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Load of parasites Helminthes in wastewater used in irrigation of Settat city (Morocco)

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ABSTRACT

The Morocco is facing demographic challenges and drought (less 1000m3/habitant/year). This causes people to use wastewater in the rough. These wastewaters contain fertilizing substances however its use in the raw could threaten the environment.

For the city of Settat, a Morocco city which is located 60 km south of Casablanca, the wastewater is discharged without prior treatment in the river of Bou Moussa. Thus, in this work, we contribute to characterize the parasitological status of the wastewater in two main collectors A and B.

The results showed that the average concentration of nematode eggs at the collector A is 13.50 eggs/liter and 29.72 eggs/liter at the collector (B). Respectively, the eggs Cestoda was 5.24 eggs/liter in A and 2.68 eggs/liter in B.

The Protozoa was 11.58 cysts/l and in A and 6.75 cysts/liter in B. Nematodes are represented especially by the eggs of *Ascaris* sp, *Trichuris* sp, *Nematodirus sp*, *Enterobius vermicularis* and *Ancylostoma sp*. For the tapeworms, we have *Taenia saginata* and *Hymenolepis diminuta* and *Hymenolepis nana*. The Protozoa were *Entamoeba coli*, *Giardia lamblia* and *Entamoeba hystolitica*.

The concentrations of these parasites exceeds WHO standards. They therefore constitute a potential danger to the direct and indirect use of wastewater.

INTRODUCTION

In Morocco, the raw wastewater is reused frequently of in farm. Due to agricultural intensification and the exacerbation of drought, this practice Is Made of very old and common way to the periphery of major continental cities which have not a Sanitation Network. So, over 7200 hectares of agricultural land are irrigated directly with raw wastewater[1,2]. This practice may cause a risk of pollution of groundwater and soil degradation[3]. In this area intestinal parasitic infections are also among the most common health risks. In addition, this

ancient practice formally based on good intentions, among other water conservation and use of fertilizers, is risky and must be new practices based on wastewater treatment according to standards recognized.

In the present study, we evaluated the degree of sewage pollution by a group of parasites that pose a potential risk to the health of farmers those waters not previously treated.

MATERIALS AND METHODS

Study site

Settat is located at the center of Morocco, 60 km south of Casablanca on national road No. 9 connecting Casablanca Marrakech (Figure 1).

Figure 1 : Location of the city of Settat.

The city of Settat is marked by arid to semi-arid climate, an average annual rainfall of 284 mm with a high intra-and interannual variability (coefficient of variation greater than 30%)[4]. During the year, the rainy season lasts from October to April; June, August and September are almost dry with average monthly rainfall, almost always less than 5 mm.

The city of Settat has 120,000 inhabitants and produces about 120 tons of manure per day and rejects about 9000 m^3 of wastewater per day. This untreated wastewater is reused to irrigate over 400 hectares of agricultural land[5].

Methodology of study

The samples of raw sewage were conducted twice a month between January 2012 and October 2012 at the manifolds A and B. Following the method adopted by Bontoux (1983)[2], the samples were taken in the flow of the sewer area where water circulation is more active and a few inches above the surface the collector.

In Every sample, one liter of washwater was collected and preserved by the addition of formalin 10% (2 ml/liter) in sterile vials. Then the bottles are brought to the laboratory in a cooler (at a temperature of 4 °C).

In the laboratory, wastewater samples were left to settle during 24 h. Then, the parasitological analysis has needed different actions amplification techniques for the parasite load has been too low for a direct microscopic examination is conclusive[3].

We have used the method of Bailenger since it is strongly recommended by the WHO in 1997[6]. It is advantageous for its efficiency, simplicity, speed and repeatability for constant character reagents that are less expensive and non-hazardous. This technique is designed for the concentration of parasitic elements by removing the remains insignificant result of attractive forces caused by the two immiscible acetoacetic ether and the irritated plots patches lipophilic and hydrophilic phases.

The observation of helminth eggs was carried out at a magnification 100.X while protozoa, due to their small size, were observed at magnification 1000.X.

The recognized parasites are subsequently quantified using a blade Mac Master. Microphotographs were performed using a trinocular microscope with a digital camera.

The total number of parasites (X) per liter of waste water is calculated using the following formula:

X = A. B / C.D

X = number of parasites per liter of waste water sample.

A = number of parasites counted on the blade Mac Master

B = volume of the final product (ml).

C = capacity of the Mac Master blade (0.3 ml).

D = initial volume of wastewater to be analyzed (2 liters)

RESULTS

Microscopic observation of the wastewater has allowed to determining the presence of several groups of

parasites returning the following groups: protozoa, nematodes and cestodes.

The protozoa from raw sewage collector A were represented by *Entamoeba coli, Giardia lamblia* and *Entamoeba hystolitica*.The nematodes were represented by *Ascaris sp, Nematodirus sp* and *Ancylostoma* sp, and the cestodes were represented by *Hymenolepis nana* and *Taenia saginata*.

Quantitative characterization of parasite load sewage collector A (Figure 2)

The wastewater of the collector A contains 12.5 % of positive protozoa. The average concentration of parasites identified was 2.05 cysts/l *Entamoeba coli*, 1.52 cysts/l and 4.72 *hystolitica Entamoeba* cysts/l of *Giardia lamblia*.

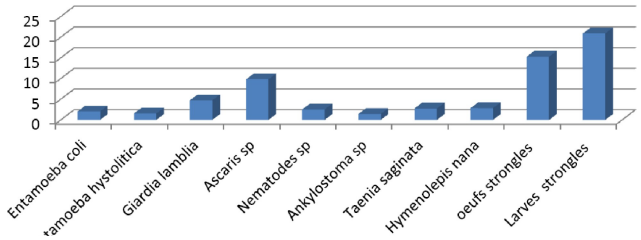

Figure 2 : Mean Concentration of parasites raw sewage collector A

For the nematodes, we have scored 20.35% of positive samples with respective average concentrations of eggs of 9.8 eggs/l of *Ascaris sp*, 2.52 eggs/l, *Nematodirus sp* and 1.40 eggs/l, and *Ankylostoma sp*.

For the tapeworms, the percentage of positive samples was 8.45% with an average concentration as eggs: 2.75 eggs/l for *Taenia saginata* and 2.84 eggs/l for *Hymenolepis nana*.

The percentage of samples with tapeworms is 8.45 % with an average concentration as eggs: 2.75 eggs/l for *Taenia saginata* and 2.84 eggs/l for *Hymenolepis nana*. For strongyles eggs, there are a percentage of positive samples in the range of 22.48% with an average concentration of 15.27 eggs/l. While, strongyles larvae were present in the samples with a positive sample percentage of 28.85% for an average larval concentration of 20.88 larvae/l.

Les protozoaires répertoriés dans les eaux usées drainées par le collecteur B sont représentés par *Entamoeba coli*. Les nématodes sont représentés par *Ascaris sp, Trichuris sp, Nematodirus sp, Enterobius vemicularis* et *Ankylostoma sp*. Les cestodes sont représentés par *Hymenolepis diminuta*.

The protozoa listed in wastewater drained by the collector B were represented by Entamoeba coli. The nematodes were represented by *Ascaris* sp, *Trichuris sp, Nematodirus sp, Enterobius vemicularis* and *Ancylostoma* sp. Tapeworms were represented by *Hymenolepis diminuta*.

Quantitative characterization of parasite load sewage collector B (Fig.ure 3)

The frequency of protozoa found in sewage collector B is 7.5% with an average concentration of 4.85 cysts/l *Entamoeba coli*. The percentage of nematodes collected at the collector B is 48.75% with respective average concentrations in eggs of *Ascaris sp* 14.24 eggs/l, 2.4 eggs/l *Trichuris sp*, 18.82 eggs/l *Nematodirus sp*, 7.8 eggs/l of *Enterobius vermicularis* and 4.24 eggs/l *Ancylostoma sp*. We recorded 4.28% of tapeworms that an average concentration of 3.24 eggs/l *Hymenolepis diminuta*. For strongyles, we spotted eggs for 12.57% of positive samples with an average concentration of 7.24 eggs/l, whereas in larvae, there were 19.77% of positive samples with 11.26 larvae/l as a mean value.

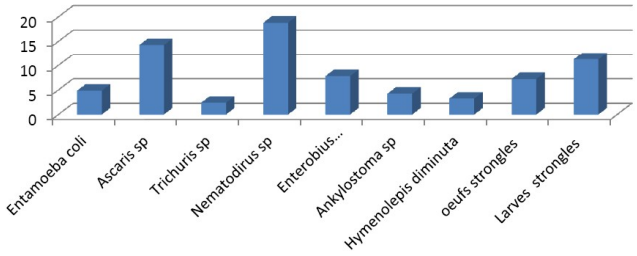

Figure 3 : Mean Concentration of parasites raw sewage collector B

The figure 3 shows the average variation of the concentration of parasites in wastewater drained in collectors (A) and (B) during cold period (October to March) and warm period (April to September).

The average concentration of protozoa during the cold period at the collector A is 3.87 and 0 cysts cyst in the collector B, while during the warmer months it is 2.50 cysts in the collector (A) and 1.8 cysts in the header of B.

The mean concentrations of nematodes during the cold period were 1.24 eggs/l in the collector A and 4.88 eggs/l in the collector B. During hot weather, they were

2.95 eggs/l in the collector A and 4.92 eggs/l in the collector B.

For Cestoda eggs, the cold period that covering September, October, November, December and February, the concentrations were 1.77 eggs/l at the collector A and 0 eggs/l at the collector B. During the warm period, the concentrations were 2.35 eggs/l in the collector A and 2.40 eggs/l in the collector B (Figure 4).

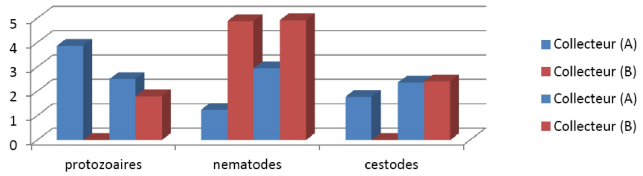

Figure 4 : Variation of the parasite concentration in wastewater collectors A and B as a function of sampling periods.

DISCUSSION

The collectors studied showed a parasitic divergence and inequality in terms of quantity and fecal pollution carried by the raw sewage. Qualitative analysis enumerated protozoa and three helminths in wastewater of samples: Nematodes, Cestodes and strongyles, with an exclusive superiority of the Nematodes compared to other.

The average concentration of helminth eggs (without the strongyles) at the collector A were 11 eggs/l and 13 eggs/l at the collector B. According Bouhoum (1996)[7], the concentration of eggs of parasitic helminths in urban wastewater is strictly tied to the demographic factor. Indeed, the manifold B has a greater extent (72 ha) that the manifold A (35 ha), so the number of people connected to the collector B is more important than people connected to the collector A. Similarly, the difference in mean parasite concentration could be explained by the importance of wastewater from industrial areas, while the collector B conducts wastewater from household charged in helminth eggs.

Therefore, the parasite concentration of urban wastewater collected in A and B has showed average loads exceed the standards recommended by the World Health Organization that was indicated by Bulmenthal et *al.* 2000[8] and the Standards Committee and standards of the Ministry of Environment of Morocco (≤ 1 viable nematode egg per liter) for waters reserved for crop irrigation)[9].

Moreover, the results showed that the highest concentrations of parasites were noted during warm periods (April to September). These results confirm those of WHO (1989)[10] reported that the increase in eggs concentration of helminths in spring and summer is due to the requirements of temperature, humidity, oxygen and adequate sunlight to ripen these parasitic helminths. These results are consistent with those on many work made in other parts of Morocco, including those of Dssouli (2001)[11] in Oujda Bouhoum et *al.* (2002)[12] in Marrakech, and Naur (1996)[13] in Beni Mellal.

Note that Schwartzbrod et *al.* (2003)[14] and Bouhoum (1996)[7] have indicated that, in wastewater, the eggs of the intestinal nematodes show greater resistance than those of Cestoda. This superiority was also announced by Belghyti et *al.* (1994)[15], Nsom et al. 2003a[16], and Nsom et *al.* 2003b[17].

CONCLUSION

A quantitative and qualitative dissimilarity in parasites of wastewater was noted. The water of the collector B was more richness in parasites than the water of the collector A. This may be due to the size of the population connected to each collector and to the difference in socioeconomic status of urban populations. The warm period (April-September) was a condition favoring parasite dispersal.

The burden of helminth eggs found exceeds the standards reported by the World Health Organization, by those of the Ministry of Environment of Morocco, and by those of the Norms and Standards Committee Moroccan waters reserved for irrigation agricultures (≤1 egg viable Nematode per liter). This brings a great hygienic hazard in reuse of wastewater without prior treatment.Thus, a treatment plant that will treat wastewater prior to their amendment is necessary.

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