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Assessment of cadmium (Cd) and lead (Pb) levels in commercial marine fish organs between wet markets and supermarkets in Klang Valley, Malaysia

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Abstract: Most investigations on heavy metals content in fish were either conducted on single markets, ports, seaside markets or direct sampling from natural habitat, and there were very few studies done on fish samples from both wet markets and supermarkets. This paper presents the assessment outcome of Cd and Pb levels in commercial fish sold between wet markets and supermarkets in Klang Valley, Malaysia. In this study, the organs of four commercial fish species (*Rastrellinger kanagurta*, *Epinephelus sexfasciatus*, *Lates calcarifer*, and *Decapterus maruadsi*) sampled from different markets within the sampling area were assessed using dry ashing-acid digestion method and Flame AAS. Results obtained concluded that Cd and Pb in fishes sampled from supermarkets are generally higher compared to wet markets, while both metals content in the edible organs fall well within the permissible limits for human consumption when compared to the Fourteenth Schedule of the Malaysian Food Regulations 1985.

Keywords: Heavy metals, wet markets, supermarkets, commercial fish, dry ashing, flame AAS

Introduction

Fish has long known for its reputation as the established health food for most of the world's population particularly developing countries in contrast to meat, poultry and eggs. The protein content in fish mostly averages from 15 to 20 percent; hence fish provides comparatively cheap and readily available protein sources in complement with long chains of n-3 fatty acids, amino acids, vitamins and minerals that further contributes to healthier nutritional options for a balance dietary intake (Hajeb *et al*., 2009; FAO, 2010). Marine fish is the important components of protein sources being incorporated into Malaysian diet which constitutes about 60 to 70 percent of protein consumed in Malaysia (Tukiman *et al*., 2006; Zuraini *et al*., 2006).

Fish which occupy top level in the aquatic food chain are notorious for its ability to bioconcentrate heavy metals in its flesh muscles and organs. Thus it is essential to study the capacity of various fish organs in bioaccumulating heavy metals since fish plays vital role in human nutrition and to ensure that unnecessary high level of several toxic trace metals are not being transfer to man through fish consumption which may directly affect human health (Zhou *et al*., 2008; Olowo *et al*., 2010).

Marine pollution indeed is a critical environmental issue of concern across the globe when growing human population increase the intensities of anthropogenic threats exert on the environment as a result of industrialisation, municipalities and agriculture activities (Raja *et al*. 2009). The negative manifestation of anthropogenic impacts from heavy metal discharge into the aquatic environment have induced disturbances to the hydrosphere equilibrium which further affects the natural structure and functions of marine biotic communities. Seafood especially marine fish are vulnerable to the effects of chemical contaminants including heavy metals which bioaccumulate and biomagnifies along the aquatic food chain (Kennish, 1998; Agusa *et al*., 2007).

Heavy metal contaminations are one of the pervasive forms of marine pollution because these metallic elements do not disintegrate rapidly in marine environment which further impairs the aquatic ecosystems due to the relatively high densities and toxicity even at low concentrations. These toxic elemental contaminants cause unhealthy effects to the fish and are transferred into human metabolism through consumption of contaminated fish that leads to serious deterioration of human health status (Virtanen, 2007; Raja *et al*., 2009; Alinnor and Obiji, 2010).

The levels of toxic contaminants in fish are of considerable interest due to its potential effects on the fish themselves and the organisms that consume them which including humans. Health advisories such as Food and Drug Administration (FDA) have recently raised concern on the safety of fish obtained from commercial sources (Burger *et al*., 2004). Therefore, this study was undertaken to compare the

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levels of hazardous heavy metals in edible marine fish purchased from wet markets and supermarkets.

Wet market generally refers to the typical local market setting whereby the market produce sold by the vendors are displayed either on the counters, stalls, or on the floor which varies upon locations (Shackleton *et al*., 2009) whereas supermarket denotes the large-format of modern retail by having a larger self-service store in selling groceries, dairy products and household goods with customers typical assumed to travel up and down the aisles of the store (Burger *et al*., 2004; Reardon *et al*., 2004; Larson *et al.*, 2005; Arda, 2006). Hence it is indispensable to investigate the level of heavy metals in commercial fish as majority of Malaysians consume fish which are purchased from commercial sources available either local wet markets or supermarkets.

Materials and Methods

Sampling

Four commercially important and commonly consumed marine fish species were used in this study: *Rastrellinger kanagurta*, *Decapterus maruadsi*, *Epinephelus sexfasciatus* and *Lates calcarifer*. All fish samples were randomly selected and purchased from nine different wet markets and supermarkets respectively in Klang Valley. Purchased samples were packed in clean zipped polythene bags and transported to the laboratory in an ice-filled polystyrene insulation box. Fish samples were transferred and stored in the laboratory freezer at -2ºC to reduce biological deterioration prior to analysis.

Sample preparation

Fish samples were de-scaled and rinsed with ultrapure water before dissection for the isolation of the following internal organs as test samples: brain, gills, intestine, kidney, liver and flesh muscles. Cares were taken during dissection of the internal organs to prevent any injuries and metal contaminations of the organ samples by using stainless steel dissecting kits.

The isolated organs were manually cut into small pieces with stainless-steel scissor and weighed accurately to 3.00 ± 0.05 g (wet weight basis) into individual sanitised porcelain crucibles and subsequently subjected to oven drying at 180ºC for 4 hours. The dried samples were later ashed at 500ºC for 12 hours inside a muffle furnace (THERMCONCEPT, Germany). The cooled ashes were digested with 1.5 mL of concentrated analytical grade 65% HNO_3 (Merck Chemicals, Germany) and subsequently diluted with ultrapure water to 30 mL. Diluted final test solution samples were filtered through Whatman® No. 595 filter paper prior to AAS analysis .

Chemical preparation

All glasswares and porcelain crucibles were soaked and sanitized in aqua regia of 1:1 analytical grade 37% HCl and 65% HNO_3 (Merck Chemicals, Germany) solution, subsequently rinsed with ultrapure water, and were air-dried for 12 hours prior to usage. Sample blanks were prepared in the similar way to the test samples for background correction. Standard solutions for Cd and Pb were prepared from stock solutions (100 ppm). The test solution samples were then analysed thrice for Cd and Pb using air-acetylene Flame AAS (Perkin Elmer AAnalyst 100). Detected metals were expressed as mg/kg wet weight (Suhaimi *et al*., 2005).

Statistical analysis

Obtained data was analysed using two-way ANOVA to determine significant differences ($p<0.05$) of statistical means of each heavy metals present within the organs of four selected fish species. Post hoc test using Scheffe's test was used to further elucidate the analysed mean differences between the organs. All data were presented in wet weight in which more useful for health risk considerations since animals as well as human consume organisms in their natural state. Moreover wet weight was chosen as it is more convenient to evaluate the safety of fisheries products in accordance with the objective of this study as to assess and evaluate the safety of marine fisheries with respect to the level of Cd and Pb detected in four species (CRESP 2006).

Results

The concentrations of Cd and Pb in the test samples were analyzed with respect to the type of markets. The average concentrations of Cd and Pb detected in the test samples of four fish species were tabulated and compared in Table 1. Table 2 showed the overall mean concentration for all paired organs samples according to the respective type of markets.

Highest mean Cd concentrations as shown in Table 2 were found in the kidney samples followed by liver tissue samples of both wet markets and supermarkets. This study therefore has shown that Cd bioaccumulate more in the kidneys than in the livers. The level of Cd examined in the kidney from wet markets and supermarkets were 1.303±0.155 mg/kg and 1.819±0.139 mg/kg respectively meanwhile the Cd level detected in liver were 1.253±0.121 mg/kg and 1.618±0.126 mg/kg.

Table 1. Concentrations of heavy metals in different fish species collected from wet markets and supermarkets in Klang Valley, Malaysia

Types of markets	Organs	[1]Cadmium mean concentrations (mg/kg)				[2]Lead mean concentrations (mg/kg)			
		Rastrellinger kanagurta	*Epinephelus sexfasciatus*	*Lates calcarifer*	*Decapterus maruadsi*	*Rastrellinger kanagurta*	*Epinephelus sexfasciatus*	*Lates calcarifer*	*Decapterus maruadsi*
Wet markets	Brain	0.489±0.099	1.246±0.044	0.654±0.163	0.657±0.067	1.007±0.049	1.344±0.159	1.386±0.146	1.278±0.161
	Gills	1.482±0.119	1.074±0.062	0.874±0.073	1.162±0.108	1.461±0.078	1.419±0.121	1.097±0.095	1.151±0.063
	Intestine	1.160±0.111	0.820±0.043	0.699±0.044	0.964±0.086	1.256±0.064	1.060±0.017	0.865±0.086	1.484±0.033
	Kidney	1.289±0.060	2.366±0.151	0.766±0.044	0.790±0.073	1.366±0.084	2.638±0.073	0.912±0.051	1.599±0.103
	Liver	1.062±0.139	1.959±0.212	0.767±0.120	1.225±0.089	1.212±0.069	2.145±0.065	0.995±0.078	1.635±0.073
	Flesh Muscle	0.863±0.042	0.536±0.024	0.497±0.031	0.119±0.007	1.506±0.095	0.616±0.046	1.045±0.088	1.419±0.070
Supermarkets	Brain	0.940±0.040	1.408±0.031	1.146±0.101	1.631±0.111	1.010±0.043	1.577±0.087	1.398±0.059	1.428±0.115
	Gills	2.080±0.052	1.201±0.031	1.138±0.056	2.460±0.116	1.795±0.085	1.434±0.144	1.183±0.075	1.554±0.130
	Intestine	1.849±0.054	1.153±0.039	1.001±0.036	1.865±0.150	1.657±0.107	1.368±0.052	1.191±0.045	1.742±0.088
	Kidney	1.767±0.087	2.667±0.097	1.044±0.040	1.796±0.142	1.475±0.084	3.124±0.217	1.092±0.074	1.747±0.078
	Liver	1.245±0.144	2.153±0.163	1.057±0.032	2.016±0.175	1.368±0.072	2.306±0.098	1.156±0.035	1.775±0.073
	Flesh Muscle	0.982±0.055	0.774±0.052	0.574±0.033	0.121±0.016	1.594±0.053	0.813±0.070	1.144±0.085	1.399±0.166

[1,2]Mean values ± SD were obtained from three replications.

Table 2. Overall mean concentration of different paired organ samples between wet markets and supermarkets in Klang Valley, Malaysia

Pairs	Organs	N	Mean (mg/kg)	
			Cadmium	Lead
Pair 1	[1]Brain	20	0.761±0.081[a]	1.254±0.071[c]
	[2]Brain	20	1.281±0.070[a]	1.353±0.061[c]
Pair 2	[1]Gills	20	1.148±0.066[a]	1.282±0.056[b]
	[2]Gills	20	1.719±0.134[a]	1.492±0.072[b]
Pair 3	[1]Intestine	20	0.910±0.053[a]	1.166±0.059[b]
	[2]Intestine	20	1.467±0.982[a]	1.489±0.062[b]
Pair 4	[1]Kidney	20	1.303±0.155[a]	1.628±0.150[b]
	[2]Kidney	20	1.819±0.139[a]	1.860±0.185[b]
Pair 5	[1]Liver	20	1.253±0.121[a]	1.497±0.106[b]
	[2]Liver	20	1.618±0.126[a]	1.651±0.106[b]
Pair 6	[1] Flesh Muscle	20	0.504±0.062[a]	1.147±0.088
	[2] Flesh Muscle	20	0.613±0.076[a]	1.238±0.082

[1]Organs collected in all four fish species from wet markets.
[2]Organs collected in all four fish species from supermarkets.
[a,b]Mean values within same pair are significantly different ($p<0.05$).
[c]Mean values within same pair are not significantly different ($p>0.05$).

The highest levels of Pb as shown in Table 2 were detected in kidney samples from wet markets and supermarkets with mean concentrations 1.628±0.150 mg/kg and 1.860±0.185 mg/kg respectively. Liver tissues also have high tendencies to bioaccumulate Pb in which the concentrations reported for wet markets and supermarket samples were 1.497±0.106 mg/kg and 1.651±0.106 mg/kg respectively.

Overall, there were significant differences ($p<0.05$) of Cd detected in all six test organs meanwhile there were no significant differences ($p>0.05$) of Pb level in brain and flesh muscles samples examined in fish

Table 3. Guidelines on heavy metals permissible limits (mg/kg) for food safety based on wet weight basis

Organisations	Permissible limits (mg/kg)	
	Cadmium	Lead
Fourteenth Schedule (Regulation 38), Malaysian Food Regulation (1985)	1.00	2.00
United States Food and Drug Administration (USFDA, 1990)	3.70	1.70
Hong Kong Environmental Protection Department (HKEPD, 1997)	2.00	6.00
Food and Agriculture Organization (FAO 1983)	0.50	0.50

collected from both wet markets and supermarkets. This variation was believed due to differences in levels of fish exposure to heavy metal contaminants. The concentrations sequence of Cd and Pb found in all test organs used in this study were as follows: kidney > liver > gills > intestine > brain > flesh muscles. Based on Table 2, higher Cd and Pb were detected in all of the test organs sampled from supermarkets fish as compared to wet markets fish samples.

Outputs from ANOVA test showed significant differences in the concentrations of Cd and Pb between wet markets and supermarkets fish. The concentrations of Cd and Pb obtained in this research were compared with several permissible guidelines shown in Table 3. The outcomes of this study are still appreciable within the permissible limits when compared with USFDA 1990 and HKEPD 1997, apart from the Malaysian Food Regulation 1985.

Discussion

Fish is among the dominant bioindicator species used for acute toxicity assay of pollutants such as heavy metals since much attention has been drawn due to the wide occurrence of metal pollution in aquatic system. The rapid development of industries and agricultures have promote the increase of environmental pollution although heavy metals in aquatic system can be naturally produced by slow leaching from rocks and soil into water which occurs at low levels. Cd and Pb are among the aquatic metal pollutants which usually present at significant levels in water system which may pose high toxicities on the aquatic organisms (Zhou *et al*., 2008).

Cd and Pb have higher tendencies to bioaccumulate in the fish kidney and liver tissues due to the similar functions of kidney and liver as the organs that involve in the detoxification process. The presence of free protein-thiol group content and metallothioneins binding proteins in kidneys and livers forms strong fixation with the heavy metals (Iwegbue, 2008). Fish kidney located along the dorsal wall of the fish body mainly contains excretory tissues meanwhile fish liver acts as major site for homeostasis (Dallinger *et al*., 1987; Reynders *et al*., 2006).

Marine fish are exposed to waterborne heavy metal fractions when marine fish drinks considerable amount of sea water. Therefore, gills serve as the important route of heavy metal cationic exposure from surrounding sea water. The large surface area of gills further facilitates the adsorption of Cd and Pb onto the surface of gills during respiration and osmoregulation processes. Metallothioneins binding proteins were also found in fish gills which trapped heavy metals compounds (Dallinger *et al*., 1987; Reynders *et al*., 2006; Di Giulio and Hinton, 2008).

Fish intestine compared to the other tested organs acts as a transient site for heavy metal bioaccumulations in fish body. Fish intestine involved in the uptake of particulate heavy metal fractions via alimentary tract in which the rate of heavy metals uptake being controlled by specific transport system through simple diffusion mechanism across the intestinal epithelium. Cd and Pb form complexation with the intestinal amino acids and small peptides with respect to high affinity for protein thiol-group which present within the fish intestine (Filipovic and Raspor, 2003; Marijic and Raspor, 2007).

The presence of blood brain barrier in fish brain serves to protect the vulnerable brain tissues from toxic metal perturbations which further prevents fish against neurotoxicity effects. Moreover, the metallothioneins found within the fish brain tissues are not as inducible as compared to the metal binding proteins found in fish kidney and liver. This further supported the lesser amount of Cd and Pb being detected in the fish brain compared to kidney and liver (Filipovic and Raspor, 2003; Marijic and Raspor, 2007).

Fish flesh muscle is the edible part of fish and frequently employed in assessing human health risks in relation to marine fish consumptions. The concentrations of Cd and Pb detected in fish flesh muscle tissues were the lowest compared to other organs although higher Cd and Pb levels were detected in supermarkets fish flesh muscle than of fish purchased from wet markets. However, the Cd and Pb concentrations in fish flesh muscles examined in this study were well situated within the permissible limits as stipulated in the Fourteenth Schedule (Regulation 38) of the Malaysian Food Regulation 1985.

The presence of mucous layer coating the fish

skin surface served as a barrier which protects the integrity of fish flesh muscle tissues from surrounding contaminants. The mucous layer serves as the first line of defence against the entrance of heavy metals into fish flesh muscle tissues by forming complexes with the heavy metals. Therefore fish flesh muscle tends to bioaccumulate lesser metals compared to the other fish organs (Schlenk and Benson, 2001; Altindag and Yigit, 2005; Uysal *et al*., 2008).

However, scientific explanations for the outcomes of higher Cd and Pb discovered in supermarket samples are unknown in this present research as similar studies have yet to be done elsewhere to date. However, the closest explanations can be link to the following postulations and hence further investigations are needed for justifications. Moreover, the main objective of this present research was to study on the distribution of Cd and Pb contents from commercial fishes sold in wet markets and supermarkets which did not impart into detailed investigations due to budget, time and instruments constraints.

The effects of freezing on cells, storage temperatures, fish stress and rigor mortis with relation to cellular metabolism during perimortem and post-mortem are speculated for higher Cd and Pb contents in supermarket samples based on the findings of this study. Freezing method is important in determining the size of ice crystals that can rupture cell walls which allow movement of cytoplasmic fluid that contain metals when thawed. Quick freezing method reduces the formation of large ice crystals which aims to yield good quality products in terms of textures, colouration, freshness and tenderness of meat. Contrastingly, slow freezing method causes the fluid in fish tissues to form large ice crystals that damages the delicate tissue cells.

Freezing also results in concentration of dissolved organic and inorganic salts increases while liquid water is converted into ice (Love and Haraldsson, 1958; Potter and Hotchkiss, 1995; Tull, 1996). The common practice of refreezing or intermittent freezing of unsold fishes overnight by some supermarket retailers due to the availability of cold storage facilities in their premises might increase Cd and Pb contaminants that originate from adjacent fishes or surrounding environment. The conditions of supermarket fish observed during field sampling have supported the fact that the retailers have mixed the new batch of fish stocks with the remainder unsold fishes. Consumers normally are able to observe that re-sale fish often dull in appearance, torn, shrunk abdomen, loss of meat firmness and elasticity, white eyes, darken gills and also liquid drippings from bodily orifices when the fish is held upright. Contradictory to the conditions of supermarkets, the ambient wet market temperatures hasten microbiological and enzymatic spoilage making it not safe for re-storage although the practice of putting and spreading chunks of ice blocks or by sprinkling treated pipe water on the fish is very common. Moreover, selling activities in wet markets begin as early as pre-dawn and recedes by noon taking advantage of the cool surrounding.

Storage temperatures might affect the enzymatic activities of ATPase ionic pumps. Cd and Pb movements across cells via active transport with the utilisation of ATP are facilitated by P-type ATPase ionic pump (mainly type IB ATPase subfamily) as both metals are divalent soft Lewis acids (Gatti *et al*., 2000). Hence, the process of phosphorylation and dephosphorylation continues in perimortem and post-mortem in dead fish is subjected to the availability of ATP, temperature and pH.

Studies by Harada (1988), Abe and Okuma (1991), Cappeln and Jessen (2002) and Erikson and Misimi (2008) on perimortem and post-mortem with regards to rigor mortis of fish flesh muscles subjected to temperatures ranges from 0 to 30°C indicated that ATP metabolism does occur. Hence, ambient temperature variations and fluctuations between supermarkets and wet markets might facilitate the movements of heavy metals in dead fish. Warmer ambient temperatures (as in wet markets) contribute to shorter ATPase activities as the half-life of the reactions shortens significantly due to optimal conditions for enzymatic reactions as substrates are completely consumed in a shorter period of time, while cooler temperatures (as in supermarkets) prolongs it, rendering more metals to move across cell membranes until ATPs are depleted. Supermarkets are able to maintain and regulate in-house ambient temperature through air-conditioning systems to slow bacterial and enzymatic spoilage. Heavy metals also bind to the cell-surface functional groups found on both gram-positive and negative bacteria which are thought to be an important step of intracellular accumulation of trace metals required for enzymatic functions when toxic metal species are being used as electron acceptors under anaerobic conditions. Most microorganisms produce extracellular polysaccharides (EPS) often containing proteins which strongly bind metals as EPS-metal interactions has the ability to mobilize and transports metals in aquatic systems (Ford and Ryan, 1995).

Thus, the selection of final freezing temperatures is a critical factor to minimize microbial and enzymatic spoilage as fish, by body weight contains about 75% water which 90 – 95% of this water freezes at temperature of -25^{o}C; this exclude the chemically bound water to specific sites such as carbonyl and

amino groups of proteins and hydrogen bonds which never available for freezing (Hall, 1997). The use of -18°C provides a reasonable safety measures against pathogenic microorganisms in most storage and transportation practices. However, the temperature of -18°C is not exceptionally low for nonenzymatic reactions to occur even at a very slow rate due to the availability of bound water in the cells which acts solvents (Potter and Hotchkiss 1995). Hence this could further explain higher Cd and Pb found in supermarkets as compared to wet markets in this study.

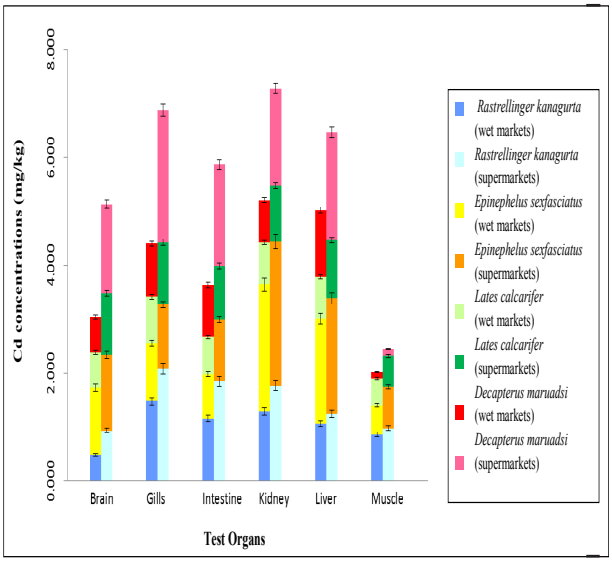

Figure 1. Comparison of Cd concentrations (mg/kg) in different organs of four fish species between wet markets and supermarkets

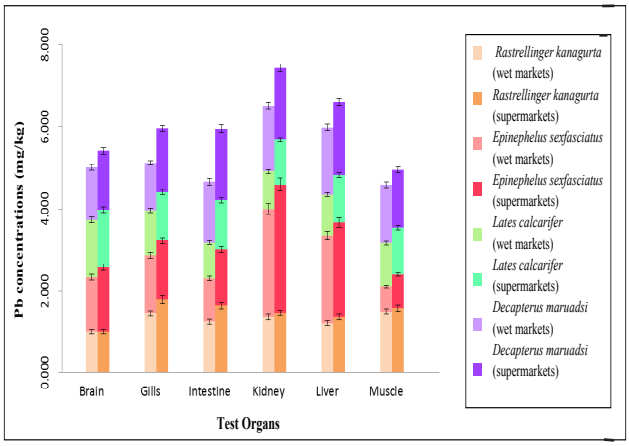

Figure 2. Comparison of Pb concentrations (mg/kg) in different organs of four fish species between wet markets and supermarkets

Conclusion

This present study provides new information and serves as a baseline reference for future continuous studies in regards to the comparison of heavy metals in fish between wet markets and supermarkets around Klang Valley. Cd and Pb concentrations found in different organs of four selected fish species were significantly higher in fish collected from supermarkets compared to wet markets. However the edible fish flesh muscle in all four fish species purchased from wet markets and supermarkets were not heavily contaminated with Cd and Pb as the concentrations were well situated within the permissible safety limits.

Factors from post-harvest handling such as freezing on pre-mortem and post-mortem fish that can affect cellular metabolic activities might be attributable to the significant difference of Cd and Pb contents in fish sampled from wet markets and supermarkets. Furthermore, the scenario of the actual freezing and storage practices by fish trawlers were unknown and this can also influence both metal concentrations, Cd and Pb in fish captured.

However, the potential of dietary hazards due to Cd and Pb exposures upon consumption of these selected fish species sold in wet markets and supermarkets within Klang Valley are possible to occur in the future as further study is highly recommended since toxic heavy metals have high tendency to bioaccumulate in various organs of marine organisms especially fish which reflects the pollution level of marine environment.

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