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Assessing foliar application of chitosan andhumic acid on soil-solution partitioning of *Pisum sativum*

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Key words: Soil Solution, Humic Acid, Metals and Chitosan

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Abstract

An experiment was carried out at Nursery of University of Agriculture Peshawar during 2016-201 for the assessment of foliar application of Chitosan and Humic acid on Soil solution partitioning , so as to see the indirect effects that they put forth on the chemical and biological properties of the soil. Trial was planned in CRD design with three replications. Various combinations of Humic acid (0, 1, 2, 3g.L^{-1}) and Chitosan (0 , 40, 60, 80 mg.L $^{-1}$). *Meteor* verity was under consideration during trial. Combinations of Humic acid (0, 1, 2, 3g.L^{-1}) and Chitosan (0, 40, 60, 80 mg. L $^{-1}$)were under consideration . Three foliar application were done first at vegetative stage (2nd week) second at flowering stage (4th week) and third at pods formation (8th week). . Decrease in Soil pH (6 @ 3g.L^{-1} HA 40mg.L^{-1} CHT), increase in SMN(57 ml.L^{-1} @ 2g L^{-1} HA 40mg.L^{-1} HA) and LOI(4.067%) as effected in combination((@CHT 40mg.L^{-1} and Humic acid @1g.L^{-1})in post-harvest soil was observed. Soil Solution weekly results for pH showed pH of 5.4 (@3g.L^{-1} HA and 0mg.L^{-1} CHA in week 5) ,mg concentration of 2.57 ml.L^{-1} (@ 3g.L^{-1} HA in week 9), Cu concentration was 0.08 ml.L^{-1}(@ 80mg.L^{-1}CHT in week 12). NO_3 result showed concentration of 0.52 ml L^{-1}(@ 3g L^{-1} HA and 0.15 ml.L^{-1}).C2H3 contributed towards decrease in soil pH in post-Harvest Soil significantly at $P \leq 0.05$ however soil solution pH was influenced again by C0H3 mostly after second foliar application ,soil-solution pH was influenced more at HA@3 gL^{-1},SMN as available NO_3 in soil was significantly affected by C3H2 and NO_3 in solution phase mineralized more with C0H2,overall HA @2g.L^{-1}released available NO_3 in soil solution Partitioning. The effects which HA and CHT put forth on plant growth can be grouped into indirect effects on chemical as well as biological properties of the soil, thus HA and CHT important effect on plant development may be credited to the promoting effects on uptake of some important nutritional status of soil in particularly N, P and K which are needed for the growth of plant .

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Introduction

Despite intensive research on foliar methods and its effect on Plant yield, Quantitative prediction of Nitrates, pH and metals fluxes at the field scale is still very difficult. The research described in this article identify the ultimate relationships between changes in temperature, changes in foliar interaction types, changes in pore water pH, Nitrate concentrations and metal concentrations. We feel this paper adds some significant additional evidence and the result may help to focus the attention to the key processes which must be quantified in soil pore water in realistic conditions with the given priority to adopt foliar methods than soil application. therefore the current research was undertaken to study effect of both Chitosan and Humic acid individually and their combinations on yield attributes (which is not shown in this article) and to see how above soil chemistry could have effected below soil physiological and chemical functions in terms of its sole pore water changes based on weekly basis and changes brought in pre harvest soil and post-harvest soil.

Chitosan is a natural, less toxic and cheap compound that is biodegradable and environmental friendly with different kind of applications in agriculture sector; it is produced by de acetylation of chitosan. Soil solution partitioning exerts a major control on pH,nitrates and on the transport and retention of metals in soil– water systems. The best of knowledge there has also been no previous report on regarding the effect of chitosan and humic acid combination on Pea Plant generally and the effect of foliar applications has not been related to below soil chemistry specifically. to further contribute to understanding of the factors influencing Foliar applications and its secondary benefits on below soil changes with the view that when nutrients are provided to foliage it causes the plants to exude more sugars and other compounds into the root zone which can increases microbial activity around the root zone, thus in turn enhances the uptake of nutrients by the plant from the soil. This important activity has been barely recognised in any type of agriculture but our research has demonstrated that this is a major benefit of foliar spraying.

HA and CHT has been reported to increase in plant augmentation and improve the yield it is also noticed that it helps in improving physiological processes in plant system (farouk *et al* 2011).

Humic substances has been reported to increase plant growth , it has been reported to increase shoot dry weight helps in the root growth, increase height of plants and uptake of macronutrient in oat plants (Rosa *et al.*, 2004) ;improves growth in wild olive and Nitrogen as well as Chlorophyll in wild olives.(Murillo *et al.*, 2005).

Specific Objective of the experiment was to characterize the soil solution partitioning of metals, pH and mineral N from a macrocosm experiment and to identify relationship between soil & soil-solution metal concentrations ,pH and Nitrates via installation of rihzone samplers in the pots and extraction of soil solution for analysis.

Material and methods

The experiment was conducted during the winter season from 2016-2017 in Peshawar with a view to find out the effect of foliar application Chitosan & Humic acid on yield attributes of pea and to analyze the change in soil solution during the process.

Experimental design

The experiment was conducted in completely randomized design (CRD) two factor full factorial with fifteen levels along with one control & three replications.

Factor A: Humic Acid Altitudes

HA_0 = 0g.L^{-1}, HA_1=1g.L^{-1}, HA_2=2 g.L^{-1}, HA_3=3 g.L^{-1}

Factor B : Chitosan Altitudes

CHT_0 = 0mg L^{-1}, CHT_1 =40mg L^{-1}

CHT_2 =60mg L^{-1}, CHT_3=80mg L^{-1}

The following are the treatment combinations which were employed during the course of experiment.

T_1=CHT_0 + HA_0, T_2=CHT_0 + HA_1, T_3=CHT_0 + HA_2, T_4=CHT_0 + HA_3, T_5=CHT_1+ HA_0, T_6=CHT_1+ HA_1, T_7=CHT_1+HA_2, T_8=CHT_1 +HA_3, T_9=CHT_2+HA_0, T_{10}=CHT_2+HA_1, T_{11}=CHT_2+ HA_2, T_{12}=CHT_2+ HA_3, T_{13}=CHT_3+ HA_0, T_{14}=CHT_3+ HA_1, T_{15}=CHT_3 + HA_2, T_{16} =CHT_3 + HA_3

Plastic pots of about one liter volume with the width of 4 cm and height of 10 cm were used. In the base of the pots three to four equivalent holes were made to keep the soil moist. Soil mixture was taken and mixed properly in the farm yard manure the pots were then filled with proper amount of soil. Then the pots were placed in such a way that the pots could not slide from its place. Cores of soil were placed in the plastic pots drainage holes were made at 2cm from the base to keep the soil moist ,soil pore water were sampled by installing the Rhizon samplers (Eijkelkamp, Agrisearch Equipment, Netherlands) 5cm from the bottom of each core. The Rhizon samplers were connected to a syringe, which was pulled out to create a vacuum. Sampling was done on weekly basis for soil pore water. Sampling was continued for a period of 12 weeks. Foliar application was done once in the vegetative stage (second week) and the second foliar application was done when the flowering appeared followed by third one soon after pods emergence at eighth week. pH meter was used to analyze soil pore water for its pH the pH on weekly basis.

Table 1. Shows the concentration of Chitosan (CHT) and Humic acid (HA) and its combinations (CxH) and its acid being used as foliar spray.

Level	CHITOSAN	HA	Application/plant
C0H0	-	-	0ml
C0HI	-	1g.L^{-1}	10ml
C0H2	-	2g.L^{-1}	10ml
C0H3	-	3g.L^{-1}	10ml
C1H0	40mg.L^{-1}	-	10ml
C1H1	40mg.L^{-1}	1g.L^{-1}	10ml
C1H2	40mg.L^{-1}	2g.L^{-1}	10ml
C1H3	40mg.L^{-1}	3g.L^{-1}	10ml
C2H0	60mg.L^{-1}	-	10ml
C2H1	60mg.L^{-1}	1g.L^{-1}	10ml
C2H2	60mg.L^{-1}	2g.L^{-1}	10ml
C2H3	60mg.L^{-1}	3g.L^{-1}	10ml
C3H0	80mg.L^{-1}	-	10ml
C3H1	80mg.L^{-1}	1g.L^{-1}	10ml
C3H2	80mg.L^{-1}	2g.L^{-1}	10ml
C3H3	80mg.L^{-1}	3g.L^{-1}	10ml

Soil pore solution were taken through rhizome sampled connected to 5mm syringes which was collected in small plastic bottles properly labeled according to the treatment.

The day of harvest were recorded in the note book, morphological characteristics of the pea plants such as number of flowers/plant, number of pods per plant, and plant height were assessed. Furthermore, the total above-ground biomass of each plant were determined by totaling the dry weight of all the harvested plant components separately.

Each component was placed in to paper or plastic beg according to the need (i.e. one for pod and one for remaining above-ground biomass per plant), dried to constant weight (ideally at approx. 80°C for approx. 48 hours in an electric oven) and will be cooled in desiccators. Pods were harvested at one stage of maturity.

The seeds were air-dried and then oven- dried at 65 oC for seventy-two hours.

Table 2. Soil pH as affected by Chitosan and Humic acid foliar application and their interaction (CxH) on *Pisum sativum.*

HA		Chitosan (mg L-1)				H x BandAF
		0	40	60	80	
Before	0	7.8167	7.7800	7.8400	7.7900	7.8067
Before	1	7.7700	7.8767	7.8367	7.8133	7.8242
Before	2	7.8833	7.9667	8.0267	7.5633	7.8600
Before	3	7.9300	7.9833	7.7233	7.6033	7.8100
After	0	7.7600	7.8267	6.6933	7.8833	7.5408
After	1	7.8467	6.3510	7.9457	7.5373	7.4202
After	2	7.7033	7.1713	6.7867	6.3867	7.0120
After	3	6.1123	6.0087	6.2867	6.1047	6.1281
Before		7.8500	7.9017	7.8567	7.6925	7.8252 a
After		7.3556	6.8394	6.9281	6.9780	7.0253 b
Humic acid (g L-1)						
0		7.7883	7.8033	7.2667	7.8367	7.6738 a
1		7.8083	7.1138	7.8912	7.6753	7.6222 a
2		7.7933	7.5690	7.4067	6.9750	7.4360 a
3		7.0212	6.9960d	7.0050	6.8540	6.9690 b
CxH		7.603	7.371	7.392	7.335	
	Humic acid LSD (p≤0.01)	0.291048				
	Band AF LSD (p≤0.01)	0.205802				

Soil analysis

After twelve weeks each core was divided and cut into three 5cm portions (the lower, middle and upper soil layers) to get soil samples. Soil were homogenized by hand, well mixed to avoid internal variation between sub samples, and roots and shoots were removed before analysis, as described below and kept in the refrigerator before further analysis. Approximately 10g duplicate sub-samples of each field moist soil were weighed into pre-weighed dried aluminum dishes, which were then oven dried over night at 105^{o}C, after wards cooled in desiccators, and reweighed to determine the moisture content from the loss of mass, expressed on an oven-dry weight basis. The organic content of the soils sampled were determined as percentage loss on ignition (LOI %) after burning the soils for 5 hours at 550 ^{o}C.

Table 3. Mean values of soil mineral nitrogen as affected by Chitosan and Humic acid foliar application and their interaction (CxH) on Pisum sativum.

	HA	Chitosan (mg L-1)				H x BandAF
		0	40	60	80	
Before	**0**	49.2500	43.9333	46.9767	42.9233	45.7708
Before	**1**	48.6033	39.2833	40.7833	44.9833	43.4133
Before	**2**	44.8250	47.3167	39.3333	36.3500	41.9563
Before	**3**	46.9717	34.0167	48.3817	46.5733	43.9858
After	**0**	46.0400	31.7767	28.4700	30.0900	34.0942
After	**1**	34.8600	30.3700	30.4107	33.3067	32.2368
After	**2**	46.3850	57.5100	46.4667	54.6733	51.2588
After	**3**	55.3867	55.0133	55.1197	56.2000	55.4299
Before		47.4125	41.1375	43.8688	42.7075	43.7816
After		45.6679	43.6675	40.1168	43.5675	43.2549
Humic acid (g L-1)						
0		47.6450	37.8550	37.7233	36.5067	**39.933bc**
1		41.7317	34.8267	35.5970	39.1450	**37.825c**
2		45.6050	52.4133	42.9000	45.5117	**46.608ab**
3		51.1792	44.5150	51.7507	51.3867	**49.708a**
		46.540	42.403	41.993	43.138	
Chitosan LSD (p≤0.01)		5.505193				
Humic acid LSD (p≤0.01)		5.505193				
Band AF LSD (p≤0.01)		3.89276				
CxH LSD (p≤0.01)		11.01039				
CxBandAF (p≤0.01)		7.785519				
HxBandAF (p≤0.01)		7.785519				
CxHxBandAF (p≤0.01)		15.57104				

The pH of soil was measured in both pore water and extracted soil samples. The soil pH were determined after mixing fresh soil with deionized water (1:2 w:w) and shaking for 1h at 100 rpm. The pH meter (MP220 Basic/mV/°C Meter, Mettler Toledo International Inc.) was pre-calibrated with commercial standard buffer solutions at pH 4.0, 7.0 and 10.1. The pH 7 buffer was used after every 8-10 samples to confirm instrumental stability.

Table 4. Mean values of LOI of soil as affected by Chitosan and Humicacidfoliar application and their interaction (CxH) on Pisumsativum.

	HA	Chitosan (mg L-1)				H x BandAF
		0	40	60	80	
Before	0	3.4300	3.3733	3.5833	3.7510	3.5344
Before	1	2.7667	4.0200	3.9500	3.4567	3.5483
Before	2	3.6947	3.5500	3.5133	3.3133	3.5178
Before	3	3.7800	3.6267	3.5867	3.4300	3.6058
After	0	4.0607	3.7700	3.5567	3.8367	3.8060
After	1	3.4767	3.4567	3.6533	3.9417	3.6321
After	2	3.0067	3.2833	3.4733	3.6533	3.3542
After	3	2.8233	3.2400	3.6367	3.9133	3.4033
Before		3.4178	3.6425	3.6583	3.4878	3.5516
After		3.3418	3.4375	3.5800	3.8363	3.5489
Humic acid (g L-1)						
0		3.7453	3.5717	3.5700	3.7938	3.6702
1		3.1217	3.7383	3.8017	3.6992	3.5902
2		3.3507	3.4167	3.4933	3.4833	3.4360
3		3.3017	3.4333	3.6117	3.6717	3.5046
		3.380	3.540	3.619	3.662	
Chitosan LSD (p≤0.01)		6.892889				
Humic acid LSD (p≤0.01)		6.892889				
Band AF LSD (p≤0.01)		4.874008				
CxH LSD (p≤0.01)		13.78578				
CxBandAF (p≤0.01)		9.748017				
HxBandAF (p≤0.01)		9.748017				
CxHxBandAF (p≤0.01)		19.49603				

The moisture content was determined by oven drying method at 105°c till a constant weight is obtained. Proteins in each sample was determined by micro kjeldhal apparatus. The samples were digested in kjeldhal flask by heating with concentrated sulphuric acid in the presence of digestion mixture (K_2SO_4 +$CuSO_4$) for two hours.

The digest was treated with NAOH and NH_3 so released was collected in 45 boric acid solution and will be titrated against standard 005N HCL Total proteins was calculated by multiplying the amount of nitrogen with appropriate factor. The percent protein will be determined as:

% crude proteins = % nitrogen × 6.25

Analysis of pea plant was performed on flame photometer and atomic absorption spectrophotometer methods (A.O.A.C. 2000). Sodium (Na) and Potassium (K) was analyzed on (Copper, Manganese).

Atomic absorption spectrophotometer was used for micro minerals determination i.e, Copper (cu) and Manganese (Mn) however nitrates concentration was determined using spectrophotometer.

Statistical analysis

Since the experiment follows a full factorial Completely Randomized design with 3 replicates, CRD factorial statistics was used to analyze the results for significant differences. Least significant differences (LSD) test was used for means in case of comparison between pre and post soil harvest, soil pH, soil mineral Nitrogen, LOI and soil moisture Bivariate correlation analysis were used to determine relationships between variables i.e, soil solution pH, soil solution Nitrates, soil solution Cu and Mn, based both on the mean data determined in each week, and the values for all the individual cores on all measurement dates. An analysis of variance were carried out to assess the effects of Foliar application on soil concentrations at the end of the experiment. All analyses were carried out in SPSS 20.

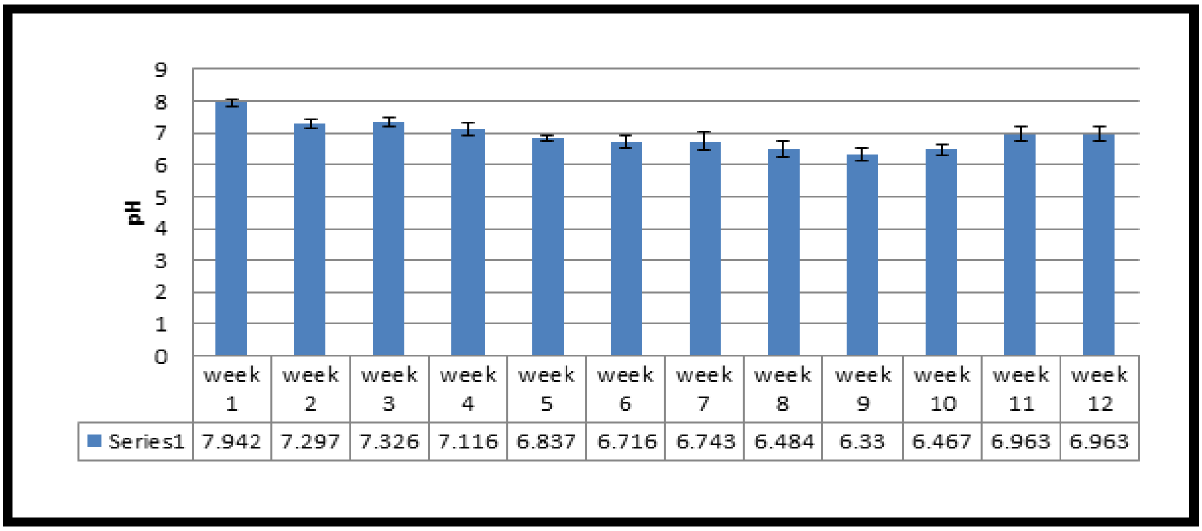

Fig. 1. Average mean values pH in soil pore water recorded over time (12 weeks).

pH variation during experiment

Figure.1. shows the changes in pH over the experiment from November 2016 to February 2017 (12 weeks), pH was recorded on a weekly biases for every treatment from the figure it can be seen that in first week all the pH values starts from almost the same point i.e7.6-8.3 Figure.2 shows that highest pH was recorded in first week with the average mean of 7.94 pH drop was seen in a steady manner during the course of experiment, lowest pH was recorded in week 5 with the average mean pH of 6.26. Figure2 shows average mean pH of soil pore water as affected by application of Chitosan and Humic acid as well as interactive effect of Chitosan and Humic acid (CxH), lowest pH(5.91) was recorded in Control Chitosan with Humic acid application at 3g L^{-1} .Highest pH was recorded (7.53) with control (0g.L^{-1}) Humic acid application and Chitosan at 40mg.L^{-1}.

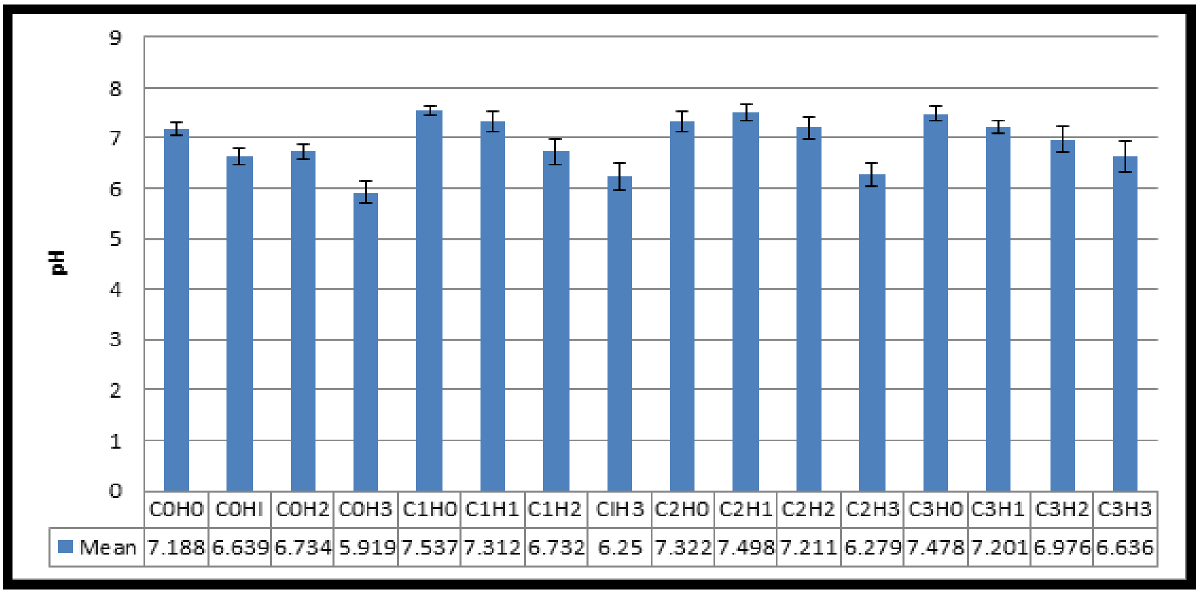

Fig. 2. Treatment (Humic acid, 1gL^{-1} 2gL^{-1}3gL^{-1})(Chitosan, 40mgL^{-1}60mgL^{-1}80mgL^{-1})and their interaction(CxH) on changes in the mean value of pH over time(12 weeks).

Variation in Metal Concentrations overtime

Concentration of Manganese

Concentration of manganese was recorded in the soil pore water over twelve weeks in Figure.3 shows that the lowest concentration was recorded in the control pots throughout however in the fifth weak in control plot, highest concentration of 1.01 µ g.ml^{-1} was recorded in the ninth week with highest application rate of Humic acid.

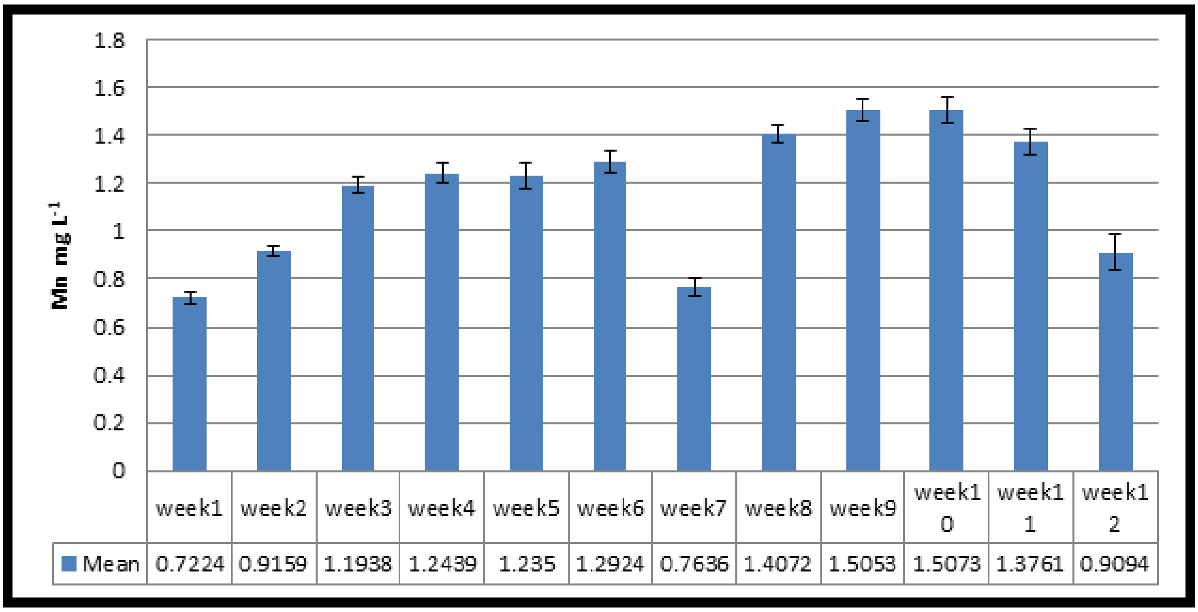

Fig. 3. Average mean value Manganese concentration in soil pore water recorded over time (12 weeks).

Concentration of Copper

Figure 4 shows that concentration of copper was lowest in the first week with the average mean of 0.022 mg.L^{-1} which increased in the 2nd week when foliar application was done the average mean value was 0.03 at that time this trend remained steady for five weeks however in the eighth week the copper concentration was seem to increase with the average mean value of 0.044 mg.L^{-1} soon after third the trend was steady with only a little change in the concentration however at the 12th week of experiment with mean value of 0.050 mg.L^{-1}.

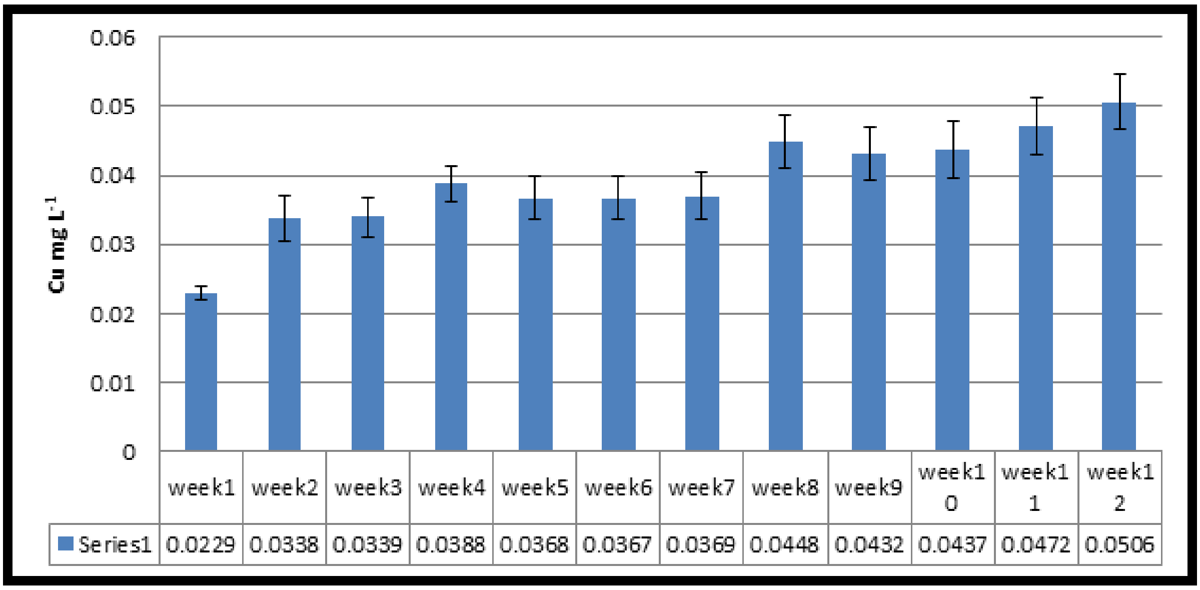

Fig. 4. Average mean values of Copper concentration in soil pore water recorded over time (12 weeks).

Effect of treatment on nitrates

Figure 5 shows foliar application was done thrice during the experiment ; In first application highest concentration was observed in control group. Figure 7 indicates when Chitosan with Humic acid applied at 3g.L^{-1}while lowest concentration was observed in control , in second application highest concentration was seen in treatment where Chitosan was applied at 60mg.L^{-1} and Humic acid at 3 g.L^{-1}while lowest was observed in control group , in final application highest concentration was reported in treatment where Chitosan was applied at 60mg.L^{-1} and Humic acid at 3g.L^{-1}while lowest in the treatment where Chitosan was applied at 80mg.L^{-1} and Humic acid at 1g.L^{-1}.Figure 6 shows concentration of Nitrates in the soil pore water in first second and third week of application; which shows that the level of nitrogen was lowest in the second week of experiment when first foliar application was conducted the concentration of nitrates increased in the second foliar application in soil pore water and almost doubled in the final application of Chitosan and Humic Acid. Figure 5c shows average mean value of nitrates on weekly basis. From the graph it is clear that on the first foliar application (week 2) nitrates became high with average mean value of 0.368mg.L^{-1} while which remained steady until 6th and 7th week when the nitrate concentration dropped (0.274 and 0.215 mg.L^{-1}least of all weeks),however in the fifth week the concentration increased (0.438 mg.L^{-1})at the week of 2nd foliar application , this value increased in the ninth week (0.375 mg.L^{-1}) which then decreased towards the end of experiment. Figure 7 shows concentration of Nitrates in soil pore water during the course of experiment as effected by different level of treatment, highest concentration of nitrates was recorded when Chitosan and Humic acid were applied in the ratio 80 g.L^{-1} and 3 g.L^{-1} in week 8, 0.21 mg.L^{-1} of nitrates was recorded when Chitosan was applied at the rate of 60 mg.L^{-1} separately in week 8.

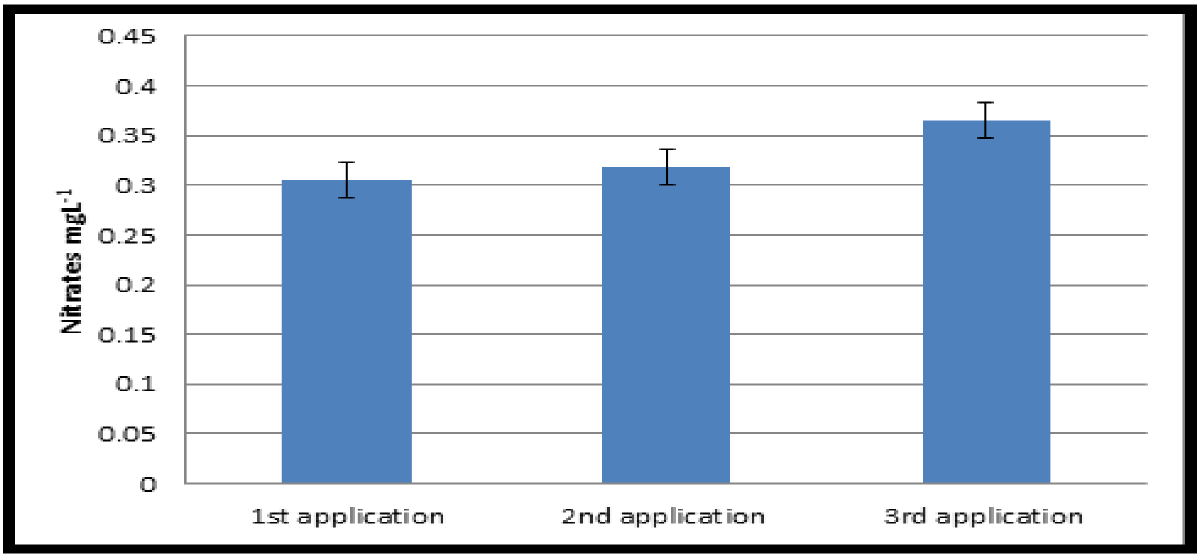

Fig. 5. Treatment (Humic acid, 1gL^{-1} 2gL^{-1}3gL^{-1}) (Chitosan, 40mgL^{-1}60mgL^{-1}80mgL^{-1})and their interaction (CxH) on changes in the mean value of the concentration Nitrates in soil pore water after 1st 2nd and 3rd application during the course of experiment (12 weeks).

Correlation between components of soil chemistry

The correlation coefficients and its significance, among the different soil solution parameters, including metals copper and manganese calculated form the mean weekly values for treatment. The analysis identify relationships among the changes throughout in the values of different parameters as shown in Figure 8 The negative correlation between pH and copper with a r^2 value of 0.53 indicates that with a drop in pH rise in the amount of copper can be expected, when the pH was correlated with manganese the correlation came out to be negative with the r^2 value of 0.335 which shows the same trend between pH and manganese as that of pH and copper. Similarly when pH was correlated with nitrate mean value from weekly data the result showed a negative correlation with the r^2value of 0.15.When the average mean weekly value of manganese was correlated with pH a negative correlation was recorded with r^2= 0.335 which shows that pH of the soil pore water was influenced by the concentration of manganese.

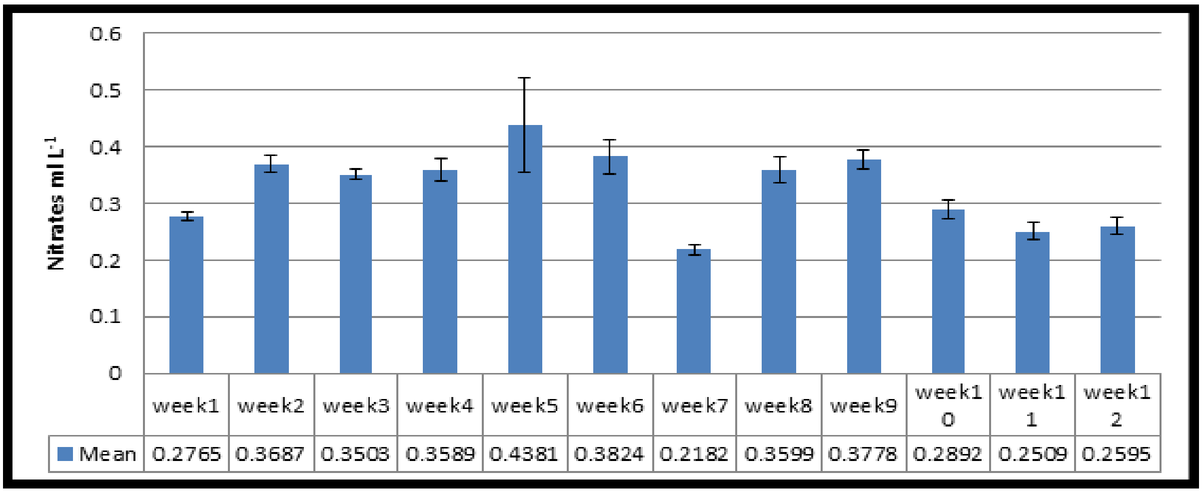

Fig. 6. Average mean values Nitrates concentration in soil pore water recorded over time(12 weeks).

Effect of treatments pre &post soil chemistry

Soil pH before and after harvesting

The recorded data shows the fluctuation in soil pH with the entire course of time for research trail. Anova table 2 shows that soil pH was not significantly affected by foliar application with chitosan. Humic acid significantly affected soil pH at p≤0.01pH was significantly different before sowing and after harvesting.

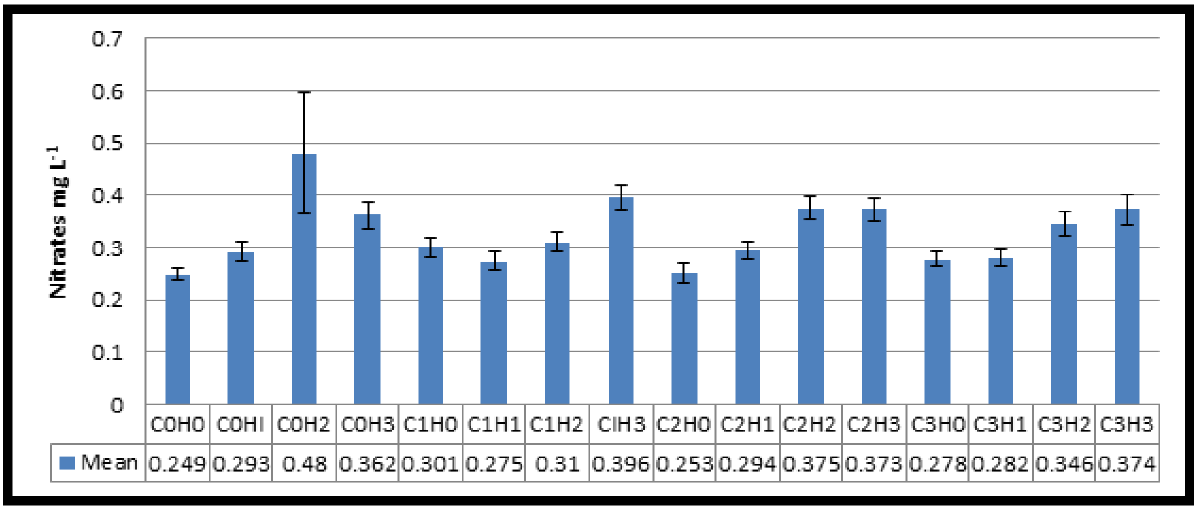

Fig. 7. Treatment (Humic acid, $1gL^{-1}$ $2gL^{-1}3gL^{-1}$) (Chitosan, $40mgL^{-1}60mgL^{-1}80mgL^{-1}$) and their interaction (CxH) on changes in the mean value of the concentration Nitrates in soil pore water over time(12 weeks).

Humic acid in combination with Chitosan (CxH) foliar application also significantly affected the soil pH. Anova table also shows that soil pH was also significantly different when analyzed before sowing and after harvesting the plants (CxHxB and AF) of pea. In table 2 ANOVA for the mean data of pH of soil shows that highest soil pH (8.0267) was recorded in soil of treatment with humic acid control while lowest soilpH (6.9690) was recorded in soil of plants that were treated with humic acid at 3 g L^{-1}.

Highest soil pH (7.8252) that was statistically different from that after harvesting (7.0253) of pea plants (table 5). A trend of lowering pH was noted for the chitosan and humic acid interaction (CxH) as shown in Figure 9.Figure shows that chitosan and humic acid in combination lowered the pH as the chitosan and humic acid increases. A sudden increase was seen when chitosan application .Figure 7a shows that pH was higher before sowing that was gradually reduced after harvesting. pH was lowest with chitosan and humic acid(60 mg L^{-1} and 2 g L^{-1}). Chitosan and humic acid lowered soil pH after harvesting that was higher before sowing (CxHxB and AF) as shown in Figure 9.Accoding to table 2 the pH was higher before Sowing that was gradually reduced after harvesting. pH was lowest with chitosan and humic acid 60 mg L^{-1} and 2 g L^{-1}).

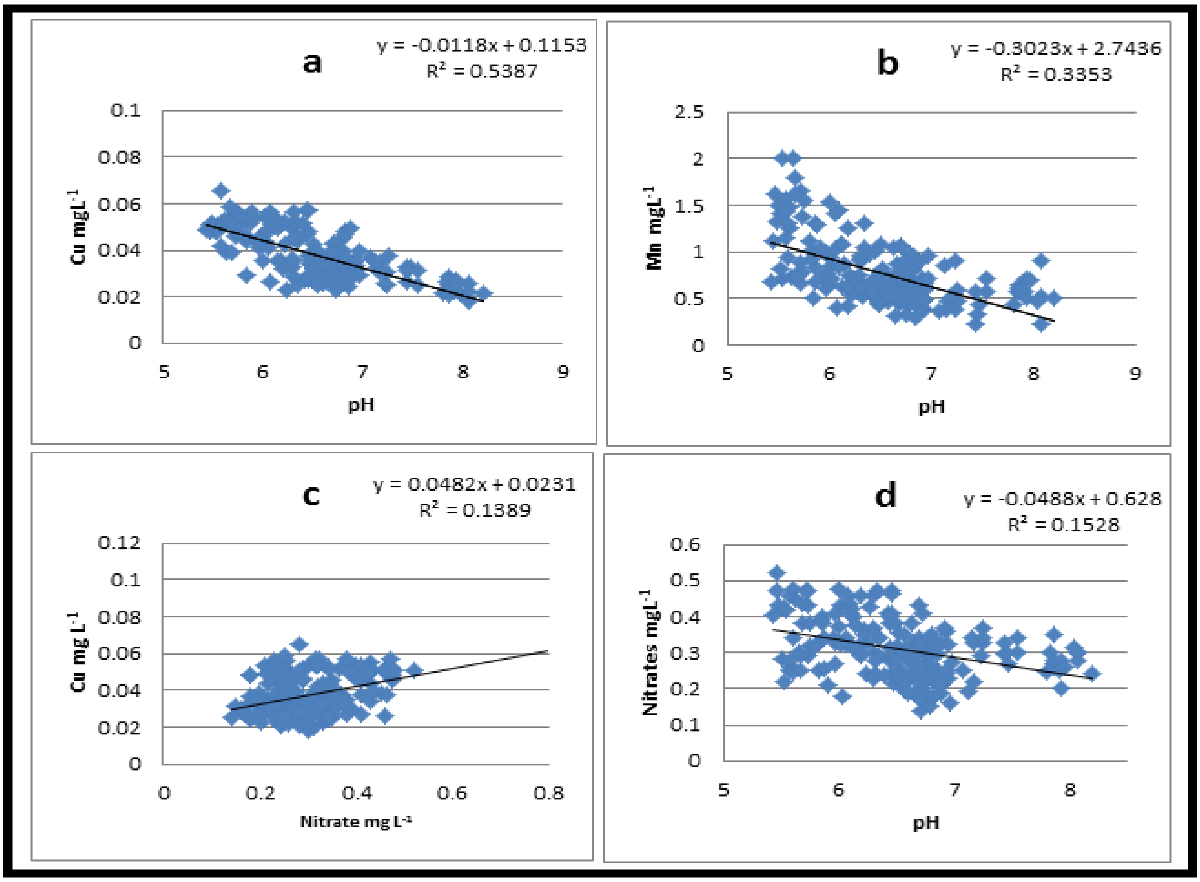

Fig. 8. Relationship(r^2) between pH and copper(a) pH and Manganese (b) Nitrate and Cu (c) pH and Nitrates (d)based on weekly mean basis.

Pre & post-harvest status of soil mineral nitrogen

Anova Table 3 shows that soil mineral nitrogen was not affected by application of Chitosan however it was significantly affected by Humic acid application with p≤ 0.01. Soil mineral nitrogen was not affected by Chitosan and Humic acid in combination and proved nonsignificant, in term of before and after harvest soil Humic acid was proved significant at p≤0.01. when Chitosan and Humic acid were applied together it was recorded that the after harvest soil was affected non significantly.

Soil mineral nitrogen was found highest (46.708 mg kg^{-1}) with foliar application of humic acid at at 3g L^{-1} that was not statistically different from (46.608mg kg^{-1}.) that of humic acid @ 2g L^{-1}.

Pre & post-harvest status of soil loss on ignition

According to appendix 4 ANOVA after harvest soil ash content was nonsignificantly affected by Chitosan as well as Humic acid when applied separately. LOI was significantly affected by combination of Chitosan and Humic acid (CxH) after harvest soil at p≤0.05.

Interactive effect of Chitosan and Humic acid (CxH) was found to be significantly effective in altering the LOI of soil in after harvest soil as compared to pre sowing results. Figure 10 shows that before sowing the soil LOI was less in control group which was significantly increased after harvesting. Highest LOI (4.067%) was recorded for foliar application C:H @ 40mg L^{-1}, 1g L^{-1} respectively.

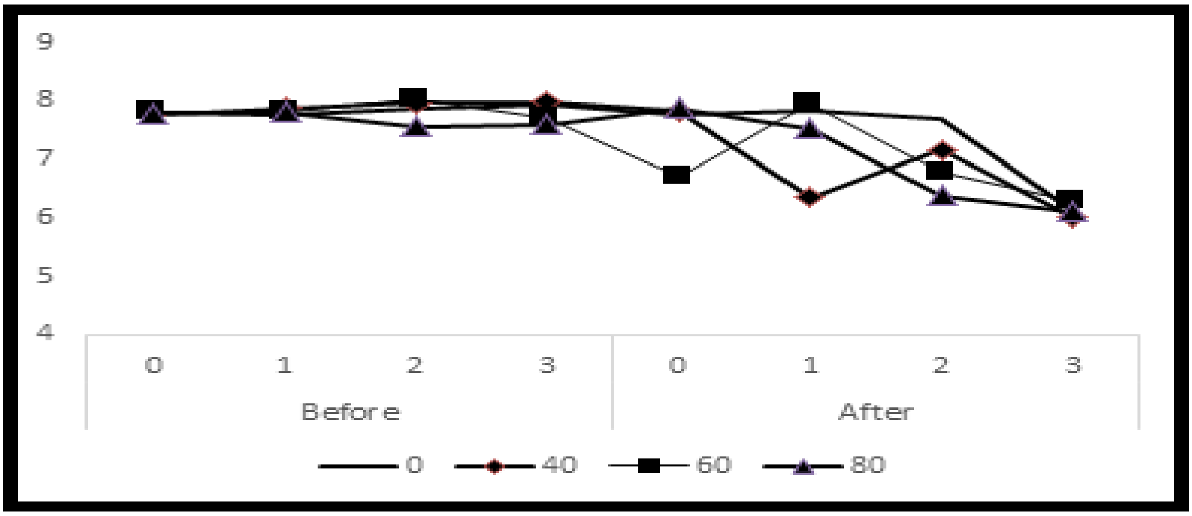

Fig. 9. Treatment (Humic acid, 1gL^{-1} 2gL^{-1}3gL^{-1}) (Chitosan, 40mgL^{-1}60mgL^{-1}80mgL^{-1}) interactive (CxH) effect on changes in the mean value of before sowing and after harvesting pH of soil over time(12 weeks).

Discussion

For every treatment there was a huge amount of fluctuation in the pH i.e application of different chemicals and different rate of the respective chemicals affected the soil chemistry especially in terms of pH. According to analysis of variance of Humic acid showed significant effect on before sowing and after harvesting soil chemistry of pea plants. Organic matter decomposition results in a stable product that is humic acid. Humic acid might affect plant growth by releasing unavailable nutrients in soil as it buffers the soil pH (Mackowiak, 2001).Chitosan and Humic acid interaction (CxH) also resulted significant difference term of soil pH when were applied together. The statistics shows that the soil pH of pea was significantly reduced with foliar application of 3 g L^{-1}Humic acid and 40 mg L^{-1} Chitosan., the overall average mean pH of soil of all the treatments was 7.8, when the soil after harvesting of plants were tested the overall mean temperature of all the treatment came out to be 7.02. For every treatment there was a huge amount of fluctuation in the pH i.e application of different chemicals and different rate of the respective chemicals affected the soil chemistry especially in terms of pH. According to analysis of variance Humic acid showed significant effect on before sowing and after harvesting soil chemistry of pea plants. Organic matter decomposition results in a stable product that is humic acid. Humic acid might affect plant growth by releasing unavailable nutrients in soil as it buffers the soil pH (Mackowiak, 2001).Chitosan and Humic acid interaction (CxH) also resulted significant difference term of soil pH when were applied together. The statistics shows that the soil pH of pea was significantly reduced with foliar application of 3 g L^{-1}Humic acid and 40 mg L^{-1} Chitosan.

Soil mineral nitrogen was non significantly affected by Chitosan. Humicacid was proved significant in altering the soil mineral nitrogen, soil mineral nitrogen was non significant in changing soil mineral nitrogen after harvest. Humic acid altered the soil mineral nitrogen in after harvest soil significantly at $p \le 0.01$, however when Humic acid and Chitosan were applied together the result for post-harvest soil for soil mineral nitrogen was proved to be non-significant.

Highest soil mineral nitrogen was found when 3g L^{-1}Humic acid (49.70mg kg^{-1}) second highest mineral nitrogen was recorded when Humic acid was applied at 2g L^{-1} (46.60mg kg^{-1}) lowest mineral nitrogen was recorded when humic acid was applied at 1g L^{-1} (37.82 mg kg^{-1}). A. V. Katkat *et al.,* (2009) reported humic acid raised the dry weight and N, P, K, Ca, Mg, Na, Fe, Cu, Zn and Mn uptake of plants.

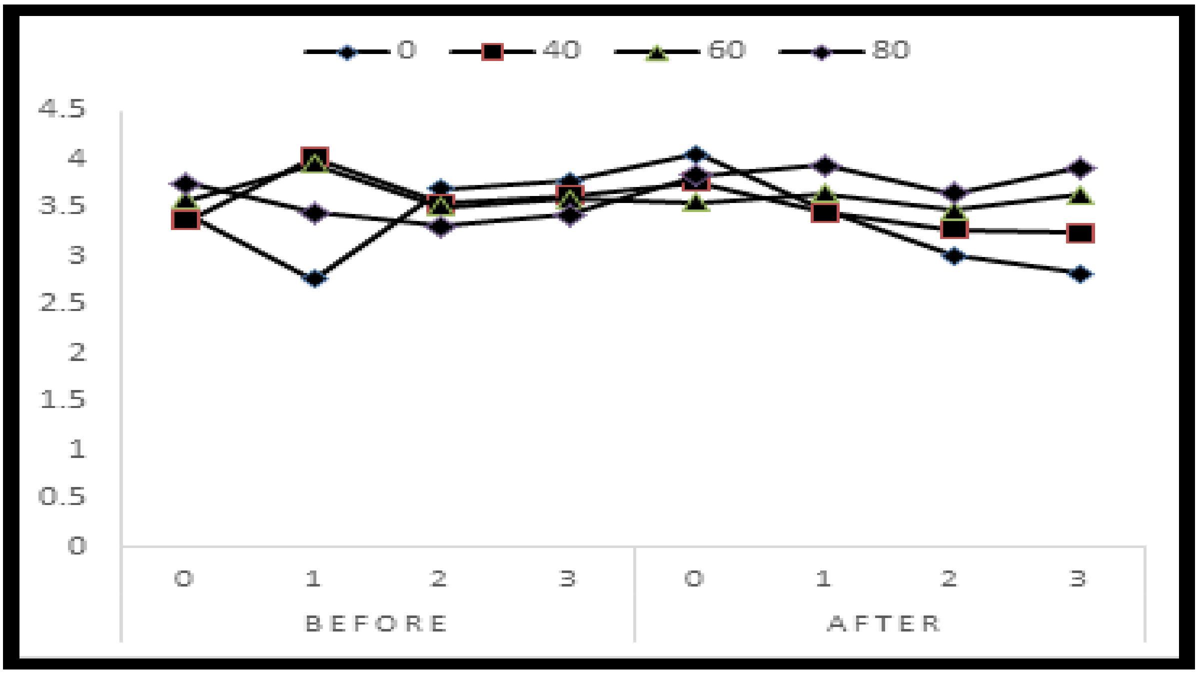

Fig. 10. Treatment (Humic acid, 1gL^{-1} 2gL^{-1}3gL^{-1}) (Chitosan, 40mgL^{-1}60mgL^{-1}80mgL^{-1}) interactive (CxH) effect on the mean value of LOI in before sowing and after harvesting soil over time(12 weeks).

Conclusion

HA and CHT interaction contributed towards decrease in soil pH in post-Harvest Soil significantly, however soil solution pH was also influenced by interaction and after second foliar application was performed, soil-solution pH was influenced more at HA@3 gL^{-1} .SMN as available NO_3 in soil was significantly increased and Nitrates in solution phase mineralized more as well, with Humic acid @2gL^{-1} released available N^2 in soil solution Partitioning. As Photosynthesis accelerated with chlorophyll content and has produced soluble carbohydrates, which accumulated in the root system of this plant and facilitated nodule production and thus has enable microbes to increase mineralization to improve soil fertility as well.

Metals showed inconsistency with treatment effect however increase observed in both metals after third application. HA and CHT contains some important acidic groups such as carboxyl, phenolic and hydroxyl groups that have provided organic macromolecules with an important function in the transportation, bioavailability and solubility of some metals.

Recommendations

The availability of store of metals and their concentration in soil pore water will change if environmental factor and foliar application (CHT, HA, CxH) influence key variables that determine the partitioning in soil solution and the post-harvest soil. Chitosan , Humic acid andits interaction (CxH) have positive and growth promoting effects on *Pisum sativum* by providing supplemental doses, so it should be used in practice to increases its growth and yield. The effects which HA and CHT put forth on plant growth can be grouped into indirect effects on physiological, chemical as well as biological properties of the soil, plant system that has been seen to improve with direct physiological output. Thus HA and CHT important effect on plant development may be credited to the promoting effects on uptake of some important nutrient and nutritional status of soil in particular N, P and K which are needed for the growth of plant .

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