clay	(%)	9	14
Texture class	----	Loam	Silt loam
Fe	(mg kg^{-1})	65.9	38.4
Mn	(mg kg^{-1})	5.3	3.0
Zn	(mg kg^{-1})	3.1	2.7
Cu	(mg kg^{-1})	1.6	1.8

Experimental set-up: An experiment was conducted in the greenhouse of The Department of Soil and Environmental Sciences, The University of Poonch Rawalakot during 2011–12. Thoroughly cleaned earthen pots of 30 cm height and 15 cm width were taken, filled with 7.5 kg of respective soil collected from two different locations. Five levels of HA (0, 50, 100, 150 and 200 mg kg^{-1} designated as HA_0, HA_{50}, HA_{100}, HA_{150} and HA_{200}) with two application methods (soil application; soil+foliar application) were used. In case of foliar application, HA was applied at the rate of 100 mg L^{-1} (in addition to soil applied HA). A basal dose of 100–90–60 mg kg^{-1} NPK was also added in the form of urea, SSP and SOP, respectively. The pots were labeled according to their respective treatments and arranged in a completely randomized design with three replications having three factors (HA levels, soils, application methods).

All the treatments were applied and well mixed into the soil prior to sowing. Wheat variety Shafaq-2006 was grown as a test crop. Seeds were collected from the seed section, National Agriculture Research Center (NARC) Islamabad. Ten healthy and uniform sized seeds were sown in each pot at a depth of 2 cm and tap water was applied to bring the soil into a field capacity level. After complete germination, thinning was carried out to leave six plants in each pot. The pots were irrigated regularly to maintain a proper moisture level of approximately 60% of soil's water holding capacity. In case of foliar application, HA was dissolved in distilled water as 100 mg L^{-1}, and sprayed by using a knapsack sprayer at three growth stages (early tillering, before head emergence and milking stage).

Agro- morphological and chemical analysis: For plant morphological characteristics, two plants from each pot were uprooted at two growth stages i.e., tillering and spike formation, with minimal damage to the root system. In the laboratory, shoots were separated from roots by cutting. The roots were then washed gently with tap water to remove all the adhering soil particles. Root length was measured as described by Farrell *et al*. (1993). Shoot length was also measured. Root and shoot dry weight was recorded after oven drying for three days at 70°C. Chlorophyll content readings were taken by following the method of Lichtenthaler & Wellburn (1985). To determine the leaf macronutrient concentration, two diagnostic leaves were taken from each plant before head emergence stage. At complete maturity the remaining two plants in each pot were harvested and data was recorded for no of spikes, spike length, 1000-grain weight, biological yield, dry matter yield and grain yield. Bulked plant parts (shoot+ leave) and roots were rinsed with deionized water, cleaned, air dried and then oven dried at 70 C° for 48 hours. The dried root, shoot and grain samples were ground to pass through a 1–mesh sieve in an ED-5 Wiley mill (Arthur H. Thomas Co) and then digested in a diacid mixture of nitric and perchloric acid (HNO_3:$HClO_4$ 2:1 v/v ratio) for the determination of P and K (Ryan *et al*., 2001). Kjeldhal method (Keeney & Nelson, 1982) was used to determine the total N content of plants. At the end of the experiment, composite soil samples were collected from each pot, air dried and sieved (2-mm). Soil samples were then stored in a cool and dry place until analyzed for OM, pH, electrical conductivity, (ECe), P, K and total N.

Statistical analysis: The data collected were subjected to statistical analyses. Two-way analysis of variance (ANOVA) was performed to compare variations in soil properties and plant growth characteristics for each HA application rate/treatment and for pooled means of soils and method of HA application. Least significant difference (LSD) multiple range test (p≤0.05) was used to indicate the significant differences among the treatments, soils and application methods. All analyses were performed using the Statistical Analysis Systems (Anon., 2002) software.

Result and Discussion

Growth characteristics of wheat: Response of plant growth characteristic to HA rates, methods of HA application and different soils is presented in Table 2. Results indicated that application of different rates of HA significantly ($p \leq 0.05$) increased growth traits of wheat (except shoot length in HA_{50}) over the control treatment. Among different HA application rates, the maximum values for most of the traits were recorded under HA_{200}. However, the difference between HA_{150} and HA_{200} was non-significant. The relative increase in shoot length, shoot dry weight, root length, root dry weight and chlorophyll contents due to HA application was 11–15%, 29–73%, 18–32%, 30–89% and 57–97%, respectively over the control.

The increase in growth characteristics of wheat in response to HA may be due the presence of growth promoting substances like indole acetic acid (IAA), gibberellins and auxin in its structure that are directly involved in cell respiration, photosynthesis, oxidative phosphorylation, protein synthesis, and various enzymatic reactions (Ulukan, 2008). This increase may also be owing to the effect of HA on root development. Stimulation of root hairs and enhancement of root initiation by HA may increase nutrients uptake that eventually affected the growth characteristics of plant as reported earlier (Nikbakht *et al*., 2008; Shahrayri *et al*., 2011; Tahir *et al*., 2011; Saruhan *et al*., 2011).

The maximum increase in shoot characteristics i.e. shoot length and shoot dry weight due to HA application was 15 and 73%, respectively compared to 32 and 89% increase in root length and root dry weight showing the dominating effect of HA on root development compared to the shoot. This finding is in accordance with the observations reported earlier (Atiyeh *et al*., 2002; Nardi *et al*., 2002).

Application of HA at the rate of 200 mg kg^{-1} showed highest value for most of the growth characteristics. However, in most of the cases the values were at par with HA rate of 150 mg kg^{-1} showing that the highest dose of 200 mg kg^{-1} did not show any increasing effect. These results are in conformity with the findings of Sharif *et al*. (2002) who reported that increasing levels of HA above 100 mg kg^{-1} had no significant effect on maize yield.

With regards to methods of HA application, both shoot dry weight and root dry weight were significantly higher under soil applied HA while shoot and root length showed non-significant response to the methods of HA application. Soil effects showed significant response and except chlorophyll content, all the growth traits in Mollisols (Tolipir) was significantly higher than Inceptisols (Rawalakot). This higher response of Mollisols/loamy soil may be attributed to higher initial organic matter content and other plant nutrients present in this soil (Table 1).

Yield and yield components: Yield and yield components of wheat i.e. 1000-grain weight, biological yield, dry matter yield, grain yield and harvest index was significantly affected by HA (Table 3). By taking the average values across methods and soils (treatments effect) and comparing them to those recorded from control, application of HA increased 1000-grains weight by 9–17%., biological yield by 18–36%, dry matter yield by 15–25%, grain yield by 19–58% and harvest index by 3–14%. Among different HA rates, the highest values were recorded either under HA_{150} or HA_{200} treatments. However, the difference between the two rates was non-significant. The positive influence of HA on the yield and yield characteristics seems to be concentration specific. Results revealed that the lower concentrations/rates of HA i.e., 50 and 100 mg HA kg^{-1} were less effective and a significant reduction in yield was observed compared to the higher rates i.e. 150 and 200 mg kg^{-1}. These results are in contrast to the findings of Sharif *et al*. (2002) who reported that lower doses of 50 and 100 mg HA kg^{-1} soil were either more effective in promoting yield of maize or at par with higher doses (150 to 300 mg kg^{-1}).

The observed increase in yield and yield components of wheat recorded in this study is in consonance with previous findings (Chen *et al*., 2004; Tahir *et al*., 2011). An increase in grain yield of different crops due to HA application is reported earlier by Hai & Mir (1998) i.e., wheat 8 – 20%, rice 14%, vegetables 8%, and radish 44%. Under field conditions, Sharif *et al*. (2002) found a significant increase in wheat grain yield due to HA by 20–69% while Delfine *et al*. (2005) reported 23 – 26% increase in grain yield of wheat by HA application. Celik *et al*. (2011) concluded that the application of HA at the rate of 0.1 and 0.2% significantly increased maize dry mater yield by 14 and 13%, respectively. The increase in yield and yield traits of wheat could be attributed to direct or indirect effects of HA on plant growth and development. Humic acid could stimulate root growth and affecting root morphology by exudation organic acid that led to increase nutrient uptake and consequently improve growth and yield of crops (Canellas *et al*., 2008). The correlation analysis displayed a significant correlation between root length and root mass with N-uptake (r^2 = 0.92 and 0.68), P-uptake (r^2 = 0.95 and 0.69), and K-uptake (r^2 = 0.98 and 0.77) (data not shown) confirmed the role of root development towards nutrient uptake. Humic substances have been considered as agents endowed with auxin-like activities which promote cell elongation, apical dominance and rooting that ends with high crop yield (Nardi *et al*., 2002).

With regard to methods of HA application, soil applied HA exhibited higher grain yield (6.2 g $plant^{-1}$**)** compared to soil+foliar applied HA (5.3 g $plant^{-1}$) while the remaining traits showed non-significant response to the method of HA application. Soil effect showed that Mollisols (Tolipir) exhibited significantly higher biological and dry matter yield while 1000-grain weight was significantly higher in Inceptisols (Rawalakot). Grain yield and harvest index exhibited non-significant response to soils.

Nutrient accumulation and uptake in plants: Application of HA showed promising effects on nutrient contents and nutrient uptake in different components of wheat (shoot and grains) (Tables 4 and 5). The relative increase in N contents due to HA application in shoot and grains ranged between 7–11% and 6–15%, P contents 32–63% and 19–31% and K contents 38–63% and 7–17%, respectively. The increase in total NPK uptake (shoot+grains) due to HA application varied between 21–57% for N, 44–96% for P and 32–62% for K.

Table 2. Effect of different levels of HA on shoot length, shoot dry weight, root length, root dry weight and chlorophyll content of wheat grown in two soils under greenhouse conditions at Rawalakot Azad Jammu & Kashmir.

Factors	Shoot length (cm)	Shoot dry weight (g plant^{-1})	Root length (cm)	Root dry weight (g plant^{-1})	Chlorophyll content (mg cm^{-2})
			Treatment effect		
HA_0 [a)]	67.73 b [b)]	3.60e	12.25 c	0.27 d	3.85 d
HA_{50}	68.72 b	5.31 b	14.59 b	0.46 b	6.04 c
HA_{100}	76.03 a	4.64 d	14.48 b	0.35 c	6.67 b
HA_{150}	75.28 a	5.01 c	15.89 a	0.51 a	7.58 a
HA_{200}	78.09 a	6.21 a	16.11 a	0.49 a	6.09 c
LSD (≤0.05)	2.54	0.13	0.72	0.03	0.10
			Method effect		
Soil applied	72.75	5.09 a	14.89	0.44 a	6.15 a
Soil + Foliar	71.99	4.82 b	14.44	0.39 b	5.95 b
LSD (≤0.05)	NS	0.08	NS	0.02	0.06
			Soil effect		
Tolipir	76.50 a	5.18 a	15.59 a	0.38 b	6.07
Rawalakot	68.24 b	4.72 b	13.74 b	0.46 a	6.03
LSD [c)] (≤0.05)	1.60	0.08	0.46	0.02	NS

[a)] HA_0 control (without HA); HA_{50} , 50 mg kg^{-1} soil,; HA_{100}, 100 mg kg^{-1} soil; HA_{150}, 150 mg kg^{-1} soil and HA_{200}, 200 mg kg^{-1} soil; [b)] Values followed by the same letter in a column are not significantly different at $P \cdot 0{:}05$; [c)] Least significant difference

Table 3. Effect of different levels of HA on 1000-grains weight, biological yield, dry matter yield, grain yield and harvest index of wheat grown in two soils under greenhouse conditions at Rawalakot Azad Jammu & Kashmir.

Factors	1000-grain weight (g)	Biological yield (g plant^{-1})	Dry matter yield (g plant^{-1})	Grain yield (g plant^{-1})	Harvest index (%)
			Treatment effect		
HA_0 [a)]	47 c [b)]	11.6 d	7.3 c	4.3 d	37 d
HA_{50}	51 b	13.7 c	8.4 b	5.1 c	38 c
HA_{100}	54 ab	14.1 b	8.4 b	5.8 b	40 b
HA_{150}	55 a	15.8 a	9.1 a	6.7 a	42 a
HA_{200}	53 ab	15.8 a	9.0 a	6.8 a	42 a
LSD (≤0.05)	3.23	0.45	0.31	0.31	1.46
			Method effect		
Soil applied	52	14.3	8.5	6.2 a	40.2
Soil + Foliar	52	14.1	8.3	5.3 b	40.7
LSD (≤0.05)	NS	NS	NS	0.20	NS
			Soil effect		
Tolipir	51 b	15.2 a	9.1 a	5.8	40.5
Rawalakot	54 a	13.2 b	7.8 b	5.7	40.4
LSD [c)] (≤0.05)	2.05	0.29	0.20	NS	NS

[a)] HA_0 control (without HA); HA_{50} , 50 mg kg^{-1} soil,; HA_{100}, 100 mg kg^{-1} soil; HA_{150}, 150 mg kg^{-1} soil and HA_{200}, 200 mg kg^{-1} soil; [b)] Values followed by the same letter in a column are not significantly different at $P \cdot 0{:}05$; [c)] Least significant difference

The wide variation in nutrient contents and nutrient uptake was associated with HA rates. Generally, concentration and uptake increased with increasing HA rates (Table 5). The increase in nutrient concentration and uptake in response to HA may be due to the fact that humic substances may stimulate microbiological activity (Mayhew, 2004), and enhances nutrients uptake (Daur, 2014). Delfine *et al*. (2005) documented that enhanced uptake of macronutrients (N, P, K) was due to the stimulatory effect of humic substances. Many researchers reported that soil or foliar application of HA significantly increased the macro (N, P, K, Ca, Mg) and micro nutrient (Fe, Cu, Zn Mn) contents of different crops i.e., in gerbera (Nikbakht *et al*., 2008; Haghighi *et al*., 2014); in maize (Celik *et al.,* 2011); in wheat (Taha *et al*., 2006); in cucumber (El-nemer *et al*., 2012).

The increased P content in different wheat parts with HA application may be due to the fact that HA increases P availability and uptake by decreasing calcium phosphate (Ca-P) precipitation rates (Inskeep & Silvertooth, 1988), competing for adsorption sites (Sibanda & Young, 1986), and decreasing the number of adsorption sites by promoting dissolution of metal solid phases by chelation (Guppy *et al*., 2005). Similarly, increase in wheat K content and K-uptake recorded in this study may be due to the reduced K fixation with the addition of HA. Tahir *et al.* (2011) reported that HA significantly ($p \leq 0.05$) improved wheat K contents of the non-calcareous soil and P and NO_3-N contents in calcareous soil.

Table 4. Effect of different levels of humic acid on diagnostic leaves and grains NPK content of wheat grown in two soils under greenhouse conditions at Rawalakot Azad Jammu & Kashmir.

Factors	Leaf N (%)	Leaf P (%)	Leaf K (%)	Grain N (%)	Grain P (%)	Grain K (%)
	Treatment effect					
HA_0 [a]	3.23 c [b]	0.19 e	1.43 d	2.28 d	0.32 b	0.58 c
HA_{50}	3.56 a	0.25 d	2.20 b	2.41 c	0.38 a	0.62 bc
HA_{100}	3.60 a	0.27 c	1.98 c	2.51 b	0.40 a	0.65 ab
HA_{150}	3.53 a	0.29 b	2.33 a	2.62 a	0.42 a	0.68 a
HA_{200}	3.44 b	0.31 a	2.31 a	2.62 a	0.42 a	0.67 a
LSD (≤0.05)	0.069	0.016	0.077	0.07	0.031	0.052
	Method effect					
Soil applied	3.50 a	0.26	2.12 a	2.50	0.39	0.64
Soil + Foliar	3.44 b	0.26	1.98 b	2.46	0.39	0.64
LSD (≤ 0.05)	0.044	NS	0.049	NS	NS	NS
	Soil effect					
Tolipir	3.55 a	0.27	2.14 a	2.58 a	0.39	0.63
Rawalakot	3.39 b	0.25	1.96 b	2.39 b	0.38	0.65
LSD [c] (≤ 0.05)	0.044	NS	0.049	0.04	NS	NS

[a] HA_0 control (without HA); HA_{50} , 50 mg kg^{-1} soil,; HA_{100}, 100 mg kg^{-1} soil; HA_{150}, 150 mg kg^{-1} soil and HA_{200}, 200 mg kg^{-1} soil; [b] Values followed by the same letter in a column are not significantly different at $P \cdot 0{:}05$; [c] Least significant difference

Table 5. Effect of different levels of humic acid on the total NPK uptake (shoot + root + grain) of wheat grown in two soils under greenhouse conditions at Rawalakot Azad Jammu & Kashmir.

Factors	Total N uptake (mg $plant^{-1}$)	Total P uptake (mg $plant^{-1}$)	Total K uptake (mg $plant^{-1}$)
	Treatment effect		
HA_0 [a]	214 d [b]	27 d	119 d
HA_{50}	260 c	39 c	158 c
HA_{100}	287 b	45 b	166 b
HA_{150}	334 a	53 a	189 a
HA_{200}	337 a	53 a	193 a
LSD (≤0.05)	11.49	3.14	7.49
	Method effect		
Soil applied	279 b	44	166
Soil + Foliar	294 a	43	164
LSD (≤0.05)	7.27	NS	NS
	Soil effect		
Tolipir	319 a	47 a	174 a
Rawalakot	254 b	40 b	156 b
LSD [c] (≤0.05)	7.27	1.98	4.74

[a] HA_0 control (without HA); HA_{50} , 50 mg kg^{-1} soil,; HA_{100}, 100 mg kg^{-1} soil; HA_{150}, 150 mg kg^{-1} soil and HA_{200}, 200 mg kg^{-1} soil; [b] Values followed by the same letter in a column are not significantly different at $P \cdot 0{:}05$; [c] Least significant difference

Among the three macronutrients studied (NPK), response of plant P to HA was substantially higher than the response shown for N and K. Averaged over N rates, the relative increase in plant N, P and K uptake was 42, 76% and 48%, respectively over the control treatment showing about 2-fold higher uptake of P due to HA compared to N and K.

Changes in soil properties/nutrient status: Soil analysis (after crop harvest) indicated that soil organic matter (OM), ECe and NPK content was significantly increased with HA application (Table 6). The relative increase in soil OM, N, P and K content after HA application was in the range of 9%, 30%, 166% and 51%, respectively. The increase in OM content of soil following HA application was in agreement with Sharif *et al*. (2002) who observed 7 to 14% increase in soil OM after HA application. Generally HA contains a substantial amount of OM i.e., 50–-90% and its application to soil is expected to increase soil OM as observed in this study.

Increase in soil NPK content due to HA application was also been reported earlier (Sharif *et al*., 2002; Tahir *et al*., 2011). Tenshia *et al*., (2005) stated that N content of soil treated with HA at the rate of 20 kg ha^{-1} increased by 28% and 29%. This increase in soil N content is probably due to the presence of 7% N in HA derived from lignitic coal (Hai & Mir, 1998; Sharif *et al*., 2002). Vaughan & Ord (1991) found that inhibition of urease activity by HA led to reduced N losses thereby increase N concentration in soil.

Table 6. Effect of different levels of humic acid on changes in soil nutrient status i.e. NPK content, organic matter (OM), ECe and pH after harvesting wheat.

Factors	N (g kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	OM (g kg^{-1})	ECe (dSm^{-1})	pH
	Treatment effect					
HA_0 [a)]	2.7 d [b)]	1.90 d	92 e	36.0 c	0.32 c	6.84
HA_{50}	3.0 c	3.01 c	107d	37.9 b	0.38 b	6.79
HA_{100}	3.3 b	4.46 b	123 c	37.6 b	0.36 b	6.81
HA_{150}	3.4 a	4.70 b	130 b	37.8 b	0.42 a	6.82
HA_{200}	3.5 a	5.05 a	140 a	39.1 a	0.43 a	6.84
LSD (≤0.05)	0.01	0.30	3.68	0.05	0.02	NS
	Method effect					
Soil applied	3.3 a	3.93 a	115 b	3.75 b	0.37 b	6.86 a
Soil + Foliar	3.1 b	3.73 b	121 a	3.78 a	0.39 a	6.78 b
LSD (≤0.05)	0.19	0.19	2.32	0.03	0.02	0.03
	Soil effect					
Tolipir	4.2 a	1.97 b	125 a	5.93 a	0.44 a	5.76 b
Rawalakot	2.1 b	5.68 a	111 b	1.60 b	0.31 b	7.88 a
LSD [c)] (≤0.05)	0.19	0.19	2.32	0.03	0.02	0.03

[a)] HA_0 control (without HA); HA_{50} , 50 mg kg^{-1} soil,; HA_{100}, 100 mg kg^{-1} soil; HA_{150}, 150 mg kg^{-1} soil and HA_{200}, 200 mg kg^{-1} soil; [b)] Values followed by the same letter in a column are not significantly different at *P* · 0:05; [c)] Least significant difference

The available P content of the soils increased significantly with HA application. Humic acid has the ability to reduce P fixation and solublize insoluble P, thereby increasing P concentration of soil (Sibanda & Young, 1986). Hua *et al.* (2008) reported that humic substances in soil can decrease P fixation and increase the P uptake of plants. Zhen-Yu *et al*. (2013) stated that addition of HA to monocalcium phosphate (MCP) resulted in the increased concentration of water–extractable P, acid-extractable P and Olsen P. Similarly, the increased soil available K observed in this study may be attributed to the reduced K fixation as well as release of fixed K by HA. According to Chenghua *et al.* (2005), humic acids stimulate fixation and release of K in soil by dissolving K-bearing minerals or blocking interlayers and adsorbing K.

The non-significant effect of HA on soil pH recorded in this study is in agreement with the findings of Tahir *et al*. (2011). This is due to the buffering effect of HA which resisted the change in soil pH. The buffering capacity and properties of HA was explained in detail by the study of Boguta & Sokotowska (2012). However, contrasting results are also reported by some researchers. Sharif *et al*. (2002) reported decrease in soil pH value by 0.2 to 0.3 units with HA addition. Similarly, Kutuk *et al*. (2000) reported a significant decline in pH of a soil supplemented with 200 mg kg^{-1} HA. In contrast to pH, the ECe was increased by 34% (at the highest HA rate) over the control. Similar increasing trend in soil ECe was reported by Kutuk *et al*. (2000). Methods of HA application showed significant effect on changes in soil properties. Soil+foliar application was found more effective in case of OM, ECe and available K while soil applied HA showed the highest total N and available P content. Similarly, soil effect indicated that the Tolipir soil showed the highest OM, ECe and soil N and K content with relative increase of 271%, 42%, 19% and 12%, respectively over the Rawalakot soil while P content and pH of Rawalakot soil was higher.

Conclusions

The present study demonstrates a significant effect of HA on improving agro-morphological characteristics, yield and yield attributes of wheat and stimulating the nutrient contents and nutrient uptake both in shoot and grains. Effect of HA depended on HA rates and in general response was higher at higher rates of HA application i.e. HA_{150} and HA_{200}. However, difference between .HA_{150} and HA_{200} was non-significant showing that application of HA beyond 150 mg kg^{-1} soil did not show any positive effect. Root development i.e. root length and root dry weight showed substantially higher response to HA application compared to shoot characteristics and significant correlation exists between root length/mass and NPK uptake showing that by improving plant root development, HA may affected nutrient uptake thereby increased yields and growth of wheat. Application of HA also improved the nutrient/fertility status of soil by increasing the organic matter content, total N, available P and K contents. Among different nutrients studied, response of soil P to HA was substantially higher than that recorded for OM, N and K. Similar response of plant to P-uptake was also observed by a substantial uptake of P compared to N and K. Phosphorus in soil is often present in unavailable form. Adding humic substances as chelating agents break the Fe, Al or Ca bonds between the organic matter and the phosphate, thereby releasing P into the soil solution (Turgay *et al*., 2011). The improvements in soil properties recorded in the present study are critical in the degraded and eroded soils normally exist in the State of Azad Jammu and Kashmir and other parts of Himalayans regions. The quality of these degraded and eroded soils may be improved by using HA continuously in our cropping systems. The experiment was conducted in pots under controlled conditions. In order to confirm these findings, long term studies are recommended under field conditions to examine the HA benefits for improving soil fertility and increasing crop productivity.

References

Anonymous. 2002. Statistical analysis software guide for personal computers. Release 9.1 SAS Institute Inc., Cary, NC, USA.

Atiyeh, R.M., N.Q. Arancon, C.A. Edwards and J.D. Metzger. 2002. The influence of earthworm-processed pig manure on the growth and productivity of marigolds. *Bioresour. Technol*., 81: 103-108.

Bastida, F., E. Kandeler, T. Hernandez and C. Garcia. 2008. Long-term effect of municipal solid waste amendment on microbial abundance and humus associated enzyme activities under semiarid conditions. *Microbial. Ecol*., 55: 651-661.

Boguta, P. and Z. Sokotowska. 2012. Influence of phosphate ions on buffer capacity of soil humic acids. *Int. Agrophys*., 26: 7-14.

Canellas, L.P., L.R.L. Teixeira-Junior, L.B. Dobbss, C.A. Silva, L.O. Medici, D.B. Zandonadi and A.R. Facanha. 2008. Humic acids cross interactions with root and organic acids. *Ann. Appl. Biol*., 153: 157-166.

Çelik, H., A.V. Katkat, B.B. Asik and M.A. Turan. 2011. Effect of foliar applied humic acid to dry weight and mineral nutrient uptake of maize under calcareous soil conditions. *Commun. Soil Sci. Plant Anal.,* 45: 29-38.

Chen, Y., C.E. Clapp and H. Magen. 2004. Mechanisms of plant growth stimulation by humic substances: The role of organic-iron complexes. *Soil Sci. Plant Nutr.,* 50: 1089-1095.

Chenghua, L., L. Lei and D. Liyu. 2005. Effect of humic acids on fixation and release of potassium in cultivated brown soil. *Acta Pedolog. Sinica,* 42: 472–477.

Daur, L. 2014. Effect of humic acid on growth, protein and mineral composition of pearl millet [*Pennisetum glaucum* (L.) r.br.] fodder. *Pak. J. Bot.,* 46: 505-509.

Daur, I. and A.A. Bakhashwain. 2013. Effect of humic acid on growth and quality of maize fodder production. *Pak. J. Bot*., 45: 21-25.

Delfine, S., R. Tognetti, E. Desiderio and A. Alvino. 2005. Effect of foliar application of nitrogen and humic acids on growth and yield of durum wheat. *Agron. Sustain. Develop*., 25: 183-191.

El-Nemr, M.A., M. El-Desuki, A.M. El-Bassiony and Z.F. Fawzy. 2012. Response of growth and yield of cucumber plants (*Cucumis sativus* L.) to different foliar applications of humic acid and bio-stimulators. *Aust. J. Basic Appl. Sci*., 6: 630-637.

Eyheraguibel, B., J. Silvestre and P. Morard. 2008. Effects of humic substances derived from organic waste enhancement on the growth and mineral nutrition of maize. *Bioresour. Technol*., 99: 4206-4212.

Farrell, R.E., F.L. Walley, A.P. Lukey and J.J. Germida. 1993. Manual and digital line-intercept methods for measuring root length: a comparison. *Agron. J*., 85: 1233-1237.

Guppy, C.N., N.W. Menzies, P.W. Moody and F.P.C. Blamey. 2005. Competitive sorption reactions between phosphorus and organic matter in soil: A review. *Aust. J. Soil Sci.,* 43: 189-202.

Haghighi, M., A. Nikbakht, Y.P. Xia and M. Pessarakli. 2014. Influence of humic acid in diluted nutrient solution on growth, nutrient efficiency, and postharvest attributes of gerbera. *Commun. Soil Sci. Plant Anal.*, 45: 177-188.

Hai, S.M. and S. Mir. 1998. The lignitic coal derived HA and the prospective utilization in Pakistan agriculture and industry. *Sci. Technol. Develop*., 17: 32-40.

Hua, Q.X., J.Y. Li, J.M. Zhou, H.Y. Wang, C.W. Du and X.Q. Chen. 2008. Enhancement of phosphorus solubility by humic substances in ferrosols. *Pedosphere*, 18: 53-538.

Inskeep, W.P. and J.C. Silvertooth. 1988. Inhibition of hydroxyapatite precipitation in the presence of fulvic, humic, and tannic acids. *Soil Sci. Soc. Am. J*., 52: 941-946.

Jones, C.A., J.S. Jacobsen and A. Mugaas. 2007. Effect of low-rate commercial humic acid on phosphorus availability, micronutrient uptake, and spring wheat yield. *Commun. Soil Sci. Plant Anal*., 38: 921-933.

Karaca, A., O.C. Turgay and N. Tamer. 2006. Effects of a humic deposit (gyttja) on soil chemical and microbiological properties and heavy metal availability. *Biol. Fertil. Soils,* 42: 585-592.

Keeney, D.R. and D.W. Nelson. 1982. Nitrogen-inorganic forms. In: *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*. Page, A.L., R.H. Miller and D.R. Keeney.American Society of Agronomy Madison, WI, pp. 643-693.

Kirn, A., S.R. Kashif and M. Yaseen. 2010. Using indigenous humic acid from lignite to increase growth and yield of okra (*Abelmoschus esculentus* L.). *Soil Environ*., 29: 187-191.

Kutuk, C., G. Caycy, A. Baran and O. Baskan. 2000. Effect of humic acid on some soil properties. In: International Symposium on Desertification. Soil Sci. Soc. Turkey, Konya, Turkey.

Lichtenthaler, H.K. and A.R. Wellburn. 1985. Determination of total carotinoids and chlorophyll A and B of leaf in different solvents. *Biochem. Soc. Trans.,* 11: 59-592.

Mayhew, L. 2004. Humic substances in biological agriculture [Online]. Available at www.acresusa.com/toolbox/reprints/Jan04_Humic%20Substances.pdf (2004).

Mikkelsen, R.L. 2005. Humic materials for agriculture. *Better Crops*., 89: 6–10.

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

Nikbakht, A., M. Kafi, M. Babalar, Y.P. Xia, A. Luo and N. Etemadi. 2008. Effect of humic acid on plant growth, nutrient uptake, and postharvest life of gerbera. *J. Plant. Nutr*., 31: 2155-2167.

Pinton, R., Z. Varanini and P. Nannipieri. 2007. The rhizosphere: Biochemistry and organic substances at the soil–plant. 2nd ed. CRC Press Madison, WI. pp. 447.

Ryan, J., G. Estefan and A. Rashid. 2001. Soil and plant analysis laboratory manual. International Center for Agricultural Research in the Dry Areas (ICARDA). Aleppo Syria.

Saruhan, V., A. Kusvuran and K. Kokten. 2011. The effect of different replications of humic acid fertilization on yield performances of common vetch (*Vicia sativa* L.). *Afr. J. Biotechnol*., 10: 5587-5592.

Selim, E.M. and A.A. Mosa. 2012. Fertigation of humic substances improves yield and quality of broccoli and nutrient retention in a sandy soil. *J. Plant Nutr. Soil Sci*., 175: 273-281.

Shahryari, I., M. Khayatnezhad and N. Bahari. 2011. Effect of two humic fertilizers on germination and seedling growth of maize genotypes. *Adv. Environ. Biol*., 5: 114-117.

Sharif, M., R.A. Khattak and M.S. Sarir. 2002. Effect of different levels of lignitic coal derived humic acid on growth of maize plants. *Commun. Soil Sci. Plant Anal*., 33: 3567-3580.

Sibanda, H.M. and S.D. Young. 1986. Competitive adsorption of humus acids and phosphate on goethite, gibbsite, and two tropical soils. *J. Soil Sci*., 37: 197-204.

Taha, A.A., A.S. Modaihsh and M.O. Mahjoub. 2006. Effect of some humic acids on wheat plant grown in different soils. *J. Agric. Sci.,* 31: 4031-4039.

Tahir. M.M., M. Khurshid. M.Z. Khan, M.K. Abbasi and M.H. Kazmi. 2011. Lignite-derived humic acid effect on growth of wheat plants in different soils. *Pedosphere*, 21: 124-131.

Tenshia, J.S.V. and P. Singaram. 2005. Influence of humic acid on yield, nutrient availability and uptake by tomato. *Madras Agric. J.,* 10: 670-676.

Turgay, O.C., A. Karaca, S. Unver and N. Tamer. 2011. Effects of coal- derived humic substance on some soil properties and bread wheat yield. *Commun. Soil Sci. Plant Anal*., 42: 1050-1070.

Ulukan, H. 2008. Effect of soil applied humic acid at different sowing times on some yield components in wheat (*Triticum* spp.) hybrids. *Int. J. Bot*., 4: 164-175.

Vaughan, D. and B.G. Ord. 1991. Influence of natural and synthetic humic substances on the activity of urease. *J. Soil Sci.,* 42: 17-23.

Zhen-Yu, D., W. Qing-Hua, L. Fang-Chun, M. Hai-Lin, M. Bing-Yao and S.S. Malhi. 2013. Movement of phosphorus in a calcareous soil as affected by humic acid. *Pedosphere*, 23: 229-235.

(Received for publication 29 October 2014)