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Irrigation with domestic wastewater: Responses on growth and yield of ladyfinger *Abelmoschus esculentus* and on soil nutrients

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Abstract

Domestic wastewater is generated continuously and in large quantities. It can serve as an alternative water nutrient source for irrigation. In the present study *Abelmoschus esculentus* L. (Ladyfinger) was irrigated using untreated wastewater (T1), treated wastewater (T2) and rainwater (T3) in pot experiments. The effect was seen on nutrient fortication, growth and yield of the plant and the nutrient status of the soil. Additionally the build up of Cr, Cu and Zn from the irrigation water were anlayzed in different parts of the plant biomass and in the soil. The sapling survival rate was found to be 87% in T1 followed by T2 and T3. Root shoot ratio under different treatments was found in the order T3 (0.46) >T2 (0.35) >T1 (0.31). The chlorophyll a, b and carotene content in the leaves (mg g^{-1}) was found to be 6.3, 0.5, 0.9 under T1, 4.8, 0.4, 0.8 under T2 and 3.2, 0.3, 0.5 under T3 respectively and all the three varied in the order T1>T2>T3. The same trend was found in case of total dry matter (g) T1 (6.3) >T2 (3.7) >T3 (2.3) at $p \leq 0.05$. There was a considerable increase in nutrients in the soil under T1 and T2 as compared to T3 after final harvest. The organic matter (%), NO_3-N and PO_4^{3-} (mg kg^{-1}) content post harvest soil was found to be 3.4, 71, 90 under T1 and 2.9, 52, 63 under T2 respectively. Also, there was an increase in cations Na, K, Ca and Mg in the soil irrigated with T1 and T2 after the final harvest. Thus irrigation with wastewater generally increased soil fertility. Only a small percentage of the heavy metal was bioaccumulated by the plant parts from the irrigation water. There was hardly any metal accumulation in fruits. Bulk of the metal ions remained in the soil.

Key words

Domestic wastewater, Ladyfinger, Metal ion accumulation

Introduction

Rapid growth of urban population results in generation of huge quantities of wastewater perennially. In India only 30% of the wastewater is treated before its discharged. Thus, untreated water finds its way into water systems such as rivers, lakes, groundwater and coastal waters, causing serious water pollution (GOI, 2002). Good quality water resources are becoming scarce and are allocated for urban water supply. On the other hand large amounts of water are needed for irrigation in agriculture. If the wastewater can be used as an alternative water source for irrigation both the problems can be solved. Application of wastewater to cropland and forested lands is an attractive option for disposal because it can improve physical properties and nutrient contents of soils (Kiziloglu *et al.,* 2007). Wastewater irrigation not only provides water, N, and P but also organic matter (OM) to the soils (Siebe, 1998). Thus, its use would help in water conservation, recycling nutrients (NPK) in wastewater, reducing direct fertilizer inputs and minimizing pollution loads to receiving water bodies (Vasudevan *et al*., 2010; Thapliyal *et al*., 2009; Hylander *et al*., 2006). Vegetables are a good source of vitamins, minerals and fiber which are beneficial for health. Growing these requires fertile land, water and other inputs for better yield (Akan *et al.,* 2009). Domestic wastewater is often used by farmers to grow vegetables and salad crops for nearby urban markets. Such vegetables include carrot, lettuce, cabbage and others some of which are consumed raw as salad (Kiziloglu *et al.,* 2008). But there is always a concern about the contamination and bioaccumulation of potentially toxic elements such as Cd, Cu, Fe, Mn, Pb, and Zn from both domestic and industrial sources (Kiziloglu *et al.,* 2007) by the vegetables. Besides, contamination of food chain mainly through the vegetables grown on such soils, loading of heavy metals often leads to degradation of soil health (Rattan *et al.,* 2002). However, treated wastewaters can still be used for irrigation under controlled conditions which minimize hazard from pathogenic and toxic contaminants to agricultural products, soils, surface, and groundwater (Kiziloglu *et al.,* 2007). For this purpose studies are needed on the effect of nutrient availability in wastewater on growth and yield of different crops needs. At the same time the possibility of bioaccumulation of hazardous chemicals such as heavy metals which vary from crop to crop in plant parts and soil needs to be looked into. *Abelmoschus esculentus* L. is a commonly grown vegetable in suburban areas of Indian cities. Various studies on *A. esculentus* related to seed germination with distillery effluent (Pandey *et al.,* 2007), hyperaccumulation and mobility of heavy metals (Kumar *et al.,* 2009, Hashmi *et al.,* 2007; Sharma *et al.*, 2009), impact of sewage irrigation on speciation of nickel in soils and its accumulation in crop (Khurana and Bansal, 2008), influence of wastewater application on water, soil and crop (Khurana and Aulakh 2010) and responses to elevated levels of Zn and Cd in the soil (Sharma and Agrawal, 2010) have been reported. Since in villages and periurban areas farmers practice wastewater irrigation in depth studies are needed to see the effect of wastewater use on crops.

In view of the above, the present study was carried out to assess the effects of untreated and treated (phytoremediated) domestic wastewater on morphological, biochemical and growth characteristics of ladyfinger, *A. esculentus*. Heavy metal accumulation in soil and different plant parts was also quantified.

Materials and Methods

Study site: Pot experiments were conducted at the campus of Indian Institute of Technology (IIT), Delhi, India, using local garden soil. The experimental site is situated between 77.09' E longitude, 28.45' N latitude and 228 m altitude above sea level. The maximum and minimum temperature during the study period varied between 26-31 and 10-25°C, respectively.

Untreated wastewater (T1): Domestic run off was collected from open storm water drain on IIT Delhi campus and served as untreated wastewater.

Treated phytoremediated wastewater (T2): A subsurface wetland was set up for phytoremediation of wastewater. Ten horizontal beds 1 m wide, 0.6 m deep, and 10 m long were lined with polyethylene sheets and filled with gravel. These were connected at one end to an inlet channel for untreated wastewater and at the other end to an outlet channel for collecting treated water. The gravel beds were planted with Canna (*Canna indica*). After a residence time of 24 hr, the treated water collected from the 10 beds gets drained into a storage tank. This water was termed as phytoremediated water.

Rainwater (T3): Rainwater was collected from the rooftop of a household at IIT, Delhi campus, and served as control.

Water and soil analysis: Untreated (T1), treated (T2) and control (T3) water samples were analyzed for different physico-chemical parameters as per the methods given in APHA (2005). Soil samples from local garden were analyzed for pH (soil: water, 1:5) using pH meter. Na and K were extracted from soil in ammonium acetate solution following repeated leaching procedure and then concentrations were determined with the help of Flame photometer (Jones, 2001). The available phosphorous in soil were quantified by $NaHCO_3$ extraction method given by Olsen and Sommers (1982). NO_3^- N was analyzed spectrophotometrically (Jones, 2001), and organic matter (%) by acid digestion method (Houba *et al*., 1989). The metals Cr, Cu and Zn in different samples were digested using aqua regia and analyzed by atomic absorption spectrophotometer, Perkin Elmer AAnalyst 200 (Baker and Amacher, 1982).

Experimental design: Seeds of *A. esculentus* L. (ladyfinger) were sterilized with mercuric chloride (0.1%) for 5 min and then soaked for 8 hr in distilled water and sown in pots containing potting soil. Seedlings were transplanted at the two or three leaf stage to experimental pots containing 5 kg of garden soil. Five replicates per species per treatment were established. Irrigation treatments included untreated wastewater (T1), treated or phytoremediated water (T2), and rain water (T3). Pots were watered as needed to maintain field capacity.

Three pots out of five were selected randomly from each treatment at the end of the experiment (120 days after sowing), and plants were harvested. The biomass was washed with double distilled water to remove soil and other contaminants. Each plant was separated into leaves, stems, and roots. Plant height (shoot length), root length, and leaf area were recorded. All plant parts were than oven dried at 60°C. Dry root:shoot length ratio and dry mass percentages of plant parts and total dry plant were measured (Evans, 1972). Fruits were gathered as and when they matured during the period.

Biochemical parameters: For chlorophyll analysis, 25 mg of freeze dried leaf samples were immersed in 5 ml of dimethyl sulphoxide and kept in darkness for 8 hr. Optical density (OD) of the solution was recorded at four wavelengths (663, 645, 510 and 480 nm). Chlorophyll *a, b* and total carotene were calculated (Arnon, 1949).

0.5 g of each replicate of oven dried plant samples (root, shoot and leaves) were ground and dry ashed at 550°C for 6 hr in a muffle furnace. Ash was dissolved in 5 ml of 2M hot HCl, filtered into a 50 ml volumetric flask and diluted to 50 ml with distilled water. Na and K were analyzed by flame photometry and Cr, Cu and Zn

Table - 1: Physicochemical characteristics of untreated (T1), treated phytoremediated (T2) and rainwater (T3) used for irrigation of ladyfinger plant (*A. esculentus)*

Irrigation water	pH	TDS (ppm)	BOD (ppm)	Nutrients (ppm)					Salts (ppm)		Heavy metal (ppm)		
				NO_3	PO_4^{3-}	K^+	Ca^{++}	Mg^{++}	Na^+	Cl^-	Cr	Cu	Zn
T1	7.3 ±0.1[a]	560 ±23.1[a]	130 ±15.4[a]	11.3 ±1.3[a]	16.8 ±2.5[a]	173.5 ±12.2[a]	59.4 ±5[a]	47.08 ±5[a]	177 ±12.8[a]	107 ±7.7[a]	0.072 ±0.01[a]	0.256 ±0.015[a]	0.047 ±0.001[a]
T2	7.2 ±0.12[a]	480 ±12.5[b]	70 ± 8.6[b]	8.3 ±1.1[b]	12.5 ±1.4[a]	169.5 ±10.7[a]	53.68 ±3[a]	18.05 ±8[b]	172 ±10.1[a]	94 ±4.3[b]	0.064 ±0.006[a]	0.238 ±0.013[a]	0.03 ±0.005[b]
T3	6.1 ±0.13[b]	30 ±5[c]	0 ±0[c]	0.7 ±0.02[c]	0.2 ±0.01[b]	0.4 ±0.03[b]	8 ±1.4[b]	5 ±0.8[c]	1.8 ±0.2[b]	5 ±0.7[c]	0.016 ±0.002[b]	0.048 ±0.003[b]	0.014 ±0.002[c]
Irrigation standards*	None	1500	None	None	None	None	230	100	230	400	0.1	0.2	2

* = WHO, 1989. All values except pH in mg l^{-1}, Means in columns of same parameter followed by same letters are not significantly different ($p<0.05$, Duncan's multiple range test)

Table - 2: Effect of different irrigation treatments on morphological parameters of *A. esculentus*

Irrigation water	No. of fruits plant^{-1}	Length of fruit fruit^{-1} (cm)	Height (cm)	Root shoot ratio	Roots (g)	Shoot (g)	Leaves (g)	Total biomass(g)	Leaf area/ Leaf (cm^2)
T1	8± 1[a]	13.5± 1[a]	43.8±2.5[a]	0.31±0.04[c]	1.32± 0.15[a]	2.91±0.18[a]	2.10±0.29[a]	6.3±0.32[a]	7.3±1.2[a]
T2	5 ± 2[b]	11±0.5[a]	38.9±1[b]	0.35±0.03[b]	0.98 ±0.11[b]	1.96±0.28[a]	0.80±0.16[b]	3.7±0.18[b]	3.3±0.8[b]
T3	3± 1[c]	7.4±1.5[b]	37±1[c]	0.46±0.03[a]	0.61±0.12[c]	1.04±0.20[b]	0.60±0.003[b]	2.3±0.05[c]	1.2±0.3[c]

All values are ± SD. Means in columns of same parameter followed by same letters are not significantly different ($p<0.05$)

Table - 3: Effect of different irrigation treatments on metal concentration (MC) in different parts of *A. esculentus*

Metals	Irrigation water	Metal accumulated root (µg g^{-1})	Metal accumulated shoot (µg g^{-1})	Metal accumulated leaves (µg g^{-1})	Total metal uptake unit^{-1} total biomass (µg g^{-1})
Cr	T1	18.1±0.004	3.7±0.001	21.9±0.001	13±0.004
	T2	22.0±0.003	8.0±0.002	36.3±0.002	18±0.003
	T3	31.1±0.002	10.2±0.002	38.3±0.002	23±0.002
Cu	T1	37.2±0.001	1.8±0.002	20.8±0.004	16±0.004
	T2	31.7±0.003	2.3±0.001	62.5±0.002	23±0.002
	T3	39.3±0.002	1.3±0.002	59.4±0.005	27±0.001
Zn	T1	53.6±0.0042	21.2±0.005	21.9±0.007	28±0.003
	T2	56.9±0.002	29.2±0.002	52.9±0.002	42±0.002
	T3	75.4±0.005	40.6±0.003	102.8±0.006	65±0.005

All values are +SD

Table - 4: Change in nutrient status of soil (post harvest)

Soil samples	Irrigation treatment code	pH	Organic matter (%)	Nutrients (mg kg^{-1})					Salt (mg kg^{-1})	Heavy metals (mg kg^{-1})		
				NO_3^-	PO_4^{3-}	K^+	Ca^{++}	Mg^{++}	Na^+	Cr	Cu	Zn
Initial		7.1 ±0.01	1.2 ±0.2	6.10 ±1.2	4.36 ± 0.54	110 ±3.4	810 ±9	340 ±6.3	1080 ±15	0.007 ±0.001	0.016 ±0.002	0.015 ±0.001
Post harvest	T1	7.2 ±0.02	3.4 ±0.8	71 ±2.5	90 ±2.3	1143 ±10	1160 ±21	1746 ±26	2140 ±20	0.356 ±0.02	1.436 ±0.005	0.11 ±0.002
	T2	7.21 ±0.02	2.9 ±0.4	52 ±1.6	63 ±1.8	1119 ±5.3	1127 ±15	877 ±15	2108 ±16	0.334 ±0.05	1.312 ±0.005	0.064 ±0.003
	T3	7.2 ±0.01	0.9 ±0.2	1.4 ±0.3	0 ±0	107 ±2.9	854 ±5.6	366 ±10	1085 ±13	0.0684 ±0.004	0.253 ±0.003	0.01 ±0.001
Agricultural standard*		4.5-8.5	>0.86	-	>7	>80	-	-	-	100	100	200

*Source: Alloway,1990. All values are ± SD

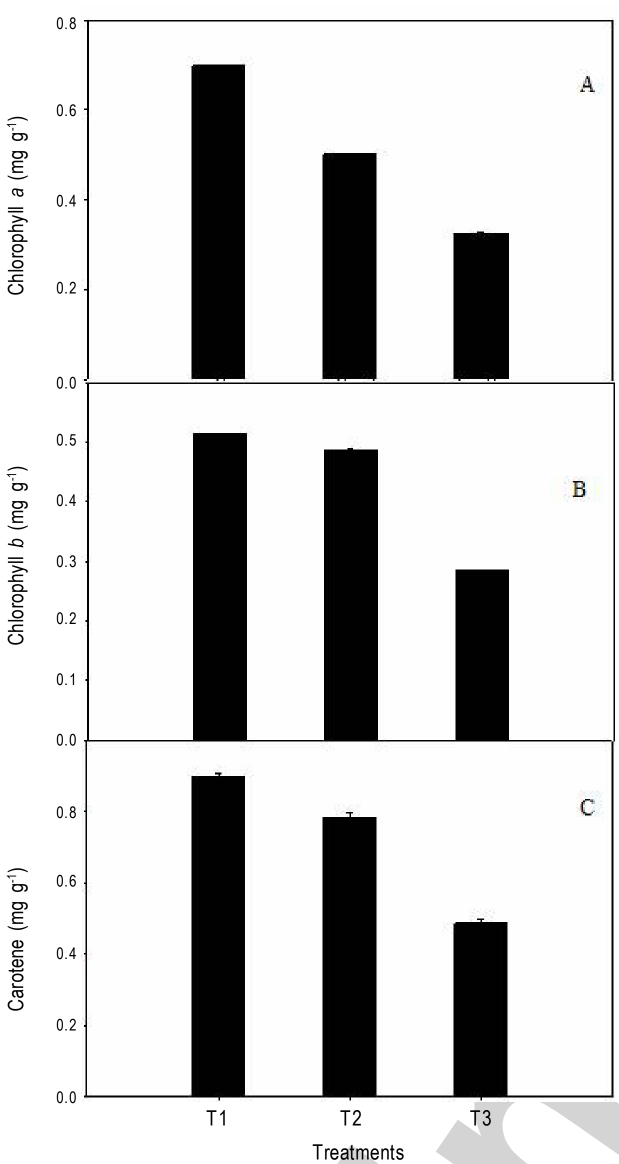

Fig. 1: Effect of different treatment on (A) Chlorophyll *a (B)* Chlorophyll *b* and (C) Carotene in ladyfinger (*A. escluentus*) plant

by atomic absorption spectrophotometer (Perkin Elmer AAnalyst 200). Ca and Mg were determined titrimetrically (Jones, 2001).

Statistical analysis: Mean values of three measurements with standard deviation (SD) were taken. Data were statistically analyzed by analysis of variance (ANOVA). Means were separated using Duncan's multiple range test at $p \leq 0.05$ level of significance using SPSS software (Version 10).

Results and Discussion

Water and soil characteristics: The quality of all the three water samples fall within permissible limits for irrigation water as per quality standards. T3 (control) was significantly ($p \leq 0.05$) different than T1 and T2 with respect to all measured parameters. There was a significant reduction in TDS, BOD, NO_3^-, Mg, Cl, and Zn in T2 as compared to T1 (Table 1). The chemical characteristics of untreated (T1) as well as treated (T2) were in general satisfactory. On an average basis the pH and TDS were higher in T1 and T2 compared to T3. Nutrient concentrations and heavy metals in T1, T2 and T3 were below the irrigation standards (WHO 1989). Phytoremediation of wastewater significantly ($p \leq 0.05$) reduced TDS (77%), BOD (47%), NO_3^--N (27%), PO_4^{3-} (26%) Mg (61%), Zn (36%) and to a lesser extent Cr (11%) and Cu (7%). This demonstrates that phytoremediation is an eco-friendly method by which domestic wastewater can be treated to reduce various contaminants which would be otherwise carried into the environment. It has been reported that primary mechanisms for removal of BOD, TDS and dissolved chemicals in a constructed wetland are sedimentation, adsorption, and microbial metabolism. However, the performance is contingent upon microbial activity, hydraulic retention time, hydraulic loading rate, temperature and vegetation type (Karathanasis *et al.*, 2003).

The experimental garden soil used for the study was found to be relatively fertile. The pH of the soil was neutral. The major macronutrients in the soil essential for a plant growth were, nitrate nitrogen (6 mg kg^{-1}), available phosphorous (4.4 mg kg^{-1}), potassium (110 mg kg^{-1}), calcium (810 mg kg^{-1}) and magnesium (340 mg kg^{-1}) and organic matter (0.7%). These were above the minimum international agricultural standards. Concentration of heavy metals, Cr (0.016 mg kg^{-1}), Cu (0.007 mg kg^{-1}) and Zn (0.015 mg kg^{-1}) were below the permissible limits (Alloway, 1990).

Morphological and biochemical parameters: ANOVA was performed on the results for each water treatment separately. Significant differences were seen in survival rate of plants under T1, T2 and T3 irrigation. After transplantation the survival percentage for ladyfinger was found to be maximum in T1 (87%), followed by T2 (80%) and T3 (67%). Survival was better in T1 and T2 as compared to T3 possibly due to more availability of nutrients in the former. Height (shoot length) was in the order of T1>T2>T3 ($p \leq 0.05$) (Table 2). The increase in root length in T3 can be attributed to absence of nutrient in rainwater leading to the plants rooting deeper into soil for nutrient (Ekanayake *et al*., 1985). Overall, the nutrient availability in T1 has positive influence on the plant survival, height, number of fruits and leaf area on *A. esculentus.* Root to shoot length ratio was significantly more in T3 at $p \leq 0.05$ and varied in the pattern T3>T2>T1 indicating that the root length increased under nutrient stressed conditions (Table 2). Increase in shoot length and decrease in root length of *A. esculentus* under high nutrient application (40% sludge amended soil) has been reported by others (Singh and Agrawal, 2009). Maximum number of fruits (Table 3) were obtained under T1 (8 $plant^{-1}$) and minimum under T3 (3 $plant^{-1}$). The length of the fruit also varied in the order T1>T2>T3 with maximum of 13 cm under T1 and minimum of 7.5 cm under T3 (Table 2). Plants given T1 treatment had 31% more total dry biomass than T3. Under all the three treatments maximum biomass was allocated in shoot.

Table - 5: Effect of different irrigation treatments on metal accumulation in soil

Irrigation water	Cr		Cu		Zn	
	Total Cr input* (mg)	Total Cr uptake by plant (mg)	Total Cu input* (mg)	Total Cu uptake by plant (mg)	Total Zn input* (mg)	Total Zn uptake by plant (mg)
T1	2.195±0.002	0.0805±0.004	7.782±0.03	0.098±0.002	1.48±0.08	0.178±0.008
T2	1.955±0.008	0.0662±0.006	7.242±0.04	0.086±0.003	0.98±0.05	0.155±0.002
T3	0.515±0.004	0.0527±0.007	1.53±0.03	0.061±0.001	0.48±0.02	0.150±0.003

All values are ± SD. * = Total input corresponds to input from 30 lit. water + 5 kg soil

The leaf area under T1 was maximum and minimum under T3 giving more area for photosynthetic activity in the former (Table 2). The chlorophyll (total, *a, b*) and carotene content observed in the leaves, varied in the order T1>T2>T3 (Fig. 1A-C). Chlorophyll content was enhanced in T1 and T2 which may be due to higher availability of magnesium. Similar findings were reported in *A. esculentus* where total chlorophyll and carotene increased significantly with increasing sludge amount (Singh and Agrawal, 2009). Quantitative estimation of the chlorophyll may be considered as an index of primary productivity in the ecosystem. Carotenoids are non-enzymatic antioxidants, which protect the chlorophyll molecules against oxidative stresses. Increase in carotenoid content may thus be attributed to the plant defense strategy to overcome the heavy metal stress (Singh and Agrawal, 2010). Nutrients and organic matter enhance photosynthetic activity and chlorophyll content (Kaushik *et al*., 2005). Wastewater supplies nutrients and enhances leaf area and chlorophyll content (Chandra *et al*., 2009) and photosynthetic activity (Jarvis *et al*., 1976). Better availability of nutrients to the plant results in more leaf area, better CO_2 fixation and photosynthetic activity (Singh and Bhati, 2004).

Metal concentration in *A. esculentus*: The study of heavy metal accumulation from wastewater in the crops is important as this would affect human and animal health directly through the food chain. Cr, Cu and Zn uptake were measured in the study. Heavy metal concentrations (MC) in various plant parts (μg g^{-1}) are recorded (Table 3). This was determined by taking a weighed sample of the dry biomass and determining the metal content by AAS. The amount of metal in the sample is divided by the weight of the smaple to give the metal concentration (MC). This is an important parameter as in the subsequent use of any plant part or total biomass as food, feed etc. the amount of metal introduced can be calculated as MC multiplied by weight of plant biomass used in the given application. It should be noted that this ratio which shows translocated metal concentration would depend on the concentration of metal in the wastewater as well as total biomass produced. While biomass produced is generally in the order T1>T2>T3, the ratio depends on metal concentration under different treatments. The partitioning of metals among different plant parts in *A.esculentus* was seen to vary both with the metal and treatment and showed differences in magnitude and relative distribution. As the concentration of the metals in the fruit was below detection limit, it has not been shown in Table 3. The total metal per unit total biomass (μg g^{-1}) was found in the order Zn > Cu >Cr for all treatments (Table 3). This variation in MC may be due to the fact that Cu and Zn are essentially required by the plant as they are integral to oxidases and anhydrases in proteins and carbohydrate metabolism.

The trend in variation of MC between different plant parts of *A. esculentus* depended on treatments. The concentration of all the studied metals was found to be the least in shoot and was in the order leaves>root>shoot in most of the cases (Table 3). This in agreement with the findings that concentration of the metals is generally much higher in roots and leaves, and the smallest in flower buds and fruit (Smical *et al*., 2008). Sometimes roots can act as barriers to the transfer of toxic metals through soil-plant system (Kumar *et al*., 2009). But once there is sufficient metal build up in the root, metal mobility to shoot and finally to the leaves of the crop could be high. Moreover, different vegetable species accumulate metals differently depending on environmental conditions, plant species and type and available forms of heavy metals (Lokeshwari and Chandrappa, 2006). It is however interesting to observe that the concentration of metals for unit weight of total biomass (μg g^{-1}) varied in the order T3>T2>T1 for Cr, Cu and Zn, indicating increased concentration of metal with less nutrient input.

Soil nutrient status: It may be noted that 5 kg of soil was taken in each of the pots and 30 lit. of water (T1, T2 and T3) was applied during the whole period of study. There was a slight increase in the soil pH value in T1 and T2 after the final harvest as compared to the values obtained before planting (Table 4). This finding is similar to that of Purdom (1980) and Shatanawi (1994) who proposed that the use of treated wastewater to irrigate crops can increase soil pH. In T3 there was a decrease in soil pH possibly because the pH of rainwater was slightly lower than that of initial pH of the soil. Under T3 there is a considerable decrease in organic matter and N and P values in the soil after harvest. This is attributed to low availability of nutrients in the control water. In T1 and T2 availability of N and P in water was high and hence there was a build up in the soil while this was not the case in T3. Thus, the nutrient status of the soil improved with wastewater irrigation.

Similar results have been reported by Burun *et al*. (2006) who studied the effect of urban wastewater on growth and soil properties on irrigating *Hordeum vulgare*. It has been reported that application of wastewater irrigation resulted in about 4, 10 and 8 fold increases in N, P, K, respectively (Bums *et al*., 1985). Kiziloglu *et al.* (2008) also reported an increase of organic matter, N, P, K, exchangeable Na, K, Ca, Mg, available phosphorus and microelements after irrigation with wastewater.

The total metal input to the soil (from irrigation water and soil) and total metal uptake by the plant biomass is shown in Table 5. The total metal uptake was calculated as metal concentration (MC) multiplied by the weight of biomass (dry weight). It is seen that only a small percentage of the total input of metal ions was taken up by plant biomass and distributed among its parts and the rest is accumulated in the soil. The percentage of metal transferred to the plant to the total input was in the order T3>T2>T1. Thus, under wastewater irrigation appreciable levels of all ions including heavy metals accumulated in the soil and the amount transferred to the plants during their growth was small.

A. esculentus (ladyfinger) was able to meet its nutrient requirements from wastewater either treated or untreated and established good growth showing no signs of toxicity at any stages of growth. The wastewater also increased the organic matter, N, available P and exchangeable nutrient contents in soil enhancing the growth of plants.

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