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ORIGINAL PAPER

Dispersion model and bioaccumulation factor validating trace metals in sea bream inhabiting wastewater drain outfalls

A. H. Bu-Olayan · B. V. Thomas

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Abstract Our study was based on the recent increase in wastewater pollution and its deleterious effects to the marine ecosystem. Using numerical simulation (DESCAR-3.2 software program), we investigated the orientation and quantification of trace metals in wastewater discharges from permanent and semi-permanent drain outfalls constructed along the Kuwait Coastline encompassing six Kuwait Governorates (GI-GVI). This study was related to trace metals toxicity and bioaccumulation effects on the commercial yellow fin Sea bream, *Acanthopagrus latus* fish using probit program and bioaccumulation factor (BAF), respectively. Observations from wastewater discharges showed high trace metals concentrations in the sequence of Zn > Cr > Cu > Fe > Ni > Pb > Hg during winter compared to summer and in GI and GIV compared to drain outfalls in the other Governorates. Seasonally, trace metals in *A. latus* revealed the sequence of Zn > Fe > Cu > Ni > Cr > Pb > Hg in GI, GII and GIV indicating the significance of toxic metals that bioaccumulated from their surrounding untreated wastewater. Toxicity test revealed *A. latus* highly sensitive to Hg even at low lethal concentrations (LC_{15}) compared to other metals. BAF in *A. latus* body parts was >1 indicating significant accumulation of trace metals from wastewater. However, BAF was <1 in Cr suggesting that *A. latus* could absorb trace metals from multiple sources over lengthy exposure period and not necessarily from wastewater containing rich Cr levels. Thus, the present findings validate *A. latus* as bioindicator to pollution more authentically by numerical simulation, toxicity and bioaccumulation tests compared to the traditional method of labeling *A. latus* as a pollution indicator.

Keywords *A. latus* · Bioaccumulation · Toxicity · Trace metals · Wastewater

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Introduction

Ocean is a basin for water borne wastes discharged from the land. Domestic and industrial effluents are the major waste that discharges from the drain outfalls into the open sea of many Coasts. In shallow seas, effluents dilute the pollutants to certain extent but fail to achieve the equilibrium level in the deep sea and form fewer dense layers than the salt water in the ocean bed. Thus, effluents were found to disperse due to the differences in the density besides natural turbulence. Wastewater is buoyant and tends to rise when they enter the marine environment from the drain outfalls (Wood et al. 1993; Oh et al. 2000). The effluent gradually ascends on a specific path from the drain outfalls or ports and mixes with seawater. Generally, marine outfall needs to be located in the least environmentally sensitive area. Hence, an open sea is always preferred over estuary or bay (Wood et al. 1993). Furthermore, the discharges from the polluted sea not only depend on the effluent volume, but also to the velocity and the direction of the water current affecting the discharge point to a great extent. Hence, the point of discharge is of great importance for defining the area. The coastal ecology is likely to be disturbed due to accumulation of waste in the estuarine region if the effluent is disposed near the shore as sufficient dilution, and dispersion is not available in such areas. Occasionally, these discharged effluents may float on the surface, if heavy or no circulation of waves and water current are experienced. The disposed effluent gets accumulated at the bottom of the seabed and becomes buoyant and rises to the surface as buoyant jet (Panigrahi and Tripathy 2011). This phenomenon was observed in Kuwait and hence, the detailed study.

The total shoreline of Kuwait is 195 km. Forty kilometers of the shoreline indents the Kuwait Bay, the most prominent geographic feature of Kuwait's, marine ecosystem. The Bay experiences low water current, poor mixing of effluents that discharge into the sea and high concentrations of pollutants. Additionally, environmental variables (temperature, salinity and pH), seasonal changes and anthropogenic inputs were found to alleviate the organic and inorganic pollutant's levels. The 'stressed ecosystem' of a Bay in Kuwait influencing the entire coastal environment to physiochemical and sedimentological variables, degradation of organic matter, enhanced primary productivity due to nutrient loadings in semi-closed eutrophication system and influence in domestic and industrial waste disposal via sewage outfalls showed similarities in marine Bays elsewhere the globe (Jean et al. 2013; Yanguang et al. 2013; Yesim 2012). When these pollutants containing trace metals reach beyond the threshold limits, they become toxic to the marine organisms and the surrounding environment. Coastal or Bay dwelling demersal commercial fishes in 'stressed' marine environment revealed high trace metals levels due to their life history characteristics and accumulation of pollutants from sediment and possible anthropogenic sources than the metals levels in pelagic, deep-sea and migratory fishes (Shenwen et al. 2012; Asante et al. 2010; Roach et al. 2008; Demirak et al. 2006; Mormede and Davies 2001). This directed our studies to determine the trace metals pollution in wastewater and also in *Acanthopagrus latus* fish observed near the wastewater drain outlet mouth. *A. latus* is a demersal schooling species, found in shallow coastal waters often feeds mainly on echinoderms, worms, crustaceans and molluscs (Fish Base 2011). Earlier studies (Jaleel et al. 1995; Nussey et al. 2000; Hosseinkhezri and Tashkhourian 2011; Fei-Dang et al. 2012) describing the characteristic features of *A. latus* to dietary habits and environmental stress could be related to the accumulation of trace metals in their body tissues. Most essential and non-essential trace metals were found to accumulate over time and specific seasons especially in liver, gills and skin of selected fish species (Hamilton and Mehrle 1986; Wong et al. 1999; Eleftheriadou and Skoullos 2003; Bu-Olayan and Thomas 2005; Tetsuro et al. 2005; Fernandes et al. 2007). However, no literature is available to quantify the possible dispersion route of trace metals concentrations from point sources especially from wastewater drain outfalls in Kuwait and in similar marine environment elsewhere other countries. The dispersion route along with the determination of trace metals in wastewater and in fish will enable environmentalists to (a) track the direction of trace metals dispersed that will serve as a potential tool to quantify the magnitude of trace metals for a given distance and their role in abiotic and biotic components (b) ascertain the cause of pollutant dispersion such as flow rate, depth, wind and wave action, water current mixing and seasonal attributes and (c) deduce future metals remediation and preventive measures to the consumption of fish caught from such 'stressed' ecosystem. In view of the above features, we focused our studies during the years 2010–2012 and utilized: (a) the DESCAR program (ver. 3.2: Canarina Environmental Software, Spain) of numerical simulation following buoyant jet model to map the dispersion of trace metals from permanent concrete and semi-permanent wastewater drain outfalls sited along the Kuwait Coastline (b) Probit Program (USEPA 1993) to determine the trace metals toxicity in *A. latus* fish and (c) bioaccumulation factor (BAF) to validate the trace metals in gills, liver and muscle tissues of *A. latus* and correlate the impact of untreated wastewater discharges in the marine ecosystem.

Materials and methods

Sampling sites

We selected four permanent concrete drain outfalls, each constructed along the Kuwait Coast encompassing the six Kuwait Governorates areas (GI-GVI) that let domestic and industrial wastewater into the marine environment during summer and winter seasons (Fig. 1a, b). We chose semi-permanent drain pipe outfalls (SP) that were installed to discharge low volume of wastewater and in locations where no permanent drains were constructed especially in the southern region of Kuwait (Fig. 1a, b).

Wastewater sample collection and analysis

Wastewater samples (3360 nos.) were collected employing Vandorn's water sampler (2 L) from three loci at the entry point of each drain outfall (4 drain outfalls × 3 loci/drain × 10 replicates × 7 locations × 2 seasons/year × 2 years). Wastewater was filtered using a 0.45-µm membrane filter and analyzed for trace metals analysis.

Following the standard methodology of APHA (1998) with slight modifications in perfection analysis, trace metals were pre-concentrated from the wastewater by adding 30 ml ammonium-pyrrolidine-dithiocarbonate (2 % v/v), 15 ml HCl and 40 ml methyl isobutyl ketone to one liter filtered wastewater in a separatory funnel, shaken for 5 min and left undisturbed for 25 min. Two separate phases, namely upper organic and lower aqueous solutions,

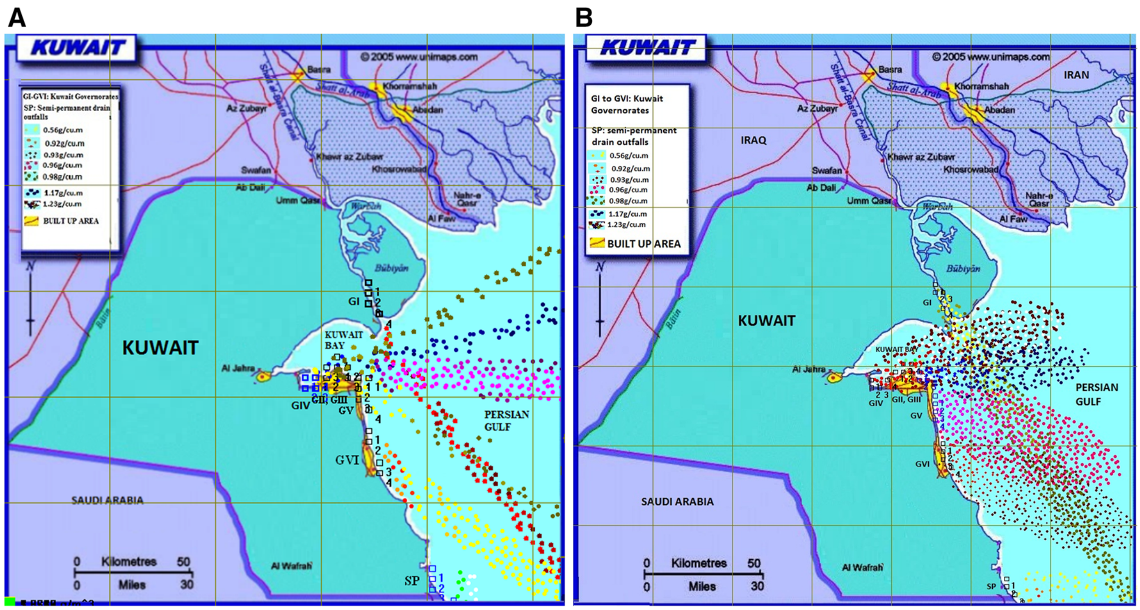

Fig. 1 **a** Dispersion of wastewater containing trace metals from permanent and semi-permanent drain outfalls of the six Kuwait Governorates during summer. **b** Dispersion of wastewater containing trace metals from permanent and semi-permanent drain outfalls of the six Kuwait Governorates during winter

were obtained. The lower solution was eluted in another separatory funnel. To the upper solution, one liter of wastewater was added and the above chemicals and the process repeated. The upper organic solutions obtained from the 2 l of wastewater were collected in a 50-ml volumetric flask, and the lower solutions discarded. Trace metals concentrations in the organic samples were measured in the ICP-MS. Quality control and assurance described by APHA (1998) were carried out by using the respective trace metals standards (ICP grade), blanks and Certified Reference Materials (CRM-403: marine water).

Numerical simulation of trace metals discharges from wastewater drain outfalls

Kuwait map indicating the sampling sites was incorporated with the corresponding coordinates present in the graphical interface of DESCAR-3.2 program. Effluent velocity, discharge flow rate, water depth at discharge location, discharge density and mean trace metals concentrations of each drain outfall constructed along the Kuwait coastline were determined using channel flow meter, water depth finder, water density meter and ICP-MS, respectively. The above data were incorporated in the graphical interface of the DESCAR-3.2 program to obtain the direction and intensity of trace metals concentrations that dispersed into the sea from the wastewater outfalls.

DESCAR-3.2 Program (Canaria, Spain) software with graphical user interface was used to create robust numerical simulations of discharges from wastewater containing trace metals into the coastal waters. The present study used buoyant jet model. This was based on time-dependent Gaussian equation that simulated the pollutant dispersion located near the coast with little depth (Jirka 2004; Zhang and Adams 1999). The pollutant concentration at a distance × (m) in the X-Axis and at a distance y (m) in the Y-axis was given by:

$$c = c_c \exp\left[-(r/b)^2\right] \tag{1}$$

wherein, 'c' is the trace metals concentration, 'r' the distance from the point to the center of line that forms the polluting plume, 'c_c' the pollutant concentration in the center of plume line and 'b' is the plume half-width. Further, the software simulates the buoyancy dominated regimes and transitions linked relationship to obtain solutions for a vertical buoyant jet in a cross flow, and buoyant jets discharged horizontally perpendicular to cross flow. The software calculates this simulation by:

$$z/L_b = c_{xy}(x/L_m)^{1/3} \tag{2}$$

wherein, 'z' is the vertical coordinate, 'L_b' is the plume–cross-flow length scale, 'C_{xy}' constant of proportionality, 'x' is the horizontal downstream coordinate in global coordinate system and 'L_m' is jet to cross-flow length scale.

Analysis of trace metals in *Acanthopagrus latus* fish

Acanthopagrus latus is commonly found inhabiting in and around the drain outfalls. They were caught using drag and trap nets. *A. latus* (10 replicates) body parts (gills, liver and muscle tissues) of uniform size (20 ± 0.5 cm) were dissected. Tissues (each 2 g) were cleaned in deionized distilled water and dried in a hot air oven (GallenKamp II) at 45 °C until dryness. Samples were individually treated in 5 % nitric acid and digested in an automated microwave digester (Ethos 1, Milestone, Italy). The digested samples were determined for trace metals in the ICP-MS (APHA 1998).

Trace metals toxicity and bioaccumulation in *Acanthopagrus latus* fish

Toxicity tests (LC_{50}) in the laboratory involved the acclimation of *A. latus* (10 replicates) for 24 h. In seven tanks, stock solution of each trace metal (Cu, Fe, Zn, Cr, Pb, Ni, Hg: ICP grade) was added separately to filtered wastewater to produce the respective LC_{50} test concentrations. Trace metals were renewed every 24 h to prevent reduction in the toxicant levels (Abel and Axiak 1991). Using Probit Program (USEPA 1993), LC_{15}, LC_{50} and LC_{99} at 72 h were calculated. During toxicity tests, the sub-lethal concentration (SL) that caused behavioral and primary sensitivity reaction of *A. latus* fish was found to coincide with trace metals concentration at LC_{15}. Thus, the present study labeled SL at LC_{15} test concentrations.

Bioaccumulation of trace metals was assessed by exposing A. *latus* for 30d to trace metals at LC_{15} (following the labeling of LC_{15} concentrations range) in each tank (Fernandes et al. 2007). Ten fish from a tank containing wastewater without the addition of trace metals were killed over 1–2 days to serve as control. The bioaccumulation factor (BAF) for the trace metals accumulation in fish tissues to that of the wastewater was calculated by:

$$\mathrm{BAF}=\frac{\mathrm{Concentration of trace metals in fish tissue}\left(\mu g g^{-1}\right)(b)}{\mathrm{Concentration of trace metals in wastewater}\left(\mu g l^{-1}\right)(a)} \quad (3)$$

wherein, ‘*b*’ is the metals concentration in each fish tissue (liver, gills and muscles) and ‘*a*’ trace metals concentration in wastewater. In the present study, *A. latus* was fed to satiety with brine shrimp larvae to facilitate complete consumption and assimilation of feed instead of unconsumed food traces in the tank, as obtained in standard methods. Every day, excreta in the tanks was removed, water exchange (5 %) was provided and the metals concentrations were replenished at the respective trace metals dosage. Fish reared in control and experimental setup tanks were killed after 30d of exposition. Fish tissues namely muscles, liver and gills were dried in an oven (GallenKamp II) at 60 °C overnight until constant weight. The dried tissues (0.2 g) were added with 5 % nitric acid and digested in the automatic microwave digester (Ethos1, Milestone Italy). Fish tissues were measured for trace metals in the ICP-MS. Quality assurance using reference material (CRM: DORM-4 fish protein, National Research Council, Canada) assessed the precision of the instrument. Recoveries of samples ranging from 97 to 98 % in agreement with the certified values were alone considered for quality trace metal concentration measurements.

Results and discussion

Our study on the 24 concrete and four semi-permanent drain outfalls (Fig. 1a, b) was undertaken in the light of the frequent wastewater discharges into the sea without adequate treatment and suspected deleterious effect on the marine ecosystem. Few semi-permanent drains that were dry and sparingly used by residents were not considered for this study.

An overall view revealed elevated trace metals concentrations in wastewater drain outfall samples during winter than in summer (Fig. 2). Water velocity causing wastewater containing trace metals to concentrate and disperse near the drain outfall, low water current in the Kuwait Bay causing stagnation of wastewater, direction of water current causing specific pattern of wastewater dispersal in the Bay and occasional untreated domestic wastewater discharges may be attributed to the high trace metals concentration during winter compared to summer. This observation was validated by DESCAR-3.2 program in revealing a specific seasonal pattern of wastewater dispersal, defined the magnitude of dispersal and total mean volume of trace metals concentrations from each permanent and semi-permanent drain outfalls encompassing the six Kuwait Coasts (Fig. 1a, b). Similar observations were found in agreement with the earlier studies (Wood et al. 1993; Oh et al. 2000; Mormede and Davies 2001; Roach et al. 2008; Asante et al. 2010). Trace metals concentrations were high in the sequence of Zn > Cr > Cu > Fe > Ni > Pb > Hg irrespective of the two seasons (Fig. 2). High Zn, Cr and Cu levels in wastewater outfall samples could be attributed to the effluents discharged from power plants, automobiles, paint industries, lubricants and domestic wastes from residential areas. Governorate-wise analysis revealed high trace metals concentrations in wastewater in the sequence of GI > GIV > GII > GIII > GVI > GV > SP irrespective of the seasons (Fig. 2). High trace metals levels from GI drain outfalls may be the result of desalination, thermal plant and accidental or untreated domestic wastewater discharges. The impact of low water current in the Bay could be related to the elevated trace metals concentration in wastewater sampled from GII and GIV. Such phenomenon of water current causing high trace metals was evidenced by earlier investigators (Yesim 2012; Yanguang et al. 2013). Despite the presence of oil industries, moderate trace metals concentrations were observed in GVI and GV because of more precautionary measures taken to discharge treated wastewater from this region. Trace metals concentrations were very low in samples collected from semi-permanent drain outfall due to: (a) their sparing usage (b) elevated flow rate causing the water to be diluted in the open sea and (c) effective treatment of wastewater before being discharged into the open sea.

Trace metals concentrations were high in *A. latus* than the metals concentrations in wastewater indicating a clear evidence for bioaccumulation in their body system. An overall analysis of the three body parts of *A. latus* revealed high trace metals concentrations in the sequence of Zn > Fe > Cu > Ni > Cr > Pb > Hg (Fig. 3). The high concentrations of Zn, Fe and Cu in *A. latus* may attribute to the accumulation of these metals from the industrial and domestic wastewater discharges. This support the earlier evidences of Demirak et al. (2006), Shenwen et al. (2012) and Jean et al. (2013). Parts-wise analysis showed variations in the mean concentrations of metals in the muscle, liver and gills of *A. latus* which may be related to the differences in ecological requisites,

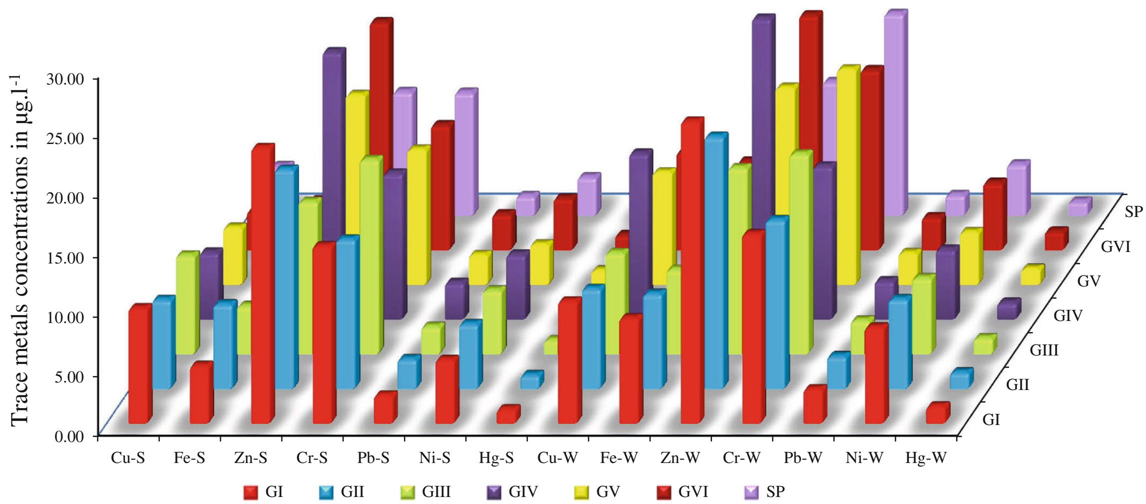

Fig. 2 Overall seasonal and Governorate-wise trace metals concentrations from wastewater drain outfalls in Kuwait GI–GVI: Drain outfalls from Kuwait Governorates, *SP* semi-permanent drains, *S* summer, *W* winter

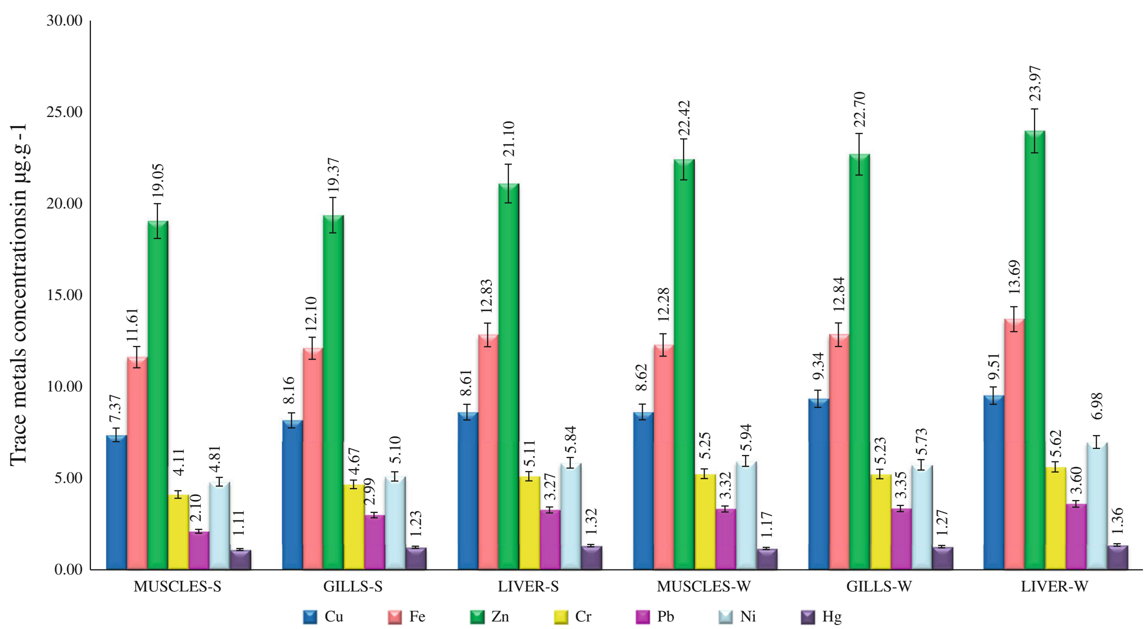

Fig. 3 Season-wise trace metals concentrations in *A. latus* body parts *S* summer, *W* winter

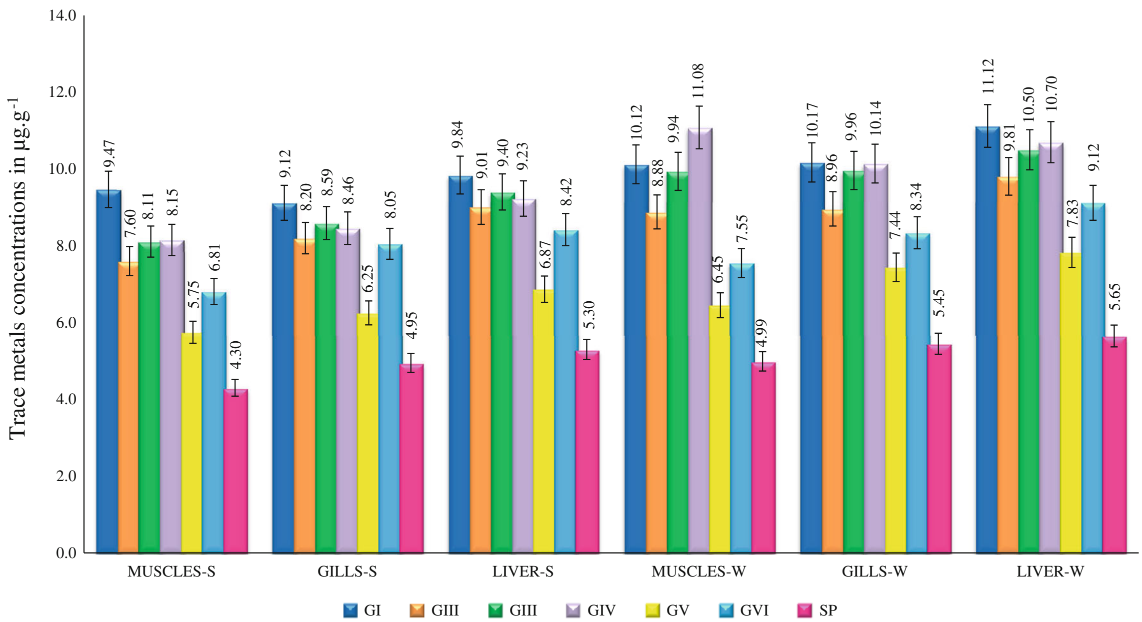

Fig. 4 Governorate-wise trace metals concentrations in *A. latus* body parts GI-GVI: Drain outfalls from Kuwait Governorates, *SP* semi-permanent drains, *S* summer, *W* winter

assimilation, adsorption and accumulation process of trace metals in their body parts. High trace metals concentrations in *A. latus* were observed in the sequence of liver > gills > muscles irrespective of the seasons and location of the drain outfalls (Fig. 3). High trace metals concentrations in liver and gills in the present study may be attributed to reasons validated by earlier investigators who showed large quantity of metallothioneins induction in the liver tissue and adsorption of metals in gill surface of fishes (Hamilton and Mehrle 1986; Hosseinkhezri and Tashkhourian 2011).

Governorate-wise analysis revealed *A. latus* containing high trace metals concentrations in the sequence of GI > GIII > GIV > GII > GVI > GV > SP during summer whereas, during winter, Governorate-wise trace metals concentrations in *A. latus* varied in the sequence of GIV > GI > GIII > GII > GVI > GV > SP (Fig. 4). Fish caught from GI and GIII drain outfalls during summer revealed high trace metals in their body parts as a result of their accumulation from untreated and voluminous wastewater discharges that were occasionally let into the marine environment from the power, desalination and treatment plants. High metals concentrations in GIV during winter may be related to the clandestine and untreated domestic wastewater discharges (Fig. 4). The overall season-wise analysis revealed high trace metals concentrations during winter than in summer. Earlier findings of Eleftheriadou and Skoullos (2003) that highlighted bioaccumulation and consumption of phytoplankton cells that bloomed during early summer and low assimilation of trace metals from

Table 1 Lethal concentrations of trace metals to *A. latus* (10 replicates) using Probit program

Metals	Exposure concentration (µg l^{-1})	LC point	95 % CI limits		χ^2 calculated
			Lower	Upper	
Cu	9.50	15	11.77	15.07	1.47*
	13.27	50	16.09	24.82	
	28.12	99	22.00	49.81	
Fe	12.55	15	11.73	13.62	1.56*
	15.38	50	14.05	18.86	
	19.79	99	16.89	29.49	
Zn	18.67	15	23.06	49.08	1.63*
	25.68	50	30.70	56.17	
	52.54	99	40.89	100.84	
Cr	7.96	15	4.82	9.99	3.56*
	13.56	50	11.09	16.38	
	44.86	99	30.42	117.39	
Pb	4.22	15	3.14	4.88	1.15*
	5.91	50	5.19	6.73	
	12.54	99	9.79	22.67	
Ni	5.57	15	4.32	6.30	1.85*
	7.50	50	6.73	8.36	
	14.67	99	11.84	24.18	
Hg	1.09	15	0.86	1.23	1.71*
	1.45	50	1.31	1.64	
	2.74	99	2.20	4.71	

Table value for all samples = 11.07

LC lethal concentrations, *CI* confidence interval, χ^2 chi-square

* χ^2 significant at 0.05 level

Table 2 Trace metals levels in wastewater, control in *A. latus* and related studies

Metals/expt.	Concentration ($\mu g\ l^{-1}$)	Exposure	Metal levels in *A. latus* body parts ($\mu g\ g^{-1}$)			Mortality
			Muscles	Gills	Liver	
Wastewater (a)						
Cu	7.41 ± 1.25					
Fe	6.29 ± 1.12					
Zn	18.16 ± 1.62					
Cr	13.94 ± 1.50					
Pb	2.39 ± 0.56					
Ni	5.13 ± 0.73					
Hg	1.09 ± 0.02					
Toxicity test (b)						
Cu	Control	30	7.99 ± 1.33	8.75 ± 1.46	9.06 ± 1.51	–
LOEC	6.26	30	8.30 ± 1.38	9.16 ± 1.52	9.22 ± 1.52	–
SL	9.50	30	8.71 ± 1.46	9.45 ± 1.56	10.08 ± 1.67	1
Fe	Control	30	11.95 ± 1.98	12.47 ± 2.07	13.26 ± 2.21	–
LOEC	7.96	30	12.24 ± 2.04	12.92 ± 2.15	13.91 ± 2.32	–
SL	12.55	30	12.52 ± 2.09	13.36 ± 2.23	14.04 ± 2.34	–
Zn	Control	30	20.74 ± 3.44	21.03 ± 3.50	22.54 ± 3.72	–
LOEC	12.55	30	21.12 ± 3.52	21.57 ± 3.59	22.97 ± 3.65	–
SL	18.67	30	21.46 ± 3.57	23.16 ± 3.85	24.95 ± 4.15	1
Cr	Control	30	4.68 ± 0.78	4.95 ± 0.82	5.36 ± 0.89	–
LOEC	4.10	30	4.94 ± 0.82	5.21 ± 0.87	5.43 ± 0.91	–
SL	7.96	30	5.29 ± 6.05	5.84 ± 0.96	6.05 ± 1.02	1
Pb	Control	30	2.71 ± 0.47	3.17 ± 0.53	3.44 ± 0.55	–
LOEC	2.78	30	2.94 ± 0.50	3.83 ± 0.65	3.99 ± 0.68	–
SL	4.22	30	3.27 ± 0.53	4.06 ± 0.68	4.27 ± 0.72	1
Ni	Control	30	5.38 ± 0.90	5.43 ± 0.90	6.41 ± 1.05	–
LOEC	3.84	30	5.87 ± 0.97	5.90 ± 0.98	6.85 ± 1.13	–
SL	5.57	30	6.13 ± 1.03	6.22 ± 1.04	7.29 ± 1.21	–
Hg	Control	30	1.14 ± 0.20	1.25 ± 0.20	1.34 ± 0.21	–
LOEC	0.77	30	1.27 ± 0.22	1.54 ± 0.25	1.59 ± 0.26	–
SL	1.09	30	1.48 ± 0.25	1.61 ± 0.28	1.73 ± 0.29	1
References[1]						
Cu[a]			0.85	1.60	1.80	
Fe[b]			19.98	20.94	20.94	
Zn[a]			2.22	4.10	6.86	
Cr[b]			0.34	0.61	1.13	
Ni[b]			0.34	0.51	0.73	
Pb[a]			1.39	2.09	2.44	
Hg[a]			0.07	0.09	0.10	

References[1]: metals levels in *A. latus*; No records on *A. latus* BAF

LOEC least observed effective concentration (LC_5), *SL* sub-lethal concentration (LC_{15})

[a] Hosseinkhezri and Tashkhourian (2011)

[b] Zahra et al. (2012)

food due to low metabolic rate may be validated for the high trace metals in *A. latus* during winter in the present study.

Toxicity tests revealed *A. latus* highly sensitive to Hg ($1.09\ \mu g.l^{-1}$) at LC_{15} compared to the other trace metals investigated in this study (Table 1). A 72 h Probit analysis (USEPA 1993) confirmed LC_{50} in the sequence of Hg ($1.45\ \mu g\ l^{-1}$) > Pb ($5.91\ \mu g\ l^{-1}$) > Ni ($7.50\ \mu g\ l^{-1}$) > Cr ($13.56\ \mu g\ l^{-1}$) > Cu ($13.27\ \mu g\ l^{-1}$) > Fe ($15.38\ \mu g\ l^{-1}$) > Zn ($25.68\ \mu g\ l^{-1}$) (Table 1). Statistical test in the Probit Program using chi-square (χ^2) distribution showed significant difference in all the calculated exposed concentrations against the χ^2 table value (Table 1).

Analysis revealed trace metals bioaccumulation factor (BAF) in the three body parts (liver, gills and muscles) of *A. latus* exposed for 30d to be > 1 except chromium (Cr), confirming the trace metals bioaccumulation in this fish from wastewater (Tables 2, 3). BAF <1 for Cr indicated:

Table 3 Trace metals bioaccumulation factor in *A. latus* exposed for 30d

Metals	Test concentration ($\mu g\ l^{-1}$)	$BAF = \frac{\text{Concentration of trace metals in fish tissue} (\mu g\ g^{-1})\ (b)}{\text{Concentration of trace metals in wastewater} (\mu g\ l^{-1})\ (a)}$			Mean BAF
		Liver	Gills	Muscles	
Cu	Control	1.22	1.18	1.08	1.16
LOEC	6.26	1.25	1.24	1.12	1.20
SL	9.50	1.36	1.28	1.18	1.27
Fe	Control	2.11	1.98	1.90	1.99
LOEC	7.96	2.21	2.05	1.95	2.07
SL	12.55	2.23	2.12	1.99	2.11
Zn	Control	1.24	1.16	1.14	1.18
LOEC	12.55	1.26	1.19	1.16	1.20
SL	18.67	1.37	1.28	1.18	1.28
Cr	Control	0.38	0.36	0.34	0.36
LOEC	4.10	0.39	0.37	0.35	0.37
SL	7.96	0.43	0.42	0.38	0.41
Pb	Control	1.44	1.33	1.13	1.30
LOEC	2.78	1.67	1.60	1.23	1.50
SL	4.22	1.79	1.70	1.37	1.62
Ni	Control	1.25	1.06	1.05	1.12
LOEC	3.84	1.34	1.15	1.14	1.21
SL	5.57	1.42	1.21	1.19	1.28
Hg	Control	1.23	1.15	1.05	1.14
LOEC	0.77	1.46	1.41	1.17	1.35
SL	1.09	1.59	1.48	1.36	1.47

LOEC least observed effective concentration(LC_5), *SL* sub-lethal concentration (LC_{15}), *BAF* bioaccumulation factor

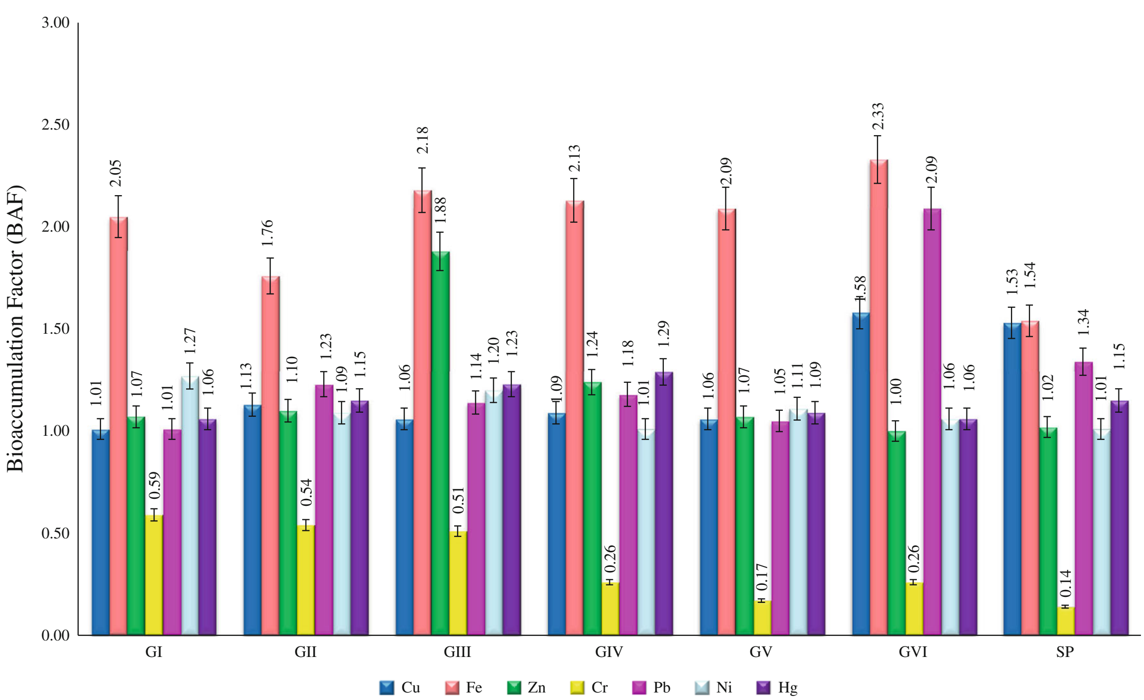

Fig. 5 Governorate-wise trace metals bioaccumulation factor in *A. latus* GI–GVI: Drain outfalls from Kuwait Governorates, *SP* semi-permanent drains, *S* summer, *W* winter

(1) Cr in *A. latus* tissues in trace amounts could have been the outcome of Cr absorption from feed or trace sources and possibly not by absorption from the wastewater that has fairly rich Cr levels in Kuwait's drain outfalls (2) Cr resistant to fish in its hexavalent form (Cr-VI) and (3) immune resistance of Cr in this fish compared to Cr in other aquatic organisms from the surrounding medium (Nussey et al. 2000). Parts-wise analysis showed high BAF in liver followed by gills and muscles. High BAF in liver may be attributed to the effects of metals detoxification in fish. These results were in agreement with the earlier observations of Wong et al. (1999). It is interesting to note that a comparison between an earlier observed surface dwelling habitat fish to that of the presently studied demersal fish revealed the major cause of trace metals bioaccumulation in both the fish as an outcome from the 'stressed' ecosystem although trace metals bioaccumulation rates varied between their body parts and with different species (Bu-Olayan and Thomas 2005; Nussey et al. 2000; Fernandes et al. 2007; Fei-Dang et al. 2012). Comparatively, BAF was low in gills followed by muscles (Table 3). BAF in gills attributes to the metal complex formation with mucus on the gill lamellae. A low degree of BAF was met with muscles as a result of residual absorption through the intestinal walls in comparison with BAF in liver and gills. BAF of trace metals was high in fish reared in wastewater collected from GVI, GIII and GIV than drain outfalls located in the other Governorates (Fig. 5). Reasons for the high BAF in fish collected from these drain outfalls may be attributed to the influence of oil and industrial waste discharges.

Conclusion

In an overall view, the present study using DESCAR-3.2 program revealed (a) the dispersed angle of wastewater pathway containing trace metals from the drain outfall mouth (b) the approximate distance (Fig. 1a, b) determining the intensity of trace metals concentrations from the mouth of the drain outfalls off the Persian Gulf (c) seasonal variation pattern in the dispersion of wastewater discharges and (d) location-wise variation of wastewater dispersion. The above factors linked with toxicity studies using probit program and evaluating the bioaccumulation factor (BAF) validated *A. latus* to stressed marine ecosystem due to wastewater discharges and labeled this species as biomonitoring tool to trace metals pollution. Although trace metals concentrations in wastewater and bioaccumulation in the *A. latus* body parts were high (BAF > 1), no catastrophic effect such as 'fish kill' or humans subjected to illness due to the consumption of such fish was observed during the course of our investigations. Reasons may attribute to: (a) the adaptive features of this fish to withstand contaminants from wastewater (b) dilution of wastewater in the open sea causing the fish to physiologically remove its toxic wastes by excretion (c) constant migration of the fish to waters away from the drain channels and (d) the absence of sickness in humans because of seafood consumption as they cook fish with ingredients that detoxify contaminants. Besides the above factors, trace metals concentrations in nature and in fish exposed for 30d ranged between 2.71 and 21.46 $\mu g\ g^{-1}$. Except Hg concentrations ($> 1\ \mu g\ g^{-1}$), trace metals concentrations were found within the permissible limits (0.5–40 $\mu g\ g^{-1}$) for human consumption (FAO 1983; WHO 1989). Thus, the outcome of this study revealed the following: (a) utilizing DESCAR-3.2 program—indirectly validates evidences to the quantification of trace metals concentrations in wastewater discharged into the marine ecosystem (b) characterization of demersal fish—a potential tool to label such fish as trace metals bioindicators, (c) toxicity database—that may prevent the consumption of contaminated fish and contagious diseases in humans during their recreational activities in the Bay or open sea and (d) authentic method—to formulate guidelines on the pollution level for a given marine location or similar stressed ecosystem elsewhere the globe.

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