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Influence of Wastewater Application and Fertilizer use on Growth, Photosynthesis, Nutrient Homeostatis, Yield and Heavy Metal Accumulation in Okra (*Abelmoschus esculentus* L. Moench)

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Abstract: The scarceness of freshwater assets is a serious problem in semi-arid zones and marginal quality water is increasingly being used in agriculture. This study aimed at evaluating the physico-chemical and biological risks on irrigated soils of treated wastewater, the nutrient supply and the effect on okra plant. A pot experiment based on completely randomized block design was conducted with Treated Wastewater (TW) and inorganic fertilizers to observe a comparative effect on biochemical characters using Okra var. Nidhi. The physico-chemical analysis of the TW showed that it was rich in total suspended and dissolved solids with large amount of BOD and COD. The higher amount of Cl^-, Ca^{++}, Mg^{++} and K^+ were also present in the effluent. The heavy metal (Cd, Cr, Ni and Pb) content in TW is comparatively more than groundwater (GW). The values of these heavy metals were slightly higher in the soil irrigated with TW. The effluent severely affects crop plants and soil properties when used for irrigation. The growth parameters, photosynthetic characteristics, chlorophyll content, yield and nutrient homeostatis were analyzed during different growth periods in all treatments. All the parameters were found to increase due to wastewater application. Among the fertilizer treatments, N_{120} proved optimum, N_{90} deficient and N_{150} proved as luxury dose. The seeds accumulated Cd and Ni but their level was under permissible limits. Thus, it may be concluded that wastewater may be used profitably for the cultivation of okra.

Key words: Wastewater, fertilizer, growth, nutrient homeostatis, photosynthetic rate, yield

INTRODUCTION

The shortage of freshwater resource is a major problem in arid and semi-arid regions and hence, resulting in increased reuse of marginal quality water in agriculture (Cisse, 1997). Besides the scarcity of freshwater, the population explosion is a global problem. There was a twofold increase in human population i.e., from 0.6-1.2 billion in the 150 years from 1700 A.D., however, it increased fivefold (6.1 billion) during the next 150 years (2000 A.D.). Hence, to meet the rising demand for food urban agriculture is developing rapidly.

On the other hand, the agriculture sector is the largest user of water (70%), whereas, only 1.1% of water is accounted for domestic and municipal supplies, while the rest of the water is used up by various industries. Therefore, there is a need for the use of secondary quality waters due to the fact that urban agriculture cannot depend merely on the reducing freshwater resources. The lack of a sufficient number of sewerage systems has led to the disposal of unrefined wastewater from the houses, market, hotels, hospitals and industries directly into the natural canals and also, get mixed with runoff rainwater (Akponikpe *et al*., 2011). The surroundings of these canals have been spontaneously invaded by market gardeners who use the water to irrigate their vegetables without any kind of treatment. Several studies have reported the influence of the extensive use of untreated wastewater on vegetable quality, population health and soil quality. Akhtar *et al*., (2012) and Tak *et al*. (2010, 2012) reported the heavy contamination of soil, crop leaves and fruits by faecal coliforms and helminth eggs. Similar vegetable contamination was reported in other areas by Amoah *et al*. (2006), Erdogrul and Sener (2005), Amahmid *et al*. (1999) and Blumenthal *et al*. (2001). Besides microbiological contamination of crop, one of the serious problems caused by raw wastewater irrigation is the accumulation of suspended matters in soil which are present in higher quantity in untreated wastewater resulting in clogging and hence, reduced soil aeration and hydraulic conductivity (Viviani and Iovino, 2004; Toze, 2006). In addition to this, the presence of excessive

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sodium and nitrate in untreated wastewater may also create salination and groundwater contamination, respectively (Ayers and Wescot, 1985; Oron *et al*., 1999). Therefore, to reduce the health risks of raw wastewater use on crop, soil quality as well as on the consumers, many research works have been carried out focusing on treatment possibilities. Reuse of treated wastewater for agricultural irrigation may be beneficial for different reasons: First of all, the excessive amount of wastewater is available throughout the year for irrigation. Secondly, water pollution is reduced as wastewater is not drained into the fresh water bodies. Lastly, wastewater reuse provides an economic benefit to the farmers as the nutrients present in it reduces the fertilizer expenses. Even though, wastewater has essential nutrient-elements for the requirement of plant but these concentrations are not sufficient for the productivity, hence, plants need a simultaneous application of chemical fertilizers also along with wastewater. The aim of this study was to evaluate (1) The physico-chemical and biological risks to wastewater irrigated soil (2) The nutrients supply and (3) The effect on the yield of okra crop irrigated with treated wastewater.

MATERIALS AND METHODS

Experimental site: Pot experiments were conducted in a naturally illuminated Photosynthetic Active Radiation (PAR)>900 μ moL^{-1} photons/m^2/S; temperature 22°C net house at the Department of Botany, Aligarh Muslim University, Aligarh, India.

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 13-15°C. The mean annual rainfall is about 850 mm and more than 85% of the total downpour is normally delivered during July to September while remaining occurs in winter. The soil texture of the area is sandy loam.

Experimental design, irrigation waters and crop material: Randomized Complete Block Design (RCBD) was adopted and each treatment was set simultaneously in triplicate with four different nitrogen treatments (0, 90, 120 and 150 kg ha^{-1}) supplied as urea along with 60 kg P ha^{-1} as single super phosphate and 60 kg K ha^{-1} as muriate of potash being watered by Ground Water (GW) and treated wastewater (100% ww).

Treated wastewater was collected from municipal wastewater treatment plant Peela Khar Sewage Treatment Plant, Agra, Uttar Pradesh (India), whereas the regular tap water was used as a source of ground water.

The experiments were carried out on okra crop (*Abelmoschus esculentus* L.) during dry seasons from April 2008 to July 2008. The crop was protected against insects, nematodes and fungi by application of carbofuran, cypermethrin and dimethoate periodically.

Measurements and statistical analysis: During the experimental period, the quality of the two irrigation waters (GW and WW) was monitored. Physico-chemical characteristics (biochemical oxygen demand BOD, chemical oxygen demand COD, electrical conductivity EC, suspended matter SM, pH, nitrate NO_3^- and other cations) and biological characteristics (faecal coliforms and helminth eggs) were assessed. The irrigated soils (0.0-0.2 m depth) were also sampled for biological contaminations. Water and soil samples were collected and analyzed according to the recommendations of APHA (1998) (Table 1, 2). Total Suspended Solids (TSS) are solids in water that can be trapped by the filter. TSS can include a wide variety of material, such as silt, decaying plant and animal matter, industrial wastes and sewage. Total Solids (TS) is a measure of all the suspended, colloidal and dissolved solids in a sample of water. This includes dissolved salts such as sodium chloride (NaCl) and solid particles such as silt and plankton. An excess of total solids in rivers and stream is a very common problem.

All the growth, photosynthetic and yield parameters of the crop were observed at pre flowering, flowering and post flowering stages i.e. 45, 75 and 105 days after sowing respectively. For obtaining plant biomass, fresh plant samples were dried in hot air oven at 80° C for two days and then weighed on an electronic balance. Leaf area was recorded by leaf area meter (LA-211, Systronics, India).

Net photosynthetic rate is the rate of conversion of dissolved carbon dioxide and bicarbonate ion to photosynthetic product. Stomatal conductance is the measure of the rate of passage of carbon dioxide entering, or water vapour exiting through the stomata of a leaf. Photosynthetic water use efficiency is the ratio of CO_2 assimilation into the photosynthetic biochemistry of water lost, via transpiration through the stomata. Net photosynthetic rate, stomatal conductance and photosynthetic water use efficiency at each sampling was measured in fully expanded leaves of plants by using LICOR-6400, portable photosynthetic system (Nebraska, USA). The measurements were made on the cloudless clear day between 11:00 and 13:00 solar time.

Chlorophyll content (Chl. a, Chl. b and total chlorophyll) in the fresh leaf was estimated following the method worked out by MacKinney (1941). For the estimation of N, P and K content, the dried leaf powder was first digested according to Linder (1944). Then from the peroxide digested material, nitrogen content was estimated following the method of Linder (1944), phosphorus content was estimated by the method of Fiske and Subbarow (1925) and potassium content was estimated with the help of flame photometer.

For the determination of heavy metals, the samples of soil, irrigant and crop produce were digested according to Van Loon and Lichwa (1973). A well-mixed, acid preserved samples were transferred to a beaker containing 5 mL concentrated nitric acid and a few boiling chips. These samples were heated on a water bath until digestion is complete, as shown by the light colored clear solution. Solutions were filtered with Whatman filter paper No. 42. These filtrated were diluted with distilled water and used for determination of different metals using SENSAA GBC Avanta Ver. 2.02 atomic absorption spectrophotometer.

In order to reach a definite conclusion, ANNOVA was carried out to check the efficacy of treatments while the inter-treatment differences were sorted out by using Duncan's Multiple range test (Steel and Torrie, 1996).

RESULTS AND DISCUSSION

Physico-chemical quality of irrigation waters: Table 1 shows the average physico-chemical characteristics of the two irrigation waters. Overall the load of most of the analyzed parameters was much higher for WW than for the GW. The physical characteristics (pH, EC, TDS, TS and TSS) of treated wastewater were in agreement with the recommendations of Food and Agriculture Organization, FAO (Ayers and Wescot, 1985). In terms of chemical quality, high values (with high standard deviation) were observed for WW (Ayers and Wescot, 1985; Pescod, 1992). BOD of WW was in average higher than the 25 mg L^{-1} FAO limit (Pescod, 1992). Clogging (due to high organic content) and pH related risks as well as the negative effect of infiltration and hydraulic conductivity could be considered low for both water sources. In several investigations, Lubello *et al*. (2004) reported the antagonistic effect of high concentrations of ammonia on crop root growth. In the bulk of the soil, ammonia goes through nitrification process. Nitrates indeed migrate into deeper soil layers and can be hazardous for shallow groundwater.

Nutrients supply of irrigation waters: The supply of nutrients through wastewater was not only much higher in comparison to GW but may also fulfil the most of the nutrient requirements of the crop. For example, nitrate nitrogen was 116.67% higher in wastewater as compared to groundwater, however, supply of potassium through GW was not negligible (only 51.6% less than ww). Average NO_3-N was above the limit of 10 mg L^{-1} and average ammonium was in the recommended range (Feigin *et al*., 1991). Hence, it can be concluded that

Table 1: Average physico-chemical characteristics of groundwater and wastewater, all determinations in mg L^{-1}

Determinants	Groundwater	Wastewater	Normal range	Indian standards
Physical characteristics				
Colour		Light black		
Odour		Slightly unpleasant		
pH	7.5	8.2	6.5-8.4[a]	8.5 (max)
Electrical Conductivity (EC) (dS mL^{-1})	0.74	1.32	0.25-3.0[a]	-
Chemical characteristics				
Total dissolved solids (TDS)	542	1421	<2000[a]	2100
Total solids (TS) (g L^{-1})	947	1288	-	-
Total suspended solids (TSS) (g L^{-1})	431	694	-	-
Biological oxygen demand (BOD)	16.75	52.8	<25[b]	-
Chemical oxygen demand (COD)	62.34	145.23	30-160[b]	-
Calcium (Ca^{++})	19.24	42.24	<400[a]	-
Magnesium (Mg^{++})	26	132	<61[a]	-
Carbonate (CO^{3-})	52.13	132.59	-	-
Bicarbonate (HCO^{3-})	84.27	94.22	<610[a]	-
Chloride (Cl^{-})	74.66	130.7	<350[a]	600
Potassium (K^{+})	5	11	<2.0[a]	-
Nitrate Nitrogen (NO_3-N)	0.6	1.3	<10.0[a]	-
Ammonia Nitrogen (NH_4-N)	0.17	4.12	5.0[a]	-
Phosphorus (PO_4^{-})	0.07	0.89	<2.0[a]	-
Cadmium (Cd) (μg L^{-1})	ND	0.012		
Chromium (Cr) (μg L^{-1})	0.011	0.04		
Nickel (Ni) (μg L^{-1})	0.25	0.36		
Lead (Pb) (μg L^{-1})	0.014	0.047		

[a]Ayers and Wescot (1985) (FAO) , [b]Pescod (1992) (FAO)

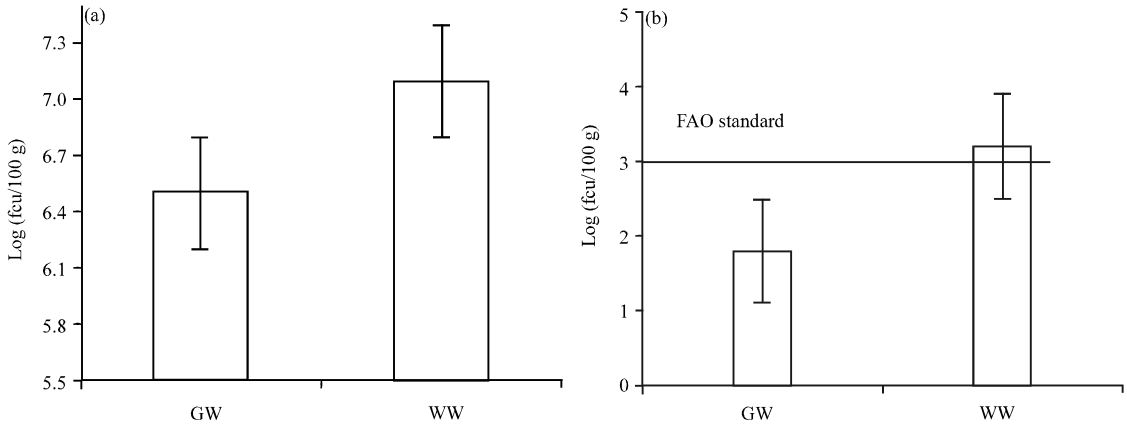

Fig. 1(a-b): Average rate of faecal coliforms in (a) Irrigated soil cropped with okra and (b) Groundwater (GW) and treated wastewater (WW). Error bars denote standard deviation

Table 2: Average physico-chemical characteristics of soil irrigated with groundwater and treated wastewater

Determinants	GW	Treated (ww)
Texture	Sandy loam	Sandy loam
pH	7.74	8.2
Cation Exchange Capacity (CEC) (meq 100 g^{-1}soil)	3.30	3.98
Electrical conductivity (EC) (dS m^{-1})	0.82	0.99
Total dissolved solids (TDS)	779.00	835.00
Calcium (Ca^{++})	29.3	32.06
Magnesium (Mg^{++})	14.62	23.86
Carbonate (CO_3^{-})	18.08	22.16
Bicarbonate (HCO_3^{-})	185	196
Chloride (Cl^{-})	28.79	39.16
Potassium (K^{+})	9.4	12.50
Nitrate nitrogen (NO_3-N) (g kg^{-1}soil)	0.352	0.42
Phosphorus (PO_4^{--}) (g kg^{-1}soil)	0.115	0.28
Cadmium (μg g^{-1})	0.85	1.45
Chromium (μg g^{-1})	27.25	28.55
Nickel (μg g^{-1})	31.64	34.22
Lead (μg g^{-1})	19.22	21.85

All determinations in mg L^{-1} in 1:5 (Soil: Water) extract or as specified

wastewater application was more suitable for irrigation due to its higher nutrition value as compared to the groundwater (Table 1).

Biological quality of irrigation waters: According to WHO recommendations on irrigation water quality (Blumenthal *et al*., 2000; WHO, 2006), the average load of faecal coliforms for crops likely to be eaten uncooked is $\leq 10^3$ faecal coliforms in 100 ml i.e., 3 decimal logarithmic units per 100 mL or log (CFU/100 mL^{-1}), however, the average load of WW was habitually higher than this standard (Fig. 1). In comparison to groundwater, which is free from helminth eggs, the treated wastewater appeared to be having 9.5±11.4 helminth eggs L^{-1} which is higher than the recommended value of the revised WHO standards (less than 0.1 egg L^{-1}) for crops susceptible to be eaten uncooked. However, it is pertinent to point that the fruits of okra were helminth egg free regardless of irrigation water. On the other hand, several workers like, Amahmid *et al*. (1999) and Bouhoum and Amahmid (2002) indicated a strong contamination of the vegetables, in particular the cysts of Giardia and eggs of helminths (Ascaris).

Physico-chemical quality of soil: The data for soil analysis (Table 2) revealed that the soil was sandy loam having a favorable pH (8.2) for root growth and nutrient uptake. Soils, watered with groundwater as well as wastewater presented a contamination in faecal coliforms and helminth eggs. Although groundwater was helminth eggs free, the latter was found in related irrigated soils.

Heavy metal content in irrigation water and soil: For heavy metal content in each irrigant, a total of 12 samples were analysed to obtain their mean values (Table 1). It is evident from the table that concentrations of heavy metals were more in treated wastewater than groundwater. Among the heavy metals, nickel concentration was highest followed by that of lead, chromium and cadmium. But level of all the heavy metals was under permissible limit. It is obvious that the higher heavy metal content in treated wastewater than groundwater will result in their accumulation in the soil; however, their levels are under the permissible limit (Table 2). The heavy metal content was found maximum for nickel, followed by chromium, lead and cadmium. The absence of correlation between the heavy metal content of the treated wastewater or groundwater with that of the soil and plant has clearly demonstrated that the fate of a particular metal in soil might depend upon several factors including the interaction of the metal with the plant (Juste and Mench, 1992) as well as with the organic matter of the soil.

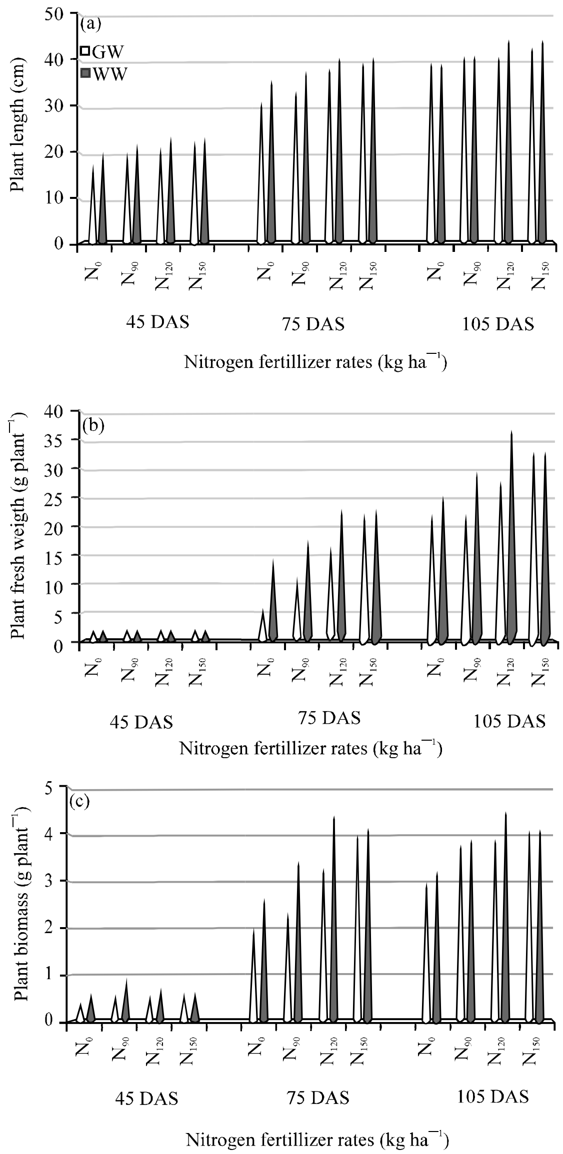

Fig. 2(a-c): Effect of wastewater irrigation and different levels of nitrogen (N) on (a) Plant length (cm), (b) Plant fresh weight (g plant^{-1}) and (c) Plant Biomass (g plant^{-1}) in Okra at 45, 75 and 105 days after sowing

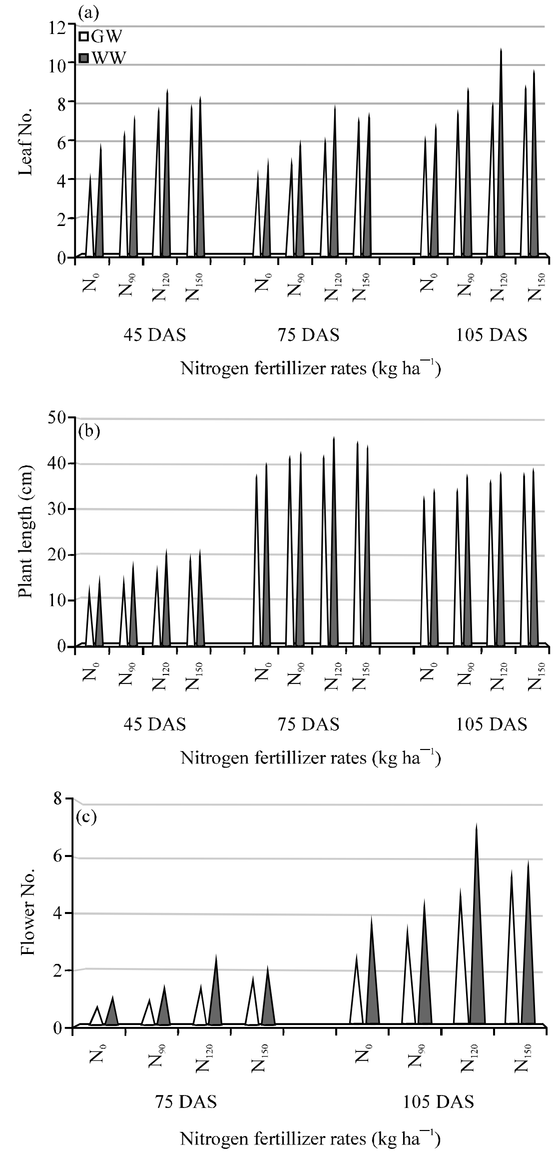

Fig. 3(a-c): Effect of wastewater irrigation and different levels of nitrogen (N) on (a) Leaf number (b) Leaf area (cm^2) and (c) Flower number in Okra at 45, 75 and 105 days after sowing

Effect on plant characteristics: The treated wastewater was found to be beneficial because of the presence of the nutrients which are considered necessary for sustaining the soil fertility as well as for raising the plant growth and productivity (Akponikpe *et al*., 2011). The interaction of 100%WW×N_{120} proved beneficial in increasing the plant growth characteristics in comparison to groundwater (Fig. 2, 3). This may be predicted to be due to the presence of NH_4^+ and NO_3^-, the two ionic forms of nitrogen which is responsible for increasing the number of meristematic cells. Similar reports of the favorable effects on growth are given by many workers (Akhtar *et al*., 2012; Tabassum *et al*., 2007; Javid *et al*., 2006).

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 for the synthesis of the

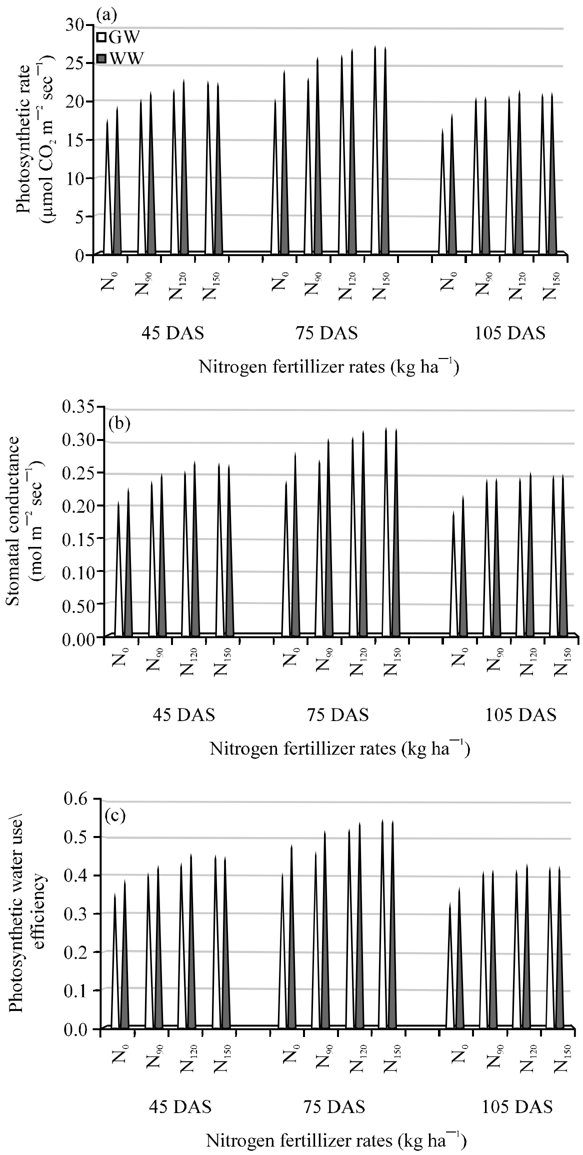

Fig. 4(a-c): Effect of wastewater irrigation and different levels of nitrogen (N) on (a) Photosynthetic rate (µmol CO_2 m^{-2} s^{-1}) (b) Stomatal conductance (mol m^{-2} s^{-1}) and (c) Photosynthetic water use efficiency (WUE) in Okra at 45, 75 and 105 days after sowing

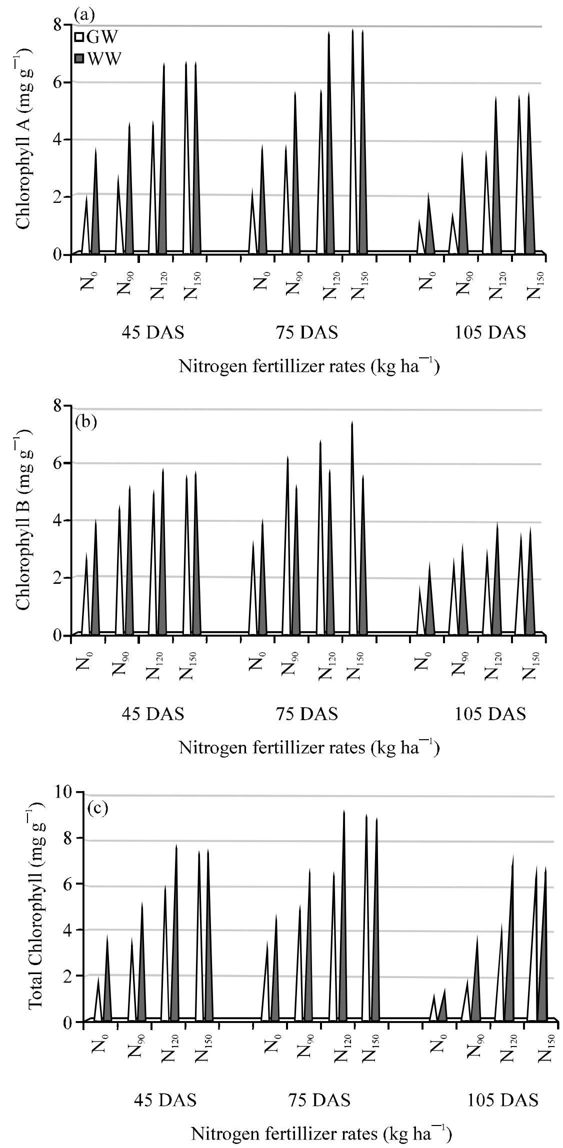

Fig. 5(a-c): Effect of wastewater irrigation and different levels of nitrogen (N) on (a) Chlorophyll A (mg g^{-1}) (b) Chlorophyll B (mg g^{-1}) (c) Total chlorophyll content (mg g^{-1}) in Okra at 45, 75 and 105 days after sowing

photosynthetic apparatus. Maximum chlorophyll content and the best photosynthetic activity with the maximum net photosynthetic rate (P_N), stomatal conductance (g_s) and photosynthetic water use efficiency (WUE) were recorded under 100% WW×N_{120} (Fig. 4, 5). These parameters were found maximum at flowering stage (75 DAS) and decreased subsequently. The treatment of 100%WW×N_{150} creates a toxic level which is sufficient to damage photosynthetic apparatus and a concomitant reduction in photosynthetic activity which is a main driving force for the production of dry matter. Explanation for the increase in dry matter is possibly because of the increase in leaf area and expansion which might have influenced the light absorption within a plant causing stimulation of P_N, g_s and WUE thereby optimizing the CO_2 assimilation and photosynthetic production. The increase in the leaf area brought about by N supply causing the

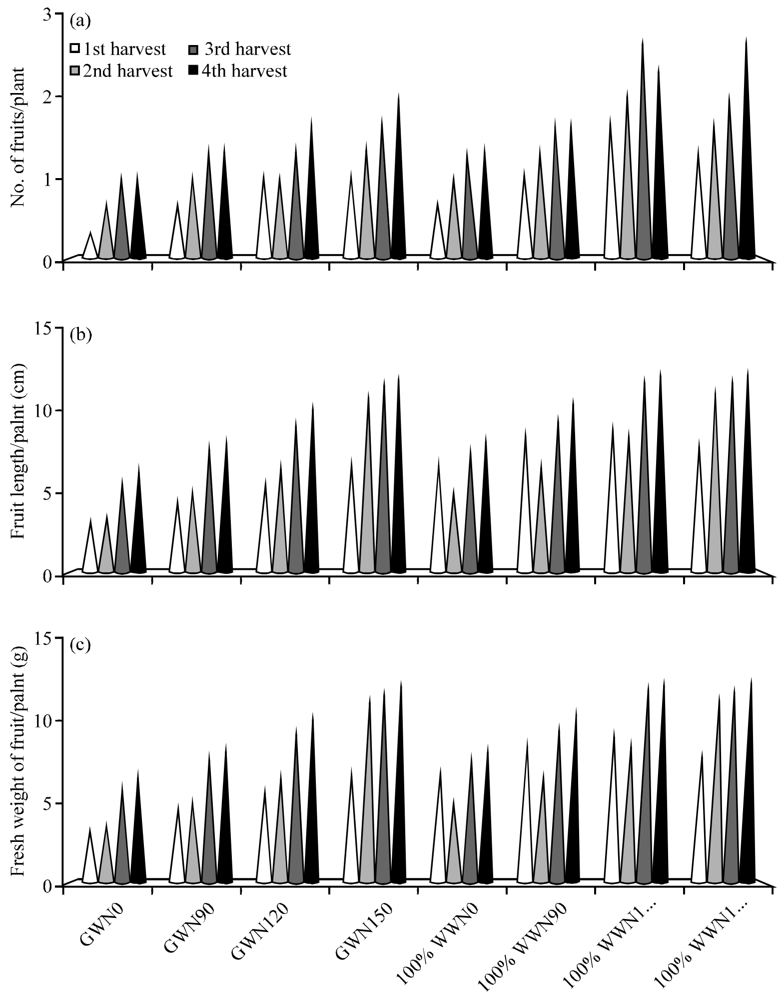

Fig. 6(a-c): Effect of 100% WW and GW along with N doses on (a) No. of fruits plant^{-1} (b) Fruit length plant^{-1} (cm) and (c) Fresh weight of fruit plant^{-1} (g) of okra at four harvestings

expansion of the individual leaves was also reported by Gastal and Lemaire (2002) and Taylor *et al.* (1993) which may be through its effect on cell division and cell expansion (Lemaire, 2001).

The maximum chlorophyll a, chlorophyll b and total chlorophyll content were observed in plants receiving 100% wastewater as a source of irrigation water over groundwater at 45, 75 and 105 DAS respectively (Fig. 5). Nitrogen treatments also affected significantly with N_{120} proved to be optimum, N_{90} deficient while N_{150} proved toxic. Increase in chlorophyll content was recorded from vegetative (45 DAS) to flowering (75 DAS) and then decline was observed at the last sampling stage (105 DAS). This is possibly due to the role of nitrogen in cell division, elongation, expansion and differentiation and also in biochemical reactions (Gardner *et al.*, 1985), leading to increased growth and leaf area and thus allowing the plant to trap maximum solar energy and enhancing the photosynthetic rate for bio-mass production.

For yield attributing characteristics, it was noted that wastewater proved superior as it out yielded all other treatments. Plants grown with wastewater recorded higher values for number of fruits plant^{-1}, fruit length and fresh weight of fruit (Fig. 6). All yield parameters were significantly affected by the application of fertilizers among which N_{120} proved best for increasing fruit yield. Overall the combinations of 100%WW×N_{120} proved best in case of growth, physiology and yield of the plant at all the growth stages. As photosynthesis is the main driving force for the production of dry matter, plant dry matter was increased as a result of an enhanced leaf area which provided a larger surface for the interception of solar radiation and thus the higher photosynthetic rate, thereby

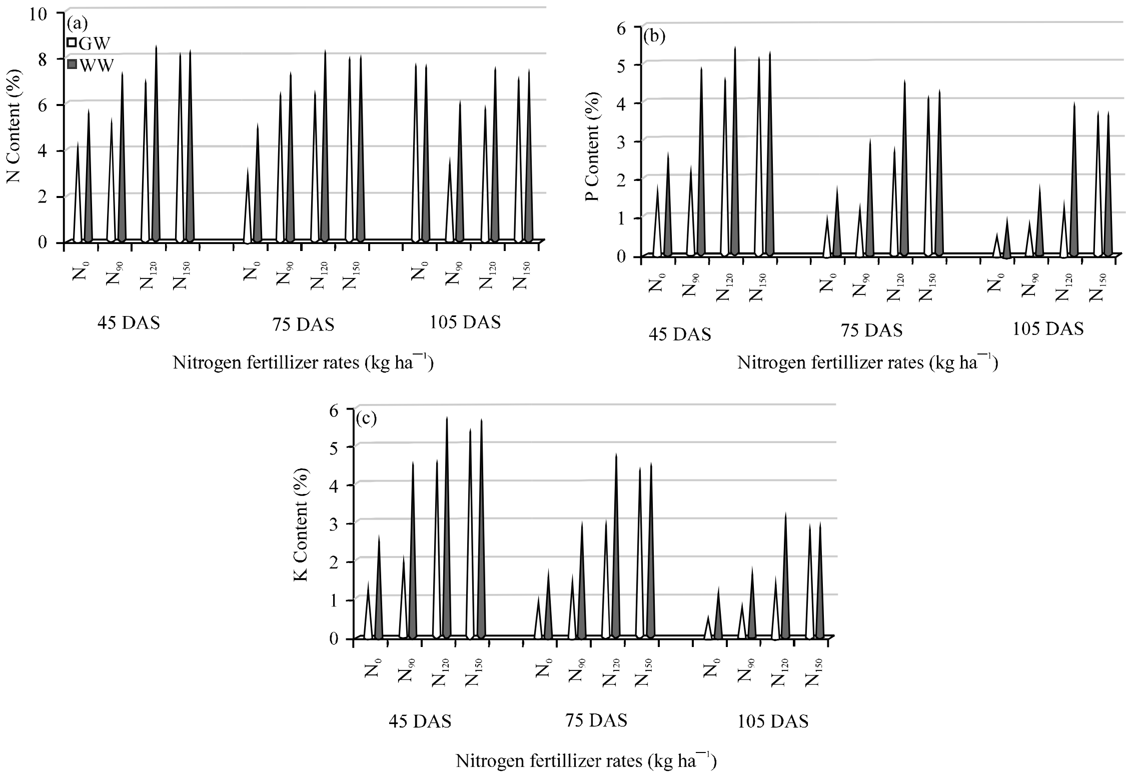

Fig. 7(a-c): Effect of 100% WW and GW along with N doses on (a) Nitrogen content (%), (b) Phosphorus content (%) and (c) Potassium content (%) of Okra at 45, 75 and 105 days after sowing

improving most yield attributes including number of fruits, fruit length and fresh weight as compared to control. The increase in the yield of wheat, barley, oat and mustard characteristics due to wastewater application has been also reported by Akhtar *et al*. (2012), Akhtar *et al*. (2008) and Tabassum *et al*. (2007). The same effect was found in several vegetables by Bouhoum and Amahmid (2002).

Leaf NPK contents were noted to be enhanced as a result of irrigation with wastewater, like the earlier reports of Tabassum *et al*. (2007), Shah *et al*. (2004) and Javid *et al*. (2003) for other plants. This could be due to the additional nutrients present in wastewater which cause the development of the larger leaf area and ultimately leads to extract more nutrients and water (Javid, 2002). The crop was fertilized with urea which is readily converted to NO_3^- and thus remains as an available form of nitrogen in soil which results in higher N uptake by roots and finally leads to higher concentrations in leaves (Fig. 7). While the increase in leaf P and K content may be explained to be due to the synergistic interplay of these nutrients which are known to accelerate root proliferation, thus, extracts more nutrients present near the root zone leading to higher dry matter (Fig. 2, 7). Synergistic interaction of N and P was reported by Russell (1973) and between N and K by Murphy (1980).

The constant availability of other essential nutrients through wastewater also played beneficial role in the growth and productivity of the okra crop (Table 1). Phosphorus, the backbone of the RNA and DNA structures, also aids in root development, flower initiation and seed and fruit development. Potassium being an enzyme activator promotes metabolism and it is vital for plant growth and also controlling the opening and closing of leaf stomata. Calcium plays a major role in the formation of cell wall membrane and its plasticity and indirectly assists in improving crop yields by reducing soil acidity when soils are limed. Magnesium is a major content of chlorophyll molecule and therefore, actively involved in photosynthesis, hence, enhancing overall growth and yield of wastewater irrigated crop plants (Uchida, 2000; Mengel and Kirkby, 1996).

Heavy metal content in seeds of the plant, irrigated with treated wastewater and groundwater are presented in Fig. 8. It is evident that accumulation of Cd and Ni are recorded in the seeds of plants grown and it is, however, noteworthy that all values for heavy metals were below the permissible limits (Annan *et al*., 2010).

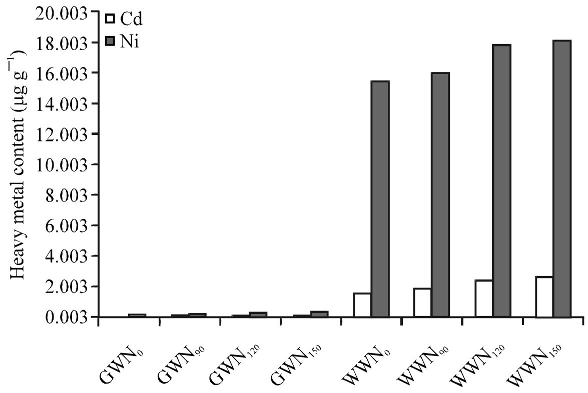

Fig. 8: Heavy metal content (µg g^{-1}) in seeds of okra irrigated with 100% WW and GW along with N doses

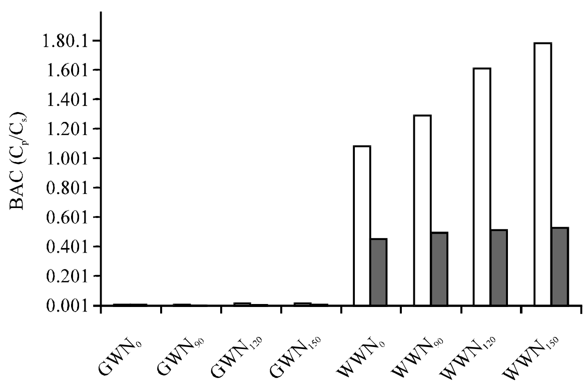

Fig. 9: Biological absorption coefficient (BAC = C_P/C_S) of Okra (C_P is concentration in plants; C_S is concentration in related soil)

In order to determine the influence of heavy metal levels in soils on uptake by plants irrigated with groundwater and treated wastewater, the biological absorption coefficient (BAC) was calculated and plotted in Fig. 9. The BAC is the ratio of the element concentration in the crop to that in soil (Kabata-Pendias and Pendias, 1984).

CONCLUSION AND RECOMMENDATION:

During the experiments, we used treated wastewater and groundwater as control, in combination with or without mineral fertilizer application to evaluate the physico-chemical and biological risks on irrigated soils and treated wastewater, the nutrients supply and the effect on okra production. We found that:

- The physico-chemical quality of treated wastewater was acceptable whereas bacteriological quality (faecal coliforms) and helminths quality were not satisfactory according to the directives of FAO and WHO on the quality of the water intended for irrigation of crops to be consumed uncooked
- The reuse of treated wastewater to irrigate okra significantly improved (less than fertilizer) okra yield compared to that of the groundwater
- As the nutrients N, P_2O_5 and K_2O have been already present in the treated wastewater but their supply did not satisfy the whole crop need during the experiment, therefore, we recommend that moderate N, P_2O_5 and K_2O fertilizer be applied in the soil when irrigating with wastewater
- Present results clearly show that treated wastewater can be used as both as a nutrient source and crop water supply in the areas of freshwater shortage. However, vegetable consumers should properly cook or disinfect before eating and farmers should avoid the direct contact with treated wastewater because of the remaining biological risks

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