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A Mini Review on Prevalence of Protozoan Cysts in Sewage Sludge

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Abstract: *ThePioneering spirit should continue, not to conquer the planet or space, but rather to improve the quality of life. The uncontrolled sewage discharges and the poorly managed wastewater treatment plants are the two major source of water pollution and risk of infectious diseases transmission. It is the order of the day that we need to think of permanent solution of safe disposal methodology for wastewater sludge and mitigate the health risk form it. The importance of microbiological and parasitological criteria for controlling the contamination of recycled water and sludge generated thereby has been repeatedly emphasized. In industrialized countries, the most common human parasitic protozoa transmitted by water belong to the genera Giardia and Cryptosporidium. Cryptosporidium and Giardia are infectious protozoan parasites capable of causing gastrointestinal illnesses in both animals and humans. The release of contaminated effluents into the environment could increase the risk of human infection with these Pathogens. This study provides evidences that Cryptosporidium oocysts and Giardia cysts are present in the raw sewage sludge and the treated sewage sludge, which is the end product from wastewater treatment processes with the negative consequences for public health. Also, the various methods for sludge treatment and its efficiency in safely discharging the sludge into environment are being discussed.*

Keywords: *Cryptosporidium oocysts, Giardia cyst, Sewage sludge, Wastewater treatment plant.*

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I. INTRODUCTION

Sewage and sewage sludge have been recognized as potential sources of two important waterborne pathogenic protozoa: Giardia cyst and Cryptosporidium oocysts. The reuse of domestic wastewater and sludge is increasing around the world, which has environmental and economic purposes. However, many questions regarding public health risks associated with his practice deserve more attention from governmental authorities as it may facilitate the circulation of Cryptosporidium spp. and Giardia spp. in the environment. Furthermore, wastewater and sludge reuse for agriculture is commonly reported in the literature as a significant cause of infections with giardiasis and cryptosporidiosis to humans. In the case of developing countries like India, where there are no legal norms to establish criteria of maximum values for the presence of the protozoa Giardia spp. and Cryptosporidium spp. in wastewater and sludge for reuse (such as agricultural applications) or even disposal in the environment or landfills, the situation becomes an issue of major public health concern.

Studies including analysis of sewage for the parasites Cryptosporidium and Giardia have been performed throughout the world. Cryptosporidium spp. And Giardia lamblia have been detected in effluents and bio solids from wastewater treatment plants, but so far only a little is known about wastewater treatment and how it may affect their survival and removal. Many authors report a high reduction of protozoan (oo) cysts as a result of sewage treatment processes like Digestion, Heat Drying, Composting, Lime Stabilization etc., and the (oo) cysts are resistant to conventional routine disinfectants. The prevalence of Giardia infection in people was estimated at 2 – 5% in industrialized countries and 20 – 30% in developing countries.

Present paper reviews different case studies on the occurrence of Cryptosporidium oocysts and Giardia cysts in raw and treated sludge from wastewater treatment plants and to evaluate the levels of (oo) cysts present in them.

II. PATHOGENS IN THE SEWAGE SLUDGE

A pathogen is an organism or substance capable of causing disease. Pathogens infect humans through several different pathways including ingestion, inhalation, and dermal contact. The pathogens in domestic sewage are primarily associated with insoluble solids. Primary wastewater treatment processes concentrate these solids into sewage sludge, so untreated or raw primary sewage sludge has higher quantities of pathogens than the incoming wastewater. Biological wastewater treatment processes such as lagoons, trickling filters, and activated sludge treatment may substantially reduce the number of pathogens in the wastewater. These processes may also reduce the number of pathogens in sewage sludge by creating ad- verse conditions for pathogen survival. Nevertheless, the resulting biological sewage sludge may still contain sufficient levels of pathogens to pose a public health and environmental concern.

If improperly treated sewage sludge was illegally applied to land or placed on a surface disposal site, humans and animals could be exposed to pathogens directly by coming into contact with the sewage sludge, or indirectly by consuming drinking water or food contaminated by sewage sludge pathogens. Insects, birds, rodents, and even farm workers could contribute to these exposure routes by transporting sewage sludge and sewage sludge pathogens away from the site. Principal pathogens of concern in domestic sewage and sewage sludge are given in the below table. (Table.1)

Table.1 Principal Pathogens of concern in sewage and sewage sludge

SN	Microorganism/Pathogen	Disease/Symptoms
	Bacteria:	
1	Salmonella species	Salmonellosis
2	Shigella species	Typhoid fever
3	Yersinia species	Acute gastroenteritis
4	Vibrio cholerae	Cholera
5	Campylobacter jejuni, Escherichia coli.	Gastroenteritis
	Enteric Viruses:	
6	Hepatitis A virus	hepatitis
7	Norwalk and Norwalk-like viruses, Rotaviruses	Epidemic gastroenteritis with severe Diarrhea
8	Coxsackieviruses	Meningitis, pneumonia, hepatitis, fever, cold-like symptoms, etc.
9	Reovirus	Respiratory infections, gastroenteritis
	Protozoa:	
10	Cryptosporidium	Gastroenteritis
11	Giardia lamblia	Giardiasis (including diarrhea, abdominal cramps, weight loss)
12	Toxoplasma gondii	Toxoplasmosis
	Helminthic Worms:	
13	Ascaris lumbricoides, Ascaris suum	Digestive and nutritional disturbances, abdominal pain, vomiting, restlessness.
14	Taenia saginafa	aches, neurological symptoms, Nervousness, insomnia, anorexia,
15	Hymenolepis nana	Taeniasis

(Source: Report by U.S. Environmental Protection Agency Office of Research and Development on Control of Pathogens and Vector Attraction in Sewage Sludge)

Wastewater generally contains significantly high concentrations of pathogens which may enter the wastewater system from industries, hospitals, and infected individuals. The wastewater treatment process tends to remove pathogens from the treated wastewater, thereby concentrating the pathogens in the sewage sludge. Like any other living organisms, pathogens thrive only under certain conditions. Outside of these set conditions, survivability decreases. Each pathogen species has different tolerance to different conditions; pathogen reduction requirements are therefore based on the need to reduce all pathogenic populations. Some of the factors which influence the survival of pathogens include pH, temperature, and competition from other microorganisms, sunlight, and contact with host organisms, proper nutrients, and moisture level.

III. CRYPTOSPORIDIUM OOCYSTS: AS A PATHOGEN IN SEWAGE SLUDGE

Cryptosporidiosis is a parasitic infection caused by Cryptosporidium parvum. Once a person is infected by the protozoan, the parasite resides in the intestine and then is passed into the stool of the infected person. "Crypto" as the parasite and disease are commonly known as, is a diarrheal disease; symptoms include watery diarrhea, dehydration, cramps and nausea. Crypto has gained particularly notoriety during the past two decades as it has become one of the most common causes of waterborne diseases in the United States. It is spread easily by contaminated food and water thus making cleanliness vitally important in its prevention and control. The first widely publicized outbreak of Cryptosporidiosis occurred in 1987 in Carrollton, Georgia, where approximately 13000 people became ill with the disease. The source of the outbreak was traced to a contaminated municipal water system. Six years later, in Milwaukee, Wisconsin, drinking water was again contaminated, causing approximately 400000 people to become ill. Cryptosporidium lives in the intestines of infected individuals and is released with bowel movements. Because the parasite is passed with the stool of an effected individual, any surfaces that may come in contact with human or animal feces is suspect to Crypto contamination. A person must ingest the parasite to become infected but, interestingly, cannot be infected through the contact of blood.The most common symptom of the disease is watery diarrhea. Other symptoms usually include: Abdominal cramps, Nausea, Low-grade fever, Dehydration, Weight loss. Interestingly, some people will have absolutely no symptoms at all.

To date, there is no established specific treatment of patients with cryptosporidiosis. One drug, nitazoxanide, however, has been approved by the US Food and Drug Administration for treatment of immune competent patients. For patients with compromised immune systems, such as HIV/AIDS patients, anti-retroviral therapy, which has been shown to reduce oocyst excretion, is recommended. Because diarrhea results in the rapid loss of fluids, it is also recommended that patients drink plenty of fluids to prevent dehydration. Antidiarrheal medicine may help slow down fluid loss.Effective prevention involves practicing good hygiene. Individuals with compromised immune systems should take particular care to avoid contact or exposure with Cryptosporidium.

IV. GIARDIA CYSTS: AS A PATHOGEN IN SEWAGE SLUDGE

Giardiasis is an infection in your small intestine. It’s caused by a microscopic parasite called Giardia lamblia. Giardiasis spreads through contact with infected people. Pet dogs and cats also frequently contract giardia. Giardia is found in animal and human feces as the cysts. They also thrive in contaminated food, water, and soil. They can survive outside a host for long periods of time. Accidentally consuming these parasites can lead to an infection. Infectious cysts may be found in water, food, or passed person-to-person through the fecal-oral route. Contaminated water sources include wells ponds, lakes, and streams. They may also occur in city reservoirs, as cysts are resistant to water treatment. Giardia is the leading cause of infectious waterborne diarrhea outbreaks.Infection occurs by the ingestion of cysts in contaminated water, food, or by the fecal-oral route (hands or fomites). In the small intestine, each cyst releases two trophozoites, which absorb their nutrients from the lumen of the small intestine, and are anaerobes. Trophozoites multiply by longitudinal binary fission, remaining in the lumen of the proximal small bowel where they can be free or attached to the mucosa by a ventral sucking disk. IgA blocks attachment to intestinal wall. Encystation occurs as the parasites transit toward the colon. The cyst is the stage found most commonly in non-diarrheal feces. Giardia infections can remain asymptomatic, though they can also result in acute or chronic diarrhea, which may be severe. Acute giardiasis develops after an incubation period of 1-14 days and usually lasts 1-3 weeks.

Giardiasis is diagnosed by the identification of cysts or trophozoites in the feces, using direct mounts as well as concentration procedures. Repeated samplings may be necessary, and three negative tests should be carried out before declaring a patient negative. Other techniques include enzyme immunoassays, enteroscopy and immunofluorescence. Lastly, duodenal fluid or duodenal biopsy may be done, if necessary, to demonstrate trophozoites. Most physicians will recommend that you get treated with antiphrastic drugs, rather than leaving it to heal on its own. Certain antibiotics are commonly used to treat giardiasis like, Metronidazole is an antibiotic that can cause nausea and leave a metallic taste in your mouth, Tinidazole often treats giardiasis in a single dose and is as effective as metronidazole, Nitazoxanide is a popular option for children because it’s available in liquid form and Paromomycin has a lower chance of causing birth defects than other antibiotics, although pregnant women should wait until after delivery before taking any medication for giardiasis.

V. SEWAGE SLUDGE TREATMENTS

Sewage sludge treatment describes the processes used to manage and dispose of sewage sludge produced during sewage treatment. Sludge is mostly water with lesser amounts of solid material removed from liquid sewage. Primary sludge includes settleable solids removed during primary treatment in primary clarifiers. Secondary sludge separated in secondary clarifiers includes treated sewage sludge from secondary treatment bioreactors.Sludge treatment is focused on reducing sludge weight and volume to reduce disposal costs, and on reducing potential health risks of disposal options. Water removal is the primary means of weight and volume reduction, while pathogen destruction is frequently accomplished through heating during thermoplilic digestion, composting, or incineration. The choice of a sludge treatment method depends on the volume of sludge generated, and comparison of treatment costs required for available disposal options. Considering the processes to significantly reduce the pathogens in the sewage sludge may be adopted depending on the detailed categorization of the pathogens present in that waste water treatment plant sludge. The below Table.2 describes the different sludge treatment process under this category.

Table 2. Processes to Significantly Reduce Pathogens

Sludge treatment method	The brief process involved
Aerobic Digestion	Sewage sludge is agitated with air or oxygen to maintain aerobic conditions for a specific mean cell residence time (i.e., solids retention time) at a specific temperature. Values for the mean cell residence time and temperature shall be between 40 days at 20°C (68°F) and 60 days at 15°C (59°F).
Air Drying	Sewage sludge is dried on sand beds or on paved or unpaved basins. The sewage sludge dries for a minimum of 3 months. During 2 of the 3 months, the ambient average daily temperature is above 0°C (32°F).
Anaerobic Digestion	Sewage sludge is treated in the absence of air for a specific mean cell residence time (i.e., solids retention time) at a specific temperature. Values for the mean cell residence time and temperature shall be between 15 days at 35°C to 55°C (131°F) and 60 days at 20°C (68°F).
Composting	Using either the within-vessel, static aerated pile, or windrow composting methods, the temperature of the sewage sludge is raised to 40°C (104°F) or higher and remains at 40°C (104°F) or higher for 5 days. For 4 hours during the 5 day period, the temperature in the compost pile exceeds 55°C (131°F).
Lime Stabilization	Sufficient lime is added to the sewage sludge to raise the pH of the sewage sludge to 12 for ≥2 hours of contact.

(Source: Report by U.S. Environmental Protection Agency, Office of Research and Development on Control of Pathogens and Vector Attraction in Sewage Sludge)

Further, there are new processes to further reduce pathogens. Composting, heat drying, heat treatment, thermophilic aerobic digestion, beta ray irradiation, gamma ray irradiation, and pasteurization.Composting is the controlled, aerobic decomposition of organic matter which produces a humus-like material. Sewage sludge which is to be composted is generally mixed with a bulking agent such as wood chips which increases porosity in the sewage sludge, allowing air to more easily pass through the composting material and maintain aerobic conditions. There are three commonly used methods of composting: windrow, static aerated pile, and within vessel.Heat drying is used to reduce both pathogens and the water content of sewage sludge. Properly conducted heat drying will reduce pathogenic viruses, bacteria, and helminthic ova to below detectable levels. Four processes are commonly used for heat drying sewage sludge: flash dryers, spray dryers, rotary dryers, and steam dryers.Heat treatment processes are used to disinfect sewage sludge and reduce pathogens to below detectable levels. The processes involve heating sewage sludge under pressure for a short period of time. The sewage sludge becomes sterilized and bacterial slime layers are solubilized, making it easier to dewater the remaining sewage sludge solids.Thermophilic aerobic digestion is a refinement of the conventional aerobic digestion processes. In this process, feed sewage sludge is generally pre-thickened and an efficient aerator is used. In some modifications, oxygen is used instead of air. Because there is less sewage sludge volume and less air to carry away heat, the heat released from biological oxidation warms the sewage sludge in the digester to as high as 60°C (140°F).Beta Ray and Gamma Ray Radiation: Radiation can be used to disinfect sewage sludge. Radiation destroys certain organisms by altering the colloidal nature of the cell contents (protoplasm). Gamma rays and beta rays are the two potential energy sources for use in sewage sludge disinfection. Gamma rays are high-energy photons produced by certain radioactive elements. Beta rays are electrons accelerated in velocity by electrical potential in the vicinity of 1 million volts. Both types of radiation destroy pathogens that they penetrate if the doses are adequate.Pasteurization involves heating sewage sludge to above a predetermined temperature for a minimum time period. For pasteurization, the temperature of the sewage sludge is maintained at 70°C (158°F) or higher for 30 minutes or longer. Pasteurization reduces bacteria, enteric viruses, and viable helminthic ova to below detectable values. Sewage sludge can be heated by heat exchangers or by steam injection. Although sewage sludge pasteurization is uncommon.

VI. OCCURRENCE AND REMOVAL OF GIARDIA CYSTS AND CRYPTOSPORIDIUM OOCYST

Lack of studies about the occurrence of these pathogens in sewage and sludge in Brazil, an investigation was conducted at various stages of a municipal wastewater treatment plant (WWTP) aiming to assess the occurrence of Giardia spp. cysts and Cryptosporidium spp. oocysts, their removal by the treatment processes, which are up flow anaerobic sludge blanket (UASB) reactor and dissolved air flotation process, and also the correlations between protozoa and indicator microorganisms. Significant quantities of cysts were detected in 100% of the analyzed wastewater samples, while oocysts were detected only in 39.0% of all wastewater samples. The overall removal of Giardia spp. cysts from the WWTP was on average 2.03 log and the UASB reactor was more efficient than flotation. The sludge samples presented high quantities of (oo) cysts, implying the risks of contamination in the case of sludge reuse or inadequate disposal. Giardiasis prevalence was estimated between 2.21% and 6.7% for the population served by the WWTP, while cryptosporidiosis prevalence was much lower. Significant positive correlation was obtained only between cysts and Clostridium spores in anaerobic effluent.(Priscila and Luiz Daniel, 2016).

The microscopy results of this study revealedthat Giardia cysts occurred more frequently in the effluentscollectedwhereasCryptosporidium oocysts occurred less frequently, or were absent altogether.The infective dose for Cryptosporidium and Giardia isas low as 1 cyst or oocyst, which is quite sufficient to causeinfection. One of the sources of these parasites can be treated wastewater from wastewater treatment plants (WTPs). Presence of oocysts andcysts (largely viable) in effluents from WTPs imply a risk of transmission of waterborne protozoan parasites to humans.Therefore, additional wastewater purification procedures are necessary (Jacek Sroka et. al., 2013)

Study was initiated to determine the occurrence and levels of Cryptosporidium parvum oocysts in wastewater and surface watersin north-eastern Spain.Two clear maxima were observed during spring and autumn in raw sewage, showing a seasonaldistribution and a correlation with the number of cryptosporidiosis cases and rainfall events. (M. Montemayor et. al., 2005)Despite the fact that occurrence of Cryptosporidiumoocysts in sewage was similar in all treatment plants studied,oocysts concentrations have been described as beingdependent upon both the size of the contributing communityand the levels of infection and disease within thecommunity and infected livestock (Bukhari et al. 1997).

Among the sludge treatments studies in this work, aerobic digestion and lime stabilization showed higher average concentration of (oo) cysts in the treated sludge than in the raw sludge. Further, Cryptosporidium oocysts counts in treated sludge were higher than Giardia cysts counts in four of the wastewater treatment plants. Only in one, higher counts of cysts than oocysts were found. Composting has proved to be a better sludge treatment process out of conventional ones such as landfills, incineration. Composting is known to decrease the load of human pathogenic microorganism potentially present in bio-solids. Also, its product can be of good nutritive value to be used for agricultural purposes. Author here infers that (oo) cysts are heat resistant but hemophilic composting reduced their number greatly. Giardia cysts have been found in both digested and undigested sludge, and there were no significant differences between their occurrence in raw and treated sludge, concluding that Giardia cysts did not appear to be reduced by anaerobic digestion (A.M. Nasser et. al., 2012). In this study, the anaerobically digested sludge from WWTP-1 also contained up to 102/g (oo) cysts. It is possible that during the sludge treatment, such as anaerobic digestion where temperature is generally more than 35°C, (oo) cysts might lose viability and infectivity rapidly. In most of the studies, (A.M. Nasser et. al., 2012) viability assessment of oo (cysts) is either not carried out or was inconclusive. Regarding aerobic digestion, results obtained in our study in both WWTP-2 and WWTP-4 showed that the average of (oo) cysts present in sludge after stabilization were higher than in raw sludge. In a study about efficiency of a wastewater treatment plant for removal of pathogens in Spain, (I. Marín et. al., and 2014)Giardia was detected in the sludge after aerobic digestion and no Cryptosporidium was detected. Limited information is available on the fate of protozoan pathogens in biosolids. Moreover, interpretation and comparison of data are difficult due to inconsistencies in sampling, concentration and recovery procedure. Landfill leachate and sewage sludge contained high of Cryptosporidium and Giardia numbers of potentially viable, human-virulent species (Inmaculada et. al., 2016)

VII. CONCLUSIONS

At present there are many proven cases of prevalence of protozoan cysts in the wastewater as well as in the sewage sludge across the globe. Occurrence and levels of pathogen and its indicators in bio solids is necessary to be characterized and anticipate the risks associated with the treated sewage sludge. An evaluation of the risks associated with the disposal or use of bio solids for soil conditioning and its proper regulations are both necessary from public health perspective. Further research is needed to establish whether treatment options could render the sewage sludge into pathogen-free bio solids, which can be safely disposed into the environment.

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