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Detection and enumeration of pathogenic microorganisms associated with fresh vegetables and their implication for food safety

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Abstract

Background: Nowadays, food safety becomes an environmental and public health concern due to the increasing demand of vegetable diets observed from the last decades. This has stipulated research regarding the risks associated with consumption of food stuffs contaminated with pathogenic microorganisms. **Objective:** The aim of this study was to detect and enumerate pathogenic microorganisms associated with fresh vegetables and their implications for food safety among consumers. **Methods:** Fresh samples of vegetables including tomato, onion, carrot and spinach were collected immediately after harvest for the bacterial characterization and identification using Bergey's Manual of Systematic Bacteriology while the fungi were identified by their morphology through examination of their microscopic structures. **Results:** The results show higher number of *Vibrio cholerae* in the vegetables, a high mean score of 8.7×10^8 CFU/g for AMB, and 5.6×10^5 for TCB in tomatoes and 3.9×10^6 CFU/g for FCB in carrot sample. There were no statistically significance difference within and between groups in the AMB, FCB and TCB using one-way ANOVA at $p = 0.05$ and 95% confidence interval ($F = 2.065$; 0.152 and 4.045 respectively). Spinach contained 495 MPN/g of *Aspergillus flavus* (ASPf) while onion has 518 MPN/g of *Aspergillus niger* (ASPn) per each 25 plates of samples. **Conclusion:** This study concluded that microbial loads on vegetables were above ICMSF limits for vegetables and therefore, consumers should ensure that the raw vegetables are thoroughly washed and adequately processed to prevent chances of food borne diseases.

Keywords: *Detection, Enumeration; Microorganisms; Fresh vegetables; Food safety*

1. Introduction

Recent shifts in promoting a healthy lifestyle around the globe has contributed to the increased consumption of fruits and vegetables in fresh, raw or undercooked forms (Abadias *et al.*, 2008; and Feroz *et al.*, 2013). Although the health benefits of fresh produce are great, the proportion of foodborne disease outbreaks linked to contaminated produce has increased over the past few decades (Ailes *et al.*, 2008). Contamination of fresh

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vegetables during handling process is a common problem and it is usually ignored the use appropriate techniques of decontamination (Ankita *et al.,* 2014). If consumed, contaminated fruits and vegetables can lead to food poisoning, because of the presence of intestinal infectious microbes on the outer surfaces.

The majority of diseases associated with fresh fruits and vegetables are primarily those transmitted by the fecal-oral route, and this is as a result of contamination at some points in their processing (DeRoever, 1998). Therefore, the endemic of gastroenteritis, giardiasis, hepatitis A, hepatitis E, shigellosis (bacillary dysentery), typhoid fever, vibrio parahaemolyticus infections and vholera is an evident example of fecal-oral route transmitted diseases (Ankita *et al.,* 2014). Food spoilage is a metabolic process that causes foods to be undesirable or unacceptable for human consumption due to changes in sensory characteristics. Spoiled foods may be unsafe to eat, and may cause illnesses because of presence of pathogens or toxins. Other spoilage associated changes in texture, smell, taste, or appearance may cause them to be rejected under normal circumstances (Burkepile *et al.,* 2006).

Vegetables, like other food stuffs can become contaminated with pathogenic organisms. This may occur during growth, harvest, postharvest handling, and preservation or during cookery (McMaho and Wilson, 2001; and Beuchat, 1999). The use of untreated wastewater in irrigation represents an important route for transmission of these pathogenic organisms. The common pathogens associated with the use of highly polluted water are the *faecal coliforms, E. coli, Klebsiella species, Vibrio cholerae* etc. and eggs of some eggs of fungi such as A*spagillus* and whose resistant eggs can be found in the wastewater (Amoah *et al.,* 2007). Some of these microbes can cause food poisoning in through infecting the intestines, leading to inflammation, difficulty in absorbing nutrients and water, and may result in diarrhea (Sabiena *et al.,* 2000).

Other microbes produce chemicals in foods (toxins) that are poisonous to the human digestive system and when eaten, these chemicals can lead to nausea and vomiting, kidney failure, and even death (Mara *et al.,* 2007; and Uzeh *et al.,* 2009). Food safety is a major public health concern worldwide (Beuchat, 2002). During the last decades, the increasing demand for food safety has stipulated research regarding the risk associated with consumption of food stuffs contaminated with pathogenic microorganisms and studies have revealed that pathogenic contamination of vegetables increases risk of food borne diseases among the consumers (D'Mello 2003; and Zandstra and De Kryger, 2007). This study was therefore designed to detect and enumerate pathogenic microorganisms associated with fresh vegetables and their implications for food safety among consumers.

2. Materials and methods

One hundred and sixty samples of fresh vegetables were collected immediately after harvest at River Getsi irrigation site, in Kano metropolis, Nigeria. The vegetable include of 40 tomatoes, 40 onions, 40 carrots and 40 spinaches respectively. These samples were collected using sterile hand gloves in order to avoid cross contamination, put into new polythene bags and transported to laboratory. The samples (30 g each) were washed thoroughly with fresh water in order to remove the adhering dirt and finally washed with deionized water. Each of the vegetable samples was dried separately at room temperature in a sun free environment. The dried samples were grinded into homogenous fine powder mass with the aid of ES-314 blender (220 V, 50 HZ and 300 W) of SONCAP approved, put into airtight polythene bags and kept in a refrigerator for further analysis.

The vegetable homogenate was prepared by mixing 15g of each vegetable sample with 225ml of buffered peptone water (BPW) in a sterile beaker and blended for about 2-3 min. Vegetable homogenates in three test tubes each containing 9 ml MacConkey broth with an inverted Durham were used for coliforms presumptive test (FAO/WHO, 2008a). Bacterial colonies in pure form were collected using streak plate method. Based on phenotypic colony characters on media plates, total 127 different bacterial colonies were isolated in pure forms, which were further identified. This detection was done by visual colony characters, microscopy (Gram and Endospore straining) and some other tests like motility test, oxidase test, catalase test, indole test, Gram's reaction, citrate utilization test, and Voges-Proskauer test, while, the secondary identification of the isolates was carried out on the basis of biochemical tests (IMViC tests) and carbohydrate utilization test.

For the detection of fungi, a drop of methyl blue was placed on a clean slide; a sterile needle was used to introduce a small amount of the growth from incubated plate into the stain. A cover slip was gently applied with little pressure to prevent air bubbles. The slide was mounted and observed under × 40 objective lens (Fawole, 1988).

The isolation of fungi was done with Potato Dextrose Agar media, after isolation of all fungal colonies in pure culture, all of them were visually examined for phenotypic characters like color, texture, exudates, growth zones, aerial/submerged hyphae, and macroscopic structures such as ascocarps, pycnidia, sclerotia, sporodochia, and synnemata.

The final identification of fungi was performed by morphological examination of microscopic structures, particularly the spores and the conidia, using lactophenol and cotton blue. The data obtained were analyzed using frequency distribution tables including number and percentages for the identified bacteria, descriptive statistics using mean based on the bacterial colony forming units per gram as well as one-way analysis of variance (ANOVA) for between and within group differences in bacterial counts per samples of the vegetables. For the fungal species, descriptive statistics using mean was employed based on the fungal most probable number per gram (MPN/g) in 25 plates of each sample of the vegetables. All the statistical analyses were performed using SPSS software version 25 at significance level of $p = 0.05$ and 95% confidence interval.

3. Results and discussion

This experimental study was conducted to find any pathogenic microbes (bacteria and fungi) on vegetables collected from river Getsi irrigational farm site, which could be responsible for contamination of these vegetables and ascertain their potential implications for food safety, Different techniques were used for isolation and characterization was done with help of phenotypic and biochemical characters. The total number of the identified bacterial colonies from all vegetables (Spinach, Tomato, Onion and carrot) were 127 as shown in Table 1. The strains of the identified bacterial species were counted and *Vibrio cholerae* was found to be the highest specie (31 stains) while *Klebsiella* (6 strains) and *Pseudomonas* (3 stains) were the least species among all the detected bacteria as shown in Table 2.

Table 1: Cultural, biochemical and morphological characteristics of the isolated bacteria			
Bacterial culture	**Reactive tests (+)**	**Cell type**	**Inference**
NA: Small slightly raise area colonies	Gram's reaction Catalase test, Oxidase test	Cocci	*Staph. Aureus*
MSA: Yellowish colonies	Catalase test Citrate utilization Voges proskauer	Cocci	*Klebsiella species*
EMB: Pink-blue mucoid with a metallic sheen	Catalase test Motility, Indole	Cocci	*E.ColiPseudomonas species*
BA: Large flat colonies greenish blue colorSmall gray transluscent drop like colonies	Gram's reaction Catalase test, Motility Vogesproskauer	Rod	*Listeria species*
KIA: Red pink slope and yellow butt.	Motility Voges proskauerIndole Citrate utilization Oxidase test	Bacilli	*Vibrio cholera*
KIA: Red pink slope and yellow butt.	Catalase test Citrate utilization	Rod	*Citrobacter species*
Note: NA: Nutrient agar; MAC: MacConkey agar; MSA: Mannitol salt agar; BA: Blood agar; KIA: Kligler iron agar: E.coli: Escherichia coli; Staph. Aureus; and Staphylococcus aureus.			

Table 2: Number of bacterial species found to be present on vegetables	
Bacterial species	**No. of identified strains (%)**
Citrobacter spp	13 (10. 2)
Eschericia coli	9 (07. 1)
Faecal coliforms	26 (20. 5)
Staphylococcus aureus	20 (15. 7)
Klebsiella spp.	6 (04. 7)
Listeria monocytogene	19 (14. 9)
Pseudomonas spp	3 (02. 4)
Vibrio cholera	31 (24. 4)

From Table 3, the bacteria species were further grouped into aerobic mesophillic bacteria (AMB), total coliform bacteria (TCB) and *faecal coliform* bacteria (FCB) base on their morphological features and the numeric index of each was determined using the colony forming units per gram (CFU/g). Table 4 shows the results of the one-way ANOVA for statistical difference between and within the groups for the AMB, FCB and TCB. These were found to be insignificant for AMB ($F = 2.065$, $P = 0.05$ at 95% confidence interval), TCB ($F = 4.045$, $P = 0.05$ at 95% confidence interval) and FCB ($F = 0.151$, $P = 0.05$ at 95% confidence interval) respectively.

Table 3: Mean number of isolated bacteria in the tested vegetables					
Sample ID	**Indicator bacteria**	**Vegetable types (CFU/g)**			
		Tomato	**Onion**	**Spinach**	**Carrot**
1		9.3×10^4	1.7×10^6	2.2×10^4	1.6×10^3
2	AMB	8.0×10^2	1.6×10^8	2.0×10^2	1.4×10^5
3		8.7×10^6	1.9×104	2.1×10^3	1.8×10^7
Mean veg		8.7×10^4	1.7×10^6	2.1×10^3	1.6×10^5
1		5.8×10^2	3.7×10^5	6.6×10^5	5.2×10^7
2	TCB	5.3×10^5	4.0×10^3	5.4×10^5	3.1×10^5
3		5.6×10^2	3.4×10^7	4.1×10^5	4.1×10^3
Mean veg		5.6×10^3	3.7×10^4	5.4×10^5	4.1×10^5
1		3.1×10^3	3.7×10^5	5.6×10^1	4.5×10^2
2	FCB	3.2×10^3	3.2×10^6	3.8×10^3	4.1×10^5
3		3.0×10^6	3.0×10^4	3.0×10^5	3.2×10^2
Mean veg		3.1×10^4	2.9×10^5	3.3×10^3	3.9×10^3
Note: 1, 2 and 3 = Sampling in first, second and third months respectively, AMB = Aerobic mesophilic bacteria, TCC = Total coliform count, FC = Faecal coliform and CFU/g = Colony forming unit per gram.					

Table 4: One-way ANOVA of isolated bacteria in the tested vegetables

Isolated bacteria		Sum of Squares	df	Mean square	F	Sig.
Aerobic mesophilic	Between Groups	73457.450	57	4179.856	1.065	0.376
Bacteria	Within Groups	4049.000	2	3354.500		
	Total	**78966.250**	**59**			
	Between Groups	74768.050	47	4398.121	3.045	0.256
Total coliform count	Within Groups	2174.500	2	1087.250		
	Total	**76942.550**	**49**			
	Between Groups	31134.050	57	1762.238	0.151	0.732
Feacal coliform	Within Groups	26356.800	2	13458.250		
	Total	**67371.350**	**59**			

Note: df = degree of freedom, $p = 0.05$ at 95% confidence interval.

Table 5 shows the morphological features of the identified fungal species. These were obtained from the potato dextrose agar medium and were of typical *Aspergillus flavus* (ASPf) and *Aspergillus niger* (ASPn) respectively. Table 6 shows the MPN/g of the ASPf and ASPn in 30 plates of each vegetable (tomato, onion,

Table 5: Cultural and morphological characteristics of the isolated fungi

Cultural appearance	Microscopic characteristics	Inference
PDA: Irregular size, shape, black colonies	Conidia arise from stigma and bore in chain	*Aspergillus niger*
PDA: Grey to green irregular colonies	Conidiophore bore laterally on the hyphae	*Aspergillus flavus*

Note: PDA = Potato Dextrose Agar

Table 6: Mean number of isolated fungi in the tested vegetables

Sample ID	No. of plates (*N*)	Indicator Fungi	Vegetable types (MPN/g)			
			Tomato	Onion	Spinach	Carrot
1	30		244	401	621	543
2	30	ASPf	514	437	543	332
3	30		500	399	321	514
Mean value			419.3	412.3	495	463
1	30		442	633	399	484
2	30	ASPn	387	592	408	365
3	30		542	328	537	501
Mean value			457	517.7	448	450

Note: 1, 2 and 3 = Number of sampling in first, second and third month respectively, ASPf = *Aspergillus flavus*
ASPn = *Aspergillus nigger*, MPN = Most probable number and *N* = No. of plates.

spinach, carrot) in three samples. The mean value of ASPf was higher in spinach (495 MPN/g) while ASPn was found to be higher in onion sample. We detected and enumerated an array of pathogenic bacteria and selected fungi associated with post-harvest fresh vegetables that are irrigated with wastewater in river Getsi irrigation site.

The objective of our study is to determine the contaminating microbes, their implications for food safety and effect on food borne illnesses or infections outbreak. Presence of microorganisms in vegetables is directly related to the water used and the hygienic conditions practiced during their cultivation, harvesting, postharvest handling, processing and distribution of the produce (Halablab *et al.*, 2011). Consequently, these microorganisms on vegetables may act as a reservoir which may be responsible for further post-harvest contamination, if not reduced or eliminated (Barth *et al.*, 2010; and Joint FAO/WHO Codex Alimentarius Commission, 1994). Eight different bacteria were identified which include *Eschericia coli, Listeria monocytogene, Staphylococcus aureus, Klebsiella spp, Citrobacter spp, Faecal coliforms, Pseudomonas spp* and *Vibrio cholera* respectively. In this study, nine cell of *Eschericia coli* were detected in the 25 plates of the vegetables.

Although, this number is lower than the infectious dose of 10-1000 cells per plate, it is still a concern, as it may cause symptomatic food-borne infection (Beuchat, 1996). Enterotoxigenic *E. coli* is a common cause of travelers' diarrhea, an illness sometimes experienced when visiting developing countries. Raw vegetables are thought to be a common cause of travelers' diarrhea. A prospective study comprising of 121 participants that were attending a conference in Mexico City revealed that enterotoxigenic *E. coli* caused illness in 44% of the participants as a result of eating salads containing raw vegetables. The typical incubation of *E. coli* is 2-5 days, causing symptoms comprising of bloody diarrhea, abdominal pain, leading hemolytic uremic syndrome and kidney failure especially in children and the elderly (Merson *et al.*, 1976).

Listeria monocytogene was found to be 19 cells in the 25 plates of the vegetables, this pathogenic microorganism is implicated in febrile gastroenteritis in healthy adults, may lead to spontaneous abortion or stillbirth pregnant women; severe septicemia and meningitis in neonates and immune compromised adults and mortality may be 20 to 40. Infectious dose of *Listeria monocytogene* is unknown, it depend upon the health of individual at an incubation period of 1 day to 5 or more weeks. It is widely distributed on raw fruits and vegetables and on plant material (Beuchat, 1996). Factors affecting its presence or persistence have yet to be determined. Vegetables and plant parts used as vegetables recipes may play an indirect role in disseminating the pathogen from natural habitats to the human food supply.

Moreover, 20 cells of *staphylococcus aureus* have been detected on these fresh and ready-to-eat vegetables. This organism is known to be carried by food handlers and survives in low temperature between 4 to 8 °C (39.2 to 46.4 °F). Therefore, survival of this organism may cause disease outbreak. This is because vegetables are mostly processed at lower temperature which impinges on their growth and proliferation. Similarly, *Klebsiella spp* was also detect and differentially confirmed. This organism is implicated in the aetiology of gastroenteritis with secondary abdominal cramp, discomfort, systemic fever and local septicemia is well established (WHO 2009), but exact ratio of its flora loads is debatable despite its well-known association with food borne disease.

Citrobacter spp, faecal coliform, pseudomonas spp and *vibrio cholerae* were also isolated. These entroinvasive microbes are implicative in the aetiology of urinary tract infection, cholera, bloody and non-bloody diarrhoae, acute abdomen, food poising and listeriosis (WHO, 2009). More many decades, food borne disease outbreaks specifically associated with leafy vegetables is been on records in USA and a recent study indicates that between 1973 and 2006, 502 (4.8%) outbreaks, 18.242 (6.5%) illnesses and15 (4.0%) deaths were associated with "leafy greens", describe as lettuce, cabbage, onions, Spanish or a salad item containing one or more of these leafy vegetables (Herman *et al.*, 2008). Obviously, there is an observed paucity of data in Nigerian based public health consequences and reliable statistics on microbes induced food borne diseases out-brakes.

The bacteria species were further grouped into AMB, TCB and FCB base on their morphological features and the numeric index of each was determined using the colony forming units per gram (CFU/g). Table 3 below showed that, spinach contained the least CUF/g mean index of AMB (2.1×10^3) and higher for TCB (5.4×10^5), tomato was found to contain higher CUF/g for AMB (8.7×10^4) and FCB (3.1×10^4), while, onion contained the least CFU/g index of TCB (3.7×10^4) and FCB (2.9×10^5) respectively. The AMB, TCB and FCB levels were above the International Commission on Microbiological Specifications for Foods (ICMSF) recommended level of 3 log CFU ·g–1 fresh weight. Carrots, tomato, onion and spinach are while harvested from the farm, and may become contaminated by pathogenic organisms that were harbored in the soil. The

high bacterial load in the vegetables can be attributed to the large surface area of the leaves suitable for water contact, making them susceptible to bacterial contamination or could be due to poor hygiene practices by handlers.

Furthermore, ASPf and ASPn were isolated from the vegetables. This outcome is concordant with other findings (Akinmusire, 2011), which also isolated ASPf and *Phytopthora sp.* from irrigated products. These are fungi of grey to green morphological appearance and conidiopore bore on microscopic outlook which proliferate in a favorable pH, water activity, atmospheric composition. In this study, we determined the most probable number (MPN) of the fungi. We obtained moderately higher values and imply that there are chances that the contaminated vegetables can harbor pathogens potential of causing foodborne communicable diseases associated with fungal contaminants.

4. Conclusion

Fresh vegetables obtained from farm site can be associated with pathogenic microbial load due to use of wastewater irrigational practice and should be which should be thoroughly wash before consuming them. This study shows that microbial loads on vegetables in the river Getsi irrigation farm were well above ICMSF recommended limits for vegetables. Consumers of these vegetables stand a chance of contacting food-borne diseases if the vegetables are not thoroughly wash and adequately processed. To prevent an endemic outbreak, efforts have to be made by the concern bodies in ensuring that standard of microbiological guidelines are stick to by both the farmers and marketers, and to promote campaigns, awareness and enlightenment on general hygiene and public food safety.

References

Abadias, M., Usall, J., Anguera, M., Solsona, C. and Vinas, I. (2008). Microbiological quality of fresh minimally processed fruit and vegetables, and sprouts from retail establishments. *International Journal of Food Microbiology.* 123(1-2), 121-9. doi: 10.1016/j.ijfoodmicro.2007.12.013. Epub 2008 Jan 30

Akinmusire, O.O. (2011). Fungal species associated with the spoilage of some edible fruits in Maiduguri Northern Eastern Nigeria. *Advances in Environmental Biology.* 5(1), 157-161.

Ailes, E.C., Leon, J.S., Jaykus, L.A., Johnston, L.M., Clayton, H.A., Blanding, S., Kleinbaum, D.G., Backer, L.C. and Moe, C.L. (2008). Microbial concentrations on fresh produce are affected by postharvest processing, importation and season. *J. Food Prot.* 71, 2389-2397.

Amoah, P., Drechsel, P., Abaidoo, R.C., Henseler, M. (2007). Irrigated urban vegetable production in ghana: Micro-biological contamination in farms and markets and associated consumer risk groups. *Journal for Water and Health.* 5(3), 455-466. doi:10.2166/wh.2007.041

Ankita, M., Akshay, J., Dharmesh, H. (2014). Microbial contamination of raw fruits and vegetables. *Internet Journal of Food Safety.* 16, 26-28.

Barth, M., Hankinson, T., Zhuang, H. and Breidt, F. (2010). Microbiological spoilage of fruits and vegetables. Compendium of the Microbiological Spoilage of Foods and Beverages, New York: Springer.

Beuchat, L. (1996). Pathogenic microorganisms associated with fresh produce. *J. Food Prot.* 59, 204-216.

Beuchat, L.R. (1999). Food safety issues: surface decontamination of fruits and vegetables eaten raw: a review. Food Safety Unit, WHO. Geneva.

Beuchat, L.R. (2002). Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Microbes Infect.* 4, 413-423. DOI: https://doi.org/10.1016/s1286-4579 (02)01555-1

Burkepile, D.E., Parker, J.D., Woodson, C.B., Mills, H.J., Kubanek, J., Sobecky, P.A. and Hay, M.E. (2006). Chemically-mediated competition between microbes and animals: microbes as consumers in food webs. *Ecology.* 87, 2821-2831.

DeRoever, C. (1998). Microbiological safety evaluations and recommendations on fresh produce. *Food Control.* 9(6), 321-347. https://doi.org/10.1016/S0956-7135(98)00022-X

D'Mello, J.P.F. (2003). *Food safety: Contaminants and toxins.* CABI Publishing, Wallingford, Oxon, UK: Cambridge.

FAO/WHO. (2008a). Microbiological risk assessment series. Pre-publication version. microbiological hazards in fresh fruits and vegetables. Meeting Report Available at: http://www.fao.org/ag/agn/agns/files/FFV_Final.pdf (accessed 18th June, 2008).

Fawole, M.O. (1988). Laboratory Manual of Microbiology. Spectrum Books Limited Ibadan. 12.

Feroz, F., Senjuti, J.D. and Noor, R. (2013). Determination of microbial growth and survival in salad vegetables through in vitro challenge test. *International Journal of Nutrition and Food Sciences.* 2(6), 312-319 doi: 10.11648/j.ijnfs.20130206.18

Halablab, A., Sheet, H. and Holail, M. (2011). Microbilogical quality of raw vegetables grown in Bekaa Valley, *Lebanon. Am. J. Food Technol.* 6(2), 129-139.doi:10.3923/ajft.2011.129.139

Herman, K.M., Ayers, T.L. and Lynch, M. (2008). Foodborne disease outbreaks associated with leafy greens, 1973–2006 Abstract. International Conference on Emerging Infectious Diseases, 16–19 March 2008, Atlanta, Georgia, USA. See: http://www.cdc.gov/ncidod/EID/announcements/I eid_2008.htm

Joint FAO/WHO Codex Alimentarius Commission (1994). Codex Alimentarius, Rome, Food and Agriculture Organisation.

Mara, D.D., Sleigh, P.A., Blumenthaland, U.J., 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.doi:10.2166/wh.2006.055

McMaho, M.A.S. and Wilson, I.G. (2001). The occurrence of enteric pathogens and aeromonas species in organic vegetables. *International Journal of Food Microbiology.* 70(1-2), 155-162. doi:10.1016/S0168-1605(01)00535-9.

Merson, M.H., Morris, G.K., Sack, D.A., Wells, J.E., Feeley, J.C., Sack, R.B., Creech, W.B., Kapikian, A.Z. and Gangarosa, E.J. (1976). Travelers' diarrhea in Mexico. *N Engl J Med.* 294(24), 1299-305.

Sabiena, F., Raheela, H., Van der, W. (2000). Health risks of irrigation with untreated urban wastewater in the Southern Punjab, Pakistan, Institute of Public Health (IPH) and International Water Management Institute (IWMI) Research Report No. 107.

Uzeh, R.E., Alade, F.A., Bankole, M. (2009). The bacterial quality of pre-packed mixed vegetable in some retail outlets in Lagos, Nigeria. *African Journal of Food Science.* 3, 270-272.

WHO (2009). *Weekly epidemiologic record.* 83(31), 309-324.

Zandstra, B.H., De Kryger, T.A. (2007). Arsenic and lead residues in carrot from foliar applications of monosodium methanearosonate (MSMA): A comparison between mineral and organic soils, or from soil residues. *Food Addit. Contam.* 24, 34-42.