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RESEARCH ARTICLE

Sludge nematodes, cestodes, and trematodes eggs variation from lagooning, activated sludge and infiltration-percolation wastewater treatment system under semi-arid climate

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Abstract

The prevalence and the identification of the helminth eggs load of raw sewage sludge was assessed of three different wastewater treatment systems. The results showed a variety of parasite species with following average concentrations; five taxa belonging to three classes nematodes, cestodes and trematodes were inventoried. The class of nematodes is the most diverse with 5 taxa. It is represented by the eggs of *Ascaris sp.*, *Capillaria sp.*, *Trichuris sp.*, *Toxocara sp.*, and *Ankylostome sp.*, then comes the cestodes class, this is represented by the eggs of *Tænia sp*. The trematode class is represented by *Schistosoma sp*. The lagooning station of Chichaoua shows the highest load 7 species with *Ascaris* 21 eggs/g; *Capillaria sp.*, 11 eggs/g; *Trichuris sp.*, 6 eggs/g; *Toxocara sp.*, 2 eggs/g and *Ankylostome sp.*, 1 egg/g; *Taenia sp.*, 2eggs/g; and *Schistosoma sp.*, 1 egg/g. Infiltration-percolation sludge show the presence of 4 species of helminths eggs in sludge from anaerobic settling with different rates: 15 eggs/g for *Ascaris sp.*, 15 eggs/g for *Trichuris sp.*, 13 eggs/g for *Capillaria sp.*, and 8 eggs/g for *Taenia sp.* However, in sand filter pool, the sludge helminth eggs load was decreased by 47% of *Ascaris sp.*, 85% of *Capillaria sp.*, and 75% of *Taenia sp.*, Nevertheless, an increase of *Trichuris* eggs load was noted in the second sludge by 17%. Five helminth eggs was detected in primary sludge coming from decantation pools in activated sludge plant in Marrakech, that is *Ascaris sp.*, with a load of 16 eggs/g; *Capillaria sp.*, with 3 eggs/g, *Trichuris* eggs with 2 eggs/g; *Taenia sp.*, with 4 eggs/g; and *Schistosoma sp.*, with 2 eggs/g. The abatement load of *Ascaris sp.* with 81% and *Schistosoma* and *Taenia sp.*, with 100% was noted in biological sludge. Nevertheless, an increase load of *Capillaria* and *Trichuris* eggs 81% and 75% respectively was observed in this sludge coming from biological pools. The distribution of parasitic helminth eggs is linked to the differences in demographic and socio-economic status, seasonal variation, physico-chemical characteristic of helminth eggs, and the purification wastewater system performance.

Keywords Lagooning · Activated sludge · Infiltration-percolation · Nematodes · Cestodes · Trematodes

Introduction

Morocco has launched a several projects to build wastewater treatment plants on a national scale since 2005 such as national sanitation program (NSP). The objective of this program was to build 260 sewage treatment plants between 2006 and 2020, which will be added to the 80 stations already in service. Currently, the volume of wastewater discharged by Moroccan cities is estimated at 550 million m^3 per year, of which 45% is purified considering to 117 wastewater treatment plants (Ministry Delegate in charge of Water, Morocco, 2017).

The wastewater contains a significant amount of the biological contaminant such as helminth eggs that originate from fecal matter. The rate of parasites in wastewater and by consequence in sludge depends on the activities connected

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to the treatment plant, the health status of the populations, the wastewater treatment system (lagooning, activated sludge …), and the shape, density, and the sedimentation rate of helminth eggs (Stien 1989).

The effectiveness of wastewater treatment systems for helminth eggs depends on the treatment system. Several abiotic and biotic factors contribute to this purification (sedimentation, temperature, solar radiation, pH, protozoan predation, phytoplankton, adsorption on suspended particles) (Keffala et al. 2012). Shuval (1977) has estimated the sedimentation speed of helminth eggs at 0.65 m/h for *Ascaris* eggs; 0.39 m/h for *Ankylostome*; 0.26 m/h for *Teania*; 1.53 m/h for *Trichuris*; and 12.55 m/h for *Schistosoma*. Thus the sedimentation experiments of Sengupta et al. (2011) in tap water showed that *Ascaris* eggs had the slowest settling velocity compared to the settling velocities observed for eggs of *Trichuris* and *Oesophagostomum*. Infestation by helminth eggs is responsible for human and animal parasitic diseases, usually two groups of helminth eggs are present in wastewater and sludge: platyhelminthes and nemathelminthes. The platyhelminth species include cestodes and trematodes, such as *Diphyllobothrium sp.* Hymenolepis (nana and diminuta), Taenia (*saginata and solium*), *Clonorchis sinensis*, *Fasciola* (*hepatica and busci*), *and Schistosoma*. The group of nemathelminthes is represented by nematodes (Keffala et al. 2012). In the case of intestinal nematodes, the infestation can be carried out orally following ingestion of eggs such as *Ascaris Lumbricoides*, *Trichuris trichiura*, or transcutaneously in the form of larvae (*Ancylostoma duodenale*, *Necator americanus*, *Strongyloides stercoralis*). But *Ascaris sp.* remains the most common in the field of sanitation (Konaté et al. 2010). The eggs of helminths are different in form, size, and density; these differences are according to the species. The diameters of the majority of the most important helminth eggs in the sanitary field frequently measure between 20 and 80 μm, except Schistosoma the diameter measures more than 185 μm. According to their high density, which exceeds that of the water (1056 to 1237) the helminth eggs can decant (Keffala et al. 2012). Helminth eggs are resistant to aggressions from the extra-intestinal environment. This resistance is due to the presence of a cuticle composed of several layers that prevent the passage of certain substances (strong acids and bases, oxidizing agents, reducing agents, and detergents) (Wharton 1983). The permeability of the eggs is limited to the passage of water, certain solvents and certain gases. Thus the favorable factors for survival of helminth eggs in the environment are low temperature, high humidity, and poor exposure to the sun (Gaspard and Schwartzbrod 2003; Keffala et al. 2012).

This study focused on the identification and quantification of helminth eggs in sewage sludge of three sewage treatment plants of three cities on the scale of Morocco (Chichaoua, Marrakech, and Agadir) with different treatment systems.

Material and methods

Descriptions of the activated sludge plant of Marrakech

The wastewater treatment plant (WWTP) of mixed wastewater (industrial/urban) using activated sludge system has a hydraulic capacity of 110,000 m^3/day, to treat a polluting load of 58,000 kg BOD_5/day.

The plant possesses seven circular settling tanks of which three are for primary decantation. The heavier particles that sink to the bottom by gravity make the primary sludge that is first pumped to the thickeners, followed by four pools of ventilation flow piston for the bacterial activation and four others for secondary precipitation; the organic matter precipitated make the secondary sludge.

The usual principle of activated sludge is as follows: decanted wastewater was ventilated by effective turbines at the surface of water or by area bleached-blond ramps placed at the far end of the pool. After decantation, sludge is sent back in the activation pool to maintain the microbial populations, the remaining is in excess. The biological sludge underwent a thickening then anaerobic mesophilic digestion at 35 °C. The sludge of this phase (150 m^3/day) was stocked after a mechanical desiccation by filters band.

The sludge underwent a static thickening at room temperature of about 30 °C in drying pools; this has for goal to increase their dryness before their discharge. UV treatment was used to ensure a tertiary wastewater purification.

Chichaoua lagooning purification plant

The Chichaoua urban wastewater treatment plant has a flow of 3456 m^3/d. The resort covers an area of 7 ha. The wastewater treatment process in the Chichaoua lagooning plant is successively: a physical pre-treatment (screening and sandblasting), followed by an anaerobic treatment in three anaerobic pools and two others for maturation. The sludge was recovered after decantation of the precipitated material in these three pools. The drying beds were used to ensure dehydration of sludge produced in the anaerobic pools. A total volume of cured sludge estimated at 3609 m^3 for the three anaerobic pools was recovered after a periodic cleaning of 7 years.

Infiltration-percolation Agadir treatment plant

The method of purification in the M'Zar site consists of three successive stages of mixed wastewater treatment: first stage occurring in anaerobic settling with 75,000 m^3/j of hability, these anaerobic basins are used for sludge settling and the primary sludge was recovered after precipitation. Second stage is the infiltration percolation on sand with a flow of 10,000m^3/j, the recovered sludge from infiltration-percolation pools is the secondary sludge. From secondary treatment, 2 to 5 t of sludge per day was recovered from the surface of the filters. A tertiary stage consists of a wastewater purification by UV.

The main physico-chemical features of primary and secondary sludge from three wastewater treatment plant are presented in Table 1.

Method of extraction and identification of helminth eggs

Because of their weak density to carry out a direct microscopic examination (Thevenot et al. 1985), the parasites analysis needs different concentration techniques. For this reason, the analysis of the helminth eggs was carried out on 5 g of fresh matter. The concentration of the parasitic elements was achieved according to the applied flotation method for the analyses of biowastes (Bowman et al. 2003; US EPA Protocol 1999) modified by Schwartzbrod (2003); Koné et al. (2007). After particles scattering by ammonium bicarbonate (11.9%) for few minutes, and because of the weaker density of *Ascaris* and *trichuris* eggs, 1.10 and 1.15 respectively (David and Lindquist 1982), and after centrifugation to 1389 g/3 min, the pellet was re-suspended in 40 mL of $ZnSO_4$ (56.81%), after 10 min, the mixture was centrifuged at 617 g/3 min, and the supernatant was recovered in several centrifuge tubes sand washed by distilled water, then centrifuged at 964 g/3 min. The precipitates were combined in a test tubes using water to recover all the eggs from the other tubes. Then, there was a final centrifugation at 964 g for 3 min to obtain a pellet ready for analysis. The identification of helminth eggs is carried out at 400 times magnification after being located at × 100 magnification.

The quantification of the identified helminth eggs is carried out using a MacMaster slide with two chambers with a capacity of 0.3 ml (0.15 ml is held under each grid). Photomicrographs are realized using a binocular microscope with a numerical photographic device (Moticam 1000, 1,3 M Pixel USB 2.0, lens 16 MM, ø28) the total number of helminth eggs is calculated per gram of fresh sample.

Results and discussion

Parasitic helminths contain in raw sewage sludge from the different wastewater treatment system (Marrakech, Chichaoua, and Agadir)

Five taxa belonging to three classes nematodes, cestodes, and trematodes were inventoried (Fig. 1). The class of nematodes is the most diverse with 5 taxa. It is represented by the eggs of *Ascaris sp.*, *Capillaria sp.*, *Trichuris sp.*, *Toxocara sp.*, and *Ankylostome sp.*, then comes the cestodes class, this is represented by the eggs of *Tænia sp*. The trematode class is represented by *Schistosoma sp.*

The distribution of the identified helminth eggs shows a predominance of nematode eggs showing their easy transmission and their high contamination power. Many studies showed that raw wastewater presents evident contents of parasites in several taxons (Bouhoum et al. 2002; Sylla and Belghyti 2008; Akpo et al. 2013). Schwartzbrod and Banas (2003) reported that the analysis of 110 samples of untreated sludge shows a contamination of 72.6% in helminth eggs, the eggs belong mainly to the nematode family and to the genus *Toxocara*, *Capillaria*, *Trchuris*, and *Ascaris*. The same authors show that the eggs of the class of intestinal nematode are more resistant than those of cestode in wastewater. By direct adsorption on suspended matter of the effluent, the eggs accumulated in sludge by decantation phenomenon. These eggs originate mainly from fecal matter and animals. Thus, this high concentration of eggs reflects the rate of infestation of the human and animal populations of the districts served by the sewer system. The different kinds of parasites encountered in sludges can reflect the polyparasitism that plagues Morocco. All three classes are found in the sludge of the station of Chichaoua and Marrakech city. However, the trematode class is absent for the case of sludge of Agadir. That may be linked to the absence of this parasitic class in the population of Agadir city. The predominance of nematode eggs according to trematode is also demonstrated in other studies carried out in Tunisia (Alouini et al. 1995), France (Stien and Schwartzbrod 1988), and Morocco (Bouhoum et al. 2002).

Table 1 Physico-chemical parameters of primary and secondary sludge from activated sludge plant, infiltration-percolation plant, and lagooning plant

Parameters	Activated sludge plant		Infiltration-percolation plant		Lagooning plant
	Primary sludge	Secondary sludge	Primary sludge	Secondary sludge	Lagooning sludge
pH	7.09 ± 0.2	6.45 ± 0.09	8.15 ± 0.4	7.72 ± 0.3	7.3 ± 0.4
Moisture/(% FWt)	42 ± 1.5	46.46 ± 0.26	29 ± 1.8	5 ± 1	14 ± 0. 2
Ash content (% DWt)	44.6 ± 1.2	43.80 ± 0.02	28.49 ± 0.2	41.55 ± 1.3	59.2 ± 0.1
TOC (% DWt)	29.8 ± 1.3	31.20 ± 0.11	20.3 ± 1.03	29.6 ± 0.8	24.75 ± 0.6
NTK (% DWt)	1.14 ± 0.7	1.50 ± 0.2	1.54 ± 0.1	1.78 ± 0.03	1.02 ± 0.014
C/N	26.14	20.8	13.18	16.62	24.26

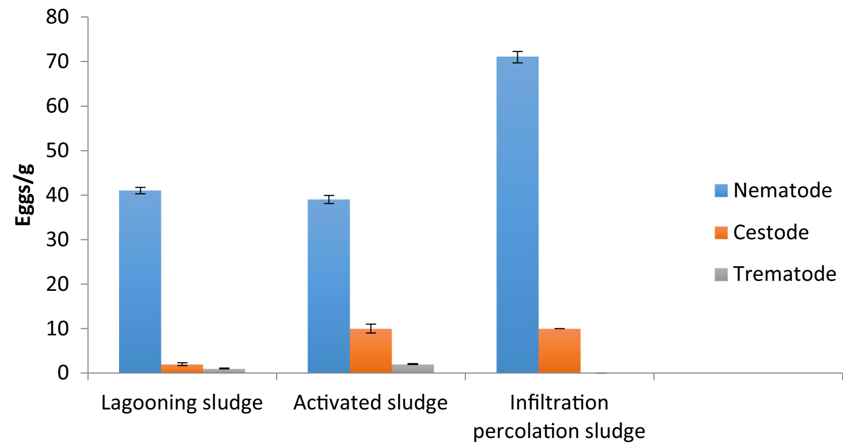


Fig. 1 Distribution of the average concentration of different classes of helminth eggs parasites in different wastewater treatment system

Firadi (1991) reported that nematodes are present with a proportion of 65% whereas cestodes are present with a proportion of 35%. The same authors reported that parasitological analysis of alfalfa showed nematodes (52%) and cestodes (48%) and total absence of trematodes. This absence is justified by the fact that the latter pass a phase of their life in an intermediary which is the mollusk. Akpo et al. (2013) explained that the absence of trematode eggs would be related to the density of these eggs, reflecting the modest performance of the ARTHER-FITZGERALD-FOX method for eggs in this class.

Enumeration of helminth eggs in different wastewater treatment plants

The comparison of results of parasitological analyses of different wastewater stations, show that the lagooning station of Chichaoua shows the highest diversity of helminth eggs (Fig. 2) with the presence of 7 species of helminths eggs with different rates: *Ascaris* 21 eggs/g; *Capillaria sp.*, 11 eggs/g; *Trichuris sp.*, 6 eggs/g; *Toxocara sp.*, 2 eggs/g and *Ankylostome sp.*, 1 egg/g; *Taenia sp.*, 2 eggs/g and *Schistosoma sp.*, 1 egg/g.

The helminth eggs prevalence in infiltration-percolation sludge in Agadir city (Fig. 3) show the presence of 4 species of helminth eggs in primary sludge from anaerobic settling with different rates: 15 eggs/g for *Ascaris sp.*, 15 eggs/g for *Trichuris sp.*, 13 eggs/g for *Capillaria sp.*, and 8 eggs/g for *Taenia sp.* However, in secondary sludge from sand filter pool the helminth eggs load was decreased by 47% of *Ascaris sp.*, 85% of *Capillaria sp.*, and 75% of *Taenia sp.* Nevertheless, an increase of *Trichuris* eggs load in secondary sludge was noted, that is of about 17% (Fig. 3).

The same helminthes eggs evolution profile was noted in activated sludge system in Marrakech city (Fig. 4). Five species of helminthes eggs was detected in primary sludge coming from decantation pools, that is *Ascaris sp.*, with a load of 16 eggs/g; *Capillaria sp.*, with 3 eggs/g, *Trichuris* eggs with 2 eggs/g; *Taenia sp.*, with 4 eggs/g and *Schistosoma sp.*, with 2 eggs/g. The abatement load of *Ascaris sp* with 81% and *Schistosoma* and *Taenia sp.*, with 100% was noted in secondary

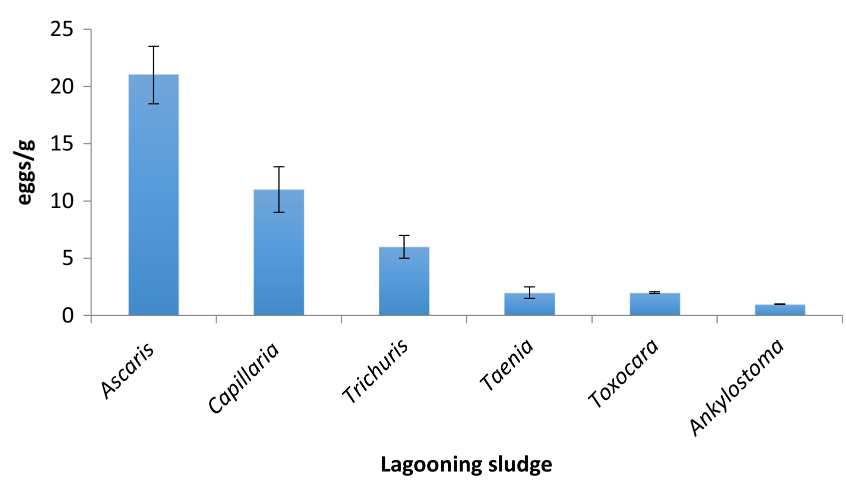
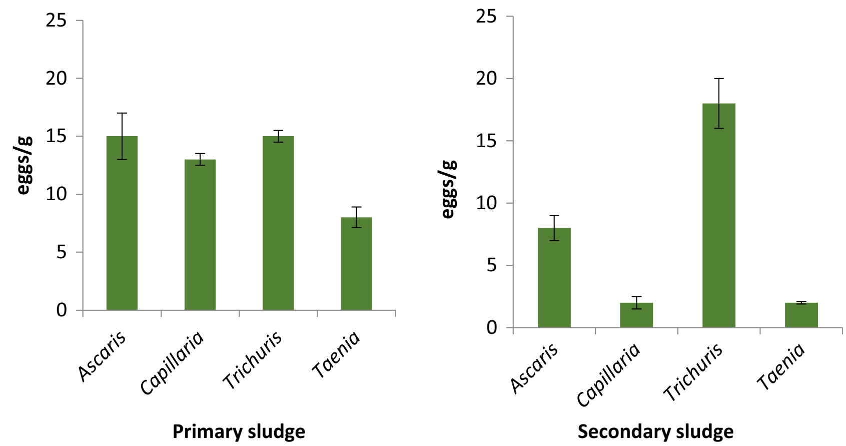


Fig. 2 Total helminth eggs per g of sewage sludge from lagooning system of Chichaoua city

Fig. 3 Total helminth eggs per g of sludge from infiltration/percolation system in Agadir city



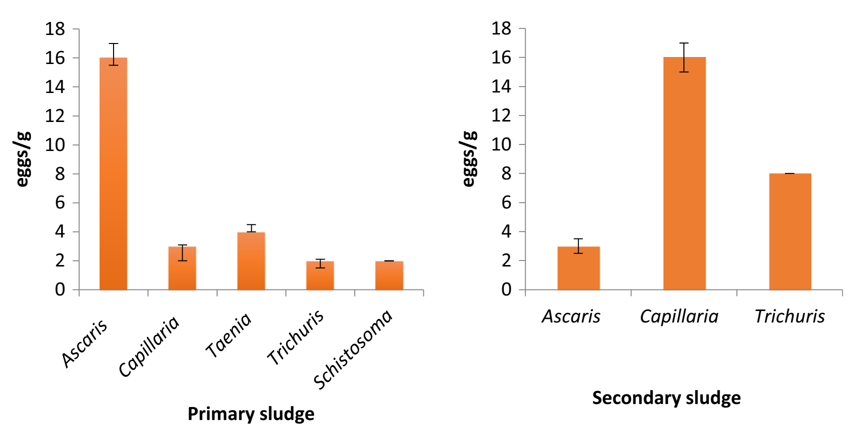
sludge recorded after biological treatment. Nevertheless an increase load of *Capillaria sp.*, and *Trichuris sp.*, eggs by 81% and 75% respectively, was observed in this secondary sludge (Fig. 4).

The results show the predominance of *Ascaris* eggs in all studied sludge, this highest load is explained by their resistant nature. Feachem et al. (1983) explained the predominance of *Ascaris* in the environment by their extremely abundant eggs production and their ability to survive. The same authors show that the female of *Ascaris* produces 200,000 eggs a day, in contrast to *Trichuris* that produces 2000–10,000 eggs a day which explain their high concentration in the environment. The difference in concentration and diversity of helminth eggs is very significant between three sludges. That is linked to the differences in demographic and socio-economic status of each connected manifold surveyed populations (Salama et al. 2013). On the other hand, besides being of human origin, the helminths eggs most common parasites in animals, particularly dogs, cats, and badgers. Bugg et al. (1999) showed that the prevalence of *Toxocara canis* and *Ancylostoma caninum* in dogs from refuges and pups from pet shops was higher (up to 7%). While early studies in dogs from Australia found high levels of infection with *Toxocara canis* of up to 38% (Kelly 1975). The absence of nematode eggs, *Toxocara* and *Ankylostoma*, in the sludge from Agadir and Marrakech station was due to the dilution by industrial effluents. Periodic cleaning of decantation pools which is shorter in Marrakech and Agadir wastewater treatment plant could affect the concentration of *Toxocara* and *Ankylostoma* in sludge.

Seasonal variation infects widely the load helminth eggs. Khnifi (1987) has reported that the increase in the prevalence of parasitic worm infections is recorded in Spring. WHO (1989) reported that the abundance of helminth eggs is noted during spring-summer during which the variation of temperature is important.

The abatement rate in helminth eggs load for secondary sludge could explained by the wastewater system treatment performance, and by the accumulation rate of helminth eggs in primary sludge. Several authors showed high elimination rate of nematode eggs during wastewater treatments (Feachem et al. 1983, Lakshminarayana and Abdulappa 1969, Sengupta et al. 2012; Maya et al. 2012; Konaté et al. 2013). Amahmid et al. (2002) attributed this reduction of helminths eggs during the treatment of wastewater to their accumulation in the sludge. According to (Feachem et al. 1983; Keffala et al. 2012) the logooning system allows a good elimination of

Fig. 4 Total helminth eggs per g of sludge from activated sludge system in Marrakech city



bacteria and viruses. For helminth eggs elimination, Ouazzani et al. (1995) have noted a 100% removal efficiency in a lagooning system in Morocco for an initial concentration of about three eggs per liter and a residence time of 0.4 days. Konaté et al. (2010) have calculated an elimination efficiency of 90% of helminth eggs in a lagooning basin with and initial entry concentration of ten eggs per liter and residence time of 3 days. Jimenez-Cisneros and Maya-Rendon (2007) have cited the percentage of elimination of helminth eggs in sewage treatment systems of about 78 to 99% for stabilization basins; 90 to 99% for primary chemical basins; 85 to 95% for activated sludge and sedimentation ponds; 99% for secondary treatment: coagulation/flocculation and 100% for infiltration-percolation beds on sand.

Sand filtration of wastewater removes about one logarithmic unit of fecal coliforms, pathogenic bacteria (*Salmonella* and *Pseudomonas aeruginosa*) and enteroviruses, 50 to 80% of protozoal cysts (*Giardia*, *Entamoeba coli*, and *Entamoeba histolytica*) and 90 to 99% of helminth eggs. Under these conditions, effluents consistently contain less than 0.1 helminth eggs per liter (Jiménez 2007). Keffala et al. (2012) and El Hayany et al. (2018) explain that humidity, pH, temperature, and ammonium can contribute to the abatement of the helminth eggs load. Other compounds can play a role in the inactivation of pathogens, including organic acids, aldehydes, and alcohols (Reimers et al. 2001). Pecson and Nelson (2005) showed that the presence of ammonia at concentrations usually found in sludge led to 99% of eggs inactivation.

However, the increase in *Trichuris* and *Capillaria* eggs loads in secondary sludge of Marrakech (activated sludge) and the *Trichuris* eggs in case of sludge from Agadir wastewater treatment plant (percolation-infiltration) could be explained by their high load in wastewater and by their later decantation. Particle size, organic matter content of sludge, moisture content, and inconsistent sampling procedures could explain the high level of this helminth eggs in secondary sludge. Stien (1989) showed that the sewage treatment system (lagooning, activated sludge …) and the shape, density, and rate of sedimentation of helminth eggs could influence their concentration in the sludge. The performance treatment system could also contribute to the accumulation of helminth eggs in sludge. The recycling of secondary sludge towards the ventilation pools of bacterial activation allowed to the concentration of helminth eggs.

Conclusion

Parasitological analysis of raw sewage sludge show a large difference in the concentration and diversity of helminths parasites identified eggs during different wastewater treatment system. In sand filter pool, the sludge helminths eggs load was decreased by 47% of *Ascaris sp.*, 85% of *Capillaria sp.*, and 75% of *Taenia sp.*, in contrast that is of about 81% of *Ascaris sp.*, 100 of *Schistosoma* and *Taenia sp.*, in biological sludge; that explained by their accumulation in primary sludge. Nevertheless, the increase of *Trichuris* eggs load by 17% in the second sludge coming from infiltration percolation system, and the rise of *Capillaria sp.*, and *Trichuris sp.*, eggs by 81% and 75% respectively in sludge from biological pools are linked to the performance of system. However in lagooning sludge, all identified species are present.

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