



Short communication

Reclamation of municipal domestic wastewater by aquaponics of tomato plants

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ABSTRACT

In search of low-cost eco-tech for the reclamation of municipal domestic wastewater, tomato plants (*Lycopersicon esculentum*) were cultivated on the floating bed of pulp-free coconut fiber over four different concentrations of wastewater (25%, 50%, 75% and 100%) and groundwater as control, in 10 L plastic bucket for two months. The study revealed that $\text{PO}_4\text{-P}$ was removed by 58.14–74.83% with maximum removal at 50% wastewater. More than 75% removal of $\text{NO}_3\text{-N}$ was observed in all treatments. Both COD and BOD were reclaimed highest at 100% wastewater by 61.38% and 72.03%, respectively. Ammonium-N concentration was subsided below the toxic level in all the treatments. The population of coliform bacteria (*Escherichia coli*) was reduced to 91.10–92.18% with maximum efficiency at 100% wastewater. Growth performance was observed relatively better at 100% wastewater. Crop production as the value addition of this technology was also recorded maximum at 100% wastewater. The bioaccumulation of Cd and Ni in tomato crop was far below the threshold level, but the bioaccumulation of Pb and Cr was above the safe level by 80 times and 660 times, respectively. The aquaponically reclaimed water can be reused in agriculture, aquaculture and industries.

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1. Introduction

Wastewater reclamation by low-cost ecotechnology is one of the challenging issues at present in wastewater management. In wastewater-fed agriculture, nutrients flow from wastewater into plants that expedite nutrient removal from wastewater coupled with the production of crop, leaf or flower as a value addition for human consumption or human aestheticism.

Many economically important vegetables and flowering plants can utilize the major nutrients ($\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ and $\text{H}_2\text{PO}_4/\text{HPO}_4\text{-P}$) for their growth from the nutrient-rich wastewater upon proper management or suitable amendments. Plant growth and crop and flower yield eventually may lead to nutrient removal from the wastewater that results in the reclamation of sewage water. This concept may be applied as a method for ecological treatment of wastewater.

Traditional hydroponics has been primarily used for increasing crop production under controlled conditions by supplying balanced nutrients in solutions. A large number of vegetables and crops such as beets, radishes, carrots, potatoes, cereal crops, fruits, ornamentals and seasonal flowers can be grown on inert supporting medium instead of soil (gravel, sand, peat, rock-wool, perlite, vermiculite, coconut fiber, sawdust, crushed rock or bricks, shards of cinder

blocks and even Styrofoam) wetted with nutrient media prepared by mixing all essential elements and water (Jensen and Collins, 1985; Runia, 1995).

Domestic wastewater is potentially rich in nutrients and has traditionally been used as an important source of fertilizers for either agriculture or aquaculture in many developed and developing countries (Edward, 2000; Jana, 1998; Jana and Jana, 2002). Boyden and Rababah (1996) used a commercial hydroponic system for the cultivation of lettuce, capsicum, corn and tomatoes in settled primary domestic wastewater but no attempt has so far been made to couple the processes of eco-reclamation of wastewater with biological production of some economically important vegetables and crops that will not only reduce the cost of wastewater treatment process but also yield some revenues in the form of agricultural crops. The beauty of this approach is due to the fact that wastewater provides all the essential elements required for plant growth, and the wastewater, in turn, is reclaimed by the removal of excess nutrients through the bio-process of crop production. The net outcome of this modified system is the reduction of fertilizer cost for crop production as well as the cost of wastewater treatment. As a result, a large number of eutrophic wetlands and water bodies which remain unutilized can be exploited by farmers for the cultivation of various economically important crops using wastewater. Morin (1996) has provided different strategies for the cultivation of commercial greenhouse crops in sewage water. However, the probability of infection for a single ingestion event of NFT (Nutrient Film Technique) in growing lettuce on primary

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treated municipal effluent was about 1.7% for viruses (Rababah and Ashbolt, 2000).

The infestation of faecal coliform bacteria is a great concern in wastewater agriculture and aquaculture. The strain of *Escherichia coli* (0157: H7) can cause serious illness in human. A recent outbreak of colitis disease by this strain generated much public disquiet. Though most of the cases were reported from eating under cooked hamburger (New York State Department of Health, 2005), yet there is a possibility of transmission of the strain through the plant parts. Drinking water contaminated by this bacterium through water supplies is rare (New York State Department of Health, 2005).

But the heavy metals in wastewater (Cd, Pd, Cr, Ni, etc.) can be accumulated within leaf and crop tissue as organo-metallic compounds that can create metal toxicity in human body when the leaves and crops are consumed. Even this accumulation depends directly upon the concentration of heavy metal in wastewater. So, the feasibility of implementing this eco-tech for wastewater reclamation massively must be cautions of heavy metal accumulation in leaf and crops{AQ clarity}.

The present experiment was conducted to evaluate the efficiency of the eco-tech “tomato plant aquaponics” in wastewater reclamation coupled with crop yield as a by-product.

2. Materials and methods

The present study was performed in fifteen 10 L plastic buckets (4 treatments and one control in triplicate). Buckets were filled with several concentrations of domestic wastewater (25%, 50%, 75% and 100%). Concentrations were prepared with groundwater. The domestic grey wastewater was collected from the Grit Chamber of Sewage Treatment Plant of Kalyani Municipality.

The tomato plant was selected for the study because the crop tomato was enriched with anticancer compound “lycopene”. Healthy tomato plants (*Lycopersicum esculentum*) of ten days old were procured from a local nursery and were then implanted in the matrix of pulp-free coconut fiber (3 in. × 3 in. × 1 in.) as per one plant in one bed and three plants in one bucket. Mats were supported with rectangular piece (4 in. × 4 in. × 2 in.) of thermocol as a float. Plants were allowed to float on the surface of bucket water. The growing plants were supported externally by bamboo sticks to hold the shoot upright. Thus the tomato plants were cultured for two months (December, 2004–January, 2005) outdoors under natural conditions. After two months, the crops were harvested from the plants. Index leaves and roots were collected and finally all the plants were removed from treatment water.

Dead leaves that fell on the treatment water were removed instantly. Algal slag and Spirogyra that grew during culture were also removed carefully. Water loss due to sampling and evaporation was replenished with the addition of distilled water. The water level was monitored to keep it constant at every moment during the period of investigation.

The growth performance and yield of the tomato plant were investigated by measuring biomass, number of leaves, chlorophyll content in index leaf, number of twig, stem length, shoot–root ratio, nitrate reductase (NR) activity in root, crop number and crop mass. Nutrient removal from different treatments by the capacity of tomato plant was determined by monitoring the different nutrient quality parameters ($\text{PO}_4\text{-P}$, $\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$) of bucket water before and after tomato plantation. The state of ion adsorption and respiration in root was reflected from the water quality of temperature, pH, alkalinity and dissolved oxygen (DO). Ion arrest through adsorption by organic matter in wastewater has been assessed by the quantification of chemical oxygen demand (COD). Possibility

of faecal coliform transmission from wastewater to human and colitis disease in human have been investigated through the quantification of population size of *E. coli* in wastewater as it is the major species in the faecal coliform group and is the best indicator of faecal pollution and possible presence of pathogens. Heavy metals were estimated from crop to depict the potency of bio-accumulation, whether it is safe for human consumption.

Parameters were measured following standard methods cited in Appendix A. Heavy metals (Cd, Cr, Pb and Ni) were estimated using a Flame Atomic Absorption Spectrophotometer (Company: Varian, Australia and Model: AA 240).

All tabulated data were represented as arithmetic mean (\bar{x}) of three samples ($n=3$) for water quality and of nine samples ($n=9$) for biological parameters. The error due to sampling was measured as standard error of mean ($\pm\text{SE}$). The variances among different treatments were analyzed by one way ANOVA and their multiple comparisons were analyzed by Tukey's test. One way ANOVA and Tukey's test were performed in statistical package (SPSS-10.0). Variances were accepted when the probability of variance (P) < 0.05.

3. Results

The reclamation efficiency of the eco-tech is the direct function of the growth performance of tomato plants. So all findings have been taken from the aspect of growth performance of plant, pollutant reclamation efficiency and the feasibility (pathogenic and heavy metal bioaccumulation) for human consumption of its products (crop).

3.1. Plant growth

3.1.1. Shoot length

The average shoot length of tomato plants grown in different strengths of wastewater ranged from 11.81 ± 1.07 cm (in control) to 55.0 ± 2.38 cm (in 100% wastewater) (Table 1). Shoot length increased with the rise of strength of wastewater. The maximum length observed in 100% wastewater was 4.7 times of that of control. The variances in shoot length in all pair combination of treatments were statistically significant (one way ANOVA, $P < 0.05$; Tukey's test, $P < 0.05$).

3.1.2. Internode length

The average internode length of full-grown tomato plants varied from 2.8 ± 0.21 cm (in 25% wastewater) to 3.1 ± 0.23 cm (in 100% wastewater). The internode length was proportional to the strength of wastewater, but in the 25% strength this length declined by 0.03% from the control (Table 1). But all the variations in the length among the strength of wastewater and control were statistically insignificant (one way ANOVA, $P > 0.05$). The maximum length (3.1 cm) was about 7% higher than that of control (2.9 cm) (Table 1).

3.1.3. Number of twigs

The average number of twigs per plant varied from 2 (in 25% wastewater) to 6 (in 100% wastewater) (Table 1). No twig formation was observed in control. The number of twigs in 75% wastewater was not different from the number in 50% wastewater.

3.1.4. Leaf number

The average leaf number of full grown tomato plants varied from 6 to 21 in control and 100% wastewater respectively (Table 1). The number in 100% wastewater was 3.5 times of that in control. The number of leaf was more with the increasing strength of wastewater and the variances in number among different strengths of wastewater were statistically distinct (one way ANOVA, $P < 0.05$;

Table 1
Growth performance and crop yield of tomato plants in different strengths of domestic wastewater in the tomato aquaponics experiment. Data represented as arithmetic mean of nine samples ($n=9$) with standard error of mean (\pm SE). Unlike superscripts (a–e) denote significant differences between treatment means at 5% level ($P<0.05$) and vice versa.

Parameter	Treatment									
	Control		25% wastewater		50% wastewater		75% wastewater		100% wastewater	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Shoot length (in cm)	6.2 \pm 0.78	11.81 ^e \pm 1.07	6.2 \pm 0.42	37 ^d \pm 1.27	6.2 \pm 0.31	46 ^c \pm 1.29	6.2 \pm 0.39	52 ^b \pm 2.13	6.2 \pm 0.42	55 ^a \pm 2.38
Internode length (in cm)	1.5 \pm 0.23	2.9 ^a \pm 0.33	1.5 \pm 0.13	2.8 ^a \pm 0.21	1.5 \pm 0.14	3.0 ^a \pm 0.21	1.5 \pm 0.11	3.0 ^a \pm 0.23	1.5 \pm 0.12	3.1 ^a \pm 0.23
Number of twigs per plant	0	0	0	2 ^c \pm 0.09	0	4 ^b \pm 0.28	0	4 ^b \pm 0.31	0	6 ^a \pm 0.85
Number of leaf per plant	5 \pm 0	6 ^c \pm 0.17	5 \pm 0.33	14 ^b \pm 0.07	5 \pm 0.11	15 ^b \pm 0.18	5 \pm 0.23	20 ^a \pm 1.01	5 \pm 0.12	21 ^a \pm 1.28
Surface area of index leaf 5th (in cm ²)	3.11 \pm 0.21	5.52 ^d \pm 0.26	3.11 \pm 0.17	30.86 ^c \pm 1.47	3.11 \pm 0.22	36.52 ^a \pm 1.93	3.11 \pm 0.33	34.18 ^b \pm 1.71	3.11 \pm 0.27	37.12 ^a \pm 2.12
Chlorophyll-a content in index leaf 5th (mg g ⁻¹)	0.029 \pm 0.001	0.035 ^d \pm 0.005	0.029 \pm 0.006	0.091 ^c \pm 0.012	0.029 \pm 0.008	0.119 ^a \pm 0.012	0.029 \pm 0.003	0.106 ^b \pm 0.012	0.029 \pm 0.007	0.120 ^a \pm 0.011
Chlorophyll-b content in index leaf (mg g ⁻¹)	0.018 \pm 0.002	0.024 ^c \pm 0.004	0.018 \pm 0.003	0.031 ^b \pm 0.006	0.018 \pm 0.004	0.064 ^a \pm 0.009	0.018 \pm 0.002	0.060 ^a \pm 0.008	0.018 \pm 0.003	0.068 ^a \pm 0.012
Root dry biomass per plant (in g)	0.119 \pm 0.023	0.255 ^d \pm 0.037	0.119 \pm 0.031	1.466 ^b \pm 0.231	0.119 \pm 0.035	1.889 ^a \pm 0.341	0.119 \pm 0.020	1.746 ^a \pm 0.224	0.119 \pm 0.021	1.358 ^c \pm 0.227
Shoot dry biomass per plant (in g)	0.231 \pm 0.031	0.495 ^d \pm 0.037	0.231 \pm 0.031	3.556 ^c \pm 0.417	0.231 \pm 0.061	5.874 ^a \pm 0.171	0.231 \pm 0.031	5.645 ^b \pm 0.311	0.231 \pm 0.032	5.921 ^a \pm 0.214
Crop yield per plant (in g)	–	0	–	32 ^d \pm 2.0	–	100 ^c \pm 6.12	–	105 ^b \pm 4.71	–	125 ^a \pm 7.21

Tukey's test, $P < 0.05$) except between 75% and 100% wastewater treatments.

3.1.5. Surface area of index leaf

The average surface area of index leaf 5th that was 5th leaf from the base of the plant 5.52 ± 0.26 to $37.12 \pm 2.12 \text{ cm}^2$ (Table 1). This surface area became bigger with the increasing strength of wastewater. At the termination of the experiment, the surface area in control (5.52 cm^2) was magnified by about 12 times in 100% wastewater (37.12 cm^2). The variances in surface area among different strengths of wastewater and control were statistically significant (one way ANOVA, $P < 0.05$; Tukey's test, $P < 0.05$) except between 100% and 50% wastewater treatments.

3.1.6. Chlorophyll-a and -b content in index leaf

The final average concentration of chlorophyll-a and -b in index leaf (5th from base) is recorded in Table 1. In Chlorophyll-a, the variations in the average concentration among different treatments including control also were statistically significant (one way ANOVA, $P < 0.05$; Tukey's test, $P < 0.05$) except in between 100% and 50% wastewater treatments (Table 1) but in chlorophyll-b, the variations among 50%, 75% and 100% were statistically insignificant (one way ANOVA, $P > 0.05$). The responses in the concentration of both chlorophyll-a and -b in index leaf were increased with the strength of wastewater, though this concentration in both decreased 10.92% and 6.25%, respectively, from 50% wastewater treatment and 75% wastewater treatment {AQ}.

3.1.7. Biomass

The average root and shoot final dry mass ranged from $0.255 \pm 0.037 \text{ g}$ to $1.889 \pm 0.341 \text{ g}$ and $0.495 \pm 0.037 \text{ g}$ to $5.921 \pm 0.214 \text{ g}$ respectively in four treatments and in control (Table 1). These dry biomasses were statistically distinct from each other in both the cases (one way ANOVA, $P < 0.05$; Tukey's test, $P < 0.05$) except in-between the 50% and 75% wastewater treatments for root and the 100% and 50% for shoot (Table 1). The root mass tended to grow bigger up to 50%, but in turn, it started to decline to extreme strength. By contrast, the shoot dry mass increased with the strength of wastewater and the highest shoot mass (5.921 g) was obtained in 100% wastewater, which was 12 times that of the control (0.495 g).

3.1.8. Nitrate reductase activity in root

Nitrate catalyzation by nitrate reductase (NR) enzyme in root of tomato plant varying from 0.0521 ± 0.001 to $0.8695 \pm 0.087 \mu\text{MNO}_2 \text{ min}^{-1} \text{ g tissue}^{-1}$ was statistically distinct in all strength of wastewater (one way ANOVA, $P < 0.05$; Tukey's test, $P < 0.05$) except between control ($0.1594 \mu\text{MNO}_2 \text{ min}^{-1} \text{ g tissue}^{-1}$) and 75% wastewater treatment ($0.1594 \mu\text{MNO}_2 \text{ min}^{-1} \text{ g tissue}^{-1}$) (Fig. 1). This rate was accelerated to optimum ($0.8695 \mu\text{MNO}_2 \text{ min}^{-1} \text{ g tissue}^{-1}$) at 50% wastewater and in turn was decelerated to minimum ($0.0521 \mu\text{MNO}_2 \text{ min}^{-1} \text{ g tissue}^{-1}$) with the further increase in strength of wastewater up to 100% (Fig. 1).

3.2. Crop yield

The crop yield in all treatments varied from $32 \pm 2.0 \text{ g}$ (in 25% wastewater) to $125 \pm 7.21 \text{ g}$ (in 100% wastewater) that were statistically distinct (one way ANOVA, $P < 0.05$; Tukey's test, $P < 0.05$) (Table 1). It was also observed that the crop yield was increased with the rise of the strength of wastewater. No crop was yielded in control. The highest yield was recorded in 100% wastewater that was about 4 times of the yield in 25% wastewater.

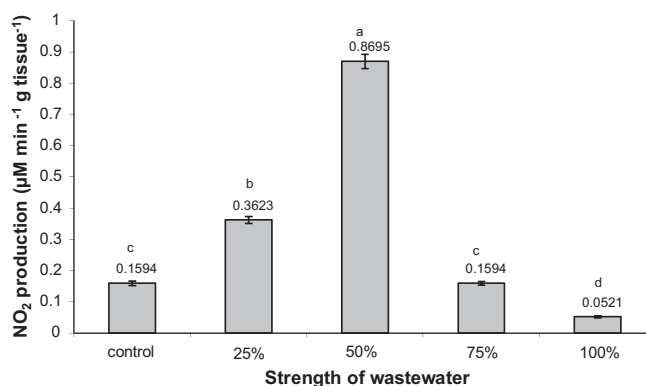


Fig. 1. Nitrate reductase activity in root tissue of tomato plant in different strengths of wastewater of aquaponic system. Bar (T) represents the standard error of mean (\pm SE) of nine samples ($n=9$). Unlike superscripts (a, b, c and d) indicate their significant difference between treatment means in multiple comparisons of five treatments at 5% level ($P < 0.05$) and vice versa.

3.3. PO₄-P removal efficiency from wastewater

In this aquaponics, PO₄-P removal was observed from 58.14% (control) to 74.83% (50% wastewater) (Fig. 2). The removal followed the order – 50% wastewater > 100% wastewater > 75% wastewater > 25% wastewater > control (Fig. 2). This removal in 50%, 75% and 100% wastewater and in 25% wastewater and control was the same (one way ANOVA, $P > 0.05$), though the strength of wastewater was increased (Fig. 2).

3.4. NO₃-N removal efficiency from wastewater

The values of NO₃-N removal from different strengths of wastewater are shown in Fig. 2. These varied from 74.7 ± 5.44 to $100 \pm 8.57\%$. Highest removal was observed in control and lowest in 25% wastewater. It followed the order – control > 100% wastewater > 75% wastewater > 50% wastewater > 25% wastewater. These removals were same in 25%, 50%, 75% and 100% wastewater (one way ANOVA, $P > 0.05$; Fig. 2).

3.5. COD removal efficiency

The removal of COD ranged from 20% (25% wastewater) to 61.38% (100% wastewater) irrespective of the value of control (-20%). These COD removals increased with the strengths of the wastewater (Fig. 3). The COD removal in control, 25%, 50%, 75%

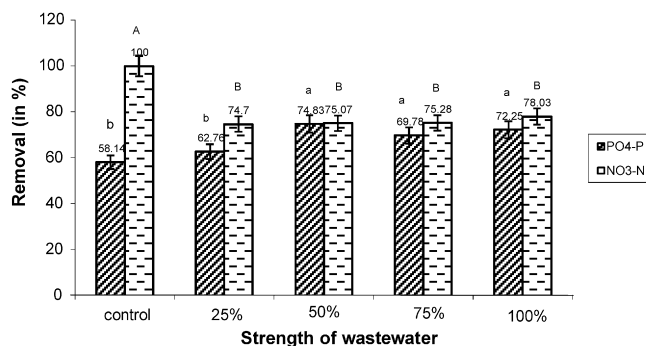


Fig. 2. Removal of PO₄-P and NO₃-N from wastewater by tomato plant in aquaponic system. Bar (T) represents the standard error of mean of three samples ($n=3$). Like superscripts (a and b or A and B) denote significant mean difference between treatments at 5% level ($P < 0.05$) and vice versa.

Table 2
Water quality in different strengths of domestic wastewater in the tomato aquaponics experiment. Data represented as arithmetic mean of three samples ($n = 3$) with standard error of mean (\pm SE). Unlike superscripts (a–e) denote significant differences between treatment means at 5% level ($P < 0.05$) and vice versa.

Parameter	Treatment									
	Control		25% wastewater		50% wastewater		75% wastewater		100% wastewater	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Temperature ($^{\circ}\text{C}$)	22.5 ^a \pm 0	21.5 \pm 0	22.5 ^a \pm 0	21.5 \pm 0	22.5 ^a \pm 0	21.5 \pm 0	22.5 ^a \pm 0	21.5 \pm 0	22.5 ^a \pm 0	21.5 \pm 0
Concentration of hydrogen ion (pH)	8.14 ^a	8.3	7.82 ^b	8.47	7.74 ^b	8.4	7.71 ^b	8.32	7.49 ^c	8.26
Total alkalinity (mg $\text{CaCO}_3 \text{ L}^{-1}$)	178.0 ^a \pm 8.67	211.64 \pm 10.32	158.98 ^b \pm 9.23	306.0 \pm 13.27	128.99 ^c \pm 7.54	287.0 \pm 13.29	124.32 ^c \pm 7.77	220.14 \pm 12.31	62.1 ^d \pm 3.37	198.23 \pm 9.87
Dissolved oxygen (DO) (mg L^{-1})	4.01 ^a \pm 0.17	12.67 \pm 0.75	3.97 ^a \pm 0.18	14.67 \pm 0.89	3.74 ^a \pm 0.18	12.01 \pm 0.56	3.11 ^a \pm 0.19	11.74 \pm 0.86	1.32 ^b \pm 0.061	9.54 \pm 0.29
Inorganic phosphate-phosphorus ($\text{PO}_4\text{-P}$) (mg L^{-1})	0.043 ^e \pm 0.005	0.018 \pm 0.002	0.094 ^d \pm 0.011	0.035 \pm 0.002	0.147 ^c \pm 0.042	0.037 \pm 0.001	0.182 ^b \pm 0.027	0.055 \pm 0.008	0.209 ^a \pm 0.018	0.058 \pm 0.006
Nitrate nitrogen ($\text{NO}_3\text{-N}$) (mg L^{-1})	0.001 ^e \pm 0.000	0	0.842 ^d \pm 0.091	0.213 \pm 0.019	7.14 ^c \pm 0.084	1.78 \pm 0.152	8.13 ^b \pm 0.761	2.01 \pm 0.213	8.83 ^a \pm 0.875	1.94 \pm 0.213
Ammonium ion (NH_4^+) (mg L^{-1})	0.01 ^e \pm 0.001	0.087 \pm 0.093	0.72 ^d \pm 0.084	0.41 \pm 0.052	1.81 ^c \pm 0.201	0.53 \pm 0.067	4.0 ^b \pm 0.53	0.61 \pm 0.078	5.91 ^a \pm 0.641	0.76 \pm 0.112
Nitrite nitrogen ($\text{NO}_2\text{-N}$) (mg L^{-1})	0.001 ^d \pm 0.000	0.006 \pm 0.001	0.005 ^c \pm 0.001	0.013 \pm 0.006	0.008 ^c \pm 0.001	0.043 \pm 0.007	0.015 ^b \pm 0.003	0.052 \pm 0.008	0.034 ^a \pm 0.005	0.087 \pm 0.014
Chemical oxygen demand (COD) (mg $\text{O}_2 \text{ L}^{-1}$)	0	20 \pm 3.12	100 ^d \pm 11	80 \pm 9.5	212 ^c \pm 17.28	140 \pm 11.23	307 ^b \pm 23.51	160 \pm 13.28	518 ^a \pm 27.89	200 \pm 17.78
Biological oxygen demand ($\text{BOD}_{5\text{at}20^{\circ}\text{C}}$) (mg $\text{O}_2 \text{ L}^{-1}$)	0	4 \pm 0.81	60.7 ^d \pm 6.17	23.3 \pm 1.89	90.1 ^c \pm 8.41	34.2 \pm 3.11	147.3 ^b \pm 11.32	48.7 \pm 4.17	211.4 ^a \pm 22.37	59.12 \pm 6.31
<i>E. coli</i> population ($\text{cfu} \times 10^7 \text{ ml}^{-1}$)	0	0	87 ^d \pm 7	7 \pm 2	153 ^c \pm 15	12 \pm 3	192 ^b \pm 18	17 \pm 3	243 ^a \pm 21	19 \pm 3
Total dissolved solid (TDS) (mg L^{-1})	0	4 \pm 0.78	269 ^d \pm 23.56	9 ^a \pm 1.23	779 ^c \pm 62.38	9 \pm 2.14	1983 ^b \pm 153.7	10 \pm 1.03	3749 ^a \pm 271.31	12 \pm 0.97
Total suspended solid (TSS) (mg L^{-1})	0	16 \pm 1.21	547 ^d \pm 43.28	17 \pm 1.18	1581 ^c \pm 129.37	19 \pm 2.11	3375 ^b \pm 311.27	21 \pm 1.97	4971 ^a \pm 437.28	28 \pm 2.31
Total solid (TS) (mg L^{-1})	0	20 \pm 1.88	816 ^d \pm 47.66	26 \pm 2.13	2360 ^c \pm 211.37	28 \pm 2.13	5358 ^b \pm 521.52	31 \pm 2.74	8720 ^a \pm 633.28	40 \pm 8.17

Table 3
Heavy metal concentration in wastewater at the beginning and in crop (tomato) at the harvest. Data represented as the arithmetic mean of three samples ($n=3$) for water and nine samples ($n=9$) for crop with standard error of mean (\pm SE).

Metal	Treatment			
	Control		25% wastewater	
	Water (ppm)	Crop (mg/g)	Water (ppm)	Crop (mg/g)
Cd	0.000	–	0.000	0.000
Pb	0.000	–	0.057 \pm 0.002	0.001 \pm 0.000
Cr	0.000	–	0.003 \pm 0.001	0.011 \pm 0.001
Ni	0.000	–	0.000	0.000
	50% wastewater		75% wastewater	
	Water (ppm)	Crop (mg/g)	Water (ppm)	Crop (mg/g)
Cd	0.000	0.000	0.001 \pm 0.000	0.000
Pb	0.104 \pm 0.003	0.010 \pm 0.001	0.149 \pm 0.004	0.016 \pm 0.001
Cr	0.009 \pm 0.001	0.017 \pm 0.001	0.016 \pm 0.001	0.023 \pm 0.001
Ni	0.000	0.000	0.001	0.000
	100% wastewater			
	Water (ppm)	Crop (mg/g)	Water (ppm)	Crop (mg/g)
Cd	0.003 \pm 0.000	0.000	0.003 \pm 0.000	0.000
Pb	0.268 \pm 0.005	0.024 \pm 0.001	0.268 \pm 0.005	0.024 \pm 0.001
Cr	0.027 \pm 0.001	0.033 \pm 0.001	0.027 \pm 0.001	0.033 \pm 0.001
Ni	0.001 \pm 0.000	0.000	0.001 \pm 0.000	0.000

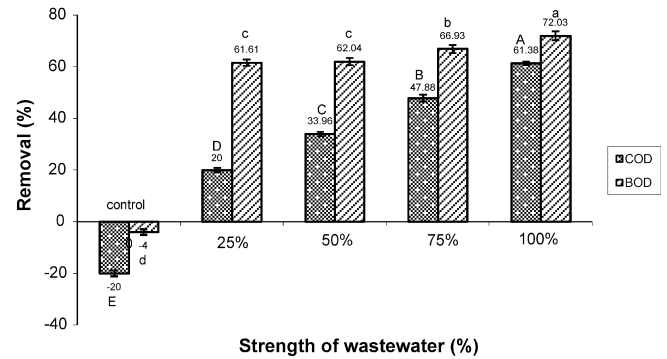


Fig. 3. Removal of COD and BOD in different strengths of wastewater in tomato aquaponics at the termination of the experiment. Unlike superscripts (a–d or A–E) denote significant difference between treatment means at 5% level ($P < 0.05$) among different strengths of wastewater and vice versa.

and 100% wastewater was significant (one way ANOVA, $P < 0.05$; Tukey's test, $P < 0.05$; Fig. 3).

3.6. BOD removal efficiency

BOD as a dependent variable of COD also declined in all strengths of wastewater. Its average removal ranged from 61.61% (25% wastewater) to 72.03% (100% wastewater) irrespective of control (–4%) (Fig. 3). The removal efficiency increased significantly (one way ANOVA, $P < 0.05$; Tukey's test, $P < 0.05$) with increasing the strength of wastewater, except between 25% and 50% wastewater (Fig. 3).

3.7. Coliform bacteria (*E. coli*) removal efficiency

The population of *E. coli* bacteria was reduced in all treatments of wastewater, including control (Table 2). The reduction ranged from 91.10% to 92.18% and the maximum removal was observed in 100% wastewater treatment (Table 2). The removal efficiency of all concentrations (25%, 50%, 75% and 100% wastewater) was almost the same (one way ANOVA, $P > 0.05$; Table 2).

3.8. Heavy metal accumulation in crop

The accumulation of Cd, Pb, Cr and Ni in tomato crops grown in different strengths of wastewater is recorded in Table 3. This shows that the accumulation of Cd and Ni was absolutely below the safe limit whereas, the accumulation of Pb and Cr was higher than the safe limit maximally by 80 times and 660 times respectively (safe limit of Cd: 0.1 mg kg dry wt⁻¹, WHO, 1992; Pb: 0.3 mg kg dry wt⁻¹, WHO, 1995; Cr: 0.05 mg kg dry wt⁻¹, WHO, 1991; Ni: 0.01–0.10 mg g dry wt⁻¹, Havlin et al., 1999).

3.9. Water quality

In this aquaponics, the temperature of the wastewater ranged from 21.5 °C to 22.5 °C (Table 2). The pH range (7.49–8.14) was changed to 8.26–8.47 (Table 2) but the maximum change was observed in 100% wastewater (10.28%). Total alkalinity was initially low (62.1–178.0 mg CaCO₃ L⁻¹; Table 2) that finally was increased by 18.5–220.8% and this increase was highest in 100% wastewater treatment. The poor DO content was promoted to above saturation level (9.54–14.67 mg L⁻¹; Table 2) (saturation level: >9.0 mg L⁻¹ at 21 °C; APHA, 1995). The concentration range of NO₂-N (0.006–0.087 mg L⁻¹; Table 2) was below the toxic level (<1 mg L⁻¹; Parker, 2002). The toxicity did not occur in the initial concentration of NH₄⁺ (0.01–5.91 mg L⁻¹; Table 2) (NH₄⁺ toxicity

concentration in soil: >1.8 – 9.0 mg L^{-1} ; Van Katwijk et al., 1997). But these concentrations were reduced in all treatments and highest reduction was observed in 100% wastewater (87%). In 25%, 50%, 75% and 100%, TDS, TSS and TS were extremely high but these values were reduced greatly at the end of cultivation.

4. Discussion

4.1. Ion uptake and plant growth

In the study, tomato plants were grown well with good health in all treatments except in the control. NO_3^- deficiency was well marked in the control ($\text{NO}_3\text{-N}$: 0.001 mg L^{-1} ; Table 2) that inhibited the growth of tomato plant. But high concentration of $\text{NO}_3\text{-N}$ (0.842 – 8.83 mg L^{-1} ; Table 2) enhanced the growth of plant in all treatments and highest concentration offered highest growth and yield in 100% treatment. High concentration of $\text{NH}_4^+\text{-N}$ (0.72 – 5.91 mg L^{-1} ; Table 2) was also a growth promoter of the plants through rapid HPO_4^{2-} uptake and nitrification. Alkaline condition (pH: 7.49 – 7.82 ; Table 2) of water favoured these ion uptake and nitrification process. High NO_3^- concentration also alleviated the ammonia (aqueous NH_3) toxicity in root of tomato plant in all treatments and that was highest in 100% wastewater treatment. Total alkalinity was increased due to excess uptake of NO_3^- s by the plants that caused to release HCO_3^- s into culture water. And this uptake was the highest in 100% treatment. Kochy and Wilson (2001) showed that tomato plants were grown excellently on NO_3^- rich soil. Gloser and Gloser (2000) reported that higher NO_3^- uptake increased the absorption of essential cations such as K^+ , Ca^{2+} and Mg^{2+} . Highest concentration of phosphorus (0.209 mg L^{-1} ; Table 2) offered highest yield in 100% wastewater (standard for maximum yield: 0.003 – 0.3 mg L^{-1} ; Havlin et al., 1999). Very high concentration $\text{NO}_3\text{-N}$ ($>8 \text{ mg L}^{-1}$) might have caused inhibition in nitrate reductase (NR) activity in 75% and 100% wastewater. Hyper saturation of O_2 in all the treatment of wastewater could not create any respiratory stress in root tissue that promoted ion absorption and root development in the media.

4.2. Ion absorption, removal efficiency and reclamation

Plant needs more nitrogen (N) than phosphorus (P) for normal growth and crop yield. Therefore, tomato plants absorbed more NO_3^- than H_2PO_4^- in all treatments and this absorption removed N and P from wastewater. In 100% wastewater, $\text{NO}_3\text{-N}$ removal was maximum due to highest absorption of NO_3^- and maximum removal of $\text{PO}_4\text{-P}$ in 50% wastewater was also due to highest absorption of H_2PO_4^- . Sedimentation of suspended and undissolved organic matter of wastewater caused the removal of COD and static state of water favored this. As total solid was maximum in 100% wastewater the COD removal was highest. Algal photosynthesis made the bucket water highly aerobic (9.54 – 14.67 mg l^{-1} Table 2, above the saturation level) that caused the die off of *E. coli* bacteria and this was highest in 100% wastewater. According to WHO (2006) competition with heterotrophic bacteria may be one of the cause of the die off.

4.3. Organo-metallic complex and heavy metal bio-accumulation in crop

Heavy metals (Cd, Cr, Pb and Ni) were transported to crop through ascent of sap and were accumulated within tissue of crop (tomato) due to the formation of metal coordinate complexes with biomolecules.

5. Conclusion

Tomato plants grew better in 100% domestic wastewater. The N, P, BOD, coliform bacteria and heavy metal load of 100% domestic wastewater were reclaimed significantly through aquaponic cultivation of tomato plant. Crop yield was secondary in this low-cost eco-tech. Heavy metal accumulation in crop was dependent upon the concentration of heavy metal in wastewater. The tomato crop yielded in the study was not safe for human consumption. Possibility of colitis disease transmission from wastewater to human was very low in this approach. So, this eco-tech can be recommended for the management of raw wastewater. However, further research is needed for the possible transmission of other pathogenic bacteria that are wastewater-borne and of the accumulation of heavy metals like Hg and As in tomatoes in order to make the tomato safe for human consumption.

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Appendix A. Methods for measuring different parameters used in this study

Parameters	Methods
Stem length	Simple scale measuring method
Number of twig, crop and leaf	Visual counting method
Biomass	Dry weight measuring method
Crop mass	Wet weight measuring method
Shoot Root ratio	Algebraic dividing method (shoot/root)
Leaf surface area	Graph paper method
Removal efficiency	Simple algebraic method (initial-final) concentration \times 100/initial concentration
Chlorophyll content	Colorimetric method (APHA, 1995)
Nitrate reductase (NR) activity	Spectrophotometric method (Gilliam et al., 1993)
$\text{PO}_4\text{-P}$	Stannous chloride method (APHA, 1995)
$\text{NH}_4\text{-N}$	Phenate method (APHA, 1995)
$\text{NO}_3\text{-N}$	Spectrophotometric screening method (APHA, 1995)
$\text{NO}_2\text{-N}$	Colorimetric method (APHA, 1995)
Temperature	Partial immersion method (APHA, 1995)
pH	Electrometric method (APHA, 1995)
Total alkalinity	Titration method (APHA, 1995)
Dissolved oxygen (DO)	Azide modification Winkler's method (APHA, 1995)
Chemical oxygen demand	Closed reflux titrimetric method (APHA, 1995)
Biological oxygen demand	5-Day dilution BOD test method (APHA, 1995)
Total solid (TS)	Thermal evaporation method (APHA, 1995)
Total suspended solid (TSS)	Filtration and thermal evaporation method (APHA, 1995)
Total dissolved solid (TDS)	$\text{TDS} = \text{TS} - \text{TSS}$
Enumeration of coliform bacteria through count of colony forming unit (cfu)	Conventional spread plate technique for 48 h of aerobic incubation at 37°C (APHA, 1995)
Digestion of heavy metals (Cd, Cr, Pb and Ni)	Di-acid digestion method (APHA, 1995)
Estimation of heavy metals (Cd, Cr, Pb and Ni)	Spectrophotometric method (APHA, 1995)

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