

“Helminth eggs and faecal coliforms in the sewage treatment plant of Aïn taoujdate (Morocco): Interest of lagoon treatment.”



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ABSTRACT

In Morocco, the presence of Helminth eggs and faecal coliforms are considered as a limit to the reuse of wastewater because of sanitary risks. So, the overall objective of this study is primary, to evaluate the efficiency of the Aïn taoujdate conventional wastewater treatment plant in the elimination of Helminth eggs, their bacterial treatment efficiency, also to seek a total inactivation treatment of the bacteria that are more resistant to the treatments envisaged, so as to ensure correct safety of the purified water and for a possible reuse of these purified water. Moreover, the results show that raw wastewaters of Aïn taoujdate is heavily loaded in faecal coliform bacteria, which are in the order of 10^6 to 10^8 NNP / 100 ml, and rates the reduction are in the order of 3 to 4Ulog. While, the acidification treatment of these purified water with a dose of 15.08 ml of phosphoric acid up to a pH of 2 units allows for a total elimination of the aforementioned bacteria. In addition and to attenuate the impacts of eutrophication on the environment, a dose of 88 ml of calcium oxide is recommended for the neutralization of this phosphoric acid which is a fertilizer.

Also, the concentrations of Helminth eggs in raw wastewater at the entrance to the WWTP recorded during the months (January 2013-2014 and July 2013-2014) are respectively about 06 eggs / l, 11 eggs. 21 eggs / l and 32 eggs / l. While at the exit, we note a complete absence of Helminth eggs. Therefore, the Aïn taoujdate WWTP of the natural lagoon type with a correction of its pH shows its efficiency in the complete elimination of bacteria and parasites (Helminth eggs). The results are conclusive and widely in line with the standards recommended by the World Health Organization and the Moroccan Standards and Standards Committee for water intended for crop irrigation, which recommends a content of no more than one (1) viable egg Nematode per liter and a content less than or equal to 1000 NPP faecal coliforms.

Keywords :

Helminth eggs,
faecal coliforms,
wastewater,
natural lagoon,
WWTP

I. INTRODUCTION

Morocco is classified among the countries with high water stress. In addition to its scarcity, water is subject to increasing and continuing pressure for water needs due to increasing density of population, the rapid development of industrial activities, the improvement of the living environment and the extension of water of irrigated agriculture. Also, the wastewater domestic can be considered as a secondary source of water and fertilizer [1]. Indeed, the reuse of wastewater will be a real asset in the future if it is implemented and is taken in time the necessary safeguarding measures [2-3]. It can alleviate the overexploitation of conventional waters resources that can no longer meet the needs of intensive agriculture and reduce the water deficit [4-5-6]. However, raw wastewater contains pathogenic microorganisms, parasitic protozoa or metazoans [7-8-9-10-11-12], which represent a potential danger to public health [13-14-15]. Therefore, the present study is a contribution to the evaluation of the efficiency of Ain taoujdate WWTP for the elimination of faecal coliform bacteria and parasites (Helminth eggs) [16] and also, the originality of this study is to search for a treatment of complete inactivation of the aforementioned bacteria which are more resistant to the treatments envisaged, so as to guarantee a correct safety of the purified water and it, for possible reuse of these waters.

II. MATERIAL AND METHODS

2.1 Enumeration of faecal coliforms

2.1.1 Sampling and retention

The flasks of water for bacteriological analysis are collected with all the aseptic conditions in pre-sterilized 500 ml glass vials in an autoclave with a temperature of $121^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and a pressure of 1 bar 15min. As a general rule, the samples taken were conditioned and transported in a cooler at a temperature of $\pm 4^{\circ}\text{C}$ to the laboratory and analyzed immediately without exceeding 24 hours [17].

The bacteriological parameters (faecal coliforms) and parasitologies (helminth eggs) are monitored at the entrance of Ain taoujdate WWTP, at the exit of anaerobic basins and at the exit of Ain taoujdate WWTP.

2.1.2 Principle.

The term "faecal coliforms" includes several bacterial species of the enterobacteria family, they are facultative aerobic and anaerobic microorganisms, rod-shaped, gram negative, non-sporogenous.

For wastewater, the search and enumeration of faecal coliforms or thermotolerants was performed using the liquid seeding method or the most probable number (MPN), which consists to estimate the concentration of viable microorganisms in a sample by means of replicate liquid A1 broth growth in ten-fold dilutions. It is commonly used in estimating microbial populations in, wastewaters, and the incubation is carried out at $44^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 24H. A turbid culture medium and a gas production indicate the presence of fecal coliforms [18].

2.1.3 Preparation of culture medium A1 Broth

31.5 g of dehydrated A1 Broth medium are dissolved in one liter of distilled water, the mixture is stirred slowly until complete dissolution, 10 ml of medium thus prepared is distributed in each tube containing a Durham bell, then sterilized in the autoclave at $121^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 15 min. After cooling. The pH measured should be 6.9 ± 0.2 at 25°C .

2.1.4 Preparation of the dilutions

We put in a rack the number of sterile tubes that correspond to the number of dilutions chosen, for our case we chose 10^{-7} . Shake the sample vigorously to obtain a homogeneous distribution of the microorganisms and immediately transfer with a sterile pipette 1 ml of this sample homogenized in the first of the tubes containing 9 ml of dilution water. This dilution represents the 10^{-1} dilution. With a new pipette, 1 ml of this dilution, homogenized, is transferred into the second tube (10^{-2}). From the second tube, proceed to the preparation of the following dilution (10^{-3}) and Continue thus until 10^{-7} (Figure 1).

2.1.5 Seeding the waste water sample

5 tubes of A1 broth medium prepared beforehand are transferred to each of these tubes, with a pipette, 1 ml of the homogenized dilution 10^{-1} . Then, take a second series of 5 tubes of medium A1 broth and transfer into each of these tubes, with a pipette, 1 ml of the homogenized dilution 10^{-2} . For each of the following dilutions (10^{-3} to 10^{-7}) take 5 tubes of medium and transfer to each of them 1 ml of the appropriate dilution of the sample and change the pipette for each dilution. The contents of the tubes are then mixed so as to obtain a homogeneous distribution of the inoculum and the medium.

2.1.6 Calculation method

For the reading of positive thermotolerant coliform tubes, the strongest choice of dilutions, that is the one containing the lowest concentration of the sample, which reveals 3 positive tubes, as well as the two highest dilutions which follow immediately.

The number of faecal coliforms per 100 ml of sample is given by the following expression:

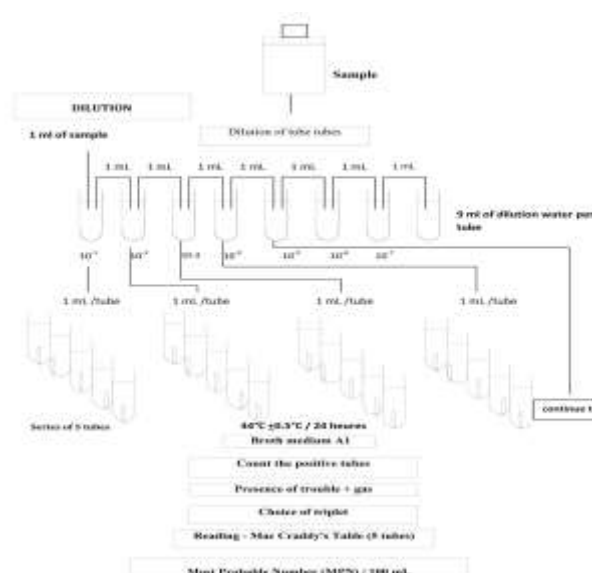
$$N = \frac{\text{NPP}}{\text{Tx}}$$

N = number of indicators sought

NPP = most likely number read in the table of Mac Craddy 5 tubes.

Tx = dilution ratio corresponding to the highest dilution retained.

Research and enumeration of faecal coliforms in wastewater (MPN method - series of 5 tubes)



2.1.7. Results and discussions

Faecal coliform bacteria concentrations in the raw sewage effluent at the entrance of Ain taoujdate WWTP measured during the months (January 2013-2014 and June 2013-2014) are respectively of the order of $8.0 \cdot 10^6$; $3.0 \cdot 10^6$; 1.310^7 ; and $3.0 \cdot 10^8$ NNP / 100 ml. Therefore, they do not show significant fluctuations. This bacterial load is of the same order of magnitude as that found in urban raw sewage [19-20-21-22-23-24-25]. In the effluents treated, the faecal coliform contents recorded are respectively of the order of $2.5 \cdot 10^5$; 5.010^4 ; $3.0 \cdot 10^5$ and $9.0 \cdot 10^6$. The abatement rates for fecal contamination indicators recorded for the Ain taoujdate treatment plant are consistent with the values found in the literature, which show that biological processes can reduce coliform abundance by 3 to 4 log units [26-27-28-29].

In addition and with the aim of seeking a treatment of inactivation or complete elimination of the bacteria resistant to the treatments envisaged, in order to guarantee a sanitary safety correct of the purified water at the exit of the of Ain taoujdate WWTP for possible reuse of these treated waters. We performed tests in the laboratory by injecting 1% phosphoric acid on samples of purified waste water to acidify them in such a way as to have pH values (7, 6, 5, 4, 3 and 2) (Figure 2) and perform fecal coliform analyzes because the pH of the medium directly influences the growth of microorganisms (Table 1).

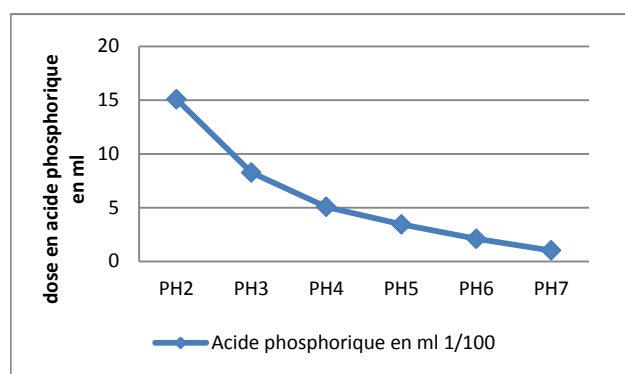


FIG. 2: Measured pH of the purified water samples of Ain taoujdate WWTP as a function of the ml contents of the phosphoric acid.

Table 1: Fecal coliform bacterial load as a function of the pH of the purified waste water sample

Dilutions \ pH	1	10^{-1}	10^{-2}	10^{-3}	10^{-4}	Number of faecal coliformes in NNP / 100 ml
7	5	5	5	4	2	$1.6 \cdot 10^6$
6	5	5	5	3	2	$9.0 \cdot 10^5$
5	5	5	4	3	1	$3.3 \cdot 10^5$
4	5	4	2	2	1	$2.2 \cdot 10^4$
3	3	2	1	0	0	$1.7 \cdot 10^3$
2	0	0	0	0	0	0

We find that the bacterial load in faecal coliforms in the purified waste water of Ain taoujdate WWTP is reduced proportionally by 1 to 3 Ulog with the decrease in pH until complete absence of coliform bacteria faeces with pH equal to 2 units. So the pH it an important role in the growth of bacteria (Table 1).

Also and despite the fact that phosphoric acid is a fertilizer, we have neutralized it by adding calcium oxide; so that there is no impact of eutrophication on the environment and especially the proliferation of algae Cyanobacteria, Chlorophyceae, Euglenophyceae and Diatoms [30-31-32-33-34-35-36-37-38]. Indeed, phosphorus will be assimilated by algae by implementing temporary bonds between the PO_4^{3-} ion and transport enzymes at the level of the cell membrane [39-40-41-42]. In our study, a 88 ml dose of calcium oxide at a concentration of 10% to neutralize 15.08 ml of phosphoric acid in the sample at pH 2 (Figure 3).

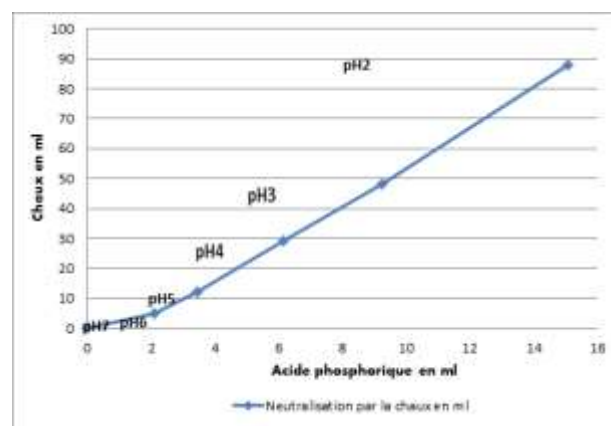


Figure 3: Curve of neutralization of phosphoric acid with calcium oxide

2.2 Enumeration of Helminth eggs parasite

2.2.1 Principle.

Helminths refer to parasitic worms in the gut of humans or certain animals. Nematelminths (roundworms) and Plathelminths (flatworms) are distinguished. Intestinal helminthiasis are animal and human parasitic diseases due to Helminth infestation or direct or indirect contact by the consumption of crops irrigated with these raw sewage. Consequently, they constitute a health risk for the human population when they are reused in the raw state in agriculture [43-44]. Numerous studies have demonstrated the effectiveness of lagoon water treatment systems for Helminth eggs [45-16].

Therefore, the objective of this study is qualitative research and quantitative enumeration of Helminth eggs at the entry and exit of the natural lagoon Ain taoujdate WWTP to evaluate the performance of the said STEP as for the folding of the eggs of Helminthes.

2.2.2 Material and methods

The wastewater samples were collected in appropriate vials, stabilized at pH 2 and stored in the dark at (5 ± 3) °C for 48 hours up to one week. The method applied by coupling sedimentation-centrifugation supplemented by a flotation phase. The recovery of viable and / or non-viable Helminth eggs was made by flotation in a solution of Zinc Sulfate at a density of 1.35. Then counting is done immediately under a microscope using the Mac Master cell at magnification ($\times 10$, $\times 20$) immediately to avoid crystallization of zinc sulfate [46].

2.2.2 Expression of results

The concentrations are expressed in number of Helminth eggs per 1 liter of sample analyzed using the formula below: $N = AX / PV$

Or :

N = number of eggs per liter of sample

A = number of eggs counted on the McMaster blade or average of numbers found in two or three blades
X = volume of the final product (ml)

P = Capacity of the Mac Master blade (ml)

V = volume of the initial sample (liters).

2.2.3 Results and discussions

The concentrations of Helminth eggs in the raw sewage water entering the Ain taoujdate WWTP recorded during the months (January 2013-2014 and July 2013-2014) are of the order of respectively 06 eggs /l, 11 eggs /l, 21 eggs /l and 32 eggs /l d'Helminth (Figure 4).

Therefore, we observe a significant increase in Helminth eggs levels during the warm period compared to the cold period and a significant variation during the two years of study. These results are comparable to those found in wastewater in some Moroccan cities such as Marrakesh with variations from 4 eggs /l to 32 eggs /l [45-47], Ouarzazate with 18 eggs /l [48-49-50] and Beni Mellal with 25,3 eggs /l [51], kenitra with 21,56 ceufs/l [52].

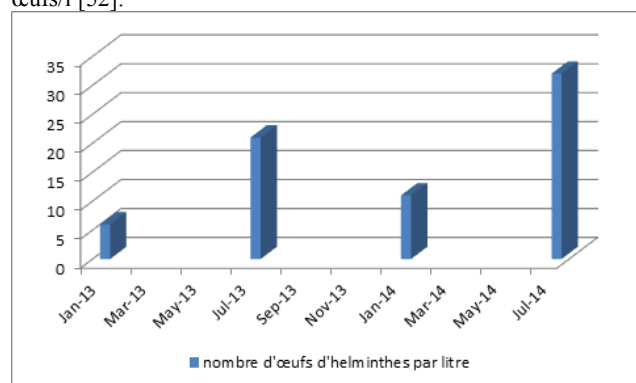


Figure 4: Number of eggs of helminthes by litre

Also, they are almost similar and of the same order as those found in Egypt's wastewater with 6 eggs / l to 42 eggs / l [53-54]. Ain taoujdate wastewater is lightly loaded compared to the wastewater of some Latin American countries such as Brazil with 1490 eggs / l [55] and heavily loaded with wastewater from some African cities as Dakar in Senegal [56] and Yaoundé in Cameroon [57] and Europeans like Nancy in France with 8 eggs / l [58].

While there is complete absence of helminth eggs at the exit of the Ain taoujdate WWTP during study periods. Therefore, the treatment of wastewater by natural lagooning in arid climate proves its effectiveness in the total elimination of helminth eggs. These results are largely in line with the recommendations of the OMS and the Moroccan Standards and Standards Committee for Crop Irrigation Waters which advocates one (1) viable Nematode egg per liter.

III. CONCLUSION

The present work is a contribution to the study of the microbiological and parasitological quality of the waste water from the Ain Toujdate purification plant of the natural lagoon type, which was intended, on the one hand, to control the efficiency of this wastewater treatment plant. STEP regarding the elimination of faecal coliform bacteria and to seek a treatment of total inactivation of the aforementioned bacteria which are more resistant to the treatments envisaged and this; for a possible reuse of these purified waters without harming the environment. In addition, the results show that the raw sewage is heavily loaded with fecal coliform bacteria which are in the range of 106 to 108 NNP / 100 ml. While the treatment of purification of these waters made it possible to have a reduction of 3 to 4Ulog.

Moreover, and for the total elimination of these resistant bacteria, the treatment which has been envisaged is an acidification of water purified by phosphoric acid which is a fertilizer. The injected dose is of the order of 15.08 ml to have a pH of 2 units. In addition and for its neutralization, we applied an 88 ml dose of calcium oxide in order to mitigate the impacts of eutrophication on the environment. On the other hand, the parasitological characterization of raw sewage water at the entrance of Ain taoujdate WWTP shows that Helminth egg levels are high and vary according to the seasons, they are high during the hot and humid period. low in cold period. This difference in concentration would be related to seasonal variations in the infestation. The levels of Helminth eggs evidenced in the wastewater samples analyzed are comparable to those found in previous work carried out in other WWTPs in Morocco. The treatment of domestic wastewater by natural lagooning demonstrates its effectiveness in the total elimination of Helminth eggs. Consequently, these results are largely in line with the standards recommended by the World Health Organization and the Moroccan Standards and Standards Committee for water intended for crop irrigation, which recommends a content of not more than one (1) viable eggs Nematode per liter.

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