IJARSE
ISSN 2319 - 8354

An Assessment of *Ascaris* Eggs and *E.Coli* Contamination and Related Health Risk Associated With the Wastewater Irrigation of Lettuce in Titagarh, West Bengal, India

Gupta, N.[1], Khan, D.K.[2], Santra, S.C.[3]

[1] *Department of Environmental Science, Bir Bikram Memorial College, Tripura (West), Tripura, India*

[2, 3] *Department of Environmental Science, University of Kalyani, Kalyani-741235, Nadia, West Bengal, India*

ABSTRACT

A total of 108 wastewater samples (Untreated and Treated), 62 soil samples and 106 lettuce samples were collected during dry and wet seasons from the wastewater-irrigated area of Titagarh to assess its contamination level with *E.coli* and *Ascaris* eggs. The study revealed that 53.57% and 80.35% of untreated wastewater, 34.61% and 65.38% of treated wastewater, 61.29% and 53.22% of soil and 60.37% and 25.47% of lettuces were found positive for *E.coli* and *Ascaris* eggs respectively. Contamination levels of these faecal pathogens in all samples were significantly higher during wet season than the dry season. To assess the health risk associated with the wastewater farming and consumption of wastewater-irrigated lettuce, quantitative microbial risk assessment (QMRA) models combined with 10,000 montecarlo simulations were used and compared with tolerable risk guidelines. This assessment identified that the microbial risks involved in wastewater- irrigated vegetable farming to farmers and consumers in the study area were high and exceeded the tolerable risk limit.

Key Words: *Wastewater-irrigation, Microbial contamination, Health risk, E.coli, Ascaris eggs*

INTRODUCTION

An estimated 20 million hectares worldwide are irrigated with wastewater, most of it were untreated wastewater (Scott et al. 2004). The increasing global scarcity of good quality water will turn wastewater irrigation from an undesirable phenomenon into a necessity wherever agricultural water demand is not met by supply. This is not only the case in drier regions, but anywhere where farmers seek land and water to address market demand. Common examples are urban and peri-urban areas in most developing countries where clean water sources are hardly sufficient even to meet domestic demand (Bos et al. 2010). While the nutrients contained in the wastewater is considered as beneficial to agriculture, the contaminants present in the wastewater pose health risks directly to agricultural workers and indirectly to consumers of the wastewater grown produce. Use of sewage effluent for irrigation exposes the public to the dangers of infections with a variety of pathogens such as bacteria, viruses, protozoa and helminthes. Transmission of diseases may occur through direct physical contact of farmers with wastewater, consumption of products irrigated with effluent and contaminated ground and surface waters (Minhas and Sharma, 2004; Scott et al.,2004). The problem of microbial quality of agricultural produce especially vegetables consumed raw is more severe than when the produce is cooked at home or processed in factories before sale. Microbial infections of foodborne origin are a major public-health problem internationally and a significant cause of death in developing countries (WHO 2006). The WHO standards for intestinal nematodes and faecal coliform in irrigation water are <1 egg/L and≤1000 faecal coliform /100ml (WHO 1989). These health guidelines are difficult to follow, sometimes even canal water supplies in India exceeds these limits (Minhas et al. 2006).

The Titagarh area was selected for this study as the entire agricultural produce cultivated in this area caters to the need of Kolkata city and its suburbs including Titagarh for salad vegetables, and since this area is generally irrigated with untreated and treated wastewater, the vegetables are likely to pose a threat to public health.

Previously the impact of wastewater irrigation on soil and vegetables in Titagarh has been assessed for heavy metal and helminth contamination (Gupta et al. 2008, 2009) but so far no study has been reported on microbial contamination and related risk to consumers and farmers of this region. The main purpose of the study was to assess the extent of microbial contamination (*E coli* and *Ascaris* eggs) in wastewater-irrigated soil and lettuce and to quantify the health risk associated with this wastewater irrigation practice using the Quantitative Microbial Risk Assessment (QMRA) approach. QMRA has been widely used to establish the health risks associated with wastewater reuse in both developed and developing regions under different scenarios. It has been applied to establish the health risk associated with consuming wastewater-irrigated food crops (Tanaka et al. 1998) and vegetables (Hamilton et al. 2006; Shuval et al. 1997). Mara et al. (2007) have also applied QMRA to assess the health risks for farmers using wastewater under different irrigation and technology regimes. The choice of *Ascaris* was due to its predominance in the study area and its parasitic infection worldwide. *Ascaris* can persist for months to years in soils under harsh conditions thus making it an ideal reference organism for Quantitative Microbial Risk Assessments (QMRA) in a developing country (Hamilton et al. 2006) like India. In this study, mean *E.coli* concentrations found in soil and on lettuce were translated to rotavirus concentrations to run the QMRA models as applied by Mara et al. 2007. Rotavirus is a major cause of gastroenteritis (Chandran et al. 2006) and has been widely used as a representative organism for enteric viruses in QMRAs of wastewater reuse (Hamilton et al. 2006; Shuval et al. 1997).

MATERIALS AND METHODS

Study Area: The study area, Titagarh is a suburban Industrial town located in 24-Parganas District (North), West Bengal, India. It is situated in the east side of the river Hooghly (a tributary of the river Ganges) 22 km North of Kolkata, the capital city of West Bengal. Titagarh Municipality has an old conventional Sewage Treatment Plant. The system is for treatment of raw sewage from commercial as well as domestic sources as discharged by the people of Titagarh and parts of Barrackpore municipality. In the Sewage Treatment Plant, raw sewage is being treated and the final effluent is directly taken to the adjoining agricultural land through concrete pipes. Both untreated and treated sewage water are used for irrigation purpose in the study area. Sewage treatment plant operators are often to release raw wastewater due to pressing demand of the farmers of the surrounding agricultural land. The common vegetables grown in the study area were Lettuce, Coriander, Parsley, Pudina, etc. Most of these vegetables cultivated in this site are supplied to wholesale vegetable markets in Kolkata and the rest enters the local markets. For a worst case scenario, we chose lettuce over other vegetables. Lettuce is reported to have a higher *Ascaris* and faecal coliform contamination compared to other vegetables irrigated with wastewater (Obuobie et al. 2006) mainly due to its morphology for water retention (Shuval et al. 1997). In addition, Lettuce also selected for the study because it was generally eaten uncooked. The present study was carried out during January and September 2010.

SAMPLE COLLECTION

Water: A total of 108 wastewater samples (untreated -56 and treated-52) used for irrigation were collected from the wastewater irrigated area of Titagarh. For bacterial analysis, composite wastewater samples consisting of three individual 1L samples were collected over a 4 hr period. Samples were collected in 1 L sterile glass bottles. For *Ascaris* egg analysis, 2L of untreated and 10L of treated composite wastewater samples were collected. Samples were collected in sterile 2L and 10L containers with lids and left for sedimentation for 2-3 hours. Samples were stored in a cool box and transported to the laboratory for further analysis on the day of sampling. Analysis of water samples for *E.coli* was always initiated on the day of collection. Samples for *Ascaris* egg analysis were stored at 4^0C until further processing.

Soil: 62 soil samples were collected randomly from the wastewater- irrigated area of Titagarh at 0-30cm and 30-60 cm depths. Each composite sample consisted of three soil samples. Sample collection was done using a sterile manual auger. Soil samples were brought to the laboratory in sterile plastic bags and stored at 4^0C. Microbiological analysis was done within 24 hours of soil collection. Soil moisture was determined on the same soil samples by drying at 105^0C for 48 hours.

Vegetables: A total of 106 lettuces (*Lactuca sativa*) were collected randomly from the peri urban area of Titagarh during dry and wet seasons. Lettuce samples were collected with sterile disposable gloves and cut into pieces using alcohol sterilized scissors and then they were packed in sterile plastic bags and finally transported in cold packs to the laboratory where they were analyzed immediately or stored at 4^0C until analysis within 24 hours. Steps were taken to avoid contamination of the lettuces by soil or other contamination sources. Each sample was collected in triplicate to prevent sampling error.

SAMPLE ANALYSES

Enumeration of E.coli: *E. coli* was enumerated in treated and untreated wastewater by the membrane filter procedure (APHA, AWWA, WEF, 1995). Water samples were subjected to suitable dilutions and filtered through cellulose nitrate membrane filters with 0.45 µm pore size. Trypton bile X-glucouronide (TBX) agar medium (Oxoid) was used for *E. coli* analysis. Plates were incubated for 24 hrs at 44 ^{0}C and blue colonies were counted as *E. coli*. The density of *E. coli* was reported as CFU/ 100 ml.

E. coli in soil and vegetables was counted using the Most Probable Number (MPN) method following the 3 replications x 5 dilutions scheme (APHA, AWWA, WEF 1995; Woomer 1994).

100 grams of vegetables were homogenised with 900 ml of tryptone water (0.1%) by stomacher. Thereafter 10-fold dilutions were carried out within the same medium. For soil analysis, 10gm of soil was homogenized for 30s in a blender (Stomacher®) followed by addition of 90ml tryptone water (0.1%). 1ml aliquot of the 10 fold

dilutions of soil and vegetables were inoculated into tubes filled with 10 ml of Lauryl Tryptose Broth (Oxoid) containing inverted vials. After incubation for 24–48 hrs at 35 ^{0}C, a 0.1-mL aliquot of any broth showing gas bubbles was transferred to tubes containing EC Broth (Oxoid) and incubated for 24–48 h at 45 ^{0}C. A 0.1-ml aliquot was taken from tubes showing gas production (considered a positive reaction) and placed in a tube of Tryptone Water (Oxoid) to perform the indol test. After 48 h at 45 ^{0}C, several drops of Kovac's reagent were added to the broths agitated slightly. A cherry red colour, visible at the surface of the broth, was considered positive for indol and thus confirmed the presence of *E. coli.*

Enumeration of Helminth Eggs: For Ascaris egg estimation, 100gm of soil sample, 2L of treated and 10L of untreated wastewater were analyzed. The water and soil samples were examined by the technique of Bailenger (1962). For analysis of lettuce, a portion of the vegetable was weighted (100 gm) into plastic bag and washed with 2 L of physiological saline solution (0.95% NaCl). The washed water was then left for about 10 h for sedimentation to take place. The top layer was discarded and the remaining washed water was centrifuged at 2164g for 15 min. The supernatant was discarded and the residue was collected carefully. The concentration of helminth eggs in the residue was determined using the technique of Téléman Rivas modified by Bailenger (1962). Microscopic observation of helminth eggs in samples was performed in Mac Master Counting cell at 100-fold magnification.

Health Risk Assessment: The guidelines developed by the World Health Organization in 2006 for the safe use of wastewater in agriculture are based on a tolerable additional disease burden from working in wastewater-irrigated fields and consuming wastewater –irrigated crops of 10^{-6} disability-adjusted life year (DALY) loss per person per year (pppy).The tolerable burden of disease is translated into a tolerable annual risk of infection which for an episode of diarrhoeal disease has been determined as an annual disease risk of 7.7×10^{-4}.

To ascertain the health risk of farmers engaged in wastewater-irrigation and consumers of wastewater-irrigated produce, quantitative microbial risk assessment (QMRA) models with 10,000 montecarlo simulations were applied. These models assessed the disease risk as a result of the accidental ingestion of wastewater irrigated soil by farmers and for the consumption of contaminated produce (Mara et al. 2007; WHO 2006).

E.coli and *Ascaris* egg concentration of irrigation water and soil and locally appropriate exposure scenarios were used to run the models. To account for the rotavirus in our risk assessment models, geometric mean count of *E.coli* found in irrigation water, wastewater-irrigated soil and lettuce were converted to rotavirus concentrations using a ratio of 1 (rotavirus) to 10^5 (*E.coli*) as applied by Mara et al. 2007. Exposure scenarios included days and hours spent in irrigated fields, daily consumption pattern of lettuce, degree of mechanization in the irrigation schemes and protective measures. The exposures were identified by on site survey of the farming site and its surroundings. Vegetable farming activities in Titagarh are labour intensive and farmers do not use any protective clothes (boot, mouth cover, gloves etc) thus they are putting themselves into direct contact with irrigation water and contaminated soil. In this study, a uniformly distributed accidental ingestion of 10-100mg of soil per daily exposure for a total of 150 days per year was used for the farmers (Haas et al. 1999; Mara et al. 2007). The amount of lettuce consumed per person per day was taken as 20 to 30 gm and the frequency of consumption was taken to be once in a week based on the survey carried out in this study. Also it was taken that 10 to 15 ml of irrigation water would be left on a 100gm of lettuce after harvest (Mara et al. 2007; WHO 2006).

Diarrhoea was used as a proxy for infections related to rotavirus. For comparative analyses, we used the WHO (2006) benchmark for annual tolerable risk for diarrhea per person of $7.7x10^{-4}$ for developing countries. For *Ascaris* infection, a tolerable risk of $1x10^{-2}$ (Mara et al. 2007) was used to account for its high prevalence in developing countries and in wastewater reuse (WHO 2006).

DATA ANALYSIS

Data were compiled in a spreadsheet (Microsoft Excel, version 2007) and analyzed as appropriate using descriptive statistics. Student's t-test was used to compare the mean counts of *E.coli* and *Ascaris* for two seasons. Results of analysis are quoted at $p< 0.05$ level of significance.

RESULTS AND DISCUSSION

A total of 108 wastewater (56 untreated and 52 treated), 62 soil and 106 lettuce samples were examined to ascertain microbial contamination in the wastewater- irrigated area of Titagarh, West Bengal, India. The presence and concentration of *E. coli* and *Ascaris* eggs were examined in wastewater and soil samples for two seasons, dry (January- May) and wet (June-September). Student's t-test was used to compare mean *E.coli* and *Ascaris* egg contamination of all the samples between seasons and the result showed higher contamination in the wet season than the dry seasons and the difference was significant ($p<0.05$). High level of *E.coli* and *Ascaris* eggs during the wet rainy season were obviously due to favourable temperature, high turbidity and the addition of more sewage and faecal matter through surface run-off.

IRRIGATION WATER QUALITY

Wastewater can contain a wide variety of microorganisms that are pathogenic to humans (Toze 2006). Table 1 summarized the concentration of *E.coli* and percentage of *E.coli* positive untreated and treated sewage water used for irrigation. In wet seasons, *E.coli* was detected in 20/32(62.5%) and 12/30 (40%) of the untreated and treated wastewater samples respectively. During dry months10/ 24 (41.66%) of untreated wastewater and 6/22(27.27%) of treated wastewater were found E.coli positive. The untreated wastewater from the study area had *E.coli* counts ranging from $4.20x10^6$ to $7.4x10^6$ cfu/100ml and $2.33x10^7$ to $4.30x10^7$ cfu/100ml during dry and wet season respectively. The *E.coli* count in treated wastewater ranged from$2.8x10^6$ to $6.1x10^6$ cfu/100ml and $1.5x10^7$ to $1.82x10^7$ during dry and wet season respectively (Table1). In all the *E.coli* positive untreated and treated wastewater samples of the wastewater-irrigated study area, contamination level far exceeded the WHO recommended value of 10^3cfu/100ml (WHO 1989) for "irrigation of crops likely to be eaten uncooked".

Table 1. Contamination of *E.coli* in irrigation water, soil and lettuce collected from the wastewater irrigated area of Titagarh, West Bengal, India

Sample type	season	Total number of samples	Number and percentage of E.coli positive samples	Geometric mean count of E.coli (cfu/100ml or cfu/100gm wet

				weight)[range]
Untreated wastewater	Dry	24	10 (41.66)	$6.06x10^6$ [$4.2x10^6$-$7.4x10^6$]
	Wet	32	20(62.5%)	$3.27x10^7$ [$2.33x10^7$-$4.30x10^7$]
	Total	**56**	**30(53.57%)**	-
Treated wastewater	Dry	22	6(27.27%)	$4.83x10^6$ [$2.8x10^6$-$6.1x10^6$]
	Wet	30	12(40%)	$1.67x10^7$ [$1.5x10^7$-$1.82x10^7$]
	Total	**52**	**18(34.61%)**	-
Soil	Dry	37	21(56.75%)	$1.47x10^6$ [$1.1x10^6$-$1.74x10^6$]
	Wet	25	17(68%)	$1.87x10^6$ [$1.5x10^6$-$2.67x10^6$]
	Total	**62**	**38(61.29%)**	-
Lettuce	Dry	62	34(54.83%)	$2.59x10^5$ [$1.6x10^5$-$3.7x10^5$]
	Wet	44	30(68.18%)	$4.76x10^5$ [$2.5x10^5$-$6.21x10^5$]
	Total	**106**	**64(60.37%)**	-

Studies in Pakistan by Feenstra et al. (2000) and van der Hoek et al (2002) on the health risks of irrigation with untreated urban wastewater showed potential health risk for both farmers and crop consumers when the wastewater contained *E.coli* count beyond WHO recommended limit. The mean *E.coli* count of irrigation water (both treated and untreated wastewater) recorded during the present study were higher than those reported in Italy (0-$7.8x10^4$ cfu/100ml) (Palse ET al.2009) and in Mexico ($4x10^4$- $29x10^4$cfu/100ml) (Rosas et al.1984). In a similar study, Srikanth and Naik (2004) studied the health effects of wastewater reuse for agriculture in the Asmara city, Eritrea. They reported the *E.coli* level in the range from $4x10^3$cfu/100ml to$13x10^8$cfu/100ml in the wastewater used for irrigation purposes which was slightly higher than the wastewater of our study area.

For *A. lumbricoides* contamination, it is noted that 80.35% of untreated wastewater and 65.38% of treated wastewater samples were found positive for *Ascaris* eggs (Table 2). After treatment of the raw wastewater in the Titagarh Sewage Treatment Plant, the load of *Ascaris* eggs decreased to some extent but considerable level of *Ascaris* egg contamination still remained in the treated wastewater used for irrigation. A study in India by

Ensink et al. (2006) and the study in Turkey by Ulukanligil et al. (2001) showed 64% and 60.8% of wastewater samples were contaminated with *Ascaris* ova respectively which were lower than the percentage of *Ascaris* egg positive irrigation water (untreated- 80.35% and treated- 65.38%) of the present study. The mean concentrations of *Ascaris lumbricoides* eggs (eggs/L) in untreated wastewater were 15.63 eggs/L and 24.07 eggs/L during dry and wet season respectively (Table 2). In treated wastewater, the mean count of *Ascaris* eggs observed during dry season was 7.14 eggs /L while 15.20 eggs /L mean concentration was found during wet season (Table 2). The result showed that the mean *Ascaris* egg concentration in untreated and treated wastewater used for irrigation of lettuce were much higher than the WHO recommended value of ≤1 helminth egg/L (WHO 2006).A study in Pakistan by Ensink et al. (2004) reported that nematode egg concentrations in the wastewater used for irrigation were very high with an average *Ascaris* eggs concentration of 142 eggs /L which was much higher than the average *Ascaris* egg concentration in untreated and treated wastewater of our study area. Another study in Marrakech by Bouhoum et al. (1997) also showed much higher *Ascaris* egg count (75.6 eggs/ l) in the irrigation water compared to the *Ascaris* egg concentration in irrigation water of Titagarh wastewater-irrigated area. The *Ascaris* eggs present in wastewater beyond threshold level constitute a risk when used in irrigation because these eggs are very resistant in the environment and they can survive in water, soil and crops for several months or years (Feachem et al. 1983).

SOIL

In the wastewater –irrigated area of Titagarh, *E.coli* was detected in 56.75% (21/37) and 68 % (17/25) of the soil samples during dry season and wet seasons respectively (Table 1). The role that soil plays in the bacteriological contamination of vegetables, due to direct contact between the soil and plants, is well known, especially when they are irrigated with wastewater containing $4.83x10^6$to $3.27x10^7$ *E.coli* per 100 ml of water, as observed in this case (Table 1). In the present study, *E.coli* counts in the wastewater- irrigated soil samples ranged from $1.1x10^6$ to $1.74x10^6$ cfu/100gm and $1.5x10^6$ to $2.67x10^6$ cfu/100gm during dry and wet seasons respectively. Prevalence of *E.coli* was detected especially in the upper fractions of the soil samples and negligible or no contamination was observed in the lower fractions of the soil samples. Palese et al. (2009) explained that the soil acted as a filter, reducing bacterial concentration in the deeper soil layers. The findings of our study were also in agreement with the results of El Hamouri et al. (1996) and Oron et al (2001). In particular El Hamouri et al. (1996) observed a gradual reduction of faecal coliform concentration through the soil profile.

Table2. Status of *Ascaris* egg contamination in analyzed samples of wastewater, soil and lettuce

Sample type	Season	Total number of samples	Number and percentage of Ascaris egg posive samples	Mean number of helminth eggs (egg/L or egg/100gm wet weight)[range]
Untreated	Dry	24	19(79.16%)	15.63 [11-23]

wastewater	Wet	32	26(81.25%)	24.07[13-45]
	Total	**56**	**45(80.35%)**	-
Treated wastewater	Dry	22	14(63.63%)	7.14[4-14]
	Wet	30	20(66.66%)	15.2[10-27]
	Total	**52**	**34(65.38%)**	-
Soil	Dry	37	16(43.24%)	6.9 [3.2-11.8]
	Wet	25	17(68%)	10.37[3.6-14]
	Total	**62**	**33(53.22%)**	-
Lettuce	Dry	62	12 (19.35%)	3[1-7]
	Wet	44	15(34.09%)	7.06[1-12]
	Total	**106**	**27(25.47%)**	-

Data obtained by analyzing wastewater-irrigated soil for *Ascaris* eggs are summarized in table 2. Of the 62 soil samples tested from the study area, 33 (53.22%) were found positive for *Safaris lumbricoides* eggs. Embryonated *Ascaris* eggs were found in most of the soil samples in average of 6.9 eggs /100gm and 10.37 eggs/100 gm during dry and wet season respectively. A study on soil-transmitted helminths in Sanliurfa, Turkey showed 84.4% of soil samples were contaminated with *Ascaris* eggs (Ulukanligil et al. 2001) which were higher than the percentage of *Ascaris* positive (53.22%) wastewater-irrigated soil samples of the present study. A similar study in Vietnam by Toan (1998) showed that the prevalence of soil transmitted helminth infection was high and all of the soil samples were positive for *Ascaris* eggs.

VEGETABLES

Lettuce samples irrigated with untreated and treated wastewater were analyzed for the presence and concentration of *E.coli* and Ascaris eggs on the surface of this leafy vegetable. Of the 106 vegetables tested 64 (60.37%) were found positive for E.coli. The result showed that the mean concentration of E.coli in Lettuce was $2.59x10^5$ cfu/100gm and $4.76x10^5$ cfu/100gm during dry and wet season respectively (Table 1).

Minhas et al. (2006) reported contamination for different types of crops irrigated with sewage water characterized by average faecal coliform counts of $1.5x10^8$ /100ml. They observed faecal coliform counts of vegetables, fodder and grain crops ranged between <2 and $9x10^5$, $9x10^2$ and $2x10^5$ and <2 MPN/100gm respectively. Another study in South Africa by Gemmell and Schmidt (2012) indicated that the microbes present in contaminated irrigation water can be transferred via irrigation to fresh produce. They reported that the faecal coliform count in the fresh produce was up to $1.6×10^5$ per gram when irrigated with polluted river water containing faecal coliform load of $1.6×10^6$ per 100 ml of irrigation water. Studies in Ghana, Mexico and Pakistan found faecal coliform concentrations in vegetables much lower than those in Titagarh (Amoah ET al.2005; Ensink et al. 2007; Rosas ET al.1984). The bacteriological result of the present study indicated that all wastewater irrigated lettuce from the study area were heavily contaminated with *E.coli* and mean *E.coli* counts exceeding the ICMSF, 1974 (International Commission on Microbiological Specification for Foods) recommended level of 10^3 faecal coliform per 100 gm fresh weight. This high concentration of *E.coli* in lettuce could be attributed to the fibrous roots and large surface areas of this vegetable. .For this reason, bacteria from the soil could easily be deposited by rain splash or other mechanisms (Rosas et al. 1984). Besides, Lettuce also have foliar surfaces with many folds and fissures that provide good shelter for microorganisms (Kowal & Pahren 1980), and the leaves are fragile and thus allow the penetration and reproduction of bacteria in their inner tissue (Ercolani 1976).

Of the 106 lettuce samples that were collected, processed and examined, 27 (25.47 %) of them were contaminated with embryonated *Ascaris* eggs (Table 2). This *Ascaris* egg contamination found in Lettuce could be explained by the fact that broad leaved vegetables such as Lettuce, Spinach, and Cabbage etc are having large surface areas which are in direct contact with the sewage contaminated soil surface (Larkin et al. 1978). In the study period, the mean concentration of *Ascaris* eggs (eggs /100gm wet weight) observed in lettuce was ranged from 3 to 7.06 (Table 2). *Ascaris* egg numbers as recorded in this study were much higher compared to the study by Erdogrul and Sener (2005) who reported 1 to 3 eggs per 100 gm of produce in farms of Turkey. The difference could be due to the quality of the irrigation water being used for the vegetable by fruit farmers in Turkey. Another study in Turkey by Ulukanligil et al. (2001) showed that nearly half of the wastewater irrigated vegetables including lettuce sold in the market were contaminated with *Ascaris*. Amoah et al. (2006) reported similar findings in Ghana where he showed that *Ascaris lumbricoides* contamination was the most predominant and was observed in 85% of the contaminated vegetables. This could be attributed to its high level of persistence and resistance in diverse environments (Feachem et al.1983).

HEALTH RISK ASSESSMENT

The infection risks of rotavirus and *Ascaris* for the accidental ingestion of wastewater irrigated soil and the consumption of wastewater irrigated vegetables are presented in Table 3. The annual risk of infection for all pathogens for both ingestion of soil and consumption of lettuce exceeded the tolerable risk of $7.7 x 10^{-4}$ for rotavirus and 10^{-2} for *Ascaris* per person per year (Mara et al. 2007; WHO 2006). The result showed that the risk of rotavirus infection associated with the possible ingestion of wastewater irrigated soil was $3.1 x 10^{-1}$ which was higher than the risk of rotavirus infection ($7.6 x 10^{-2}$) reported by Seidu et al. (2008). The rotavirus infection risk recorded in the present study due to exposure of contaminated soil was above the recommended annual risk of infection by a 3 order magnitude. The risk of *Ascaris* infection due to the accidental ingestion of contaminated wastewater irrigated soil was $1.07 x 10^{-1}$ which was higher than the tolerable risk of *Ascaris* infection ($1 x 10^{-2}$) but the estimated risk was lower than the risk of *Ascaris* infection ($9.9 x 10^{-1}$) to farmers of Ghana as reported by Seidu et al. (2008) in their study in Ghana.

Table 3 Disease risk (Rota virus and *Ascaris*) for farmers and consumers (DALY/person/year) as a result of exposure to wastewater irrigated soil and consumption of lettuce

Disease risk of	Consumption of lettuce		soil
Pathogens	Untreated	Treated	

	wastewater	wastewater	
Rotavirus	$9.9x10^{-1}$	$9.9x10^{-1}$	$3.1x10^{-1}$
Ascaris	$1.2x10^{-1}$	$7x10^{-2}$	$1.07x10^{-1}$

Present study indicated that there was a substantial risk of rotavirus and *Ascaris* infection due to the consumption of untreated and treated wastewater irrigated lettuce from the study area. In terms of rotavirus infection, the risk of infection was $9.9x10^{-1}$ for consumers irrespective of the quality of the irrigation water. According to the study in Ghana by Seidu et al. (2008), the annual risk of rotavirus infection to consumers of lettuce irrigated with different qualities of wastewater were ranged from 10^{-3} to 10^{-4} which were lower than the rotavirus infection risk to consumers of the present study. In Titagarh for both untreated and treated wastewater irrigation, the estimated rotavirus infection risk of consumers far exceeded the WHO guideline of permissible disease risk.

The health risk assessment result showed that the risk of *Ascaris* infection to the consumers from consumption of untreated and treated wastewater-irrigated lettuce were $1.2x\ 10^{-1}$ and $7x10^{-2}$ respectively. In this study, the recorded *Ascaris* infection risks to the consumers of lettuce were above the tolerable risk (10^{-2}). A study in Ghana by Ackerson and Awuah (2012) reported the annual risk of *Ascaris* infection of $7.51x10^{-2}$ which was lower than the estimated risk of *Ascaris* infection for consumption of untreated wastewater –irrigated lettuce in our study area.

The study showed that both the consumption of wastewater -irrigated lettuce and the accidental ingestion of wastewater irrigated soil posed substantial health risk to farmers and consumers. In this assessment, the estimated risks of *Ascaris* and rotavirus infections have to be interpreted with some caution. For *Ascaris*, our assessment only reported the probability of infection and did not reflect the worm load of the infected farmers and consumers. Therefore, the probability of infection even though the same for consumers and farmers is not tantamount to both groups having the same level of worm load. All things being equal, the level of worm load will be higher for farmers than consumers due to their annual dose of *Ascaris* ingestion. The worm load within the farming as well as the consumer populations will also differ due to the characteristic over-dispersed distribution of *Ascaris* load burden by age among infected populations (Bundy et al. 2004). This over dispersed distribution will reflect in children engaged in wastewater irrigation and eating wastewater irrigated lettuce harbouring a disproportionately higher *Ascaris* burden than adults (Bundy 1988). For wastewater irrigation of crops that are eaten uncooked, WHO (1989) recommended a value of ≤1 helminth egg/L but recent epidemiological research work showed that a limit of <0.1 helminth egg/L is needed if children under 15 years are exposed (Blumenthal et al 2000). Also, the rotavirus health risks presented here should not be misconstrued as a representative for all the possible enteric virus infections because of the diverse symptoms of enteric virus infections (Wastrel 2004).

CONCLUSION

Despite increasing pressure to make more efficient use of water resources, irrigation of food crops with reclaimed water still remains a contentious issue, primarily because of risks of human health arising from

IJARSE
ISSN 2319 - 8354

infectious diseases. The present study indicated that the microbial risk in wastewater irrigated vegetable farming to farmers and consumers in the study area was high (annual risk in the range of 10^{-1} for rotavirus and 10^{-1} to 10^{-2} for *Ascaris*) and exceeded the tolerable risk limit mainly due to the high levels of pathogens in the irrigation water, soil and on the lettuce. Microbiological quality of irrigation water and lettuce of the study area were above WHO (10^3 E coli/100ml and ≤1helminth egg/L) and ICMSF (10^3 faecal coliform/100gm) recommendations respectively and therefore unsafe for use in the production of green leafy vegetables generally eaten uncooked. In conclusion, in this study the public health risk of using contaminated water to irrigate lettuce is established. The prevention of this risk can be achieved by an integrated set of approach which may include proper wastewater treatment, crop restriction and appropriate wastewater application techniques. Beside these, for consumers, the practice of basic food safety rules in the use of wastewater irrigated lettuce may ensure safety. Prior to consumption, thorough washing and disinfecting of vegetables are highly advisable. To be more precise, the vegetables should be carefully rinsed for 30 sec. Finally, the rinsed vegetables should be soaked in a disinfectant solution before consumption (Rosas et al. 1984).The farmers and the local people surrounding Titagarh were unaware of the health risk associated with wastewater farming and consumption of contaminated vegetables. There is need, therefore, to undertake comprehensive health education programmes for public and farm workers in particular on the risk involved in the use of untreated and partially treated wastewater for irrigation of agricultural crops.

ACKNOWLEDGEMENT

The authors gratefully acknowledge University of Kalyani for providing financial support and necessary facilities for the research work.

REFERENCES

Ackerson Nana, O.B. and Awuah, E. (2012). Microbial risk assessment of urban agricultural farming: A case study on Kwame Nkrumah University of Science and Technology campus, Kumasi, Ghana. International Journal of Engineering and Technology, 2, 356-363.

Amoah, P., Drechsel, P. and Abaidoo, R.C. (2005). Irrigated urban vegetable production in Ghana: sources of pathogen contamination and health risk elimination. *Irrigation and Drainage*, 54, 49-61.

Amoah, P., Drechsel, P., Abaidoo, R.C. and Now, W.J. (2006). Pesticide and pathogen contamination of vegetables in Ghana's urban markets. Archives of Environmental Contamination and Toxicology, 50, 1-6.

APHA, AWWA, WEF.(1995). Standard Methods for the Examination of Water and Wastewater. 19th edition (Washington DC: USA).

Bailenger, J. (1962). Valeur comparée des méthodes d'enrichissement en coprologie parasitaire. Pharmaceutical Biology, 3,249-259.

Blumenthal, U.J., Mara, D.D., Peasey, A., Ruiz-Palacios, G. and Stott, R. (2000). Guidelines for the microbiological quality of treated wastewater used in agriculture: recommendations for revising WHO guidelines. Bulletin of the World Health Organization, 78, 1104 –1116.

Bos, R.,Carr ,R. and Keraita, B. (2010). Assessing and Mitigating Wastewater-Related Health Risks in Low-Income Countries: An Introduction. (In P. Drechsel, C.A. Scott, L. Raschid-Sally, M. Redwood, & A. Bahri (Eds.), Wastewater irrigation and health: assessing and mitigating risk in low-income countries (pp. 29-47). London, UK: Earthscan; Ottawa, Canada: International Development Research Centre; Colombo, Sri Lanka: International Water Management Institute.)

Bouhoum, K., Amahmid, O., Habbari, K.H.and Schwartzbrod, J. (1997). Fate of helminth eggs and protozoan cysts in an open channel receiving raw wastewater from Marrakech. Revue des sciences de l'eau, 10,217-232.

Bundy, D.A.P. (1988). Population ecology and intestinal helminth infections in human communities. Philosophical Transactions of the Royal Soceity of London, Series B, 321, 405–420.

Bundy, D.A.P., Chan, M.S., Medley, G.F., Jamison ,D. and Savioli, L. (2004). Intestinal nematodes infections. (In C.J.L. Murray , A.D. Lopez, & C.D. Mathers (Eds.), The Global Epidemiology of Infectious Diseases (pp. 243–300). Geneva, Switzerland: WHO.)

Chandran, A., Heinzen, R.R., Santosham, M. and Siberry, G.K..(2006). Non- socomial rotavirus infections: a systematic review. The Journal of Pediatrics, 149,441–447.

El Hamouri, B., Handouf ,A., Mekrane ,M., Touzani, M., Khana, A., Khallayoune, K. and Benchokroun, T. (1996). Use of wastewater for crop production under arid and saline conditions: yield and hygienic quality of crop and soil contaminations. Water Science and Technology, 33,327-334.

Ensink, J.H.J., Mahmood, T., van der Hoek, W., Raschid-Sally, L. and Amerasinghe, F.P. (2004). A nation-wide assessment of wastewater use in Pakistan: an obscure activity or a vitally important one? Water Policy, 6, 1–10.

Ensink, J.H.J., Brooker, S., Cairncross, S.and Scott, C.A. (2006). Wastewater use in India: the impact of irrigation weirs on water quality and farmer health. In Proceedings of 32nd WEDC International Conference; Colombo (Sri Lanka); p.15-18.

Ensink, J.H.J., Mahmood, T. and Dalsgaard, A. (2007). Wastewater-irrigated vegetables: market handling versus irrigation water quality. Tropical Medicine and International Health, 12, 2–7.

Ercolani, G.L. (1976). Bacteriological quality assessment of fresh-marketed lettuce and fennel. Applied and Environmental Microbiology, 31,847-852.

Erdogrul, O.R. and Sener, H. (2005). The contamination of various fruit and vegetable with Enterobius vermicularis, Ascaris eggs, Entamoeba histolytica cysts and Giardia lamblia cysts. Food Control, 16, 557-560.

Feachem, R.G., Bradley, D.J., Garelick, H. and Mara, D.D. (1983). Sanitation and disease: Health aspects of excreta and wastewater management. (Chichester,England: Wiley).

Feenstra, S., Hussain, R.and van der Hoek, W. (2000). Health risk of irrigation with untreated urban wastewater in South Panjab, Pakistan. IWMI Pakistan report 107.(Lahore, Pakistan: International Water Management Institute).

Gemmell, M.E. and Schmidt, S.(2012). Microbiological assessment of river water used for the irrigation of fresh produce in a sub-urban community in Sobantu, South Africa. Food Research International, 47,300–305.

Gupta, N., Khan, D.K. and Santra, S.C. (2008). An assessment of heavy metal contamination in vegetables grown in wastewater-irrigated areas of Titagarh, West Bengal, India. Bulletin of Environmental Contamination and Toxicology, 80,115–118.

Gupta, N., Khan, D.K. and Santra, S.C. (2009). Prevalence of intestinal helminth eggs on vegetables grown in wastewater-irrigated areas of Titagarh, West Bengal, India. Food Control, 20, 942–945.

Haas, C.N., Rose, J.B. and Gerba, C.P. (1999). Quantitative Microbial Risk Assessment. (New York (USA): John Wiley and Sons).

Hamilton, A.J., Stagnitti, F., Premier, R., Boland, A.M. and Hale, G. (2006). Quantitative microbial risk assessment models for consumption of raw vegetables irrigated with reclaimed water. Applied Environmental Microbiology, 72, 3284–3290.

The International Commission on Microbiological Specification for Foods. (1974). Microorganisms in Food: Sampling for Microbiological Analysis: Principles and Scientific Applications.(Canada: University of Toronto Press).

Kowal, N.E. and Pahren, H.R. (1980) . Health effects associated with wastewater treatment and disposal. Journal (Water Pollution Control Federation), 52, 1312-1325.

Larkin, E.P., Tierney, J.T., Lovett, J., Van Donsel, D., Francis, D.W. and Jackson, G.J.(1978). Land application of sewage wastes: potential for contamination of foodstuffs and agricultural soils by viruses, bacterial pathogens and parasites. (In H.L. Mc Kim (Eds.), State of knowledge in land treatment of wastewater (pp. 215-223). Hanover, NH: US Army Corps of Engineers, CRREL, 2.)

Mara, D.D., Sleigh, P.A., Blumenthal, U.J. and Carr, R.M. (2007). Health Risks in wastewater irrigation: comparing estimates from quantitative microbial risk analyses and epidemiological studies. Journal of Water and Health, 5, 39–50.

Minhas, P.S.and Samra, J.S. (2004). Wastewater use in peri-urban agriculture: impacts and opportunities. Bulletin No. 1/2004. Central Soil Salinity Research Institute, Karnal. pp.78.

Minhas, P.S., Sharma, N., Yadav, R.K.and Joshi, P.K. (2006). Prevalence and control of pathogenic contamination in some sewage irrigated vegetable, forage and cereal grain crops. Bioresource Technology, 97,1174–1778.

Obuobie, E., Keraita, B., Danso, G., Amoah, P., Cofie, O.O., Raschid-Sally, L., and Drechsel, P.(2006). Irrigated Urban Vegetable Production in Ghana: Characteristics, Benefits and Risks. Accra(Ghana): IWMI-RUAF-IDRC-CPWF;p.150. www.cityfarmer.org/GhanaIrrigateVegis.html

Oron, G., Armon, R., Madelbaum, R., Manor, Y., Campos, C., Gillerman, L., Saigot, M., Gerba, C., Klein, I.and Enriquez, C.(2001). Secondary wastewater disposal for crop irrigation with minimal risks. Water Science and Technology, 43, 139-146.

Palese ,A.M., Pasquale, V., Celano, G., Figliuolo, G., Masi, S.and Xiloyannis, C.(2009). Irrigation of olive groves in Southern Italy with treated municipal wastewater: Effects on microbiological quality of soil and fruits. Agriculture Ecosystem and Environment, 129, 43–51

Rosas, I., Baez, A.and Coutino, M. (1984) . Bacteriological quality of crops irrigated with wastewater in the Xochimilco plots, Mexico City, Mexico. Applied Environmental Microbiology, 47, 1074-1079.

Scott, C.A., Faruqui, N.I. and Raschid-Sally, L. (2004). Wastewater use in irrigated agriculture: management challenges in developing countries. (In C.A. Scott, N.I. Faruqui, & L. Raschid-Sally (Eds.), Wastewater use in irrigated agriculture: confronting the livelihood and environmental realities (pp 1-10). Wallingford: CABI Publishing.)

Seidu, R., Heistad, A., Amoah, P., Drechsel, P., Jenssen, P.D. and Stenström, T.A. (2008). Quantification of the health risk associated with wastewater reuse in Accra, Ghana: A contribution toward local guidelines. Journal of Water and Health , 6, 461–471.

Shuval, H., Lampert, Y.and Fattal, B. (1997). Development of a risk assessment approach for evaluating wastewater reuse standards for agriculture. Water Science and Technology , 35, 15–20.

Srikanth, R. and Naik, D. (2004). Health effects of wastewater reuse for agriculture in the suburbs of Asmara City, Eritrea. International Journal of Occupational and Environmental Health, 10, 284-288.

Tanaka, H., Asano, T., Schroeder, E.D. and Tchobanoglous, G. (1998). Estimating the safety of wastewater reclamation and reuse using enteric virus monitoring data. Water Environment Research, 70, 39–51.

Toan, D.N. (1998). Contamination of soil with Ascaris and Trichuris eggs in a rural area of Vietnam. (In M. Yokogawa (Eds.), Collected paper on the control of soil transmitted helminthiases (pp.87-91). Tokyo: APCO,Vol.VI.)

Toze, S.(2006). Reuse of effluent water-benefits and risks. Agricultural Water Management, 80, 147–159.

Ulukanligil, M., Seyrek, A., Aslan, G., Ozbilge, H. and Atay, S. (2001). Environmental pollution with soil-transmitted helminths in Sanliurfa, Turkey. Memorias do Instituto Oswaldo Cruz, 96,903-909.

van der Hoek, W., Hassan, M.U., Ensink, J.H.J., Feenstra, S., Raschid-Sally, L., Munir, S., Aslam, R., Ali, N., Hussain, R. and Matsuno, Y. (2002). Urban Wastewater: A Valuable Resource for Agriculture- A case study from Haroonabad, Pakistan. IWMI Research Report 63. Colombo: International Water Management Institute.

Westrell, T. (2004). Microbial risk assessment and its implications for risk management in urban water systems, Phd thesis, Linkoping University, Sweden.

WHO. (1989). Health guidelines for the use of wastewater in agriculture and aquaculture, Technical Report Series No.778. Geneva: World Health Organization.

WHO. (2006). Guidelines for the Safe Use of Wastewater, Excreta and Grey water, volume 2: Wastewater Use in Agriculture. Geneva: World Health Organization.

Woomer, P.L. (1994). Most probable number counts. (In R.W. Weaver, J.S. Angle , & P.S.Bottomley (Eds.), Methods of soil analysis. Part 2. Microbiological and biochemical properties (pp. 59–79). Madison, WI: Soil Science Society of America.)