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FACTORS AFFECTING THE PARASITIC CONTAMINATION OF EDIBLE LOCALLY PRODUCED DRY SEASON LEAFY VEGETABLES CULTIVATED IN SOUTH EAST ENUGU, NIGERIA

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ABSTRACT

Background/Aim: Outbreaks of human infections associated with consumption of fresh fruits and vegetables have occurred with increased frequency during the past decade. This study evaluated parasitic contamination of locally produced dry season vegetables cultivated in Enugu metropolis, Enugu State, Nigeria.

Materials/Method: Vegetables, water and soil samples selected from vegetable farms were investigated. A total of 160 vegetable samples, 15 soil samples and 15 water samples were investigated. The samples were washed separately in saline and the resulting solution was subjected to standard sedimentation and flotation techniques. Physiochemical analysis of soil and water samples was done using the standard DREM technique. Furthermore, the water samples were investigated for C. parvum using the modified Ziehl-Neelsen staining technique.

Results: Parasitic contamination of the different vegetables were *Teifeairia occidentalis* 242 (18.1%), *Amaranthus hybridus* 193 (15.1%), *Cucurbita maxima* 192 (15.0%), *Solanum* species 191 (14.9%), *Ocimum grattisimum* 136 (10.6%), *Solanun nigrum* 114 (8.9%), *Gongronema latifolium* 101 (7.9%), *Talinum triangulare* 73 (5.7%) and *Murraya koenigii* 39 (3.0%). The parasites identified included; *Ascaris lumbricoides, Taenia* spp, *Fasciola hepatica, Schistosoma spp, Trichuris* spp, *Enterobius vermicularis, Toxocara.spp, Giardia lamblia, Entamoeba* spp and *Cryptosporidium parvum. Entamoeba* species was the highest contaminating parasite 529 (41.3%) while *Schistosoma haematobium* was the least,14 (1.1%). Physiochemical analysis revealed a strong positive correlation between parasite load in water and total suspended solutes (TSS) (r = 0.59, p = 0.021) and turbidity (r = 0.55, p = 0.032) and also a strong positive correlation between parasite load in the soil and organic matter (OM), (r = 0.71, p = 0.003) and silt (r = 0.63, p = 0.011).

Conclusion: These vegetables should be considered as potential sources of parasitic infections, especially when they are not properly cooked (the type called 'Half Done') or consumed raw like the local salad (abacha) because of the high risk of parasitic infections such as giardiasis, amoebiasis, enterobiasis, cyptosporidiosis, ascariasis, etc. Public enlightenment is necessary to enhance the adoption of effective food safety approaches by agronomic practices of vegetable farmers which will in turn reduce foodborne illnesses.

Keywords: Parasite, contamination, vegetables, Enugu.

FACTEURS AFFECTANT LA CONTAMINATION PARASITAIRE DE SAISON SÈCHE PRODUITE LOCALEMENT COMESTIBLES LÉGUMES FEUILLUS CULTIVÉS DANS LE SUD-EST DE ENUGU AU NIGÉRIA

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RÉSUMÉ

Fond/Aim: foyers d'infections humaines associées à la consommation de fruits et légumes se sont produits avec l'augmentation de la fréquence au cours de la dernière décennie. Cette étude a évalué la contamination parasitaire de saison sèche légumes cultivés en métropole, l'état d'Enugu Enugu, Nigéria.Matériaux/Méthode: légumes, des échantillons d'eau et de sol sélectionné dans les fermes maraîchères ont été étudiés. Un total de 160 échantillons de légumes,

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15 échantillons de sol et 15 échantillons d'eau ont été étudiés. Les échantillons ont été lavés séparément dans une solution saline et la solution résultante a été soumis à des techniques de flottation et de sédimentation standard. L'analyse physicochimiques des échantillons de sol et d'eau a été réalisé en utilisant la technique standard de DREM. En outre, les échantillons d'eau ont été étudiées du C. parvum en utilisant la technique de coloration de Ziehl-Neelsen modifiée.

Résultats: La contamination parasitaire des légumes différents ont été Teifeairia occidentalis 242 (18,1 %), l'Amaranthus hybridus 193 (15,1 %), Cucurbita maxima, 192 (15,0 %), 191 espèces de Solanum (14,9 %), Ocimum grattisimum 136 (10,6 %), Solanun nigrum 114 (8,9 %), Gongronema latifolium 101 (7,9 %), Talinum triangulare 73 (5,7 %) et de Murraya koenigii 39 (3,0 %). Les parasites identifiés inclus ; Ascaris lumbricoides, Taenia spp, Fasciola hepatica, Schistosoma spp., Trichuris spp., Enterobius vermicularis, Toxocara.spp, Giardia lamblia, Entamoeba spp et Cryptosporidium parvum. Espèce Entamoeba a été la plus forte contamination parasite 529 (41,3 %) tandis que Schistosoma haematobium était le moins,14 (1,1 %). L'analyse physico-chimiques a révélé une forte corrélation positive entre la charge parasitaire dans l'eau et de entre la charge parasitaire dans le sol et la matière organique (MO), (r = 0,71, p = 0,003) et de limon (r = 0,63, p = 0,011).

Conclusion: Ces légumes doivent être considérées comme des sources potentielles d'infections parasitaires, surtout quand ils ne sont pas bien cuits (le type appelé "Demi-Fait") ou consommés crus comme la salade locale (abacha) en raison du risque élevé d'infections parasitaires telles que l'amibiase, giardiase, entérobiase, cyptosporidiosis, l'ascaridiase, etc. l'information du public est nécessaire pour améliorer l'adoption d'approches efficaces de sécurité sanitaire des aliments par les pratiques agronomiques de maraîchers qui, à son tour, réduire les toxi-infections alimentaires.

Mots-clés: Parasite, contamination, légumes, Enugu.

INTRODUCTION

One of the most important needs of man is to be healthy. Over half of the world's population are affected by parasites, especially in underdeveloped countries like Nigeria. There are so many foodborne illnesses caused by intestinal parasites that remain a public health problem in the developing countries [1]. Intestinal parasites are among the major public health problems around the globe, particularly in tropical and subtropical countries [2]. Several enteric parasites cause severe morbidity and death rate in both man and animals worldwide. In developed settings, enteric parasites (helminths and protozoa) are frequently dismissed as a case of diarrheal illness due to apparently better hygienic conditions, and as such, very little effort is produced toward the laboratory diagnosis of these beings. Although these parasites contribute to the high burden of infectious diseases, estimates of their true prevalence are sometimes struck by the lack of sensitive diagnostic techniques to observe them in clinical and environmental specimens and documentation. Despite recent proper improvements in the epidemiology, molecular biology, and treatment of parasitic infections, health problems involving foodborne infections of parasitic origin still soars.

Intestinal parasites such as *Cryptosporidium spp.*, *Giardia lamblia*, *Entamoeba histolytica*, *Ascaris lumbricoides*, *hookworms*, *Enterobius vermicularis*, *Trichuris trichiura*, *Toxocara spp.*, *Hymenolepis spp.*, *Taenia spp.*, *Fasciola spp.*, etc., can infect humans as a consequence of consumption of contaminated, uncooked (raw), or improperly cooked vegetables [3]. The outbreak of human infections associated with ingestion of fresh fruits and vegetables has occurred with increased frequency during the past ten years. Factors contributing to this increase may include changes in agronomic and processing practices, an increase in per capita consumption of raw or minimally processed fruits and vegetables, increased international trade and distribution, and an increase in the number of immunocompromised consumers. A general lack of efficacy of sanitizers in removing or killing pathogens on raw fruits and vegetables has been attributed, in character, to their inaccessibility to locations within structures and tissues that may harbor pathogens [4]. Vegetables are essential for good health, and they form a major component of human diet in every family. They are vital energy contributors which are depended upon by all levels of humans as food supplements or nutrients and their regular consumption is associated with a reduced risk of cardiovascular diseases, stroke and certain cancers [5].

Epidemiological studies have also indicated that, in areas of the world where parasitic diseases are endemic and where wastewater is used to irrigate vegetables, which are eaten raw, the consumption of wastewater irrigated vegetables without proper washing may lead to parasitic infection [5]. Different parasitic stages can contaminate vegetables. Most contamination occurs in vegetables before harvest, either by contaminated manure in soil, sewage, irrigation water, and wastewater from livestock operations or directly from wild and domestic animals [1]. Other contaminations occur during harvesting, transport, processing, distribution, and marketing or even at home [6]. Therefore, the main aim of the study was to investigate the parasitic contamination of locally produced dry season leafy vegetables cultivated in Enugu metropolis with specific objectives of

determining the parasite load on such vegetables, soil and water samples where the vegetables are cultivated.

MATERIALS AND METHODS Study area

This study was undertaken in the expanding and residential areas of Enugu metropolis and its periurban zones in south east, Nigeria, during the dry season, when vegetables are grown. Enugu metropolis was grouped into five zones, and each zone consists of three prominent dry season leafy vegetable farming locations. The areas were as follows; Zone one (Maryland, Ugwuaji and New Artisan), Zone two (Trans-Ekulu, Abakpa (1) Iji Nike and Abakpa (11) Ugbele Nike), Zone three (Thinkers' Corner, Emene and Independence Layout) Zone four (Uwani, Achara Layout and Garikki) and Zone five (GRA, Artisan, and New Haven). Samples for the control study were obtained from the University of Nigeria, Enugu Campus (UNEC)'s gardens.

Sample collection

This study was carried out in Enugu metropolis and its peri-urban zones (October 2014 to February 2015) after an initial visitation to the different vegetable farming areas. In addition to the sample collection, interactions with the gardeners on their agronomic practices with regards to the water and manure sources they used for the vegetable cultivation was obtained., The leafy vegetables collected for the study included Amaranthus hybridus commonly known as green, Teifeairia occidentalis (fluted pumpkin or ugu in Igbo), Ocimum grattisiumum (scent leaves or nchuanwu in Igbo), Talinum triangulare (waterleaf or mgbolodi in Igbo), Cucurbita maxima (morning glory or Ugbogulu in Igbo), Solanum specie (anara in Igbo) Solanum nigrums (Awa in Igbo), Gongronema latifolium (Utazi in Igbo) and Murraya koenigii (curry). These vegetables were picked at random from the gardens. Three (3) samples of a particular vegetable constituted a sample from a farm. Nine different types of vegetable samples were collected from each of the vegetable farm locations listed above. A total of 160 samples of vegetables, fifteen (15) soil samples on which the vegetables were grown and fifteen (15) samples of the water used for irrigating the vegetables were collected and taken to the laboratory in sterile polythene bags, jars and plastic bottles respectively.

Sample processing

Macroscopic and Microscopic examination of samples were conducted. Macroscopically, the vegetables were carefully examined for the presence of adult forms of parasites according to a standard technique by [7]. Microscopic examinations were done following the standard sedimentation and floatation techniques that involve washing the vegetable and soil samples. Physiochemical analyses of the soil and water samples from the various gardens were also done.

Microscopic Examination of the Samples

A Simple Sedimentation Method which involves the concentration of parasite eggs or ova and cysts as sediments and used to get operculated helminth eggs.

The vegetables, soil and water samples

The vegetable samples were washed using the standard washing technique [8, 9]. The filtrate was used to carry out both sedimentation and floatation techniques on each of the soil samples. The preparation was examined under the light microscope for various stages of intestinal parasites such as helminth eggs, larvae, cysts and protozoan using x10 and x40 objectives [8, 10]. The processed samples from vegetables, soil and water that were not centrifuged, were used to carry out the floatation technique according to standard method [11], especially for the sand samples. Apart from the sedimentation and floatation techniques for investigating the samples, the water samples from some of the farms situated near piggeries were also stained with the modified Ziehl Neelsen staining technique for Cryptosporidium organisms.

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Conflict of Interest: I hereby state that there was no conflict of interest among the authors that participated in this research study

Data analysis: The mean parasite load was calculated according to vegetable types, soil and water samples of the different farms. The data obtained were subjected to descriptive statistics involving frequencies and percentages. Analysis of variance (ANOVA), Chi-square tests of the SPSS software version 17, Pearson's Product Moment Correlation and Fishers LSD (Least Significant Difference) tests. Probability less than 0.05 was considered significant, *P*-values < 0.05 [12].

RESULTS

All the 15 samples of each vegetable were parasitized except one sample of *Murraya coenigii*; hence, only *Murraya coenigii* had prevalence less than 100%. The results also show the mean intensity and paraistic loads of the vegetables, soil and water samples from various zones (Table 1). When comparing the parasitic load in the vegetables, a Chi-Square Test revealed a significant difference between them, p < .001; with *Teifeairia occidentalis* having the highest parasitic load. The parasitic load for the different vegetables in the descending order was as follows: *Teifeairia occidentalis* (242), Amaranthus hybridus (193), Cucurbita maxima (192), Solanum species (191), Ocimum grattisimum (136), Solanum nigrum (114), Gongronema latifolium (101), Talinum triangulare (73) and Murraya coenigii (39) Figure 1.

No. of Vegetables Sampl		No. parasitized sample	Prevalence (%)	No. of Parasites	Mean Parasitic Load	Range (Min- Max)	
Teifeairia occidentalis	15	15	100.00	242	16.13 ±8.43	5-37	
Amaranthus hybridus	15	15	100.00	193	12.87±7.35	4-31	
Ocimum grattisimum	15	15	100.00	136	9.07±4.88	2-20	
Cucurbita maxima	15	15	100.00	192	12.80±7.10	3-25	
Talinium triangulare	15	15	100.00	73	4.87±4.09	1-15	
*Murraya coenigii	15	14	93.33	39	2.6±1.76	0-6	
Gongronema latifolium	15	15	100.0	101	6.73±3.97	1-16	
Solanum species	15	15	100.00	191	12.73±4.88	4-21	
Solanum nigrum	15	15	100.00	114	7.60±5.64	2-23	

TABLE 1: PREVALENCE AND MEAN PARASITIC LOAD IN VEGETABLES

All the 15 samples of each vegetable were parasitized except one sample of **Murraya coenigii*; hence, only *Murraya coenigii* had prevalence less than 100%.

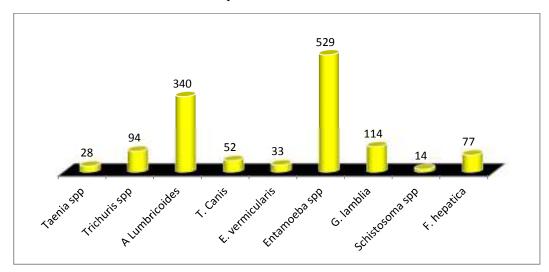


FIGURE 1: PARASITIC LOAD IN VARIOUS VEGETABLES

Four (4) vegetables, *Teifeairia occidentalis, Amaranthus hybridus, Cucurbita maxima* and *Solanum* species were contaminated by all the parasites. In nine vegetables examined, *Entamoeba spp.* was the highest, followed by *Ascaris lumbricoides* while *Talinum triangulare* was the least contaminated with 4 parasites. In 7 vegetables examined, *Schistosoma* spp load was least in 7 vegetables (Ocimum grattisimum, Cucurbita maxima, Talinum triangulare, Murraya coenigii, Solanum species, Solanum nigrum and Gongronema latifolium), followed by Taenia, which was least in 4 vegetables (Teifeairia occidentalis, Talinum triangulare, Murraya coenigii and Solanum nigrum)(Table 2).

Parasites													
Vegetables	Taenia	Trich	Ascaris	Тохо	Entero	Entam	Gia	Sch	Fas	Total			
*Teifeairia occidentalis	4	19	54	11	7	102	21	5	19	242			
*Amaranthus hybridus	4	11	46	6	3	83	19	6	15	193			
Ocimum grattisimum	3	12	30	5	4	56	12	0	14	136			
*Cucurbita maxima	8	16	48	12	7	75	15	2	9	192			
Talinum triangulare	0	0	25	0	0	41	4	0	3	73			
Murraya coenigii	0	3	16	0	1	17	2	0	0	39			
*Solanum species	5	17	54	6	5	76	16	1	11	191			
Solanum nigrum	0	3	37	1	3	47	17	0	6	114			
Gongronema latifolium	4	13	30	11	3	32	8	0	0	101			
Total	28	94	340	52	33	529	114	14	77	1281			

TABLE 2: PARASITE DISTRIBUTION ACCORDING TO VEGETABLES

Four vegetables **Teifeairia occidentalis, *Amaranthus hybridus, *Cucurbita maxima* and **Solanum* species were contaminated by all the parasites

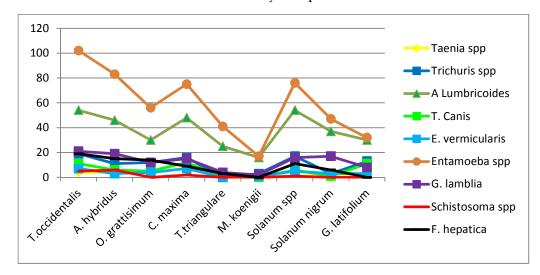


FIG 2: PARASITE-DISTRIBUTION ACCORDING TO VEGETABLES

Ascaris lumbricoides, Entamoeba spp and Giardia lamblia contaminated all the vegetables, while Schistosoma spp was the least contaminated parasites followed by Taenia spp

The diversity level of the vegetables is as follows: Gongronema latifolium (0.782), Cucurbita maxima (0.767), Ocimum grattisimum (0.758), Teifeairia occidentalis (0.752), Solanum species (0.745), Amaranthus hybridus (0.741), Solanum nigrum (0.704), Murraya coenigii (0.649) and Talinum triangulare (0.570). This implies that the probability of randomly getting two parasites that were different from a vegetable was highest in Gongronema latifolium than the other vegetables (figure 2).



FIGURE 3, WASTE PIPE CONNECTED TO CHANNEL WASTE INTO VEGETABLE FARMS

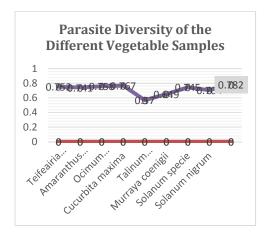


FIGURE 4.PARASITIC DIVERSITY OF DIFFERENT VEGETABLES

Pearson Correlation revealed a strong positive relationship between the parasite load in the vegetables in a given location and parasite load in the water source in that location, which was statistically significant, r = 0.532, n = 15, p = 0.041. A Pearson Correlation revealed a strong positive relationship between the parasite load in the vegetables in a given location and parasite load in the soil in that location, which was statistically significant, r = 0.718, n = 15, p = 0.003. In the physiochemical analysis of both soil and water samples, the result revealed a moderate positive correlation between the level of parasite load and water parameters, Pearson revealed a strong positive relationship between parasite load in the soil and the soil organic matter (OM) with r = 0.712, p = 0.003. A strong positive relationship also existed between parasite load and silt with r = 0.632, p =0.011.

DISCUSSION

Vegetables are essential for good health, and they form a major component of human diet in every family. They are vital energy contributors which are depended upon by all levels of humans as food supplements or nutrients and their regular consumption is associated with a reduced risk of cardiovascular diseases, stroke and certain cancers [5]. Parasitic infections, caused by intestinal helminths and protozoan parasites, are among the most prevalent infections in humans in developing countries. It was observed that the rate of contamination also varied probably according to whether the vegetable leaf surfaces were very fine (smooth) e.g. the leaves of Murraya koenigii, Gongronema latifolium, Talinum triangulare, etc or very coarse (rough) e.g. the leaves of Cucurbita maxima, Teifeairia occidentalis, Amaranthus hybridus, etc. Vegetable samples with coarse or rough leaves had higher parasitic load when compared with the vegetable samples with fine or smooth leaves. Going by this assumption, our study revealed that

Teifeairia occidentalis (fluted pumpkin) had the highest level of parasitic contamination while Murraya koenigii had the least level of contamination. This finding agrees with other research works [13], on the detection and enumeration of parasitic eggs. There was high incidence of intestinal parasites found in communities that consume raw vegetables, especially where those vegetables are cultivated on farms fertilized with untreated human and animal wastes as fertilizers (figure 4). This only confirmed in our study the different types of parasitic contaminants (helminth and protozoan) on the vegetables from the different zones which were examined and the parasites identified based on the morphological characteristics of their eggs and cysts respectively.

Among the helminth eggs and protozoan cysts identified in the analyzed leafy vegetable samples were *Taenia* spp, *Ascaris, Toxocara* spp, *Trichuris* spp, *Schistosoma* spp, *Enterobius vermicularis,* and *Fasciola* hepatica eggs (helminths) and *Entamoeba sp* and *Giardia lamblia* (protozoa).

It was remarkable to note in our study that among the nine different intestinal parasites identified in the study, Entamoeba species recorded the highest prevalence 529 (41.3 %) while Schistosoma spp were the least 14 (1.1%). This high prevalence of Entamoeba species especially E. histolytica agrees with a previous study in South West of Saudi Arabia which demonstrated that eggs of Ancylostoma and Ascaris together with cysts of *Entamoeba* species were the most common parasites found in the 5 leafy vegetable plants investigated [14]. However in contrast to this finding, Robertson and Gjerde [15] did not encounter Ascaris in their investigations. The contrasting reports can be explained by different environmental factors including some physiochemical parameters of the soil on which the vegetables were grown or in the water used in irrigating the vegetables. .

In this study, *Cryptosporidium parvum* was the only parasite whose investigation involved a staining technique and yielded a level of contamination which was as high as 91% in the water samples. This finding is in close agreement with a study done in Alexandria in Egypt [14], on the detection of Cryptosporidium oocysts in irrigation water from El Mahmoudeya canal. Here, a high rate of irrigation water contamination with this parasite was (100%). Another study in USA demonstrated that Cryptosporidium oocysts were capable of strongly adhering to spinach plants after contact with contaminated water and also internalized within the leaves [16]. *Cryptosporidium* is a common cause of acute diarrhea in the immunocompetent but severe and prolonged diarrhea in the immunocompromised hosts, particularly patients with HIV/AIDS [14].

Findings from this study show that sources of contamination on these vegetables were the soil on which the vegetables were grown and the water from where the vegetables were irrigated. The organic fertilizers used by these vegetable farmers often consist of both animal and human waste even though some of the farmers, during a little interaction with them claimed that the name of the organic fertilizer they applied is called 'black soil'.

To identify vegetables as a source of parasitic infection, it has been proposed that it is necessary to provide the following three conditions: firstly, they must be highly contaminated, secondly they must be cultivated in a warm season when the parasite eggs can develop, and thirdly they must be eaten uncooked [17].

According to Abougraina et al [10], the most likely hypothesis of contamination is that it must have occurred before harvest, either by contaminated manure, manure compost, sewage sludge, irrigation water, runoff water from livestock operations or directly from wild and domestic animals. These potential contaminants are all plausible and consistent with the assumption that the level of contamination must have been high. In contrast, Ettehad et al, [18] reported slightly lower level of contamination of consumed native garden vegetables with intestinal parasites (29%) in Ardabil city, Iran [19]. It was also reported that in Abha, most of the vegetables in the market sampled, were irrigated with well water which revealed high contamination levels of Entameba species. However, in this study, our findings is not consistent with any of the above reports because 169 parasites were recorded from the soil samples while 139 parasites were recorded from the water samples. The analysis showed that there was no statistically significant difference in the parasitic load of the soil and water samples to have had any of them tagged as the only or major source of parasitic contamination of the vegetables here in Enugu metropolis.

During field visits in this study, evidence of indiscriminate human defecation and piggery waste pipes channeled to flow directly into these vegetable farms were observed (figure 3). Physiochemical analysis of both soil and water samples revealed a moderate positive correlation between the parasite load and water parameters such as total dissolved solutes (TDS) and turbidity. Also there was a strong positive correlation between parasite load and soil parameters like organic matter (OM) and silt. The results of this study show that the higher the levels of these

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The diversity level of the vegetables was high in our study and this implies that the probability of randomly getting two parasites that were different from a vegetable was highest in *Gongronema latifolium* than the other vegetables (figure 4).

The physiochemical analysis revealed a high positive correlation between the parasitic load in the water samples and the organic matter such that the more the organic matter content of the soil the more the parasitic load will be. The correlation was as high as r = 0.71, p < 0.05. This shows that soil organic matter affects the chemical and physical properties of the soil (soil physiochemical parameters), soil structure and porosity, the water infiltration rate and moisture holding capacity of soils for the diversity and biological activities of soil organisms, and plant nutrient availability. As they break down the organic matter, any excess nutrients (N, P and S) are released into the soil in forms that plants can use. This release process is called mineralization. The waste products produced by micro-organisms are also soil organic matter. This waste materials are less decomposable than the original plant and animal material, but it can be used by a large number of organisms for their biological activities. From this study, our findings show that the soil of the vegetable farms investigated must be rich in organic matter hence sustaining such level of parasitic load. Each species and group of organisms exists where it can find appropriate food supply, space, nutrients and moisture.

However, the results of this study showed good evidence of parasitic contamination of the dry season leafy vegetables cultivated here in Enugu metropolis and the levels of contamination were significant. These vegetables should be considered as potential sources of parasitic infections especially when they are not properly cooked (the type called 'Half Done') or consumed raw in our foods like the local salad (Abacha, etc) because of the high risk of contracting parasitic infections such as giardiasis, amoebiasis, enterobiasis, cryptosporidiosis, ascariasis, etc. Certain physiochemical parameters of soil and water are positively correlated with the level of parasitic contamination on the vegetables. Therefore, increased awareness and overall education of the public is necessary to enhance the adoption of effective food safety approaches which will in turn reduce foodborne illnesses.

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