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OCCURRENCE AND POTENTIAL HEALTH RISKS OF INDICATOR POLYCHLORINATED BIPHENYLS (PCBs) IN SELECTED MARINE FISH SPECIES, *RASTRELLIGER KANAGUTRA* AND *SIGANUS SUTOR*. A CASE OF DAR ES SALAAM

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ABSTRACT

The occurrence of polychlorinated biphenyls (PCBs) in marine fish and their potential health risks was assessed in Dar es Salaam region. Six indicator PCBs were assessed in selected marine fish species, *Rastrelliger kanagutra* and *Siganus sutor* collected from the Kivukoni-Feri, the major fish market and fishermen landing site in Dar es Salaam. Fish samples were extracted using the QuEChERS method and quantified by GC-MS/MS. The concentration of detected PCBs congeners in the analysed fish species was relatively low, ranging from <LOD to 3.71 ng/g w/w in *R. kanagutra* and from <LOD to 0.58 ng/g w/w in *S. sutor* species. However, the sum of mean PCBs concentration levels was below the maximum residue limit (MRL) of 75 ngg^{-1}w/w set by the European Commission (EC) in the muscle of fish and fishery products. Estimated lifetime cancer risks were categorised as very low in both children and adults, with indices values ranging from 1.24E-08 to 6.14E-07 for children and 5.14E-09 to 2.55E-07 for adults, while non-carcinogenic risks were insignificant as the estimated hazard indices were less than 0.1 for both children and adults.

Keywords: Polychlorinated biphenyls (PCBs), marine fish, persistent organic pollutants (POPs), risk assessment, biomagnification.

1. INTRODUCTION

- PCBs were detected in both marine fish species
- There was a significant difference in concentration levels between species
- Estimated health risks were very low below recommended health guidelines

Polychlorinated biphenyls (PCBs) are synthetic industrial chemicals widely used worldwide from the 1920s until the ban on their commercial production was imposed in the late 1970s (Montano et al., 2022; Shi et al., 2019; Munawar et al., 2021). PCBs are characterized by their resistance to degradation (Wenaty et al., 2019b), high lipophilicity (Mwakalapa et al., 2018; Lin, et al., 2020; Munawar *et al*., 2021), and ability to transport over a long distance far from the point of production or release (Umasangaji et al., 2020) remaining in the environment for a long time and accumulate along the food chains (Lin, et al., 2020). PCBs had many uses in applications such as flame retardants, heat-resistant lubricants, paints, electrical transformers, and electronic appliances (Shen, et al., 2017; Wenaty et al., 2019b).

Improper handling of the PCBs-containing products during use, storage, and maintenance (Umasangaji et al., 2020) leads to the release of PCBs into the environment and then distributed to other locations far from the site of release through runoff water, underground water from

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contaminated area and emissions from open-burning municipal waste landfills (Jafarabadi et al., 2019; Hellar-Kihampa et al., 2013; Arinaitwe et al., 2018; Aslam et al., 2019). Most PCBs end up in oceans sediments which is the stable reservoir for PCBs, remaining there for a long time and continuously being released in small quantities into the water and other fish feeding grounds over time (Ibeto *et al*., 2019; Obanya et al., 2019; Umasangaji et al., 2020). Human exposure to PCBs is primarily through the consumption of food of animal origin namely fish, meat, and dairy products (WHO, 2016; Duedahl-Olesen et al., 2020; Ravenscroft and Schell, 2018). Exposure to PCBs can result in adverse health effects including endocrine system disruption, cancer, infertility, developmental effects in breastfed infants, and immunological effects (Hulin et al., 2020; Lin et al., 2020; Muller et al., 2017; Quijano et al. 2018; WHO, 2016; Panseri et al., 2019; Montano et al., 2022).

Tanzania imported and used products containing PCBs including electrical transformers, oil circuit breakers, and switch gears before they were banned, with some of them still in use and others in storage across the country (URT/NIP, 2005, 2018). Studies (Hellar-Kihampa et al., 2013; Mahugija, 2015; Polder, 2014; Wenaty et al., 2019b; Mwakalapa et al., 2018; Muller et al., 2017) have documented the presence of PCBs contamination in various locations in Tanzania. As most of the PCBs from contaminated sited end up in the ocean, marine organisms, particularly fish are vulnerable to contamination by PCBs (Duedahl-Olesen et al., 2020; Montano *et el.*, 2022).

Fish is one of the main sources of food in Tanzania, as an important source of essential compounds such as iodine, vitamin D, and essential fatty acids, making up to 30% of the country's animal protein intake (URT 2019; Duedahl-Olesen et al., 2020). However, there is limited information on PCBs contamination status in the marine environment and seafood in Tanzania. This study aimed to assess the occurrence of indicator PCBs (PCB 28, PCB 52, PCB 101, PCB 138, PCB 153, and PCB 180) in selected marine fish species *Rastrelliger kanagutra* (Indian mackerel) and *Siganus sutor* (spine-foot rabbitfish) and assess their probable health effects in Dar es Salaam city, Tanzania.

2. MATERIALS AND METHOD

2.1 Study area description

This study was conducted in Dar es Salaam city located along the coast of mainland Tanzania. Dar es Salaam is the most populous and industrialized region in Tanzania and is the major hotspot for the discharge of industrial and urban wastes into the Indian Ocean.

2.2 Sample collection and preparation

Fish samples were collected from fishermen and fish vendors at the Kivukon Feri fish market in Dar es Salaam. Sixty fish samples from two different species (30 fish samples from each species) *R. kanagutra* and *S.sutor* were randomly collected. The collected samples were placed in the container, marked appropriately, immediately transferred into a cool ice box, and transported to the laboratory where they were held at -18^{0}C until the time of analysis. During sample preparation, fish were thawed and skin-free fillets were cut and placed on clean aluminium foil, then homogenized using a laboratory blender. The homogenized sample was placed in a sample container and stored at -18^{0}C until the time of analysis. Contamination during sample preparation was avoided by cleaning the laboratory blender, knives, spatula, and forceps thoroughly with soap, and water was then wiped with ethanol between samples.

2.3 Sample extraction and clean-up
Sample extraction was conducted by using a slightly modified Quick, Easy, Cheap, Efficient, Rugged, and Safe (QuEChERS) extraction method described in detail by Norli et al. (2011), where 5 gram of fish filets were weighed into 50mls centrifuge tubes marked M for *R. kanagutra* and R for *S. sutor* samples. Five millilitres of water were added and vortexed for 1 min to homogenize the mixture. Ten millilitres of acetonitrile were then added to the mixture and vortexed for 2 min then sonicated for 30 min to enhance extraction. The mixture was then centrifuged for 5 minutes at 4000rpm and placed into a freezer and kept at – 20 ^{0}C for 1 hour, then immediately centrifuged for 5 minutes at 4000rpm. The supernatant was then transferred into a tube containing 300 mg $MgSO_4$ and 50 mg PSA and the mixture was vortexed and centrifuged for 5 min the supernatant was transferred into the vials for GC analysis.

2.4 Instrumental analysis
Detection and quantification of indicator PCBs were carried out using a gas chromatography-tandem mass spectrometer (GC-MS/MS – Agilent 7890B – 7000D) operating in multiple reaction monitoring (MRM/SRM) with HP-5MS capillary column (30 m x 0.25 mm x 025 µm). The carrier gas used was helium flowing at a constant flow rate of 1.2 ml/min. Injection volume was 1 µL in splitless mode into the GC column. Inlet temperature was set at 280^{0}C and the program for oven temperature was, 80 ^{0}C hold for 1 min, 80 – 170^{0}C at 50^{0}C min^{-1}, then 170 – 240 ^{0}C at 5 ^{0}C min^{-1}, hold for 1 minute, finally 240 – 280^{0}C at 20^{0}C min^{-1}.

2.5 Quality control and assurance
Cross-contamination and contamination from outside environments were controlled by conducting analyses in a controlled environment. In-house quality control samples (spiked sample, blank sample, and blank solvent) which were processed after a run of each 10 samples were used for monitoring and recovery analyses. Recoveries were estimated from spiked samples of the 6 indicator PCBs standards (PCB 28, 52, 101, 138, 153, 180) and analysed with the same analytical method used to analyse the samples. The concentration (in ngg^{-1}) obtained in samples was corrected by using the blank values obtained for each batch.

2.6 Risk Characterisation
The potential human health risks related to human exposure to PCBs through consumption of contaminated fish were estimated by using a risk assessment model adopted from (Wenaty et al., 2019b) in which lifetime cancer risk (C_R) was calculated using Equation 1;

$$C_R = SF \times CDI \quad \ldots (1)$$

where SF is the cancer oral slope factor (2 per mg/kg/day) used for food ingestion and early life exposures (ATSDR, 2018), CDI is the estimated chronic daily intake, and the CDI is estimated using equation 2;

$$CDI = \frac{C \times IR \times EF \times ED}{BW \times AT} \quad \ldots (2)$$

Where C is the measured concentration of contaminant in fish (mg/kg), IR is the fish consumption rate, 23 gday^{-1} (URT 2021), EF is the exposure frequency (365 days/year), ED is the exposure duration (60 years for adults and 12 years for children (Wenaty et al., 2019a), BW is the hypothetical average body weight ((70kgs for adults and 29kgs for children for Africans and Europeans consumers (Yu et al., 2010) and AT is the average time per year (For carcinogenic risk for both adults and children, AT was set at 70 hrs × 365 days and for non-carcinogenic risks AT was set at 30hrs × 365 days for adults and 8 × 365 days for children, (Lin et al., 2020). Non-carcinogenic risks were estimated using the hazard quotients (HQs) for each indicator PCBs by using Equation 3;

$$HQ = \frac{CDI}{RfD} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(3)$$

Where RfD is the Reference dose (estimate of daily oral exposure, 0.02 mg/kg-day (Lin et al., 2020)) and the overall non-carcinogenic risk was estimated using Equation 4;

$$HI = \sum HQs \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(4)$$

Where ∑HQs is the sum of the hazard quotients (HQs) estimated for each of the indicator PCBs.

Description of lifetime cancer risks was based on (ATSDR, 2018) standards as follows; very low when the estimated value is Cr ≤ 10E-06, low when 10E-06<value≤10E-04, moderate when 10e-04<value≤10E-03, high when 10E-03<value≤10E-01 and very high when estimated value is Cr >10E-01. For non-carcinogenic risks, no carcinogenic health risk will occur when HI < 0.1, low potential for non-carcinogenic risks to occur when HI value range from 0.1 to 1, and when HI > 1 there is a high potential for non-carcinogenic risks to occur (Wenaty et al., 2019b; Lin et al., 2020).

2.7 Data analysis

Statistical analysis of data was conducted using IBM SPSS software v.20 in which data were presented in mean. One sample t-test was used to compare obtained ∑PCBs levels to the set maximum residue level (MRL) and Independent t-test was used to compare concentration between species. A level of $p \leq 0.05$ was chosen as a criterion for statistical significance.

3. RESULTS

3.1 Characteristics of analysed fish samples

The mean length for *R. kanagutra* was 25.79 cm with lengths ranging from a minimum length of 23.5 cm to a maximum length of 27.5 cm while the mean weight was 300.54 grams with weights ranging from a minimum weight of 214.74 grams to the maximum weight of 389.79 grams. The mean length for *S. sutor* was 28.07 cm with lengths ranging from a minimum length of 24.0 cm to the maximum length of 33.0 cm, while the mean weight was 448.22 grams with weights ranging from a minimum weight of 280.41grams to the maximum weight of 717.77 grams.

3.2 Occurrence and Levels of PCBs in fish muscles

Sixty fish samples were analysed, 30 samples from each species, *R. kanagutra*, and *S.sutor*. Results showed the presence of indicator PCBs in both fish species with 73.3% of R. *kanagutra* samples and 20% of *S.sutor* samples contaminated with PCBs (Table 2.1). The concentration levels of the six indicators PCBs were relatively low in both fish species with the highest mean concentration ($\sum_6$PCBs) of 3.71 ngg^{-1} w/w observed in *R. kanagutra*. For *R. kanagutra* analysed samples, the individual concentration levels of congeners, PCBs 28 and 52 were below detection level (<LOD), PCB 101 concentration ranged from 0.11 to 0.12 ngg^{-1} w/w, PCB 138 ranged

from 0.12 to 1.07 ngg^{-1} w/w, PCB 153 ranged from 0.1 to 1.55 ngg^{-1} w/w and PCB 180 concentration ranged from 0.11 to 1.09 ngg^{-1} w/w, and the sum of the six indicator PCBs ($\sum_6$PCBs) levels ranged from 0.12 to 3.71 ngg^{-1} w/w.

Table 2.1: Mean concentration of PCBs contaminants in ngg-1 wet weight (ng/g w/w) in fish species *R. kanagutra* and *S. sutor* from Kivukoni-Feri fish market.

		Mean Concentration (ngg^{-1} w/w)				
PCB Congener	N	*R. kanagutra*	Sample detected (%)	*S. sutor*	Sample detected (%)	EC-MRL
PCB 28	30	<LOD	0	<LOD	0	-
PCB 52	30	<LOD	0	<LOD	0	-
PCB 101	30	0.008	7	<LOD	0	-
PCB 138	30	0.220	56	0.008	7	-
PCB 153	30	0.396	73	0.026	13	-
PCB 180	30	0.216	63	0.016	13	-
$\sum_6$PCBs	**30**	**0.840**		**0.050**		75

Key: N: Total number of samples analyzed for each species, EC-MRL: Maximum residue limit set by European Commission (EC, 2011), w/w: Wet weight, LOD: Limit of Detection

For *S. sutor* analysed samples, the individual concentration levels of congeners, PCBs 28, 52, and 101 were below detection level (<LOD), PCB 138 concentration ranged from 0.1 to 0.14 ngg^{-1} w/w, PCB 153 ranged from 0.13 to 0.28 ngg^{-1} w/w and PCB 180 ranged from 0.12 to 0.13 ngg^{-1} w/w and the sum of six indicator PCBs ($\sum_6$PCBs) concentration levels ranged from 0.12 to 0.54 ngg^{-1} w/w.

3.3 Risk Characterization

The potential human health risks to the general population as a result of consumption of PCBs-contaminated fish consumption were assessed using the equations stated above for both children and adults. The estimated lifetime cancer risks, C_R, were between 1.24E-08 and 6.14E-07 for children and between 5.14E-09 and 2.55E-07 for adults (Table 2.2), while the estimated non-carcinogenic health risks (HI), were between 1.95E-06 and 3.26E-05 for children and 8.07E-07 and 1.35E-05 for adults (Table 2.2).

Table 2.2. Estimated lifetime cancer risks for children and adults as results of intake of detected PCBs in *R. kanagutra* and *S. sutor* from Kivukoni-Feri fish market.

	Estimated lifetime cancer risks (C_R)			
	R. kanagutra		*S. sutor*	
PCB Congener	Adults	Children	Adults	Children
PCB 28	-	-	-	-
PCB 52	-	-	-	-
PCB 101	5.14E-09	1.24E-08	-	-
PCB 138	1.41E-07	3.41E-07	5.14E-09	1.24E-08
PCB 153	2.55E-07	6.14E-07	1.68E-08	4.07E-08
PCB 180	1.39E-07	3.35E-07	1.03E-08	2.48E-08

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Table 2.3. Non-carcinogenic risks for children and adults as a result of intake of detected PCBs in R. kanagutra and S. sutor from Kivukoni-Feri fish market

	Estimated Non-carcinogenic risks (HI)			
	R. kanagutra		*S. sutor*	
PCB Congener	Adults	Children	Adults	Children
PCB 28	-	-	-	-
PCB 52	-	-	-	-
PCB 101	1.29E-07	3.10E-07	-	-
PCB 138	3.54E-06	8.53E-06	1.29E-07	3.10E-07
PCB 153	6.36E-06	1.54E-05	4.21E-07	1.02E-06
PCB 180	3.47E-06	8.38E-06	2.57E-07	6.21E-07
Hazard Indices (∑HQs)	**1.35E-05**	**3.26E-05**	**8.07E-07**	**1.95E-06**

Key: ∑HQs: Sum of hazard quotients, HI: Hazard indices

4. DISCUSSION

4.1 Occurrence and levels of PCBs in fish muscles

The estimated PCBs concentration levels in the analysed fish species were dominated by the three congeners PCBs 138, 153, and 180. PCB 153 was the most prevalent congener in the present study for both fish species followed by PCB 138 and PCB 180 congeners. The prevalence of the three congeners is explained by their higher chlorination which makes them more resistant to degradation in the environment, ability to bioaccumulate due to their higher lipophilicity and low biodegradation rate in organisms (Lin et al., 2020; Wenaty et al., 2019b; Mwakalapa et al., 2018). This trend is similar to that observed by (Mwakalapa et al., 2018, Haar et al., 2021; Shang et al., 2016) where PCB 153 congener was more dominant but slightly different from studies from other locations in Tanzania (Wenaty et al., 2019b; Polder et al., 2014) where PCB 138 congener was more dominant congener, which suggests contamination originated from different sources of PCBs (Mwakalapa et al., 2018). The absence of PCB 28 and 52 in both species and the very small contamination level of PCB 101 only in *R. kanagutra* species suggests that contamination is not from the fresh release of PCBs into the environment. This can be explained due to fact that PCBs 28, 58, and 101 have relatively higher volatility, water solubility, and biodegradability than PCBs 138, 153, and 180 (Lin et al., 2020; Wenaty et al., 2019b; Hellar-Kihampa et al., 2013).

Statistical analysis showed a significant difference in the mean PCBs concentration levels between the analysed species samples at ($p< 0.05$). The mean PCBs concentration of indicator PCBs congener in *R. kanagutra* was significantly higher than that of *S. sutor* with the sum of indicator PCBs mean concentration ($\sum PCB_6$) ranging from 0.12 to 3.71 ng/g w/w for *R. kanagutra* and 0.12 to 0.54 ng/g w/w for *S. sutor*. However, the PCBs contamination levels observed were below the European Commission (UC) maximum residue limits (MRL) set values of 75 ng/g w/w for fish meat and fishery products (EC, 2011). The higher concentration of PCBs in *R. kanagutra* fish species than in *S. sutor* species is probably due to the difference between their feeding habit, and also the fact that *R. kanagutra* has a higher trophic level than *S. sutor* (Fishbase, 2017). *R. kanagutra* are omnivorous feeding on seagrasses and phytoplankton (diatom/algae) but also feed on zooplanktons and other small organisms such as polychaetes, ostracods, cladocerans, and small fishes such as anchovy and sardines which results to biomagnifacation (Mziray and Kimirei, 2016; Fishbase, 2017) while the *S. sutor* are herbivorous fish feeding on seaweed and seagrasses benthic algae and associated flora (Mziray and Kimirei, 2016; Fishbase, 2017). These findings have a similar trend to results obtained by other studies (Shen et al., 2017; Panseri et al., 2019), that showed omnivorous fish and other predator fish (carnivorous) have more contamination levels of PCBs than herbivorous fish because of biomagnification.

Fish habitation may have also influenced the difference in PCBs contamination levels obtained in *R. kanagutra* and *S. sutor* fish species. *R. kanagutra* are oceanodromous fish and habit may have contributed to its higher PCBs contamination levels compared to *S. sutor* which live in shallow water but are not oceanodromous. This finding is in line with those obtained in a study by Shang et al. (2016) where oceanodromous fish living in shallow coastal water were found to have higher PCBs contamination than non-oceanodromous species that live in shallow water. Fish age is also one of the factors influencing the concentration level of PCBs in fish (Wenaty et al., 2019b; Polder *et al*., 2014). The age of the collected fish sample in the present study was not recorded but the maximum age of *R. kanagutra* is reported to be 4 years while the reported maximum age of *S. sutor* is 2.5 years (Fishbase, 2017) which may explain why *R. kanagutra* have higher PCBs concentration levels than *S. sutor* due to difference in time of accumulation of PCBs in corresponding fish species taking into account the average size of the analysed samples (Fishbase, 2017).

Findings in the present study showed the concentration of PCBs levels obtained in marine fish in this study is relatively higher than that of fish from freshwater in Tanzania, where Polder et al. (2014) reported that tilapia from Lake Tanganyika (0.57 ngg^{-1} w/w), Lake Nyasa (0.02 ngg^{-1} w/w) and Lake Victoria (0.08 ngg^{-1} w/w) had lower PCBs concentration levels than that obtained in the present study (0.84 ngg^{-1} w/w) with the same feeding habits(*R. kanagutra*). This trend is similar compared to other reported studies where PCBs contamination levels in marine fish were found to be higher than that obtained from freshwater fish (Ahmed, et al., 2016; Shang et al., 2016) probably due to the vast amount of waste discharged into the ocean from multiple source channels and rivers from different parts of the country far from the ocean. However, the PCBs concentration levels obtained in this study were lower than that obtained by other studies for marine fish with the same feeding habits in India 65.8 ngg^{-1} w/w by Ahmed, et al. (2016) and China 3.82 ngg^{-1} w/w by Shang et al. (2016) involving *R. kanagutra* fish species which suggests a higher level of contamination because to a higher level of industrialization of the areas which may lead to higher environment pollution.

4.2 Risks characterization

Estimated lifetime cancer risks estimated for adults and children were observed to be below the critical values of 10E-6 for adults and 10E-04 for children (Lin et al., 2020), which indicates that consumption of the *R.kanagutra* and *S. sutor* fish species from the Kivukoni Feri fish market will have negligible adverse health effects. The hazard indices (HI) values used to estimate the non-carcinogenic health risks were lower than 0.1 indicating that there are no non-carcinogenic risks that are likely to occur as a result of consumption of the *R.kanagutra* and *S. sutor* fish species from the study area.

5. CONCLUSION

Based on the finding of this study, there is a presence of PCBs contamination in the study area. However, the detected PCBs concentration levels were below the maximum residue limits (MRL) set by European Commission (UC), while the estimated values for potential health effects using the detected contamination levels were observed to have insignificant adverse health effects for both children and adults. Despite the very low risks estimates of potential health risks in the present study, further regular monitoring of PCBs contamination of marine fish is recommended to include a wider range of all coastal regions in Tanzania to establish the status of contamination that can lead to the formulation of best strategies to prevent further build-up of PCBs in the food chain.

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