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Effect of Inorganic Pollutants on Body Structures of the Pomfret, *Pampus argenteus* (Stromatidae: Perciformes)

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Abstract: The intensification of environmental stress greatly contributes to the significant rise of trace metal pollution in Kuwaiti water resources making threats on aquatic life. This instigated us to determine the trace metals (Pb, Ni, V, Cd and As), on the commercially relished Pomfret, *Pampus argenteus* and also in relation to nutrient levels in seawater. The sequence of Pb>Cd>Ni>V>As levels was observed in both Kuwait Bay and coastal waters sites. Nutrient levels were observed in the sequence of Silicate>Nitrate>Phosphate in both the sites. Observations showed high trace metals and nutrient levels in Kuwait Bay than in the coastal waters. Among the three body parts, observation showed high trace metal concentrations in otolith followed by dorsal fin and eye lens. This study showed significant correlation between fish length (15-30 cm) and the body parts. The overall bioaccumulation factor (BAF) in their body parts was in the sequence of As>V>Ni>Cd>Pb and high in Kuwait Bay than in the coastal waters. High trace metals bioaccumulation was observed in dorsal fins followed by otolith and eye lens. These results validate hard parts of *P. argenteus* as an indicator of trace metal pollution other than its use in the aging studies.

Key words: Trace metals, hard parts, bioaccumulation, Pomfret.

Introduction

Investigations on the marine ecosystems revealed trace metals validate significant role in marine pollution over the recent years. Trace metals occur in aquatic ecosystems, but deposits of anthropogenic origin increase their levels creating environmental problems in coastal zones and rivers (Saad et al., 1981; Lemus and Chung, 1999; Bu-Olayan et al., 2001a). In the marine ecosystem, contamination is mainly from untreated industrial and sewage discharges (Mance, 1987; Abel and Axiak, 1991). Similar sources, especially from the power, thermal, desalination and water treatment plants and leakage from oil wells that contained high metal levels were observed in Kuwait marine environment (Bu-Olayan and Thomas, 2001b; Al-Sarawi et al., 2002). Most metals are essential for the physiological function processes in fish (Khalaf et al., 1985). Above tolerable limits, these metals cause deaths while sub-lethal concentrations may lead to behavioral, biochemical and histological changes in fish as well as alter primary production that indirectly changes the dynamics of seawater (Heath, 1987; Al-Ghadban et al., 1998; Al-Yamani et al., 2006). The main routes of trace metals accumulation by fish are through the gills, skin and food (Chan, 1995; Wong et al., 1999). Body structures such as otolith, eye lens and dorsal spines of fish that determined their age, showed significant accumulation of trace metals in the marine environment (Jaffar et al., 1995; Dove and Kingsford, 1998; Conides and Al-Hasan, 2000; Burkholder and Edwards, 2001; Gillanders, 2001; Raza et al., 2003; Forrester, 2005). No publications showed evidences on the synergistic effect of trace metals bioaccumulation and nutrients to the body structure that relates growth of *P. argenteus* collected from Kuwaiti waters, and hence the study.

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Materials and Methods

Commercially important and widely relished Pomfret, *P. argenteus*, were collected at random from Kuwait marine sites off the Arabian Gulf (Figure 1). For analytical purpose, fish samples from Sites I-IV and Sites V-VII were pooled into two main categories namely Kuwait Bay and Kuwait Coastal water samples respectively, as the samples were subjected to similar nutrient, hydrological variables and ecosystem. Fish replicates (10 nos.), each weighing 700 ±10 g and total length ranging 15-30 cm were segregated and reared in seawater collected from Kuwait's coast (Sites: I-VII). Seawater parameters like temperature, dissolved oxygen, salinity and pH were measured using a multiple sensor (Hach Incorp. Ltd., US).

One-litre seawater added with 25 ml ammonium-pyrrolidine-dithiocarbonate (APDC 2% v/v), 10 mL HCl (0.5 M) and 35 mL methyl-isobutyl-ketone (MIBK 99.5%, Sigma-Aldrich) was shaken for two minutes in a separatory funnel and left undisturbed for 15-20 minutes. Obtained were two separate phases: upper and lower solutions (A and B). To one litre of fresh seawater, was added the upper solution (A) with APDC, HCl and MIBK and the process repeated. In another separatory funnel, the lower solution (B) was eluted with the above chemicals. Analytik Jena System (HS-55) measured the trace metals concentration collected from both the upper solutions (A and B) in 50 ml volumetric flask and the lower solutions discarded. Certified Reference Material (BCR-403) with recoveries ranging 96-97%, standard metals solution (1000 ppm-HPLC Grade, Sigma-Aldrich) and blanks were used to maintain quality assurance adopting the earlier methodology (APHA, 1998).

Cleaned and processed body structures of Pomfret, namely the first dorsal fin, eye lens and otoliths, were removed as described by Secor et al. (1991). They were dried in an oven (GallanKamp II) at 60°C overnight until constant. Dried hard parts (2 g) were predigested in Aristar grade 3% HNO_3 (v/v) and 1% HCl (v/v) overnight, in polystyrene sterile centrifuge tubes. Samples diluted in de-ionized water (50 ml) and digested in an automatic microwave digester (Spectroprep CEM) were measured in the Analytik Jena System (HS-55) to determine the metals bioaccumulation. Quality assurance employing replicates, ICP-standard trace metals, blanks and standard reference material and bovine liver (SRM 1577b) from National Institute Standard Technology (NIST), assessed the precision of the instrument. Recoveries (ranging 96%-98.78%) were in agreement with certified values. Statistically (SPSS-15), Pearson's correlation coefficient was used to correlate significance between the different body structures to that of the total body length and weight in relation to their trace metals concentrations.

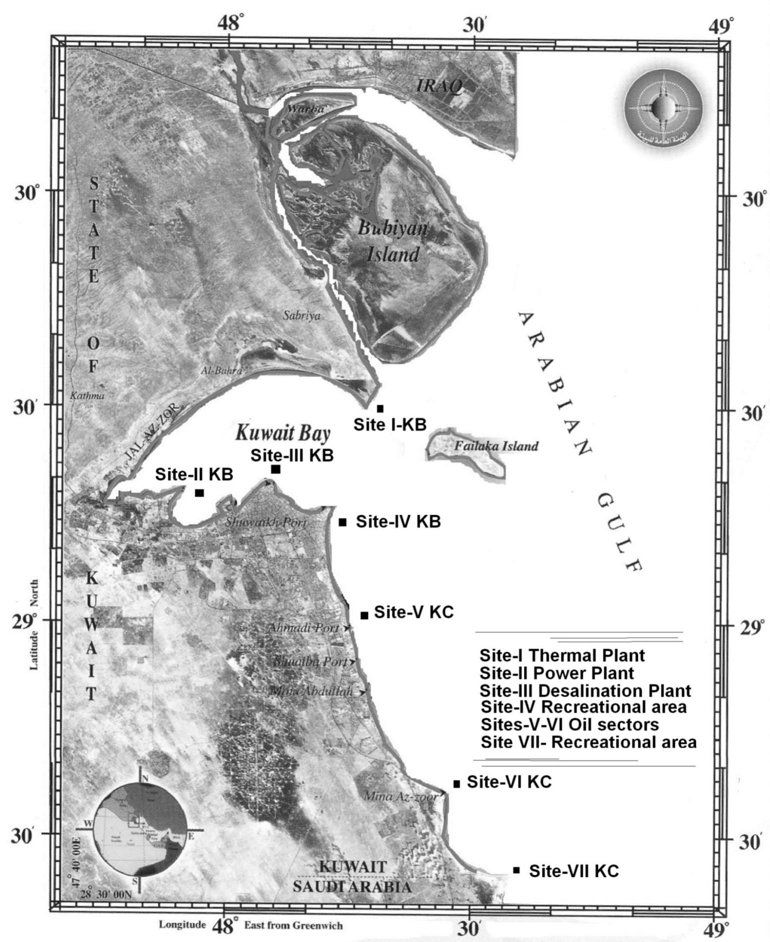

Figure 1: Sampling sites in Kuwait Bay (KB) and Coastal waters (KC).

Trace metals bioaccumulation factor (BAF) in *P. argenteus* were determined by using the formula described by ASTM (1990) as given below:

BAF = Concentration of metals in fish tissue (μg kg^{-1}) (*b*) /Concentration of metals in seawater (μg L^{-1}) (*a*).

wherein, BAF is the ratio of metals concentration in the fish tissue (*b*) (in the present study, the hard parts such as dorsal fins, eye lens and otoliths were substituted for tissues) to its concentration in the seawater (*a*). Trace metals bioaccumulation was measured using Analytik Jena System (HS-55).

Following the modified methods (APHA, 1998; Kramer et al., 1972; Koroleff, 1983), nutrients such as phosphate and silicate in seawater samples and pre-treated for chloride interferences were estimated by spectroscopy method with absorbencies measured at 880 and 810 nm, respectively. For nitrate, the potential measurement of NO_3-N concentration standards and samples were recorded against a semi-logarithmic graph with a slope of +57 ± 3 mV/decade at 25°C and concentrations measured from the calibration curve (Synott et al., 1984).

Results and Discussion

The present study chose trace metals such as Pb, Ni, V, Cd and As, since they were: (a) significant to marine pollution, (b) abundant in Kuwait seawater than other metals and (c) within the detectable limits of Analytik Jena System (HS-55) (Al-Sarawi et al., 2002).

Comparatively, trace metals levels were high in Kuwait Bay than in the Kuwait coastal waters. This could be attributed to their concentration in the Bay due to low mixing of water, high evaporation rate and shallowness of the Bay when compared to the thorough mixing of water and dilution in the open sea through Shatt-al-Arab River from the north of Kuwait (Al-Ghadban et al., 1998). Observations revealed high Pb levels among the metals, irrespective of sites (Table 1). This may be due to multidimensional marine pollution sources. Trace metals were high in all the three body structures (first dorsal fin, eye lens and otolith) of fish caught from Kuwait Bay than in Kuwait coastal waters (Table 1). Metal-wise analysis in Pomfret dorsal fin and eye lens revealed high arsenic (As) levels than other metals irrespective of seasons. This attributes to (1) oil spills in the Bay, (2) extensive land use, (3) effect of pesticides, (4) rapid industrialization and (5) accidental spills of arsenic compounds in the sea and supports evidences to the earlier studies (Bu-Olayan and Thomas, 2001; Yamani et al., 2006). Contrary to the above, arsenic was moderately low in otolith when compared to the other metals. This attributes to its low assimilation and accumulation of arsenic in internal part like otolith than external tissues or other hard parts that are constantly in direct contact with the pollutants in the sea. Further, statistical analysis showed that there exists definite correlation significance (*r*) between seawater, body structures and seasonal analysis (Table 1).

Observation showed a proportional increase in the length and weight of first dorsal fin and otolith respectively to that of the increasing total length of the Pomfret fish. However, in the case of eye lens diameter, the increase was marginal to that of the total length of the fish (Table 2). This supported the earlier views of Conides and Hasan (2000). Statistical analysis showed significant correlation between the total fish length to that of the hard parts length and weight as well as to trace metal levels (Table 3).

Table 1: Mean trace metals levels in seawater and body structures of *P. argenteus* and nutrients from Kuwait Bay and Coastal waters

Metals	*Kuwait Bay*	*(r)*	*Kuwait Coastal waters*	*(r)*
(a) Seawater (μg L^{-1})				
Pb	1.99 ±0.19	(*0.92*)	1.64 ±0.10	(*0.98*)
Ni	0.23 ±0.02	(*0.95*)	0.12 ±0.01	(*0.88*)
V	0.12 ±0.01	(*0.97*)	0.02 ±0.001	(*0.43*)
Cd	0.24 ±0.03	(*0.90*)	0.15 ±0.01	(*0.88*)
As	0.05 ±0.01	(0.89)	0.02 ±0.001	(*0.43*)
(b) First Dorsal fin (μg g^{-1})				
Pb	0.20 ±0.06	(*0.99*)	0.18 ±0.05	(*0.88*)
Ni	0.94 ±0.18	(*0.97*)	0.82 ±0.17	(*0.85*)
V	0.53 ±0.12	(*0.91*)	0.43 ±0.11	(*0.82*)
Cd	0.55 ±0.15	(*0.93*)	0.54 ±0.13	(*0.87*)
As	0.65 ±0.16	(*0.86*)	0.57 ±0.14	(*0.83*)
(c) Eye lens (μg g^{-1})				
Pb	0.19 ±0.02	(*0.93*)	0.17 ±0.03	(*0.97*)
Ni	0.42 ±0.10	(*0.94*)	0.36 ±0.10	(*0.85*)
V	0.18 ±0.05	(*0.91*)	0.16 ±0.02	(*0.90*)
Cd	0.13 ±0.01	(*0.88*)	0.10 ±0.01	(*0.45*)
As	0.49 ±0.15	(*0.87*)	0.39 ±0.12	(*0.84*)
(d) Otolith (μg g^{-1})				
Pb	0.86±0.17	(*0.94*)	0.21 ±0.01	(*0.98*)
Ni	1.54±0.20	(*0.90*)	0.61 ±0.15	(*0.97*)
V	0.83±0.16	(*0.91*)	0.19 ±0.09	(*0.88*)
Cd	1.49±0.19	(*0.97*)	0.48 ±0.12	(*0.89*)
As	0.15±0.12	(*0.93*)	0.14 ±0.01	(*0.92*)
(e) Nutrients in seawater (μg L^{-1})				
Phosphate	0.016 ±0.001	(*0.32*)	0.012 ±0.001	(*0.45*)
Silicate	0.643 ±0.14	(*0.93*)	0.617 ±0.03	(*0.96*)
Nitrate	0.250 ±0.01	(*0.92*)	0.207 ±0.01	(*0.91*)

r: correlation coefficient, ± : Standard Error

Chan (1995) opined that trace metal levels in fish tissue increase proportionally to their levels in water. This phenomenon was supportive to the fish in the present study. High trace metals bioaccumulation was observed in *P. argenteus* collected from Kuwait Bay than from the Kuwait coastal waters. Bioaccumulation of trace metals were in the sequence of As>V>Ni>Cd>Pb (Table 4).

Table 2: Relationship between Pomfret fish length to body structure and trace metal levels in Kuwait Bay and Coastal water samples

No. Body parts/ Metals level	Fish Length (cm) 15-20	20-25	25-30
1. Dorsal Fin (cm)	1.914 ±0.52	3.080 ±0.49	3.630 ±0.59
2. Eye diameter (cm)	0.790 ±0.28	0.795 ±0.32	0.798 ±0.41
3. Otolith weight (g)	0.010±0.001	0.022 ±0.02	0.030 ±0.03
4. KB-T-DF (μg/g)	0.262 ±0.11	0.510 ±0.20	0.826 ±0.31
5. KB-T-EY (μg/g)	0.137 ±0.07	0.188 ±0.10	0.423 ±0.19
6. KB-T-OT (μg/g)	0.327 ±0.14	1.150 ±0.39	2.110 ±0.47
7. KC-T-DF (μg/g)	0.153 ±0.07	0.417 ±0.18	0.680 ±0.22
8. KC-T-EY (μg/g)	0.120 ±0.05	0.170 ±0.09	0.336 ±0.16
9. KC-T-OT (μg/g)	0.097 ±0.27	0.320 ±0.05	0.736 ±0.13

KB: Kuwait Bay; KC: Kuwait coastal waters; T: Trace metals; DF: Dorsal fins; EY: Eye; OT: Otolith; ± : Standard error.

Table 3: Correlation coefficient between fish length to body structure and trace metals in Pomfret caught in Kuwait Bay and Coastal waters

No.	1	2	3	4	5	6	7	8	9	10
1	1.00									
2	0.97	1.00								
3	0.99	0.99	1.00							
4	0.99	0.99	0.99	1.00						
5	0.99	0.96	0.99	0.98	1.00					
6	0.93	0.84	0.91	0.89	0.95	1.00				
7	0.99	0.96	0.99	0.98	0.99	0.95	1.00			
8	0.99	0.97	0.99	0.99	0.99	0.93	0.99	1.00		
9	0.95	0.87	0.93	0.91	0.97	0.99	0.96	0.95	1.00	
10	0.98	0.92	0.97	0.95	0.99	0.98	0.99	0.98	0.99	1.00

1: Fish length (cm); 2: Dorsal fin length (cm); 3: Eye lens diameter (cm); 4: Otolith weight (g); 5-10: Trace metals (μg/g) in dorsal fin, eye lens and otolith (5-7 in Kuwait Bay and 8-10 in Kuwait Coastal waters).

Significant variation in Ni and V was observed in BAF. This supports Wong et al. (1999) views of: (a) effective accumulation of Ni in the tissue and (b) the antagonistic action of Ni and V with organic and inorganic constituents that eliminated from the fish tissues. Among the five metals, mean BAF was least in Pb (Table 4). This attributes to (a) complex formation of Pb in the liver with protein constituents and later eliminated, (b) Pb chelating with other trace metals and (c) assimilation of Pb in the body tissues (Saad et al., 1981). Heath (1987) described varied pattern of metals bioaccumulation in different fish tissues. Observations revealed a high trace metals accumulation in the first dorsal fin followed by otolith and eye lens. Trace metals such as As and V revealed high BAF in dorsal fin. This may be due to the surface contact of such metals by the dorsal fin from the surrounding environment to a certain extent as well as metals, complex formation with mucus on the skin when compared to eye and otolith. Metals such as As and V were comparatively low in otolith than Pb, Ni and Cd because the former metals were not found to assimilate or enhance the accumulation process rapidly in the hard parts. Further, As and V showed no major interaction with other organic and inorganic pollutants to produce synergistic effects in comparison to Pb, Ni and Cd. The above features supported evidences to the earlier studies (Burkholder and Edwards, 2001; Gillanders, 2001; Raza et al., 2003; Forrester, 2005).

Table 4: Trace metals bioaccumulation in *P. argenteus* body parts from Kuwaiti waters

Metals (μgL^{-1})	BAF: C= (b)/(a) Dorsal fins	Eyes	Otolith
I. Kuwait Bay			
Pb	0.10 ±0.01	0.09 ±0.01	0.43 ±0.08
Ni	4.08 ±0.41	1.82 ±0.26	6.69 ±0.98
V	4.41 ±0.43	1.50 ±0.25	6.91 ±1.01
Cd	2.29 ±0.33	0.54 ±0.11	6.20 ±0.75
As	13.0 ±1.56	9.80 ±1.22	3.0 ±0.37
II. Kuwait coastal waters			
Pb	0.10 ±0.01	0.10 ±0.01	0.12 ±0.05
Ni	6.83 ±1.0	3.0 ±0.38	5.08 ±0.68
V	21.5 ±2.10	8.0 ±1.30	9.50 ±1.35
Cd	3.60 ±0.49	0.66 ±0.12	3.20 ±0.42
As	28.6 ±2.12	19.5 ±1.97	7.0 ±1.03

BAF: Bioaccumulation factor, C= Concentration, *(b)*: concentration in fish body structures (i.e. b, c and d from Table 1), *(a)*: Concentration in seawater, *(b)/(a)*: Calculated BAF from Table 1, ±: Standard error.

Nutrients were in the sequence of silicate>nitrate> phosphate irrespective of the two sites. Mean nutrient levels were higher in Kuwait Bay than in coastal waters (Table 1). This may be attributed to: (1) the deposition of nutrients in the Bay by the action of water current, (2) inputs of nutrient from different sources such as surface run off, atmospheric deposition, shoreline erosion, discharges from domestic sewages and power plants, oil spills, and (3) thermocline stratification that enhances primary productivity and algal enrichment. Further, the comparative increase of nutrient levels in Kuwait Bay than Kuwait coastal water sites indicates their synergism to trace metals levels. These evidences support the earlier studies (Bu-Olayan et al., 2001; Al Yamani et al., 2006).

The above findings deduce that body structure from the Pomfret fish caught from Kuwait Bay have the capability to bio-accumulate high trace metals levels than body structures of fish collected from the coastal waters as well as metals levels described in the present study can elevate in relation to nutrient levels. Therefore, future ecological investigations recommend the use of Pomfret body structures as a tool to determine trace metals levels besides its utility in age and growth estimation.

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