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Fate of pathogenic parasites in sewage sediments and environmental components

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ABSTRACT: The purpose of the current study was the assessment of the persistence of *Ascaris eggs* and *Giardia* cysts in sewage products and environmental components commonly implicated in the transmission of these pathogen parasites. In fact, wastewater and sewage sediments reuse for agricultural purposes has the potential to contaminate water supplies, soil and crops. The ability of parasites cysts to persist in the environment may threaten public health. So, destruction rates of parasite cysts and eggs in stored sediments, crops and soil were examined to help understand the fate of these agents in sewage products and environmental components. In lagoon stored sewage sediments, *Ascaris* eggs disappeared after approximately 180 days of storage versus 49 days for sediments stored under ambient laboratory conditions, and less than 18 days in dried sediments. *Giardia* cysts persisted less than one month lagoon-stored sediments, up to 24 days under laboratory conditions and less than 9 days in dried sediments. For parasites persistence on crops, *Ascaris* eggs persisted on lucerne for 6 days while *Giardia* cysts have not been detected for longer periods than 3 days after contamination. In soil, *Ascaris* eggs were not isolated in periods greater than 90 days, while *Giardia* cysts were not discovered 3 days after contamination induced by wastewater.

KEYWORDS: *Ascaris*, *Giardia,* persistence, storage, drying, sewage sediments, soil, crops.

1 INTRODUCTION

Global climate change is expected to exacerbate current and future stresses on water resources from population growth and irrigation [1]. Consequently, sewage reuse for irrigation purposes attempts to reduce the pressure over the fresh water resources, at the same time it carries other benefits such as improving crop yields and soil fertility [2], [3], [4]. The main concern of sewage reuse seems to be the health implications to exposed populations, primarily due to the possible presence of wide range of pathogenic organisms, such as parasites, that may engender a health hazard to the humans [5], [6]. Several researches have demonstrated that sewage reuse for irrigation purposes led to environmental waters, soil and crops contamination; and epidemiological studies revealed an increase of parasitic infections prevalence among the exposed populations [7], [8], [9]. The current rules for sewage reuse recommend parasitological examination for detection of the indicator parasites. The geohelmith *Ascaris* is one of the most significant enteric pathogens often used as a parasitological indicator. In fact, Ascariasis is an infection of global distribution, considered as worldwide public health problem affecting more than 1.4 billion people [10], [11]. *Giardia* is another pathogen protozoa of primary public health concern for sewage reuse, causing gastrointestinal disease Giardiasis [12]. It is a cosmopolitan parasite, which may infect human beings and animals causing diarrhea and weight loss, and was

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responsible for many outbreaks of waterborne disease in many countries [13], [14]. Infected humans and animals pass large amounts of eggs and cysts in feces, therefore their presence in sewage and environmental components. How long an excreted pathogen can persist in the environment is the property most indicative of the health hazard it engenders [15]. Yet information on the persistence of these pathogen parasites in environmental components commonly implicated in their transmission is sparse and scarce. The current study aimed to assess the persistence of *Ascaris* eggs and *Giardia* cysts in sewage sediments (stored and dried), soil and crops under arid climate of Marrakesh, Morocco. The study findings could therefore contribute to advise farmers and exposed groups, and gain insight on methods of disposing waste and precautions to be taken when handling or consuming the agricultural products so as to reduce or eliminate environmental contamination and limit the potential health hazards for humans and animals.

2 MATERIALS AND METHODS

2.1 INVESTIGATION OF PARASITES PERSISTENCE IN SEDIMENTS

In order to assess parasites persistence in sewage sediments under different storage conditions, batches of sewage sediments with initially known concentrations of *Ascaris* eggs and *Giardia* cysts, were placed into plastic bottles in lagoon bottom, and were examined monthly. Other amounts of sewage sediments were stored in closed bottles under ambient laboratory conditions. To investigate the impact of drying process on the persistence level of parasites, sewage sediments were dried outdoor subjected to normal temperature fluctuations (in Summer), which represented an attempt to simulate conditions in drying beds. Samples of 10 ml (stored sediments) and 5g (dried sediments) were analysed periodically for eggs and cysts quantification, and the relative humidity (RH) of sediments was assessed along the drying process.

2.2 INVESTIGATION OF PARASITES PERSISTENCE IN SOIL AND CROPS

The assessment of *Ascaris* eggs and *Giardia* cysts persistence in soil and crops was investigated by a study of their concentrations after the last contamination induced by raw sewage application (in Summer and Autumn). For crops, lucerne was retained as it is largely cultivated for animal feeding and frequently handled by farmers and their families. In addition, it would aid to highlight the effect dense foliage may have on pathogen persistence.

To ensure eggs and cysts desorption from soil particles, 40 ml of hypochlorite solution were added to soil samples of 10g and 5g, respectively, for 30 min. Samples were centrifuged at 2164xg (3500 rpm.) for 15 minutes. The supernatant was discarded and the residue carefully collected. Lucerne samples were washed in saline solution (0.95% NaCl) and the washing water was left for about 10 hours for sedimentation to take place. The top layer was discarded and the remaining washing water was centrifuged at 2164xg for 15 minutes. The supernatant was discarded and the residue carefully collected [16].

Concentration of *Giardia* cysts in the centrifugation residues as well as in stored and dried sediment samples was realized by a technique of Bailenger [17] and a similar procedure was used for *Ascaris* eggs concentration in sediment samples. *Ascaris* eggs concentration in soil and lucerne samples, was performed with a flotation technique according to the protocol described by Bouhoum and Schwartzbrod [18]. For eggs and cysts identification and enumeration, microscopic observation was done in a Thoma counting cell at 400x magnification for *Giardia* cysts and in a MacMaster counting cell at 100x magnification for *Ascaris* eggs.

3 RESULTS

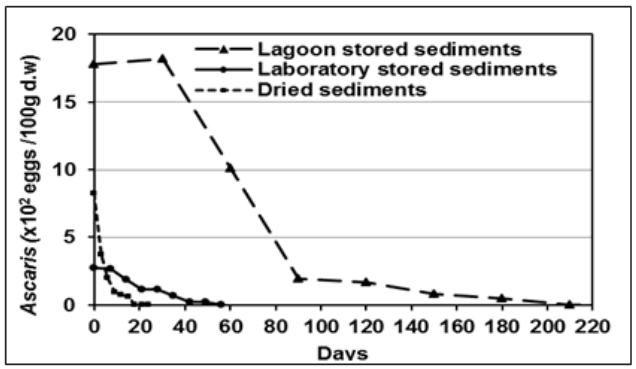
3.1 PERSISTENCE IN SEWAGE SEDIMENTS

Destruction rates of parasite eggs and cysts in stored sediments were examined monthly to help understand the fate of these agents of enteric diseases in sewage sludge. For sediments stored at lagoon conditions, the number of *Ascaris* eggs and *Giardia* cysts discovered in sediment samples decreased with storage time. *Ascaris* eggs occurred for longer periods in stored sediments. In fact, 60 days after the onset of storage, the initially concentration of $17.8x10^2$ eggs/100g dry weight (d.w) declined to $10.12x10^2$ eggs/100g d.w. After 180 days of storage they were highly reduced in sediments and were isolated with a number of 47 eggs/100g d.w. Thereafter, they were not detected in analysed samples (Fig. 1). Similarly, for sediments stored under ambient laboratory conditions, a relatively rapid reduction of *Ascaris* eggs. The original detected *Ascaris* number at the onset of storage $2.71x10^2$ eggs/100g d.w highly declined within 21 days and reached a level of $1.17x10^2$ eggs/g d.w. A very lower eggs concentration of 23 eggs/100g d.w was detected in sediments stored for 49 days, while *Ascaris* eggs were not discovered for greater periods of storage.

With respect to the impact of drying process on *Ascaris*, the survey, carried out in Autumn, resulted in a faster decrease in eggs numbers as compared to the storage impact. The parasite initially recorded number was $8.25x10^2$ eggs/100g d.w and within 6 days of exposure the detected level declined to $2x10^2$ eggs/100g d.w (75.75% reduction rate). The analysis carried out after 15 days of drying resulted in a lower egg level of 65 eggs/100g d.w. Sediments dried for 18 days and longer periods did not contain *Ascaris* eggs (Fig. 1).

Regarding *Giardia* cysts, the effect of sediment storage and drying process was more marked and shorter persistence periods were observed. Indeed, *Giardia* cysts with an initial number of $3.4x10^4$ cysts/100g d.w were not discovered in lagoon-stored sediment after one month (30 days) of storage. For sediment stored under laboratory conditions, while the initial cyst number was $16.4x10^3$ cysts/100g d.w, within 8 days of storage it highly declined to $2.29x10^3$ cysts/100g d.w (86.03% reduction rate). After 20 days of storage *Giardia* cysts have been discovered in stored sediments with a concentration of $1.05x10^3$ cysts/100g d.w, and no cyst was isolated later**.** The drying process resulted in a faster decrease of *Giardia* cysts concentrations. The original recorded number of $12.6x10^3$ cysts/100g d.w declined to $9.4x10^2$ cysts/100g d.w within 6 days of exposure, and cysts were not isolated in sediments dried for 9 days (Fig.2).

The assessment of sediment RH variation along the drying process revealed a decline in the initial sediment RH from 75.8% to 2.8% and strong positive correlation with parasite numbers was recorded (R^2=0.99).



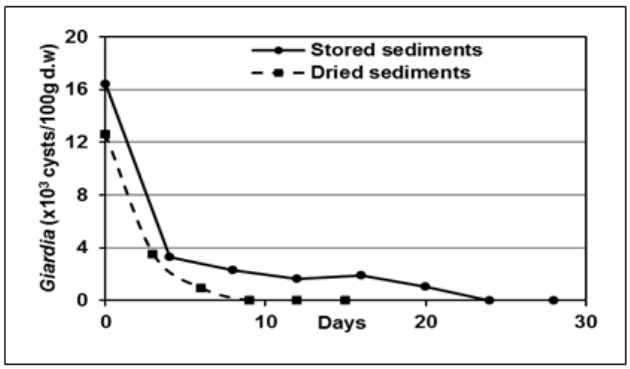


Fig. 1. Occurrence of Ascaris eggs in stored and dried sediment. ***Fig. 2. Occurrence of Giardia cysts in stored and dried sediment.***

3.2 PERSISTENCE IN SEWAGE SOIL AND LUCERNE

The initial level of *Ascaris* eggs in sewage-contaminated soil was 52.7 eggs/100g d.w. After 60 days, eggs count decreased considerably reaching 36 eggs/100g d.w, corresponding to a reduction rate of 31.7%. Only 17 eggs/100g d.w were found in soil after 90 days of soil exposure, while eggs were not isolated in soil samples analysed after 120-day period. For *Giardia* cysts, the carried out analyses showed an initial concentration of $4.76x10^3$cysts/100g d.w in contaminated soil. Within two days, a significant decrease occurred in cyst numbers with a recorded concentration of $1.19x10^3$cysts/100g d.w (43.64% as reduction rate). Thereafter sediment samples were found to be free of *Giardia* cysts (Fig. 3).

In order to assess the degree of persistence of helminth eggs and protozoan cysts on lucerne, a daily monitoring of parasite numbers was conducted after the last contamination induced by sewage (in Autumn). The obtained results showed a reduction in recovered *Ascaris* eggs and *Giardia* cysts counts over time. The initially recorded numbers were 3.76 eggs/kg and $4.11x10^3$ cysts/kg for *Ascaris* and *Giardia,* respectively. After three days of exposure to hostile environmental factors, eggs and cysts numbers decreased significantly reaching 0.53 eggs/kg and 119 cysts/kg, respectively. *Giardia* cysts were not detected after 4 days of exposure while *Ascaris* eggs were more persistent and were discovered at a level of 0.56 eggs/kg, within 6 days after crop contamination. Thereafter, eggs were not isolated in lucerne samples (Fig. 4).

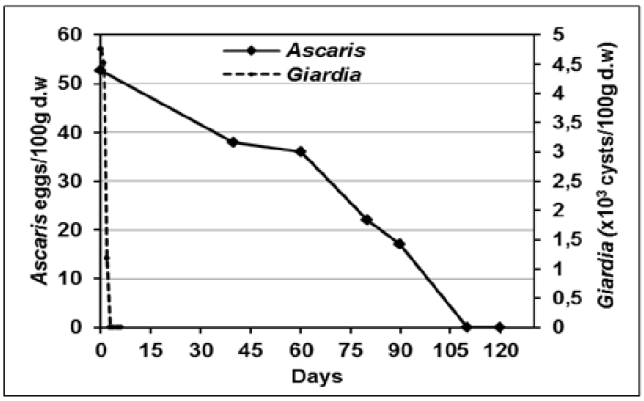


Fig. 3. Occurrence of Ascaris eggs and Giardia cysts in soil.

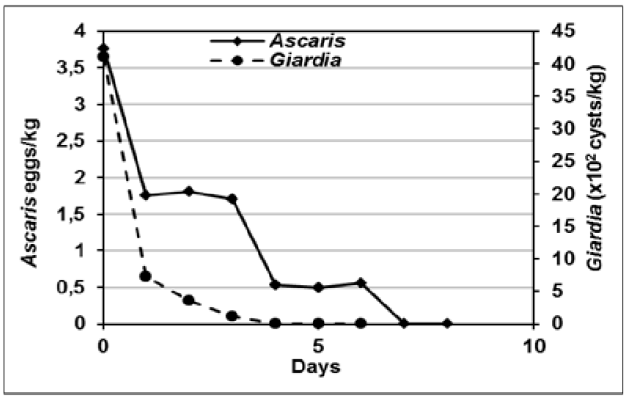


Fig. 4. Occurrence Ascaris eggs and Giardia cysts on Lucerne.

4 DISCUSSION

4.1 PERSISTENCE IN SEWAGE SEDIMENTS

The presence of both helminth eggs and protozoan cysts in sediment suggested that sedimentation is the main process which may be involved in the reduction of pathogens in sewage treatment systems. Several studies have found that parasite cysts and eggs tend to concentrate in sediments of polluted surface waters [19], [20], [21]. Thus, the bottom sediments of constructed systems could potentially serve as a reservoir of human pathogens, which could be released into the water column, and may contaminate soil and crops when they are used as fertilizers [22], [23]. However, once sewage material is placed on the land, parasite eggs and cysts could become infectious agents, thus posing a potential threat to the health of humans and animals and the degree of risk is associated with pathogens persistence in the environment and sewage products. The current survey results showed that eggs and cysts numbers detected in stored sediment samples decreased with storage time. The more the storage time is extended the less parasite cysts and eggs were discovered. After 24 days of storage it would be possible to get cyst-free sediment while longer storage period of 180 days (6 months) is required for *Ascaris* eggs destruction for lagoon-stored sediment. So, the effect of storage appears more destructible on *Giardia* cysts as compared with *Ascaris* eggs. These findings concur with data extracted from the literature [24], [25]. It has been reported that *Ascaris* may persist in sludge stored for several weeks or even months [26], and when sludge contained *Ascaris* eggs, it should be stored for at least three months before it is applied to agricultural land [15]. Longer resistance periods, ranging from 1 to 3 years, were reported for *Ascaris* eggs in stored sediment [27]. A study was carried out to investigate the destruction rate of parasite eggs stored in sludge under controlled conditions and to gain insight on their destruction rates in lagoons. Helminth eggs commonly found in sludge, including *Ascaris,* were seeded into sludge and samples were stored at 4ºC, 25ºC and in ground, under ambient outdoor conditions and subject to normal temperature fluctuations. Destruction of eggs occurred, especially within the first 3 months, and the recovery rate decreased inversely with the storage temperature [28]. For protozoan cysts, the persistence of *Entamoeba histolytica* cysts in sludge has been reported to be up to 30 days, but often lesser than 15 days [29]. However, when studies are carried out under controlled laboratory conditions they may not precisely reflect the conditions that occur in actual lagoons currently used for storage of sludge [28].

Sediment drying process ensured total destruction of *Ascaris* eggs and *Giardia* cysts within relatively shorter periods compared to sediment storage. *Ascaris* eggs were more resistant than *Giardia* cysts as they were isolated in dried sediment within 6 and 15 days of drying, respectively. Elsewhere, *Ascaris* eggs were found to persist in sediments for 14 days drying period and the highest decrease in number was observed during the first two days [30]. Other studies showed that *Ascaris* eggs destruction in drying beds required at least 8 months and sediment moisture reduction during the drying process would be harmful for eggs and cysts [31]. The *Ascaris suum* die-off in sludge drying beds were found to increase with increasing exposure time. The drying bed temperature as well as air temperature significantly affected eggs, and their destruction may occur as a result of desiccation [32], [33]. In conditions where the daily temperatures are considerably lower the persistence is considerably longer [24.

4.2 PERSISTENCE IN SOIL AND LUCERNE

The occurrence of *Ascaris* eggs in sewage irrigated soil reveals the risk of transmission to the human population, especially farm workers and children, and raises the issue of hygiene standards and public health risks at sites sewage products disposal

and reuse [34]. Of all the investigated components, the longest persistence of *Ascaris* eggs was observed in soil as they were detected up to 90 days (3 months) after contamination. *Giardia* cysts were not isolated in soil samples within 3 days. It is apparent from these results that the persistence of cysts on soil is similar to that recorded on crops, but eggs persisted for longer periods. In fact, soil is an important environmental component for *Ascaris* transmission as it is a soil-transmitted helminth (geohelminth), requiring an essential phase of its life cycle in the soil during which the infective stage is protected and preserved. *Ascaris* can persist for months to years in soils under harsh conditions [35]. Further reports quoted that the longest time that protozoan cysts may resist in soil was about 180 days versus 7 years for helminth eggs [36], while other authors indicated that *Ascaris* eggs can persist for over a year in soil that has been irrigated with sewage sludge [37]. Several environmental factors could affect the persistence of intestinal parasites in soil and pathogens may respond differently to variations in environmental conditions to which they are exposed [38], [39]. Among these conditions, temperature and relative humidity have been regarded as the most critical factors in the cysts and eggs persistence in the environment which is extended in moist soils at cool temperatures [40], [41]. The gradual recorded disappearance of eggs and cysts with time is mainly due to desiccation associated high temperatures with moisture lost mainly during dry hot period with high sunlight exposure (Summer and Autumn). Researches conducted on *Ascaris* eggs persistence resulted in 27-35 days during hot dry Summers versus 5-6 months during the Winter season [42]. In addition, it has been demonstrated that different environmental pressures influence the degradation of *Giardia* cysts, which are much more susceptible to environmental constraints than helminth eggs and studies have revealed that *Giardia* cysts are vulnerable to rapid degradation in soil and by freezing and high and diurnal changes in temperature [43], [44]. It has been suggested that organic matter content and vegetation may affect eggs and cysts persistence [45].

Raw sewage reuse led to contamination of lucerne with *Ascaris* eggs and *Giardia* cysts. These parasites are pathogenic agents for humans and animals and consumption or handling of such contaminated agricultural crops is considered unsafe and might constitute a risk for farmers and the whole population [46]. Epidemiological studies have revealed an excess of parasitic infestations associated with sewage reuse in irrigation [47]. Reports indicated that irrigation with raw sewage caused an excess prevalence of *Ascaris* infection, both in workers in raw-wastewater-irrigated fields and in consumers of raw-wastewater-irrigated crops eaten uncooked [29]. The incidence of parasitic diseases in consumers and handlers of sewage-irrigated crops was found to be higher than that of the control population [8].

The persistence of *Ascaris* eggs and *Giardia* cysts assessment on lucerne showed a decrease of their concentrations over time. After contamination induced by raw sewage application, 6 and 9 days were sufficient for cysts and eggs disappearance, respectively. In comparison with the findings recorded by other authors, a dispersion in reported persistence period was recorded. According to the USEPA [36] the longest time that protozoan cysts may survive on crops was about 5 days, and it was reported that *Giardia* cysts can resist up to 10 days on crops [24]. An early survey indicated that protozoan cysts are extremely sensitive to desiccation and may resist from 3 days to 2 weeks, depending on climate conditions and type of crops [48]. For *Ascaris* eggs, a persistence period of 10 days was reported [49], while other investigations mentioned that *Ascaris lumbricoides* eggs may persist on crops up to 60 days but usually less than 30 days [50]. The persistence of parasites on crops tends to be much shorter. Factors such as intense solar radiation, high temperature and low humidity would be in favor of cysts and eggs desiccation reducing the persistence of microorganisms [51], [52]. Helminth eggs persistence on crops is affected by surface properties. Smooth surface vegetables tend to harbour very small numbers. In contrast low growing, hairy, sticky, rough or crops with crevices and dense foliage tend to show higher contamination levels and have the ability to hold on to water creating a more favorable environment to ensure protection from hostile environmental factors [9], [53]. Since sewage reuse for crops irrigation is source of contamination with pathogens parasites, harvesting, and processing of contaminated crops involves significant risks for agricultural workers and their families as well as to crops handlers and consumers [54]. The current study findings confirm that contaminated crops may be considered safe for humans and animals if a natural decontamination period is maintained between the last application of contaminating material (sewage, sludge ...) and harvesting.

5 CONCLUSION

Land application of sewage products is a source of contamination of soil and crops. The ability of parasites eggs and cysts to persist in the environment may engender public health hazards. The persistence may depend on several factors including the type of pathogen, the concerned environmental component, climate conditions… The greatest resistance to hostile environmental factors was observed for *Ascaris* eggs, isolated within 3 months of exposure in soil and 6 days on lucerne. *Giardia* cysts were less resistant and persisted for few days in soil and lucerne. Therefore, a natural decontamination period has to be maintained between the last application of sewage and crop harvesting. Sewage products have to be treated to eliminate pathogens before reuse. Storage and drying processes may be used for sewage sediments treatment. Drying process resulted in destruction of *Ascaris* eggs and *Giardia* cysts in shorter periods as compared to storage which required longer durations.

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