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Assessment of Cadmium and Lead in the Barnacle (Semibalanus balanoides) and Oyster (Crassostrea gasar) in Port Harcourt, Nigeria: Risk Assessment of Oyster Consumption

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ABSTRACT

The barnacle (*Semibalanus balanoides*) and oyster (*Crassostrea gasar*) collected from Ikpukula-Ama in Borikiri Port Harcourt were used for heavy metal (Cd and Pb) analysis using flame atomic absorption spectrophotometer (FAAS) CBC Avanta PM in 2014. The concentration of Pb varied between 0.044 - 1.167 ppm in *S. balanoides* and 0.163 - 0.74 ppm in *C. gasar* with means of 0.75 + 0.08 ppm and 0.55 + 0.06 ppm respectively. Cd concentration ranged from 0.033 - 0.108 ppm in *S. balanoides* and 0.01 – 0.28 ppm in *C. gasar* with mean values of 0.07 ± 0.008 ppm and 0.14 ± 0.04 ppm respectively. The concentration of Pb was significantly higher than that of Cd in both animals, and the concentration of Pb in *S. balanoides* was significantly higher than that of *C. gasar*. The Pb concentrations in both organisms were higher than the FAO permissible limit in seafood of 0.5 ppm. Both organisms accumulated Pb more than Cd. *S. balanoides* appeared to be a better indicator organism to monitor Pb in the environment as concentrations of the heavy metals were higher. Risk assessment for Cd in *C. gasar*, the daily maximum allowable limit as well as the meal consumption limit over a month as a function of meal was calculated.

INTRODUCTION

In Nigeria, fish and shellfish are very important sources of protein and they also provide a source of livelihood for artisanal and small scale fisheries in the coastal communities. Some work has been done in the area on local species like *Penaeus notialis* [1], *Synodontis* and *Oreochromis* [2], *Crassosstrea gasar* [3] and in neighboring Ghana on some fish samples [4]. However, it is necessary to have frequent monitoring of the quality of fish and shellfish that is available for human consumption. Oysters which are readily harvested from coastal waters and are widely consumed need to be monitored for nonessential heavy metals as they are abundant, sizeable, of wide distribution and easy to collect [5]. Marine oysters are known to accumulate heavy metals in their soft tissues to potentially high concentrations [6]. *Crassostrea* spp have been proposed as sentinel organisms for marine Eco toxicological tests for the purpose of assessing coastal water quality because they are sensitive to pollutants [7]. Studies by Mora et al., Raposo et al., Yap et al., Chaharlang et al. and Casteneda–Chavez et al. [6,8-11] have shown significant amount of heavy metals in oysters. For example Castenada-Chavez et al. [11] recorded concentrations of Pb and Cd as high as 1.37 ± 0.77 µg/g and 63.74 ± 8.446 µg/g respectively in oysters from the Lagoon systems in Southern Gulf of Mexico and attributed the high concentration to the presence of great industrial activity and oil spills from Petrochemical industries while [10], recorded concentrations of Pb and Cd as 58.23 µg/g and 12.03 µg/g in *Saccostrea cucullata*. This indicates a large diversity of heavy metal accumulation in oysters as well as the ability to accumulate certain metals in high concentrations.

Barnacles often have high heavy metal body concentrations that vary with local availabilities and represent integrated measures of the supply of bioavailable metals. Philips and Rainbow [12] in a pioneering work in Hong Kong showed the potential for the use of barnacles as heavy metal bio monitors. Barnacles are fouling organisms found on piers and other hard substrates in the intertidal and sub littoral habitats and are typically situated in areas with the potential to be affected by industrial waste and other anthropogenic pollution. Bio monitoring studies on heavy metals in *S. balanoides* are very limited [13].

Cd and Pb are considered non – essential metals to animals. All heavy metals are potentially toxic to all organisms above a certain threshold when the rate of metal uptake is greater than the combined rate of excretion plus detoxification for storage in an organism [14]. Therefore the objective of this study was to determine the bioavailabilities of Cd and Pb in the soft tissues of barnacle (*Semibalanus balanoides*) and oyster (*Crassostrea gasar*) collected from Ikpukulu – Ama in the mangrove areas of Borikiri, Port Harcourt in Nigeria and to explore the human risk associated with the consumption of locally sourced *C. gasar* contaminated with Cd.

MATERIALS AND METHODS

Study area: The samples were collected from mangrove prop roots of a red mangrove swampy forest around a small fishing settlement called Ikpukulu - Ama in Borikiri, Port Harcourt. Borikiri lies at latitude 4.7490N and 7.0350E bounded by Okrika Island (across Aboturu Creek) to the east. It is estuarine with salinities of 5 to 8 ppt. Some of the factories located around Borikiri are NLNG Dock in Trans Amadi, Almarine Base and John Holt Plc in Ebani Island. John Holt is a boat building company engaged in other activities associated with marine technology, oil and gas, fishing and transportation. The mangrove forest is on a smaller creek (Ikpulu - Ama Creek) off the Bonny River which links Borikiri to the fishing settlement.

Sample collection and analysis: The oyster and barnacle samples used in this study were obtained from the mangrove prop roots in Ikpukulu – Ama between March and May 2014. All samples were collected from the wild during low tides when they were all exposed. The samples were brought back to the laboratory and preserved in the refrigerator until analysis.

The soft tissues of 20 barnacles of approximately 2 cm shell length were removed from their shells and used as a composite sample. The soft tissues of 3 oysters of about 10 cm shell length were used for the composite sample. Three sets of composite samples were made for each organism and three replicates from each composite sample were used for analysis. The samples were dried to a constant weight at 100 0C and ashes at 500°C. To determine the heavy metal concentration, the samples were subjected to an acid digestion process (5 mL of 10% HCl) and warmed on a water bath. The solution was transferred through a funnel into a clean and sterilized 50 mL volumetric flask. The resulting solution was analyzed for Cd and Pb by flame absorption spectroscopy. The results for each metal concentration are expressed in ppm dry weight.

Single factor ANOVA was conducted on the results of Cd and Pb concentrations in the oysters and the barnacles using Microsoft Excel 2010. The average daily intake dose of exposed individuals was calculated and compared with a Reference Dose (RfD) obtained from IRIS [15]. Pb has no RfD but that of Cd is 1×10^{-3} mg/kg/day based on a chronic intake that would result in a kidney concentration of 200 μg/g wet weight. Risk assessment for Cd in *C. gasar* was determined by calculating the Average Daily Dose (ADD) and Hazard Quotient (HQ) for children, youths, women of child bearing age and adults following the methods in [16,1] using the equation:

$ADD = C_{oyster} \times IR_{oyster} \times EF \times ED/BW \times AT$ [16]

Where:

ADD: Average Daily Dose (ingestion of contaminated oyster mg/kg/day)

Coyster: Concentration of contaminants in oyster (mg/kg fish)

IR oyster: Per capita intake rate of oyster (kg oyster/meal)

EF: Exposure frequency (meals/day)

ED: Exposure duration (days)

BW: Body weight (kg)

AT: Averaging time (days)

Input parameters were RfD of Cd, body weight of consumer, per capita intake of 4oz (0.113 g) of oyster for less than 15 years and 8oz (0.226 g) of oyster for adults, 95% CI of mean, EF of 1 meal a day, ED of 3 meals a week and average time of 1 week (7 days). Then the hazard quotient was calculated by:

Hazard quotient = ADD/RfD

Furthermore, the daily maximum allowable limit as well as the meal consumption limit over a month as a function of meal was estimated according to Ugbomeh and Jaja [1] from the equation:

$CRlim = RfD \times BW / C_{oyster}$

Where:

CRlim: Maximum allowable oyster consumption rate (kg/d)

RfD: Reference dose (mg/kg-day)

BW: Consumer body weight (kg)

Coyster: Measured concentration of contaminants in the oyster (mg/kg)

This daily consumption limit in kilograms was converted to meal consumption limits over a given time period (month) as a function of meal size:

CRmm = CRlim × AT/IRoyster

Where:

CRmm: Maximum allowable oyster consumption rate (meals/month)

CRlim: Maximum allowable oyster consumption rate (kg/d)

AT: Average time (30 days/month)

IRoyster: Per capita intake rate of oyster (kg oyster /meal)

The summary of heavy metal concentration in *S. balanoides* and *C. gasar* presented in **TABLE 1** showed that the values of Pb in *S. balanoides* ranged from 0.435 - 1.167 ppm with a mean of 0.75 + 0.08 ppm.

TABLE 1. Lead (Pb) and Cadmium (Cd) concentrations (ppm dry weight) in *S. balanoides* and *C. gasar,* along with FAO (1983) permissible limits of hazardous substances in fish products (ppm).

Metals/ Species	Mean	SE	FAO 1983
Pb			
S.balanoides	0.75	0.08	0.5
C. gasar	0.55	0.06	0.5
Cd			
S.balanoides	0.07	0.008	0.5
C.gasar	0.14	0.04	0.5

In *C. gasar* the Pb concentration ranged from 0.163 - 0.735 ppm with a mean of 0.55 ± 0.06 ppm. These values were seen to be very high when compared to the FAO [17] permissible limit of 0.5 ppm. Cd concentration ranged from 0.033 - 0.108 ppm in *S. balanoides* with a mean of 0.07 + 0.008 ppm and 0.011 to 0.28 ppm with a mean of 0.14 + 0.04 ppm in *C. gasar*. The Cd concentrations in *S. balanoides* and *C. gasar* were below the FAO [17] permissible limit of 0.5 ppm while the Pb in *S. balanoides* and *C. gasar* were significantly higher than that of Cd in the same sample. The F values from ANOVA in **TABLE 2** show no significant difference between the concentration of Cd in *C. gasar* and *S. balanoides*. Pb concentrations in *C.gasar* and *S. balanoides* were significantly different (p = 0.03).

TABLE 2. F values and p values of comparisons between means of Pb and Cd concentrations in soft tissues of *S. balanoides* and *C. gasar*.

Groups	F values	p (significant p < 0.05)
Cd in *C. gasar* and *S. balanoides*	0.57	0.46
Pb in *C.gasar* and *S. balanoides*	5.38	0.034
Cd in *C.gasar* and Pb in *C. gasar*	23.07	<0.001
Cd in *S.balanoides* and Pb in *S.balanoides*	62.3	<0.001

The estimated Average Daily Dose (ADD) in mg/kg/day and Hazard Quotient (HQ) ranged from 4.8 x 10-3 to 0.016 and 3.0 to 21.8 respectively **(TABLE 3).** The monthly oyster consumption limits for non-carcinogenic health endpoints **(TABLE 4)** ranged from 37.9 oyster meals of 0.14 kg/day for children < 5 years to 91 oyster meals of 0.34 kg/day for teenagers between 12 – 18 years.

TABLE 3. Estimated Average Daily Dose (ADD) and Hazard Quotient (HQ) of Cd in *C. gasar* with a RfD (reference dose) of 1 x 10^{-3} mg/kg/day.

Subgroup (Age in years/weight in Kg)	ADD (mg/kg/day)	HQ (range)
1 – 5 yrs (20 kg)	4.8×10^{-3} – 2.2×10^{-2}	4.8 – 21.8
6 – 11 yrs (30 kg)	0.004 – 0.018	4.0 – 18.19
12 – 18 yrs (48 kg)	0.003 – 0.011	3.0 – 11.37
Women of child bearing age. (60 kg)	0.004 – 0.017	4.0 – 17. 06
Adults (70 kg)	0.0033 – 0.016	3.3– 60

Table 4. Monthly oyster consumption limits for non – carcinogenic health endpoints for Cd.

Subgroup	Oyster meals/month	Climit (Max. Daily allowable kg/day)
1 – 5 yrs	37.9	0.14
6 – 11 yrs	56.9	0.21
12 – 18 yrs	91	0.34
Women of Child bearing age	61	0.46
Adults 0.50	66	0.5

DISCUSSION

Philip and Rainbow [12] stated that all organisms accumulate trace metals, whether essential or not, but there are striking differences between the accumulated metal concentrations in the tissue and in the whole bodies of different organisms. The results show that Pb was accumulated more readily than Cd in both *S. balanoides* and *C. gasar* in agreement with this statement. This difference in accumulation pattern could also depend on the concentration of the different metals available in the environment. Barnacles are keystone inhabitants of the hard substrates of littoral and sub littoral coastal habitats and are also major fouler of man-made structures such as piers, which are usually located in areas with the potential to be affected by industrial waste and other anthropogenic pollution. Concentrations of Pb were higher in *S. balanoides* probably because of the metal accumulation patterns adopted by barnacles which according to Rainbow [14] include strong net accumulation with either very low or no excretion of accumulated metal together with the very high uptake rates of the metals involved. It has been known for some time that barnacles take up trace metals such as zinc and cadmium from seawater at rates well in excess of those of other crustaceans [18-20]. Barnacles can be model organisms for comparing metal bio availabilities from animal tissue food sources. However, it remains unclear whether there is a significant relationship between the Cd concentration in barnacles and the bioavailability of Cd concentration in the ambient water. Measuring metals in barnacles is also relevant to human health (although this species is not consumed as food) because it is also an indication of the bioavailability of the metals in the environment.

Considerable variations in metal concentrations have been documented among different individuals collected from the same location [14]. We noticed this as well in the Pb and Cd concentrations of the oyster and barnacle collected from the same prop root in this study. The concentration of Cd in the edible oyster was below the FAO [17] permissible limit, but Pb concentrations were higher than the 0.5 ppm limit. This poses a health risk for consumers of the contaminated oysters from this particular water body in Port Harcourt as oysters are consumed daily in soups either fresh or sun dried. Pb is a neurotoxin and is a health risk when taken or accumulated in human body especially in children [21].

The HQ for Cd in *C. gasar* ranged from 3.0 to 21.8. A HQ of > 1 means the study population has excess non – carcinogenic health risk from the exposure frequency of the oyster [16]. The maximum daily allowable kg/day (C limit) of the oyster to reduce this risk ranged from 0.14 kg/day for children < 5 years to 0.5 kg/day for adults with weight of 70 kg and above. In conclusion, an oyster meal from the study site has health risks for the consumer because of the Pb concentrations and also for Cd when meals exceed the C limit.

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