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Effects of city wastewater on the characteristics of wheat with varying doses of nitrogen, phosphorus, and potassium

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Abstract

Rapidly growing India is not only facing the problem of water scarcity, but also the mismanagement of tremendous amount of wastewater produced every day. Moreover, food sufficiency has also become challenge to feed the ever increasing population leading to excessive use of chemical fertilizers in agriculture. Therefore, the study was carried out in Aligarh City of India on wheat crop (*Triticum aestivum* L.) var. PBW 343 to check the suitability of city wastewater as a source of irrigation water as well as source of nutrients. Three pot experiments were conducted in the winter season of 2006-2008. In Experiment I, nitrogen (N) at the rate of 0, 40, 80, 120 kg ha⁻¹; Experiment II, phosphorus (P) at the rate of 0, 20, 40, 60 kg ha⁻¹; and in Experiment III, potassium (K) at the rate of 0, 15, 30 and 45 kg ha⁻¹ were applied along with the basal doses under the three levels of water; ground water (GW), 50% wastewater (WW) and 100% WW. Lower fertilizer doses, 80 kg N ha⁻¹, 40 kg P ha⁻¹ and 30 kg K ha⁻¹ together with 100%WW proved optimum in three experiments, respectively, enhancing tiller number plant⁻¹, fresh mass plant⁻¹, dry matter plant⁻¹, leaf area, total chlorophyll content, photosynthetic rate (P_N), nitrate reductase (NR) activity, yield parameters (ear number plant⁻¹, length ear⁻¹, spikelet number ear⁻¹, grain number ear⁻¹ and 1000 grain weight), ultimately resulting in improved grain yield as well as grain carbohydrate and protein content as compared to control as well as higher fertilizer doses. Thus wastewater application not only provided stable supply of water, but also saved fresh water and contributed to environmental security. Moreover, it reduced the use of chemical fertilizers without showing any adverse effect on the yield and quality of wheat. Physicochemical characteristics of wastewater along with microbiological and some heavy metals were analyzed, and most of them were within the permissible limits set by Food and Agriculture Organization (FAO).

Keywords: City wastewater, fertilizer treatments, grain yield, grain quality, wheat

INTRODUCTION

More than 40% of the world's population is already reeling under the problem of water scarcity and according to United Nations, water is one of the most serious crisis the world is facing today. Globally, agriculture is the dominant user of water, accounting for 70% of total fresh water for irrigation. India's agricultural sector, which is the backbone of Indian economy, currently uses about 90% of total water resources; however, with the increasing competition between agriculture, industry and domestic demand, agriculture is beginning to receive less water [1]. Moreover, fast depletion of ground water reserves, coupled with severe water pollution, has put India in difficult position to provide sufficient fresh water for irrigation.

On the contrary to scarcity of fresh water in India, there is a substantial increase in the volume of urban wastewater production from the burgeoning cities. Today, the urban India has already become a massive and perhaps a frightening reality as far as wastewater management is concerned. According to the comparative studies of Central Pollution Control Board of India [2] on

wastewater generation, collection and treatment indicates that the quantity has increased from 7,007 million litres day⁻¹ (ML/d) in 1978-79 to 16,622 ML/d in 1994-95 in class I cities. However, the treatment capacity has increased only from 2755.94 ML/d in 1978-79 to 4037.20 ML/d in 1994-95. More recently, Bhardwaj [3] reported that the municipal wastewater treatment capacity in the year 2004-05 could handle only 27% of the total generated domestic sewage from urban centre's including 921 class I cities and class II towns in India. Hence, on the one hand, demand for water is increasing day by day resulting in rapid shrinkage of water resources, while the continuous discharge of the wastewater in water bodies is polluting them badly. Therefore, both the need to conserve fresh water and to safe and economically dispose of wastewater makes its use in agriculture a very feasible option.

Since agriculture involves the consumptive use of water, therefore, the use of additional water resources of marginal quality like, the wastewater can increase the volume of water available for irrigation. It has a high potential for reuse in agriculture as a source of irrigation, especially in arid and semiarid areas. It is currently used to irrigate crops in Middle East, North and South Africa, South America, Asia, Australia, and in parts of Europe [4]. Besides serving as a source of irrigation water, it contains many essential nutrients which may increase the yield of the crop and at the same time may substitute or even lower the fertilizer requirement of the crop [5] and may also contribute to environment security by reducing the pollution level of surface waters as well as of ground water.

Besides wastewater management and fresh water

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conservation, India's current concern is to meet the food demand of the increasing population. Wheat (*Triticum aestivum* L.) is the most important food grain in the world. It is a staple food for millions of people and major supplement in the human diet containing carbohydrate, protein, minerals and amino acids. Approximately two-thirds of the wheat produced in the world is used for human food and about one-sixth is used for livestock feed. In India, wheat is grown on the largest acreage of about 26.6 million hectares, and in 2010 its production reached a record 80.7 million metric tons [6]. However, the present level of average productivity of wheat is much lower than the other countries and its cultivation has not increased in proportion to the population growth resulting in consumption exceeding production for the past 10 years. Given the current population growth rate, India's ability to meet domestic demand will significantly be under strain in the future. To improve the productivity, application of huge amounts of chemical fertilizers is continuously increasing in the agriculture sector which in addition to being uneconomical, is potentially harmful to the environment as these get leached through the soil beyond the root zone eventually reaching the ground water or may escape because of the surface runoff into the nearby water bodies and cause eutrophication.

Therefore, considering the fact that strategies for the future must be based first and foremost on the conservation of water and careful management of chemical fertilizers for food production, the study was conducted to assess the suitability of city wastewater as a source of irrigation and nutrients for the cultivation of wheat. However, studies have revealed that wheat makes high demand for nutrient elements from the soil and if the yield of wheat is to be augmented or maintained, nutrients supplied only through the wastewater may not be sufficient. But the problem could be overcome by using adequate amount of effluent and fertilizer management [7]. It is not easy to provide plants with exactly sufficient amount of nutrients until they are properly worked out. Hence, in the experiments, optimum levels of nitrogen (N), phosphorus (P), and potassium (K) fertilizers at individual levels were also analyzed when applied with wastewater.

MATERIALS AND METHODS

Three pot experiments were conducted on wheat (*Triticum aestivum* L.) var. PBW 343 (semi-dwarf variety) during the winter seasons of 2006-2008 in the naturally illuminated net house of the Department of Botany, Aligarh Muslim University-Aligarh, India.

Agro-climatic conditions of experimental site

Aligarh city is situated in Western Uttar Pradesh, India. It is located at 27°52'N latitude and 78°51'E longitude and has an elevation of 187.45 m above sea level. The climate is semi-arid and subtropical with severe hot dry summers, temperatures reaching up to 47°C in the months of May and June. The intense cold winters start from October and ends in March, average temperature ranging between 13°C to 15°C. The mean annual rainfall is about 850mm and more than 85% of the total down pour is normally delivered during July to September while remaining occurs in winter. The soil texture of the area is sandy loam.

Pot preparation and seed treatment

The experiments were conducted in the earthen pots of 10" diameter. The N/P/K fertilizers were calculated on the basis of their composition and that one hectare of land contains 2×10^6 kg effective soil [8]. Each pot had 5 kg soil mixed with farmyard manure in the ratio of 3:1. Urea, single super phosphate and muriate of potash were used as the sources of nitrogen, phosphorus and potassium, respectively. Seeds were surface disinfected with 0.01% aqueous solution of mercuric chloride followed by repeated washing with double distilled water (DDW).

Experimental scheme

The treatments in each experiment were arranged in completely randomized block design. Each treatment was replicated three times and 26 pots were maintained for each treatment. The three water treatments were ground water (GW), 100% wastewater (100%WW) and 50% wastewater (50%WW). The 50%WW was obtained after dilution of 100%WW with GW in 1:1 ratio. Respective water treatment was started after the seedling emergence and each pot was given 300 ml of water on the alternate days uniformly up to the maturity of the crop.

Experiment I (wastewater and nitrogenous fertilizer) was conducted during the winter season of 2006-2007 to assess the comparative effect of 100%WW, 50%WW and GW in the presence of four levels of nitrogen, N at the rate of 0, 40, 80 and 120 kg ha⁻¹ to study the performance of wheat. A uniform basal starter dose of 40 kg P ha⁻¹ and 30 kg K ha⁻¹ was also applied to maintain the fertility of soil. Experiment II (wastewater and phosphatic fertilizer) was conducted simultaneously with Experiment I. Water treatments were the same as in Experiment I but applied with four levels of phosphorus, P at the rate of 0, 20, 40 and 60 kg ha⁻¹ along with a uniform basal starter dose of 40 kg N ha⁻¹ and 30 kg K ha⁻¹. Experiment III (wastewater and potassic fertilizer) was conducted during the winter season of 2007-2008 under the same three levels of water i.e. 100%WW, 50%WW and GW on the same crop and variety, grown under different levels of potassium, K at the rate of 0, 15, 30 and 45 kg ha⁻¹. Crop was supplied with uniform basal starter dose of 80 kg N ha⁻¹ and 40 kg P ha⁻¹.

Water and soil analysis

City wastewater, which includes municipal wastewater and sewage water mixed with wastewater from local lock and electroplating industries, was collected from the drain running along the Aligarh Mathura road. For analysis, water was collected twice i.e. at the beginning (I) and before the end (II) of each experiment. Tap water was used as a source of ground water. Both GW and WW were monitored for various physicochemical characteristics (Table 1) following the procedures listed in standard methods [9]. The wastewater used for crop irrigation was also assessed microbiologically and the mean values were obtained from three random samples (Table 2). Heavy metals (Cd, Cr, Ni, Pb) in the GW and WW were also analyzed using the atomic-absorption spectrophotometer (GBC-SensAA) (Table 3).

Table 1. Physicochemical characteristics of ground water and wastewater given in mg l⁻¹ or as specified.

Determinations	Experiment I & II				Experiment III				Optimum range*
	Ground water		Wastewater		Ground water		Wastewater		
	I	II	I	II	I	II	I	II	
pH	7.2	7	8.1	7.9	7.3	7.4	7.7	8	6.5-8.4
Electrical conductivity (dS/m)	0.81	0.57	1.01	1.27	0.92	0.77	1.36	1.41	0.25-3.0
Total solids	955	970	1450	1340	1020	925	1505	1412	NA
Total dissolved solids	560	610	1051	945	630	540	1124	1003	< 2000
NO ₃ -N	0.81	0.94	2.66	2.95	0.83	0.74	3.32	2.71	< 10.0
NH ₄ -N	0.17	0.11	0.87	0.91	0.18	0.15	0.96	0.82	< 5.0
PO ₄	0.28	0.33	1.24	1.58	0.32	0.37	1.49	1.13	< 2.0
Na	19.26	22.52	52.35	44.61	21.45	24.37	61.48	49.26	< 460
Ca	18.24	15.42	32.24	57.36	16.45	18.51	62.12	56.48	< 400
Mg	27.21	36.24	41.72	47.57	32.41	30.12	51.56	43.41	< 61
Cl	45.42	56.71	108.42	132.41	48.75	40.64	127.62	115.42	<350
K	7.35	6.94	16.24	18.04	8.42	7.21	15.44	17.25	<2.0
CO ₃	55.13	69.45	121.59	156.74	60.28	53.48	132.85	149.44	NA
HCO ₃	102.13	96.45	100.59	83.74	82.28	97.48	79.85	86.44	< 610

*FAO, Ayers and Westcot (1994); NA: Not available.

Table 2. Microbiological analysis of the city wastewater.

Bacteria	Method used	Experiment I & II		Experiment III
		Bacterial count (CFU 100 ml ⁻¹)		
Coliforms	MPN method	1.8×10 ³		2.1×10 ³
Faecal coliforms	MPN method	8.6×10 ²		9.2×10 ²
<i>Salmonella-Shigella</i> sp.	Spread plate method	1.3×10 ²		1.8×10 ²
Total heterotrophic bacteria	Spread plate method	2.01×10 ⁷		21.2×10 ⁶

MPN=Most Probable Number; CFU=Colony Forming Unit.

Table 3. Heavy metal analysis of ground water (GW) and wastewater (WW) given in mg l⁻¹.

Heavy metals	Experiment I & II		Experiment III		Optimum range*
	GW	WW	GW	WW	
Cd (cadmium)	ND	0.006	ND	0.005	<0.01
Cr (chromium)	ND	0.005	ND	0.007	<0.10
Ni (nickel)	ND	0.407	ND	0.391	<0.20
Pb (lead)	ND	0.034	ND	0.021	<5.0

*FAO, Ayers and Westcot (1994); ND: Not detected.

In each experiment, soil samples were collected randomly before sowing from different pots and analyzed for various physicochemical characteristics (Table 4) following the procedures of Ghosh *et al.*, [10]. The texture of the soil was sandy loam, and it also

contained some essential nutrients, like N, P, K, Mg, Ca, and Cl. The pH was alkaline which is considered suitable for the availability of essential macro nutrients.

Table 4. Physicochemical characteristics of soil. All determinations in mg l⁻¹ in 1:5 (soil:water) extract or as specified.

Determinations	Experiments		
	I	II	III
Texture	Sandy Loam		
pH	7.4	7.6	7.8
Electrical conductivity (dS/m)	0.78	0.81	0.88
Organic carbon (%)	0.449	0.352	0.506
NO	1.52	1.07	1.98
P	0.115	0.109	0.132
K	18.4	15.2	20.4
Ca	25.37	27.14	31.21
Mg	41.49	38.33	49.96
Cl	37.79	35.52	29.47
Na	53.11	47.98	61.32
HCO	155.46	132.43	188.45

Biometric and biochemical Observations

For investigating the comparative effect of WW, GW and fertilizers on wheat, observations were carried out at tillering- 60 days after sowing (DAS), heading- 90 DAS, and milky grain- 120

DAS stages of the crop. Six pots were randomly selected to study growth and physiological parameters separately at each sampling stages. Growth characteristics observed were tiller number plant⁻¹, fresh mass (g plant⁻¹), dry matter (g plant⁻¹), and leaf area (cm² plant⁻¹). Leaf area was measured by using leaf area meter (LA

211, Systronics, India). Among physiological parameters, total chlorophyll content was estimated following the method of McKinney [11]. Net photosynthetic rate (P_N) was measured in upper fully expanded leaves of intact plants using portable photosynthesis system LI-6400 (LI-COR, Lincoln, Nebraska, USA). The measurements were made on cloudless clear days between 11:00 and 13:00 solar time. The activity of nitrate reductase (NR) (E.C. 1.6.1.1) was determined in fresh leaf samples by the procedure explained by Jaworski [12].

Plants left after three samplings from each treatment were taken at the time of harvest and the yield characteristics like, ear number plant⁻¹, length ear⁻¹ (cm), spikelet number ear⁻¹, grain number ear⁻¹, 1000 grain weight (g) and grain yield (g plant⁻¹) were observed. The grain of each sample was chemically analyzed for its carbohydrate and protein contents. Extraction of grain carbohydrate was done according to the method of Yih and Clark [13] and estimated by the method of Dubois *et al.*, [14]. To estimate the total protein content, the method of the Lowry *et al.*, [15] was followed.

Statistical analysis of the collected data

The data recorded from the experiments was subjected to two way analysis (ANOVA) and the means were compared following the method given by Gomez and Gomez [16]. The 'F' test was applied to assess the significance of data at 5% level of probability ($P=0.05$).

Table 5. Effect of GW, 50%WW and 100%WW on the growth characteristics of wheat (*Triticum aestivum* L.) grown under different levels of nitrogen at 60, 90 and 120 DAS.

Treatments	Tiller number plant ⁻¹			Fresh mass (g plant ⁻¹)			Dry matter (g plant ⁻¹)			Leaf area (cm ² plant ⁻¹)		
	60 DAS	90 DAS	120 DAS	60DAS	90DAS	120DAS	60DAS	90DAS	120DAS	60DAS	90DAS	120DAS
GW												
N0	2.7	3.7	3.7	11.6	15.6	19.8	3.0	4.9	7.0	144.5	200.3	151.3
N40	3.7	4.3	4.7	14.1	19.0	24.1	4.0	6.0	8.0	162.0	232.1	181.2
N80	4.3	5.0	5.3	15.6	21.4	27.0	5.2	7.0	9.5	185.0	272.9	204.2
N120	5.0	6.0	6.0	18.0	23.9	30.2	6.0	8.1	12.0	206.8	312.2	213.9
50%WW												
N0	3.3	4.0	4.0	12.5	17.7	21.9	3.2	5.4	7.5	150.5	211.9	160.1
N40	4.0	4.7	4.7	14.9	20.1	25.5	4.3	6.5	9.0	172.4	244.3	190.0
N80	4.3	5.3	5.3	16.2	22.5	29.0	5.8	7.6	11.0	201.0	300.1	220.2
N120	5.0	5.7	5.7	18.1	23.1	30.3	6.1	8.0	12.1	202.5	306.0	221.0
100%WW												
N0	3.7	4.3	4.3	13.3	18.0	23.2	3.2	5.6	7.9	156.5	222.7	172.1
N40	4.3	5.0	5.0	15.2	21.2	26.2	5.0	6.9	9.2	179.5	268.0	199.2
N80	5.3	6.0	6.0	18.3	24.0	31.7	6.2	8.5	12.3	207.1	323.1	230.1
N120	5.3	6.0	6.0	18.1	24.0	31.0	6.1	8.2	12.2	204.2	319.8	225.3
LSD at P = 0.05												
Water (W)	0.19	0.22	0.18	0.57	0.60	0.82	0.24	0.28	0.56	NS	7.06	4.33
Nitrogen (N)	0.16	0.19	0.15	0.49	0.52	0.71	0.20	0.24	0.49	10.02	6.11	3.75
Interaction	0.32	0.37	0.30	0.96	1.00	1.38	0.40	0.47	0.95	NS	11.91	NS

NS: Non significant

Nitrate reductase levels have been shown to fluctuate in response to changes in environmental conditions, including availability of N. In present study, NR activity of leaf tissue was highly influenced by nitrogen fertilization as it increased with the increase in fertilizer dose up to N120 with GW, recording maximum at 90 DAS thereafter declined (Table 6). Similar observations were made in

RESULTS AND DISCUSSION

Wastewater and nitrogenous fertilizer

In Experiment I, among the four nitrogen treatments, 120 kg N ha⁻¹ (N120) proved best for the growth and yield parameters, recording maximum grain yield and improved grain quality, followed by lower doses on N and minimum values were recorded under control. Expectedly, improved growth was due to the supply of adequate N which increased the number of meristematic cells leading to the formation of tillers in addition to leaf expansion. However, when its supply is suboptimal, the growth may remain retarded [17] as observed in the present study also, where N40 proved deficient recording comparatively lower values for tiller number, leaf area, plant fresh mass and dry matter accumulation (Table 5). However, when interactions of wastewater and nitrogenous fertilizer were observed, medium dose, N80 proved optimum for growth as well as grain yield, as the values recorded under this treatment were statistically similar to the values given by N120 with GW. The possible explanation could be presence of N in both ionic forms in wastewater, however, suitability of NH₄⁺ or NO₃⁻ depends upon many factors. Normally the combined supply of both ions result in the highest growth rate and increased yield which was also observed in the present study where wastewater had supplemented nitrogen in both the ionic forms throughout the growing season (Table 1). Application of NH₄⁺ -N in the presence of NO₃⁻ -N has also been reported to benefit wheat by Silberbuh [18] and rice by Duan *et al.*, [19].

wheat by Khedr *et al.*, [20]. Nitrogen concentration is indirectly related to one of the basic plant physiological process, the photosynthesis, as 70% of N in plant leaves exists in chloroplast and most of it is used to synthesize photosynthetic apparatus. During the present study, maximum chlorophyll content and the best photosynthetic activity with maximum P_N were recorded either under

the highest N dose, 120 kg ha⁻¹ with GW or N80 with 100%WW (Table 6). The stimulation of photosynthesis due to nitrogen may have positive effect on plant growth. Similarly, plant dry matter accumulation was increased as a result of enhanced leaf area which provided larger surface for the interception of solar radiation and thus higher photosynthetic rate, thereby improving most yield attributes including higher 1000 grain weight, grain yield (Table 7) and also

carbohydrate content (Figure 1b) as compared to control [21, 22]. On the contrary to the optimum N level, decreased grain yield was recorded under N deficient doses (N40 and N0) as it had an adverse effect on crop growth, chlorophyll content and photosynthesis which ultimately affected the assimilate supply to the developing ears [23, 24].

Table 6. Effect of GW, 50%WW and 100%WW on the physiological parameters of wheat (*Triticum aestivum* L.) grown under different levels of nitrogen at 60, 90 and 120 DAS.

Treatments	Chlorophyll content (mg g ⁻¹ fresh mass)			Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)			NR activity (nmol NO ₂ g ⁻¹ (leaf FM) h ⁻¹)		
	60 DAS	90DAS	120 DAS	60 DAS	90 DAS	120DAS	60 DAS	90 DAS	120 DAS
GW									
N0	1.27	1.73	1.57	11.23	13.80	9.00	282.27	334.07	310.17
N40	1.43	1.97	1.77	13.53	16.83	12.13	316.63	389.43	348.53
N80	1.67	2.23	1.97	15.67	18.00	14.03	377.23	436.80	416.23
N120	1.80	2.50	2.10	17.13	21.00	15.23	429.73	500.07	452.57
50%WW									
N0	1.37	1.83	1.67	12.17	14.57	11.00	293.53	357.63	329.03
N40	1.57	2.13	1.87	14.57	17.23	12.87	339.07	418.57	381.80
N80	1.83	2.50	2.47	16.60	19.53	15.10	416.80	496.20	472.23
N120	1.77	2.53	2.27	17.30	20.47	15.03	422.03	504.10	466.20
100%WW									
N0	1.47	1.90	1.73	12.90	15.63	11.27	302.60	368.30	336.17
N40	1.63	2.20	1.93	15.23	17.93	13.90	362.37	435.03	394.90
N80	1.93	2.73	2.53	17.87	22.00	15.80	433.03	513.63	474.73
N120	1.73	2.60	2.20	17.07	20.60	15.03	421.30	506.37	470.50
LSD at P = 0.05									
Water (W)	0.09	0.09	0.09	0.18	0.46	0.46	7.49	6.20	8.98
Nitrogen (N)	0.07	0.08	0.08	0.16	0.40	0.40	6.49	5.37	7.78
Interaction	NS	0.15	0.15	0.31	0.78	0.78	12.65	10.47	15.15

NS: Non significant

Table 7. Effect of GW, 50%WW and 100%WW on the yield parameters including grain yield of wheat (*Triticum aestivum* L.) grown under different levels of nitrogen.

Treatments	Harvest					
	Ear number plant ⁻¹	Length ear ⁻¹ (cm)	Spikelet number ear ⁻¹	Grain number ear ⁻¹	1000 Grain weight (g)	Grain yield (g plant ⁻¹)
GW						
N0	3.67	10.33	14.33	30.00	40.03	4.12
N40	4.33	10.83	15.33	33.33	40.77	4.96
N80	5.00	11.30	17.33	35.00	41.21	5.95
N120	6.00	12.60	19.67	41.67	43.03	7.15
50%WW						
N0	4.00	10.37	14.67	31.67	40.21	4.62
N40	4.67	10.97	16.00	34.00	40.97	5.21
N80	5.33	11.63	18.00	37.33	41.91	6.28
N120	5.67	12.17	19.33	41.33	42.96	7.00
100%WW						
N0	4.33	10.67	15.00	32.33	40.56	4.82
N40	5.00	11.23	17.00	34.67	41.07	5.86
N80	6.00	12.87	20.00	42.33	43.11	7.20
N120	6.00	12.80	20.33	42.00	43.00	7.12
LSD at P = 0.05						
Water (W)	0.22	0.34	0.51	1.60	0.40	0.28
Nitrogen (N)	0.19	0.30	0.44	1.38	0.35	0.24
Interaction	0.37	0.58	0.85	2.70	0.68	0.46

Besides yield, nitrogen treatment N120 with GW proved better than all other treatments for grain quality also, however, N80 with 100%WW proved optimum as it recorded values which were at par with the values recorded under N120 with GW; and N120 with 100%WW proved luxurious dose as no further increase or adverse effect on yield or quality of wheat was observed. Among the three waters, 100%WW proved superior, recording an increase of 5.90% protein and 1.63% carbohydrates over GW (Figure 1a, b). The increase in grain protein content under these treatments might be

due to higher reserves of N stored in vegetative parts, which may later be remobilized to the grains of lower sink strength. Moreover, regular wastewater application provided adequate moisture in addition to N which was readily available from soil at later growth stages and might be responsible for higher protein as this N would be directly translocated to the developing grains besides the remobilization of N from the source at later phase [25, 26]. Similarly, late application of N has also been reported to improve carbohydrate content in wheat [27].

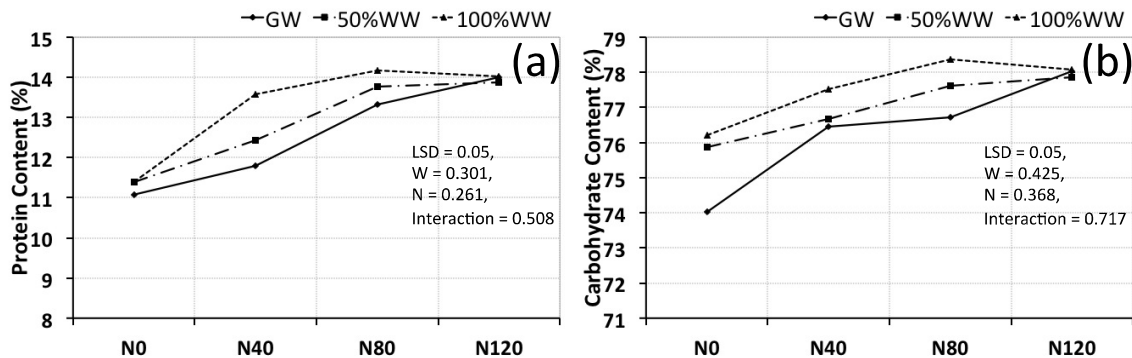


Fig 1. Effect of wastewater irrigation and different levels of nitrogen (N) on grain (a) protein and (b) carbohydrate content.

Wastewater and phosphatic fertilizer

Phosphorus has many roles in cell division, stimulation of early root growth, hastening plant maturity, fruiting and grain production [17]. The data confirmed the well-established role of phosphorus, as highest phosphorus dose P60 with GW or medium dose P40 in combination with 100%WW which in turn proved to be optimum treatment being at par with P60 with GW as well as P60 with 100%WW, promoted tissue and organ formation thereby augmenting growth, culminating in enhanced tiller formation and leaf area (Table 8). Expectedly, the increased leaf area has a higher leaf area index (LAI) which is a determinant of radiation interception will

result in the higher photosynthetic rate producing more dry matter as observed in present study (Table 9) and has also been reported by Rodriguez *et al.*, [28]. On the contrary, the most striking effects of phosphorus deficiency are poor growth, reduction in leaf expansion, and also the photosynthetic activity which was also observed under P20, a deficient dose and P0. Stark *et al.*, [29] explained the lower rate of photosynthesis under P limitation is primarily due to depression in Calvin cycle and because of reduction in the amount and activity of Rubisco (ribulose-1, 5-biphosphate carboxylase/oxygenase) and several other enzymes. Low chlorophyll content was also recorded under insufficient P which could have also resulted in declining of the P_N [30].

Table 8. Effect of GW, 50%WW and 100%WW on the growth characteristics of wheat (*Triticum aestivum* L.) grown under different levels of phosphorus at 60, 90 and 120 DAS.

Treatments	Tiller number plant ⁻¹			Fresh mass (g plant ⁻¹)			Dry matter (g plant ⁻¹)			Leaf area (cm ² plant ⁻¹)		
	60 DAS	90DAS	120DAS	60DAS	90 DAS	120 DAS	60DAS	90DAS	120DAS	60 DAS	90 DAS	120DAS
GW												
P0	2.67	3.00	3.00	11.22	15.50	19.67	2.98	4.79	6.75	146.33	201.13	149.17
P20	3.33	3.67	4.00	13.13	18.90	24.05	4.00	5.66	7.90	160.03	227.77	177.13
P40	4.00	4.33	4.67	14.76	20.87	27.46	5.08	6.90	9.22	182.10	268.80	200.10
P60	5.33	6.00	6.00	16.87	22.17	29.94	5.50	7.93	11.62	199.50	312.17	218.53
50%WW												
P0	3.00	3.67	3.67	12.13	17.13	21.53	3.19	5.27	7.20	151.57	207.20	157.10
P20	3.67	4.00	4.00	13.96	19.50	25.27	4.26	6.13	8.96	170.37	238.83	186.27
P40	5.00	5.33	5.33	15.63	21.82	29.02	5.63	7.45	10.76	198.40	292.47	217.07
P60	5.67	6.00	6.00	16.47	22.20	29.27	5.80	7.96	11.72	200.13	307.17	215.23
100%WW												
P0	3.33	3.67	3.67	13.03	18.16	23.30	3.28	5.42	7.57	157.87	217.67	168.30
P20	4.00	4.33	4.33	14.33	20.62	26.07	4.94	6.63	9.09	177.50	262.10	195.87
P40	5.67	6.00	6.00	17.82	23.68	31.03	5.91	8.14	12.27	202.63	310.07	221.03
P60	5.33	5.67	5.67	17.00	22.46	30.09	5.87	8.00	11.73	200.34	304.53	217.47
LSD at P = 0.05												
Water (W)	0.19	0.21	0.21	0.66	0.61	0.78	0.19	0.24	0.42	4.77	10.88	5.67
Phosphorus(P)	0.16	0.18	0.18	0.57	0.53	0.68	0.16	0.20	0.37	4.13	9.43	4.91
Interaction	0.32	0.35	0.35	1.11	1.03	1.32	0.31	0.40	0.71	8.05	18.37	9.57

Table 9. Effect of GW, 50%WW and 100%WW on the physiological parameters of wheat (*Triticum aestivum* L.) grown under different levels of phosphorus at 60, 90 and 120 DAS.

Treatments	Chlorophyll content (mg g ⁻¹ fresh mass)			Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)			NR activity (nmol NO ₂ g ⁻¹ (leaf FM) h ⁻¹)		
	60 DAS	90 DAS	120 DAS	60 DAS	90 DAS	120 DAS	60 DAS	90 DAS	120 DAS
GW									
P0	1.33	1.80	1.63	12.13	13.57	7.30	286.33	340.17	314.47
P20	1.47	1.93	1.83	13.10	15.90	10.47	306.47	385.03	349.60
P40	1.60	2.20	2.13	14.43	17.23	12.20	378.17	448.20	408.10
P60	1.70	2.50	2.33	15.80	22.00	13.80	401.23	489.30	440.77
50%WW									
P0	1.37	1.90	1.70	12.87	14.20	9.50	300.50	362.40	330.13
P20	1.50	2.20	1.87	13.30	16.83	10.50	330.27	408.67	376.43
P40	1.63	2.43	2.30	15.13	19.10	13.60	414.07	486.33	444.60
P60	1.73	2.50	2.38	15.80	18.50	13.07	405.43	470.10	449.70
100%WW									
P0	1.40	1.97	1.77	13.00	15.50	10.00	310.10	375.53	342.20
P20	1.57	2.27	1.97	14.67	17.00	11.83	356.73	426.80	395.87
P40	1.83	2.67	2.57	16.20	21.53	14.10	421.30	500.13	458.33
P60	1.90	2.73	2.40	16.00	21.00	13.67	412.07	492.47	450.30
LSD at P = 0.05									
Water (W)	0.08	0.07	0.08	0.22	0.25	0.24	9.17	12.68	9.92
Phosphorus(P)	0.07	0.06	0.07	0.19	0.22	0.20	7.94	10.98	8.59
Interaction	NS	0.12	0.14	0.37	0.42	0.40	15.47	21.41	16.74

NS: Non significant

Leaf NR activity increased with the fertilizer doses, recorded maximum at P60 and lowest at P20 and P0 (Table 9). NR activity is indirectly affected by P application as it plays role in phosphorylation and diversion of simple sugars towards respiration as a result of which release of photosynthates from chloroplast and their oxidation subsequently produces more reducing power for NO₃⁻ reduction. Similar explanation was given by Moinuddin *et al.*, [31] while studying NR activity in triticale. The effect of P60 on yield characteristics including grain yield was significantly higher than the other doses, recording 57.05% increase over control; and the combination of 100%WW and P40 was equally effective, proving to be optimum treatment (Table 10). In case of adequate P supply, roots retain a small quantity of the total P applied and distribute the rest to the developing parts in addition to its translocation from stem

and leaves to developing grains. The increased ear production under these treatments indicates the involvement of P in the production of fertile tillers [32, 33] and proper partitioning of photosynthates between vegetative and reproductive parts of the plant. Similarly, an increase in ear length with higher phosphorus dose or combination of 100%WW and P40 compared to P20 may be an indicator of its promotory role. Likewise, it has also played an important role in enhancing spikelet number and grain number in addition to 1000 grain weight, confirming the role of phosphorus in swinging the partitioning of photosynthates towards grain filling as noted in cereals by Hussain *et al.*, [34] and Hussain *et al.*, [35]. In the present study also, the effectiveness of P60 and 100%WW along with P40 in promoting vegetative growth was reflected in yield attributes finally resulting in higher grain yield.

Table 10. Effect of GW, 50%WW and 100%WW on the yield parameters including grain yield of wheat (*Triticum aestivum* L.) grown under different levels of phosphorus.

Treatments	Harvest					
	Ear number plant ⁻¹	Length ear ⁻¹ (cm)	Spikelet number ear ⁻¹	Grain number ear ⁻¹	1000 Grain weight (g)	Grain yield (g plant ⁻¹)
GW						
P0	3.00	10.40	13.67	30.33	39.90	4.06
P20	3.67	10.93	15.33	33.33	40.87	4.94
P40	4.33	11.30	17.33	34.67	41.22	5.90
P60	6.00	13.03	20.00	42.67	42.86	7.06
50%WW						
P0	3.67	10.57	14.67	31.33	40.36	4.50
P20	4.00	11.10	16.33	33.67	41.07	5.32
P40	5.33	11.77	18.33	37.00	41.72	6.51
P60	6.00	12.37	19.67	42.00	42.73	6.93
100%WW						
P0	3.67	10.73	15.33	33.00	40.68	4.87
P20	4.33	11.37	17.00	34.33	41.15	5.63
P40	6.00	12.97	20.33	43.00	42.97	7.12
P60	5.67	12.90	20.00	42.33	42.98	7.08
LSD at P = 0.05						
Water (W)	0.21	0.35	0.65	1.82	0.37	0.26
Phosphorus(P)	0.18	0.31	0.57	1.57	0.32	0.23
Interaction	0.35	0.60	1.10	3.06	0.62	0.44

Adequate supply of phosphorus as P60 and P40 with 100%WW equally proved beneficial in enhancing the grain quality.

100%WW proved superior over GW recording a significant increase of 3.18% protein and 0.94% carbohydrate (Figure 2a, b).

Phosphorus is required for absorption and assimilation of N by wheat plants, and translocation of N from vegetative parts to grain which may indirectly affect the protein content. Moreover, P supply seemed to delay whole plant and tiller senescence, which would contribute to both high grain yields and high protein concentration. Similarly, the same optimum doses also increased the carbohydrate content significantly due to the importance of phosphorus through its role in energy-rich ATP (Adenosine-5'-triphosphate) and NADP (Nicotinamide adenine dinucleotide phosphate) which are involved not only in the production of carbohydrate in the source but also in its

conduction towards the sink (grains). It further needs emphasis that P applied to the soil very rapidly changes to less soluble form and therefore, becomes less available with time. Hence, due to its limitations, long season crops, like corn and wheat, may show only slow growth responses and much lesser effect at seed formation and maturity. However, regular supply of the wastewater have ensured availability of P at all growth stages, thus improved growth and development which ultimately led to higher grain yield and better quality

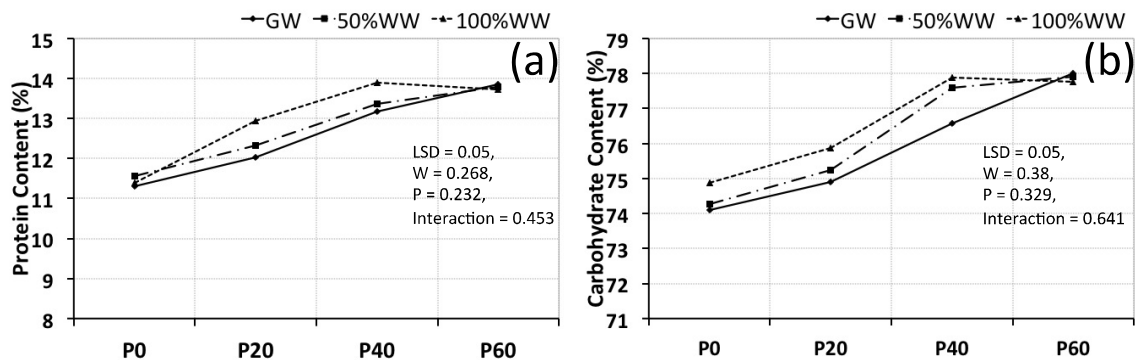


Fig 2. Effect of wastewater irrigation and different levels of phosphorus (P) on grain (a) protein and (b) carbohydrate content.

Wastewater and potassic fertilizer

K is a major osmotically active cation and it is essential for attaining full activity of enzymes which have an impact on numerous physiological processes, and some of them are of major relevance for the plant growth and development. In Experiment III, the enhanced growth characteristics under K45 were observed while with wastewater comparatively lower dose (K30) proved beneficial as compared to lower dose of K15 which proved deficient (Table 11). Similar findings were reported by Sweeney *et al.*, [36] recording

maximum growth and vigor when wheat was supplied by adequate K. Moreover, K also plays a major role in the process of photosynthesis as the mechanism of stomatal opening and closure is known to depend on K⁺ fluxes in the guard cells. Thus, a considerable decrease in the photosynthetic rate was observed in the plants grown without potassium (K0) while the enhanced leaf area, P_N and higher dry matter accumulation was observed under adequate K treatments (Table 11, 12) which was also parallel to the findings of Khalid *et al.*, [37]; Alderfasi and Refay [38].

Table 11. Effect of GW, 50%WW and 100%WW on the growth characteristics of wheat (*Triticum aestivum* L.) grown under different levels of potassium at 60, 90 and 120 DAS.

Treatments	Tiller number plant ⁻¹			Fresh mass (g plant ⁻¹)			Dry matter (g plant ⁻¹)			Leaf area (cm ² plant ⁻¹)		
	60 DAS	90DAS	120DAS	60DAS	90 DAS	120DAS	60DAS	90DAS	120DAS	60 DAS	90 DAS	120 DAS
GW												
K0	2.67	3.00	3.00	11.12	14.80	19.58	2.90	4.70	6.64	147.13	201.20	150.10
K15	3.33	3.67	4.00	13.26	18.96	24.10	3.92	5.58	7.82	163.27	230.17	179.27
K30	4.67	5.00	5.00	14.35	20.82	26.88	5.10	6.59	9.12	183.17	265.05	197.70
K45	5.33	5.67	5.67	15.68	21.77	27.13	5.34	7.02	9.76	192.33	292.22	210.60
50%WW												
K0	3.00	3.33	3.33	12.20	17.30	21.60	3.12	5.21	6.96	153.60	209.13	158.24
K15	4.00	4.33	4.33	13.98	19.38	25.16	4.23	6.08	8.90	173.40	240.30	188.40
K30	4.67	5.00	5.33	15.72	21.71	28.00	5.55	7.00	10.10	194.10	289.00	206.34
K45	5.00	5.33	5.33	16.35	22.10	28.81	5.72	7.37	10.60	197.07	297.02	212.28
100%WW												
K0	3.00	3.67	3.67	13.00	18.08	23.25	3.25	5.37	7.50	159.47	219.60	170.30
K15	4.33	4.67	4.67	14.80	21.00	27.02	5.00	6.65	9.02	180.30	268.47	200.13
K30	5.67	6.00	6.00	17.70	23.12	30.96	5.84	7.89	12.13	200.50	307.13	218.87
K45	5.67	6.00	6.00	17.64	22.85	31.02	5.92	7.77	12.04	198.03	308.10	216.07
LSD at P = 0.05												
Water (W)	0.19	0.21	0.20	0.44	0.62	0.38	0.18	0.18	0.44	5.79	7.36	3.47
Potassium(K)	0.17	0.18	0.17	0.38	0.53	0.33	0.15	0.15	0.38	5.02	6.37	3.01
Interaction	0.32	0.35	0.33	0.75	1.04	0.64	0.30	0.30	0.74	NS	12.42	5.86

NS: Not significant

Table 12. Effect of GW, 50%WW and 100%WW on the physiological parameters of wheat (*Triticum aestivum* L.) grown under different levels of potassium at 60, 90 and 120 DAS.

Treatments	Chlorophyll content (mg g ⁻¹ fresh mass)			Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)			NR activity (nmol NO ₂ g ⁻¹ (leaf FM) h ⁻¹)		
	60 DAS	90 DAS	120 DAS	60 DAS	90 DAS	120 DAS	60 DAS	90DAS	120 DAS
GW									
K0	1.20	1.60	1.53	11.70	12.90	8.50	290.10	332.17	304.77
K15	1.37	1.87	1.73	13.30	16.07	11.70	326.50	378.23	350.43
K30	1.60	2.27	2.00	14.00	17.40	13.70	373.60	434.77	415.93
K45	1.70	2.43	2.17	14.78	18.20	14.56	395.27	490.10	436.10
50%WW									
K0	1.27	1.73	1.60	12.54	14.40	10.60	300.03	350.83	326.30
K15	1.47	2.07	1.87	13.70	16.92	12.23	348.80	417.33	379.20
K30	1.73	2.50	2.23	15.04	18.80	14.68	408.30	489.60	448.27
K45	1.77	2.53	2.23	15.60	20.23	15.00	417.63	488.43	453.80
100%WW									
K0	1.33	1.80	1.67	12.96	15.62	11.35	314.73	364.70	335.47
K15	1.53	2.23	2.07	13.82	17.60	13.54	370.90	438.90	420.50
K30	1.80	2.67	2.40	16.00	20.30	15.03	427.20	500.23	466.33
K45	1.83	2.60	2.34	15.90	20.00	15.12	423.60	493.37	461.83
LSD at P = 0.05									
Water (W)	0.06	0.06	0.05	0.26	0.40	0.48	6.35	14.80	9.57
Potassium(K)	0.05	0.05	0.05	0.23	0.34	0.41	5.50	12.82	8.29
Interaction	NS	0.09	0.09	0.44	0.67	0.80	10.72	24.98	16.16

NS: Non significant

Potassium is also involved in peptide bond synthesis and in other energy releasing processes, thus an energy shortage in plants poorly supplied with K may induce a delay in protein synthesis which in turn may indirectly affect enzyme activity. This has been shown for nitrate reductase by Pfluger and Wiedemann [39] and similar results were obtained in Experiment III where poor NR activity under deficient dose (K15 and K0) was recorded at all the three growth stages (Table 12). While observing yield physiology, it is desirable to consider not only the tissues involved in the storage of food (sink) but also the plant parts producing these photosynthates (source). In our study, K45 was the highest dose recording maximum values for growth parameters, photosynthesis and yield attributing characters;

however, 100%WW along with K30 performed optimum being at par with K45 giving statistically similar values for most of the parameters including yield. The logical expectation that this augmented source under adequate K treatments would lead to better development of the components of the sink was corroborated by the data in Table 13, wherein above mentioned treatments proved best for yield parameters including 1000 grain weight and grain yield. Similar results were also reported by Sweeney *et al.*, [36] and Singh *et al.*, [40], according to which sufficient rates of K improved the quality of wheat plants, assist in grain filling, ultimately recording better grain weight and yield as compared to no-potassium.

Table 13. Effect of GW, 50%WW and 100%WW on the yield parameters including grain yield of wheat (*Triticum aestivum* L.) grown under different levels of potassium.

Treatments	Harvest					
	Ear number plant ⁻¹	Length ear ⁻¹ (cm)	Spikelet number ear ⁻¹	Grain number ear ⁻¹	1000 Grain weight (g)	Grain yield (g plant ⁻¹)
GW						
K0	3.00	10.13	14.00	29.00	39.63	4.15
K15	3.67	10.77	15.33	32.67	40.80	5.02
K30	5.00	11.10	16.67	34.33	41.46	6.01
K45	5.67	11.30	18.00	36.33	42.17	6.57
50%WW						
K0	3.33	10.33	14.33	30.33	40.12	4.76
K15	4.33	10.83	15.67	33.00	41.01	5.57
K30	5.00	11.37	17.33	36.00	42.02	6.42
K45	5.33	11.43	18.67	37.00	42.41	6.78
100%WW						
K0	3.67	10.53	14.67	32.00	40.35	4.97
K15	4.67	11.03	16.00	34.00	41.63	5.78
K30	6.00	12.13	19.33	40.33	42.76	7.00
K45	6.00	12.20	19.67	40.00	42.60	7.01
LSD at P = 0.05						
Water (W)	0.21	0.21	0.49	0.96	0.20	0.14
Potassium(K)	0.18	0.18	0.42	0.83	0.17	0.12
Interaction	0.35	0.36	0.82	1.62	0.33	0.24

Potassium is often described as “quality element” producing grains of higher quality under sufficient K application. In present study, K45 recorded maximum value for protein and carbohydrate content, followed by K30, K15 and K0. 100%WW again proved better

recording an increase of 5.83% protein and 0.87% carbohydrate over GW (Figure 3a, b). However, combination of K30 with 100%WW proved optimum being at par with K45 with GW or with WW. K not only plays a key role in the synthesis of protein and carbohydrates, it

also maintains a normal balance between them [41]. Moreover, cereals receiving good K supply absorbed more N during grain filling [42] and the presence of K in wastewater was nearly double the amount present in ground water (Table 1) and therefore, the crop under study was benefitted not only due to physiological role of K but

also by enhancing the effect of N. In addition, potassium promotes the translocation of newly synthesized photosynthates as well as plays a beneficial role in the mobilization of stored material, hence producing grains of higher quality.

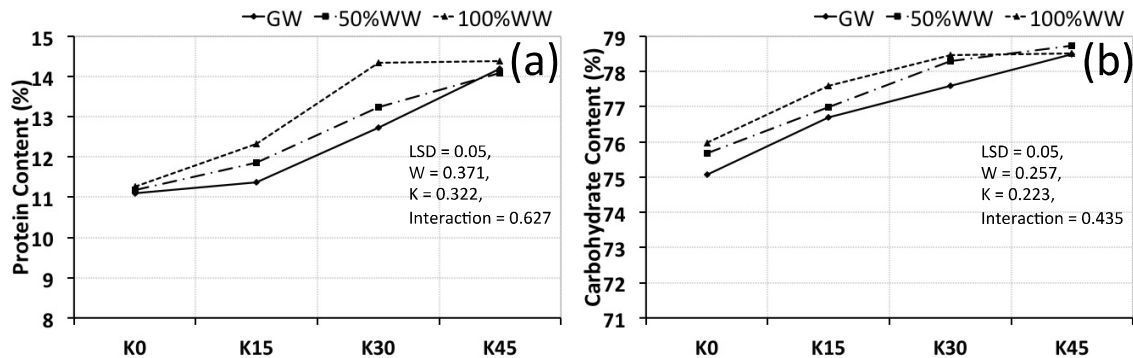


Fig 3. Effect of wastewater irrigation and different levels of potassium (K) on grain (a) protein and (b) carbohydrate content.

Nutrients present in wastewater and their role

The characteristics of wastewater used for irrigation varied with and among the years of this study (Table 1). In general, it was alkaline with basic pH ranging from 7.7 to 8.1. The average EC, pH, TDS and the observed nutrients including some heavy metals were within the permissible limits of FAO guidelines for irrigation water quality except for K⁺ [43]. Among the three water treatments, 100%WW proved more effective, recording an increase of 12.61, 12.39 and 13.79% grain yield over GW in three experiments, respectively. 100%WW also proved beneficial in significantly improving the quality of grains. Superiority of wastewater over ground water in terms of yield and quality of crop has also been reported by Day *et al.*, [44]; Singh [45]; Tabassum *et al.*, [7]; and Tak *et al.*, [5]. Even 50%WW proved better than GW, probably because of comparatively higher nutrient contents. In various studies, dilution of wastewater has been reported effective in minimizing the toxicity; however, under present experimental conditions, the city wastewater was sufficiently diluted due to the mixing of the household wastewater to an extent that when analyzed for various physicochemical characteristics was found suitable for irrigation and therefore, no further dilution was required.

In addition to three major macronutrients N/P/K in wastewater as explained earlier, presence of other essential nutrient like sulphur could have also played a vital role in enhancing the plant metabolism as it is known to take part in many reactions in all living cells. Sulphur is a building block of protein and is a key ingredient in the formation of chlorophyll [46]. The application of nitrogen in the form of urea is ineffective unless sulphur is applied simultaneously. Moreover, it also increases the availability of phosphorus and potash enhancing catalase activities and assimilation rate [47]. When wastewater is applied, it becomes available to plants at critical stages of growth and is incorporated readily into proteins and enzymes, resulting in improved performance of the crop. Significant increase in the yield quality of winter wheat in response to sulphur application has been reported by Zhao *et al.*, [48]; Riley *et al.*, [49]; and Girma *et al.*, [50].

The presence of Mg²⁺ in wastewater could have played essential role in enhancing the chlorophyll content and the photosynthetic rate in plants as Mg²⁺ is a central atom of chlorophyll and is required for structural integrity of chloroplast on which the rate

of photosynthesis is directly dependent. Application of Mg²⁺ has been proved important for the winter wheat plants by El-Metwally *et al.*, [51]. Similarly, Ca²⁺ an essential component of cell wall is involved in the cell division and its presence in wastewater could have also proved beneficial for plant growth. In addition, essential micronutrients were also present in wastewater like, Cl⁻ which plays an important role in stomatal regulation. The average chloride content of 120.96 mg l⁻¹ was low and may not cause toxicity problem. Ni deficiency leads to depressed seedling vigour, chlorosis and necrotic lesions in leaves [52]; however, it was also readily available to plants through wastewater. Therefore, the ensured supply and availability of above mentioned nutrients might have played a cumulative role in boosting the metabolic activities leading to enhanced growth and photosynthetic capacity which ultimately led to higher grain number, 1000 grain weight and finally the grain yield, carbohydrate and protein content.

Non-judicious use of chemical fertilizers and continuous use of effluents may result in the accumulation of nutrients in the soil, ultimately polluting the environment [53]. Hence, a balance between wastewater application and fertilizer dose has to be identified which was systematically obtained in present study. Although heavy metal concentration (Table 3) were well within the permissible limits except Ni [43], the continuous application of wastewater may lead to the buildup in soil and plants, hence, regular monitoring must be maintained for safe use in crop cultivation [54]. The presence of some pathogenic bacteria in wastewater, like coliforms, *salmonella* and *shigella* (Table 2) may be a serious concern. On the basis of microbiological quality assessment and considering the present guideline of WHO [55], the wastewater requires treatment to meet the quality guidelines for the crops to be eaten uncooked, however, it may be recommended for irrigating cereals and fodder crops. There are no chances of harm to the consumers, however, the farmers have to be informed to take due care while irrigating the fields with wastewater.

CONCLUSIONS

Wastewater is an effective source of nutrients, though it could not supplement the whole nutrient requirement of the crop, it reduced the quantity of N/P/K fertilizer doses and thus acted not only as a

source of irrigation water bridging the gap between water demand and water availability, but of nutrients also. In three experiments, 80 kg N ha⁻¹, 40 kg P ha⁻¹ and 30 kg K ha⁻¹ in combination with 100%WW proved optimum doses, respectively. Further study needs to be done on the combined effect of obtained optimum doses from the three experiments to manage the nutrient requirement of the wheat crop under wastewater treatment, hence obtaining its better productivity and quality.

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