Occurrence of Escherichia coli O 157 in a river used for fresh produce irrigation in Nigeria

ARTICLE *in* AFRICAN JOURNAL OF BIOTECHNOLOGY · FEBRUARY 2010

Impact Factor: 0.57

3 AUTHORS, INCLUDING:

Vincent N Chigor
University of Nigeria
23 PUBLICATIONS **172** CITATIONS
SEE PROFILE

Stella Ifeanyi Smith
Nigerian Institute of Medical Research
101 PUBLICATIONS **683** CITATIONS
SEE PROFILE

African Journal of Biotechnology Vol. 9 (2), pp. 178-182, 11 January, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Occurrence of *Escherichia coli* O157 in a river used for fresh produce irrigation in Nigeria

V. N. Chigor[1*], V. J. Umoh[2] and S. I. Smith[3]

[1]Department of Microbiology, University of Nigeria, Nsukka, Enugu State, Nigeria.
[2]Department of Microbiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.
[3]Molecular Biology and Biotechnology Division, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria.

Accepted 17 February, 2009

Concerns about the persistence of *Escherichia coli* O157 in irrigation waters and its transmission to fresh produce makes investigation of irrigation waters imperative. The prevalence of this pathogen and seasonal levels of water quality parameters in Kubanni River were studied, using standard methods, over a 10-month period. Detection rate for *E. coli* O157 confirmed by slide agglutination was 2.1%. Faecal coliform counts (FCC) exceeded acceptable limits and was significantly higher in the dry season than during the rainy season ($p<0.05$). Remarkably, nitrate level was significantly higher in the rainy season than dry season ($p<0.05$). A significant ($p<0.05$) correlation was established between FCC and each of nitrate ($r = 0.25$), biochemical oxygen demand ($r = 0.51$) and electrical conductivity ($r = 0.55$). It was concluded that the Kubanni River represents a potential public health risk, being unfit for fresh produce irrigation. Perhaps, this is the first report on the isolation of *E. coli* O157 from water sources in Nigeria.

Key words: *Escherichia coli* O157, contamination, irrigation water, nitrate, fresh produce, surface waters.

INTRODUCTION

Escherichia coli O157 is an important agent of food- and water-borne illnesses in humans globally (Chalmers et al., 2000; Bettelheim and Beutin, 2003; Duffy, 2003). The first reported outbreak of *E. coli* O157 infection in the developing world, which occurred in southern Africa in 1992 (Effler et al., 2001) has been followed up with outbreaks in Central African Republic in 1996 and Cameroon in 1997 (Cunin et al., 1999). Although not in outbreak proportions, *E. coli* O157 illness has been reported in Nigeria since 1994 (Ogunsanya et al., 1994; Olorunshola et al., 2000).

Available information indicates that the carriage of *E. coli* O157 in cattle was an important factor in the emergence of this pathogen in Africa (Effler et al., 2001; Renter et al., 2003). Reported outbreaks have been linked to contaminated drinking water and irrigation with water contaminated by animal faeces, discharges from sewage treatment facilities or surface run off (Chalmerset al., 2000; Payment and Riley, 2002; Solomon et al., 2002). In Nigeria, as in most developing countries, surface waters which constitute an important source of water for drinking, domestic and agricultural purposes are vulnerable to pollution (Shuval, 1990; Umoh et al., 2001; Cisse et al., 2002; Okafo et al., 2003, Agbogu et al., 2006).The potential risks related to the use of contaminated water in agriculture, recent reports on the persistence of *E. coli* O157 in irrigation waters and its transmission through fresh produce (Solomon et al., 2002; Renter et al., 2003) as well as the absence of reports of Nigeria studies on the prevalence of *E. coli* O157 in water sources made this study imperative. The aim was to assess the quality of Kubanni River, a major source of water for large scale fresh produce irrigation and herd watering in northern Nigeria and to screen the water samples for the presence of *E. coli* O157.

*Corresponding author. E-mail: vnchigor@yahoo.com or nnamdiva@yahoo.co.uk. Tel.: +2348036922106.

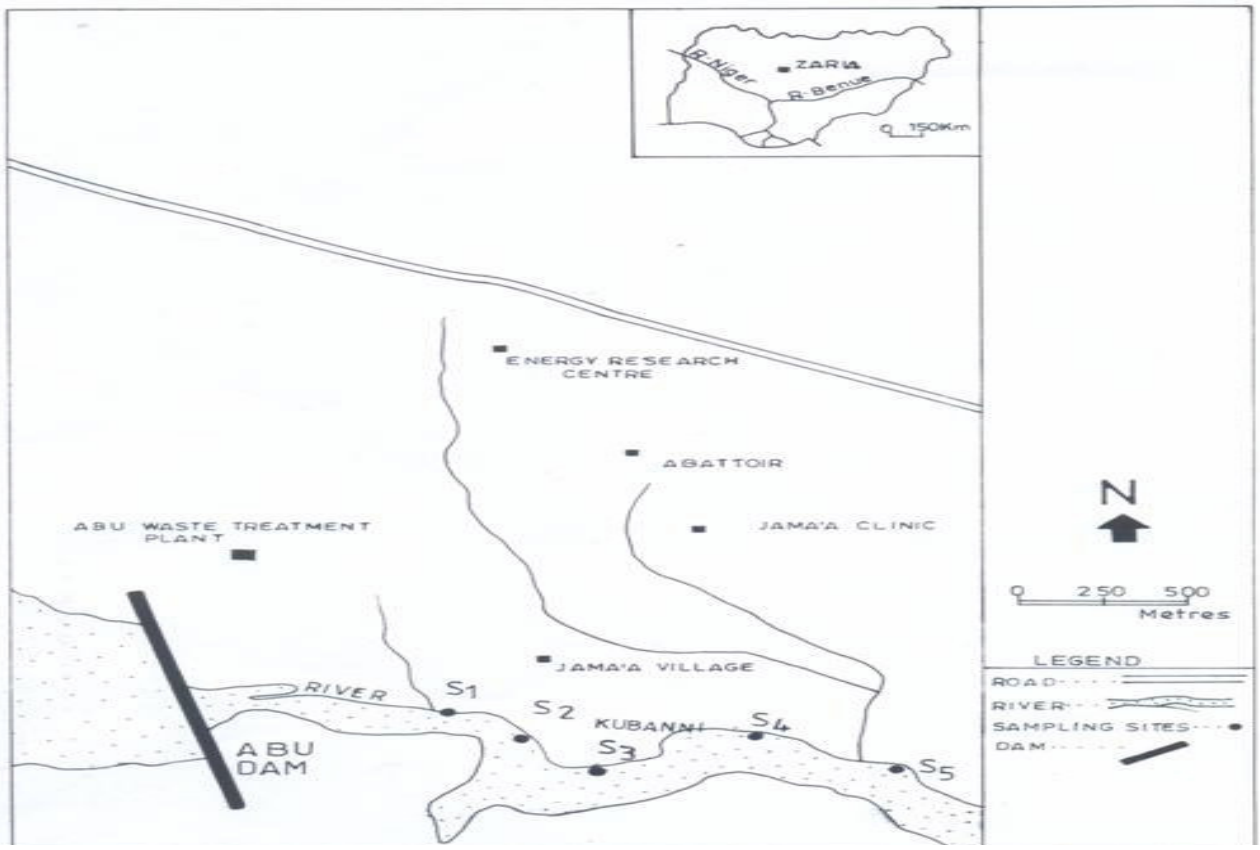

Figure 1. The study area and sampling sites, Zaria, Kaduna State, Nigeria.

MATERIALS AND METHODS

Source and collection of water samples

The Kubanni River drains Zaria SW (11°7' - 11°15' N; 7°30' - 7° 45' E) and meets with River Galma before emptying into Zaria dam. The stretch of the river studied (Figure 1) is located downstream from Ahmadu Bello University (ABU) dam. Local farmers use Kubanni River water for the irrigation of commercial crops (including tomatoes, lettuce, cabbage, onions, spinach and sugar cane) and for herd watering. The river is strongly influenced by discharges from ABU sewage treatment plant (STP), Zango abattoir and Jama'a domestic sewage. The first of the five sampling sites selected, S1, was about a metre downstream of the point where the STP effluents drains into Kubanni River; additional sites, S2, S3, S4 and S5 (Figure 1) were located at 300 m intervals downstream of S1. Sampling was according to the procedure recommended by the American Public Health Association (APHA, 1992). On a biweekly basis, and for a period of 10 months (March – December, 2002), water samples were taken from S1 to S5 using sterile, wide-mouthed crew-capped 250 ml glass bottles for bacteriological analysis. A 2.5n L screw-capped plastic can was used to collect samples for physicochemical analysis per site at a depth of 15 cm in the direction of water flow. Samples were transported to the laboratory in an ice pack and analyzed within 4 h of collection.

Isolation and identification of *E. coli* O157

E. coli O157 was isolated from water samples using the tryptic soya broth (TSB) enrichment and high temperature (44.5°C) incubation method described by LeJeune et al. (2001). Briefly, 20 ml of each water sample was combined with equal volume of double strength TSB (Difco, Detroit, USA) in a cotton–plugged 150 ml conical flask and incubated at 44.5°C for 24 h. The resulting suspension (50 µl) was then spread plated into a Sorbitol MacConkey agar (SMAC) plate and incubated at 44.5°C) for 24 h. Three to four sorbitol-negative colonies exhibiting typical *E. coli* O157 colony phenotype (gray or pale with a darker centre) were selected. They were subcultured on freshly prepared SMAC and identified using bio-chemical tests as described by Farmer (1999). All selected SMAC colonies that were found to be sorbitol-negative and indole-positive were streaked onto blood agar plates, incubated at 37°C for 24 h and then checked for O157 antiserum agglutination using the manufacturer's instructions (Robert Koch Institute, Burgstrasse, Germany).

Determination of faecal coliform count (FCC)

FCC was determined using the most probable number (MPN) technique (APHA, 1992). Samples were inoculated into sterile MacConkey broth (Biotec, Suffolk, UK) containing an inverted Durham tube. The cultures were incubated at 44.5°C for 24 - 48 h in a dry incubator (Gallenkamp, Britain). Gas and acid productions were read as positive for faecal coliforms and the combinations of positive tubes were recorded. Confirmatory tests were carried out on the lactose-positive cultures by transferring 1.0 ml into brilliant green lactose broth tubes containing Durham tubes and finally streaked on eosin methylene blue (EMB) agar for characteristic

Table 1. Seasonal levels of water quality parameters at Kubanni River.

Parameter*	Dry Season	Rainy Season
BOD_5 (mg/1)	1.62 ± 0.51	1.89 ± 0.33
FCC (MPN/100 ml)	$2.8 \times 10^4 \pm 1.1 \times 10^{4a}$	$3.5 \times 10^3 \pm 1.4 \times 10^{3b}$
WT (^oC)	22.68 ± 1.06^b	27.73 ± 6.49^a
pH	6.63 ± 0.06^b	6.81 ± 0.03^a.
EC (MS/CM)	221.27 ± 10.23	225.95 ± 14.21
Turbidity (NUT)	29.44 ± 4.60^b	157.10 ± 31.96^a
TDS (mg/1)	25.00 ± 2.69	27.00 ± 2.36
TSS (mg/1)	16.50 ± 1.96	20.33 ± 2.01
Chloride (mg/1)	29.13 ± 3.37	28.94 ± 2.40
PO_4 -P (mg/1)	0.14 ± 0.02	0.17 ± 0.02
NO_3 -N (mg/1)	$1.70 \pm -.18^b$	2.23 ± 0.21^a

*For each parameter, mean values with different superscripts are significantly different (P<0.05) using Students t-test.
SE = standard error.

colonies. The MPN/100 ml for each sample was determined according to standard methods using McGrady's probability table (APHA, 1992).

Physicochemical analysis of water samples

Temperature was measured in the field using a thermometer and pH was determined using pre-calibrated portable pH meter (Norylab; PM8). Conductivities were measured at 25°C directly in µs/cm using a digital conductivity meter (NorLab; LM8). Turbidities were measured with a digital turbidimeter (Hach Chemicals; 2100P). The total suspended and total dissolved solids were separated by filtering the water through a 0.45 µm filter paper and determined according to standard procedures (APHA, 1992). Chloride was determined by the argentometric (Mohr's) method. Nitrate (NO_3 - N) and phosphate (PO_4 - P) were determined photometrically by measuring the intensities of the colour developed following the phenoldisulphonic acid and the phosphomolybdate methods respectively. Five-day biochemical oxygen demand (BOD_5) was determined after incubation in tightly stoppered BOD bottle in the dark at 20°C and titrimetrically determining oxygen consumed (APHA, 1992).

Statistical analysis

Student t-test was used to compare the means of physicochemical parameters for the 2 seasons and one-way analysis of variance and Duncan's multiple range tests were used to compare the means of parameters for the 5 sites. Correlation between the counts and physicochemical parameters were determined using Pearson's correlation coefficient values.

RESULTS

Of the 96 water samples screened for the presence of *E. coli* O157, two were positive giving an isolation rate of 2.1%. Table 1 shows the results of student t-tests comparing the water quality parameters for the dry and rainy seasons. Nitrate, turbidity, pH and water temperature were significantly higher during the rainy season than in the dry season ($p< 0.05$). However, the mean faecal coliform count was significantly higher during the dry season than in the rainy season ($p< 0.05$).

Two sites, S1 and S5, which received effluents from the ABU sewage treatment plant and Zango abattoir respectively, had higher FCC values (Table 2). All the sites however, were contaminated with unacceptable levels of faecal coliforms for use in domestic activities (<0 faecal coliforms/100 ml) and water for irrigation of vegetable and salad crops (<1000 faecal coliforms/100 ml). There exists a significant ($p<0.05$) positive correlation between faecal coliform concentrations and each of conductance, TDS, TSS, chloride, PO_4^- P, NO_3 - N and BOD_5 (Table 3). No apparent correlation was observed between FCC and each of pH, temperature and turbidity.

DISCUSSION

The study revealed that culturable *E. coli* O157 were present in water samples from Kubanni River with an isolation rate of 2.1% (2 of 96 samples). This finding, in itself, is not surprising since it is well documented that cattle is the chief reservoir of *E. coli* O157 (Renter et al., 2003; LeJeune et al., 2004). The stretch of Kubanni River studied is strongly influenced by cattle excrement due to *in situ* herd watering and discharges from the abattoir. Moreover, the use of enrichment media and high temperature incubation had earlier been shown by LeJeune et al. (2001) to increase the sensitivity of *E. coli* O157 isolation from water. The prevalence of 2.1% of confirmed *E. coli* O157 in this study was similar to the report of Sergeant et al. (2000), which detected *E. coli* O157 in 1.5% (3 of 199 samples) of water sources. *E. coli* O157 illnesses have been reported in southern Nigeria since 1994 (Ogunsanya et al., 1994; Olorunshola et al., 2000). However, there are no records linking *E. coli* O157 infections to contaminated food or water sources in

Table 2. Comparison of the mean value of water quality parameters for 5 different sites at Kubanni River.

Parameter*	Sampling site S1	Sampling site S2	Sampling site S3	Sampling site S4	Sampling site S5
FCC (MPN/100 ml)	$2.9 \times 10^{4a} \pm 1.8 \times 10^{4}$	$4.5 \times 10^{3a} \pm 3.4 \times 10^{3}$	$1.2 \times 10^{3a} \pm 4.0 \times 10^{2}$	$1.7 \times 10^{3a} \pm 1.1 \times 10^{3}$	$3.0 \times 10^{4a} \pm 1.5 \times 10^{4}$
WT (°C)	$25.10^{a} \pm 1.42$	$25.60^{a} \pm 1.50$	$25.75^{a} \pm 1.47$	$25.90^{a} \pm 1.49$	$26.20^{a} \pm 1.32$
pH	$6.68^{a} \pm 0.5$	$6.84^{a} \pm 0.05$	$6.68^{a} \pm 0.08$	$6.69^{a} \pm 0.07$	$6.76^{a} \pm 0.06$
EC (µs/cm)	$266.70^{a} \pm 29.27$	$239.51^{a} \pm 13.44$	$187.92^{b} \pm 11.42$	$186.19^{b} \pm 15.81$	$243.08^{a} \pm 2.37$
Turbidity (NTU)	$19.98^{a} \pm 4.36$	$78.31^{a} \pm 35.74$	$131.93^{a} \pm 51.77$	$169.31^{a} \pm 64.97$	$130.65^{a} \pm 49.41$
TDS (mg/l)	$29.00^{a} \pm 3.14$	$24.00^{a} \pm 1.63$	$23.50^{b} \pm 3.17$	$19.50^{b} \pm 2.41$	$35.00^{a} \pm 6.37$
TSS (mg/l)	$16.00^{a} \pm 2.21$	$13.00^{a} \pm 1.53$	$19.00^{a} \pm 3.14$	$21.00^{a} \pm 2.77$	$25.00^{a} \pm 4.77$
Chloride (mg/l)	$29.99^{b} \pm 4.16$	$27.89^{b} \pm 3.98$	$21.29^{b} \pm 1.99$	$22.17^{b} \pm 3.11$	$43.74^{a} \pm 6.27$
PO_4 - P (mg/l)	$0.22^{a} \pm 0.04$	$0.14^{a} \pm 0.03$	$0.14^{a} \pm 0.03$	$0.18^{a} \pm 0.04$	$0.13^{a} \pm 0.03$
NO_3 - N (mg/l)	$1.90^{a} \pm 0.17$	$1.65^{a} \pm 0.23$	$1.82^{a} \pm 0.21$	$1.87^{a} \pm 0.15$	$2.06^{a} \pm 0.23$
BOD_5 (mg/l)	$3.66^{a} \pm 1.03$	$0.93^{b} \pm 0.23$	$0.96^{b} \pm 0.24$	$0.96^{b} \pm 0.27$	$2.66^{a} \pm 0.48$

Values are mean ± SE.
*For each parameter, means with the different letters (superscripts) are significantly different ($p<0.05$), using Duncan's Multiple Range test.
Abbreviations: SE, standard error; FCC, faecal coliform counts; WT, water temperature; EC, electrical conductivity; NTU, nephelometric turbidity units; TDS, total dissolved solids; TSS, total suspended solids; PO_4 - P, phosphate-phosphorus; NO_3 - N, nitrate- nitrogen; BOD_5, biochemical oxygen demand.

Table 3. Correlation coefficients and p-values for the correlation between faecal coliform counts and physicochemical water quality parameters.

Parameter	Pearson correlation	
	Coefficient (r)	p - value
pH	0.06	0.5158
Electrical conductivity	0.55	0.0001*
Turbidity	-0.13	0.1529
Total dissolved solids	0.31	0.0005*
Total suspended solids	0.26	0.0035*
Chloride	0.51	0.0001*
Phosphate – phosphorus	0.29	0.0011*
Nitrate – Nitrogen	0.25	0.0061*
Biochemical Oxygen Demand	0.51	0.0001*

* Significant p – values.

Nigeria. Since the studied river serves as irrigation for commercial crops like tomatoes, cabbage, lettuce, pepper, onions, amaranth and sugar cane, which could be eaten uncooked, the epidemiological importance of *E. coli* O157 in this water source cannot be overlooked. The concern here is heightened by the recent study of Solomon et al. (2002) which demonstrated the transmission of *E. coli* O157: H7 from irrigation water to lettuce plant and its subsequent internalization in plant tissues that protected the pathogen from the action of sanitizing agents. The results demonstrate that Kubanni river water is not safe for fresh produce irrigation, as none of the sampled sites met international standards. Faecal coliform count should be less than 1000 FC/100 ml for water used in unrestricted irrigation (WHO, 1996; Bouwer et al., 1999).

The significantly higher counts of faecal coliform recorded during the dry season could be attributable partly to reduced water volume and flow in the river due to the dam upstream and most significantly to raw sewage discharge into the river from residential houses and abattoirs. Earlier studies in this environment also confirmed significantly higher coliform organisms in the dry season than in the wet season, corresponding to a significantly higher contamination rate for lettuce, amaranths and other vegetables during the dry season (Okafo et al., 2003; Agbogu et al., 2006). The faecal coliform counts for both seasons were higher than World Health Organization limits (1000 FC/100 ml) for water used in irrigation of food crops. Therefore the use of Kubanni River water for human consumption and irrigation of vegetables and salad crops, without treatment could pose serious health risk. The significantly higher levels of nitrate observed during the rainy season could be due to water from farmlands containing nitrate residues from manure and fertilizer used in farming at the

banks of the river and adjourning lands.

Conclusion

In the present study, we demonstrated the existence of culturable *E. coli* O157 in surface waters in Nigeria, where a large part of rural and semi-urban population consume such water for their daily needs and fresh produce irrigation. In conclusion the use of Kubanni River for the irrigation of soils used for planting vegetables that are consumed raw could present a public health risk. This concern is heightened by the isolation of *E. coli* O157 from the water samples. The construction of a mini-stabilization pond at the abattoir is recommended. There is also the need for performance check on the University sewage treatment plant to prevent pollution. Public health enlightenment on the risk of consuming raw vegetables harvested from *E. coli*-polluted fields is imperative.

REFERENCES

Agbogu VN, Umoh VJ, Okuofu CA, Ameh JB, Smith SI (2006). Study of the bacteriological and physicochemical indicators of pollution of surface waters in Zaria, Nigeria. Afr. J. Biotechnol. 5 (9): 732-737.

APHA (American Public Health Association) (1992). Standard Methods for the Examination of Water and Wasterwater. 18thEdn. APHA: Washington DC, USA.

Bettelheim KA, Beutin L (2003). Rapid laboratory identification and characterization of verocytotoxigenic (shigatoxin – producing) *Escerichia coli* (VTEC/STEC). J. Appl. Microbiol. 95: 205-217.

Bouwer H, Fox P, Westerhoff P, Drewes J (1999). Integrated water management and reuse: causes for concern? Water Quality Indices, January/February: p. 19-22.

Chalmers RM, Aird A, Bolton FJ (2000). Waterborne *Escherichia coli* O157. J. Appl. Microbiol. 88: 124-132.

Cisse G, Kienta M, Ouedraogo B, Tanner M (2002). Health risk of urban agriculture development around small dams in Ouagadougou. Cashiers/Agricultures 11: 31-38.

Cunin P, Tedjouka E, Germani Y, Ncharre C, Bercoin R, Morvan J (1999). An epidemic of blood diarrhoea: *Escherichia coli* O157 emerging in Cameroon? Emerging Infectious Diseases, 5: 285-290.

Duffy G (2003). Verocytotoxigenic *Escherichia coli* in animal faeces, manures and slurries. J. Appl. Microbiol. 94: 94S-103S.

Effler P, Isaacson M, Arntzen L, Heenan R, Canter P, Barrett T, Lee L, Mamba C, vine W, Zaidi A, Griffin PM (2001). Factors contributing to the emergence of *Escherichia coli* O157: H7 in Africa. Emerging Infectious Diseases, 7: 812-819.

Farmer JJ (1999*). Enterobacteriaceae*: Introduction and identification. In: Murray PR, Barron EJ, Pfaller MA, Tenover FC, Yolken RH (Eds). Manual of Clinical Microbiology (7th Edn) ASM Press, Washington D. C. USA. p. 442-458.

LeJeune JT, Besser TE, Rice DH, Itancock DD (2001). Methods for the isolation of waterborne *Escherichia coli* O155: H7. Lett. Appl. Microbiol. 32: 316-320.

LeJeune JT, Besser TE, Rice DH, Stilborn RP, Hancock DD (2004). Longitudinal study of faecal shedding of *Escherichia coli* O157: H7 in feedlot cattle: Predominance and persistence of specific clonal types despite massive cattle population turn over. Appl. Environ. Microbiol. 70: 377-384.

Ogunsanya TL, Rotimi VO, Adenuga A (1994). Study of the aetiological agents of childhood diarrhoea in Lagos, Nig. J. Med. Microbiol. 40: 10-14.

Okafo CN, Umoh VJ, Galadima M (2003). Occurrence of pathogens on vegetables harvested form soils irrigated with contaminated streams. Sci. Total Environ. 311: 49-56.

Olorunshola ID, Smith SI, Coker AO (2000). Prevalence of EHEC O157: H7 in patients with diarrhea in Lagos, Nigeria. Acta Pathologica Microbiologica et Immunologica Scandinavica (APMIS), 108: 761-763.

Payment P, Rilley SM (2002). Resolving the global burden of gastrointestinal illness: a call to action. A Report from the American Academy of Microbiology. American Academy of Microbiology. Washington DC., USA. pp. 1-25.

Renter DG, Sargeabt JM, Oberst RD, Samadpour M (2003). Diversity, Frequency and persistence of *Escherichia coli* O157: H7 in Cow-Calf farms. Appl. Environ. Microbiol. 69: 542-547.

Shuval HI (1990). Wastewater irrigation in developing countries: health effects and technical solutions. Summary of World Bank Technical Paper No. 51. The World Bank, Washington D. C., USA. pp. 1-9

Solomon EB, Yaron S, Mathews KR (2002). Transmission of *Escherichia coli* 0157: H7 from contaminated manun and irrigation water to lettuce plant tissue and its subsequent internalization. Appl. Environ. Microbiol. pp. 397-400.

Umoh VJ, Okafo C, Galadima M (2001). Contamination of vegetables cultivated on land irrigated with urban wastewater in Zaria and Kaduna, Nigeria. Nig. J. Parasitol. 22: 95-104.

World Health Organization (WHO). (1996). Guidelines for Drinking Water Quality (2nd Edn) (Vol.1-3) WHO, Geneva, Switzerland.