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Bioaccumulation of heavy metals, lipid profiles, and antioxidant status of snails (*Achatina achatina*) around cement factory vicinities

Regina Ngozi Ugbaja[1,2], MA Enilolobo[1], AS James[1], TF Akinhanmi[3], AJ Akamo[1], DO Babayemi[1] and O Ademuyiwa[1]

Abstract

Some snails (*Achatina spp*) can be used as a biosensor of heavy metal poisoning. This study thus estimated some heavy metal levels, antioxidant markers, and lipid profiles of snails handpicked around cement factory vicinities in Ogun State, Nigeria. Snails and soil samples were collected from Oke, Ewekoro, Papalanto, and Mowodani Imeko-Afon (control site). Lead (Pb), cadmium (Cd), and arsenic (As) levels were estimated in the soil, snail foot, hemolymph, and shell using Atomic Absorption Spectrophotometry. Triacylglycerol (TAG), phospholipids (PHOL), cholesterol (CHOL), malondialdehyde (MDA), and reduced glutathione (GSH) levels, as well as glutathione-*S*-transferase (GST), lactate dehydrogenase (LDH), and arylesterase (AR) activities in the hemolymph, were estimated spectrophotometrically. The snails collected from the Oke site had the highest foot Pb (274.66 $\pm$ 13.50 mg/g tissue), CHOL, TAG, PHOL levels, and GST activity when compared with other sites. Snails collected from Papa had the highest Cd levels (1.79 $\pm$ 0.74 mg/kg), As (1206 $\pm$ 18.87 mg/g tissue) in the foot, and LDH activity, while Ewekoro snails had highest MDA levels and AR activities but the lowest GSH levels. Additionally, there were negative correlations between the heavy metal levels and the activities of GST and AR as well as GSH levels, while positively correlating with LDH activity and MDA level. Workers and the general public around cement factories are at a greater risk of heavy metal-induced pathologies. More so, consumption of snails around these sites may be deleterious to health.

Keywords

Heavy metals, biosensor, *Achatina achatina*, hemolymph, antioxidant, lipid profiles

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Introduction

"Heavy metals" describe metals and metalloids including some trace elements that are essential for enzymatic catalysis and have functional roles in various metabolic processes although some are environmental pollutants (Shotuyo et al., 2016). Pollution of the ecosystem may occur through human activities via effluent from automobiles, fossil fuel burning, sewage sludge, weathering, fertilizer application, and manufacturing industries (Mahmoud and Abu Taleb, 2013). The potential health hazards posed by heavy metals remain a global concern especially in the developing

[1]Department of Biochemistry, Federal University of Agriculture, Abeokuta, Nigeria
[2]Department of Chemical Sciences (Biochemistry Program), Augustine University, Ilara-Epe, Nigeria
[3]Department of Chemistry, Federal University of Agriculture, Abeokuta, Nigeria

Corresponding author:
Regina Ngozi Ugbaja, Department of Biochemistry, Federal University of Agriculture, P.M.B. 2240, Abeokuta, Ogun State, Nigeria; Department of Chemical Sciences (Biochemistry Program), Augustine University, Ilara-Epe, Nigeria.
Email: ugbajarn@funaab.edu.ng

countries, where treatment and elimination of effluents are inadequate or nonexistent (Banaee and Taheri, 2019). Cement kiln dust is a particulate pollutant that is generated from cement production factories during blasting of raw materials, grinding of cement clinker, and packaging or loading of finished cement (Kolo et al., 2018). The cement kiln contains heavy metals, such as lead (Pb), cadmium (Cd), and arsenic (As) (Gupta and Singh, 2011; Schemback, 1998), thus making it a source of environmental pollutant through which the workers and public in and around the cement factories are exposed to heavy metals (Gupta and Singh, 2011). When these metals are released with the kiln, they spread over wide areas aided by the wind and finally settle on soil through which human exposure could ensue (Kolo et al., 2018). Indeed, exposure to Pb may disrupt cellular redox state, inhibit heme biosynthesis (Mani et al., 2018), and cause mitochondrial apoptosis (Jin et al., 2017). Cd may disrupt the metabolism of lipids by altering the levels of triacylglycerol (TAG), cholesterol (CHOL), and lipoproteins via the inhibition of lipogenic enzymes (Yang et al., 2013). Keratosis, mitochondrial damage, disruption of glycolysis, dyslipidemia, and carcinogenicity are hallmarks of As toxicity (Afolabi et al., 2015; Gupta and Singh, 2011). Furthermore, intoxication of heavy metals may inhibit secondary antioxidant enzymes (such as arylesterase (AR)), increase lipid peroxidation, lower endogenous antioxidant molecules, such as metallothionein, and reduced glutathione (GSH) levels (Gupta and Singh, 2011; Izah et al., 2017), and induce oxidative stress (Bannae and Taheri, 2019). With these incipient dangers posed by heavy metals in mind, it is expedient to identify an organism that can be used as sentinel in studying the physiological and biochemical derangements arising from the accumulation of heavy metals in living organism (Banaee and Taheri, 2019).

Snails (*Achatina spp*) are good biomonitors of metal toxicity (Regoli et al., 2006) as they can accumulate heavy metals from their immediate milieu over a long period (Notten et al., 2005; Viard et al., 2004). Wide distribution, adaptability to environmental stress, ability to procreate easily, and relatively long lifespan (15–25 years) (Banaee and Taheri, 2019; Hodasi, 1979) make *Achatina achatina* a suitable snail species to study heavy metal bioaccumulation around cement factories. Other snail species have a shorter lifespan and thus may not be suitable for biomonitoring of long-term exposure to heavy metals (Hodasi, 1979).

Accumulation of heavy metals in snails' edible and nonedible parts may promote their long residence time in the food chains, thus making their consumption a potential risk for humans (Gupta and Singh, 2011). We, therefore, posed that snails picked around cement factories may be used to assess the risk of long-term deposition of heavy metals and that heavy metal accumulation can be associated with altered antioxidant and lipid metabolism in snails. Despite the sufficient information on the toxic effects of heavy metals on human and nonhuman species, there is an insufficient investigation on the correlation between heavy metal accumulation, antioxidant, and dyslipidemia of snails around cement factory vicinities. This study, therefore, evaluated the bioaccumulation of heavy metals in hard and soft tissues of snails collected around cement factories as well as their antioxidant and lipid profiles.

Materials and methods

Study location

The four experimental sites where snails and soil samples were pooled are as follows:

- Oke village latitude (7° 01′ 25 N and longitude 3° 02′ 31E) near Dangote cement factory, Ibese, Ogun State, Nigeria.
- Ewekoro (latitude 6° 54′ 24 N and longitude 3° 11′ 12 E) near Lafarge cement factory, Ogun State, Nigeria.
- Papalanto (latitude 6° 53′ 05 N and longitude 3° 11′ 41 E) near Lafarge cement factory, Ogun State, Nigeria.
- Mowodani, Imeko-Afon (control site), (latitude 7° 37′ 25.23 N and longitude 2° 44′ 54.75 E) 60 and 57 km away from Lafarge cement factory and Dangote cement factory, respectively (Figure 1).

Chemicals, reagents, and equipment

All the chemicals used in this study were pure and of analytical grade products purchased from British Drug Houses Chemicals Limited (Poole, Dorset, England). CHOL, TAG, phospholipids (PHOL), and lactate dehydrogenase (LDH) laboratory kits were from Cypress diagnostics Limited, England.

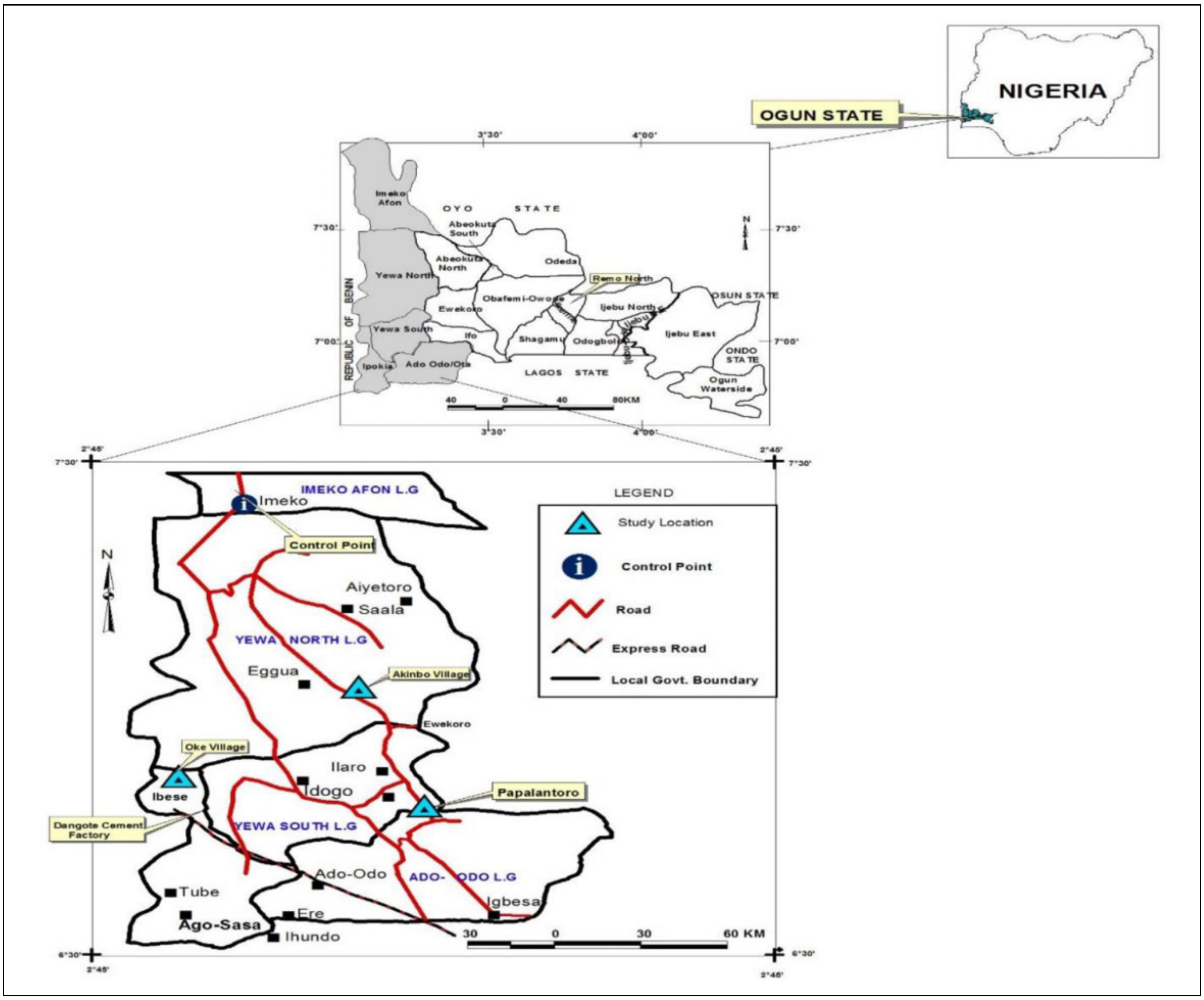

Figure 1. Map showing the cement factory vicinities and control site (Ogun State, Nigeria). Control site (Imeko-Afon), test sites [(Papalanto site (Lafarge cement factory) Oke site (Dangote Cement factory), and Ewekoro site (near Lafarge cement factory)].

Snails, soil sampling, and collection

We took 10-cm deep topsoil and handpicked 30 big snails from Oke village, 20 from Ewekoro, 6 from Papalanto, and 20 from Mowodani Imeko-Afon (control site), respectively. The control site was chosen based on the distance from the cement factories. A total of 76 snails (with an average weight of 151.96 $\pm$ 7.70 g) and soil samples were collected out of which 5 samples were randomly analyzed from each group ($n = 5$).

Metal determination

Heavy metal levels in the soil, snails, and shell samples were determined, as described by Mahmoud and Abu Taleb (2013), and in the snail hemolymph, as described by Onunkwor et al. (2004). The digested samples were analyzed for Pb, Cd, and As levels.

Pb, Cd, and As were analyzed in hemolymph, shell, foot of snails, and soil samples using Atomic Absorption Spectrometer (Buck Scientific AAS model 200, Connecticut, USA).

Digestion and estimation of Pb, Cd, and As in the soil and snail tissues. Briefly, fine soil samples/tissues (1.0 g) were digested using 20 mL of concentrated nitric acid at 100°C for 3 h. This was done after drying the soil samples at 80°C in a digestion microwave oven. The samples were then filtered using cotton wool, the solution was carefully transferred to a 20-mL

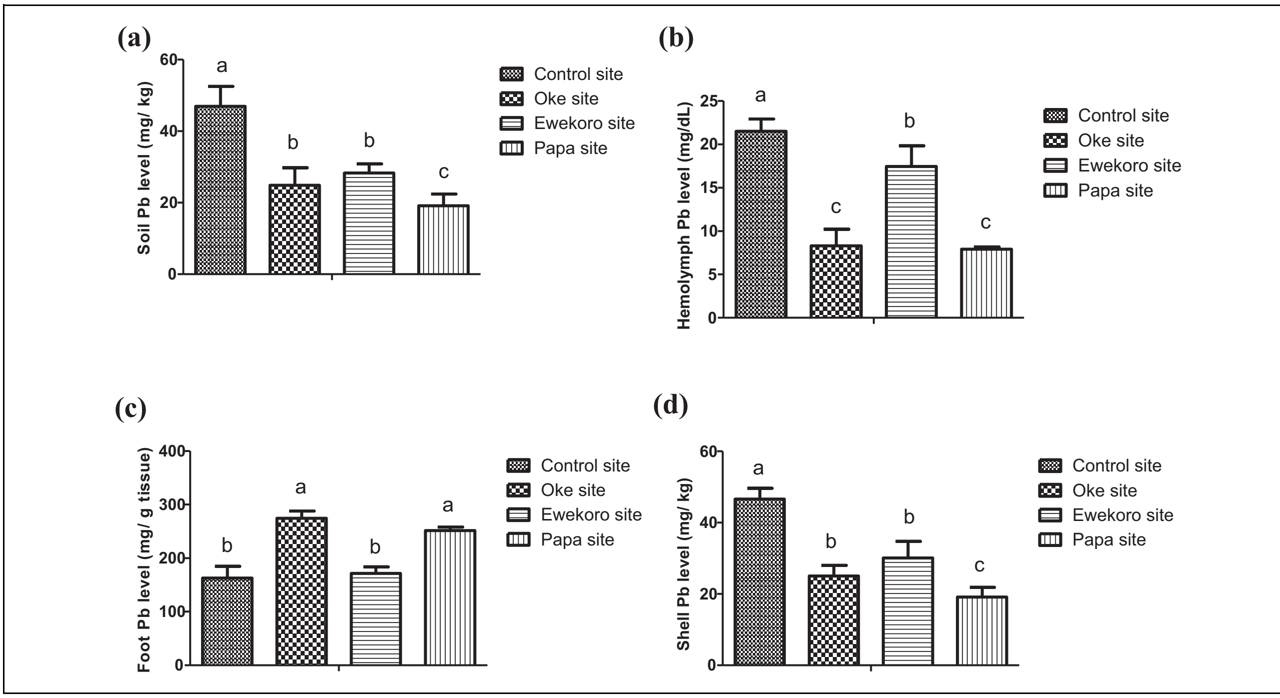

Figure 2. Lead (Pb) levels in the soil, hemolymph, foot, and shell of snails from three cement factory vicinities (Oke, Ewekoro, and Papa) in Ogun State, Nigeria. Values are expressed as mean $\pm$ SEM ($n = 5$). Bars with different letters (a–c) are statistically distinct ($p < 0.05$). SEM: standard error of the mean.

volumetric flask and was made up to mark with 5% (v/v) nitric acid and was kept in sample bottles. The digested samples were analyzed for As, Pb, Cd concentration. Metal concentrations were recorded in parts per million.

Calculations:

$$\text{Part per million(PPM)mineral} = \frac{\text{Reading from AAS} \times \text{volume of digest}}{\text{Weight of sample}}$$

Hemolymph (0.2 mL) in 100-mL conical flask was added to 10 mL of concentrated nitric acid. The solution was heated on a hot plate in a fume cupboard until the solution became clear. The solution was then carefully transferred into a 20-mL volumetric flask and made up to mark with 5% (v/v) nitric acid. The solutions were stored in nitric-acid washed plastic tubes until analyzed for the metals. External standardization for the metals was done by analyzing their certified Spex Standard (Spex Industries, Inc., Edison, New Jersey, USA) along with the samples.

Determination of biochemical parameters in the hemolymph and foot of snails. Total CHOL, TAG, PHOL, total protein concentrations, and LDH activity were assayed according to the standard protocols, as instructed by the kit manufacturers. GSH levels according to the method of Beutler (1963), glutathione-*S*-transferase (GST) activity by Habig et al. (1974), lipid peroxidation (malondialdehyde (MDA)) by Buege and Aust (1978), and AR activity with phenyl acetate as the substrate, as described by Junge and Klees (1984), were determined.

Statistical analysis

Data are expressed as mean $\pm$ standard error of the mean (SEM). The levels of homogeneity among the groups were assessed using one-way analysis of variance. Where heterogeneity occurred, the groups were separated using Tukey's *post hoc* test. Association between and among the parameters was evaluated using Pearson's correlation with p values <0.05 considered statistically significant. All analyses were done using Statistical Package for Social Science (SPSS) version 16.

Results

Figure 2 shows Pb levels as estimated in the soil, foot, shell, and hemolymph of snails collected around cement factories. Interestingly, in the control site soil (Figure 2(a)), Pb level was significantly ($p < 0.05$) higher (47%,

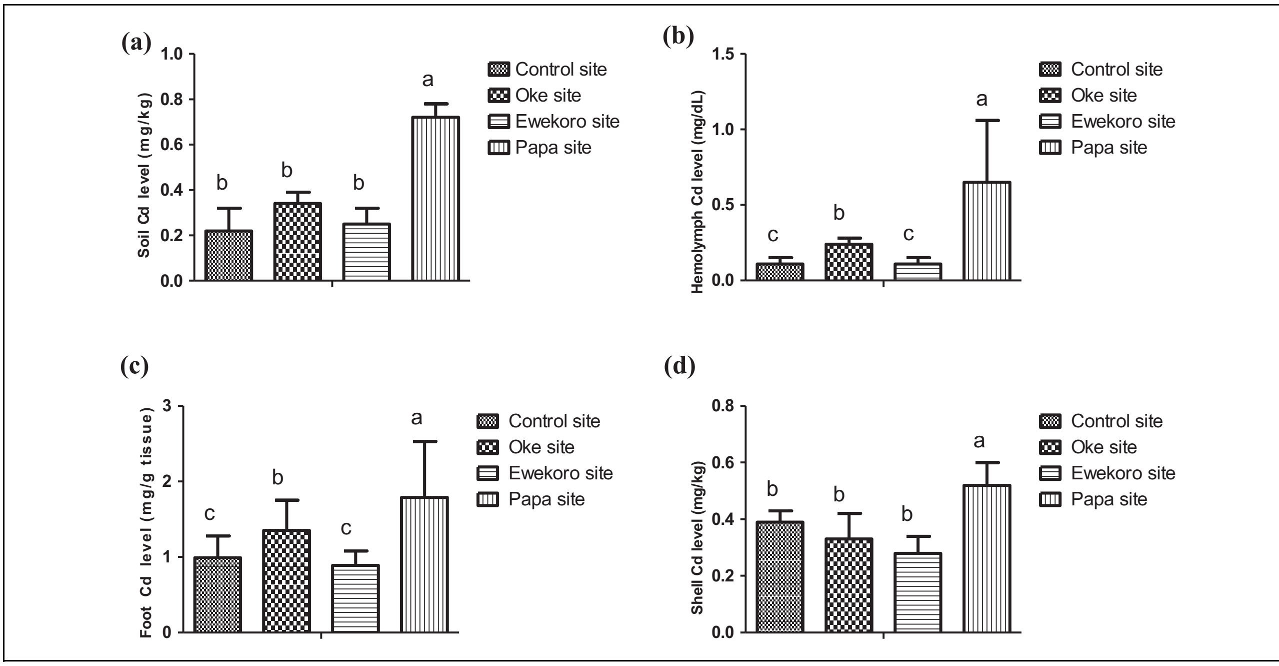

Figure 3. Cadmium (Cd) levels in the soil, hemolymph, foot, and shell of snails from three cement factory vicinities (Oke, Ewekoro, and Papa) in Ogun State, Nigeria. Values are expressed as mean ± SEM ($n = 5$). Bars with different letters (a–c) are statistically distinct ($p < 0.05$). SEM: standard error of the mean.

40%, and 59%) than in the Oke, Ewekoro, and Papa sites, respectively. Similarly, higher Pb levels in the hemolymph (Figure 2(b)) (61.4%, 18%, and 53%) and the shell (Figure 2(d)) (46%, 35%, and 59%) were recorded. However, the Pb level in the foot (Figure 2(c)) was higher at the Oke site (68%), Ewekoro site (5%), and Papa site (35%) when compared with the control site.

Cd levels in the soil (Figure 3(a)), hemolymph (Figure 3(b)), foot (Figure 3(c)), and shell (Figure 3(d)) were significantly ($p < 0.05$) higher in the Papa site by 54.5%, 1.2 fold, 36.4%, and 33.33%, respectively, relative to the control site (Figure 3). There was no significant difference ($p > 0.05$) in the Cd levels (soil and shell) among the other two test sites (Oke and Ewekoro) although a slight increase was observed in the foot Cd at the Oke site when compared with the control site.

As level was significantly ($p < 0.05$) higher at the control and Papa site (in the hemolymph and shell) (Figure 4(b) and (d)). In fact, As level in the soil (Figure 4(a)) of the control site was higher than other sites (Oke, Ewekoro, and Papa sites) while the As level in the foot (Figure 4(c)) was significantly higher at the Papa site when compared with the control site (Figure 4).

Hemolymph MDA level increased significantly ($p < 0.05$) in the snails from Ewekoro (27.59%), Papa (3.45%), and Oke sites (31.03%) when compared with the control site. AR activity (75%) in the hemolymph of the snail at Ewekoro site snails was the highest when compared with the control site. LDH activity of snails found at Papa (1.25 fold) was significantly greater than at every other site. GSH concentration was highest in the hemolymph of the snails collected from Papa (23.08%) but 5.77% lower at the Oke site and 32.69% at the Ewekoro site when compared with the control site. GST activity was significantly increased in the snails collected from the Oke site (1.6 fold), and it was decreased by 69.23% and 61.54% at the Ewekoro and Papa sites, respectively, when compared with the control site (Figure 5).

To investigate the effect of accumulation of heavy metals on snails lipid metabolism, we assayed for CHOL, TAG, and PHOL concentrations in the digestive gland and hemolymph of the snail, as shown in Figures 6 and 7. Hemolymph and digestive gland CHOL were significantly ($p < 0.05$) higher in snails collected from Oke (1.4 fold and 28%), Ewekoro (65% and 12%), and Papa (51% and 32.7%), respectively, when compared with the control site.

The levels of hemolymph and digestive gland TAG were lower in the snails collected from the Ewekoro site (53% and 33%) and Papa site (42% and 11%). Although snails collected from the Oke site had 1.4-

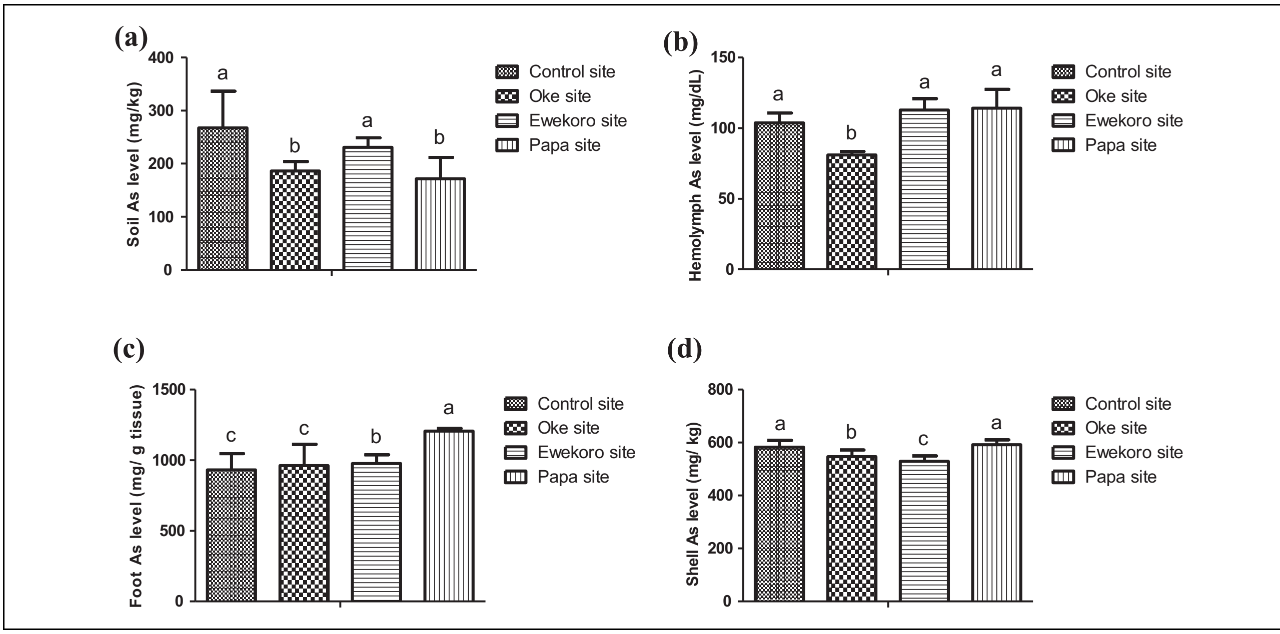

Figure 4. Arsenic (As) levels in the soil, hemolymph, foot, and shell of snails from three cement factory vicinities (Oke, Ewekoro, and Papa) in Ogun State, Nigeria. Values are expressed as mean $\pm$ SEM ($n = 5$). Bars with different letters (a–c) are statistically distinct ($p < 0.05$). SEM: standard error of the mean.

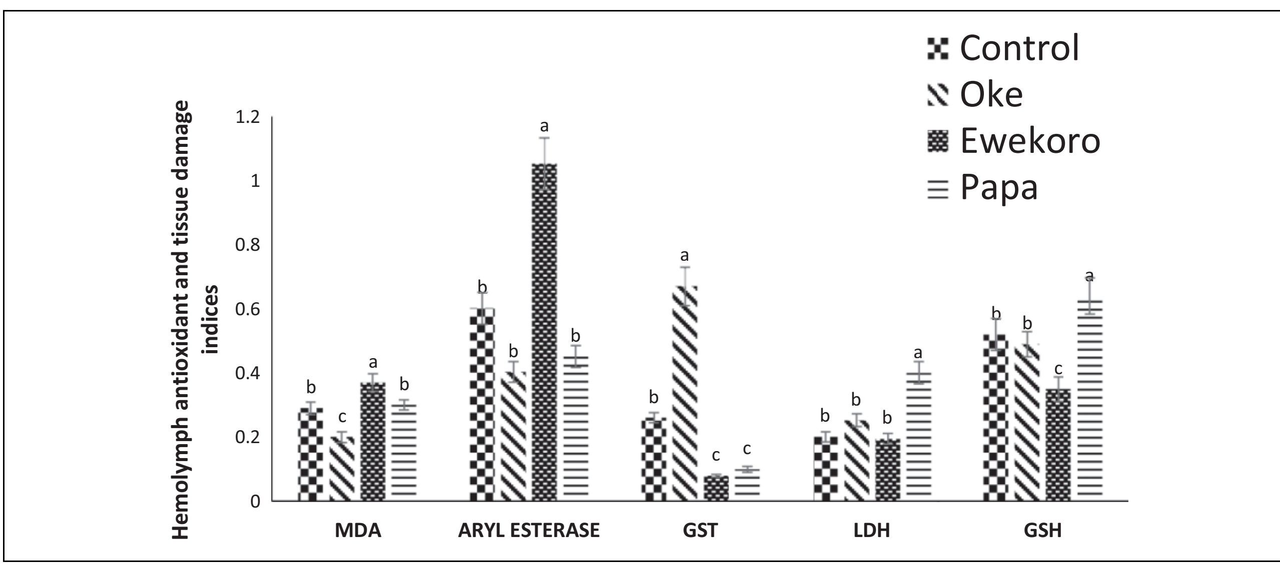

Figure 5. Hemolymph antioxidant and tissue damage indices of snails from three cement factory vicinities (Oke, Ewekoro, and Papa) in Ogun State, Nigeria. Values are expressed as mean $\pm$ SEM ($n = 5$). Bars with different letters (a–c) are statistically distinct ($p < 0.05$) for each parameter. Malondialdehyde (MDA) and reduced glutathione (GSH) levels are expressed as nmol/L; arylesterase, glutathione-*S*-transferase (GST), and lactate dehydrogenase (LDH) activities are expressed as U/mg protein $\times$ 10^2. SEM: standard error of the mean.

fold higher hemolymph TAG, the level in the digestive gland was 49.4% lower when compared with the control.

Hemolymph and digestive gland PHOL levels were significantly ($p < 0.05$) lower in the snails collected from the Ewekoro (17% and 10%) and the Papa sites (13% and 30%). Interestingly, snails from the Oke site had 7% more PHOL in the hemolymph in contrast to a 51% decrease observed in the digestive gland when compared with the control.

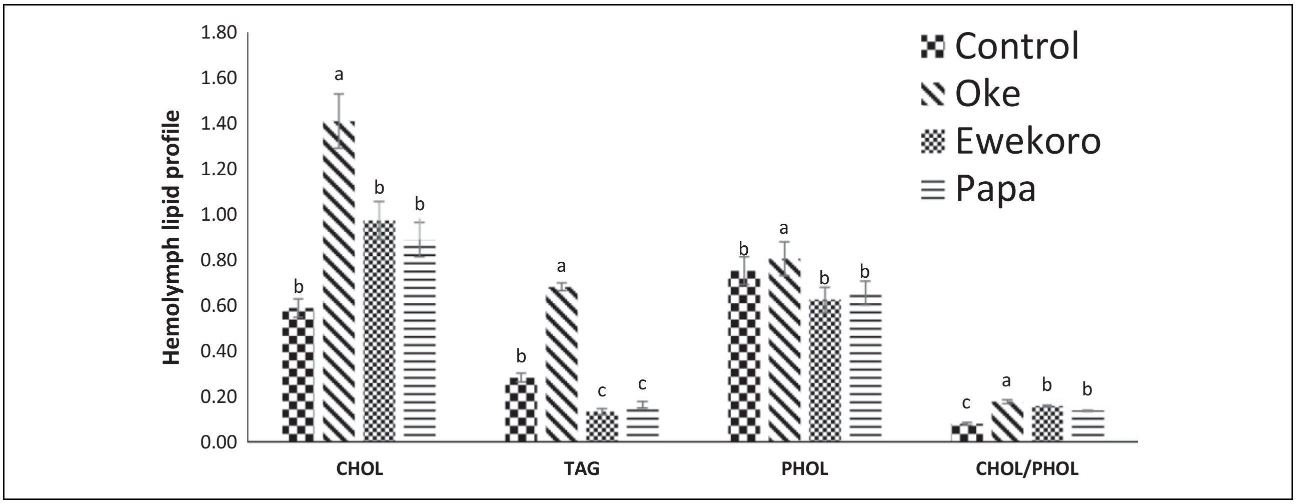

Figure 6. Hemolymph lipid profile of snails from three cement factory vicinities (Oke, Ewekoro, and Papa) in Ogun State, Nigeria. Values are expressed as mean $\pm$ SEM ($n = 5$). Bars with different letters (a–c) are statistically distinct ($p < 0.05$). Cholesterol (CHOL), triacylglycerol (TAG) and phospholipids (PHOL) levels are expressed as mg/dL $\times$ 10^2. SEM: standard error of the mean.

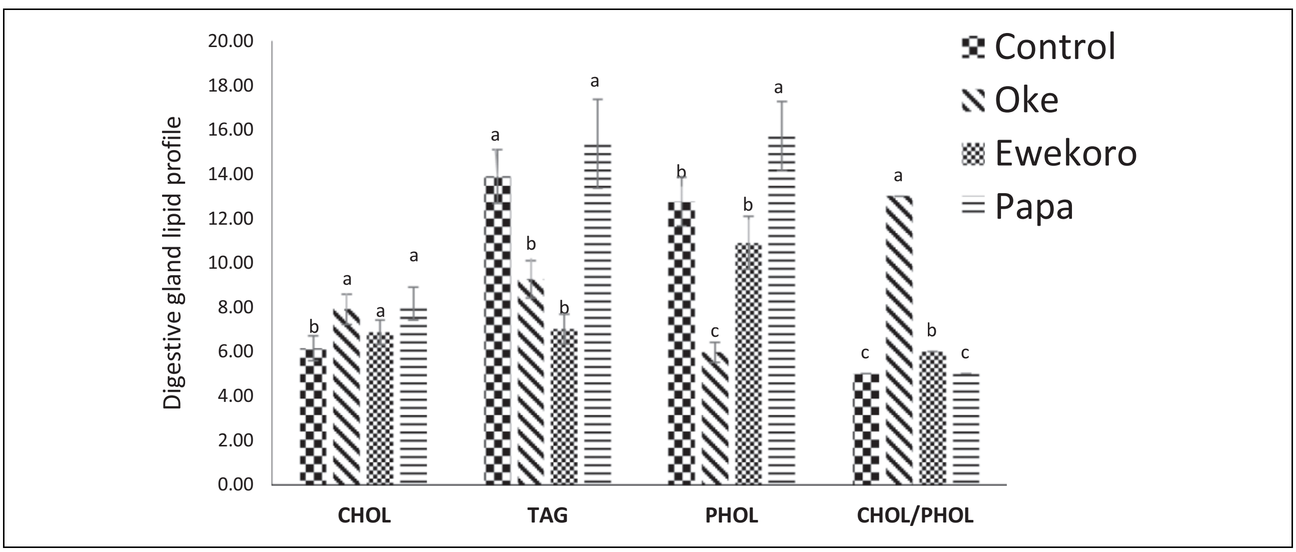

Figure 7. Digestive gland lipid profile of snails from three cement factory vicinities (Oke, Ewekoro, and Papa) in Ogun State, Nigeria. Values are expressed as mean $\pm$ SEM ($n = 5$). Bars with different letters (a–c) are statistically distinct ($p < 0.05$). Cholesterol (CHOL), triacylglycerol (TAG) and phospholipids (PHOL) levels are expressed as mg/g tissue $\times$ 10^2. SEM: standard error of the mean.

In Table 1, the association between heavy metals in the soil, foot, and shell of the snails collected around cement factory vicinities are shown. The Pb level in the hemolymph correlated positively with Pb level in the soil ($r = 0.523$, $p = 0.018$) and the shell ($r = 0.545$, $p = 0.013$) while there was an inverse correlation between Pb in the hemolymph and Pb in the foot ($r = -0.671$, $p = 0.001$). Similarly, Pb in soil correlated positively with Pb in shell ($r = 0.760$, $p = 0.000$) but negatively with Pb in the soil ($r = -0.569$, $p = 0.009$). Cd in hemolymph correlated positively with Cd level in the shell ($r = 0.516$, $p = 0.020$) while Cd in the soil associated positively with As in the foot ($r = 0.482$, $p = 0.031$). Negative correlation, however, existed between Pb in the soil and Cd in the soil ($r = -0.569$, $p = 0.009$). In addition, there were positive correlations among Cd in the soil and As in the soil ($r = 0.516$, $p = 0.020$), As in the soil, and Pb

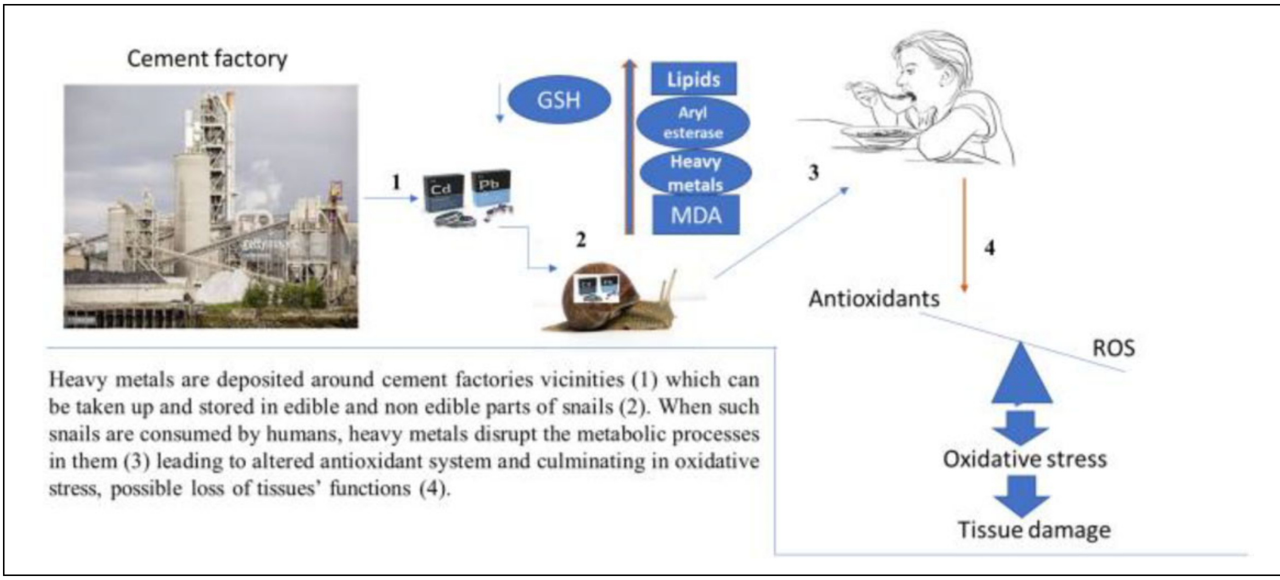

Figure 8. Graphical abstract depicting the study.

Table 1. Associations among metals in the soil, hemolymph, foot, and shell of snails collected around cement factories.[a]

Parameter	Correlation coefficient (r)	p Value
Pb in hemolymph vs. Pb in soil	0.523	0.018[b]
Pb in hemolymph vs. Pb in foot	−0.671	0.001[b]
Pb in soil vs. Pb in foot	−0.526	0.017[b]
Pb in soil vs. Cd in soil	−0.569	0.009[b]
Pb in shell vs. Pb in foot	−0.507	0.023[b]
Pb in shell vs. Cd in soil	−0.575	0.008[b]
Cd in hemolymph vs. Cd in shell	0.516	0.020[b]
Cd in soil vs. As in foot	0.482	0.031[b]

Pb: lead; Cd: cadmium.
[a]Correlation of heavy metals between the soil, hemolymph, foot and shell of snails collected around cement factories. The p value <0.05 is considered statistically significant.
[b]Statistically significant.

Table 2. Association among the heavy metals.

Parameter	Correlation coefficient (r)	p Value
Lead in soil vs. cadmium in soil	−0.569	0.009
Cadmium in soil vs. arsenic in soil	0.516	0.020
Arsenic in soil vs. lead in soil	0.482	0.031

in the soil ($r = 0.482$, $p = 0.031$). However, negative correlation exists between Pb in the soil and Cd in the soil ($r = 0.569$, $p = 0.009$; Table 2).

Biochemical parameters are correlated, as presented in Table 3. Reduced GSH level correlated negatively with AR in the hemolymph ($r = -0.322$, $p = 0.005$). In addition, digestive gland GSH level correlated negatively with MDA level in the hemolymph ($r = -0.316$, $p = 0.005$). Indeed, GSH correlated positively with CHOL in the digestive gland ($r = 0.265$, $p = 0.021$) and with triglyceride in the

Table 3. Association among biochemical parameters of snails collected around cement factory vicinities.[a]

Parameter	Correlation coefficient (r)	p Value
GSH hemolymph vs. arylesterase hemolymph	−0.322	0.005[b]
GSH digestive gland vs. arylesterase hemolymph	−0.450	0.000[b]
GSH digestive gland vs. MDA hemolymph	−0.316	0.005[b]
Arylesterase hemolymph vs. cholesterol hemolymph	−0.323	0.004[b]
Arylesterase hemolymph vs. triglyceride hemolymph	−0.396	0.000[b]
Arylesterase hemolymph vs. phospholipid digestive gland	0.407	0.000[b]
LDH hemolymph vs. triglyceride digestive gland	0.235	0.041[b]
Cholesterol hemolymph vs. triglyceride hemolymph	0.354	0.002[b]

GSH: reduced glutathione; MDA: malondialdehyde; LDH: lactate dehydrogenase.
[a]Correlation of biochemical parameters of the snails collected around cement factories. The p value <0.05 is considered statistically significant.
[b]Statistically significant.

Table 4. Association among heavy metals and biochemical parameters of snails collected around cement factory vicinities.[a]

Parameter	Correlation coefficient (r)	p Value
GST in hemolymph vs. arsenic in foot	−0.606	0.005[b]
GST in digestive gland vs. lead in foot	0.523	0.018[b]
GST in digestive gland vs. cadmium in hemolymph	0.445	0.049[b]
GSH in hemolymph vs. arsenic in soil	−0.524	0.018[b]
GSH in digestive gland vs. lead in hemolymph	−0.664	0.001[b]
Arylesterase in hemolymph vs. lead in hemolymph	0.576	0.008[b]
Arylesterase in hemolymph vs. lead in foot	−0.592	0.006[b]
Cholesterol in hemolymph vs. arsenic in foot	0.456	0.044[b]
Triglyceride in hemolymph vs. arsenic in hemolymph	−0.444	0.050[b]
Phospholipid in digestive gland vs. lead in foot	−0.472	0.036[b]

GST: glutathione-S-transferase; GSH: reduced glutathione.
[a]Correlation of biochemical parameters of the snails collected around cement factories with heavy metals levels. The p value <0.05 is considered statistically significant.
[b]Statistically significant.

hemolymph ($r = 0.325$, $p = 0.004$). AR in hemolymph, on the other hand, correlated negatively with CHOL in the hemolymph ($r = -0.323$, $p = 0.004$) and triglyceride in the hemolymph ($r = -0.396$, $p = 0.000$). LDH in hemolymph showed positive association with triglyceride in the hemolymph ($r = 0.235$, $p = 0.041$) while both CHOL in the hemolymph and digestive correlated inversely with PHOL in the digestive gland ($r = -0.285$, $p = 0.013$) and in the hemolymph ($r = -0.240$, $p = 0.037$).

As given in Table 4, correlations existed between the heavy metals and the biochemical parameters. GST activity in the digestive gland correlated negatively with Pb in the soil ($r = -0.662$, $p = 0.001$) and the shell ($r = -0.491$, $p = 0.028$). Similarly, GSH level in the hemolymph showed a negative relationship with Pb in the soil ($r = -571$, $p = 0.009$) and shell ($r = -0.707$, $p = 0.000$). Furthermore, AR activity in the hemolymph associated positively with Pb in the soil and shell ($r = 0.456$, $p = 0.043$ and $r = 0.574$, $p = 0.008$, respectively), but a negative correlation was observed between the AR activity in the hemolymph and Pb in the foot of the snail ($r = -0.592$, $p = 0.006$). Notably, GST activity (digestive gland) and GSH level (hemolymph) showed an inverse relationship with As level in the hemolymph and soil ($r = -0.524$, $p = 0.018$ and $r = -0.664$, $p = 0.001$, respectively).

Discussion

We demonstrated in this study, how *A. achatina* is used as a biosensor for passive and active biomonitoring for hazard and risk assessment to heavy metals around cement factories. This was done by the evaluation of heavy metal (Pb, Cd, and As) levels in the soil and snail samples as well as evaluation of the antioxidant system, and lipid profiling in the snails. Metals such as As, Cd, and Pb may induce toxicity by preferentially interacting with thiol-containing groups of biomolecules, oxygen, or sulfur-containing compounds to induce oxidative stress, causing tissue damage (Izah et al., 2017; Lemire et al., 2013). Heavy metals can bioaccumulate in tissues of human and nonhumans and wreck great health havoc. Indeed, metal-induced pathologies remain a global public health concern (Izah et al., 2017). Cd has been reported to be nephrotoxic and hepatotoxic, and Pb intoxication could cause convulsion, encephalopathy, and hypertension (Iweala et al., 2014). Heavy metals are also known disruptor of lipid homeostasis and the antioxidant system: for instance, Cd in crab (Yang et al., 2013), Pb and As in rats (Ademuyiwa et al., 2010), and Cd and Pb in snail and fish (Banaee and Taheri, 2019; Sarah et al., 2019). This study, therefore, investigated the heavy metal levels, the lipid metabolism, and oxidative status indices of snails handpicked around cement factory vicinities in Ogun State, Nigeria.

In this study, soil, hemolymph, and shell Pb and As levels were unexpectedly higher in the control site than the test sites, although the reverse was the case for Cd despite the distance from the experimental sites (Figure 1). A plausible reason could be due to anthropogenic activities, mining, automobiles effluents, and industrial activities that may have occurred at this site before the commencement of this study (Figure 2(a), (b), and (d)) (Mohammadein et al., 2013).

Higher Cd (Figure 3(a) to (d)) level found at Oke village and Ewekoro sites may be expected because Cd is one of the heavy metals found in and around the cement factories. The metals from the cement kiln may found their way into the surrounding soil by the disposal of wastes and effluents from the factory

(Yadegarnia et al., 2019). As level was equally found to be high especially at the Papa and control sites (Figure 4(a), (b), and (d)). This observation is consistent with the study of Vimercati et al. (2017), where Cd and As levels in the cement factory area were found to be higher than the control site. All the metals found in the edible parts of the snails in this study were alarmingly high. This is a great concern from the toxicological standpoint because the values obtained exceeded permissible levels (5 μg/kg for Pb and 15 μg/kg for Cd) in food and food products by the Food and Drug Administration (FDA, 2019). In a study by Malekirad et al. (2019), it was shown that the long-term exposure to dust from cement factories might induce oxidative stress and neurological impairment in the workers. Furthermore, Bannae and Taheri (2019) observed that snails exposed to sewage containing heavy metals had significantly reduced total antioxidant capacity and increased lipid peroxidation with elevated activities of LDH, alanine transaminase (ALT), and aspartate transaminase (AST). In a similar study to ours, evaluation of soil around Ashaka cement factory PLC, in Northern Nigeria, Kolo et al. (2018) found chromium, Pb, and nickel levels to be remarkably high. Blood analysis of the adult and children around the Ashaka cement factory was found to contain a high level of chromium (carcinogenic metal). In this study, Pb level in the soil was positively associated with that of the shell and hemolymph. In contrast, negative correlations existed between Pb in the soil and foot as well as the hemolymph. This suggests that the amount of Pb found in the shell may have bioaccumulated over time. The negative correlation may also be due to deposition of Pb into storage tissues of the snails over time. Because of the slimy nature of the snail foot, they are capable of accumulating heavy metals in the soil into their soft tissues as they glide and then distributing it to other parts, such as the shell (Table 1). Indeed, our study showed a positive association between Pb in the soil and Pb in the shell. Another finding from this study was that Cd level correlated negatively with that of Pb. This may be suggestive to the fact that snail might not uptake both metals together at the same time, perhaps because Pb being more reactive than Cd, may displace Cd. The present study also shows that bioaccumulation of heavy metals may have disrupted the snail antioxidant systems in a similar manner.

GSH level and AR activity decreased while the MDA level was high in snails from the Ewekoro site. GST activity was equally high in the snail from the Oke site (Figure 5). GSH is an endogenous thiol-containing tripeptide antioxidant molecule that is necessary for xenobiotic detoxification. It is a cosubstrate for GST, a phase II xenobiotic, detoxifying enzyme. Reduction in GSH level may be expected in the snails due to heavy metal affinity for thiol-containing molecules. Heavy metals may combine with GSH to form GSH, metal complex, thus reducing their availability. Another plausible reason for GSH depletion may be due to utilization by GST during the detoxification process (Radwan et al., 2010). Indeed, we found negative correlations between the GSH level in the hemolymph and Pb in the hemolymph. In addition, GST activity correlated negatively with the Pb level (Table 4). These observations are suggestive of metal-induced enzymatic deactivation. GST requires GSH for conjugation of xenobiotics and proper detoxification. Therefore, depletion of GSH may, in turn, lead to disruption in the activity of the GST (Abdel-Halim et al., 2013). AR may be classified as a secondary antioxidant enzyme because it helps to metabolize organophosphates. Studies have shown that the exposure to heavy metals, such as Cd, As, and Pb, may cause the inhibition of AR and increased susceptibility to metal toxicity (Dzobo and Naik, 2013). Reduced level of GSH and AR activity accompanied by elevated MDA level in snails suggests possible heavy metal-induced oxidative stress. Oxidative stress occurs in a sequence wherein oxidative insults lead to the depletion of endogenous antioxidant molecules and also initiate a chain of reactions affecting macromolecules (lipids, proteins, and DNA), causing them to become unstable (Malekirad et al., 2019). Heavy metals may also initiate the production of free radicals via Fenton's reaction. The free radicals produced may thus cause oxidation to lipids and other macromolecules (Abdel-Halim et al., 2013). MDA level is an index of lipid peroxidation *in vivo* and *in vitro* (Somade et al., 2018). In a study to assess the health status of cement factory workers in Nigeria, MDA level increased significantly in the bricklayers (that use cement), and the level correlated positively with heavy metal levels in their blood. This may be due to oxidative stress induced by heavy metals (Richard et al., 2016).

AR plays an important role in cardiovascular- and oxidative stress-related diseases and is capable of modulating susceptibility to stress but may be inhibited by heavy metals (Costa et al., 2017). AR activity was remarkably high in the snails collected from the

Ewekoro site while it decreased at other sites. It is difficult to predict the trend of the response of antioxidant enzymes during oxidative stress. While the enzymatic activity may be increased in some tissues as a homeostatic response to the stress, it may be reduced in others, suggesting an overwhelmed enzymatic activity (Dzobo and Naik, 2013). It may even be due to reactive species (RS) induced enzymatic deactivation because the active site of most enzymes possesses catalytic residue that are susceptible to proton abstraction by the RS (Ercal et al., 2001). The enhanced activity of AR in snails from the Ewekoro site may be a detoxification response by the snails due to the heavy metals. In fact, AR activity correlated negatively with Pb and As levels in the present study. This indicates that there may be an interactive effect between heavy metals and AR activity in the snail.

LDH activity in the hemolymph of snails collected from the Papa site increased significantly when compared with the control site. Richard et al. (2016) showed in their study that cement factory workers in Calabar, Nigeria, and the residents of the factory vicinity presented with derangements in the activity of LDH, AST, and ALT because of long-term exposure to the dust from the factory. LDH is a cytosolic enzyme the activity of which when found to be increased in the blood is suggestive of a leakage from the cell due to damage (Chang et al., 2020). LDH activity may also be used as a measure of stress level in snails (Abdel-Halim et al., 2013). It may be safe to infer that the oxidative stress induced by the metals present in the dust from the factories is a likely cause of the observed altered intermediary metabolism.

The levels of CHOL, TAG, and PHOL (Figures 6 and 7) measured in the hemolymph and digestive gland indicated that snails from the Oke village showed higher TAG, CHOL, and PHOL levels (in the hemolymph) but snails from Papa had the highest TAG, CHOL, and PHOL contents (in the digestive glands). Dic-Ijiewere Ebenezer et al. (2018) observed in their study that the accumulation of heavy metals in cement factory workers correlated positively with reduced CHOL level. Although the observation was in human subjects, our study, on the other hand, showed that metals might cause an increase in triglyceride, CHOL, and PHOL in snails. Increment changes in the TAG level as observed in our study is in consonance with the observations of Malekirad et al. (2019), where TAG levels increased significantly in cement factory workers relative to the control group. The inconsistent trend in the CHOL level suggests that workers and public around cement factories are prone to dyslipidemia—a primary risk factor for cardiovascular diseases (Costa et al., 2017). This study did not evaluate the humans (workers or public) around these cement factories; therefore, it may be difficult to extrapolate our results to humans. However, using snails around the cement factories, the assessment of the risk to exposure to soil and dust provides an insight into the incipient dangers. Notably, our results show a negative relationship between the TAG and As levels as well as PHOL correlated negatively with the Pb level in the foot of the snail (Table 4).

Heavy metal-induced dyslipidemia is indeed dangerous to snails and other animals of similar physiology. Cd dysregulated the TAG levels in the hemolymph of crabs via the upregulation of hepatopancreas lipase and downregulation of ATP synthesis (Yang et al., 2013). Snails require lipids during their period of hibernation for some reason. Firstly, the formation of epiphragm plays a critical role in the dynamics of the hemolymph. Hemolymph serves as the medium for the transport of materials akin to blood in humans. Epiphragm also serves as a deterrent to predators and pathogens against mechanical and infectious damage to soft tissue (Ademolu et al., 2016). Therefore, it is safe to infer that disruption in snail lipid metabolism is a threat to their sustainability. In the same vein, exposure to heavy metals from the cement factories to their immediate environment may cause life-threatening conditions to the workers and public alike.

Conclusion

Snails around the cement factory vicinities may bioaccumulate heavy metals from their milieu into their edible and nonedible parts. Heavy metals may cause the perturbation of an antioxidant system, dysregulate lipid metabolism, and increase susceptibility to oxidative damage. Our study also shows that there exists a negative correlation between the antioxidant markers (GST, GSH, and AR) and the heavy metals while a positive correlation exists between tissue damage markers (MDA and LDH activities) and the heavy metals. Therefore, consumption of these snails may be risky to humans and must be discouraged to avert future health casualties (Figure 8).

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ORCID iDs

Ngozi Ugbaja Regina https://orcid.org/0000-0003-1456-8900
AS James https://orcid.org/0000-0003-3276-8914
AJ Akamo https://orcid.org/0000-0002-4582-4615

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