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# OCCURRENCE AND CONSUMER HEALTH RISK ASSESSMENT OF HEAVY METALS IN FROZEN DEMERSAL FISH AND CEPHALOPOD PRODUCTS FROM BENOA PORT, BALI PROVINCE

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## *Abstract*

***Introduction:*** *Heavy metal pollution has become an important environmental issue today. This study was aimed to identify the heavy metals of the two fishing products namely demersal fish and cephalopods landed at Benoa Port, Bali Province and their health risk effects on consumers.* ***Methods:*** *Demersal fish and cephalopod products were obtained from local fishermen at Benoa port. Atomic Absorption Spectrophotometry (AAS) was used to specify the (Pb, Cd, and Hg) in 34 fish fillet products weighing an average of 500 grams each. Statistical software was used to conduct an analysis of the collected data, while THQ, TTHQ, and TCR were calculated and compared with USEPA regulations.* ***Results and Discussion:*** *The results of this research showed that the content of heavy metals in demersal fish and cephalopod products was lower than the levels suggested by official agencies in a number of countries. In populations of various ages, the target Hazard Quotient (THQ) for the heavy metal was less than 1. At this point, the Total THQ result did not imply any risk to human health from ingestion of these two fishery products. When these two products were ingested, the target cancer risk (TCR) did not represent a cancer risk.* ***Conclusion:*** *The outcomes of this study showed that the bioaccumulation levels of both fishery products with low levels of heavy metals are safe for human consumption.*

## INTRODUCTION

Fish becomes a commodity that is widely traded and its availability is critical to global food and nutrition security programs (1–3). Since the 1960s, there is an increase of global consumption of fish per capita, from 9.9 kg in the 2000s to approximately 20.4 kg in 2019 (4-5). Indonesia is a tropical archipelagic country known as the "maritime axis of the world" because of its abundant maritime resource potential, especially in the fisheries sector (6). Particularly, coastal residents always seek out fish as a food supply because it is relatively inexpensive, nutrient-dense, and their primary source of income (7). Despite the fact that a number of communities located far from the coast have implemented cultivation systems, Indonesia's annual production of fisheries products continues to increase significantly. It is necessary to supplement the aquaculture industry with trawling in natural marine waters, which are abundant in fish species.

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Not only does the prevalence of fish consumption trends stem from its flavor, but also from its nutritional content, which can be determined by specific fish species (8). Meat of the fish contains a high-protein, polyunsaturated fatty acid, mineral, vitamin, and omega-3 fatty acid supply, as well as a low-cholesterol dietary source that is beneficial to human health and reduces the risk of cardiovascular disease (9), cancer (10), joint inflammation (11), and anti-inflammatory (12). Fish protein is highly digestible because its amino acid composition contains more cysteine than the majority of other protein sources (13). Sea fish, furthermore to serving as an excellent source of protein from animals, include minerals that help to regulate the equilibrium of acids and bases and aid to hemoglobin formation (14).

Despite the various health benefits of fish intake, the potential of fish to accumulate environmental contaminants and transfer them to people defines a health concern in some cases (15-16). Metals can accumulate in fish tissues and induce toxicity by interfering with essential and reproductive activities, weakening the body's defenses, and causing structural and pathological changes (17-18). The level of toxicity is determined by various factors, including i) exposure period, ii) type of metal, iii) organism, iv) tissue of an organism, and v) physico-chemical properties of water (19). The same is true of fishing endeavors in the wild, which can pose a threat in and of themselves because these water bodies are contaminated with pesticides or other pollutant agents from agricultural land, oil spills, microplastics, and heavy metals from industry (20–23). Metal pollution in waters has been linked to detrimental effects on biodiversity induced by metals and metalloids, ranging from population decline to extinction (24).

To understand heavy metals in aquatic species, one can monitor their levels in fisheries products and their bioavailability to calculate the quantity of absorbed heavy metals in human body (25). The bioaccumulation capacity of aquatic species for these metals, on the other hand, is an effective exposure bioindicator and has been utilized for monitoring environmental and food quality in polluted habitats. The selection of demersal fish and cephalopod products for this research was based on their nutritional value and high export production in several region such as Mediterranean and European. Heavy metals are quantified based on their essential and non-essential importance in the environment as trace and dangerous metals, as well as their potential damage to human health (26).

Heavy metals are measured based on their essential and non-essential significance in the natural world as trace and dangerous metals, as well as their potential damage to human health (27). In assessing the health risk, the THQ method is utilized to evaluate contamination exposure and toxicity data. Because there are no mathematical criteria for evaluating possible non-cancerous consequences of toxin exposure (28-29). The published research findings on heavy metal levels in fish products, as well as risk assessments (30–32).

Benoa Port, Bali Province, is a port of entrance to the city of Denpasar via the sea route that has been in operation since 1924. Benoa Port is also used as a trade route for captured fishery products such as yellowfin tuna, cephalopods, demersal fish products, and crustaceans from various Indonesian regions. On the other hand, many marine tourism and small-scale fishing and industrial activities are conducted out in the Benoa Port area. Until now, there have been no reports regarding the monitoring of contamination (Cd, Pb, and Hg), particularly in fishery products (demersal fish and cephalopods) landed at Benoa Port in Bali Province. This research was aimed to identify the levels of heavy metals (Pb, Cd, and Hg) in frozen demersal fish and cephalopod meat, compare those levels to Food and Agriculture Organization (FAO), World Health Organization (WHO), Standar Nasional Indonesia (SNI), Regional Organization for the Protection of the Marine Environment (ROPME), Food and Drug Administration (FDA), Balai Besar Pengawas Obat dan Makanan (BBPOM), and European Commission (EC) standards, and assess the health risks (THQ, HI, and CR values) associated with the consumption of these heavy metals by children and adults.

## METHODS

### Sampling Procedure

In June of 2022, 34 commercial fish fillet products (17 frozen demersal products and 17 frozen cephalopod products) were collected from fishery product distributors in Benoa Port, Bali Province. After being sliced into fillets by distributors, demersal fish and cephalopod products are collected (Figure 1). Each product averages 500 grams in weight. The fish products are then transported in isoprene crates filled with ice gel for transportation to the Environmental Health Laboratory, PT Intimas Surya to be analyzed for heavy metal concentrations (Pb, Cd and Hg).

*(a)* *(b)* *(c)*

**Figure 1. Benoa Port in Bali Province *(a)*, Cephalopods *(b)*, and Frozen Demersal Fish *(c)* as Products Analyzed in this Study**

### Determination of Heavy Metal Concentrations

A 0.5 g fillet of fish tissue was measured and destroyed with aqua regia (a 3:1 mixture of hydrogen nitrate (Merck, USA) and HCl (Merck, USA) (33-34). Following that, 5 mL of $HNO_3$ (Merck, USA) and 15 mL of HCl (Merck, USA) were then added to each sample, and the mixture was allowed to react gently for an hour before being heated at 60°C until nearly dry. 3 mL pure $HNO_3$ was added again to thoroughly dissolve the sample, followed by 10 mL of distilled water boiled to eliminate excess acid. After repeating the method, the amount of water in the volumetric vessel was lowered to around 5 mL by heating on a hot plate. After cooling, the solution was filtered and transferred to a volumetric flask, which was then filled using distilled water to the 50 mL mark and deposited in a sterile specimen vial for AAS analysis. Heavy metals were evaluated using an atomic absorption spectrophotometer (AAS) AA-7000 (Shimadzu Co, Japan) (35). The heavy metal levels found were then compared with quality standards from various relevant authorities in various countries (FAO, WHO, ROPME, FDA, EC, and BBPOM).

### Quality Control

To prevent sample mixing during the analysis phase, each sample container was labeled with the corresponding product name. Using a pro analysis (p.a) reagent, a determination was made, and a duplicate analysis was conducted. Before conducting an analysis, laboratory equipment was sterilized with an autoclave, and plastics were immersed in 10% $HNO_3$ for 24 hours. Meanwhile, the concentration of the chemical solution contained in the acid room was measured. Standard heavy metal solutions with a total concentration of 20,000 ng/L were introduced to fish fillet specimens for precision technique analysis. The limit of detection (LOD) of the instrument was 1 ng/g for (Pb and Cd) and 0.005 ng/l for (Hg).

### Health Risk Determination

### Target Hazard Quotient (THQ)

Non-carcinogenic exposure risk limits for heavy metal pollution were determined using this calculation. A risk table created by the USEPA was used to quantify the human health danger presented by consuming heavy metal-contaminated fisheries products. The following equation was used to perform the calculations:

$$THQ = \frac{EF\ x\ ED\ x\ FIR\ x\ C}{RfD\ x\ W\ x\ ATn}\ x\ 10^{-3}$$

Description:

EF = exposure frequency (365 days/year)

ED =Length of time of contact (71.5 years) is similar -in comparison to Indonesia's average lifetime.

FIR =food consumption (in grams per person each day) rate

C = (ppm) metal content in food

RfD =- each major metal's dietary standard intake (ppm/day)

W = -Indonesia's average body weight (70 kg for adulthood, 10 kg, 15 kg, 20 kg, and 44 kg for kids aged one, three, six, and twelve years, respectively.)

ATn = Mean non-carcinogen time of exposure (365 days each year, or the number of years of interaction assuming 71.5 years of involvement in the current study, adjusted for life length)

THQ score less than one suggests that this fisheries product is safe to consume and unlikely that may cause concern (23).

### Total Target Hazard Quotient (TTHQ)

On the basis of the 2011 USEPA, the total THQ for THQs was calculated by adding the hazard quotients.

$$TTHQ = THQ(Pb) + THQ(Cd) + THQ(Hg)$$

Description:

TTHQ = Total hazard quotient of the target
THQ (Pb) = The hazard quotient for Pb ingestion.
THQ (Cd) = The desired hazard quotient for Cd ingestion.
THQ (Hg) = The hazard quotient for Hg ingestion.

### Target Cancer Risk

Target cancer risk (TCR) is the word used to describe probable carcinogenic hazards. The USEPA Region III Risk-Based Concentration Tables provide TCR measuring methodologies in addition to characterizing them. The TCR estimate model is depicted below:

$$TCR = \frac{CM \times ED \times FIR \times CPSo}{WAB \times ATc} \times 10^{-3}$$

Description:

TCR = Cancer risk target
CM = Heavy metals (Pb, Cd, fan Hg) concentration in each fishery product (ppm)
ED = Exposure time (71.5 years) is similar to the average age in Indonesia.
FIR = Indonesian seafood intake (grams per day)
CPSo = Oral cancer slope factor (1.5 ppm/day)
WAB = average body weight in Indonesia (70 kg for adults, 10 kg, 15 kg, 20 kg, and 44 kg for children aged 1, 3, 6, and 12, respectively.)
ATC = the exposure to carcinogens (365 days 71.5 years). CPSo values for Pb = 0.38 ppm, Cd = 0.01 ppm, and Hg = 0.0003 ppm

### Statistical Analysis

Heavy metal concentration levels gathered in this study were evaluated using the SPSS 23.0 (IBM, USA) software. The quantities of heavy metals in demersal seafood and cephalopod products were calculated using the mean and standard deviation (SD). The T test was performed to examine if there had been a difference in heavy metal content between the two fishery products. A p0.05 probability criterion revealed a significant difference. Ms. Excel was used to compile data for health risk assessments (Microsoft, USA).

## RESULTS

### Heavy Metals Content

The average concentration of metallic elements in each kind of fisheries product varies significantly (Table 1).

The mean Cd metal concentration was determined to be greater in Cephalopod products (0.1917±0.256 mg/kg) than in frozen demersal fish products (0.00741±0.009 mg/kg; p≤0.05). Similar findings were observed for Hg metal, which was substantially higher in Cephalopod products (0.0866±0.075 mg/kg) than in frozen demersal fish products (0.0390±0.054 mg/kg). In contrast to the two heavy metals, a higher average Pb concentration was found in frozen demersal fish products (0.1936±0.162 mg/kg) when compared to cephalopods (0.0557±0.040 mg/kg).

The mean Cd metal concentration in Cephalopod products was found to be higher (0.19170±256 mg/kg) compared to refrigerated demersal fish products (0.007410±009 mg/kg; *p* 0.05). Similarly, Hg metal levels were significantly higher in Cephalopod products (0.08660±075 mg/kg) than in cold demersal fish products (0.03900±054 mg/kg). In contrast with the two heavy metals, frozen demersal fish products had a greater average concentration (0.19360±162 mg/kg) of Pb than cephalopods (0.05570±040 mg/kg).

**Table 1. Concentration (mg/gr) of Heavy Metal in Demersal Fish and Cephalopod of the Frozen Products**

| Heavy Metals | Demersal Fish | Cephalopods | p value | t value |
|---|---|---|---|---|
| Cd | 0.00741±0.009 | 0.1917±0.256 | 0.000 | -2.962 |
| Pb | 0.1936±0.162 | 0.0557±0.040 | 0.000 | 3.396 |
| Hg | 0.0390±0.054 | 0.0866±0.075 | 0.006 | -2.107 |

Heavy metal concentrations in each fisheries product (demersal fish and cephalopods) are lower than the recommendations of governmental agencies such as FAO, WHO, ROPME, FDA, EC, and BBPOM that establish quality criteria for heavy metal levels in fishery goods. As a result, heavy metal levels in demersal fish and cephalopod fillets do not exceed the legal limitations of each agency (Table 2).

### Assessment of Health Risks

In the evaluation of health risks potential, assumptions are used to determine the target hazard distribution results (for each heavy metal) based on human age. The probability of the occurrence of non-cancer diseases during the age period of 70 years is used to convey the health hazards linked with the cancerous effects of every heavy metal found in fish flesh. The age of an average consumer of fisheries products is used to determine the outcomes of THQ value assessment.

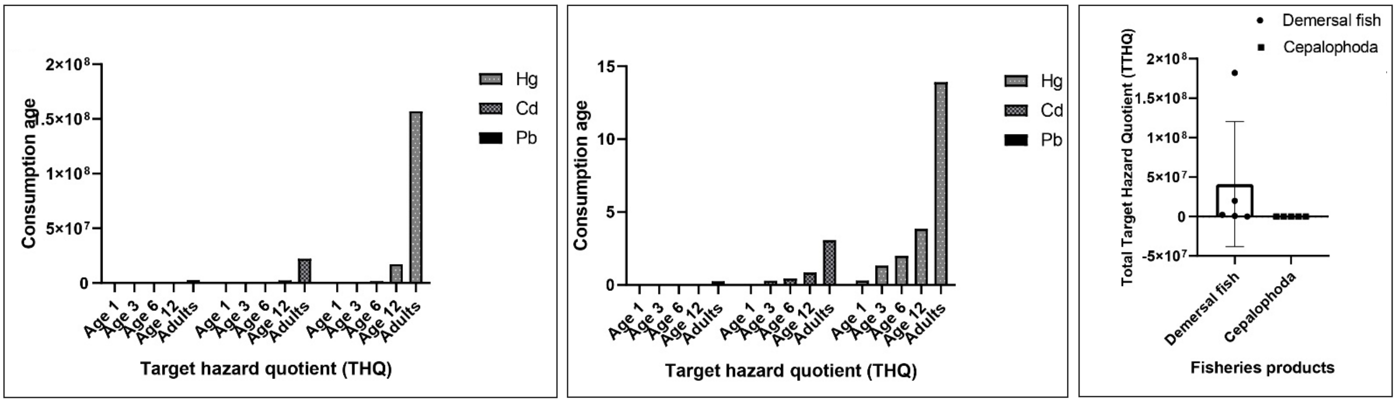
THQ = 1 is a valid THQ value. The THQ score for both frozen demersal and cephalopods is 1 for each age group and heavy metal, suggesting that there is no proof of non-carcinogenic health concerns related with Pb, Cd, and Hg ingestion through this fish product. Pb

(0.00983 for 1-year olds and 0.0000973 for 3-year-olds) was found in demersal fish, whereas Cd (0.00853 for 12-year-olds) was found in cephalopods. Similarly, the TTHQ value for all metals at all consumer ages is 1, suggesting that there is no danger of non-carcinogenic disorders in these two items (Figure 2). The average amount of TTHQ in demersal seafood, on the other hand, is greater compared to Cephalopoda products.

The TTHQ value for every metal at all consumer ages is 1, indicating that these two items are not carcinogenic (Figure 2). The average level of TTHQ in demersal fish products is higher than in Cephalopoda goods.

**Table 2. Acceptable Maximum Limits of Heavy Metals in Various Standards**

| Heavy Metals | Standards | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Demersal Fish | Cephalopods | FAO | WHO | ROPME | FDA | EC | BBPOM |
| Cd | 0.00741±0.009 | 0.1917±0.256 | 0.5 | 0.5 | 0.01-0.75 | 4 | 0.5 | 0.10 |
| Pb | 0.1936±0.162 | 0.0557±0.040 | 2 | 0.5 | 0.01-1.28 | 1.7 | 1 | 0.2 |
| Hg | 0.0390±0.054 | 0.0866±0.075 | 0.5 | - | - | < 1 | <1 | 0.50 |
| References | This study | This study | (63) | (64) | (65) | (66) | (67) | (68) |




*(a)* *(b)* *(c)*

**Figure 2. Target Hazard Quotient (THQ) of demersal fish *(a)* and cephalopod frozen products *(b)* and Total Target Hazard Quotient (TTHQ) for each Heavy Metal from ingestion of both fishery products *(c)***

The exposure of heavy metals of Pb, Cd, and Hg through consumption of contaminated demersal and cephalopod fish fillets (mg/kg/day) was estimated using FIR and CPSO data, as described in the methodologies section may result the high TCR. The TCR values resulting from exposure to Pb, Cd, and Hg through consumption of these two fishery products are listed in Table 3. It reveals that the TCR Pb for demersal fish is highest at 12 years of age and in the adult population ($1.75 \times 10^{-7}$ and $6.33 \times 10^{-7}$), whereas the highest TCR value for cephalopod products was found for Pb metal in the adult population, namely $1.82 \times 10^{-7}$. Nevertheless, the TCR levels for all detected elements remained under the dietary criterion numbers, showing that there was indeed no danger of cancer from Pb, Cd, and Hg intake of these fishery products to the general population of each age group.

**Table 3. Target Cancer Risk of Demersal Fish and Cephalopod Frozen Products at Benoa Port, Bali Province, Based on Selected Heavy Metals**

| Demersal fish | | | | |
|---|---|---|---|---|
| Age | FIR Fresh (kg/person)[1] | Pb | Cd | Hg |
| Age 1 | 0.681 | 1.37306E-08 | 1.38285E-11 | 2.18293E-12 |
| Age 3 | 1.498 | 6.04064E-08 | 6.08374E-11 | 9.60362E-12 |
| Age 6 | 1.498 | 9.06096E-08 | 9.12561E-11 | 1.44054E-11 |
| Age 12 | 3.196 | 1.75742E-07 | 1.76996E-10 | 2.79401E-11 |
| Adults | 3.14 | 6.33098E-07 | 6.37615E-10 | 1.00652E-10 |
| **Cephalopods** | | | | |
| Age | FIR Fresh (kg/person)[1] | Pb | Cd | Hg |
| Age 1 | 0.681 | 3.95072E-09 | 3.57731E-10 | 4.84953E-12 |
| Age 3 | 1.498 | 1.73809E-08 | 1.57381E-09 | 2.13351E-11 |
| Age 6 | 1.498 | 2.60713E-08 | 2.36071E-09 | 3.20026E-11 |
| Age 12 | 3.196 | 5.05667E-08 | 4.57872E-09 | 6.20709E-11 |
| Adults | 3.14 | 1.82163E-07 | 1.64945E-08 | 2.23605E-10 |

## DISCUSSION

### Heavy Metals Content

Fishery products are a source of animal protein and unsaturated fatty acids which are vital in fulfilling human nutrition. Demersal fish and cephalopods are crucial components of Indonesia's marine fishery landing products. However, alterations in the quality of fishery product fillets are a significant concern for consumers and producers. The results showed that the average concentration of heavy metals in each fishery product (demersal fish and cephalopods) was still within the safe range established by the relevant authorities in each nation. However, the amount of Cd and Hg was greater in cephalopod products, whereas Pb concentrations were found to be greater in demersal fish products. Similar results were also found in low levels of Pb, Cd, and Hg in fish meat from four farms in Algeria (36).

In contaminated environments, lead (Pb) and cadmium (Cd) are two heavy metals that are frequently found together. Both forms of heavy metals are commonly found in water habitats polluted by lead paint, car emissions, industrial pollution, and mine extraction byproducts (37). Ecotoxicological studies showed that *Alburnus mossulensis* fish that collect Cd in their bodies had hepatotoxic consequences, such as a decrease in total antioxidant capacity and an increase in oxidative damage to lipids and proteins (38). Ecotoxicological studies showed that *Alburnus mossulensis* fish that collect Cd in their bodies had hepatotoxic consequences, such as a decrease in total antioxidant capacity and an increase in oxidative damage to lipids and proteins (39). Mercury can also disrupt important pathways in apoptosis and glucose metabolism, as well as promote the expression of metallothionein in the muscle and liver tissue of *Cichla* sp., *Brachyplatystoma filamentosum*, and *Semaprochilodus* sp. from the Brazilian Amazon (40). A patent suggested a method to reduce heavy metals content in fish by soaking it in low acid and metal-binding ligands solutions under vacuum conditions with further depth rinse before commercialization (41). While this method seems promising, further effects the fish quality and overall cost of processing.

### Assessment of Health Risks

Compared to the quality standards established by food and health regulatory organizations such as FAO, WHO, ROPME, FDA, EC, and BBPOM, the heavy metals concentration in demersal and cephalopod fish fillets originating from Benoa Port, Bali Province, is relatively low. Therefore, these fish products are fit for consumption by humans. This data comparison is a significant reference for monitoring potential environmental pollution. Although the quantity of these toxic substances is still under acceptable limits for consumption by humans, their presence in the environment might have a little impact on the ecosystem's state.

Changes in the physicochemical characteristics of water quality can have a detrimental influence on the physiological systems of aquatic creatures that are more susceptible to environmental changes (41-42). Heavy metals have an effect on aquatic ecosystems either directly by producing toxicity in living organisms or indirectly by disrupting the stability of the food chain (43–45). A variety of other factors influence heavy metal bioavailability and uptake, including heavy metal quantity, exposure period, interactions with other metals, fish age and size, detoxification processes, fish metabolic procedures, feeding behavior, ecological physicochemical variables, and so on. Low amounts of contaminants may not be detrimental to fish due to their detoxifying or bioaccumulation capacities (46-47). They may, however, impede their reproductive capabilities (48-49), resulting in a reduction in their populations in the maritime environment (50-51).

Heavy metal concentrations in demersal fish and cephalopod fillets were utilized to determine possible health hazards in this investigation. This research emphasizes the dangers of heavy metals in fish products landed at Benoa Harbor, as well as their negative impact on the environment and human health. THQ and TTHQ-based evaluation approaches have proven useful in predicting health problems associated with consumption of seafood containing heavy metals (52–54). THQ and TTHQ levels measured in demersal and mollusk fish products are less than one in all consumer age groups, demonstrating that heavy metal absorption from these two forms of fish food poses no health concern. THQ levels were less than one in industrial fish, Coptodon zillii, and Parachanna occult from the Osu reservoir (26).

The TCR value can be used to predict the likelihood of developing cancer following consumption of fillets containing heavy metal contaminants. The TCR values found in this study are still within safe consumption limits for populations of heavy metals in varying ages. For comparison, The Pb metal value discovered in this investigation significantly lower than the TCR level of Pb in cabbage and tomato vegetables gathered in Mojo Area, Ethiopia, specifically $9.70 \times 10^{-6}$ and $2.02 \times 10^{-5}$, respectively, but these values are considered safe ($10^{-4}$-$10^{-6}$) (55). The same thing was also reported on the TCR value of heavy metals in fish tissue from the Eleyele and Ogun rivers, Nigeria which was safe for all heavy metals, except Arsenic (As) which had the highest TCR value and had the potential for cancer risk (56). If the TCR value is less than $10^{-6}$ , it is not significant and the risk of cancer is minimal, whereas values greater than $10^{-4}$ are hazardous and pose a risk of cancer (57–59).

These three heavy metals (Pb, Cd, and Hg) have severe health consequences. Cadmium (Cd) is toxic to the kidneys, causes lung injury (lung cancer), and causes skeletal changes in populations exposed to it in the workplace. Cd is generally absorbed and can be slowly detoxified; however, it can accumulate in the kidneys, causing chronic kidney injury (52,60). Bioaccumulation of Pb metal has a number of deleterious effects on human tissues and organs, including inhibition of growth, proliferation, differentiation, damage-repair processes, and apoptotic performance (17). Mercury (Hg) can also damage the kidneys (proximal tubules), injure the peripheral nervous system, and diminish liver function (increased ALT, AST, and GGT enzymes) in the elderly (61). Additionally, chronic Hg toxicity causes birth defects in infants born to mothers contaminated with this metal (62).

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## CONCLUSION

Overall, the average heavy metals concentration in fishery products, including demersal fish and cephalopods landed at Benoa Port, Bali Province, is still within safe limits for consumption, in accordance with standards set by various health regulatory agencies in various countries. The health risk values (THQ, TTHQ, and TCR) for demersal fish and cephalopod products are still within the safe range for all ages. However, it should be noted that health risks may arise if these fishery products are ingested for an extended period of time. As a result, attempts to detect the existence of other hazardous metals in fish-based goods remain critical. Testing for persistent pollutants like microplastics, antibiotics, and pesticides can be considered in the future.

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