Applied Research in Science and Technology
Vol. 01 No. 01, 2021 (26-35)
ISSN (Print) : 2776-7213
ISSN (Online) : 2776-7205

Toxic Elements and Microbial Loads in African Giant Land Snail (*Archachatina margenata*) Reared with Waste Contaminated Soil

Oguh Collins Egwu[1*], Umezinwa Ogochukwu Jennifer[2], Adinnu Chiamaka MariaGoretti[3], Okekeaji Uchechukwu[4], Ejiofor Uchechukwu Marksydney[5]

[1]Department of Surveillance and Epidemiology, Nigeria Centre for Disease Control, Abuja, Nigeria
[2]Department of Science Laboratory Technology, University of Nigeria, Nigeria
[3]Department of Food science and technology, Michael Okpara University of Agriculture, Nigeria
[4]Department of Pharmaceutical Microbiology and Biotechnology, University of Nigeria, Nigeria
[5]Department of Biochemistry, University of Nigeria, Nigeria

*E-mail:collinsoguh@gmail.com

Received: 28 March 2021; Revised: 3 April 2021; Accepted: 25 April 2021

Abstract: The use of dump soils for the rearing of African giant land snail (AGLS) leads to the bioaccumulation of metals and microbial loads in AGLS, which is a major food chain route for the human body. This study investigated the concentrations of heavy metals (As, Cd, Cr, Hg, and Pb) and microbial load in AGLS reared with dumpsite and control soil and also to ascertain if they are within permissible limits. Soil samples; dump soil (A), and Control soil (B) were collected at 0-30 cm depth with the aid of a soil auger and were used for AGLS farming, to ascertain whether the Toxic elements (TEs) concentration was within the permissible limits on AGLS consumption. A total of 18 juvenile snails of similar weights was used for the study. The experiment lasted for three months (90 days), during which the snails were subjected to similar dietary reign and equal quantity of feed. The soil samples were analyzed for TEs before and after farming, and a snail was also analyzed for TEs after farming using an atomic absorptions spectrophotometer (AAS). Standard methods of APHA were used to determine microbial loads such as Total heterotrophic bacteria, E. coli, total coliform, fecal coliform, Staphylococcus aureus, Salmonella, and intestinal parasites. Results indicated that bacterial counts recorded in this study exceeded the recommended levels by WHO and ICMSF, standards (i.e. 10 to 102 coliforms g-1, 10 fecal coliform g-1, and 4.9×106 aerobic count g-1). The result shows a significant difference ($P<0.05$) between the dump and the control soil The concentration of TEs (As, Cd, Cr, Hg, and Pb) in snails reared with dump soil were 2.20, 2.68, 1.08, 2.23, and 2.89 mg/kg respectively. The control recorded 0.28, 1.89, 0.36, 0.16 and 0.24 mg/kg. The values were greater than the maximum permissible limit of 0.5, 2.0, 0.3, 0.1, and 0.1 mg/kg respectively recommended by FAO/WHO compared to the control. The study concludes that snails bioaccumulate toxic elements and microbial loads from the soil used in rearing them which is deleterious to human health when consumed.

Keywords: *Archachatina margenata*, Dump, Microbial load, Soil, Toxic elements.

How to Cite: Egwu, O. C., Jennifer, U. O., Goretti, A. C. M., Uchechukwu, O. & Marksydney, E. U. (2021). Toxic Elements and Microbial Loads in African Giant Land Snail (*Archachatina margenata*) Reared with Waste Contaminated Soil. *Applied Research in Scince and Technology*, 1(1), 26-35.

Applied Research in Science and Technology
Vol. 01 No. 01, 2021 (26-35)
ISSN (Print) : 2776-7213
ISSN (Online) : 2776-7205

INTRODUCTION

Generally, most pollutants are introduced into the environment as solid waste and as compounds used to protect plants and animals. Solid waste has been introduced into the environment and it is suspected that this must have elicit pollution of the environment. Dump soil consists of faeces, garbage, refuse, rubbish, dead animals, broken glasses, plastics, metals, food remnants, paper, wood, cloth etc., which may either be biodegradable or non-biodegradable. Due to the multidimensional nature of waste and its negative effect on humans, animals, plants, microorganisms and environment, its management is difficult and requires high effort. Snails are important habitat in dumpsites. This is because decayed and composted wastes enhance soil fertility and increases nutrients to the snails. Despites the important snails are readily exposed to heavy metals, which is bio accumulated in human when consume through food chain (Oguh et al., 2019). Snails thrive better in soils that are rich in organic matter. The decay of these solid wastes releases substances that can affect the soil, increase the concentration of heavy metals in the soil, altering the natural balance of nutrients available for snail's growth and development thereby affecting species diversity.

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, 1993). *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., 2019b), but with a side effects on snails. *A. margenata* is known as Dodon kodi in Hausa, Igbin in Yoruba and Ejule in Igbo. Nutritionally, snails are of paramount important 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 regard to lysine, leucine, isoleucine and phenylalanine (Imebrove, 1990).

The term heavy metal refers to any metallic chemical element that has a relatively high density greater than 5 g/cm3 and is toxic or poisonous even at low concentrations. Recent researchers have found that even low levels of metals such as mercury, cadmium, lead, aluminum and arsenic can cause a wide variety of health problems (Hassaan et al., 2016). Heavy metals toxicity can result in damaged or reduced mental and central nervous function, lower energy levels and damage to blood composition, lungs, kidneys, liver and other vital organs. Long-term exposure may result in slowly progressing physical, muscular and neurological degenerative processes that mimic Alzheimer's disease, Parkinson's disease, muscular dystrophy and multiple sclerosis. Allergies are not common and repeated long-term contact with some metals may cause cancer. Metals such as cadmium, mercury, arsenic and lead are non-essential and therefore have toxic effects on living organisms such as damage to the renal and

nervous systems of fish as well as gill damage (severe destructive pathological changes, i.e. structural lesions) (Velcheva et al., 2010; Deore & Wagh 2012).

A wide range of microbial pathogens have been found in contaminated soil 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. Snails are widely exposed to microbial contamination through contact with soil, they therefore harbor a diverse range of microorganisms including plant and human pathogens (Oguh et al., 2019c). Use of contaminated soil for rearing of snails is considered to be responsible for transmission of several outbreaks of disease following consumption of such snail. Several cases of typhoid fever outbreak have been associated with eating contaminated snails reared with contaminated soil or sewage soil. A wide range of microbial pathogens have been found in contaminated soil and can be transferred to snails during farming. Survival of pathogens in the soil 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 contamination could be the unavailability of hygienic soil use for farming. Pathogens can be transmitted to snails and cause outbreaks of illnesses when these are consumed (Oguh et al., 2020).

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. This may lead to the bioaccumulation of metals in AGLS, which is a major food chain route for the human body. Snails 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. Many snails ingest small amount of soil particles and rasp larger rocks or snail shells in order to obtain the calcium Ca essential to reproduction, shell development (snail shells are composed mostly of calcium carbonate CaCO3), and other physiological needs. In times of Ca demand, such as egg laying, snails mobilize Ca from their own internal organs and shells (Foumie & Chetail, 1984).

Snails thrive 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, despites 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 the concentrations of heavy metals (As, Cd, Cr, Hg and Pb) and microbial load in AGLS reared with dumpsite and control soil and also to ascertain if they are within permissible limits.

The introduction section must contain (in sequence) a general background, a previous literature study (state-of-the-art) as a basis for the statement of scientific novelty of the article, a statement of scientific novelty of science, and a research problem or hypothesis. At the end of the introduction, the purpose of the article should

Applied Research in Science and Technology
Vol. 01 No. 01, 2021 (26-35)
ISSN (Print) : 2776-7213
ISSN (Online) : 2776-7205

be clearly written. In the scientific article format, it is not permissible to review the literature as in the research report, but it is manifested in the form of a previous study review (state-of-the-art) to demonstrate the scientific novelty of the article.

METHOD

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^2 in 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.

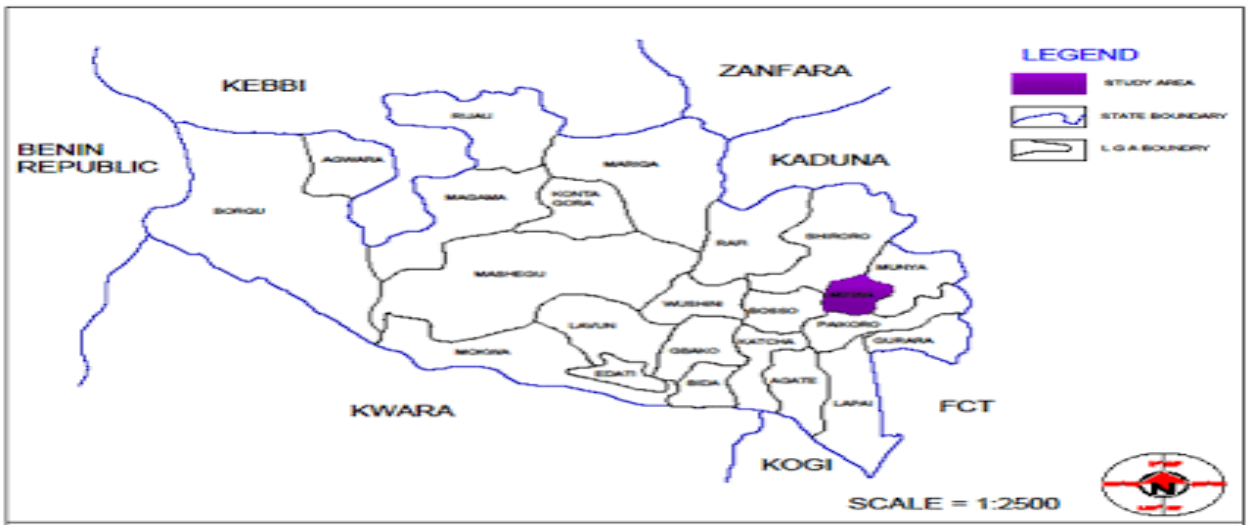

Figure 1. Map of Niger State showing study area in purple

Collection of Samples

Soil samples from dump, and control site (where no activities) was collected at 0-30 cm depth with the aid of soil auger. The total of 18 juvenile snails of similar weights were used for the study. This 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; while treatment B consists of control soil with no activities and was filled with 10 kg of soil samples. All the snails were randomly assigned to the 2 plastic containers at the rate of 9 per container. The container measures 22.80 cm in diameter and 12.7 cm in height as recommended by (Okonkwo et al., 2000). 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 soil was changed on monthly basis with similarly treated soil to avoid build up micro-organisms. 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 two treatments and three replicate groups for each. The concentrations of the TEs both in soils and snail sample, were done in two groups, group 1 and 2, which are samples from dump site, and a control site (where no activities) respectively. Both soil and snail sample were randomly collected and analyzed for TEs As, Cd, Cr, Hg and Pb.

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^{o}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 Toxic Elements

The soil samples (dump and control site) were spread on glass plates and then dried in an oven at 105^{o}C for six hours. The dried soil was ground and sieved through 0 – 5 cm mesh sieve. The pH values of the soils were determined with a digital pH meter. Exactly 1.0 g of the dried powdered sample (soil and snail) was weighed accurately into a 50ml beakers separately, to which 15ml of tri-acid mixture (70% high purity HNO_3, 65%, $HClO_4$ and 70% H_2S0_4 in 5:1:1 ratio) was added. The mixture was digested at 80^{0}C till the solution became transparent. The resulting solution were filtered and diluted to 50ml using deionized water and analyzed for As, Cd, Cr, Hg and Pb, by atomic absorption spectrophotometry.

Microbiological Analysis

All samples were processed following standard methods (APHA, 2012). 10 g of snail samples were weighted and added into 90 ml of sterile normal saline in a blender and homogenized for 1-2 minutes. 1 ml of the homogenate was mixed with 9 ml of sterile distilled water in a test tube. 1 ml of it was transferred aseptically into another test tube containing 9 ml of sterile distilled water and mixed. The dilution was done in series to the fifth dilution (10^{-5}) following a 10-fold serial dilution technique. Inocula of 0.1 ml was taken from the third (10^{-3}) and inoculated onto a sterile Nutrient Agar (NA) medium, Eosin methylene blue agar, *Salmonella shigella* agar, violet red bile agar (oxoid) medium, and Manitol Salt Agar (MSA) to determine total heterotrophic bacteria/Aerobic mesophilic bacterial count, *Escherichia coli* count and total coliform counts, *Salmonella shigella* count, faecal coliforms, and *Staphylococcus aureus*. Respectively. All plates showing 30-300 colonies were used for quantitation of bacterial load as cfu/g (Kabir et al., 2014).

Applied Research in Science and Technology
Vol. 01 No. 01, 2021 (26-35)
ISSN (Print) : 2776-7213
ISSN (Online) : 2776-7205

RESULTS AND DISCUSSION

Toxic Elements Concentration of Soil before Farming

The levels of TEs concentration Arsenic (As), Cadmium (Cd) Cromium (Cr), Mercury (Hg) and Lead (Pb) in soil samples (Dump, and a Control site) before snail farming are presented in Table 1. The concentration of As, Cd, Cr, Hg and Pb in the dumpsite soil were 2.21, 3.56, 4.58, 4.07, 3.25 mg/kg, and control site soil (1.32, 1.46, 0.16, 0.61, 0.79 mg/kg) respectively.

Table 1. Concentration of Toxic Elements in Soil before Farming

Elements (mg/kg)	Dump soil	Control soil	PL(mg/kg) in soil by WHO/FAO (2001)
As	2.21 ± 0.05	1.54 ± 0.02	20
Cd	3.56 ± 0.06	1.82 ± 0.03	3.0
Cr	4.58 ± 0.03	0.87 ± 0.04	100
Hg	4.07 ± 0.03	0.20 ± 0.02	2.0
Pb	3.25 ± 0.08	0.79 ± 0.07	50

Results expressed as Mean ± SD. Mean values with different superscript letters on the rows are considered significant (P<0.05). PL=Permissible limit. n=3

Toxic Elements Concentration of Soil after Farming

The means concentration (mg/kg) of TEs (As, Cd, Cr, Hg, and Pb) on soil after treatment were recorded in table 2. The result indicated that heavy metals were drastically reduce after treatment compared to the soil before treatment. The TEs concentrations (As, Cd, Cr, Hg, and Pb) in dump site soil were 1.40, 1.58, 1.64, 1.46 and 0.83 mg/kg, and control site were 0.56, 0.46, 0.08, 0.05 and 0.08 mg/kg respectively .The two activity sites recorded high concentration of PTEs, which is as a result of the high concentration of TEs present in the soil before treatment. This result indicates that some amount of element were transferred to the snails in Table 2.

Table 2. Concentration of Potentially Toxic Elements in Soil after Farming

Elements (mg/kg)	Dump site soil	Control site soil
As	1.40 ± 0.04	0.56 ± 0.01
Cd	1.58 ± 0.06	0.46 ± 0.02
Cr	1.64 ± 0.08	0.08 ± 0.02
Hg	1.46 ± 0.02	0.05 ± 0.02
Pb	0.83 ± 0.02	0.08 ± 0.02

Results expressed as Mean ± SD. Mean values with different superscript letters on the rows are considered significant (P<0.05).

Toxic Elements Concentration in Snail

Table 3 presents the mean concentrations (mg/kg) of TEs (As, Cd, Cr, Hg and Pb) in the snail samples treated with dump site soil, and a control soil where no activities. The mean concentration of TEs in the snail indicate bioaccumulation from the soil use in treatment. The concentration of TEs (As, Cd, Cr, Hg, and Pb) in snails treated with

Applied Research in Science and Technology
Vol. 01 No. 01, 2021 (26-35)
ISSN (Print) : 2776-7213
ISSN (Online) : 2776-7205

dump site soil were 2.99, 3.45, 3.26, 3.31, 2.87 and the snail treated with control soil were 0.24, 0.20, 0.66, 0.09 and 0.38 mg/kg respectively.

Table 3. Concentration of Toxic Elements in Snail

Elements (mg/kg)	Dump site snail	Control site snail	PL(mg/kg) snail WHO/FAO (2016)
As	2.99 ± 0.03	0.24 ± 0.02	0.5*
Cd	3.45 ± 0.08	0.20 ± 0.03	2.0*
Cr	3.26 ± 0.06	0.66 ± 0.01	0.3*
Hg	3.31 ± 0.02	0.09 ± 0.02	0.1*
Pb	2.87 ± 0.21	0.38 ± 0.01	0.1*

Results expressed as Mean ± SD. Mean values with different superscript letters on the rows are considered significant (P<0.05). PL=Permissible limit. n=3

Microbiological Analysis on Vegetable

Total bacterial load in snail reared with dump site ranged between 8.1×10^5 to 11.3×10^6 cfu/g with faecal coliform more dominate while the control site ranged between 1.0×10^5 to 2.2×10^5 cfu/g with *Salmonella* more dominant. There were more microbial loads on snails reared with dump site than the control site which significantly difference ($p<0.05$) between sites.

Table 4. Bacterial loads in snail reared with dump and control site

Microbial load (cfu/g)	Test Samples	
	Dump soil (A)	Control (B)
Total Heterotrophic B	$8.1\times10^5 \pm 8.8\times10^3$	$2.1\times10^5 \pm 5.8\times10^3$
E.coli	$9.2\times10^5 \pm 3.3\times10^3$	$1.5\times10^5 \pm 5.8\times10^3$
Total Coliform	$11.1\times10^6 \pm 5.6\times10^3$	$1.0\times10^5 \pm 5.8\times10^3$
Faecal Coliform	$11.3\times10^6 \pm 5.8\times10^3$	$8.0\times10^4 \pm 5.8\times10^3$
Staphylococcus aureus	$10.1\times10^6 \pm 5.8\times10^3$	$8.0\times10^4 \pm 5.8\times10^3$
Salmonella	$7.1\times10^5 \pm 5.8\times10^3$	$2.2\times10^5 \pm 5.8\times10^3$

B = bacteria; A = dump soil; B = Control soil; n = 3. Results expressed as Mean ± SE: Column mean values carrying different letter are significantly different (P<0.05).

As a consequence of growing human activities, heavy metals in soils have become an alarming threat to both the ecosystem and human health. The results of the soils showed that the concentration of heavy metals in the soil before treatment increased at the dumpsites soil than the control sites for all the metals (As, Cd, Cr, Hg, and Pb). Results of the soils TEs after treatment decreased at the dump soil, and control sites for all the metals (As, Cd, Cr, Hg, and Pb) when compared to the initial record before treatment or rearing of snails. The concentrations of the TEs recorded were below the WHO/FAO (2001) permissible limit. This decease indicates that snail's bioaccumulate some amount of trace metals from the test and control soil. The level of TEs can be attributed to the environment and kind of waste deposited in the area. Heavy metals are considered the most important constituents of pollution from the terrestrial environment due to toxicity and accumulation by land organisms, such as snails. The entire snail samples reared with dumpsite soil, and a control soil contained detectable levels of the elements studied. The accumulation of these heavy metals in snails may represent a

health risk, especially for populations with high consumption rates of snail (Onuoha *et al.*, 2016). Heavy metals and nutrients absorbed by snails are usually translocate to different parts of the snail which could limit the concentrations in the soil. However, availability of metals in the soil and continuous absorption by the snail could lead to higher concentration in the snail.

This study indicates that dump soil were highly contaminated with TEs than the control soil. The study also indicates that Pb in the dump site soil among the metals had the highest concentration, while As had the lowest concentration. The result of different soil before farming shows a significant different (P<0.05) between the activities sites and the control site. There were significant increase of metals on the activities site than that of the control site. The concentration of Cd and Hg in the activities were above the WHO/FAO (2001) permissible limits of 3.0 mg/kg Cd and 2.0 mg/kg Hg except for control soil which recorded a mean value that was below the permissible limit in all the metal analyzed. The result shows that snails treated with dump and mining soil were more contaminated with TEs compared to the control. The concentration of TEs on the snail treated with dump soil were all greater than the maximum permissible limit recommended by WHO/FAO (2016;2013), of metals in snail while the snail treated with the control soil were below the maximum permissible limit except Cr and Pb which were slightly higher than the WHO/FAO (2016) recommended values of 0.3 and 0.1 mg/kg respectively in Table 3.

Arsenic affects almost all organs during its acute or chronic exposure. Liver has been reported as target organ of arsenic toxicity. Toxicity is due to arsenic's effect on many cell enzymes, which affect metabolism, DNA repair and brain problem. Cadmium is a dangerous element because it can be absorbed via the alimentary track; penetrate through placenta during pregnancy and damage membrane and DNA (Maobe, 2012). Ndukwu *et al.*, (2008) reported that cadmium causes both acute and chronic poisoning, adverse effect on kidney, liver, vascular and the immune system. High dose of chromium is observed to cause Bronchopneumonia, chronic bronchitis, diarrhea, emphysema, headache, irritation of the skin, itching of respiratory tract, liver diseases, lung cancer, nausea, renal failure, reproductive toxicity, and vomiting. Mercury poisoning symptoms include blindness, deafness, brain damage, digestive problems, kidney damage, lack of coordination and mental retardation. The ability of snails to accumulate essential metals equally enables them to acquire other nonessential metals from the soil. Lead has no beneficial biological function and is known to accumulate in the body. Basapor & Ngabaza (2015) reported that lead causes both acute and chronic poisoning and thus, poses adverse effects on kidney, liver, vascular and immune system. Lead can cause serious injury to the brain, nervous system, red blood cells, low IQ, impaired development, shortened attention span, hyperactivity, mental deterioration, decreased reaction time, loss of memory, reduced fertility, renal system damage, nausea, insomnia, anorexia, and weakness of the joints when exposed to high lead.

It was observed that the microbial load on snails reared with dump soil were higher when compared to the control. The sequence of occurrence is Faecal coliform (FC)> Total coliform (TC)> *Staphylococcus aureus*> *Escherichia coli* (E-coli)> Total Heterotrophic bacteria (THB)> *Salmonella* in snails from dump soil. Snails from dump soil were mostly contaminated with faecal coliform TT (11.3 x 10^6cfu/g) due to faeces which is the main content. The data further showed that the bacterial counts recorded in this study exceeded the recommended levels by WHO and International Commission on

Microbiological Specifications for Food (1998) standards (i.e. 10 to 10^2 coliforms g-1, 10 fecal coliform g-1 and 4.9×10^6 aerobic count g-1) wet weight. 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 feaces or untreated sewage.

CONCLUSION

This research has shown that snails reared with dump soil are contaminated with Heavy metals and microbes. The microbial loads and TEs were above recommended limits for snails. People who rear snails with dump soils expose consumers to 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 discourage farmers from using dump or contaminated soil for snail farming.

REFERENCES

Adediran, J.A., De Baets, N., Mnkeni, P.N.S., Kienkens, L., Muyima, N.Y.O & Thys, A. (2003). Organic waste materials for soil fertility improvement in the border region of the eastern cape, South Africa. *Biological Agriculture & Horticulture*. 20, 283-300.

American Public Health Association (APHA). (2012). Standard Methods for the Examination of Water and Wastewater, 22nd ed. American Water Works Association (AWWA) & Water Environment Federation (WEF).

Awah A. A. (2000) Introduction to min livestock development as a sustainable Agricultural Business. Workshop Paper presented at Agricultural Development Project (ADP) of Anambra, Imo, Rivers and Cross River States.

Basapor N & Ngabaza T (2015). Toxicological effect of chlorpyrifis and lead on the acquatic snail Helisoma duryi. *Advances in Biological Chemistry*. 5, 225 - 233.

Buck, J. W., Walcott, R. R. & Beuchat, L. R. (2003). Recent trend in microbiological safety of fruits and vegetables. *Plant Health Progress*. 10, 1094.

Cobbinah J. B. (1993). Snail farming in West Africa: A practical guide: Technical Centre for Agriculture and Rural Development (CTA). 40.

Deore, S.V. & Wagh. S.B. (2012). Heavy metal induced histopathological alterations in liver of Channa gachua (Ham). *Journal of Experimental Science*. 3(3), 35-38.

Egwu, O. C., Victor U. C., Casmir, U. C., Sa'adat, U., Chibueze, N. C., &Musa, A. (2019a). Toxicity Impact on Bioaccumulation of Potentially Toxic Elements in African Giant Land Snail (*Archachatina margenata*) treated with different soils and Its Ecological Risk Assessment. *Asian Journal of Research in Biochemistry.*, 4(4): 1-15.

Fournie, J, & Chetail M. (1984). Calcium dynamics in land gastropods. *American Zoologist*. 24, 857-870.

Hassaan, M. A., El Nemr, A. & Madkour, F. F. (2016). Application of ozonation and UV assisted ozonation on decolorization of direct yellow 50 in sea water. *The Pharmaceutical and Chemical Journal*. 3(2), 131-138.

Imebvore, E. A. (1990). Management techniques in rearing of giant African Land Snail (*Archachatina marginata*). *Journal of Animal Production Research*. 8,76-87.

International Commission for the Microbiological Specifications of Foods .(1998). Microbial Ecology of Food Commodities. Microorganisms in foods. Blackie Academic and Professional.

Kabir, A, Ashish, K. D. & Shahidul, K. (2014). Incidence of antibiotic resistant pathogenic bacteria in vegetable items sold by local and super shops in Dhaka city. *Stamford Journal of Microbiology*. 4(1), 13-18.

Maobe, M.A.G., Gatebe, E., Gitu, L., & Rotich, H. (2012). Profile of heavy metals in selected medicinal plants used for the treatment of diabetes, malaria and pneumonia in Kisii region, Southwest Kenya. *Global Journal of Pharmacology*. 6(3), 245–251.

Ndukwu, B.C., Obute, G.C., & Eze E. (2008). Uptake and accumulation of heavy metals by plants on abandoned refuse dumpsites in parts of Rivers State, Nigeria. *Scientia Africana*. 7, 130-140.

Nkop, EJ, Ogunmolasuyi, A.M., Osezua, K.O., & Wahab, N.O. (2016). Comparative study of heavy metals in the soil around waste dump sites within University of Uyo. *Archives of Applied Science Research*. 8(3),11-15.

Oguh C.E, Okpaka O.C, Ibubeleye T.V, Nwizia B.P & Cookey J. (2019b). Trace metals bioaccumulation index and risk assessment in African giant land snail (*Archachatina margenata*) around University of Nigeria Nsukka sewage dumpsite, Enugu State Nigeria. *Direct Research Journal of Public health and Environmental Technology*. 4(5), 30-41.

Oguh, C.E, Ubani, C.S, Osuji C.A, & Ugwu, V.C. (2019c). Heavy metal risk Assessment on the Consumption of Edible Vegetable *Talinum triangulare* Grown on Sewage Dump Site in University of Nigeria, Nsukka. *Journal of Research in Environmental Science and Toxicology*. **8**(2), 104-112.

Oguh, C.E, Oniwon, W.O, Obiwulu, E.N.O., Mohammed, Y. M., Bakare V. O., & Farouk, J. M. (2020). Effect of *Archachatina marginata* feces and synthesize fertilizer on bioaccumulation and translocation of trace metals in some edible vegetables; a major threat to food safety. *Direct Research Journal of Biology and Biotechnology*, 6(5), 75-82.

Okonkwo, A.C., Isaac, L.J., Nkanga, I & Usoro, O.O. (2000). Effect of Various Feeding Regimes on the Performance of Snails (*Archachatina marginata*). Proceed. Of 25th Ann. Conf. of Nig. Soc. For Ani. Prod. pp: 314-315.

Onuoha S.C., Anelo P.C., & Nkpaa, K.W. (2016). Human Health Risk Assessment of Heavy Metals in Snail (*Archachatina marginata*) from Four Contaminated Regions in Rivers State, Nigeria. *American Chemical Science Journal*. 11(2), 1-8.

Velcheva, E., D. Tomova, D. Arnaudova & A. Arnaudov. (2010). Morphological investigation on gills and liver of fresh water fish from Dam Lake "Studen Kladenets". *Bulgarian Journal of Agriculture Science.* 16(3), 364-368.

WHO/FAO. (2001). Codex alimentarius commission. Food additives and contaminants. Joint FAO/WHO Food Standards Programme, ALINORM 10/12A. Retrieved from www.transpaktrading.com/static/pdf/research/ achemistry/introTofertilizers.pdf

WHO/FAO. (2013): Joint FAO/WHO food standard programme codex committee on contaminants in foods, fifth session pp 64-89Agriculture. (Coag) Meeting from Jan. 25–26. FAO, Rome.

WHO/FAO. (2016). Joint FAO/WHO Food Standard Programme Codex Alimentarius Commission 10th Session. Working document for information and use in discussions related to contaminants and toxins in the gsctff (prepared by japan and the Netherlands) 4 – 8.

Wosu L.O. (2003). Commercial snail farming in West Africa – A guide. Ap express publisher, Nsukka-Nigeria.

Yoloye V.L. (1994). Basics in vertebrate zoology. 3rd Edn., Ibadan, University Press. 139-145.