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Parasitological Toxicological Risks Associated with Vegetable Irrigation Using Untreated Wastewater in Arid Environments: The Example of the Biskra Region (Algeria)

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ABSTRACT: The city of Biskra, located in an arid region of south-eastern Algeria, faces chronic water shortages, exacerbated by extreme climatic conditions and rapid urban growth. In this context, the uncontrolled reuse of wastewater for the irrigation of vegetable crops that are often consumed raw is a common practice, despite the health risks it poses. A study conducted in this region has revealed significant contamination of raw wastewater, particularly in the Oued Biskra, by various parasites, including helminth eggs. The analyses identified several species of nematodes (Ascaris sp., Trichuris sp., Enterobius vermicularis, etc.), cestodes (Hymenolepis nana, Taenia sp.) and a trematode (Fasciola hepatica), with high concentrations, representing a significant risk to public health and the environment. In light of these results, the study recommends a rigorous health assessment of wastewater reuse, as well as the implementation of treatment, monitoring and risk management policies to protect public health and ensure the sustainable use of non-conventional water resources.

INTRODUCTION

In regions facing significant water stress, such as arid and semi-arid areas, the reuse of wastewater in agriculture is a strategic alternative to mitigate the growing water deficit (Bouarafa & Mellah, 2019; Taha *et al.,* 2019; Al-Lahham *et al.,* 2023). In Algeria, particularly in desert areas such as the Biskra region, this practice is widespread due to low rainfall and growing demand for irrigation water (Boudjenoun *et al*., 2022). However, although beneficial in terms of water and agronomy , the reuse of wastewater without prior treatment raises significant health concerns.

Wastewater can contain a variety of pathogens, including viruses, bacteria, protozoa and, above all, helminths, whose eggs are particularly resistant in the environment (Lalami *et al.,* 2014; Qureshi *et al*., 2021). The World Health Organisation (WHO, 2022) emphasises that the presence of intestinal nematodes such as *Ascaris lumbricoides*, *Trichuris trichiura* and *Ancylostoma duodenale* is one of the main obstacles to the safe reuse of wastewater, due to their low infectious dose and their ability to survive for long periods in soil and on crops.

The toxicological and parasitological management of wastewater is therefore a priority issue, particularly when it is used to irrigate vegetables that are consumed raw, such as celery, carrots and spinach. Despite these well-documented risks, in Biskra, wastewater is still used in its entirety for agricultural irrigation without undergoing prior sanitary treatment, which considerably increases the risks of exposure for human and animal populations (El Halaouani, 1995; Tsama *et al.,* 2010; Derouiche *et al.,* 2021).

In this context, the present study aims to assess the parasitic toxicological load, in particular helminth egg contamination, of a few vegetable crops irrigated with raw wastewater in Biskra, with the objective of establishing a health assessment of these practices and issuing recommendations for safer management of unconventional water resources.

2. MATERIALS AND METHODS

2.1. Presentation of the study area

Biskra is a city in south-eastern Algeria, located at an altitude of 115 metres, at geographical coordinates 34°51'01" North and 5°43'40" East (Boudjenouia *et al.,* 2022). This arid region, with extreme climatic conditions, is home to an estimated population of 176,048 inhabitants, with a density of 1,485 inhabitants per square kilometre, according to the most recent data available (Office National des Statistiques d'Algérie [ONS], 2022). The city is experiencing rapid urban growth, characterised by a juxtaposition of modern buildings, traditional dwellings, semi-rural areas and informal settlements (Agence Nationale d'Aménagement du Territoire [ANAT], 2014; Berretah, 2019).

Daily domestic wastewater production is estimated at 265 litres per capita, which places significant pressure on sanitation systems, particularly in a context of chronic water scarcity (National Water Resources Agency [ANRH], 2022, cited by Berretah, 2019). This situation is all the more worrying given that the uncontrolled reuse of this wastewater is commonly observed in the region for the irrigation of agricultural crops.

In terms of climate, Biskra is classified as a semi-arid to arid region according to the Gaussen and Bagnouls ombrothermic diagram, with a prolonged dry season lasting seven months, from April to October (Boudemagh *et al.,* 2019). Average maximum temperatures reach 34.65 °C in July, while minimum temperatures drop to 12.25 °C in January. Average annual rainfall is low, around 291.8 mm, which accentuates the region's water vulnerability (Dib, Belarbi, & Remini, 2019). Relative humidity generally increases during the winter period, correlating with lower temperatures (Zegrar, Mansouri, & Ghriss, 2022).

These climatic and demographic characteristics make Biskra particularly vulnerable to the effects of climate change, including reduced rainfall and increased evapotranspiration. Several recent studies have highlighted these changes, emphasising the urgent need to adopt integrated water management strategies based on better use of unconventional resources such as treated wastewater (Kehila *et al.*, 2021; Benmoussa *et al.,* 2023; Bessaoud & Frija, 2022).

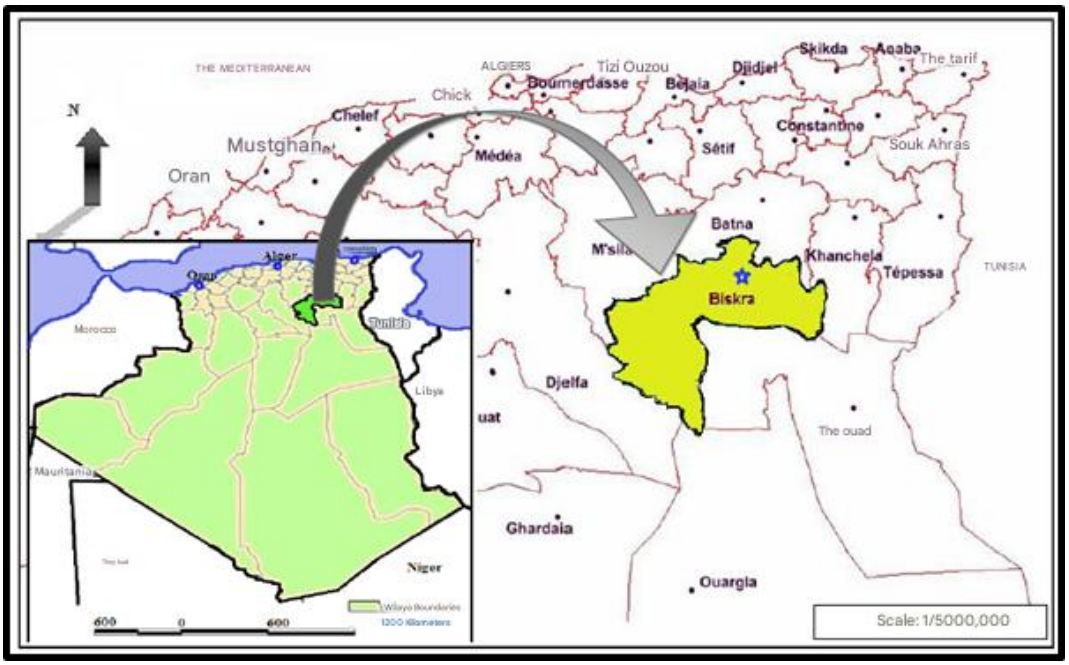


Figure 01: Map showing the location of the wilaya of Biskra and its climatic variables (National Agency for Land Use Planning [ANAT], 2015).

3. SAMPLING OF MARKET GARDENS IRRIGATED WITH RAW WASTEWATER FROM THE BISKRA WADI:

Samples were taken at random from the Oued Biskra, the main outlet for domestic effluent from the north and centre of the city. This watercourse receives inflows from several peripheral wadis, notably El Hai, Djamoura, Branis, Lefrahi, El Besbas

and Lakhdar. It has a diameter of 1,500 mm and a gradient of 2.5‰, which facilitates the flow of wastewater to peripheral agricultural areas (Brinis *et al.,* 2018). The choice of April for sampling is explained by the increase in consumption of certain vegetables during hot periods, in particular *Apium graveolens* (leaves), *Spinacia oleracea* (leaves and stems) and *Daucus carota* (roots), which are commonly grown and consumed in this region (Khelifa *et al.,* 2020; Bouteldja *et al.,* 2023). Each plant species was packaged in a numbered bag and then transported under appropriate conditions to the laboratory for physical, chemical and microbiological analysis (Zerrouki *et al*., 2019; Hadj-Mahammed *et al*., 2022).

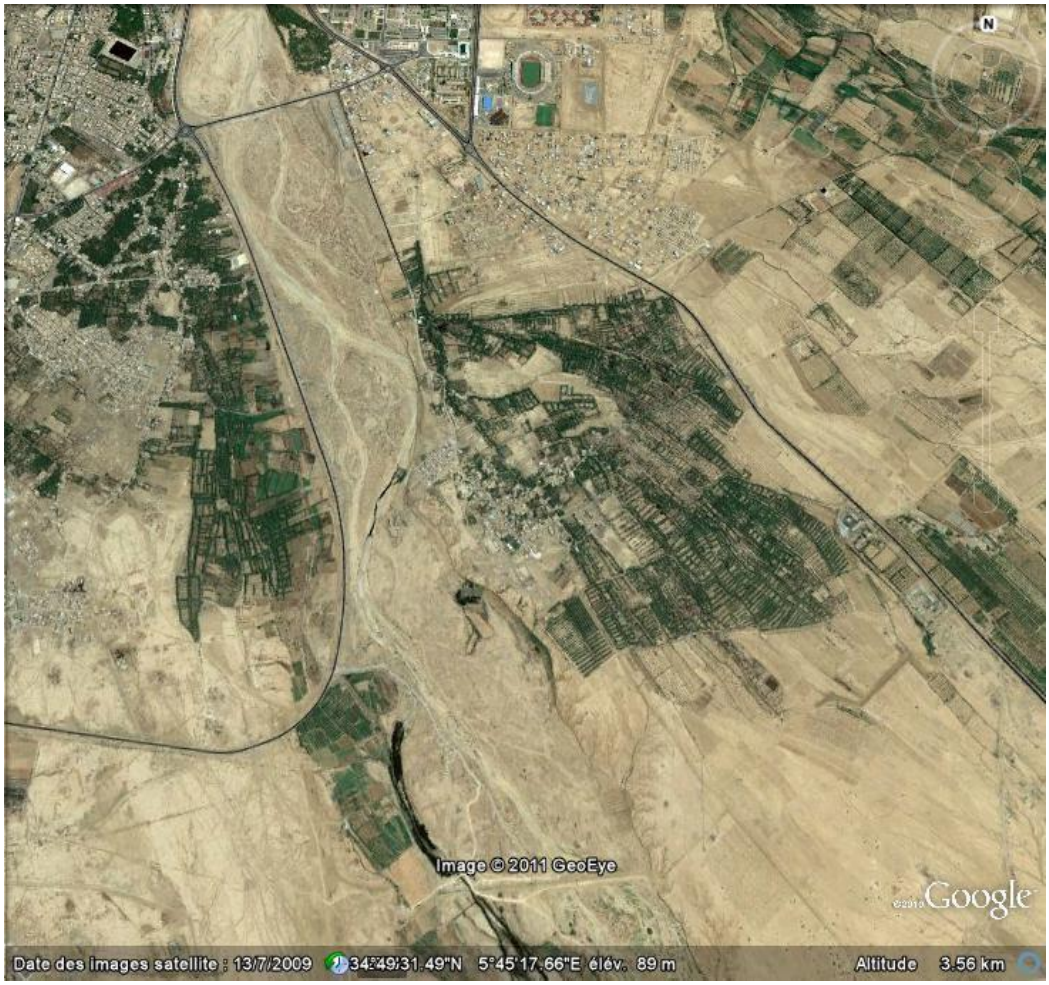


Figure 02: Location of the study site

Toxicological and parasitological analyses of raw wastewater

Due to the morphological and taxonomic diversity of helminth eggs present in raw wastewater, a concentration step is essential to ensure reliable quantification. The Bailenger method, recommended by the World Health Organisation (WHO, 1997), was adopted due to its simplicity, low cost and reproducibility, particularly in resource-limited settings. This technique involves centrifuging a mixture consisting of the sample, an acetoacetic buffer and ether. The sediment obtained is then suspended in a 33% zinc sulphate solution (density = 1.18), which allows the parasite eggs and cysts to float to the surface. These then adhere to the McMaster slide, facilitating their identification and microscopic counting (Bouzid *et al.,* 2019; Aït-Saïd *et al*., 2022; El Ghazali *et al*., 2025)

Toxicological and parasitological analyses of market garden crops:

Toxicological analysis was conducted on three species of vegetable crops frequently consumed in the Biskra region and irrigated with wastewater from the Oued Biskra: celery (*Apium graveolens*), spinach (*Spinacia oleracea*) and carrots (*Daucus carota*). Only the edible parts of each vegetable were selected for analysis.

1. Celery (Apium graveolens) and spinach (Spinacia oleracea)

For each species, 20 g of leaves were carefully washed with tap water to remove surface contamination. The wash water was filtered through a fine mesh sieve and then left to settle. After removing the supernatant, the sediment was centrifuged at 2200 g for 15 minutes. The pellet was then resuspended in a sucrose enrichment solution, according to the viral concentration protocol developed by Cheong *et al.* (2009) and adapted for leafy plant matrices by Said *et al.* (2021). This technique has recently been validated for the detection of parasitic forms on leafy vegetables irrigated with wastewater (Bougherira *et al.,* 2020; Kadi *et al.*, 2023).

2. Carrot (Daucus carota)

The carrot roots were manually extracted from the soil, weighed, and then cleaned by mechanical brushing with distilled water to remove pathogens attached to the surface. The resulting suspension was centrifuged at 1600 g for 5 minutes. The pellet obtained was treated using the same sugar solution enrichment protocol as that applied to leafy vegetables, in accordance with Cheong *et al.* (2009) and the specific adaptation proposed by Said *et al.* (2021) for root vegetables. Recent work has also confirmed the relevance of this approach in the context of parasitic biomonitoring of horticultural products irrigated with contaminated water (Mokhtari *et al.*, 2024; Benali *et al.,* 2025).

4. RESULTS

Toxicological and parasitological results for wastewater

Analysis of urban wastewater in Biskra, specifically from the Oued Biskra, revealed significant contamination with helminth eggs, including nematodes, cestodes and trematodes. These parasites pose a major health risk, particularly in areas with inadequate sanitation infrastructure. This situation raises concerns for public health and the environment, and wastewater management and treatment measures are urgently needed to prevent possible parasitic epidemics (Boudra *et al.*, 2017; El-Sheikh *et al.,* 2015).

With regard to nematodes, six species were identified, with the following egg concentrations per litre: *Ascaris sp.* (38.33 eggs/l, ±21.30), *Strongyles* (34.5 eggs/l, ±10.54), *Trichuris sp.* (31.83 eggs/l, ±18.03), *Enterobius vermicularis* (23.83 eggs/l, ±10.53), *Ankylostoma sp.* (18.5 eggs/l, ±4.59), and *Nematodirus sp.* (24.5 eggs/l, ±9.13). These results are consistent with those reported in other studies on nematode contamination of wastewater in similar regions (Ali *et al.*, 2019; Zúñiga *et al.,* 2021).

With regard to cestodes, the concentrations of eggs per litre were also notable: *Hymenolepis nana* (23 eggs/l, ±7.61), *Moniezia expansa* (25.83 eggs/l, ±9.13) and *Taenia sp.* (21.33 eggs/l, ±10.07). The presence of these cestodes in wastewater is well documented in the literature, where similar levels of contamination have been observed in urban water management systems (Chiarini *et al.*, 2020).

Finally, contamination by trematodes was relatively low, with only one species detected, *Fasciola hepatica*, at a concentration of 3.16 eggs/l (±1.83). This low concentration is consistent with observations from other studies on *Fasciola hepatica* contamination in untreated wastewater (Boudra *et al.,* 2017).

The results of this study highlight the importance of monitoring and treating wastewater in urban areas to reduce the risk of parasitic disease transmission. The data collected indicate the need to adopt preventive measures against the untreated reuse of wastewater and to implement health risk management policies in these vulnerable areas.

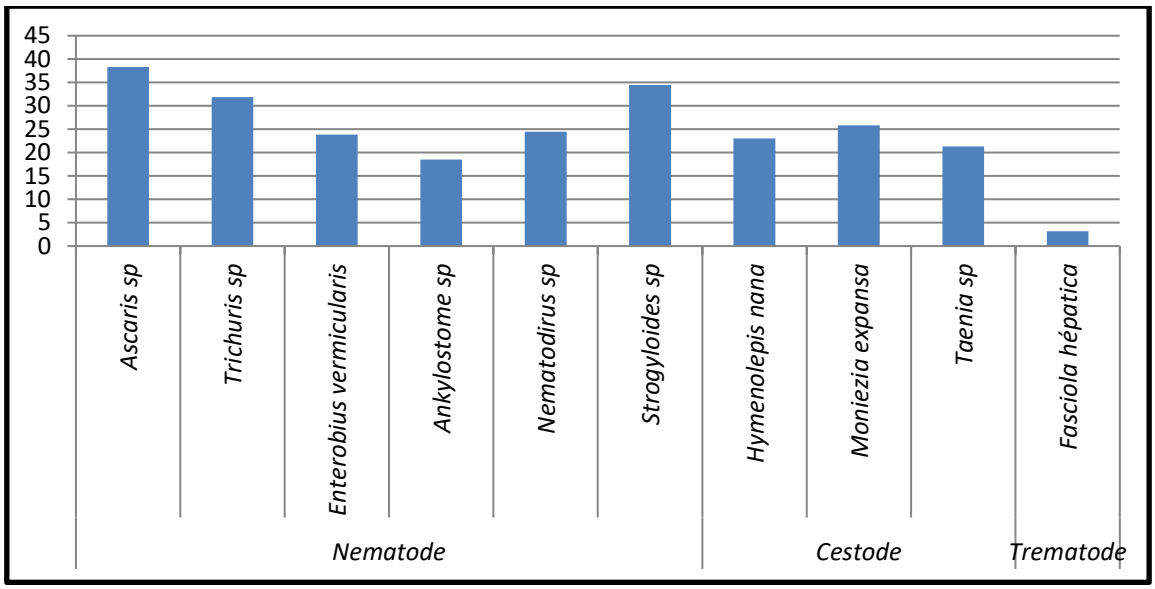


Figure 03: Average concentration of parasitic helminth eggs in wastewater from Oued Biskra.

Parasitological analyses of vegetable crops irrigated with raw wastewater (Oued Biskra).

Analysis of the parasitic load of helminth eggs in the wastewater of the city of Biskra, specifically that from Oued Biskra, reveals a predominance of nematodes and cestodes. Among the nematodes, the main species identified are *Ascaris sp.*, *Strongyloides sp.*, *Enterobius sp.*, and *Ankylostoma sp.*, while the cestodes are represented by *Taenia sp.* and *Hymenolepis sp.*. This parasitic contamination poses a significant health risk, particularly in areas irrigated by this wastewater.

Vegetable crops irrigated with this wastewater showed varying concentrations of helminth eggs. Carrots had an average parasite load of 11.96 eggs per 100 grams, mainly dominated by nematodes. The species identified were: *Ascaris sp.* (4.01 eggs), *Strongyloides sp.* (0.2 eggs), *Trichuris sp.* (1 egg), and *Enterobius sp.* (0.05 eggs), as well as cestodes, notably *Taenia sp.* (4 eggs) and *Hymenolepis sp.* (2.7 eggs). Celery, on the other hand, showed an average concentration of 5.4 eggs per 100 grams, with contamination by *Ascaris sp.* (2.1 eggs), *Strongyloides sp.* (0.1 eggs), *Trichuris sp.* (0.1 eggs), *Taenia sp.* (1.5 eggs) and *Hymenolepis sp.* (1.7 eggs). Spinach had the lowest parasite load, with an average of 2.25 eggs per 100 g grams, consisting mainly of *Ascaris sp.* (2 eggs), *Strongyloides sp.* (0.1 egg), *Enterobius sp.* (0.05 egg) and *Ankylostoma sp.* (0.1 egg).

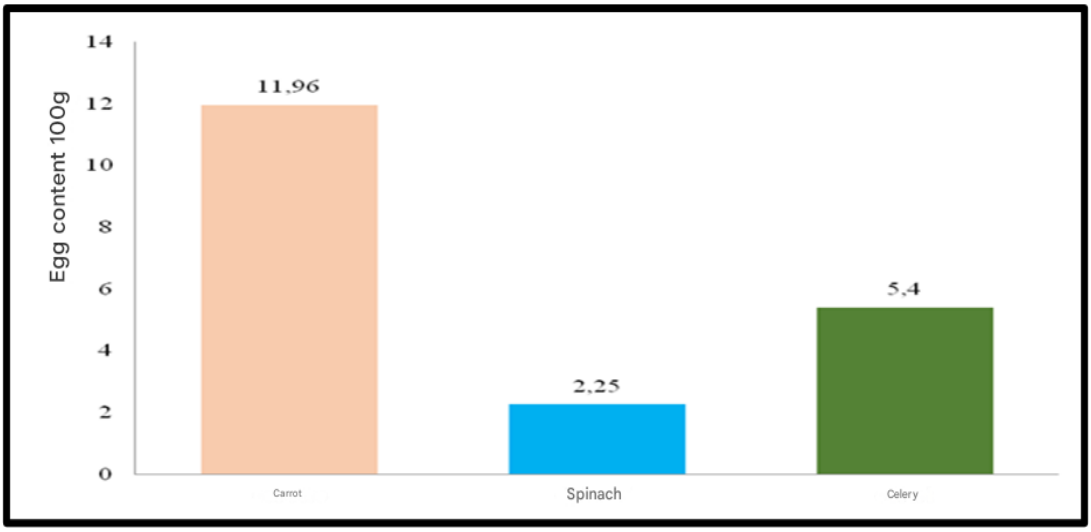


Figure 04: Helminth egg counts (eggs/100) in vegetable crops irrigated with raw sewage.

The histogram shows that carrots have the highest parasite load, followed by celery and finally spinach, which has the lowest concentration of helminth eggs. Nematodes, particularly *Ascaris sp.*, are the most prevalent in all crops, with cestodes also

detected, notably *Taenia sp.* and *Hymenolepis sp.*. This indicates that vegetables irrigated with wastewater from the Oued Biskra are contaminated, highlighting the need for adequate wastewater management to limit health risks.

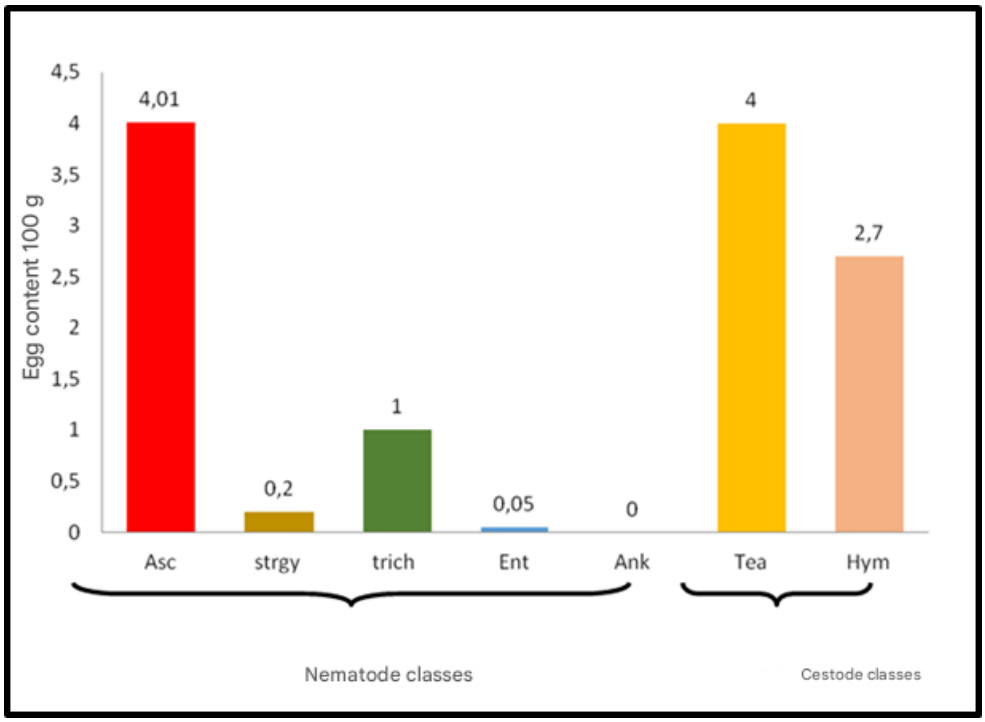


Figure 05: Representation of helminth eggs identified on carrots irrigated with raw wastewater

The graph shows an uneven distribution of egg content among different categories. One category has a much higher concentration than the others, while some are almost absent or have very low values. This difference in content suggests that some categories are much more frequent or present in greater quantities than others. The notable gap between the highest and lowest values could reflect environmental or biological factors influencing the presence of these elements. In short, the graph highlights a marked variability in the distribution of eggs among the different categories.

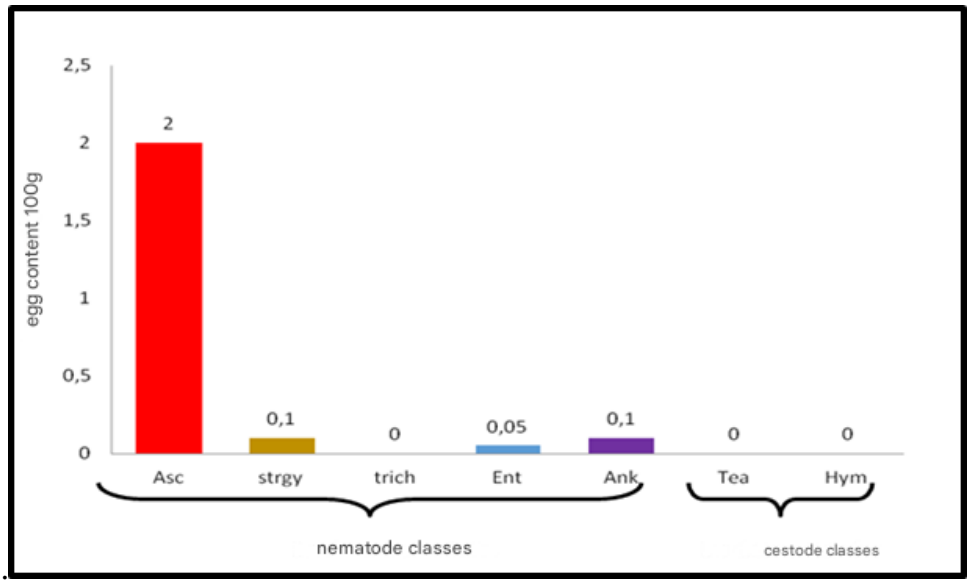


Figure 06: Representation of helminth eggs identified in spinach crops irrigated with raw sewage.

This histogram shows an uneven distribution of egg content for different categories, expressed as the number of eggs per 100 g.

Only one category stands out clearly with a high value of 2 eggs/100 g, while all the others have very low values, ranging from 0 to 0.1, with the exception of one case at 0.05. Several categories even show a total absence, with a value of zero.

This representation highlights the very marked predominance of a single element over the others, which appear to be either weakly present or completely absent in the sample considered. The low diversity of high levels suggests contamination limited to a single main source.

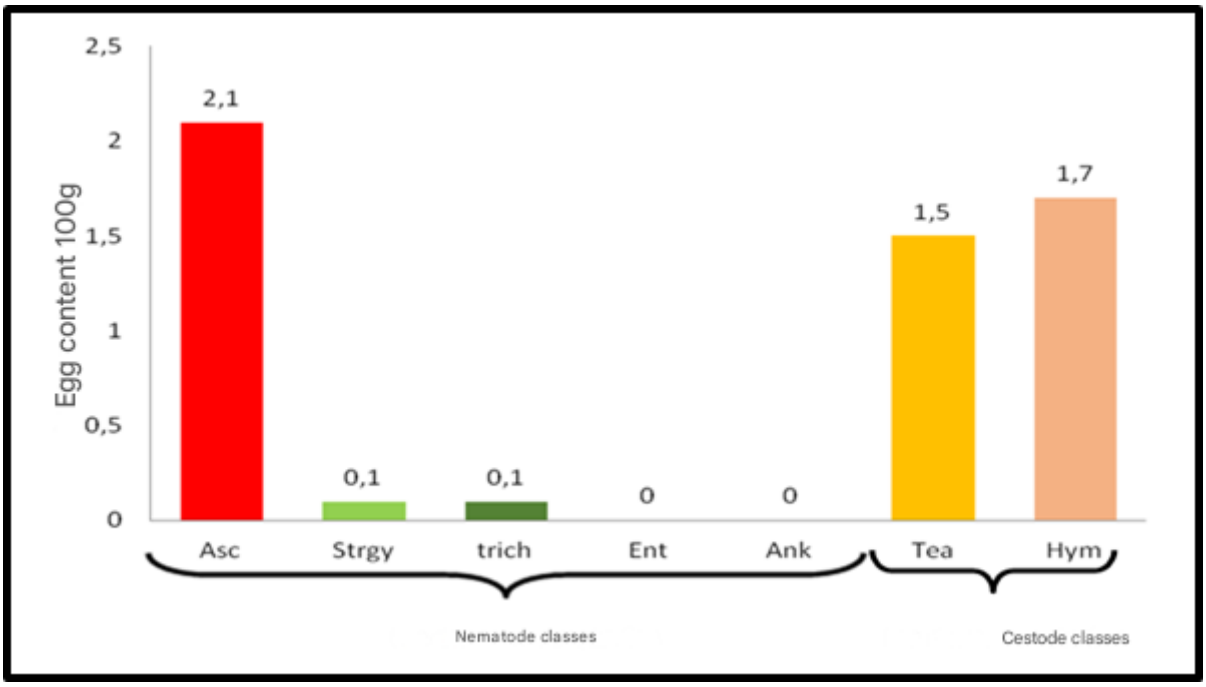


Figure 07: Representation of helminth eggs identified on celery grown using raw sewage water**.**

The histogram highlights a very contrasting distribution of the measured values. A few categories have relatively high levels, exceeding 1.5 eggs per 100 g, while the majority have very low or even zero levels.

The concentration therefore seems to be strongly dominated by a small number of categories, indicating a marked and targeted presence. Conversely, several categories are poorly represented or completely absent, reflecting low contamination or a lack of detection in these cases. This distribution suggests marked heterogeneity in the distribution of values.

DISCUSSION

Parasitological toxicological analysis of raw wastewater in the city of Biskra, in Algeria, revealed significant contamination by helminth eggs, with an average concentration of 18.47 ± 5.74 eggs/L, well above the standard recommended by the World Health Organisation (WHO), which is ≤1 egg/L for agricultural irrigation (Guemmaz *et al*., 2021; Thebo *et al*., 2017). Three major groups of helminths were identified: nematodes, which were the most prevalent, followed by cestodes, and finally trematodes, which were present in negligible quantities or absent (*Guemmaz et al*., 2021).

Among the most common species are intestinal nematodes such *as* ***Ascaris sp.***, ***Trichuris sp.***, ***Strongyloides sp.***, ***Enterobius sp.*** *and* ***Ankylostoma sp.***, as well as the cestodes ***Hymenolepis sp.*** *and* ***Taenia sp.*** (Guemmaz et al., 2021). The presence of Ascaris sp. and *Trichuris sp*. in wastewater poses a high health risk to exposed populations and compromises the safety of agricultural reuse of this water (Amoah *et al*., 2016).

The contamination of vegetable crops by helminth eggs is directly linked to the quality of irrigation water. **Carrots (*Daucus carota L.*)** have the highest loads with 11.96 eggs/L, followed by **celery (*Apium graveolens L.*)** with 5.4 eggs/L, and **spinach (*Spinacia oleracea L.)*** with 2.25 eggs/L (Guemmaz *et al.,* 2021). This difference is attributed to the underground position of the edible part of the carrot, which benefits from a microclimate favourable to the persistence of helminth eggs, particularly due to stable humidity, low exposure to UV rays and desiccation (Tsafack *et al*., 2019). Previous studies have also

shown that the underground organs of vegetable crops are most susceptible to contamination because they remain in prolonged contact with wastewater during irrigation cycles (Firadi, 1996; Dssouli *et al.*, 2006; Al Manasfi, 2020).

Although celery and spinach grow close to the ground, their overlapping leaves partially protect the eggs from adverse climatic conditions such as direct sunlight and desiccation. However, the frequency of their direct contact with wastewater during irrigation remains a significant source of contamination (Bouzou *et al.*, 2018).

In addition, the uncontrolled reuse of raw wastewater in agriculture significantly increases health risks. A recent meta-analysis showed that agricultural workers exposed to this water had a five times higher risk of contracting parasitic infections (odds ratio = 5.49) compared to those who were not exposed (Adegoke *et al.*, 2018).

Conclusion

At the end of our study, based on the toxicological and parasitological analysis of several vegetable crops irrigated with wastewater from the city of Biskra, we reached the following conclusions:

The helminth egg parasite loads in the various vegetable crops (celery, spinach and carrots) are highly dependent on the level of contamination of the irrigation water. Carrots have the highest content with 11.96 eggs/litre, followed by celery with 5.4 eggs/litre and finally spinach with 2.25 eggs/litre.

A comparison of the helminth egg counts in these three crops shows that spinach has the lowest levels, followed by celery, while carrots have the highest levels.

According to the World Health Organisation and Algerian standards for water used to irrigate crops, this poses a huge health risk if the water is reused without prior treatment. This is the case in the peri-urban area of Oued Biskra, where the practice of reusing this raw wastewater in agriculture without any precautions has been observed.

According to the results of this study, carrot cultivation, due to its high helminth egg load and raw consumption by humans, is likely to be a vector for a large number of parasitic diseases. Therefore, wastewater treatment and crop restrictions remain a requirement before any reuse in agriculture without health risks to humans and livestock.

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