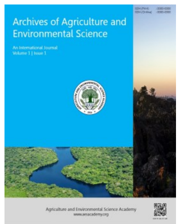
Archives of Agriculture and Environmental Science 2 (1): 36-43 (2017)

This content is available online at AESA

Archives of Agriculture and Environmental Science

Journal homepage: www.aesacademy.org

e-ISSN: 2456-6632



ORIGINAL RESEARCH ARTICLE

Heavy metals and microbial contamination of certain leafy vegetables grown in abattoir effluent disposal province of Saharanpur (Uttar Pradesh), India

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ARTICLE HISTORY

Received: 12 Dec. 2017
Revised received: 20 Jan. 2017
Accepted: 26 Feb. 2017

Keywords

Abattoir effluent
Heavy metals
Leafy vegetables
Microbial contamination

ABSTRACT

The present investigation was carried out to study the heavy metals and microbial contamination of four selected leafy vegetables viz., cabbage, lettuce, coriander and spinach grown in abattoir effluent irrigated soil. The results revealed that the values of various parameters of abattoir effluent viz., TDS (2840 mg L^{-1}), BOD (2480.50 mg L^{-1}), COD (2890.00 mg L^{-1}), total N (195.80 mg L^{-1}), Fe (18.48 mg L^{-1}), Mn (2.88 mg L^{-1}), total bacteria (6.97×10^{8} CFU ml $^{-1}$), coliform bacteria (3.24×10^{4} MPN 100 ml $^{-1}$) and total fungi (7.78×10^{5} CFU ml $^{-1}$) were found beyond the prescribed limit of Indian irrigation standards. The abattoir effluent irrigation significantly (*$p<0.05/p<0.01$*) increased the EC, total N, available P, OC, Ca, Mg, K, Na, Fe, Cd, Cr, Cu, Mn, Zn, total bacteria, coliform bacteria of the soil used for the cultivation of cabbage, lettuce, coriander and spinach in comparison to their respective controls. The most numbers of bacteria (8.67×108 CFU ml^{-1}), coliform bacteria (7.80×105 MPN 100 ml^{-1}) and total fungi (9.85×105 CFU ml^{-1}) were noted in the lettuce after abattoir effluent irrigation. Therefore, the higher contents of heavy metals and microbial population in cabbage, lettuce, coriander and spinach might be related to their contents in the soils irrigated with abattoir effluent. Therefore, the agronomical practices with abattoir effluent should be regularly monitored to avert environmental problems and attendant health hazards.

Citation of this article: Kumar V., Srivastava, S. Chauhan, R.K., Thakur, R.K. and Singh, J. (2017). Heavy metals and microbial contamination of certain leafy vegetables grown in abattoir effluent disposal province of Saharanpur (Uttar Pradesh), India. *Archives of Agriculture and Environmental Science,* 2(1): 36-43.

INTRODUCTION

Wastewater disposal is a common phenomenon in many countries including India due to generation of huge volume of effluent and lack of treatment facilities (Chopra *et al.,* 2013; Kumar and Chopra, 2014b, c). The untreated or partially treated wastewater is frequently used in the cultivation of agricultural crops due to its free availability, high cost of chemical fertilizers and presence of nutrients in the effluent required by crop plants (Chaturvedi *et al.,* 2013). The wastewater irrigation is known to contribute the significant contents of heavy metals and microbial population of the soils (Francis *et al.,* 1999; Mapanda *et al.,* 2005). The abattoir can be defined as premises approved and registered by the regulatory authorities for hygienic slaughtering, assessments, processing, efficient maintenance and storage of meat products for human use (Alorge, 1992). The effluents from abattoir and meat processing industries have been classified as industrial waste in the category of agricultural and food industries by Environmental Protection Agency (EPA), and one of the most damaging to the environment (Nwachukwu *et al.,* 2011). The meat processing effluents exhibit high organic, inorganic load, suspended solids content, dark color offensive odour and high microbial population. The discharging of abattoir effluent without treatment contributes to greatly degrading the aquatic environment and pollution of irrigation water (Adesemoye *et al.,* 2006).

The abattoir effluent has a complex composition and can be very destructive to the environment. For example, release of animal blood into the water streams would deplete the dissolved oxygen (DO) of the aquatic environment (Halablab *et al.,* 2011; Chaturvedi *et al.,* 2013). The improper disposal of paunch manure may exert oxygen demand on the getting environment or breed large population of decomposers (micro-organisms) which may be pathogenic (Nwachukwu et *al.,* 2011). Furthermore, improper disposal of animal faeces may cause oxygen depletion in the in receipt of environment. It could also lead to nutrient in excess of strengthening of the receiving system and increase rate of toxins accumulation in biological systems (Nwachukwu *et al.,* 2011). Mohammed and Musa (2012) reported that the inappropriate discarding of abattoir effluent might lead to broadcast of pathogens to human, which may cause outbreaks of water and food borne diseases e.g. diarrhea, pneumonia, typhoid fever, asthma, wool sorter diseases, respiratory and chest diseases, etc. The studies have shown that *E. coli* infection source was reported to be from undercooked beef which had been contaminated, in the abattoirs, with faeces containing the bacterium (Chaturvedi *et al.,* 2013). It had also been reported that the abattoir activities were responsible for the pollution of surface and underground waters, air quality as well as the reduction quality of health of residents within the surrounding environment (Halablab *et al.,* 2011).

The vegetables are an important part of human's diet and a potential source of important nutrients. The vegetables constitute important functional food components by contributing protein, vitamins, iron and calcium which have marked health effects (Chopra *et al.,* 2013; Kumar and Chopra, 2014a, b, c). The vegetables, especially those of leafy vegetables grown in the heavy metals contaminated soils, accumulate higher amounts of heavy metals than those grown in the uncontaminated soils because of the fact that they absorb these metals through their leaves (Al Jassir *et al.,* 2005). Keeping above in view the present study was carried out in the abattoir effluent disposal province of Saharanpur, where irrigation of vegetable crops with wastewater is a very common practice. Information on the contamination of vegetables with heavy metals and microbial pathogens from abattoir effluent of Saharanpur is not yet established. Therefore, the present investigation was undertaken to study the heavy metals and microbial contamination of certain leafy vegetables grown in the abattoir effluent disposal province of Saharanpur (Uttar Pradesh), India.

MATERIALS AND METHODS

Collection of abattoir effluent and soil samples; The abattoir effluent samples of ALM Industries Ltd. Saharanpur, India (29°58'09.63" N and 77°35' 09.20" E) were collected from the effluent disposal channel used for the irrigation of agricultural fields. The soil samples were collected before and after harvesting of cabbage, lettuce, coriander and spinach grown in the abattoir effluent irrigated agricultural fields located in the province of the industry. The samples were brought to the Agro-ecology and Pollution Research Laboratory, Department of Zoology and Environmental Science, Gurukula Kangri University Haridwar (Uttarakhand), India, and analyzed for various parameters.

Analysis of abattoir effluent and effluent irrigated soil: The physico-chemical characteristics of the abattoir effluent and soil samples were analyzed separately for pH, total dissolved solids (TDS) and conductivity (EC) using pH meter (pH System 362 Systronics, India), TDS meter (TDS meter 661E Systronics, India) and conductivity meter (Conductivity meter 306 Systronics, India), respectively. The biochemical oxygen demand (BOD) was analyzed by 5 days incubation method, while the chemical oxygen demand (COD) was determined by Open Reflux Method using potassium dichromate as oxidative agent. The total nitrogen (TKN) was determined by Kjeldahl method, phosphorus (P) by Olsen method and organic carbon (OC) by gravimetric method. The sodium (Na) and potassium (K) were measured by Stanford and English method (1949) using the flame photometry (Flame photometer 128, Systronics, India) while the calcium (Ca) and magnesium (Mg) were analyzed using versenate titration method.

Collection of vegetables and analysis of heavy metals: The vegetable samples of cabbage, lettuce, coriander and spinach were procured from the local farmers during the harvesting of the crops. The vegetable samples were washed with distilled water to remove the dust particles. The samples were then hand cut to separate the roots, stems and leaves using a sharp knife. The different parts (roots, stems and leaves) of these vegetables were air-dried separately and then placed in a dehydrator at 80 °C for 2-3 days and then dried in an oven at 100 °C. The dried samples of different parts of the vegetables were ground into a fine powder (80 mesh) using a commercial blender (Red berry, Mumbai, India) and stored in the polyethylene bags, until they are used for the acid digestion. For the acid digestion, 10 ml sample of abattoir effluent, 1.0 g sample of air dried soil or vegetables were taken separately in the digestion tubes. In each sample 3 ml of concentrate HNO_3 was added and digested on the electrically heated block for 1 hour at 145°C. Then 4 ml of $HClO_4$ was added and heated to 240 °C for an additional hour. The aliquots were cooled, filtered through Whatman # 42 filter paper and adjusted to a volume of 50 ml with double distilled water. The metals, i.e. cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn) and zinc (Zn) were analyzed by using the atomic absorption spectrophotometer (PerkinElmer Analyst 800 AAS) following standard methods (AOAC, 1990; Chaturvedi and Sankar, 2006).

Microbiological assay of effluent, soil and vegetables: For the microbiological analysis 10g of soil and vegetables samples were crushed, air dried and diluted in 90ml of sterile distilled water, while 10ml of effluent samples were diluted in 90ml of sterile distilled water instead. The serial diluents (10^{-1} - 10^{-9}) for the effluent, soil and vegetable samples were prepared aseptically using sterilized distilled water. The numbers of total bacteria and total fungi were determined by Petri plate culture method while the numbers of coliforms bacteria were recorded by culture tube method. For the estimation of total bacteria in the effluent, soil and vegetables, sterile nutrient agar media (NAM) were aseptically inoculated with aliquot of the serial diluents (10^{-4} - 10^{-9}) of the samples and incubated at 37°C for 24 hours. To numerate the total fungi, potato dextrose agar (PDA) plates supplemented with streptomycin (100ug/ml) to inhibit bacteria growth were aseptically inoculated with serial diluents (10^{-2} - 10^{-6}) and incubated at 30°C for 72 hours. After incubation the numbers of colonies on the Petri plates with distinct growth were counted using digital colony counter (Microprocessor colony counter 1362, Systronics, India). To determine the coliform bacteria, the samples were aseptically inoculated with serial dilution (10^{-2} - 10^{-6}) in the culture tubes contained MacConkey broth and incubated at 30°C for 72 hours. The culture tubes were conferred as positive of negative on the basis of gas produced in the tubes and the numbers of coliform bacteria were estimated using the MPN table (Aneja, 1996).

Quality assurance and statistical analysis: The appropriate quality assurance procedures and precautions were carried out during the study. The coefficient of variation of replicate analyses was determined for the measurements to calculate analytical precision. All the reagents and standards were of analytical grade. The data was analyzed using one way analysis of variance (ANOVA) to determine the difference between the soil parameters before and after effluent irrigation. The Duncan's test was used to compare the mean values. The mean and standard deviation for different parameters of the abattoir effluent, soil and vegetables were calculated with the help of MS Excel 2013.

RESULTS AND DISCUSSION

Characteristics of abattoir effluent: The mean ± SD values of various physico-chemical and microbiological parameters of abattoir effluent along with bore well water are presented in Table 1. The results revealed that the abattoir effluent was acidic (pH 6.75) in nature. During the present study, the values of various parameters of abattoir effluent viz., TDS (2840 mg L^{-1}), BOD (2480.50 mg L^{-1}), COD (2890.00 mg L^{-1}), total N (195.80 mg L^{-1}), Fe (18.48 mg L^{-1}), Mn (2.88 mg L^{-1}), total bacteria (6.97×10^8 CFU ml^{-1}), coliform bacteria (3.24×10^4 MPN 100 ml^{-1}) and total fungi (7.78×10^5 CFU ml^{-1}) were found beyond the prescribed limit of Indian irrigation standards (BIS, 2010) (Table 1). The higher values of BOD, COD indicated higher inorganic and organic load of the abattoir effluent. Thus, according to BIS irrigation standards any effluent contaminated to this level is neither good for the domestic use nor it is supposed to be discharged directly into the environment without treatment (BIS, 2010). Adesemoye *et al.* (2006) also reported the higher values of TDS (1800 mg L^{-1}), total bacteria (3.32×10^7 CFU ml^{-1}) and total fungi (1.6×10^5 CFU ml^{-1}) in the abattoir wastewater discharge from the slaughterhouses in Lagos, Nigeria.

Effect of abattoir effluent on soil characteristics: The mean ± SD values of different physico-chemical and microbiological characteristics of the soil before and after abattoir effluent irrigation are shown in Table 2. The soil is a major part of the earth ecosystem. It has been reported that disposal of abattoir effluent with out proper treatment may pose reflective impact on the soil characteristics, which are related to the soil fertility (Adesemoye *et al.,* 2006). In the present study, the ANOVA indicated that abattoir effluent irrigation significantly (*p<0.05/p<0.01*) increased the EC, total N, available P, OC, Ca, Mg, K, Na, Fe, Cd, Cr, Cu, Mn, Zn, total bacteria, coliform bacteria of the soil used for the cultivation of cabbage, lettuce, coriander and spinach in comparison to their respective controls (Table 2). The remarkable significant increase in the EC of abattoir effluent irrigated soil is likely due to the presence of more cationic and anionic species in the abattoir effluent. Moreover, higher values of total N, P, OC, Ca , Mg, Na, K and Fe, Cd, Cr, Cu, Mn and Zn was recorded in the soil after abattoir effluent irrigation and it might be due to the presence of more contents of these parameters in the abattoir effluent. Kumar and Chopra (2014a) also reported the higher values of OC (12.84 mg Kg^{-1}), NO_3^{2-} (99.61 mg Kg^{-1}), PO_4^{3-} (145.09 mg Kg^{-1}), Fe (12.75 mg Kg^{-1}), Cd (8.59 mg Kg^{-1}), Zn (9.36 mg Kg^{-1}), Cu (14.28 mg Kg^{-1}), Pb (6.75 mg Kg^{-1}) and Cr (5.88 mg Kg^{-1}) in the sewage sludge amended soil used for the cultivation of French bean (*Phaseolus vulgaris* L.).

Across the present study, a wide range of heavy metals contents was recorded in the abattoir effluent irrigated soil. The results showed that the contents of heavy metals were noted below the permissible limit for Cd (6.0 mg kg^{-1}), Cr (10.0 mg kg^{-1}), Cu (270 mg kg^{-1}) and Zn (600 mg kg^{-1}) prescribed for the agricultural soil in India (BIS 2010). However, there was considerable build up of Cd (4.870 mg Kg^{-1}), Cr (1.895 mg Kg^{-1}), Cu (8.72 mg Kg^{-1}), Mn (5.79 mg Kg^{-1}) and Zn (9.08 mg Kg^{-1}) in the abattoir effluent irrigated soils used for the cultivation of coriander compared to bore well water irrigated soil (Table 2). It is likely to occur due to the presence of significant quantity of these metals in the abattoir effluent. The OC and pH are important parameters that determine the availability of heavy metals in the soil and plants. Raising the soil organic matter can increase the soil EC, which in turn is a factor that may affect both the soluble and exchangeable metals (Kumar and Chopra, 2014b). However, in the present study, the slightly acidic pH (6.75) of abattoir effluent neutralizes the alkaline pH (8.45) of the soil upto slightly alkaline (pH 7.24-7.68) and it seemed to be one of the promising factor, which affects the availability, and accumulation of Cd, Cr, Cu, Mn and Zn in the soil and further in the vegetables grown.

During the present investigation, among the various soils used for the cultivation of cabbage, lettuce, coriander and spinach, the most population of bacteria (3.21×10^8 CFU ml^{-1}), coliforms (5.19×10^4 MPN 100 ml^{-1}) and fungi (9.58×10^5 CFU ml^{-1}) was recorded in the soil used for the cultivation of cabbage. Adesemoye *et al.* (2006) reported that the abattoir effluent significantly increased the microbial population particularly bacteria, and fungi in the soil in appreciable quantity. Additionally, Cd (r = + 88), Cr (r = + 80), Cu (r = + 92), Mn (r = + 90), Zn (r = + 96), total bacteria (r = + 98), coliform bacteria (r = + 94) and total fungi (r = + 98) of the soil showed significant and positive correlation with Cd, Cr, Cu, Mn, Zn, total bacteria, coliform bacteria and total fungi of the abattoir effluent.

Although, the contents of metals in the soil was recorded higher and this may be toxic to the microorganisms. But due to the accumulation of more OC in the soil after abattoir effluent irrigation, the higher population of bacteria, coliforms and fungi in the soil as earlier reported by Nwachukwu *et al.* (2011). During the investigations, the mean microbial population obtained from the abattoir effluent contaminated soil was more than that of the soil without effluent contamination (control soils). This could be regarded as deterioration of soil ecological balance arising from the contamination. The environmental stresses brought about by the contamination could be adduced for the reduction in the microbial species diversity but increasing the population of few surviving species (Adesemoye *et al.,* 2006).

Contamination of heavy metals in vegetables: The mean ± SD values of heavy metals in the roots, stem and leaves of cabbage, lettuce, coriander and spinach grown in abattoir effluent irrigated soil are presented in Tables 3-5. The ANOVA indicated that the mean contents of Cd, Cr, Cu, Mn and Zn in different parts (roots, stem and leaves) of cabbage, lettuce, coriander and spinach were recorded to be significantly (*p<0.05/p<0.01*) different to the control. The maximum contents of Cd (0.564 mg Kg^{-1}), Cr (0.384 mg Kg^{-1}) in the root of cabbage, Cu (4.456 mg Kg^{-1}) and Mn (1.489 mg Kg^{-1}) were recorded in the roots of spinach, and Zn (2.276 mg Kg^{-1}) in the roots of lettuce while the least contents of Cd (0.367 mg Kg^{-1}), Cr (0.128 mg Kg^{-1}) were observed in the roots of coriander, Cu (2.754 mg Kg^{-1}) and Zn (1.832 mg Kg^{-1}) in the roots of spinach and Mn (1.384 mg Kg^{-1}) in the roots of lettuce after abattoir effluent irrigation. The contents of metals in the roots of these vegetables were observed in the order of Cu < Zn < Mn < Cd < Cr after abattoir effluent irrigation (Table 3). The accumulation of Cd in the roots of different vegetables was recorded in the order of cabbage > lettuce > coriander > spinach; Cr in the roots of cabbage > lettuce > spinach > coriander; Cu in the roots of spinach > lettuce > coriander > cabbage; Mn in the roots of spinach > coriander > cabbage > lettuce and Zn in the roots of lettuce > cabbage > coriander > spinach after abattoir effluent irrigation (Table 3).

During the investigations, the ANOVA showed a significant (*p<0.05/p<0.01*) progressive accumulation of heavy metals in the stem of different vegetables. The most contents of Cd (1.782 mg Kg^{-1}) and Mn (2.134 mg Kg^{-1}) were recorded in the stem of cabbage, Cr (0.832 mg Kg^{-1}) in the stem of lettuce, Cu (4.980 mg Kg^{-1}) and Zn (4.880 mg Kg^{-1}) in the stem of spinach after abattoir effluent irrigation. The magnitude of heavy metals was recorded in the order of Cu < Zn < Mn < Cd < Cr in the stem of different kinds of vegetables after abattoir effluent irrigation (Table 4). The magnitude of Cd and Cr in the stems of different vegetables was noted in the order of cabbage > lettuce > spinach > coriander; Cu in the stems of spinach > lettuce > coriander > cabbage; Mn in the stems of cabbage > lettuce > spinach > coriander and Zn in the stems of spinach > lettuce > cabbage > coriander after abattoir effluent irrigation. The accumulation of heavy metals is administered by several environmental factors such as pH, solubility, and chemical speciation of the metals, organic matter, salinity, soil mineralogy, texture, and amorphous Fe and Al contents (Itanna, 2002; Kumar and Chopra, 2015). Though, distinct accumulation of Cd, Cr, Cu, Mn and Zn in the different parts of these plants suggests diverse cellular mechanisms of these metals accumulation, which may control their translocation and partitioning in the plants. As per accumulation mechanism, plants accumulate higher amounts of Cd, Cu, Mn and Zn in their tissues and only small contents of these metals is stored in their roots and the rest is translocated to the shoots (Kumar and Chopra, 2014a). The poor translocation of Cr to the shoots could be due to the sequesterization of most of Cr in the vacuoles of root cells to render it non-toxic, which may be a natural protective response of the plants (Itanna, 2002; Mapanda *et al.,* 2005). It must be noted that Cr is a toxic and non-essential element to plants and hence the plants may not possess any specific mechanism to transport the Cr in the plant cells (Ismail *et al.,* 2005; Kumar and Chopra, 2012a, b). The contents of Cr were higher in the roots than in the aerial parts, indicating that the roots act as a barrier for the translocation of Cr and protect the edible parts from the toxic contamination (Itanna, 2002; Sharma *et al.,* 2006). Moreover, the least accumulation of Cr was noted in the roots of cabbage, lettuce, coriander and spinach and it might be due to that the cells of xylem or phloem selectively translocate the Cr in the stems and leaves of the plants (Muchuweti *et al.,* 2006). Thus, the tolerant mechanism of the plants appears to be compartmentalization of the metal ions, i.e. sequestration in the vacuolar compartment, which excludes them from cellular sites, where processes such as cell division and respiration occur, thus proving an effective protective mechanism (Fytianos *et al.,* 2001; Kumar and Chopra, 2015).

The most contents of Cd (3.506 mg Kg^{-1}), Cr (1.734 mg Kg^{-1}), Cu (6.658 mg Kg^{-1}), Mn (3.540 mg Kg^{-1}) and Zn (7.892 mg Kg^{-1}) were recorded in the leaves of spinach after abattoir effluent irrigation. The contents of Cr, Cu, Mn and Zn except Cd in cabbage, lettuce, coriander and spinach were noted below the permissible limit of FAO/WHO standards for Cd (0.20 mg Kg^{-1}), Cr (2.30 mg Kg^{-1}), Cu (40.00 mg Kg^{-1}) and Zn (60.00 mg Kg^{-1}) (FAO/WHO, 2011). The accumulation of metals were observed in the order of Zn > Cu > Mn > Cd > Cr in the leaves of cabbage, lettuce, coriander and spinach after abattoir effluent irrigation. The accumulation of these metals was noted to be significantly (*p<0.05/p<0.01*) different to their respective controls after abattoir effluent irrigation. The contamination of Cd in the leaves of cabbage, lettuce, coriander and spinach were in the order of cabbage > lettuce > spinach > coriander; Cr in the leaves of cabbage > spinach > lettuce > coriander; Cu in the leaves of cabbage > spinach > lettuce > coriander; Mn in the leaves of cabbage > lettuce > spinach > coriander and Zn in the leaves of cabbage > lettuce > spinach > coriander after abattoir effluent irrigation (Table 5). Interestingly, five metals (Cd, Cr, Cu, Mn and Zn) were examined in the present study and have different chemical properties, and as a result each metal has peculiar accumulation. The diverse accumulation of these metals might be due to the number of electrons in the d-levels of the atom. Although, metals with completely filled d orbitals such as ($3d^{10}$ of Cu and Zn and $4d^{10}$ of Cd) may be least incorporated compared to ($3d^{5}$ of Cr and Mn) in these plants due to the lower reactivity and the more stability imparted by the completely filled d orbitals. The lower reactivity and stability of these metals reduce the rate of various reactions such as absorption, ionic exchange, redox reactions, precipitation and dissolution through which plants take metals from the soils (Davies, 1992; Kumar and Chopra, 2014b). However, the bioavailability of these metals increased due to the ionization in the aqueous environment which increases their reactivity and instability as earlier reported by Kumar and Chopra (2014a).

Microbial contamination of vegetables: The mean ± SD values of total bacteria, coliform bacteria and total fungi in cabbage, lettuce, coriander and spinach grown in abattoir effluent irrigated soil are shown in Table 6. The results of the microbial contamination of different vegetables indicated that the most number of bacteria (8.67×10^{8} CFU ml $^{-1}$), coliform bacteria (7.80×10^{5} MPN 100 ml $^{-1}$) and total fungi (9.85×10^{5} CFU ml $^{-1}$) were noted in lettuce after

Table 1. Physico-chemical and microbiological characteristics of abattoir effluent.

Parameter	Bore well water (Control)	Value	Irrigation standards (BIS, 2010)
TDS (mg L^{-1})	145±1.57	2840±8.94	1900
EC(dS cm^{-1})	0.03±0.00	4.50±0.11	-
pH	7.46±1.10	6.75±1.08	5.5-9.0
BOD (mg L^{-1})	8.56±0.23	2480.50±5.80	100
COD (mg L^{-1})	18.85±0.45	2890.00±6.89	250
Total N (mg L^{-1})	12.25±1.36	195.80±3.55	100
Ava. P (mg L^{-1})	5.46±0.25	22.94±2.10	-
Ca (mg L^{-1})	18.50±2.45	32.75±1.85	200
Mg (mg L^{-1})	78.69±2.15	175.60±1.77	-
K (mg L^{-1})	96.80±3.48	244.70±2.84	-
Na (mg L^{-1})	65.75±2.10	265.35±3.45	-
Fe (mg L^{-1})	4.30±0.11	18.48±1.20	1.00
Cd (mg L^{-1})	1.02±0.00	1.345±0.01	2.00
Cr (mg L^{-1})	0.04±0.1	0.558±0.04	2.00
Cu (mg L^{-1})	2.19±0.12	5.67±0.12	3.00
Mn (mg L^{-1})	1.26±0.15	2.88±0.50	1.00
Zn (mg L^{-1})	3.24±0.54	8.94±0.02	15
Total bacteria (CFU ml $^{-1}$)	215±12	$6.97\times10^{8}\pm27$	10000
Coliform bacteria (MPN 100 ml $^{-1}$)	65±5	$3.24\times10^{4}\pm20$	1000
Total fungi (CFU ml $^{-1}$)	105±10	$7.78\times10^{5}\pm34$	5000

Values are mean ± SD of three samples; - Values are not given in standards.

Table 2. Physico-chemical and microbiological characteristics of soil before and after irrigation of different vegetables with abattoir effluent.

Parameter	Before irrigation	Cabbage	Lettuce	Coriander	Spinach
EC(dS cm^{-1})	0.64±0.02	$1.36^{*}\pm0.04$	$1.48^{*}\pm0.06$	$2.04^{**}\pm0.10$	$2.25^{**}\pm0.15$
pH	8.45±1.10	$7.24^{ns}\pm0.02$	$7.32^{ns}\pm0.03$	$7.40^{ns}\pm0.05$	$7.68^{ns}\pm0.07$
Total N (mg Kg^{-1})	24.50±2.36	$115.24^{**}\pm2.10$	$120.36^{**}\pm1.95$	$148.50^{**}\pm2.28$	$164.30^{**}\pm2.14$
Ava. P (mg Kg $^{-1}$)	4.02±0.48	$5.08^{*}\pm0.04$	$7.15^{*}\pm0.12$	$9.75^{*}\pm0.45$	$15.20^{**}\pm0.80$
OC (mg Kg $^{-1}$)	5.37±1.02	$10.25^{**}\pm0.12$	$8.54^{*}\pm0.10$	$12.85^{**}\pm0.62$	$9.87^{*}\pm0.25$
Ca (mg Kg $^{-1}$)	24.50±1.64	$64.50^{*}\pm1.25$	$75.86^{*}\pm1.10$	$82.24^{**}\pm1.60$	$94.00^{**}\pm2.36$
Mg (mg Kg $^{-1}$)	68.90±2.14	$124.50^{**}\pm0.55$	$136.80^{**}\pm0.68$	$145.67^{**}\pm0.94$	$158.75^{**}\pm1.00$
K (mg Kg $^{-1}$)	136.00±2.85	$182.36^{*}\pm1.57$	$198.30^{*}\pm2.12$	$210.50^{**}\pm2.00$	$224.70^{**}\pm1.98$
Na (mg L^{-1})	12.49±1.70	$98.67^{*}\pm1.20$	$114.75^{*}\pm1.30$	$134.50^{**}\pm2.10$	$140.80^{**}\pm2.04$
Fe (mg Kg $^{-1}$)	3.68±0.45	$12.36^{**}\pm0.08$	$14.50^{**}\pm1.10$	$16.80^{**}\pm0.85$	$10.32^{**}\pm1.00$
Cd (mg Kg $^{-1}$)	0.541±0.08	$2.206^{**}\pm0.02$	$3.580^{**}\pm0.03$	$4.870^{**}\pm0.03$	$3.694^{**}\pm0.04$
Cr (mg Kg $^{-1}$)	0.120±0.02	$0.975^{*}\pm0.01$	$1.284^{*}\pm0.04$	$1.895^{*}\pm0.05$	$1.575^{*}\pm0.07$
Cu (mg Kg $^{-1}$)	2.10±0.09	$4.25^{**}\pm0.06$	$5.67^{**}\pm0.09$	$8.72^{**}\pm0.07$	$6.87^{**}\pm0.08$
Mn (mg Kg $^{-1}$)	1.14±0.03	$2.80^{*}\pm0.05$	$3.68^{*}\pm0.07$	$5.79^{*}\pm0.03$	$4.67^{*}\pm0.06$
Zn (mg Kg $^{-1}$)	2.48±0.19	$5.78^{**}\pm0.09$	$4.94^{**}\pm0.08$	$9.08^{**}\pm0.06$	$7.14^{**}\pm0.08$
Total bacteria (CFU ml $^{-1}$)	$2.45\times10^{6}\pm12$	$3.21\times10^{8**}\pm20$	$4.25\times10^{7**}\pm14$	$2.10\times10^{5**}\pm10$	$3.65\times10^{6**}\pm24$
Coliform bacteria (MPN 100 ml $^{-1}$)	$1.40\times10^{3}\pm10$	$5.19\times10^{4**}\pm12$	$1.25\times10^{4**}\pm20$	$1.10\times10^{3*}\pm10$	$1.34\times10^{4**}\pm25$
Total fungi (CFU ml $^{-1}$)	$3.80\times10^{3}\pm16$	$9.58\times10^{5*}\pm25$	$8.45\times10^{5*}\pm19$	$5.68\times10^{5*}\pm24$	$7.62\times10^{5*}\pm30$

Values are mean ± SD of three samples; ns- Not significant; *, ** significantly different at P>0.05 or P>0.01 level of ANOVA, respectively.

Table 3. Contamination of heavy metal in roots of different vegetables grown in abattoir effluent irrigated soil.

Vegetables	Cd (mg Kg $^{-1}$)	Cr (mg Kg $^{-1}$)	Cu (mg Kg $^{-1}$)	Mn (mg Kg $^{-1}$)	Zn (mg Kg $^{-1}$)
Cabbage	$0.564^{*}\pm0.08$ (0.125±0.02)	$0.384^{*}\pm0.02$ (0.085±0.01)	$2.754^{**}\pm0.13$ (0.348±0.11)	$1.430^{*}\pm0.12$ (0.450±0.02)	$2.174^{**}\pm0.10$ (0.694±0.07)
Lettuce	$0.432^{*}\pm0.06$ (0.109±0.01)	$0.265^{*}\pm0.01$ (0.064±0.00)	$3.023^{**}\pm0.15$ (1.059±0.08)	$1.384^{*}\pm0.11$ (0.351±0.06)	$2.276^{**}\pm0.15$ (0.780±0.05)
Coriander	$0.367^{*}\pm0.04$ (0.105±0.02)	$0.128^{*}\pm0.01$ (0.042±0.01)	$2.875^{**}\pm0.12$ (0.841±0.15)	$1.459^{*}\pm0.15$ (0.297±0.03)	$1.958^{**}\pm0.17$ (0.568±0.04)
Spinach	$0.340^{*}\pm0.07$ (0.102±0.01)	$0.224^{*}\pm0.02$ (0.021±0.00)	$4.456^{**}\pm0.17$ (1.261±0.10)	$1.489^{*}\pm0.02$ (0.245±0.01)	$1.832^{**}\pm0.11$ (0.429±0.03)
FAO/WHO standard limit	0.20	2.30	40.00	-	60.00

Values are mean ± SD of three samples; *, ** significantly different at P>0.05 or P>0.01 level of ANOVA, respectively; Values of control are given in parenthesis; - Value is not given in standards.

Table 4. Contamination of heavy metal in stems of different vegetables grown in abattoir effluent irrigated soil.

Vegetables	Cd (mg Kg $^{-1}$)	Cr (mg Kg $^{-1}$)	Cu (mg Kg $^{-1}$)	Mn (mg Kg $^{-1}$)	Zn (mg Kg $^{-1}$)
Cabbage	$1.782^{*}\pm0.07$ (0.507±0.06)	$0.712^{*}\pm0.04$ (0.348±0.03)	$2.899^{**}\pm0.15$ (0.865±0.07)	$2.134^{**}\pm0.10$ (0.869±0.06)	$3.580^{**}\pm0.13$ (1.796±0.17)
Lettuce	$1.538^{*}\pm0.09$ (0.429±0.03)	$0.832^{*}\pm0.05$ (0.120±0.04)	$3.865^{**}\pm0.19$ (1.298±0.12)	$1.670^{*}\pm0.14$ (0.748±0.05)	$3.975^{**}\pm0.13$ (1.580±0.10)
Coriander	$0.970^{*}\pm0.05$ (0.084±0.01)	$0.501^{*}\pm0.03$ (0.085±0.01)	$2.967^{**}\pm0.10$ (0.984±0.08)	$1.520^{*}\pm0.10$ (0.579±0.03)	$2.145^{**}\pm0.14$ (1.469±0.11)
Spinach	$1.468^{*}\pm0.06$ (0.396±0.02)	$0.621^{*}\pm0.06$ (0.094±0.01)	$4.980^{**}\pm0.18$ (1.368±0.14)	$1.543^{*}\pm0.17$ (0.697±0.04)	$4.880^{**}\pm0.15$ (1.679±0.15)
FAO/WHO standard limit	0.20	2.30	40.00	-	60.00

Values are mean ± SD of three samples; *, ** significantly different at P>0.05 or P>0.01 level of ANOVA, respectively; Values of control are given in parenthesis; - Value is not given in standards.

Table 5. Contamination of heavy metal in leaves of different vegetables grown in abattoir effluent irrigated soil.

Vegetables	Cd (mg Kg $^{-1}$)	Cr (mg Kg $^{-1}$)	Cu (mg Kg $^{-1}$)	Mn (mg Kg $^{-1}$)	Zn (mg Kg $^{-1}$)
Cabbage	$3.506^{**}\pm0.54$ (1.752±0.02)	$1.734^{*}\pm0.04$ (0.598±0.09)	$6.658^{**}\pm0.78$ (2.136±0.15)	$3.540^{**}\pm0.21$ (1.064±0.11)	$7.892^{**}\pm0.56$ (2.648±0.17)
Lettuce	$2.578^{*}\pm0.09$ (0.758±0.09)	$1.580^{*}\pm0.05$ (0.675±0.03)	$4.970^{**}\pm0.80$ (1.368±0.26)	$2.875^{**}\pm0.43$ (1.025±0.10)	$5.732^{**}\pm0.40$ (1.875±0.14)
Coriander	$1.453^{*}\pm0.07$ (0.678±)	$0.986^{ns}\pm0.07$ (0.115±0.04)	$3.542^{**}\pm0.95$ (1.184±0.13)	$1.965^{**}\pm0.76$ (0.967±0.08)	$3.405^{**}\pm0.59$ (1.890±0.12)
Spinach	$2.456^{*}\pm0.06$ (0.847±0.07)	$1.687^{*}\pm0.38$ (0.106±0.03)	$5.237^{**}\pm0.66$ (2.068±0.16)	$2.760^{*}\pm0.56$ (0.724±0.06)	$4.640^{**}\pm0.77$ (1.987±0.15)
FAO/WHO standard limit	0.20	2.30	40.00	-	60.00

Values are mean ± SD of three samples; NS- Not significant; *, ** significantly different at P>0.05 or P>0.01 level of ANOVA, respectively; Values of control are given in parenthesis; - Value is not given in standards.

Table 6. Microbial contamination of different vegetables after irrigation with abattoir effluent.

Vegetables	Total bacteria (CFU ml $^{-1}$)	Coliform bacteria (MPN 100 ml $^{-1}$)	Total fungi (CFU ml $^{-1}$)
Cabbage	$6.12\times10^{6**}\pm32$ ($2.81\times10^{2}\pm9$)	$4.65\times10^{4**}\pm20$ ($1.96\times10^{2}\pm7$)	$7.65\times10^{5*}\pm28$ ($4.67\times10^{2}\pm6$)
Lettuce	$8.67\times10^{8**}\pm30$ ($1.54\times10^{3}\pm12$)	$7.80\times10^{5**}\pm19$ ($3.49\times10^{2}\pm8$)	$9.85\times10^{5*}\pm43$ ($3.10\times10^{2}\pm5$)
Coriander	$5.40\times10^{5**}\pm54$ ($6.32\times10^{3}\pm10$)	$3.80\times10^{4**}\pm20$ ($1.64\times10^{2}\pm10$)	$5.40\times10^{5**}\pm27$ ($2.40\times10^{2}\pm9$)
Spinach	$7.30\times10^{7**}\pm31$ ($3.64\times10^{2}\pm16$)	$5.20\times10^{5*}\pm23$ ($2.30\times10^{2}\pm6$)	$6.10\times10^{5**}\pm35$ ($3.21\times10^{2}\pm8$)
FAO/WHO standard limit	$10\text{-}10^{5}$	$3\text{-}10^{2}$	-

Values are mean ± SD of three samples; *, ** significantly different at P>0.05 or P>0.01 level of ANOVA, respectively; Values of control are given in parenthesis; - Value is not given in standards.

abattoir effluent irrigation.

The ANOVA indicated that microbial contamination of vegetables was found to be significantly ($p<0.05/p<0.01$) different compared to the control (Table 6). The contamination of total bacteria and coliform bacteria in different vegetables were observed in the order of lettuce > spinach > cabbage > coriander; while contamination of total fungi was in the order of lettuce > cabbage > spinach > coriander (Table 6).

Thus, among the different vegetables, the least contamination of total bacteria (5.40×10^5 CFU ml $^{-1}$), coliform bacteria (3.80×10^4 MPN 100 ml $^{-1}$) and total fungi (5.40×10^5 CFU ml $^{-1}$) were noted in coriander and it might be due to the emission of aromatic fragrance which is likely inhibited the number of microbial population as earlier reported by Chaturvedi *et al.* (2013). Moreover, coriander has lower foliar surface area compared to lettuce, spinach and cabbage, which provide the least habitation for the microbial population (Chaturvedi *et al.,* 2013; Chopra *et al.,* 2013).

Conclusions

The present study concluded that the abattoir effluent was highly loaded with different nutrients (total N, available P, Na, K, Ca and Mg), heavy metals (Cd, Cr, Cu, Mn, Zn) and microbial contaminants (Total bacteria, coliform bacteria and total fungi). The abattoir effluent irrigation significantly ($P<0.05/P<0.01$) increased the contents of Cd, Cr, Cu, Mn, Zn, total bacteria, coliform bacteria and total fungi in the soil and cabbage, lettuce, coriander and spinach as well. The concentration of different heavy metals in the roots and stems of cabbage, lettuce, coriander and spinach was recorded in the order of Cu < Zn < Mn < Cd < Cr while in the leaves of these vegetables was in the order of Zn > Cu > Mn > Cd > Cr after abattoir effluent irrigation. The most numbers of bacteria, coliform bacteria and total fungi were noted in the lettuce after abattoir effluent irrigation. Although, contents of the metals in cabbage, lettuce, coriander and spinach were recorded within the FAO/WHO prescribed limit but it may pose a risk factor for the consumption of these vegetables. Therefore, regular monitoring of these soils and vegetables should be required to prevent any potential hazard due to consumption of heavy metals and microbial contaminate vegetables. Further studies are required on the effects of biochemical and nutritional composition of these vegetables after irrigation with abattoir effluent irrigation.

ACKNOWLEDGEMENT

University Grants Commission, New Delhi, India is acknowledged to provide Meritorious Fellowship (F.7-70/2007-2009 BSR) to VK.

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