

Tutorial 5

(Reading and understanding a scientific article)

Part I. Structure questions

1. Which format or system is used to write this paper?
2. What is the type of the paper?
3. What is the title of the journal?
4. What does mean the abbreviation DOI of the paper?
5. What is the purpose of the abstract?
6. What kind of information is provided in the acknowledgments section?
7. What style of referencing is used (APA, MLA, Chicago, Harvard, etc.)?

Part II. Content questions

II.1. Introduction

- a. What is the main objective of this paper?
- b. What research problem is the paper trying to solve?
- c. What is the research gap identified by the authors?

II.2. Methodology

- a. What methods were used to measure the concentraion of parasiic helminths eggs in wastewater?
- b. Why do you think the researchers chose this methodology?
- c. Where was the study conducted?
- d. What is the population or sample size used?

II.3. Results

- a. What are the main findings of the study?
- b. Are the results presented using figures, tables, or graphs?

II.4. Discussion

- a. How do the authors interpret the results?
- b. Do the findings agree or disagree with previous studies?

II.5. Conclusion

- a. What are the main conclusions and recommendations?



REGULAR ARTICLE

Parasite load of raw wastewater from the city of Biskra's discharge outfalls - Z'mor and Biskra valleys (Algeria)

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ABSTRACT

Algeria, like other arid and semi-arid African countries, is confronted by problems related to rapid population growth and water stress (water shortage). This stress fosters raw wastewater reuse in market gardening and horticulture. The advantages of this wastewater consist of their nutrients whose organic materials contribute to soils enrichment with humus, as well as their availability in large quantities. However, raw wastewater reuse entails a potential risk of environmental and water resources pollution. The purpose of this work was to evaluate the parasite load of raw wastewater from the city of Biskra's discharge outfalls (Biskra valley and Z'mor valley), while considering the demographic and socio-economic profile of populations connected to these outfalls. Our results revealed a variety of parasites in the form of helminths eggs at the following average concentrations: *Ascaris* sp. (33.66 eggs/L), *Trichuris* sp. (22 eggs/L), *Enterobius vermicularis* (19.58 eggs/L), *Ancylostoma* sp. (17.41 eggs/L), *Nematodirus* sp. 17.83 (eggs/L), *Hymenolepis nana* (21.66 eggs/L), *Moniezia expansa* (16.5 eggs/L), *Taenia* sp. (18.5 eggs/L), *Fasciola hepatica* (0.58 eggs/L) and *Strongyloides* spp (17.11 eggs/L). The parasitic helminths eggs distribution varies according to the size of urban areas and the socio-economic level of populations connected to each outfall. This study also highlighted the qualitative and quantitative seasonal variations of helminths eggs in wastewater from Biskra's two outfalls.

1.Introduction

Water is a vital and essential element for all living

beings. The importance of this natural resource is paramount in arid or semi-arid countries running

up against water deficit and erratic rainfall in time and space (Guemmaz *et al.* 2019). After its use, water is loaded with various elements modifying its physical, chemical and biological properties. Thus, the initially clear and drinkable water turns into grayish and waste water (Gashaye 2020). According to Rejsek (2002), urban wastewater or wastewater is water containing soluble or insoluble pollutants, mainly from human activity. They generally consist of a mixture of pollutants dispersed or dissolved in domestically or industrially-used water.

Up to date, foremost among worrying environmental problems is sanitation. In fact, anthropogenic activity generates wastewater that is daily discharged into the environment. Raw wastewater discharge into the environment is a common practice in most developing countries (Lam *et al.* 2015). This situation is even more exacerbated in urban areas due to their typical galloping demography (Sylla *et al.* 2019). This is the case of Biskra, a city in southeastern Algeria, which located in southeastern Algeria and characterized by an arid to semi-arid climate. Every day, an average of 265 L/d/h of wastewater are discharged in the city's various outfalls at a rate of 44.73 Hm³/yr, without any prior treatment (ANRH 2014).

Coming into direct contact with the natural environment, raw wastewater generates adverse effects. In particular, a nuisance related to this water stagnancy and the release of putrid odors, and a disruption of the natural environment ecological balance, especially through the pollution of surface water and groundwater (Nellyat 2016, Guemmaz *et al.* 2019). In addition, raw wastewater causes health risks due to microbiological or parasitic contamination (Akpo *et al.* 2013, Zacharia *et al.* 2018). To mitigate raw wastewater negative impacts on the environment and on human and animal health, it is necessary to set up traditional and/or modern wastewater treatment methods before their release or possible reuse for agricultural purposes.

However, various pathogenic microorganisms, mainly from feces, can be present in this raw

wastewater. Among which, we can find the group of helminths worms. The most pathogenic, present in wastewater and belonging to different taxonomic groups, are the following *Nematoda*, *Cestoda* and *Trematoda* (Kostyla *et al.* 2015). In the aquatic environment, these parasites are found as larvae or eggs which ensure their both resistance in water and their dissemination. Humans are commonly infected by ingesting these parasites through contaminated water and food or by direct penetration of infesting larvae through the skin upon contact with contaminated water (Habbari *et al.* 2000).

Wastewater parasitic contamination is therefore a serious problem, especially in arid regions, because wastewater is very used in the irrigation of certain crops due to water scarcity, which presents a great risk to the health of the consumers. So far, there are no studies that assess the degree of water contamination by parasites in the regions of South Algeria, which characterized by an arid climate and a remarkable lack of clean water, including the Biskra region. Thus, the objective of this paper is the evaluation of the parasitological pollution degree of Biskra's raw wastewater, by the temporal monitoring and analysis of helminths eggs load at two urban liquid waste outfalls.

2. Environment, equipment and methods

2.1. Study Site

The city of Biskra is located in southeastern Algeria, 115m above sea level, 34°51'01" North latitude and 5°43'40" East longitude. Biskra's population is 176.048 with a density of 1485 people/km² who discharge more than 265m³/inhabitant/day of wastewater (DSA 2010). Climate of Biskra is semi-arid to arid. The highest average maximum temperature is recorded in July (34.65°C) and the lowest average minimum temperature in January (12.25°C), with precipitation around 291.8 mm/year (Table 01).

The municipality of Biskra's typology varies from modern to traditional, semi-rural to slum; thus, bringing about a mixture of different styles build-

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec	Average
T (°C) 2014	12.95	15	16.3	22.65	26.5	30.15	34.2	30.7	31.1	25.55	18.7	13.15	23.1
P (mm) 2014	8.2	3.2	16	0	2.1	4	0	0	7.6	0.8	2.7	0.7	3.77
H % 2014	59	49	48	36	34	29	27	30	38	37	51	60	41.5

Table 1: Average values of climate variables for the city of Biskra.

Categories	Zones
Construction in Good condition	East Zone <ul style="list-style-type: none"> Essaada district, El Fadjer district, The 17 Cooperatives, University residence, buildings in the Parcs area.
	Equipment Zone (North) <ul style="list-style-type: none"> All existing constructions.
	Other Districts (Downtown) <ul style="list-style-type: none"> El Moudjahidine district, El Istikbal district, Boukhari district, Housing estates Sayhi 1 and 2, Eddali district. Almost all constructions are in good condition. El Oukhoua district, El Badr district, EL Ferdous district, M'cid, Bab El-Darb, Medjiniche, Gueddacha, Sidi Barkat, Sidi Guezal. Almost half constructions are in good condition. Frotture. In good condition. Feliache. Some constructions are in good condition.
Construction in medium to acceptable condition	West Zone <ul style="list-style-type: none"> Military City in its entirety. Ennas district Damier colonial, North River and South River, Essalam district, Essalam district, El Fardous district, Star Moulik, El Houria city, Sidi Ghezal. Less than half of the constructions. Edalia district, El Oukhoua district, M'cid, Bab El Darb, Medjeniche, Gueddacha, Sidi Barkat. About one third of the constructions. Feliache, in its entirety.

Table 2: Typology of the city of Biskra.

Sewer System	Specifications
Downtown	The checkerboard system is cleaned by an ovoid-type collector; then connected to a pipe near the hotel Les Ziban and flows into a farmland in M'cide and Biskra valley.
Southern area of Biskra	Is drained by collectors to flow into Biskra valley.
West Zone	This zone was initially equipped with a separate rainwater/sewage system, but since the intensive urban development and extension of this western zone, part of the wastewater is drained into a rainwater culvert system. The final wastewater discharge occurs on agricultural lands in the municipality of El Hadjeb (location of the future landfill), north-west of Sidi Ghazel, near Z'mor valley.
Industrial Zone	Rainwater is collected in open channels. Wastewater is drained into pipes and its discharge takes place on agricultural lands north-west of Sidi Ghazel (Z'mor valley).
East Zone and El Alia	They are equipped with collectors and wastewater is discharged directly in the environment, whereas it was initially drained towards Biskra valley.
Parks area	Wastewater from this zone is discharged into the environment (release originally planned in Biskra valley).
Feliache	Sanitized by collectors, while wastewater is discharged in Biskra valley.

Table 03: Representation of the sewer System.

ings. The update of some documents (ANAT 2015) has developed plates showing building condition by category (Table 02).

90% of the population is connected to the 233 km-long linear type sewer system that is adapted to the province topography. The study was undertaken at the Province of Biskra's two main outfalls: Biskra and Z'mor valleys.

Biskra valley: Taking its source from the confluence of El Hai and Djamoura, this valley is fed upstream by several brooks, including Branis valley, Lefrahi valley, El Besbas valley and Lakhdar valley. It is the

most important outfall, characterized by pipes of $\Phi=1500\text{mm}$ diameter and a slope of $I=2.5\%$. This river collects the North Zone and Downtown wastewaters (ANAT 2015, Guemmaz *et al.* 2019).

Z'mor valley: Located west of the city of Biskra, it crosses some hills and El Corab at the place called Fom Maouia. It is fed along its course by the tributaries of Hammam, Hassi Mebrouk valley, El Tera valley and Leham valley. It is characterized by pipes with $\Phi=1500\text{mm}$ diameter and a slope of $I=1.5\%$. It collects wastewaters discharged by the city's West Zone (Industrial Zone – Vocational Training

Center - 726 Logts- Former Souk El Fellah, etc.) (ANAT 2015, Guemmaz *et al.* 2019).

2.2 Sampling

Sampling of wastewater from the city of Biskra was performed monthly from January to June 2019 at the two studied outfalls (Biskra and Z'mor valleys) for parasitological study purposes. Two-liters each simple were taken and preserved in sterile flasks by the addition of 10% Formaldehyde (2mL per liter), then transported to the Biology Laboratory of Mohamed Khidar University, Biskra-Algeria.

Considering the large dispersion of parasitic helminths eggs in wastewater, their concentration was necessary to ensure a better count. For this reason, the BAILENGER technique, highly recommended by the WHO in 1997, was chosen for its usability, low cost and reliability. The pellet, obtained after centrifugation in a mixture of Aceto-acetic buffer and ether, is added to a solution of 33% zinc sulfate (density = 1.18) to allow the parasitic elements to adhere to the upper surface of the McMaster slide and thus facilitate their microscopic count.

The helminths eggs identification was carried out at magnifications 100 (in register), either in fresh state (placing a pellet drop between slide and coverslip), or after concentration WHO in 2015.

Microscopic observation of helminths eggs was based on their size, shape and content in accordance with the bibliographic descriptions (Golvan and Ambroise-Thomas 1986).

In cases where we could not identify the species, we only focused on identifying the genus. *Strongyloides* eggs have not been considered in the overall count, because of their diverse origins (animal, soil *Nematoda*, washing of contaminated plants, etc.) and their identification-related difficulties. The total quantification of helminths eggs, per liter (N), present in two liters of analyzed wastewater is calculated using the following formula: $N = A \cdot X / P \cdot V$

A: Number of eggs counted on the McMaster slide or average of numbers found in 2 or 3 slides.

X: Volume of the final product (mL).

P: Capacity of the McMaster slide (0.3 mL).

V: Volume of the initial wastewater sample to be analyzed.

2.3 Statistical study

All the obtained parasitological results were processed using GraphPad Prism software Version 7.04. The analysis of these results was performed by using one-way analysis of variance (ANOVA), followed by Newman-Keuls post hoc comparison. The difference between groups is considered significant when $p < 0.05$.

3. Results

3.1. Qualitative characterization of wastewater parasitic load in the city of Biskra

Parasitological analyzes of the wastewater from Biskra's two waste outfalls (Biskra valley and Z'mor valley) have exhibited a contamination by eggs belonging to three groups of parasitic helminths: *Nematoda*, *Cestoda* and *Trematoda*.

3.2. Quantitative characterization of wastewater parasitic load in the city of Biskra

3.2.1. Helminths Groups Study

In the present study, the parasitological monitoring of wastewater from Biskra's two outfalls (Biskra valley and Z'mor valley) shows that they are contaminated by parasitic helminths eggs with an average concentration of 18, 47 eggs/L (± 5.74). These eggs are split into three classes: *Nematoda* eggs, *Cestoda* eggs and *Trematoda* eggs, with average concentrations of 22.1 eggs/L (± 8.93), 17.11 eggs/L

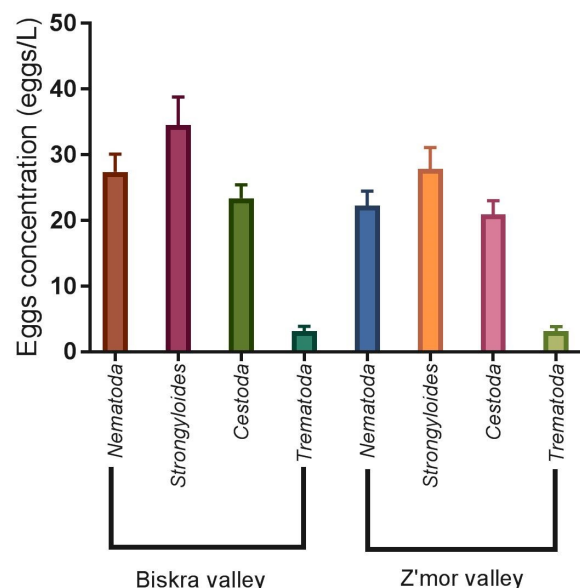


Figure 1: Distribution of the average concentration of Helminths eggs in raw wastewater from the city of Biskra's two outfalls (Biskra and Z'mor valleys). There was no significant difference between groups.

(± 6.42) and 0.58 eggs/L (± 0.51). The average egg concentration of intestinal *Strongyloides* is about 17.11 eggs/L (± 6.42). The different average concentrations of helminths eggs found in wastewater from the city of Biskra's two outfalls (Biskra valley and Z'mor valley) are shown in Figure 01.

3.2.2. Study by helminths species

The variations of helminths eggs average concentrations in the wastewater of Biskra (**Site I**: Biskra valley and **Site II**: Z'mor valley) are represented below:

The ANOVA statistical test for helminths egg average concentrations showed that the difference between the two sampling periods was significant for both outfalls ($P < 0.05$), except for Trematoda (Figures 02, 03).

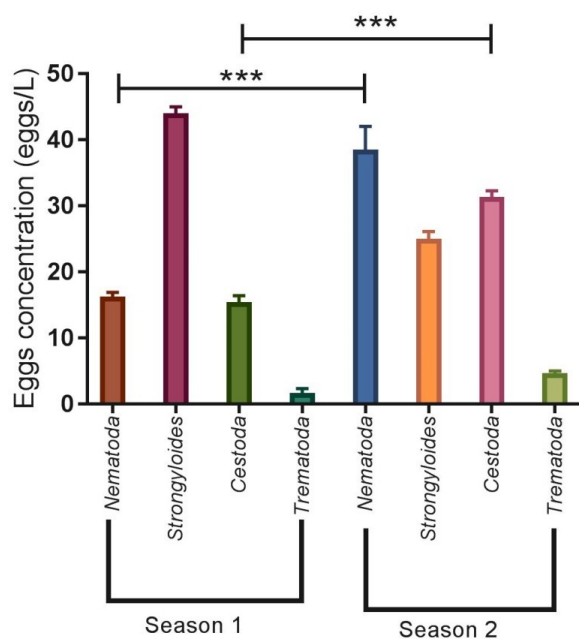


Figure 2: Seasonal variation of the parasitic load of wastewater outfall (Biskra valley).

*** $P < 0.001$.

The ANOVA statistical test for helminths egg average concentrations in the wastewater from both outfalls shows the following (Figures 04, 05):

For Nematoda: Biskra valley and Z'mor valley: the difference is not significant with $p < 0.05$. **For Cestoda:** Biskra valley and Z'mor valley: the difference is not significant with $p < 0.05$.

For Strongyloides: Biskra valley and Z'mor valley: the difference is significant with $p < 0.05$.

For Trematoda: Biskra valley and Z'mor valley: the difference is not significant with $p < 0.05$.

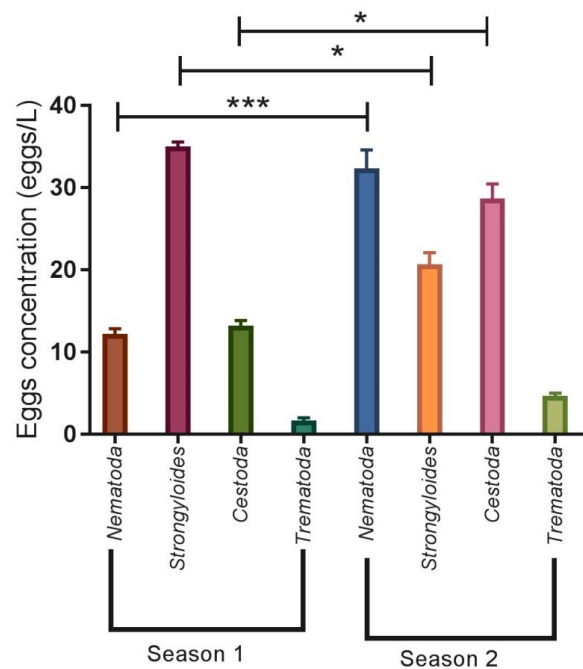


Figure 3: Seasonal variation of the parasitic load of wastewater outfall (Z'mor valley).

* $P < 0.05$; *** $P < 0.001$

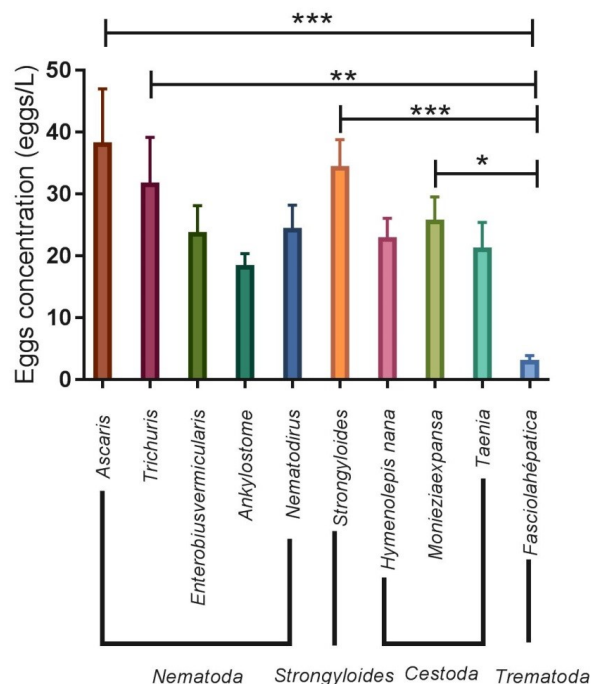


Figure 4: Helminths eggs average concentrations in Biskra's wastewater (Site I: Biskra valley). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

4. Discussion

In this work, we focused on the study of raw wastewater parasitological pollution by committing to characterize the parasitic load of wastewater from urban waste outfalls in the city of Biskra (Biskra valley and Z'mor valley) (Algeria).

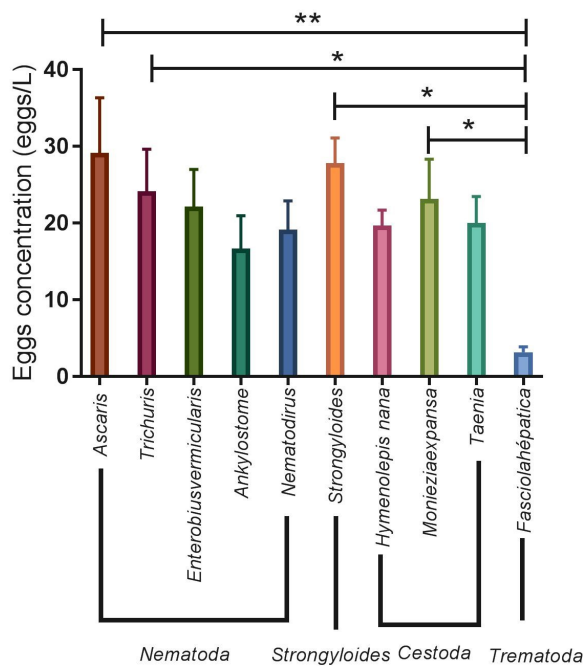


Figure 5: helminths eggs average concentrations in Biskra's wastewater (Site I: Z'mor valley).

*P < 0.05; **P < 0.01

The results of wastewater sampling exhibited a wide variety of concentrations. These data reveal a fecal pollution conveyed by these raw effluents. In addition, the results of our study on Biskra's wastewater (Algeria) are in line with those obtained by (Raweh *et al.* 2010) except for *Trematoda* that were not found at these proportions. We have detected them, but at too low concentrations. Wastewater from the city of Biskra is lightly laden (18.47 eggs/L), compared to wastewater from some Latin American countries, such as Brazil, with 1490 eggs/L (Mara and Sleight 2010), and some African cities like Dakar, Senegal, with 180 eggs/L (Akpo *et al.* 2013), but they are heavily laden compared to the wastewater of some European cities (Levantesi *et al.* 2010). The qualitative analysis identified three groups of helminths in wastewater samples: *Nematoda*, *Cestoda*, *Trematoda* and *Strongyloides* eggs, with a clear predominance of *Nematoda* compared to the others and a very low concentration of *Trematoda*, which is almost nil. The predominance of *Nematoda* eggs is also highlighted in other studies carried out in Tunisia (Ayed *et al.* 2009), in Morocco (Taha *et al.* 2019) and Senegal (Akpo *et al.* 2013).

The source of contamination is particularly related to the concerned population lifestyle, whose culinary habits do not favor the transmission of *Cestoda*, whereas the eggs of intestinal *Nematoda* class are more resistant than *Cestoda*, in

wastewater. This predominance has been reported by several studies in Morocco (Taha *et al.* 2019), and elsewhere in the world (Sobh *et al.* 2014).

The parasitic helminths isolated from wastewater in the city of Biskra are mainly represented by *Ascaris* sp., *Trichuris* sp., *Enterobius vermicularis*, *Nematodirus* sp., *Ankylostoma* sp., *Strongyloides* sp., *Taenia* sp., *Hymenolepis nana*, *Moniezia* sp. and *Fasciola hepatica*, with a predominance of *Ascaris* sp. eggs (Figures 4 and 5). This parasitic diversity reported by other studies shows that contamination sources are of human and animal origin (Morand and Lajaunie 2017, El Fels *et al.* 2019).

This study also highlighted the qualitative and quantitative seasonal variations of helminths eggs at Biskra's two waste outfalls (Figure 2 and 3). This variation results in high levels of helminths eggs, mainly for *Nematoda* eggs and *Cestoda* eggs, during hot seasons and low levels in cold ones. On the other hand, there are very high levels of *Strongyloides* eggs during cold seasons and low levels during hot ones. These observations are consistent with the work of (Taha *et al.* 2019) in Meknes (Morocco). Several authors have reported that this concentration difference between these two periods is due to the increase in verminosis parasite prevalence in spring (El Fels *et al.* 2019). While the WHO, in 1989, reported that, this abundance of helminths eggs during hot weather is due to the conditions of temperature, humidity, oxygen and solar radiation favorable to the maturation of these parasitic helminths.

Strongyloides' eggs are more abundant in cold weather wastewater samples. This is mainly due to digestive *Strongyloides* which are seasonal and their infestations occur particularly in the rainy season due to the high desiccation susceptibility of infesting *Strongyloides* larvae (Buonfrate *et al.* 2018). As for *Trematoda*, there is a very low to zero load in both seasons. This is due to their geographical distribution (ambient environment), to wit in the north-east of Algeria (Mekroud *et al.* 2004). The average concentrations of helminths eggs found in Biskra's wastewater vary according to the explored waste outfalls.

Comparing the parasitological analyzes results of the different wastewater outfalls in the city of Biskra, we can see that Biskra valley has the highest load with 23.19 eggs/L for *Nematoda*, 18.72 eggs/L for *Cestoda* and 0.66 eggs/L for *Trematoda*, while in Z'mor valley, we have found 20.86 eggs/L for

Nematoda, 11.11 eggs/L for *Cestoda* and 0.5 eggs/L for *Trematoda*. This difference in content can be explained by the fact that Biskra valley drains more than 60% of the city's wastewater. As a result, the density of populations connected to Biskra's outfall waste is higher than that of those connected to Z'mor's waste outfall. According to the demographic and socio-economic data provided by ANAT in 2015 (See Table 2 and 3).

Eggs of digestive *Strongyloides* were also observed in the two analyzed samples from the two outfalls. Their presence depends to a large extent on the release of the municipal slaughterhouse and the lifestyle of some populations living on goat, cattle and sheep farming. Therefore, the resulting wastewater is highly concentrated in helminths eggs.

According to the city of Biskra's demographic and socio-economic data, provided by ANAT in 2014, the population of neighborhoods connected to the outfalls of Biskra valley and Z'mor valley has socio-economic profiles that are generally very low to medium, with a slight high-profile population. Also reported is the difference in dwelling type, number of dwellings, dwelling density (dwelling/ha) and population density (hab/ha) for all neighborhoods connected to these two urban waste outfalls of Biskra valley and Z'mor valley (Table 2 and 3).

Statistically, the difference in helminths eggs concentration is not significant ($P < 0.05$) for *Nematoda* eggs, *Cestoda* eggs, *Trematoda* eggs between the two outfalls (Biskra valley and Z'mor valley). For *Strongyloides* eggs, the difference is statistically significant with ($P < 0.005$) between the two sites. It is therefore clear that the abundance of this parasitic load in the wastewater of the two outfalls (Biskra valley and Z'mor valley) originates in the differences in the demographic and socio-economic status of the populations connected to each outfall. This finding has also been reported in numerous studies around the world (Jiménez *et al.* 2002).

5. Conclusion

At the end of our study and based on the parasitological analysis of wastewater from the main outfalls of the city of Biskra, we reached the following conclusions:

- A great difference in the diversity of identified parasite helminths eggs, where the abundance in the *Nematoda* load is high, compared to *Cestoda* and *Trematoda*, which are extremely rare in the

two main outfalls. This is related to populations' way of life and culinary habits.

- The concentration of helminths eggs varies with the seasons and depends on populations connected to each wastewater outfall.

- The concentrations of parasitic helminths eggs found in Biskra's wastewater samples far exceed the standards recommended by the World Health Organization and the Algerian standards for crops irrigation water (≤ 1 viable *Nematoda* egg per liter). This entails a serious health risk in case of reuse without prior treatment. This is the case of Biskra valley and Z'mor valley suburban perimeters, where reuse of raw wastewater, without the slightest precaution, in agriculture has been observed.

In order to eliminate the ecological and health risks caused by raw wastewater discharge into the environment, such as Biskra valley and Z'mor valley, it is imperative to build a wastewater treatment plant.

Conflict of interest statement

We declare that we have no conflict of interest.

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