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Diversity Profiling of Helminth Eggs in Waste Stabilisation Ponds in the Tamale Metropolis, Ghana

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ABSTRACT

Eggs of intestinal nematodes are of great health risk, they are mostly released from human excreta and recognised as causative agents of excreta-associated infections. Engineered waste stabilisation ponds serve as treatment vessels for inactivation of such helminths. The study determined the diversity profile and level of concentration of helminth eggs in waste stabilisation ponds in the Tamale Metropolis landfill site. The results of the study indicated concentration levels of 23.6, 20.8, 13.3 and 10.7 eggs/litre for the anaerobic, primary facultative, secondary facultative and maturation ponds, respectively. Concentration of helminth eggs among the various ponds varied significantly (p < 0.001). Eggs of Strongyloides stercoralis, Ascaris lumbricoides and Necator americanus were identified as the most abundant with average concentrations of 295, 124 and 78 eggs/litre, respectively. Diversity profile analyses showed that helminth eggs were highly diverse in all ponds and recording various levels especially in the maturation ponds. Taenia *spp, Diphyllobothrium latum, Paragonimus westermani, Fasciola spp, Metagonimus yokogawi and Enterobius vermicularis all recorded no egg in samples collected from the maturation pond. Results also showed a concentration reduction serially of helminth eggs from the anaerobic pond to the maturation pond. Three species were dominant in all treatment ponds out of 11 identified species during the study. The primary facultative pond recorded the highest values for all the indices indicating a high level of diversity whilst the secondary facultative pond recorded the lowest thus indicating a low level of diversity.*

Keywords: *Helminth, Eggs, Concentration, Diversity, Waste, Stabilisation Pond*

INTRODUCTION

Helminth eggs according to WHO (1989) are intestinal nematodes noted for the greatest health risk. They are mostly the causative agents of excreta-associated infections which are released from infected persons in their excreta (Bos *et al.*, 2010). A higher concentration of helminth eggs is expected from poor neighbourhoods than wastewater from middle - or high-income areas (Stolk *et al.* 2016). WHO (2006) identified Agriculture at the greatest risk, especially where untreated excreta and wastewater are used. Transmission of these intestinal parasites, according to Santamaria and Toranzos (2003) is via the faecal-oral route. A guideline value of < 1 helminth egg/L is proposed by WHO (2006) for wastewater for

unrestricted agriculture to reduce risks of infections. Wastewater and sludge reuse have been noted by Fuhrimann *et al.* (2014); Fuhrimann *et al.* (2016); Contreras, *et al.* (2017) and Gyawali, (2017) to be associated with high levels of soil-transmitted helminths (STH) globally.

Also, eggs of *Ascaris* spp. have been reported by Feachem *et al.* (1983) to be able to survive for long periods under adverse environmental conditions.

Helminth infections are reported to aggravate nutritional deficiencies and worsen the rates of anaemia, most especially in children (Munisi, 2012). The occurrence of helminths, particularly nematode infections was noted by Koné and Peter (2008) to be highly prevalent in many areas of Africa, Asia and Latin America. The most common infections worldwide, according to Santamaria and Toranzos (2003) occur in the most deprived communities. In Ghana, Obuobie *et al.* (2006) identified *Ascaris lumbricoides, Necator americanus, Trichostrongylus, Schistosoma heamatobium* and *Trichuris trichiura* as types of helminth eggs in wastewater irrigated urban vegetables in the major urban cities of Accra, Kumasi, Takoradi and Tamale. Abagale *et al.* (2013) identified thirteen (13) different types of helminths contained in wastewater used for irrigation of crops in peri-urban areas of the Tamale Metropolis. According to Hill *et al.* (2003) a suite of the diversity profile of species in a community can be obtained through the application of diversity index measures. Bartram and Ballance (1996) mention that diversity indices are best applied to situations of toxic or physical pollution, which impose general stress on the organisms, thus stable ecosystems are generally characterised by high species diversity. In 2015, WHO reported of over 1.5 billion people worldwide being affected by Soil-Transmitted Helminths (STHs) with Pullan *et al.* (2014) indicating that *Ascaris*

spp., hookworm, and *Trichuris spp.* infections are the most common and these infections are especially most common in low-income countries, mainly in Sub-Saharan Africa, Asia, and South America (WHO, 2015).

A diversity index may be categorized as richness, evenness or dominance measure that incorporates information of species richness and evenness for statistical analysis (Magurran, 2004). Stabilization ponds are designed to remove microbiological contaminants from faecal waste to reduce its effect on human and environment. Costs of construction and operation of wastewater treatment plants have been reported as a challenge in poor settings (Massoud *et al.* 2009) with most widely used decentralized wastewater treatment technologies being constructed wetlands, anaerobic baffled reactors (ABRs), up-flow anaerobic sludge blankets (UASBs), waste stabilization ponds, aerated lagoons, and oxidation ditches (Istenic *et al.,* 2014; Masi *et al.* 2015).

The information available, especially related to pathogen reduction and in particular soil-transmitted helminths (STHs) and other helminths have been noted by von Sperling *et al.* (2003) and Foxon *et al.* (2004) to be limited. Species diversity is most commonly expressed using indices such as the number of species (or *S*) and evenness. The identification and characterisation of helminth eggs is therefore very important in the design of removal systems of these eggs. The study was conducted to determine the concentration and diversity profile of helminth eggs in the stabilisation ponds.

MATERIALS AND METHODS
Study Area

The Tamale Metropolitan Assembly (TaMA) in Ghana geographically lies between latitude 09°26'3. 41"N to 09°26'4. 90" N and longitude 000°45'24. 13" W to 000° 45'28.30" W and located approximately 180

metres above sea level with a total estimated land size of 550 km^2. The Metropolis experiences one rainy season starting from April/May to September/October with a peak season in July/August (TaMA, 2013). The mean annual rainfall is 1,100 mm, mean day temperatures range from 33 to 39 ˚C while mean night temperature range from 20 to 22 ˚C and with mean annual day sunshine being approximately 7.5 hours (Cobbina *et al.,* 2013).

Faecal Sludge Collection and Helminth Eggs Isolation/Identification
The faecal sludge stabilisation pond in the Tamale landfill site is made up of two anaerobic, two primary facultative and two secondary facultative ponds connected to a maturation pond. Each pond was divided into three equal sections, i.e.; inlet, mid and outlet. Three faecal sludge samples were taken from each section per sample time at two weeks interval for a period of five months (November 2013 to March 2014) in the dry season to avoid rainwater dilution. A total of 120 samples were collected in sterile containers and subsequently transported to the laboratory in an icebox.

The laboratory determination of helminth eggs was done using the Schwartzbrod (1998) concentration method for analysis of helminth eggs and cysts in sludge and wastewater based on the principles of floatation and sedimentation. The samples were allowed to settle for three hours to enable the helminth eggs settle. Much of the supernatant as possible was sucked up and the sediment transferred into 10 ml centrifuge tubes and was centrifuged at 1,450 rpm for three minutes. The supernatant was poured away and the deposit was re-suspended with ZnSO$_4$ of density 1.3 by three times the volume of the sediments. The mixture was homogenized with a spatula and centrifuged at 1,450 rpm for three minutes. At a density of 1.3 (ZnSO$_4$), all helminth eggs float leaving other sediments at the bottom of the centrifuge tube. The ZnSO$_4$ supernatant was poured into a conical flask and diluted with 22.5 ml water and allowed to settle for three hours for the eggs to settle again. As much supernatant as possible was sucked up and the deposit was re-suspended by shaking. The resuspended deposit was centrifuged at 1600 rpm for three minutes. The deposit was re-suspended in 3 ml acid/alcohol (H$_2$SO$_4$ + C$_2$H$_5$OH), after sucking much of the supernatant, and 2 ml ethyl ether was added. The mixture was shaken and the centrifuge tube occasionally opened to let out gas before centrifuging at 1,900 rpm for three minutes. With a micropipette, as much of the supernatant as possible was sucked up leaving about 1 ml of deposit. The deposit was poured onto a microscope slide and the eggs counted. The Most Probable Number (MPN) was used for the determination of helminth egg concentration (Equation 1).

$$N = AX/PV \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.(1)$$

Where: N = number of eggs per litre of sample, A = number of eggs counted on slide, X = volume of the final product (ml) P = volume of the slide (ml), V = original sample volume (litres)

Helminth Eggs Diversity Indices
The helminth eggs diversity profile was generated for each of the faecal sludge stabilisation ponds using the following indices:

Shannon-Weiner Index
The Shannon-Weiner index (1948) is a heterogeneity measure that incorporates

information on richness and evenness and it is given by the relation:

$$H' = -\sum_i (n_i/N) \times log(n_i/N)\ldots\ldots..(2)$$

Where: $_i$ are the individuals, N is the total number of individuals.

Shannon-Weiner Evenness (Pielou J) Index

Shannon-Weiner evenness index was employed to describe the variability in helminth egg abundances in the faecal sludge stabilisation ponds and it is given by:

$$J' = \frac{H'}{log(S)}\ldots\ldots\ldots\ldots\ldots..(3)$$

Where: H' is the Shannon-Weiner index and S is the total number of species.

Simpsons Index (D)

The Simpsons index (1949) relation was used to determine the extent to which helminth eggs species dominate the faecal sludge stabilisation ponds:

$$D = \sum_i \frac{\{n_i \times (n_i-1)\}}{(N \times (N-1))}\ldots\ldots\ldots\ldots\ldots.(4)$$

Where: D is expressed in the output as both 1-D and 1/D

The Simpson's index places emphasize on species dominance as a strong dominance of one species in the community will result in an expected rarity of others.

The Log Series α Index

The Log series α index relation was also used for helminth eggs species richness assessment of the faecal sludge stabilisation pond and this is given by:

$$S = \alpha In(1 + N/\alpha), \left(\frac{\alpha x^n}{n}\right)\ldots\ldots\ldots\ldots(5)$$

Where N is the total number of individuals and α is the fitted constant.

Statistical Analysis

Statistical analysis was done using Minitab 16 software. Analysis of Variance (ANOVA) was performed for variation among mean concentrations of helminth eggs in the various stabilisation ponds and as well, for the average diversity indices tested for the study. Tables and graphs were generated using Microsoft Excel 2010.

RESULTS AND DISCUSSION

Concentration of Helminth Eggs

Helminth eggs were more concentrated in the anaerobic pond compared to the other ponds with significant variation in concentrations of 23.6, 20.8, 13.3 and 10.7 eggs/litre (F pr < 0.001 and LSD = 6.43) for the anaerobic, primary facultative, secondary facultative and maturation ponds, respectively (Figure 1). Despite the reduction in helminth egg concentration from the anaerobic pond through to the maturation pond, the concentrations in the various stabilisation ponds did not meet the WHO limit of < 1 egg/litre for unrestricted irrigation (WHO, 2006). This faecal sludge effluent could pose a threat to human health and the environment if used for agricultural purposes. According to UNICEF (2000), fresh faeces are noted to have high concentrations of pathogenic contaminants and supported by field observation by Jimenez-Cisneros (2007) who noted that most helminth eggs are retained in the first anaerobic pond. Since treated effluent from stabilisation ponds is mostly reused, a lower level than the 10.7 eggs/litre recorded in the present study is required to reduce related health risks.

The observation of reducing egg counts from the anaerobic ponds to the maturation was

attributed to the designed considerations of the ponds aimed at removing the helminth eggs and the higher amount of sunlight penetration in the maturation pond. Desiccation and exposure to UV radiation from direct sunlight are known to facilitate rapid pathogen inactivation (Amoah, 2008). UV radiation inactivates pathogens by transfer of electromagnetic energy from the sun to the organism thereby destroying its genetic material (DNA and RNA) and and 100 % of STH eggs by sedimentation.

prevents replication and consequently causing death. On the other hand, lower levels of helminth eggs in the final maturation pond may be due to sedimentation and the accumulation of helminth eggs in sludge (Shanta *et al*., 2001). Rose *et al.* (1996) and Chaoua *et al.* (2018) reported that centralized WWTPs with activated sludge and trickling filter processes potentially removed between 75
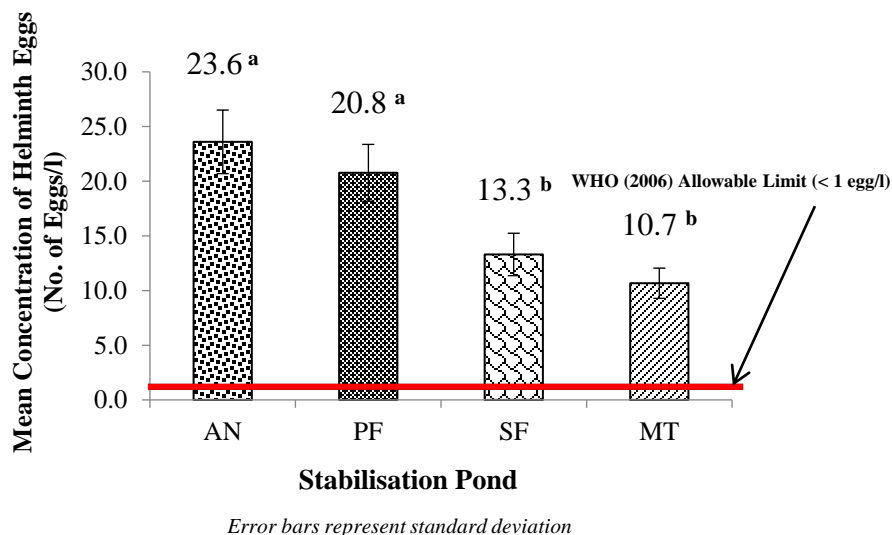
Error bars represent standard deviation

AN= Anaerobic Pond   PF=Primary Facultative Pond SF=Secondary Facultative   MT=Maturation Pond
Data labels that do not share a letter (a or b) are significantly different

FIGURE 1. **Mean Concentrations of Helminth Eggs in Stabilisation Ponds**

Helminth Species Richness and Egg Abundance

The treatment effect of the ponds resulted in the concentration of *Taenia spp, Diphyllobothrium latum, Paragonimus westermani, Fasciola spp, Metagonimus yokogawi and Enterobius vermicularis* recording no helminths at the maturation ponds. Also, *Schistosoma mansoni, Ascaris lumbricoides, Necator americanus* and *Strogyloides stercoralis* recorded reduction in

concentration from 7 to 2 eggs/l (71 % reduction), 204 to 62 eggs/l (70 % reduction) 102 to 53 eggs/l (48 % reduction), and 376 to 200 eggs/l (47 % reduction) respectively for the period. An increase in concentration of *Ternidens deminutus* eggs was observed for the period as presented in Table 1.

TABLE 1: **Helminth Species and Their Respective Egg concentrations (egg/l) in Faecal Sludge Stabilisation Ponds**

Helminth Species	AN	PF	SF	MT
		(eggs/litre)		
Ascaris lumbricoides	204	144	84	62
Strogyloides stercoralis	376	331	273	200
Necator americanus	102	118	38	53
Schistosoma mansoni	7	4	2	2
Taenia spp	11	13	0	0
Diphyllobothrium latum	4	0	0	0
Ternidens deminutus	0	2	0	4
Paragonimus westermani	2	4	2	0
Fasciola spp	0	7	0	0
Metagonimus yokogawi	0	2	0	0
Enterobius vermicularis	2	0	0	0
Species Richness	8	9	5	5

**AN= Anaerobic Pond   PF=Primary Facultative Pond SF=Secondary Facultative   MT=Maturation Pond
SS=*Strongyloides stercoralis* AL =*Ascaris lumbricoides*   NA = *Necator americanus***

Species richness (*S*) was observed to be 9 and 8 for primary facultative and anaerobic ponds, respectively, while secondary facultative and maturation ponds recorded the same level of species richness of 5 (Table 1). Higher species richness indicates the presence of more helminth species within the ponds whereas lower richness presents lower levels of helminth species within the ponds. Typical *Strongyloides stercoralis, Ascaris lumbricoides* and *Necator americanus* eggs were the most abundant in the faecal sludge stabilisation ponds. Abagale *et al.* (2013) observed dominant species of *Ascaris lumbricoides* and *Strongyloides stercoralis* in wastewater used for peri-urban vegetable crop production in the Tamale Metropolis. Obuobie *et al.* (2006) recorded *Strogyloides stercoralis* as the most occurring helminth egg species in all contaminated vegetable samples from the major urban cities in Ghana. The dominance of helminth egg species (especially *Ascaris lumbricoides*) has been associated with their persistence to the prevailing environmental conditions and high survival time rate (Obuobie *et al.* 2006; Abagale *et al.,* 2013). Kwashie (2009) classified roundworm (*Ascaris lumbricoides*), the hookworm (*Ancylostoma duodenale* or *Necator americanus*), the causative agent of strongyloidiasis (*Strongyloides stercoralis*) and the whipworm (*Trichuris trichiura*) as the intestinal parasites of significant health risk. Strauss (2000) explained that in principle, all pathogens die-off when excreted. Temperature, dryness and UV-light were noted as the major factors influencing pathogen die-off. Strauss (2000) added that pathogens have varying resistance, with worms being the more resistant and *Ascaris lumbricoides* surviving the longest. Figure 2 presents the dominant helminth eggs species in the stabilisation ponds.
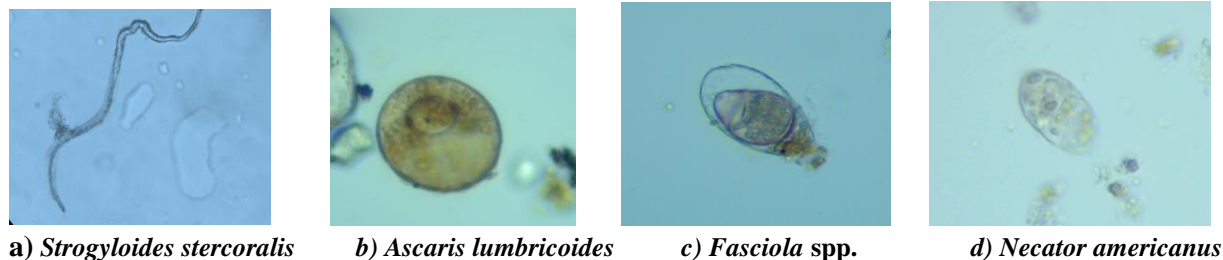
a) *Strogyloides stercoralis*   **b)** *Ascaris lumbricoides*   **c)** *Fasciola* **spp.**   **d)** *Necator americanus*

FIGURE 2. **Observed Helmith Eggs Species Under Light Microscope**

Diversity Profile of Helminth Eggs

Diversity indices provide relevant information about the composition of species within a community which determines the rarity and commonness of species present and taking into consideration the relative abundances of the different species (Beals *et al.,* 2000).

Shannon-Weiner (H') index results as in Table 2 indicate varying diversity in the various ponds. The results of Shannon-Weiner (H) index imply that the situation where fewer species dominate the ponds is relatively greater for the primary facultative pond and lesser for the secondary facultative pond. Hill *et al.* (2003) explained that Shannon-Weiner H' index gives more weight per individual of rarer than the common species and outwardly seems a good general measure to use with diverse communities. Faecal sludge from the primary facultative pond might thus, exhibit fewer helminth eggs species diversity relative to the other ponds.

The Shannon-Weiner (J') evenness index revealed a higher helminth eggs species of relative abundance for the maturation pond with an index value of 0.62. The species relative abundance of primary facultative pond emerged to be higher than that of the anaerobic pond with an index value of 0.56 and 0.55 respectively. The secondary facultative pond had the least helminth eggs species of relative abundance with an index value of 0.54 (Table 2). Magurran (2004)

explained that the more nearly equal the species relative abundance the higher the diversity. Faecal sludge from the anaerobic, primary and secondary facultative ponds might relatively exhibit higher helminth eggs species diversity than the maturation pond.

The primary facultative pond, as revealed by the Log series alpha (α) analysis was the richest in the number of helminth eggs species with an index value of 1.22 and followed by the anaerobic pond with an index value of 1.05. The maturation pond recorded an index value of 0.66 whilst the lowest index value of 0.60 was realised for the secondary facultative pond. Hill *et al.* (2003) explained that the alpha (α) is a fitted constant in the sequential equations of the log series that predict the number of species per abundance category and responds exponentially to changes in *S/N* ratio where *S* is the total number of species and *N* is the total number of individuals.

The results of Simpson (1-D) indicate that there exists a little discrepancy in helminth species diversity between the anaerobic pond and the primary facultative pond with respective index values of 0.62 and 0.63. The score for the maturation pond was relatively lower with an index value of 0.55. The secondary facultative pond was ranked the least with an index score of 0.48. Kwak and Peterson (2007) stated that the result of Simpson (1 – D) is the probability, without units, that two individuals selected randomly

from the sample will be different species. Thus, there is a higher probability of sampling two different helminth eggs species in the primary facultative pond and relatively a lower probability in the secondary facultative pond.

On the whole the primary facultative pond recorded the highest of all the indices with the exception of Shannon-Weiner J, which indicates a relatively high complexity of helminth eggs species diversity. Variation among mean diversity indices was statistically significant (p = 0.004). However, Shannon-Weiner J and Simpson 1-D were significantly different from Shannon-Weiner H (Table 2). It is thus evident that the different levels of faecal sludge treatments have significant impact on the diversity of helminth eggs species in the stabilisation ponds.

TABLE 2: **Helminth Eggs Diversity in Treatment Pond**

Diversity indices	Stabilisation Pond				Mean
	AN	**PF**	**SF**	**MT**	**Mean**
Shannon-Weiner H	1.15	1.23	0.87	1.01	1.07 [a]
Shannon-Weiner J	0.55	0.56	0.54	0.62	0.57 [b]
Log series alpha (α)	1.05	1.22	0.60	0.66	0.88 [ab]
Simpson 1-D	0.62	0.63	0.48	0.55	0.57 [b]
ANOVA					
p-value		0.004			
SED		0.123			
LSD		0.269			
CV (%)		22.6			

Means within the same column that do not share a letter are significantly different. LSD- Least significant differences, SED-Standard errors of differences, CV-Coefficient of variation

Conclusion

The concentration of helminth eggs was noted to have reduced from the anaerobic pond through to the maturation pond during the study period. Three species were dominant in all treatment ponds out of 11 different species identified. The primary facultative pond recorded the highest values for all the indices indicating a relatively high complexity of helminth eggs species diversity whilst the secondary facultative pond recorded the lowest and indicating a low level of helminth egg diversity. Concentrations of helminth eggs in the various stabilisation ponds were noted to be above WHO (2006) limit of < 1 egg/litre in wastewater for unrestricted irrigation of crops. The results of the study will therefore lead to the identification of the methods necessary for increasing the die-off rates of the helminth and also reducing the high level of the concentration.

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