Trace Metals Toxicity and Bioaccumulation in Mudskipper *Periophthalmus waltoni* Koumans 1941 (Gobiidae: Perciformes)

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Abstract

The stressed ecosystem and the accumulation of inorganic pollutants in Kuwait Bay instigated us to conduct toxicity and bioaccumulation tests on trace metals (Zn, Cu, Cd and Fe) to mudskipper, *Periophthalmus waltoni*, a bio-indicator fish to marine pollution. Among the four metals, Cd had the lowest observed effective concentration (LOEC) at 1.5µg L^{-1}. Probit analysis which showed toxicity tests (96 h) on *P. waltoni* reared in filtered seawater in the laboratory showed Cd with maximum effect at median lethal concentration (LC_{50}) followed by Fe, Cu and Zn. Bioaccumulation factor (BAF) was in the sequence of Cd > Fe > Cu > Zn. For fish exposed for 60 d, bioaccumulation exhibited increasing metal levels in liver followed by muscle tissues and gills. These results will support ecologists to use *P. waltoni* as a bioindicator to metal pollution in the future.

Key words: Trace metals, mudskipper, bioaccumulation, toxicity

Introduction

Coastal pollution has been increasing significantly over the recent years and found expanding environmental problems in many developing countries. The discharges of industrial wastes have resulted in high metal concentrations in the local marine environment, especially in the coastal sediments (Saad *et al*., 1981; Mance, 1987; Ni *et al*., 2005). Al-Sarawi *et al*. (2002) reported high levels of metal discharges from power, thermal, desalination and water treatment plants and leakage from oil wells in Kuwait marine environment. Fish are exposed to such discharges containing metals in both dissolved and dietary phases. Most metals are essential for the physiological function processes in fish (Khalaf *et al*., 1985). However, above tolerable limits and environmental changes may in turn affect the metals biokinetics of the fish leading to mortality, while sub-lethal concentrations may lead to behavioral, biochemical and histological changes in fish (Heath, 1987; Wang, 2002; Amin *et al*., 2003).

Mudskipper, *Periophthalmus waltoni* is distributed in the northern region of Kuwait Bay's tidal mudflats. They are the prey for many predators and thus, it is essential to evaluate the bioaccumulation of metals toxicants in this fish. The main routes of accumulation of metals by fish are through the gills, skin and food (Hein *et al*., 1993; Ni *et al*., 2005). Trace metals such as Copper (Cu), Zinc (Zn), Cadmium (Cd) and Iron (Fe) were found to bioaccumulate in liver followed by gills and muscles in fish (Taylor *et al*., 1985; Chan, 1995; Wong *et al*., 1999; Somer, 2003; Ni *et al*., 2005). Besides the direct impact of trace metals, changes in seawater variables and biological factors enhanced the trace metals toxicity in fish. The objectives of this study were to determine the acute toxicity (LC_{50}) and bioaccumulation factor (BAF) of trace metals in *P. waltoni* since no records reported the trace metals levels in the body parts of *P. waltoni* as well as a biomonitoring tool to determine metals toxicity in Kuwait Bay's mudflats.

Materials and Methods

Mudskipper, *P. waltoni* were collected from the tidal mudflats of Kuwait Bay, off the Persian Gulf. Ten *P. waltoni*, each weighing 8±2 g and total length 10±3 cm in four 250 L glass tanks (100x50x50 cm) were acclimated for 60 d in the laboratory. Filtered seawater (0.45 µm) was used to avoid suspended particulates and micro-invertebrates. *P. waltoni* were fed (2% body weight) with *Artemia salina* (brine shrimp) nauplii (2 g d^{-1}) without trace metals (< 0.001 µg g^{-1}). Seawater (5%) was exchanged every three days. Seawater variables like temperature (22±3°C), dissolved oxygen (8.1 mg L^{-1}), salinity (38‰) and pH (8.2) were maintained using multiple regulators in the laboratory respectively. A backwash polystyrene filter (40 µm) removed the trace debris from the tank.

Seawater Analysis

One-liter 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 %) was shaken for two minutes in a separatory funnel and left undisturbed for 15-20 minutes. Obtained were upper organic and lower aqueous layers respectively. To the organic layer, one liter of fresh seawater was added with APDC (25ml), HCl (10 ml) and MIBK (20 ml) and the process repeated. In another separatory funnel, the aqueous layer was eluted with the APDC (5 ml) and MIBK (20 ml) for further recovery of organic layer containing the trace metals. The organic layers were measured for trace metals concentration using Analytik Jena Zeenit 650. The aqueous solution was discarded. Quality assurance followed the methodology of APHA (1998) using certified reference material (BCR-CRM 403 trace metals in seawater).

Acute Toxicity Test

Ten *P. waltoni* adults were acclimated for 24 h under semi-static method before conducting toxicity tests (LC_{50}) in the lab. Trace metal (Zn, Cu, Cd and Fe: SpectrosoL) stock solution (1 g L^{-1}) was added individually to the filtered seawater in the four tanks (each 250 L capacity) to produce the required LC_{50} test concentration ranging 1.5-16 µg L^{-1} respectively (Table 1). Trace metal solutions were renewed every 24 h to prevent lowering of toxicant levels (Abel and Axiak, 1991). Probit Program (USEPA, 1993) determining the 96 h LC_5 - LC_{99} was calculated.

Bioaccumulation Test

Ten *P. waltoni* were exposed to trace metals (Zn, Cu, Cd and Fe) at LOEC and LC_{15} for 60 d in each tank. Simultaneously, five fish were sacrificed on the second day to determine the initial metal levels (control). Bioaccumulation factor (BAF) by the formula given below, determined the actual metal accumulation in fish tissues from that of seawater.

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

Where in, BAF is the ratio of metals concentration (C) in the fish tissue (*b*) to its concentration in the seawater (*a*). Fish were fed to satiation. Trace metal levels were measured using ICP-MS during each water exchange. Live fish from the control and tests were sacrificed after 60 d exposure. Cleaned fish muscle, liver, and gill tissues were dried in an oven (GallanKamp II) at 60°C overnight until constant. Dried tissues (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) digested in an automatic microwave digester (Spectroprep CEM) and measured in the Analytic Jena Zeenit 650 to determine the metals bioaccumulation. Quality assurance using bovine liver (1577b) standard reference material (National Institute Standard Technology) assessed the precision of the instrument. Recoveries (ranging 96 %-98.78 %) were in agreement with certified values.

Results and Discussion

Seawater Analysis

The present study chose trace metals such as Zn, Cu, Cd and Fe since they were found within the detectable limits of ICP-MS and abundant in Kuwait seawater (Al-Sarawi *et al*., 2002).

Acute Toxicity Tests

Toxicity in *P. waltoni* revealed Cd sensitive at LOEC (1.5µg L^{-1}) than other trace metals described in this study (Table 1) and supports the earlier attributes of Cd toxicity to their dependent function of water quality and specific body parts in a variety of fish (Irwin *et al*., 1997). Probit program (USEPA, 1993) revealed 96 h LC_{50} to *P. waltoni* in the sequence of Cd (3.25 µg L^{-1}) > Fe (6.48 µg L^{-1}) > Cu (7.56 µg L^{-1}) > Zn (12.21 µg L^{-1}). Comparatively, LC_{50} values in *P. waltoni* were found higher than LC_{50} in other fish (Irwin *et al*., 1997; Somer, 2003). Statistical analysis revealed significance in all the metals by Chi-square heterogeneity test (Table 1).

Bioaccumulation Tests

Chan (1995) opined trace metal levels in fish tissue increases proportionally to their levels in water. The present study showed similar phenomenon. Bioaccumulation of trace metals in *P. waltoni* after 60 d of exposure was in the sequence of Cd>Fe>Cu>Zn (Table 2). High Cd and Fe levels in *P. waltoni* may be attributed to the accumulation of these metals brought into the surface water by natural upwelling process. This supports the earlier findings in Oman fish samples (Somer, 2003). BAF was inversely proportional to metal toxicity levels in *P. waltoni*. Observations revealed low Zn and Cu BAF in the three tissues (liver, muscles and gills) of *P. waltoni* exposed for 60 d with increasing toxicity levels (Table 2). This supports the views of Wong *et al.* (1999) and Saad *et al.* (1981): (a) effective accumulation of Cu and Zn and their interaction with organic or inorganic constituents in the tissues, (b) complex formation of Zn in the liver with protein constituents, (c) Zn chelating with other trace metals and (d) assimilation of Zn in the body tissues. Taylor *et al.* (1985) and Hein *et al.* (1993) described varied patterns of metal bioaccumulation in different fish tissues after 30 d of exposure. High trace metal accumulation in: (1) liver could occur during metal detoxification in fish, (2) muscle tissue may be because of absorption of residues through the intestinal walls and (3) gills could be due to metals

Table 1. Lethal concentrations of trace metals to *P. waltoni* (10 replicates) using Probit Program (USEPA 1993)

	Metals Conc.[1]	Conc.[2] (µg L^{-1})	LC Point	95% C.I. limits		χ^2 Calculated
				Lower	Upper	
Zn	8.0	7.43	05	4.12	9.02	0.269*
	10.0	8.92	15	6.06	10.31	
	12.0	12.21	50	10.68	14.13	
	14.0	17.98	90	15.15	29.64	
	16.0	24.65	99	18.84	57.99	
Fe	3.0	2.69	05	0.93	3.96	0.558*
	5.0	3.73	15	1.81	4.88	
	7.0	6.48	50	4.99	8.19	
	9.0	12.85	90	9.67	28.14	
	11.0	22.45	99	14.31	89.20	
Cd	1.5	1.50	05	0.63	2.06	1.270*
	2.5	2.00	15	1.10	2.54	
	3.5	3.25	50	2.56	3.99	
	4.5	5.92	90	4.62	10.97	
	5.5	9.67	99	6.60	28.45	
Cu	4.0	3.59	05	1.49	4.86	0.430*
	6.0	4.73	15	2.61	5.93	
	8.0	7.56	50	6.08	9.22	
	10.0	13.48	90	10.60	25.92	
	12.0	21.61	99	14.78	68.06	

Conc.[1]: Test concentration,
Conc.[2]: estimated exposure concentration;
C.I: Confidence interval;
χ^2: Calculated Chi square for heterogeneity;
*: Chi square significance

Table 2. Trace metal levels in seawater, control, body tissues of *P. waltoni* (60 d exposure) and in related studies

Metals/ Expt.		Conc.(µg L^{-1})	Metal Levels in Tissues (µg Kg^{-1})		
			Muscle	Gills	Liver
Seawater *(a)*					
Zn		2.94 ±0.05			
Cd		0.24 ±0.02			
Fe		0.38 ±0.03			
Cu		2.42 ±0.05			
Toxicity test *(b)*					
Zn		Control	171 (58.1)	160 (54.4)	180 (61.2)
	LOEC	7.43	182 (61.9)	173 (58.8)	188 (63.9)
	SL	8.92	189 (64.2)	182 (61.9)	193 (65.6)
Cd		Control	118 (491.6)	110 (458.3)	141 (587.5)
	LOEC	1.50	136 (566.6)	129 (537.5)	152 (633.3)
	SL	2.00	142 (591.6)	135 (562.5)	168 (700.0)
Fe		Control	125 (328.9)	123 (323.6)	163 (428.9)
	LOEC	2.69	140 (368.4)	131 (344.7)	167 (439.4)
	SL	3.73	152 (400.0)	142 (373.6)	173 (455.2)
Cu		Control	152 (62.8)	140 (57.8)	171 (70.6)
	LOEC	3.59	158 (65.2)	149 (61.5)	179 (73.9)
	SL	4.73	163 (67.3)	156 (64.4)	186 (76.8)
References (µg L^{-1})					
Zn*			840	542	916
Cd*			160	140	220
Fe**			750	---	682
Cu†			1260	243	358

(a): mean metals concentration in seawater from Kuwait Bay tidal flats;
(b): tissue concentration; Control: fish reared in seawater without metals addition in the laboratory;
LOEC: Lowest observed effective concentration; SL: sub lethal concentration; Values in parenthesis: Bioaccumulation factor (BAF) = (b)/(a);
* USEPA (1999)
**Wong *et al.* (1999);
†Hein *et al.* (1993).

complex formation with mucus on the gill lamellae. Thus, low metals accumulation was noted in the gills than other tissues of *P. waltoni* and supported the studies of Wong *et al.* (1999).

The above findings deduce that *P. waltoni* caught from Kuwait tidal mud flats have the capability to bioaccumulate metals and the trace metals described in the present study can give toxic effects. Therefore, toxicological investigations that use *P. waltoni* as bioindicator are recommended.

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