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RESEARCH ARTICLE

TYPES AND SEASONAL DIVERSITY OF HELMINTH EGGS IN WASTEWATER USED FOR PERI-URBAN VEGETABLE CROP PRODUCTION IN TAMALE METROPOLIS, GHANA

[1*]Abagale F. K., [2]Kyei-Baffour, N., [2]Ofori, E., and [2]Mensah, E.

[1]Faculty of Agriculture, University for Development Studies, Tamale, Ghana
[2]Department of Agricultural Engineering, College of Engineering, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

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ABSTRACT

Thirteen (13) different types of helminths were identified for both the wet and dry seasons with the dry season recording eight (8) different helminths and the wet season recording nine (9) different helminths. The typical fertile *Ascaris lumbricoides* and *Strongyloides stercoralis* as well as *Schistosoma mansoni* were observed to be the most predominant types of helminths in both seasons and this may be due to their environmental tolerance and resistance. *Ascaris lumbricoides* was the most predominant species recorded with arithmetic mean population of 12 and 17 for wet and dry season respectively. Exceeded the recommended level of <1 egg/liter for unrestricted irrigation (WHO, 1989). Variable concentrations of helminth eggs were recorded for the dry season and wet season for the period of study. During the current study, all the recorded helminths were noted to have a population density ranging from 2 to 17 eggs with a coefficient of variation being between 0 and 85%. Commonly observed dominant species of *Ascaris lumbricoides* and *Strongyloides stercoralis* for both seasons, a higher dominance index of 0.58 for the wet season was recorded compared to 0.52 for the dry season. Also, environmental factors such as temperature, sunshine amount and duration, rainfall, etc have been noted to impact greatly on the occurrence and concentration of helminth eggs in wastewater of a particular locality. These were noted to have contributed largely to a high number of helminth eggs in the wet season as compared to the dry season.

INTRODUCTION

Population growth especially in Africa and Sub-Saharan Africa is increasing at a very fast rate especially in the urban areas. This is exerting a lot of social and infrastructural pressure on the facilities of the urban cities. It has been reported that urban population since 2002 is experiencing a 4.2% annual growth rate. This consequently puts a lot of pressure on the limited resources especially land and water. Keraita *et al.* (2003) reported that, less than 10% of the urban dwellers in Ghana are connected to a piped sewerage system and the wastewater is channelled from street gutters to larger drains and inner-city streams. This usually results in open and poor urban sanitation systems especially in unplanned areas or slums. In northern Ghana especially, Tamale, the city currently reports a population of 371,351 as per the 2010 Population and Housing Census of the Ghana Statistical Service (2010). Also with an increasing adult population as reported by the 2010 GSS Census especially within the age bracket of 15 to 64 years, it implies the availability of more labour force. Due to the limited jobs in the Cities, many people have resorted to the use of 'polluted' water for their crop production purposes especially vegetable salad. This presents a very high risk system of vegetables for the consumer market. The situation is also aggravated by the unimodal rainfall system that characterise the area and the impact of climate change which has also affected the planting periods of most crops. With an estimated population of over 40 % of urban dwellers in Ghana without good quality drinking water, most farmers in the urban and peri-urban areas often use low-quality water from open drains, shallow wells, streams and broken down sewer systems.The risk of producing fresh vegetable crops contaminated with eggs of helminths cannot be discounted however. Reports indicate that Armar-Klemesu *et al.* (1998) and Cornish *et al.* (1999) raised concerns regarding the potential health risks to consumers of vegetables produced with water from these sources.This study therefore discusses the common type of helminth eggs that occur in wastewater used for peri-urban vegetable crop production in the Tamale Metropolis of Ghana.

***Corresponding author: Abagale F. K.,**
Faculty of Agriculture, University for Development Studies, Tamale, Ghana

MATERIALS AND METHODS

Study Area

The Tamale Metropolitan area is located at the centre of the Northern Region of Ghana. It occupies 750 km^2 which is 13 % of the total area of the Northern Region. The population of

Tamale Metropolis is said to be 371,351. In the Metropolis there are several sites where wastewater vegetable farming takes place and crops cultivated include cabbage, lettuce, *amaranthus, chochorus,* etc. This study was done in the Zagyuri community where community farmers use wastewater from a broken down sewer of the Kamina Military Barracks for vegetable crop production. Figure 1 shows the map of Ghana and the Tamale Metropolitan Area.

contained in the wastewater. It quantifies the uncertainty (entropy or degree of surprise) associated with this prediction. It is most often calculated as follows:

$$H' = -(\ln P_1^{P1} + \ln P_2^{P2} + \ln P_3^{P3} + \ ... + \ln P_R^{PR})$$

Where; p_i is the proportion of individuals belonging to the i^{th} species in the dataset.

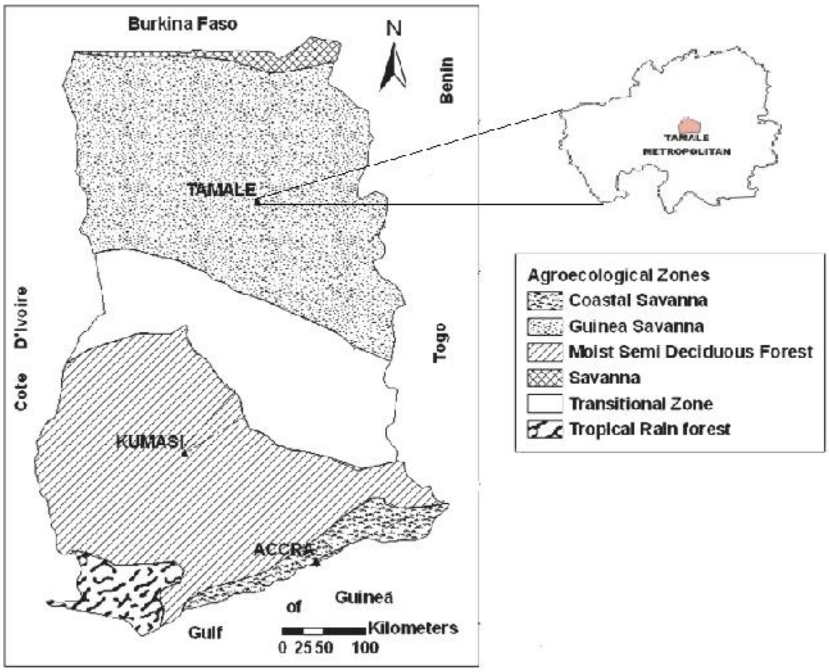


Figure 1. Map of Ghana showing Tamale Metropolitan Area

Water Sampling

Sampling of water was done in both the dry and wet seasons for two (2) years i.e. 2010 and 2011. Sampling was done once a day at weekly interval for a period of two (2) months in each season. The months of August and September were used for the wet season and December and January for dry season.

Laboratory Procedures

Helminth eggs were enumerated using the concentration method as described by Schwartzbrod (1998). This is a modified US-EPA method, but the same principle of floatation and sedimentation as in the method of Ayres and Mara (1996) was followed. During all sampling and analysis periods, sterile sampling containers, hand gloves, water and chemical reagents were used. An ice box was used for the storage and transportation of samples from the field to the laboratory.

METHODS

The following methods were used in the calculation of some most important indices during the study.

Shannon-Wiener Index

Use widely in ecological studies and also known as Shannon's diversity index, the Shannon-Weaver index and the Shannon entropy, the Shannon-Wiener Index was used in the determination of the diversity of the types of helminth eggs

Simpson Index

The Simpson index measures the degree of concentration when individuals are classified into types. The measure equals the probability that two entities taken at random from the dataset of interest represent the same type. The Simpsons index is presented as:

$$l = \frac{\sum_{i=1}^{R} n_i(n_i - 1)}{N(N-1)}$$

Where; = n_i Number of individuals of i^{th} species, and N = Total number of individuals of all species. The value of this Index ranges from 0 – 1 with 1 showing high diversity and 0 no diversity.

Berger-Parker Index

The Berger-Parker index equals the maximum p_i value in the dataset, i.e. the proportional abundance of the most abundant type. This corresponds to the weighted generalized mean of the p_i values when q approaches infinity, and hence equals the inverse of true diversity of order infinity ($1/^{\infty}D$).

Data Analysis

The concentration of the helminth eggs used the Most Probable Number (MPN) and indices were calculated using the above relations. Microsoft Excel 2007 was also used in results presentation.

RESULTS AND DISCUSSIONS

Contamination and Risk of Wastewater Use

It is common in urban and peri-urban areas of Ghana to see resource poor farmers using contaminated or polluted wastewater for especially dry season vegetable production. Resource poor farmers use wastewater mainly because crop yields are higher as the wastewater contains not only water for crop growth, but also important plant nutrients necessary for crop growth and performance. Wastewater treatment has been proposed severally and the WHO (2006) guidelines as well as other measures as a way to minimise the health risk of wastewater irrigation to farmers and consumers are being encouraged. However, there is the risk that wastewater irrigation may facilitate the transmission of excreta-related diseases. In 1989, the World Health Organization (WHO) drew attention to diarrheic diseases caused mainly by helminths contained in sludge and wastewater re-used for agriculture, and in agreement, set the guidelines for safe reuse. According to UN (2003), globally there are 5 million people suffering helminthiases, mainly in developing countries. Helminthiases are particularly common in regions where poverty and poor sanitary conditions are dominant, reaching incident rates of up to 90% (Bratton and Nesse, 1993). Ascariasis prevails among poor people and is widespread in warm climates, being one of the most common infections, in the developing countries. According to Bratton and Nesse (1993), ascariasis, is endemic in Africa, Latin America, South America, and the Far East, with an incidence up to 90% in specific sectors of the population. The most important factors responsible for the high prevalence of ascariasis in the world are closely related to poverty.

Identified Helminth Eggs

There are numerous texts available with drawings and photographs of eggs and larvae of all the major parasitic helminths, which may be found in raw or treated wastewater (Jeffry and Leach, 1975; Fox *et al.*, 1981; Thienpont *et al.*, 1986). During the study helminth eggs were identified using the WHO (1994) Bench Aid for the Diagnosis of Intestinal Parasites. Thirteen (13) different helminths were identified for both the wet and dry seasons with the dry season recording eight (8) different helminths and the wet season recording nine (9) different helminths. The identified helminths of both seasons include *Ascaris lumbricoides, Strongyloides stercoralis, Schistosoma mansoni, Schistosoma haematobium, Clonorchis sinensis, Paragonimus uterobilateralis, Paragonimus westermani, Schistosoma japonicum, Diphyllobothrium latum, Trichuris trichiura, Fasciola hepatica, Enteroblus vermicularis and hookworm.* The typical fertile *Ascaris* and *Strongyloides stercoralis* as well as *Schistosoma mansoni* were observed to be the most predominant types of helminths in both seasons and this may be due to their environmental tolerance and resistance.

Seasonal Concentrations

In the dry season, the commonest occurring type of helminths in a reducing order was *Ascaris lumbricoides, Hookworm, Strongyloides stercoralis, Schistosoma mansoni* and *Schistosoma japonicum. Fasciola hepatica, Clonorchis sinensis* and *Paragonimus westermani* however recorded the same level of helminths in the dry season. Also observed in a decreasing order during the wet season were *Ascaris lumbricoides, Strongyloides stercoralis, Schistosoma haematobium, Hookworm* and *Schistosoma mansoni* but with

Ascaris lumbricoides ***Diphyllobothrium latum*** ***Paragonimus uterobilateralis***

Strogyloides stercoralis ***Trichostrongylus*** ***Schistosoma mansoni***

Figure 2. Pictorial Presentation of Observed Helminths Eggs

the last two being at the same level. *Paragonimus uterobilateralis Diphyllobothrium latum Trichuris trichura* and *Enteroblus vermicularis* were also observed in very low levels during the wet season. It is observed from both seasons that *Ascaris lumbricoides* was common and as reported by Nolf, (1932) that *Trichuris* eggs required a more highly saturated atmosphere before they could develop than *Ascaris* eggs, and that under fractional relative humidity's, the eggs of *Trichuris* succumbed more readily than those of *Ascaris.* The explanation for the difference may lie in two basic differences in the eggs: the comparative sizes: *Ascaris* eggs are larger and have a considerably greater surface of the fibrous membrane through which the diffusion of gases occurs; and the difference in time required to complete embryonation under optimum conditions: *Trichuris* eggs require more time to complete their development than *Ascaris*.

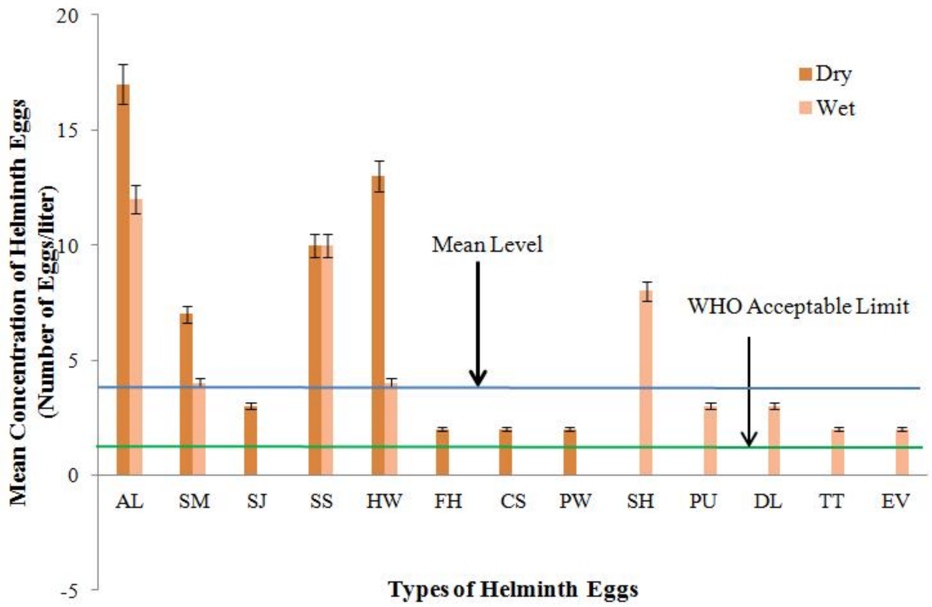
The wet season was observed to have recorded much more different helminth eggs as compared to the dry season and this could be attributed to a favourable environmental condition during the period. As a result of favourable environmental temperature as reported by Arene (1986), *Ascaris* develop in the egg between temperatures 16°C and 34°C. Ovicidal fungi are capable of attacking and destroying *Ascaris lumbricoides* eggs under experimental conditions during several days or weeks (Lysek and Bacovsky, 1979). Also reported by Sobenina (1978), *Cylindrocarpon radicola* is known to penetrate and destroy *Ascaris* eggs. Invertebrates, particularly insects and gastropods, can also destroy helminth eggs by mechanically breaking the eggs and ingesting them (Miller *et al.*, 1961). The conditions during the wet season may therefore be seen as being favourable for the development of these parasites in attacking and destroying the helminth eggs.
Largely, the dry season recorded a high level of concentration of helminth eggs compared to the wet season and this can be attributed to the absence of rainfall and thus resulting in the concentration of the eggs. The effect of the dilution factor from rainfall is therefore eliminated in the dry season and increasing the egg population per liter of wastewater as presented in Table 1.

Table 1. Arithmetic Mean and Coefficient of Variation of Seasonal Concentration of Helminth Eggs in Wastewater Used for Peri-Urban Vegetable Crop Irrigation in Tamale

Type	Dry Season		Wet Season	
	[illegible])	CV (%)	[illegible])	CV (%)
Ascaris lumbricoides	17 (9)	85	12 (8)	66
Schistosoma mansoni	7 (3)	11	4 (2)	4
Schistosoma japonicum	3 (1)	0	0 (-)	0
Strongyloides stercoralis	10 (8)	67	10 (6)	32
Hookworm	13 (7)	44	4 (1)	1
Fasciola hepatica	2 (-)	0	0 (-)	0
Clonorchis sinensis	2 (-)	0	0 (-)	0
Paragonimus westermani	2 (1)	0	0 (-)	0
Schistosoma haematobium	0 (-)	0	8 (5)	25
Paragonimus uterobilateralis	0 (-)	0	3 (1)	1
Diphyllobothrium latum	0 (-)	0	3 (1)	0
Trichuris trichura	0 (-)	0	2 (2)	4
Enteroblus vermicularis	0 (-)	0	2 (2)	2

Figures in parentheses are standard deviation; values were rounded to the nearest whole number.

In a study by Amoah *et al*. (2005) from different irrigation water sources in Kumasi and Accra of Ghana, identified eggs included *Ascaris lumbricoides*, *Hymenolepis diminuta*, *Trichuris trichura*, *Facsiola hepatica* and *Strogyloides* larvae.
During the current study, all the recorded helminths were noted to have a population density ranging from 2 to 17 eggs with a coefficient of variation being between 0 and 85% (Table 1). In this study *Ascaris lumbricoides* was the most predominant species recorded with arithmetic mean population of 12 and 17 for wet and dry season respectively. Largely, all the observed eggs concentrations as presented in Table 1 and Figure 3 exceeded the recommended level of <1 egg/liter for unrestricted irrigation (WHO, 1989). In a study by Cornish *et al.* (1999) in urban and peri-urban irrigation water sources 1–5 helminth eggs per l^{-1} was recorded.



Ascaris lumbricoides (AL), Strongyloides stercoralis (SS), Schistosoma mansoni (SM), Schistosoma haematobium (SH), Clonorchis sinensis (CS), Paragonimus uterobilateralis (PU), Paragonimus westermani (PW), Schistosoma japonicum (SJ) Diphyllobothrium latum (DL), Trichuris trichiura (TT), Fasciola hepatica (FH), Enteroblus vermicularis (EV) and hookworm (HW).

Figure 3. Mean Concentration of Helminth Eggs

Diversity of Helminth Eggs in Wastewater

Attributes of helminth egg seasonal variation is as presented in Table 2 and the Simpson index (λ) was 0.48 for the dry season and 0.42 for the wet season. Even though the values were in the same range, the Simpson index was realised to be high in the dry season compared to the wet season. The Shannon-Wiener index (H') was however observed to be high in the wet season with a value of 1.76 compared to a dry season value of 1.57. Similarly, Berger-Parker Dominance index (D_{BP}) of 2.29 and 1.88 were recorded for the wet and dry season respectively. These results indicate clearly that the dry season largely have low diversity of helminth eggs compared to the wet season even though it may have a high concentration of helminth eggs. Margalef Richness index (D_{Mg}) indicates that the wet season was helminth egg richer than the dry season with richness index values of 2.29 and 1.88 respectively. Commonly observed dominant species of *Ascaris lumbricoides* and *Strongyloides stercoralis* for both seasons, a higher dominance index of 0.58 for the wet season was recorded compared to 0.52 for the dry season. Details of the diversity of helminth eggs studied is as presented in Table 2.

Table 2. Seasonal Variation in Diversity Indices of Helminth Eggs

Characteristics	Season	
	Dry	Wet
No. of Species	8	9
Simpson Index (λ)	0.48	0.42
Shannon-Wiener Index (H')	1.57	1.76
Berger-Parker Dominance Index (D_{BP})	0.37	0.40
Margalef Richness Index (D_{Mg})	1.88	2.29
Equitability Index (E_H)	0.75	0.80
Dominance Index	0.52	0.58
Dominant Species	AL, SS	AL, SS

AL = *Ascaris lumbricoides*SS= *Strongyloides stercoralis*

The study results reveals that helminth egg type diversity is very useful in looking at their distribution seasonally and as influenced by environmental factors. It is clear that the wet season shows high species diversity compared to the dry season. The Shannon-Wiener index is controlled largely by equitability than by species richness as indicated by Routledge (1979) and Wolda (1981). The high level of diversity observed for the wet season can be largely attributed to the favourable environmental conditions of low temperature, high humidity and other conditions necessary for egg development.

Factors Influencing Helminth Egg Occurrence

Factors that affect the occurrence and concentrations of helminth eggs and protozoan cysts observed in raw wastewater, include the endemicity of disease within the indigenous animal and human population, the size and socio-economic status of the population, the percentage of population sewered, the percentage or wastewater contributed by industry, the volume of influent sampled and the recovery efficiency of the sampling method (Grimason *et al*, 1995). Table 3 presents the prevailing environmental factors during the sampling of the wastewater for analysis. Helminths are ranked high risk (Shuval *et al.*, 1986) among pathogens present in soils and wastewater. Also, environmental factors such as temperature, sunshine amount and duration, rainfall, etc have been noted to impact greatly on the occurrence and concentration of helminth eggs in wastewater of a particular locality. These were noted to have contributed largely to a high number of helminth eggs in the wet season as compared to the dry season.

Table 3. Environmental Factors Influencing Helminth Egg Concentration

Environmental Factor	Season	
	Dry	Wet
Temperature (°C)	23.5 – 32.8	21.8 – 23.9
Relative Humidity (%)	20.5 – 48	72.5 – 92.5
Sunshine Duration (Hours)	6.3 – 10.3	0 – 10.2
Rainfall (mm)	0 – 1.5	0 – 32.6
pH	5.6 – 8.5	4.0 – 9.1

Conclusions

Various types of helminth eggs occurred in the wastewater used by resource poor farmers in peri-urban areas of the Tamale Metropolis. Seasonal variation in the number of eggs was observed and this was mainly due to the effect of the environmental factors. Limited water supply as a result of irregularity of flow of domestic pipe water especially during the dry season was said to influence greatly the concentration of the helminth eggs per litre of sampled wastewater. The use of protective clothing during crop watering and performance of other crop production cultural practices is expected to help reduce contamination of vegetables produced. Farmer education and adoption of simple on-farm techniques will play a key role in the creation of awareness and also in helping to reduce irrigated vegetable crop contamination.

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