Journal of Applied Sciences

ISSN 1812-5654

Journal of Applied Sciences 7 (23): 3756-3761, 2007
ISSN 1812-5654

The Effect of Sludge Application-to-Planting Interval on the Number of Coliforms Recovered from Vegetables Grown on Sludge-Amended Soils

Veronica M. Ngole
Department of Geography, Environmental Science and Planning,
University of Swaziland, Private Bag 4, Kwaluseni M201, Swaziland

Abstract: Studies were carried out to determine whether there is any difference in the health risk involved in growing carrots and spinach on sludge-amended soils when a 90-day sludge application-to-planting interval and sludge application-to-harvest intervals are used for further pathogen reduction. The health risk was determined by enumerating the Most Probable Number (MPN) of Faecal Coliform (FC) recovered from both vegetables and identifying the different types of enteric bacteria recovered at harvest. The spinach and carrots were grown on four different soil types unto which a 3 year old sludge (type 1 sludge) and three month old sludge (type 2 sludge) had been separately applied at different rates. Two sludge application-to-planting and sludge application-to-harvest interval were used. A higher number of FC were recovered from carrots (1.5 $\log_{10}$ MPN/10 g-1.8 $\log_{10}$ MPN/10 g) than spinach (<1.5 $\log_{10}$ MPN/10 g) grown on same soil type with identical type of sludge applied at the same rate. More FC was recovered when a 0±3 day sludge application-to-planting interval was used as opposed to a 90 day sludge application-to-planting interval. Soil type, sludge age and sludge application rate affected the amount of FC recovered. The implications of these results on the specification of time interval in Regulations guiding the growth of vegetables on sludge-amended soil are discussed.

Key words: Faecal coliform, vertisol, luvisol, arenosol, carrots, spinach, health risk

INTRODUCTION

Application of sludge to soil is one of the many ways through which pathogens could be introduced into vegetables grown on such soil. Though different stabilization techniques are used to reduce the pathogen load in sewage sludge, complete elimination of pathogens is rarely achieved. Most guidelines have therefore proposed a minimum time interval between when sludge is applied to soil and when vegetables and other food crops grown on such soil should be harvested to allow for further pathogen reduction. The United States Department of Agriculture National Organic Program for example has specified a minimum non-composted manure application-to-harvest time interval of 120 days for organic crops with edible parts exposed to soil particles. Though unpublished data have suggested that an interval of 90 days would adequately minimize the risk of contamination of leaf and root crops (Natvig *et al*., 2002), studies by Ingham *et al*. (2005) have suggested that the 120 day standard should not be reduced. Whether an application-to-planting or application-to-harvest interval is stressed may depend on several factors which all influence the survival period of enteric bacteria in the soil environment and on vegetables.

The survival period of enteric pathogens in sludge-amended soil depends on pathogen species (Windfield and Groisman, 2003; Epstein, 1998; Lau and Ingham, 2001), sludge application rate (Ngole *et al*., 2006; Jiang *et al*., 2002; Guan and Holly, 2003), sludge age (Ngole *et al*., 2006a; Epstein, 1998), soil type (Ngole *et al*., 2006a; Tate, 1978; Gagliardi and Karns, 2000; Ingham *et al*., 2004; Kirby *et al*., 2003), soil management (Gagliardi and Karns, 2000) and the prevailing climatic conditions. According to Islam *et al*. (2004b) the type of vegetable grown influences the survival of *E. coli* O157:H7 on the vegetable and in the soil on which the vegetable is grown. Carrot for example has been shown to have a toxic or inhibitory effect on the growth of *Listeria monocytogenes* (Beuchat and Brackett, 1990). Islam *et al*. (2004b) observed lower *E. coli* reduction (1.7 log cfu g^{-1}) on carrot roots and in soils around the carrot root (2.3 log cfu g^{-1}) compared to onions (2 log cfu g^{-1}) and soils around the onion bulb (3 log cfu g^{-1}).

For sludge-borne enteric pathogens to present any health risk, they must be taken up by the vegetables and survive within the tissues of the vegetables or on the vegetables until when they are eventually consumed. Conclusions from studies that have investigated the uptake of pathogens from manure-amended soils by vegetables vary. Whereas studies by Watchtel *et al.* (2002) and Johannessen *et al.* (2005) suggest that pathogens are not taken up by roots of vegetables, Solomon *et al.* (2002) have demonstrated systemic uptake of *E. coli* O157:H7 from manure-amended soil into lettuce plants through the roots. The specific time during the growth of the vegetables when they are exposed to bacteria also influences uptake and internalization. Warriner *et al.* (2003) and Jablasone *et al.* (2005) have indicated that the susceptibility of vegetables to invasion by pathogens varies throughout the growth cycle of the vegetable.

Considering that all these factors interplay to determine how long a pathogen may survive in the soil and on the vegetables, the suitability of an application-to-harvest or application-to-planting interval may vary with soil type, sludge age, vegetable type, pathogen load in soil at time of planting, sludge application rate and the prevailing climatic condition. This study was therefore designed to investigate whether there is any difference in the health risk when a sludge application-to-planting and sludge application-to-harvest intervals are used in growing carrots and spinach on four different soil types onto which anaerobically-stabilised sewage sludge of two different ages have been applied at different rates. The health risk was determined by comparing the number of Faecal Coliforms (FC) recovered from both vegetables at time of harvest. It is anticipated that the results from this study will determine which interval may be more appropriate and whether soil type, sludge age and Sludge Application Rate (SAR) should be taken into consideration when such intervals are emphasized.

MATERIALS AND METHODS

Experimental design: This research was carried out in Botswana between March 2004 and May 2005. Two categories of anaerobically-stabilized sewage sludge, three years old and three months old (henceforth referred to as type 1 and type 2 sludge, respectively) were collected from a wastewater treatment plant in Gaborone, Botswana. The different soils namely luvisol 1, luvisol 2, arenosol and vertisol used in this study were collected from the main agricultural regions in Botswana. Details of the methods used to sample the sludge and soils and their characteristics are reported in Ngole *et al.* (2006a, b). Each category of sludge was mixed with each soil type at volume per volume (v/v) percent ratios of 0: 100, 5: 95, 10: 90, 20: 80 and 40: 60 (sludge: soil). Mixtures of the 0: 100 ratio of each soil-sludge mixture served as the control for that soil type. After homogenizing each mixture, samples were collected from each to determine the number of FC as described in Ngole *et al.* (2006a). Each of the different soil-sludge mixtures was then split into two sets; ST1 and ST2. Mixtures in ST1 were transferred into separate plastic plant pots and taken to a greenhouse where they were watered and allowed to acclimatize for three days before vegetables were planted. All soil-sludge mixtures in ST2 were exposed to natural conditions for 3 months (about 90±3 days), after which the enumeration of FC in the soil-sludge mixtures was repeated. The soil-sludge-mixtures in ST2 were then transferred to identical plant pots and taken to the same green house where they were also watered and allowed to acclimatize for three days before vegetables were planted. The sludge application-to-planting interval for both carrots and spinach grown on soil-sludge mixtures in ST1 was three days whereas those grown on mixtures in ST2 had a sludge application-to-planting interval of 90±3 days.

Each soil-sludge mixture in ST1 and ST2 was divided into two portions for the growth of carrots and spinach, respectively. Whereas four relatively healthy shoots were allowed to grow to maturity in each pot containing carrots, only three spinach seeds were allowed to grow in each pot. This design was repeated for both sludge types and the four different soil types. The growth of vegetables was carried out twice using freshly mixed soils and sludge collected from the same areas and piles, respectively. Whereas spinach was harvested after nine weeks, carrot was harvested after 13 weeks. Spinach and carrots grown on soil-sludge mixtures in ST1 therefore had a sludge application-to-harvest interval of 63 and 91 days, respectively. This interval was longer for vegetables grown on mixtures in ST2 being 153 days for spinach and 181 days for carrots.

Enumeration of coliforms and identification of Escherichia coli in spinach and carrot: At the end of the growth period, the leaves of spinach and storage roots of carrot from the different soil-sludge mixtures in ST1 and ST2 were collected in sterile bags and washed using sterile water by manually shaking in the sterile bags to simulate consumer washing of vegetables before consumption or use (Natvig *et al.*, 2002). Ten grams of each sample of carrot root and spinach leaves were shredded using a sterile blade and combined with 90 ml of sterile water before blending at low speed in a Waring Laboratory blender with a sterilized cup (Islam *et al.*, 2004a; Natvig *et al.*, 2002). The enumeration of FC in these homogenized samples was done using the Multiple Tube Fermentation technique described by US EPA (1999), Josephson *et al.* (2000) and Coyne (1999) with serial dilutions of up to 10^3. The presence of *E. coli* was confirmed using Eosin Methylene Blue Agar (EMBA) (Oxoid CM69). In an endeavour to identify the different enteric bacteria recovered from the vegetables, subcultures were made from plates that were positive on

EMBA by streaking on sterile MacConkey agar (Oxoid CM7) and incubating them for 24 h. A bioMérieux Sa API 20 E identification system, Software Package and an Analytical Profile index Library were used as recommended by the manufacturers to identify the different bacteria recovered in each positive sample.

Statistical analyses: The mean of values obtained for the MPN of FC in spinach and carrots from both growth cycles were reported as the MPN in spinach from the respective soil-sludge mixtures. To determine if there was any significance in the difference between means, the data was subjected to analyses of variance (ANOVA) tests. Using an IRRISTAT version 4.4 of 2004 statistical software package. Using the same software package, the significance of the role of soil type, sludge age and sludge application rate on the FC numbers in both spinach and carrots was also determined and Tukey's test was carried out to determine the significance of treatment means at p = 0.05.

RESULTS

Faecal coliform load in sludge and soil-sludge mixtures: Type 2 sludge had a higher FC load than type 1 sludge and this was reflected in the different soil-sludge mixtures, where mixtures with type 2 sludge had higher numbers of FC than mixtures with type 1 sludge applied at the same rate (Fig. 1).

Ninety days after sludge addition, there was a decrease in FC load in all soil-sludge mixtures in ST2 especially in those with type 2 sludge. As a result, the numbers of FC in type 2 sludge-soil mixtures were similar to those in type 1 sludge-soil mixtures with the same SAR after 90 days (Fig. 2). Though there was a significant difference between the MPN of FC in the soil-sludge mixtures in ST1 and ST2, this difference was not shown in the MPN of FC recovered from vegetables grown on both sets of soil-sludge mixtures.

Coliforms in spinach: The MPN of Total Coliform (TC) in spinach grown on soil-sludge mixtures in ST1 and ST2 increased with increase in SAR. Spinach grown on mixtures in ST1 had significantly higher numbers of TC than those grown on corresponding mixtures in ST2 ($p>0.05$) with values ranging from 2.0-3.3 $\log_{10}$MPN TC/10 g in spinach from mixtures with type 2 sludge in ST1 and 1.6-2.5 lo g_{10} MPN TC/10 g in spinach from mixtures with type 2 sludge in ST2. More TC was recovered from spinach grown on the vertisol and arenosol than the luvisol ($p>0.05$) (Table 1).

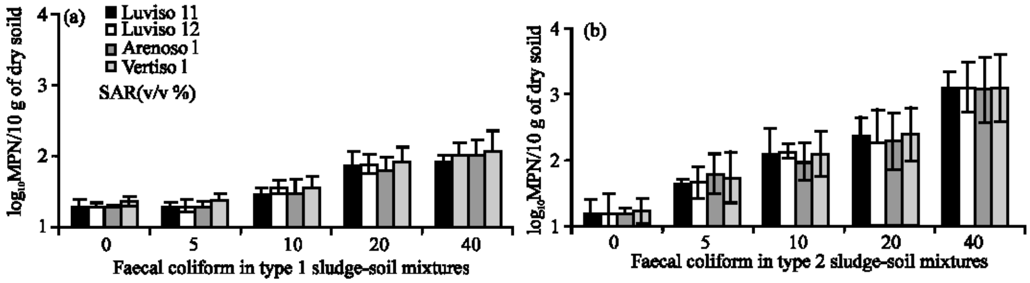

Fig. 1: Faecal coliform numbers in soil-sludge mixtures in ST1 at time of planting. Bar indicate standard deviation. modified after Ngole *et al.* (2006a)

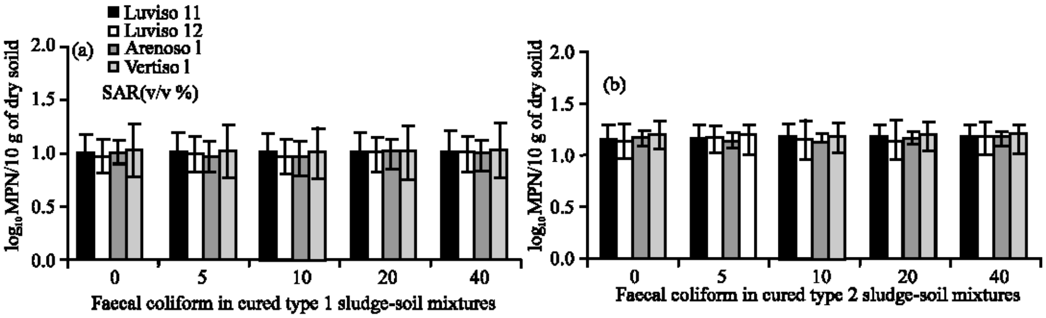

Fig. 2: Faecal coliforms numbers in soil-sludge mixtures in ST2 at time of planting. Bar indicate standard deviation. modified after Ngole *et al.* (2006a)

Table 1: Total coliforms recovered in spinach grown on type 2 sludge-soil mixtures in ST1 and ST2

	Log$_{10}$ MPN TC/10 g of spinach							
	Luvisol 1		Luvisol 2		Arenosol		Vertisol	
Sludge application rate (%)	ST1	ST2	ST1	ST2	ST1	ST2	ST1	ST2
5	1.92	1.56	1.88	1.48	2.01	1.52	2.27	1.87
10	2.32	1.78	2.10	1.56	2.59	2.04	2.41	1.87
20	2.60	1.87	2.60	1.87	2.78	2.04	3.32	2.58
40	2.65	1.87	2.65	1.87	2.95	2.18	3.36	2.58

Table 2: Total coliform recovered in carrots grown on soil-sludge mixtures in ST1

	Log$_{10}$ MPN TC/10 g of carrots							
	Luvisol 1		Luvisol 2		Arenosol		Vertisol	
Sludge application rate (%)	Type 1	Type 2	Type 1	Type 2	Type 1	Type 2	Type 1	Type 2
5	1.40	1.96	1.20	1.40	1.40	1.50	1.50	1.87
10	1.48	2.36	1.56	2.04	2.18	2.48	1.96	2.36
20	2.43	2.56	1.96	2.32	2.32	2.56	2.04	2.63
40	2.63	2.88	2.43	2.66	2.48	2.63	2.18	2.97

The results of the confirmatory test for FC in all spinach grown on the mixtures containing type 1 sludge in both ST1 and ST2 and on those containing type 2 sludge in ST2 were negative (0-0-0) indicating a load of $<1.5 \log_{10}$ MPN FC/10 g of spinach. Spinach from mixtures with type 2 sludge in ST1 had higher values for FC (1.6 $\log_{10}$ MPN FC/10 g) than those from corresponding mixtures in ST2 ($<1.5 \log_{10}$ MPN FC/10 g). However, API 20 E analyses were negative indicating that no *E. coli* were identified in the spinach leaves. Natvig *et al.* (2002) and Nguz *et al.* (2005) obtained similar results with lettuce and Hora *et al.* (2005) with spinach. The effect of sludge age on the number of FC recovered from spinach was insignificant ($p<0.05$) especially when a 90 day application-to-planting interval was observed.

Coliforms in carrots: Carrots generally had a higher number of TC than spinach grown on corresponding mixtures in both ST1 and ST2. The MPN of TC in carrots also increased with increase in SAR with no significant difference between carrots grown on mixtures with type 1 and those grown on mixtures with type 2 sludge (Table 2). Soil type also had no effect on the number of TC recovered from carrots grown on mixtures from both set of samples ($p<0.05$).

The MPN of FC recovered in carrots grown on mixtures with type 1 sludge in ST1 and ST2 and type 2 sludge in ST2 were all $< 1.5 \log_{10}$ MPN/10 g. Whereas the MPN of FC recovered from carrots grown on mixtures with type 2 sludge applied at the rate of 5 and 10% in ST1 were also $< 1.5 \log_{10}$ MPN FC/10 g, the MPN of FC recovered in carrots grown on mixtures with same sludge type applied at the rate of 20 and 40%, ranged between 1.6 and 1.8 $\log_{10}$ MPN FC/0 g. Except for carrots grown on the arenosol-type 2 sludge mixtures with an SAR of 40% in ST1 where API 20 E analyses identified *E. coli*, API 20 E results were negative for all the other carrot samples. The rate at which the sludge was applied to the soil and the age of the sludge significantly affected the number of coliforms recovered from the carrots ($p>0.05$).

Table 3: Faecal coliform recovered from carrots and spinach grown on mixtures with type 2 sludge in ST1 and ST2

	Spinach		Carrots	
Sludge application rate	ST1	ST2	ST1	ST2
5	1.49	1.49	1.50	1.50
10	1.50	1.49	1.60	1.50
20	1.57	1.49	1.68	1.50
40	1.60	1.49	1.80	1.50

Effects of the various intervals on faecal coliform numbers recovered from vegetables: There were differences observed in the number of FC recovered from vegetables grown on both set of mixtures especially with the young sludge. The number of FC recovered from both carrots and spinach were lower when a 90 day sludge application-to-planting interval rather than a 90 day sludge application-to-harvest interval was observed especially when the younger type 1 sludge was used (Table 3). No difference was observed in the FC numbers recovered from both vegetables when the older sludge was used irrespective of the interval observed.

DISCUSSION

Effect of soil type, sludge type, age and application rate on number of FC recovered from vegetables: Increase in pathogen load in vegetables with increase in SAR has been reported by Islam *et al.* (2004) and is attributed to an increase in the load of pathogens applied to the soil with increase in the amount of sludge added. The observations in this study are therefore explained. Luvisol, arenosols and vertisols vary in their physico-chemical properties especially Organic Matter (OM) content, textural properties and nutrient status which all affect the survival of bacteria in soil (Ngole *et al*., 2006a; Kirby *et al*., 2003; Tate, 1978; Gagliardi and Karns, 2000). The OM content in young sludge is usually higher than in older sludge and OM content also increase with increase in SAR. The recovery of a higher number of coliforms in carrots and spinach grown on the vertisol with type 2 sludge applied at higher rates compared to those grown on luvisol and arenosol is therefore attributed to the high OM content and fine texture of the vertisol which according to Saini *et al*. (2003) and Sessitsch *et al*. (2001) provide stable aggregates that can successfully shield bacteria from harsh environmental conditions and predation by other organisms in the soil. Considering that carrots are in direct contact with the sludge-amended soil whereas spinach is not, the higher numbers of FC recovered from carrots compared to spinach is explained.

Effects of the various intervals on the health risks in growing vegetables on sludge amended soil: Result obtained have indicated that there is a reduction in the health risk involved in growing vegetables on sludge amended soil when a 90 day interval is employed for application-to-planting rather than application-to-harvest. The 90 day application-to-planting interval provides a minimum of at least 153 days between sludge application and harvesting of spinach which allows for further pathogen reduction compared to the 120 days specified by some Guidelines for the use of sludge in vegetable production. The advantage of a 90 day sludge application-to-planting interval is further supported by studies carried out by Epstein (1998) and Ngole *et al.* (2006a) where *E. coli* and *Salmonella* species were recovered from manure- and sludge-amended soils more than 90 days after manure application. Natvig *et al*. (2002) have also recovered faecal coliforms (*Salmonella enterica* serovar *Typhimurium* and *E. coli*) on root and leaf vegetables harvested 17 weeks (119 days) after manure application, which indicates that 120 day application-to-harvest interval may not offer enough health protection.

Though carrot has a longer growth cycle compared to spinach, it is in direct contact with the sludge-amended soil and depending on the soil type a longer period may be required to guarantee the microbial safety of consuming carrots grown on sludge-amended soil. The 90 day application to-planting interval will imply at least a 181 day sludge-application-to-harvest interval which will further reduce the pathogens in the soil and consequently reduce the chances that these bacteria will be taken up by the carrots. The 90 day application-to-planting interval becomes even more vital when the sludge applied to the soils is relatively young. Studies by Jablasone *et al.* (2005) and Warriner *et al*. (2003) on bacterial colonization and internalization by vegetables including carrot, cress, lettuce, radish, spinach and tomato suggests that internalisation is greatest when plants are seedlings. The population of pathogens in the seedling stage of the vegetables should therefore be at its lowest to avoid systemic uptake. It is therefore more appropriate to reduce the bacterial population to which seedlings are exposed by allowing more time between sludge application and planting of vegetables. Though some unpublished data may be in support of a 90 day application to harvest interval, this study in agreement with that carried out by Natvig *et al.* (2002) has shown that *E. coil* and other enteric pathogens could be recovered from vegetables long after 90 days. The 90 day application-to-harvest interval suggested by other studies may not be appropriate especially for root vegetables grown on soil amended with young sludge or other organic manure.

CONCLUSIONS

This study has shown that an application-to-planting interval of 90 days significantly reduces the health risk involved in growing both root and leaf vegetables on vertisol, luvisol and arenosol amended with sludge. Though and application-to-harvest interval of 120 days could be appropriate when relatively older sludge is applied to soils on which leafy vegetables are grown, this period is shorter than the survival period of some pathogenic enteric bacteria in soil. It is recommended that programs designed for the use of sludge in organic vegetable farming should consider an application-to-planting rather than an application-to-harvest interval. The type of soil and age of sludge used should also be taken into consideration. Further studies are needed to investigate how these intervals may vary under different soil management practices which are some of the factors affecting the survival of enteric pathogens in soil and on vegetables

REFERENCES

Beuchat, L.R. and E.R. Brackett, 1990. Inhibitory effect of raw carrots on *Listeria monocytogenes*. Applied Environ. Microbiol., 56: 1734-1742.

Coyne, M.S., 1999. Lab Manual to Accompany Soil Microbiology: An Exploratory Approach. Delmar Publishers Albany, New York.

Epstein, E., 1998. Pathogenic health aspects of land application. Biocycle Emm., 39: 62-67.

Gagliardi, J.V. and J.S. Karns, 2000. Leaching of *Escherichia coli* O157:H7 in diverse soils under various agricultural management practices. Applied Environ. Microbiol., 66: 877-883.

Guan, Y.T. and R.A. Holley, 2003. Pathogen survival in swine manure environments and transmission of human enteric illness. A review. J. Environ. Qual., 32: 383-392.

Hora, R., K. Warriner, B.J. Shelp and M.W. Griffiths, 2005. Internalization of *Escherichia coli* O157:H7 following biological and mechanical disruption of growing spinach plants. J. Food Prot., 68: 2506-2509.

Ingham, S.T., J.A. Losinski, M.P. andrews, J.E. Breuer, J.R. Breuer, T.M. Wood and T.H. Wright, 2004. *Escherichia coli* contamination of vegetables grown in soils fertilized with noncomposted bovine manure: Garden scale studies. Applied Environ. Microbiol., 70: 6420-6427.

Ingham, S.C., M.A. Fansleu, R.A. Engel, J.R. Breuer, J.E. Breuer, T.H. Wright, J.K. Reith-Rozelle and J. Zhu, 2005. Evaluation of Fertilizer-to-planting and fertiliozer-to-harvest intervals for safe use of noncomposted bovine manure in wisconsin vegetable production. J. Food. Prot., 68: 1134-1142.

Islam, M., M.P. Doyle, S.C. Phatak, P. Millner and X. Jiang, 2004a. Persistence of enterohemorrhagic *Escherichia coli* O157:H7 in soil and on leaf lettuce and parsley grown in fields treated with contaminated manure composts or irrigation water. J. Food Prot., 67: 1365-1370.

Islam, M., J. Morgan, M.P. Doyle and X. Jiang, 2004b. Fate of *Escherichia coli* O157:H7 in manure compost-amended soil and on carrots and onions grown in an environmentally controlled growth chamber. J. Food Prot., 67: 574-578.

Jablasone, J., K. Warriner and M. Griffiths, 2005. Interactions of *Escherichia coli* O157:H7, *Salmonella typhimurium* and *Listeria monocytogenes* plants cultivated in a gnotobiotic system. Int. J. Food Microbiol., 99: 7-18.

Jiang, X., J. Morgan and M.P. Doyle, 2002. Fate of *Escherichia coli* O157:H7 in manure-amended soil. Applied Environ. Microbiol., 68: 2605-2609.

Johannessen, G.S., B.G. Bengtsson, B.T. Heier, S. Bredholt, Y. Wasteson and L.M. Rørvik, 2005. Potential uptake of *Escherichia coli* O157:H7 from organic manure into crisphead lettuce. Applied Environ. Microbiol., 71: 2221-2225.

Josephson, K.L., C.P. Gerba and I.L. Pepper, 2000. Cultural Methods. In: Environmental Microbiology, Maier, R.M., I.L. Pepper and C.P. Gerba (Eds.). Academic Press. San Diego, California, pp: 213-232.

Kirby, R.M., J. Bartram and R. Carr, 2003. Water in food production and processing: Quantity and quality concerns. Food Control, 14: 283-299.

Lau, M.M. and S.C. Ingham, 2001. Survival of faecal indicator bacteria in bovine manure incorporated into soil. Lett. Applied Microbiol., 33: 131-136.

Natvig, E.E., C.S. Ingham, H.B. Ingham, L.R. Cooperband and T.R. Roper, 2002. *Salmonella enterica* serovar Typhimurium and *Escherichia coli* contamination of root and leaf vegetables grown in soils with incorporated bovine manure. Applied Environ. Microbiol., 68: 2737-2744.

Ngole, V.M., S. Mpuchane and O. Totolo, 2006a. The survival rate of coliforms in four different soil types in Botswana amended with sewage sludge. Eur. J. Soil Biol., 42: 208-218.

Ngole, V.M., S. Mpuchane and O. Totolo, 2006b. The effect of ageing on the fertilizer value of sludge from Botswana. JASEM., 10: 109-115.

Nguz, K., J. Shindano, S. Samapundo and A. Huyghebaert, 2005. Microbiological evaluation of fresh-cut vegetables produced in Zambia. Food Control, 16: 623-628.

Saini, R., L.J. Halverson and J.C. Lorimor, 2003. Rainfall timing and frequency influence on leaching of *Escherichia coli* RS2G through soil following manure application, J. Environ. Qual., 32: 1865-1872.

Sessitsch, A., A. Weilharter, M.H. Gerzabek, H. Kirchmann and E. Kandeler, 2001. Microbial population structures in soil particle size fractions of a long-term fertilizer field experiment. Applied Environ. Microbiol., 67: 4215-4224.

Solomon, E.B., S. Yaron and K.R. Matthews, 2002. Transmission of *Escherichia coli* O157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalisation. Applied Environ. Microbiol., 68: 397-400.

Tate, III R.L., 1978. Cultural and environmental factors affecting the longevity of *Eschericha coli* in Histosols. Applied Environ. Microbiol., 35: 925-929.

US EPA, 1999. Method 1680: Faecal Coliforms in Biosolids by Multiple-Tube Fermentation and Membrane Filter Procedures. EPA No. EPA-821-R-98-003.

Warriner, K., F. Ibrahim, M. Dickenson, C. Wright and W.M. Waites, 2003. Interaction of *Escherichia coli* with growing salad spinach plants. J. Food Prot., 66: 1790-1797.

Watchtel, M.R., C.L. Whitehand and R.E. Mandrell, 2002. Prevalence of *Escherichia coli* associated with a cabbage crop inadvertently irrigated with partially treated sewage wastewater. J. Food Prot., 65: 471-475.

Windfield, D.M. and E.A. Groisman, 2003. Role of nonhost environments in the lifestyle of *salmonella* and *Escherichia coli.* Applied Environ. Microbiol., 69: 3687-3694.