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Influence of Habitat Affinity on the Bioaccumulation of Copper, Cu and Zinc Zn in Coastal Fishes of Bintulu, Sarawak

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Abstract: Anthropogenic activities are increasingly growing in Bintulu area where residues and runoffs from the activities would consequently affect its adjacent aquatic systems and organisms. An investigation on the trace metal accumulation (copper, Cu and zinc, Zn) was conducted in coastal fishes off Bintulu, Sarawak of three habitat affinities: pelagic, benthopelagic and benthic. Samples of fish gills, livers and muscles from the fishes were analyzed to examine the accumulation of Cu and Zn. The study found that habitat affinity affected the accumulation level of Zn and Cu in the fishes examined. Benthic fishes, living in the bottom area of water column accumulated the highest trace metals as compared pelagic fish (surface dwellers) which accumulated the least trace metals. In almost all tissues analyzed, muscles accumulated the least concentration of Zn and Cu while livers accumulated the highest. The study exhibited a significant interplay in trace metal accumulation among fishes, their organs and habitat within water columns.

Key words: Bioaccumulation, trace metals, coastal fishes, benthic fishes, Zn

INTRODUCTION

Accumulation of undesirable anthropogenic substances in coastal areas is closely related to discharge from nearby towns as its point source (Vystavna *et al*., 2013). Accumulated wastes from the urbanization can have impacts on natural resources especially with the growing population and industries. Over time, anthropogenic inputs, especially trace metal residues from human activities discharged into the coastal areas can deplete natural resources in the surrounding areas (Fabure *et al*., 2015; Villanueva *et al*., 2015).

Trace metals cannot be degraded once they enter the aquatic ecosystems. Eventually they are deposited, and incorporated in water, sediment and aquatic animals, thus, causing pollution in water bodies (Wu *et al*., 2016; Kibria *et al*., 2016). The levels of metals can be traced in organisms in the aquatic food chain through the effects of called bioaccumulation. Depending on species and types of organ/tissues implicated, substantial high accumulation can become toxic to the organisms in the food chain (Carranza *et al*., 2016; Monferran *et al*., 2016).

Commercial fishes are a group of aquatic organism that is often examined for metal concentration levels. The foraging affinity of marine organisms in the bottom (benthic) and open (pelagic) water column have been shown to affect metal accumulation in aquatic organisms (Canli and Atli, 2003; Jonathan *et al*., 2015; Liu *et al*., 2015). Previous studies also exhibited that bioaccumulation in fish tissue is organ-dependent, where the more exterior organs such as gills contain higher metals than the more interior organs such as livers (Liu *et al*., 2015; Bustamante *et al*., 2003; Monikh *et al*., 2013). For instance, benthic fishes such as mudskippers (*Periophthalmodon schlosseri*) that are abundant in tide-affected mudflats contain the highest metal accumulation as they mainly feed on mostly organisms in the area if they are to be compared to pelagic fishes (Marcus *et al*., 2013; Diop *et al*., 2016). Indeed, owing to their greater home range and feeding area, pelagic fishes accumulate the least metal concentration as compared to fishes living in other parts of the marine water columns (Canli and Atli, 2003; Monikh *et al*., 2013; Dural *et al*., 2007). However, with the current climate trends across the globe, the predicted effects of bioaccumulation on aquatic organisms especially in the marine coastal waters are prone to drastic changes. Therefore, the impact of remobilization and dispersal of antropogenic disposals such as trace metals cannot be overlooked.

The need for monitoring level trace metal in Malaysian coastal areas is important considering their potential adverse effects on the local and regional biota. Bintulu was selected as the study site. A fishing village

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until 1969, Bintulu has now become the center of energy-intensive industries ever since oil and gas reserves were discovered offshore the town. Since then, Bintulu becomes the center of energy-intensive industries. In light of that, the objectives of the study were to determine the amount of Zn and Cu in different tissues of marine coastal fishes sampled collected from the coastal areas of Bintulu, and to determine whether the Zn and Cu accumulation reflect habitat affinity in the water column for bethic, betho-pelagic and pelagic fishes.

MATERIALS AND METHODS

A total of 120 fish samples were purchased from fish markets in Bintulu, Sarawak (Pasar Tamu Bintulu, Tanjung Kidurong and Kampung Baru) in December 2014 and February 2015 (Fig. 1). Fishes were purchased based on their presumed habitat affinities and feeding trophic: benthic, bentho-pelagic and pelagic. Two species from each habitat affinity group were selected for this study. In order to remove the effect of size on metal accumulation, samples purchased in both sampling months were of the same class size. Upon purchase, the samples were placed immediately in insulated polystyrene boxes and then brought to the laboratory at the University Putra Malaysia Campus Bintulu, Sarawak for permanent storage.from each habitat affinity group were selected for this study. In order to remove the effect of size on metal accumulation, samples purchased in both sampling months were of the same class size. Upon purchase, the samples were placed immediately in insulated polystyrene boxes and then brought to the laboratory at the University Putra Malaysia Campus Bintulu, Sarawak for permanent storage.

Livers, gills and muscular tissues were dissected out of the fish samples and homogenized. Due to insufficient minimum weights required for the metal concentration analysis, the organ samples were pooled and then divided into three replicates. Then, samples were kept in crucible jars, and oven-dried at 80°C until constant weight was achieved. Dried samples were crushed with mortar and pestle. Approximately, 2.0 g of crushed sample was weighed and burned to ash in the furnace at 550°C for 90 min. The ash was dissolved in 5 mL of concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a ratio of 3:1 (HNO_3: $HClO_4$)made up to 25 mL volume. Following a standard procedure, Atomic Absorption Spectrometer was then used to determine the presence of zinc, Zn and copper, Cu.

Statistical procedures were performed using the statistical software SAS Version 9.2 for Windows. Correlation analysis was conducted to determine if the accumulation is related to size. One-way analysis of covariance ANCOVA was used to indicate significant differences in metal levels among species and different water columns ($\alpha = 0.05$). One-way analysis of covariance ANCOVA was also used to compare metal accumulation among tissue types ($\alpha = 0.05$). Prior to analysis, all data were checked for the homogeneity of variances and normality data that are not normally distributed or not homogeneous were log-transformed.

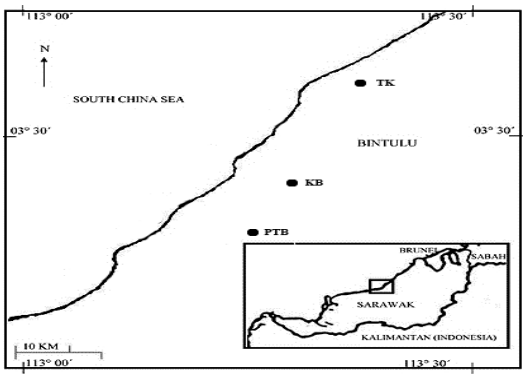

Fig. 1: Sampling sites in Bintulu, redrawn to scale; PTB = Pasar Tamu Bintulu, KB = Kampung Baru, TK = Tanjung Kidurong

RESULTS AND DISCUSSION

Total Lengths (TL) variation among samples did not show significant differences, indicating effect of size has been eliminated from the determination of trace metal accumulation in the analyzed samples. Elimination of size factor is of paramount because there is a significant association between organisms tend to accumulate organic matters and metals at different levels given their ages and lengths (Al-Yousuf *et al*., 2000; Farkas *et al*., 2003). Gills and muscles accumulations for Cu and muscle accumulation for Zn marked significant differences ($p<0.05$). On contrary, the rest of the trace metal concentration in body tissues did not show significant difference with size correction among analyzed species.

Due to insufficient amount of ashed samples, some of the analyses for the fish tissues were not performed. Despite that, among the three body tissues analyzed, muscles accumulated the lowest Zn and Cu concentrations within each species. Conversely, livers accumulated the highest trace metals as compared to gills (Table 1). Relative observation from the results indicated that fishes living in the upper section of water column especially pelagic fishes accumulated lesser trace metals than fishes living in the lower section of water column. Such patterns were relatively observed in all body tissues for both Cu and Zn accumulations. Fish of different water column significantly accumulate metals differently in their tissues.

Table 1: Specimens collected for the study, n = number of samples. Scientifc names are given below vernacular names. Size distribution are mentioned in standard deviation, SD from the mean. The last two columns indicate descending order of trace metal accumulation in different types of tissues examined. Measurement units are given in parentheses. *Comparison not available

Species	Total length (cm) Mean±SD	Body weight (g) Mean±SD	Trace metal accumulations Cu (μg g^{-1})	Zn (μg g^{-1})
Pelagic fish				
Yellow-banded scad *Selaroides leptolepis*	20.63±0.24	99.75±4.030	Gills>Liver>Muscle	Muscle*Gills>Liver>Muscle
Malabar red snapper *Lutjanus malabaricus*	34.66±0.38	289.92±33.06	Muscle*	Muscle*
Bentho-pelagic fish				
Malabar grouper *Epinephelus tauvina*	23.60±0.73	224.55±27.18	Gills>Liver>Muscle Liver*	Gills>Liver>Muscle Liver*
Giant catfish *Arius thalassinus*	26.49±0.78	289.92±33.06	Liver*	Liver*
Benthic fish				
Indian halibut *Psettodes erumei*	21.35±0.43	83.99±11.62	Liver>Gills	Liver>Gills
Large-scaled tongue sole *Cynoglossus macrolepidotus*	24.71±0.31	95.46±2.350	Muscle*	Muscle*

The accumulation depends on aquatic environments of pelagic, benthopelagic, and benthic. The differences in concentration amount of metals between fishes are attributed to feeding habits, the bio-concentration capacity of each species and to the biochemical characteristics of the metal (Romeo *et al*., 1999). Additionally, the ability of fish to accumulate metals depends on ecological needs, metabolism, and degree of pollution in sediment, water and food, as well as salinity and temperature of water.

There were significant differences in Zn and Cu accumulations in different fish body tissues. However, the accumulation in terms of tissues varied among species. Such an accumulation trend that was found highest in the liver is linked to its role in metabolism. Zn and Cu in hepatic tissues are usually related to a natural binding proteins such Metallothioneins (MT). They act as essential metal stores to complement enzymatic and other metabolic activities (Agusa *et al*., 2007; Moselhy *et al*., 2014).

Pelagic fishes in the present study accumulated the highest Cu concentration in the gills among the three habitat affinities (Fig. 2a). The range of Cu contents (0.8-8.0 μg g^{-1}) overlapped with the lower range of Zn in sea sediments in the vicinity of present study area (5.7- 30.6 μg g^{-1}, unpublished Bintulu Port environmental monitoring program). The highest Cu accumulation was almost double the amount of the the second highest Cu accumulation in the benthic fish in the study. On contrary, Cu accumulations in livers and muscles of benthic and bentho-pelagic fishes were higher than the pelagic fishes (Fig. 2). Cu is an essential element that plays an important role in biological systems. The low levels found in the present study (0.1-5.2 μg g^{-1} w.w.) are in agreement with its homestatic control below 50 μg g^{-1} d.w (Weber *et al*., 2013).

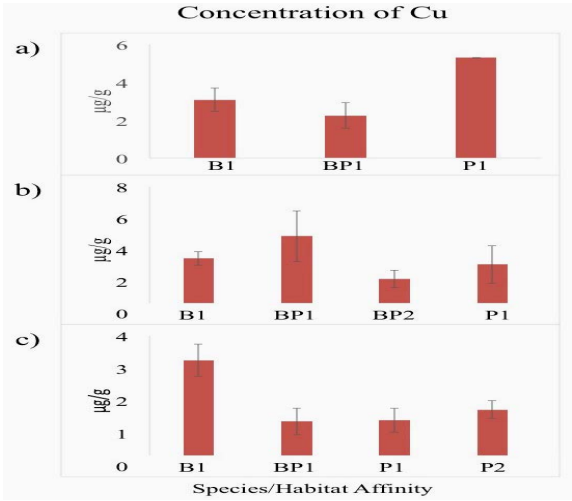

Fig. 2: Concentration of copper, Cu in benthic (B), Bentho-Pelagic (BP) and pelagic fishes based on different organs: a) gills, b) liver, c) muscles. B1 = *C. macrolepidotus*, BP1 = *E. tauvina*, BP2 = *A. thalassinus*, P1 = P. erumeri, P2 = *C. macrolepidotus*

Similar patterns of higher trace accumulation were observed for Zn. Gills of a pelagic fish analyzed in the study exhibited the highest accumulation and almost double than the rest of the analyzed benthic and bentho-pelagic samples (Fig. 3a). Zn accumulation in benthic and bentho-pelagic species were higher than pelagic fishes for livers and muscles which live more on the upper part of water column (Fig. 3b and c). The range of Zn contents (5-90 μg g^{-1}) overlapped with Zn in sea sediments in the vicinity of present study area (47.4-231.8 μg g^{-1}, unpublished Bintulu Port

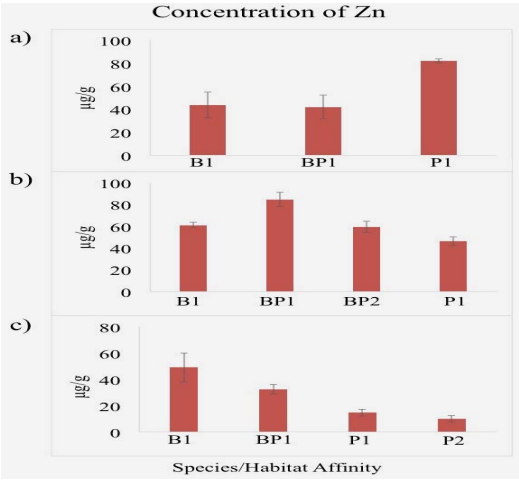

Fig. 3: Concentration of zinc, Zn in benthic (B), Bentho-Pelagic (BP) and pelagic fishes based on different organs: a) gills, b) liver, c) muscles. B1 = *C. macrolepidotus*, BP1 = *E. tauvina*, BP2 = *A. thalassinus*, P1 = *P. erumeri*, P2 = *C. macrolepidotus*

environmental monitoring program). High exposure of Zn (>250 mg L^{-1}) can expose fishes to physiological disturbance such as reduced oxygen consumption and enzyme that controls their stress (McRae *et al*., 2016).

In terms of metal accumulation in fish organs, the present study found the concentrations of metals in more internal tissues were lower than those more external ones, consistent with previous findings in benthic organisms (Bustamante *et al*., 2003; Weber *et al*., 2013). This is because each organ undergoes unique mechanisms for sequestering metals. Additionally, induction of metallothioneins in liver is the main form of storage and detoxification of metals in fish (Weber *et al*., 2013).

Although it was not accounted in the present study, fish development can be retarded with the presence of high concentration of metals in water, especially the early life stages such as larval and juvenile (Canli and Atli, 2003). Accumulation of metals will consequently implicate the ecology as well as food resources.

CONCLUSION

Metal contents in the fishes analyzed in this study were below the safe guideline (65 µg g^{-1} for Cu and 200 µg g^{-1} for Zn, Interim Sediment Quality Guideline). A metal accumulation monitoring program is vital as the accumulation run at different rates before their effects can become detectable (Vystavna *et al*., 2013). Besides the coastal development activities, global climate change is also responsible for where alteration of the sea currents increases the chances of anthropogenic materials and consequently add additional threats to the overall ecosystem. Therefore, the need for monitoring trace metal in Malaysia especially the coastal areas, is important considering their potential adverse effects on the local and regional biota. If fishes are involved in the environmental monitoring or assessment programs, they must not be species-specific. But rather focusing on fishes from a broad range of habitat affinities. Habitat affinities in this study indicated different ability of fishes to ingest metals from the environment as shown by the metal concentration in different types of organs/tissues.

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