



Decentralised approach of wastewater treatment and impact assessment of treated and untreated wastewater irrigation on agriculture land and its produce

SUMIT PAL^{1*}, NEELAM PATEL², ANUSHREE MALIK³ and D K SINGH⁴

Centre for Rural Development and Technology, Indian Institute of Technology, New Delhi 110 016, India

Received: 9 December 2019; Accepted: 24 December 2019

ABSTRACT

A field experiment was conducted from October, 2015 – March, 2017 to investigate the effects of drip irrigation with wastewater, and treated wastewater on crop quality at the 12-C Research farm of Indian Agriculture Research Institute, New Delhi. A bioreactor was installed at 12-C field for the purpose of cleaning the wastewater for the irrigation. After wastewater irrigation, the level of metals in the wastewater irrigated field got increased with time in the soil. While in treated water irrigated soil, concentration of metals was very less. In case of crops, the level of heavy metals was found more in wastewater irrigated crops than the treated water irrigated crops. From the study, it can be concluded that unlike treated wastewater, untreated wastewater cannot be used in agricultural land for a long time. Further, use of treated wastewater for irrigation does not have a negative impact on the consumers' health as well.

Key words: Bioreactor, Drip irrigation, Heavy metal, Treatment, Wastewater

Due to rapid industrialization, urbanization, and subsequent change in lifestyles, amount of wastewater generation is gradually increasing. In many cases, they are disposed either in the river or on open ground without adequate treatment. Therefore, use of contaminated water to sustain farmers' livelihoods has been a common practice in urban and peri-urban areas (Saravanan *et al.* 2011). Such waters are typically polluted by excessive quantities of nutrients as well as contaminated with pathogens and toxic chemical substances that affect both the ecosystem and the public's health (Okereke *et al.* 2016). In many urban and peri-urban areas, these discharges are used by local farmers for irrigating crops, thus introducing these pollutants to the crops and then to the food chain. It's because, they are relatively easily available, have issues for proper disposal, and freshwater for irrigation are not abundantly available (Khan *et al.* 2008, Sharma *et al.* 2008). Continuous irrigation of agricultural land with such wastewater may cause heavy metal accumulation in the soil and vegetables. Intake of heavy metals through the food chain by human populations has been widely reported throughout the world (Muchuweti *et al.* 2006). Due to the non-biodegradable and

persistent nature, heavy metals are accumulated in vital organs of the human body such as kidneys, bones and liver are associated with numerous serious health disorders, i.e. liver damage, kidney disorders, amyotrophic, spinal disorder and muscular dystrophy (Duruibe *et al.* 2007). With the health hazards, wastewater irrigation is expected to cause changes in the soil and agricultural produce quality. (Rusan *et al.* 2007). While some of the changes in soil properties such as increase in micronutrients and organic carbon are beneficial, the risk of metal accumulation and microbial contamination needs to be carefully considered. Metal contamination from wastewater leads to damage of soil quality and these accumulated metals are further taken up by the crops grown with wastewater.

This study aims to evaluate effectiveness of treated wastewater and drip irrigation method for sustainability of agriculture as well as in minimizing health risks by means of carrying out field experiments. The experiment was carried out at the 12-C Research farm of Indian Agricultural Research Institute (IARI), New Delhi.

MATERIALS AND METHODS

Site selection and design/installation of water conveyance system

12C Field is situated at IARI, New Delhi at 28°38'30" N, 77°08'59" E. The site is chosen due to easy availability of wastewater, i.e. Lohamandi Drain throughout the year which ultimately flows into Yamuna river. Polyvinyl chloride (PVC) pipe system has been designed and installed

* Corresponding author: sumitiitdelhi2013@gmail.com

^{1,2}Water Technology Centre, Indian Agricultural Research Institute, New Delhi, India. ³Centre for Rural Development and Technology, Indian Institute of Technology, New Delhi, India.

⁴Department of Zoology, University of Delhi, New Delhi, India.

for the supply of water. A power supply and control panel with two 3-hp motors and a 2-hp motor with pump is also installed and connected with 3-inch pipe inlet and 2-inch pipe outlet. The headworks and pump house were also constructed at the 12C field. Three different pipe systems, namely, untreated wastewater, treated waste water (bio-remediated), and groundwater for the treatment were also constructed.

Bioreactor

A bioreactor has been installed for the treatment of wastewater with support from Indian Institute of Technology (IIT) Delhi and University of Delhi. Design of the wastewater treatment reactor consists of a motor system to uplift the wastewater into a storage tank (2000 l). The collected wastewater was connected to the small 1-hp motor to transfer the wastewater to the pilot plant (1000 l). In this pilot plant, the consortium was processed so that treatment of water with indigenous microorganisms was performed. Initially, the wastewater was treated for seven days, and afterwards, it became a continuous bioreactor. It was connected to the settler through the pipes so that the solid waste mass which was coming with the treated water is settled and remaining clean water is stored in a tank (750 l). Then this water was used for irrigation.

Layout

The layout and associated details are given in Table 1.

Treatments

The 12C field has been divided into two main plots of untreated wastewater irrigation and treated wastewater irrigation with drip irrigated and flood irrigated sub-plots. All the treatments are in triplicates with split-plot design. Each plot is of 24 m² of size. The selection of crop and its varieties has been done by choosing the crops locally grown in the Delhi-NCR regions. Details of crops and their varieties are listed in Table 2, geometry of the crop in Table 3, and crop growing stages in Table 4.

Water samples digestion and analysis for heavy metals

Water samples (100 ml) were digested after adding 15ml of Di acid mixture (HNO₃ and HClO₄ in ratio 9:4)

Table 1 Layout of the treatments

Treatment type	Irrigation type	Crop
Wastewater	Drip	Okra
Treated wastewater	Drip	Okra
Wastewater	Flood	Okra
Treated wastewater	Flood	Okra

Table 2 Details of crop

Crop	Varieties	Season
Okra	PUSA A-4	Summer

Table 3 Geometry of crops

Crop	Crop spacing		Dripper to dripper spacing	Lateral to lateral spacing	Dripper discharge rate (lph)
	Row to row (cm)	Plant to plant (cm)			
Okra	60	30	30	120	1.07

Table 4 Crops growth stages

Crop	Initial (Days)	Developmental (Days)	Middle (Days)	Late (Days)	Total
Okra	20	20	30	20	90

at a temperature of 80°C until a transparent solution was obtained (standard protocols of waste water analysis by APHA (American Public Health Association), AWW & WEA (Arkansas Water Works and Water Environment Association). After cooling, the digested sample was filtered using Whatman no. 42 filter paper and the filtrate was finally maintained to 100 ml with double distilled water.

Soil samples digestion and analysis for heavy metals

Soil samples were collected in triplicate from different depths (0-15cm, 15-30cm and 30-45cm) and at varying distance (5m, 100m and 300m) from the river bank. Soil samples were air dried, crushed and passed through 2 mm mesh size sieve and stored at ambient temperature before analysis (Singh *et al.* 2010).

Soil (1 g) was digested after adding 15 ml of tri-acid mixture (HNO₃, H₂SO₄ and HClO₄ in 5:1:1 ratio) at 80°C until a transparent solution was obtained. After cooling, the digested sample was filtered using Whatman No. 42 filter paper and the filtrate was finally maintained to 25 ml with distilled water.

The analysis for heavy metals was conducted using AAS4141 ECIL Atomic absorption spectrophotometer. The instrument was fitted with specific lamp of particular metal. The instrument was calibrated using manually prepared standard solution of respective heavy metals as well as drift blanks. Standard stock solutions for all the metals were obtained from Sisco Research Laboratories Pvt Ltd, India. These solutions were diluted for the desired concentrations to calibrate the instrument. Acetylene gas was used as the fuel and air as the support. An oxidising lamp was used in all cases (Pal *et al.* 2015).

Crop samples digestion and analysis for heavy metals

Plant samples (1 g) were digested after adding 15 ml of tri acid mixture (HNO₃, H₂SO₄ and HClO₄ in the ratio 5:1:1) at 80°C until a transparent solution was obtained. After cooling, the digested sample was filtered using Whatman no. 42 filter paper and the filtrate was finally maintained to 25 ml with double distilled water. Triplicate digestion of each sample was carried out together. The analysis was conducted using AAS4141 ECIL Atomic absorption spectrophotometer.

Quality control analysis

Blank and drift standards (Sisco Research Laboratories Pvt Ltd, India) were run after five determinations to calibrate the instrument. The coefficients of variation of replicate analysis were calculated for different determinations for the precision of analysis and variations below 10% were considered correct.

Microbial community analysis

Soil samples from untreated wastewater irrigated soil and treated wastewater irrigated soil were collected from 0-15cm depth at the field and analysed for the microbial community. After collection, samples were stored at - 20°C until further analysis.

Sampling for DNA extraction: For DNA extraction, the soil sample was collected in sterilized polythene bags. DNA was isolated from 1 g of the soil sample using manufacture's protocol via FastDNA SPIN kit (for soil) (MP Biomedical, Cambridge, United Kingdom). The concentration of DNA after extraction was quantified with Eonc Take 3 microliter plate (Biotek).

Amplification of 16s rRNA gene: Primer set of F1 (5'-ATTACCGCGGCTGCTGG-3') and R2 (5'-ATTACCGCGGCTGCTGG-3') targeting conserved V3 domain of 16s rRNA was employed for the partial amplification of 16s rRNA gene (Muyzer and Waal 1993). A 40-nucleotide GC-rich sequence (GC-clamp) was added to the 5'-end of the forward primer (F1) (Muyzer and Waal 1993). The PCR reaction mixture of total volume 50µL was prepared [PCR supermix™- 25 µL (Gene Direx), Forward primer (F1)-4 µL, R2-4 µL, DNA template- 6 µL, MQ water- 11 µL] for amplification. The PCR cycling conditions used was: Initial denaturation: at 94°C for 1 min; 35 cycles of denaturation at 94°C for 1 min; Annealing at 55°C for 1 min; Extension at 72°C for 1 min. It was followed by final elongation at 72 °C for 7 min.

DGGE (Denaturing gradient gel electrophoresis): DGGE was performed for PCR amplicons obtained with a DCode system (Bio-rad, CA). PCR products were loaded onto a 10% (w/v) acrylamide gel having a 30-60% denaturing gradient. Chemicals used in preparation of DGGE gel is listed in Table 5. Electrophoresis was performed at 60 volts for 16 h in 1x TAE buffer. Composition of 1x TAE buffer was: 40mM tris acetate, 1 mM Sodium ethylene diaminetetraacetic acid, pH: 8). The DGGE gel was stained with 1x TAE buffer containing EtBr (Ethidium Bromide: 10mg mL⁻¹) for 45 min using gel rocker (REMI). Image of gel was captured by Gel Doc imaging system (Bio-rad, CA). Visible bands on gel were excised using a sterile blade and stored in TE buffer. To elute out the DNA bands from excised gel to TE buffer, bands were incubated at 95°C for 5 min. The eluted DNA bands were amplified using the same primer set and PCR conditions as described above. PCR reaction obtained were purified with QIAquick™ purification kit (Qiagen, Germany) as per the protocol provided by the manufacturer. After purification, sequencing of the amplicons were performed

Table 5 Chemical constituent of denaturing gradient gel electrophoresis (DGGE) gel

Chemical	30% denaturing gel	60% denaturing gel
40% Bis acrylamide	5 mL	5 mL
50 x TAE buffer	0.4 mL	0.4 mL
Formamide	2.4 mL	4.8 mL
Urea	2.52 gm	5.04 gm
Final volume	20 mL	20 mL
APS (10%) solution	120 µL	120 µL
TEMED	12 µL	12 µL

using dye termination reaction (MicroSEQ™M500 16S rDNA Identification, applied bio-system) and products were loaded into the genetic analyser (Applied Biosystems™ 3500 Series Genetic Analyzers — Use MicroSEQ™ ID Analysis Software Version 3.0) to determine the sequence of the PCR products (Green *et al.* 2010).

Phylogenetic analysis: Sequence similarity search for the obtained sequence was performed using BLAST programme, whereas alignment was performed by CLUSTALW. The evolutionary history was inferred using the UPGMA method (Unweighted Pair Group Method with Arithmetic Mean). The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA5.

RESULTS AND DISCUSSION

Monthly variation of heavy metals in raw and treated wastewater

Studying the monthly variation from 2015-2017 of Lohamandi drain water and treated Lohamandi drain water, the following (Table 6) average variation in heavy metal concentration has been observed in a particular year, which are above the permissible limits of FAO (permissible limits of heavy metals in irrigation water: Zn:2ppm, Cu:0.2ppm, Ni:0.2ppm, Pb:5ppm and Cr: 0.1ppm). The metals like chromium and nickel are above the permissible limits in most of the months, while other metals were not above limits but high in concentrations in wastewater. On the other hand, in treated wastewater (2016-2017) the concentration of all the studied metals was very low and chromium was below the detectable range in the treated water. Hence, the treated wastewater is safe to use over untreated wastewater.

Impacts of drip and flood wastewater irrigation on soil and crop

Impacts of drip irrigation and flood wastewater irrigation on soil and crop were assessed. The studied soil was sandy loam and the crop was okra. The physical properties of the soil are shown in Table 7. In terms of chemical properties,

Table 6 Average monthly variation (year-wise) of heavy metals in wastewater

Years	Zn (ppm)	Zn (ppm)	Cu (ppm)	Cu (ppm)	Cr (ppm)	Cr (ppm)	Ni (ppm)	Ni (ppm)	Pb (ppm)	Pb (ppm)	Cd (ppm)	Cd (ppm)
	WW	TW	WW	TW	WW	TW	WW	TW	WW	TW	WW	TW
2015	0.838	-	0.158	-	0.155	-	0.143	-	0.633	-	Nd	-
2016	1.083	0.170	0.138	0.025	0.088	Nd	0.155	0.033	1.727	0.068	Nd	Nd
2017	1.185	0.267	0.149	0.017	0.128	Nd	0.204	0.033	2.006	0.071	Nd	Nd
Permissible Limit (ppm) FAO	2	2	0.2	0.2	0.1	0.1	0.2	0.2	5	5	0.01	0.01

WW : Wastewater, TW: Treated wastewater

Table 7 Physical properties of soil

Depth (cm)	Particle size distribution			Textural class	Hydraulic conductivity (cm h ⁻¹)	Bulk density (gm cm ⁻³)	FC (%)	PWP (%)
	Clay (%)	Silt (%)	Sand (%)					
0-15	12	16	72	Sandy loam	1.32	1.56	29	9.6
15-30	14	16	70	Sandy loam	1.29	1.55	28.9	9.7
30-45	15	21	64	Sandy loam	1.22	1.58	30.5	10.2
45-60	15	25	60	Sandy loam	1.21	1.58	30.4	10.5
60-75	17	25	58	Sandy loam	1.12	1.59	30.8	10.9
75-90	18	24	58	Sandy loam	0.99	1.61	30.5	10.8

nitrogen, phosphorus and potassium in soil of wastewater and treated wastewater in drip and flood irrigated soil is increasing with time. This study also identified that the amount of nitrogen and phosphorus was increasing with the depth of soil. The concentration of nutrients was increasing by wastewater irrigation and also the amount of nutrients in drip irrigated plots was more than flood irrigated ones. The percentage increase of nutrient was 50%-66% in the final year. Heavy metals present in wastewater irrigated soil increase with time and get accumulated in the soil. The value of copper, chromium, nickel, zinc and lead in okra is under permissible limit in soil of 1st year. But the range has been increased in the last year of study. The amount of lead is 129 mg/kg and nickel 74.59 mg/kg in flood irrigated soil. It proves that the accumulation of metal crosses the permissible limits provided by European Union (i.e. Cu-100 mg/kg, Cr-100 mg/kg, Ni- 50 mg/kg, Pb-100 mg/kg and Zn- 300 mg/kg).

Table 8 Impacts of drip and flood wastewater irrigation on crop

Element	Concentration in the beginning (mg/kg)		Concentration in the final year (mg/kg)	
	Flood irrigation	Drip irrigation	Flood irrigation	Drip irrigation
Cr	1.02	1.4	1.07	1.97
Cu	7.71	3.13	8.90	4.09
Ni	7.06	2.40	9.07	2.22
Pb	7.69	8.96	11.52	9.03
Zn	52.89	17.9	76.77	28.47

FAO/Codex Permissible Limits: Cu: 30 mg/kg, Cr: 2.3 mg/kg, Ni: 1.5 mg/kg, Pb: 2.5 mg/kg, Zn: 50 mg/kg

In crop, the initial year of study has shown that heavy metals, i.e. chromium, nickel and lead was above the permissible limits of FAO while copper was below the permissible limits in okra. The concentration of metal accumulation in crops was higher in flood than drip irrigated ones. The results of heavy metal concentration in okra is presented here under.

The concentrations in the beginning

In flood irrigation: Nickel (7.06 mg/kg), Lead (7.69 mg/kg) and Zinc (52.89 mg/kg),

In drip irrigation: Nickel (2.40 mg/kg) and Lead (8.96 mg/kg), which are above the permissible limits of FAO/Codex. (i.e. Cu: 30 mg/kg, Cr: 2.3 mg/kg, Ni: 1.5 mg/kg, Pb: 2.5 mg/kg, Zn: 50 mg/kg)

Concentrations in the final year,

In flood irrigation: Nickel (9.07 mg/kg), Lead (11.52 mg/kg) and Zinc (76.77 mg/kg).

In drip irrigation: Nickel (2.22 mg/kg) and Lead (9.03 mg/kg), which are above the permissible limits of FAO.

Here, the accumulation has been increased by 20 to 33% in flood irrigated crops and 1.25 to 24% in drip irrigated crops. Also, the metal accumulation causes fast degradation of crops then the normal grown (Table 8).

Impacts of drip and flood irrigation of treated wastewater on soil and crop

In treated water irrigated soil, the concentration of heavy metals was found to be very low. The amount of nitrogen, phosphorus and potassium is in medium range. The concentration of nutrients was also decreasing with depth in the soil. In treated water irrigated okra, all the five

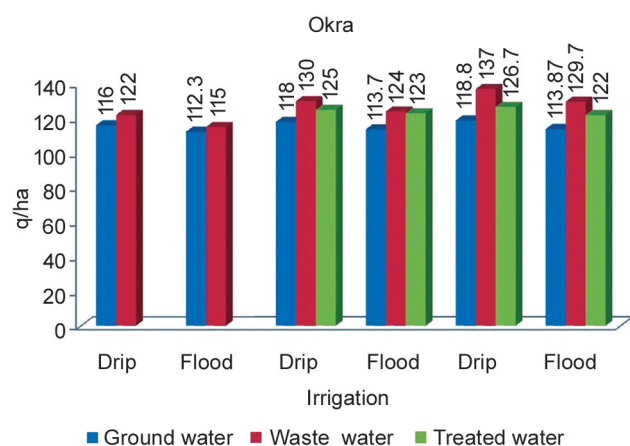


Fig 1 Year-wise yield of okra.

metals are below the permissible limits of FAO. Also, the degradation rate was less than that of wastewater irrigated okra. These metals in okra have been estimated as;

In flood irrigation: Copper (2.8 mg/kg), Chromium (1.4 mg/kg), Nickel (2.06 mg/kg), Lead (2.01 mg/kg) and Zinc (40.08 mg/kg).

Drip: Copper (1.3 mg/kg), Chromium (1.3 mg/kg), Nickel (1.9 mg/kg), Lead (1.9 mg/kg) and Zinc (16.6 mg/kg).

Impacts of drip and flood irrigation of wastewater and treated wastewater on crop yield

The yield of okra, was more in drip irrigated wastewater and treated water than the flood irrigated. In treated water, yield was less than wastewater but the contamination was very less than waste water irrigated crops.

Microbial communities in wastewater and treated wastewater irrigated soil

Species found in soil	Waste water	Treated waste water	Comment
<i>Acidobacteria bacterium</i> clone PBM3_H10,	Present	Present	Biodegrade
<i>Alcaligenes faecalis</i> strain 3d,	Present	Absent	Opportunistic microorganism
<i>Alcaligenes faecalis</i> strain ABE1a,	Present	Absent	Opportunistic microorganism
<i>Alcaligenes faecalis</i> strain C_9,	Present	Absent	Opportunistic microorganism
<i>Alcaligenes faecalis</i> strain DBT18,	Present	Absent	Opportunistic microorganism
<i>Alcaligenes pakistanensis</i> strain DBT26,	Present	Present	Heavy metal tolerant
<i>Alcaligenes</i> sp. clone K2DN258,	Present	Present	Heavy metal tolerant
<i>Alcaligenes</i> sp. ST3-13,	Present	Absent	Heavy metal tolerant
alpha proteobacterium IICTSVMME1,	Present	Present	Endo symbiont of pathogens
bacterium clone 3c08,	Present	Absent	-
bacterium clone RM157,	Present	Absent	-
bacterium gene.	Present	Absent	-
Bacterium T-2-48-9A	Present	Present	-
bacterium isolate DGGE gel band B23,	Present	Present	-
Bacterium MS-AsIII-61,	Present	Present	-
<i>Burkholderia cepacia</i> gene,	Present	Present	Opportunistic microorganism
<i>Burkholderia vietnamiensis</i> strain KNL-16,	Present	Present	Opportunistic microorganism
<i>Burkholderia vietnamiensis</i> strain KNL-16,	Present	Absent	Opportunistic microorganism
<i>Candidimonas</i> sp. UCM-F49,	Present	Present	Opportunistic microorganism
<i>Gemmatimonadetes bacterium</i> partial 16S rRNA gene clone UMAB-cl-124,	Present	Absent	Heavy metal tolerant
<i>Halochromatium</i> sp. isolate DGGE gel band 17BAC,	Present	Absent	-
<i>Hymenobacter</i> sp. RP-2016a strain CCM 8649,	Present	Absent	-
<i>Micrococcus</i> sp. strain YM47,	Present	Present	Non-pathogenic
proteobacterium clone Upland_40_6285,	Present	Absent	-
<i>Pseudomonas</i> sp. DGGE band 5,	Present	Present	Pathogenic
<i>Pusillimonas</i> sp. N12,	Present	Present	Pathogenic
<i>Shewanella putrefaciens</i> strain DHS01,	Present	Present	Bioremediant
<i>Shewanella</i> sp. FS8-2,	Present	Present	Bioremediant
<i>Sphingomonas</i> sp. strain A835,	Present	Absent	Bioremediant
<i>Telluria mixta</i> strain 58-Y97,	Present	Absent	Biodegrade
Uncultured bacterium clone OTU33,	Present	Present	-

Impacts of wastewater and treated water on soil microbiological biodiversity

After studying the DGGE (Denaturing gel gradient electrophoresis) of wastewater and treated water soil, following community has been observed.

In Lohamandi drain irrigated soil has more pathogenic microorganisms than the treated water irrigated soil. Some uncultured microorganisms were also present in soil which could behave both pathogenic and non pathogenic.

In 12C IARI field, water of Lohamandi drain are polluted with chromium and nickel, while other heavy metals are at the borderline of permissible limits in wastewater, which got accumulated in the soil and then to crops. In most of the months, chromium and nickel was above the permissible limits in Lohamandi drain. In soil, the concentration of metal was increasing with time and in the final year of study the concentration of nickel and lead was above the permissible limits in soil irrigated with wastewater. While in treated water all the metal limits are below. In case of crop, the wastewater irrigated crops had higher concentration of chromium, nickel, lead and zinc.

The flood irrigated crops had increase of total metal percentage by 28% in okra. While in drip irrigation, the concentration of metal has been increased by 24% in okra. This study also reveals that in the 3rd year, drip accumulates 27.1%, less total metals than flood in edible part of okra. Nearly the same trend follows in the 1st year and 2nd year of crops also. Yield is also better in drip irrigated crops with wastewater, treated water and groundwater than the flood irrigated. Drip irrigation accumulates less metal with better crop yield than flood irrigated one. In treated water and wastewater, the metal accumulation got reduced with treated water with very less reduction in yield of crops. Also, the microbiological diversity study reveals that, pathogenic microorganisms were more in the wastewater irrigated soil. This study recommends that the drip irrigation with treated water is cost effective, less hazardous and prominent for sustainable agriculture. Farmers will be highly benefited and this eco-friendly approach of treatment of waste water will be helpful in reduction of environmental and health risk.

ACKNOWLEDGEMENTS

This research is funded by National Agricultural Science fund, ICAR, New Delhi and research and technical support from IIT Delhi and University of Delhi.

REFERENCES

- Duruibe J O, Ogwuegbu M O C and Egwurugwu J N. 2007. Heavy metal pollution and human biotoxic effects. *International Journal of Physical Sciences*. 2(5): 112-118.
- Green S J, Leigh M B and Neufeld J D. 2010. Denaturing gradient gel electrophoresis (DGGE) for microbial community analysis. (In) *Handbook of Hydrocarbon and Lipid Microbiology*, pp 4137-4158. Springer, Berlin Heidelberg.
- Khan S, Aijun L, Zhang S, Hu Q and Zhu Y G. 2008a. Accumulation of polycyclic aromatic hydrocarbons and heavy metals in lettuce grown in the soils contaminated with long-term wastewater irrigation. *Journal of Hazardous Materials* 152(2): 506-515.
- Muchuweti M, Birkett J, Chinyanga E, Zvauya R, Scrimshaw M and Lister J. 2006 Heavy metal content of vegetables irrigated with mixtures of wastewater and sewage sludge in Zimbabwe: implication for human health. *Agri. Ecosys. Environ.* 112: 41-48.
- Okereke J N, Ogidi O I and Obasi K O. 2016. Environmental and health impact of industrial wastewater effluents in Nigeria-A Review. *Int. J. Adv. Res. Biol. Sci* 3(6): 55-67.
- Pal S, Patel N, Malik A and Singh D K. 2015. Heavy metal health risk assessment and microbial menaces via dietary intake of vegetables collected from Delhi and national capital regions peri urban area, India. *Journal of Food, Agriculture & Environment* 13 (2): 82-88.
- Rusan, M Hinnawi, Sami and Rousan, Laith. 2007. Long term effect of wastewater irrigation of forage crops on soil and plant quality parameters. *Desalination*: 215 143-152. 10.1016/j.desal.2006.10.032.
- Saravanan V S, Mollinga, P P and Bogardi J J. 2011. Global change, wastewater and health in fast growing economies. *Current Opinion in Environmental Sustainability* 3(6): 461-466.
- Sharma R K, Agrawal M and Marshall F M. 2008. Heavy metal (Cu, Zn, Cd and Pb) contamination of vegetables in urban India: A case study in Varanasi. *Environmental Pollution* 154 (2): 254-263.
- Singh A, Sharma R K, Agrawal M and Marshall F M. 2010. Health risk assessment of heavy metals via dietary intake of foodstuffs from the wastewater irrigated site of a dry tropical area of India. *Food and Chemical Toxicology* 48(2): 611-619.