Journal of Materials and Environmental Sciences
ISSN : 2028-2508
CODEN : JMESCN

J. Mater. Environ. Sci., 2020, Volume 11, Issue 2, Page 274-282

http://www.jmaterenvironsci.com

Evaluation of the diversity and the parasitic load of the waters of the Bouishak Wadi in the city of Meknes (Central Morocco)

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Received 22 Oct 2019,
Revised 10 Jan 2020,
Accepted 12 Jan 2020

Keywords
- ✓ *Wastewater*
- ✓ *Bouishak Wadi*
- ✓ *Parasites,*
- ✓ *Meknes ,*
- ✓ *Morocco.*

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Abstract

The objective of this study is to evaluate the diversity and abundance of the forms of dissemination of helminths and protozoa present in the waters of wadi Bouishak (Meknes, Morocco), and to elucidate the risks that these parasites present for health. The wastewater sampling has carried out monthly during a period from January to December 2017 between 10 am and 11 am at the three stations (B1, B2 and B3) that flow into the wadi. The 36 samples analyzed were positive with a predominance of protozoan cysts on Helminth eggs (29.08 10^5 cysts/L against 4573 eggs/L). Thirteen parasite taxa has detected: *Giardia lamblia, Entamoeba histolytica, Entamoeba coli, Blastocystis hominis, Ascaris lumbricoides, Trichuris trichiura, Ancylostoma diudinal, Capillaria sp Toxocara canis, Enterobius vermicularis, Taenia saginata, Hymenolepis nana and Hymenolepis diminuta.* The results obtained reveal a high parasite contamination of the watercourse, which could have negative effects on the quality of the environment in general and on human and animal health in particular.

1. Introduction

Demographic, economic and climatic constraints lead to an increase in the demand for water, which causes a great pressure on the surface water. Yet the surface waters has also polluted by industrial, domestic and agricultural waste. In Morocco wastewater is used as an important resource for agriculture that suffers from water deficit [1-2]. Without prior control, these effluents pose such a significant threat to the environment, human and animal health [3-4]. According to WHO, serious health problems has caused by waterborne diseases [5]. In the region of Meknes, wastewater has discharged without prior treatment into the watercourses crossing the city (Ouislane wadi, Boufakrane wadi and Bouishak wadi). As a result, these rivers has considered as open sewers through urban and peri-urban areas of the city. The reuse of these raw or mixed effluents in irrigation is a real practice. Previous studies have examined the impact of liquid effluents on the water quality of these rivers [6-7-8].

The choice of the Bouishak valley lies in the fact that it constitutes an extreme case since the raw sewage represents 93% of the water used for irrigation [9]. The bed of the Bouishak wadi drains wastewater from densely populated areas (Hay El Mansour, Kamilia and Toulal). Agriculture is mainly oriented towards market gardening and arboriculture. In the current state of knowledge, no parasitological study has carried out. The objective of this study is to evaluate the diversity and abundance of the forms of dissemination of Helminths and Protozoa that are present in the waters of the Bouishak wadi and to elucidate the health risk emanating from these parasites. To do this, spatio-temporal monitoring of the parasitological quality of sewage discharged into the Bouishak wadi has carried out.

2. Material and Methods

2.1. Study are

The city of Meknes is located in the northern part of Morocco. It occupies an area of about 79210 km^2. The study area belongs to the Fez-Meknes region according to the new 2015 administrative division (Figure 1). It is the second city of the plain of Sais, after the city of Fez. This area has characterized by geological diversity consisting of hard lake limestones and tawny sands. The climate is Mediterranean, with an average rainfall of 660 mm / year [10]. According to the 2014 General Census of Population and Housing [11], the population of the city of Meknes is in the order of 835,695 inhabitants against 715,284 in 2004. As a result, the average annual growth rate reached 1.6% for the period 2004-2014.

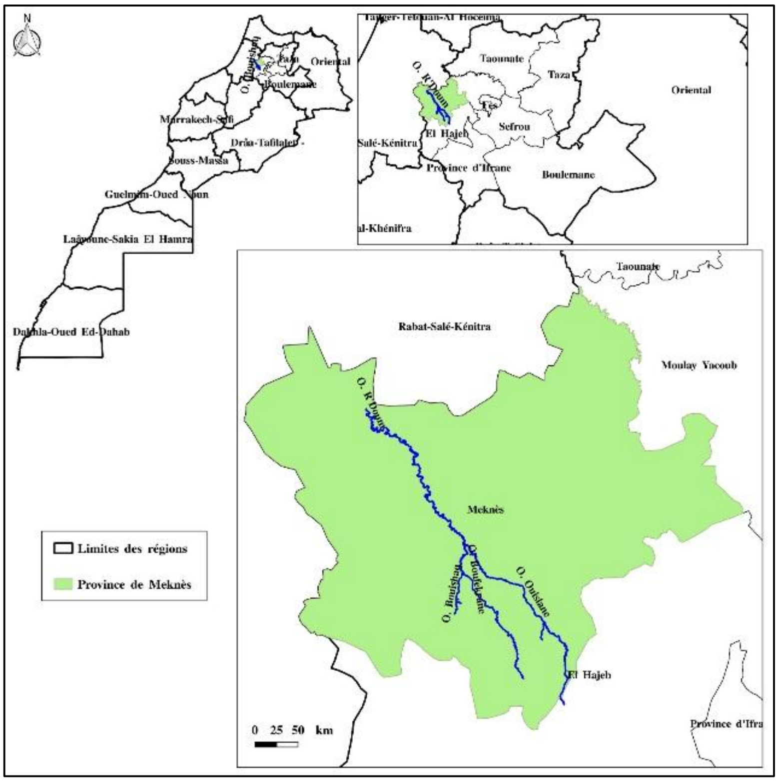

Figure 1: Geographical location of the study area

2.2. Sampling and analysis methods

For a more representative sampling of the watercourse, we have chosen three stations on the Bouishak Wadi bed (Figure 2): Station B1 is located upstream of watercourses in a zone of great anthropogenic influence (domestic, industrial and agricultural), stations B2 and B3 are located downstream of the agglomeration of Meknes (Table1). Monthly samples were taken between 10 am and 11 am, when the parasite load is closely related to human activity [12-13- 14], during a period from January to December 2017. À chaque station, un échantillon de 1 litre d'eaux usées a été prélevé et conservé en ajoutant du formol, 10% (2 ml / l d'eaux usées) étiqueté et transporté dans un refroidisseur (à une température de + 4 ° C) au laboratoire d'hydrobiologie à la faculté des sciences de Meknès.

Table1: Characteristics of the different sampling sites

	Stations	Types of pollution	Lambert Contact Information		
			Latitude	Longitude	Altitude (meters)
Bouishak watercourse	B1	Domestic, agricultural and industrial	482120,6	364713,8	488
	B2	Domestic, agricultural and industrial	482205,4	365004,53	481
	B3	Domestic and industrial	482202,5	365900,2	458

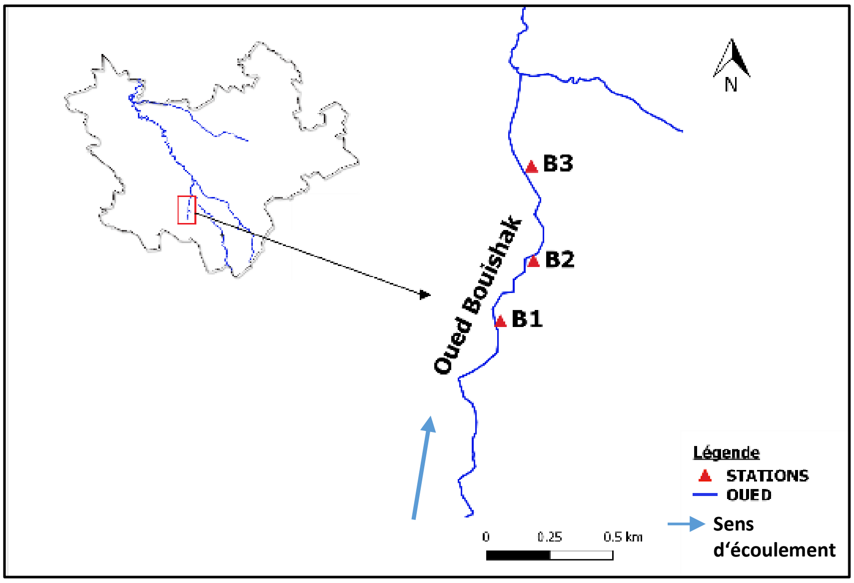

Figure 2: Location of the study stations

The evaluation of the parasite load was determined by two techniques: modified Bailenger concentration technique strongly recommended by [15] and Faust technique (flotation method with zinc sulphate solution (33%, density, d = 1,18) [16]. Egg identification has done using the WHO protocol (2004), the diagnostic manual for verminosis by coprology examination [17] and the manual of medical parasitology by Viviane GUILLAUME [18]. The criteria used for the identification of parasites are essentially: the size, shape, thickness of the shell, the presence or absence of polar plugs and a larva inside the egg. Quantification has done using a Mac Master slide under a light microscope. The total number per liter of Helminth eggs and protozoan cysts present in the wastewater sample is calculated using formula (1):

$$N = X / P \times V / S \quad (1)$$

With:
N = number of eggs per liter of sample
A = number of eggs counted on the McMaster blade or average number of eggs found in three blades
X = volume of the final product (ml)
P = McMaster Blade Capacity (0.3 ml)
V = volume of the initial sample (1 liter).

3. Results and discussion

The results of parasitological analysis of wastewater from Bouishak wadi reveal a significant specific diversity of parasitic species. This is in agreement with other works [19-20].

This diversity made it possible to highlight the presence of thirteen parasite taxa (Table 2). Protozoan cysts (amoebae and intestinal flagellates) and Helminth eggs has divided into two classes: NEMATODES and CESTODES. The NEMATODES class is the most diversified with six taxa, a prevalence of 46% (Table 2) (Figure 3).The eggs of represent it: *Ascaris lumbricoides*, *Capillaria sp*, *Trichuris trichiura*, *Ancylostoma diudinal*, *Enterobius vermicularis*, and *Toxocara canis*. On the other hand, the class of CESTODES has a prevalence of 23% (3 taxa); the species collected are *Hyminolipis nana*, *Hyminolipis diminuta*, and *Taenia saginata*. The group of protozoa has represented by four taxa (31%): *Giardia lamblia*, *Entamoeba histolytica*, *Entamoeba coli* and *Blastocystis hominis*. It has noted that in all wastewater samples from different stations, protozoan cysts on helminth eggs are prominently predominant, consistent with previous work [20-21] (Figure 4). The number of Protozoan cysts is relatively high, ranging from 6.49 10^5 cysts / L (station B3) to 12.97 105 cysts / L (station B1), while station B2 contains 9.62 10^5 cysts / L.

Table2: Inventory of the different species encountered in the urban wastewater of Bouishak collector.

Splitter	*Classes*	Species
Helminth	*Nematodes*	Ascaris lumbricoides Trichuris trichiura Capillaria sp Toxocara canis Enterobius vermicularis Ancylostoma diudinal
	Cestodes	Hyminolipis nana Hyminolipis diminuta Taenia saginata
Protozoan	*Flagellated*	Giardia lamblia,
	Amoebas	Entamoeba coli Entamoeba histolytica Blastocystis hominis

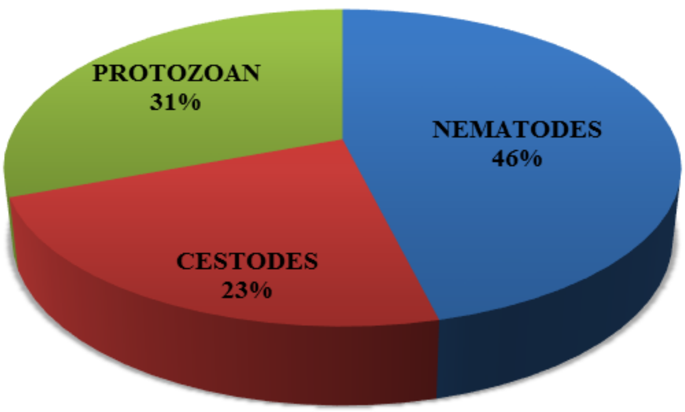

Figure 3: Frequency of occurrence of parasite groups

The parasite load of the Helminth classes ranges from 1930 to 2651 eggs / L. The highest strength has recorded at station B1 while the lowest has obtained at station B3. Helminthes collected in Station B2 are in the order of 2314 eggs / L. On the other hand, the Helminths identified has characterized by the predominance of nematodes with a frequency of 66% against 34% for the Cestodes (Figure 5). Our results are consistent with other work done in Morocco, Tunisia and France [21-22-23-24]. This unequal distribution of Nematodes with respect to Cestodes has explained by the fact that the first class is the most resistant in wastewater [21-25-26-27].

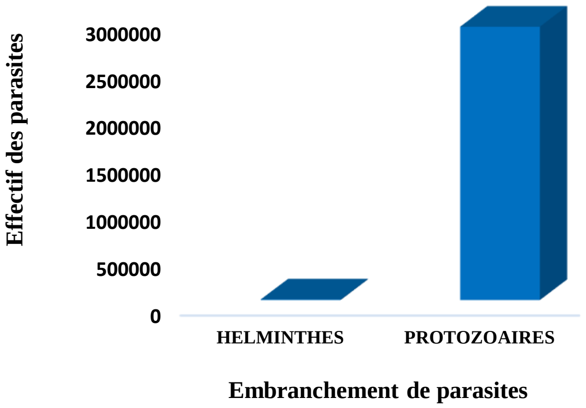

Figure 4: Distribution of parasites in the waters of Bouishak wadi

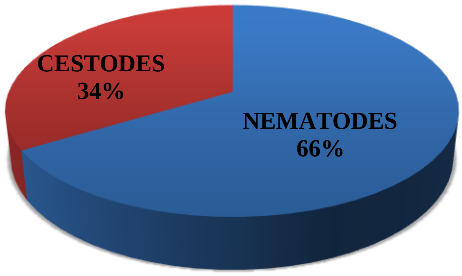

Figure 5: Frequency of occurrence of Helminth parasite classes

The high parasite load has detected at station B1. We has observed a decreasing slope from upstream (B1) to downstream (B3). This could explained by the fact that the B1 collector drains all domestic wastewater from a large agglomeration (Mansour, Camilia and Toulal) and the wastewater from the B2 collector is used by pumping for irrigation of the surrounding land, which has an impact on the parasitic load, noted low, at the B3 station (Figure 6). Within the Nematode class, *Ascaris lumbricoides* is the most dominant species in all stations (B1, B2 and B3) with an average concentration of about 114 eggs / L (Figure 6). Qualitatively, our results has corroborated with other works, this preponderance being due to the strong resistance of this kind to environmental conditions [28-29]. On the other hand, the presence of intestinal nematodes and particularly *Ascaris lumbricoides* and *Trichuris triciura* in wastewater intended for irrigation constitutes a potential impact on human and animal health [28-36-37-38]. Quantitative analysis of protozoan cysts in wastewater from Bouishak wadi revealed flagellates (*Giardia lamblia)* and amoebae (*Entamoeba coli, Entamoeba histolytica and Blastocystis hominis)* (Figure 7).

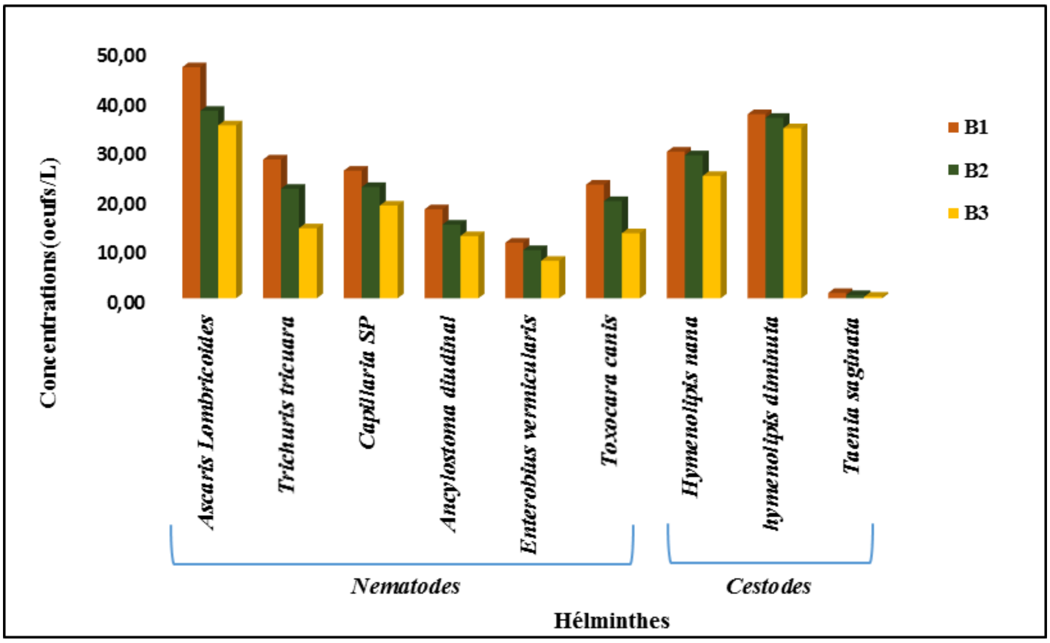

Figure 6: Egg concentration by class and taxon of wastewater from Bouishak wadi

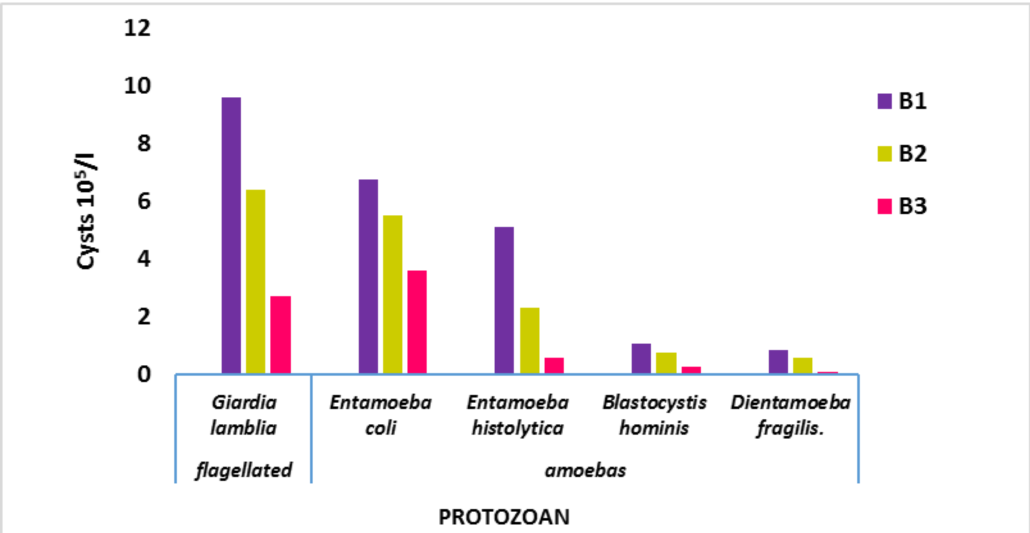

Figure 7: Concentration of identified protozoa in wastewater from Bouishak wadi

We noted a predominance of *Giardia lamblia* cysts in the different stations and during the study period with average contents of 39.6 10^3 cysts/L (B1), 36.93 103 cysts/L (B2) and 35.90 10^3 cysts/L (B3) (Figure 7). While

Entamoeba coli cysts have the lowest concentration, from 16.75 10^3 cysts/L (B1) to 10.75 10^3 cysts/L (B3).From a quantitative point of view, the parasite load of the waters studied is large compared to that found by other researchers in other sites [21-30]. From a spatial point of view, mean cyst levels were high at station B1 with a total of 10.8 10^4 cysts / L and increased to 80.17 10^3 cysts / L then 54.09 10^3 cysts/L respectively to B2 and B3 (Figure 8). This concentration, which decreases considerably from upstream to downstream, has explained by the decrease in the population size. The prevalence of *Giardia lamblia* and *Blastocystis hominis* in surface water and wastewater could lead to serious health risks, in this case giardiasis, which has manifested by watery diarrhea, steatorrhea and malabsorption, is a common infection in children [34].

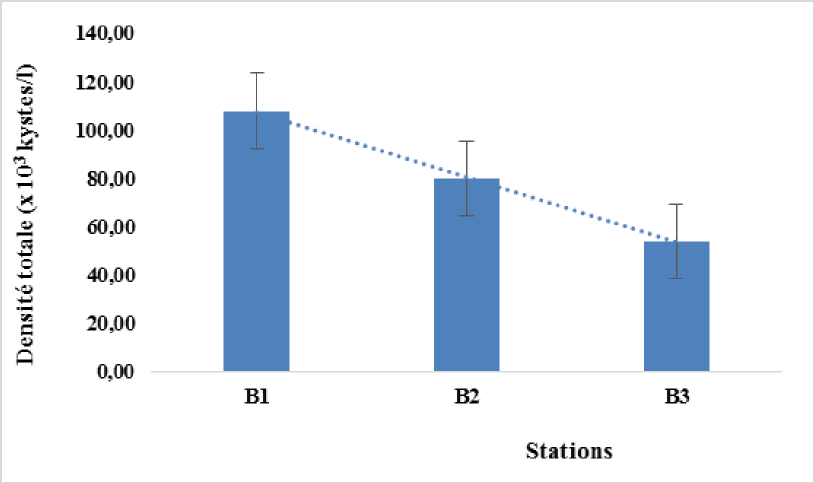

Figure 8: Spatial variation of protozoan cyst densities

For example, the presence of intestinal nematodes, particularly *Ascaris lumbrocoides*, and *Trichuris triciura* in wastewater intended for irrigation has considered a major health and environmental hazard [35-36-37]. The World Health Organization (WHO) [38] estimated the number of cases of Ascaridiosis due to *Ascaris lumbricoides* and 500 million cases of *Trichuris trichiura* infection in the world at 1,000 million. In 1994, Chan et al., [39] estimated that there were 1471 million and 1048 million cases of A. *lumbricoides* and *T. trichiura* infections in the world, respectively. Clinically, *A. lumbricoides* can cause bowel obstruction and *T. trichiura* has been associated with dysentery [40]. Both Helminths have also been associated with stunting [41-43]. Massive infestations of these two nematodes are more common in children than in adults.

Conclusion

The results of our study show that helminth protozoan egg and cyst concentrations far exceed the standards recommended by the WHO guidelines and Moroccan water standards for irrigation described in the decree. Ministerial Meeting No. 1276-01 of the year 2002. Indeed, it claims a parasite load less than or equal to a viable egg per liter for water for irrigation crops. A qualitative and quantitative divergence of sewage pests has noted. Sewage upstream of Bouishak wadi was more parasitic than downstream water. This difference in concentration may be due to the size and socio-economic level of the urban population, the geographical context, the arid and semi-arid climate of the region, and the urban effluents that has discharged daily into the river. wadi. Therefore, the control of waterborne diseases becomes an essential strategy to reduce risk factors.

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