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Ecotoxicological Assessment of Cadmium and Lead Exposure to Terrestrial Sentinels - Snails (*Archachatina marginata*)

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Abstract: A major threat to environmental and human health is caused by anthropogenic inputs of heavy metals to soils. In the present study, the deleterious effect on African land snails (*Archachatina marginata*) exposed to soils spiked with cadmium and lead was evaluated. The research was aimed with the view of assessing the likely environmental impact the metals could have on soil dwelling sentinels when released on soils. The standard protocol of the International Organization for Standardization (ISO) #15952 was adopted for the 14-day exposure to varying concentrations of the test metals (0.3125, 0.625, 1.25, 2.5 and 5.0 mg l⁻¹), resulting in 0.3125, 0.625, 1.25, 2.5 and 5.0 mg kg⁻¹ after been placed in 1.0 kg of natural soils from the habitat of the organisms. The toxicity end point indicator used for the assessment was survival and exposure duration. The organisms exposed to the spiked soils with concentrations of 0.3125, 0.625, 1.25, 2.5 and 5.0 mg kg⁻¹ recorded for cadmium was 80, 67, 43, 40 and 17% mean % survival while lead was 83, 70, 60, 50 and 40% respectively. It was therefore evident that the effects of lethal toxicity of the test metals was concentration-related; the higher the concentration, the greater the effect. Using the arithmetic method of analysis by Spearman Karber, the estimated mean 14-day LC₅₀ concentration calculated for cadmium and lead were 2.052 and 2.734 mg kg⁻¹ respectively. There was significant difference between the exposed species and the control at levels of P < 0.05. Similarly, the metals were found to accumulate in the body tissues of the organisms with the concentrations in the dead species higher than the surviving species. The effect was also concentration-dose dependent. The study revealed that the juvenile snails were adversely affected. Thus, contact with cadmium, lead and similar heavy metals with such hazardous effects could harm snails and other terrestrial biota and subsequently humans, who are the end consumer of this edible and viable source of protein.

Keywords: cadmium, lead, African land snail (*Archachatina marginata*), terrestrial sentinels, survival, physiological impairment, contamination.

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Introduction

Several potentially harmful chemicals (organic and inorganic), derived from anthropogenic activities of urban centres, rural properties and industries are released indiscriminately into terrestrial environment. Terrestrial ecosystems poisoning by metals has increased during the last decades due to their extensive use in agricultural, chemical, and industrial processes thus resulting in threat to living organisms. As a result, inorganic contamination has increased the level of transfer across food chain / web leading to environmental and health hazards ^[1,2].

Historical and modern industrial activities accounts for most heavy metals in anthropized soils. Anthropogenic inputs of heavy metals to soils are associated primarily with mining, smelting, uncontrolled exploitation for energy, mineral resources of some heavy metals, obsolete technology, inadequate and unprofessional use of agrochemicals, burning of fossil fuels, aerial deposition, sewage sludge, manure and phosphate fertilizer application, large and uncontrolled urbanization, lack of water treatment plants, lack of engineered landfills, etc. ^[3].

Cadmium is a metal that has several modern applications, including some dyes, certain types of batteries and electronics, plastics and paints and in the protective coating applied in steel and iron manufacturing. Cadmium is an environmental toxicant in Japan that resulted to itai-itai (ouch-ouch) disease, a disease from long-term cadmium poisoning. The name comes from Japan, where a large number of people in the 1950s developed the condition because of the widespread presence of cadmium in one province's water. The people who lived near the Jinzu basin farmed rice fields located downstream from the pollution. Cadmium entered the rice they ate, and then proved toxic to their bodies. The condition is quite painful and prolong increase uptake interferes with the function of the kidneys, causes softening of bones by disturbing the metabolism of calcium, attacks the lungs on acute inhalation and the gastrointestinal tract on acute ingestion and also decreases productivity^[4-6].

Soil contamination of lead may be more likely if the site has or had any of the following: lead paint, lead mine / shooting ranch, mineral deposit, heavy or dense traffic of old cars with incomplete combustion of fuel, use of fertilizers or pesticides, industrial / commercial activity, treated lumber, petroleum spills, automobile / machine repair, battery waste dumps, furniture refinishing, landfills, garbage dumps and so on^[7]. Lead poisoning can also be gotten from 'Tiro' eye pencil. Tiro is a powdery Nigerian cosmetic that is applied to infant and adult eyelids as a remedy for promoting visual development, relieving eyestrain or pain, preventing infections in children's umbilical cord stump and circumcision wound^[8]. Lead is highly toxic and could damage body organs including the brain, kidneys and bone marrow. High levels of lead exposure could result in behavioural and learning problems such as hyperactivity or slow growth. Lead could also cause reproductive problems, abnormal response to the opposite sex (chemoreceptor alteration), nervous disorders, respiratory and memory problems. In severe cases, it could lead to seizures, coma and death. Young children are especially susceptible to lead poisoning because they tend to put substances they find on the floor into their mouths, and because of the differences in their body functions, such as greater absorption in their guts and underdeveloped central nervous systems^[8-10].

Repeated release of heavy metals into environmental media could lead to loss of biota. Since heavy metals are recalcitrant that is not easily degradable, they persist in the media they are present in. Heavy metals have the potential to bioaccumulate at all trophic levels, accumulating in the tissues, livers and kidneys of different organisms in food chains and consequently influence human health^[11,12].

Snails are pertinent ecological indicators for assessing the quality of soils, as they are characteristic of the soil surface layer (saprophagous and phytophagous) of which a large part of their biological cycle takes place in the soil (egg-laying, hatching, initial stages of development, hibernation, etc). During the other phases of their cycle, they eat soil and are in contact with the soil via their moist pedal sole (foot) covered with mucus and participate in the permanent exchanges with the soil (water, mineral salts, excrement, shell and organic matter when they die)^[13]. Thus, some criteria for choosing the species for this study include the fact that they are easy to sample and identify; abundant all the year round; their ecological and physiological characteristics are well-known; and they are now easy to breed under controlled conditions. In addition, they are daily consumed by man and other higher organisms since they are used as a rich source of protein^[14-17].

Osemeobo,^[18] listed fifteen (15) health conditions that are believed to be curable with the fluid, shells and tissue of African land snails. Some of these ailments are listed in the Table 1. Similarly, the shiny secretion from the species as they move on surfaces has been used in the cosmetic domain and beauty health care for facial and acne related problems. The secretion has a masking effect and is also known to have healing and medicinal effects.

In this study, soil dwelling biota snails (*Archachatina marginata*) were exposed to varying concentrations of cadmium and lead in the laboratory and survival and exposure duration were used as the ecotoxicological endpoint indicator to assess the likely environmental consequence of exposure to these viable species consumed by human.

Material and Methods

Experimental animals

African land snails (*Archachatina marginata*) (length 1.2 ± 0.2 cm, weight 0.87 ± 0.07 g) were collected from farms at Ugbo-mro in Ughelli area of the Niger Delta ecological zone of Nigeria at latitude $5^{\circ} 34'$ N and longitude $5^{\circ} 50'$ E. The snails were cleaned by removal of algal mass, debris and well-cleaned animals were transferred to large plastic holding troughs. All snails were acclimated under laboratory conditions for a period of seven days prior to the experiments.

Toxicant and animal toxicity

Cadmium nitrate [$\text{Cd}(\text{NO}_3)_2$] and lead nitrate [$\text{Pb}(\text{NO}_3)_2$] of Analar grade was used for the toxicity assessment. Pre-determined amounts of the test compounds were weighed, dissolved in a small quantity of deionised water and the solution made up to a fixed volume by adding appropriate volume of deionised water as diluents to achieve a stock solution of known strength (1000 mg l^{-1} Cd and Pb). The

resultant stocks were then serially diluted to obtain solutions of the required concentrations.

Experimental bioassay procedure

The 14-day experimental procedure was carried out using the International Organization for Standardization (ISO) protocol # 15952^[17]. The experiment began with a range-finding test to determine the concentrations to be used in the definitive test. Toxicity endpoint indicator considered was survival and exposure duration. From the prepared stock solutions of the test chemicals, serial dilutions were made to obtain concentrations in the range of 100, 10 and 1.0 mg l⁻¹ Cd and Pb for the range-finding. The definitive concentrations (0.3125, 0.625, 1.25, 2.5 and 5 mg l⁻¹ Cd and Pb) were chosen after the range-finding experiment. When spiked with 1.0 kg of natural soil, the concentrations obtained were (0.3125, 0.625, 1.25, 2.5 and 5 mg kg⁻¹ Cd and Pb).

The snails were acclimated in unspiked soil for seven days before commencement of the bioassay. For each of the test concentrations, 1000 g (1 kg) of natural soil (from organisms' habitat) was placed into the bioassay container to form a thick layer of substrate. Five (5.0 g) of *Carica papaya* (pawpaw) leaves was then placed on each bioassay container. The *Carica papaya* (pawpaw) leaves was added to the soil as food for the organisms (*alternatively fresh prepared cellulose could also be used*). Feeding with the leaves was to ensure that the snails were not starved during the experimental period.

Each test medium was spiked with 100 mL (test solution) containing the pre-determined concentration of the test chemicals (Cd and Pb) homogenized in a 2000 mL amber glass vessel to obtain a moisture content of 35%. The snails were kept on moist filter paper for few hours to void contents of the stomach and intestinal tract before being placed in the test jars. Thereafter, ten voided snails were cleaned, weighed and transferred to the soils spiked with different concentrations of test chemicals. Three replicates per treatment were prepared for five exposure concentrations for the test chemicals.

The control setup was prepared in conjunction with the test chemicals as described above except that the leaves and clean substrate was sprinkled with 100 mL of water before being homogenized. The setup was covered with wire mesh to prevent the test medium from drying^[17,19]. The soils used for the study was air dried, crushed and sieved through a 2 mm sieve, and tested for physico-chemical properties. The entire test was conducted at a temperature of 28 ± 1°C in natural soils with pH 5.83 ± 0.03. The soil was loamy sand soil (44.3% sand, 37.2% silt, 18.5% clay) containing 0.31 ± 0.03% organic carbon and 15.6% moisture content.

Cadmium and lead concentration in the unspiked natural soil was <0.001 mg kg⁻¹.

Toxicological response to snails (survival)

Snail survival was evaluated on day 7 and 14 of the experiment in all the triplicates in the natural soil. Direct contact was avoided so as not to induce stress on snails. Percentage survival (endpoint of lethal toxicity) was estimated while physical changes (morphology) were also noted. Some organisms were observed to hibernate secreting a mucus calcareous substance. Snails were considered dead if there is no movement when the foot region of the animal was prodded with a metal rod (platinum wire) or if there is no activity after sprinkling the snail with water and then placing on moist white filter paper for five minutes.

Analysis of metals

In addition to assessing the effects of the test chemicals on the snails, the internal concentration of the chemicals in the tissues of the species were also measured. The snails were killed by freezing at -4°C and after thawing, the soft tissues (foot or pedal) were removed from the shells before and after exposure to the test concentrations for analysis. These were dried at 310°C for 6 hours and the dried tissues were ground and known weights were digested in a mixture of nitric acid (5 mL), sulphuric acid (3 mL) and perchloric acid (3 mL). The level of the test metals in the digest (soft tissues of test species) was determined using atomic absorption spectrophotometer (AAS, Shimadzu 6701 F model).

Statistical analysis

Survival and LC₅₀ at day 14 of lead and cadmium exposure was calculated using the arithmetic method of analysis by Spearman-Kärber in Finney,^[20]. All experiments were conducted in triplicate and used for the calculation of mean LC₅₀. The analysis of variance (ANOVA) test was performed using Statistical Package for Social Science (SPSS) statistical software in Version 21.0 to determine the significant difference between effects resulting from exposure of the test chemicals and controls at significance levels of P < 0.05. Line and scattered graphs were used for the pictorial representation of the measurement endpoint.

Results

The results of the acute toxicity of snails exposed to varying concentrations of the test metals (cadmium and lead) in spiked soils are presented in Tables 2 to 4 with pictorial representation in Figures 1 and 2.

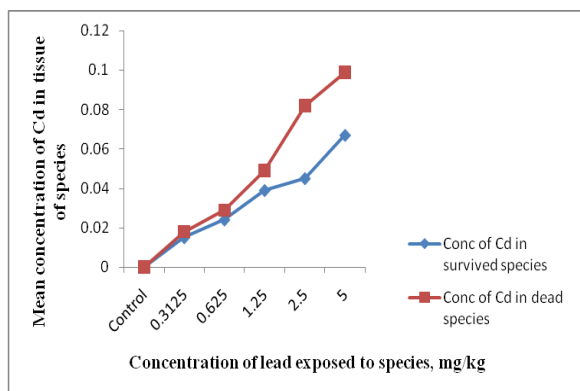


Figure 1: Internal concentrations of cadmium in the survived and dead snails (*A. marginata*) at day 14

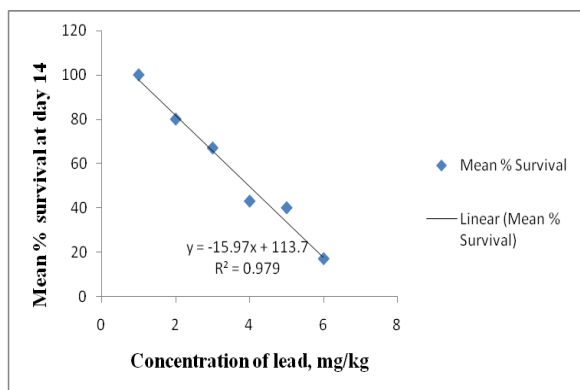


Figure 2: Mean % survival for snails (*Archachatina marginata*) exposed to lead at day 14

After the 7th day, an assessment of the exposed snails revealed that the organisms were moderately affected by the chemicals. At the same period, the movement of some of the surviving snails was slower than before the commencement of the experiment, while some were weak; others were completely immobilized though not dead. The colour of the snail shells was adversely affected after cadmium intoxication and changed from brownish black to whitish in the test tanks. Similar observations were also noted on the last day of the bioassay, though some organisms were alive but not active, the movement was slower than that observed at the first half of the experiment. Some species that survived used calciferous materials to cover themselves within the shell. Snails were considered dead if there was no movement when the foot region of the animal was prodded with a metal rod (platinum wire) or if there was no activity after sprinkling the snail with a few drops of water and then placing them on moist white filter paper for about five minutes.

The test organisms exposed to varying concentration of cadmium at day 7, survived in all test concentrations

except the last two concentrations of 2.5 and 5.0 mg kg⁻¹ recording 70 % and 30% respectively. However, on day 14 of the test, concentrations of 0.3125, 0.625, 1.25, 2.5 and 5.0 mg kg⁻¹ recorded 80, 67, 43, 40 and 17% mean percentage survival respectively, indicating that survival decreased not only with increased concentration but with exposure duration (Figure 1).

The internal concentration of cadmium in snail tissues was linearly correlated with cadmium concentrations in the spiked soils. The internal concentrations of the surviving snails exposed to cadmium were 0.012, 0.020, 0.039, 0.045 and 0.067 mg kg⁻¹ in 0.3125, 0.625, 1.25, 2.5 and 5.0 mg kg⁻¹ respectively. In the dead organisms, the internal concentrations of cadmium were 0.018, 0.033, 0.049, 0.067 and 0.082 mg kg⁻¹ in the above order. The concentrations of cadmium absorb by the dead organisms was higher than that accumulated in the species that survived for all test concentration, indicating that it was concentration-dose dependent, that is survival was dose related. The organisms used for the metal analysis were selected randomly from the three replicates at the end of the experiment to represent each test vessel for the different concentrations.

The results showed that no death or morphological alterations were observed in the controls on day 7 and 14 of the test. The control groups were healthy and did not show any sign of physical or behavioural alteration. Movement was normal and the colour of the shell was unaltered in all ten survivors. The LC₅₀ determination based on arithmetic method of Karber is shown below:

$$LC_{50} = LC_{100} - \frac{\Sigma \text{Conc. Diff} \times \text{Mean Death}}{\text{No. of org. per group}}$$

$$LC_{50} = \frac{5 - 27.97}{10}$$

$$LC_{50} = 2.203 \text{ mg kg}^{-1}$$

Using the three replicates, the mean estimated 14 day LC₅₀ value using the Spearman Karber arithmetic method of analysis for cadmium was 2.052 mg kg⁻¹ (Table 2). Based on the OECD, [21] rating for contaminants in soil matrix, cadmium could be classified as extremely toxic (Table 3). However, in order to arrive at environmentally tolerable concentrations, safety factors are randomly obtained around the LC₅₀ values. The concentration of a chemical in the environment should not exceed 10% of the LC₅₀, thus, cadmium could be said to have a safe limit of 0.205 mg kg⁻¹ [21].

Table 1: Use of fluid, shells and tissue of African land snails to treat different ailments

Parts of the body used	Ailments
Fluid:	<ul style="list-style-type: none"> * Cure of headache * Prepared in concoction for curing new babies sickness * Cure of malaria * Used for blood clotting during circumcision and to stop bleeding in a cut or wound. * Treatment of dysentery * Suppression of high blood pressure. * To cure eye problems * Cure of smallpox
Shell:	<ul style="list-style-type: none"> * Treatment of dysentery * Treatment of stomach ache * Anti-rheumatic * Storage of magical charms and against body pains
Meat (tissue):	<ul style="list-style-type: none"> * Used to prepare talisman for protection * Treatment of infertility in woman * Cure of convulsion in new babies * Treatment of bone fracture. * Cure of anemic patient

Table 2: Mean LC₅₀ determination of Cd exposure based on Arithmetic method of Karber

Conc., mg kg ⁻¹	Conc. Difference	No alive Tank 1	No dead	Mean dead	Mean death dose difference
Control	-	10	0	-	0
0.3125	0.3125	8	2	1	0.3125
0.625	0.3125	7	3	2.5	0.7813
1.25	0.625	5	5	4	2.5
2.5	1.25	4	6	5.5	6.88
5.0	2.5	2	8	7	17.5
					∑ =27.97

Table 3: Soil toxicity rating

Rating	Designation	LC ₅₀ (mg kg ⁻¹)
1	Super toxic	<1.0
2	Extremely toxic	1.0-10
3	Very toxic	10-100
4	Slightly toxic	100-1000
5	Practically non-toxic	>1000

Abbreviations: LC₅₀ median lethal concentration. Data from OECD (2003)

Table 4: Mean LC₅₀ determination of Pb exposure based on Arithmetic method of Karber

Conc., mg kg ⁻¹	Conc. difference	No alive Tank 1	No dead	Mean dead	Mean death dose difference
Control	-	10	0	-	0
0.3125	0.3125	8	2	1	0.3125
0.625	0.3125	7	3	2.5	0.7813
1.25	0.625	6	4	3.5	2.188
2.5	1.25	5	5	4.5	5.63
5.0	2.5	4	6	5.5	13.8
					∑ =22.71

Toxicological Alterations after Intoxication of Lead

The results of the acute toxicity of snails exposed to varying concentrations of lead in spiked soils are presented in Table 4 and Figure 2. The test organisms exposed to lead at the first half experimental duration revealed that there were survivors in all the test tanks except the highest concentration of 5 mg kg⁻¹ (70%). However, at test termination on day 14, the snails exposure to 0.3125, 0.625, 1.25, 2.5 and 5.0 mg kg⁻¹ Pb concentrations recorded 83%, 70%, 60%, 50% and 40% mean % survival respectively implying that survival decreased not only with increased concentrations but with exposure duration (Figure 2).

As in the cadmium bioassay, the internal concentration of lead in snail tissues was linearly correlated with the lead concentrations spiked in the soils. The internal concentrations of the surviving snails exposed to lead were 0.010, 0.014, 0.015, 0.044 and 0.065 mg kg⁻¹ for 0.3125, 0.625, 1.25, 2.5 and 5.0 mg kg⁻¹ while in the dead organisms, 0.011, 0.031, 0.061, 0.073 and 0.088 mg kg⁻¹ respectively was recorded.

Similar observations as in the cadmium assay were also noted at the test termination on the fourteenth day. In summary, the behavioural response observed in the surviving organisms include: slow or sluggish movement and adverse morphological / physiological alterations (hibernation and rigidity). The effect of the test chemical resulted in reduction in sizes, affected appearance and the behaviour of the organisms.

The LC₅₀ determination based on arithmetic method of Karber is shown below:

$$LC_{50} = LC_{100} \frac{\Sigma \text{Conc. Diff} \times \text{Mean Death}}{\text{No. of org. per group}}$$

$$LC_{50} = \frac{5 - 22.71}{10}$$

$$LC_{50} = 2.729 \text{ mg kg}^{-1}$$

Using the three replicates, the mean estimated 14 day LC₅₀ value obtained from the Spearman Karber arithmetic method of analysis for lead was 2.734 mg kg⁻¹ (Table 4). Lead could be classified as extremely toxic (Table 3) with a safe limit of 0.273 mg kg⁻¹.

Discussion

Land snails are soil invertebrates that are well known for their ability to concentrate substances through ingestion and other metabolic processes. The species are pertinent ecological indicators for assessing metal pollution in soil and since their biological cycle takes place in the soil (egg-laying, hatching, feeding, initial stages of development, hibernation, etc), the toxicant

exposure could cause detrimental effects on them and others members of the food chain / web [22-24].

The main point of entry of cadmium in the organs of animals is the digestive tract and alveolar absorption. Cadmium stimulates the formation of metallothioneins and reactive oxygen species (ROS), thus causing oxidative damage to erythrocytes and various tissues resulting in loss of membrane functions and subsequently death. It is generally agreed upon that oxidative stress plays important roles in acute Cd poisoning [25,26].

The concentration of heavy metal in aquatic or terrestrial media affects numerous phenomena involved in the development and maintenance of mollusca physiology as feeding, growth, reproduction, and maturity of the animal [27,28]. Some findings show that snails in polluted areas have high levels of cadmium in the digestive glands, kidney, gills, and muscle as well as in the foot and shell [29,30]. According to Arimoro, [31], cadmium levels found in snails tissues in Southern Nigeria was between 0.08 – 0.22 mg kg⁻¹. The results from this study were corroborated with Arimoro's study and also in agreement with similar studies carried out by Otitolaju *et al.* [19], Russell *et al.* [27], Tanhan *et al.* [30] and Włostowski *et al.* [32]. Since, cadmium is known to stimulate the formation of metallothioneins and reactive oxygen species (ROS), damage would be done to various vital organs in the specie, which would affect a number of membrane functions and eventually cell death of species, thus causing disruption of the food chain / web and loss of biodiversity [25,26].

In 2010, lead poisoning caused by illegal gold mining killed 163 Nigerians, most of which were children, in remote villages in Zamfara State. The existence of gold deposits in Zamfara State along the border of Niger had been long known. The villages affected, Daretta and Giadanbuzu in Arika Local Government Area are in the poor, arid Sahel region on the southern fringe of the Sahara, where the people work as miners and subsistence farmers. The poisoning was caused by the illegal extraction of the ore by villagers, who took the crushed rock home to extract. The ore brought back to the villages contained extremely high levels of lead. Miners return from work dusted with lead, which then pollutes their homes. The men carried the precious rocks home to store inside their mud-walled compounds, sometimes leaving them on sleeping mats, while the women often broke the rocks and ground them, sending dust and flakes into the air, water and soil, Rain, wash water and runoffs carries these particles into soils and receiving water bodies which then poison people through hand-to-mouth contamination while others were contaminated by contact with work / mining tools [33].

The level of metals retained in the body tissues could be attributed to the effect observed for the alteration in morphology, behaviour and reduced survival in the species exposed to soils spiked with the test metals. The effect could also result in accumulation of the toxicant in the organisms leaving the higher organisms that would feed on them at a high risk from a myriad of environmental and health related issues^[23,34].

Sometimes, the amounts of soil elements and other substances may exceed levels recommended for the health of humans, animals, or plants. Soils in some of the affected areas in Zamfara State, Nigeria have lead concentrations above 1000 ppm. A level that is too high for the survival of most soil dwelling organisms except very tolerant species. The Department of Petroleum Resources (DPR) recommended target and intervention limits for lead in soils is 35 mg kg⁻¹ and 210 mg kg⁻¹ respectively^[35]. This study was also to check the amount of lead and cadmium absorbed in the snail tissues so as to corroborate the findings from the Zamfara region. Access to the area is quiet restricted and volatile and as such assessment of live land snails from the region could not be carried out, hence the study considered only the laboratory experiment, however, findings from this study could be correlated with actual effects from the wild or field.

Research has shown that metal accumulation is more rapid than metal elimination, probably due to the presence of metal binding proteins in tissues. Since the body struggles to rid itself of the metals, which are very slow to leave the body, they accumulates in the tissues and other vital organs over time since the rate of accumulation is higher than elimination. Hence, over time, metals bioaccumulate in lipid tissues of the species they find themselves to such toxic levels that contributed to their deleterious effect in humans and organisms^[4,5,6,36,37].

For some decades, health issues have been predominant and health advisers are advocating the use of snails to combat some health related problems and as such most disease suffers are consuming snails than ever before. However, some of these species are consumed contaminated without the knowledge of the consumers be it humans or other lower organisms in the environment and this could heighten more health issues down trophic levels as a result of bioaccumulation of the metals in the species systems.

Conclusion

Knowledge of the safe limits helps to reduce likely environmental consequence on these viable organisms that are rich in protein and used for a lot of ailments. Protecting these species would help in safe guarding the environment, its components and man since harm done to one is harm done to all.

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