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Prevalence and control of pathogenic contamination in some sewage irrigated vegetable, forage and cereal grain crops

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Abstract

A total of 344 samples comprising of different vegetables, fodder and grain crops were obtained from a long-term experiment under sewage irrigation. The aerobic bacterial plate counts for vegetables, fodder and grain crops ranged between 2×10^6 and 3.5×10^7, 6×10^6 and 3×10^8, 2×10^5 and 3.8×10^{10}, respectively, while the corresponding *Faecal coliform* ranged between <2 and 9×10^5, 9×10^2 and 2×10^5 and <2, indicating that the pathogenic loads got reduced below permissible level in the produce that was harvested after sun drying in the field itself, whereas the parts coming in direct contact were the most severely contaminated. The health hazards could be markedly lowered with adoption of some of the low cost practices such as repeated washings, exposure of the produce to sunlight and raising the crops on beds. The coliform counts in vegetables were within permissible limits by two washings with water, exposing these to sunlight for about 4 h and removing the two outmost leaves of cabbage. Also, cutting above some height from ground level (0.10 m) in sorghum reduced the pollution load in fodder crops.

Keywords: *Faecal coliform*; Pathogenic contamination; Wastewater; Health hazards; Management practices

1. Introduction

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 (FAO, 1993; Minhas and Samra, 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. The WHO standards for intestinal nematodes and *Faecal coliform* in irrigation water are <1 egg/l and <1000 *Faecal coliform*/100 ml (Hespanhol and Prost, 1994). These health guidelines are difficult to realize as sometimes even canal water supplies in India exceed these limits. Though appropriate control measures are recommended especially for helminth eggs and *Faecal coliform* through high cost waste water treatment plants but some of the precautions can be low cost interventions like information about the hygiene to the farmers, wearing of shoes and gloves while working in wastewater irrigated fields, crop restrictions with waste water irrigation, regular treatment of farmers and their families with anti-helmintic drugs and specialized growing practices to induce minimum contact with wastewater (FAO, 1993; Minhas and Samra, 2004; Scott et al., 2004). Still the possible accumulation of pathogens in soils and their entering into food chain due to irrigation with sewage effluent cannot be disregarded. In fact outbreaks of human gastro-enteritis and food poisoning

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linked to consumption of contaminated vegetables are being increasingly reported.

Although partial and sporadic information on the pathogenic contamination of the sewage irrigated crops have been periodically attempted, no agency in India seems to have a systematic program for such monitoring on a regular basis. The associated disease causing micro-organisms (pathogens) are commonly assessed using measurement of faecal indicator bacteria such as *Faecal coliform*. Therefore, we monitored the populations of *Faecal coliform* on edible parts of grain, fodder and vegetable crops grown with sewage water and attempted for some of the alternatives those can result in minimization of disease risks.

2. Methods

2.1. Sampling procedures for plant parts

A total of 344 samples of edible parts were collected from a long-term experiment being conducted at the research farm of Central Soil Salinity Research Institute at Karnal where different cropping systems viz. vegetable (cabbage–ridge gourd), forage (sorghum–Egyptian clover), grain (paddy–wheat) and agro-forestry (paddy–wheat with poplar) were being irrigated with sewage water, following the recommended package of agronomic practices. The *Faecal coliform* counts in sewage water being used for irrigation of these crops averaged 1.5×10^8/100 ml. Eight samples were drawn from the each of the edible parts of the produce of above crops i.e., bolls of cabbage, fruits of okra and ridge gourd, plants of Egyptian clover and sorghum and grains in case of paddy and wheat. These were analyzed for total bacterial counts, *Faecal coliform*, fungi, *Salmonella* and *Shigella*.

Eight boll samples were also drawn randomly from the harvested lot of cabbage. Leaves from these bolls were removed starting from the outmost and then remaining boll was tested for coliform. Sampling for sorghum was done by cutting at an interval of 5 cm from ground, and 48 samples were drawn from eight randomly selected plants. Similarly, Egyptian clover was harvested at a height interval of 2.5 cm from 12 plants and total number of samples was 48. Fifty-six fruits in ridge gourd were sampled, either randomly from ridge-sown crop, or from the sides and inner areas of bed ($6\ \text{m} \times 2\ \text{m}$) sown crop. These samples were drawn in the morning hours (8.00 AM) and for sub-sampling at regular intervals 2 h, and were either kept under the shade of trees, or in the open sunlight. A part of these samples were given repeated washings with a good quality water to monitor the effect of washings on pathological pollution load. Similarly samples of ridge gourd were also drawn during the evening (5.00 PM) and half of these were kept overnight for monitoring of coliform during the next morning (9.00 AM). At desired time, the samples were put in paper bags, brought to the laboratory and stored in refrigerator.

2.2. Monitoring for pathogen counts

The determinations for coliform were initiated within 2 h of collection. The plate counts for aerobic bacteria were analyzed using standard nutrient agar medium while fungi were monitored by Martin rose Bengal medium as described by Rao (1986). Detections for *Salmonella* and *Shigella* were done with xylose lysine desoxycholate (XLD) agar and *Faecal coliform* were tested by multiple tube fermentation procedure as described by APHA (1995). The coliform analysis was done using 10-fold serial dilution for 10 g of each sample in 90 ml of 0.1% peptone dilution water. The test was performed in two stages where enrichment in presumptive medium was done using lauryl tryptose broth in presumptive portion of multiple tube test (tryptose 20 g, lactose 5 g, K_2HPO_4 2.74 g, KH_2HPO_4 2.74 g, NaCl 5 g, sodium lauryl sulfate 0.1 g, distilled water 1 l, pH 6.8 ± 2 after sterilization). Formation of gas in any amount in inverted tubes/vials within 48 ± 3 h at 35 °C constitute positive presumptive reaction. This is followed by confirmatory test using EC medium (tryptose and trypticose 20 g, bile salt mixture or bile salt no. 3 1.5 g, lactose 5 g, KH_2PO_4 1.5 g, NaCl 5 g, distilled water 1 l, pH 6.9 ± 2 after sterilization). Make transfer of presumptive total coliform tube and 48 h negative tube showing growth to the EC medium by using sterile metal loop and these were incubated at 44.5 ± 0.2 °C in water both. Gas production in fermentation tube within 24 h was considered as the positive reaction. The density of *Faecal coliform* has been reported as most probable number (MPN/100 g).

3. Results and discussion

Aerobic plate counts and the coliform counts for different marketable parts of the crops and vegetables are given in Table 1. Aerobic bacterial plate counts for vegetables, fodder and grain crops ranged between 2×10^6 and 3.5×10^7, 6×10^6 and 3×10^8, 2×10^5 and 3.8×10^{10}, respectively. Their corresponding coliform counts ranged between <2 and 9×10^5, 9×10^2 and 2×10^5 and <2. These data indicate that the chances of contamination get reduced in the produce e.g., grains harvested after sun drying in the field itself, whereas parts of crops coming in direct contact are the most severely contaminated. Earlier also, there are similar reports of high aerobic and coliform counts on the vegetables produced from sewage irrigated farm (Ofosu et al., 1999). The storage of harvested vegetables further

Table 1
Effect of sewage water irrigation on micro flora of crops

Crop	Part	Bacteria (cfu/g)		*Faecal coliform* (MPN/100 g)		Fungi (cfu/g)	
		Range	Mean	Range	Mean	Range	Mean
1. Food crops							
Wheat	Grain	2.9×10^{10}–4.2×10^{10}	3.8×10^{10}	<2	<2	Nil	Nil
Paddy	Grain	25×10^2–16×10^6	2×10^5	<2	<2	Nil	Nil
2. Fodder crops							
Egyptian clover	Plant	6×10^6–9×10^7	6×10^6	21×10^2–21×10^6	2×10^5	1×10^6–11×10^6	4×10^6
Sorghum	Plant	2×10^7–4×10^8	3×10^8	<2–9×10^2	9×10^2	3×10^2–1×10^4	2×10^3
3. Vegetables							
Cabbage	Boll	10×10^6–6×10^7	3.5×10^7	<22.6×10^5	2.3×10^2	1×10^4–8.9×10^5[a]	9×10^4[a]
Okra	Fruit	32×10^6–10×10^7	7.2×10^7	<2	<2	1×10^6–8×10^6	4×10^6
Ridge gourd	Fruit	7.3×10^4–15×10^6	2×10^6	<2–9×10^5	9×10^5	1×10^1–2×10^6	43×10^3

[a] Fungi nil, values given for *Shigella*.

attributes to an increase in their microbial loads. Hazard analysis and critical control points–total quality management (HACCP–TQM) technical guidelines (1998) has laid down the limits for microbial quality. Food containing <10^4 cfu/ml of organisms is rated "good" and those containing >5×10^7 cfu/ml as "spoiled food". As the vegetable samples showed aerobic counts in the range of 10^6–10^7 cfu/g, these can be considered as belonging to spoiled grade and hence unsafe for consumption. HACCP–TQM technical guidelines also give threshold and quality levels for food borne illness hazards. The suggested level of coliform for purchaser is <10 cfu/g. The tested high counts of coliform on forage and vegetables thus also suggest the possible potential hazards to cause food borne illness. Similarly, the fungal population was more on shoot of Egyptian clover and sorghum where as the population of other pathogens like *Salmonella* and *Shigella* was nil except for cabbage where *Shigella* was monitored to average 9×10^4 cfu/g.

Keeping in view the high pathogenic contamination in the produce from sewage irrigated crops; some of the alternatives were tried so that the consumed parts should hold the minimum pathogens. Since the major source of contamination is the direct contact with sewage water itself and with soil during post-harvest handling, the coliform counts were made after leaving such parts which have chances of coming in direct contact with sewage e.g., in cabbage bolls ready for the market. Ingress of coliform contamination was also tested after removing outmost leaflets of cabbage. As is observed from data in Table 2, the coliform contamination was monitored to reduce drastically in the inner boll and was within the safe limits after the removal of two outmost leaflets. Therefore, the consumer can possibly avoid hazards due to pathogens if the outmost two leaves can be left and only inner cabbage boll is consumed.

The ridge gourd was grown on both the ridge as recommended and on broader bed. As is presented in Table 3, the coliform count was more in ridge gourd fruits harvested from the sides of bed and the ridges while it was quite low in fruits harvested from beds. Thus one of the alternatives to minimize the contamination can be to avoid direct contact with sewage by sowing on beds. The general practice with the vegetable growers is to harvest vegetables during day and these are sent to the market during evening or next day morning. Since there are reports, that exposure to sunlight enhances the die-off rate of some contaminating organisms like *Leptospira*, *Brocella*, *Mycobacteriom*, *Salmonella*, *E. coli* (Ellis and McCalla, 1976; Gerba et al., 1975; Odeymi, 1990), the coliform contamination was monitored during different intervals after keeping the fruits in shade as well as sunlight. The data showed that the coliform contamination could be brought with in permissible limits after about 4 h of exposure to sunlight although not much reduction was monitored in the evening time harvested vegetables and left overnight. Geldreich and Bordner (1971) also reported similar observations on different fruits/vegetables where as Nicholson et al. (2000) observed that the survival of microorganism's increased with decrease in temperature. Less reduction of indicator organisms on ridge gourd kept in shade as compared to those kept in sunlight was observed as also reported earlier by VanDonsel et al. (1967) and Tannock and Smith (1971). Moreover, there is a general practice in the households to wash the

Table 2
Coliform contamination on sewage irrigated cabbage bolls ($n = 8$) after removal of outmost leaves

No. of leaves removed	*Faecal coliform* (MPN/100 g)	
	Range	Mean
Nil	<2–3.4×10^3	1.8×10^2
1	<2–2.6×10^3	2.9×10^1
2	<2	<2
3	<2	<2
4	<2	<2

Table 3
Effect of planting method, time of plucking and keeping period on coliform contamination of ridge gourd sampled ($n = 8$) from sewage irrigated plots

Main treatment	Sub-treatment	*Faecal coliform* (MPN/100 g)	
		Range	Mean
Planting method			
Bed planting	Middle	<2–5.0 × 10^3	8.1 × 10^1
	Sides	2.1 × 10^2–2.6 × 10^4	3.4 × 10^2
Ridges	Overall	<2–2.4 × 10^4	8.5 × 10^2
Morning plucking (8.00 AM) with Faecal coliform determined at (hours) kept in sunlight			
	900	<2–9.0 × 10^5	8.9 × 10^4
	1100	<2–3.5 × 10^5	4.1 × 10^3
	1300	<2–7.0 × 10^4	2.2 × 10^1
	1500	<2–2.8 × 10^3	0.5 × 10^1
	1700	<2–5.0 × 10^2	0.4 × 10^1
Morning plucking (8.00 AM) with Faecal coliform determined at (hours) kept in shade			
	900	16 × 10^4–16 × 10^5	46 × 10^4
	1100	16 × 10^3–50 × 10^4	23 × 10^4
	1300	90 × 10^2–90 × 10^4	13 × 10^3
	1500	<2–17 × 10^4	21 × 10^3
	1700	<2–34 × 10^3	70 × 10^1
Evening plucking with Faecal coliform determined at (hours)			
Evening (1700)		<2–5.0 × 10^5	2.8 × 10^2
Next morning (900)		<2–3.0 × 10^5	1.4 × 10^2
Faecal coliform determined after washings (nos.)			
Nil		1.1 × 10^3–2.6 × 10^5	1.3 × 10^4
1		<2–3.0 × 10^3	1.3 × 10^2
2		<2–1.4 × 10^2	1.1 × 10^1
3		<2	<2

vegetables before their consumption. Thus the possibilities of washing of surface infections cannot be ignored. Monitoring of coliform after giving the ridge gourd different washings indicated that the risks of contamination could be avoided after two washings when the coliform was reduced to permissible limits. However, Rosas et al. (1984) found that common rinsing of vegetables with tap water though reduced the indicator coliform but not to safe level.

Several cases of animal sickness due to *Salmonella aberdeen* have been reported when fresh sewage was applied to fodder crops or when sewage accidentally over flow into pastures fields (Bicknell, 1972). Monitoring of coliform in case of fodder crops of sorghum and Egyptian clover showed that the maximum contamination was prevalent in the lowest parts coming in direct contact with sewage. The contamination was considerably lowered with height above the ground level. Thus it seems that chances of contamination can be minimized if these are cut about 10 cm above the ground level (Table 4).

The present study reveals the potential hazards associated with pathogenic contamination of edible parts of vegetables, fodder and grain crops grown using sewage water. Considering some of the options tested for minimizing the health risks for the consumers, washing and exposure to sunlight seem to be low cost strategies while such risks in animals can be avoided by cutting the fodder at some height above ground level. The other alternative of raising the sewage irrigated crops with bed planting may be feasible for the trailing type vegetables only.

Table 4
Faecal coliform contamination in sewage irrigated sorghum ($n = 8$) and Egyptian clover ($n = 12$) fodder at different heights above ground level

Height above ground level (cm)	*Faecal coliform* (MPN/100 g)	
	Range	Mean
Sorghum		
0–5	1.7 × 10^5–3.3 × 10^7	1.8 × 10^6
5–10	5.0 × 10^4–3.3 × 10^7	1.5 × 10^6
10–15	1.3 × 10^2–2.6 × 10^5	2.0 × 10^3
15–20	<2–2.6 × 10^7	1.2 × 10^3
20–25	<2–2.1 × 10^7	6.3 × 10^2
25–30	<2–4 × 10^1	0.4 × 10^1
Egyptian clover		
0–2.5	<2–2.6 × 10^4	6.1 × 10^2
2.5–5.0	<2–2.3 × 10^4	1.2 × 10^2
5.0–7.5	<2–4.0 × 10^1	0.3 × 10^1
7.5–10.0	<2–9.0 × 10^1	0.3 × 10^1

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