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

Felix K ABAGALE* and Richard A. OSEI

West African Center for Water, Irrigation and Sustainable Agriculture, University for Development Studies, Tamale-Ghana

*Corresponding Author: fabagale@uds.edu.gh

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 yokogawai 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 km². 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 ZnSO₄ 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 (ZnSO₄), all helminth eggs float leaving other sediments at the bottom of the centrifuge tube. The ZnSO₄ 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 (H₂SO₄ + C₂H₅OH), 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).

\[ N = \frac{AX}{PV} \]  

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:

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

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:

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

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:

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

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 \( \alpha \) Index**

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

$$S = \alpha \ln(1 + N/\alpha), \left(\frac{\alpha e^N}{n}\right)$$

Where \( N \) is the total number of individuals and \( \alpha \) 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 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%

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 (eggs/litre) | PF (eggs/litre) | SF (eggs/litre) | 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 *Strongyloides 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.
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 |
|----------------------------|--------------------|
|                            | AN    | PF    | SF    | MT    | Mean   |
| Shannon-Weiner H           | 1.15  | 1.23  | 0.87  | 1.01  | 1.07   |
| Shannon-Weiner J           | 0.55  | 0.56  | 0.54  | 0.62  | 0.57   |
| Log series alpha (α)       | 1.05  | 1.22  | 0.60  | 0.66  | 0.88   |
| Simpson 1-D                | 0.62  | 0.63  | 0.48  | 0.55  | 0.57   |
| **ANOVA**                  |        |       |       |       | 0.004  |
| 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.
REFERENCES
Abagale, F. K., Kyei-Baffour, N., Ofori, E. and Mensah, E. (2013). Types and Seasonal Diversity of Helminth Eggs in Wastewater Used for Peri-Urban Vegetable Crop Production in Tamale Metropolis, Ghana. International Journal of Current Research. 5(11): 3354-3359.

Amoah, P. (2008). Wastewater Irrigated Vegetable Production: Contamination Pathway for Health Risk Reduction in Accra, Kumasi and Tamale – Ghana. PhD Thesis, Kwame Nkrumah University of Science and Technology (KNUST).

Bartram, J. And Ballance, R. (1996). Water Quality Monitoring – A Practical Guide to the Design and Implementation of Freshwater Quality Studies and Monitoring Programmes. Published on behalf of United Nations Environment Programme and the World Health Organisation. ISBN 0419223207.

Beals M., Gross L., and Harrell S. (2000). Diversity Indices. Available at http://www.tiem.utk.edu/~gross/bioed/bealsmodules/shannonDI.htm. Retrieved on 29/12/2020,

Bos, R., Carr, R., and Keraita, B. (2010). Assessing and Mitigating Wastewater-Related Health Risks, in Low-Income Countries: An Introduction, in Jiménez, B., Drechsel P., Koné, D., Bahri, A., Raschid-Sally, L. and Qadir, M. (2010). Wastewater, Sludge and Excreta Use in Developing Countries: An Overview in IWMI/IDRC 2010 Report: Wastewater irrigation and Health: Assessing and Mitigating Wastewater-Related Health Risks in Low-Income Countries.

Chaoua, S., Boussaa, S., Khadra, A. and Boumezzough, A. (2018). Efficiency of two sewage treatment systems (activated sludge and natural lagoons) for helminth egg removal in Morocco. J Infect Public Health. 11(2). DOI: https://doi.org/10.1016/j.jiph.2017.07.026

Cobbina, S. J., Michael, K., Salifu, L., Duwiejua, A. B. (2013). Rainwater Quality Assessment in the Tamale Municipality. International Journal of Scientific and Technology Research Volume 2, Issue 5, ISSN 2277-8616.

Contreras, J. D., Meza, R., Siebe, C., Rodríguez-Dozal, S., López-Vidal, Y. A., Castillo-Rojas G, Amieva RI, Solano-Gálvez SG, Mazari-Hiriart, M., Silva-Magaña, M. A., Vázquez-Salvador, N., Pérez, I. R., Romero, L. M., Cortez, E. S., Riojas-Rodríguez, H. and Eisenberg, J. N. S. (2017). Health risks from exposure to untreated wastewater used for irrigation in the Mezquital Valley, Mexico: a 25-year update. Water Res 123:834 – 850

Feachem, R. G., Bradley, D. J., Garlick, H. and Mara, D. D. (1983). Sanitation and disease: health aspects of excreta and wastewater management. JohnWiley, Chicester

Foxon, K. M., Pillay, S., Lalbahadur, T., Rodda, N., Holder, F., Buckley, C. A. (2004). The anaerobic baffled reactor (ABR): an appropriate technology for on-site sanitation. Water SA 30:44–50

Fuhrimann, S., Winkler, M. S., Pham-Duc, P., Do-Trung, D., Schindler, C., Utzinger, J. and Cissé, G. (2016) Intestinal parasite infections and associated risk factors in communities exposed to wastewater in urban and peri-urban transition zones in Hanoi, Vietnam. Parasit Vectors 9: 537. https://doi.org/10.1186/s13071-016-1809-

Fuhrimann, S., Winkler, M. S., Schneeberger, P. H. H, Niwagaba, C. B., Buwule, J., Babu, M., Medlicott, K., Utzinger, J. and Cissé, G. (2014) Health risk assessment along the wastewater and
faecal sludge management and reuse chain of Kampala, Uganda: a visualization. Geospat Health 9: 251–255

Gyawali, P. (2017) Infectious helminth ova in wastewater and sludge: a review on public health issues and current quantification practices. Water Sci Technol. https://doi.org/10.2166/wst.2017.619

Hill, T. C. J., Walsh A. K., Harris, J. A. and Mofett, B. F. (2003). Using Ecological Diversity Measures with Bacterial Communities. Elsevier Science B.V FEMS Microbiology Ecology 43 1-11.

Istenic, D., Bodík, I. and Bulc, T. (2014) Status of decentralised wastewater treatment systems and barriers for implementation of nature-based systems in central and eastern Europe. Environ Sci Pollut Res 22: 12879–12884

Jimenez-Cisneros, B. E. (2007). Helminth Ova Control in Wastewater and Sludge for Agricultural Reuse, in Water and Health, [Ed. W. O.K. Grabow], in Encyclopaedia of Life Support Systems (EOLSS), Developed under the Auspices of the UNESCO, Eolss Publishers, Oxford, UK. Available at www.eolss.net.

Koné, D. and Peter, S. (2008). Faecal Sludge Management (FSM) Sandec Training Tool 1.0 – Module 5. EAWAG/SANDEC, Department of Water and Sanitation in Developing Countries.

Kwak, T. J., and Peterson, J. T. (2007). Community indices, parameters, and comparisons. In Guy, C. S. and Brown, M. L. (Eds.), Analysis and Interpretation of Freshwater Fisheries Data. Bethesda, Maryland: American Fisheries Society. pp. 677-763.

Kwashie, K. C. (2009). Microbial Analysis of Soil Samples in A Wastewater Irrigated Vegetable Production Site: Case Study atAtonsu, Kumasi. MSc Thesis Kwame Nkrumah University of Science and Technology (KNUST) Kumasi, Ghana.

Magurran, A. E. (2004). Measuring Biological Diversity. Blackwell Science Ltd, Oxford.

Masi, F., Rochereau, J., Troesch, S., Ruiz, I. and Soto, M. (2015). Wineries wastewater treatment by constructed wetlands: a review. Water Sci Technol 71:1113–1127

Massoud, M. A., Tarhini, A. and Nasr, J. A. (2009) Decentralized approaches to wastewater treatment and management: applicability in developing countries. J Environ Manag 90:652–659

Munisi, D. Z. BVM (2012). Soil-Transmitted Helminths Infections, Malnutrition and Anaemia among Primary School Children in Same District. MSc Thesis, Muhimbili University of Health and Allied Sciences, Tanzania.

Nasr, F. A., Doma, H. S. and Nassar, H. F. (2009). Treatment of domestic wastewater using an anaerobic baffled reactor followed by a duckweed pond for agricultural purposes. Environmentalist 29:270–279

Obuobie, E., Keraita, B., Danso, G., Amoah, P., Cofie, O.O., Raschid-Sally, L. and Drechsel, P. (2006). Irrigated urban vegetable production in Ghana: Characteristics, benefits and risks. IWMI-RAUF-CPWF, Accra, Ghana: IWMI, 150 pp.

Pullan, R. L., Smith, J. L., Jasrasaria, R. and Brooker, S. J. (2014) Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. Parasit Vectors 7:37–56

Rose, J. B., Dickson, L. J., Farrah, S. R. and Carnahan, R. P. (1996). Removal of pathogenic and indicator
microorganisms by a full-scale water reclamation facility. Water Res 30:2785–2797

Santamaria, J. and Toranzos, A. (2003). Enteric Pathogens and Soil: A Short Review. IntMicrobiol. 6: 5-9. Saunders Co, Philadelphia, USA.

Schwartzbrod, J. (1998). Methods of Analysis of Helminth Eggs and Cysts in Wastewater, Sludge, Soils and Crops. University Henry Poincare, Nancy: France.

Shannon, C. E. (1948). A mathematical theory of communication. The Bell System Technical Journal. 27:379-423.

Shanta, S., Chaudhari P. R. and Kaul, S. N. (2001). Environmental Significance of Helminth Parasites and their Removal in Wastewater Treatment Processes. Environment Conservation Journal. 2: 1-23

Simpson, E. H. (1949) Measurement of diversity. Nature. 1949: 163:688.

Strauss, M. (2000). Human Waste (Excreta and Wastewater) Reuse. ETC/SIDA, Bibliography on Urban Agriculture.

Tamale Metropolitan Assembly (TaMA) (2013). The Composite Budget of the Tamale Metropolitan Assembly for the 2013 Fiscal Year. Available at www.mofep.gov.gh. Accessed on 16/9/2014

UNICEF (2000). Sanitation for All: Promoting Dignity and Human Rights. UNICEF, New York.

don Sperling, M., Chernicharo, CAL., Soares, A. M. E. and Zerbini, A. M. (2003). Evaluation and modelling of helminth eggs removal in baffled and unbaflled ponds treating anaerobic effluent. Water Sci Technol 48: 113–120

WHO (1989). Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture. World Health Organization, Geneva. Technical Report Series, No. 778.

WHO (2006). Guidelines for the Safe Use of Wastewater, Excreta and Greywater Use in Agriculture Volume 4. World Health Organization, Geneva.

WHO-World Health Organization (2015) Investing to overcome the global impact of neglected tropical diseases third who report on neglected tropical diseases. WHO Document Production Services, Geneva, Switzerland (WHO/HTM/NTD/2015.1).