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Toxicity and Accumulation of Biocides to Body Structures of Mullet Fish *Liza klunzingeri* (Mulletidae: Perciformes)

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Abstract: Stressed ecosystem due to rapid industrialization, installation of mega-desalination plants and suspected biocides (glutaraldehyde: $C_5H_8O_2$, formaldehyde: HCHO and sodium hypochlorite: NaOCl) discharges in the Kuwait marine environment, instigated us to determine their toxicity on the body structures (1st dorsal fin ray, eye lens, otolith and scales) of the commercially relished mullet fish, *Liza klunzingeri*. 96 h toxicity test on biocides, HCHO, $C_5H_8O_2$ and NaOCl showed *L. klunzingeri* to be effective at test concentrations of 0.10 μg l^{-1}, 0.07 μg l^{-1} and 0.029 μg l^{-1}, respectively to produce LOEC (lowest observed effective concentration). *L. klunzingeri* showed toxicity effect at low LC_{50} to confirm NaOCl as a toxic biocide to fish, followed by HCHO and $C_5H_8O_2$. Among the body structures, observation showed high biocides concentration in scales followed by 1st dorsal fin, otolith and eye lens irrespective of the sampling sites. The overall bioaccumulation factor (BAF) in body structures was high in the sequence of HCHO> $C_5H_8O_2$ > NaOCl. Further, biocides analysis showed high bioaccumulation in Kuwait Bay than in the coastal waters. These results characterize body structures of *L. klunzingeri* not only a tool to validate aging of the species but also to be an indicator of biocide pollution.

Key words: Body structures · bioorganic pollutant · toxicology · fish

INTRODUCTION

Marine pollution is one major concern of oil producing states surrounding the Arabian Gulf due to their dependence on this semi-enclosed highly stressed water body [1]. Pollutants that contaminate the environment are from untreated industrial and sewage discharges [2]. Thermal, power and desalination plants as well as from domestic sewage discharges into the marine environment were suspected to cause deleterious effects to the biota [3-4]. Glutaraldehyde ($C_5H_8O_2$), formaldehyde (HCHO), sodium hypochlorite (NaOCl), methanol and ethanol used in the thermal and desalination plants as biocides, disinfectant, in medical application, oil and as solvents in various industries were found discharged accidentally in the aquatic environment without any safety measures [5-6]. This effect was observed in the Kuwait marine environment. Observations showed mass mortality to sensitive fish, *L. klunzingeri* in Kuwait Bay and in coastal waters. The cause was evident to untreated effluent discharges, fluctuating seawater variables and inorganic pollutants [7]. Earlier findings [8-11] showed the influence of site specificity to organic pollutants that caused toxicity in various species of fish. Probit program [12] calculated the acute toxicity levels in fish exposed from 1d to 96 h on pollutants. This led investigators [2, 13-16] to determine Bioaccumulation Factor (BAF) of pollutants to fish. Varied pattern of inorganic pollutants accumulate in different fish tissues such as liver, muscles and gills [17]. Body structure, such as scales, spines and otoliths that determines the age and growth of fish was influenced by environmental factors and relative to water chemistry [2, 18-19]. Based on the above, the present study focused to determine the toxicity and bioaccumulation effect of biocides on the body structures of *L. klunzingeri* in Kuwaiti waters.

MATERIALS AND METHODS

Sampling sites: Seawater collected from commercially important Kuwait Bay and Kuwait coastal water sites (Sites I-VII) that represented stressed ecosystem is described below (Fig.1).

Kuwait Bay Sites: Site I (Subiyah): It is in the Northern part of Kuwait Bay. It has a large tidal flat with silt and clay profile. The thermal plant in this site is subjected to effluent discharges.

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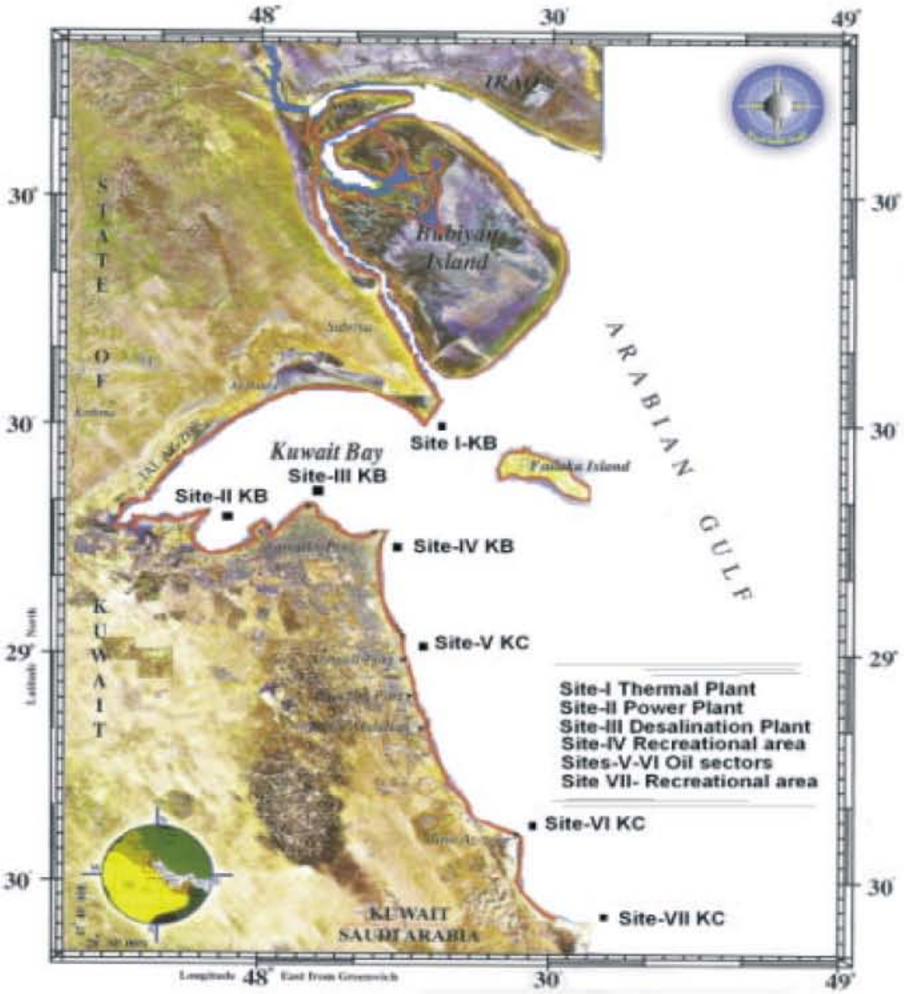

Fig. 1: Sampling sites in Kuwait Bay (KB) and Coastal waters (KC)

Site II (Doha): It was affected by major oil spills discharged during the Gulf War by the Iraqis. The seabed is shallow. This site has a power plant and various industries and thus, demarcated as an industrialized zone of Kuwait.

Site-III (Kuwait Towers): It has a desalination plant, few beach rocks and inter-tidal flats. This site is prone to domestic and industrial effluents. This location is a center for recreational activities and thus, found polluted with sewage wastes in the aquatic system.

Site-IV (Salmiya): This beach is an artificially nourished recreational area. This site prone to domestic and sewage effluents was chosen for the present study.

Kuwait Coastal waters: Site-V (Fahaheel): Beach rocks are exposed in this area. The site near the crude oil loading terminal was chosen for the study as this area is subjected to severe oil pollution.

Site-VI (Mina Al-Zour): The site is near Al-Zour power and desalination plants. The rocky surface of the intertidal zone is covered by oolithic sand. The presence of oil pollution in this area formed the criteria for the present investigation.

Site-VII (Al-Kiran): It is located on the southern region of the country and it represents the discharges of seawater from tidal creeks. The sea is shallow with fine sandy beach and observed for its recreational activities.

Formaldehyde determination in seawater: Based on the concentration ranges of formaldehyde (HCHO) employed in desalination plants, test solutions ranging 0.10-0.40 $\mu g\ L^{-1}$ was prepared and added to seawater in the aquarium tanks. After preparing the test solution, samples of 10 ml were subjected to 4-amino-3-hydrazine-5mercapto-1, 2, 4-triazole (AHMT) method [20] using a HP Spectrophotometer. Standard curves of HCHO (37% v/v) reagent was obtained to calculate their concentrations ($\mu g\ L^{-1}$) in seawater.

Glutaraldehyde determination in seawater: Stock solution of glutaraldehyde ($C_5H_8O_2$) ranging 0.07-0.10 $\mu g\ L^{-1}$ was prepared based on the biocide use in desalination and power plants. The test method employs a titrimetric chemistry using sulfuric acid and sodium sulfite with phenolphthalein indicator [14].

Sodium hypochlorite determination in seawater: Test solution of sodium hypochlorite (NaOCl: 5%) with concentrations range of 0.023-0.042 $\mu g\ L^{-1}$ were prepared based on their concentrations used in desalination and power plants. Following DPD (N-N Diethyl-P-Phenylenediamine) method, hypochlorite concentrations was determined as DPD compounds reacted with hypochlorite ions [21, 22].

Fish samples: Fish (10 replicates), each weighing 20 ±3 g and total length 9 ± 3 cm were acclimated separately in aquarium tanks in the laboratory containing filtered disinfected seawater (250 L) collected from Kuwait Bay and coastal sites (Fig. 1). Besides the filters provided inside the tanks, seawater (5 %) was exchanged every two days. Antibiotics and fungicides were used in both test samples and in control before the initiation of toxicity tests.

Acute toxicity test to *L. klunzingeri*: *L. klunzingeri* were acclimated for 24 h in the lab and tested for toxicity (LC_{50}). In each tank, stock solution (1 g/l) of each biocide was added to the filtered seawater collected from the Kuwait Bay and coastal sites (Fig. 1) to produce the required LC_{50} test concentration ranges. Biocide solution was renewed every 24 h as described to prevent lowering of toxicant levels [13]. Fish reared in the respective seawater sampling sites (Sites I-VII) without the addition of biocide, served as control. The 96 h LC_5-LC_{99} values using the Probit Program were calculated [12].

Bioaccumulation tests on *L. klunzingeri*: In each tank, *L. klunzingeri* (10 Nos.) were exposed for 180 d to biocides at lowest observed effective concentration (LOEC: LC_5) and sub-lethal concentrations (LC_{15}). The initial biocides level was determined by sacrificing ten fish on the second d. Biocides bioaccumulation factor (BAF) in *L. klunzingeri* was calculated by the formula given below [23]:

BAF = Concentration of biocides in fish body structures ($\mu g\ kg^{-1}$) (*b*) /Concentration of biocides in seawater ($\mu g\ l^{-1}$) (*a*).

Wherein, BAF is the ratio of biocide concentration in the fish body structures (*b*) (1^{st} dorsal fin, eye lens, otoliths and scales) to its simulated biocide concentration at LOEC and LC_{50} in the culture tanks on second day (*a*). Fish were fed to satiation and the unconsumed food removed after 45 minutes. Live fish from the tests were sacrificed after 180 d exposure. Cleaned body structures such as first dorsal fin, eye lens, otoliths and scales were removed [24-25]. Biocides bioaccumulation in fish at LOEC was assessed following the method described [10]. The body structures were freeze-dried in an oil-free freeze-drier and Soxhlet extracted with (4:1) *n*-hexane-dichloromethane for 18 h. An aliquot (10%) of this extract was used to measure total extractable lipid weight after evaporation to dryness. Water content was determined by weight difference before and after freeze-drying. Then, it was reduced to 2 ml of *n*-hexane and cleaned up by agitating with sulfuric acid. After vigorous stirring in a Vortex (2 min), the two layers were decanted by centrifugation and sulfuric acid removed. The *n*-hexane extract was washed three times with Milli-Q water for pH neutralization. The *n*-hexane was concentrated under vacuum to a small volume for instrumental analysis. GC analyzed the accumulation of biocides in the fish body structures.

Hydrological variables: Seawater variables like temperature, dissolved oxygen, salinity and pH were measured on board as well as in the laboratory using a multiple sensor (Hach Incorp, US).

RESULTS AND DISCUSSION

The present study chose biocides, $C_5H_8O_2$, HCHO and NaOCl since they were found discharged from commercial desalination, water treatment and power plants into the marine environment untreated along with other pollutants, sporadically [4]. In general, these biocides concentrations were found below detectable levels in seawater collected from the Kuwait Bay and coastal sites. Reasons could be attributed to: (1) their highly photo and biodegradable nature and upwelling water current [1] and (2) the effect of seawater treatment used in the industrial plants before they were let out in the Bay or open sea [3].

Table 1: Acute biocides toxicity tests on *L. klunzingeri* (10 replicates) using Probit Program (USEPA 1993)

Biocides	Test Conc. (µg l^{-1})	† Est. Conc.	LC Point	95 % C.I. limits lower	upper	$X2$ calculated
Kuwait Bay						
$C_5H_8O_2$	Control	BDL				
	0.07	0.048	05	0.025	0.058	0.212*
	0.08	0.056	15	0.035	0.064	
	0.09	0.072	50	0.061	0.079	
	0.10	0.128	99	0.104	0.255	
HCHO	Control	BDL				
	0.10	0.040	05	0.006	0.072	1.339*
	0.20	0.066	15	0.018	0.103	
	0.30	0.157	50	0.101	0.222	
	0.40	1.092	99	0.540	11.78	
NaOCl	Control	BDL				
	0.029	0.016	05	0.007	0.021	1.717*
	0.036	0.019	15	0.010	0.024	
	0.039	0.027	50	0.020	0.030	
	0.042	0.053	99	0.043	0.112	
Kuwait coastal waters						
$C_5H_8O_2$	Control	BDL				
	0.07	0.051	05	0.022	0.062	0.293*
	0.08	0.061	15	0.036	0.070	
	0.09	0.083	50	0.074	0.100	
	0.10	0.165	99	0.123	0.640	
HCHO	Control	BDL				
	0.10	0.041	05	0.002	0.082	1.001*
	0.20	0.077	15	0.011	0.124	
	0.30	0.222	50	0.145	0.424	
	0.40	2.430	99	0.848	380.2	
NaOCl	Control	BDL				
	0.029	0.022	05	0.008	0.027	1.384*
	0.036	0.027	15	0.015	0.031	
	0.039	0.038	50	0.033	0.048	
	0.042	0.081	99	0.057	0.436	

†conc.: estimated exposure concentration; C.I: Confidence interval; $X2$: Calculated Chi square for heterogeneity *: significant Chi square; BDL: Below detectable limits

Using Probit program [12], the 96-h LC_{50} tests on *L. klunzingeri* in seawater collected from Kuwait coastal sites (Fig. 1) revealed the sequence of NaOCl (0.038 µg L^{-1}) < $C_5H_8O_2$ (0.083 µg L^{-1}) < HCHO (0.222 µg L^{-1}) (Table 1). This concentration was high in Kuwait coastal water samples when compared to the 96-h LC_{50} tests on *L klunzingeri* reared in Kuwait Bay sites (0.027 µg L^{-1}, 0.072 µg L^{-1} and 0.157 µg L^{-1} in NaOCl, $C_5H_8O_2$ and HCHO, respectively). Observation revealed *L. klunzingeri* sensitive to NaOCl at LOEC (0.027-0.038 µg L^{-1}) than other biocides described in this study (Table 1). This may be attributed to the effect of chlorine

Table 2: Biocides levels in seawater and body structures of *L. klunzingeri* (180 d exposure) from Kuwait Bay and Coastal water

Biocides		Conc. µgL^{-1}	Kuwait Bay Conc^2 µg g^{-1}	Kuwait Bay BAF	Kuwait Coastal waters Conc^2. µg g^{-1}	Kuwait Coastal waters BAF
(a)1st Dorsal fin						
$C_5H_8O_2$:	LOEC	0.07	0.25	3.5	0.22	3.1
	SL	0.08	0.27	3.3	0.24	3.0
HCHO:	LOEC	0.10	2.18	21.8	1.52	15.2
	SL	0.20	2.59	12.9	1.76	8.8
NaOCl:	LOEC	0.029	0.12	4.1	0.09	3.1
	SL	0.036	0.11	3.0	0.14	3.8
(b)Eye lens (µg g^{-1})						
$C_5H_8O_2$:	LOEC	0.07	0.20	2.8	0.16	2.2
	SL	0.08	0.23	2.8	0.21	2.6
HCHO:	LOEC	0.10	1.98	19.8	1.36	13.6
	SL	0.20	2.20	11.0	1.41	7.0
NaOCl:	LOEC	0.029	0.09	3.1	0.05	1.7
	SL	0.036	0.10	2.7	0.03	0.8
(c)Otolith (µg g^{-1})						
$C_5H_8O_2$:	LOEC	0.07	0.23	3.2	0.20	2.8
	SL	0.08	0.25	3.1	0.23	2.8
HCHO:	LOEC	0.10	2.01	20.1	1.41	14.1
	SL	0.20	2.48	12.4	2.35	11.7
NaOCl:	LOEC	0.029	0.11	3.7	0.06	2.0
	SL	0.036	0.12	3.3	0.09	2.5
(d)Scales (µg g^{-1})						
$C_5H_8O_2$:	LOEC	0.07	0.27	3.8	0.23	3.2
	SL	0.08	0.29	3.6	0.25	3.1
HCHO:	LOEC	0.10	2.26	22.6	1.83	18.3
	SL	0.20	2.61	13.0	1.98	9.9
NaOCL	LOEC	0.029	0.14	4.8	0.10	3.4
	SL	0.036	0.15	4.1	0.12	3.3

Conc.: Experimental concentration; Conc^2: concentration in body structure after 180d exposure; $C_5H_8O_2$: glutaraldehyde, HCHO: formaldehyde, NaOCl: sodium hypochlorite), BAF: bioaccumulation factor = C= *(b)/ (a)* wherein *(b)* is concentration in fish body structures (i.e. a, b,c and d), *(a)*: LOEC and SL concentration in medium respectively, LOEC: least observed effective concentration, SL: sub lethal concentration.

Table 3: Hydrological variable in Kuwait Bay and Kuwait costal waters

Description	Kuwait Bay	Kuwait coastal waters
Water temperature (°C)	18-32	15-28
Dissolved oxygen (mg L^{-1})	5.2-7.0	4.2-8.0
Salinity (‰)	35-46	33-41
pH	7.9-8.2	8.0-8.2
Conductivity (m/Scm)	53-59.6	53-55
Turbidity (NTU)	10-45	6-10

compounds in the body tissues of fish when compared with other biocides and supports the earlier findings [2, 6]. Statistical analysis revealed significance in all the biocides by Chi-square heterogeneity test (Table 1).

Site wise analysis showed biocides to be highly effective at LOEC (correspondingly estimated exposure at LC_5) and LC_{50} to *L. klunzingeri* tested with seawater collected from Kuwait Bay that stations thermal, power and desalination plants when compared to sampling sites of Kuwait Coastal waters (Table 1). 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 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 [7]. These chemicals were found photo and biodegradable in seawater [13]. Mass mortality of this fish is expected to occur when large quantities of these biocides are untreated and accidentally discharged into the Kuwait Bay.

Biocides were high in body structures (1st dorsal fin, eye lens, scales and otolith) of fish reared in Kuwait Bay sites water than in all the three body structures of fish caught from Kuwait Coastal waters (Table 2). Parts-wise analysis in *L. klunzingeri* revealed high biocides in scales followed by dorsal fin, otolith and eye lens irrespective of the sampling sites.

Environmentalists opined pollutants level in fish tissue increases proportionally to their levels in water [15, 19]. High biocides bioaccumulation was observed in *L. klunzingeri* reared in Kuwait Bay samples than from the Kuwait Coastal waters. This could be related to the significant variations in their hydrological variables (Table 3). Bioaccumulation of biocides were in the sequence of HCHO> $C_5H_8O_2$ and NaOCl. Among the three biocides, BAF was least in NaOCl (Table 2). This may be attributed to: (a) complex formation of NaOCl in the body structure with organic and inorganic constituents and later eliminated [10] and (b) low accumulation and assimilation of NaOCl in the body structure than other biocides [6, 8]. Further, high photo and biodegradable properties of pollutants that have low molecular weight showing toxicity effects at low concentrations for a short period could also add to the low bioaccumulation phenomenon in NaOCl [3]. Biocides, HCHO and $C_5H_8O_2$ 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 [5, 9, 11] and (b) its chelating properties with other pollutants in the environment [20].

Observations revealed a high biocide accumulation in the scales followed by first dorsal fin, otolith and eye lens. Biocides revealed high BAF in dorsal fin. This may be due to adsorption of biocides by the scales and dorsal fin from the surrounding environment to a certain extent as well as biocide complex formation with mucus on the skin when compared to biocides BAF in eye lens and otolith samples and supports the earlier views [17-19].

The above findings imply that body structures from the mullet fish caught from Kuwait Bay can bio-accumulate biocides than body structure of fish collected from the coastal waters. Biocides 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 biocides discharged into the Bay and coastal waters and to a certain extent of seasonal variations. Thus, future ecological investigations recommend the use of *L. klunzingeri* body structures as a tool to determine biocide level besides its use in age and growth validation.

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REFERENCES

1. Beg, M.U., M. Al-Bahloul, P.G. Jacob, K. Al-Matrouk and E. Abdel, 2001. Biomarker response in Sheim (*Acanthopagrus latus*) exposed to polycyclic aromatic hydrocarbons. Bulletin of Environment Contamination and Toxicology, 67: 210-216.
2. Wayne, G.L. and H.Y. Ming, 1998. Introduction to environmental toxicology: Impacts of chemicals upon ecological systems. CRC Press Inc./Lewis Publishers, Boca Raton, Florida, pp: 134-138.
3. Manna, A.J., 1994. Environmental impact of dual-purpose plants. Desalination Water Reuse, 4: 46-49.
4. Al-Sarawi, A., M.S. Massoud, S.R. Khader and A.H. Bu-Olayan, 2002. Recent trace metals in coastal waters of Sulaibhikhat Bay, Kuwait. Technology, 8: 27-38.

5. Laopaiboon, L., S.J. Hall and R.N. Smith, 2003. The effect of an aldehyde biocide on the performance and characteristics of laboratory-scale rotating biological contractors. Journal of Biotechnology, 102: 73-82.
6. Emmanuel, E., G. Keck, J.M. Blanchard, P. Vermande and Y. Perrodin, 2004. Toxicological effects of disinfections using sodium hypochlorite on aquatic organisms and its contribution to AOX formation in hospital wastewater. Environment International, 30: 891-900.
7. Bu-Olayan, A.H. and B.V. Thomas, 2004. Effects of trace metals, harmful algal blooms, nutrients and hydrological variables to mullet *Liza klunzingeri* in Kuwait Bay. Bioscience Biotechnology Research Asia, 2: 1-8.
8. Saad, M.A.H., A.A. Ezzat, O.A. El-Rayis and H. Hatez, 1981. Occurrence and distribution of chemical pollutants in Lake Mariut, Egypt II Heavy metals. Water Air and Soil Pollution, 16: 401-407.
9. Sijm, D.T.H.M., C.K. Flenner and A. Opperhuizen, 1991. The influence of bio-chemical species differences on acute fish toxicity of organic chemicals. Comparative Biochemistry and Physiology, 100 C (1-2): 33-35.
10. Svecivicius, G., J. Syvokiene, P. Stasiunaite and L. Mickeniene, 2005. Acute and chronic toxicity of Chlorine dioxide (ClO_2) and chlorite (ClO^{2-}) to rainbow trout (*Onycorhynchus mykiss*). Environmental Science and Pollution Research, 12(5): 302-305.
11. Bu-Olayan, A.H. and B.V. Thomas, 2006. Assessment on biocides bioaccumulation in Mullet *Liza klunzingeri* in Kuwaiti waters, off the Arabian Gulf. American Journal of Environmental Sciences, 2(3): 109-113.
12. USEPA (United States Environment Protection Authority), 1993. Statistical analysis for biological methods, http://www.epa.gov/nerleerd/stat2.htm#probit, 1.
13. Abel, P.D. and V. Axiak, 1991. Ecotoxicology and the marine environment. England, Ellis Horwood Publisher, 39-43.
14. Arnold, E.G., Lenore, S.C. and A.E. Eaton, 1992. Standard method for the examination of water and wastewater, American Public Health Association, Washington, 4-75.
15. Chien, Y.H. and Y.H. Chou, 1989. The effects of residual chlorine, temperatures and salinities on the mortality of larval milkfish *Chanos chanos*. Journal of the Fisheries Society of Taiwan, 16(4): 271-280.
16. Franco, A., S. Malavasi, M. Zucchetta, P. Franzoi and P. Torricelli, 2006. Environmental influences on fish assemblage in the Venice Lagoon, Italy. Chemistry and Ecology, 22(1): 105-118.
17. Heath, A.G., 1987. Water pollution and fish physiology. Boca Raton FL, CRC Press, 245.
18. Campana, S.E. and S.R. Thorrold, 2001. Otoliths, increments and elements: keys to a comprehensive understanding of fish populations? Canadian Journal of Fisheries and Aquatic Sciences, 58: 30-38.
19. Wells, B.K., B.E. Rieman, J.L. Clayton and D.L. Horan, 2003. Relationships between water, otoliths and scale chemistries of West slope cut throat trout from the Coeur d'Alene river, Idaho: The potential application of hard part chemistry to describe movements in freshwater. Transaction American Fisheries Society, 132: 409-424.
20. Jung, S.H., J.W. Kim, I.G. Jeon and Y.H. Lee, 2001. Formaldehyde residues in formalin-treated olive flounder (*Paralichthys olivaceus*) black rockfish (*Sebastes schlegeli*) and seawater. Aquaculture, 194: 253-262.
21. APHA (American Public Health Association), 1998. Standard methods. 20th Ed, 4500-Cl G, 4-63.
22. USEPA (United States Environment Protection Authority), 1983. Methods for chemical analysis of water and wastes method. 330.5: 3.
23. ASTM (American Society for Testing Materials), 1990. Standard practice for conducting bioconcentration tests with fishes and saltwater bivalve mollusks, Standard E 1022. Philadelphia, PA, 33.
24. Summerfelt, R.C. and G.E. Hall, 1987. Age and Growth of Fish. Ames, Iowa: Iowa University Press.
25. Secor, D.H., M. Dean and E.H. Laban, 1991. Otolith Removal and Preparation for Microstructural Examination: A Users Manual. Technical publications-1991-01, the electric power research institute, the Belle W Baruch institute for marine biology and coastal research. http://www.cbl.umces.edu/~secor/manual/intro.pdf, 84.