

Full length Research paper

# Parasitological Analyses and Soil physicochemical properties in African Giant Land Snail (*Archachatina marginata*) reared with dump soil

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Snail drive better in waste dump areas, due to the fact that decayed and composted wastes enhance and increase nutrients to the snails, despite the fact that snails are readily exposed to contaminants. The study investigate some parasite and soil physicochemical properties in African giant land snail (*Archachatina marginata*) reared with dump soil. Dump contaminated soil samples were collected at 30 cm depth with the aid of soil auger for snail farming and soil where no activities served as control. A total of 18 juvenile snails of similar weights was used for the study. The experiment lasted for three month (90 days), during which the snails were subjected to similar dietary regime and equal quantity of feed. The soil samples were analyzed for physicochemical properties such as pH, total nitrogen, total phosphorus, organic matter, total carbon and exchangeable cations (*i.e.*,  $K^+$ ,  $Mg^{2+}$  and  $Na^+$ ) before and after farming using standard methods. Standard methods was used to determine intestinal parasites such as *Ascaris lumbricoides* eggs, *Entamoeba histolytica* cyst and *Giardia intestinalis* cysts on the snail after farming. The result showed a significant difference ( $p < 0.05$ ) in soil physicochemical properties between soil from dump area and snail farm soil, except  $K^+$  which is not significant. *Ascaris lumbricoides* ova and *Entamoeba histolytica* cyst parasites were mostly found in snails reared with dump soil than the control snails with normal soil (no activities). This study showed that snails reared with dump soil highly accumulate parasite and there was a change in the physicochemical properties within the two soils which indicate the sources of parasite.

**Keywords:** *Archachatina marginata*, parasites, physicochemical, dump soil, snail

## INTRODUCTION

*A. marginata* (AM) belongs to the group Phylum Mollusca and Family Achatinidae belonging to the class Gastropoda (Nkop et al., 2016). Aside from insects, mollusca are the largest invertebrates group in the animal kingdom (Yoloye, 1994). *A. marginata* is bilaterally symmetrical invertebrates with soft segmented exoskeleton, inhabiting mostly marine environments, tolerating varied environmental conditions and thrive best in temperate and tropical areas, where soil pH ranges from 4.5-8.0 (Adediran et al., 2003).

Organic manure and dead decay plant, and sewage soil ultimately maximize snail productivity and economic returns (Oguh et al., 2019a), but with a side effects on snails. *A. marginata* is known as Dodonkodi in Hausa, Igbin in Yoruba and Ejule in Igbo. Nutritionally, snails are of paramount importance as source of high profile protein, low in fat and rich in iron food ideal for human nutrition especially for diabetic patients, as well as animal (Cobbinah, 1993, Awah, 2000). Snails also serve as valuable sources of nutrition to human and animals with high levels of protein, iron, calcium, phosphorus and amino acid such as lysine, leucine, and arginine, relatively low amount of sodium, fat and cholesterol compared to poultry and other livestock (Wosu, 2003). Snail meat compares favourably with whole egg in all essential amino acids especially with

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regard to lysine, leucine, isoleucine and phenylalanine (Imebvore, 1990).

African giant land snail (AGLS) are often found in many locations and have a very diverse type of habitat especially dump and dead decay sites and this may lead to the bioaccumulation of metals in AGLS, which is a major food chain route for the human body. Snails are not able to hear at all, but rely on their sense of touch to interact with each other and use their sense of smell to help them find food. Snails are more active at night than day time and may come out during the early morning hours as well. Land snails are particularly well adapted to changes in moisture and dry conditions and are able to remain sealed within their thick shells for two or more years. Most snails have thousands of microscopic tooth-like structures located on a ribbon-like tongue called a radula used to cut food into small pieces. Many snails are herbivorous, eating plants or rasping algae from surfaces with their radula. Snail farming is a lucrative business in most part of the world especially in Nigeria (oguh et al., 2019b). However while some farmers in Asia and Europe have developed a technology for soilless snail farming, in Nigeria and most part of Africa snails are raised mainly in soil medium. Many snails ingest small amount of soil particles and rasp larger rocks or snail shells in order to obtain the Ca essential to reproduction, shell development (snail shells are composed mostly of calcium carbonate  $\text{CaCO}_3$ ), and other physiological needs. In times of Ca demand, such as egg laying, snails mobilize Ca from their own internal organs and shells (Fournie and Chetail, 1984).

A wide range of microbial pathogens have been found in sewage water and can be transferred to snail in such area. Survival of pathogens in the water and surrounding environment is mainly dependent on factors such as nutrient availability, temperature, organic matter content, competition with other

microorganisms, pH and radiation. One major cause of snail's contamination could be the unavailability of hygienic environment. Pathogens can be transmitted to snail and cause outbreaks of illnesses when these are consumed. Risk assessment are series of calculation to estimate the nature and probability of adverse health effects in humans who may be exposed to chemicals in contaminated environmental media especially heavy metals.

Human health risk assessment is the process to estimate the nature and probability of adverse health effects in humans who may be exposed to chemicals in contaminated environmental media, now or in the future (United States Environmental Protection Agency (USEPA), 2017).

Snailsthrive better in soils that are rich in organic matter and snails are important habitat in dumpsites due to the fact that decayed and composted wastes enhance soil fertility and increases nutrients to the snails, despite the important snails are readily exposed to heavy metals, which is bio accumulated in human when consume through food chain. This research aimed to investigate *some parasite and soil physiochemical properties in African giant land snail (Archachatina marginata) reared with dump soil.*

## MATERIALS AND METHODS

### Experimental Site

The study was carried out in Makolo farm, Chanchaga Minna Niger State, Nigeria. Chanchaga is situated at 9°34 North latitude, 6°33 East longitude, with an area of 72km<sup>2</sup> (Figure. 1) and a population of 201,429 at the 2006 census. It has a moderate climate with a very high temperature during the dry season and average rainfall during the rainy season.



Figure 1: Map of Niger State showing study area in purple

## Collection of Samples

Soil samples from dump site, and a control site (where no activities) was collected at 0-30 cm depth with the aid of soil auger and were use in snail farming. The total of 18 juvenile snails of similar weights was collected from Makolo farm Niger state Nigeria were used for the study. The snails were allowed to acclimatize with the environment for seven days before the onset of the experiment.

## Housing of Experimental Animals

Two containers (housing pens for the snails) was labelled A, and B. Treatment A consists of dump soil; and treatment B contain soil sample with no activities (control) and was filled with 10 kg of soil samples of A, and B. All the 18juvenile snails of similar weights were randomly assigned to the 6 plastic containers at the rate of 3 per container. The container measures 22.80 cm in diameter and 12.7 cm. The containers were covered on top with wire netting to allow ventilation and prevent flies while the bottom of each container was drilled in a number of places to allow water drainage and was kept in a cool environment.

The experiment lasted for three month (90 days), during which the snails were subjected to similar dietary reign and equal quantity of feed (Paw-paw leaves, pumpkin leaves, potatoes leaves, and water leaves) and water. Leftover feeds were removed to avoid buildup of microorganisms and the pens cleaned out every morning. The environment in each container was humidified by sprinkling water 3 times weekly into the container for easy mobility and to prevent the snails from injury. While water logging was avoided in all cases.

## Experimental Design

The experiment was carried out under a Completely Randomized Design (CRD) with three treatments and three replicate groups for each. The physicochemical properties of soil before and after rearing and snail parasite was determine and in two groups, from group 1 to 2, which are samples from dump site and a control site (where no activities).

## Preparation of Snail Sample

The snail samples were sacrificed by striking with a wooden material on the shell carefully. The flesh/foot of the snail was carefully removed from the shell and washed with distilled de-ionized water, dried in an oven at the temperature of 105°C to constant weight in three days.

After drying, samples were crushed to fine powder using porcelain mortar and pestle, then sieved using a 0.4 mm mesh. The powdered samples were stored in 100 mL air tight bottles prior to digestion/analysis.

## Determination of the Physico-chemical Properties

The physicochemical properties measured were soil texture, pH, TN, TOC, OM, TP, and EI (Na<sup>+</sup>, Mg<sup>2+</sup> and K<sup>+</sup>). The Physico-chemical properties of the soil were analysed in order to evaluate the biodegradable process. Physicochemical parameters of the contaminated soil and the control soil samples were determined using standard method.

## Soil pH

Triplicate quantities of air-dried (20 g) of the soil samples were weighed into two separate groups of six 50 mL beaker and 20 mL of distilled water was added to one group and 30 ml of 1M KCl<sub>2</sub> was added to the other group. The mixtures were allowed to stand for 30 minutes with occasional stirring using a glass rod. The electrode of the calibrated pH meter, MI 806 pH/EC/Temperature Portable Meter was inserted into mixtures and the pH value was read using pH meter. The results were reported as soil pH in 1M KCl<sub>2</sub> and soil pH in water (H<sub>2</sub>O). Three readings were recorded and then mean of it was calculated.

## Organic Matter

The soil samples were grounded to pass through 0.5mm sieve after which 1g was weighed in triplicate and transferred to 250 mL Erlenmeyer flasks. Exactly 10 mL of 1M potassium dichromate was pipetted into each flask and swirled gently to disperse the soil. Then 20 mL of concentrated, tetraoxosulphate (IV) acid was added.

The flask was swirled gently until soil and reagents were thoroughly mixed. The mixture was then allowed to stand for 30 minutes on a glass plate to allow for the oxidation of potassium dichromate to chromic acid. Distilled water (100 mL) was added then 3- 4 drops of ferroin indicator or 1ml of diphenylamine indicator was added, after which the mixture was titrated with 0.5 M ferrous sulphate solution or ferrous ammonium sulphate till the colour flashes from blue-violet to green or bright green.

A blank titration was similarly carried out.

The percentage of organic matter is given by the following equation:

$$\% \text{ organic matter} = (M1V1K_2Cr_2O_7 - M2V2FeSO_4) \times 0.0031 \times 100 \times F/\text{Mass(g) of air dried soil}$$

F = correction factor (1.33), M1 = mole of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, V1 = volume of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, M2 = mole of FeSO<sub>4</sub>, V2 = volume of FeSO<sub>4</sub>.

## Total Organic Carbon

Exactly 1g soil sample was accurately weighed into a 500 mL conical flask. Then 10 mL of 1Mpotassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) solution was added to the sample

using a bulb pipette, followed by 20 mL of concentrated sulphuric acid ( $H_2SO_4$ ) while gently swirling the flask in a fume cupboard. It was allowed to stand and cool slowly on insulated pad like sheet of asbestos for about 25 minutes after which 200 mL of distilled water was added using a measuring cylinder. After this, 1 g of crystal sodium fluoride (NaF) was added to avoid interference by complexing  $Fe^{3+}$ , obtaining a black colour mixture which was shaken vigorously. Finally, 1 mL of 1 % diphenylamine was added as an indicator and the mixtures were titrated immediately with 1 M ferrous sulphate ( $FeSO_4$ ) solution in the burette. A blank without soil was prepared alongside the sample and titrated same way. End point was indicated a colour change from deep purple to green.

$\% \text{ Total Carbon content} = \frac{(B-T) \times M \times 0.003 \times 100 \times 1.33}{\text{weight of soil sample taken}}$

Where; B = Blank titre, T = Test sample titre, M = Molarity of  $FeSO_4$ , 1.33 = Correction factor, 0.003 = mg equivalent of carbon.

### Total Phosphorus

Air-dried soil 2 g was weighed and dispensed in 20 ml of (0.025N HCl + 0.03N  $NH_4F$ ) solution, shaken for 5 minutes and then filtered using whatman filter paper. After filtration, 3ml of the clear filtrate was put into a test tube, and 3 ml of (0.87N HCl, 0.38N ammonium molybdate, and 0.05%  $H_3BO_3$ ) solution and 5 drops of (2.5g of 1-amino 2- tetraoxosulphate (vi) acid, 5.0g  $Na_2SO_3$ , 146 g  $Na_2S_2O_5$ ) solution were sequentially added to the prepared clear sample. A colorimeter (at wave length of 660 nm) was then used to take readings.

### Total Nitrogen

Total Nitrogen was determined using the kjeldahl digestion method. Than 20 ml of concentrated tetraoxosulphate (VI) acid was added to a 1g measurement of air dried soil. A catalyst known as Kjeldahl TAB was also added and the solution was digested. After digestion, a clear solution was observed; this clear solution was distilled and subsequently titrated with 0.01M HCL.

### Sodium, Magnesium and Potassium ion ( $Na^+$ $Mg^{2+}$ and $K^+$ )

The exchange ions was determined calorimetrically

using Flame photometer. Soil sample (5g) was accurately weighed into No. 1 filter paper fitted into a funnel on a leaching rack with 100 mL volumetric flask for collecting the leachate. The soil sample was leached with 1 N  $NH_4OAC$  solution obtaining 100 mL volume of leachate. The Optical density readings for  $Na^+$   $Mg^{2+}$  and  $K^+$  were obtained from the flame photometer.

$Na^+/mg^{2+}/K^+meq/100g = \text{Optical density} \times \text{correction factor} \times 100/5$

### Parasitological Analyses of Vegetables

In the laboratory, 100 g of each fresh snail sample put into a clean beaker containing physiological saline solution (0.85 % NaCl), enough to wash the sample. Fragments of the snail sample was removed from the washing saline using sterile forceps and was kept for about 24h for sedimentation to take place. After 24h sedimentation, the top layer of the washing saline was carefully discarded leaving 5 ml of the sediment. This was finally centrifuged at 2000 rotations per minute for 5min and the supernatant discarded. The pellets/residue was mounted on slides, stained with Lugol's iodine solution and examined under the compound light microscope to examine the samples for intestinal parasites. *Ascarislumbricoides* eggs, *Entamoebahistolytica* cyst and *Giardia intestinalis* cysts with the following characteristics, oval or spherical in shape and are 45-75  $\mu m$ , oval or spherical in shape 10-20 micro meter in diameter, pear shape with two nuclei, and four pairs of flagella 10-12  $\mu m$  long respectively. (Abougrain et al., 2009).

## RESULT

### Physicochemical Properties of Soil

The physicochemical properties of the soil was presented in (Table 1). The texture of the dump soil is loamy soil because of the decayed organic matter which contain more nutrients and humus than the control soil which is sandy loam. The pH of the soil in  $H_2O$  and  $KCl_2$  were (4.64 and 5.04) and (4.98 and 4.01) in dump soil after and before farming respectively. The nitrogen, organic matter, organic carbon, total phosphorus, and exchangeable cation ( $K^+$ ,  $Mg^{2+}$ , and  $Na^+$ ) of sewage dump and control soil were significantly different.

**Table 1: Physicochemical properties of soil samples before and after farming**

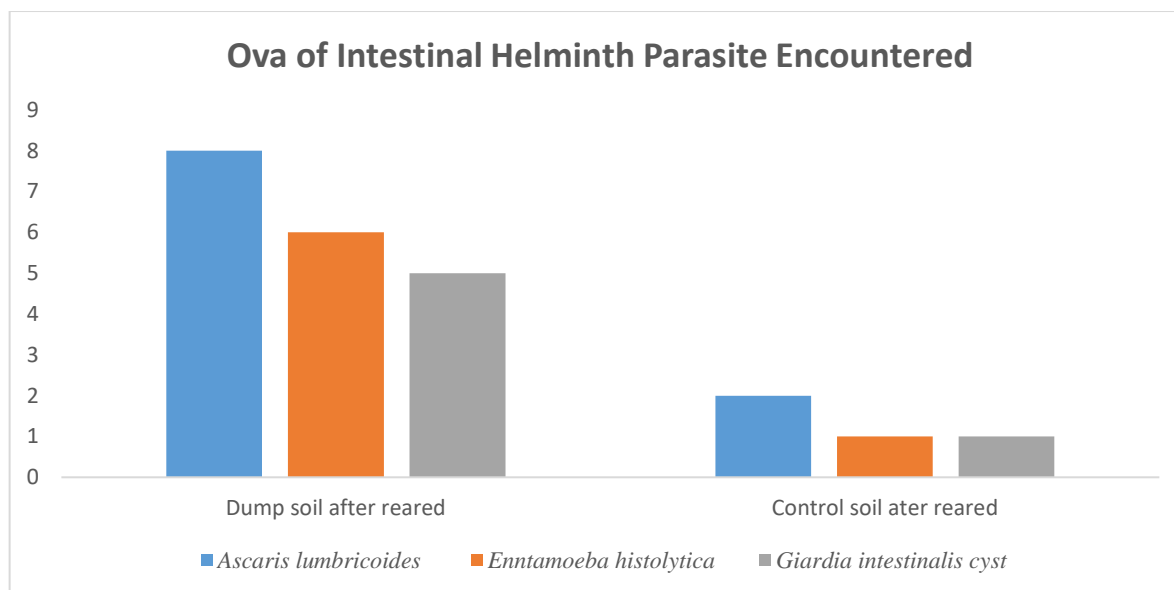
Soil Properties	Physicochemical properties			
	Dump before	Control before	Dump after	Control after
pH H <sub>2</sub> O	4.64 ± 0.10	6.78 ± 0.15	4.98 ± 0.15	6.56 ± 0.10
pH KCl	5.04 ± 0.16	7.01 ± 0.07	4.01 ± 0.07	6.45 ± 0.16
TN %	7.81 ± 0.10	2.06 ± 0.08	5.06 ± 0.08	3.69 ± 0.10
TP %	13.51 ± 0.19	11.47 ± 0.13	10.47 ± 0.13	12.51 ± 0.09
TOC %	5.13 ± 0.11	1.82 ± 0.07	4.82 ± 0.07	2.13 ± 0.11
OM %	31.18 ± 0.03	12.43 ± 0.10	15.43 ± 0.10	12.18 ± 0.03
K <sup>+</sup> meq/100g	3.60 ± 0.07	2.54 ± 0.05	2.68 ± 0.05	2.60 ± 0.07
Mg <sup>2+</sup> meq/100g	3.24 ± 0.12	6.89 ± 0.07	5.89 ± 0.07	5.24 ± 0.12
Na <sup>+</sup> meq/100g	5.73 ± 0.08	5.24 ± 0.04	5.24 ± 0.04	5.73 ± 0.08
Texture	Loamy	Sandy loam	Loamy	Sandy loam

Results expressed as Mean ± SD. n=3

### Parasitological Analyses of Vegetables

The results of this study showed that the parasitic stages recovered from snails presented in Table 2. Out of 9 samples of snail collected from Dump soil, 8 (88.8 %), 6 (66.6%) and 5 (55.5%) were found to be positive for *Ascaris lumbricoides*, *Entamoebahistolyticacyst* and

*Giardia intestinalis* cysts respectively. While the control soil were 2(22.2 %), 1(11.1 %) and 1(11.1%) of *Ascaris lumbricoides*, *Entamoebahistolyticacyst* and *Giardia intestinalis* cysts respectively.



### Ova of Intestinal Helminth Parasite Encountered After rearing

Samples	No of sample	<i>Ascaris lumbricoides</i>	%	<i>Entamoebahistolytica</i>	%	<i>Giardia intestinalis</i> cyst	%
DS	9	8	88.88	6	66.66	5	55.55
CS	9	2	22.22	1	11.11	1	11.11

### DISCUSSION

The low pH value recorded in H<sub>2</sub>O and KCl<sub>2</sub> (4.64, 5.04) at dump soil before farming were as a result of the

waste materials, and high content of organic matter which moves toward acidity. The pH values before farming was below the recommended target limits (6.5 - 8.5) for agriculture ( WHO-World Health Organization,

2006), while the control site is within the recommended limits. The value of pH in the soil after farming were more acidic compared with the soil before farming and this shows that snails increase the acidity in the soil. This has also shown that organic matter are more mobile at pH < 7 than at pH > 7 which has influence in accumulation of microorganism and parasite (Fonge et al., 2017, Oguh et al., 2019c). In connection with this findings, (Oguh et al., 2019a) also reported low pH in water (5.64) in sewage dump soil. However, low pH can pose a significant health risk, leading to various chronic diseases and infection, particularly in increased concentrations or in prolonged snail exposure (Tirima et al., 2016).

Solubility of parasite tends to increase at lower pH value and decrease at higher pH values. The pH values obtained in this study are similar to that reported for dumpsites by other researchers (Elaigwu et al., 2007, Uba et al., 2008). The high total nitrogen in the dump soil is as a result of the waste nitrogenous substances such as decay plant and animals materials. The results reported by (Osazee et al., 2013) had the range 3.476 to 4.522 % which is within range of the present research with 3.81 in sewage soil. Organic matter and total organic carbon in the dump soil were higher than that of the control sample. Decrease in total phosphorus content in the dump soil can be attributed to low phosphorus content in the dump site areas. The increase of Cations exchange capacity is due to the presence of clay content and increase in organic matter. The Analysis of variance (ANOVA) carried out shows significant differences ( $p < 0.05$ ) in the dump site soil and control soil (Table 1). The result of physicochemical carried out on the soil changes the properties of soil, especially the sewage site soils. The alteration of the physicochemical properties of the soil is therefore expected to affect the survival of certain species of animal such as snails which depends on soil for survival and hence their diversity (Table 1).

It was observed that the parasite load on snail reared with dumpsite soil were higher when compared to the control from non-dumpsoil. The result was in accordance with the findings of Samuel et al., 2013 who recorded highest level of contamination of parasite on lettuce grown with dumpsoil. Dump soil snails samples were mostly contaminated with *Ascarislumbricoides*. The data further showed that all the parasite counts recorded in this study exceeded the recommended levels by WHO and International Commission on Microbiological Specifications for Food (ICMSF) standards. Similarly (Drechsel et al., 2006) has reported that fresh poultry manure used for vegetable production in Kumasi recorded high fecal coliform counts ranging from  $3.6 \times 10^4$  to  $1.1 \times 10^7$ . The result correspond to the findings of (Buck et al., 2003) who reported that the presence of many pathogens in the soil was thought to be from historical application or environmental presence of faeces. Intestinal parasites are common in fresh vegetables. Hence, consumption

of raw vegetables plays an important role in the transmission of human parasitic infection (Tiimub et al., 2012; Farahat et al., 2017). Epidemiological studies have shown that the actual risk of infection for people exposed to wastewater is highest for intestinal nematodes such as roundworm (*Ascarislumbricoides*).

## CONCLUSION

This research has shown that snails reared with dump soil accumulate more parasite. Farmers are advice not to use dump soil to reared snails despite its impact on the snails. People who eat these snails, stand a high chance of contracting gastrointestinal diseases like typhoid, cholera and dysentery and disruption of numerous biochemical processes. To prevent an eminent outbreak efforts have to be made to discouraged farmers from the use of dump soil for snail rearing. The community needs to be informed that consumption of this snails can be a mediator for contamination of the pathogen and health problems.

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