HEALTH RISK OF URBAN WASTEWATER REUSE IN LETTUCE IRRIGATION IN FEZ, MOROCCO

Hakima Talouizte[a], Mohammed Merzouki[a], Fatima Zahra El Hassani[a], Laila Bennani[b], Abdelhakim El Ouali Lalami[b], Khadija Bekhti[c], Mohamed Benlemlih[a]*

a. Laboratory of Biotechnology, Faculty of Sciences Dhar El Mahraz, University Sidi Mohamed Ben Abdellah, PO Box 1796, Atlas, Fez,Morocco.
b. Laboratory of Epidemiologiy, EL Ghassani Hospital, Fez, Morocco.
c. Laboratory of Biotechnology, Faculty of Science and Techniques, Fez, Morocco.
**: corresponding author.*
E-mail: merzoukimo@yahoo.fr

Abstract

In this study the health risk of urban wastewater reuse in lettuce (*Lactuca sativa*) irrigation was investigated. Samples were collected from wastewater and lettuce crops and analyzed for selected quality parameters of health significance. Results were compared to national and international guidelines for water use in irrigation. Wastewater COD averaged 1190 mg l^{-1}, while electric conductivity ranged from 1480 to 2012 µS cm^{-1} and was less than the Moroccan limit value of 8700 µS cm^{-1}. Faecal coliforms abundance of urban wastewater was high (2.1 10^7 CFU 100 ml^{-1}) compared to the WHO and Moroccan limits for water use in irrigation of 1000 CFU 100 ml^{-1} and 100 CFU 5000 ml^{-1}. Lettuce microbial load was 6.4 10^4 CFU g^{-1} for total coliforms and 3.2 10^4 CFU g^{-1} for faecal coliforms. In addition, *Salmonella typhi* was identified in lettuce and wastewater. Parasitological analysis of wastewater and lettuce showed the presence of protozoa cysts (*Entamoeba histolytica*, *Entamoeba coli* and *Giardia lamblia*) and helminthes eggs (*Ascaris sp*, *Trichuris sp*, *Strongiloides sp* and *Taenia sp*).

Keywords: *Wastewater reuse, Urban wastewater, Health risk, Irrigation, Lettuce*

INTRODUCTION

The demand for water is continuously increasing in arid and semi-arid countries. Therefore, application of sewage to agricultural land may provide an economical way to dispose the increasing amounts of wastewater generated in many areas of these countries. A recent estimation shows that around the world, there are at least 20 million hectares in 50 countries that are irrigated with raw or partially treated wastewater [1,2]. In Israel, Egypt, Tunisia, Greece, South Africa, Japan and a number of developing countries in South America, wastewater reuse is a primordial source for agricultural production [3]. The lack of financial and technical resources in many developing countries makes wastewater collection and treatment an interesting long-term future strategy [4]. Morocco, a semi-arid country, suffers from shortage in water supply for domestic, industrial and agricultural purposes. Therefore, reusing wastewater in agriculture is a common practice. Indeed, wastewater is a valuable source of plant nutrients and organic matter needed for maintaining fertility and productivity of arid soils. However, the reuse of wastewater for irrigation may potentially create environmental problems if not properly treated and managed [5,6]. The benefits of wastewater reuse may be limited by its potential health hazards associated with the transmission of pathogenic organisms from irrigated soil to crops, grazing animals and humans [7].

In addition, there is a concern about the accumulation of potentially toxic heavy metals such as Cd, Cu, Fe, Mn, Pb and Zn from both domestic and industrial sources. Maximum permissible concentrations of heavy metals in soils irrigated with wastewater are normally given as total concentrations [8]. Current guidelines do not specify the types of soils, plants and other factors having a bearing on how much heavy metals a plant can take up. It is also well known that polycyclic aromatic hydrocarbons (PAHs) are recalcitrant and mutagenic/ carcinogenic pollutants, and there is serious concern about their presence in the environment, especially their tendency for bioaccumulation in food chains [9,10]. Vegetables cultivated on the wastewater-contaminated soils may take up these pollutants in sufficient quantities to cause health problems for the consumers. According to Fryer and Collins [11] and Wild et al [12], several mechanisms, including uptake through transpiration stream, volatilization and subsequent deposition on leaves and sorption from direct contact with soil particles are responsible for the transfer of organic pollutants from soil to plant tissues.

In many areas of Fez city, untreated wastewater flows are diverted by farmers to small plots of vegetables and salad crops, grown for nearby urban markets. Such vegetables include carrots, lettuce, cabbage and others which are easily consumed raw as salad. The public health risks of using such contaminated streams for irrigation are obvious [13,14].

The main objective of this study is to assess the potential public health hazard of using urban effluents for lettuce (*Lactuca sativa*) production. Specifically, the study aimed at determining the effluent quality, its suitability for lettuce irrigation and to estimate the lettuce quality where wastewater is applied. Lettuce was chosen because it is regularly cultivated in farms and routinely used as uncooked green salad. The quantitative human health risk of consumption of contaminated lettuce was determined. Results were compared to the Moroccan and international guidelines for effluent use in irrigation and lettuce consumption.

2. Materials and methods

2.1. Description of the study site

The study site consisted in a suburban field of El Batha locality neer Fez-Morocco, which is the principal site of wastewater reuse in crops irrigation in Fez. The experimental area site position is 4° 98' West and 34° 05' North. The weather is typical Mediterranean, semiarid to arid, with an average rainfall of 420 mm year^{-1} and an average annual temperature of 20-24°C. The soil is a lime constituted by 51% of sand, 32.5% of silt and 16.2% of clay.

2.2. Water irrigation sampling

Wastewater samples were carried out from a wastewater storage basin using glass sterile bottles of 500 ml, stored at 4°C and taken to the laboratory for analysis [15]. The sampling and analysis of wastewater were carried out at a biweekly frequency.

2.3. Lettuce sampling

Composite samples of lettuce were collected from the study site. The sampling of lettuce leaves was conducted under sun-light. The plant samples were then packed into sterile plastic bags, stored at 4°C and taken to the laboratory for analysis.

2.4. Physicochemical analysis of water irrigation

pH, electrical conductivity and dissolved oxygene were determined at the studied site, using a Senti X 22 pH meter, WTW CF 92 conductimeter and an OXI 315i WTW Oxymeter respectively. Rest of the physicochemical parameters (total suspended solids (TSS), COD, BOD_5, total nitrogen (T-N), total phosphorus (T-P) and $N\text{-}NO_3^-$) were determined after bringing the samples in the laboratory of biotechnology, Faculty of Sciences Dhar El Mehraz Fez, using standard methods of Water and Wastewater analysis [16].

2.5. Bacteriological and parasitological analysis of water irrigation

2.5.1. Bacteriological analysis of water irrigation

Wastewater samples were screened out for the enumeration of faecal coliforms, total coliforms and faecal streptococci using the method of filtration with membrane filter (0.45 μm porosity) [16]. The volume of water filtered was 100 ml.

Wastewater samples were serially diluted in sterile distilled water before filtration. Colony counts were converted into log10 CFU l^{-1}. Concerning pathogens, it included isolation and identification of *Salmonella* and *Vibrio cholera* [16]. *Salmonella* identification included isolation of suspicious colonies (colonies lactose negative with black center) from Hektoen medium and their transplantation on Kligler agar medium and incubation at 37 °C for 24 hours. Biochemical identification of suspicious strains of *Salmonella* was done using API 20E system. *Salmonella* strains isolated and purified from wastewater were also sequenced using an automatic sequencer Brand: Applied (genetic analysis by capillary electrophoresis separation module). For *Vibrio cholera* identification, suspicious colonies sucrose positive in TCBS agar medium (with 1 to 2 mm in diameter) are transplanted on Kligler agar medium and incubated at 37 °C for 24 hours. Suspicious strains are submitted to biochemical identification.

2.5.2. *Parasitological analysis of wastewater*

For parasitological analysis, we used the method of Faust [16]. The supernatant collected after centrifugation at 1500 rpm for 1 to 2 minutes was diluted in solution of zinc sulfate at 33 % (1.18 Density). The surface layer of supernatant was taken over blade Malassez for identification and enumeration. The microscopic observation of oocysts, eggs, and parasites that were found on the samples was done using an Olympus microscope. The total number of helminths eggs and protozoa cysts per liter (N) in the wastewater sample was calculated using the following formula:

$$N = X/ P \times V / S$$

(*N*: number of eggs or cysts per liter; *X*: the number of eggs or cysts counted; *P*: suspension volume introduced into the cell numeration (ml); *V*: total volume of the suspension (ml); S: volume of water irrigation (1 liter)).

2.5. *Bacteriological and parasitological analysis of lettuce*

2.5.1. *Bacteriological analysis of lettuce*

10 g of lettuce leaves were weighed into sterile stomacher bags and homogenized with 90 ml of sterile peptone buffered water (BPW) for 2 min. Decimal dilutions (from 10^{-1} to 10^{-5}) were prepared from the suspension of BPW. One milliliter of each dilution was applied in the center of sterile Petri dishes then 15 ml of Nutritive agar medium (Oxoid, England) previously melted were well mixed with the inoculum for enumeration of total mesophilic aerobic bacteria. Plates were incubated at 37°C for 24 h. Likewise, 1ml of each decimal dilution was mixed with 15 ml of Desoxycholate Lactose agar medium (Oxoid, England) previously melted to count total coliform and faecal coliform. Plates were incubated at 37°C and 44°C for 24 h for total coliform and faecal coliform respectively. Baired Parker agar medium (Oxoid, England) was used for the enumeration of *Staphylococcus aureus*, the plates were incubated at 37°C for 48h. Typical black colonies with a transparent outline were enumerated and colony counts in 1 g sample were determined. The identification of *Salmonella* in lettuce was done using the same identification protocol used for *Salmonella* identification in wastewater.

2.5.1. *Parasitological analysis of lettuce*

The parasitological contamination degree of lettuce irrigated with wastewater was determined using method of water washing lettuce described by Dsouli et al [17]. 20 grams of lettuce were washed with one liter of tap water, the washing water was then centrifuged at 2200 g for 15 minutes. The supernatant was carefully collected and was mixed with zinc sulfate solution using Faust technique described previously [16].

3. Results and discussion

3.1 Wastewater irrigation quality

3.1.1 Physicochemical quality of wastewater

The urban wastewater physicochemical caracteristics are presented in Table 1 (total number of samples, n = 8). Apparently, the wastewater samples collected from the studied site during the study period presented moderate temperature (average = 23 °C), neutral pH (average = 7.2) and a high electric conductivity (average = 1117 µS cm^{-1}). TSS in the urban effluent was low (47.8 mg l^{-1}). There was observed a high load of COD (average = 1190 mg l^{-1}) and high load of BOD (average = 887 mg l^{-1}). Total nitrogen and total phosphorous averaged 33 mg l^{-1} and 24.4 mg l^{-1} respectively. N-NO_3^- mean value was found to be 0.5 mg l^{-1}.

Physicochemical status of urban wastewater used in lettuce irrigation revealed a reasonably high load of pollution indicators. Temperature of effluent ranged from 19 to 28 °C which is below the limit set by the Moroccan guidelines for water use in irrigation (35 °C) [18]. It is reported that temperature affects the metabolic rates, electric conductivity and dissolved oxygen [16]. Generally, urban wastewater presented a neutral pH and ranged from 6.95 to 7.2. A pH range of 6.5-8.4 is desirable for effluent quality for irrigation according to the FAO guidelines [8] and Moroccan guidlines for water use in irrigation [18]. According to WHO [19], a pH range of 6,5-7,5 is the optimum for bacteria growth, while a pH<5 or a pH>8.5 destroys bacteria and can temporarily inhibit movement of heavy metals through the soil. Irrigation water with a pH outside the normal range causes a nutritional imbalance thereby affecting plant growth.

Table 1
Physicochemical characteristics of urban wastewater used in lettuce irrigation compared with Moroccan guidelines for water use in irrigation.

Parameter	Urban wastewater		Moroccan guidelines
	Average	Range	
Temperature (°C)	23	19-28	35
pH	7.2	6.95-7.2	6.5-8.5
E.C (µS cm^{-1})	1717.75	1328-2012	8700
TSS (mg l^{-1})	47.8	35-56.3	2000
COD (mg l^{-1})	1190	502-1612	
BOD_5 (mg l^{-1})	887	320-1134	
T-N (mg l^{-1})	33	21.5-46.11	
$N\text{-}NO_3^-$ (mg l^{-1})	0.5	0.13-2.03	50
T-P (mg l^{-1})	24.4	12.55-49	

Electric conductivity noted in urban wastewater was considerably high and ranged from 1328 µS cm^{-1} to 2012 µS cm^{-1}. Ayers and Westcot [20] recommend an electric conductivity of 0-2000 µS cm^{-1} for wastewater to be used for irrigation. According to Richards [21] calassification of water irrigation, electric conductivity of urban wastewater is belonging to the class III with high salinity and health risk. Foth [22] reported that the concentration of specific ions (which is mainly mesured by the EC) may cause an accumulation of one or more trace elements in the soil and plant and at a long-term cause plant phytotoxicity or build up in animal and human bodies. Concerning Moroccan guidelines [18], electrical conductivity of urban wastewater was less than the guideline value of 8700 µS cm^{-1}.

Urban effluent presented high load of organic matter. The ratio DCO/DBO_5 was 1.34 which is characteristic of urban wastewater [16]. This ratio can appreciate the degradability of organic matter. In addition to its adverse effects on the aquatic environment (oxygen consumption), the organic matter is benefic for irrigation by providing fertilizers.

Organic matter affects also soil chemical (mineral reserves), physical (soil aeration and water retention) and biological (nutrient for microorganisms and plants) properties and the overall soil fertility. We can say that urban wastewater is a good fertilizer for lettuce irrigation.

Urban effluent showed low concentration of $N\text{-}NO_3^-$ (average = 0.5 mg l^{-1}) and was below the Moroccan guideline limit for irrigation of 50 mg/l [18]. Those results are in agreement with the findings of El Halouani et al [23] who demonstrated that the oxided forms of nitrogen in urban wastewater exist in low to negligible concentrations. This is due to the low concentration of oxygen in wastewater.

In agriculture, phosphorus as nitrogen is a main constituent of living matter. It is closely related to plant growth and is an important nutrient. Hence, urban wastewater with a T-P range of 12.55-49 mg l^{-1} is a no negligeable source of phosphorus.

3.2.4. Bacteriological quality of wastewater

Variation of faecal coliforms (FC), total coliforms (TC) and faecal streptococci (FS) abundance in urban wastewater during study period is shown in Table 2. The mean values of FC, TC and FS abundances were found to be 2.1 10^7 CFU 100 ml^{-1}, 1.85 10^8 CFU 100 ml^{-1} and 1.1 10^7 CFU 100 ml^{-1} respectively. The WHO guideline [19] and the Moroccan guideline limits for water use in irrigation [18] allow a mean value of FC below 1000 CFU 100 ml^{-1} and 100 CFU 5000 ml^{-1} respectively. High coliform counts recorded are probably due to the high contamination of urban wastewater by human manure.

Table 2
Microbial load of urban wastewater used in lettuce irrigation.

TC (CFU 100 ml^{-1})		FC (CFU 100 ml^{-1})		FS (CFU 100 ml^{-1})	
Mean	Range	Mean	Range	Mean	Range
1.85 10^8	4.2 10^6-8.35 10^8	2.11 10^7	1.56 10^6-1.09 10^8	1.2 10^7	6.06 10^5-8.23 10^7

The ratio FC/FS of urban wastewater irrigation was around 1.88 (FC/FS>1), what suggests that the faecal pollution in urban wastewater has a human origin [16]. In addition, *Salmonella typhi* was identified during the study period in three samples. The WHO [19] and Moroccan guidelines of water irrigation [18] require total absence of *Salmonella* in these waters. Hence, urban wastewater in El Batha locality (Fez, Morocco) does not respect the guidelines of water use in irrigation. However, *Salmonella typhi* can be considered as a potential danger to human health. The danger resides in the persistence of this pathogen on crops irrigated with water containg this bacterium. Asano [24] reported that lifespan of *Salmonella* is 1 month in wastewater, 2 months in soil and 1 to 2 months on crops. Concerning *Vibrio cholera*, this pathogen was absent in urban wastewater during all the study period.

3.2.5 Parasitological quality of wastewater

Parasitological analysis of urban wastewater has revealed the presence of protozoa cysts and helminths eggs. Table 3, summarizes the qualitative results and shows the presence of various parasites such as *Ascaris* eggs, *E. histolytica* cysts and *Giardia* cysts in urban wastewater. The most common protozoa species identified in wastewater are *Entamoeba histolytica*, *Entamoeba coli* and *Giardia lamblia* with means counts of 5 10^3 cysts l^{-1}; 3.9 10^3 cysts l^{-1} and 1.2 10^3 cysts l^{-1} respectively.

Table 3
Qualitative results of parasitological analysis of urban wastewater

Pathogens	Species parasites	Urban wastewater
Protozoa	*Entamoeba histolytica*	+
	Entamoeba coli	+
	Giardia lamblia	+
Helminths		
Nematodes	*Ascaris sp*	+
	Ankylostoma sp	+
	Trichuri sp	+
	Strongiloides sp	+
Cestodes	*Taenia sp*	+
	Shistosoma mansoni	-
Trematodes	*Shistosoma haematobium*	-

(+: Presence -: absence)

Quantitative analysis has distingued three groups of helminths in wastewater samples: nematodes, cestodes and trematodes, with a clear predominance of nematodes. Helminths eggs identified during the study period are eggs of *Strongiloides sp*, *Ascaris sp*, *Trichuri spp*, *Ankylostoma sp* and *Taenia sp* with mean values around: 11.35 eggs l^{-1}; 5 eggs l^{-1}; 2.1 eggs l^{-1}; 1.7 eggs l^{-1} and 0.2 eggs l^{-1} respectively (Fig. 1). These results are substantially in agreement with the findings of Dssouli et al [17] in their study of wastewater reuse in irrigation of vegetables in Oujda city, Morocco. The results of Dssouli et al [17] also show a high prevalence of nematodes in wastewater irrigation with an average value of 29.31 eggs l^{-1} represented mainly by *Ascaris sp*, *Trichuri sp*, *Ankylostoma sp*, *Enterobius sp* and *Strongiloides sp*. It can therefore be concluded that the urban wastewater parasitological load is far to respond to WHO [19] parasitological limit (≤1 egg l^{-1}) and Moroccan limit [18] (total absence of parasites) of water use in irrigation.

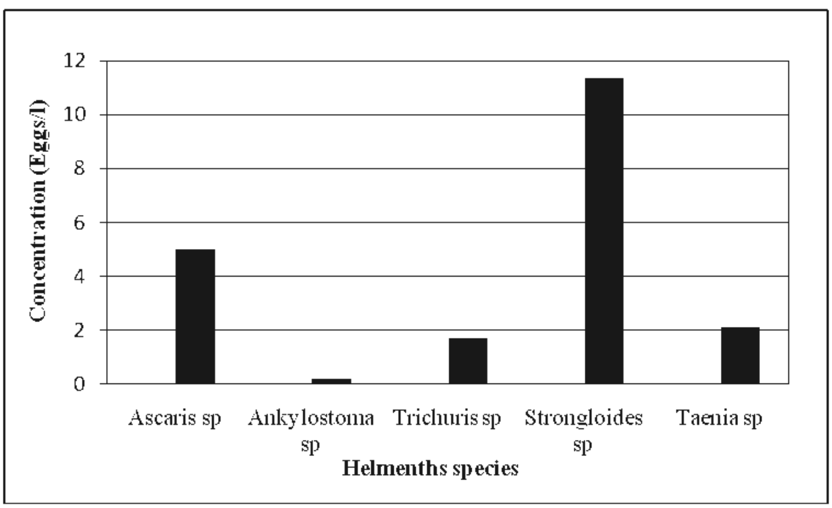

Fig. 1.
Concentration of helminths eggs in urban wastewater.

3.2 Lettuce quality

3.2.1. Bacteriological quality of lettuce

Abundances of total aerobic mesophilic microorganisms (TAMM), total coliforms (TC), faecal coliforms (FC) and *Staphylococcus aureus* in lettuce leaves are shown in Table 4. As urban wastewater, lettuce irrigated with this water presented high microbial load. TAMM, TC and FC mean abundances in lettuce irrigated with urban wastewater are 7.86 10^4 CFU g^{-1}, 6.4 10^4 CFU g^{-1}, 3.2 10^4 CFU g^{-1} respectively. Concerning *Staphylococcus aureus*, we found complete absence of this pathogen in lettuces irrigated with wastewater during the study period.

Table 4
Microbial load of lettuce irrigated with urban wastewater.

TAMM (CFU g^{-1})		TC (CFU g^{-1})		FC (CFU g^{-1})		*S. aureus* (CFU g^{-1})	
Mean	Range	Mean	Range	Mean	Range	Mean	Range
7.86 10^4	10^4-2.3 10^5	6.4 10^4	3,3 10^4-1,3 10^5	3.2 10^4	2.1 10^3-5.9 10^4	0	0

Salmonella typhi was isolated and identified from lettuce irrigated with urban wawtewater for three sampling times. Referring to Larpent guidelines [25] fixing the limit value of TAMM to 5 10^3 CFU g^{-1}, FC to 10 CFU g^{-1} and total absence of *Salmonella* in lettuce as a vegetable consumed crude, we can say that lettuce irrigated with urban wastewater is not conform to consumption and constitutes a major threat to consumer health. Those results are in agreement with findings of many authors. Solomon et al [26] noted that lettuce grown in soil contaminated with manure (10^4–10^8 CFU g^{-1}) or irrigated with contaminated water results in contamination of the edible portion of the lettuce plant. Moreover, authors' results suggested that the edible portion of the plant could become contaminated without direct exposure to a pathogen but through transport of the pathogen into the plant by the root system [26]. Goyal et al [27] established the relationship between coliform levels and the incidence of pathogenic bacteria like *Salmonella*. WHO [28] has recommended that crops to be eaten should be irrigated only with biologically treated effluent that has been disinfected to reach a coliform level of not more than 100 CFU 100 ml^{-1} in 80% of the samples [29,30]. The use of untreated wastewater and water supplies contaminated with sewage in irrigation has been implicated as one of the important sources of vegetables pathogenic contamination [27,30].

3.2.2. Parasitological quality of lettuce

Results have demonstrated the contamination of lettuce with protozoa cysts and helminths eggs consequently to urban wastewater application. The protozoa identified in lettuce are *Entamoeba histolytica*, *Entamoeba coli* and *Giardia lamblia* with mean values of 2.1 10^3 cysts g^{-1}, 1.3 10^3 cysts g^{-1} and 10^3 cysts g^{-1} respectively. The mean values of helminthes eggs in lettuce are about 11.01 eggs 100 g^{-1}. Fig. 2 presents the prevalance of the main helminths species identified. We noticed the dominance of *Strongiloides sp* with 76%, *Ascaris sp* in second position with 18%, in third position *Trichuri sp* and *Taenia* sp with 4% and 2% respectively. The mean abundances of helminthes eggs in lettuce are 2 eggs 100 g^{-1}, 8.4 eggs 100 g^{-1}, 0.41 eggs 100 g^{-1} and 0.2 eggs 100 g^{-1} for *Ascaris sp*, *Strongiloides sp*, *Trichuri sp* and *Taenia sp* respectively. These parasites are considered as pathogenic agents for humans. The consumption or manipulation of such contaminated agricultural crops is considered unsafe and might constitute a risk for farmers and the whole population [31]. These eggs are very resistant in the environment and they can survive in water, soil and crops for several months and years [32,2]. Bryan [33] observed that field vegetables were directly contaminated with irrigation water or indirectly by contact with contaminated soil. These results are in agreement with the findings of Dssouli et al [17].

The mean abundances of helminths eggs in lettuce irrigated with raw sewage in Oujda city is 10.5 eggs 100 g^{-1}. Many epidemiological studies have revealed parasitic infestations associated with raw wastewater reuse in irrigation [29,34,35,36]. Bryan [33] reported three epidemics of ascariasis in Germany and two of amoebiosis in the United States associated with food contamination by wastewater. Pound and Crites [37] demonstrated that the incidence of parasitic diseases in consumers of sewage irrigated crops was higher than that of a control population. Lettuce and strawberry were found to be relatively more heavily contaminated than the other crops. In addition, WHO [19], describes the presence of helminths, especially intestinal nematodes (*Asearis*, *Trichuris*, *Ankylostoma*) as the main injury for wastewater reuse in agriculture because of their low infective dose and their longterm survival in the environment. Hence, lettuce irrigated with urban wastewater in El Batha locality, Fez-Morocco is likely to be a vector of a large number of parasitic diseases and presents a real health danger for consumers.

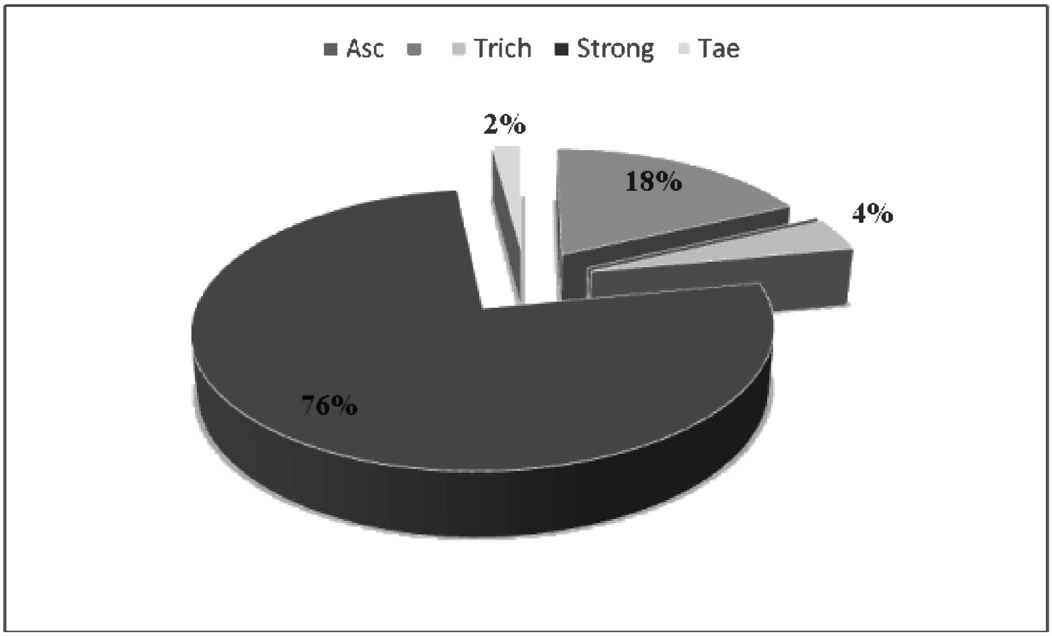

Fig. 2.

Prevalence of helminths eggs in lettuce irrigated with urban wastewater.

4. Conclusion

It emerges from this study that urban wastewater of Fez city is far to responde to the WHO and the Moroccan guidelines for irrigation water. Therefore, this wastewater should not be reused for irrigation of vegetables eaten crude and especially lettuce. The high bacterial load registered in lettuce leaves (3.2 10^4 CFU g^{-1} of feacal coliforms) is consistent with the one recorded in wastewater. The identification of *Salmonella typhi* in lettuce leaves and wastewater irrigation has been achieved simultaneously for three times. This result confirms the direct contamination of lettuce by wastewater irrigation. The presence of *Salmonella typhi* in lettuce leaves is a real danger for consumers health. Therefore it may cause typhoid fever and serious health complications for consumers. Moreover, parasites load in lettuce is important as in wastewater. The presence of helminths eggs (*Ascaris sp*, *Trichuris sp*, *Strongiloides sp*) is considered the main danger of lettuce leaves consumption. The danger resides mainly in the emergence of serious parasitic diseases such as ascariasis. So, sanitary quality of lettuce irrigated with urban wastewater of El Batha locality in Fez city, Morocco is very bad and presents a danger for public health. Therefore, the risk is intensified when lettuce is consumed without rinsing or not enough washing. As a result, wastewater treatment should be required to avoid any likely health risk for humans.

References

[1] I. Hussain, L. Raschid, M. Hanjra, F. Marikar, W. Van der Hoek, A framework for analyzing socioeconomic, health and environmental impacts of wastewater use in agriculture in developing countries. Working paper, Vol. 26. International Water Management Institute (IWMI). Colombo, Sri Lanka, 2001.

[2] N. Gupta, D.K. Khan, S.C. Santra, Prevalence of intestinal helminth eggs on vegetables grown in wastewater-irrigated areas of Titagarh, West Bengal, India. J. Food Control. 20 (2009) 942–945.

[3] E. Cifuentes, M. Gomez, U. Blumenthal, M.M. Tellez-Rijo, I. Romieu, G. Ruiz-Palasios, S. Ruiz-Valazco, Risk of *Giardia intestinalis* infection in agricultural villages practicing wastewater irrigation in Mexico. J. Am. J. Trop. Med. Hyg. 62 (2000), 388-392.

[4] M. Qadir, D. Wichelns, L. Raschid-Sally, P.G. McCornick, P. Drechsel, A. Bahri, P.S. Minhas, The challenges of wastewater irrigation in developing countries. Agr. Water Manage. 97 (2010) 561-568.

[5] A. Bahri, F. Brissaud, Wastewater reuse in Tunisia: assessing a national policy. J. Water Sci. Technol. 33 (1996) 87–94.

[6] B. Weber, Y. Avnimelech, M. Juanico, Salt enrichment of municipal sewage: new prevention approaches in Israel. J. Environ. Manag. 20 (1996) 487–495.

[7] L. Korentajer, A review of the agricultural use of sewage sludge: Benefits and potential hazards. Water SA, 17 (1991) 189–196.

[8] M.B. Pescod, Wastewater Treatment and Use in Agriculture. FAO Irrigation and Drainage Paper, Rome, 1992.

[9] Y. Jian, L. Yong, P.F. Peter, H.T. Yu, Photomutagenicity of 16 polycyclic Aromatic hydrocarbons from the USEP Apriority pollutant list. J. Mutat. Res. 557 (2004) 99–108.

[10] S. Khan, L. Aijun, S. Zhang, Q. Hu, Y. Zhu,. Accumulation of polycyclic aromatic hydrocarbons and heavy metals in lettuce grown in the soils contaminated with long term wastewater irrigation. J. Haz. Mat. 152 (2008) 506–515.

[11] M.E. Fryer, C.D. Collins, Model intercomparison for the uptake of organic chemicals by plants. J. Environ.Sci. Technol. 37 (2003) 1617–1624.

[12] E. Wild, J. Dent, J.L. Barber, G.O. Thomas, K.C. Jones, Anovelanalytical approach for visualizing and tracking organic chemicals in plants, Environ. J. Sci.Technol. 38 (2004) 4195–4199.

[13] P.S. Mead, P.M. Griffin, Escherichia coli O157: H7. The Lancet, 352, 1998.

[14] WHO, Guidelines for Drinking-Water Quality. Third edition, vol. 1: Recommendations. World Health Organization, Geneva, 2004.

[15] A. Azizi, M. Lamqaddam, M. Jad, Guide pour les activités d'hygiène du milieu en zone rurale. Fonds des nations unies pour l'UNICEF, Rabat, 1990.

[16] J. Rodier, C. Bazin, J. P. Broutin, P. Chambon, H. Champsaur, L. Rodi, L'analyse de l'eau: eaux naturelles, eaux résiduaires, eau de mer, 8ème ed. Dunod, Paris, 1996.

[17] K. Dssouli, H. El Halouani, A. Berrichi, L'impact sanitaire de la réutilisation des eaux usées brutes de la ville d'Oujda en agriculture : étude de la charge parasitaire en œufs d'helminthes au niveau de quelques cultures maraîchères. J. Biologie et Santé. 6 (2006) 51-56.

[18] Moroccan Standards of water use for irrigation. Minesterial Decree no. 1276-01, Morocco, 2002.

[19] WHO, Wastewater use in agriculture and aquaculture recommendations: to health wise. World Health Organization, a series of technical reports n° 778, Geneva, 1989.

[20] R.S. Ayers, D.W. Westcot, Water Quality for Agriculture. F.A.O Irrigation and Drainage paper 29, 1985.

[21] L. Richards, Diagnosis and improvement of saline and alkali soils. U.S. Salinity laboratory Stoff, Agr. Handbooks n° 60, 1969.

[22] H.D. Foth, Fundamentals of Soil Science, seventh ed. John Wiley and Sons, New York, USA, 1984.

[23] H. EL Halouani, B. Picot, C. Casellas, G, Pena, J. Bontoux, Elimination de l'azote et du

phosphore dans un lagunage à haut rendement. Revue des sciences de l'Eau, 6 (1993) 47-61.

[24] T. Asano, Wastewater reclamation and reuse. Water quality management library, 1998.

[25] J. Larpent, Techniques et documentations. Microbiologie alimentaire, Paris, 1997.

[26] E.B. Solomon, S. Yaron, K.R. Matthews, Transmission of Escherichia coli

O157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. Appl. Environ. Microbiol. 68 (2002) 397–400.

[27] S.M. Goyal, C.P. Gerba, J.L. Metnick, Occurrence and distribution of bacterial

indication and pathogens in canal communities along Texas Coast. Appl. Environ.

Microbiol. 34 (1977) 139–149.

[28] WHO, Reuse of Effluent : Methods of wastewater treatment and health safeguards.

World Health Organization, Technical Report Series No. 517. WHO, Geneva, Switzerland, 1975.

[29] U.J. Blumenthal, D.D. Mara, A. Peasey, G. Ruiz-Palacios, R. Stott, Guidelines

for the microbiological quality of treated wastewater used in agriculture: recommendations for revising WHO guidelines. Bull. World Health Org. 78 (2000) 1104–1116.

[30] H. Aysisek, U. Oguz, K. Karci

,Determination of total aerobic and indicator

bacteria On some raw eaten vegetables from wholesalers in Ankara, Turkey. Int. J. Hyg. Environ. Health. 209 (2006) 197–201

[31] O. Amahmid, S. Asmama, K. Bouhoum, The effect of waste water reuse in

irrigation on contamination of food crops by Giardia cysts and Ascaris eggs. Int. J. Food Microbiol. 49 (1999) 19–26.

[32] R.G. Feachem, D.J. Bradely, D. Garelick, D.D. Mara, Sanitationand disease: Health

aspects of excreta and wastewater management. John Wileyand Sons, Chichester, 1983.

[33] F.L. Bryan, Diseases transmitted by foods contaminated by wastewater. J. Food

Prot. 40 (1977) 45–56.

[34] R. M. Bradely, S. Hadidy, Parasitic infection and the use of untreated sewage for

irrigation of vegetables with particular reference to Aleppo. J. Syrian Public Health

Engineering. 9 (1981) 154–157.

[35] E. Cifuentes, U. Blumenthal, G. Ruiz-Palacios, S. Bennet, Biological health

risks associated with the composting of wastewater plant sludge. J. Water Pollut. Control Fed. 56 (1992) 1269–1276.

[36] S. Ruiz-Valazco, Risk of *Giardia intestinalis* infection in agricultural villages

practicing wastewater irrigation in Mexico. J. Am. J. Trop. Med. Hyg. 62 (2000), 388-392.

[37] C.E. Pound, R.W. Crites, Wastewater Treatment by land application, vols. I and

II. EPA-600/2-73-006, Raisanen, Washington, DC, 1973.