EVALUATION OF QUALITY AND IMPACT OF UNTREATED WASTEWATER FOR IRRIGATION

Mousumi Debnath*, Vijay Chejara, Brij Kishore Vijaya, Dalip Singh Bika, Sajal Kumar , Anil Dixit, Renu Jain and Vishwas Jain

CEG Test house & Research Centre , Malaviya Industrial area, Malaviya nagar , Jaipur-302017, India
*Corresponding author: mousumi.debnath@gmail.com

Abstract: Wastewater generated from industries is widely reused for irrigation in agricultural fields. The purpose of this study was to examine the wastewater generated from textile industries and study the impact of the wastewater irrigation on crops. The microbial load, physico-chemical characteristics and the heavy metals in wastewater was found to be on higher side and predominant presence of iron (Fe), copper (Cu) and chromium (Cr) poised a major concern. Pesticide residues were found to exceed the quality goal of 0.1ug/L. On examining the vegetables produced by the plants, *Lagenaria siceraria* and *Abelmoschus esculentus* confirmed accumulation of heavy metal, pesticide residue and microbial contamination, indicating a possibility of recycling the toxicants and microbes through the wastewater. Overall assessment indicated a potential risk of heavy metal, microorganisms and pesticides on agriculture and human health.

Key words: Wastewater, irrigation, heavy metals, pesticides, microbial communities, Sanganer, textile effluents

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1. Introduction

Concern for human health and the environment are the most important constraints in the reuse of wastewater. The irrigation method used and the type of crop grown determine the magnitude of the contact between the wastewater and crops and affect the nature of the human hosts exposure to the contaminated soil, wastewater, and crops.

The untreated sewage water and wastewater from textile industries containing variety of chemicals such as aniline, caustic soda, acids, bleaching powder including heavy metals, are used in irrigating agricultural fields, for growing vegetables and other crop plants. The effluent water takes the dissolved toxicants to crop plants and its consumers (Khan *et al.,* 2009). Vegetables grown in the agricultural fields using untreated textile wastewater for irrigation are adversely affected. Use of wastewater alters the nutritional value of the vegetables grown there and in long run consumption of such vegetables may impose health hazards in human beings, which is a matter of concern. Irrigating crops with wastewater containing high nutrient load often leads to prolific weed growth and undesirable growth of microorganisms (Modasiya *et al.*, 2013**)**. Pests are habitually controlled by blanket-spraying with insecticides however, the hot climate provides opportunistic breeding conditions by quickening insect reproductive cycles, which then enables pests to build up pesticide resistance faster (Bradford *et al.*,2003). To overcome such situations, Danso *et al.,* (2002) described that farmers usually use a mix of many pesticides to control the situation. Such residue of pesticide has been reported in vegetables from these irrigated lands (Amoah *et al.,* 2006; Jiries *et al.*, 2002). Because of their highly toxic and persistent nature, the residues still appear as pollutants in food as well as in the environment (Bempah *et al.*, 2011). However, residues of pesticides could affect the ultimate consumers especially when these commodities are freshly consumed (Bempah *et al.*,2012).

It certainly makes the part of food chain as people in and around Sanganer in Jaipur district consumes these vegetables and products of other crop plants (Marwari *et al.*, 2009). Jaipur is well known for the colourful Calico block printing textiles of Sanganer town. Textile industry requires large volume of water (Sharma *et al.*,2007) and use a variety of complicated, stable, non biodegradable chemicals and azo dyes (Mathur and Kumar, 2013, Sethi *et al.,* 2012). To meet the growing domestic and agricultural demands, untreated textile dye wastewater (10,000 - 15,000 kl/day) is used for irrigation purpose and is released into surface waters of Amanishah Nalla through the nalla systems, which seeps into the ground water and adjoining water bodies and then flows through Sanganer (Joshi and Kumar, 2011; Gangal, 2005).

Wastewaters contain various toxic salts, acids, dissolved gases, heavy metals, pesticides, persistent organic pollutants, etc. (Garg and Kaushik, 2006). In textile industries, pesticides are sometimes used for the preservation of natural fibers and are transferred to wastewaters during washing and scouring operations. Pesticides are also used for moth proofing, brominated flame retardants for synthetic fabrics, and isocyanates for lamination (Goel and Goel, 2012). The fate of the pesticides in the soils is extremely important because of their impact on ecosystem (Jiries *et al.,* 2002). A number of pesticides are highly toxic and exposure to very small quantities can result in the death of humans and animals (Hussain and Siddique, 2010). The textile effluent is rich in color and characterized by extreme fluctuations in many parameters such as chemical oxygen demand (COD), biochemical oxygen demand (BOD), pH , salinity (Talarposhti *et al.,* 2001) and heavy metals like Cr, Cu, Pb, Zn, Fe (Jaishree and Khan, 2013). This water quality decay is a result of toxicants in wastewater. The water pollution problem particularly due to toxic heavy metals has become a menacing concern. (Lokhande *et* *al.*, 2011). Heavy metals are extremely persistent in the environment as they are non biodegradable and nonthermodegradable. Heavy metals can accumulate in the soil at toxic levels due to the long-term application of wastewater (Bohn *et al.*, 1985).

Through irrigation, the microorganisms present in the water can contaminate crops, then pass into the food chain and eventually infect humans (Pianetti *et al.*, 2004). The enteric bacteria are perhaps the most common pathogens present in wastewater and *Salmonella* species occur most frequently. Coliform bacteria have served as indicators of faecal contamination of water for many years, and their densities have been utilized as criteria for the degree of pollution (Teltsch *et al.*, 1980). This is because of the variable survival rates of faecal streptococci species, variations in detection methods, and variable sensitivity to water treatments (Clesceri *et al.*,1998). The total and faecal coliforms (MPN/100 mL), faecal coliforms (CFU/100 mL), faecal streptococci and *Clostridium perfringes* are the four most common indicator bacteria presently in use (Feigning *et al.*, 1991; FAO, 1992; Cooper and Olivieri, 1998**)**. Actual risk of infection due to bacteria (cholera, typhoid and shigellosis), and protozoan infections (amoebiasis, giardiasis) are lower, and viral infections (viral gastroenteritis and infectious hepatitis) pose the least health risk (Amoah, 2008).The detection of the pathogens in wastewater, soil, and on crops are an indication of potential health risks to the populations consuming crops irrigated with wastewater and agricultural workers exposed occupationally (Strauss, 1994).

Several studies on crop contamination with pathogenic microorganisms using wastewater or partially treated wastewater have been carried out in different parts of the world. The vegetables and soil irrigated by wastewater accumulate heavy metals such as Cd, Zn, Cr, Ni, Pb, and Mn. When the capacity of the soil to retain heavy metals is reduced due to repeated use of wastewater, soil releases heavy metals into ground water or soil solution available for plant uptake (Sharma *et al.*, 2007). In the long run these may contaminate the surface and ground water, soil, crops and vegetation (food/fruits/vegetables) causing considerable adverse impact on health of the consumers/local population as a result of environmental exposure. A number of previous studies from Sanganer have reported heavy metal contamination in wastewater (Mathur and Kumar, 2013; Joshi and Santani, 2012; Kumar *et al.*, 2009; Singh and Chandel, 2006).

Therefore, to evaluate the impact of the wastewater on irrigation, a detailed analysis of the physiochemical characterization of wastewater, the microbial and toxicants load and the hygienic quality of vegetables produced in those fields irrigated with the same wastewater was performed.

2. Experimental

2.1 Sampling material and location: Wastewater used for irrigation of vegetables growing in the fields was collected from inflow and effluent regions of Amanishah Nalla (drainage basin), Sanganer, Jaipur. Sanganer town is situated nearly 20 km away from the main city of Jaipur. The total area of Sanganer is about 635.5 sq km. This town lies between 26°49' to 26°51'N latitude and 75°46' to 75°51'E longitude. Three sampling locations (N1, N2 and N3) along the Amanishah Nalla were selected with reference to the point receiving maximum wastewater (Fig 1).Two commonly cultivated vegetables, Bottle gourd (*Lagenaria siceraria*) and Okra (*Abelmoschus esculentus* Moench) growing in the nearby fields (L1, L2, L3) were selected for the study as test plant species.

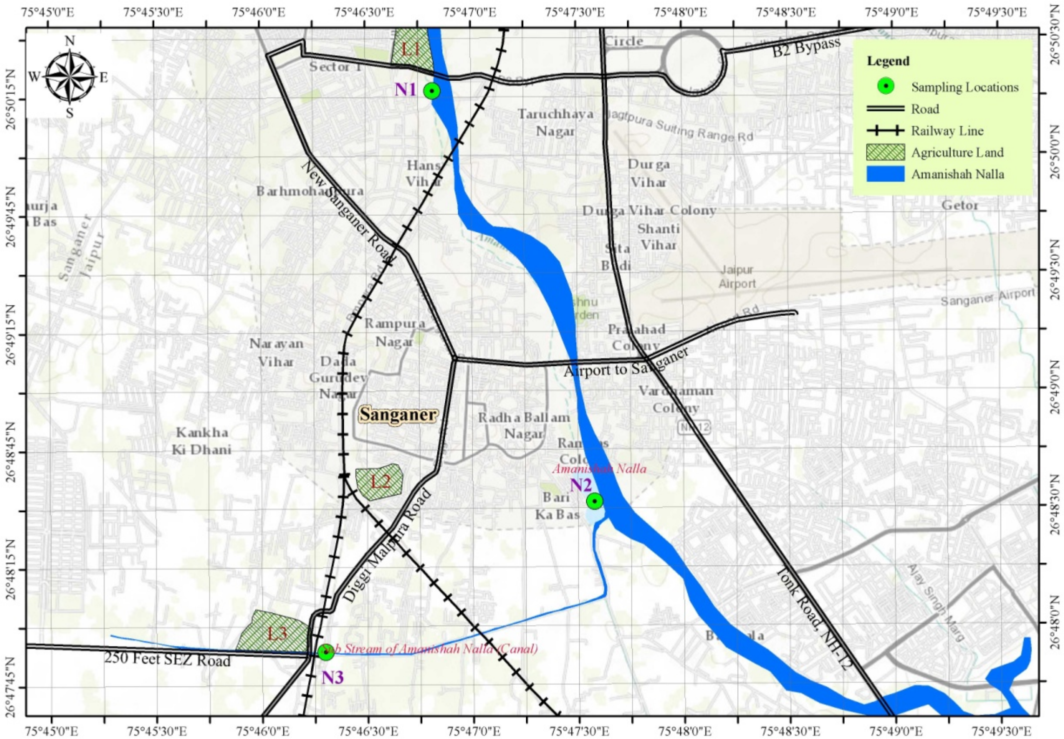

Fig 1 : Study region and sampling sites at and near Amanishah Nalla, Sanganer, Jaipur.

2.2 Standards and chemicals: Pesticide analytical standards of high purity (> 98%) were purchased from Sigma- Aldrich (Steinheim, Germany). Individual stock solutions of all pesticides were prepared in HPLC grade Ethyl acetate and stored at 4^oC. All the solutions were stored in a refrigerator at 4^o C. Distilled water was provided by a Milli-Q water purification system from Millipore (Bedford, MA, USA).

For accurate determination of concentration of metals in water and vegetables, the multi element mix of 24 elements was purchased from Merck, Germany. A stock solution of 10mg/mL was made and stored at 4^oC.
Certified strains were procured from Microbial type culture collection (MTCC), Institute of Microbial Technology (IMTech), Chandigarh. They were stored as glycerol stock at -20^0C. All tester strains were stored and maintained according to the prescribed standard methods. Microbiological media and supplements were purchased from HiMedia. All glasswares, reagents, media and petriplates were sterilised before any use.

2.3 Physico-chemical characterisation of wastewater: The wastewater from three sampling locations along the Amanishah Nalla in Sanganer area was collected in airtight plastic containers. The physio-chemical properties like pH, electrical conductivity, TDS, nitrate, TSS, COD, BOD, DO, Sodium (Na), Potassium(K), Calcium (Ca), Magnesium (Mg), Phosphorus (P), oil and grease were analysed immediately in the laboratory as per the Bureau of Indian standards (IS codes) as enlisted in Table 1.

2.4 Preparation of microbiological media: All the media used in this study were prepared and sterilized according to manufacturer's (Himedia) instructions. Methods according to Bureau of Indian Standards were followed for total bacterial count, yeast and mould count, *Salmonella, Shigella, Vibrio cholerae*, *Staphylococcus aureus*, *Faecal streptococci*, *Pseudomonas aeruginosa*, *E. coli*, sulphate reducing bacteria (*Clostridium* sp.) and coliforms. To detect the presence of microorganisms in the vegetables, 25 g of each of the sample was weighed and blended in sterile peptone solution under sterile condition. The homogenates were collected in sterile bottles and stored at -20^0C until needed. Aliquots (1.0mL) of each homogenate were serially diluted in serial dilution**.** Sterile nutrient agar plates were aseptically inoculated with aliquot of serial dilutions (10^{-1} to 10^{-9}) of the wastewater and vegetable samples as per the standard IS methods mentioned in Table 2 for the specific microorganism.

2.5 Heavy metal analysis of wastewater and vegetables: For wastewater, 50 mL of the samples was mixed with 10 mL of concentrated Nitric acid (Suprapure) in 250 mL beaker and digested at 100^0C for 2 hours on water bath as instructed in APHA, 2012. The vegetables were washed with distilled water, and cut into small pieces. The minced vegetable samples were accurately weighed and dried in an oven at 60^0C overnight. Vegetable samples underwent pressurised digestion with HNO_3 /H_2O_2 in a microwave heated system (SINEO microwave, China). All samples were analyzed for heavy metals in wastewater and vegetable samples with external calibration using ICP MS (XSERIES 2, Thermo Scientific, USA).

Guidelines for maximum limit (ML) of metals in vegetables and irrigation water were adopted from FAO /WHO and USEPA guidelines. The heavy metals present in the vegetables content obtained from ICP-MS analysis in mg/L were converted into mg/kg using Temminghoff and Houba's formula (2004)

$$\frac{\{(a - b) \times v\}}{w}$$

where, a is the concentration of the heavy metal in the sample (mg/L); *b* is the concentration of the heavy metal in the blank (mg/L); *v* is the total volume of digest (mL) and *w* is the weight of the plant material (g). Counts were recorded and analyte concentration was calculated with Plasma lab software.

2.6 Pesticide analysis:

2.6.1 ***Extraction of pesticides from wastewater:*** One litre of wastewater sample was taken in a separating funnel; 50 g of sodium chloride was added and shaken vigorously to dissolve salt. The aqueous phase was liquid-liquid extracted with dichloromethane following the standard method of AOAC official method 2007.01. The contents were evaporated to near to dryness using turbo evaporator (PCi Analytics, India) and later reconstituted with ethyl acetate.

2.6.2 ***Extraction of Pesticides from vegetable:*** The extraction procedure of pesticides from vegetables employed in the present study was based on a previously developed method (AOAC official method 990.06).

2.6.3 ***Instrument condition for GC-MS/MS:*** Analytes in the sample extracts were analysed on GC-MS/MS (Thermo Scientific, San Jose, CA, USA). The determination of target pesticides were carried out in timed SRM mode as programmed in the GC-MS/MS method. A minimum of two transitions for each pesticide and their collision energy were selected. Matrix matched calibration standard was prepared by spiking a mix of pesticide into the blank wastewater and vegetables extract and then followed by extraction into the GC-MS/MS analysis. The GC condition and the detector response were adjusted so as to match the relative retention times and responses.

The GC conditions used for the study included helium as the carrier gas at constant flow of 1mL/min in splitless mode. The oven temperature was programmed as follows: 100^0C/min, 1.0 min hold time, ramp at 100^0 C/min. to 160^0C, held for 1 min, ramp at 5.0^0 C/min to 300^0C and held for 2.0 minutes. The injection volume of the GC was 2µL. The column type used was TG 5MS (30m x 0.25mm x 0.25 µm).TSQ Quantum GC mass spectrometer operated with an ion source temperature of 250^0C and transfer line temperature of 315^0C . An external method was employed in the determination of the quantities of residues in the sample extracts. A standard mixture of known concentration of pesticide was run and response of the detector for each compound was ascertained. Quantification was based on linear least square calibration of analyte where peak areas were plotted versus the analyte concentration. Detection limits of the method were also assessed based on the lowest concentration of the residues in each of the matrices that could be reproducibly measured at the operating conditions of the GC, which were 0.001mg/kg. Blank analysis was also carried in order to check any interfering species in the reagent. Instrument control and data acquisition was performed using Xcalibur 2.1 software (Thermo Scientific, San Jose, CA, USA).

2.7 Statistical analysis

All the analysis was carried out in triplicate and mean concentration computed accordingly. Plate count data of wastewater and vegetable samples were expressed as the Log_{10} mean CFU/g value standard deviations while those of the water samples were expressed as the log10 mean CFU/mL values ± standard deviations, obtained from duplicated plates. Significant differences among the different locations and vegetables collected from different fields in plate count, physiochemical, heavy metal and pesticide data was established using one way analysis of variance (ANOVA). Results were expressed by means of ±SD. Means were separated according to Duncan's multiple range analysis ($P < 0.05$) using IBM software SPSS 20.0.

3. Results and Discussion

3.1 Characterisation of wastewater

3.1.1 *Physico-chemical characterisation*

The physico-chemical properties of wastewater used for irrigation of agricultural region in Sanganer area of Jaipur were analysed in the present study and results are shown in Table 1. The observed pH in this study was within the recommended target limits (6.5 - 8.5) for agriculture (FAO, 1992; WHO, 2006). pH values ranging from 3 to 10.5 favour the growth of indicator and pathogenic microorganism (Amxaka *et al.*, 2004). The mean conductivity values for all the samples from the three sampling locations were higher than the WHO guideline values of 1000µScm^{-3} for the discharge of wastewater into the Nalla (drainage basin). Dissolved oxygen (DO) levels in this study were below the acceptable limit (5 mg/L) at all the locations (Fatoki *et al.*, 2003). The total dissolved solids (TDS) in drinking water reveal the saline behavior of water, which indicates the organic pollution level of water (Jain *et al.*, 2006). These values in the wastewater samples were high and varied from 790 to 1110 mg/L. According to the Environment protection (EPA) rules (1986), the permissible limit for TDS is 500 mg/L for effluents from dye and dye intermediate industry. The total suspended solids (TSS) concentrations were in the range of 92-275 mg/L. Literature review classified wastewater TSS as follows: TSS less than 100 mg/L as weak, TSS greater than 100mg/L as medium (but less than 220 mg/L) and TSS greater than 220 mg/ l as strong wastewater. Results of the present study show that wastewater from the Amanishah Nalla of Sanganer, can be classified as strong wastewater with high TSS. The values for EC, TDS, TSS and DO varied significantly with sampling locations ($P<0.05$). The required oxygen demand expressed as BOD and COD is an important parameter for the evaluation of wastewater. COD value is often greater than BOD value (Speer, 1995). This has also been observed in our study and all the values have been found to be above the acceptable limits. The concentrations of BOD and COD in all the sampling point were higher than the WHO values of 50 mg/L and 250mg/L respectively for the discharged wastewater into stream. High BOD and COD concentration observed in the wastewater might be due to the use of chemicals, which are organic or inorganic caused by the inflow of the industrial waste containing elevated levels of organic pollutants (Salem *et al.*, 2011). The values for BOD and COD varied significantly with sampling locations ($P<0.05$). In the present investigation, the oil and grease content varied between 64 mg/L (minimum) and 93mg/L (maximum) which is well above the US EPA permissible limit of 10mg/L. It is important to note that such water with high oil and grease content can be a potential threat to the vegetables growing in the fields. Many workers have reported earlier the harmful effects of oil and grease content of wastewater on agriculture and environment (Mathur and Kumar, 2013; Lokhande *et al.*, 2011).

The concentration of phosphorus and nitrogen in wastewater samples ranged from 1.2 mg/l -2.86 mg/L and 0.56 mg/l -1.68 mg/L and were within limits as specified by WHO, 2006. However, the values for phosphorous and nitrogen varied significantly with sampling locations ($P< 0.05$). Presence of increased sodium ion concentration of wastewater can be attributed to the abundance of minerals in the wastewater. Soil irrigated with wastewater contains high amount of available phosphorus and nitrate concentration increase productivity in agriculture (WHO, 2006**;** Ahmad *et al.*, 2012; Odjadjare *et al.*, 2011)**.** Further, the higher levels of nitrogen (N), sodium (Na), phosphorous (P) and potassium (K) in the treated wastewater result in positive impact on the crop productivity in the areas receiving it (Singh 2004). The calcium in contaminated soil ranged between 36.35 mg/kg to 47.05 mg/kg and magnesium between 14.08 mg/kg to 23.88 mg/kg. High amounts of sodium ions can result in precipitation of calcium and magnesium ions from the soil.

There was a high statistically significant difference ($P<0.05$) among all the physico-chemical parameters between the group and within the group in the sampling locations. Location N3 had the highest EC, pH, TDS, TSS, BOD, phosphorus and magnesium. This may be due to the presence of high concentration of ions and dyes contributed by numerous printing houses located near the drain (Mathur and Kumar, 2011). Long-term irrigation with such effluents can increases EC, organic carbon content and heavy metals accumulation in soils (Brar and Arora 1997). Nitrate, oil and grease and dissolved oxygen were highest at location N2 and COD, sodium and potassium was highest in location N1. The present investigations were in agreement with the results of the survey conducted by Gupta *et al*. (1994), Joshi and Kumar (2011) and Mathur and Kumar (2013).

Table 1: Physico-chemical analysis of waste water collected from agricultural fields, located near Amanishah nalla, Sanganer, Jaipur

Parameters	Test Method	Unit	Permissible limit**	SITE N1*	SITE N2*	SITE N3*
pH	IS:3025 Part-11: 1983	-	5- 9.0	7.11±0.21	7.48±0.21	7.63±0.04
EC	IS:3025 Part-14: 1984	mmhos/cm	300	1240±0.57	1658±3.5	1962±1.52
TDS	IS:3025 Part-16:1964	mg/L	<500	790±1.73	980±0.5	1110±5.77
TSS	IS:3025 Part-17:1984	mg/L	100	270±5.13	92±0.5	275±4.3
Nitrate Nitrogen	IS:3025 Part-34:1988	mg/L	10	1.68±0.17	1.71±66	0.56±0.005
COD	IS:3025 Part-58:2006	mg/L	250	528±4.61	456±0.57	300±5.6
BOD	IS:3025 Part-44:1993	mg/L	100	109±5.93	182±0.57	208±0.577
Phosphorous	IS:3025 Part-31:1988	mg/L	23-56	2.77±0.03	1.2±0.057	2.86±0.01
Oil and grease	IS:3025 Part-39:1989	mg/L	10	83±0.577	93±0.57	64±1.527
Dissolved oxygen	IS:3025 Part-38:1989	mg/L	5	2.02±0.16	2.04±0.12	1.8±0.04
Sodium	IS:3025 Part-45:1993	mg/L	50-60	297.61±0.5	181.85±0.92	136.77±0.92
potassium	IS:3025 Part-45:1993	mg/L	20	6.01±0.005	1.02±0.005	1.04±0.005
Calcium	IS:3025 part 40:1991	mg/L	75-200	36.35+1.485	37.09± 1.61	45.05±1.19
magnesium	IS:3025 Part-46:1994	mg/L	30-150	17.1 ±1.63	14.08±1.41	23.88±0.96

*Values are Means± SD of three repeated sampling of three replicates
** Reference FAO/WHO; WHO; USEPA, Environment Protection rules (1986)

3.1.2 *Microbial assessment*

The total yeast and mould count, faecal contamination indicators (Coliform, *E. coli*, Faecal streptococci), *Salmonella, Vibrio cholerae, Shigella, Staphylococcus aureus, Pseudomonas aeruginosa, Clostridium* were investigated in wastewater samples used for irrigation. The data was compared statistically for all the microbes from the three sampling locations of wastewater collection .The results as shown in Table 2 revealed a high microbial counts and varieties of microorganisms most of which are pathogenic in nature in the soil contaminated with effluents. There was a substantial difference ($P<0.05$) of the means among the microbes at the collection locations. A comparison was also made between the similar microorganisms from the three locations which showed there was no significant difference ($P<0.05$) among total bacterial count, coliforms, *Salmonella, Pseudomonas aeruginosa* and *S.aureus.* However a noteworthy difference was observed in the number of yeast and mould count, *E.coli*, *Faecal Streptococci, Vibrio cholerae* in the three sampling locations. The location N2 showed the high predominance of yeast and mould count, coliform bacteria, *Vibrio cholerae, Pseudomonas aeruginosa, S.aureus* and *Clostridium sp.*, whereas location N3 confirmed high abundance of *Salmonella and Shigella.*

The coliforms dominated the wastewater samples and demonstrated abundant presence in the order Feacal streptococci > *E.coli*> Coliforms. The enteric bacteria showed a similar high presence in the order *Staphylococcus aureus >Salmonella >Shigella.* A high population density of *Vibrio cholerae, Pseudomonas aeruginosa and Clostridium sp.* were also noted from the wastewater along with a very high total yeast and moulds count. This was because the effluents contain many growth factors that could be easily utilized. Also, it may be attributable to the destabilization of the soil ecological balance as a result of the contamination due to the discharges of the wastewater into the soil ecosystem.

Physico-chemical parameters such as pH, TSS, BOD and COD have a major influence on bacterial population growth (Amxaka *et al.,* 2004). At the same time, as wastewaters often have high nutrient loads, high number of pathogens can be present, increasing the risk of infections occurring from them. In the present study, total coliforms levels ranged from 3.2 log 10CFU/mL - 3.98 log 10CFU/mL in all the wastewater samples. It is known that surface water can transmit microbial pathogens directly and indirectly. Among the indirect ways, irrigation of the crops with the possible passage of the agents into the food chain can be included. In the present investigation, faecal contamination indicators were present in almost all of the locations. Palese *et al*. (2009) also conducted a similar study and concluded that the reuse of wastewater for irrigation could increase the soil microbial load. On the other hand, due to the presence of pathogens in wastewater effluent, irrigation by this water resource could be associated with health hazards and increasing the risk of intestinal infections. Urban wastewater contains numerous pathogenic microorganisms and a high content of organic matter; therefore, it poses a number of potential risks for public health and the environment. Indicators of pollution are numerous (Modasiya *et al.,* 2013**).** The enteric bacteria are perhaps the most common pathogens present in wastewater, and of these, *Salmonella* species occur most frequently. Coliforms bacteria have served as indicators of faecal contamination of water for many years, and their densities have been utilized as criteria for the degree of pollution. Since coliforms organisms are universally accepted as indicators of water contamination, they were examined as possible indicators of contamination from wastewater irrigation fields. (Teltsch *et al.,* 1980).

Disease-causing microbes (pathogens) in wastewater can cause diarrhoea, cramps, nausea, headaches, or other symptoms. These pathogens may pose a special health risk for infants, young children, and people with severely compromised immune systems. The results of this study should invite us to implement management guidelines for pathogenic bacteria that can affect human health. In particular assessment of wastewater treatments need to take into account the enterobacterial pathogens as potential pathogens that should be correctly controlled. Results of the present study were in conformity with that of Adesemoye *et al.*, (2006) who reported similar high counts of microorganisms from soil samples contaminated with wastewater at Agege and Odo in Lagos, Nigeria abattoirs. However, the presence of *E.coli* and faecal streptococci in the contaminated soil may also be attributable to the high load of animal excreta in the wastewater. It is also an indication of recent faecal pollution. The presence of these organisms is a pointer to possible pollution and may have an effect on the soil ecological balance.

Table 2: Microbial analysis of waste water and vegetables collected from agricultural fields, located near Amanishah nalla, Sanganer, Jaipur

Microbes	Waste water sampling sites			*Lagenaria siceraria*			*Abelmoschus esculentus*		
	N1*	N2*	N3*	L1*	L2*	L3*	L1*	L2*	L3*
TBC	4.24±0.027	4.26±0.0189	4.0±0.01	4.26±0.012	4.22±0.0311	4.21±0.002	4.22±0.081	4.22±0.01	4.3±0.01
Y&M	3.8±0.050	4.2±0.0118	4.12±0.01	3.69±0.0369	3.04±0.0084	3.71±0.0105	3.66±0.009	2.5±0.018	3.64±0.005
E.coli	3.11±0.04	3.98±0.0191	3.18±0.0121	3.73±0.03	3.6±0.027	3.6±0.221	2.84±0.910	2.84±0.029	3.40±0.032
Coliforms	3.81±0.0674	3.98±0.0115	3.2±0.0099	3.72±0.043	3.5±0.029	3.3±0.035	3.64±0.112	3.66±0.045	3.62±0.22
F.streptococci	2.3±0.036	4.08±0.0113	3.6±0.0882	3.73±0.067	3.9±0.045	3.7±0.045	3.67±0.022	3.64±0.056	3.62±0.63
Salmonella	3.67±0.0177	3.76±0.0193	3.83±0.0124	3.33±0.066	3.0±0.032	3.2±0.065	3.12±0.031	3.10±0.036	3.11±0.043
Shigella	3.4±0.063	3.69±0.0281	3.79±0.0214	3.45±0.075	3.4±0.012	3.4±0.291	3.2±0.011	3.2±0.032	3.24±0.074
V.cholerae	2.48±0.3177	3.38±0.0396	2.48±0.1512	2.96±0.12	2.5±0.035	2.9±0.870	3.11±0.039	3.33±0.020	3.3±0.063
P.aeruginosa	3.71±0.0217	3.87±0.0245	3.68±0.0227	3.03±0.034	3.1±0.045	3.1±0.022	3.89±0.322	2.41±0.022	2.42±0.068
S.aureus	3.89±0.020	3.96±0.0191	3.85±0.0181	3.46±0.098	3.8±0.034	3.9±0.054	3.8±0.467	3.5±0.014	3.5±0.075
Clostridium	+++	++	++	++	+	++	+	++	++

*Values are mean of Log 10 CFU/mL ± SD of three repeated sampling of three replicates for water samples and are mean of Log 10 CFU/mL ± SD of three repeated sampling of three replicates for vegetable samples.
++ indicate the intensity of the growth; + less, ++ medium, +++ maximum

3.1. 3 *Heavy metal assessment*

Table 3: Heavy metal content of waste water and vegetables collected from agricultural fields, located near Amanishah nalla, Sanganer, Jaipur

Waste water			**Waste water sampling site (N1)**			**Waste water sampling site (N2)**			**Waste water sampling site (N3)**		
S.No.	**Metal**	**Permissible limits(µg/L)**	**Amount (µg/L)**	**Standard Deviation**	**% RSD**	**Amount (µg/L)**	**Standard Deviation**	**% RSD**	**Amount (µg/L)**	**Standard Deviation**	**% RSD**
3	Cr	550	253.9	0.588	0.231	238.3	3.891	1.633	233.6	1.122	0.481
4	Mn	200	74.87	0.346	0.462	81.01	1.309	1.616	80.48	0.494	0.614
5	Fe	500	2035	5.772	0.284	1932	33.21	1.719	1295	8.189	0.632
6	Co	50	2.483	0.023	0.926	2.227	0.036	1.625	2.302	0.02	0.885
7	Ni	1400	120.8	0.534	0.442	105.4	1.827	1.734	126	0.629	0.499
8	Cu	17	36.05	0.351	0.974	39.98	0.83	2.077	2.621	1.779	0.679
9	As	100	0.564	0.024	4.247	0.652	0.036	5.474	0.4	0.009	2.367
12	Cd	10	3.427	0.006	0.162	0.182	0.002	1.119	0.766	0.008	0.98
13	Sb	-	0.078	0.001	1.222	0.071	0.003	3.722	0.131	0.002	1.272
14	Pb	65	24.51	5.497	22.43	9.152	0.095	1.036	25.73	5.401	20.99
Lagenaria siceraria			**Agricultural field (L1)**			**Agricultural field (L2)**			**Agricultural field (L3)**		
S.No.	**Metal**	**Permissible limits (mg/kg)**	**Amount (mg/kg)**	**Standard Deviation**	**% RSD**	**Amount (mg/kg)**	**Standard Deviation**	**% RSD**	**Amount (mg/kg)**	**Standard Deviation**	**% RSD**
3	Zn	99.4	17.98	0.38	1.06	5.49	0.14	1.30	7.4	0.01	0.78
4	Cr	2.3	21.35	0.01	0.03	30.64	0.03	0.04	28.15	0.89	0.01
5	Mn	500	1.56	0.04	1.27	4.1	0.02	0.21	3.7	1.47	0.04
6	Fe	425.5	68.2	2.69	1.97	163.3	0.68	0.21	222.15	0.07	0.06
7	Co	50	22.87	0.01	0.02	0.27	0.01	1.35	0.25	0.01	0.98
8	Ni	67.9	25.98	0.01	0.02	16.4	0.12	0.37	14.57	0.95	0.08
9	Cu	73.3	8.35	0.04	0.02	10.44	0.11	0.51	11.64	0.76	0.85

12	As	0.43	1.86	0.05	1.32	0	0	0	0	0	0
13	Cd	0.2	1.46	0.05	0.01	0.02	0.01	0.3	0.55	0.06	0.44
14	Pb	0.3	0	0	0	1.56	0	0.06	1.54	0.34	0.52
Abelmoschus esculentus			**Agricultural field (L1)**			**Agricultural field (L2)**			**Agricultural field (L3)**		
3	Zn	99.4	11.04	0.36	1.61	14.80	0.08	0.54	11.03	0.31	0.03
4	Cr	2.3	2.21	0.05	1.19	0.67	0.22	0.39	0.23	0.05	1.97
5	Mn	500	2.88	0.05	0.90	7.39	0.03	0.42	11.08	2.07	0.66
6	Fe	425.5	64.1	1.04	0.81	222.30	0.14	0.03	67.28	1.91	1.03
7	Co	50	0.02	0.01	1.27	0.50	0.01	0.49	0.06	0.01	0.05
8	Ni	67.9	1.21	0.03	1.28	14.57	0.14	0.49	2.37	0.54	0.80
9	Cu	73.3	6	0.12	0.96	11.64	0.19	0.80	6.3	1.52	0.85
12	As	0.43	0	0	0	0	0	0	0.02	0.02	5.27
13	Cd	0.2	0.03	0	3.79	0.55	0.01	1.15	0.04	0.01	0.90
14	Pb	0.3	0.98	0	1.07	1.54	0.01	0.40	0	0	0

The composition of metals in the wastewater samples from the three sampling locations from Amanishah Nalla was assessed (Table 3). It ranged between 233 to 253 µg/L for chromium and lower than the permissible limit of 550 µg/L (Jaishree and Khan, 2013). The maximum concentration of manganese comparing the three locations were 81µg/L and below the permissible limit of 200 µg/L. In the present study, the maximum concentration of lead recorded in wastewater from one of the sampling locations was 25.51 µg/L, whereas the permissible limits of lead is 65 µg/L. Desirable limits of lead (Pb) is 50 µg/L beyond this limit water becomes toxic. The maximum concentration of copper found in one of the sampling locations was 39.98 µg/L, while the permissible limits is 17 µg/L. Maximum concentration of cadmium was 3.427 µg/L. N1 location showed the highest concentration of 2035 µg/L ferrous, which was well above the permissible limits of 500 µg/L. The concentration of nickel ranged from 105 µg/L to 126 µg/L which is below the permissible limit. Arsenic was present in the range of 0.4 µg/L to 0.65 µg/L in all the samples of wastewater but below the permissible limit of 100 µg/L (WHO/EU, 1993).

Selenium was found only at one location in trace amount of 0.131 µg/L. Zinc and antimony was also below the permissible limit. The high standard deviation of iron, copper, lead suggests that they are not uniformly present in all locations. The concentration of the heavy metals in industrial effluents industries was higher than the permissible limit.

Presence of heavy metals in industrial effluents followed the decreasing order of Cu>Fe >Cr>Zn>Ni>Pb>Mo>Cd>Ar>Sb>Se. It is interesting to note that in all the wastewater samples, copper and iron were present above the permissible limit whereas the rest of the heavy metal were below the permissible limits. A comparison was made between these metals at all the three locations and a high statistical significance ($P< 0.05$) was observed in chromium content between the groups of metals and among the groups of metals. Among the three locations, a high level of heavy metal accumulation was found in all the three locations, notably more in location N1. Jaishree and Khan (2013) also reported similar high accumulation of heavy metals. This result suggests that textile based heavy metal industries using heavy metals such as Cd, Cu, Zn, Pb, Ni, and Cr, in colours and pigments are the main source of elevated heavy metal concentrations in wastewater except Nickel. The data presented here revealed that the water quality of Amanishah Nalla is a great matter of concern. Most of the water parameters were quite high as per WHO (1989) and US EPA (1992) standards for irrigation. Overall findings are in agreement with earlier studies and indicated that the wastewater from the dyeing and printing textiles industries of the Sanganer industrial areas of Jaipur city used for irrigation contains heavy metals and should not be used for irrigation without prior treatment. Similar conclusions were also reported by Singh and Chandel, (2006); Marwari *et al*. (2009); Esabela *et al.*, (2011); Joshi and Shrivastava, (2012) and Jaishree and Khan, (2013).

3.1.4 *Pesticide content*

Analysis of wastewater for pesticide residue by GC-MS/MS revealed the presence of a variety of pesticides, some having potential toxicological significance. Mass spectral identifications were made for 20 pesticides namely, Dichlorovas, Monocrotophos, Lindane, β-HCH, Paraxon methyl, Methy Parathion, Dieldrin, Malaxon, Alachor, Malathion, Aldrin, Butachlor, 4,4 DDE, Endrin , 2,4 DDD, 4,4, DDD, Ethion, Phorate sulphone and Deltamethrin. The tentative identifications reflect the most reasonable match in the NIST MS search 2.0 spectral libraries for detectable concentrations of the above mentioned pesticides. Detection limit (LOD; Signal-to-Noise Ratio S/N=3) and quantification limit (LOQ; S/N=10) were calculated on the values of the blank at the retention times of analytes (ten injections). The linearity (R^2), percentage recoveries, limit of detection and limit of quantification, accuracy and range are shown in Table 4. In the present study, the detected concentration of pesticide residues in the wastewater below the accepted maximum residue limit (MRL) value of 0.1 µg/L was considered as also adopted by the FAO/WHO Codex Alimentarius Commission (1986).

The response of SRM transitions were used for quantification analysis and the ratio of the two SRM transition for each compound were used for confirmation. Calibration curves from 0.01 µg/L - 0.200 µg/L were created using matrix matched standard calibration solutions. A summary of the linearity of calibration standards, average recovery and precision data from five replicated QC samples at 0.050 µg/L are given in Table 4 for all the pesticides in wastewater. The correlation coefficient (R^2) for most pesticides

Table 4: Pesticide residue analysis of waste water collected from agricultural fields, located near Amanishah nalla, Sanganer, Jaipur

Name of pesticide	Units	Waste water sampling sites			Limit of detection	Limit of Quantification	Linearity (R^2)	RSD (%)	Accuracy (%)	Range
		N1	N2	N3						
Dichlorvas	µg/L	0.1439	0.6014	N.D	0.015	0.05	0.999	4.71	106.6	0.01 – 0.200
Monocrotophos	µg/L	N.D	N.D	N.D	0.065	0.215	0.995	16.7	128.4	0.01 – 0.200
Lindane	µg/L	0.0048	0.0046	N.D	0.016	0.052	0.999	4.77	109.2	0.01 – 0.200
B HCH	µg/L	0.005	0.0092	0.0075	0.005	0.017	0.999	1.7	98.4	0.01 – 0.200
Paraxon methyl	µg/L	N.D	N.D	N.D	0.044	0.148	0.998	12.69	116.2	0.01 – 0.200
Methyl parathion	µg/L	N.D	0.046	0.0579	0.037	0.122	0.994	10.69	114.2	0.01 – 0.200
Dieldrin	µg/L	N.D	N.D	N.D	0.056	0.186	0.997	17.75	104.8	0.01 – 0.200
Malaxon	µg/L	0.0882	0.0286	0.0172	0.016	0.219	0.998	17.22	127	0.01 – 0.200
Alachlor	µg/L	0.0344	0.032	N.D	0.009	0.029	1	2.92	98.4	0.01 – 0.200
Malathion	µg/L	0.0148	0.249	1.027	0.048	0.162	0.991	13.22	122.2	0.01 – 0.200

Aldrin	µg/L	0.0135	N.D	N.D	0.014	0.045	0.998	4.34	103.6	0.01 – 0.200
Butachlor	µg/L	0.0183	N.D	N.D	0.023	0.078	0.999	7.39	105.8	0.01 – 0.200
4, 4 DDE	µg/L	0.0092	0.0104	N.D	0.028	0.093	0.999	9.49	98	0.01 – 0.200
Endrin	µg/L	0.0081	0.0074	N.D	0.007	0.024	0.998	2.57	93.6	0.01 – 0.200
2, 4 DDD	µg/L	0.0079	0.0076	N.D	0.021	0.071	0.998	7.44	95	0.01 – 0.200
4,4 DDD	µg/L	0.0003	0.0104	N.D	0.019	0.063	1	6.16	102	0.01 – 0.200
Ethion	µg/L	N.D	N.D	N.D	0.03	0.101	0.998	9.73	103.6	0.01 – 0.200
Phorate sulfone	µg/L	N.D	N.D	N.D	0.039	0.129	0.996	11.8	109.8	0.01 – 0.200
phorate	µg/L	N.D	0.0066	N.D	0.039	0.129	0.996	11.8	109.8	0.01 – 0.200
Deltamethrin	µg/L	0.0116	0.0018	0.0077	0.046	0.152	0.995	13.1	116	0.01 – 0.200

was nearly 0.99. The signal to noise ratios for all the pesticides at lower calibration label was more than 10:1. The average recoveries for most pesticides at 0.050 µg/L in matrix were within the range of 73% to 110% with an average precision of 10.3% CV.

The main pollutants of surface and ground water are organophosphorus pesticides such as chloropyrifos, diazinon, malathion, parathion, pirimiphos methyl, azinphosmethyl and so on (Real *et al*. 2007). Malathion is the most widely used among them (Zang and Pagilla, 2010) and can be regarded as a potential mutagen/carcinogen. In our present investigation, wastewater from all the three collecting locations contained malathion and in one location it was more than the permissible limit. Furthermore, Dichlorvos is also an organophosphate insecticide widely used in developing countries. Because of its high acute toxicity and the consequent danger to workers, there are concerns whether safe use is possible under such conditions. In our study, Dichlorovas also exhibited a higher concentration above the permissible limit. Among the three locations of study, N2 location showed more accumulation of pesticide residues whereas location N3 showed the presence of toxic levels of Dichorovas and Malathion. There was no statistical difference in the pesticide content among the three sampling locations. Increased usage of organophosphorus pesticides on agricultural and urban areas has resulted in polluting natural water resources such as surface water and groundwater (Gilliom 2007). There are many similar reports about contamination of surface water by pesticides (Tsuda *et al*., 2009; Sun *et al*., 2006; Shayegh *et al*., 2001; Hela *et al*., 2005).

3.2 Impact assessment of wastewater on the environmental quality of the disposal area

3.2.1 Microbial analysis in vegetables

Many studies have shown that the microbial pollution in the recycled effluent could contaminate the soil as well as the crops and develop the risk of disease in both consumers and the farm workers (Baghapour *et al.,* 2013). ‘‘Faecal coliforms’’ are the indicator bacteria most commonly used in discussions of wastewater reuse. They are broadly equivalent to ‘‘thermotolerant coliforms’’. The preferred grouping would be ‘‘thermotolerant coliforms/*E. coli*’’ which would eventually allow *E.coli* to be used as the preferred, and exclusively faecal indicator bacterium.Thus the indicator organisms used to detect faecal pollution are total coliforms and *Escherichia coli* (Osamwonyi, *et al.,* 2013).

All vegetables sampled during the present study recorded high levels of coliforms bacteria and varieties of pathogenic microorganisms. The faecal contamination indicators (Coliforms, *Escherichia coli*, *Faecal streptococci*) and the genera *Salmonella*, *Vibrio cholerae, Shigella, Staphylococcus aureus , Pseudomonas aeruginosa, Clostridium sp.* and total yeast and mould count were isolated from vegetables that might have recycled through the wastewater for irrigation. All these bacteria are of public health significance and their presence in any food items indicates that such food has been contaminated with faecal materials and it is unsafe for human consumption. The mean microbial loads and the aerobic count on vegetables irrigated with wastewater in the present study are presented in Table 2. The bacterial counts from all the three locations of Sanganer were not statistically different. The coliforms dominated the vegetables and followed the same pattern as the wastewater samples. Highest level of contamination of total coliform, faecal streptococci and *E. coli* was recorded in *Abelmoschus esculentus* in comparison to *Lagenaria siceraria. E. coli* detected in *Abelmoschus esculentus* from all the three locations showed a significant level of difference and the range is 2.8 - 3.4 $\log_{10}CFUg^{-1}$ fresh weight. Coliforms counts suggest a potential risk of gastrointestinal diseases at all locations. *E. coli* levels on *Lagenaria siceraria* showed a high count of 3.75 log CFUg−1. Equally, wastewater used for vegetable production in the study area contained *E.coli* ranging from 3.18 to 3.98 log CFU/ mL (Table 2). The coliform bacteria, *E.coli* and faecal streptococci recorded in wastewater used for watering of the vegetables were higher than that recorded on the vegetables. Location L1 dominated for both the vegetables and showed high microbial content. World Health Organization (WHO) guidelines for faecal coliforms for wastewater reuse for the irrigation of crops exceeded the permissible limit in the present case. Several factors may account for the high levels of total and faecal coliform contamination recorded in both the analyzed vegetables. Among these, one could be the use of polluted irrigation water and fresh poultry manure. Both the irrigation water and the manure are applied on top of the crops. Another contamination source is market-related handling, especially where provision for better sanitary standards is lacking (Amoah *et al.,* 2006). In the present study the source of contamination of vegetables on the farm seems to arise primarily from wastewater used in watering vegetables. The transported pathogens from wastewater may survive in soil and crops which will in turn be transported to consumers and may potentially be responsible for numerous diseases(Halablab *et al.*, 2011). In the present study the abundance of the coliform bacteria were more than the enteric bacteria. The order of prevalence and abundance in both the vegetables were in the decreasing order of Coliform> *E.coli*> Feacal streptococci. The total coliform count can be considered as the hygiene indicator and their presence might be due to faecal contamination or irrigation water (Vishwanathan and Kaur, 2001). In our study, *E. coli* was isolated with a quite high CFU, therefore underlining the importance of this parameter as a specific indicator of faecal pollution. This microorganism is therefore essential in a habitat where it is necessary to discriminate between environmental and potentially pathogenic flora belonging to humans or animals. The enteric bacteria showed similar high abundance in the decreasing order of *Staphylococcus aureaus >Shigella>Salmonella.* Coliform, *E.coli* and *S.aureaus* were the predominant microorganisms in both the vegetables. A high population density of *Vibrio cholerae and Clostridium* were also noted in vegetables with a very high count for total yeasts and mould. The presence of *V.cholerae* in the wastewater shows that the groundwater supplies are potentially at risk from contamination, with a resultant potential cholera risk because this bacterium is almost exclusively transmitted by water. The results of this study therefore support the previous findings that irrigation of food crops with contaminated water resulted in contamination of the crops (Steele *et al.*, 2005; Afolabi and Oloyede, 2010).

3.2.2 Heavy metal analysis in vegetables

Heavy metals are known as non-biodegradable and persist for long durations in aquatic as well as terrestrial environments. They might be transported from soil to ground waters or may be taken up by plants, including agricultural crops (Ekmekyapar *et al.,* 2012). Similar bio-accumulation of heavy metals by the crops irrigated with wastewater of Amanishah Nalla has been reported by many authors (Marwari *et al.*, 2009; Kala and Khan, 2009; .Marwari and Khan, 2012).

The total concentrations of heavy metals in the *Abelmoschus esculentus* and *Lagenaria siceraria* samples from Sanganer field are shown in Table 3. The range of the concentrations of heavy metals in *Abelmoschus esculentus* decreased in the order of Fe (64.1 to 222.30 mg/kg) > Zn(11.03 to 14.80 mg/kg) > Mn(2.88 to 11.8 mg/kg)> Cu(6.0 to 11.64 mg/kg) >Ni (1.21-14.57 mg/kg) > Cr (0.23- 2.21 mg/kg) > Pb (0- 1.54mg/kg) > Cd (0.03 -0.55 mg/kg)> Co(0.02-0.06mg/kg) > As (0-0.02mg/kg). All the heavy metals found in *Abelmoschus esculentus* were below the FAO/WHO maximum permissible limits except chromium and lead in one of the samples. The most important toxic effects, of chromium compounds are dermatitis, allergic asthmatic reactions, bronchial carcinomas and gastro-enteritis (Baruthio, 1992). Depending on the amount of exposure, lead can adversely affect the nervous system, kidneys, the immune system, reproductive and developmental systems and the cardiovascular system. Its toxic effects vary from subtle changes in neurocognitive function in low-level exposures to a potentially fatal encephalopathy in acute lead poisoning (Gillis *et al.,* 2012). The levels of cadmium in the experimental vegetable *Abelmoschus esculentus*, ranged from 0.67 mg/kg to 2.21mg/kg and the levels of lead in only one sample was 0.98mg/kg whereas the permissible limit is 0.3mg/kg. Cd is primarily toxic to the kidney, especially to the proximal tubular cells, the main location of accumulation. Cd can also cause bone demineralization, either through direct bone damage or indirectly as a result of renal dysfunction. In the industry, excessive exposures to airborne Cd may impair lung function and increase the risk of lung cancer (Bernard, 2008). Accumulation of lead and cadmium by *Abelmoschus esculentus* has also been reported earlier (Marwari and Khan, 2012). Fatoba *et al.,* (2012) also reported accumulation of heavy metal like accumulation of Pb, Cd and Hg in a similar study on heavy metal content in *Abelmoschus esculentus* when irrigated with wastewater. In another study by Lawal and Audu, 2011, the mean concentrations of Co, Cr, Cu, Ni, Pb and Zn increased in *Abelmoschus esculentus* samples from the three effluent irrigated locations of Nigeria. Muazu *et al.,* (2010) also reported high concentrations of heavy metals in vegetables irrigated along the bank of River Challawa and found the mean concentrations of these metals in the *Abelmoschus esculentus* were found to be in the order Mn > Zn >Cr > Ni > Pb > Cu . They reported that *Abelmoschus esculentus* has higher retention capacity for the essential metals (Cu, Zn and Mn) than the toxic ones (Pb, Cr and Ni). These results were similar to the earlier ones obtained by Audu and Lawal (2002). A high concentrations of heavy metals observed in the vegetable samples from the effluent irrigated gardens might be related to the concentrations of the metals in the soil (Al Jassir *et al.,* 2005; Akinola and Ekiyoyo, 2006). In comparison to the three fields, high heavy metal content was found in *Abelmoschus esculentus* growing in agricultural field L2.

In the present study, heavy metal accumulation was also noted in bottle guard, *Lagenaria sinceria*. The range of concentration of heavy metal in *Lagenaria siceraria* growing in all the three agricultural locations decreased in the order of Fe (68.2-222.15 mg/kg) > Cr (21.35- 30.64 mg/kg)>Ni(14.57-25.98mg/kg) > Cu (8.35-11.64mg/kg)> Zn(5.49-17.98 mg/kg) > Co(0.25-22.87 mg/kg)> Mn(1.56-4.1 mg/kg) > Cd(0.02-1.46 mg/kg)> As(0-1.86 mg/kg) > Pb(0-1.56 mg/kg). All the heavy metals concentration found in the sampling locations were below the FAO/WHO maximum permissible limits except chromium, cadmium, arsenic and lead. This concentration of chromium was very high when compared to the permissible limit of 2.3mg/kg. In comparison to the three fields, high heavy metal content was found in *Lagenaria sinceria* growing in agricultural field L1.

Cadmium can cause severe gastrointestinal irritation, vomiting, diarrhoea, and excessive salivation, and doses of 25 mg of Cd/kg body weight can cause death (Kumar *et al.,* 2007). The consumption of such vegetables and cereals may result into serious health hazards such as kidney damage, anaemia, disorder of central nervous system and renal failure (Reddy, 1984). Intake of toxic metallic ions by the autotrophs results in their bio-accumulation in our delicate food web. This establishes that use of industrial effluents for irrigation practices may create various health hazards to human beings and damaging effects on crop plants. The reuse of such industrial effluents without proper treatment should be strictly prohibited. Some metals such as Cd, Cr, and Ni are highly toxic even in trace amounts (Agarwal *et al.*, 1961; Bisht and Agarwal, 1982). High Ni concentration in the nutrient medium reduces the uptake of most other nutrients (Crooke, 1995, Pandey 2006). Cu levels in all the vegetables were below both the FAO/WHO maximum permissible limits.

Further, the critical levels of the heavy metals for agricultural crops are much higher than those observed in our study areas irrigated with wastewater and there seems no adverse impact of metals and pesticides on agricultural crops in these areas. However, the enhanced yield in later case may be due to more irrigation water availability with high nutrient/fertilizer (N, P, K, and organic carbon) value water (Singh *et al.,* 2004). Study reveals that vegetable crop *Lagenaria siceraria* has the ability to uptake the heavy metals through their roots and transport them to the edible portion of the plant. Seshabala *et al.,* 2007 also noted uptake of Ni and Pb by *Lagenaria siceraria,* above the prescribed concentration. Singh *et al.,* 2010 also noted the high concentrations of heavy metals viz. Cd, Cr, Cu, Ni, Pb and Zn in both *Abelmoschus esculentus* and *Lagenaria siceraria* as well as in wastewater used for irrigation The high concentration of Co and Cu content was recorded in Lagenaria *siceraria* by Kumar *et al.*, 2007.The heavy metals may accumulate in the edible parts of the crops that are consumed by people or fed to the animals. Their increased concentration in human food chain over a long period can provoke detectable damage to health (carcinogenic and mutagenic effects). This depends not only on the type of heavy metals but also the prevailing soil and other growing conditions. There is no regular testing of heavy metals in vegetables by the designated authorities in India (Seshabala *et al.*, 2007).The observation regarding the accumulation of heavy metals in vegetables is in good agreement with other studies elsewhere (Sharma *et al.,* 2006; Sawidis *et al.*, 2001) which suggested that uptake of metals by plants is proportional to their concentrations and availabilities in soils. Absorption and accumulation of heavy metals in vegetables and fruits are influenced by many factors, including: concentration of heavy metals in soil, composition and intensity of atmospheric deposition, including precipitations, phase of plant vegetation (Vontsa *et al.*, 1996). To all of these, can be added other sources generated by agricultural technologies such as: irrigation with wastewater, the administration of organic and mineral fertilizers with the load of heavy metals, or application of pesticides, which contain in their structure such chemical elements (Singh *et al.*, 2006; Sharma *et al.*, 2006). The present study indicates that wastewater-irrigated vegetables accumulate heavy metals beyond prescribed toxic limits and may cause serious health hazards to people who consume these vegetable products regularly (Gupta *et al.*,2011; Lawal and Audu, 2011)

3.2.3 Pesticide content in vegetables

Twenty pesticides with various chemical structures were selected to monitor their presence in the two vegetables collected from L1, L2, L3 agricultural fields irrigated with N1, N2 and N3 wastewater respectively. To validate the GC–MS/MS method, linearity and regression coefficient (R^2) were investigated under optimized experimental conditions. The linearity of the method was evaluated using vegetable samples spiked with the selected pesticides compounds at various concentrations. Each calibration level was analyzed in two replicates. The determination coefficients in linear range of each analyte are presented in Table 5. In summary, the (R^2) coefficients varied between 0.994–0.999. The limits of detection (LOD) and the limits of quantification (LOQ) for each compound were also calculated for the optimized methods. The LOD and LOQ were calculated as the concentration of pesticides with signal-to-noise (S/N) ratios of about 3:1 and 10:1, respectively. The LOQ ranged from 0.37 to 9.056 μg/kg. The LODs of GC–MS were lower than 20 μg/kg except for Dieldrin. Precision (expressed as relative standard deviation) was lower than 10% for all pesticides and finally, limits of detection were also 10–20 times lower than maximum residue levels (MRLs) established by European Regulation. The relative standard deviation (RSD) % ranged from 0.47 to 13.84. Using triple quadrapole mass spectrometry, very low detection limits and good confirmation (1 precursor ion and 2 or more product ions) are achieved simultaneously (Sheredin and Meola, 1999). The entire list of pesticides was screened with 2 injections per sample. The method allowed determination of μg/kg of pesticide residues in vegetables.

Most of the pesticides are non-biodegradable (Babu *et al.*, 2011). In the present study, a multi residue analysis of twenty pesticide mix was monitored and determined in the two vegetable crops growing in the fields. According to the detected pesticides, two groups of pesticides, organochlorines and organophosphorus compounds clearly emerged. The proposed method shows good sensitivity and recovery and allows for rapid analysis. Data in Table.5 shows the amounts of the detectable resides in *Abelmoschus esculentus* and *Lagenaria siceraria* from the farms at Sanganer, Jaipur irrigating with the wastewater from textile industries. The LODs achieved with the method are similar to those previously obtained by other authors in vegetables (Gelsomino *et al.*, 1997; Osman *et al.*, 2010). The data showed that only 25% of the samples had monocrotophos residue above the limit in *Lagenaria siceraria.* In *Lagenaria siceraria,* trace amounts of Alachor, malathion , paraxon methyl, phorate sulphone, dichlorovas, lindane , β HCH, paraxon, methyl parathion, malaxon, phorate , aldrin, butachlor, 4,4, DDE, and lindane, β HCH, paraxon, methyl parathion, malaxon, phorate sulphone, lindane, malathion, aldrin, butachlor, 4,4, DDE, and endrin pesticide residues were found . In *Abelmoschus esculentus* many pesticides residue like lindane, β HCH, paraxon, methyl parathion, malaxon, phorate sulphone, lindane, malathion, aldrin, butachlor, 4, 4, DDE, and endrin levels were present in trace amounts. There was a significant difference among the samples growing in the three fields with high pesticide residue found in vegetables growing in location L1. The health risk analysis for the systemic effects associated with the pesticide residues in vegetables, Monocrotophos showed a high risk while the rest of the pesticide were within the safe limits . The results of the detectable amounts of pesticide in selected vegetables clearly explains the pattern of pesticide residues present (Saeid and Selim, 2013). `

Table 5 : Pesticide residue analysis of vegetables collected from agricultural fields , located near Amanishah nalla, Sanganer, Jaipur

Name of pesticide	Units	*Lagenaria siceraria*			*Abelmoschus esculentus*			Limit of detection	Limit of Quantification	Linearity (R^2)	RSD (%)	Accuracy (%)	Range
		L1	L2	L3	L1	L2	L3						
Dichlorvas	µg/kg	0.7959	0.39795	0	0	0	0	0.765	2.55	0.999	1.22	103.8	10 - 200
Monocrotophos	µg/kg	20.1066	0	0	0	0	0	0.449	1.49	0.995	0.77	96.4	10 - 200
Lindane	µg/kg	0.535	0.7944	0	1.1326	0	1.1549	2.116	7.05	0.999	3.18	110.56	10 - 200
B HCH	µg/kg	0.8293	0.8348	0.7896	0.95775	0.9431	0	0.829	2.76	0.999	1.33	103.15	10 - 200
Paraxon methyl	µg/kg	2.7103	0	0	0	0	0	0.529	1.76	0.998	0.92	94.9	10 - 200
Methyl parathion	µg/kg	6.3271	6.7171	0	6.15175	6.0811	0	2.352	7.84	0.994	2.9	134.76	10 - 200
Dieldrin	µg/kg	0	0	0	0	0	0	9.056	30.18	0.997	13.84	109.01	10 - 200
Malaxon	µg/kg	1.15045	1.1357	0	1.1453	1.1396	1.14245	0.43	1.43	0.998	0.74	95.6	10 - 200
Alachlor	µg/kg	0.1319	0.2987	0	0.2153	0.2934	0.2784	0.625	2.08	0.9996	1.03	100.88	10 - 200
Malathion	µg/kg	0	0	0	0.5647	0.5511	0.5783	0.591	1.96	0.991	0.99	99.06	10 - 200
Aldrin	µg/kg	1.4171	0.8442	0.9272	0.88895	0.9855	0.7924	0.506	1.68	0.998	0.81	103.61	10 - 200

Butachlor	µg/kg	1.2917	1.4187	1.383	1.59665	0	1.6773	0.8	2.68	0.999	1.31	101.4	10 - 200
4, 4 DDE	µg/kg	0	1.3831	0.69155	1.39495	1.3914	0	0.654	2.18	0.999	1.07	101.8	10 - 200
Endrin	µg/kg	2.3606	2.3241	2.2549	2.3606	2.465	2.2562	3.374	11.24	0.998	5.19	108.28	10 - 200
2, 4 DDD	µg/kg	0	0	0	0	0	0	0.294	0.98	0.998	0.47	103.63	10 - 200
4,4 DDD	µg/kg	0	0	0	0	0	1.3831	1.223	4.07	0.999	1.89	107.29	10 - 200
Ethion	µg/kg	1.9866	1.9369	1.9522	0	1.93175	0	1.268	4.22	0.998	2.03	104.05	10 - 200
Phorate sulfone	µg/kg	6.6209	3.8165	3.8815	3.8165	3.37885	3.3757	0.379	1.26	0.996	0.623	101.41	10 - 200
Deltamethrin	µg/kg	0	0	0	0	0	0	5.297	17.65	0.995	6.098	124.77	10 - 200

3.2.4 Comparison of the toxicant and microbial levels at water collection locations and vegetable grown in agricultural locations :

In comparison to other locations, wastewater from location N1 of Amanishah Nalla, showed the highest microbial contamination, more accumulation of heavy metals and high sodium and potassium content and COD. The location L1 irrigated field showed similar highest abundance of various microbes and high accumulation of heavy metals and more pesticide content in *Lagenaria siceraria* and high content of monocrotophos in *Abelmoschus esculentus*. Wastewater from location N2 showed highest nitrate and oil and grease content and the location L2 receiving irrigation from this location showed the highest metal accumulation in *Abelmoschus esculentus.* Wastewater irrigating location N3 showed the maximum pesticide content. Changes in physicochemical characteristics in comparison to the standards probably favoured the growth of enteric bacteria, *Shigella* and *Salmonella.*

4. Conclusion

Based on this research study, the physicochemical parameters monitored at three locations showed different levels of all the assessed parameters. Due to this, faecal and enteric bacteria got a conducive environment to grow. To overcome the effect of high nutrient load of the wastewater and the wide range of weeds and pest problem arising due to it, farmers use a high concentration of a pesticides mixture. The sample irrigation water and vegetables collected from the Sanganer area also showed substantial high levels of heavy metals and pesticide residue. From the study it is concluded that, untreated industrial effluents are the main source of pollution in soil and irrigation with the same water containing variable amounts of heavy metals leads to increase in concentration of metals in soil and vegetables. All these results lead to high nutrient and bacterial load, heavy metal and pesticide contamination in environment and vegetables growing in the Sanganer area. This highlights an urgent need for proper- management practices of wastewater for irrigation purpose.

Risks. Our results suggest that pathogens are priority agents as compared to chemicals for groundwater contamination. By the coliforms criterion, a potential risk of gastrointestinal disease can be identified with total coliforms and *E.coli* counts above the recommendable level. The trace amounts of heavy metals and pesticide residues found in both the vegetables pose serious health problems as these vegetables are consumed regularly by the population.

Recommendation. The wastewater used for irrigation cause microbial contamination of the soil, where vegetables are grown. Therefore, periodic bacteriological monitoring of the wastewater and the soil are recommended. Concentration of metals in vegetables will provide baseline data and there is a need for intensive sampling for quantification of results throughout the year. Washing the vegetables thoroughly before cooking is highly recommendable. This could decrease or eliminate much of the microbiological and pesticide residues if done more consciously. It is highly recommendable to treat the effluents before they are released. Continuous monitoring of soil, plant and water quality together with prevention of metals entering vegetables is a to be taken care in order to prevent potential health hazards to human beings. On the basis of the above findings, the results recommend the need for continuous survey and monitoring programs for faecal and enteric bacteria, heavy metals and pesticide in all vegetables and food commodities. The potential harmful effects could be minimized through enforcement of legislation on the release of the toxic effluents in the Amanishah Nalla. This will help to protect the end user against the indiscriminate exposure of all toxicants and pathogens.

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