Internet Journal of Food Safety, Vol.14, 2012, p.23-29

Assessment of Health Risk Associated With Reuse of Treated Wastewater

Nadia Oubrim[1, 2*], Nozha Cohen[1], Abouddihaj Barguigua[3], Kaoutar Hajjami[1, 2], Brahim Bouchrif [1] and My Mustapha Ennaji[2]

[1]*Laboratoire de Microbiologie et d'Hygiène des Aliments et de l'Environnement, Institut Pasteur du Maroc.*
[2]*Laboratoire de Virologie, Hygiène et Microbiologie, Faculté des Sciences et Techniques, Université Hassan II-Mohammedia, Maroc.*
[3]*Laboratoire de Microbiologie et Biologie Moléculaire, Institut Pasteur du Maroc.*

Abstract

To determine the microbiological quality of wastewater treatment effluents, samples of raw water (n = 72), treated wastewater (n=72) taken from both Wastewater Treatment Plants Settat (33°00'N, 7°37'W) and Soualem (34°26'N, 5°53'W), (n=168) of crops samples irrigated by treated wastewater taken from two stations cited previously. The results showed that average concentration of fecal coliforms in treated water is 4.4± 0.4 Log MPN/100ml ; 7.9± 0.4 Log MPN/100ml for raw water and 3.2±0.3 Log CFU/g for crops, (n=170) strains of E. coli were isolated from these samples. Serotyping of E. coli have shown the presence of the four serotypes O26, O111, O128 and O157. Four pathogenic genes, stx1, stx2, eae and hlyA genes, were tested in E. coli isolates by Polymerase Chain Reaction (PCR), four were positive for stx2 gene and are all four of serotypes O157:H7.

Key words: Wastewater Treatment Plant, fecal coliforms, E. coli, serotyping, PCR.

Introduction

To achieve effective pathogen removal requires a very careful selection of treatment processes since several pathogen groups – viral, bacterial, protozoan and helminthic – have to be removed to varying degrees and, in developing countries, at the lowest possible cost (Blanca et al., 2010), Wastewater treatment process, such as stabilization ponds or lagooning is well adapted to the climate conditions in tropical and subtropical zones. Removal of helminthes eggs, bacteria and viruses is commonly achieved by wastewater stabilization ponds and other 'natural' treatment processes. The removal efficiencies required are of the order of 95–99.99 per cent for helminthes eggs and 3–6 log units for fecal coliforms (Blanca et al., 2010). Escherichia coli and to a lesser extent thermo tolerant coliforms bacteria are considered to best fulfill the criteria to be an ideal indicator (Campos, 2008), these are universally present in large numbers in the faeces of humans and warm-blooded animals, readily detected by simple methods, do not grow in natural waters and persistence in water and removal by water treatment similar to waterborne pathogens.

*Corresponding author mailing address : Laboratoire de Microbiologie et d'Hygiène des Aliments et de l'Environnement. Institut Pasteur,
E-mail : n.oubrim@yahoo.fr

However, pathogenic strains of these bacteria are an important cause of bacterial infections. In humans, these strains are the foremost cause of urinary tract infections, as well as a major cause of neonatal meningitis, nosocomial septicemia, and surgical site infections. Infection with shiga toxin–producing E.coli (STEC) may also result in complications including thrombocytopenic purpura, severe hemorrhagic colitis, and hemolytic uremic syndrome (Carl et al., 2002). STEC include serotype O157:H7 and more than 100 non-O157 serotypes such as O111 and O26. Recently, several of these non-O157 STEC serovars have been linked to an increasing number of gastroenteritis infections and HUS in humans. Shiga toxins (stx) are considered the major virulence factors of STEC, which are responsible for vascular endothelial damage. These toxins, encoded by lysogenic bacteriophages, are classified into two main types, stx1 and stx2. STEC strains may produce stx1 or stx2, or both types. Another virulence factor of STEC is intimin, essential for cellular attachment; it is encoded by an eae gene. An additional virulence marker carried by some STEC strains is enterohemorraghic hemolysin (EHEC-Hly), encoded by EHEC-hlyA gene which seemed to be associated with severe clinical disease in humans (Badri et al., 2011). The aims of this study were: (i) enumeration of fecal coliforms and E. coli in raw, treated

wastewater and crops (ii) serotyping E. coli by agglutination (ii) determining the occurrence of stx1, stx2, eae and hlyA genes in shiga toxin-producing E. coli O157:H7 and non-O157 in different samples by Polymerase Chain Reaction (PCR).

Material and Methods

Raw water, purified and vegetable crops and fodder sampling. Sampling was done monthly over a period of two years from July 2007 to August 2009 in Waste Water Treatment Plants (WWTP) Settat (33°00'N, 7°37'W) and Soualem (34°26'N, 5°53'W) located in west of Morocco; The climate in these areas is classified as semi-arid, the annual rainfall of 41 mm^3 (mean 2007–2009) falls mostly in the winter, the mean annual temperature ranges from 16 to 42 ℃. A total of (n= 72) raw water, (n=72) treated water taken monthly from both WWTPs, samples were collected in sterile flasks 1000 ml and stored at 4-8℃ before microbiological analysis. Four types of crops were harvested and analyzed (forage, herbs, cereals and vegetables), a total of (n=168) of crops samples irrigated by treated water taken from both WWTPs; Samples of cultures were directly irrigated by treated wastewater as they are grown on farmland alongside around sewage treatment plants, sampling was done according to the availability of such crops; (n=24) crops irrigated with freshwater were also analyzed as control samples. Quantities more than 100g were collected from vegetable crops and fodder in sterile bags at 4℃ and transported to the laboratory before microbiological analysis. Samples were collected on time between 11h and 13h and analyzed within six hours after collection.

Microbiological analysis. The detection and enumeration of fecal coliforms and *Escherichia coli* in water were done by MPN (Most Probable Numeration) according to standard NF T 90-413. Buffered Peptone Water -BPW- (Oxoid) is used as a presumptive medium with incubation at 37 ° C for 24 hours and the broth lactose bile brilliant green –BLBVB- (Oxoid) as a confirmation medium with incubation at 42℃ for 24h, a 0.1 ml aliquot was taken from tubes showing gas production (considered a positive reaction) and placed in a tube of peptone water free of indole –EPPI- (Oxoid) to perform the indol test. After 48 h at 45℃, several drops of Kovac's reagent were added to the broths agitating slightly: a cherry red colour visible at the surface of the broth was considered positive for indol confirming the presence of *E. coli*. In parallel, *E.coli* was performed using Violet Red Bile Lactose Agar -VRBL- (Biorad) , Plates were incubated for 24 h at 44℃. The density is reported as log MPN 100 ml-1 for both fecal coliforms and *E. coli*.

Regarding the microbiological analysis of vegetable and fodder, a weight of 25g homogenized with 225ml of BPW and then pummeled with a MIX I mixer (AES Laboratory, Combourg, France), one milliliter of this suspension and decimal dilution were streaked onto VRBL and incubated for 24 h at 44℃. The density is reported as log CFU per g.

The use of a medium containing sortibol instead of lactose like Sorbitol MacConkey Agar (SMAC) provides a way to differentiate *E. coli* non fermenting sorbitol from most other strains of *E. coli*. On this medium, the colorless colonies of sorbitol non fermenting *E. coli* O157:H7 can be differentiated from the sorbitol fermenters, which are pink after the specimen is incubated for 18-24 hours at 35-37 ^{o}C. The β-D-glucuronidase (GUD) activity of the isolates was examined on TBX agar (Biokar), the colorless colonies of GUD non producing *E. coli* O157:H7 can be differentiated from GUD producing which are blue/green colonies.

Serotyping of STEC. Determination of the serogroups was performed by agglutination tests using polyvalent and monovalent sera against O antigens (O26, O55, O86, O111, O119, O124, O125, O126, O128, O157) and flagella H antigens (H7, H8, H9, H11, H19, H25) according to the instructions of the manufacturer (Bio-Rad Co and Statens Serum institute, Copenhagen, Denmark, respectively).

Preparation of DNA template for PCR. DNA templates for Polymerase Chain Reaction (PCR) process were generated by suspending 5 colonies of overnight culture of *Enterobacteriaceae* isolates growing on Luria Bertani agar (Bio-Rad, Marnes-la-Coquette, France) in 500µl of DNase- and RNase-free water (Invitrogen, England). The suspension was boiled at 100℃ for 10 min in thermal block (Polystat 5, French), then centrifuged at 15000 rpm for 5min. An aliquot of 1 µl of the supernatant was used as DNA template for PCR. Primers were selected based on previously published information for *stx1* and *stx2*, *hly*A and *eae*A genes (Table 1).

Table 1. The primers used for detection of the various genes by PCR

Gene	Primer	Oligonucleotid sequence (5'-3')	Fragment size(bp)	References
stx1	Stx1F Stx1R	ATA AAT CGC CAT TCG TTG ACT AC AGA ACG CCC ACT GAG ATC ATC	180	(Paton & Paton 1998)
stx2	VT2 425(VT2a) VT2 952(3'II)	TTA ACC ACA CCC CAC CGG GCA GT GGA TAT TCT CCC CAC TCT GAC ACC	524	(Pollard, Johnson &Lior, 1990)
eae A	SK1 SK2	CCC GAA TTC GGC ACA AGC ATA AGC CCC GGA TCC GTC TCG CCA GTA TTC G	864	(Karch & Bielaszewska, 2001)
hly A	hlyAF hlyAR	GCA TCA TCA AGC GTA CGT TCC AAT GAG CCA AGC TGG TTA AGC T	534	(Paton & Paton 1998)

Table 2. Microbiological hygienic quality of raw sewage and treated in Wastewater Treatment Plants Settat and Soualem

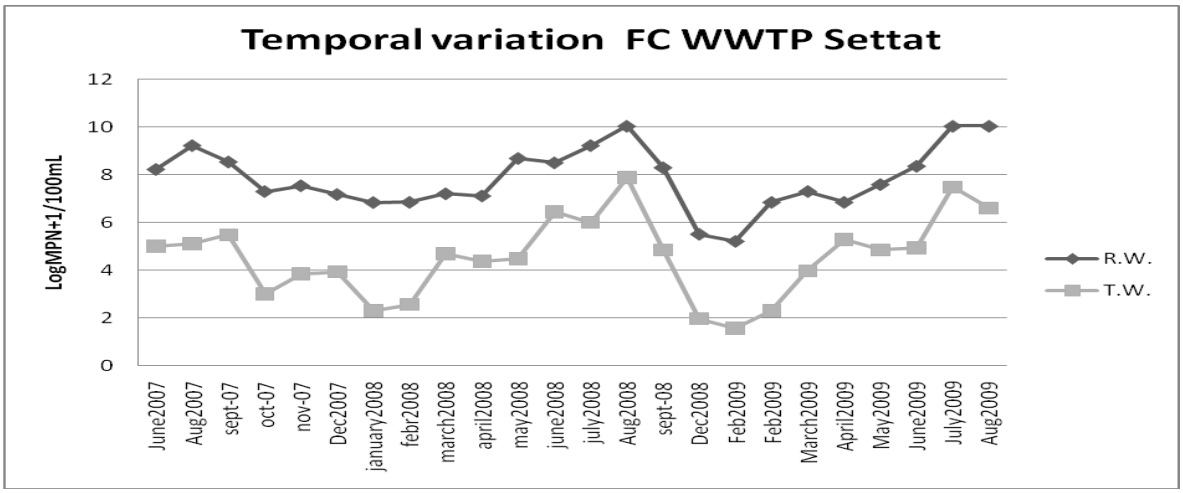
		WWTP Settat				WWTP Soualem			
		1st year		2nd year		1st year		2nd year	
Germ searched	Sample	Cold season	Hot season	Cold season	Hot season	Cold season	Hot season	Cold season	Hot season
Fecal coliforms	Raw water	6.8±0.3	9.3±0.3	6.7±0.4	9.9±0.3	6.8±0.5	8.1±0.5	6.8±0.5	9.3±0.5
logMPN100 ml^{-1}	Treated water	2.3±0.4	6.0±0.4	3.7±0.4	6.1±0.3	2.3±0.4	6.3±0.4	3.7±0.4	6.9±0.4
	Rate abattement	4	3	3	3	4	2	3	3
Escherichia coli	Raw water	4.6±0.3	8.0±0.3	4.8±0.4	8.6±0.3	2.9±0.4	8.0±0.4	2.6±0.4	9.3±0.4
logMPN100 ml^{-1}	Treated water	2.1±0.3	5.3±0.3	1.0±0.4	4.7±0.3	1.6±0.3	4.6±0.3	1.3±0.3	4.7±0.3

Note. Mean log MPN 100 ml-1 ± standard deviation and Mean log CFU 100 ml-1 ± standard deviation

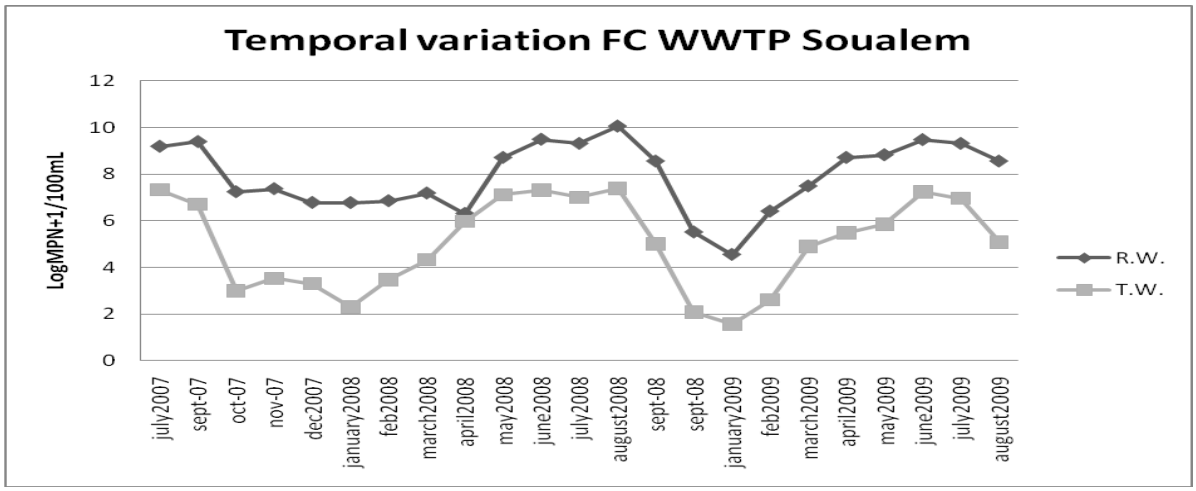
Results

Microbiological quality and effect of season of water samples. The table 2 summarizes the results of the bacterial load and the cumulative rate reduction at the entrance and exit of the two stations during the 48 months of sampling;

Temporal variation is illustrated in graphs 1 and 2. The rate abatement varies between 3 and 4 log units for WWTP Settat, and between 2 and 4 log units for WWTP Soualem.



Graph 1. Temporal variation of fecal coliforms (FC) in raw and treated wastewater in WWTP Settat.



Graph 2. Temporal variation of fecal coliforms (FC) in raw and treated wastewater in WWTP Soualem

Table 3. Microbiological quality of crops analyzed

	For (*n*=30)		C.For	Cer(*n*=64)		C.Cer	Her(*n*=56)		C.Her	Veg (*n*=18)		C.Veg
	H.S. *n*=13	C.S. *n*=17	*n*=4	H.S. *n*=29	C.S. *n*=35	*n*=4	H.S. *n*=26	C.S. *n*=30	*n*=10	H.S. *n*=9	C.S. *n*=9	*n*=6
No. of samples positive F.C. (%)	9 (69%)	6 (35%)	2 (50%)	13 (45%)	7 (20%)	1 (25%)	14 (54%)	6 (20%)	3 (30%)	0 (0%)	0 (0%)	0 (0%)
Average load F.C. (log CFU g^{-1})	4.2±0.3	2.7±0.3	3.3±0.4	4.3±0.3	2.6±0.4	3.7±0.3	3.7±0.3	2.0±0.4	3.0±0.4	0.0	0.0	0.0
No. of samples positive E.C. (%)	6 (46%)	3 (17%)	0 (0%)	8 (27%)	4 (11%)	1 (25%)	9 (34%)	2 (6%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)
Average load E.C. (log CFU g^{-1})	3.6±0.3	2.0±0.3	0.0	3.4±0.3	2.4±0.3	2.4±0.3	3.6±0.3	1.9±0.3	1.2±0.3	0.0	0.0	0.0

Note. Mean log CFU 100 g^{-1} ± standard deviation

H.S.: Hot Season, C.S.: Cold Season; For: Forage, C.For: Control Forage, Her: Herbs, C.Her: Control Herbs, Cer: Cereals, C.Cer: Control Cereals, Veg: Vegetables, C.Veg: Control Vegetables; F.C.: *Fecal Coliforms*, E.C.: *Escherichia coli*.

Table 4. The *E. coli* serotypes found in each station

Serotypes	WWTP Settat			WWTP Soualem		
	Origin (no. of isolates)			*Origin (no. of isolates)*		
	Raw water (*n*=70)	Treated water (*n*=5)	Crops (*n*=3)	Raw water (*n*=74)	Treated water (*n*=6)	Crops (*n*=4)
O26: H8	-	-	-	5	0	0
O26 :H11	21	0	0	7	0	0
O55 :H8	-	-	-	7	0	0
O55 :H11	-	-	-	6	0	0
O55 :H7	-	-	-	7	0	0
O86 :H8	20	2	1	9	0	0
O111 :H8	7	0	1	5	0	1
O111 :H9	1	0	0	-	-	-
O119 :H7	-	-	-	8	0	0
O124 :H19	4	0	0	-	-	-
O124 :H25	-	-	-	6	0	0
O125 :H8	7	0	0	-	-	-
O125 :H9	7	0	0	7	2	1
O126 :H7	1	1	0	-	-	-
O126 :H8	-	-	-	3	0	0
O128 :H8	1	1	1	1	1	1
O128 :H9	-	-	-	2	2	1
O157 :H7	1	1	0	1	1	0

(-) Absence

Microbiological quality and effect of season of crops samples. Table 3 summarizes the results of the bacterial load of crops analyzed and controlt. With regard to the vegetables, no germ was detected in both seasons.

Serotyping results. During this study, 170 strains of *Escherichia coli* have been isolated from different samples (Table 4), all of these strains has been tested for hemolysis, the results showed that 144 (84.7%) strains were hemolytic.

The serotypes found in untreated wastewater are very diverse; however they are much less in samples of treated wastewater and crops. The number of serotypes identified in Soualem WWTP remains quite high (n=84) compared to that found in WWTP Settat (n=78) for waters and crops samples. There are serotypes that persist even after treatment in the two stations such as O86:H8, O125:H9, O128:H8 and O157:H7.

Table 5. Genotypic characteristics of STEC isolates

Pathovars	No positive /tested	Virulence genes detected by PCR			
		stx 1	*stx 2*	*eae* A	*hly* A
O26	0/33	-	-	-	-
O111	0/15	-	-	-	-
O128	0/11	-	-	-	-
O157	4/4	-	4	-	-

Molecular results. Table 5 shows the results of molecular assays. Sixty three pathovars were isolated: O26 (n=33), O111 (n=15), O128 (n=11) and O157 (n=4); Four were positive for stx2 gene by PCR, and they are all O157:H7. None of the four genes researched has been detected for the non-O157 strains.

Statistical analysis. For each organism, duplicate plates were enumerated and the means calculated; the mean log *(X)* value and standard deviation *(SD)* were calculated on the assumption of a log normal distribution. Statistical treatment of data is based on analysis of the correlation; performing calculations were performed using *XLSTAT* Software version 2011.1.05. Pearson's correlation coefficient (*r*) was used to show correlation between microbiological data on the one hand and rainfall and temperature on the other.

Discussion

The presence and removal of the fecal coliforms and E. coli were monitored over two different sampling periods, there were significant differences between the two seasons hot (April to September) and cold (October to March) for raw and treated water as shown in graphs 1 and 2, similar results to those obtained in our study were reported by other authors (Bezuidenhout et al., 2002) which showed that temperature, UV-light and rainfall are key factors of bacterial growth in water.

As shown in table 2, the abatement of fecal coliforms and E. coli is in the same order of magnitude, identical results were obtained in a study in India on the performance of stabilization pond with respect to the reduction of indicator bacteria (Ministry of environment & forests, 2008).

According to the results obtained, the rate of reduction varies between 3 and 4 log units at the Settat WWTP, contrary to the WWTP Soualem which has been a fall in the rate of reduction equal to 2 log units during the first year to stabilize at a rate of 3 log units, this can be explain for two reasons, first: the residence time applied in the WWTP Settat ranging from 40 to 46 days, against 22days for the WWTP Soualem , indeed several studies have shown the effectiveness of the storage and prolonged solar radiation in decontamination, during daylight, lethal solar radiation greatly accelerates the bacterial die- off; the ultraviolet light is the most lethal (United Nations, 2004); The second reason is the large number of ponds, 12 units which are all functional in the resort of Settat , while for station Soualem containing 6 units, one of the maturation ponds is non-functional; The barrier efficacy is thus determined by its 'weakest link', because when a treatment step consists of several parallel treatment units, the poorest performing unit will dominate the pathogen removal (Smeet et al., 2006).

Regarding the control of crops and fodder, the higher rates were obtained during the hot season ranging from 3.4 ± 0.3 and 4.3 ± 0.3 log CFU g^{-1}, while during the cold season charges range from 1.9 ± 0.4 and 2.7 ± 0.3 log CFU g^{-1}, this variation is due to the high temperature that promotes the growth of bacteria, the values obtained for control cultures ranged from 1.2 ± 0.3 to 3.7 ± 0.3 log CFU g^{-1}, bacteria detected in these samples can be explained by the fact that they are from the ground, in this sense, studies have shown that *E. coli* O157: H7 can be transmitted from a contaminated soil with compost or irrigation water contaminated with to crop plants such as lettuce (Ibenyassine et al., 2007). Analysis of samples of vegetables showed the complete absence of germ; According to another study, organic acids naturally present in fruits and vegetables or accumulated as a result of fermentation are relied upon to retard the growth of some microorganisms and prevent the growth of others; Some organic acids naturally found in or applied to fruits and vegetables behave primarily as fungistatic; while others are more effective at inhibiting bacterial growth; Acetic, citric, succinic, malic, tartaric, benzoic and sorbic acids are the major organic acids that occur naturally in many fruits and vegetables, the mode of action of these acids is attributed to direct pH reduction, depression of the internal pH of microbial cells by ionization of the undissociated acid molecule, or disruption of substrate transport by alteration of cell membrane permeability (Beuchat, 1998).

Pathogenic strains of *Escherichia coli* are an important cause of bacterial infections; In humans, strains of serotypes O26, O111, O128 and O157 are the foremost cause of urinary tract infections, and have been implicated in infections with Shiga toxin–producing *E. coli* (STEC) (Carl et al., 2002). Other studies have conducted a review of research on *E. coli* O157:H7 strains in water sources in South Africa and they did not find any evidence of Enterohemorrhagic *Escherichia coli* EHEC O157 virulence factors presence in the 96% of analyzed samples (n=196) (Müller et al., 2001); Several authors put emphasis on the importance of this kind of investigation, and the risk of the EHEC virulence factors could constitute, because there have been incidents involving different kind of waters sources drinking water and recreational lakes, bays or rivers due a fecal contamination; These evidences should

stimulate researchers to investigate *E. coli* O157 occurrence in different waters sources to provide data about this important emerging pathogen, special attention to wastewater sources with potential agricultural irrigation (Razzolini et al., 2002). *E. coli* O157:H7 has also been associated with a number of outbreaks from contaminated produce. In 2006, a large, multi-state outbreak of *E. coli* O157: H7 in the USA was linked to the consumption of fresh spinach and involved over 200 laboratory-confirmed cases (Wendel et al., 2009).

Conclusion

As presented here, the efficiency of this treatment system in removing the various bacteria is clear when the results obtained from the series of samples are considered, so it's possible to examine the potential risks of infection associated with the consumption of food crops that are irrigated with treated wastewater using a quantitative microbial analysis; it can be a tool to test the usefulness of international guidelines and standards for acceptable levels of pathogens in treated wastewater. These results revealed the importance of this research and also show how it is fundamental to continue these investigations to know better the frequency and circulation in the environment of these emerging pathogens. The irrigation techniques like drip systems, and the cessation of irrigation two weeks prior to harvest crops are suggested as good practices for minimizing risk of contamination (Choukr-Allah et Hamdy, 2005).

Acknowledgements

The authors are grateful to all collaborators in this study especially: i) Mr Hassoune Zakaria and all employees who work at the wastewater treatment plant in Settat and Soualem for their collaboration, ii) We would like to thank RADEEC for their collaboration, iii) Dr Timinouni and Mrs Karraouane Bouchra for their collaboration of the molecular analysis.

References

Badri S., Fassouane A., Filliol I., Hassar M. & Cohen N. 2011. Detection of shiga toxin- producing Escherichia coli in meat marketed in Casablanca (Morocco). Cellular and Molecular Biology, 15 Suppl: OL1476-OL1479.

Bezuidenhout C.C., Mthembu N., Puckree T., & Lin J. 2002. Microbiological evaluation of the Mhlathuze River, KwaZulu-Natal (RSA). Water SA, 28(3), 281-286.

Beuchat L.R., 1998. Surface decontamination of fruits and vegetables eaten raw: a review, Food Safety Issues, WHO, pp. 21-22.

Blanca Jiménez, Duncan Mara, Richard Carr, & François Brissaud. 2010. Wastewater Treatment for Pathogen Removal and Nutrient Conservation: Suitable Systems for Use in Developing Countries. Wastewater irrigation and health, 149-169.

Campos. 2008. New perspectives on microbiological water control for wastewater reuse. Desalination, 218, 34–42.

Carl, M. Schroeder, Jianghong Meng, Shaohua Zhao, Chitrita DebRoy, Jocelyn Torcolini, Cuiwei Zhao, Patrick F. McDermott, David D. Wagner, Robert D. Walker, & David G. White. 2002. Antimicrobial Resistance of Escherichia coli O26, O103, O111, O128, and O145 from Animals and Humans. Emerging Infectious Diseases, 8(12), 1409-1414.

Choukh-Allah, R. & Hamdy, A. 2005. Wastewater treatment and reuse as a potential water resource for irrigation. International center for advanced mediteranean agronomic Studies, Options mediteranéennes, Series A: Mediteranean seminars, 66, 101-124.

Ibenyassine, K., AitMhand, R., Karamoko, Y., Anajjar, B., Chouibani, MM., & M. Ennaji. 2007. Bacterial pathogens recovered from vegetables irrigated by wastewater in Morocco. Journal of Environmental Health, 69, 47-51.

Karch, H., & Bielaszewska, M. 2001. Sorbitol-fermenting Shiga toxinproducing Escherichia coli O157:H(-) strains: epidemiology, phenotypic and molecular characteristics, and microbiological diagnosis. Journal of Clinical Microbiology, 39, 2043-2049.

Ministry of environment & forests-A Govt. of India Organisation-. 2008. Central pollution control board Performance of Sewage Treatment Plants - Coliform Reduction. Control of urban pollution. Series: CUPS/ 69 /2008, pp.17-36.

Müller,E.E, Ehlers,M.M. & Grabow,W.O.K. 2001. The occurrence of E. coli O157:H7 in South Africa water sources intended for direct and indirect human consumption. Water Research, 35(13), 3085 – 3088.

Paton, A. W., & Paton, J. C. 1998. Detection and characterization of Shiga toxigenic Escherichia coli by using multiplex PCR assays for stx1, stx2, eaeA, enterohemorrhagic E. coli hlyA, rfbO111, and rfbO157. Journal of Clinical Microbiology, 36, 598-602.

Pollard, D. R., Johnson, W. M., Lior, H., Tyler, S. D., & Rozee, K. R. 1990. Rapid and specific detection of verotoxin genes in Escherichia coli by the polymerase

chain reaction. Journal of Clinical Microbiology, 28, 540-545

Razzolini M., Dd Santos, F., Matté, M., Matté G. & Günther, W. 2002. Occurrence of Escherichia coli O157 in raw and treated domestic sewage from stabilization ponds with potential reuse in agricultural irrigation. Congresso Interamericano de Ingeniera sanitariay Ambiental, Mexico, 27-31 Octobre.

Smeets Patrick, Luuk Rietveld, Wim Hijnen, Gertjan Medema, Thor-Axel Stenström. 2006. Efficacy of water treatment processes, In: Microrisk-Microbiological risk assessment: a scientific basis for managing drinking water safety from source to tap, p10.

United Nations environment programme Mediterranean action plan. 2004. Guidelines on sewage treatment and disposal for the Mediterranean region. MAP Technical Reports Series No. 152. Athens, p47.

Wendel, A., Johnson, M. D.H., Sharapov, U., Grant, J., Archer, J.R., Monson, C., Koschmann, T. & Davis, J.P. 2009. Multistate outbreak of Escherichia coli O157: H7 infection associated with consumption of packaged spinach, August–September 2006: The Wisconsin investigation. Clinical Infection Diseases, 48, 1079–86.