J. Mater. Environ. Sci. 7 (5) (2016) 1614-1623
ISSN : 2028-2508
CODEN: JMESC

Evaluation of the qualitative and quantitative of parasitic load and Environmental Risks of Raw Sewage Rejected on the Coast of the City of El Jadida (Morocco)

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Received 24 Dec 2015, Revised 13 Feb 2016, Accepted 28 Feb 2016
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Abstract

The main objective of the study was the assessment of the prevalence and the identification of the parasitic load of Raw Sewage Rejected in the seashore of El Jadida City. The results showed a variety of parasite species with following average concentrations: *Hymenolepis nana* (4.21 eggs/L), *Taenia sp*. (3.6 eggs/L), *Moniezia expansa* (3.02 eggs/L), *Ascaris lumbricoides* (14.71 eggs/L), *Capillaria sp.* (5.92 eggs/L), *Trichuris trichiura* (4.13 eggs/L), *Ankylostome sp.* (6.01 eggs/L), *Enterobius vermicularis* (1.13 eggs/L) *Necator americanus* (4.3 eggs/L), *Nematodirus sp.* (2.07 eggs/L) and *Strogyloides sp.* (3.63 eggs/L). Station S1 and S3 (Lower Town station and AB station respectively) have the highest level of parasitic load, while, station S2 (Station El Manar) had a lower level of contamination. The distribution of parasitic helminth eggs varies with the size of the urban and socio-economic level of the urban population connected to each station.

Keywords: Assessment, Parasitic contamination, Wastewater, Helminth Eggs, El Jadida, Morocco.

1. Introduction

Sewage is the major source of harmful microorganisms including bacteria, viruses and parasites. The wastewater contains several pollutants like chemicals and microorganisms [1, 2]. This contamination is directly evident in serious intoxication, skin problems, and intestinal parasites [3, 4]. The inadequate disposals of urban waste residues result in serious contamination of soil, ground water [5-8] and agricultural crops and consequently affect both human and animal health adversely [9, 10].

In the context of Morocco water, the wastewater potential should be considered a valuable resource to develop within a framework of controlled reuse. Indeed, treatment of these unconventional resources would protect the receiving environment and offer the potential of significant recovery. According to the Department of Environment Moroccan [11], 25,000 Ha could be irrigated around the coastal towns around 2020.

The annual volume of wastewater discharges tripled from 148 to 370 million m^3 from 1960 to 1990. These releases will reach 500 million m^3 in 2000 and 900 million m^3 in 2020 (Superior Council of Water and Climate,

1994). This is explained by the increase in the urban population, increasing supply and individual consumption of drinking water as well as the extensive use of water by industry.
Given the importance of parasitic contamination in wastewater, this research has focused on the identification and quantification of helminth eggs in raw sewage of the city of El Jadida. We propose in the present study to make a qualitative and quantitative evaluation of the parasitic load of the wastewater.

2. Materials and methods

2.1. The study area

The city of El Jadida is the second industrial pole of Morocco is located on the Atlantic coast of Morocco between Casablanca (90 km southwest of Casablanca) and Jorf Lasfar (one of the largest ports in Africa) (Fig. 1). It covers an area of 2480 hectares, with latitude of 27 m and it has 4 urban districts [12]. It is delimited to the north by the Atlantic Ocean and to the east by the rural municipality Haouzia. To the west of El Jadida, the rural commune found Mly Abdellah and the Atlantic Ocean and south by the rural commune Oueld Hcine.

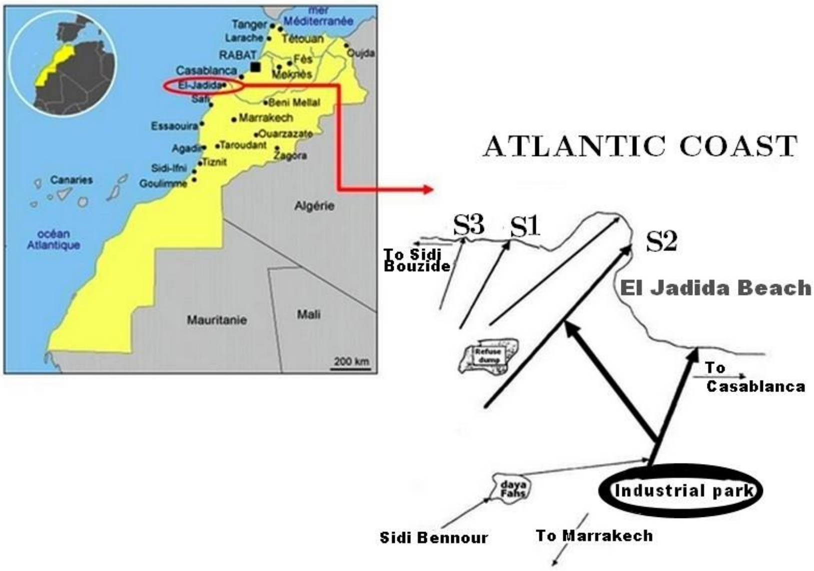

Figure 1: Location of sampling sites in the study zone: S1 (Lower town station), S2 (Station El Manar), S3 (AB station)

2.2. Sampling of Urban Wastewater

Two wastewater samples were collected at each station (the 1st and 15th of each month of 2014) between 8 h and 10 h morning. This sampling will allow determining afterwards the variation in concentrations of the seasonal load helminth eggs in each station. At these times, the domestic wastewater discharges are more important due to the abundance of household activities [13] and the two samples were used to average the concentrations of the parasite load per month. At each sample, 5 L of sewage was collected by the station using sterile vials PVC. These samples were fixed at 10 % formalin, labelled and transported to the laboratory Maghreb Rabat in Morocco for parasitological analysis.

2.3. Concentration of Parasitic Elements

The concentration of parasitic elements was made according to the method Bailenger [14]. After decantation of samples 24 h, the pellet was transferred to tubes and centrifuged for 15 minutes at 1000 rev/min. The resulting pellet was treated with a buffer solution acetoacetic to pH 4.5 by adding an amount equal to the volume of the pellet. Then, ether is added to an amount equal to twice the volume of buffers and the mixture is stirred for several minutes by using a vibrating Vortex-type mixer. The resulting mixture was further centrifuged at 1000 rev/min for six minutes. After the last centrifugation, the sample has three distinct phases. Once the supernatant removed, the pellet was suspended in a solution of zinc sulphate ($ZnSO_4$) 33% of volume equal to five times the pellet.

2.4. Microscopic Examination

The identification of helminth eggs was carried out at magnifications x100 (in register) after concentration by following the technique of Amir H. Mahvi [16] with the use of Sheater's solution as flotation liquid. We opted for this technique to its ease of implementation, low cost and risk for the handler and reliability. Microscopic observation of helminth eggs was based on the size, form and content of such eggs in accordance with the bibliographic descriptions. In case we could not identify the species has been limited only to identify gender [15, 16].

2.5. Enumeration of Helminth Eggs

The eggs were counted using the McMaster slide with a grid (McM 0.15 mL). The total number of helminth eggs (N) per litre of wastewater is calculated from the formula [16]:

$$N = (A*X) / (P*V)$$

N = Number of eggs per litre of wastewater;
A = Number of eggs counted in the McMaster slide;
X = Volume of the final product (ml);
P = Volume of the McMaster slide (0.3 mL);
V = volume of the initial wastewater sample (litres).

2.6. Statistical Studies

The results of parasitological study of wastewater are analysed by a statistical comparison of averages. The data is captured and analysed on software Statistica 11. A comparison of the average is performed by the ($\chi 2$) test with a risk of error of 0.05. A test is being taken from a significance level of $p <0.05$.

3. Results and discussion

3.1. Qualitative characterization of the parasitic load of wastewater from the city of El Jadida

The parasitological analysis of all wastewater samples showed high concentrations of digestive strongyles larvae. These larvae are not addressed in this first study. Parasitological analyses of three wastewater stations helped highlight eggs from two groups of parasitic helminths: Nematodes and Cestodes and several digestive Strongyle eggs (Table 1).

Table 1: Parasitic helminths contain in raw sewage from the city of El Jadida ((+) Present; (-) absence)

			Stations sampling		
Classes	**Taxa**	**Acronyms**	***Station 1***	***Station 2***	***Station 3***
Cestodes	*Hymenolepis nana*	Hym	+	+	+
	Taenia sp.	Tae	+	+	+
	Moniezia expansa	Mon	+	+	+
Nematodes	*Ascaris lumbricoides*	Asc	+	+	+
	Capillaria sp.	Cap	+	+	+
	Trichuris trichiura	Tri	+	+	+
	Ankylostome sp.	Ank	+	-	+
	Enterobius vermicularis	Ent	+	-	+
	Necator americanus	Nec	+	+	+
	Nematodirus sp.	Nem	+	-	+
Strongyles	*Strogyloides sp.*	Str	+	+	+

3.2. Quantitative characterization of the parasitic load of wastewater from the city of El Jadida

In this study, parasitological monitoring of urban wastewater stations in the city of El Jadida shows that they are infected with helminth eggs with an average concentration of 28.08 eggs/L (± 12.3). These eggs are divided into two classes: Nematode and Cestode eggs with respective average concentrations of 22.98 eggs/L (± 16.6) and 5.10 eggs/L (± 4.3).

The average concentration in gastrointestinal strongyle egg is around 14.66 eggs/L (± 10.21). The mean concentrations found in various helminth eggs in wastewater at three stations are illustrated in Figure 2.

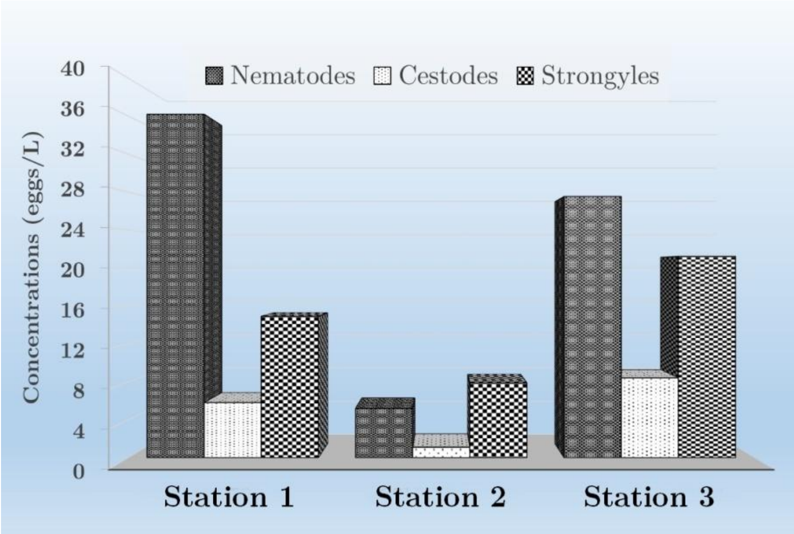

Figure 2: Distribution of the average concentration of helminth eggs parasites in raw sewage from the city of El Jadida

The results of parasitological loads of wastewater stations 1, 2 and 3 according to the seasons are summarized in figure 3.

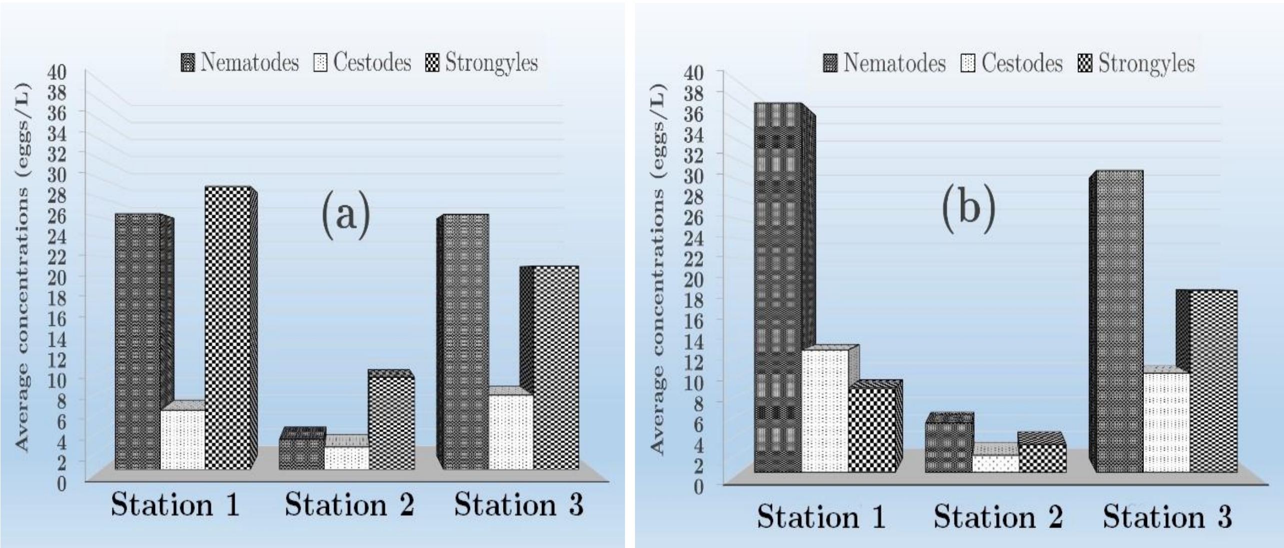

Figure 3: Seasonal variation in parasites loads of wastewater from the city of El Jadida ((a)= Autumn-Winter season; (b)= Spring-Summer season)

The statistical test (χ2) average concentrations in helminths eggs shows that the difference between the two sampling periods is significant only for strongyle eggs, at sewage station 3 ($P < 0.05$).

ISSN : 2028-2508
CODEN: JMESC

3.3. Study by Species of Helminths

The changes in average concentrations of helminth eggs in raw wastewater at the level of stations 1, 2 and 3 are shown in Figure 4.

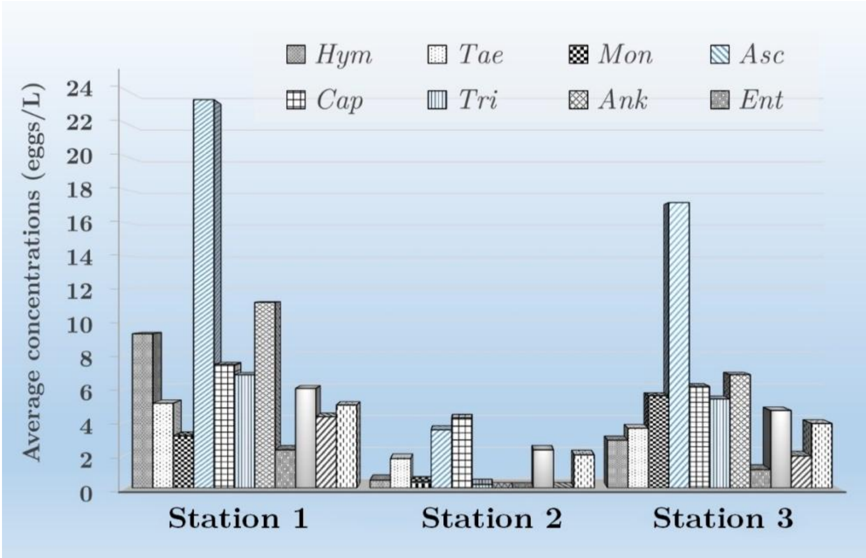

Figure 4. Average concentration of parasitic helminth eggs in wastewater from the city of El Jadida

The statistical test ($\chi 2$) mean concentrations in helminth eggs in various sewage stations shows that:

For Nematodes:

- Station 1 and Station 2: the difference is very significant with $p < 0.00001$
- Station 2 and Station 3: the difference is very significant with $p < 0.00001$
- Station 1 and Station 3: the difference is not significant.

For Cestodes:

- Station 1 and Station 2: the difference is significant with $p < 0.01$
- Station 2 and Station 3: the difference is significant with $p < 0.01$
- Station 1 and Station 3: the difference is not significant.

For Strongyles:

- Station 1 and Station 2: the difference is not significant
- Station 2 and Station 3: the difference is very significant with $p < 0.00001$
- Station 1 and Station 3: the difference is not significant.

The results of wastewater samples show very varied of concentrations in parasitic load. This data indicate faecal pollution conveyed by these raw sewage. In addition, our results are consistent with those of [17, 18] found in studies performed in El Jadida (Morocco). They are the same as those identified in the wastewater from the cities of Tehran and Isfahan in Iran [16]. The wastewater from the city of El Jadida are lightly loaded compared to wastewater some Latin American countries like Brazil with 1490 eggs/L [19] and very loaded compared to wastewater some African cities like Dakar in Senegal [20] and Yaoundé in Cameroon [21].

The qualitative analysis has identified three groups of helminths in wastewater samples: Nematodes, Cestodes and Strongyles, with a clear predominance of Nematodes in relation to others. No eggs of Trematodes were identified during the study period. The predominance of nematode eggs is also demonstrated in other studies carried out in Tunisia [22], France [23] and Morocco [24, 15].

It is particularly related to the lifestyle of the population in question whose eating habits (eating meat) do not support the transmission of cestodiasis [25]. Schwartzbrod and other authors [26-28, 22] reported that the eggs of the class of intestinal Nematodes are more resistant than those of Cestodes in wastewater. This predominance has been reported by several authors in Morocco [4.19 to 23] and elsewhere [22, 23].

The parasitic helminth, isolated from wastewater El Jadida are represented mainly by *Ascaris sp.*, *Capillaria sp.*, *Trichuris sp.*, *Enterobius vermicularis*, *Nematodirus sp.*, *Necator americanus*, *Ancylostoma sp.*, The Strongyles (*Strogyloides sp.*), *Taenia sp.*, *Hymenolepis nana* and *Moniezia sp.*, with a predominance of eggs of *Ascaris sp.* (Fig. 4).

This parasite diversity reported by other authors shows that the sources of contamination are original human and animal [29].

This study also highlighted the qualitative and quantitative seasonal variations of helminth eggs in wastewater from different stations in the city of El Jadida (Fig. 3). This change results in high contents of helminth eggs, mainly for nematode and tapeworm eggs, during the spring-summer and low levels of Autumn-winter period.

However, we note the very high levels of strongyle eggs during the fall-winter and low levels during the Spring Summer. These observations are consistent work [30, 15] in Marrakech; [31] in Rabat; [32] and [25] in Oujda; [33]; and [34] in Beni-Mellal.

However, Firadi [30] reported very high concentrations in helminth eggs during the Fall-Winter season in wastewater of Ouarzazate, this result is attributed by the author to the scarcity of rainfall in this arid city. In addition, several authors have reported that the difference in concentration between the two periods is due to the increase in the prevalence of parasitic worm infections in Spring [35, 36]. While the [37] reported that the abundance of helminth eggs during spring-summer is due to the conditions of temperature, humidity, oxygen favourable to the maturation of these helminths sunlight. Strongyle eggs are more abundant in wastewater samples of autumn-winter period at the station (3).

According to a survey conducted at the municipal slaughterhouse of El Jadida, was asked about the degree of infestation in cattle slaughtered at the abattoir. They are highly infested with these parasitic worms during the autumn-winter period. This is also been demonstrated in previous studies [25]. The $\chi 2$ test gave an F value of 6.74.

This difference is statistically significant ($P < 0.05$). Animals slaughtered in abattoirs during this period is highly infested. In particular, digestive strongyles are seasonal and infestations are particularly during the rainy season due to the high sensitivity of infective larvae of strongyles desiccation [38].

The average concentration of helminth eggs found in the wastewater of the city of El Jadida varies stations surveyed. The same observation was made by [17, 18] in El Jadida. Our results are similar to those found in Oujda by [25] and [32].

The comparison of results of parasitological analyses of different wastewater stations in the city of El Jadida, we can see that the station 1 shows the highest load with 32.2 eggs/L and 9.88 nematode eggs/L for Cestodes and station 3 second with 29.14 eggs/L for Nematodes and 8.38 eggs/L for Cestodes.

The station 2 has the lowest parasite load with 3.36 eggs/L for nematodes and 1.09 eggs/L for Cestodes. This content difference can be explained by the fact that the station 1 drains more than 70 % of the wastewater from the city and, therefore, the number of people connected to the station 1 is higher than that served by other stations 2 and 3. According [27], the content of helminth eggs in wastewater is strongly linked to factor in demographic development. The digestive strongyle eggs were also observed in the samples analysed station 3, the presence of these parasitic elements depends largely rejecting municipal slaughterhouse. Thus the resulting highly concentrated wastewater is in helminths eggs.

Moreover, the wastewater effluent may contain this protozoan cyst other than eggs intestinal helminths.

In addition, wastewater drained by the station 2 show load helminth eggs qualitatively and quantitatively very low compared with those of the stations 1 and 3.

Low levels of helminth eggs recorded at the station will be mainly due to the dilution by industrial effluents and the small number of inhabitants connected to the station 2.

Statistically, the difference in concentrations of helminth eggs is very significant for the nematode eggs between the stations 1, 2 and 3 ($P < 0.00001$). For cestode eggs, this difference is significant between the two stations 1 and 2 and between stations 2 and 3 ($P < 0.01$).

For eggs of strongyles, the difference is highly statistically significant with ($P < 0.00001$) only between the stations 2 and 3. It is therefore clear that the abundance of the parasite load in the wastewater station 1 and 3 compared to the station 2 originates on the differences in demographic and socio-economic status of each connected manifold surveyed populations. This was also reported in many studies worldwide [39, 40] and Morocco [41, 18, 42]. A study conducted in Yaoundé (Cameroon) showed that liquid sanitation affects the socio-health and environmental levels of planned housing area [40].

3.4. Potential Effects of Environmental Contamination

Moreover, the release of these urban wastewater (domestic and industrial) without prior treatment can have a significant environmental impact [12, 43]. These raw sewage causing contamination of receiving waters and consequently causing significant disturbance to residents, consumers and wildlife resources (Fig. 5) [44]. So the rational management of water resources and the construction of a sewage treatment plant in the city of El Jadida, will minimize health risks and environmental dangers of sewage.

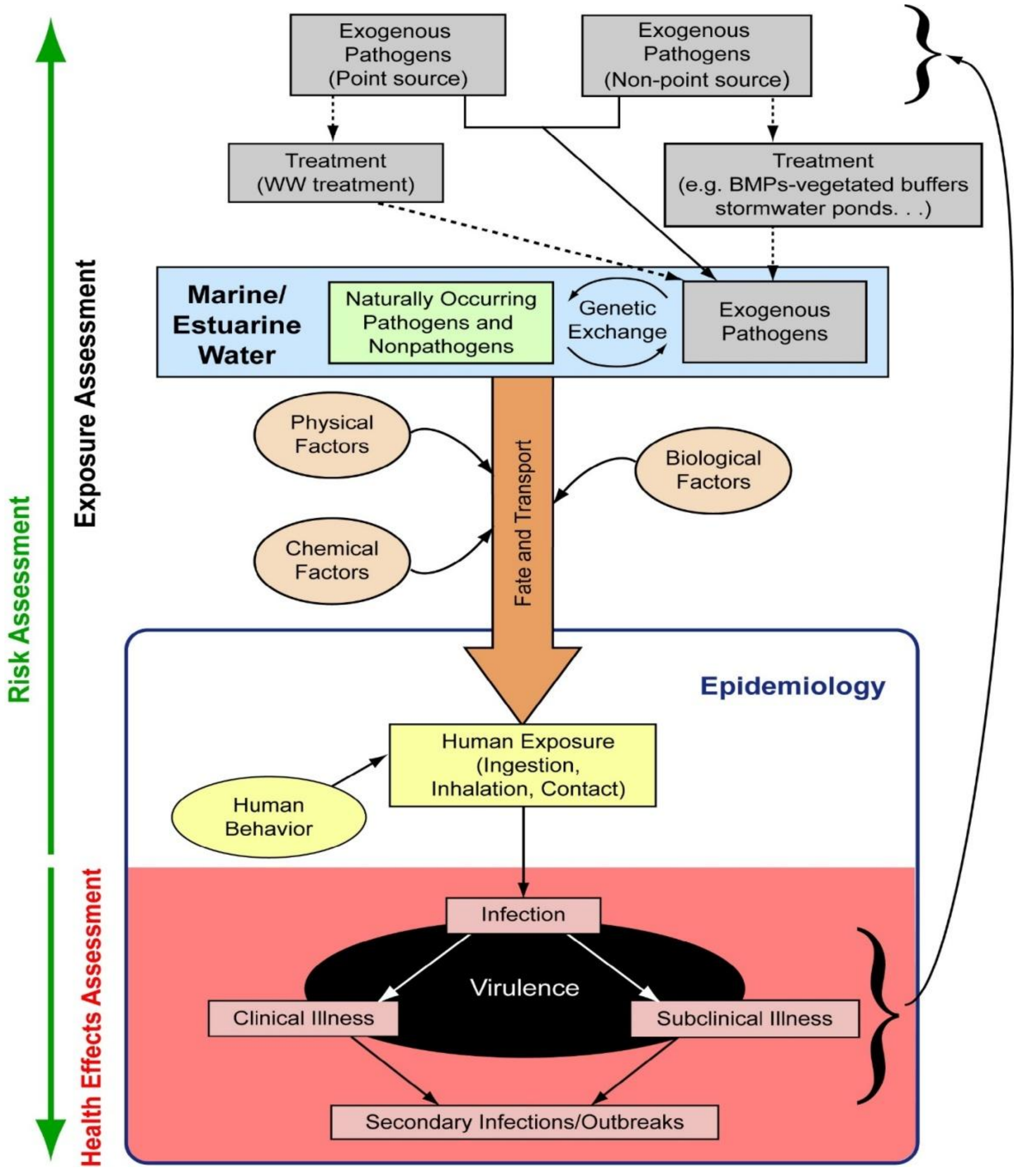

Figure 5: Relationships between pathogens, the environment and human health Relationships between pathogens, the environment and human health. WW treatment = wastewater treatment; BMPs = best management practices

Marine organisms, particularly marine mammals, can also be sensitive sentinel species to warn of impending human health problems from ocean-borne pathogens.

Causes of mass mortality events in marine mammals have included viruses, bacteria and protozoa [45]. Monitoring for either emerging or recurring health problems in marine animals may provide information that can be used as a measure of ocean health that could also indicate the potential for future human health issues. The ability to use marine mammals as sentinels for pathogens important to the ecosystem and human health requires appropriate tools and protocols to accurately test for and track those pathogens in sentinel populations and the ecosystem. In addition to being useful for detecting zoonotic diseases that can affect human health, marine mammals are also shown to be useful sentinel species for assessing health risks from natural toxins (i.e. algal toxins) and persistent chemical contaminants (e.g. polybrominated diphenyl ethers (PBDEs) and other organochlorines).

An estimated 75% of emerging infectious diseases are zoonotic [46], and anthropogenic influence on ecosystems appears to be a common factor in the emergence and re-emergence of zoonotic pathogens [47]. In the marine environment proper, bacterial, viral, fungal and protozoal pathogens that can infect humans have been detected in a range of marine animals, including pinnipeds, dolphins, cetaceans and otters.

A study cited in EPA's draught guidance document on water quality found that surfers and divers are at greater risk of illness from contact with contaminated beach water than are swimmers or waders. In addition, an epidemiological study in Santa Monica Bay found that there is an increased health risk when swimming within 400 yards of a flowing storm drain. In Southern California you will be hard pressed to find a stretch of surf that isn't near a storm drain [48].

Once pathogens enter the marine environment, they can be further concentrated by the action of the filter feeding organisms such as mussels, clams and oysters. Mussels and oysters, in particular, are implicated more than any other marine animal in seafood illnesses. Since they are sessile filter feeders inhabiting the benthic environment, they bioaccumulate both metal and organic contaminants [49, 50], as well as concentrate microbial organisms including human pathogens [51, 52]. The evaluation of the presence and distribution of pathogens among marine bivalves is critical to determining the present and future risk to human health by better understanding the nature of the interaction between pathogens and shellfish.

Conclusion

At the end of following performed on three stations urban areas of the city of El Jadida, the results of parasitological analysis of raw sewage show a large difference in the concentration and diversity of helminths parasites identified eggs.

The present study also draw the following conclusions:

The concentration of helminth eggs vary from one station to another and depending on drained areas, this is due to the size of the population connected to each manifold prospected and socioeconomic status of urban populations;

- Also, the parasite load in the wastewater of the city of El Jadida is a function of the sampling period, which shows that the concentration of raw sewage in eggs varied according to the season’s helminths;
- The contents of helminth eggs highlighted in the wastewater samples analysed are comparable to those encountered in previous work to the city of El Jadida [17, 18].
- The concentrations of eggs of parasitic helminths encountered in wastewater samples of El Jadida far exceed recommended by the World Health Organization [53] and the Norms and Standards Committee Moroccan water standards for the crop irrigation (≤ 1 viable nematode egg per litre). This represents an enormous health risk in case of reusing without prior treatment.

The activities of urban areas of the city of El Jadida were originally an increase in wastewater discharges and consequently, environmental pollution varying severity. In terms of the fight against this pollution efforts on treatment devices are still missing in the city of El Jadida. To eliminate the environmental and health hazards caused by the discharge of sewage in the rough environments to stations, it is imperative to build a wastewater treatment plant.

Acknowledgements-The authors wish to thank the Hassan II Academy of Science and Technology (Rabat, Morocco) for the financial support of this study. We would also like to give our thanks to all the colleagues from Lab Maghreb Rabat who provided a lively atmosphere all throughout the research.

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