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Risk assessment of cancer from dioxin and aflatoxin contamination in dried seafood sold in Port Harcourt, Nigeria

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Keywords: Aflatoxin, *Aspergillus* species, dried seafoods, dioxin, food poisoning, food safety

Abstract: Contamination of seafood by toxic environmental pollutants and fungal metabolites is a growing public health concern in developing countries. This study assessed dioxin and aflatoxin levels in dried seafood sold at Creek Road Market, Port Harcourt, Nigeria, and evaluated the associated non-carcinogenic and carcinogenic health risks. Twelve samples, comprising dried Bonga fish, crayfish, shrimp, and periwinkle (three replicates each), were randomly collected from different vendors and analyzed using standard methods. Dioxins were determined by Gas Chromatography-Mass Spectrometry, aflatoxins by Enzyme-Linked Immunosorbent Assay (ELISA), while *Aspergillus* species were isolated using Potato Dextrose Agar and identified microscopically. Data were expressed as mean ± standard deviation. Detectable levels of dioxins and aflatoxins were found in all samples. Dried Bonga fish recorded the highest dioxin concentration (5.82 ± 0.31 pg TEQ/g fat), whereas crayfish had the highest aflatoxin concentration (20.63 ± 1.25 μg/kg). *Aspergillus flavus*, *Aspergillus niger*, and *Aspergillus fumigatus* were isolated from several samples, indicating fungal contamination during storage and handling. Hazard Quotient and Hazard Index values exceeded the acceptable threshold of 1.0 for all seafood types. At the same time, estimated cancer risks ranged from 2.1×10^{-4} to 5.2×10^{-4}, suggesting potential adverse health effects from long-term consumption. The findings stress the need for increased food safety examination, improved processing and storage practices, and effective environmental pollution control to reduce consumer exposure and safeguard public health.

Introduction

Over the years, food contamination has been recognized as a major global public health challenge, especially in developing urban areas [1]. These areas are characterized by high levels of environmental pollution and poor food safety practices [1, 2]. In these situations, contaminated food can become a major pathway for exposure to toxic chemicals. These toxic chemicals can then cause either short-term health effects, such as food poisoning [3, 4], or long-term health effects, such as cancer [5]. Across Africa, particularly in Nigeria, a lot of people usually consume seafood because of its high nutritional value. These seafoods are nutritionally composed of indispensable proteins, vitamins, minerals, and unsaturated fatty acids necessary for human growth and development, repair of worn-out tissues, and proper functioning of the body systems [6-8]. Interestingly, dried seafood and seafood products are among the commonly sold consumable foodstuffs in open markets in Rivers State. Rivers State is one of the 36 states in Nigeria, located in the oil-rich Niger Delta region, where many households rely heavily on this dried seafood and its products due to the fact that they are moderately affordable, readily available, and possess prolonged shelf lives [9, 10]. Despite the advantages of this dried seafood and its products, they are still vulnerable to contaminants during the stages of processing, transporting, storing, and marketing them, hence making them unsafe for consumption. Several concerns regarding food safety in Port Harcourt have increased recently due to poor hygiene and the high level of environmental pollution resulting from illegal or artisanal crude oil exploration, gas flaring, industrial emissions, open domestic waste burning, and very poor waste management practices [11-13]. These anthropogenic activities release toxic pollutants like dioxins and aflatoxins directly into the food or indirectly into the environment, which may contaminate aquatic ecosystems and bioaccumulate in seafood consumed by humans [14, 15].

Dioxins are a class of chlorinated aromatic hydrocarbons comprised mostly of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans [16]. These toxic organic pollutants possess high chemical stability, lipophilicity, and persistence in the environment. Their high lipophilic nature enhances their easy bioaccumulation in fatty tissues of aquatic organisms and biomagnification along the food chain [16, 17]. Mechanistically, dioxins exhibit their toxic effects by activating the aryl hydrocarbon receptor [18], leading to modifications in gene expression [19], oxidative stress [20], and the destabilization of cellular homeostatic processes [21]. Long-term exposure to dioxins has been associated with several health challenges, such as hepatic toxicity, endocrine disruption, reproductive disorders, immune suppression, and carcinogenesis [22]. Aflatoxins, on the other hand, are difuranocoumarin mycotoxins produced mainly by Aspergillus flavus and Aspergillus parasiticus [23]. Like dioxins, they exhibit high thermal stability and may persist in food despite processing [24]. At the molecular level, aflatoxins undergo hepatic metabolic activation by cytochrome P450 enzymes, forming reactive epoxide intermediates that bind to DNA, inducing mutations and initiating carcinogenic processes [25]. Among the different aflatoxins, aflatoxin B_1 is the most potent and has been strongly associated with hepatocellular carcinoma [26]. Long-term exposure to aflatoxins may also result in liver damage [27], oxidative stress [28], impaired immune function [29], and impaired growth and decreased nutritional status [30].

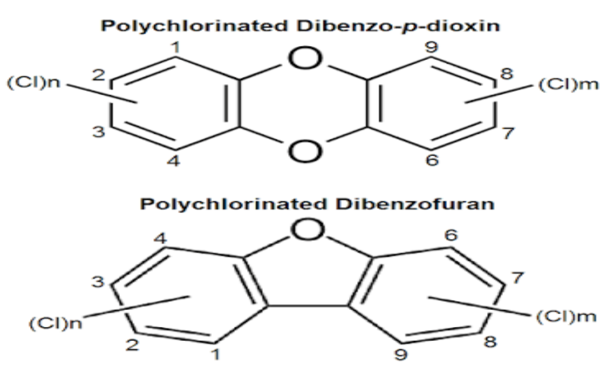

Figure 1: Chemical structures of polychlorinated dibenzo-p-dioxin and polychlorinated dibenzofuran https://share.google/s2xdMpcolQOhu3O1i

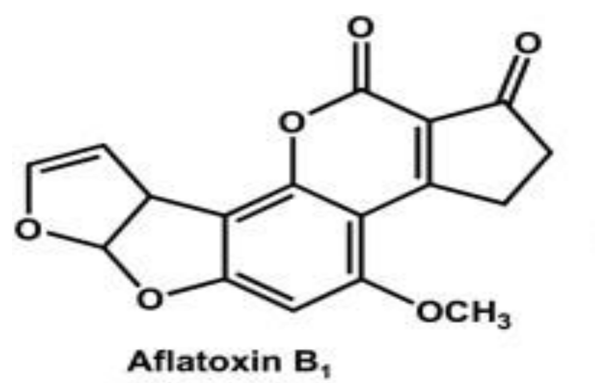

Figure 2: Chemical Structure of Aflatoxin B_1 https://share.google/4Y1brmxNFZcBlWDpV

Even though seafood consumption is prevalent in Port Harcourt, there is limited data on the levels of dioxin and aflatoxin contamination in dried seafood sold in local markets, as well as the associated health risks. Existing studies have mostly focused on individual contaminants, with a limited combination of acute and chronic risk pathways. Therefore, this study was designed to evaluate the levels of dioxins and aflatoxins in dried seafood sold in Port Harcourt, Nigeria, and to assess the associated risks of food poisoning and cancer among consumers using established risk assessment models.

Materials and methods

Study area: This study was conducted at Creek Road Market, Port Harcourt, Rivers State, Nigeria. The market is one of the major commercial centers for seafood trading within the metropolis.

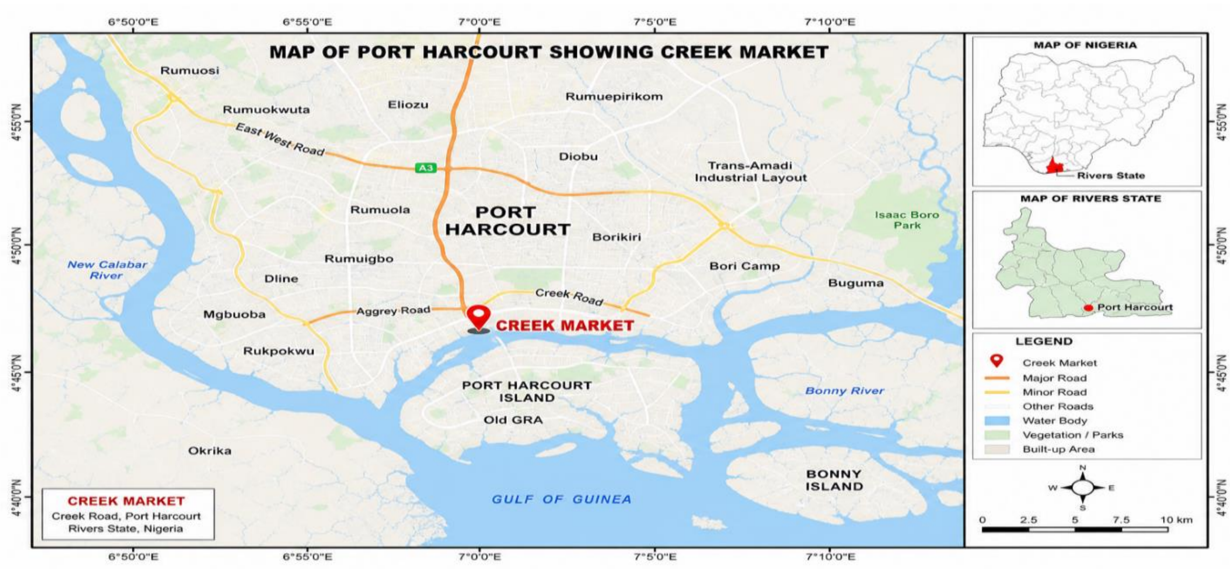

Figure 3: Map of the study area (Port Harcourt)

Study design and sample collection: A cross-sectional analytical study design was adopted to evaluate dioxin and aflatoxin levels in dried seafood samples and assess associated health risks among consumers. A total of 12 samples, comprising three replicate samples each of dried Bonga fish (*Ethmalosa fimbriata)*, crayfish (*Procambarus clarkia)*, shrimp (*Penaeus monodon)*, and periwinkle (*Tympanotonus fuscatus)*, were randomly purchased from different vendors within Creek Road Market. Samples were collected in sterile polyethylene bags, appropriately labelled, and transported in insulated containers to the laboratory for analysis.

Figure 4: Pictures of dried seafood from Creek Road Market, Port Harcourt

Sample preparation: The seafood samples were cleaned to remove extraneous materials and debris before being homogenized using a sterile laboratory grinder. The homogenized samples were transferred into sterile airtight containers and stored at 4°C until laboratory analyses were performed.

Determination of dioxin concentration: About 10.0 g of each homogenized seafood was mixed with anhydrous sodium sulphate and extracted using Soxhlet extraction with a hexane–acetone solvent mixture. The extracts were concentrated under reduced pressure and purified using silica gel column chromatography before instrumental analysis. Dioxin concentrations (expressed in pg TEQ/g fat) were determined using Gas Chromatography-Mass Spectrometry (GC-MS) (Agilent Technologies, Santa Clara, CA, USA) following established analytical procedures. Chromatographic separation was achieved on an HP-5MS fused silica capillary column (30 m × 0.25 mm internal diameter × 0.25 µm film thickness; Agilent Technologies). Helium (99.9% purity) was used as the carrier gas at a constant flow rate of 1.0 mL/min. The injector temperature was maintained at 280°C with spitless injection of a 1.0 µL sample volume. The oven temperature programme was set at 80°C (held for 2 min), ramped at 15°C/min to 200°C, then at 5°C/min to 300°C and held for 10 min. The mass spectrometer operated under electron ionization (EI) mode at 70 eV, with data acquired in selected ion monitoring (SIM) mode to enhance sensitivity and selectivity for target analytes. Quantification was performed using external calibration curves prepared from certified dioxin reference standards with calibration coefficients (R^2) exceeding 0.995. Method validation included procedural blanks, duplicate analyses, matrix spike recoveries, and analysis of certified reference materials where available. The limit of detection (LOD) and limit of quantification (LOQ) were established based on signal-to-noise ratios of 3:1 and 10:1, respectively, and were determined to be 0.01 ng/g and 0.03 ng/g, respectively. Recoveries for fortified ranged from 85% to 110%, and all measured concentrations were corrected for procedural recovery where appropriate.

Determination of aflatoxin concentration: Aflatoxins were extracted from homogenized seafood using a 70% methanol-30% distilled water (v/v) extraction solution. Briefly, 5 g of each homogenate was mixed with 25 mL of the extraction solvent and shaken vigorously for 10 min using an orbital shaker. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was diluted as required before analysis. Quantitative determination of total aflatoxins (expressed in µg/kg) was performed using a commercial competitive Enzyme-Linked Immunosorbent Assay (ELISA) kit according to the manufacturer's instructions. Absorbance was measured at 450 nm using a Microplate Spectrophotometer, and aflatoxin concentrations were calculated from a calibration curve generated using the supplied standards. Quality control procedures included the analysis of reagent blanks, duplicate samples, and kit-provided quality control standards. The assay had a limit of detection (LOD) of 1.75 µg/kg and a limit of quantification (LOQ) of 5.0 µg/kg, as specified by the manufacturer.

Isolation and enumeration of Aspergillus species: One gram of each homogenized seafood was aseptically transferred into 9.0 mL of sterile distilled water and thoroughly mixed to obtain an initial 10^{-1} dilution. Serial ten-fold dilutions were subsequently prepared using sterile distilled water. Aliquots (0.1 mL) from appropriate dilutions were spread-plated in duplicate onto Potato Dextrose Agar (PDA) supplemented with chloramphenicol (100 mg/L) to inhibit bacterial growth. The inoculated plates were incubated at 28.0 ± 2.0°C for 3-5 days in an incubator. Following incubation, distinct Aspergillus colonies were counted and expressed as colony-forming units per gram (CFU/g) of sample. Representative colonies were sub-cultured on fresh PDA plates to obtain pure isolates. The identification of *Aspergillus* species was performed based on macroscopic colony characteristics, including colony color, texture, pigmentation, and growth pattern, as well as microscopic morphology following staining with lactophenol cotton blue. Microscopic examination was carried out using a CX23 binocular light microscope. Identification focused on the detection of *Aspergillus flavus*, *Aspergillus niger*, and *Aspergillus fumigatus*, using standard mycological identification keys.

Estimation of daily intake (EDI): EDI was calculated using: EDI = C x IR / BW, where (C) = contaminant concentration, (IR) = ingestion rate, and (BW) = body weight.

Estimation of non-cancer risk: Non-cancer risk was estimated using Hazard Quotient (HQ): HQ = EDI / RfD, where RfD = Oral reference dose. Hazard Index (HI) was calculated as the sum of individual HQ values.

Estimation of cancer risk: Cancer risk (CR) associated with the consumption of contaminated seafood was estimated using the standard equation: $CR = EDI \times CSF$, where (CR) = Cancer risk and (CSF) = Cancer slope factor $(mg/kg/day)^{-1}$.

Data presentation: Data were analyzed using SPSS version 25 and expressed as mean ± standard deviation (SD).

Results

Dioxin levels in dried seafood: **Table 1** shows the dioxin levels in dried seafood sold in Creek Road Market, Port Harcourt. Dioxins were detected in all seafood analyzed, indicating widespread contamination across the different seafood types examined. Dried Bonga fish recorded the highest dioxin concentration (5.82 ± 0.31 pg TEQ/g fat), followed by crayfish (4.96 ± 0.28 pg TEQ/g fat), shrimp (3.41 ± 0.19 pg TEQ/g fat), and periwinkle (2.87 ± 0.15 pg TEQ/g fat).

Table 1: Dioxin concentrations in dried Seafood sold at Creek Road Market, Port Harcourt, Nigeria

Seafood	Dioxin level (pg TEQ/g fat)	EFSA limit (pg TEQ/g fat)
Dried Bonga fish	5.82 ± 0.31	2.00
Crayfish	4.96 ± 0.28	2.00
Shrimp	3.41 ± 0.19	2.00
Periwinkle	2.87 ± 0.15	2.00

Values are presented as mean ± standard deviation (SD) of three replicate determinations.
Limits are expressed in picograms (pg) of Toxic Equivalent (TEQ) per gram of fat.

Aflatoxin levels in dried seafood: **Table 2** presents the aflatoxin concentrations in dried seafood sold in Creek Road Market, Port Harcourt. Aflatoxins were detected in all seafood analyzed, indicating widespread contamination among the seafood products examined. Crayfish recorded the highest mean aflatoxin concentration (20.63 ± 1.25 μg/kg), followed by dried Bonga fish (18.45 ± 1.12 μg/kg), shrimp (11.27 ± 0.83 μg/kg), and periwinkle (9.15 ± 0.64 μg/kg).

Table 2: Aflatoxin concentrations in dried seafood sold at Creek Road Market, Port Harcourt, Nigeria

Seafood	Aflatoxin Level (μg/kg)	EFSA Limit (μg/kg)
Dried Bonga fish	18.45 ± 1.12	10.00
Crayfish	20.63 ± 1.25	10.00
Shrimp	11.27 ± 0.83	10.00
Periwinkle	9.15 ± 0.64	10.00

Values are expressed as mean ± standard deviation (SD)

Qualitative occurrence of Aspergillus species in dried seafood: **Table 3** indicates the presence of different *Aspergillus* species in the dried seafood analyzed. *A. flavus* was detected in dried Bonga fish, crayfish, and shrimp, while *A. niger* was present in dried Bonga fish, crayfish, and periwinkle. *A. fumigatus* was identified in crayfish and shrimp but was absent in dried Bonga fish and periwinkle. Crayfish showed the presence of all the identified *Aspergillus* species in the sample.

Table 3: Qualitative occurrence of *Aspergillus* species in dried seafood

Seafood	*A. flavus*	*A. niger*	*A. fumigatus*
Dried Bonga fish	Present	Present	Absent
Crayfish	Present	Present	Present
Shrimp	Present	Absent	Present
Periwinkle	Absent	Present	Absent

Quantitative occurrence of Aspergillus species in dried seafood: **Table 4** presents the quantitative enumeration of *Aspergillus* species isolated from the dried seafood. Crayfish exhibited the highest total Aspergillus count ($13.0 \pm 0.7 \times 10^3$ CFU/g), followed by dried Bonga fish ($8.0 \pm 0.5 \times 10^3$ CFU/g), shrimp ($6.1 \pm 0.4 \times 10^3$ CFU/g), and periwinkle ($2.6 \pm 0.2 \times 10^3$ CFU/g). *A. flavus* recorded the highest count in crayfish ($5.9 \pm 0.5 \times 10^3$ CFU/g), while A. niger was most abundant in crayfish ($4.3 \pm 0.4 \times 10^3$ CFU/g) and dried Bonga fish ($3.2 \pm 0.3 \times 10^3$ CFU/g). *Aspergillus fumigatus* was detected only in crayfish and shrimp, with counts of $2.8 \pm 0.2 \times 10^3$ CFU/g and $2.4 \pm 0.2 \times 10^3$ CFU/g, respectively.

Table 4: Fungal counts of *Aspergillus* species isolated from dried seafood products sold at Port Harcourt

Seafood sample	*A. flavus* ($\times 10^3$ CFU/g)	*A. niger* ($\times 10^3$ CFU/g)	*A. fumigatus* ($\times 10^3$ CFU/g)	Total *Aspergillus* count ($\times 10^3$ CFU/g)
Dried Bonga fish	4.8 ± 0.4	3.2 ± 0.3	ND	8.0 ± 0.5
Crayfish	5.9 ± 0.5	4.3 ± 0.4	2.8 ± 0.2	13.0 ± 0.7
Shrimp	3.7 ± 0.3	ND	2.4 ± 0.2	6.1 ± 0.4
Periwinkle	ND	2.6 ± 0.2	ND	2.6 ± 0.2

ND = Not Detected. Values are presented as mean ± standard deviation (SD) of three replicate determinations

Estimated daily intake of dioxins and aflatoxins: **Table 5** reveals the estimated daily intake (EDI) of dioxins and aflatoxins from the consumption of dried seafood. Dried Bonga fish recorded the highest dioxin EDI value (4.85 pg/kg/day), followed by crayfish (4.13 pg/kg/day), shrimp (2.84 pg/kg/day), and periwinkle (2.39 pg/kg/day). For aflatoxins, crayfish showed the highest EDI value (0.69 µg/kg/day), followed by dried Bonga fish (0.62 µg/kg/day), shrimp (0.38 µg/kg/day), and periwinkle (0.31 µg/kg/day).

Table 5: Estimated daily intake of dioxins and aflatoxins

Seafood	Dioxin EDI (pg/kg/day)	Aflatoxin EDI (µg/kg/day)
Dried Bonga fish	4.85	0.62
Crayfish	4.13	0.69
Shrimp	2.84	0.38
Periwinkle	2.39	0.31

Non-cancer risk assessment of dioxin and aflatoxin exposure: **Table 6** presents the non-carcinogenic risk associated with dietary exposure to dioxins and aflatoxins through the consumption of dried seafood. Crayfish recorded the highest Hazard Quotient (HQ) value (3.08) and Hazard Index (HI) value (3.71), followed by dried Bonga fish with HQ and HI values of 2.91 and 3.44, respectively. Shrimp and periwinkle exhibited comparatively lower values; however, HQ and HI exceeded the acceptable threshold value of 1.0.

Table 6: Non-cancer risk assessment

Seafood	Hazard quotient (HQ)	Hazard index (HI)
Dried Bonga fish	2.91	3.44
Crayfish	3.08	3.71
Shrimp	1.82	2.10
Periwinkle	1.44	1.88

Estimated cancer risk associated with seafood consumption: **Table 7** shows the estimated cancer risk values associated with the consumption of contaminated dried seafood. Crayfish recorded the highest cancer risk value (5.2×10^{-4}), followed by dried Bonga fish (4.7×10^{-4}), shrimp (2.9×10^{-4}), and periwinkle (2.1×10^{-4}).

Table 7: Estimated cancer risk associated with seafood consumption

Seafood	Cancer risk value
Dried Bonga fish	4.7×10^{-4}
Crayfish	5.2×10^{-4}
Shrimp	2.9×10^{-4}
Periwinkle	2.1×10^{-4}

Discussion

Dioxin levels in dried seafood: Dried Bonga fish had the highest dioxin concentration, followed by crayfish, shrimp, and periwinkle. These findings suggest that dried Bonga fish and crayfish may accumulate greater quantities of dioxins than the other seafood analyzed, possibly due to differences in lipid content and processing methods. The concentrations observed in this study are generally higher than those reported in several seafood monitoring studies from Europe and Asia, where mean dioxin concentrations in fish and shellfish were typically below 2.00 pg TEQ/g fat, although elevated values have been reported in samples collected from industrialized and highly polluted environments [31, 32]. Furthermore, all the measured values exceeded the European Commission action threshold of 2.00 pg TEQ/g fat for dioxins in certain fishery products, indicating potential contamination that may warrant further investigation and monitoring [33]. The relatively high contamination levels observed may be attributed to multiple sources, including environmental deposition of dioxins from industrial activities [34], open burning and other combustion processes [22, 35], as well as contamination introduced during traditional smoke-drying practices [12, 13, 36]. Dioxins are persistent organic pollutants that resist environmental degradation, bioaccumulate in aquatic organisms, and biomagnify through the food chain, making seafood an important route of human exposure [33, 37].

Aflatoxin levels in dried seafood: Aflatoxins were detected in all seafood analyzed, indicating widespread contamination among the seafood products examined. Crayfish had the highest mean aflatoxin concentration, followed by dried Bonga fish, shrimp, and periwinkle. Although the European Union has established a maximum level of 10.0 µg/kg for the sum of aflatoxins in certain food commodities, including some dried products and spices, no specific maximum level has been prescribed for dried seafood under Commission Regulation (EU) 2023/915 [38]. Nevertheless, the aflatoxin concentrations detected in crayfish, dried Bonga fish, and shrimp exceeded this reference value, whereas the concentration observed in periwinkle remained below it. The elevated aflatoxin levels in crayfish and dried Bonga fish may be attributable to inadequate drying practices, poor storage conditions, prolonged exposure to humid environments, and fungal proliferation during processing, transportation, and marketing, as reported in previous studies [39-41].

Qualitative occurrence of Aspergillus species in dried seafood: A. flavus was detected in dried Bonga fish, crayfish, and shrimp, while *A. niger* was present in dried Bonga fish, crayfish, and periwinkle. *A. fumigatus* was identified in crayfish and shrimp but was absent in dried Bonga fish and periwinkle. Crayfish showed the highest fungal occurrence, as all the identified *Aspergillus* species were present. The detection of *A. flavus* supports the aflatoxin contamination observed in the seafood and suggests possible fungal colonization during storage, handling, and marketing under humid environmental conditions [39, 42].

Quantitative occurrence of Aspergillus species in dried seafood: Crayfish exhibited the highest total *Aspergillus* count, followed by dried Bonga fish, shrimp, and periwinkle. *A. flavus* recorded the highest count in crayfish, while *A. niger* was most abundant in crayfish and dried Bonga fish. *Aspergillus fumigatus* was detected only in crayfish and shrimp, with counts of $2.8 \pm 0.2 \times 10^3$ CFU/g and $2.4 \pm 0.2 \times 10^3$ CFU/g, respectively. The relatively high fungal loads observed in crayfish and dried Bonga fish are consistent with the elevated aflatoxin concentrations detected in these samples and may reflect inadequate storage conditions and prolonged exposure to humid environments during processing and marketing [39-42].

Estimated daily intake of dioxins and aflatoxins: Dried Bonga fish recorded the highest dioxin EDI value (4.85 pg/kg/day), followed by crayfish (4.13 pg/kg/day), shrimp (2.84 pg/kg/day), and periwinkle (2.39 pg/kg/day). Regarding aflatoxins, crayfish showed the highest EDI value (0.69 µg/kg/day), followed by dried Bonga fish (0.62 µg/kg/day), shrimp (0.38 µg/kg/day), and periwinkle (0.31 µg/kg/day). The estimated daily intake values suggest continuous exposure among consumers, especially individuals who frequently consume dried seafood. Prolonged exposure may increase the accumulation of these contaminants within the body and contribute to adverse health effects over time [18, 26, 36, 40].

Non-cancer risk assessment of dioxin and aflatoxin exposure: Crayfish recorded the highest HQ value (3.08) and HI value (3.71), followed by dried Bonga fish with HQ and HI values of 2.91 and 3.44, respectively. Shrimp and periwinkle exhibited comparatively lower values; however, HQ and HI exceeded the acceptable threshold value of 1.0. Hazard Quotient and Hazard Index values greater than unity indicate the possibility of adverse non-carcinogenic health effects following prolonged exposure. Such effects may include liver dysfunction, immunotoxicity, oxidative stress, and metabolic disturbances [44].

Estimated cancer risk associated with seafood consumption: Crayfish recorded the highest cancer risk value, followed by dried Bonga fish, shrimp, and periwinkle. The estimated cancer risk values exceeded acceptable safety thresholds, suggesting possible carcinogenic risks among frequent consumers of the seafood analyzed [45, 46]. The relatively high contamination levels observed in dried Bonga fish and crayfish may result from prolonged smoke exposure, environmental pollution, and poor storage conditions [35, 36, 40, 47]. The occurrence of *Aspergillus* species further indicates fungal contamination during handling and marketing [12]. The toxic effects associated with dioxins and aflatoxins may be linked to oxidative stress, enzyme disruption, reactive metabolite formation, and DNA damage [20, 22, 29]. Dioxins mainly exert their effects through activation of the aryl hydrocarbon receptor pathway [18], while aflatoxins go through metabolic activation in the liver to produce reactive intermediates capable of inducing cellular mutations and liver injury [25].

Conclusion: This study confirmed the presence of dioxins, aflatoxins, and potentially toxigenic *Aspergillus* species in dried seafood at Port Harcourt, Nigeria. Although periwinkle exhibited comparatively lower contaminant concentrations and risk estimates than the other seafood, its hazard Quotient, hazard index, and estimated cancer risk values remained above recommended safety thresholds, suggesting that cumulative exposure through regular consumption may pose public health concerns. The findings indicate that environmental pollution, smoke-drying practices, and inadequate storage conditions may contribute substantially to seafood contamination. Consequently, prolonged dietary exposure to these contaminants may increase the likelihood of oxidative stress, hepatotoxicity, immunological impairment, and carcinogenic effects.

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