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Research article

Nitrogen level affects growth and reactive oxygen scavenging of fenugreek irrigated with wastewater

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Abstract: Today, due to the constraint in availability of the freshwater for irrigation, wastewater is being used for irrigation of agriculture fields. Wastewater contain plant nutrients that favour crop growth but leave a burden of heavy metals which can enter the food chain and is a cause of great concern. This research work aimed at evaluating the potential utilization of wastewater as an alternative source of water and nitrogen (N) for fenugreek. For this purpose, a pot experiment was conducted with wastewater and four doses of nitrogen (0, 20, 40 and 60 $kg.ha^{-1}$) to observe a comparative effect on growth and biochemical characteristics using fenugreek as the test crop. Wastewater and soil used in the experiment were analyzed for their physicochemical properties. Results indicate that wastewater was richer in essential plant nutrients but contained some heavy metals in amounts well below the permissible limits for its use as irrigation water. Use of wastewater with 20 $kg.N.ha^{-1}$ improved growth, proline and antioxidant enzymes of the plant as compared to application of groundwater with no fertilizer. Lipid peroxidation increased with wastewater but decreased with the increasing nitrogen doses, so the best combination ($WW \times N_{20}$) has negative impact on this parameter. These results concluded that wastewater can efficiently substitute groundwater and also works as a nutrient source whereas application of nitrogen could provide protection against the oxidative stress by increasing the antioxidant protective system.
Keywords: *Trigonella foenum-graecum* - Proline - Lipid peroxidation - Catalase - Peroxidase - Superoxide dismutase.

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INTRODUCTION

In many parts of the world freshwater resources is scarce, and there is with gradual destruction and increased pollution of fresh water resources, requiring alternatives to supply water for agriculture need to be considered (Yoshida & Ishii 1993). One solution to insufficient water supplies is water reuse. Sewage water for irrigation is a way to dispose of sewage; provide the reliability of large volumes of water for irrigation; to reduce discharge into potable water bodies; serve as an alternative source to chemical fertilizers, provide organic matter; improve soil infiltration capacity, permeability, cation and anion exchange capacities, water holding capacity and texture (Schneider & Erickson 1972, FAO 1992, Avemelech 1993, Brady & Weil 1999, Van der Hoek *et al.* 2002). However, there is a concern about contamination and bioaccumulation in plants of potentially toxic elements such as chromium (Cr), lead (Pb), nickel (Ni), cadmium (Cd), copper (Cu) and zinc (Zn) from domestic and industrial sources (Chen & Chia 2002, Kiziloglu *et al.* 2007).

In urban areas, especially in developing countries, raw wastewater for irrigation is used to grow vegetables for nearby urban markets (Lucho-Constantino *et al.* 2005). If the wastewater is used after proper dilution for irrigation hazards of heavy metals can be minimized (Pathak *et al.* 1999). The water used in the proposed research work has already been diluted from the source (Tak *et al.* 2012, Akhtar *et al.* 2012, Iqbal *et al.* 2012, 2017, Chalkoo *et al.* 2014, Sahay *et al.* 2015) and the contents of all the heavy metals except Ni were well within the permissible limits of Awasthi (2000) (Table 1). The presence of pathogenic microorganisms in wastewater poses an additional health-hazard. In order to avoid this problem, side-on-soil-surface watering was

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used with watering can and practiced in pots to avoid direct contact with crop foliage. However, two weeks before the harvest, this technique was replaced by direct watering on the foliage (Cisse 1997, Akponikpe *et al.* 2011). Wastewater provides variable nutrients and improves attributes of the plant compared to fresh water, but the nutrient concentrations are not sufficient to fulfill requirements of the crop. External inputs need should be guided by the efficiency of their use, as the use of fertilizers beyond limits, in addition to being uneconomical, is potentially harmful to the environment by leaching through the soil beyond the root zone, eventually reaching ground water, or through surface runoff be deposited into nearby water bodies and cause eutrophication.

Table 1. Physicochemical characteristics of ground water (GW) and 100% wastewater (100%WW). All determinations in $mg.l^{-1}$ or as specified.

Determinations	GW	WW	FAO acceptable level
pH	7.30	8.00	6.5–8.4[a]
EC (µ mhos cm^{-1})	700.00	1310.00	0.25–3.0[a]
TS	955.00	1550.00	2200[c]
TDS	560.00	1060.00	<2000[a]
TSS	400.00	576.00	-
BOD	16.25	105.55	<25[b]
COD	65.45	150.25	30-160[b]
NO_3-N	0.83	2.75	<10.0[a]
NH_4-N	0.11	4.45	5.0[a]
Na^+	20.56	59.35	-
K^+	4.94	20.44	<2.0[a]
Ca^{++}	15.45	52.35	<400[a]
Mg^{++}	27.35	118.72	<61[a]
Cl^-	55.25	113.22	<350[a]
CO_3^{--}	54.54	147.74	-
HCO_3^-	105.33	80.48	<610[a]
SO_4^{--}	30.86	88.95	-
PO_4^{---}	0.36	1.24	<2.0[a]
Cu	-	0.209	0.20[d]
Cd	-	0.009	0.01[d]
Ni	-	0.331	0.20[d]
Zn	-	0.153	2.0[d]
Cr	-	0.039	0.10[d]
Pb	-	0.028	5.0[d]

Note: [a]Ayers and Westcot, (1994); [b]Pescod, (1992); [c]Indian Standard Institution (1974); [d]Awasthi, (2000).

In India, vegetable consumption is many times more than the European countries due to the vegetarian food habit of a large proportion of the population, and the lower cost of the common vegetables compared to meat, fish, egg and milk products. It is also important that, compared to other crops, many vegetables can be grown throughout the year and be marketed regularly, due to a short life-cycle.

Fenugreek (*Trigonella foenum-graecum* L.) is used fresh or dried, as spice (seeds) and vegetables (fresh leaves, sprouts and microgreens). Fenugreek leaves contain calcium, phosphorus and iron. Seeds are bitter in taste and have been known over 2500 years for their medicinal qualities (chronic dysentery, diarrhoea, chronic cough, enlargement of liver and spleen).The seeds contain the steroidal substance “diosgenin” which is used as a starting material in the synthesis of sex hormone as oral contraceptive. The study examined application of urban wastewater for irrigation and its effect on Fenugreek supplemented with nitrogen.

MATERIALS AND METHODS

Wastewater (WW) was collected in 50 L Jerry cans for watering the pots, as and when required, from the drain running along the Aligarh Mathura road, 6 km away from the Department. The water analyzed at the start and end of each experiment. Tap water was used as the control (GW) pumped directly from under the earth’s surface and stored in syntax water tanks. The water types were analyzed for various physicochemical characteristics (Table 1) following standard procedures (APHA 1998). Determination of heavy metals in the wastewater samples was with an Atomic Absorption Spectrophotometer (SENSAA GBC Avanta var. 2.02, Aligarh, India) according to the method of Ademoroti (1996). Microbiological examination of wastewater was conducted in the Microbiology Laboratory, Department of Agricultural Microbiology, Aligarh Muslim University, Aligarh, following quality guidelines of WHO (1989) and FAO (1992).

Before sowing, samples of the sandy loam soil were randomly accumulated from each pot, air-dried, mixed well, ground in a mortar and pestle, and passed through a 2 mm sieve before analysis. Soil pH and electrical conductivity were determined following the procedure of Jackson (1958). Organic matter was measured using the method described by Walkley & Black (1934). Estimation of carbonates and bicarbonates was followed Richards (1954). Nitrate nitrogen was estimated according to Ghosh *et al.* (1983). Phosphorus was measured following Olsen *et al.* (1954); potassium was estimated following Jackson (1958). Soil cation exchange capacity was determined following Ganguly (1951). Soil heavy metals were determined following Lindsey & Norwell (1978).

The experiment was conducted in the November 2010 to March 2011. The experiment was a completely randomized design replicated 3 times with main treatment of 100% urban wastewater (WW) and groundwater (GW). Doses of NPK fertilizer ($N_0P_0K_0$, $N_{20}P_{30}K_{30}$, $N_{40}P_{30}K_{30}$ and $N_{60}P_{30}K_{30}$) were mixed into the soil with the fertilizers from urea, single super phosphate and muriate of potash.

Effects of treatment were determined by measurement of plant: length, fresh and dry weights, numbers of leaves, leaf area, leaf fresh and dry weights, proline content, malondialdehyde content and enzymatic antioxidants (catalase, peroxidase and superoxide dismutase) at vegetative, flowering and fruiting stages at 30, 60 and 90 DAS, respectively. Plants were uprooted and lengths of the main tap root and shoot were measured. To assess dry mass, plants were dried for about 72 hrs in a hot air oven (forced air oven) maintained at 80°C and then weighed. After separating all the leaves at the petiole and stem junction, leaf area was measured using a leaf area meter (LA 211, Systronics, India). Proline content in fresh leaves was estimated following the procedure of Bates *et al.* (1973). The level of lipid peroxidation products in leaves was determined as malondialdehyde content by a modified version of the method described by Cakmak & Horst (1991). Catalase activity (EC 1.11.1.6) was measured according to Aebi (1984). SOD activity (EC 1.15.1.1) was estimated by recording the decrease in absorbance of superoxide nitroblue tetrazolium complex by the enzyme (Sen Gupta *et al.* 1993). The method of Bergmeyer *et al.* (1974) was used to measure oxidation of pyrogallol to purpurogallin by peroxidase.

The data were analyzed according to Panse & Sukhatme (1985) using a two-way analysis of variance after Steel & Torrie (1962) SPSS (ver. 11.0, Chicago, IL). If interactions were significant they were used to explain results using Duncan Multiple Range Test (Duncan 1955).

RESULTS

The physicochemical and microbiological properties of water types varied (Table 1). Electrical conductivity, total solids, total dissolved solids, BOD, COD, nitrate nitrogen, ammonia nitrogen, sodium, calcium, magnesium, chlorine, carbonate, sulphate and phosphate had higher values in wastewater than in groundwater (Table 1). Contents of Cu, Cd, Zn, Cr and Pb were within permissible limits; the Ni content exceeded permissible limits. The presence of microorganisms in wastewater from highest to lowest was faecal coliforms (7.2×10^2 cfu/100 mL), total heterotrophic bacteria (2.83×10^6 cfu/100 mL), total coliforms (1.9×10^3 cfu/100 mL) and *Salmonella-Shigella* sp. (1.3×10^2 cfu 100/mL).

Soil characteristics prior to the study and after the study varied (Table 2). The pH of the pre-sowing soil was desirable in agricultural soil. In pots irrigated with wastewater, the pH of soil extract decreased from before sowing to after harvesting. The organic carbon of wastewater irrigation was higher than well water and soil irrigated with wastewater had higher organic carbon than pre-sowing soil. This indicates that wastewater irrigation helps improve fertility after crop harvest. Wastewater provided essential nutrients to the soil and the crop because wastewater irrigated soil had more nitrogen, phosphorus and potassium than pre-sowing soil. Concentrations of heavy metals were within permissible limits (Awasthi 2000).

The wastewater and nitrogen combination, $WW\times N_{20}$, was most effective as it produced the longest plants, higher plant fresh and dry weights, more leaves, higher leaf area, and higher leaf fresh and dry weight at the 3 growth stages (Figs. 1, 2 & 3). There was an increase of 65.57% at 30 DAS, 35.06% at 60 DAS and 35.03% at 90 DAS in plant length over the $GW\times N_0$. For plant fresh weight, interactions were significant only at fruiting stage, where WW×N_{20} and WW×N_{40} had similar values with increases of 35.14 and 29.13% over GW×N_0, respectively. The best combination increased 54.89, 52.13 and 51.81% in plant dry weight over GW×N_0 at the 3 growth stages. Among interactions, WW×N_{20} increased leaf number by 35.16, 35.58 and 35.53% over GW×N_0 at the successive stages. For leaf area, WW×N_{20}, produced increases of 34.95, 35.29 and 35.37% over GW×N_0 at the 3 growth stages, respectively. Interactions were significant at vegetative and flowering stages in leaf fresh weight. The WW×N_{20} had the best causing increases of 40.27 and 39.13% over GW×N_0 at the vegetative and flowering stages, respectively. This optimum combination recorded an increase of 39.2, 35.95 and 37.06% in leaf dry weight over GW×N_0 at the 3 growth stages, respectively. The combinations WW×N_{20} and WW×N_{40} were similar. The WW×N_0 combination was similar with GW×N_{20}, GW×N_{40} and GW×N_{60}. The linear regression curves worked out for plant height with leaf number and leaf area with leaf fresh weight favour the present findings (Fig. 4).

Table 2. Physicochemical characteristics of soil collected before sowing. All determinations in mg.l^{-1} in 1:5 (soil-water extract) or as specified.

Determinations	**Pre-sowing soil**	**After harvest (soil layer irrigated with wastewater)**	**Normal Range**
Texture	Sandy Loam	Sandy Loam	-
CEC (meq 100 g^{-1} soil)	3.32	3.92	-
pH	7.74	6.85	-
Organic Carbon (%)	0.419	1.15	-
EC (µ mhos cm^2)	242	-	-
TDS	758	-	-
NO_3^-N (g kg^{-1} soil)	0.352	0.283	-
Phosphorus (g kg^{-1} soil)	0.109	0.151	-
Potassium	8.2	11.6	-
Calcium	27.14	23.89	-
Magnesium	16.49	12.94	-
Chloride	29.47	-	-
Carbonate	19.34	-	-
Bicarbonate	92.43	-	-
Sodium	14.11	15.02	-
Sulphate	17.32		-
Cu	-	1.11	135–270
Cd	-	0.22	3–6
Ni	-	0.75	75–150
Zn	-	2.09	300–600
Cr	-	0.053	-
Pb	-	0.72	250–500

Proline content increased with increasing nitrogen dose as well as with wastewater; the WW×N_{60} interaction caused an increase of 24.09, 27.27 and 22.84% over GW×N_0 at 3 stages, respectively. Wastewater treated plants had increased malondialdehyde content over GW irrigated plant. However, malondialdehyde content declined with increase in nitrogen. Among combinations, WW×N_0 and WW×N_{20} and WW×N_{40} were best and similar (Fig. 5).

Wastewater irrigation increased the activity of catalase, peroxidase and superoxide dismutase. Nitrogen dose also positively influenced the enzyme activity with most activity under N_{60}. The WW×N_{60} combination produced increases of 25.19, 20.74 and 22.58% in catalase over GW×N_0 at the 3 samplings, respectively. However, this combination increased peroxidase by 24.56% at the vegetative stage, and 20.04 and 18.91% in superoxide dismutase at vegetative and flowering stages over GW×N_0, respectively. The interactions were not significant at flowering and fruiting stages for peroxidase but were significant at the flowering stage for superoxide dismutase (Fig. 6). The proline content and antioxidant enzymes increased with the combination of WW×N_{60}. The proline content was positively correlated with CAT (r^2=0.9479), POD (r^2=0.8991) and SOD (r^2=0.9056) (Fig. 7).

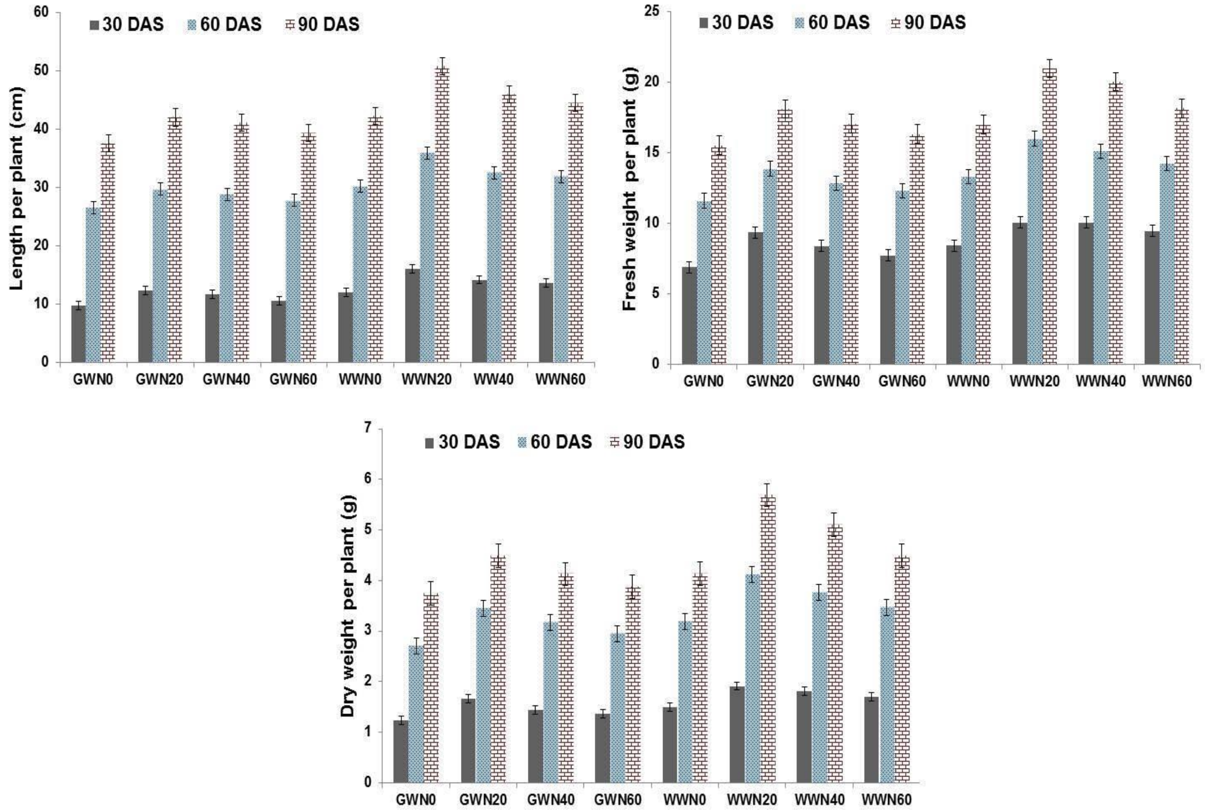

Figure 1. Effect of waste water on length, fresh weight and dry weight/plant of fenugreek supplemented with different nitrogen at 30, 60 and 90 DAS.

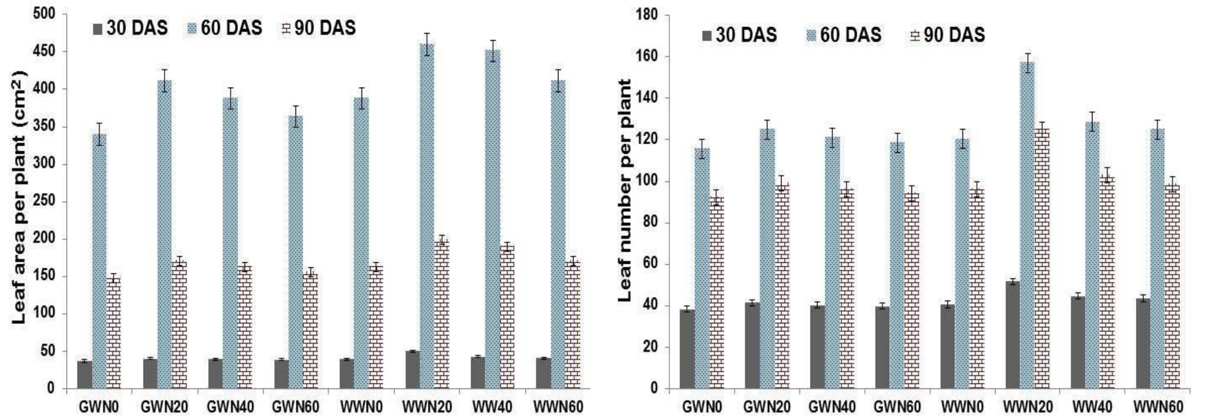

Figure 2. Effect of waste water on leaf number and leaf area/plant of fenugreek supplemented with nitrogen at 30, 60 and 90 DAS.

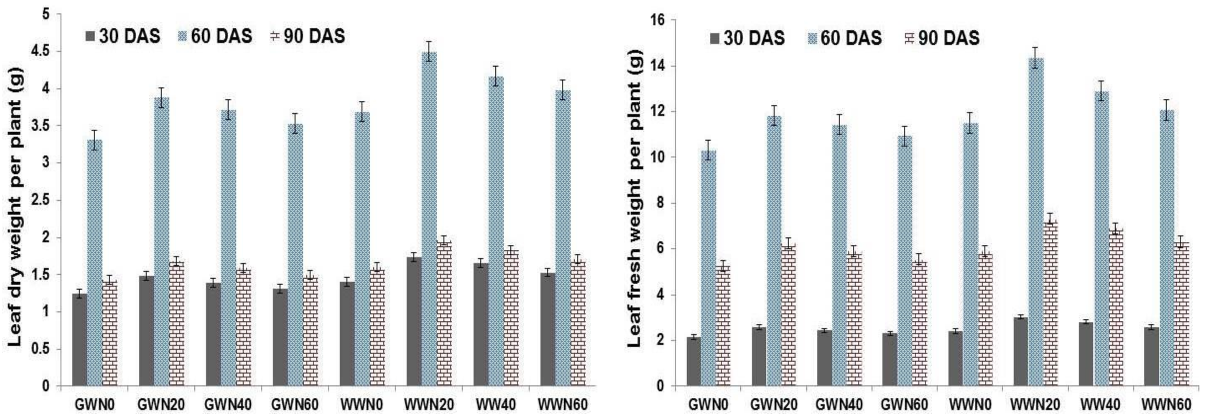

Figure 3. Effect of waste water on leaf fresh weight and leaf dry weight/plant of fenugreek supplemented with nitrogen levels at 30, 60 and 90 DAS (at 30 DAS, leaf dry weight×10).

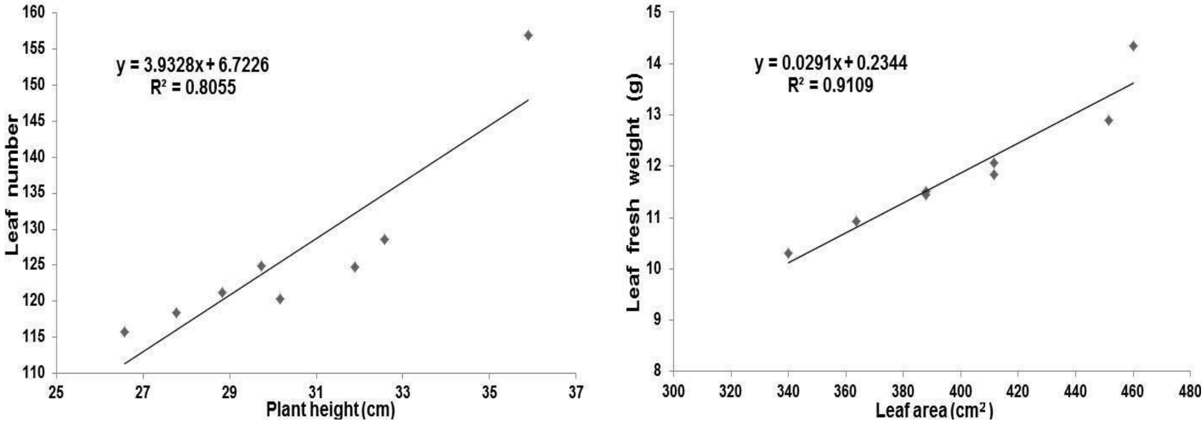

Figure 4. Linear regression curve of fenugreek showing correlation of plant height with leaf number and leaf area with leaf fresh weight at 60 DAS.

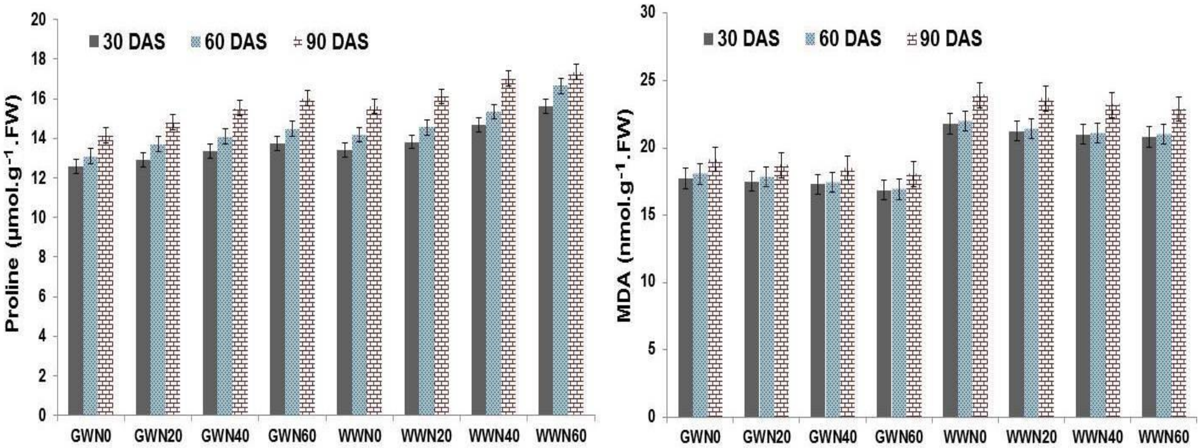

Figure 5. Effect of waste water on proline and malondialdehyde contents of fenugreek supplemented with nitrogen at 30, 60 and 90 DAS.

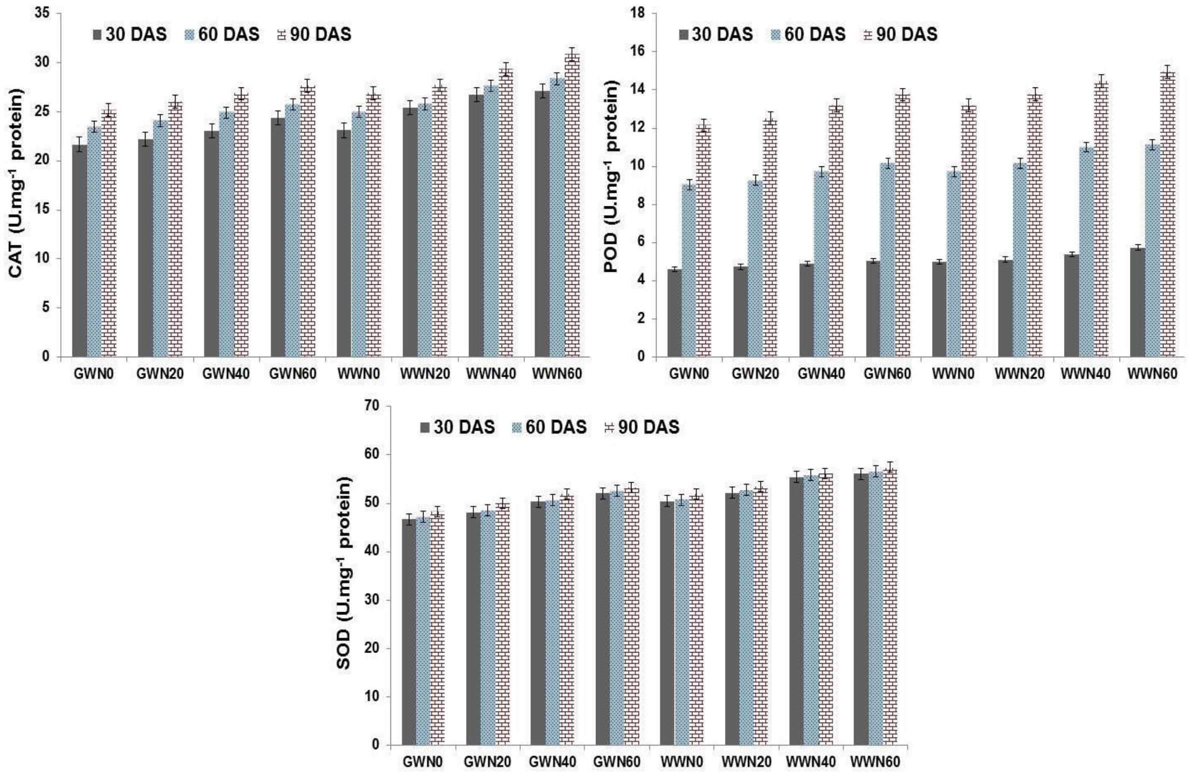

Figure 6. Effect of waste water on catalase, peroxidase and superoxide dismutase activity of fenugreek supplemented with nitrogen.

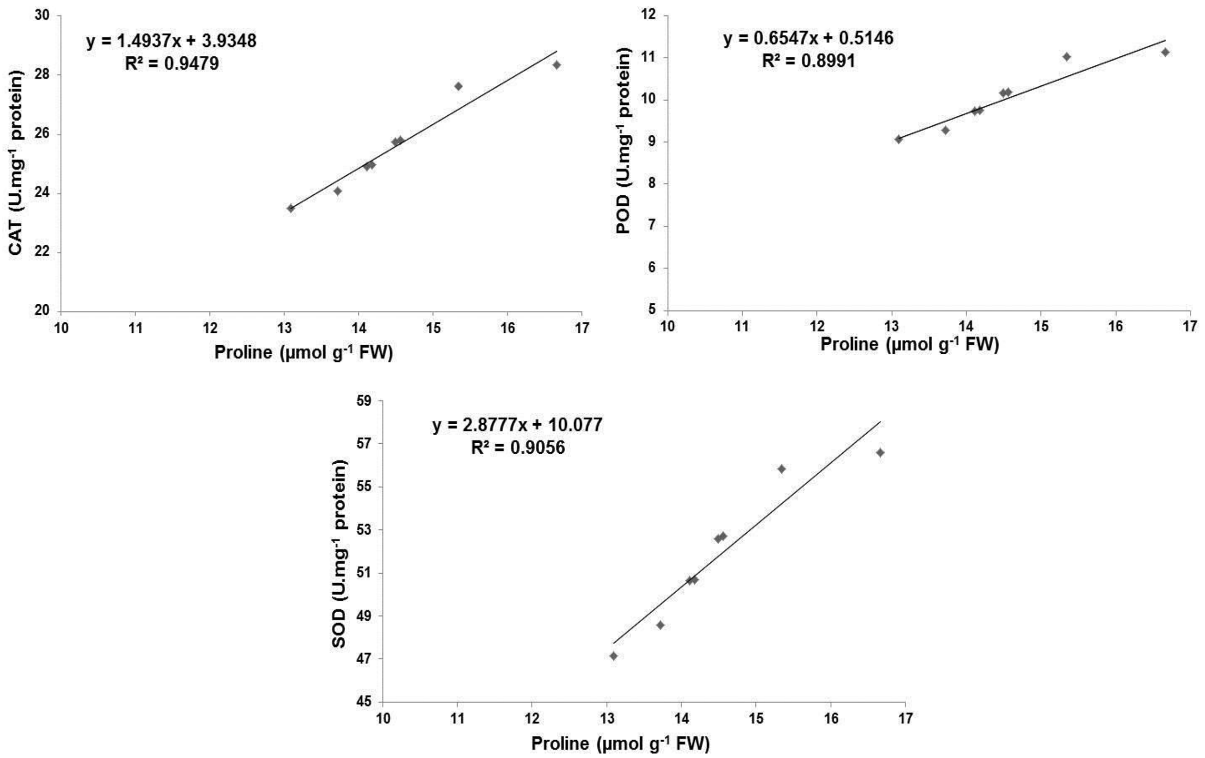

Figure 7. Linear regression curve of fenugreek showing correlation of proline with catalase, peroxidase and superoxide dismutase at 60 DAS.

DISCUSSION

The maximum values of various parameters were recorded with 100% concentration of wastewater which was black in color. The higher value of TDS is associated with higher EC of wastewater (Bhargava *et al.* 2008). The Cl^-, CO_3^{-2} and HCO_3^- were within the permissible limits (ISI 1974, Pescod 1992, Ayers & Westcot 1994). High BOD, COD, NO_3^- and PO_4^{-3} might be due to the presence of high oxidizable organic matter and rapid consumption of dissolved inorganic materials (Biswas *et al.* 2009, Kumar & Chopra 2012). The TSS content contributed to high salinity. Heavy metals at high levels in water bodies could cause toxicity to aquatic life (Zyadah & Abdul-Bakky 2000, Indra & Sivaji 2006) and buildup in river sediments (Singh *et al.* 1997). Total bacterial count and most probable number in wastewater are likely due to higher organic load of wastewater (Kumar & Chopra 2012). The pH is an important parameter as many nutrients are available for plant uptake only at a particular pH range. A pH value of 6.0–8.2 supports bacterial activity and is favourable for maximum yield. A shift in pH outside that range renders nutrients less available, even though they remain in the soil. Under acidic conditions, iron, aluminium, manganese, and heavy metals become highly soluble and may create problems for plants (Charman & Murphy 1991). The higher concentration of Na in the soil after effluent irrigation may be associated with the presence of high concentration of carbonate and bicarbonate in the effluent (Thompson *et al.* 2001). Reduction in water infiltration can occur when irrigation water contains high sodium relative to the calcium and magnesium content. This condition is called sodicity which reduces soil permeability (Kelley 1951). The overall increase in nitrogen is likely due to the nitrate and ammonium ions in wastewater. Irrigation with wastewater generally adds significant quantities of salts to the soil.

The beneficial effect on growth may be attributed to the nutrients present in wastewater (Table 1). However, reduced growth at higher concentrations may be due to the accumulation of salt which causes increased electrical conductivity (EC) with a link between EC and reduced plant growth (Kumar & Chopra 2012). The source of wastewater was diluted urban sewage which appears to be beneficial for the development of fenugreek because properly diluted wastewater can minimize the hazards of heavy metals (Pathak *et al.* 1999). Use of diluted wastewater from the same source produced beneficial effects on *Triticosecale* Wittm. (Shah *et al.* 2005), *Lens culinaris* Medikus (Tabassum *et al.* 2007a), *Brassica juncea* L. (Tabassum *et al.* 2007b), *Abelmoschus esculentus* (L.) Moench (Kausar 2009, Faizan *et al.* 2014, Kausar & Faizan 2015), *Cicer arietinum* L. (Tak *et al.* 2010, 2012, 2013), *Triticum aestivum* L. (Akhtar *et al.* 2012), *Capsicum annuum* L. (Iqbal *et al.* 2012, Chalkoo *et al.* 2014, Iqbal *et al.* 2015).

Improvement in growth might be due to the presence of nitrogen. Fenugreek responded better to relatively low nitrogen dose because it is a leguminous crop and able to fulfill nitrogen needs by symbiotic nitrogen fixation. However, the process of symbiosis takes time to establish and became fully operative and the nitrogen accumulated in this manner is only available to the soil when the legume plant dies. During early plant growth, a low starter dose of nitrogen is necessary because higher doses hinder nodule formation (Mann 1968, Arrese-Igor *et al.* 1997, Ram & Verma 2001, Shahroz 2009). Other reports concerning nitrogen application on the same crop differ (Chaudhary 1999a, b, Sharma 2000, Thapa & Maity 2003, Datta *et al.* 2005, Tuncturk *et al.* 2011) where comparatively higher rates of nitrogen were reported.

Nitrogen is an important nutrient required in large amounts by plants. It is an integral constituent of proteins, nucleic acids, chlorophyll, co-enzymes, phytohormones and secondary metabolites. The availability of N to roots is therefore a decisive factor for plant growth (Marschner 2012). The urban wastewater used for irrigation contained nitrogen in the ionic forms, NH_4^+ and NO_3^- and further supplementation of nitrogen in the form of urea yielded nitrate rapidly within a few days which is absorbed by plants possibly contributing to a cumulative effect on growth (James 2010).

Proline is an amino acid that easily accumulates in plants under stress and is a marker of imbalance in a metabolic pathway. It also functions as an osmolyte to alleviate damage caused by biotic or abiotic stresses (Ashraf & Foolad 2007, Chandra *et al.* 2009, Myriam *et al.* 2009, Bedouh & Bekhouche 2012, Kumar *et al.* 2012a, b). Resistant plants accumulate more proline than less resistant plants (Naidu *et al.* 1992). Accumulation of proline preserves the structure and activity of protein, reduces enzyme denaturation and protects biomembranes from damage by inactivating hydroxyl radicals or other reactive chemical species (Pollard & Wyn Jones 1979, Smirnoff & Cumbes 1989, Saradhi *et al.* 1995). Proline accumulates in plants exposed to heavy metal stress (Zhao 2011, Bauddh & Singh 2012a, b). Fenugreek irrigated with wastewater exhibited increase in the proline content. The wastewater contained heavy metals that cause stress to plants likely increasing proline content. Heavy metal induced proline accumulation has been reported (Lalk & Dorfling 1985, Bhattacharjree & Mukherjee 1994, De & Mukherjee 1998. Handique & Handique 2009, Kumar *et al.* 2015). Proline accumulation helps the plant to withstand the heavy metal stress by protecting the key enzyme such as glucose-6-phosphate dehydrogenase and nitrate reductase from being inactivated by toxic heavy metal ions by forming metal-proline complex (Handique & Handique 2009). In this study, proline content increased with increased nitrogen application. This may be because it is a nitrogen-storage compound (Ahmad & Hellebust 1988) and that synthesis and accumulation of proline are stimulated by nitrogen supply (Sanchez *et al.* 2002). This result is in agreement with the findings of many workers (Naidoo & Naidoo 2001, Sanchez *et al.* 2001, 2002, Zhao & Liu 2009, Ahmadi *et al.* 2010, Wang *et al.* 2011).

Malondialdehyde content (MDA) or thiobarbituric acid reactive substances (TBARS) is the decomposition product of polyunsaturated fatty acids of biomembranes obtained when reactive oxygen species (ROS) initiates the process of lipid peroxidation, which is considered an indication of oxidative stress in plants (Gosset *et al.* 1994, Blokhina *et al.* 2003). Production of the ROS superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) increases as a result of abiotic stress such as can occur with heavy metal toxicity, salinity and drought in plants. Under normal conditions, these ROS are well regulated by cell metabolism but under abiotic stress, they may exceed the scavenging capacity of the antioxidant system. The wastewater contained heavy metals which may enhance the level of H_2O_2 which can result in the formation of hydroxyl radicals that can cause lipid peroxidation (Loggini *et al.* 1999). Oxidative damage to bio-membranes of plants can be primarily attributed to the presence of Cd, a redox active metal catalyzing the generation of hydroxyl radicals and O_2^- (Arora *et al.* 2002). The non-redox metals Zn, Ni, Cr, Cu and Pb do not produce ROS directly but generate oxidative stress by interfering with the plant's antioxidant defence system (Garnczarska & Ratajczak 2000, Aravind & Prasad 2003, Panda & Choudhary 2005). These metals might have contributed to increased MDA levels (Olorunfemi & Lolodi 2011). Reduction of MDA content due to nitrogen application was probably due to increased activity of antioxidant enzymes (Shin *et al.* 2005, Zhang *et al.* 2007, Zhao & Liu 2009, Lin *et al.* 2011). Xiao *et al.* (1998) reported that nitrogen application can improve light reaction of photosynthesis which ultimately leads to the lower ROS production.

Biotic and abiotic stresses affect plants by causing excess accumulation of ROS, which can react with certain biomolecules, and alter or inactivate biochemical activities (Mittler 2002, Mittler *et al.* 2004, Choudhary *et al.* 2012). Even under optimal conditions, ROS are generated as by-products of normal metabolism in subcellular components (Asada & Takahashi 1987, Elstner 1991, Asada 1994, del Rio *et al.* 1998). These radicals are capable of initiating peroxidation reaction (Bandyopadhyay *et al.* 1999). The ROS also influence degeneration of DNA, oxidation of deoxyribose sugar, the reforming and breaking of DNA strands and cause mutations (Zhang & Kirkham 1996, Chirkova *et al.* 1998). To migrate and repair damage initiated by ROS, plants developed complex antioxidant systems (del Rio *et al.* 2002). This defense system includes enzymatic and non-enzymatic components. The enzymatic defense system includes the enzymes superoxide dismutase (SOD), catalase (CAT), ascorbic peroxidase (APX), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) (Yordanova *et al.* 2003). SOD is an enzyme acting as a first line of defence in combating oxidative stress in plants, which dismutates superoxide anions to H_2O_2 (Shalini & Dubey 2003, Mishra *et al.* 2006, Davies *et al.* 2009). CAT and POD are involved in building resistance against accumulation and toxicity of hydrogen peroxide by converting H_2O_2 to water and oxygen in cells (Msttes 2000). Increases in levels of all antioxidant enzymes occurred in fenugreek irrigated with wastewater. This is likely due to the pollutants present that can lead to oxidative stress causing biochemical changes (Lee *et al.* 2001, Panda & Upadhyay 2003, Singh *et al.* 2003, Fatima & Ahmad 2005, Baghel 2008, Xu *et al.* 2010, Olorunfemi & Lolodi 2011, Jain & Srivastava 2012, Sangeetha *et al.* 2012). Application of levels of nitrogen increased CAT, POD and SOD activities in fenugreek. This may be due to the alleviation of heavy metal damage by the nitrogen increased activity of antioxidant enzymes. Williams *et al.* (1967) suggested that addition of nitrogen in soil increases the formation of NO_3^- which decreases soil pH. Lowering pH increases the solubility of heavy metals and decrease the efficiency of adsorption (Moreno *et al.* 2000, Zhang *et al.* 2007, Zhao & Liu 2009, Ahmadi *et al.* 2010, Lin *et al.* 2011).

CONCLUSION

From the present study we concluded that the wastewater used for irrigation is suitable for fenugreek as the nutritive and toxic chemical elements present in wastewater were accumulated in the soil at low level and this accumulation did not cause any problem to the plants tested. Use of wastewater can provide economic benefits. Substitution of ground water by wastewater at some contents increased growth, proline, malondialdehyde content and antioxidant enzymes. Integrated application of wastewater and fertilizer increased growth and biochemical characteristics in fenugreek. Even if oxidative stress is induced in fenugreek plants irrigated with wastewater, application of N could provide protection against oxidative stress by increasing the antioxidant protective system.

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REFERENCES

Ademoroti CMA (1996) *Standard method for water and effluents analysis*. Foludex Press Ltd, Ibadan, Nigeria.

Aebi H (1984) Catalase *in vitro*. *Methods of Enzymology* 105: 121–126.

Ahmad I & Hellebust JA (1988) The relationship between inorganic nitrogen metabolism and proline accumulation in osmoregulatory response of two euryhaline microalgae. *Plant Physiology* 88: 348–354.

Ahmadi A, Emam Y & Pessarakli M (2010) Biochemical changes in maize seedlings exposed to drought stress conditions at different nitrogen levels. *Journal of Plant Nutrition* 33: 541–556.

Akhtar N, Inam A, Inam A & Khan NA (2012) Effects of city wastewater on the characteristics of wheat with varying doses of nitrogen, phosphorus and potassium. *Recent Research in Science and Technology* 4: 18–29.

Akponikpe PBI, Wima K, Yacouba H & Mermoud A (2011) Reuse of domestic wastewater treated in macrophyte ponds to irrigate tomato and eggplant in semi-arid West-Africa: Benefits and risks. *Agricultural Water Management* 98: 834–840.

APHA (1998) *Standard Methods for the Examination of Water and Wastewater*, *20th Edition*. American Public Health Association, Washington, D.C.

Aravind P & Prasad MNV (2003) Zinc alleviates cadmium-induced oxidative stress in *Ceratophyllum demersum* L.: A free floating freshwater macrophyte. *Plant Physiology and Biochemistry* 41: 391–397.

Arora A, Sairam RK & Srivastava GC (2002) Oxidative stress and antioxidative system in plants. *Current Science* 82: 1227–1238.

Arrese-Igor C, Minchin FR, Gordon AJ & Nath AK (1997) Possible causes of the physiological decline in soybean nitrogen fixation in the presence of nitrate. *Journal of Experimental Botany* 48: 905–913.

Asada K (1994) Production and action of active oxygen species in photosynthetic tissue. In: Foyer CH & Mullmeaux PM (eds) *Causes of photo-oxidative stress and amelioration of defense system in plants*. CRC Press, Boca Ratton, FL, pp. 77–107.

Asada K & Takahashi M (1987) Production and scavenging of active oxygen in photosynthesis. In: Kyle DJ, Osmond CB & Arntzen CJ (eds) *Photoinhibition*. Elsevier, Amsterdam, pp. 227–287.

Ashraf M & Foolad MR (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany* 59: 206–216.

Avemelech Y (1993) Irrigation with sewage effluents: The Israeli experience. *Environmental Science & Technology* 23: 2127–2134.

Awasthi SK (2000) *Prevention of Food Adulteration Act No. 37 of 1954*. Central and State Rules as Amended for 1999, Third edition, Ashoka Law House, New Delhi.

Ayers RS & Westcot DW (1994) *Water quality for agriculture*. FAO Irrigation & Drainage Paper 29 Rev.1. Food and Agriculture Organization of the United Nations, Rome.

Baghel RS (2008) Toxicity of distillery effluent on seed germination, seedling growth and metabolism in *Pisum sativum*. *Research in Environment and Life Sciences* 1: 29–32.

Bandyopadhyay U, Das N & Banerjee RK (1999) Reactive oxygen species: oxidative damage and pathogenesis. *Current Science* 77: 658–666.

Bates LS, Waldren RP & Teare ID (1973) Rapid determination of free proline for water-stress studies. *Plant Soil* 39: 205–207.

Bauddh K & Singh RP (2012a) Cadmium tolerance and its phytoremediation by two oil yielding plants *Ricinus communis* L. and *Brassica juncea* L. from the contaminated soil. *International Journal of Phytoremediation* 14: 772–785.

Bauddh K & Singh RP (2012b) Growth, tolerance efficiency and phytoremediation potential of *Ricinus communis* L. and *Brassica juncea* L. in salinity and drought affected cadmium contaminated soil. *Ecotoxicology and Environmental Safety* 85: 13–22.

Bedouh Y & Bekhouche F (2012) Influence of treated wastewater irrigation on some biochemical parameters of onion (*Allium cepa*). *Annals of Biological Research* 3: 4820–4827.

Bergmeyer HU, Gawehn K & Grassl, M (1974) Enzymatic assay of peroxidase, insoluble. In: Bergmeyer HU (ed) *Methods of enzymatic analysis, Vol. I, 2nd edition*. Academic Press, Inc., New York, pp. 473–474.

Bhargava RN, Chandra R & Rai V (2008) Phytoextraction of trace elements and physiological changes in Indian mustard plants (*Brassica nigra* L.) grown in post methanated distillery effluent (PMDE) irrigated soil. *Bioresource Technology* 99: 8316–8324.

Bhattacharjee S & Mukherjee AK (1994) Influence of cadmium and lead on physiological and biochemical responses of *Vigna unguiculata* (L.) Walp. Seedling germination behavior, total protein, proline content and protease activity. *Pollution Research* 13: 83–88.

Biswas AK, Mohanty M, Hati KM & Misra AK (2009) Distillery effluents effect on soil organic carbon and aggregate stability of a vertisol in India. *Soil & Tillage Research* 104: 241–246.

Blokhina O, Virolainen E & Fagerstedt KV (2003) Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Annals of Botany* 91: 179–194.

Brady NC & Weil RR (1999) *The nature and properties of soils, 12th edition*. Pretence Hall, Upper Saddle River, New Jersey.

Cakmak I & Horst WJ (1991) Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tips of soybean (*Glycine max*). *Physiologia Plantarum* 83: 463–468.

Chalkoo S, Sahay S, Inam A & Iqbal S (2014) Application of wastewater irrigation on growth and yield of chilli under nitrogen and phosphorus fertilization. *Journal of Plant Nutrition* 37: 1139–1147.

Chandra RR, Bhargava N, Yadav S & Mohan D (2009) Accumulation and distribution of toxic metals in wheat (*Triticum aestivum* L.) and Indian mustard (*Brassica campestris* L.) irrigated with distillery and tannery effluents. *Journal of Hazardous Materials* 162: 1514–1521.

Charman PEV & Murphy BW (1991) *Soils-their properties and management: A soil conservation handbook for New South Wales*. Sydney University Press/Soil Conservation Service, NSW. Sydney.

Chaudhary GR (1999a) Response of fenugreek (*Trigonella-foenum-graecum*) to N, P and *Rhizobium* inoculation. *Indian Journal of Agronomy* 44: 424–426.

Chaudhary GR (1999b) Response of fenugreek (*Trigonella foenum-graecum* L.) to seed rate and fertilizer application. *Indian Journal of Agronomy* 44: 427–429.

Chen T & Chia O (2002) Vegetable growth in polluted soils in China. *Environmental Sciences* 2: 193–195.

Chirkova TV, Novitskaya LO & Blokhina OB (1998) Lipid peroxidation and antioxidant systems under anoxia in plants differing in their tolerance to oxygen deficiency. *Russian Journal of Plant Physiology* 45: 55–62.

Choudhary SP, Oral HV, Bhardwaj R, Yu JQ & Tran LS (2012) Interaction of brassinosteroids and polyamines enhances copper stress tolerance in *Raphanus sativus. Journal of Experimental Botany* 63: 5659–5675.

Cisse G (1997) *Health impact of the use of polluted water in urban agriculture: The case of market gardening in Ouagadougou. Ph.D. Thesis*. Department of Agricultural Engineering, Federal Polytechnic School of Lausanne, Lausanne, Switzerland.

Datta S, Alam K & Chatterjee R (2005) Effect of different levels of nitrogen and leaf cutting on growth, leaf and seed yield of fenugreek (*Trigonella foenum-graecum* L.). *Indian Journal of Agricultural Science* 75: 580–581.

Davies LC, Cabrita GJM, Ferreira RA, Carias CC, Novais, JM & Martins-Dias S (2009) Integrated study of the role of *Phragmites australis* in azo-dye treatment in a constructed wetland: From pilot to molecular scale. *Ecological Engineering* 35: 961–970.

De B & Mukherjee AK (1998) Mercury induced metabolic changes in seedlings and cultured cells of tomato. *Geobios* 23: 83–88.

del Rio LA, Corpas FJ, Sandalio LM, Palma JM, Gomez M & Barroso JB (2002) Reactive oxygen species, antioxidant systems and nitric oxide in peroxisomes. *Journal of Experimental Botany* 53: 1255–1272.

del Rio LA, Pastori G, Palma JM, Sandalio LM, Sevilla F, Corpas FJ, Jimenez J, Lopez-Huertas E & Hernandez JA (1998) The activated oxygen role of peroxisomes in senescence. *Plant Physiology* 116: 1195–1200.

Duncan DB (1955) Multiple range and multiple F tests. *Biometrics* 11: 1–42.

Elstner EF (1991) Mechanisms of oxygen activation in different compartments of plant cells. In: Pell EJ & Steffen KL (eds) *Active oxygen species, oxidative stress and plant*. The American Society of Plant Physiologists, Rockville**,** MD, pp. 13–25.

Faizan S, Kausar S & Akhtar N (2014) Influence of wastewater application and fertilizer use on growth, photosynthesis, nutrient homeostatis, yield and heavy metal accumulation in okra (*Abelmoschus esculentus* L. Moench). *Pakistan Journal of Biological Sciences* 17: 630–640.

Fatima RA & Ahmad M (2005) Certain antioxidant enzymes of *Allium cepa* as biomarkers for the detection of toxic heavy metals in wastewater. *Science of the Total Environment* 346: 256–273.

FAO (1992) *Wastewater treatment and use in agriculture*. Irrigation Paper 47. Food and Agriculture Organization of the United Nations, Rome, Italy.

Ganguly AK (1951) Base exchange capacity of silica and silicates. *Journal of Physical and Colloid Chemistry* 55: 1417–1428.

Garnczarska M & Ratajczak L (2000) Metabolic responses of *Lemna minor* to lead ions II. Induction of antioxidant enzymes in roots. *Acta Physiologiae Plantarum* 22: 429–432.

Ghosh AB, Bajaj JC, Hasan R & Singh D (1983) *Soil and water testing methods: A laboratory manual*. Indian Agriculture Research Institute, New Delhi.

Gosset DR, Millhollon EP & Lucas MC (1994) Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. *Crop Science* 34: 706–714.

Handique GK & Handique AK (2009) Proline accumulation in lemongrass (*Cymbopogon flexuosus* Stapf.) due to heavy metal stress. *Journal of Environmental Biology* 30: 299–302.

ISI (1974) *Permissible limits of industrial waste waters discharge into inland surface water*. No. 2490. Indian Standard Institution, New Delhi, India.

Indra V & Sivaji S (2006) Metals and organic components of sewage and sludges. *Journal of Environmental Biology* 27: 723–725.

Iqbal S, Inam A, Inam A, Ashfaque F & Sahay S (2017) Potassium and wastewater interaction in the regulation of photosynthetic capacity, ascorbic acid and capsaicin in chilli (*Capsicum annuum* L.) plant. *Agricultural Water Management* 184: 201–210.

Iqbal S, Inam A, Inam A, Sahay S & Chalkoo S (2012) Balance use of inorganic fertilizers on chilli (*Capsicum annuum* L.) irrigated with wastewater. *Biosciences International* 1: 82–89.

Iqbal S, Inam A, Sahay S & Inam A (2015) Growth, physiological, yield and quality response in Chilli (*Capsicum annuum* L.) under wastewater irrigation and different levels of phosphorus. *American Journal of Experimental Agriculture* 5: 70–81.

Jackson ML (1958) *Soil chemical analysis*. Prentice Hall, Englewood Cliffs, N.J.

Jain R & Srivastava S (2012) Nutrient composition of spent wash and its impact on sugarcane growth and biochemical attributes. *Physiology and Molecular Biology of Plants* 18: 95–99.

James DW (2010) Urea: A low cost nitrogen fertilizer with special management requirements. In: *Fertilizer composition and reactions in soils*. Utah fertilizer guide. Utah State University Cooperative Extension (extension.usu.edu).

Kausar S (2009) *Effect of wastewater on growth and productivity of Okra, M.Phil. Dissertation*. Department of Botany, Aligarh Muslim University, Aligarh, India.

Kausar S & Faizan S (2015) Influence of inorganic fertilizers and wastewater irrigation on growth and yield of Okra (*Abelmoschus esculentus* L.). In: Siddiqui FA (ed) *Population Dynamism and Resource Utilization: Geo-Informatics and Resource Dynamics, Vol III*. Academic Publications, pp. 97–105.

Kelley WP (1951) *Alkali soils: Their formation properties and reclamation*, Reinhold, New York.

Kiziloglu FM, Turan M, Sahin U, Angin I, Anapali O & Okuroglu M (2007) Effects of wastewater irrigation on soil and cabbage-plant (*Brassica oleracea* var. *capitate* cv. yalova-1) chemical properties. *Journal of Plant Nutrition and Soil Science* 170: 166–172.

Kumar CS, Singh A, Sagar RK, Negi MPS & Maurya JN (2012a) Impact of exogenous application of indole acetic acid on accumulation of heavy metal and antioxidants in wheat (*Triticum aestivum* L.) under sewage water irrigation. *Recent Research in Science and Technology* 4: 16–22.

Kumar CS, Singh A, Sagar RK, Negi MPS & Maurya JN (2012b) Study of indole acetic acid and antioxidant defense system of wheat grown under sewage water. *International Journal of Environmental Sciences* 3: 821–832.

Kumar N, Kumar S, Bauddh K, Dwivedi N, Shukla P, Singh DP & Barman SC (2015) Toxicity assessment and accumulation of metals in radish irrigated with battery manufacturing industry effluent. *International Journal of Vegetable Science* 21: 373–385.

Kumar V & Chopra AK (2012) Fertigation effect of distillery effluent on agronomical practices of *Trigonella foenum graecum* L. (Fenugreek). *Environmental Monitoring and Assessment* 184: 1207–1219.

Lalk I & Dorfling K (1985) Hardening, ABA, proline and freezing resistance in the winter wheat varieties. *Physiologia Plantarum* 63: 287–292.

Lee HD, Kim YS & Lee CB (2001) The inductive responses of the antioxidant enzymes by salt stress in the rice (*Oryza sativa* L.). *Journal of Plant Physiology* 158: 737–745.

Lin T, Zhu X, Zhang F & Wan X (2011) The detoxification effect of nitrogen on cadmium stress in *Populus yunnanensis*. *Botany Research Journal* 4: 13–19.

Lindsey WL & Norwell WA (1978) Development of a DTPA soil test for zinc, iron, manganese and copper. *Soil Science Society of America Journal* 42: 421–428.

Loggini B, Scartazza A, Brugnoli, E & Navmi-Izzo F (1999) Antioxidation defense system, pigment composition and photosynthetic efficiency in two wheat cultivars subjected to drought. *Plant Physiology* 119: 1091–1100.

Lucho-Constantino CA, Alvarez-Suarez M, Beltran-Hernandez RI, Prieto-Garcia F & Poggi-Varaldo HM (2005) A multivariate analysis of the accumulation and fractionation of major trace elements in agricultural soils in Hidalgo state, Mexico, irrigated with raw wastewater. *Environment International* 31: 313–323.

Mann HS (1968) Manuring of pulse crop neglected aspect in poor yield. *Indian Farming* 17: 7–8.

Marschner P (2012) *Mineral nutrition of higher plants, 3rd edition*. Academic Press, London, UK.

Mishra S, Srivastava S, Tripathi RD, Kumar R, Seth CS & Gupta DK (2006) Lead detoxification by coontail (*Ceratophyllum demersum* L.) involves induction of phytochelatins and antioxidant system in response to its accumulation. *Chemosphere* 65: 1027–1039.

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science* 17: 405–410.

Mittler R, Vanderauwera S, Gollery M & Van Breusegem F (2004) Reactive oxygen gene network of plants. *Trends in Plant Science* 9: 490–498.

Moreno DA, Vfllora G, Pulgar G & Romero L (2000) Effect of nitrogen and potassium supply on concentration of iron and manganese and activities of catalase, peroxidase and aconitase in pepper plants. *Journal of Plant Nutrition* 23: 1787–1795.

Msttes JM (2000) Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. *Toxicology* 153: 83–104.

Myriam K, Houria B, Rachid R & Reda DM (2009) Biochemical changes observed in isolated roots of *Phragmites australis* treated with industrial wastewater. *American-Eurasian Journal of Toxicological Sciences* 1: 19–23.

Naidoo G & Naidoo Y (2001) Effects of salinity and nitrogen on growth, ion relations and proline accumulation in *Triglochin bulbosa*. *Wetlands Ecology and Management* 9: 491–497.

Naidu BP, Aspinall D & Paleg LG (1992) Variability in proline-accumulating ability of barley (*Hordeum vulgare* L.) cultivars induced by vapor pressure deficit. *Plant Physiology* 98: 716–722.

Olorunfemi DI & Lolodi O (2011) Effect of cassava processing effluents on antioxidant enzyme activities in *Allium cepa* L. *Biokemistri* 23: 49–61.

Olsen SR, Cole CV, Watanabe FS & Dean LA (1954) *Estimation of available phosphorus in soils by extraction with sodium bicarbonate.* Circular No. 939. United States Department of Agriculture, Washington D.C.

Panda SK & Choudhury S (2005) Chromium stress in plants. *Brazillian Journal of Plant Physiology* 17: 95–102.

Panda SK & Upadhyay RK (2003) Salt stress injury induces oxidative alterations and antioxidative defiance in the roots of *Lemna minor*. *Biologia Plantarum* 48: 249–253.

Panse VG & Sukhatme PV (1985) *Statistical methods for agricultural workers, 4th edition*. Indian Council of Agricultural Research, New Delhi.

Pathak H, Joshi HC, Chaudhary A, Chaudhary R, Kalra N & Dwiwedi MK (1999) Soil amendment with distillery effluent for wheat and rice cultivation. *Water Air & Soil Pollution* 113: 133–140.

Pescod MB (1992) *Wastewater treatment and use in agriculture*. FAO Irrigation & Drainage Paper No.47, FAO, Rome.

Pollard A & Wyn Jones RG (1979) Enzyme activities in concentrated solutions of glycinebetaine and other solutes. *Planta* 144: 291–298.

Ram D & Verma JP (2001) Effect of level of phosphorus and potash on the performance of seed crop of fenugreek (*Trigonella foenum graecum* L.) cv. Pusa Early Bunching. *Haryana Journal of Horticultural Sciences* 30: 249–250.

Richards LA (1954) *Diagnosis and improvement of saline alkali soils*. Agriculture Handbook No. 60. United States Department of Agriculture, Washington, D.C.

Sahay S, Inam A, Inam A & Iqbal S (2015) Modulation in growth, photosynthesis and yield attributes of black mustard (*B. nigra* cv. IC247) by interactive effect of wastewater and fly ash under different NPK levels. *Cogent Food and Agriculture* 1: Article no. 1087632

Sanchez E, Lopez-Lefebre LR, Garcia PC, Rivero RM, Ruiz JM & Romero L (2001) Proline metabolism in response to highest nitrogen dosages in green bean plants (*Phaseolus vulgaris* L. cv. Strike). *Journal of Plant Physiology* 158: 593–598.

Sanchez E, Ruiz JM & Romero L (2002) Proline metabolism in response to nitrogen toxicity in fruit of French bean plants (*Phaseolus vulgaris* L. cv. Strike). *Scientia Horticulturae* 93: 225–233.

Sangeetha R, Kamalahasan B & Karthi N (2012) Use of tannery effluent for irrigation: an evaluative study on the response of antioxidant defences in maize (*Zea mays*). *International Food Research Journal* 19: 607–610.

Saradhi PP, Arora S & Prasad KVSK (1995) Proline accumulates in plants exposed to UV radiation and protects them against induced peroxidation. *Biochemical and Biophysical Research Communications* 290: 1–5.

Schneider IF & Erickson AE (1972) *Soil limitations for disposal of municipal waste waters*. Michigan State University Research Report No. 195. Michigan, United States.

Sen Gupta A, Webb RP, Holaday AS & Allen RD (1993) Overexpression of superoxide dismutase protects plants from oxidative stress. *Plant Physiology* 103: 1067–1073.

Shah RA, Javid S & Inam A (2005) Effect of sewage irrigation and nitrogen rates on the growth and productivity of triticale. *Pollution Research* 24: 267–274.

Shahroz B (2009) *Response of wastewater to some vegetable crops, Ph.D. Thesis*. Department of Botany, Aligarh Muslim University, Aligarh, India.

Shalini V & Dubey RS (2003) Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plant. *Plant Science* 164: 645–655.

Sharma SK (2000) Response of nitrogen and spacing on fenugreek seed production. *The Horticulture Journal* 13: 39–42.

Shin R, Berg RH & Schachtman DP (2005) Reactive oxygen species and root hairs in *Arabidopsis* root response to nitrogen, phosphorus and potassium deficiency. *Plant and Cell Physiology* 46: 1350–1357.

Singh AB, Biswas AK & Ramana S (2003) Effect of distillery effluents on plant and soil enzymatic activities and groundnut quality. *Journal of Plant Nutrition and Soil Science* 166: 345–347.

Singh RP, Dabas S, Choudhary A & Maheshwari R (1997) Effect of lead on nitrate reductase activity and alleviation of lead toxicity by inorganic salts and 6-benzylaminopurine. *Biologia Plantarum* 40: 339–404.

Smirnoff N & Cumbes QJ (1989) Hydroxyl radical scavenging activity of compatible solutes. *Photochemistry* 28: 1057–1060.

Steel RGD & Torrie JH (1962) *Principles and Procedures of Statistics, 2nd edition*, McGraw-Hill Book Company, New York.

Tabassum D, Azad S & Inam A (2007a) Utility of city wastewater as a source of irrigation water for mustard. *Journal of Industrial Pollution Control* 23: 391–396.

Tabassum D, Azad S & Inam A (2007b) Impact of wastewater on the yield of a pulse crop. *Journal of Industrial Pollution Control* 23: 279–284.

Tak HI, Ahmad F, Babalola OO & Inam A (2012) Growth, photosynthesis and yield of chickpea as influenced by urban wastewater and different levels of phosphorus. *International Journal of Plant Research* 2: 6–13.

Tak HI, Babalola OO, Huyser MH & Inam A (2013) Urban wastewater irrigation and its effect on growth, photosynthesis and yield of chickpea under different doses of potassium. *Soil Science and Plant Nutrition* 59: 156–167.

Tak HI, Inam A & Inam A (2010) Effects of urban wastewater on the growth, photosynthesis and yield of chickpea under different levels of nitrogen. *Urban Water Journal* 7: 187–195.

Thapa U & Maity TK (2003) Green and seed yield of fenugreek (*Trigonella foenum graecum* L.) as affected by nitrogen, phosphorus and cutting management. *Journal of Interacademicia* 7: 347–350.

Thompson G, Swain J, Kay M & Forster C (2001) The treatment of pulp and paper mill effluent: a review. *Bioresource Technology* 77: 275–286.

Tuncturk R, Celen AE & Tuncturk M (2011) The effects of nitrogen and sulphur fertilizers on the yield and quality of fenugreek (*Trigonella foenum-graecum* L.). *Turkish Journal of Field Crops* 16: 69–75.

Van der Hoek W, Hassan MU, Ensink JHJ, Feenstra S, RaschidSally L, Munir S, Aslam R, Ali N, Hussain R & Matsuno Y (2002) *Urban wastewater: A valuable resource for agriculture*. A case study from Haroonabad, Pakistan. Research Report 63, International Water Management Institute, Colombo, Sri Lanka.

Walkley AJ & Black IA (1934) Estimation of soil organic carbon by the carbonic acid titration method. *Soil Science* 37: 29–38.

Wang WG, Li R, Liu B, Li L, Wang SH & Chen F (2011) Effects of low nitrogen and drought stress on proline biosynthesis of *Jatropha curcas* seedling. *Acta Physiologia Plantarum* 33: 1591–1595.

Williams CH, Singh RN & Vlamis AH (1967) Metals in sludge-amended soils: A nine-year study. *Soil Science* 2: 86–93.

WHO (1989) *Health guidelines for the use of wastewater in agriculture and aquaculture*. World Health Organization Technical Report Series 778, Geneva.

Xiao K, Zhang RX & Qian WP (1998) The physiological mechanism of senescence and photosynthetic function decline of flag leaf in wheat regulated by nitrogen nutrition. *Plant Nutrition and Fertilizer Science* 4: 371–378 [in Chinese].

Xu J, Zhang J, Xie H, Li C, Bao N, Zhang C & Shi Q (2010) Physiological responses of *Phragmites australis* to wastewater with different chemical oxygen demands. *Ecological Engineering* 36: 1341–1347.

Yordanova R, Christov K & Popora LP (2003) Antioxidative enzymes in Barley plants subjected to soil flooding. *Environmental and Experimental Botany* 51: 93–101.

Yoshida I & Ishii T (1993) *Studies on the reuse of purified wastewater: Effects of purified wastewater on soil*. Bulletin of the Faculty of Agriculture, Tottori University.

Zhang F, Wang Z & Dong J (2007) Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (*Kandelia candel* and *Bruguira gymnorrhiza*). *Chemosphere* 67: 44–50.

Zhang J & Kirkham MB (1996) Antioxidation responses to drought in sunflower and sorghum seedling. *New Phytologist* 132: 361–373.

Zhang LX, Li SX, Zhang H & Liang ZS (2007) Nitrogen rates and water stress effects on production, lipid peroxidation and antioxidative enzyme activities in two maize (*Zea mays* L.) genotypes. *Journal of Agronomy and Crop Science* 193: 387–397.

Zhao C & Liu Q (2009) Growth and physiological responses of *Picea asperata* seedlings to elevated temperature and to nitrogen fertilization. *Acta Physiologia Plantarum* 31: 163–173.

Zhao Y (2011) Cadmium accumulation and antioxidative defenses in leaves of *Triticum aestivum* L. and *Zea mays* L. *African Journal of Biotechnology* 10: 2936–2943.

Zyadah MA & Abdul-Bakky TC (2000) Toxicity and bioaccumulation of copper, zinc and cadmium in some aquatic organisms. *Bulletin of Environmental Contamination and Toxicology* 64: 740–747.