Environmental Health Engineering and Management Journal 2023, 10(1), 107-119
http://ehemj.com
doi 10.34172/EHEM.2023.12

Environmental Health Engineering and Management Journal

Review Article

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Wastewater reuse in agriculture: A review of soil and crops parasitic contamination, associated health risks and mitigation approach

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Abstract

Background: Wastewater reuse in agriculture can potentially result in adverse health implications including parasitic diseases spread. *Trichuris*, *Ascaris*, and *Giardia* are major pathogenic parasites of concern associated with this practice. This review investigated their occurrence in wastewater, and environmental components reached through wastewater application, including irrigated soil and grown crops. Exposure pathways and evidence for health risks were also explored.

Methods: Several databases (Google Scholar, PubMed, Science Direct, Scopus, Web of Science, and ResearchGate) and other sites were searched for published literature up to 2021. The searched keywords include wastewater reuse, soil-transmitted helminths (STHs), *Ascaris*, *Trichuris*, *Giardia*, crop contamination, soil contamination, health risk, epidemiological studies, exposure pathways, and risk mitigation. Overall, 160 papers have been yielded. After screening for relevance, 60 studies were considered for inclusion.

Results: *Giardia*, *Ascaris*, and *Trichuris* were frequently detected in wastewater with up to 5×10^5 cysts/L and 5.73×10^3 eggs/L. Concentrations of 750 eggs/100 g and 2.8×10^4 cysts/100 g were reported in wastewater irrigated soil. *Ascaris* was reported in irrigated crops with up to 70 eggs/kg versus 6.6×10^3 cysts/kg for *Giardia*, depending on the type of crops. Epidemiological studies provided evidence supporting the increase of ascariasis, trichuriasis, and giardiasis diseases related to the exposure to wastewater irrigated soil and crops.

Conclusion: The findings suggest that wastewater reuse in agriculture leads to contamination of soil, and crops with pathogenic parasites, increasing health risks in the exposed groups. To remedy this issue, protection measures, including a multi-barrier approach, can be applied to mitigate the health risks engendered by wastewater reuse for irrigation.

Keywords: Wastewater reuse, *Ascaris*, *Trichuris*, *Giardia*, Epidemiologic studies

Citation: Amahmid O, El Guamri Y, Rakibi Y, Yazidi M, Razoki B, Kaid Rassou K, et al. Wastewater reuse in agriculture: a review of soil and crops parasitic contamination, associated health risks and mitigation approach. Environmental Health Engineering and Management Journal 2023; 10(1): 107-119. doi: 10.34172/EHEM.2023.12.

Article History:
Received: 29 April 2022
Accepted: 16 July 2022
ePublished: 27 February 2023

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Introduction

Wastewater reuse for agricultural purposes including crop irrigation is an old and common practice (1). Currently, agriculture represents by far the highest water and wastewater consumer worldwide, with an approximate average of 70% (2). The rising freshwater shortage in many regions of the world due to climate change resulted in an increasing interest in reusing wastewater (3). This practice is considered as a form of adaptation to climate change since it provides a permanent source of water, particularly in water-scarce areas, and under drought conditions by providing an additional and sustainable source of fresh water (4). Raw wastewater is commonly reused for irrigation in developing countries, depending on the geographic and economic contexts (5). Moreover, generated wastewater with partial or no treatment is discharged into water bodies in countries lacking treatment facilities (6). In several areas, wastewater is extensively used as an available and inexpensive alternative water source for irrigation, supporting livelihoods, and generating income in urban and peri-urban areas (7). It has been reported that approximately 20 million hectares of agricultural land are irrigated with raw wastewater worldwide (8). In addition, in some urban areas, it has been reported that the majority of the locally consumed food crops are grown in wastewater-irrigated fields (9). Wastewater reuse can therefore mitigate the pressure on freshwater used for irrigation, and contribute to water and food security, as a consistent source of water and nutrients for the improvement of soil fertility and crop productivity (6,7,10). This is particularly crucial in areas with water shortage and variable precipitations expected to be exacerbated by climate change (5). However, despite many benefits wastewater reuse in agriculture may have, this practice can impose adverse health effects on humans (11). Wastewater may transport a myriad of hazardous agents that are harmful to humans, with pathogenic microorganisms being the most important (12).

Among the pathogenic agents, the major health problem related to wastewater reuse is due to the soil-transmitted helminths (STHs), *Ascaris* and *Trichuris* (13). These parasites are often used as parasitological indicators, because of their long persistence in the environment, and lower infectious dose (12). *Ascaris* and *Trichuris* represent a great public health burden globally, with over 1.5 billion affected people (14,15). For protozoa, *Giardia* is one of the main intestinal parasites of health concern, associated with recurrent outbreaks of disease in different parts of the world (16,17).

The level of occurrence of these pathogenic parasite eggs and cysts in wastewater and reached environmental components may be indicative of the potential health hazard related to the wastewater and sludge application to cultivated fields (10-12,18). The STHs, including *Ascaris* and *Trichuris*, are considered as human pathogens of primary public health concern for wastewater reuse. They are the main target in the new guidelines for the safe reuse of wastewater in agriculture, recommending a limit value of ≤ 1 egg/L (13). However, reports indicated an increase in protozoan diseases such as giardiasis, amoebiasis, and other diarrheal diseases, related to wastewater exposure and reuse (13). Moreover, helminth infections in particular (e.g. ascariasis and trichuriasis), were found to be commonly related to wastewater and sewage sludge exposure (5,19). Thus, most attention is given by scientists and health organizations to the occurrence and fate of pathogenic parasites contained in wastewater, reaching soil and crops via land application (20). The protozoa *Giardia* and the STHs, *Ascaris* and *Trichuris*, are common in countries where raw wastewater is used to a large extent (19-21). This review analyzed the available data on the occurrence status of these pathogenic parasites in wastewater, wastewater-irrigated soil, and grown crops. It also explored the exposure pathways and current evidence for health risks and the spread of parasitic infections to farmworkers and consumers through wastewater irrigation. The application of protection measures to reduce the health effects that wastewater reuse may engender, has been addressed.

Materials and Methods

The review was prepared based on a literature search in several databases for publications (Google Scholar, PubMed, Scopus, ScienceDirect and ResearchGate) and other sites. The literature search was performed using main keywords (wastewater reuse and STHs, wastewater irrigation and *Ascaris*, wastewater irrigation and *Trichuris*, wastewater reuse and *Giardia*, wastewater reuse and crop or soil contamination, wastewater reuse and parasitic infections, wastewater irrigation and exposure pathways, wastewater reuse and health risks reduction, wastewater irrigation and health risks, wastewater reuse and epidemiological studies). Article titles and abstracts were assessed to determine their suitability for inclusion in this review. The literature search results were screened manually for the relevance of the provided information for the review. Included studies are publications reporting the occurrence of *Ascaris*, *Trichuris*, and *Giardia* in wastewater reused in agriculture; articles that reported the concentration of these parasites on soil, and vegetables grown with wastewater, and papers that directly measured the impact of wastewater reuse on the three parasite infections using epidemiological methods. Studies that reported infections by other targeted parasites were excluded. Relevant full-text articles that were available and published up to 2021 were reviewed and included in the review. Full-text articles with insufficient reporting of data in article text, and unrelated title and article text, or with no access to the text of paper were excluded. Conference abstracts and papers, letters to editors, theses, and presentations were not considered (Figure 1). The extracted data of the reviewed articles include geographic location of the study, author, date of publication, concentration of *Ascaris* and *Trichuris* eggs and *Giardia* cysts in the samples studied (wastewater, soil, crops); type of parasite reported; target population investigated, and infection levels reported in each exposed group. Information retrieved from articles regarding concentrations of *Ascaris*, *Trichuris*, and *Giardia* in wastewater, soil, and irrigated crops, and the

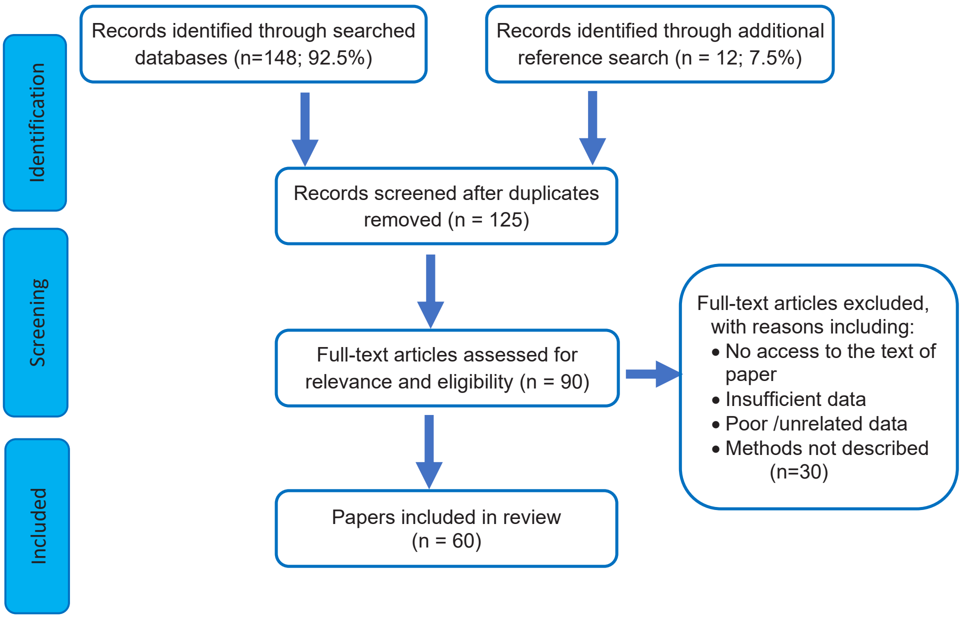

Figure 1. Searching process of studies, assessment and selection

measured impacts of wastewater reuse on the prevalence of *Ascariasis*, *Trichuriasis*, and *Giardiasis* infection, were archived in Excel software. The main data were collated and captured in tables for easy presentation and descriptive statistical analysis.

Results

A total of 160 papers and reviews had been yielded. After excluding duplicates and papers not relevant for the review, 60 articles were included in the review (Figure 1). Among these articles, there were papers reporting on the occurrence of *Giardia* and the STHs, *Ascaris* and *Trichuris*, in wastewater from different localities, those addressing the contamination level of irrigated soil. Other included papers reported parasite detection in wastewater-irrigated crops, and studies on parasite persistence in soil and crops. The review also included studies that applied epidemiological surveys for the assessment of the health risks associated with wastewater reuse, by measuring the prevalences of parasitic diseases in the exposed humans. The concentrations of STHs eggs and *Giardia* cysts in wastewater-irrigated soil and vegetables as well as data regarding the measured health effects of wastewater reuse in agriculture on the transmission of STHs infections with reported prevalences are summarized and presented in Tables 1-4. Also, information on the possible exposure pathways to pathogens through wastewater reuse in agriculture, as well as the measures for the mitigation of the health risks are illustrated in Figures 2 and 3.

Discussion

Soil-transmitted helminths and Giardia in wastewater and sludge

The nematodes, *Trichuris* and *Ascaris*, and the protozoa *Giardia*, account for a major burden of parasitic diseases with concentrations varying in wastewater between different locations. The concentration of these parasites' cysts and eggs in wastewater has been assessed by many research studies at a variety of wastewater treatment plants (22-40). Several reports showed that concentrations of STHs eggs and *Giardia* cysts in wastewater are very variable (Table 1).

For the STHs, higher levels of occurrence can be seen in endemic areas, especially in low-income countries, as compared to developed countries (14,22-24). For *Ascaris*, concentrations reaching up to 517 and 5730 eggs/L have been reported in Oman and Vietnam, respectively (24,36), whereas *Ascaris* concentrations as low as 1.7 eggs/L have been reported in other studies (31). Relatively lower concentrations were reported for *Trichuris* eggs, attaining up to 405 eggs/L (36), while the study from India reported the detection of *Trichuris* eggs with a level of 1.19 eggs/L in the examined raw wastewater samples (26). For *Giardia*, several studies have recorded variable cysts concentrations in wastewater. Studies indicated that the concentration of *Giardia* in raw wastewater can generally reach higher levels as compared to STHs (Table 1). The reported concentrations ranged from 3.75 cysts/L (34) to up to 5×10^5 cysts/L (28). It is noteworthy that even though methodologies have been highly improved, there are inefficient standard and accessible methods for the recovery and detection of STH eggs and *Giardia* cysts from wastewater samples. Methodologies employed are usually variable limiting the possibility of making comparisons between the findings (25,29,30). In fact, such comparisons should take into account that techniques for concentrating, purifying, and detecting parasite cysts and eggs in wastewater may vary between studies. Some

Table 1. Concentration of *Ascaris* and *Trichuris* eggs and *Giardia* cysts in wastewater from different locations

Parasites	Country	Concentration of eggs or cysts (no/L)		Wastewater treatment plant	Reference
		Raw wastewater	Treated wastewater		
Ascaris	Poland	0-8	0-2	Activated sludge process	(18)
	Ghana	2.82-2.62	NR	No treatment performed	(22)
	Vietnam	5730	NR	No treatment performed	(23)
	Tunisia	455	46	Activated sludge	(24)
	Kenya	17.5-133.3	0.7-88.9	Stabilization ponds	(25)
	India	38.2	23.7	Activated sludge process	(26)
	Tanzania	12-19	0	Stabilization ponds	(27)
	Morocco	1.7	0	Stabilization ponds	(31)
	Algeria	180	20	Activated sludge process	(32)
	India	70	12	Dilution in river water	(33)
	Nigeria	307	7.81	Slow sand filtration	(34)
	Iran	45.75	0.97	Activated sludge process	(35)
		29.98	0	Stabilization ponds	
		30.43	0.08	Constructed wetland	
	Oman	517	24	Activated sludge process	(36)
	Pakistan	142	21	Stabilization ponds	(37)
	South Africa	16-91	2.2-3.8	Planted gravel filters	(39)
Trichuris	India	1.19	0.39	Activated sludge plant	(26)
		4	0.3	Partially treated	(33)
	Algeria	14	0	Activated sludge	(32)
	Nigeria	5.69	3.44	Slow sand filtration	(34)
	Iran	2.49	0	Activated sludge process	(35)
	Iran	2.53	0	Stabilization ponds	(35)
	Oman	405	0	Activated sludge process	(36)
	South Africa	4.6-23	1.2-13	Planted gravel filters	(39)
Giardia	Tunisia	759	116	Activated sludge	(24)
	France	1000-25000	21-90	Stabilization ponds	(25)
	Kenya	3000	40-50	Stabilization ponds	
	Tanzania	9-22	0	Stabilization ponds	(27)
	Cayman	500 000	0	Stabilization ponds	(28)
	Morocco	2800	0	Stabilization ponds	(31)
	Algeria	26	0	Activated sludge process	(32)
	Nigeria	3.75	1.44	Slow sand filtration	(34)
	Iran	15.55	0.2	Activated sludge process	(35)
		7.6	0	Stabilization ponds	
	England	5-940	354.3	Activated sludge process	(41)
	Scotland	10-13600	40	Biological filtration	
All	NR	NR	NR	NR	(29,30,38,40)

NR: Not reported.

techniques may be more efficient and sensible, allowing detection of parasite eggs and cysts when they are present in reduced concentrations, as compared to other techniques. Even with similar methods, the detection efficiencies may differ depending on several factors such as pH, relative turbidity or other material contents in wastewater. Moreover, reports indicated that concentrations of STH eggs and *Giardia* cysts detected in wastewater influent may significantly vary according to sampling mode (grab vs composite) and time of sampling (31).

On the other hand, the variable occurrences of STH eggs and *Giardia* cysts in wastewater can depend on many potential influencing factors such as the number of people infected in the catchment (26). Infected individuals can

Table 2. Occurrence of *Giardia* cysts and the soil-transmitted helminths *Ascaris* and *Trichuris* eggs in wastewater-irrigated soil

Parasite	Concentration (Positive rate)	Country	Reference
Ascaris	19 eggs/100 g (73.3)	Morocco	(50)
	60 eggs/100 g (53)		
	75 eggs/100 g (70)		
	38.4 eggs/100 g (85)		
	290-370 eggs/100 g (NR)	Ghana	(22)
	36-63 eggs/100 g(NR)		(52)
	750 eggs/100 g(NR)	Bolivia	(49)
Trichuris	19 eggs/100 g (66.7)	Morocco	(50)
	32 eggs/100 g (65.4)		
	84 eggs/100 g (80)		
	440 eggs/100 g (NR)	Bolivia	(49)
Giardia	45.2 cysts/100 g (56)	Morocco	(50)
	88.5 cysts/100 g (65)		
	354 cysts/100 g (66.7)		
	2.8×10^4 cysts/100 g(NR)		(53)
	7.4×10^3 cysts/100 g(NR)		
All	NR	NR	(42-48,51)

NR: Not reported.

Table 3. Occurrence of *Giardia* cysts and the soil-transmitted helminths *Ascaris* and *Trichuris* eggs in wastewater-irrigated vegetables

Parasites	Concentration (Positive Rate)	Crops	Country	References
Ascaris	43 eggs/kg (50)	Mint	Morocco	(65)
	1.64 eggs/kg (33.3)	Radish		
	0.18 eggs/kg (50)	Potatoes		
	0.27 eggs/kg (25)	Turnip		
	1.1 eggs/kg (50)	Squash		
	2.1 eggs/kg (33.3)	Marrow		
	1.62 eggs/kg (8.3)	Lettuce		(50)
	2.68 eggs/kg (63)	Coriander		
	0.71 eggs/kg (44.4)	Carrots		
	0.7 eggs/kg (75)	Alfalfa		
	78 eggs/kg (NR)	Lettuce		(58)
	4 eggs/kg (33.3)	Coriander		(59)
	0 eggs/kg (0)	Alfalfa		
	1.5-2.2 eggs/kg (NR)	Lettuce	England	(66)
	0 eggs/kg (0)	Eggplant	Morocco	(65)
	2 eggs/kg (NR)	Tomato		(67)
	33.6 eggs/kg (NR)	Zucchini		(58)
	3 eggs/kg (11.1)	Mint		(59)
	0 eggs/kg (0)	Cereals		
Trichuris	2.55 eggs/kg (13.9)	Lettuce		(50)
	1.4 eggs/kg (56)	Coriander		
	2.4 eggs/kg (100)	Carrots		
	0.6 eggs/kg (68.7)	Alfalfa		
Giardia	96 cysts/kg (50)	Mint	Morocco	(65)
	59.1 cysts/kg (83.3)	Radish		
	5. cysts/kg (25)	Potatoes		
	0 cysts/kg (0)	Pepper		
	0 cysts/kg (0)	Lettuce		(50)
	1.54 cysts/kg (44.4)	Coriander		
	1.55 cysts/kg (55.5)	Carrots		
	26.5 cysts/kg (83.3)	Alfalfa		
	1-6 cysts/kg (NR)	Tomato		(67)
	6600 cysts/kg (NR)	Spinach	Cambodia	(60)
All	NR	NR	NR	(54-57,61-64)

NR: Not reported.

excrete between 10^2-10^4 eggs/g feces, and up to 1×10^6 cysts/g, resulting in high occurrences in wastewater and sewage sludge (38,41-43). Other factors such as rainy water can increase dilution levels. Moreover, a reduction of pathogen concentrations and infectivity may occur along with the sewer network due to adsorption into solids and settling, pH, and temperature changes (44). It has been reported that findings variation may be related to socio-economic factors and decreased hygienic standards, as well as to demographic characteristics since parasitic infections are more common in children than in other age groups. Studies showed that *Giardia* cysts levels detected in wastewater significantly are correlated with likely contributors, suggesting that the number of cysts in wastewater may be used to assess the prevalence of giardiasis in the served population through wastewater-based epidemiology (41,45).

To face the main issue associated with wastewater reuse in irrigation, related to public health and infection risks, various wastewater treatment processes (e.g., as stabilization ponds, activated sludge, constructed wetlands, trickling filters, sand beds or soil percolation, constructed overland flow system, reed beds, lagoon aeration, etc) can be used for pathogenic parasite eggs and cysts removal or reduction (Table 1). Different mechanisms can be responsible for parasitic egg and cysts removal during wastewater treatment processes. These mechanisms include sedimentation, filtration, adsorption, adhesion to plant roots, soil particles, and organic matter, entrapment in activated sludge flocs, inactivation due to hostile environmental factors, etc (29). Several studies have investigated the occurrence and removal of parasitic pathogens, including *Ascaris*, *Trichuris*, and *Giardia* in wastewater treatment plants (29), which reported various efficiencies (Table 1). Despite the complete removal of parasite eggs and cysts may occur through several highly efficient treatment plants (27,31,32,35,36), significant amounts of eggs (0.3-88.9 eggs/L) and cysts (0.2-354 cysts/L) could still be detected in the treated effluent, and discarded in the environment (25,29,33,35,41) (Table 1).

Through the wastewater treatment processes, STHs eggs and *Giardia* cysts occurring in raw wastewater have

Table 4. Summary of epidemiological studies that assessed health risks for parasitic infections associated with wastewater reuse in irrigation in different countries

Country	Practice	Investigated group	Health risk and conclusions	Reference
Eritrea	Wastewater	Farmers (n = 75) and residents (n = 1000)	Higher prevalence of giardiasis among farmers irrigating with wastewater compared to non-users.	(73)
India	Wastewater	Famers (n = 1078)	Farmers exposed to wastewater had a higher risk of infection with *Ascaris* and *Trichuris*.	(33)
Jordan	Wastewater	Children (n = 647)	Amoebiasis and giardiasis were significantly more prevalent in exposed children than in unexposed ones.	(74)
Mexico	Wastewater	Children and adults (n = 2257)	Farmworkers and families had more giardiasis than unexposed groups.	(75)
	Wastewater	Children and adults (n = 10489)	Higher prevalence of *Ascaris* infection among exposed children compared to unexposed children.	(76)
	Wastewater	farmworkers and families	Significantly higher occurrence of ascariasis in the exposed children (15%) compared to the control group (3%).	(77)
Morocco	Wastewater	Children (n = 610; n = 683)	*Giardia*, *Ascaris*, and *Trichuris* were significantly prevalent in exposed children compared to controls.	(78)
	Wastewater	Children (n = 1343)	Significant increase in *Ascaris* infection for exposed children.	(79)
	Wastewater	Adult farmers (n = 333)	Adults from wastewater irrigated areas had more *Ascaris* and *Trichuris* infections than unexposed groups.	(80)
Pakistan	Wastewater	Farmers (n = 1704)	Giardiasis was more prevalent in farmers and their children than in the unexposed population.	(81)
	Wastewater	Famers and families (n = 543)	Exposed farmers and families had a higher prevalence of *Ascaris*, *Trichuris*, and *Giardia*.	(82)
South Africa	Wastewater	Farmworkers and families (n = 444)	Increased prevalence of *Ascaris* in farmers and their family members as compared to unexposed populations.	(22)
	Wastewater	Farmers and children (n = 443)	An increase in giardiasis and hookworm among farmers and their children.	(83)
Vietnam	Wastewater and excreta	Farming households	Contact with wastewater was a significant risk factor for *Ascaris* infection.	(11)
	Wastewater	Workers and children (n = 1088; n = 1407)	No significant association between wastewater exposure and *Ascaris* and *Trichuris* infections in farmers and their children.	(84)
Others	NR	NR	NR	(65-72)

NR: Not reported.

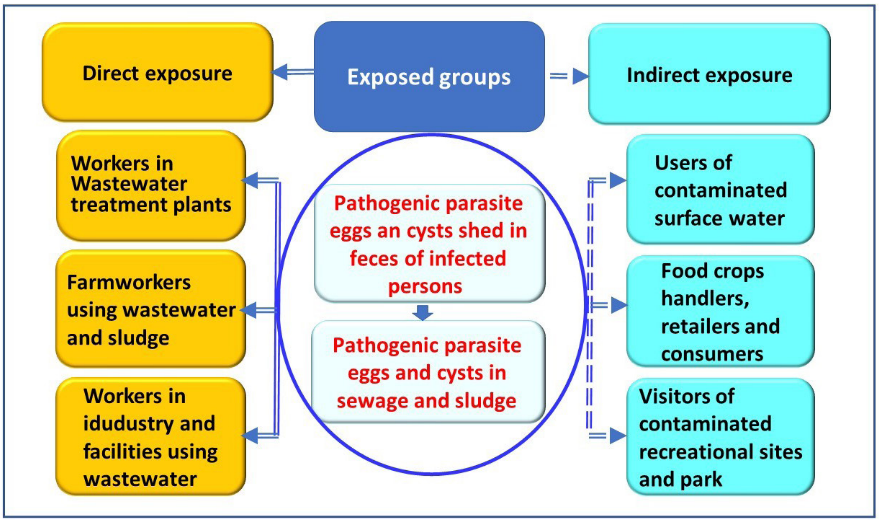

Figure 2. Exposure pathways to pathogenic parasite infections associated with wastewater reuse

been recovered in sewage sludge (solid phase) and/or in treated effluent (aqueous phase). Several reports have addressed the presence of STH eggs and cysts of *Giardia* in wastewater sediments and sludge (25,31,40). Available data are very sparse and differ according to the technique of analysis used and sludge type. Concentrations reaching up to 4140 eggs/100 g have been reported for *Ascaris*, versus 3250 eggs/100 g for *Trichuris* (40). In Brazil, *Trichuris* and *Ascaris* eggs concentrations isolated in raw sludge ranged from 900 to 4100 eggs/100 g (46), while concentrations of

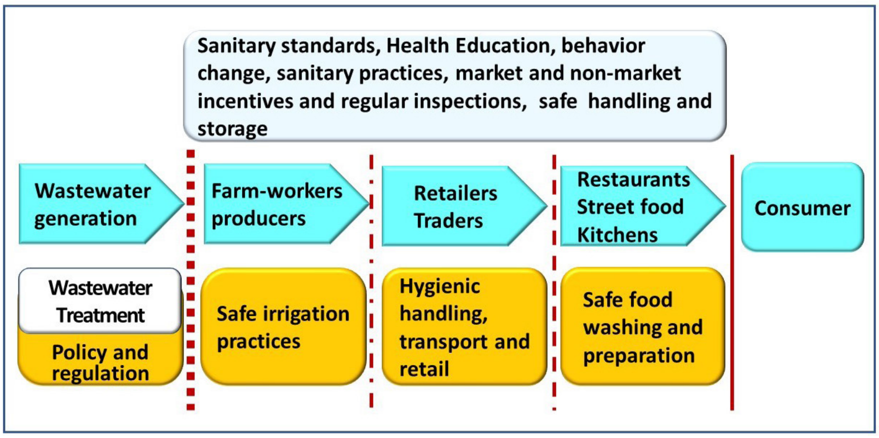

Figure 3. The multi-barrier approach to mitigate health risks associated with wastewater reuse in irrigation from wastewater source to consumers

Ascaris attaining up to 556 eggs/g sludge were reported in Burkina Faso (47). Similarly, bibliographical data on the occurrence of *Giardia* in sludge are variable. Rose et al. (48) have recorded a concentration of 2.8×10^4 cysts/100 g in raw sludge, while Amahmid et al reported significantly higher *Giardia* levels in ponds sediments, reaching up to 39×10^5 cysts/100 g (31). The high concentrations of *Giardia* and *Ascaris* in sediments suggest that sedimentation is the main phenomenon responsible for pathogen removal due to their relatively high densities.

Giardia cysts and soil-transmitted helminth eggs in wastewater-irrigated soil

The high occurrence of STH eggs and *Giardia* cysts in wastewater and sludge reflects the parasitic infection status in served population and can be a source of concern, especially when reused for agricultural purposes. Wastewater and sludge reuse in agriculture is one of the major exposure pathways to parasite cysts and eggs, affecting exposed groups mainly through the oral route. Indeed, as shown in Table 2, the analysis of soil samples taken from wastewater-irrigated and sludge-fertilized fields resulted in the detection of STH eggs of *Ascaris* and *Trichuris*, and *Giardia* cysts in considerable and various concentrations (49-53). The concentrations of *Ascaris* have been reported 19-750 eggs/100 g, while *Trichuris* was detected in levels of 19-440 eggs/100 g (40,49). Significantly higher levels were reported for *Giardia* cysts with a range of 45.2 to 2.8×10^4 cysts/100 g in wastewater-irrigated soil (50). These reports indicated that during the land application of wastewater and sludge, eggs and cysts of pathogenic parasites transfer into the soil compartment. This leads to the accumulation of parasite eggs and cysts in wastewater-irrigated soil in various concentrations (51).

The accumulation of parasite eggs and cysts in irrigated soil is related to the repeated land applications of wastewater, increased by the longer persistence of eggs and cysts in the environment. Thus, STH eggs and *Giardia* cysts may reach higher levels in on-farm soil as compared to water matrices implying greater risks of infection (22).

Studies on repeated wastewater-irrigated fields showed the occurrence of *Ascaris* eggs and *Giardia* cysts at different soil depths (10,37). The accumulation of eggs and cysts was recorded in the first soil surface layer (0-15 cm depth), supporting the transfer of pathogenic agents from water to the soil compartment through irrigation (53). Such transfer may be achieved by several processes including sedimentation, adsorption, percolation, etc. The soil compartment has a crucial role in the transmission of a myriad of human pathogens, including the STHs, *Ascaris* and *Trichuris* (54). Current estimates indicate that approximately one billion people are infected with STHs globally, especially in low-resource settings (21). The morbidity of STHs is considerable given their marked effects on nutrition and growth among infected children and adults from developing countries (55). It has been reported that around 89.9 million children harbor STHs in Africa, and many of them hosting two or more STHs species (21).

On the other hand, the persistence of parasitic eggs and cysts in the environmental components has been investigated. The persistence period of a given pathogen in the environment as an indicative property of the health risks, may engender through wastewater reuse practices (14). Upon reaching the soil, parasite eggs and cysts can either persist or be inactivated or disintegrated by the hostile physical and chemical factors occurring in the soil compartment, and potentially through predation by soil-living organisms. In addition, it was reported that *Giardia* cysts and *Ascaris* eggs can strongly adhere to the mineral particles of irrigated soils as compared to the organic fraction (10). In contrast, protozoan cysts are preferably adsorbed into soil organic fraction rather than into the mineral particles (56). Longer persistence periods in irrigated soil going from months to years were reported for *Ascaris* and *Trichuris* eggs against several days for protozoan cysts (37). Hostile environmental conditions such as increasing temperatures, sunlight exposure, and water loss result in a gradual decrease in eggs and cysts concentrations through desiccation (57).

Occurrence of Giardia cysts and soil-transmitted helminth eggs in irrigated crops

Based on the available reports, more attention is given to the microbial contamination of crops as compared to irrigated soils which accumulate wastewater pollutants including pathogenic agents. For the contamination level of crops, data on the occurrence of *Giardia* cysts and the STH eggs of *Ascaris* and *Trichuris* in crops irrigated with raw or treated wastewater are scarce and sparse, varying between studies (Table 3). The STH eggs and *Giardia* cysts have been discovered in different types of crops and vegetables. The concentration of *Ascaris*, has been reported 0 to 78 eggs/kg (58,59), while *Trichuris* concentration isolated in crops has been reported 0.6 to 2.55 eggs/kg (50). Significantly higher occurrences were recorded for *Giardia* cysts reaching up to 6.6×10^{3} cysts/kg (60). More recent research from different countries has discovered parasite eggs, including *Ascaris*, in farm crops with average concentrations up to 1000 eggs/kg (26,49). Contamination of crops with parasite eggs and cysts may occur during the pre-harvest stage through soil amendments and irrigation water, and post-harvest stage via production harvesting and handling. The contamination levels of crops depend on many factors including the quality and quantity of water applied to soil, irrigation method used, and the type of irrigated crop (61). Reports indicated that the occurrence of parasites cysts and eggs differed between leafy and non-leafy vegetables with higher levels in leafy ones as compared to root and fruit vegetables (50,62). The disparity in the contamination rate between crop varieties may be related to the physiognomy of plants as well as to the characteristics of the pathogens (63). Vegetables with broad leaves and uneven surfaces, such as lettuce and parsley, and those with dense foliage, provide large contact areas with contaminated irrigation water and soil, enhancing the trapping and attachments of cysts and eggs to the crop surface. Moreover, the morphology of some vegetables may be in favour of parasite cysts and eggs protection against hostile conditions, increasing their survival periods (64). Similarly, pathogens attachment is more likely to occur in crops growing close to the soil surface, and sticky and hairy plants, as compared to those with a smooth surface (37).

For the persistence of STH eggs and *Giardia* cysts on wastewater irrigated crops, it has been seldom assessed and reported data are scarce and sparse (50,58-60,65-67). The levels of *Ascaris* eggs and *Giardia* cysts detected on wastewater irrigated crops were found to decrease over time (53). *Giardia* cysts disappeared from wastewater-irrigated lucerne within 4 days of exposure while *Ascaris* eggs were not discovered 6 days after crop contamination. Reports from the United States Environmental Protection Agency (68), indicated that the longest period that cysts may persist on crops was about 5 days, while other reports declared 10 days for *Giardia* cysts (37). Earlier investigations showed that parasite cysts are very sensitive to desiccation, with a resistance period ranging from 3 days to 2 weeks, depending on the type of crops and climate factors (69). Longer persistence periods in crops were reported for *Ascaris* eggs ranging from 10 days to up to 60 days, but usually less than 30 days (13,70). The persistence of parasites' eggs and cysts on irrigated crops is more likely to be shortened due to hostile environmental factors (e.g. elevated temperature, intense solar radiation, low humidity, etc.) responsible for cysts and eggs desiccation (64,71).

Exposure pathways to parasitic infections through wastewater reuse

In many locations, the concentrations found for STHs in wastewater surpass the limit value of ≤ 1 egg/L recommended for wastewater/sludge reuse in agriculture (Table 1). The occurrence of the STH eggs of *Ascaris* and *Trichuris*, and *Giardia* cysts in wastewater, irrigated soil, and crops raised questions on the potential health risks of exposure to such matrices and produce may engender. For the three parasites, the route of exposure is primarily fecal-oral. Parasite transmission to farmers may occur through direct exposure to contaminated wastewater or sludge during land application. It may also happen via ingestion of wastewater, contaminated soil or crops. Agricultural workers are considered as the most affected group as they are frequently exposed to wastewater and contaminated soil (Figure 2). Other groups at risk include farmers' families, handlers and consumers of agricultural products, and people living near wastewater-irrigated land (13).

Many epidemiological surveys assessed the health impacts that wastewater reuse can have on the prevalence of parasitic diseases in groups at risk (5,72). Reports have established the association between wastewater/sludge reuse and the parasitic diseases ascariasis, trichuriasis, and giardiasis spread among exposed groups (Table 4).

Based on the available data, a large number of reports from different countries showed that wastewater and/or excreta reuse increases the STHs and *Giardia* infections among exposed populations including farmworkers and their families (Table 4). These studies have been particularly carried out in developing countries where wastewater reuse for agricultural purposes is a common practice (11,22,33,73-84). They investigated either the occupational exposure of farmworkers and households and/or children living in wastewater-irrigated areas (11,73,74,80,83). Several surveys focused on children considered as vulnerable to wastewater contaminants and their exposure has been supported by epidemiological studies (74,79). Reports from different countries indicated that farmers' children in agricultural areas exposed to raw wastewater are at high risk of infection by *Giardia, Ascaris* and/or *Trichuris* than unexposed groups (14,78). This may be due to the increased contact of children with contaminated environments while playing, or their involvement in helping parents in agricultural activities (79). However, it is noteworthy that some research studies did not find any increase in parasitic infections associated with wastewater irrigation. In the peri-urban area in Hanoi (Vietnam), wastewater reuse in agriculture had no significant effect on the risk of helminth infections (84). Moreover, a report from France found no health risks for farmers using treated wastewater as compared to non-exposed groups of farmworkers (85). Similarly, a report from Morocco found no significant difference between the prevalence of *Trichuris* in wastewater irrigation areas as compared to controls where wastewater is not reused in irrigation (79).

Health risks mitigation approach

Epidemiological and risk assessment methods have shown that wastewater reuse for irrigation purposes results in significantly increased STH and *Giardia* infections (22). This is mainly related to the high occurrences of these pathogens in reclaimed water exceeding recommended guidelines (13). To adequately manage the overall risk and attain the health-based goals, several control measures may be used together according to the multiple barrier approach (15,86). This may be possible through the implementation of these measures at various steps and stages along with the production chain from the wastewater source to food pathway including farm and market stages (Figure 1), and each stage offers another barrier of protection against pathogens spread (7). On this basis, the WHO guidelines suggested the use of the multi-barrier approach as an effective way to protect environmental and human health (13,87). This includes combined steps and measures such as the selection of appropriate wastewater treatment technology, continued health-protection, and control measures. These mainly consist of pre-farm barriers, on-farm barriers, and post-farm barriers. The suggested measures for health protection are comprised of treatment for water quality improvement (29), wastewater application techniques, restriction of crops, post-harvest handling practices, and food preparation methods (Figure 3) (52,88). Moreover, the education and training of on-site workers and families involved in agricultural irrigation are crucial for implementing and maintaining preventive measures. In addition, these technical measures can be reinforced by administrative barriers considered as an important tool for safe water reuse (89). It also should be noted that while current evidence showed that the reuse of raw or partially treated wastewater in irrigation increases health risks to exposed farmworkers and consumers, this practice is crucial for the livelihoods of smallholders, particularly in low-income communities, and water-scarce zones (90). In this context, adopted approaches to mitigate human health risks associated with wastewater reuse should take into account the need for the improvement of food security, nutrition, and livelihoods of the involved population (5-7,13).

Conclusion

Due to growing populations and food demand, the increasing use of wastewater for irrigation purposes is expected to continue, especially in developing countries. According to the epidemiological studies performed over the last decades, the reuse of untreated wastewater for crop production can result in real infection risks caused by pathogenic parasites including STHs and protozoa. The most exposed groups include agricultural workers and their families, as well as consumers of irrigated crops. The lack of awareness, and poor knowledge regarding the health impact of wastewater reuse, combined with poor sanitary practices and hygiene, may sustain the health risks. In this context, there is a requirement for a comprehensive investigation of the exposure pathways and associated burden. This may help to implement appropriate control measures to manage potential health risks under different conditions of wastewater reuse. In the final instance, the implemented interventions should be proven to protect the farmworkers and their families, crops consumers and handlers, and sustain the livelihoods of the poor producers and traders in a long term. Importantly, there is a need for particular focus on vulnerable groups such as young children facing disproportionate risks via involvement in agricultural work. At present, there is no universally applicable risk management strategy, which should be context-specific. Adopted measures should take into account economic, socio-cultural, and environmental factors, as well as standards for health risk reduction. A set of measures have been suggested and integrated into a multi-barrier approach for risk mitigation. Through the implementation of the multiple barriers, it can be possible to reduce the risks and achieve targets at the consumption stage. A combination of wastewater treatment to comply with regulation limits, safer irrigation practices, exposure control, pathogen persistence on irrigated crops, produce processing before consumption, and education campaigns for the population, can be considered to attain context-specific goals.

Acknowledgments

The authors would like to thank the officials and staff of the Regional Centre for Careers in Education and Training CRMEF Marrakech-Safi (Morocco) for their collaboration and assistance. Special thanks to Prof. Samia Boussaa for her valuable comments and guidance. The authors confirm that no funding was received to carry out this study.

Ethical issues

There were no ethical issues in writing this article. The authors certify that the article has not been published before and is not currently being considered for publication elsewhere.

Competing interests

The authors declare that there is no conflict of interests.

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