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A Study of the Toxicants and Biomarkers of Oxidative Stress in Samples from Ebubu and Elele-Alimini Communities in Rivers State

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Abstract. This study was carried out to assess the levels of heavy metals, polycyclic aromatic hydrocarbons (PAHs), and antioxidants present in pumpkin leaf (*Telfairia occidentalis*), catfish (*Clarias anguillarus*), and African land snail (*Archachatina marginata*), obtained from Ebubu and Elele-Alimini communities in Rivers State, Nigeria. The heavy metals and PAHs were analyzed using Atomic Absorption Spectrophotometry (AAS) and gas chromatography respectively, while the antioxidants were assayed by conventional methods. Soil samples at Ebubu contained significantly higher Pb, Cr, Cd, and Ni contents than that of Elele-Alimini. The pumpkin leaf from Elele-Alimini contained higher Pb, Zn, Cd, and Fe levels. Cr was undetected in the snails from both locations, while Cd and Ni contents of the snails and catfish at both communities were comparable. For the PAHs, the soil samples from Ebubu contained mostly anthracene (93.37 ppm), benzo[k]fluoranthene (74.36 ppm), fluoranthene (72.64 ppm), and acenaphthylene (47.38 ppm), while those from Elele-Alimini contained more of dibenz[a,h]anthracene (38.65 ppm) and naphthalene (20.55 ppm). Pumpkin leaves from Ebubu were mostly composed of naphthalene, indeno[1,2,3–cd]pyrene, and dibenz[a,h]anthracene, which were undetected in pumpkin leaf samples from Elele-Alimini. In the snail and catfish from Ebubu, acenaphthylene and fluoranthene respectively were the most occurring PAHs, whereas pyrene and phenanthrene respectively had the highest occurrences in snails and catfish from Elele-Alimini. Results for the antioxidant enzymes: catalase and superoxide dismutase in both snails and catfish from Elele-Alimini were significantly higher than those from Ebubu whereas samples from Ebubu contained significantly higher glutathione and malondialdehyde levels. The level of toxicants shown in the foods analyzed in this study is suggestive of potentials to pose significant health risks to the populace when consumed.

Keywords: PAHs, heavy metals, antioxidants, snail, catfish.

1. Introduction

Heavy metals and polycyclic aromatic hydrocarbons are common environmental pollutants. Heavy metal contamination usually follows a cyclic order: industry, atmosphere, soil, water, foods and human. Although the toxic effect and the further threat to human health of heavy metals are based on the degree of exposure, it has been established that increased exposure to heavy metals at even relatively low concentrations can have adverse effects on the system [1]. Polycyclic aromatic hydrocarbons (PAHs), a group of organic compounds which consist of two or more fused aromatic rings, originate mainly from anthropogenic processes, particularly from incomplete combustion of organic fuels and they are distributed widely in the atmosphere [2]. Human beings are exposed to PAH mixtures in gaseous or particulate phases in ambient air. Long-term exposure to high concentrations of PAHs is associated with adverse health problems. Two major methods can be used to measure the level of human exposure to toxic substances in the environment, the first method is the use of environmental monitoring i.e., measuring the concentration of the toxicant, and the second method requires the use of biomarkers to estimate the level of systemic exposure [3]. Biomarkers are factors that can be measured objectively to indicate a normal and functional, pathogenic process, biological process or even a pharmacological response to a therapeutic intervention [4]. Biomarkers are sensitive indicators demonstrating the penetration of a toxic substance into the organism and its distribution among tissues; they therefore are the decisive indicators of the toxic effect of xenobiotics in living systems. It is well-known that the aquatic environment is contaminated with different types of organic and non-organic pollutants [5]. As a result, fishes have been used as aquatic contamination indicators for many years. In the case of an environmental disaster, they usually are unable to leave the affected site [6]. Hence, they bioaccumulate toxic substances and since they are the last link in the food chain in the aquatic environment, they may have negative influence on the safety of food and raw materials that are produced from them [6]. Thus, understanding the reaction and response to

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the exposure to toxic substances by fishes may be very important indicators of health risks [7].

The issue of environmental pollution is very peculiar to the Niger-Delta region. This however, is not unrelated to the activities of oil exploration and, or, exploitation by some of the multinational oil companies resident in the region. Ebubu, a small community in Eleme Local Government Area, and Elele-Alimini Community in Emohua L.G.A, both in Rivers State are some of the numerous communities that have had to deal with the aftermath of environmental pollution due to oil spillages. This has led to a drop in agricultural activities including fishing and crop production. There are also cases of increased health challenges among the populace which could be related to heavy metals as well as polycyclic aromatic hydrocarbons pollution. It was therefore of interest to conduct a study to evaluate the levels of some of these heavy metals, PAHs in soil, plant, fish and snail and also to determine the level of biological changes in the fish and snail samples using some selected biomarkers of oxidative damage.

2. Materials and methods

2.1. Materials

The high purity reagents of analytical grades were obtained from BDH chemical Ltd., UK.

2.2. Sampling

Samples for this work were collected in June 2017 from Ebubu Community in Eleme L.G.A and Elele-Alimini Community in Emohua L.G.A, both in Rivers State. The results obtained are further compared with FAO/WHO reference standards for these compounds.

Three layers of the soil samples measuring 10 cm apart were bagged in perforated bags while live fish samples were collected from the rivers within the areas under study through the help of a fisherman.

The samples were kept in a 20 L container with water in order to keep the fishes alive. The snail specimens were collected from bushes within the area under study and were kept in perforated bags while the vegetables were also collected from gardens within the area under study and they were all transported to the laboratory for analysis.

2.3. Heavy metal analysis

The heavy metals (lead (Pb), chromium (Cr), zinc (Zn), cadmium (Cd), nickel (Ni), and iron (Fe)) in the soil samples were determined using an atomic absorption spectrophotometer following the method of Agomuo and Amadi [8]. One (1) gram of soil sample was weighed out and transferred into an empty 250 ml beaker. To this soil sample, a mixture of 15 ml of HNO_3, H_2SO_4, and $HClO_4$ in a ratio of 5:1:1 was added. The mixture was gently stirred and placed on a heating mantle up to a temperature of 80°C till a clear solution was obtained. The mixture was cooled and made up to 30 ml with 2% HNO_3 and filtered. The concentrations of the heavy metals were obtained using an atomic absorption spectrophotometer (Shimadzu AA-670, Japan) after the preparation of a reference solution.

2.4. Assay for polycyclic aromatic hydrocarbons

Samples (2 g) were weighed into a clean extraction centenary. Exactly 10 ml of extraction solvent (pentane) was added into the samples and mixed thoroughly and allowed to settle. The mixtures were carefully filtered into clean solvent rimed extraction bottle using filter paper fitted into Buchner funnel. The extracts were concentrated to 2 ml and then transferred for clean up/separation.

2.4.1. Clean up / separation

Moderately packed glass wool (1 cm) was placed at the bottom of 10 mm ID x 250 mm loup chromatographic column.

Slurry of 2 g activated silica in 10 ml methylene chloride was prepared and placed into the chromatographic column. To the top of the column was added 0.5 cm of sodium sulfate. The column was rinsed with additional 10 ml of methylene chloride.

The column was pre-eluted with 20 ml of pentane, this was allowed to flow through the column at a rate of about 2 minute until the liquid in the column was just above the sulfate layer. Immediately 1 ml of the extracted sample was transfer into the column. The extraction bottle was rimed with 1 ml of pentane and added to the column as well.

The stop–clock of the column was opened and the effluent was collected with a 10 ml graduated cylinder.

Just prior to exposure of the sodium sulfate layer to air, pentane was added to the column in 1-2 ml increments, and subsequently, the eluent (8-10 ml) was collected and labeled as aliphatic.

2.4.2. Gas chromatographic analysis

The concentrated aliphatic fraction was transferred into labeled vials with Teflon and rubber septum into the column. Separation occurs as the vapor constituent partition between the gas and liquid phases. The sample was automatically detected as it emerged from the column (at a constant flow rate) by the FID detector whose response is dependent upon the composition of the vapor.

2.4.3. Quality Assurance/control

A procedural blank analysis was performed with every 3 samples to monitor interferences and cross-contamination. Each analysis was performed in triplicate and the experimental detection limit was 0.01 mg/kg dry matter for individual compounds.

2.5. Assay for biomarkers of oxidative stress

2.5.1. Sample preparation

After samples were collected, fish tissue samples (liver) and snail tissue samples (foot) were weighed and immediately kept in the refrigerator. Sample homogenates were made of individual respective

tissues with chilled phosphate buffer (0.1 M, pH 7.4), and centrifuged in a centrifuge at 9,000 x *g* for 20 min at 4 ºC. The supernatant obtained was used for further analysis

2.5.2. Catalase activity assay

Catalase activity was determined according to Clairborne [9] with slight modifications.

Briefly, 0.2 ml of sample (equivalent to 50-100 µg protein) was added to 50 mM of phosphate buffer (pH 7.4) containing 100 mM (v/v) of H_2O_2 (Sigma, St Louis, USA) in a total of 1 ml. The reaction mixture was incubated for 2 min at 37 ºC and the rate of absorbance change (ΔA/min) at 240 nm was recorded, which indicated the decomposition of H_2O_2. Activities were calculated using the molar extinction coefficient of H_2O_2 at 240 nm, 43.59 l/mol/cm. One unit of catalase activity equals the amount of protein that converts 1 µmol H_2O_2/min. All samples were measured in triplicates.

Catalase activity was further calculated using the formula below:

$$\frac{\Delta \text{ in Abs/min}}{43.59 \text{ l/mol/cm}}$$

2.5.3. Superoxide dismutase (SOD) activity assay

The activity of superoxide dismutase was determined by the method of Misra and Fridovich [10].

Briefly, an aliquot of supernatant from 12,000 x *g* centrifugation of tissues homogenates was diluted in distilled water to make a 1:10 dilution. The diluted sample (200 µl) was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3 ml of freshly prepared 0.3 mM epinephrine to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5 ml buffer, 0.3 ml of substrate (epinephrine) and 0.2 ml of distilled water. The increase in absorbance at 480 nm was monitored every 30 s for 2.5 min.

2.5.4. Estimation of reduced glutathione (GSH) and glutathione *S*-transferase (GST) activity

The method of Sedlak and Lindsay [11] was followed in estimating the level of reduced glutathione.

An aliquot of the sample (0.2 ml) was added to 1.8 ml of distilled water and 3 ml of the precipitating solution and mixed. The mixture was then allowed to stand for approximately 5 min and then centrifuged at 1200 x *g* for 10 minutes (4 ºC). One milliliter of the filtrate was added to 4 ml of 0.1 M phosphate buffer. 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB) (0.5 ml) was finally added. A blank was prepared with 4 ml of 0.1 M phosphate buffer, 1 ml of distilled water and 0.5 ml of DTNB. The solution was kept at room temperature for 15 minutes and read at 412 nm on a spectrophotometer. GSH was proportional to the absorbance at that wavelength and the estimate was obtained from the GSH standard curve.

The results were expressed in µg GSH/min/mg tissue. GSH activity is further calculated using the formula below:

Abs. x 200 (dilution factor)

GST activity was assayed spectrophotometrically by monitoring the conjugation of 1-chloro-2,4-dinitro benzene (CDNB) with GSH at λ_{max} = 340 nm at 37 ºC following the method of Harbig *et al.* [12].

2.5.5. Statistical analysis

Data was expressed as mean ± SD. The data were analyzed by one way analysis of variance (ANOVA) using the least standard deviations (LSD). *p* values < 0.05 were considered as significant.

3. Results and discussion

The heavy metal concentrations of soil, pumpkin, snail, and catfish samples from Ebubu and Elele-Alimini communities are presented in Table 1.

The result showed that the Pb, Cr, Cd, and Ni levels of Elele-Alimini soil samples were significantly lower than those obtained at Ebubu community while Elele-Alimini soils produced significantly higher Zn levels than that from Ebubu, but a comparable Fe content. Among the heavy metals analyzed, only Fe exceeded the FAO/WHO permissible limits for soil samples. Some researchers have reported values above the FAO/WHO limits [13] and lower (controls) than the values reported in this study. It is well known that natural soils contain significant concentrations of iron [14, 15]. High level of iron has been reported to cause vomiting, anxiety, nausea, brain hemorrhage, cardiac arrest, metabolic disorder and tension [16].

The heavy metal concentration in the pumpkin leaf from Elele-Alimini and Ebubu shown in the table indicated that chromium was not detected in the plant samples. The result further showed that the concentration of zinc and nickel in both locations were below FAO/WHO standards. However, lead, chromium and iron had concentrations above the FAO/WHO standard limits and this trend was observed in both Ebubu and Alimini. Chromium was undetected in the snail samples of both locations while lead, zinc, cadmium, nickel and iron were found in concentrations above the FAO/WHO limits.

For the catfish samples, the Pb, Zn, and Fe contents obtained from that of Elele-Alimini locality, where significantly higher than that of Ebubu, while their Cd and Ni contents were equivalent. Also, only the Pb and Cr levels from Ebubu and Elele-Alimini samples respectively occurred below the permissible limits. This generally implies that adequate measures to prevent the predisposition of the residents of these localities from the adverse effects of these heavy metals become extremely imminent.

Table 1. Heavy metal contents of soil, pumpkin, snail, and fish samples from Ebubu and Alimini communities

Sample/ location	Lead (mg/kg)	Chromium (mg/kg)	Zinc (mg/kg)	Cadmium (mg/kg)	Nickel (mg/kg)	Iron (mg/kg)
SOIL						
Ebubu	7.69±0.49^{b}	5.98±0.11^{b}	6.67±0.45^{b}	0.77±0.05^{b}	1.62±0.08^{b}	3094.55±39.59^{a}
Alimini	2.27±0.04^{a}	5.29±0.09^{a}	15.14±0.16^{a}	0.19±0.02^{a}	0.69±0.45^{a}	3035.98±51.52^{a}
FAO/WHO	300 *	175*	260*	2.5*	10*	1.0*
PUMPKIN						
Ebubu	5.13±0.17^{b}	ND	22.38±0.89^{b}	0.38±0.03^{b}	2.39±0.14^{a}	121.39±1.21^{b}
Alimini	9.75±0.25^{a}	ND	34.22±0.32^{a}	0.75±0.06^{a}	3.09±0.17^{a}	5318.34±120.95^{a}
FAO/WHO	0.3*	102*	55*	0.2*	10*	1.0*
SNAIL						
Ebubu	10.16±0.19^{b}	ND	40.61±0.55^{b}	1.03±0.04^{a}	3.05±0.19^{a}	97.69±0.92^{b}
Alimini	7.96±0.44^{a}	ND	51.13±0.43^{a}	0.98±0.05^{a}	2.95±0.18^{a}	2142.42±54.18^{a}
FAO/WHO	2*	102*	5*	0.01*	10*	0.30*
CATFISH						
Ebubu	1.43±0.0.06^{b}	ND	15.80±0.69^{b}	0.15±0.02^{a}	0.53±0.04^{a}	25.58±1.12^{a}
Alimini	2.88±0.14^{a}	1.82±0.23	29.89±1.68^{a}	0.17±0.03^{a}	0.59±0.03^{a}	51.63±0.92^{b}
FAO/WHO	2*	102*	5*	0.01*	10*	0.30*

Values are mean ± SD of triplicate determinations. Values with different alphabets are significantly different. All comparisons were done at $p < 0.05$.

*= values are FAO/WHO standard for the corresponding samples.

Table 2. PAH concentrations of soil, pumpkin, snail, and fish samples from Ebubu and Alimini communities

Compounds	**SOIL (ppm)**		**PUMPKIN LEAF (ppm)**		**LAND SNAIL (ppm)**		**CATFISH (ppm)**	
	Ebubu	**Alimini**	**Ebubu**	**Alimini**	**Ebubu**	**Alimini**	**Ebubu**	**Alimini**
Naphthalene	ND	20.54±1.4^{a}	71.27±3.7^{b}	ND	ND	ND	ND	ND
Acenaphthene	47.48±2.7^{a}	ND	ND	ND	50.63±3.5^{a}	ND	ND	ND
Acenaphthylene	1.36±0.2^{a}	ND	2.99±0.1^{b}	3.15±0.1^{b}	3.70±0.2^{c}	3.2±0.1^{b}	4.86±0.2^{d}	6.32±0.2^{e}
Fluorene	ND	3.88±0.0	ND	ND	ND	ND	ND	ND
Phenanthrene	ND	4.78±0.1^{a}	ND	ND	ND	53.92±2.8^{b}	ND	90.46±3.1^{c}
Anthracene	93.37±3.6^{a}	ND	1.05±0.0^{b}	ND	ND	ND	1.32±0.0^{c}	ND
Fluoranthene	72.64±3.1^{a}	0.25±0.0^{b}	ND	ND	ND	83.57±4.6^{c}	72.89±3.3^{b}	ND
Pyrene	ND	0.15±0.0^{a}	1.35±0.0^{b}	ND	ND	ND	ND	ND
B[g,h,i]p	3.53±0.1	3.68±0.1	5.47±0.1	5.63±0.1	0.40±0.0	3.99±0.1	5.28±0.3	ND
Chrysene	ND	0.01±0.0^{a}	ND	ND	9.21±0.2^{b}	ND	3.35±0.0^{c}	ND
B[b]f	1.23±0.0^{a}	ND	1.48±0.0^{a}	5.32±0.1^{c}	ND	ND	2.62±0.0^{d}	2.71±0.1^{d}
B[k]f	74.36±2.6^{a}	ND	1.00±0.0^{b}	1.95±0.0^{c}	ND	51.96±3.2^{d}	ND	ND
B[a]p	1.13±0.0^{a}	2.84±0.0^{b}	1.64±0.0^{c}	9.43±0.2^{d}	5.89±0.1^{e}	1.07±0.0^{a}	2.66±0.1^{b}	1.20±0.0^{a}
I[1,2,3-cd]p	32.52±2.1^{a}	ND	47.38±2.8^{b}	ND	ND	32.17±2.4^{a}	ND	18.89±1.5^{c}
D[a,h]a	34.50±1.7^{a}	38.65±2.4^{a}	50.53±3.0^{b}	ND	2.93±0.0^{c}	ND	48.27±2.5^{d}	22.51±1.8^{e}
B[a]a	ND	ND	ND	ND	ND	31.73±2.7^{a}	1.55±0.0^{b}	ND
∑PAH	362.12	74.78	184.16	25.48	72.76	261.61	142.80	142.09
∑cPAH	143.74	41.50	102.03	16.70	18.03	116.93	58.45	45.31

Values represent mean ± S.D of triplicate determinations. Values bearing different superscript letters (a-e) across the row are significantly different ($p < 0.05$).

Abbreviations: B[b]f = benzo[b]flouranthene, B[k]f = benzo[k]fluoranthene, B[a]p = benzo[a]pyrene, I[1,2,3-cd]p = indeno[1,2,3-cd]pyrene, D[a,h]a = dibenz[a,h]anthracene, B[g,h,i]p = benzo[g,h,i]perylene, B[a]a = benzo[a]anthracene, ∑cPAHS- total carcinogenic PAHs.

The PAH concentration of the various samples from Ebubu and Elele-Alimini locations are shown in Table 2. A total of 16 PAH were assayed in all the samples. Anthracene, fluoranthene, and benzo[k] flouranthene, were the most predominant PAH in the Ebubu soil while in Alimini, dibenz[a,h]anthracene and naphthalene were the most predominant. The pumpkin leaves obtained from Ebubu showed abundance of naphthalene, a non carcinogenic PAH, followed by dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene which were all undetected in samples from Alimini. In Ebubu snails, chrysene and acenaphthene were the most abundant carcinogenic and non carcinogenic PAH respectively, which were undetected in the Elele-Alimini samples, while fluoranthene and phenanthrene were the predominant non carcinogenic PAH and benzo[k]flouranthene, indeno[1,2,3-cd]pyrene, and benzo[a]anthracene, the most abundant carcinogenic PAH in Elele-Alimini snail samples. Further, fluoranthene and phenanthrene were the most abundant non carcinogenic PAH in Ebubu and Elele-Alimini catfish respectively, while dibenz[a,h]anthracene was the most predominant carcinogenic PAH in both catfish samples. The result further showed that in all the samples analyzed soil samples from Ebubu contained the highest amount of total PAH with predominance of carcinogenic PAH. However, for the edible samples, snails from Elele-Alimini produced the highest amount of PAH, followed by pumpkin leaves from Ebubu while the least amount of PAH and carcinogenic PAH was found in Elele-Alimini pumpkin leaves and Ebubu land snails. Consumers of these edibles should be particularly cautious of the excessive amount of carcinogenic PAH in Elele-Alimini land snails and Ebubu pumpkins, having recorded the highest amount of carcinogenic PAH, and as such, are capable of producing significant health threats. More so, the total PAH and total carcinogenic PAH in all samples and locations in this study, exceeded the WHO recommended maximum permissible limit of 10 ppm [17].

Table 3. Biomarkers of oxidative stress in snail and fish samples from Ebubu and Alimini communities

	ALIMINI		**EBUBU**	
PARAMETERS	**Snail**	**Catfish**	**Snail**	**Catfish**
GSH	3.20 ± 0.87^{a}	11.13 ± 0.50^{b}	7.02 ± 0.53^{c}	18.46 ± 1.60^{d}
GST	1.58 ± 0.08^{a}	1.66 ± 0.02^{a}	1.59 ± 0.04^{a}	1.61 ± 0.073^{a}
CAT	0.14 ± 0.07^{a}	0.24 ± 0.01^{b}	0.76 ± 0.07^{c}	1.22 ± 0.16^{d}
SOD	0.59 ± 0.04^{a}	0.71 ± 0.05^{b}	0.25 ± 0.01^{c}	0.27 ± 0.08^{c}
MDA	0.45 ± 0.08^{a}	0.44 ± 0.11^{a}	1.02 ± 0.04^{b}	0.71 ± 0.08^{c}

Values represent mean ± S.D of triplicate determinations. Values bearing different superscript letters (a-d) across the row are significantly different ($p < 0.05$).

Oxidative stress enzymes from snail and fish samples obtained from Ebubu and Elele-Alimini localities were presented in Table 3. The catfish from both communities produced the higher concentrations of GSH while Elele-Alimini snails contained the least amount of GSH. However, no significant change was found for the GST levels of both snail and catfish from both locations. Farombi *et al.* [18] explained that increased GSH activity is an oxidative stress response to environmental pollutants. Also it was observed that there was a high level of GSH and GST activity in the Ebubu sample, these were also responses to oxidative stress. The increase in the GSH activity could be as a result of the interaction between the xenobiotics and cellular GSH in the process of defending the organism against free radicals. This result is in agreement with the findings of Grara [19]. The result further suggests that the snail and fish samples from Ebubu were more exposed to these environmental toxicants than the snail and fish samples from Alimini. The samples from Ebubu produced higher catalase concentrations while those from Elele-Alimini recorded higher SOD concentrations. The elevated catalase activity could be associated with an adaptive mechanism by the organisms to rid itself of reactive oxygen species (ROS) resulting possibly from accumulation of PAH and polycyclic aromatic biphenyls [19, 20]. The increased level of SOD activity observed in the snail and fish from Elele-Alimini could be associated with the enzymes response to free radicals, thus indicating the presence of toxicants in the environment. No significant difference was found between the SOD levels of snail and catfish samples obtained from Alimini, while the values were significantly lower than those from Ebubu community. Increased MDA level is associated with high level of free radicals generated by contaminants in the cell [21]. The expression of the MDA activity whether high or low as seen in the result is a pure indication oxidative stress. The result is also in line with the findings of Siwela *et al.* [22] that observed a significant increase in the level of MDA in the marine snail *Lymnaea natalensis* exposed to environmental pollutants.

4. Conclusions

This study has shown the predisposition of the soil in Ebubu community to more heavy metal contamination than at Elele-Alimini, thus, a higher risk of heavy metal toxicity. Further, the results indicated that residents of Ebubu community were more susceptible to PAH toxicity as a result of the higher total PAH contents of the soil, pumpkin leaf, and catfish. Also, majority of the enzyme markers of oxidative stress from Ebubu were consequently

higher than those at Elele-Alimini. Thus, the level of toxicants shown in the samples analyzed in this study, especially in Ebubu community, is suggestive of potentials to pose significant health risks to the populace.

Conflict of interest

The authors declare no conflict of interest regarding this article.

References

[1]. M.I. Castro-González, M. Méndez-Armenta, Heavy Metals: Implications associated to fish consumption, Environmental Toxicology and Pharmacology 26 (2008) 263-271. DOI: 10.1016/j.etap.2008.06.001

[2]. C.J. Okereke, P.U. Amadi, Evaluation of polycyclic aromatic hydrocarbons (PAH) in barbecued meat and roasted potato samples sold in Port Harcourt metropolis, Nutritional Ecology and Food Research 4 (2017) 1-5. DOI: 10.1166/jnef.2017.1150

[3]. P.J. Peterson, Assessment of exposure to chemical pollutants in food and water, In: Mineral components in foods, pp. 413-431, P. Szefer and J.O. Nriagu (Eds.), CRC Press, 2007.

[4]. National Institute of Health (NIH), Biomarkers Definitions Working Group, Biomarkers and Surrogate endpoints: preferred definitions and conceptual framework, Clinical Pharmacology and Therapeutics 69 (2001) 89-95. DOI: 10.1067/mcp.2001.113989

[5]. A. Sen, A. Kirikbakan, Biochemical characterization and distribution of glutathione *S*-transferases in leaping mullet (*Liza saliens*), Biochemistry (Moscow) 69 (2004) 993-1000.

[6]. R. Gadzala-Kopciuch, B. Berecka, J. Bartoszewicz, G. Buszewski, Some considerations about bioindicators in environmental monitoring, Polish Journal of Environmental Studies 13 (2004) 453-462.

[7]. R. Van der Oost, J. Beyer, N.P. Vermeulen, Fish bioaccumulation and biomarkers in environmental risk assessment: a review, Environmental Toxicology and Pharmacology 13 (2003) 57-149. DOI: 10.1016/S1382-6689(02)00126-6

[8]. E.N. Agomuo, P.U. Amadi, Accumulation and toxicological risk assessments of heavy metals of top soils from markets in Owerri, Imo state, Nigeria, Environmental Nanotechnology, Monitoring and Management 8 (2017) 121–126. DOI: 10.1016/j.enmm.2017.07.001

[9]. A. Clairborne, Catalase activity, in Handbook of methods for oxygen radical research, pp. 237–242, A.R. Greewald (Ed.), CRC Press, Boca Raton, 1995.

[10]. H.P. Misra, I. Fridovich, The role of superoxide anion in the auto-oxidation of epinephrine and a simple assay for superoxide dismutase, Journal of Biological Chemistry 247 (1972) 3170–5.

[11]. J. Sedlak, R.H. Lindsay, Estimation of total, protein-bound and non-protein sulfhydryl groups in tissue with Ellman's reagent, Analytical Biochemistry 25 (1968) 192–205. DOI: 10.1016/0003-2697(68)90092-4

[12]. W.H. Harbig, M.J. Pabst, W.B. Jacob, Glutathione-S-transferase. The first enzymatic step in mercapturic acid formation, Journal of Biological Chemistry 249 (1974) 7130–9.

[13]. C.J. Okereke, P.U. Amadi, Accumulation and risk assessment of heavy metals contents of school playgrounds in Port Harcourt metropolis, Rivers State, Journal of Chemical Health and Safety 24 (2017) 11-22. DOI: 10.1016/j.jchas.2017.01.002

[14]. C.M.A. Ademoroti, Environmental chemistry and toxicology, pp. 171 – 204, Foludex Press Ltd. Ibadan, 1996.

[15]. O.O. Aluko, P.A. Oluwande, Characterization of leachates from a municipal solid waste landfill site in Ibadan, Nigeria, International Journal of Environmental Health Research 2 (2003) 83-84.

[16]. S. Kaur, P. Mehra, Assessment of heavy metals in summer and winter seasons in River Yamuna segment flowing through Delhi, India, Journal of Environment and Ecology 3 (2012) 1-17. DOI: 10.5296/jee.v3i1.2675

[17]. A.S. Adekunle, O.J.A. Oyekunle, S.O. Oluwaseyi, W.M. Nobanathi, G.O. Olutona, O.R. Obisesana, Determination of polycyclic aromatic hydrocarbon levels of groundwater in Ife north local government area of Osun state, Nigeria, Toxicology Reports 4 (2017) 39-48. DOI: 10.1016/j.toxrep.2016.10.002

[18]. E.O. Farombi, O.A. Adelowo, Y.R. Ajimoko, Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (*Clarias gariepinus*) from Nigeria Ogun River, International Journal of Environmental Research and Public Health 4 (2007) 158-165.

[19]. N. Grara, Evaluation de la toxicité de certains polluants industriels sur un animal bioaccumulateur (gastéropode *Helix aspersa*): Cas des métaux. Mémoire de doctorat, Université Badji Mokhtar, Annaba Algérie, p. 280, 2011.

[20]. D.R. Livingstone, C.L. Mitchelmore, S.C. O'Hara, M. Lemaire, P. J. Sturve, L. Förlin, Increased potential for NAD(P)H-dependent reactive oxygen species production of hepatic subcellular fractions of fish species with in-vivo exposure to contaminants, Marine

Environmental Research 50 (2000) 57-60. DOI: 10.1016/S0141-1136(00)00043-X

[21]. N. Belhaouchet, M.R. Djebar, L. Meksem, N. Grara, I. Zeriri, H. Berrebbah, Evaluation of the biomarkers of the oxidative stress induced by a biopesticide. The Spinosad on an alternate model: *Helix aspersa*, Journal of Applied Sciences Research 8 (2012) 4199-4206.

[22]. A.H. Siwela, C.B. Nyathi, Y.S. Naik, A comparison of metal levels and antioxidant enzymes in freshwater snails, *Lymnaea natalensis*, exposed to sediment and water collected from Wright Dam and Lower Mguza Dam, Bulawayo, Zimbabwe, Ecotoxicology and Environmental Safety 73 (2010) 1728-32. DOI: 10.1016/j.ecoenv.2010.08.001

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