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ORIGINAL ARTICLE

Evaluation of lipid peroxidation products and heavy metals load of selected crustaceans from Ogbe-Ijoh and Main Market Watersides in Warri South

Fadairo Ekaette[1], Otite-Douglas Mfon[2], Olowoyo Niyi[3], Birma Godwin[4], Bankole Adedoyin[5]. and Alegbemi Adebayo[6]

[1, 2, 3, 6] Department of Science Laboratory Technology, Petroleum Training Institute, PMB 20 Effurun, Nigeria.

[4] Industrial Safety and Environmental Technology Department, Petroleum Training Institute, Effurun.

[5]Department of Environmental Management and Toxicology, Federal University of Petroleum Resources, PMB. 1221 Effurun, Nigeria

Correspondence: Fadairo Ekaette
Email: fadairo_e@pti.edu.ng

ABSTRACT

Contamination of sea foods by heavy metals continues to be one of the devastating environmental problems that pose serious health risk to people living in the Niger Delta. Heavy metals concentrations and lipid peroxidation levels were investigated in shrimps and crabs from watersides of Ogbe-Ijoh (OIW) and Main Market (MMW) communities. The concentrations of Pb, Cu, Cr, Cd, As and Ni, which are crude oil complimentary metals, were evaluated in shrimps (Nematopalaemon hastatus) and land crab (Cardiosoma armatum) bought from both markets using the atomic absorption spectrophotometer (ACCU SYS 212) while malondialdehyde (MDA), an index of lipid peroxidation, levels in the respective crustaceans samples were measured using the Hitachi 912 UV at an absorbance of 532 nm against blank as thiobarbituric acid reactive substances (TBARS) and calculated using the molar extinction co-efficient of 1.56 x10^5 M^{-1} cm^{-1}. The result shows that the concentrations of Pb (0.76±0.06 and 0.47±0.05) mgkg^{-1} in N. hastatus and C. armatum from MMW were higher when compared to Pb (0.25±0.05 and 0.17±0.05) mgkg^{-1} in equal amount of homogenized N. hastatus and C. armatum samples respectively from OIW. The concentrations of CU Cr, As and Ni in N. hastatus and C. armatum from MMW were also higher when compared to the concentrations in N. hastatus and C. armatum from OIW, although, their levels were not significant (P>0.05). The concentration of Cd was second with values of (0.055±0.01 mg/kg and 0.082±0.05 mg/kg) and (0.03±0.001mg/kg and 0.04±0.00mg/kg) for N. hastatus and C. armatum from MMW and OIW respectively. The MDA levels were (12.83 ± 0.10 and 3.55 ± 0.05) µmole MDA/g tissue and (5.37±0.25 and 3.79±0.09) µmole MDA/g tissue for N. hastatus and C. armatum from MMW and OIW respectively. This study indicates the presence of some levels of Pb and Cd in crustacean from MMW and OIW, however, the levels were not significant (P>0.05) since the values obtained were within WHO and NAFDAC permissible established limits for metals in sea foods. Thiobarbituric acid reactive substances (TBARS) activities, signaling lipid peroxidation in N. hastatus and C. armatum from MMW and OIW were also established by this study. The danger of environmental and food chain mediated tissue bioaccumulation of crude oil accompanying metals in significant levels in human is envisioned by this study.

Keywords: *Evaluation, Lipid Peroxidation, Heavy Metal Load, Crustaceans, Main Market Waterside, Ogbe-Ijoh Watersides, Warri South.*

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INTRODUCTION

Alterations in lipid metabolism are often linked with exposure to various environmental contaminants including heavy metals. These metals, which include lead (Pb), cadmium (Cd) and mercury (Hg), are ubiquitous toxic substances that can easily bio-accumulate in biological tissues and are extensively distributed in the environment. Studies show that chronic exposure of mice to heavy metals induced

alterations in lipid metabolism [8]. Malondialdehyde (MDA) level is use as a marker of lipid peroxidation and an indication of oxidative stress in living organisms, especially humans. Studies also show its use as an indicator of food quality, as high levels of MDA can indicate rancidity in seafood [4, 10,12]. Several studies have investigated MDA levels in seafood from the Niger Delta region, with varying results. A study done in Nigeria, demonstrates that malondialdehyde levels in biological tissues play very significant role in oxidative stress [11]. Another study carried out in the same year showed that MDA levels of Tilapia, Catfish, Bonga, and Barracuda fishes, collected from a more crude oil polluted location in the Niger Delta region were significantly higher than that of fishes from a less polluted location [1].

There are reports showing the direct correlation of MDA levels of aquatic organisms with heavy metal pollution load of the organisms and the aquatic environment [2, 3]. Another study analyzed MDA levels of aquatic organisms, collected from a river in the Niger Delta region and found that their MDA levels were significantly higher in organisms collected from the river compared to a controlled group of same species of aquatic organism raised in a controlled environment [5]. Study also found that MDA levels were positively correlated with the levels of heavy metals and pesticides in the river water [5]. Overall, the studies reviewed suggest that sea foods from the Niger Delta region may be at risk of contamination from heavy metallic pollutants, which can lead to elevated levels of MDA and reduced food quality. Main market and Ogbe Ijoh watersides are shoreline settlements in the Niger Delta.

Besides the vast crude oil deposit in the Niger Delta region, the region is also known for its vast coastline and aquatic resources, which support the livelihoods of many people in the region. However, the area is also prone to environmental pollution, including from oil spills and other industrial activities, which can impact the quality of sea foods from the region. The metals investigated in this study are crude oil accompanying metals (natural occurring elements that are found along crude oil deposits and usually present on pollution sites) with examples as Ni, Vn, Fe, Cu, Zn, Pb, Hg, As, Cd and Cr. The presence of these metals in crude oil can have both positive and negative impacts on the refining and processing of crude oil. For example, the presence of vanadium and nickel can lead to increase corrosion in refining equipment and can also reduce the quality of fuels produced from the crude oil. On the other hand, some of these metals, such as copper and zinc, can act as catalysts during refining and processing, improving the efficiency of these processes. There are numerous reports investigating MDA levels and metallic load in seafood from various regions, including Nigeria (4, 5), nonetheless, the results are specific to the species of seafood, the location, the processing and storage methods, and the analytical methods used to measure the metal concentrations and MDA levels. However, there appears to be paucity of information and data available on metallic load and lipid peroxidation products of the crustaceans from the location chosen in this study. This study therefore seeks to evaluate the concentrations of crude oil accompanying metallic compounds in shrimps and crabs from watersides of Ogbe-Ijoh and Main Market, Warri South with a view to monitoring the load of these crude oil accompanying elements as an index of health protection for every stakeholder of the community.

MATERIAL AND METHODS

This was done according to the method outlined by [6].The crustacean samples were bought from Ogbe Ijoh and Main Market watersides, Warri South Local Government Area. The samples were prepared by the market women to remove the non-edible parts. The samples were washed immediately with deionized water and stored in labeled sterile transparent sample bags and taken to the laboratory. The samples were then chopped into pieces, homogenized in 0.09% normal saline solution using a white porcelain mortar and pestle, sieved and stored in plain 40 ml sample tubes until needed for analysis.

Determination of metals in samples was done according to the AAS procedure outlined by [13] and modified by [6]. Two gram (2g) of each samples were weighed and put into a beaker. A mixture of nitric and perchloric acid (HNO_3 and $HClO_4$) in the ratio 4:1 was added to each sample. The samples were heated on a hot plate for about 1hr. few drops of hydrogen peroxide were added and the mixtures were re-heated for until almost dried. It was filtered and residue rinsed with deionized water into a 100ml volumetric flask and the mark made up to mark with more deionized waiter. Although, arsenic concentrations in the shrimp and crab samples were determined using the AAS, a slight modification was done on sample. As follows: 5ml of HCl treated sample for arsenic, was pretreated with a mixture of potassium iodide and ascorbic acid, and allowed to stand for one hour at room temperature. The concentration of arsenic in the samples were then determined at 198nm using the AAS.

MDA levels of crustacean were determined by the colorimetric method outlined by [7]. Malondialdehyde (MDA), a product of lipid peroxidation, when heated with 2-thiobarbituric acid (TBA) under alkaline condition forms a 1:2 adduct red coloured complex, which has absorption maximum at 532 nm. The intensity of colour generated is directly proportional to the concentration of MDA in the sample [7].

The reagents were reconstituted in the laboratory. Glacial acetic acid, 0.05 M NaOH (0.2g of NaOH in 100ml of distilled water),1% thiobarbituric acid (TBA) in 0.05M NaOH (w/v) (obtained by dissolving 1g of TBA in 100ml of 0.05M NaOH). The solution was then heated in a hot water bath for 10 minutes to dissolve TBA. One millilitre (1ml) of 1% thiobarbituric acid dissolved in alkaline medium was added to 0.1 ml of sample in test tube. The mixture was thoroughly mixed, and 1 ml of glacial acetic acid was thereafter, added to the mixture. The reaction mixture was also shaken thoroughly and incubated in boiling water (100 °C) for 15 minutes. It was allowed to cool and the turbidity or flocculent precipitate was removed by centrifugation at 3000 rpm for 10 minutes. The supernatant was thereafter read at 532 nm against blank using the UV spectrophotometer- Hitachi 912. The same volume of TBA and glacial acetic acid was added to a blank test tube, but 0.1 ml of distilled water was added to the blank instead of the homogenized crustacean filtrate. The level of MDA in the tissue filtrate was expressed as mmol/ml using the molar extinction coefficient for MDA ($1.56x10^5$ $M^{-1}cm^{-1}$) and calculated as follows:.

MDA (mmol/ml) = (OD X 1000000)/ E532

Where E532 = Molar extinction coefficient for MDA ($1.56x10^5$ M^{-1} cm^{-1}).
The level of thiobarbituric acid reactive substances (TBARS) which is an index of lipid peroxidation was determined by the method of [7]. Malondialdehyde, a product of lipid peroxidation, formed as a result of breakdown of polyunsaturated fatty acids reacts with thiobarbituric acid to form a 1:2 adduct, a red coloured complex, which is measured by spectrophotometer at 535nm. TCA –TBA -HCL reagent (2 ml) was added to 0.1ml of the filtrate in a test tube labeled Test, while 0.3ml of the reagent was put in a second test tube labeled Blank. The individual mixture was heated for 15mins in a boiling water bath. The test tubes were cooled and centrifuged at 3000rpm for 10mins to remove the flocculent precipitates. The absorbance of the supernatant of the centrifuged sample was read at 535nm against blank using UV spectrophotometer-Hitachi 912.

STATISTICAL ANALYSIS

Statistical significance of data in this study was defined as *p*-value of ≤ 0.05. The data analysis was conducted using SPSS Software (version 16.0, NY, USA)

RESULTS

The results of heavy metals load of selected crustaceans from Main Market and Ogbe-Ijoh watersides are represented in Table 1.0 below.

Table 1.0: Metallic load of selected crustaceans from watersides of Main Market and Ogbe- Ijoh

S/No	Metals	**Heavy metals concentrations (mg/kg)**					
S/N	Metal (s)	Main Market		Ogbe Ijoh		WHO	NAFDAC
		N. hastatus	*C. armatus*	*N. hastatus*	*C. armatus*		
i	Pb	0.76±0.06	0.47±0.05	0.25±0.05	0.17±0.05	0.1	2.5
ii	Cu	1.2±0.05	1.4±0.03	1.05±0.05	1.15±0.01	2.0	30
iii	Cr	0.095±0.00	0.07±0.01	0.87±0.04	0.59±0.05	0.05	0.1-0.5
iv	Cd	0.06±0.01	0.08±0.01	0.03±0.00	0.04± 0.00	0.05	0.1
v	As	0.09±0.00	0.07±0.00	0.04±0.00	0.03±0.01	0.1±0.003	0.1- 2.0
vi	Ni	0.03±0.003	0.065±0.005	0.01±0.00	0.089±0.00	0.02	0.5

The results of malondialdehyde levels of crustaceans from Ogbe Ijoh and Main Market watersides are represented in Table 2.0

Table 2.0: MDA levels of shrimps and crabs from watersides of Main Market and Ogbe- Ijoh

S/No	Location	Crustaceans	TBARs activities *(Umole MDA/g tissue)*
i	Main market	*N. hastatus*	*12.83±0.10*
		C. armatus	3.55±0.05
ii	Ogbe -Ijoh	*N. hastatus*	5.37±0.25
		C. armatus	3.79±0.09

N. hastatus =*Nematopalaemon hastatus*) ; *C. armatus* = (*Cardiosoma armatum*); TBARs= thiobarbituric acid reactive substances and MDA= malondialdehyde

Malondialdehyde level of N. hastatus from Main Market waterside was significantly ($p \leq 0.05$) higher when compared to the levels of same Crustacean from Ogbe Ijoh Waterside. The MDA levels of *C.armatus* from both Main market and Ogbe Ijoh watersides had no significant variation.
The relationship between the heavy metallic load and MDA levels of crustaceans from MMW and OIW is better represented in Figure 1.0 below:.

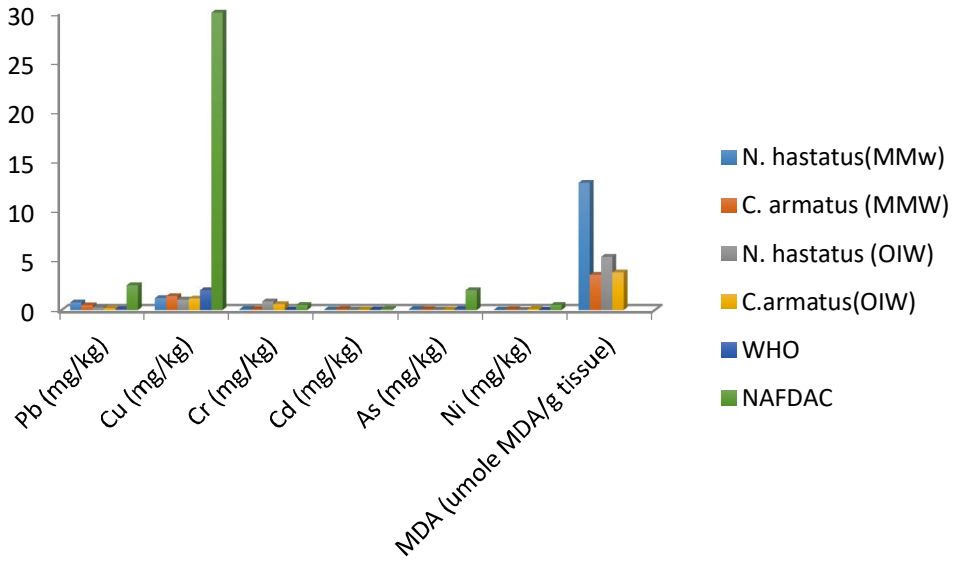

Figure 1.0: Crudeoil accompanying metal load and MDA levels of crustaceans from Ogbe Ijoh and Main market Watersides

DISCUSSION

A comparison between the heavy metal concentration and the levels of lipid peroxidation products in the crustaceans can be better understood by observation of Figure 1.0. The concentration of Pb in crustaceans from MMW is higher than Pb concentration in OIW. Also, observation of Figure 1.0 shows that the alteration of lipid (MDA levels) in the crustaceans from MMW is significantly ($p \leq 0.05$) elevated when compared to lipid alteration (MDA) in crustaceans from OIW. The finding from this study aligns with the report of Osioma *et al*., 2013, who reported that heavy metal concentrations of fishes from a certain river in the Niger Delta correlated positively with the malondialdehyde levels and [1] the finding from this study could indicate a heavier pollution load by crude oil in MMW when compared to OIW.
Observation of Figure 1.0 shows a higher concentration of Cu in crustacean, *C. armatus*, from MMW when compared to OIW, however the malondialdehyde levels of crustacean, *C. armatus*, from OIW was observed to be slightly higher than that from MMW. The finding from this study deviates from the report of Osioma *et al*., 2013.This non alignment of the finding from this study with previous reports could be due Cu exhibiting slight protective ability in the specific *C.armatus*.
Chromium in high amounts in the soft tissues of the body has been implicated in hepatotoxicity and nephrotoxicity. However within the recommended daily intake, it aids fats, protein and glucose metabolism. In this present study, the chromium load of crustaceans in OIW was higher when compared to MMW (Figure 1.0) leading to a corresponding elevation in the MDA levels of crustaceans from OIW relative to the levels found in MMW. The findings from the study agrees with the report of [8].

CONCLUSION

This findings from this study indicates the presence of seven crude oil accompany metals in concentrations that though may not be significant but can bioaccumulation overtime and cause poisoning to soft tissues. The high malondialdehyde levels observed in tissue of crustaceans from main market waterside with high Pb load when compared to the Pb load of Ogbe-Ijoh market points to the positive correlation of metallic contaminants with tissue damage**.**

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