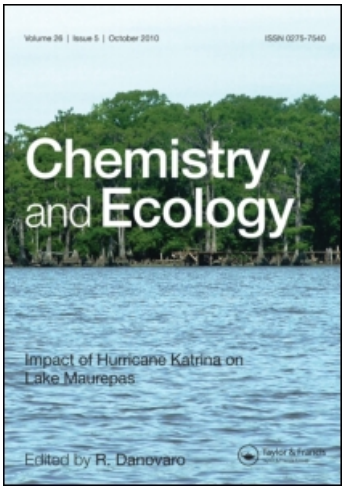

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Toxicity and bioaccumulation of heavy metals in mullet fish *Liza klunzingeri* (Mugilidae: Perciformes)

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Toxicity and bioaccumulation of heavy metals in mullet fish *Liza klunzingeri* (Mugilidae: Perciformes)

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The mullet fish, *Liza klunzingeri*, commercially important and widely relished by Kuwaiti residents, and the stressed ecosystem in Kuwait Bay instigated us to conduct toxicity and bioaccumulation tests on heavy metals (Pb, Ni, V, Cu and Fe). Among five metals, Pb had the lowest observed effect concentration (LOEC) at $1\,\mu g\,l^{-1}$. Using multi-factor Probit analysis, toxicity tests (72 h) on *L. klunzingeri* reared in filtered sea water in the laboratory showed Pb with maximum effect at median lethal concentration (LC_{50}) followed by V, Ni, Cu and Fe. Their bioaccumulation factor (BAF) was in the sequence Pb > V > Fe > Cu and Ni. For fish exposed for 30 d, bioaccumulation exhibited increasing metal levels in liver followed by gills and muscles. These results suggest the potential use of *L. klunzingeri* as a bioindicator of metal pollution in the future.

Keywords: Heavy metals; Fish; Bioaccumulation; Toxicity

1. Introduction

Heavy metals occur naturally in aquatic ecosystems, but deposits of anthropogenic origin increase their levels creating environmental problems in coastal zones and rivers [1, 2]. Environmental contamination is generally a result of untreated industrial release and sewage discharge [3]. Similar sources of contamination can be contributed by the power, thermal, desalination and water treatment industries and leakage from oil wells that contained high metal levels. These sources of contamination are observed in the Kuwaiti marine environment [4]. Most metals are essential for physiological functions in fish [5]. Above tolerable limits, these metals can be fatal to marine organisms; sub-lethal concentrations may determine behavioral, biochemical and histological changes in fish [6, 7]. Hein *et al.* [8] reported that the main routes of accumulation of metals by fish are through the gills, skin and food. Heavy metals, especially lead (Pb), vanadium (V), copper (Cu) and iron (Fe) accumulate over time and appear in high concentrations in the liver followed by the gills and muscles of fish [9–11]. Besides the direct impact of heavy metals, the synergistic action of sea water parameters and biological factors can also enhance heavy-metal toxicity in fish. No publications are available

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on the use of *L. klunzingeri* as a tool to determine metal toxicity along the Kuwait Coast. This is the first study in which the acute toxicity (LC_{50}) and bioaccumulation factor (BAF) of heavy metals have been tested in *L. klunzingeri*.

2. Materials and methods

Commercially important mullet, *L. klunzingeri*, were collected from the traditional fish traps of Kuwait Bay. Fish replicates (weighing 20 ± 3 g and 9 ± 3 cm in length) were acclimated for 30 d in 250 l glass tanks in the laboratory. Sea water was disinfected and filtered through a 0.45 μm filter to remove suspended particulates and microinvertebrates. The fish were fed (2% body weight) with *Artemia salina* (brine shrimp), nauplii ($2\,g\,d^{-1}$) and formulated fish feed ($3\,g\,d^{-1}$) without heavy metals ($<0.001\,\mu g\,g^{-1}$). The sea water (5%) was changed every 3 days, and any dead fish were removed. Antibiotics and fungicides were used before the toxicity tests. Sea water parameters such as temperature ($25 \pm 2\,^{\circ}C$), dissolved oxygen ($8.1\,mg\,l^{-1}$), salinity and pH (8.2) were maintained constant in the laboratory using multiple regulators.

2.1 *Sea water analysis*

One litre of sea water containing 25 ml of ammonium-pyrrolidine-dithiocarbonate (APDC 2% v/v), 10 ml of HCl (0.5 M) and 35 ml of methyl-isobutyl-ketone (MIBK 99.5%) was shaken for 2 min in a separator funnel and left undisturbed for 15–20 min. Thus, two separate phases were obtained: the upper and lower solutions (solutions A and B, respectively). One litre of fresh sea water was added to the upper solution (A) with APDC, HCl and MIBK, and the process was repeated. In another separator funnel, the lower solution (B) was treated with the same chemicals described above. Inductive coupled plasma-mass spectrometry (ICP-MS, Varian, Inc.) was used to measure the trace-metal concentrations collected from both upper solutions (both A and B) in a 50 ml volumetric flask, and the lower solutions were discarded. Quality assurance was tested according to the methodology of Arnold *et al.* [12].

2.2 *Acute toxicity test*

L. klunzingeri replicates were acclimated for 24 h in the laboratory for toxicity tests (LC_{50}). The stock solution ($1\,g\,l^{-1}$) of heavy metals (Pb, Ni, V, Cu and Fe: Spectrosol) was added to the filtered sea water in the tanks to produce the required LC_{50} test concentration ranging from 1 to $9\,\mu g\,l^{-1}$. The heavy-metal solution was renewed every 24 h to prevent any reduction in toxicant levels [13]. LC_{15}, LC_{50}, and LC_{99} at 71 h were calculated using the Probit Program [14] described by Finney [15]. The significance of the above tests was statistically treated using Dunnett's and Bartlett's P values [14].

2.3 *Bioaccumulation test*

L. klunzingeri (10 numbers) was exposed to LOEC and LC_{15} of heavy metals (Pb, Ni, V, Cu, and Fe) for 30 d in each tank. Simultaneously, 10 fish were sacrificed over 1–3 days and used as control to determine the initial metal levels. The bioaccumulation factor (BAF) for the actual metal accumulation in fish tissues from that of sea water was estimated as:

$$C = \frac{\text{concentration of metals in fish tissue (mg kg}^{-1})(b)}{\text{concentration of metals in sea water (mg l}^{-1})(a)}.$$

BAF is the ratio of metal concentration (C) in fish tissue (b) to its concentration in sea water (a). Fish were fed to satiety, and the unconsumed food was removed after 45 min. Heavy-metal levels were measured using ICP-MS during each water change. Fish from the control and treatments were killed after 30 d of exposition. Portions of the fish muscle, liver and gill tissues were dried in an oven (GallanKamp II) at 60 °C overnight until the weight was 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 deionized water (50 ml) and digested in an automatic microwave digester (Spectroprep CEM) were measured in the ICP-MS to determine the bioaccumulation of metals. Quality assurance using bovine liver (1577b) as standard reference material (National Institute Standard Technology) assessed the precision of the instrument. Recoveries (ranging from 96 to 98.8%) agreed with certified values.

3. Results and discussion

3.1 *Sea water analysis*

Heavy metals such as Pb, Ni, V, Cu and Fe were analysed in this study, since they were found within the detectable limits of ICP-MS and were more abundant than other metals in Kuwait sea water [4].

3.2 *Acute toxicity tests*

L. klunzingeri showed a toxicity in which Pb was more sensitive at LOEC ($1\,\mu g\,l^{-1}$) than other heavy metals investigated in this study (table 1). Using a 72-h Probit program [14], LC_{50} was found to be in the following sequence: Pb ($2.486\,\mu g\,l^{-1}$) > V ($3.33\,\mu g\,l^{-1}$) > Ni ($4.16\,\mu g\,l^{-1}$) > Cu ($4.51\,\mu g\,l^{-1}$) > Fe ($5.64\,\mu g\,l^{-1}$) in *L. klunzingeri*. A Chi-square heterogeneity test revealed significant differences in all metals (table 1). Table 2 shows the

Table 1. Lethal concentrations (LC_{15}, LC_{50} and LC_{99}) of heavy metals to *Liza klunzingeri* (10 replicates) using the Probit program [14].

Metals	Conc. ($\mu g\,l^{-1}$)	LC point	95% C.I. limits		χ^2 calculated
			Upper	Lower	
Pb	1.361	15	0.669	1.888	1.686*
	2.486	50	1.755	3.217	
	9.605	99	6.324	25.720	
V	2.037	15	0.948	2.734	1.902*
	3.333	50	2.338	4.084	
	10.069	99	7.205	24.778	
Ni	2.715	15	1.455	3.446	3.157*
	4.168	50	3.197	4.998	
	10.909	99	7.916	27.194	
Cu	2.670	15	1.230	3.496	1.586*
	4.514	50	3.419	5.594	
	14.672	99	9.623	56.610	
Fe	3.821	15	2.284	4.643	1.459*
	5.643	50	4.643	6.625	
	13.542	99	9.955	33.716	

Note: Conc.: estimated exposure concentration; C.I.: confidence interval; χ^2: calculated Chi square for heterogeneity; χ^2 table value for all the analysed heavy metals = 7.815.
*Chi square significance.

Table 2. Dunnett's test of significance between control and heavy-metal concentrations and Bartlett's equality of variance using the Probit program [14].

Metals	Concentration ($\mu g\,l^{-1}$)	n	Mean	S.D.	C.V.	Dun.	Bart.
Pb	Control	10	0.200	±0.042	210.8	0.047	0.995
	1*	10	0.080	±0.042	52.7		
	2*	10	0.270	±0.048	17.9		
	4*	10	0.670	±0.048	7.2		
	5*	10	0.870	±0.048	5.6		
	6*	10	0.970	±0.048	5.0		
V	Control	10	0.300	±0.048	161.0	0.055	0.516
	2*	10	0.170	±0.048	28.4		
	4*	10	0.470	±0.067	14.4		
	5*	10	0.770	±0.067	8.8		
	6*	10	0.880	±0.042	4.8		
	7*	10	0.980	±0.042	4.3		
Ni	Control	10	0.100	±0.031	316.2	0.037	0.645
	2*	10	0.900	±0.031	35.1		
	4*	10	0.280	±0.042	15.1		
	5*	10	0.570	±0.048	8.5		
	6*	10	0.890	±0.031	3.6		
	9*	10	0.990	±0.031	3.2		
Cu	Control	10	0.200	±0.042	210.8	0.040	0.879
	2*	10	0.900	±0.031	35.1		
	4*	10	0.290	±0.031	10.9		
	5*	10	0.480	±0.042	8.8		
	6*	10	0.780	±0.042	5.4		
	8*	10	0.880	±0.042	4.8		
Fe	Control	10	0.200	±0.042	210.8	0.049	0.473
	4*	10	0.800	±0.042	52.7		
	5*	10	0.360	±0.069	19.4		
	6*	10	0.680	±0.042	6.2		
	8*	10	0.780	±0.042	5.4		
	10*	10	0.880	±0.042	4.8		

Note: n: number of replicates; S.D.: standard deviation; C.V.: coefficient of variance; Dun.: Dunnett's test; Bart.: Bartlett's test.
*Mean > control at alpha 0.05 by Dunnett's test.

significant differences between the control and mean heavy-metal concentrations and equality of variances by Dunnett's test and Bartlett's test, respectively.

3.3 *Bioaccumulation tests*

Chan [10] reported that heavy-metal levels in fish tissues increased in proportion to their levels in the water. This phenomenon was observed in fish exposed to heavy metals (LOEC and LC_{15}) for 30 d in this study. Heavy-metal bioaccumulation in *L. klunzingeri* after 30 d was higher in Pb and followed by V, Cu, Ni and Fe (table 3). Pb and V showed the maximum BAF effect on the fish for the 30 d exposure period. A significant variation in Ni and Cu was observed in BAF. This was accorded to Wong *et al.* [11] in terms of: (a) effective accumulation of Cu in the tissue and (b) the antagonistic action of Ni with organic or inorganic constituents that were eliminated from the fish tissues. Among all five metals investigated, Fe showed the lower mean BAF (table 4), although fish tissues showed moderately high Fe levels. This can be explained by (a) the complex formation of Fe with protein constituents in the liver and later eliminated (b) the Fe chelating with other trace metals and (c) the assimilation of Fe

Table 3. Heavy-metal levels in seawater, control and body tissues of *L. klunzingeri* and in related studies.

Metals/Expt.	Concentration ($\mu g\,l^{-1}$)	Exposure (d)	Metal levels in tissues ($\mu g\,kg^{-1}$)			Mortality (%)
			Liver	Gills	Muscle	
Seawater (*a*)						
Pb	0.90 ± 0.02					
V	0.55 ± 0.02					
Ni	0.79 ± 0.03					
Cu	2.42 ± 0.05					
Fe	2.94 ± 0.05					
Toxicity test (*b*)						
Pb	Control	30	179 ± 6.24	124 ± 5.22	102 ± 6.02	–
LOEC	1.00	30	397 ± 7.61	348 ± 6.66	312 ± 6.05	–
SL	1.36	30	399 ± 7.87	352 ± 6.71	315 ± 6.09	1
V	Control	30	96 ± 5.25	88 ± 5.10	62 ± 5.03	–
LOEC	1.50	30	220 ± 7.01	216 ± 5.09	183 ± 6.12	–
SL	2.03	30	226 ± 7.90	221 ± 5.18	186 ± 6.18	1
Ni	Control	30	126 ± 6.29	110 ± 5.08	90 ± 5.11	–
LOEC	2.10	30	274 ± 7.02	230 ± 5.25	210 ± 5.62	–
SL	2.70	30	293 ± 7.06	266 ± 5.44	260 ± 5.69	1
Cu	Control	30	375 ± 7.46	359 ± 6.30	356 ± 6.28	–
LOEC	1.96	30	894 ± 8.98	778 ± 7.92	745 ± 8.26	–
SL	2.67	30	898 ± 8.93	880 ± 8.91	749 ± 8.33	1
Fe	Control	30	347 ± 7.22	320 ± 6.22	305 ± 6.07	–
LOEC	3.03	30	768 ± 6.88	697 ± 7.12	540 ± 7.22	–
SL	3.82	30	875 ± 8.21	743 ± 7.56	544 ± 7.29	–
References						
Pb*	1.50	15	450 ± 7.28	–	–	
V*	2.20	15	278 ± 2.44	–	156 ± 6.18	
Ni*	3.50	15	1180 ± 12.5	–	–	–
Cu**	3.00	30	1260 ± 13.8	243 ± 5.48	358 ± 6.31	–
Fe†	3.10	30	750 ± 7.72	–	682 ± 7.95	

Note: (*a*): mean metal concentration in seawater from Kuwait Bay sites; (*b*): tissue concentration; Control: fish reared in seawater without metals addition in the laboratory; LOEC: Lowest observed effective concentration; SL: sublethal concentration. Values in italics: standard deviation.
*Taylor *et al.* [9].
†Wong *et al.* [11].
‡Hein *et al.* [8].

in the body tissues [1]. Hein *et al.* [8] and Taylor *et al.* [9] described a different pattern of metal bioaccumulation in different fish tissues after 30 d of exposure and higher heavy-metal accumulations were reported in the liver followed by gills and muscle tissues.

The high degree of heavy-metal accumulation in liver could occur during metal detoxification in fish; accumulation in gills could be due to metal complex formation with mucus on the gill lamellae; and accumulation in muscle tissue may be due to the absorption of residues through the intestinal walls. Thus, a low degree of metal accumulation was observed in the muscles than in other tissues of *L. klunzingeri*, according to Wong *et al.* [11].

4. Conclusions

Our results suggest that fish (in particular *L. klunzingeri*) caught from the Kuwait coastal area can accumulate heavy metals, which can have toxic effects, and so future ecotoxicological investigations to better characterize the use of *L. klunzingeri* as a bioindicator organism are recommended.

Table 4. Heavy-metal bioaccumulation in *L. klunzingeri* exposed for 30 d.

Metals	Test concentration ($\mu g\,l^{-1}$)	BAF $C = \frac{\text{concentration in fish tissue } (b)}{\text{concentration in seawater } (a)}$ Liver	Gills	Muscle	Mean BAF
Pb	Control	198	137	113	149
LOEC	1.0	441	386	346	391
SL	1.5	443	391	350	394
V	Control	174	160	112	148
LOEC	1.5	400	392	332	374
SL	1.8	410	401	338	383
Ni	Control	159	139	113	137
LOEC	2.1	346	291	265	300
SL	2.7	370	336	329	345
Cu	Control	154	148	147	149
LOEC	1.96	369	321	307	332
SL	2.67	371	363	309	347
Fe	Control	118	108	103	109
LOEC	3.03	261	237	183	227
SL	3.82	297	252	185	244

Note: BAF: bioaccumulation factor; C: concentration; (b): concentration in tissue; (a): concentration in seawater; BAF: (b)/(a) calculated from table 3; Control: fish reared in seawater without metals addition in the laboratory; LOEC: Lowest observed effective concentration; SL: sublethal concentration.

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