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RESEARCH

Accumulation of potentially toxic elements in fourfinger threadfin (*Eleutheronema tetradactylum*) and black pomfret (*Parastromateus niger*) from Selangor, Malaysia

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Abstract The accumulation of potentially toxic elements (PTEs) has raised public awareness due to harmful contamination to both human and marine creatures. This study was designed to determine the concentration of copper (Cu), zinc (Zn), cadmium (Cd), and nickel (Ni) in the intestine, kidney, muscle, gill, and liver tissues of local commercial edible fish, fourfinger threadfin (*Eleutheronema tetradactylum*), and black pomfret (*Parastromateus niger*) collected from Morib (M) and Kuala Selangor (KS). Among the studied PTEs, Cu and Zn were essential elements to regulate body metabolism with certain dosages required while Cd and Ni were considered as non-essential elements that posed chronic and carcinogenic risk. The concentration of PTEs in fish tissue samples was analyzed using flame atomic absorption spectrometry (F-AAS). By comparing the concentration of PTEs in fish tissues as a bioindicator, the environmental risk of Morib was more serious than Kuala Selangor because both fish species collected from Morib resulted in a higher PTEs concentration. For an average 62 kg adult with a fish ingestion rate (FIR) of 0.16 kg/person/day in Malaysia, the estimated weekly intake (EWI) of Cd from the consumption of *E. tetradactylum* (M: 0.0135 mg/kg; KS: 0.0134 mg/kg) and *P. niger* (M: 0.0140 mg/kg; KS: 0.0132 mg/kg) had exceeded the provisional tolerable weekly intake (Cd: 0.007 mg/kg) established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and oral reference dose (ORD) values of Cd (0.001 mg/kg/day) as provided by the United States Environmental Protection Agency (USEPA) regional screening level, thus it posed chronic risks for daily basis consumption. Besides, the value of the carcinogenic risk of Cd (0.7^{-3} to 0.8^{-3}) and Ni (0.5^{-3} to 0.6^{-3}) were in between the acceptable range (10^{-6} to 10^{-4}) of the health index that indicates a relatively low possibility cancer occurrence to the consumers in both Morib and Kuala Selangor. This study recommended FIR to be 0.80 kg/person/day to reduce the possibility of posing chronic and carcinogenic risks while at the same time obtaining the essential nutrients from the fish.

Keywords Potentially toxic element (PTE) · *Eleutheronema tetradactylum* · *Parastromateus niger* · Target hazard quotient (THQ) · Carcinogenic risk (CR)

Introduction

Potentially toxic elements (PTEs), known for their mutagenic, poisonous, abundant, persistent, and bio-accumulative nature (Sonone et al., 2020; Ali et al., 2019), input from industries, and tourism activities

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along the coastal areas into aquatic system especially estuarine environment have contributed to the ongoing issues that relate to detrimental impacts on the aquatic ecosystem as well as health risk through seafood consumption (Rahman & Rahim, 2019; Vu et al., 2017). These inorganic substances are non-metabolizable and non-biodegradable in the body, leading to their accumulation over time (Lipy et al., 2021). PTEs enter the environment through natural and/or anthropogenic processes including agriculture, discharge of improperly treated industrial wastewater, metal electroplating, manufacture and disposal of batteries, circuit boards, and mining (Mehmood et al., 2019; Ng et al., 2019). Within the marine ecosystem, 80% of water bodies were polluted and the welfare of local inhabit organisms is being threatened by fast urbanization and industrial expansion (Ahmad et al., 2016; Lipy et al., 2021). Once PTEs enter the marine ecosystem, they may be deposited or adhered to sediments, providing a new source of PTEs for the water body. Eventually, the marine creatures absorbed PTEs from the water body and then accumulated in different tissues (Mehmood et al., 2019). PTE concentration can be amplified thousands of times due to the biomagnification process within a complex food chain (Salam et al., 2021). Meanwhile, the PTE will then enter and greatly accumulate in human organ tissue, since humans are positioned at the relatively higher trophic level within the food chain.

Aquatic products are virtually impossible to be prohibited from our daily meal consumption pattern, as it is undeniable that aquatic products contain essential nutrients and elements beneficial to the human body. The American Heart Association and various public health organizations have recommended at least twice weekly of fish consumption, to avail of omega-3 fatty acids that would be beneficial to cardiovascular health and potentially reduce the risk of developing chronic illnesses (Djedjibegovic et al., 2020; Gu et al., 2017; Kalantzi et al., 2016). Contrary to these health advantages of aquatic product consumption, PTE accumulation in marine fish and seafood may pose negative health impacts for the human population due to prolonged consumption of fishes that accumulate certain levels of PTEs, especially for those who consume aquatic products often. Therefore, recent risk assessments have led to potential interest on the impacts of PTEs on the consumers (Barone et al., 2015). Continuously monitoring the levels of PTE concentrations in aquatic products is crucial to strike a balance between the advantages of nutrients and the potential dangers posed by toxicants. This monitoring is essential as it enables regular updates to the maximum permissible limits and recommended ingestion rates for aquatic products. The demand and consumption of seafood has expanded over the years leading to the thriving fisheries industry in Malaysia. The contribution value of the fisheries industry has increased by 7.5%, an increase from MYR 13.84 (USD 2.95) billion in 2020 to MYR 1488 (USD 317.64) billion in 2021 (Jabatan Perikanan Malaysia, 2022). The increase in seafood consumption per capita has led to an increase in production, straining wild-caught fisheries and speeding up the aquaculture development. In the last several decades, the accumulation of PTE has been viewed as a severe threat to the environment and the general public's health (Malviya et al., 2023; Ali et al., 2019; Aghoghovwia et al., 2016; Mahboob et al., 2016).

The study encompassed essential PTEs including copper (Cu) and zinc (Zn), along with non-essential elements nickel (Ni) and cadmium (Cd), which play roles in human health. As a crucial human essential PTE, Cu is indispensable for the enzymatic synthesis of hemoglobin, the protein within red blood cells responsible for oxygen transport (Doguer et al., 2018). Despite its significance in various biological processes, excessive copper intake or exposure can have adverse health consequences particularly affecting the kidneys and liver, leading to severe outcomes such as organ damage (Zeinali et al., 2019). Additionally, an excess of Cu in the body may manifest symptoms such as seizures, high blood pressure, increased respiration rates, and damage to the central nervous system, causing convulsions, cramps, and vomiting (Altarelli et al., 2019; Myint et al., 2018). Zn is essential for the proper operation of enzymes such as insulin synthesis and secretion as well as contributing to immune function as it is required for the synthesis of protein and collagen, supporting wound healing and healthy skin (Chasapis et al., 2020; Freitas et al., 2017). Nevertheless, excessive amounts of Zn in the body can result in stomach discomfort, giving rise to symptoms like nausea and cramps, while vomiting and even anemia may occur in severe instances of excessive intake (Gunturu & Dharmarajan, 2020; Parveen et al., 2017). Moreover, elevated Zn levels may manifest externally, causing skin irritations (Chasapis et al., 2020). There was an antagonism relationship between Cu and Zn in the human body whereby Zn and Cu typically maintain a delicate equilibrium, with Zn acting as a primary antagonist to Cu. In cases of Zn deficiency, Cu tends to accumulate in various storage organs causing Cu toxicity (Royer & Sharman, 2023).

Among non-essential elements, Cd alongside arsenic, lead, mercury, and chromium, is classified as a high-toxicity PTE with no specific biochemical function in the human body (Chowdhury et al., 2018; Genchi et al., 2020a). Human exposure to Cd can lead to various adverse effects, including renal and hepatic dysfunction, pulmonary edema, testicular damage, osteomalacia, and harm to the adrenals and hematopoietic system (Mezynska & Brzóska, 2018; Mulware, 2020; Tinkov et al., 2018). On the other hand, Ni is considered a non-essential element for higher animals and humans because it does not have biochemical function nor has a low dietary intake been definitively shown to interrupt the life cycle (Nielsen, 2021). While there is currently no evidence indicating the nutritional significance of nickel for humans as higher-level organisms do not have known enzymes or cofactors containing nickel, nickel-based enzymes are well-established in Archaea, bacteria, algae, primitive eukaryotes, and plants (Desguin et al., 2018; Genchi et al., 2020b; Song et al., 2017). Ni poses health risks as an immunotoxic and carcinogenic agent, potentially leading to various health issues, including contact dermatitis, cardiovascular disease, asthma, lung fibrosis, and respiratory tract cancer (Bilandžić et al., 2021; Khan et al., 2022; Rehman et al., 2018). Apart from that, Cd and Ni were recognized as a confirmed human carcinogen, classified in Group I by the International Agency for Research on Cancer (IARC, 1993; Loomis et al., 2014; Mhungu et al., 2022). Alarmingly, prolonged exposure to certain levels of PTEs including both essential and non-essential elements has been linked to an increased risk of developing detrimental effects on the human body (Chasapis et al., 2020; Genchi et al., 2020a, 2020b; Zeinali et al., 2019). Therefore, underscoring the importance of maintaining a balance and adhering to maximum permissible levels of food safety guideline standards is crucial to avoid adverse health outcomes as suggested by the Malaysia Food Act (MFA), Food and Agriculture Organisation (FAO), World Health Organisation (WHO), Federal Environmental Protection Agency (FEPA), and the European Food Safety Authority (EFSA) in this study (Selvam et al., 2020).

Selangor, the most populous state in Malaysia, has witnessed rapid development and population growth, with approximately 7.2 million residents as of July 2023 (Statista, 2023). This remarkable growth is coupled with a robust economy, positioning Selangor as one of the most developed districts in the country. Selangor's coastal areas have long been recognized for their significance in marine fish harvesting, owing to the region's unique geographical features. The coastal zone supports various scales of fishing industry and activities, contributing significantly to both local livelihoods and the broader economy. However, the region faces environmental challenges, notably stemming from industrial expansion and the construction of shipping ports along its coastline (Ibrahim et al., 2020; Kadhum et al., 2015; Othman et al., 2018). These developments have raised concerns about the presence of PTEs in the coastal waters. The increasing prevalence of elevated PTEs concentrations in these marine settings is an escalating worry due to its potential consequences for human well-being. Given that seafood is a primary dietary component for coastal communities, there are apprehensions regarding its safety for consumption as pollution levels continue to rise.

Typically, marine organisms absorb toxins such as PTEs from the environment and have been commonly utilized in different marine pollution risk assessments (Hashempour-Baltork et al., 2023). Fishes served as ideal bioindicators of PTE accumulation due to their bioaccumulation ability in the natural environment (Lipy et al., 2021). Bioaccumulation of PTE in commercial fish species can provide important information to evaluate the health and environmental risks of PTE accumulation. To assess the environmental risk and potential health impacts on humans, the accumulation of PTEs in commercially edible fish species should be regulated to ensure compliance with food safety standards (Yap & Al-Mutairi, 2022). As a result, this study was carried out to evaluate the PTE concentrations in *Eleutheronema tetradactylum* (Fourfinger threadfin) and *Parastromateus niger* (Black pomfret), two commercially available fish species that are commonly found in local markets in Selangor and highly consumed by Malaysians. This study aimed to (i) analyze the concentration of PTEs

(Cu, Zn, Cd, and Ni) in muscles, gills, liver, kidney and intestine tissues of the *E. tetradactylum* and *P. niger* from Morib and Kuala Selangor; (ii) evaluate the environmental risk between Morib and Kuala Selangor based on the concentration of PTEs; and (iii) assess the human health risk based on the maximum permissible limits (MPLs), provisional tolerable weekly intake (PTWI), target hazard quotient (THQ), and carcinogenic risk (CR) in the studied location.

Materials and methods

Sampling sites

The two study locations of Morib and Kuala Selangor were situated on the west coast of Peninsular Malaysia. Morib (2° 45′ 7.03″ N, 101° 26′ 34.6″ E) was situated in Kuala Langat district, Selangor, as the home to a palm oil refinery whereby water canals were used to irrigate crops to avoid flooding. In addition, Morib Beach was a popular tourist destination for recreation activities that was close to several fishing communities and seafood restaurants. On the other hand, Kuala Selangor (3° 20′ 30.08″ N, 101° 14′ 46.78″ E) was situated at the estuary of the Selangor River. The seaside town of Kuala Selangor was located around 53 km from Kuala Lumpur and was positioned adjacent to a sizable estuary that feeds into the ocean also commonly known as the "Confluence of the Selangor River" (Yunus et al., 2015) (Fig. 1).

Sample preparation

The two fish species selected in this study were *Eleutheronema tetradactylum* (fourfinger threadfin) and *Parastromateus niger* (black pomfret). The fish samples were purchased from the local

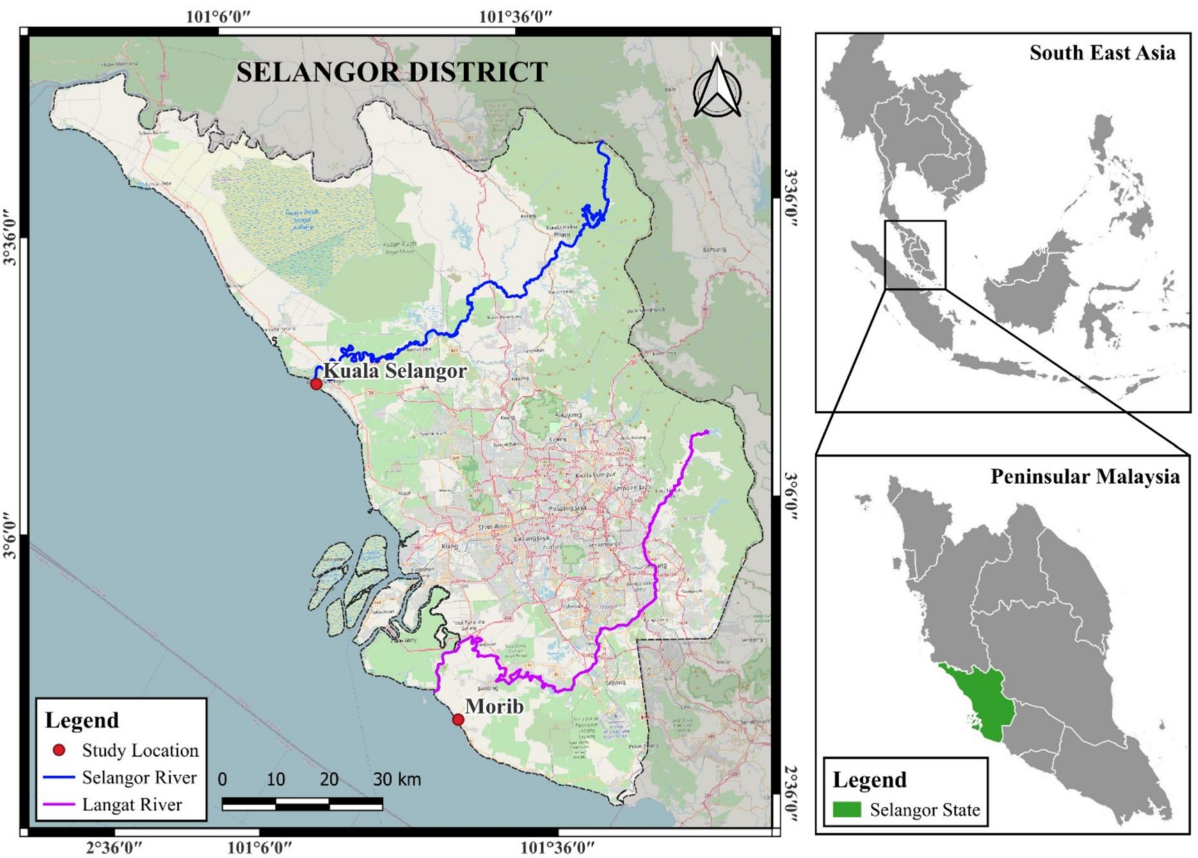

Fig. 1 The location of sampling sites

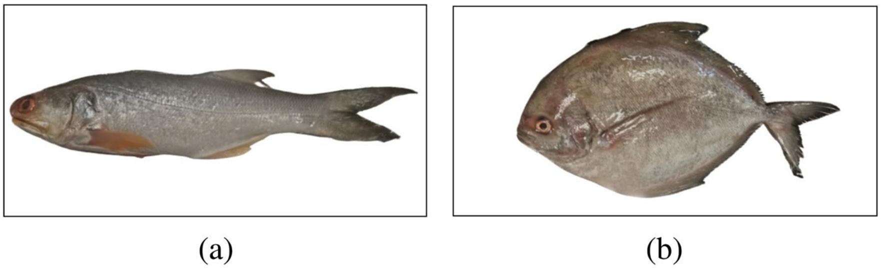

Fig. 2 Fish species selected in this study were **a** *Eleutheronema tetradactylum* (fourfinger threadfin) and **b** *Parastromateus niger* (black pomfret)

Table 1 Wet weight (g) and total length (cm) of fish samples collected

Location	Fish species	Wet weight (g)	Length (cm)
Morib	*E. tetradactylum*	215.73 ± 12.07	29.50 ± 0.30
	P. niger	348.50 ± 12.40	27.5 ± 0.10
Kuala Selangor	*E. tetradactylum*	216.76 ± 3.64	29.53 ± 0.25
	P. niger	348.75 ± 9.74	26.23 ± 0.38

Mean ± standard deviation

market Kwee Kee Fishery and Chuan Hock Fishery in Morib and Kuala Selangor, respectively. Fish samples with similar sizes in term of weight and length were preferred to minimize experimental error and biases in PTE content due to variation in fish sizes. The fish samples were rinsed with ultrapure water to remove any surface residues, subsequently wrapped in polyethylene bags, and kept frozen at − 4 °C until dissection and chemical analysis (Vu et al., 2017). The fish species were identified using "FishBase" by comparing the morphological similarity with the diagram outlook of each family's species provided by the database (Froese & Pauly, 2010) (Fig. 2).

All fish samples were thawed at room temperature (27–29 °C) whereby the total length and wet weight of each fish were measured using the measuring ruler (with 1 mm accuracy) and digital balance (Mettler Toledo, with 0.1 g accuracy), respectively (Table 1). All samples were then washed and rinsed with ultrapure water to remove any adhering particles. Each triplicate sample of the fish was dissected to obtain the white muscle tissues from the middle body part, gill filaments, liver, kidney, and intestine tissues, using a stainless steel dissecting pan and scalpel blades (Fig. 3, modified from Carolina, 2022). The fish dissection and disposal procedure were outlined by Gupta and Mullins (2010). The extracted tissues were oven-dried at 60 °C for approximately 8 h until the samples achieved a constant weight .

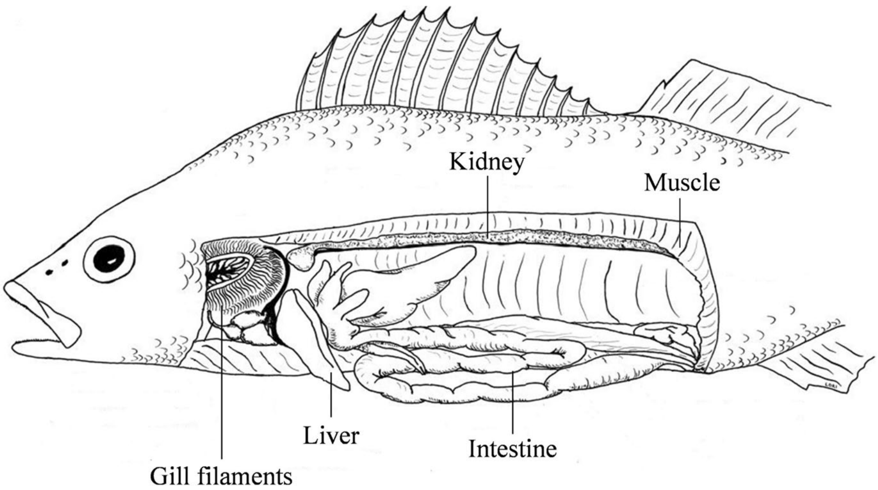

Fig. 3 General fish anatomy with labeled organ tissues (modified from Carolina, 2022)

Chemical analysis

The nitric acid (65–68%), sulfuric acid (95–98%), and hydrogen peroxide (30%) used for wet digestion were analytical reagent grade (Chemiz). The dried tissue samples were digested through the modified nitric-sulfuric-perchloric acid digestion. About 5.0 mL of concentrated HNO_3 and 5.0 mL of H_2SO_4 were added to 0.5 g dried weight of tissue samples. After the reaction of tissue samples with HNO_3 and H_2SO_4 stopped for approximately 10 min, the samples were heated at 60 °C for 30 min. Subsequently, the solution was left to cool down for 15 min, then 10.0 mL of HNO_3 was added and reheated at 95 °C for another 3 h and cooled down again for another 1 h before 2.0 mL of ultrapure water and 3.0 mL of H_2O_2 were added and stayed overnight to complete the full reaction. Lastly, the digested samples were filtered using Whatman filter paper 42 and then diluted into 50.0 mL with ultrapure water (Hashim et al., 2014; Idera et al., 2015). All glassware used were rinsed with 1% HNO_3 and kept separately from other glassware.

Elemental analysis

All reagents were analytical grade, and standard stock solutions of PTEs (1000 mg/L as nitrate salts in 0.5 mol/L nitric acid) were obtained from Merck and Scharlau. Standard solutions for each PTE were prepared by appropriate dilution of stock standards with ultrapure water to generate the calibration curve. The chemical and elemental analysis procedures were verified by evaluating the European Reference Materials (ERM-CE278k) mussel (*Mytulis edulis*) tissue with the average recovery rate 75.00% for Cu, 126.27% for Zn, 95.95% for Cd, and 104.14% for Ni while the potential contamination of samples was corrected by taring with acid blank prepared for each element. Besides, 1% nitric acid was prepared in advance for instrument washing purposes.

The flame atomic absorption spectrometer (F-AAS) (Thermo Scientific iCE 3500 Series) was employed for PTEs analysis with the air-acetylene flame and elemental hollow cathode lamps (HCL) as light sources. The limit of detection (LOD) and limit of quantitation (LOQ) for different PTEs and a series of standard solutions were determined by diluting the stock standard solution (1000 mg/L) with ultra-pure water. This study has established a highly sensitive F-AAS method for the detection and quantitation of PTEs in fish, with LOD (mg/kg) for Cu, Zn, Cd, and Ni found to be 0.085, 0.114, 0.132, and 0.193, while the LOQ (mg/kg) was determined to be 0.259, 0.346, 0.401 and 0.584, respectively, while the correlation coefficient (R^2) value of each PTEs acquired was higher than 0.995 as reported in Table 2.

The value of the dilution factor (DF), required as input to calculate the PTE concentration, was determined using the following formula (modified from Maurya et al., 2019):

$$DF(\frac{L}{kg}) = \frac{V(L)}{W(kg)}$$

where V refers to the volume of a diluted digested sample read from a volumetric flask while W represents the dry weight of a tissue sample. Subsequently, the PTE final concentration in the original tissue sample will be computed using the following formula:

$$C(\text{mg/kg}) = F - AAS\, reading\, (\text{mg/L}) \times DF(\text{L/kg})$$

where C refers to the actual PTE concentration while the F-AAS corrected concentration was obtained from the Thermo Scientific SOLAAR software.

Table 2 Quality assurance of F-AAS for detection of PTEs

PTE	Standard calibration concentration (mg/kg)	R^2	LOD (mg/kg)	LOQ (mg/kg)	Recovery rate (%)	RSD (%)
Cu	0.50, 1.00, 1.50, 2.00	0.9998	0.0854	0.2587	75.00 ± 2.11	2.81
Zn	0.25, 0.50, 0.75, 1.00	0.9987	0.1143	0.3464	126.27 ± 11.25	8.91
Cd	0.50, 1.00, 1.25, 1.50	0.9994	0.1324	0.4012	95.95 ± 62.51	65.15
Ni	1.00, 1.50, 2.50, 3.00	0.9996	0.1927	0.5838	104.14 ± 91.79	88.14

R^2 = correlation coefficient; RSD = relative standard deviation; LOD = limit of detection; LOQ = limit of quantification; Percent recovery obtained from European Reference Materials (ERM-CE278k) mussel (*Mytulis edulis*) tissue reported as mean ± standard deviation

Health risk assessment

United States Environmental Protection Agency (USEPA) developed a preliminary procedure for non-carcinogenic risk assessments with several health indexes including estimated daily intake (EDI), estimated weekly intake (EWI), THQ, as well as CR to evaluate the human health risk regarding PTE concentration in *E. tetradactylum* and *P. niger*, particularly the amount of ingestion (Smith, 1995; Vu et al., 2017; Zaghloul et al., 2022). In this study, the health index will solely consider the PTE concentration resulting in muscle tissues as the only tissue that is usually consumed.

EDI and EWI

The EDI was the approximation amount for a certain PTE consumed each day, calculated based on the following formula (Yap & Al-Mutairi, 2022):

$$EDI = \frac{EC \times FIR}{BW}$$

whereas BW refers to the body weight, with 62 kg for an adult Malaysian individual (Ahmad et al., 2016). EC represents the PTE concentration in fish tissue (mg/kg) while fish ingestion rate (FIR) covers the fish ingestion rate with about 0.16 kg/person/day for a typical Malaysian adult (Ahmad et al., 2016). On the other hand, the EWI was the approximation amount for a certain PTE consumed each week, calculated based on the following formula (Matsunuma et al., 2011):

$$EWI = EDI \times 7\, days\, (\text{in a week})$$

Then, comparing the EWI values with specified PTWI stated by Joint FAO/WHO Expert Committee for Food Additives (JECFA), the PTWI of Cu (3.5 mg/kg/week), Zn (7.0 mg/kg/week), Cd (0.007 mg/kg/week), and Ni (0.035 mg/kg/week) (JECFA, 2022; Matsunuma et al., 2011). PTWI is the amount of a chemical that may be consumed weekly during a human lifetime without causing serious harm to health (JECFA, 2022; Matsunuma et al., 2011); meanwhile, the EWI was used to evaluate whether the amount of weekly basis consumption for particular fish species from the studied location exceeded the PTWI restrictions.

Chronic and carcinogenic risk assessments

The THQ served as a health index to assess chronic risk, calculated based on the following formula (Yap & Al-Mutairi, 2022):

$$THQ = \frac{EDI}{ORD}$$

whereas the oral reference dose (ORD) values of Cu (0.04 mg/kg/day), Zn (0.3 mg/kg/day), Cd (0.001 mg/kg/day), and Ni (0.02 mg/kg/day) as provided by the USEPA regional screening level (USEPA, 2023). The ORD is an estimated amount of contaminant in daily consumption that is not anticipated to pose any negative impact on health over a lifetime (USEPA, 2000). THQ smaller than 1 indicated that the amount of fish consumption was lower than the oral reference dose specified by USEPA, thus no negative health impacts were revealed during a human lifetime (Vu et al., 2017). Nonetheless, the CR served as a health index to assess carcinogenic risk, calculated based on the following formula below (Smith, 1995; Vu et al., 2017):

$$CR = CSF \times EDI$$

whereas the cancer slope factor (CSF) of Cd (0.38 mg/kg/day) and Ni (1.7 mg/kg/day) derived from the Integrated Risk Information System as provided by USEPA, while the CSF of Cu and Zn were not available because they were categorized as essential elements (USEPA, 2000; Traina et al., 2019). The CSF is a toxicity value that quantitatively defines the relationship between dose and response which indicates a plausible upper-bound estimate of the probability that an individual will develop cancer if exposed to PTE for a lifetime over 70 years (Smith, 1995). CR is a dose–response relationship that represents the accumulative probability of cancer in an adult as the potential outcome of carcinogen exposure via ingestion (Selvam et al., 2020).

Statistical analyses

The descriptive statistics such as mean and standard deviations of the data set were calculated using Microsoft Excel (version 2019) with three replicates.

All datasets were transformed and subjected to normality using the skewness-kurtosis test (Kim, 2013; Razali & Wah, 2011). The homogeneity of variances using Levene's test showed that the variances of the groups within the four PTEs were equal with $p > 0.05$. Subsequently, a three-way analysis of variance (ANOVA) was applied to the dataset to assess the statistically significant variation in each PTE among all locations, species, and tissues, using the Statistical Package for the Social Sciences (SPSS) software (version 27 for Windows). The one-way ANOVA followed by the least significant different (LSD) post hoc test was adopted to examine the comparative outlook of PTEs concerning different location, species, and tissue separately at the 95% statistical confidence level.

Results and discussion

Trend of PTE accumulation

The average PTE concentration in fishes from Morib decreased from Zn (67.37 mg/kg) > Cu (3.02 mg/kg) > Ni (1.78 mg/kg) > Cd (1.33 mg/kg) in *E. tetradactylum* and Zn (121.75 mg/kg) > Cd (5.52 mg/kg) > Cu (5.19 mg/kg) > Ni (0.68 mg/kg) in *P. niger*. For both fishes from Kuala Selangor, the average concentration of each PTEs decreased in the same following pattern with Zn (63.26 mg/kg) > Cu (2.73 mg/kg) > Cd (1.32 mg/kg) > Ni (0.82 mg/kg) in *E. tetradactylum* while Zn (81.62 mg/kg) > Cu (5.18 mg/kg) > Cd (2.32 mg/kg) > Ni (1.20 mg/kg) in *P. niger*. The essential elements (Zn and Cu) recorded higher accumulation in both *E. tetradactylum* and *P. niger* compared to non-essential elements (Cd and Ni) in this study. Zn and Cu showed higher concentrations among the all PTEs because they are considered as a part of the micronutrients for fish (Kumar et al., 2023; Pandey & Madhuri, 2014; Ustaoğlu & Islam, 2020). Besides, the concentration of Zn (15–30 mg/kg) requirements from feed for fishes was 5 to 10 times higher than Cu (3 mg/kg feed); therefore, the PTE accumulation of Zn always higher than Cu regardless of different locations (Lall & Kaushik, 2021; Terech-Majewska et al., 2016).

In terms of the non-essential PTE accumulation, the concentration of Cd was greater than Ni in this study for both fishes, except for *E. tetradactylum* from Morib. Similarly, Ali et al. (2014) found that the concentration of Cd (non-essential element) was greater than Ni in *Cyprinus carpio* (common carp) while Alina et al. (2012) revealed that Cd has the highest concentration among the studied PTEs (Hg, Ar, Cd, and Pb) were detected in *Pampus argentus* (silver pomfret) and *E. tetradactylum* (fourfinger threadfin) collected along the Straits of Malacca, Malaysia.

PTE accumulation in fish tissues

The average PTE concentration of each tissue (intestine, kidney, muscle, gill, and liver) for a fish species from both Morib and Kuala Selangor are summarized in Table 3. In terms of essential elements, the ability for tissue of *E. tetradactylum* to accumulate Cu was decreasing following the order of liver > intestine > kidney > gill > muscle, as compared to the ability of Zn accumulation which followed the order of intestine > liver > kidney > gill > muscle. For *P. niger*, the ability of fish tissue to accumulate Cu decreases in the order of liver > gill > kidney > intestine > muscle; while the ability of Zn accumulation decreases in the order of kidney > intestine > liver > gill > muscle.

Overall, this study found that the accumulation of PTEs decreased from liver > gill > muscle for both species, but intestine > kidney for *E. tetradactylum* as well as kidney > intestine for *P. niger*. Fishes act as the receptors to PTEs, which enter into their body from the marine environment through their digestive tract by feeding food and water as well as non-dietary paths via dripping the muscle tissues and gills (Selvam et al., 2020). Furthermore, the findings from Onita et al. (2021) supported that the liver of *P. niger* and *E. tetradactylum* showed the highest Cu accumulation among the five tissues in this study. Moreover, Onita et al. (2021) also found that the Zn (essential element) and Cd (non-essential element) accumulated at the highest concentrations in the kidney of both studied species, except for Cd accumulation in *P. niger*.

Nevertheless, the PTEs accumulation in the kidney and intestine was generally greater than gill and muscle, meanwhile the accumulation of the digestion tract was greater than in the non-dietary path tissues (muscle and gill). Moreover, a significant increase ($p < 0.05$) of Zn and Cu concentrations was found in the gill compared to the muscle tissues, and this would probably be due to the PTEs complexation

Table 3 Concentration of PTEs in the tissues of fish species collected from Morib and Kuala Selangor

Fish tissue	Fish species	PTE concentration (mg/kg DW)			
		Cu	Zn	Cd	Ni
Morib					
Intestine	*E. tetradactylum*	4.41 ± 0.83	117.91*# ± 25.51	1.18*# ± 0.26	3.33# ± 1.35
	P. niger	3.80 ± 0.58	93.25# ± 19.52	2.08*# ± 1.83	0.96# ± 0.68
Kidney	*E. tetradactylum*	3.44 ± 1.27	68.85# ± 11.45	2.49*# ± 1.04	2.25# ± 1.47
	P. niger	3.87 ± 0.29	305.75*# ± 143.84	5.64*# ± 3.87	1.19# ± 0.53
Muscle	*E. tetradactylum*	0.41 ± 0.33	13.29 ± 4.03	0.75# ± 0.04	0.49 ± 0.06
	P. niger	0.36 ± 0.21	9.21 ± 1.65	0.77# ± 0.04	0.56# ± 0.42
Gill	*E. tetradactylum*	1.46 ± 0.63	62.47# ± 4.20	1.59*# ± 0.84	1.50# ± 0.30
	P. niger	8.58 ± 4.25	105.87*# ± 39.07	0.55# ± 0.11	0.63# ± 0.14
Liver	*E. tetradactylum*	5.35 ± 3.38	74.34# ± 14.65	0.63# ± 0.10	1.34# ± 0.77
	P. niger	9.32 ± 3.48	94.69# ± 23.10	18.58*# ± 10.50	0.09 ± 0.05
Kuala Selangor					
Intestine	*E. tetradactylum*	4.62 ± 3.55	102.47*# ± 40.80	2.52*# ± 0.94	0.67# ± 0.46
	P. niger	4.02 ± 0.53	93.54# ± 17.52	1.56*# ± 0.58	3.17# ± 1.83
Kidney	*E. tetradactylum*	2.51 ± 0.67	65.34# ± 3.36	2.21*# ± 0.38	2.43# ± 1.39
	P. niger	4.49 ± 1.11	165.69*# ± 55.02	2.90*# ± 3.11	0.73# ± 0.30
Muscle	*E. tetradactylum*	0.66 ± 0.17	20.85 ± 13.44	0.74# ± 0.08	0.62# ± 0.07
	P. niger	ND	9.62 ± 0.83	0.73# ± 0.05	0.54# ± 0.21
Gill	*E. tetradactylum*	0.61 ± 0.39	66.53# ± 1.82	0.71# ± 0.29	0.03 ± 0.02
	P. niger	4.31 ± 0.29	49.27# ± 4.47	0.21 ± 0.11	0.73# ± 0.42
Liver	*E. tetradactylum*	5.27 ± 2.42	61.11# ± 7.80	0.40 ± 0.13	0.33 ± 0.19
	P. niger	7.90 ± 2.93	89.99# ± 11.19	6.18*# ± 5.21	0.82# ± 0.47
Maximum permissible limit (MPL) recommended by different local and international authorities					
MFA* (1983)		30.00	100.00	1.00	NA
FAO (1983)		30.00	30.00	0.50	0.50
WHO (1995)		3.00	10.00	NA	0.50
FEPA (2003)		1.30	75.00	NA	NA
EFSA (2006, 2023)		0.50	30.00	0.05	NA
A-MPL#		12.96	49.00	0.52	0.50

Mean ± standard deviation.

DW = dry weight; ND = not detected; NA = not available; MFA = Malaysia Food Act; FAO = Food and Agriculture Organisation; WHO = World Health Organisation; FEPA = Federal Environmental Protection Agency; EFSA = European Food Safety Authority; A-MPL = average of maximum permissible limit.

*Exceeded MFA (1983) permissible level.

#Exceeded A-MPL permissible level

with the mucus, which was difficult to remove completely from the tissue (Kanda et al., 2020). Therefore, gill tissue could serve to be a suitable environmental primary indicator for water pollution as the concentration of PTEs in the gill could reflect the accumulation levels of PTEs in the water body (Aly, 2016; Kanda et al., 2020).

Environment risk assessment

To evaluate the environmental impact, PTE concentration within fish samples served as a bioindicator to assess the severity of PTE contamination at the sampling site. The PTE contamination at Morib was more severe than in Kuala Selangor because the

PTEs concentration in both *E. tetradactylum* (Zn: 67.37 ± 37.26 mg/kg, Cd: 1.33 ± 0.75 mg/kg) and *P. niger* (Zn: 121.75 ± 109.91 mg/kg, Cd: 5.52 ± 2.35 mg/kg) from Morib were greater than both *E. tetradactylum* (Zn: 63.26 ± 28.97 mg/kg, Cd: 1.32 ± 0.97 mg/kg) and *P. niger* (Zn: 81.62 ± 58.12 mg/kg, Cd: 2.32 ± 1.17 mg/kg) from Kuala Selangor in overall. The accumulation of PTEs in Morib and Kuala Selangor may have been caused by the ecological impacts resulting from the combination of both natural and anthropogenic factors. Since Malaysia has a wet tropical climate with a high level of rainfall, these PTEs would possibly introduce into the Morib coast through storm runoff of the Langat River whilst the Selangor River served as the main flow for transporting PTEs into the Kuala Selangor coastal region (Nguyen et al., 2020; Yao et al., 2022).

Among the four studied PTEs, Cu is commonly acquired by extracting and processing copper ores, which contain key copper minerals such as chalcopyrite, bornite, malachite, and azurite (Tabelin et al., 2021). Beyond the primary production from ores, copper is also obtained through recycling methods (Ettler et al., 2022). Scrap copper sourced from diverse outlets, such as electrical cables, electronic devices, and industrial waste, undergoes recycling to reclaim and repurpose the metal (Sridhar & Hammed, 2016). Similarly, the primary source of zinc is the extraction from mining and processing zinc ores. The prevalent zinc ore is typically zinc sulfide, commonly found alongside other minerals like lead, silver, and copper (Kania & Saternus, 2023; Mudd et al., 2017). Zn serves various industrial purposes, including alloy production, steel galvanization, and the manufacturing of diverse products such as batteries and pigments (Javed & Usmani, 2019; Kaya et al., 2020). The activities of zinc, lead, and copper mines contribute to the release of Cd into the atmosphere, causing environmental contamination (Genchi et al., 2020a). Among the principal uses of Cd are transportation, building materials, machinery, home appliances, and the plating of metals, as well as the utilization of pigments and paints (Ishchenko, 2018; Loomis et al., 2014). While, Ni finds extensive application in contemporary metallurgical practices, playing a crucial role in alloy production, electroplating, manufacturing nickel–cadmium batteries, and catalyzing the chemical and food industries (Genchi et al., 2020b).

Generally, Morib has a special socioeconomic and environmental significance to the south of Selangor and the west coast of Peninsular Malaysia. Besides, there were several primary sources of PTEs at Morib due to the active seaside cattle farming, agriculture, and palm oil manufacturing activities (Alam & Silpa, 2020; Embrandiri et al., 2012; Shan et al., 2013). Moreover, Kadhum et al. (2015) found the presence of PTEs including Cd and Ni at Morib seaside town (Jugra, Jenjarom, and Hulu Langat roads) that eventually converged into Langat River and subsequently entered into the Morib coastal areas. On the other hand, Kuala Selangor was formerly a major tin mining area, thus this region is rich in mineral resources (Rahman & Rahim, 2019). Additionally, Kuala Selangor was close to the districts of Tanjung Karang, Sekinchan, and Sungai Besar, which support a variety of paddy fields and agricultural activities. As a result, the excessive usage of fertilizers and pesticides to regulate the quality of the crops may cause residual leakage and flow along the riverbanks before ending up at the estuary downstream through the Selangor River (Rahman & Rahim, 2019). Similarly, Yunus et al. (2015) also found that PTEs accumulation of fish samples in Kuala Selangor might probably be due to the boating activities such as loading and offloading of fish, boat cleaning and maintenance, as well as applications of antifouling paint.

Health risk assessment

This study focused mostly on the PTEs in the muscle tissues for health risk assessment including direct comparison with MPL, PTWI, and chronic and carcinogenic risks.

Direct comparison with MPLs

The MPL served as the food safety guideline standard for each PTE by comparing with various local and international governing authorities (Table 2), including the Malaysia Food Act (MFA, 1983), Food and Agriculture Organisation (FAO, 1983), World Health Organisation (WHO, 1995), Federal Environmental Protection Agency, Nigeria (FEPA, 2003), and European Food Safety Authority (EFSA, 2006, 2023). Since the guidelines specified were slightly different among various organizations, this study considers the Malaysia Food Act (1983) as the main reference of comparison with the PTEs concentration obtained in *E. tetradactylum* and *P. niger* from Morib and Kuala Selangor. Nonetheless, the average MPL (A-MPL) specified by five different organizations served as a secondary reference comparison with the PTEs concentration obtained since the MPL of Ni was not available in the MFA (1983).

Overall, the international guidelines of PTE concentration in edible fish were much stringent than the local MFA (1983) while the non-essential elements (Cd and Ni) had a low tolerable limit than essential elements (Cu and Zn). This direct comparison with food safety guideline standards was a preliminary step before conducting any health assessment index to evaluate the overall expression of the PTE concentration in two studied fish species. The direct comparison of MPL as specified by MFA (Cu: 30.00 mg/kg; Zn: 100 mg/kg) and A-MPL (Cu: 12.96 mg/kg; Zn: 49.00 mg/kg) exhibited that the concentration of Cu and Zn for muscle tissues in *E. tetradactylum* and *P. niger* from Morib and Kuala Selangor was below the MPL. In contrast, the MPL of Cd concentration specified by MFA and A-MPL was 1.00 and 0.52 mg/kg, respectively. For both locations, the Cd concentration of muscle tissues in *E. tetradactylum* (Morib, 0.75 mg/kg; Kuala Selangor, 0.74 mg/kg) and *P. niger* (Morib, 0.77 mg/kg; Kuala Selangor, 0.73 mg/kg) have exceeded the A-MPL. Similarly, all muscle tissues exceeded the MPL of Ni concentration, except for *E. tetradactylum* from Morib which was slightly below the A-MPL (0.50 mg/kg) while no information was provided in MFA.

PTWI The EWI for a typical group of Malaysian adults was compared with respect to their PTWI established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) from Codex Committee on Contaminants in Food (CCCF) (Table 4), aiming to examine the risk of exposure and the amount of fish consumption (JECFA, 2022; Matsunuma et al., 2011). The EWI of Cu, Zn, and Ni in both *E. tetradactylum* and *P. niger* from Morib and Kuala Selangor did not exceed the PTWI. However, the EWI of Cd for both fish species exceeded the PTWI guideline (Table 4) indicating that the estimated quantity of Cd in *E. tetradactylum* and *P. niger* consumed weekly throughout a lifetime will pose a significant health risk (JECFA, 2022; Matsunuma et al., 2011). Furthermore, the findings also revealed that the EWI of Cd would pose a significant health exposure with chronic and carcinogenic risks.

Table 4 Average PTE concentration in terms of location and tissue in *E. tetradactylum* and *P. niger*

Factors	Fish species	PTE concentration (mg/kg DW)			
		Cu	Zn	Cd	Ni
Location					
Morib	*E. tetradactylum*	3.02 ± 2.05[a]	67.37 ± 37.26[a]	1.33 ± 0.75[a]	1.78 ± 1.07[a]
	P. niger	5.19 ± 3.72[a]	121.75 ± 109.91[a]	5.52 ± 2.35[a]	0.68 ± 0.42[a]
Kuala Selangor	*E. tetradactylum*	2.73 ± 2.17[a]	63.26 ± 28.97[a]	1.32 ± 0.97[a]	0.82 ± 0.29[a]
	P. niger	5.18 ± 1.83[a]	81.62 ± 58.12[a]	2.32 ± 1.17[a]	1.20 ± 1.11[a]
Tissue					
Intestine	*E. tetradactylum*	4.52 ± 0.15[a]	110.19 ± 10.91[a]	1.85 ± 0.95[a]	2.00 ± 1.88[a]
	P. niger	3.91 ± 0.15[a]	93.40 ± 0.20[a]	1.82 ± 0.36[a]	2.07 ± 1.56[a]
Kidney	*E. tetradactylum*	2.97 ± 0.66[a]	67.09 ± 2.48[b]	2.35 ± 0.19[ab]	2.34 ± 0.12[a]
	P. niger	4.18 ± 0.44[a]	235.72 ± 99.04[b]	4.27 ± 1.94[ab]	0.96 ± 0.32[a]
Muscle	*E. tetradactylum*	0.54 ± 0.17[b]	17.07 ± 5.34[c]	0.75 ± 0.01[a]	0.56 ± 0.09[a]
	P. niger	0.36 ± 0.26[b]	9.42 ± 0.29[c]	0.75 ± 0.03[a]	0.55 ± 0.01[a]
Gill	*E. tetradactylum*	1.04 ± 0.60[a]	64.50 ± 2.87[a]	1.15 ± 0.62[a]	0.77 ± 0.03[a]
	P. niger	6.44 ± 3.02[a]	77.57 ± 40.02[a]	0.38 ± 0.24[a]	0.68 ± 0.07[a]
Liver	*E. tetradactylum*	5.31 ± 0.06[c]	67.72 ± 9.36[a]	0.52 ± 0.16[b]	0.83 ± 0.71[a]
	P. niger	8.61 ± 1.00[c]	92.34 ± 3.33[a]	12.38 ± 8.77[b]	0.45 ± 0.09[a]

Mean ± standard deviation followed by the same letters are not significantly different at 0.05 levels of probability within each PTE

Table 5 Health index caused by the consumption of PTEs in *E. tetradactylum* and *P. niger*

Location	Fish species	Estimated weekly intake (EWI)				Target hazard quotient (THQ)				Carcinogenic risk (CR)	
		Cu	Zn	Cd	Ni	Cu	Zn	Cd	Ni	Cd	Ni
Morib	*E. tetradactylum*	0.0075	0.2401	0.0135*	0.0089	0.0268	0.1143	1.9326#	0.0637	0.0007	0.0005
	P. niger	0.0066	0.1664	0.0140*	0.0100	0.0235	0.0792	1.9972#	0.0717	0.0008	0.0005
Kuala Selangor	*E. tetradactylum*	0.0119	0.3766	0.0134*	0.0112	0.0426	0.1793	1.9135#	0.0801	0.0007	0.0006
	P. niger	NA	0.1738	0.0132*	0.0097	NA	0.0828	1.8906#	0.0692	0.0007	0.0005

NA = not available.

*Exceeded the provisional tolerable weekly intake (PTWI) stated by Joint FAO/WHO Expert Committee for Food Additives (JECFA) with Cu (3.5 mg/kg/week), Zn (7.0 mg/kg/week), Cd (0.007 mg/kg/week), and Ni (0.035 mg/kg/week).

#Indicated THQ > 1

Chronic and carcinogenic risks

The value of the target hazard quotient (THQ) and carcinogenic risk (CR) of each fish species from both Morib and Kuala Selangor were reported in Table 4. The THQ and CR served as health indexes to assess chronic and carcinogenic risks, with daily basis consumption of fish species (Yap & Al-Mutairi, 2022). THQ assesses the chronic risk with the ratio between EDI and ORD (Ahmed et al., 2019; Traina et al., 2019). THQ value below 1 indicated no adverse effects on human health, whereby possible negative health risks may be subjected by daily consumption if the amount exceeds the ORD. Among the PTEs, the THQ values of Cu, Zn, and Ni were < 1, except for Cd (1.89–2.00). The THQ results of Cd greater than 1 revealed that the local consumers in Morib and Kuala Selangor may face high chronic risk with the regular consumption of *E. tetradactylum* and *P. niger* in their daily diet.

CR analyses the incremental probability of developing cancer in an individual, over a lifetime, due to the exposure of a substantial carcinogen (Ahmad et al., 2016; Selvam et al., 2020). The agreed acceptable range for a lifetime exposure of CR is between 10^{-6} and 10^{-4}. CR values greater than 10^{-4} were unacceptable because this infers the feasible probability of carcinogenic risk, whereas lower than 10^{-6} indicated that carcinogenic risk is negligible (Traina et al., 2019). In this study, the CR results obtained from Cd and Ni were within the acceptable range ($10^{-6} < CR < 10^{-4}$) revealing that both fish species could pose a moderate risk of cancer occurrence to the consumers in Morib and Kuala Selangor. Therefore, consumers should be aware that both *E. tetradactylum* and *P. niger* from Morib and Kuala Selangor would likely pose a serious health risk associated with prolonged consumption.

Permissible fish ingestion rate (FIR)

Both *E. tetradactylum* and *P. niger* recorded the average concentration of Cd in fish muscle tissue at Morib (0.76 mg/kg) and Kuala Selangor (0.74 mg/kg), respectively. Considering 0.75 mg/kg as the average concentration of Cd in both fish species from Morib and Kuala Selangor, the recommended dosage of fish to minimize chronic (THQ) and carcinogen (CR) risks should not exceed 0.0022 kg/day which was not practical because there were other various nutrients beneficial to the human body. Optimally, the maximum FIR with 0.08 kg/day was calculated based on the current PTEs concentration, indicating that the consumption of *E. tetradactylum* and *P. niger* from Morib and Kuala Selangor will have almost no adverse effects on human health and a low possibility for cancer occurrence. Thereby, the optimal FIR suggested in this study with 0.08 kg/person/day for an average of 62 kg adults in Malaysia was reasonable as compared with other studies (Lipy et al., 2021; Selvam et al., 2020; Vu et al., 2017) (Table 5).

Conclusion

The study was carried out in Morib and Kuala Selangor at Selangor district, targeting on PTE accumulation (Cu, Zn, Cd, and Ni) in *E. tetradactylum* and *P.*

niger. The concentration of PTEs in *E. tetradactylum* and *P. niger* from Morib and Kuala Selangor were decreasing in the order: Zn > Cu > Cd > Ni, except for *P. niger* in Morib with Zn > Cd > Cu > Ni. It was found that the PTEs accumulation in Morib was more severe than Kuala Selangor coastal area due to the higher PTE concentration in all fish samples regardless of species from Morib. Through the health risk assessments with THQ and CR, both fish samples (*E. tetradactylum* and *P. niger*) in this study can be concluded with the probability of deleterious effects, specifically posing chronic risks mainly because of the concentration of Cd (0.73–0.77 mg/kg) in muscle tissues exceeding the provisional tolerable weekly intake established by JECFA and oral reference dose provided by the USEPA. Thus, the recommended daily fish ingestion rate of 0.08 kg/person/day should be applicable for the population from both Morib and Kuala Selangor in Selangor, Malaysia. Both studied species had a high consumption rate for the Malaysian population regularly. Therefore, this research outcome will be significant to assess the feasibility of current food safety guidelines and further contribute to food safety guideline amendment as a reference. Moreover, the research outcomes will raise public awareness regarding PTEs contamination in the surrounded environment as well as to persuade relevant agencies in Malaysia to establish a stricter waste disposal management and pay attention on the implications to industrial area especially those factories located along coastal area within the proposed studied location.

Author contribution Peggy Pei Yee Tek: conceptualization, methodology, data curation, writing an original draft. Chuck Chuan Ng: investigation, supervision, resources, reviewing and editing.

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Declarations All authors have read, understood, and have complied as applicable with the statement on “Ethical responsibilities of Authors” as found in the Instructions for Authors. Moreover, it is important to note that both data availability and animal ethics declaration are not applicable in this context.

Conflict of interest The authors declare no competing interests.

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