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Original papers

Occurrence and removal of protozoan cysts and helminth eggs in the Médéa sewage treatment plant (south-east of Algiers)

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ABSTRACT. One of the most important quality characteristics associated with wastewater reuse in discharging in water bodies is the microbial quality. This study aimed to determine the efficiency of Médéa wastewater treatment plant (conventional activated sludge system) in the removal of protozoan cysts and parasitic eggs. This study was carried out during four months and samples were collected at weekly intervals from influent and effluent of the wastewater plant. In order to determine the concentration of ova, samples were analyzed according to Bailenger method. The wastewater treatment plant (WWTP) of Médéa has removed 88.9–100% of parasite eggs and more than 95% of protozoan cysts.

Key words: helminth eggs, Médéa, Algeria, protozoan cysts, removal, wastewater

Introduction

Wastewater discharges are worldwide risk factors for the introduction of human pathogens into surface waters used as drinking and recreational resources. Microbial pathogens which can be potentially present in wastewater can be divided into three separate groups: viruses, bacteria, and protozoans/helminths [1]. Pathogenic protozoa are more prevalent in wastewater than any other environmental source [2]. These parasites are also characterized by their zoonotic transmission, low infective dose and resistance in the environment. *Entamoeba histolytica*, *Giardia intestinalis* and *Cryptosporidium* are three of the major causes of parasitic induced diarrhea disease [3,4] and the most common cause of infection worldwide [5,6]. Helminths (nematodes and tapeworms) are common intestinal parasites which, as the enteric protozoan pathogens, are usually transmitted by fecal route in humans [7].

Sewage effluent is a source of contamination of the environment, which may be of public health significance, particularly if sewage is discharged into water that is subsequently used for drinking, recreation, or agricultural purposes [8,9]. Wastewater treatment is the most important way of ensuring that it is properly handled before discharge into the environment. The wastewater must be treated in order to remove pollutants such as organic matters and pathogens. There are a number of methods through which wastewater treatment can be carried out including activated sludge [10–13]. The activated sludge process is the most widely applied biological wastewater treatment process in the world.

In Algeria, no studies have been conducted on efficiency of removal by wastewater treatment plants in protozoan cysts and parasite eggs removal.

The present study aimed to identify helminth eggs and protozoan cysts in wastewater samples to which both human and animals could be exposed when they are reused in agriculture, and evaluate the efficiency of their removal by wastewater treatment system.

Materials and Methods

Médéa is the capital city of Médéa Province

(Algieria) and is located roughly 68 km south of Algiers. The wastewater treatment plant is located at 10 km east of Médéa.

Samples (n=7 pairs of data) were taken weekly from May 30 to July11, 2010 in both the inlet and the outlet of the wastewater treatment plant. Analysed volumes were 1 L for raw wastewater samples and 5 L for treated wastewater samples; defined as one giving the most significant results [14]. The parasitic analysis was performed using the modified Bailenger method applied to wastewater [15]. Briefly, the samples were decanted in the laboratory for 24 hours. About 90% of supernatant liquid was discarded, the sediment recovered was then transferred to tubes and centrifuged for 15 min at 1000 RPM (revolutions.min^{-1}). The deposited sediment was combined, transferred into one tube, then centrifuged for another 15 min at 1000 RPM. After that, the pellet was suspended in an equal volume of acetoacetic buffer at pH=4.5, considered as the most favorable to concentrate parasites [16]. Two volumes of ether are added and the sample mixed for 10 min before being again centrifuged for 15 minutes at 1000 RPM. Three layers were formed in the tube, the black and the turbid layers were discarded, Zinc sulfate ($ZnSO_4$) was added to the tube (5 folds sediments volume, gravity 1.3, density=33%) and mixed. Fifty µL were transferred to a slide for microscopic counting (magnification ×100, ×400). The number of wastewater ova or cysts.L^{-1} was calculated using the equation: $N = (nV_1)/(V_2V_3)$, where N is the number of ova or cysts.L^{-1} of sewage, n is the number of ova or cysts counted under microscopic observation, V_1 (mL) the volume of the final product, V_2 (0.050 mL) the volume put on the slide and V_3 the original sample volume (1 L for raw wastewater and 5 L for treated wastewater).

The efficiency of the WWTP in removal of parasites is calculated using the following formula [17]:
Removal percentage (%) = $(N_{influent} - N_{effluent}) \times 100/N_{influent}$; where $N_{influent}$ = number of parasites eggs in the influent wastewater and $N_{effluent}$ = number of parasites eggs in the effluent wastewater.

Signs of egg development were not considered for confirmation of eggs viability.

Statistical analysis. The comparisons of the parameters, before and after treatment, were performed using t-test for dependent samples; the Wilcoxon matched pairs tests were using in cases of severe violations to normality or heterogeneity of variances. The statistical analysis was performed using Statistica 10 (Statsoft Inc, Tulsa, OK, USA). The results are given as mean±SD (SD: standard deviation). The differences were considered significant at $p<0.05$.

Results

The protozoa cysts (mainly *Giardia intestinalis*, *Entamoeba coli*, *Entamoeba histolytica/dispar*) and helminth ova (mainly *Ascaris* sp., *Trichuris* sp., *Hymenolepis nana*, *Hymenolepis diminuta*, *Taenia* sp., and *Toxocara* sp.) were differentiated under the microscope on the basis of their size and their shape. The used technique all over that survey did not allow differentiation of the pathogenic *E. histolytica* species from the morphologically similar but non-pathogenic *Entamoeba dispar* species, they are reported as *E. histolytica/dispar* (Table 1).

This study focused on the monitoring of wastewater contaminated with protozoan cysts and helminth eggs distributed in two classes, nematodes

Table 1. Protozoa and helminth parasites present in raw (RWW) and treated (TWW) wastewater

Parasites	Genus/Species	RWW	TWW
Protozoa	*Giardia intestinalis*	+	-
	Entamoeba coli	+	+
	Entamoeba histolytica/dispar	+	-
Nematoda	*Ascaris* sp.	+	+
	Trichuris sp.	+	-
	Toxocara sp.	+	-
Cestoda	*Taenia* sp.	+	-
	Hymenolepis nana	+	+
	H. diminuta	+	+

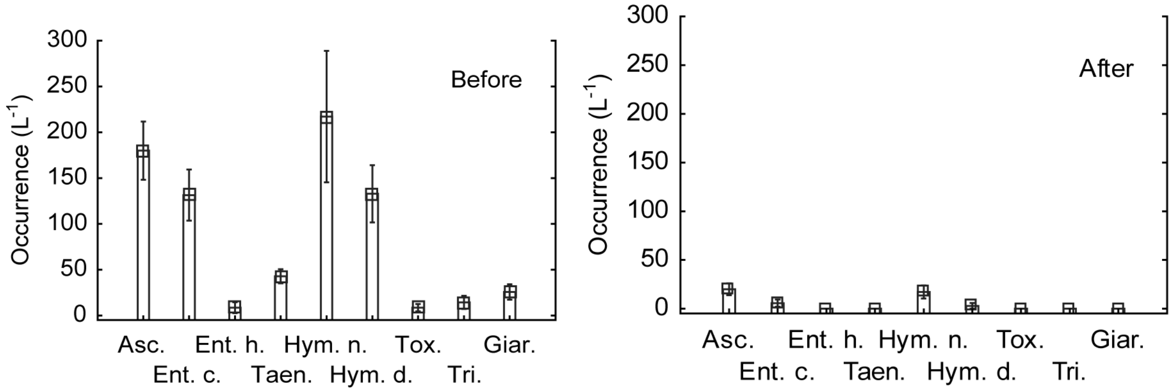

Fig. 1. Average concentration of helminth parasites eggs and protozoa cysts in incoming and outgoing waters of the WWTP (Asc: *Ascaris*; Ent.h.: *Entamoeba histolytica/dispar*; Hym.n.: *Hymenolepis nana*; Hym.d.: *Hymenolepis diminuta*; Tox.: *Toxocara*; Giar.: *Giardia intestinalis*; Ent.c.: *Entamoeba coli*; Taen.: *Taenia*; Tri.: *Trichuris*).

and cestodes, with a predominance of the second class. Three protozoan parasites were recognized distinctly by microscopy namely: *Giardia intestinalis*, *Entamoeba histolytica/dispar* and *Entamoeba coli*. Among these waterborne protozoans, *Giardia* and *Entamoeba coli* were found in most of the samples. In both raw treated wastewater, helminths concentrations are consistently higher than those of parasitic protozoa. The main species of helminth eggs encountered were *Taenia* sp., *Hymenolepis nana*, *Hymenolepis diminuta*, *Ascaris* sp., *Toxocara* sp. and *Trichuris* sp. Eggs of *Trichuris* sp. and *Toxocara* sp. were occasionally found. The mean intensity, abundance, dominance and standard deviation (SD) were determined and has been presented in the Fig. 1.

Results showed that wastewater treatment plant removed 88.9–100% of parasite eggs and more than 95% of protozoan cysts ($p<0.05$ for the majority of species) (Table 2).

Discussion

Although information on the occurrence of these pathogens in wastewater is available, there is no record of previous parasitological study in the Médéa wastewater treatment plant. This study fills this gap and reports on the occurrence of protozoa cysts and helminths eggs in raw and treated wastewater. These results are significant in assessing the health risk faced by the inhabitants of Médéa.

According to some authors, concentrations and varieties of eggs found in wastewater are based on various climatic factors, socio-economic, and

Table 2. Concentration of helminth eggs and protozoa cysts in incoming and outgoing waters in the WWTP (Mean ±SD) and percentage of reduction (cysts.L^{-1} or eggs.L^{-1})

Genus/Species	RWW	TWW	R (%)	p
Ascaris sp.	180±84	20±16	88.89	**0.001**
Entamoeba coli	131±74	5.7±9.8	95.66	**0.018**
Entamoeba histolytica/dispar	9±16	0	100.00	
Taenia sp.	43±21	0	100.00	
Hymenolepis nana	217±190	17±17	92.12	**0.022**
Hymenolepis diminuta	133±83	3±8	97.82	**0.018**
Toxocara sp.	9±11	0	100.00	
Trichuris sp.	14±19	0	100.00	
Giardia intestinalis	26±22	0	100.00	**0.043**

demographic factors and are closely linked to their origins (domestic water, industrial water, slaughter-houses, and storm waters) [18]. The kind of parasites present was varied; it was composed of parasites of man and animal parasites. In fact, qualitative analysis of the samples, identified two groups of helminths in those samples: nematodes and cestodes. This is in agreement with the observations of Alouini in Tunisia [19], with a clear predominance for cestodes. Except for *Enterobius vermicularis* (pinworms) and *Hymenolepis* eggs, many protozoan cysts are directly infective; helminth eggs are only infective after a period of maturation in the environment [20].

Conventional wastewater treatment processes may remove most gastrointestinal parasites of human. However, *Giardia* cysts are less dense and smaller in size (8–12×7–10 µm) comparing to helminthic eggs, they may penetrate through wastewater treatment systems more readily [21]. Other studies conducted in Sweden, Norway and Poland also reported a constant detection of *Giardia* in sewage [22,24]. Documented evidences indicate that cysts can pass through conventional wastewater treatment processes with reported efficiencies of cyst removal varying from 40 to 100% for *Giardia* [25]. In this study, a cyst removal of 100% was obtained noticed.

In the examined samples examined in this study, *Entamoeba histolytica/dispar* was occasionally isolated. This result is probably related to the lower frequency of these parasites in temperate zones with a high level of hygiene [26,27]. However, there was a reduction at 100% in the number of cysts which may be due to the treatment of the plant.

In this work, *Hymenolepis nana* ova were the most predominant followed by *Ascaris* sp., then *Hymenolepis diminuta*, *Taenia* sp. and finally, by *Toxocara* ova. So, concentrations of cestodes were higher than nematodes. *Ascaris* sp. and *Hymenolepis nana* were detected in raw wastewater of all cities. It may be due to high resistance of *Ascaris* eggs than other parasites such as hooking worms and *Trichiuris* sp. against unfavorable environmental conditions [28,29].

However, the risk caused by these pathogens is different from one class to another. In fact, risks evaluation model showed that the risks are higher for intestinal nematodes than for trematodes and cestodes [30,31]. This is due of their higher resistance in the environment, their simpler life cycle, and to their DI50.

Long retention time (and thus sedimentation) is considered to be the main mechanism to remove parasite eggs and protozoan cysts. In addition to the high retention time, solar radiation, high pH (due to algal biomass), and existing of hunter micro-organisms may also help the removal of parasite eggs and protozoan cysts [32]. Shanthala et al. [33] reported that an activated sludge system removed parasite eggs up to 99% [34] whereas Miranzadeh and Mahmoudi [35] found that extended aeration activated sludge can remove 100% of nematode eggs. The research conducted by Sharafi et al. [36] showed that the primary sedimentation unit of a conventional activated sludge process eliminates about 99% of parasite eggs. Caccio et al. [37] conducted an investigation in four wastewater treatment plants in Italy and revealed that the removal efficiency in the number of cysts was significantly higher when the secondary treatment consisted of active oxidation with O_2 and sedimentation instead of activated sludge and sedimentation (94.5% versus 72.1–88.0%). Casson et al. [38] showed that activated sludge system can remove more than 99% of *Giardia* cysts and the removal efficiencies reported by Wiandt et al. [39] ranged from 99.5 to 99.8%.

The results of the present study suggest that the treatment of wastewater promoted a reduction of cysts and eggs of parasites, but however, it could not properly remove all parasites properly, which reflects as, a result, reflecting a constant risk of infection if this eggs are viable.

In conclusion, parasite eggs and protozoan cysts in the raw wastewater of Médéa WWTP were almost identical and largely similar to raw wastewater of developing countries. Based on our preliminary study, it would be desirable to study the viability of oocysts and cysts in effluents from sewage treatment plants to conclude whether there is a risk of transmitting protozoan parasites that pose a risk to water and human health.

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