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Research article

Impacts of trace metals on African common toad, *Amietophrynus regularis* (Reuss, 1833) and depuration effects of the toad's enteric parasite, *Amplicaecum africanum* (Taylor, 1924) sampled within Lagos metropolis, Nigeria

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ABSTRACT

The study aimed at assessing the depuration potentials of endoparasite, *Amplicaecum africanum* on trace metals in its toad host, *Amietophrynus regularis* at sites of significant anthropogenic perturbations within the Lagos metropolis, in Nigeria. A total of 120 toads of both sexes, alongside 45 soil samples were collected from each of three (3) stations labeled Dumpsite, Lagoon front and Highrise, using hand nets and by hand-picking between February and October, 2018. The intestinal tissues sections of the toads were examined using a binocular dissecting microscope (American Optical Corporation, Model 570) and hematoxylin and eosin (H&E) stain. Oxidative stress in toad intestine was assessed by estimating the levels of glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), and lipid peroxidation (MDA). Trace metals in the water, soil, toad liver, intestine and parasite, *Amplicaecum africanum* were tested using Atomic Absorption Spectrophotometry (Philips model PU 9100). Lead (Pb), copper (Cu), nickel (Ni), cadmium (Cd), and chromium (Cr) were detected in the toads, with the infected toads having lower concentrations of most trace metals than the uninfected toads, irrespective of the locations and sex. Strong negative correlations between parasitological indices and concentrations of trace metals in the toads suggest that the parasites might have taken up significant amounts of trace metals from the host. The study demonstrated the potentials of parasite, *A. africanum* to depurate trace metal burden in *Amietophrynus regularis*. When the dominant factor impacting the toad is the parasitic infection, parasite intensity determines the trade-off between parasitological harm and depuration benefit to the host. Hence, under controlled conditions, parasites may serve as bioremediation agent in the event of pollution. Depuration potential of *A. africanum* in the study was supported by the mild tissue alterations observed in the intestine of infected toads, compared to the uninfected counterparts, which exhibited severe glandular hyperplasia, increased connective tissue, and severely stunted villi. Consistently lower activities of biochemical biomarkers which characterize the uninfected toads compared to the infected, irrespective of the sex and stations, further corroborate drawn inferences.

1. Introduction

Trace metals are regarded as persistent bioaccumulative and toxic micro-pollutants. They are bioavailable in perturbed conditions, hence they penetrate biota by passing through phospholipid cell membranes, causing harms due to inability of exposed organisms to metabolize them (Walker et al., 2001). Ecologically unacceptable concentrations of trace metals in Nigeria soil and biota have been traced to oil production activities (Isibor et al., 2016a; Isibor and Imoobe, 2017a).

The African common toad (*Amietophrynus regularis*), also known as the square-marked toad, or the African bouncing toad (due to its bouncing motion) is a good source of protein for humans in many parts of sub-Saharan Africa (Adediran et al., 2014), notably in Nigeria and Burkina Faso. Since the past two decades, ecological and ecotoxicological researches on amphibians have been on the increase (Sparling et al.,

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2000; Halliday, 2016) following the decline of the global amphibian population (Houlahan et al., 2000). International Union for the Conservation of Nature (IUCN) pointed out that there are 787 rare or endangered amphibian species (Vasconcelos et al., 2010) and about 1,900 species are threatened (Stuart et al., 2004). Frogs and toads constitute about 90% of all amphibians (Santos and Amato, 2010), therefore they are an important link between human and ecosystem health (Hayes et al., 2002). They are the main component of linkage between aquatic and terrestrial ecosystems (Unrine et al., 2007. Most adult frogs and toads feed on insects; hence they are the important energy-efficient trophic link between invertebrates and other vertebrates (Sparling et al., 2000).

Some causes of amphibian population decline include habitat loss or alteration (Icochea et al., 2002; Beebee and Griffiths, 2005), ultraviolet radiation and chemical pollution (Blaustein, and Bancroft, 2007), climate change (Pounds et al., 2001) and epidemic diseases (Pounds et al., 2006). Some of these factors may also cause allometric abnormalities such as disproportionate body mass to body length, malformations of limbs and body organs (Sparling et al., 2000), which may further reduce the viability of amphibian populations. Such deformities reduce their ability to escape from predators or achieve successful mating. It also limits their chances of competing and securing vital environmental resources necessary for their niche, thereby increasing the mortality rates and reducing the reproductive rate (Rowe et al., 1996, 1998; Pahkala et al., 2002, 2003, Johnson et al., 2003).

Toads are capable of accumulating trace metals in their cells, higher than other aquatic fauna due to the relatively higher permeability of their skin (Forstner and Wittmann, 1981). Susceptibility of amphibians to xenobiotics necessitates their use as bioindicators in pollution studies (Welsh and Ollivier, 1998; Johansson et al., 2001; Loumbourdis et al., 2007).

Amphibians have a rich parasite fauna, including viruses, protozoans, and helminths (Canning et al., 1964; Sulieman and Pengsakul, 2015). Several studies have been carried out on the protozoan and helminth parasites of Amphibia (Santos *et al.*, 2013).

Environmental contaminants reduce immunocompetence in many species (Luebke *et al.*, 1997). Pesticides with different modes of action decrease antibody responses (Gilbertson *et al.*, 2003), lymphocyte proliferation (Christin *et al.*, 2004) and levels of circulating eosinophilic granulocytes (Kiesecker, 2002). In tadpoles, the developing immune system may be more susceptible to contaminants (Carey and Bryant, 1995) and early exposure to foreign compounds could lead to long-term changes in immunocompetence (Milston *et al.*, 2003).

Amphibians are sentinel species because they have highly semi-permeable epidermis and different life cycle stages in both aquatic and terrestrial ecosystems (Alford and Richards, 1999). Hence they may be useful indicators of environmental conditions and may help in combating the trend of organismal population decline. Susceptibility of hosts to xenobiotics may enhance parasite transmission through immunosuppression (Pina-Vazquez et al., 2012). For example, tadpoles (second intermediate hosts of *Echinostoma trivolvis*) exposed to pesticides during early development suffer increased parasite susceptibility which continued after pesticide exposure was discontinued (Budischak et al., 2008). On the other hand, the concentration of the toxicant in the host may be reduced through accumulation by the parasites (Koprivnikar *et al.*, 2007; Griggs and Belden, 2008). Intricate interactions between pollutants and parasites in the host have been reported (De Denato et al., 2017; Gilbert and Avenant-Oldewage, 2017), showing parasite's ability to reduce pollutants levels in infected hosts compared with uninfected conspecifics (Sures, 2007, 2008; Sures et al., 2017).

Metal's threshold of essentiality is an important factor in the host organism. Essential metals such as iron, zinc, chromium, calcium, etc. have metabolic functions, while unessentials such as lead, cadmium, nickel may be toxic at low concentrations (Isibor and Imoobe, 2017). Even essential metals may elicit toxicity at concentrations higher than required. Hence proper moderation of metal concentrations in organism is paramount. Depuration of trace metals by enteric parasites may be disadvantageous to the host if the metals depurated are essential (Yones et al.,2015).

The toad species, *Amietophrynus regularis* is an exotic species, hunted for its acclaimed uniqueness as a seasoned delectable delicacy by subsistence and commercial hunters in specific locations within the Lagos metropolis. The uninformed and indiscriminate toad capture, in conjunction with pollution threats within the catchment areas may pose synergistic threats to the population of the species.

The study was aimed at assessing the depuration of trace metals by enteric parasites in the common toads, *Amietophrynus regularis* using histopathological and biochemical biomarkers for evaluation of toxicity gradients. The outcome of the study may contribute to knowledge on the complex interactions of parasites and pollutants in the host organisms and the resultant synergism, antagonism or supra-additive effects.

2. Materials and methods

2.1. Description of the study area

Three (3) study stations (Figure 1) were selected within the Metropolis of Lagos State, Nigeria. These stations were chosen because of anthropogenic activities within their vicinity and availability of toad hunters. The first study station was a dumpsite (6º30′51″ N, 3º23′33″ E) within the campus of the University of Lagos. The station was chosen for this study based on our observations of tremendous refuse dumped at this location by residents in the University of Lagos, Nigeria. The site is surrounded by green vegetation, with subsistent farming systems set up at different locations. The station was labeled as 'Dumpsite' (6º30′51″ N, 3º23′33″ E) .

The second was named 'Lagoon front' (6º31′7″ N, 3º24′4″ E), located at Epe Lagoon which is fed by River Ishun and lies between the Lagos and Lekki Lagoons. The Lagos, Epe and Lekki Lagoons open into the Gulf of Guinea via the Lagos Harbour. Epe Lagoon has a surface area of about 225 km^2, and a depth range of 1–6m (Balogun, 1978). The vegetation surrounding Lekki Lagoon is of the swampy mangrove type, predominated by *Rhizophora racemosa* and *Avicenia nitida.* Bioaccumulation of petrogenic pollutants have previously been reported at this location (Doherty et al., 2010; Doherty and Otitoloju, 2016; Akinsanya et al., 2018).

The third set of samples were collected from an undisturbed location within the University of Lagos, at 'Highrise' Staff quarters (6º31′46″ N, 3º23′50″ E) and this was labeled as 'Highrise'. The seasonality of the entire study area is characterized by wet and dry seasons, typical of the southern part of Nigeria.

2.2. Sample collections

A total of 120 toads (74 males, 46 females) were purchased lifeless but fresh from toad hunters within each location. The toad hunters captured the animals for sale as source of livelihood and/consumption as animal protein. The indiscriminate toad capture, in conjunction with pollution threats to the animals stresses the justification of this study.

Investigations revealed that each of the three stations was explored by different toad hunters using hand nets and by hand-picking. The procurement of toads as experimental specimens spanned the periods of February and October 2018. The lifeless but fresh toads were transported in iced chest coolers (labelled according to the stations) to the laboratory of Zoology Department, University of Lagos, where the morphometric features were measured. Afterwards, they were dissected and the intestinal tissues were excised for histopathology and trace metals analysis.

Soil sample replicates were collected from 5 scattered spots at each sampling station with the aid of Van Veen grab (30 × 15 × 28 cm) for a period of 9 months, totaling 45 samples from each station. Samples were stored immediately in sterile polythene bags and then transported

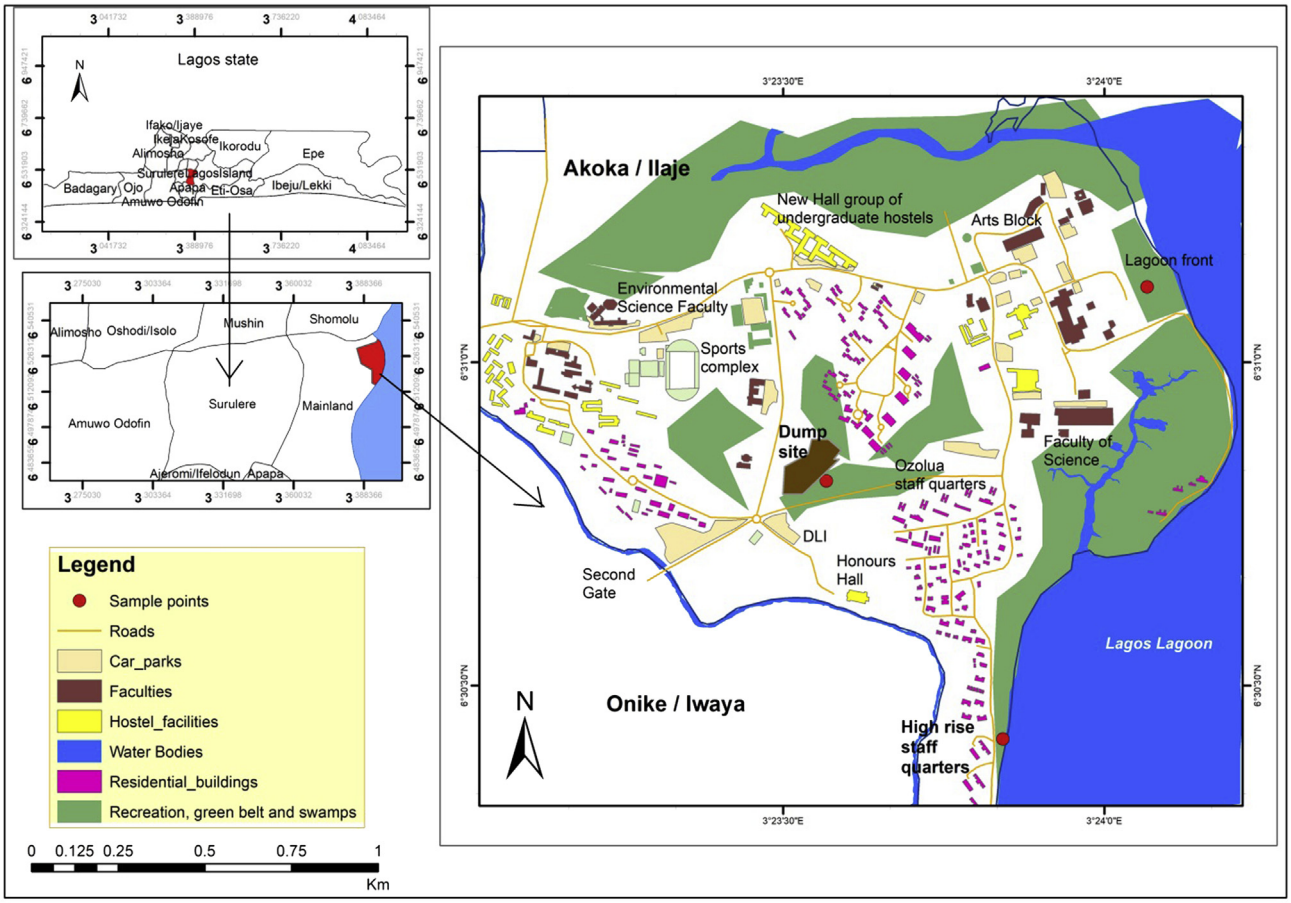

Figure 1. Map of Lagos metropolis, showing sampled stations.

immediately to the laboratory of the Department of Marine Sciences of the University of Lagos, where they were further refrigerated at 4 °C prior to laboratory analyses.

2.3. Morphometric assessment of the toad

Sex determination in the toads was first conducted through physical observation of the throat area and then confirmed using an identification guide. The males are characterized by the dark or green throat while the females are characterized by the white throat. Females also have coiled oviducts which are absent in males. A confirmatory determination was then carried on the reproductive system according to the description of Kobayashi et al. (2018).

Each toad was measured to the nearest 0.01 g using a battery-powered digital Camry weighing balance (model EK-1A SERIES). Using a thread and ruler, five measurements were taken which include:

1. Snout - urostyle length (SVL): This is the distance between the anterior tip of the snout and the posterior tip of the urostyle.
2. Length of the forelimb (LF): This is the distance between the posterior end of the humerus and the tip of the longest finger.
3. Length of the hind limb (LH): This is the distance between the tibial head and the tip of the fourth toe (which is the longest toe).
4. Width across the head (HW): This is the greatest width of the head at the level of the tympanum (Balogun, 1978).
5. Tympanic diameter (TD): This is the longitudinal distance between the outer margins of the tympanic annulus.

All measurements were taken to the nearest 0.1cm.

2.4. Laboratory analysis

2.4.1. Collection and analysis of enteric parasites

Using sterile blades, the intestine of the toad specimens were eviscerated and placed in saline solution. The intestines were then dissected to obtain the enteric parasites. The intestine was preserved in separate sampling bottles containing Bouin's fluid and the parasites were preserved in 70% alcohol. The recovered parasites were fixed in 70% alcohol, counted and recorded. The enteric parasites collected from the toad were Nematodes identified as *Amplicaecum africanum.* The identification procedure was carried out at the pathology laboratory of the Department of Veterinary Pathology, University of Ibadan, Nigeria, using identification manuals such as Colombo et al. (2005), Xing et al. (2005), and Sures (2007) and Akinsanya et al. (2008). Parasite samples were then labeled according to the sex of source toad and stations they were obtained.

2.4.2. Histopathological assessment

The intestinal tissues were placed in bottles containing Bouins fluid for 6 h, after when it was decanted and 10% buffered formalin was added to preserve the tissue. The tissues were routinely dehydrated in an ascending series of alcohol at 30 min interval; they were then embedded in molten paraffin wax and allowed to solidify. The blocked tissues were sectioned at 4–5 microns processed and stained with hematoxylin and eosin (H&E) stains. The stained tissues were washed off in tap water and the overstained ones destained in 1% alcohol (Akinsanya et al., 2018). The tissues were mounted using DPX mountant, and dried. Cover slips were then mounted over the sections and examined using a binocular dissecting microscope (American Optical Corporation, Model 570). The photomicrographs were taken with the aid of a Camera (INFINITY,

3-3URC 4.54 × 4.54 μm) in the pathology laboratory of the Department of Veterinary Pathology, the University of Ibadan, Nigeria.

2.4.3. Tissue analysis

The liver and intestine of the toad samples were excised using a sterile blade and kept separately in air-tight glass containers with lid. The containers were labeled appropriately according to sex and station. The labeled samples were then preserved in a freezer at -10 °C till further analysis (48 h).

2.4.3.1. Trace metals. Frozen tissues were thawed and two (2) grams wet-weight sample of liver and intestine was weighed separately into a PTFE beaker and digested with 25 mL of ratio 1:1 hydrogen peroxide and Nitric acid on a hot plate inside the fume cupboard. Heating continued until the volume was reduced to about 5 mL. They were allowed to cool then filtered and made up with distilled water to the 50 mL volumetric flask for the trace metals concentration analysis. Flame Atomic Absorption Spectrometer (Philips model PU 9100) was used in analysing the concentrations of Pb, Cu, Ni, Cd, and Cr with detection limits of 0.03 μg g^{-1},0.05 μg g^{-1}, 0.05 μg g^{-1}, 0.01 μg g^{-1}, and 0.5 μg g^{-1} respectively. All procedures were guided by the guidelines of Whiteside (1981).

The limits of quantification (LOQ) for the metals were empirically calculated as the mass fraction for which a certainty and uncertainty was achieved, on the basis of outputs the computed ratios between the calculated and the reference mass fractions (i.e. calculated value ÷ reference value) in the calibration curves. The LOQs for Pb, Cu, Ni, Cd, and Cr were 0.014, 0.016, 0.012, 0.017, and 0.013mg.kg^{-1}respectively. Results obtained were validated using TraceCERT® and ERM-CE27 as certified reference materials (CRMs). Adequacy of trueness was evaluated using statistical tool z-scores which indicated standard deviations from the certified reference materials was less than 6% at 95% confidence interval (ISO13528:2015, 2015).

2.4.3.2. Analysis of biochemical biomarkers. Thawed liver tissues were homogenized in a buffer of pH- 7.4. The sample was centrifuged at 10,000 rpmfor 20 min at 4 °C. Derived supernatant was drained and the pellet was rinsed with with 7.4 pH buffer and preserved in Eppendorf tube at -85 °C for 48 h, prior to analysis of the biochemical biomarkers (Siroka et al., 2005).

2.4.3.2.1. Lipid peroxidation (MDA). Lipid peroxidation was measured using the method of Jiang *et al.* (2008). 0.1 mL of intestinal homogenized supernatant was treated with 2 mL of (1:1:1 ratio) TBA-TCA-HCI reagent (thiobarbituric acid 0.37%, 0.25N HCI and 15% TCA) and placed in a water bath for 15min, cooled and then centrifuged at 3,000 rpm for 10 min at room temperature. The absorbance of clear supernatant was measured and recorded, with respect to reference blank at 535 nm. Values were expressed as μmol MDA formed/g tissue.

2.4.3.2.2. Glutathione peroxidase (GPx). Activity of glutathione peroxidase (GPx) was determined by the method of Ellman (1959). We added 10% TCA to the homogenate, centrifuged at 3,000 rpm for 10 min at room temperature. 1.0 mL of supernatant was treated with 0.5 mL of Ellman's reagent (19.8 mg of 5, 5′-dithiobisnitro benzoic acid (DTNB) in 100 mL of 0.1% sodium nitrate) and 3.0 mL of phosphate buffer (0.2M, pH 8.0).

We assayed GPx by following the rate of NADPH oxidation at 340 nm by the coupled reaction with glutathione reductase, whose activity was determined by spectrophotometrically measuring NADPH oxidation at 340 nm. Glutathione peroxidase (GPx) was then expressed in nmol/min/mg prot.

2.4.3.2.3. Catalase (CAT). Catalase (CAT) was assayed at 620nm and expressed as moles of hydrogen peroxide (H_2O_2) consumed/min/mg protein as described by Quinlan et al. (1994). The reduction of hydrogen peroxide was estimated spectrophotometrically at 240 nm, using 1.0 mL quartz cuvettes with a light path of 1.0 cm. Results were expressed as nmol H_2O_2 consumed/min/mg protein.

2.4.3.2.4. Superoxide dismutase (SOD). Assessment of SOD activity was based on the ability of the antioxidant to inhibit the autoxidation of pyrogallol. Thereafter, 970 μL of buffer (100 mMTris-HCl, 1 mM EDTA, pH 8.2), 10 μL of homogenates and 20 μL pyrogallol 13 mM were mixed. The assay was performed in thermostated cuvettes at 25 °C and changes of absorption were recorded by a spectrophotometer (Spectronic 20D) at 480nm. Evaluation of SOD activity was based on the amount of enzyme required to inhibit the auto-oxidation of 50% of the total pyrogallol in the reaction. Superoxide dismutase (SOD) was expressed in U/mg prot.

2.5. Analysis of soil

2.5.1. Physico-chemical analysis

Temperature of soil was measured in-situ using a mercury-in-glass thermometer. The pH and electrical conductivity were measured using a handheld multi-parameter probe (Horiba Checker Model U-10).

2.5.2. Trace metals

The soil sample was thawed and then sieved through a 2 mm sieve and 1g of soil sample was air-dried, homogenized and transferred into PTFE conical flask. 25 mL of ratio 3:1 Hydrochloric and Nitric acid (aqua regia) was added to the sample in a fume cupboard. The mixture was then heated at 45 °C on a hot plate until the volume reduced to about 5 mL. The mixture was filtered and made up with distilled water to 50 mL volumetric flask. Lead, copper, nickel, cadmium, and chromium were then analyzed using the Flame Atomic Absorption Spectrometer with detection limits of lead (0.03 μg kg^{-1}), copper (0.5 μg kg^{-1}), nickel (0.05 μg kg^{-1}), cadmium (0.03 μg kg^{-1}), and chromium (0.05 μg kg^{-1}). All procedures were conducted in conformity to the laboratory guide lines of Jones et al. (2001) and Estefan et al. (2013).

2.5.3. Determination of nitrates (NO_3)

Nitrate was determined through ionic strength adjusting solution (Millham et al., 1970; Watson and Isaac, 1990; Gelderman and Beegle, 1998). We prepared an ionic strength adjusting solution by weighing 67 g of $(Al_2SO_4).18H_20$, 12 g H_3BO_3, 20g Ag_2SO_4 and 19g NH_2HSO_3 into a 1000 mL volumetric flask which was made up with the aid of de-iodized water. Twenty (20) grams of sieved soil sample was placed in a 100 mL vessel and 50 mL ionic strength adjusting solution was added and thoroughly shaken for 5 min. The potential was read on a calibrated meter as the concentration of nitrate (NO_3) while stirring the suspension with the aid of a magnetic stirrer Jones et al. (2001).

2.5.4. Determination of sulfate (SO_4)

Extraction reagent was prepared by putting 2.03 g calcium phosphate $[Ca(H_2PO_4)_2.2H_20]$ into a 1000 mL volumetric flask, which was made up with de-ionized water. Ten grams of soil sample was placed in an extraction vessel. We pipetted 25 mL extraction reagent into a flask and placed on an electrical shaker and agitated for 30 min. We washed 0.15g activated carbon with extraction reagent, we then rinsed with de-ionized water and oven dried it. It was shaken for another 30 min. We filtered it and transferred 10 mL aliquot into another flask for spectrophotometric determination of sulfate Jones et al. (2001).

2.5.5. Determination of total organic compound (TOC)

Total organic compound was determined by the method of wet digestion as described by Mebius (1960). We weighed 13.072 g potassium dichromate ($K_2Cr_2O_2$) into 100 mL volumetric flask. We then added 400 mL de-iodized water to dissolve it. Afterwards, 55° mL of concentrated sulfuric acid (H_2SO_4) was added. The mixture was then allowed to cool and then made up to volume with water. We weighed 0.5 g of soil sample into a 500 mL Erlenmeyer flask and added 15 mL 0.267 N potassium dichromate reagent. The flask was connected to a reflux condenser and boiled for 30 min, allowed to cool to room temperature. Condenser was then rinsed with de-iodized water. Three (3) drops of

indicator solution was titrated with Mohr's salt solution. The colour changed from violet to green. The entire procedure was conducted simultaneously for a blank without soil added. TOC was then calculated with the titer value (Davies and Freitas, 1970).

2.6. Quality assurance and quality control

Standard reference material (Aluminum Standard for AAS Trace-CERT®, 1000 mg/L Al in nitric acid) purchased from Merck, Nigeria was used for calibration and validation of results. Multi-element Ion Chromatography Cation Standard Solution (MICCSS), certified for ion chromatography, was used in calibrating the instruments for detectability of each metal. We utilized analyses of certified reference materials (CRM), duplicates, spikes, and method blanks for correction of systemic errors.

The dissecting instruments and sampling containers used were pre-cleaned using 80% ethanol and sterilized under an autoclave at 120 °C for 2 h pending use. One surgical blade was used per tissue sample, after when it was discarded safely. To avoid hand contamination of samples, sterile laboratory gloves and nose masks were used throughout the experimental session. For quality assurance analyte grade saline water was subjected to microbial and contamination analysis prior to use. All readings were taken in triplicate to minimize error.

2.7. Statistical analysis

The descriptive statistics (mean ± SE) of readings in soil and tissues were subjected to analysis of variance (ANOVA). The outcomes were further subjected to the Duncan Multiple Range test (DMR) in order to ascertain the actual locations of significant differences.

Standard normal homogeneity (SNH) test was conducted among the various groups to ascertain homogeneity of data at 95% confidence interval.

The parasitological matrices were calculated thus:

Percentage Prevalence $= \frac{\text{Number of infected toads}}{\text{Number of toads examined}} \times 100$ (Saliu et al., 2014)

Parasite Abundance $= \frac{\text{Number of collected parasites}}{\text{Number of toads examined}}$ (Saliu et al., 2014; Cosmas et al., 2014)

Mean intensity $= \frac{\text{Number of collected parasites}}{\text{Number of infected toads}}$ (Ezewanji et al., 2005)

Descriptive statistics were presented as mean ± SE. Data was subjected to analysis of variance (ANOVA) and locations of significant differences were determined by the Duncan Multiples Range test. Spearman's rank correlation coefficient (InSat 3.0 for Macintosh) was used in analyzing correlative relationship between parasitological indices (parasite prevalence, abundance, and mean intensity) and the concentrations of trace metals in toad intestine. Thresholds of significance determined were $p > 0.05$ = not significant, $p \leq 0.05$ = significant, $p \leq 0.01$ = very significant, and $p \leq 0.001$ = extremely significant.

3. Results

The pH of soil collected from the Dumpsite was slightly acidic compared to those of Lagoon front and control station (Highrise) which were within the standard range of Canadian Council of Ministers of the Environment (CCME, 2001). Electrical conductivity (EC) detected in soil samples from Dumpsite and Lagoon front was distinctly higher than the level observed in the soil samples collected from control station which was below the established regulatory limit of CCME (2001). The values obtained in the soil samples from Highrise were quite low. This validates our observation that the station was least perturbed (Table 1). The concentrations of SO_4^{2-}, total organic compounds (TOCs), and nitrates at the Dumpsite were higher than values obtained at other stations ($p < 0.05$).

The Concentrations of trace metals such as Cd and Cu in the soil collected from Dumpsite and Lagoon front were significantly higher than the concentrations obtained at the control station and the set regulatory limits of CCME (2001). The concentration of Pb in soil sample at Dumpsite was significantly higher than that of Lagoon front ($p < 0.05$) which was not different from the concentration detected at Highrise ($p > 0.05$). Most importantly, the concentrations of Pb at all the stations were higher than the limit established by CCME (2001). The concentration of Cr was higher in the soil at Lagoon front than other stations, while Ni was in the order of Dumpsite > Lagoon front > High rise ($p < 0.05$). Cadmium and chromium were in the order of Dumpsite > Lagoon front > CCME (2001) > High rise ($p < 0.05$).

Amietophrynus regularis individuals were weighed and categorized into different weight cohorts according to the sex and sampling stations. Weights of both sexes from the three (3) sites were systematically tabulated (Table 2). Weights of female toads from Dumpsite before and after dissection were significantly higher ($p < 0.001$) than the weights of the males at the same site, which was higher ($p < 0.001$) than the weights of all specimens from the other two sites (Lagoon front and control). Snout to urostyle length (SUL) was significantly higher ($p < 0.001$) in females from Dumpsite than the SUL of females from the control station, which was significantly higher ($p < 0.001$) than the SUL of all other toads in the rest categories. No significant difference occurred in the forelimbs, hind limbs, tympanic diameter, and head width among all specimens across the stations ($p > 0.001$).

A. africanum parasites were detected only in the toads from Dumpsite and Lagoon front. None was found in the intestine of toads from Highrise.

Comparison of intestinal trace metals concentrations was conducted between the infected and uninfected toads using analysis of variance (ANOVA) and Duncan Multiple Range (DMR) test. The comparison was done among the toads on the bases of infection status, sex and location. Concentrations of Pb in the uninfected female toads at Dumpsite and Highrise and uninfected male at Highrise were significantly higher ($p < 0.05$) than the FEPA (2003) limit and the concentrations in the uninfected males at both stations, which were significantly higher ($p < 0.05$) than the concentrations in the other categories of the toads across the 3 stations (Table 3). Concentrations of Cu in the uninfected toads (both sexes) at Dumpsite and Lagoon front were significantly higher than the concentrations in the infected counterparts at both locations, which were significantly higher than the FEPA (2003) limit and the concentrations in all groups at Highrise. The concentrations of Ni in the uninfected toads at Dumpsite and Lagoon front were higher than the concentrations in the infected counterparts ($p < 0.05$), except in the infected male at Dumpsite, in which the concentrations of Ni were not significantly different from the uninfected toads ($p > 0.05$). The concentrations of Cd in the uninfected toads at Dumpsite and Lagoon front were also significantly higher than the concentrations in the infected counterparts. The concentrations in the infected males at lagoon front were not significantly different from concentrations detected in the groups at Highrise ($p < 0.05$). However, concentrations detected across all stations were lower than the established limits of FEPA (2003).

In a similar pattern, the concentrations of Cr in the uninfected toads (male and female) at Dumpsite were higher than the concentrations in the infected ones. The concentrations of Cr detected in the uninfected males at Lagoon front were higher than levels in infected males ($p < 0.05$), while no significant difference was detected among the females at the station. There was no significant difference between the concentrations in the infected male toads and all categories in the toads at Highrise ($p > 0.05$). It is noteworthy that no significant difference occurred most of the comparisons made among the toads at Higherise ($p > 0.05$).

Furthermore, comparison of the activities of the biochemical biomarkers between the intestinal tissues of infected and uninfected toads of same-sex and from the same stations revealed inferable variability. The activities of GPx in uninfected male and female *A. regularis* at Dumpsite and male at lagoon front were significantly lower ($p < 0.05$) than the activities in the infected counterparts (Table 4). The activities of SOD in the uninfected female toad at the Dumpsite and male at Lagoon front were significantly lower ($p < 0.01$) than the infected toads.

Catalase (CAT) activities in the uninfected toads of both sexes at Dumpsite ($p < 0.05$) and Lagoon front ($p < 0.01$) were significantly lower than the activities in the infected toads. However, MDA activity

Table 1. Physicochemical properties and trace metals (mean ± SD) in sediment samples between February and October 2018.

Parameters	Dumpsite	Lagoon front	Highrise	CCME (2001)
pH	6.40 ± 1.82^{c}	7.00 ± 2.02^{b}	8.01 ± 3.01^{a}	6.5–8.5
EC (μS/cm)	7876 ± 112.07^{a}	7439 ± 21.02^{a}	85 ± 4.02^{b}	100
SO_4^{2-} (ppm)	4233 ± 104.02^{a}	3528.49 ± 85.02^{a}	285.43 ± 40.02^{b}	-
TOCs (%)	2.12 ± 0.02^{a}	0.35 ± 0.04^{b}	0.25 ± 0.03^{b}	-
NO_3 (ppm)	10.20 ± 2.02^{a}	9.12 ± 2.04^{a}	5.43 ± 1.02^{b}	40
Texture	sandy loam	sandy clay	sandy	-
Pb (mg.kg^{-1})	2.08 ± 1.75^{a}	1.62 ± 0.41^{b}	1.53 ± 0.01^{b}	0.05
Cu (mg.kg^{-1})	8.06 ± 4.01^{a}	2.05 ± 0.01^{b}	0.02 ± 0.01^{c}	0.3
Ni (mg.kg^{-1})	6.25 ± 2.15^{a}	4.03 ± 2.23^{b}	0.08 ± 0.02^{c}	-
Cd (mg.kg^{-1})	2.12 ± 0.02^{a}	1.07 ± 0.02^{b}	ND	0.05
Cr (mg.kg^{-1})	4.03 ± 2.76^{a}	3.06 ± 1.32^{b}	0.07 ± 0.02^{c}	0.3

Values with different superscripts are significantly different ($p < 0.05$). ND = not detected. Sample size(n) = 45; 5 points for 9 months.

Table 2. Morphometrics (mean ± SD) of *Amietophrynus regularis* collected from Lagos metropolis.

Parameters	Dumpsite		Lagoon front		Highrise	
	Female	Male	Female	Male	Female	Male
Weight (g)	43.68 ± 10.82^{a}	31.91 ± 10.64^{b}	28.41 ± 2.21^{c}	23.13 ± 13.09^{c}	36.34 ± 10.55^{b}	24.46 ± 11.24^{c}
SUL (cm)	7.52 ± 1.52^{a}	6.61 ± 1.63^{c}	6.97 ± 1.02^{c}	6.80 ± 0.94^{c}	7.08 ± 0.90^{b}	6.54 ± 0.93^{c}
Forelimb (cm)	4.48 ± 0.53	4.14 ± 0.46	4.34 ± 0.54	4.09 ± 0.51	4.27 ± 0.99	3.85 ± 0.72
Hindlimb (cm)	8.46 ± 0.72	7.93 ± 0.83	8.30 ± 1.01	8.05 ± 1.24	8.27 ± 1.45	7.59 ± 1.44
TD (cm)	0.50 ± 0.00	0.49 ± 0.02	0.49 ± 0.09	0.54 ± 0.34	0.47 ± 0.05	0.41 ± 0.09
Head width (cm)	1.94 ± 0.09	1.77 ± 0.00	1.52 ± 0.72	1.66 ± 0.21	1.81 ± 0.23	1.64 ± 0.24

Note: SUL = snout to urostyle length, TD = tympanic diameter. Sample size N = 17. Emboldened figures are significant at a confidence interval of 99% ($p < 0.001$). Numbers with superscripts are significantly different, in order of a>b > c.

Table 3. Concentrations (mean ± SD) of trace metals (mg.kg $^{-1}$) in the intestine of infected and uninfected *A. regularis*.

Metals	Dumpsite				Lagoonfront				Highrise				FEPA (2003)
	IF	UF	IM	UM	IF	UF	IM	UM	IF	UF	IM	UM	
Pb	0.40 ± 3.3^{c}	2.50 ± 1.2^{a}	0.76 ± 0.3^{c}	1.16 ± 1.0^{b}	0.21 ± 0.1^{c}	0.65 ± 0.3^{c}	0.36 ± 0.2^{c}	1.01 ± 0.2^{b}	0.49 ± 0.3^{c}	2.31 ± 1.1^{a}	0.16 ± 0.12^{c}	2.06 ± 1.3^{a}	2
Cu	8.44 ± 9.1^{b}	29.24 ± 13.1^{a}	1.87 ± 0.1^{c}	36.49 ± 21.1^{a}	3.72 ± 2.5^{c}	21.25 ± 16.1^{a}	5.48 ± 0.53^{b}	18.75 ± 16.3^{a}	1.86 ± 1.4^{c}	1.72 ± 1.2^{c}	1.36 ± 0.1^{c}	1.18 ± 0.3^{c}	3
Ni	1.07 ± 1.0^{a}	0.59 ± 0.3^{b}	1.17 ± 0.1^{a}	1.87 ± 1.4^{a}	0.23 ± 0.1^{b}	1.01 ± 0.5^{a}	0.42 ± 0.3^{b}	2.74 ± 2.4^{a}	0.02 ± 0.01^{c}	0.36 ± 0.3^{b}	0.18 ± 0.03^{b}	0.36 ± 0.3^{b}	-
Cd	1.18 ± 0.9^{a}	0.78 ± 0.6^{b}	1.02 ± 0.2^{a}	1.87 ± 0.93^{a}	0.77 ± 0.01^{b}	1.88 ± 1.7^{a}	0.05 ± 0.01^{c}	1.05 ± 1.02^{a}	0.03 ± 0.01^{c}	0.04 ± 0.02^{c}	0.02 ± 0.03^{c}	0.06 ± 0.03^{c}	2
Cr	1.45 ± 0.6^{b}	2.65 ± 1.6^{a}	1.05 ± 0.1^{b}	2.29 ± 1.33^{a}	0.24 ± 0.02^{c}	0.54 ± 0.4^{c}	0.34 ± 0.3^{c}	1.25 ± 1.2^{b}	0.47 ± 0.4^{c}	0.50 ± 0.4^{c}	0.34 ± 0.2^{c}	0.36 ± 0.2^{c}	-

IF = infected female, UF = uninfected female, IM = infected male, UM = uninfected male. Numbers with same superscripts are not significantly different, while those with different superscripts are significantly different at $p < 0.05$. Sample size (n) = 17.

Table 4. Comparison of biochemical biomarkers (mean ± SD) in the intestine of infected and uninfected *A. regularis*.

Marker	Dumpsite				Lagoonfront				Highrise			
	IF	UF	IM	UM	IF	UF	IM	UM	IF	UF	IM	UM
GPx	0.28 ± 0.2^{b}	0.12 ± 0.2^{b}	0.48 ± 0.1^{a}	0.16 ± 0.1^{b}	0.29 ± 0.1^{b}	0.01 ± 0.0^{c}	0.48 ± 0.2^{a}	0.04 ± 0.1^{c}	0.36 ± 0.1^{a}	0.37 ± 0.2^{a}	0.38 ± 0.2^{a}	0.41 ± 0.2^{a}
SOD	0.79 ± 0.9^{b}	3.72 ± 1.8^{a}	0.19 ± 0.1^{b}	1.97 ± 0.8^{a}	0.92 ± 0.1^{b}	2.70 ± 0.3^{a}	0.82 ± 0.6^{b}	3.06 ± 1.2^{a}	0.37 ± 0.8^{c}	0.42 ± 0.4^{c}	0.42 ± 0.2^{c}	0.46 ± 0.4^{c}
CAT	2.99 ± 0.5^{c}	12.14 ± 4.5^{a}	4.98 ± 2.8^{b}	9.28 ± 0.4^{a}	2.04 ± 0.2^{c}	6.02 ± 0.2^{b}	3.02 ± 0.8^{b}	7.02 ± 0.7^{b}	2.65 ± 1.2^{c}	2.62 ± 0.3^{c}	1.92 ± 0.3^{c}	2.97 ± 1.4^{c}
MDA	0.18 ± 0.0^{c}	2.08 ± 1.2^{a}	0.13 ± 0.1^{c}	3.08 ± 0.1^{a}	0.16 ± 0.1^{c}	1.88 ± 0.7^{b}	0.06 ± 0.01^{d}	2.01 ± 0.7^{a}	0.21 ± 0.2^{c}	0.22 ± 0.2^{c}	0.25 ± 0.2^{c}	0.26 ± 0.1^{c}

IF = infected female, UF = uninfected female, IM = infected male, UM = uninfected male. Numbers with same superscripts are not significantly different, while those with different superscripts are significantly different at $p < 0.05$. Sample size (n) = 17.UNITS = GPx (nmoles/min/mg prot.), SOD (U/mg prot.), CAT (nmoles/min/mg prot.), MDA (μmol MDA/g tissue).

Table 5. Indices of sex-based parasitic infection in *Amietophrynus regularis*.

Sex	Num. examined			Num. infected			Prevalence (%)			Num. of parasites			Abundance			Mean intensity		
	D	L	H	D	L	H	D	L	H	D	L	H	D	L	H	D	L	H
Male	32	30	12	12	9	0	43.8	36.7	0	144	86	0	4.5	2.9	0	12	9.6	0
Female	29	15	2	14	7	1	48.3	46.7	0.5	185	33	3	6.4	2.2	1.3	13.2	4.7	3

D = Dumpsite, L = Lagoon front, H= Highrise.

was significantly higher in uninfected male and female toads ($p < 0.05$) at both stations than their infected counterparts. The levels of CAT and MDA in most of the infected toads at Dumpsite and Lagoonfront were not significantly different ($p > 0.05$) from the levels detected in the toads at Highrise among which no significant difference was detected ($p > 0.05$).

Percentage prevalence of parasite on toads was generally lower than 50 % (Table 5). Higher parasite prevalence, parasite load, abundance and mean intensity occurred in the female than the male counterparts from the same location. The rate of parasitic infection among in the toads with regards to the location was Dumpsite > Lagoon front > Highrise.

The percentage parasite prevalence, parasite abundance, and parasite mean intensity of different weight cohorts (18–21.9, 20–23.9, 24–27.9, 28–31.9, 32–35.9, 36–39.9, 40–43.9, 44–47.9 g) of male and female toads from all stations were obtained for determination of their effects on the concentrations of the trace metals analyzed in the intestine of the toads.

The concentrations of Pb in the toad intestine had a significant regression (Table 6) on the percentage (F (3, 17) = 0.043, p=*0.03,* R^2 = 0.5211), and parasite abundance (F (3, 7) = 0.043, p= *0.024*, R^2 = 0.4231). Copper has a significant regression on the percentage prevalence (F (3, 17) = 0.053, p= *0.05,* R^2 = 0.6521), parasite abundance (F (3, 17) = 0.053, p= *0.03,* R^2 = 0.6321), and mean intensity (F (3, 17) = 0.053, p= *0.041,* R^2 = 0.5291). The concentration of Ni showed a significant regression only on the percentage prevalence of the parasites (F (3, 17) = 0.023, p= *0.01,* R^2 = 0.5281). The concentration of Cd in the intestine of the toads had a significant regression on the percentage prevalence (F (3, 17) = 0.042, p= *0.04,* R^2 = 0.6522), parasite abundance (F (3, 17) = 0.042, p = *0.03*, R^2 = 0.6671), and mean intensity (F (3, 17) = 0.042, p = *0.05,* R^2 = 0.6533). It is noteworthy that all the regressions recorded were negative.

Further than gender-based analyses,organ-based analyses were conducted in order to estimate the mean concentrations of trace metals in the tissues of combined sexes at the different stations. Comparison was done among the intestine, liver, parasites (*A. africanum*) of toad, *A. regularis*.

Intestinal Cu was the trace metal with the highest concentration among the tissues of the toads at Dumpsite and Lagoon front. The mean concentration of Cu in the intestine (32.49 ± 2.91 mg kg^1) > the liver (29.24 ± 3.12 mg kg^{-1}) of toads at Dumpsite (Figure 2 A) were higher than the concentrations detected in the intestine (28.25 ± 6.03 mg kg^{-1}) > the liver (3.72 ± 2.51 mg kg^{-1}) at Lagoon front (Figure 2 B), which were in turn higher than the concentrations in the liver (4.02 ± 1.86 mg kg^{-1})> intestine (3.06 ± 1.49 mg kg^{-1}) at Highrise (Figure 2 C).The concentrations of nickel at Dumpsite and copper at Lagoon front (Figure 2 B) were in the order of intestine > parasite > liver.

A markedly higher concentration of copper was detected in the intestine of toads at Lagoon front than the concentrations observed in other tissues ($p < 0.05$)(Figure 2 B).

At Highrise, although far less concentration of all examined trace metals was observed in the liver and intestine of the toads than at other stations. The concentration of lead in the intestine (Figure 2 C) of the animal at this station was markedly higher than other tissues ($p < 0.05$), while Cu concentration was significantly higher in the liver than the intestine, which was higher than the concentration in the parasite ($p < 0.05$).

3.1. Histopathological analysis

Histopathological analysis of the intestinal tissues of infected and uninfected toads at all stations further implicates the trace metals. Assessments of histopathological effects of trace metal accumulation in the intestine of *Amietophrynus regularis* presented differential alteration levels consistent with the detected trace metals concentrations and oxidative stress levels (Figure 1). Histopathological analysis of the tissues of infected and uninfected toads at Highrise showed no tissue alterations (Figure 1 A). However, the tissue micrographs of uninfected toads at Lagoon front showed sever alterations such as glandular hyperplasia (characterized by proliferation in the number of cells per unit area (Ivanov et al., 2010) and infiltration of inflammatory cells. The infected counterparts on the other hand exhibited only infiltration of inflammatory cells (Figure 1 B). Infiltration of inflammatory cells to mucosa was characterized by migration of cells from their sources of origin as a result of abnormal multiplication (Väyrynen et al., 2013). This was further confirmed by well-defined foci and diffusely distributed individual cells observed in the tissue section (see Figure 3).

Outstandingly marked alterations such as focal areas of stunting of villi, glandular hyperplasia, and infiltration of inflammatory cells occurred in the uninfected toads at Dumpsite, while mild congestion in submucosa occurred in the infected counterpart (Figure 1C).

4. Discussions

High level of TOCs at Dumpsite is attributable to possibly high organic composition in the deposited biodegradable municipal wastes. Insects and other detritus feeding organisms which abound at the Dumpsite may serve as ready food for *A. regularis*. Ad-libitum feeding due to the abundance of food at the Dumpsite might have resulted in storage

Table 6. Multiple regression analysis of trace metal concentrations in the intestine of infected toads on parasitic infection indices.

Metals	Percentage prevalence		Parasite abundance		Mean intensity	
	R^2	*p-value*	R^2	*p-value*	R^2	*p-value*
Pb	**0.5211**	**0.03**	**0.4231**	**0.024**	0.2722	3.44
Cu	**0.6521**	**0.05**	**0.6321**	**0.03**	**0.5291**	**0.04**
Ni	**0.5281**	**0.01**	0.1601	0.81	0.2471	4.21
Cd	**0.6522**	**0.04**	**0.6671**	**0.03**	**0.6533**	**0.05**
Cr	0.0043	0.79	0.1434	0.85	0.2112	0.09

Emboldened figures indicate significant values.

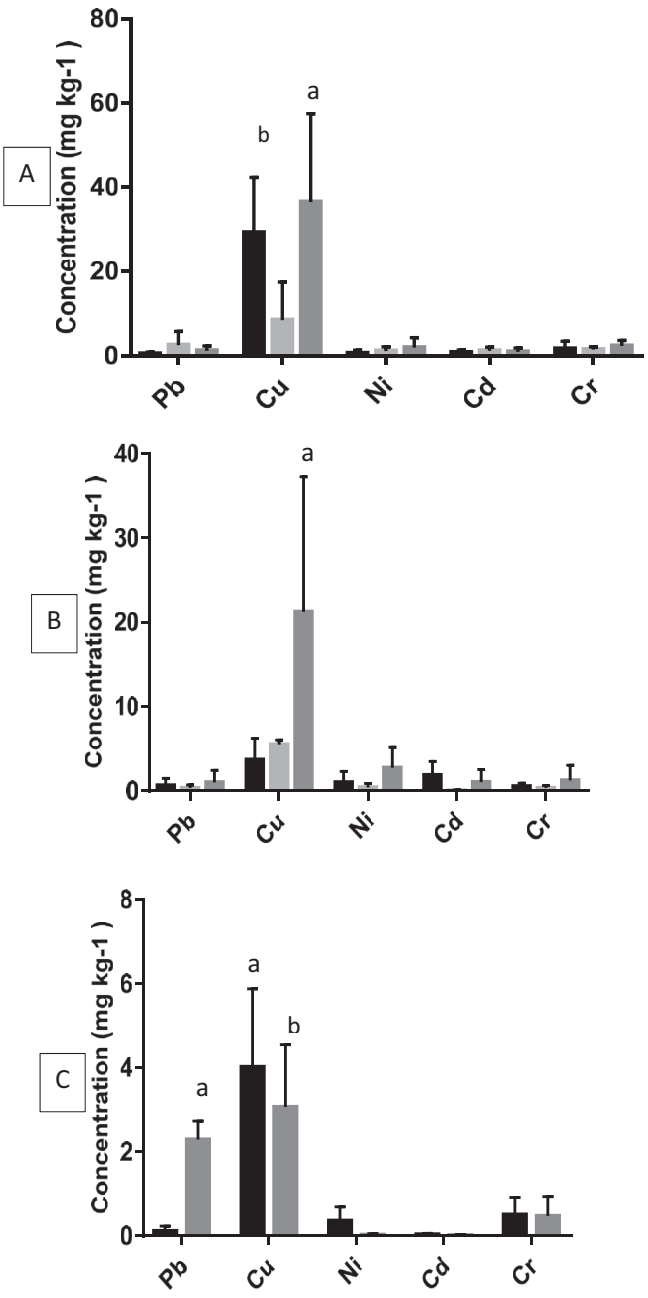

Figure 2. Spatial variation of trace metals. Legends: A = Dumpsite, B= Lagoon front, C= Highrise. Bars of a metal with same alphabets are not significantly different ($p > 0.05$), while those with different alphabets are significantly different ($p < 0.005$).

of lipids inthe toads at the Dumpsite. This might be linked to the location-based variability in weights among the toads (especially the females).

High amounts of metal-containing wastes such as tin cans, e-wastes and metal scraps at the Dumpsite might have released leachate plumes of the metals into soils, thereby increasing their bioavailability. Availability of contaminated food materials in terms of high biomass of insects and other detritivores may also enhance the accumulation of the trace metals in the toads. . This might be responsible for the significantly higher trace metals in the toads at Dumpsite than others at Lagoon front and Highrise. Suedel et al. (1994) earlier stressed the potentials of biomagnification of contaminants through trophic transfer. Loumbourdis et al. (2007) reported significant accumulation of trace metals in frog (*Rana ridibunda*) from lower trophic levels. Aderinola et al. (2012) also observed biomagnification of trace metals from *Macrobrachium macrobrachion* to *Chrysichthys nigrodigitatus* and *Tilapia zillii*.

As expected, sediments at Dumpsite, followed by Lagoon front accumulated higher Cd, Cu and Pb than at Highrise and the established limits. This is attributable to anthropogenic sources such as general use of fossil fuel, discharge of untreated sewage, tin cans, electronic wastes and iron scraps which were present particularly at Dumpsite. Such wastes may release trace metals into the soil (Don-Pedro et al., 2004; Isibor et al., 2016b). This observation corroborates that of Odiete (1999), who submitted that bottom sediment is a repository to most of the metals received by an aquatic ecosystem. At the Lagoon front, trace metals from oil exploration activities around the catchment area could have been resealed into the water column. The metals may adsorb on suspended particulates and precipitate unto the sedentary sediments (Isibor and Oluowo, 2016). With the process unabated, concentrations of the metals in sediment may rise above the concentration in the overlying water column (Omoigberale and Ikponmwosa-Eweka, 2010).

Distinctively high electrical conductivity in soil samples from Dumpsite and Lagoon front can be attributed to the preponderance of domestic wastes at these locations. Furthermore, high acidity levels in soil at Dumpsite may increase the bioavailability of trace metals in the soil, thereby elevating the exposure level of toads which constantly contact the soil with their moist and permeable skin. Combination of these factors may result in high bioaccumulation of trace metals from these locations (Umoru, 2005). The toads, particularly females at the Dumpsite stored up lead from the contaminated environment. Lead in the environmental media is traceable to the electronic components of domestic wastes at the Dumpsite. This might be responsible for the high concentration of lead in the liver and intestinal tissues of the toad. Unregulated lead exposure may lead to hampered cognitive capacity in humans and neurological dysfunctions in vertebrates (WHO, 2009). Copper accumulated in the intestine at a greater rate than in the liver of the toad from the polluted stations (Dumpsite and Lagoon front). However, the concentration of copper was higher in the liver of the toads from the less polluted environment (Highrise) despite the absence of enteric parasites. This suggests that copper was readily taken up by the intestine of the toads in the contaminated environments, but it was preferably accumulated in the liver in a less polluted environment. Copper being a co-factor for digestive and antioxidant enzymes (possibly in response to metal toxicity and/parasitism) might have contributed tos elevated level in the intestine of toads at perturbed stations. Higher levels of copper in liver of toads at Highrise may be attributed to the uptake and binding of copper to metallothioneins and other intra-hepatocellular metal ligands. These observations conform to the findings of Isibor et al. (2016a). Isibor and Imoobe (2017b) also detected the preference of copper for the intestinal tissues of *Clarias gariepinus* and *Tilapia mariae*. The observations also corroborate the report of Lindh et al. (2018) on the comparative tissue distribution and depuration of copper in rainbow trouts. Although copper is an essential metal, it can be toxic to biota at levels elevated above the ecological background levels (Suedel et al., 1994; Song et al., 2015; Xiao et al., 2015; Lindh et al., 2018).

Parasite, *A. africanum* accumulated nickel and copper from the intestine of its host even at greater levels than what was observed in the liver of the host. The indiscriminate accumulation of copper by *A. africanum* from the host may be beneficial to the host and detrimental to the parasite (Griffin and Mitchell, 2007). Although far less concentration of all examined trace metals was observed in the liver and intestine of the toads at Highrise, the concentrations of lead in the intestine of the uninfected toad at this station is traceable to the high concentration detected in the soil at this station, which might have occurred as a result of the discarded electronic devices and lead batteries observed at the vicinity. Concentrations of trace metals in uninfected toads were consistently higher ($p < 0.05$) than the concentrations in the infected ones. Furthermore, no differences occurred between the infected and uninfected toads at Highrise. These observations are attributable to the effects of the parasites on the metals which were minimal at Highrise due

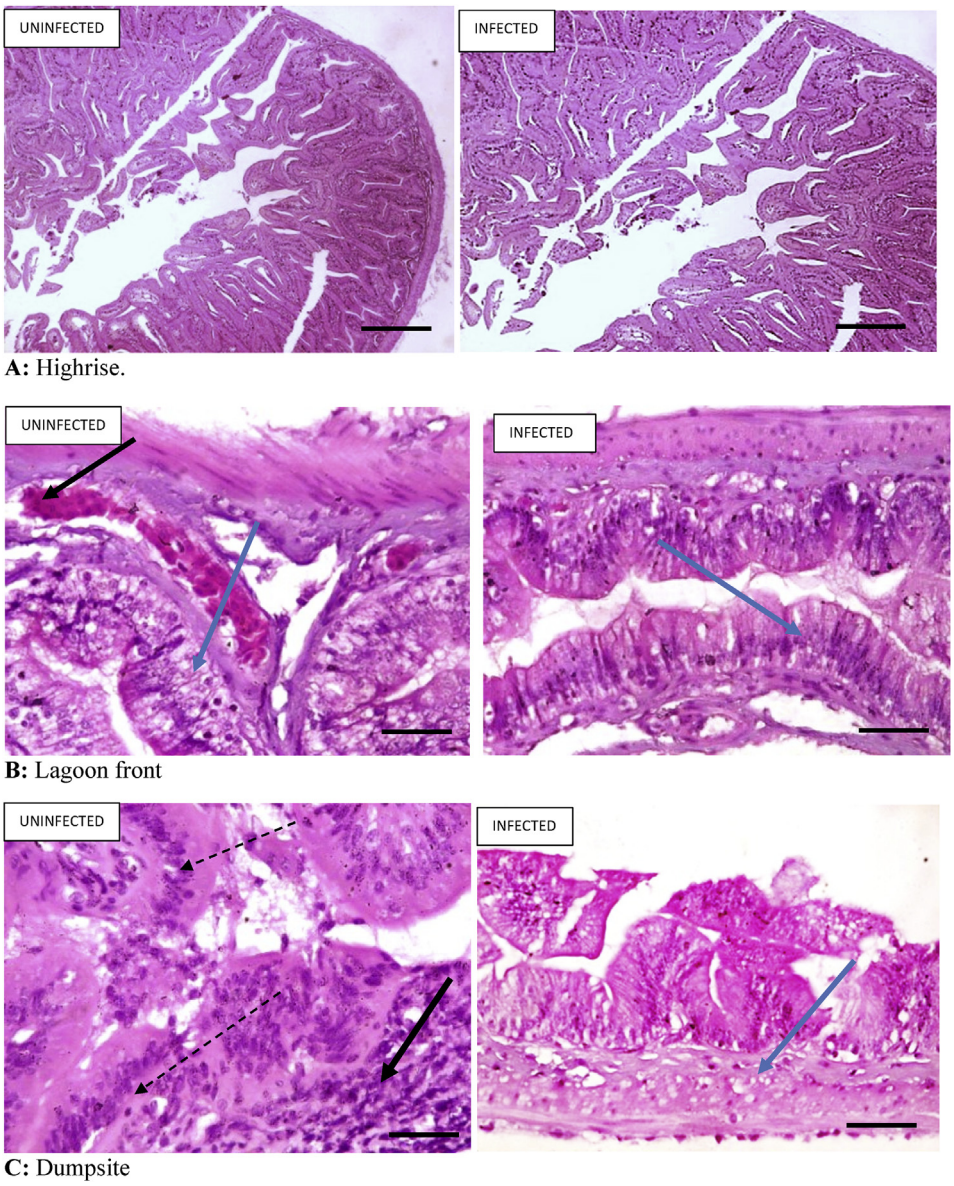

Figure 3. Photomicrographs of intestinal tissues of *A. regularis*. **A- Highrise:** Normal epithelial mucosa, **B- Lagoon front:** severe glandular hyperplasia (black arrow) and infiltration of inflammatory cells (blue arrow), **C- Dumpsite:** focal areas of stunting of villi (blue arrow), glandular hyperplasia (dashed arrow), and infiltration of inflammatory cells (black arrow), mild congestion in submucosa (blue arrow). Scale bars = 25μm.

to low metal levels, except the case of Pb which was also significantly higher ($p < 0.05$) in the uninfected toads than the infected ones. Strong negative regressions of the trace metal concentrations onmost of the parasitological indices analyzed suggest the parasites might have taken up a significant amount of the trace metals from the host. Although, the absorptive integrity of the intestinal mucosa of the host might have been disrupted by the parasites, thereby hampering ingestion of metals alongside other vital nutrients, this does not however occur without the parasite sharing from the load of ingested metal burden. As food and toxicants are ingested by a host, the enteric parasites come in contact with the materials even before absorption process in the host's gut.

In previous studies, strong positive correlations were detected between parasite abundance and metal concentration in a host. Priyadarshani *et al.* (2011) earlier reported a decrease in the immune response of the Indian green frog *Euphlyctis hexadactylus* exposed to Cu, Zn, Pb, and Cd. They demonstrated higher susceptibility of the exposed frogs to infections, in comparison to the unexposed counterparts. De Donato *et al.* (2017) linked the positive correlation between helminth infestation and trace metal concentration in green frog *Pelophylax synkl* to immunosuppression produced by the accumulation of the metal in the tissues the frog.

The detoxification potential of the parasites in the current study may be partly linked to the low parasitological indices, particularly percentage prevalence, which was generally below 50%. Relatively higher parasitological indices previously reported in green frog, *Pelophylax synkl* was associated with more immunosuppression than depuration effects (De Denato et al., 2017).

Although the level of parasitological infestation in the toad *A. regularis* was too low to have inflicted significant implications, it was however enough to muster significant depuration effects in the host. This study showed that at a low level of infection, parasites might play a net role of detoxification of trace metals. In this case, a negative correlation is formed between the parasite abundance and the concentration of the metals. On the contrary, in the event of trace metals occurring as the dominant factors, physiological consequences may ensue and a negative correlation is established.

Furthermore, selective depuration with regards to thresholds of essentiality of the trace metals may be most beneficial to the toad if only the unessential ones are depurated. Hence, insignificant absorption of lead from infected female toads at Lagoon front and cadmium from infected males at Dumpsite may be of disadvantage to the host as the metals may be toxic even at low concentrations (Isibor and Imoobe, 2017b). Absorption of copper by the parasite may have also deprived the toad of the metabolic functions and tissue maintenance the metal offers (Okolo and John, 2006; Yones et al., 2015). Alteration of copper by parasites in infected humans has been previously reported (Karakas et al., 2001; Shenkin, 2006). If excess chromium is absorbed by the parasite from the toad, normal carbohydrate and lipid metabolism may be hampered. Insufficient dietary Cr has been linked to cardiovascular diseases (Anderson, 1986; Lewicki et al., 2014).

Lipid peroxidation is a major reaction underlying tissue injuries mediated by toxicants. Hence lipid peroxidation is regarded as a key biomarker of oxidative stress (Cini et al., 1994; Bosch et al., 2015). Assessment of MDA, which is the by-product of oxidative damage to the phospholipids of cell membranes and antioxidants, indicated significant stress and cellular damage in uninfected toads at Dumpsite and Lagoon front. This was supported by the higher rate of tissue injuries observed in the histopathology of toads intestines at these stations. Generally, the activity of MDA and antioxidant enzymes in the examined toads was in the order of Dumpsite > Lagoon front > Highrise.

The inhibition of SOD activities supports the possibility of oxidative stress in the toads resulting from the toxicity of metals. Although SOD offers protection to biological systems from disruptions of free radicals, it may be overwhelmed in the event of excessive stress. The surpassed capacity of SOD therefore culminates in oxidative stress, which is characterized by its eventual inhibition (Otitoloju and Olagoke, 2011). Catalase is involved in the decomposition of hydrogen peroxide to water and oxygen is very important in protecting the cell from oxidative damage by reactive oxygen species (ROS). Significantly lower levels of catalase in the uninfected toads than the infected at the impacted stations support inferences that the former was more stressed than the latter. The only possible variance between the two groups is the levels of the trace metals.

As for GPx, which is involved in the protection of cell membranes from disruptions of lipid peroxidation, it defends the cell by scavenging oxygen radicals, thereby producing glutathione peroxidase disulfide as a by-product. Moreover, glutathione peroxidase is the cofactor of many enzymes that catalyze the detoxification and excretion of several toxic compounds. Reduction in GPx levels in the uninfected toads compared to the infected may further indicate an active uptake of free anions by cellular glutathione peroxidase.

Results of Dumpsite and Lagoon front showed consistently low activities of antioxidants and higher MDA in the uninfected toads than the infected compared to those at Highrise, irrespective of the sex. Therefore, antioxidant activities and peroxidation can be linked to the infection status of the toads. This suggests that antioxidant enzymes and MDA were possibly affected by the presence of *A. africanum* in the intestine of the host *A. regularis*, suggesting and possible depuration potentials of the parasite, *A. africanum* in the toad.

Insignificant difference in the biochemical biomarkers in the infected toads at the impacted stations and Highrise in conjunction with insignificant difference between infected and uninfected toads at Highrise further points to the suggested inferences of depuration potentials in the parasite. Differences did not occur between the parasitized and unparasitized toads at Highrise probably because little or no pollution was detected at the stations.

Unaltered tissues observed in the intestines of both infected and uninfected toads at Highrise are attributable to the insignificant amount of trace metals detected at the station. However, variability in the tissue alterations between the infected and uninfected hosts at the Lagoon front, particularly at Dumpsite further point to the inference that the parasite *A. africanum* might have offered the infected toads some level of protection from the trace metals by simply sharing in their metal burden.

5. Conclusion

The study suggests possible depurative tendency of the parasite, *A. africanum* on trace metal burden in *Amietophrynus regularis.* When the dominant factor impacting the toad is parasitic infection, parasite prevalence may determine the net effect between parasitological harm and depuration benefit to the host. Hence under controlled conditions, parasites may serve as bioremediators in the event of pollution. We hereby recommend future experiments under controlled conditions in which host *Amietophrynus regularis* and parasite *Amplicaecum africanum* are exposed to known levels of trace metals to ascertain the depuration potentials of the parasite.

Declarations

Author contribution statement

Akinsanya Bamidele and Patrick Omoregie Isibor: Conceived and designed the experiments.

Onadeko A., Benedict and Tinuade Abe: Alimi- Performed the experiments.

Patrick Omoregie Isibor: Analyzed and interpreted the data. Onadeko A., Benedict: Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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