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Wastewater Reuse for Irrigation in Morocco: Helminth Eggs Contamination's Level of Irrigated Crops and Sanitary Risk (A Case Study of Settat and Soualem Regions)

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Abstract

The present study aimed to evaluate potential risk that humans and animals are exposed, when wastewaters (raw and treated) are reused for irrigation. Helminth eggs were researched in (n=120) wastewater samples (untreated: 60 and treated: 60); collected from two Wastewater Treatment Plant (WWTP) located at Settat and Soualem in Morocco, (n=69) crops samples (mint, coriander, *alfalfa* and cereals) collected from farmland, alongside around the WWTPs that have been irrigated by the treated wastewater and other crops (coriander, parsley and radish) issued from field trials. Samples were examined by a concentration method. The analysis of vegetable samples has revealed that 50% (35/69) of crops from farmland were contaminated by helminth eggs, with an average concentration of 8.4 eggs/100 g. In the experimental study, we have found helminth eggs's mean concentration of 35.62 eggs/100 g, 9.14 eggs/100 g and 0 eggs/100 g in crops irrigated by raw wastewater, treated wastewater and fresh water, respectively. Among helminth eggs detected in vegetables, we noted *Taenia* sp*, Ascaris* sp*, Toxocara* sp and *Strongyle* eggs. In regard to results of this study, irrigation with wastewater conduct to parasitological contamination of irrigated crops, several actions may be made in order to reduce sanitary risk associated to this practice.

Keywords: Wastewater; Crops; Helminth eggs; Sanitary risk

Introduction

Wastewater reusing in agriculture is an ancient practice that has been generally implemented worldwide [1]. In Morocco, this practice allows the irrigation of 7000 hectares, mainly continental cities [2]. A part of the fertilizing elements contents, wastewater is an important vehicle of biological agents [3] that can be transmitted by direct contact, or indirectly through consumption of crops irrigated with wastewater [4]. On the other hand, although fresh vegetables are an important part of a healthy diet [5], it is reported that there have been an increase in the number of food-borne illness reported cases linked to fresh vegetables, in recent years. In fact, vegetables can become contaminated with enteric bacterial [6], viral [7] and parasitic pathogens [5,8-10], throughout the process of planting to consumption. The extent of contamination depends on several factors that include, among others, use of untreated wastewater and water supplies contaminated with sewage for irrigation [5]. In this sense, the WHO consider that helminths present the highest risk of wastewater related disease transmission due to long latency periods, with soil stage required for transmission, long persistence in the environment, low infective dose, without practical host immunity [11], and recommends for unrestricted irrigation, water containing less than one nematode egg per litre [12]. It is estimated that as much as 60% of the world's population is infected with gut parasites (pathogen and nonpathogen), which may be transmitted through direct and indirect contact, food, water, soil, vertebrate and arthropod vectors, and, rarely from mother to offspring [13-15]. Several epidemiological studies around the world have revealed an excess of parasitic infestations associated with raw wastewater reuse in irrigation [16-18]. An earlier study conducted by Bryan [19] reported 3 epidemics of Ascariasis in Germany, associated with food contaminated by wastewater. In Morocco, Amahmid and Bouhoum [20] and Bouhoum and Amahmid [21] detected that the incidence of parasitic diseases in consumers of sewage irrigated crops was higher than that of the control population, the same thing was reported by previous research [17,18,22,23].

By cons, little studies into the recovery of parasites from vegetables irrigated by RWW have been conducted in Morocco, and even less from those irrigated by TWW. Studies done show that the prevalence was high in examined vegetables irrigated by RWW, and parasites such as *Ascaris, Taenia, Moniezia, Trichuris, Capillaria* and *Toxocara* eggs have been reported [8,24-29]. The prevalence of intestinal parasites such as *Ascaris lumbricoides*, among the inhabitants of Settat city, Morocco were reported by El Kettani et al. [30], however, the parasitic prevalence in vegetables was still undetermined in this region. The aim of this study was to assess the wastewater agricultural reuse's risk on the parasitological quality of crops. Helminth eggs were researched on vegetable products collected in spreading fields in Settat and Soualem regions, and other vegetables issued from field trials realized in Settat WWTP.

Materials and Methods

Detection of helminth eggs in samples of water

A total of 120 wastewater samples (untreated: 60 and treated: 60), were collected from two Wastewater Treatment Plant (WWTP) located at Settat (33°00'N, 7°37'W) [31] and Soualem (34°26'N, 5°53'W) [32], in Morocco, to assess their contamination level with helminth eggs. The

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treatment of raw water adopted for the stations were natural lagoons; (n=16) samples of freshwater were also analyzed for control test in the experimental study.

Volumes were analyzed are 1 L for raw wastewater samples and 5 L for both treated wastewater and fresh water samples. The samples were then decanted in the laboratory for 24 hours, and the sediment recovered (100 to 300 ml) was centrifuged for 15 min at 1200 rpm (revolutions min^{-1}). The identification of helminth eggs was carried out at magnifications 100 (in register) in Mac Master counting cell, after concentration, following the technique of Arther et al. [33], with the use of Sheater's solution as flotation liquid.

Detection of helminth eggs in vegetable samples from farmland

Crops samples (n=69) directly irrigated by treated wastewater were collected from farmland, alongside around Settat and Soualem wastewater treatment plants, sampling was done according to the availability of such crops; between January 2009 and December 2010. The samples were transported to the laboratory in sterile plastic bags for parasitological analysis. Portion of vegetables (200 g) were carefully washed with tap water, and the washing water was screened, then left for 24 h for sedimentation to take place. The top layer was discarded and the remaining washing water centrifuged at 1200 rd/min for 15 min. The supernatant was discarded, the residue carefully collected and examined by following the technique of Arther et al. [33]. Microscopic observation was performed in a Mac Master counting cell at 100-fold magnification.

Detection of helminth eggs in samples of experimental study

Crop samples: Our study also included experiments with three types of water, raw wastewater taken at the entrance of Settat Wastewater Treatment Plant (WWTP), treated wastewater taken at the exit of the WWTP, and fresh water. Irrigation waters were analysed to determine helminth eggs concentrations.

A total of 9 plots of 3 m^2 each were cultivated by coriander, parsley and radish. Crops irrigation was done by gravity mode, with a water volume of 60 to 80 L per plot.

The technique used for detection of helminth eggs is the same as described for samples from farmland, except for radish's samples, because of its developing in the ground, radish tubercles were removed from the ground, weighed (200 g), then have been subject to mechanical treatment by brushing and washing with tap water, after that, the same protocol previously cited is followed.

Soil samples: For soil analysis, 10 samples of 100 g were collected from each plot in plastic bag, then three sub samples of 10 g were analyzed. Like other samples, the technique of Arther et al. [33] is followed with the use of Sheater's solution as flotation liquid.

Results

Crops from Field

In order to assess the parasitic risk associated with agricultural reuse of treated wastewater, we analyzed crops taken from fields (around Settat and Soualem WWTPs) where the treated wastewater is used for irrigation.

Water analyzing results: Treated wastewater samples (n=60), taken at the outlet of Settat and Soualem WWTPs, have been analyzed. The average concentration of helminth eggs was of 0.13 eggs/L; represented by *Ascaris* sp. for nematodes, *Taenia* sp., and *Hymenolepis* sp for cestodes and digestive *Strongyle* eggs.

Crops analyzing results: A total of 69 samples of crops collected between January 2009 and December 2010. 50% (35/69) were contaminated by different intestinal parasites, with average concentration 8.4 eggs/100 g.

Table 1 shows kind of contaminations in different crops collected.

Most frequency of pathogen parasites in vegetables was found in coriander (66.7%), *Ascaris* sp. and *Toxocara* sp. were identified in 33% each ones. Followed by mint, that 22.2% of samples were contaminated. For the *alfalfa*, 14% of samples analyzed were positives of *Taenia* sp., and concerning the cereals, no samples were contaminated by pathogen parasites.

For *Strongyle* eggs, they were observed in the four crops category analysed, 42% of samples were positives of them. The higher prevalence was in coriander (66.7%) and *alfalfa* (57%).

Experimental study

Water analyzing results: The experimental study has confirmed that irrigation of crops by RWW lead to contamination, and it reduces when TWW is used, and decrease more and more if using fresh water.

Figure 1 shows helminth eggs contamination levels in different types of water, which have been used for irrigation in the experimental study.

It is noted that helminth eggs were found at mean concentration of 8.98 eggs/L and 0.13 eggs/L in RWW and TWW, respectively. Fresh water samples were negatives for helminth eggs.

Crops and soils analyzing results: The average concentration of pathogen helminth eggs was of 1.07 eggs/100 g and 0.92 eggs/100 g in crops irrigated by RWW and TWW, respectively. *Strongyle* eggs were also decreased, they were found with respective mean concentrations of 34.54 eggs/100 g and 8.23 eggs/100 g.

On the other hand, the mean concentration of helminth eggs in soil obtained from fields irrigated by RWW and TWW was of 2 eggs/10 g and 1.67 eggs/10 g for pathogen helminth eggs, and 2eggs/10 g and 1egg/10 g for *Strongyle* eggs.

The analysis of crops irrigated by fresh water shows that they are not contaminated by helminth eggs. The same result was observed for soil samples analyzed.

Table 2 summarizes results obtained of analyzing crops and soil when RWW, TWW, or fresh water is used for irrigation. It shows that *Ascaris* sp. were isolated in crops irrigated by raw and treated wastewater at concentrations of 0.79 eggs/100 g and 0.33 eggs/100 g,

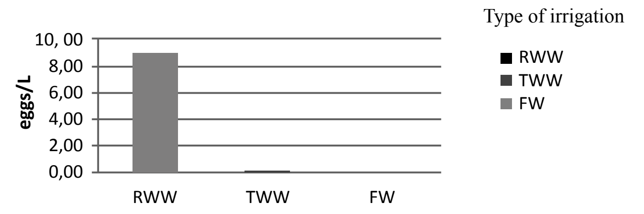

Figure 1: Occurrence of helminth eggs in waters used for crops irrigation in the experimental study RWW
RWW: Raw wastewater, TWW: Treated wastewater, FW: Fresh water

Crop Samples	n (%) positive							Total helminthes charges	
	Taenia sp	Ascaris sp	Toxocara sp	Cappilaria sp	Habronema sp	All parasite Helminthes	Strongyle eggs	C	n%
Mint (n=27)	3 (11.1)	0	0	3 (11.1)	3 (11.1)	6 (22.2)	6 (22.2)	4.33	9 (33.3)
Coriander (n=12)	0	4 (33.33)	4 (33.33)	0	0	8 (66.7)	8 (66.7)	2.17	8 (66.7)
Alfalfa (n=21)	3 (14.3)	0	0	0	0	3 (14.3)	12 (57.1)	2	13 (71.4)
Cereals (n=9)	0	0	0	0	0	0	3 (33.3)	0.33	3 (33.3)
Total (n=69)	6 (8.7)	4 (5.8)	4 (5.8)	3 (4.4)	3 (4.4)	17 (24.6)	29 (42)	8.38	35 (50.7)

n=number of positive samples; % of positive samples; C: mean concentration (eggs/100g)

Table 1: Prevalence of intestinal parasites in vegetables from field.

	C(eggs/100g)												C(eggs/10g)		
Helminths Eggs	Parsley			Coriander			Radish			Mean in Total Crop			Soil		
	PR	PT	PF	PR	PT	PF	PR	PT	PF	PR	PT	PF	PR	PT	PF
Ascaris sp.	2.12	0	0	0	0	0	0.25	1	0	0.79	0.33	0	2	1.67	0
Toxocara sp.	0.85	0	0	0	0	0	0	0.5	0	0.28	0.17	0	0	0	0
Moniezia sp.	0	0.25	0	0	1	0	0	0	0	0	0.42	0	0	0	0
Total Helminthes Parasite	2.97	0.25	0	0	1	0	0.25	1.5	0	1.07	0.92	0	2	1.67	0
Digestive Strongly Eggs	14.85	5.25	0	10.53	2.8	0	78.25	16.63	0	34.54	8.23	0	2	1	0
Total	17.82	5.5	0	10.53	3.8	0	78.25	18.13	0	35.62	9.14	0	4	2.67	0

PR: Plots irrigated by raw wastewater; PT: Plots irrigated by treated wastewater; PF: Plots irrigated by Fresh water

Table 2: Occurance of Helminthe eggs in Experimental study crops

respectively. *Toxocara* sp. with respective averages of 0.28 eggs/100 g and 0.17 eggs/100 g. *Moniezia* sp. were discovered only in parsley and coriander irrigated by treated wastewater, with a mean concentration of 0.25 eggs/100 g and 1 eggs/100 g, respectively. For soil samples, from pathogen helminth eggs, *Ascaris* sp. was identified when RWW and TWW were used with mean numbers of 2 eggs/10 g and 1.67 eggs/10 g.

Discussion

The consumption of raw vegetables plays an important role in the transmission of parasitic contaminations [34]; their recovery in vegetables used as the source of contamination, may be helpful in indicating the incidence of intestinal parasites among a community. A study realized by El Kettani et al. [30] about 3 villages in Settat region showed that prevalence of intestinal helminthiasis in a group exposed to RWW was 4.7%. The helminths identified were mainly *Ascaris lumbricoides*, with a prevalence of 4.2%. In our survey, 50.7% of crops obtained from areas in Settat and Soualem's regions irrigated by TWW were contaminated by helminth eggs, with an average concentration of 8.4 eggs/100 g. In Morocco, some authors in their studies about crops irrigated by RWW reported a values of 2 eggs/kg [35], around 4 eggs/100 g in crops got from market [8], 32 eggs/kg in *alfalfa* [36], and loads varying between 10.5 and 4.65 eggs/100 g have been noted by Dssouli [37]. Helminth eggs loads in mint, coriander, *alfalfa* and cereals have respective values of 4 eggs/100 g, 2.2 eggs/100 g, 2 and 0.3 eggs/100 g of fresh weight. Our result collaborate with those of Idrissa et al. [8], who reported that mint had the higher helminth eggs contamination load. It can be explained by the dense foliage of mint that protects eggs of parasitic cope from hostile environmental conditions, such as sunlight, desiccation and wind [8]. On the other hand, the whole development of the mint is at ground level, and thus, the leaves of mint are in direct contact with raw sewage, at each irrigation. In addition, the morphology of the mint leaves overlap on each other, protecting the eggs of helminths against unfavorable conditions.

Bryan [19] indicated that field vegetables are directly contaminated by irrigation water or indirectly by contact with soiled ground. That is confirmed by our experimental study results where crops irrigated by raw wastewater have highest helminth eggs contamination's levels, they are of 34.54 eggs/100 g, crops irrigated by treated wastewater loads are smaller (8.23 eggs/100 g), and we did not found any helminth eggs in crops irrigated by fresh water.

It is clear that wastewaters used for irrigation are the source of this pollution. Our work is in agreement with those of Peterson et al. [38], during their study about the contamination of crops irrigated with raw wastewater and drinking water, partly or totally processed, and those of Idrissa et al. [8], when they asses the parasite loads contamination of crops irrigated with waste water.

On the other hand, the analysis of the experimental study results shows that type root crops have higher helminth eggs loads than crops upright. Indeed, comparing the helminth eggs levels of the three cultivated crops showed that radish have higher charges than those recorded for the parsley and coriander.

This is explained by the facts that: i) for the cultivation of parsley and coriander, the leaves are off the ground and are only partially in contact with the irrigation water. In addition, this type of plant is exposed to multiple effects of solar radiation, desiccation, wind and leaching by rainwater; all these factors contribute effectively to the reduction of their content of helminth eggs. These results are in accordance with those reported by several authors as Bouhoum [39], about the persistence of helminth eggs on *alfalfa*, Firadi [10] in his study about contamination of soil and crops and El Hamouri [40] in his study about the helminth eggs disappearance's kinetics on *alfalfa*. ii) for the cultivation of radish, we know that edible organ of the plant is the underground part (tubercles). The high loads recorded are mainly due to the microclimate at this level, which puts helminth eggs protected from solar radiation and desiccation. In addition, at the tubercles, soil humidity is sufficient to maintain the survival of helminth eggs [41]. Norman et al. [42] and Khallaayoune and Fethi [9] reported that wet soils, low temperatures, solar radiation and high organic matter content increases the persistence of helminth eggs. These conditions are combined at radish tubercles. Also, other authors have reported that the underground part of the plant is the most contaminated by helminth eggs, because it is always in direct contact with wastewater during irrigation cycles [10,37,40]. Khallayoun et al. [3] reported that helminth eggs can survey more time in roots than in vegetables.

The raw wastewater used in irrigation in the experimental study does not respond the WHO standard, it exceeds the value recommended (<1 helminth egg/l) [12] for unrestricted irrigation, and therefore, must not be used for irrigation of green leafy vegetables, which are generally eaten uncooked. But, our result shows too that irrigation with treated wastewater having a value <1 helminth egg/l is also unsafe for their reuse for this type of crops. This result collaborates with recent epidemiological research study which shows that a limit of <0.1 helminth egg/l is needed, if children under 15 years are exposed to this wastewater [1,43].

Among identified helminth eggs in analyzed crops are those of *Taenia* sp, *Ascaris* sp, *Toxocara* sp, Cappilaria sp and *Strongyle*s eggs, in fact several authors reported that fresh vegetables can be agents of transmission of helminths eggs such as those cited above [1,5,15,44,45].

In the soil, *Ascaris* and *Strongyle* eggs were the parasites identified; according to several authors the latest ones may be have natural origin [46], or can be parasites of animals since water abattoirs are also routed to the station settat; By cons, *Ascaris* eggs owe their presence in crops, irrigated soil with RWW and TWW to the trilamellaire structure of the shell, that allows them a high resistance to weather conditions and physico-chemical environment for long-term [42]. The absence of cestode eggs in soil is due to their rapid destruction in the environment, because of their fragility and high vulnerability to unfavorable climate [47].

Conclusion

The state of unhealthiness caused by agricultural reuse of untreated wastewater in Settat and Soualem regions is a serious danger to people, animals and environment. These results clearly illustrate the sanitary and environmental risk associated with the reuse of raw wastewater. This risk would be even higher if the crops are eaten raw. So, prevention of diseases associated with wastewater would not be limited then to prohibit their reuse in the raw state or the installation of wastewater treatment plants but the restriction to irrigate crops.

Vegetables have an important role in the transmission of intestinal parasites, it is so necessary to improve the sanitary conditions of these kinds of food. Consumers have to apply good disinfection for crops in order to reduce their contamination.

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