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Survey of Parasitic Contamination of Sewage Sludges in Northern Iran

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ABSTRACT: The use of sewage sludge as fertilizer in agriculture is a convenient and economic solution, but it is essential to monitor presence of parasitic contamination. This study investigated parasites in sewage sludge of wastewater treatment plant in Gorgan, Iran. This descriptive-analytical study was performed on 18 sewage sludge samples collected from wastewater treatment plant of Gorgan within 6 months with three repeatitions per month. The samples were analyzed in the laboratory of School of Public Health at Golestan University of Medical Sciences. Analysis of parasites was done using Bailenger method of counting parasites in chamber of McMaster slides with volume of 0.3 ml. The results showed that majority of parasite eggs in the sewage sludge was related to nematodes. The dominant nematode eggs detected were related to *Ancylostoma duodenale*, *Necator americanus* and *Enterobious vermicolaris*. However, no parasite was found in dried sewage sludge. Considering the amount of parasite eggs in the returned sludge, it is necessary to modify the treatment process. However, it is permissible to use dried sewage sludge as agricultural fertilizer.

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Municipal wastewater has high levels of chemical and microbial contamination. These organisms, such as bacterial and parasitical contaminations can enter the purification process and change the treatment plant into a unique ecosystem. The microorganisms were not remove completely removed at the end of treatment process and may enter natural ecosystems via wastewater discharge. Since, municipal wastewater treatment plants are usually design to removal of organic contaminants; they seldom have been plann to removal of heavy metals, parasite eggs and pathogenic microorganisms (Tyagi *et al*., 2011; Conservation DoEa, 2010). Hence, some of these organisms, such as parasite eggs, may act as a potential threat to the human and animal health (Marín.I *et al*., 2014). Using sewage sludge in farmlands is a viable option of sewage disposal due to possibility of improving soil properties and increasing the productivity of plants by recycling valuable soil elements such as organic matter, nitrogen, phosphorus and other nutrients for plants (Dadban-Shahamat *et al*., 2017). A study in 2000 showed that 70-80% of all agricultural lands are lacking sufficient nutrients. In addition, applying organic fertilizers and overuse of chemical fertilizers lead to soil degradation, lowers cultivation levels and reduces soil water holding capacity (LIU and SUN, 2013). Presence of chemical pollutants and pathogens is a major concern for utilization of sewage. Potential pathogens in wastewater and sewage sludge include several species of bacteria, enteroviruses, rotavirus and worms' eggs, which could be harmful for human health (Marín.I *et al*., 2014). Parasitic elements such as eggs, worms and protozoan cysts are often detect more in sewage compared to other surface waters. These elements are resistant to chlorine or ozone commonly used in water and wastewater treatment systems. In general, the removal of parasites from sewage is synonymous with the removal of intestinal nematode eggs, particularly Ascaris, whipworm and hookworm, which usually occurs in a simple filtration method. In addition, there are microscopic methods for detection of their presence (Hatam-Nahavandi *et al*., 2015). Wastewater should be treat by removal of contaminants including organic materials and pathogens. There are different treatment processes such as activated sludge, wastewater stabilization ponds (WSPs), constructed wetlands (CW), aerated lagoon and trickling filters

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(Tchobanoglus GaB, 2003). The mechanisms of removing parasites during wastewater treatment processes are different. The most important methods are sedimentation (due to high density and weight), filtration, absorption by plant roots and immobilization in activated sludge, and inactivation (due to unfavorable environmental conditions) (Miranzadeh and Mahmodi, 2002; Patricia.M *et al*., 2008). Previous studies indicated that removal efficiency of parasite eggs is up to 99.9% in the aerated lagoon, 99% in the trickling filters, 100% in the WSPs (due to retention time) and CWs (with subsurface flow), and 99% in the activated sludge. In each process, wastewater removal efficiency varies based on the properties of wastewater and design criteria of the treatment plant (Patricia.M *et al*., 2008; Matteus, 2010). This study investigated the presence of parasites in returned and dried sludge from Gorgan's wastewater treatment plant.

MATERIALS AND METHODS

This descriptive analytical study evaluated the presence of parasites in sludge of wastewater treatment plant in city of Gorgan within 6 months with three replications. Wastewater treatment plant in Gorgan uses the conventional activated sludge system. In this study, 18 samples were analyzed and evaluated for the presence of parasites. Sample were taken from the returned and dried sludge and transferred to the School of Public Health in Golestan University of Medical Sciences, Gorgan, Iran. After preparation, the samples were sent for analysis to the laboratory of parasitology at School of Medicine. Parasite analysis was done using the Bailenger method and McMaster worm egg counting slides with chamber size of 0.3 mm, as described in standard methods (Eaton *et al*., 2005). First, 1 liter of sludge sample was placed at rest for about 2 hours. For dried sludge, one gram of dried sludge sample was beaten and brought to volume of 100 ml with saline (0.87g NaCl in 100 ml of distilled water). The samples were wrapped in aluminum foils and autoclaved. The samples were placed on shaker for 24 hours to solve the dried particles completely. The sample was placed at rest. Then, 90% of the supernatant was removed using a vacuum pump. Sediments were transferred to a Falcon tube and centrifuged for 15 minutes at 1000 g. The supernatant was discarded and all sediments were transferred to a Falcon tube. Bottom of the tube was washed with detergent and then added to the sample. The sample was centrifuged again for 15 min at 1000 g. The supernatant was removed and replaced with buffer (pH = 4.5). Ethyl acetate with twice the volume of sediment was added and mixed by vortexing until a uniform mixture was achieved. The sample was centrifuged again for 15 minutes at 1000 g, which divided the sample into three distinct phases. All non-fat and heavy materials such as eggs, larvae and protozoa were in the lower layer. Clear buffer layer was in the middle and fatty substances and substances solved in ethyl acetate appeared as a thick dark layer on top of the sample. The upper supernatant was discarded slowly and the sediment was mixed with zinc sulfate 33% (1.18 density), and recorded as the final volume. The mixture was quickly transferred to McMaster slide chambers using a Pasteur pipette. The slides were placed on a flat surface for 5min before laboratory testing so that the eggs float on the surface. McMaster slides were placed under a microscope with 10X or 40X magnification. It should be noted that worms are usually observed under 10X magnification, while 40X lens is used to view parasites. All parasite eggs found in the grid chamber were counted. Finally, the number of eggs per liter of sample was calculated using the following equation
(1):

$$N = \frac{AX}{PV} \quad 1$$

N: Number of eggs per liter of sample; A: The number of parasite eggs counted per slide (average of two or three slides); X: Volume of the final sample; P: Volume of McMaster slide (0.3 mL); V: Volume of the original sample (L) (Tyagi *et al*., 2011).

RESULTS AND DISCUSSION

In this study, the number of eggs from nematodes, trematodes and cestodes were counted. Majority of eggs were related to the nematodes (Table 1), hookworms and *Enterobius vermicularis*, respectively. In addition, Rhabditoid larvae, cysts of *Giardia* species, *Cryptosporidium* oocysts, free-living amoeba trophozoite, and in rare cases *Eimeria oocysts* were observed. Shapes and sizes similar to parasite eggs in the sludge samples included significant amounts of plant pollen, *Arcella discoides*, *Euglypha* spp., fertile and non-fertile *Ascaris* eggs-like species and *Hymenolepis diminuta* and *Trichocephalus*-like species, while no nematode and protozoa were observed in dried municipal sewage sludge. In May, July and August, Rhabditoid larvae have been detected among samples of parasite eggs in wet sludges. All samples of wet sludges, except two case, were contaminated by free-living amoeba trophozoite. The samples collected in May, August and September contained Cryptosporidium oocysts, while Giardia cysts were detected in the samples of September, 2016/6/20, 2016/7/25 and 2016/8/6. As shown in table 1, number of hookworm eggs was found to be 40, 50 and 80 per liter. Number of *Enterobius vermicularis* eggs was found as 40 per liter.

Table 1: Nematodes and protozoa in returned activated sludge

Rhabditoid larva	Cryptosporidium oocysts	free-living amoeba trophozoite	Giardia cysts	The number of eggs per liter	Type of parasite eggs	Date of sampling
-	▲	-	-	0	-	2016/4/4
-	▲	-	-	0	-	2016/4/12
▲	▲	▲	-	0	-	2016/4/18
▲	▲	▲	-	0	-	2016/4/24
-	▲	▲	-	0	-	2016/5/2
-	▲	▲	-	0	-	2016/5/14
-	-	▲	-	0	-	2016/5/24
-	-	▲	-	0	-	2016/6/5
-	-	▲	▲	*40*	Hookworm	2016/6/20
▲	-	▲	-	0	-	2016/6/26
▲	-	▲	-	0	-	2016/7/2
▲	-	▲	-	0	-	2016/7/17
▲	▲	▲	▲	50	Hookworm	2016/7/25
▲	▲	▲	▲	80	Hookworm	2016/8/6
▲	-	▲	-	0	-	2016/8/13
-	-	▲	▲	40	*Enterobius vermicularis*	2016/8/22
-	▲	▲	▲	0	-	2016/8/236
-	▲	▲	▲	40	*Enterobius vermicularis*	2016/8/24

▲ contamination

▲contamination

Study of Mirhosseini *et al*. (2006) on possibility of using dried sludge from wastewater treatment plants in Tehran for agriculture showed that the highest and lowest number of parasite eggs are found in autumn and spring, respectively. The absence of any type of parasite eggs in the spring in the mentioned study is in agreement with our findings except for sample 2016/6/20 (Mirhosseini, 2006). Meanwhile, no contamination with nematodes and protozoa was present in the dried sewage sludge, which is in accordance with the Engelberg guideline. This could be due to drying of sewage sludge in direct sunlight during several consecutive months in bed of treatment plant. Our results showed that most parasite eggs in sludge from wastewater treatment plant in Gorgan are related to *Enterobius vermicularis* and hookworms. In addition, microbial contamination in sewage sludge exceeds the permitted levels. However, no parasite egg was found in dried sewage sludge. In study of Nahavandi *et al*., (2015) hookworm and rhabditoid eggs, *Giardia cyst*, and *Entamoeba*, *Cryptosporidium* and *Eimeria* species were detected in wastewater treatment plant in Tehran. Similar to our study, *Entamoeba* and rhabditoid were the most frequent species detected in the study of Nahavandi *et al.* (2015). Study of Marin in Spain on the efficiency of wastewater treatment plant for potential removal of pathogens showed the presence of *Giardia* and *Acanthamoeba cysts* in the sludge samples, which is consistent with our study (Marín.I *et al*., 2014). The present study is consistent with studies of Cheng *et al.* (2009) and Tonani *et al*. (211) that detected *Giardia cysts* and *Cryptosporidium oocysts* in sewage sludge. However, our results contradict with results of Abreu-Acosta (2011) and Mosteo *et al*. (2103). Investigation of Sharafi et al. in treatment plant of Kermanshah showed that the conventional activated sludge system is able to remove parasite eggs and protozoan cysts up to 97-99% and 99-100%, respectively (Sharafi *et al*., 2012).

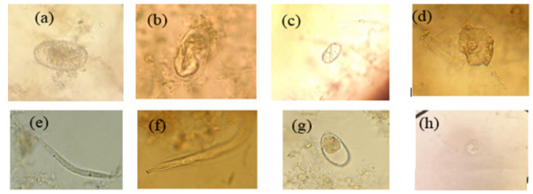

Fig. 1: (a) hookworm eggs, (b) *Enterobius* vermicularis eggs, (c) *Giardia* cysts, (d) *Amoeba Trophozoite,* (e and f) Rhabditoid larva, (g) *Eimeria* species, (h) *Cryptosporidium* oocysts

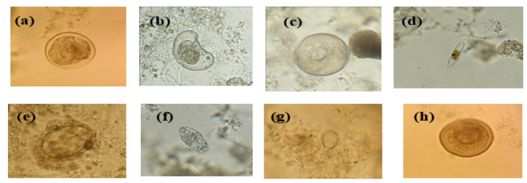

Fig. 2: Shapes and sizes similar to parasite eggs. (a&b) pollen, (c) *Arcella discoides*, (d) Trichocephalus egg-like shape, (e & f) fertile and non-fertile *Ascaris* egg-like shape (g) *Euglypha* spp., (h) *Hymenolepis diminuta*-like shape

The removal efficiency for Giardia cysts in studies of Casson *et al*. (1990) and Wiandt was 99% and 99.5-99.8%, respectively (Wiandt.S *et al*., 2000). However, in study of Miranzadeh and Mahmoudi, the removal efficiency for nematode eggs in the extended aeration activated sludge treatment process was 100% (Miranzadeh and Mahmodi, 2002). Otherwise, Gorgan wastewater treatment plant system is capable to reduce the parasite eggs up to the permissible limit of WHO (less than 1 egg/L) (Tyagi *et al*., 2011) and according to standards of France, (less than 3 viable eggs/10 g dry matter) (Gaspard and Schwartzbrod, 2003). The results show that there are any parasitic contamination in dried sludges and it is permissible to use as an agricultural fertilizer in compliance with all standards and health regulations in terms of physicochemical, microbial, and heavy metals parameters. Otherwise, wastewater treatment plant of *Gorgan* city is capable to reduce the parasite eggs up to the permissible of WHO standards and according to standards of France. However, it is essential to accurately control and monitor the function and maintenance of wastewater treatment systems due to removal and destruction of parasitic contamination in wet sludge from wastewater treatment plants. Moreover, raising health awareness and imposing major changes on attitudes and practices of families, as well as promoting correct principles of cleaning vegetables and other edible agricultural products can decrease parasitic contamination.

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