Int. J. Environ. Res., 2(3): 249-254, Summer 2008
ISSN: 1735-6865

Trace Metals Toxicity to the Body Structures of Mullet Liza Klunzingeri (Mugilidae: Perciformes)

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Received 2 Jan 2008; Revised 12 Feb. 2008; Accepted 25 Feb. 2008

ABSTRACT: Rapid industrialization, installation of mega-desalination plants and suspected trace metals discharges in the Kuwait marine environment instigated us to determine the trace metals toxicity on the body structures (first dorsal fin, otolith and scales) of the commercially relished mullet fish, *Liza klunzingeri*. 96 h toxicity test with Fe, Cd, Ni, As, showed the body structure of *L. klunzingeri* to attain LOEC (lowest observed effective concentration) at test concentrations of 10µg/L, 4.0µgL, 8.0µg/L and 2.0µg/L, respectively. Observation revealed high metal concentrations in scales followed by first dorsal fin and otolith irrespective of the sampling sites. Mean bioaccumulation factor (BAF) in body structures was high in the sequence of Ni> Fe > As >Cd. Trace metals bioaccumulation was high in samples reared in Kuwait Bay than in the Kuwait coastal waters. These findings supported evidences to body structures of *L. klunzingeri* as an indicator to trace metal pollution besides its use for species aging.

Key words: Trace metal toxicity, Bioaccumulation, Fish, Marine pollution, Kuwait

INTRODUCTION

Metals and slowly degrading chemicals threaten coastal waters. Toxic materials settle into marine sediments, where they are accumulated as hazards to organisms that live and feed on the bottom mud. Eventually, long-lasting chemicals may enter the food web and contaminate the fish and shellfish we consume (Ocean Planet, 1995). Recent investigations are in conscious of the need to ensure that the processes of urban development be applied to preserve marine resources and coastal amenities and that such development should not lead to deterioration of the marine environment (Ocean Planet, 1995). Pollution sources from domestic sewage, wastewater treatment plants, thermal, power and desalination plants, oil field fires, industrial effluent and hospital waste discharges, anti-scalants, anti-foulants, and suspected contamination of trace metals generated a highly stressed marine ecosystem in Kuwait (Khalaf *et al.*, 1985; Taylor *et al.*, 1985; Wayne and Ming, 1998; Al-Sarawi *et al.*, 2002). Most metals are essential for the physiological function processes in fish such as oxidative phosphorylation, gene regulation and free-radical homeostasis (Khalaf *et al.*, 1985). Above tolerable limits, these metals cause death, while sub-lethal concentrations may lead to behavioral, biochemical and histological changes in fish (Heath, 1987). The main routes of metal accumulation in fish are through the gills, skin and food (Hein *et al.*, 1993). Trace metals, such as Cu, Ni, Fe, and Cd are accumulated over time in higher concentrations in fish liver, gills and muscles (Taylor *et al.*, 1985). Besides the direct impact of heavy metals in fish, the synergistic action of some hydrological variables and nutrients to fish was found to enhance heavy metal toxicity in fish (Bu-Olayan and Thomas, 2004; Franco *et al.*, 2006). The acute trace metal toxicity levels in fish exposed from 24h to 96 h was statistically tested using Probit program (USEPA, 1993) by various investigators (Abel and Axiak, 1991; APHA 1992; Wayne and Ming, 1998; Franco *et al.*, 2006). Heath (1987) described varied pattern of inorganic pollutant bioaccumulation in different fish tissues

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such as liver, muscles and gills. Hard body structures, such as scales, first dorsal fin and otoliths are also influenced by environmental factors and water chemistry (Wayne and Ming, 1998; Campana and Thorrold, 2001; Wells *et al.*, 2003; Forrester, 2005; Bu-Olayan and Thomas, 2005). Based on the above findings, the present study accomplished the determination of trace metals toxicity and bioaccumulation effect using body structures of *L. klunzingeri* as bio-indicators of monitor marine pollution.

MATERIALS & METHODS

Based on the earlier investigation (Bu-Olayan and Thomas, 2005), seawater samples were collected from (1) a point source where high metal pollution was observed in Kuwait Bay (KB) and (2) a non-point source where the least metal pollution was observed in Kuwait Coastal (KC) waters (Fig.1).Seawater samples at 2m depth were collected using Vandorn's water sampler. Following the methodology of APHA (1992), filtered one-liter seawater samples were added with 25 mL ammonium-pyrrolidine-dithiocarbonate (APDC 2 % v/v), 10 mL HCl (0.5 M) and 35 mL methyl-isobutyl-ketone

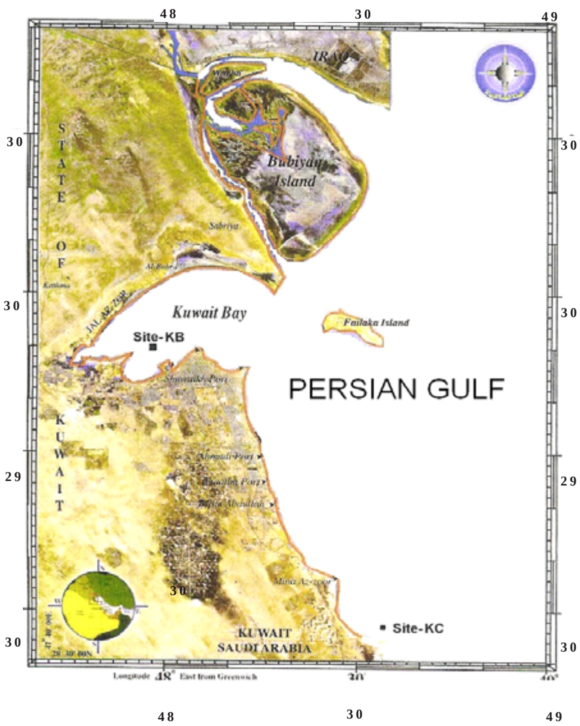

Fig.1. Kuwait Bay (KB) and Kuwait Coastal waters (KC) sampling sites

(MIBK 99.5 %), shaken for two minutes in a separator funnel and left undisturbed for 15-20 minutes.Obtained were two separate phases, upper organic and lower aqueous solutions of which the later was collected in another separator funnel. To the organic layer, one liter of fresh filtered seawater was added with APDC, HCl and MIBK (volume added as above) and the process repeated. The separator funnel containing the aqueous solution was eluted with 35mL MIBK to recover the maximum possible organic layer that had trace metals. Both the lower aqueous solutions were discarded. The eluted organic layers were collected in 50 mL Teflon sterile container and measured for trace metals in Analytical Hydra Jena (HS-55). Quality assurance followed the methods adopted earlier (APHA, 1992).

Twenty replicates of *L. klunzingeri* (each measuring 15-20cm length, 35-40g weight) were collected from Kuwait Bay (KB) and Kuwait Coastal waters (KC). They were equally distributed (10 individuals) in four aquarium tanks, each measuring100cm x 40cm x 50cm containing filtered seawater from the respective sites in the laboratory and acclimated for 24h before conducting toxicity tests. In two tanks, stock solution (1 g/L) of each trace metal (Fe, Cd, Ni, As) was added to produce the required LC_{50} test concentration ranges (4-8 µg/L, 8-10 µg/L, 2-4 µg/L,10-14µg/L) in seawater collected from the two sampling sites respectively (Fig.1). Two tanks containing seawater collected from the two sites (KB and KC) with fish tested without metals, served as control. Trace metal bioaccumulation factor (BAF) in *L. klunzingeri* was determined by using the formula (ASTM, 1990) given below: BAF= Concentration of metals in fish body structures (µg/kg) (*b*) /Concentration of metals in seawater (µg/L) (*a*).Wherein, BAF is the ratio of metals concentration in the fish body structures (1^{st} dorsal fin, otoliths and scales, respectively), (*b*), to its metals concentration in seawater from the respective Kuwait sampling sites (*a*). Fish were fed to satiety with *Artemia* nauplii (0.5 g/L). Cleaned body structures such as first dorsal fin, eye lens, otoliths and scales were removed from each fish after 180 days of exposition

(Summerfelt and Hall, 1987; Secor *et al.*, 1991). They were dried in an oven at 55°C for 72h. Samples were predigested in HCl (1%v/v) and HNO_3 (3% v/v) overnight, digested in an automatic microwave digester (Spectroprep CEM) and trace metals measured using Analytical Hydra Jena (HS-55). Quality assurance and the precision of the instrument was checked by using appropriate blanks, ICP grade metal standards and by obtaining 96-98% recovery of samples using the Certified Reference Materials (FEBS-1: otolith for trace metals from Canadian National Research Council). Trace metals bioaccumulation in each body structure of the fish at LOEC and sub-lethal concentration was assessed by calculating the BAF.Seawater variables like temperature, dissolved oxygen, salinity and pH were measured on board as well as in the laboratory using a multi-checker (Hatch Incorp, US).

RESULTS & DISCUSSION

In the present study, trace metals concentrations such as Cd, Fe, and Ni and As were chosen for the study as they were found (a) in higher concentrations in Kuwait Bay than in the Coastal waters, (b) highly detectable in the analytical instrument and (c) suspected to cause pollution in the Kuwait's marine environment (Table 1).

Table 1. Trace metals and hydrological variables in Kuwait Bay and Kuwait coastal waters

Description	Kuwait Bay	Kuwait coastal waters
Trace metals in seawater (µg/L) (a):		
Fe	2.94 ±0.09	2.66 ±0.05
Cd	1.12 ±0.08	1.07 ±0.03
Ni	0.65 ±0.03	0.58 ±0.02
As	0.58 ±0.02	0.45 ±0.02
Hydrological variables:		
Water temperature (°C)	18-32	15-29
Dissolved oxygen (mg/L)	5.2-7.0	4.2-8.0
Salinity (%)	35-46	33-41
pH	7.7-8.2	8.0-8.2
Conductivity (m S/cm)	53-59.6	53-55
Turbidity (NTU)	8-45	6-10

High metal levels in the Kuwait Bay attributes to: (1) low water current, (2) low dilution factor in the Bay than in the open sea, (3) single directional flow of seawater from north to southern regions, causing stagnation and (4) anthropogenic influences. Besides, these factors, the synergistic effect with nutrients and organic pollutants, accidental spills containing heavy metals from the neighboring countries, and rapid industrialization attributed high metal levels in this Bay (Khalaf *et al.*, 1985; Al-Sarawi *et al.*, 2002; Bu-Olayan and Thomas, 2004; Bu-Olayan and Thomas, 2005).Probit program (USEPA, 1993) on 96-h LC_{50} tests revealed *L. klunzingeri* sensitive to Ni at LOEC (1.13- 1.47 µg/L) than other metals described in this study (Table 2). This may be attributed to the effect of Ni with other inorganic and organic compounds in the body tissues of the fish when compared to other metals and supports the earlier findings (Taylor *et al.*, 1985; Wayne and Ming, 1998). Statistical analysis revealed significance in all the metals by Chi-square heterogeneity test (Table 2).

Site wise analysis showed trace metals to be highly effective at LOEC (corresponding estimated exposure at LC_5) and LC_{50} to *L. klunzingeri* tested with seawater collected from thermal, power and desalination plants in Kuwait Bay when compared to sampling sites of Kuwait Coastal waters (Table 2). This may be attributed to the fluctuating seawater: (a) salinities (35-46 ‰) (b) low mixing and stagnation of seawater, (c) high evaporation rate and shallowness of the Bay (Ocean Planet, 1995) and (d) temperatures (18°C-32°C) from the thermal plant discharges into the Bay when compared to the above variables observed in Kuwait coastal sites (Bu-Olayan and Thomas, 2004). Since, these metals were substantially low in seawater (Abel and Axiak, 1991; Hein *et al.*, 1993; Al-Sarawi *et al.*, 2002); mass mortality of this fish is expected to occur only when large quantities of these metals are accidentally discharged into the Kuwait Bay.

In the present acute toxicity and bioaccumulation tests, trace metal concentrations were found high in the body structures (scales, 1st dorsal fin and otolith) of fish reared in Kuwait Bay water than trace metal concentrations in body structures of fish reared in Kuwait Coastal water

Table 2. Acute trace metal toxicity test on *L. klunzingeri* (10 replicates) using Probit Program (USEPA 1993)

Metals	Test Conc(µg/L)	†Est. Conc. (µg/L)	LC Points	95 % C.I. limits		χ2 calculated
				lower	upper	
Kuwait Bay						
Ni	2.0	1.47	05	0.38	2.16	1.15*
	3.0	2.05	15	0.83	2.72	
	4.0	3.62	50	2.74	4.58	
Fe	4.0	3.71	05	2.32	4.43	0.24*
	5.0	4.37	15	3.15	4.99	
	6.0	5.75	50	5.05	6.46	
Cd	8.0	6.98	05	4.11	8.08	1.02*
	9.0	7.94	15	5.60	8.83	
	10.0	9.90	50	8.94	10.91	
As	10.0	8.00	05	3.64	9.95	0.58*
	12.0	9.63	15	5.66	11.30	
	14.0	13.22	50	11.23	15.03	
Kuwait coastal waters						
Ni	2.0	1.13	05	0.14	1.85	1.61*
	3.0	1.66	15	0.39	2.39	
	4.0	3.21	50	2.09	4.12	
Fe	4.0	3.08	05	1.20	3.99	0.63*
	5.0	3.84	15	2.04	4.64	
	6.0	5.58	50	4.60	6.50	
Cd	8.0	7.31	05	5.12	8.23	0.63*
	9.0	8.14	15	6.40	8.90	
	10.0	9.80	50	8.99	10.59	
As	10.0	6.95	05	2.08	9.15	0.25*
	12.0	8.54	15	3.69	10.46	
	14.0	12.13	50	9.29	13.82	

†conc.: estimated exposure concentration; LC points: (LC5: Lowest observed effective concentration, LC15: sub lethal concentration, LC50: lethal concentration at which 50% of the fish are killed); C.I: Confidence interval; χ2: Calculated Chi square for heterogeneity *: significant Chi square

samples (Table 3). This supported the earlier findings (Wells *et al.,* 2003; Franco *et al.,* 2006) who described the proportional increase of metals level in fish tissues to that of the metals level in water. Bioaccumulation of trace metals were in the sequence of Ni> Fe>As > Cd. Among the trace metals, BAF was least in Cd (Table 3). This may be attributed to: (a) complex formation of Cd in the body structure with organic and inorganic constituents and later eliminated and (b) low accumulation and assimilation of Cd in the body structure than other trace metals (Wells *et al.,* 2003). Trace metals, showed high BAF with increasing concentration levels in their body structures. This may be due to (a) increasing test concentration required to produce LOEC and LC_{50} in the laboratory, and (b) its chelating properties with other pollutants in the environment (Forrester, 2005).

Observations revealed high trace metals accumulation in the scales followed by first dorsal fin and otolith. All the four trace metals analyzed in the present study revealed high BAF in scales. This may be due to adsorption of metals on the scales from the surrounding environment to a certain extent as well as chemical complex formation with mucus on the skin when compared to trace metals BAF in dorsal fin and otolith samples and supports the earlier views (Heath, 1987; Campana and Thorrold, 2001; Wells *et al.,* 2003).

CONCLUSIONS

The above findings shows that high trace metal concentrations bio accumulate in the body structures of mullet fish reared in Kuwait Bay water than in body structures of mullet reared in the Kuwait Coastal waters. Trace metals the

Table 3. Trace metals and bioaccumulation levels in body structures of *L. klunzingeri* from control (2nd d*), Kuwait Bay and Coastal waters (180 d*)

Metals/ Expt.	Conc. (µg/L)	Metal levels in body structures (µg g^{-1})			Mean BAF
		1	2	3	
Toxicity test *(b)*					
Kuwait Bay					
Fe	Control	3.46 (1.17)	3.09 (1.05)	4.12 (1.40)	1.21
LOEC	10.0	3.95 (1.34)	3.26 (1.10)	5.63 (1.91)	1.45
SL	12.0	5.16 (1.75)	4.15 (1.41)	6.44 (2.19)	1.78
Cd	Control	0.78(0.69)	0.74(0.66)	0.96(0.85)	0.73
LOEC	4.0	0.93 (0.83)	0.80 (0.71)	1.14 (1.01)	0.85
SL	5.0	1.10 (0.98)	0.88 (0.78)	1.28 (1.14)	0.97
Ni	Control	0.86 (1.32)	0.83 (1.27)	1.04 (1.60)	1.40
LOEC	8.0	1.05 (1.61)	0.96 (1.47)	1.26 (1.93)	1.67
SL	9.0	1.28 (1.96)	1.06 (1.63)	1.40 (2.15)	1.91
As	Control	0.65 (1.12)	0.56 (0.96)	0.68 (1.17)	1.08
LOEC	2.0	0.75 (1.29)	0.59 (1.01)	0.83 (1.43)	1.24
SL	3.0	0.88 (1.51)	0.65 (1.12)	0.98 (1.68)	1.44
Kuwait Coastal waters					
Fe	Control	3.06 (1.15)	2.69 (1.01)	3.70 (1.39)	1.18
LOEC	10.0	3.41 (1.28)	2.87 (1.07)	4.90 (1.84)	1.40
SL	12.0	4.64 (1.74)	3.58 (1.34)	5.80 (2.18)	1.75
Cd	Control	0.72 (0.67)	0.69 (0.64)	0.83 (0.77)	0.69
LOEC	4.0	0.87 (0.81)	0.75 (0.70)	0.98 (0.91)	0.81
SL	5.0	1.01 (0.94)	0.82 (0.76)	1.16 (1.08)	0.93
Ni	Control	0.76 (1.31)	0.70 (1.20)	0.88 (1.51)	1.34
LOEC	8.0	0.92 (1.58)	0.83 (1.43)	1.11 (1.91)	1.64
SL	9.0	1.12 (1.93)	0.88 (1.51)	1.23 (2.12)	1.85
As	Control	0.46 (1.02)	0.42 (0.93)	0.47 (1.04)	1.00
LOEC	2.0	0.51 (1.13)	0.40 (0.88)	0.62 (1.37)	1.13
SL	3.0	0.61 (1.35)	0.47 (1.04)	0.71 (1.57)	1.32

***: Exposure period, 1: 1st Dorsal fin, 2: Otolith, 3: Scales; values in parenthesis: BAF: bioaccumulation factor = C= *(b)/ (a)* wherein *(b)* = concentration in fish body structures (1, 2 and 3), *(a)*: concentration in seawater (Table 1), LOEC: least observed effective concentration, SL: sub lethal concentration**

described in the present study can elevate in relation to seawater physiochemical parameters, synergistic effect with organic and inorganic pollutants or anthropogenic sources, extent of their mixing through water current, wind action, volume and concentrations of pollutants discharged into the Bay and coastal waters, and seasonal variations. This study recommends the use of mullet's body structures as a tool to monitor trace metal pollution besides its use in age and growth validation.

ACKNOWLEDGEMENTS

We acknowledge the Research Administration, Kuwait University for the financial support to this research project SC01/04. We thank the Faculty of Science Analytical Facilities (SAF), Kuwait University, and Mr. Talal A. Dashti, KISR, Kuwait, for his support in sample collection.

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