RESEARCH ARTICLE

Impact of a phosphate fertilizer plant on the contamination of marine biota by heavy elements

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Abstract Due to their toxicity, persistence, and bioaccumulation, metals are important marine environment pollutants, especially in low renewal rate water such as the Mediterranean Sea, receiving a lot of untreated industrial waste. The impact of a phosphate fertilizer plant on the marine biota metal contamination was studied. Several types of organisms: crabs, mussels, patella and fish were collected from two areas of the Lebanese coast, one subjected to the impact of the plant and another away from it; samples were analyzed for Zn, U, Cr, V, Mn, Ni, Co, Cu, As, Cd and Pb by ICP-MS. Higher accumulation was in crabs, patella, and mussels. Fish accumulated principally Zn, Cu, and Cd; a difference was observed between species and tissues. Cytosol metal fractionation using size-exclusion LC-ICP-MS showed principally Pb, As, Co, and Mn in the low molecular weight fraction (<1.8 Da); Cd, Zn, and Cu in the metallothionein fraction (1.8–-18 k Da), and Ni in high molecular weight fraction (>20 kDa).

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Introduction

The very low renewal rate makes the Mediterranean Sea extremely sensitive to pollution. The decline of the Mediterranean threatens the health of many millions of people living on its shore and jeopardizes the long-term development of key economic sectors such as fishing and tourism (Haffner and Sommer 2008). This fact has led to initiatives to help the countries of the southern and eastern Mediterranean reduce the waste released into the sea. The key issue is the assessment of the present contamination linked to waste discharges from plants located at the seacoast.

Heavy metals are normal constituents of the marine environment at trace levels, but their concentrations have been continually increasing due to domestic, industrial, mining, and agricultural activities (Uysal and Emre 2011). Discharges of heavy metals into the marine environment can change both aquatic species diversity and ecosystems (Funes et al. 2006). Because of their persistence, toxicity, and bioaccumulation, heavy metals may cause a risk to human and global ecosystem (Funes et al. 2006; Geeraerts and Belpaire 2009). On the other hand, some aquatic organisms such as, in particular, fish but also seaweeds and molluscs can be used to assess heavy metal contamination in the marine environment, since they accumulate metals to concentrations many times higher than those present in water or sediment (Silva et al. 2006). Studies have shown that metals accumulate in various organs of fish at different levels (Fernandes et al. 2007; Uluturhan and Kucuksezgin 2007). Heavy metals often accumulate to a higher concentration in the active metabolite parts such as the liver, gill, and kidney (Canli and Atli 2003). In general, the accumulation of heavy metals in a tissue is mainly dependent from water pollution level, time of exposure, intrinsic factors (fish age, feeding habits, metabolisms), and other environmental factors (salinity, pH, water temperature) (Canli

and Atli 2003). Mussels, crabs, and patella are excellent metal biomonitoring species available throughout the year with great affinity to pollution and important accumulation capacities (Nesto et al. 2007). Thus, metal concentration in their tissues reflects environmental levels.

A further insight into the toxicity, bioavailability, and environmental behavior of the metals can be obtained by the identification of the biomolecule targets involved in its metabolic pathways (Wallace et al. 2003; Vijver et al. 2004; Geffard et al. 2010). Indeed, the total accumulation of a metal in aquatic organisms may be insufficient to predict its toxicity since its various accumulated chemical forms are not biologically equally active (Vijver et al. 2004). For the toxicokinetics and toxicodynamics of metals, the cytosolic fraction was shown to be the most important (Perceval et al. 2006) since it contains both the metal-detoxified compounds (metallothionein-like proteins) and the metal proteins (Wallace et al. 2003; Perceval et al. 2006).

In the Mediterranean countries, most of the industries are located at the seaside due to the need for cooling and transportation (Fakhri et al. 2008). The Lebanese coastal marine zone is intensely affected by several sources of heavy metal contamination, which discharge directly in seawater without any pretreatment. The objective of this study was to probe, for the first time, the direct impact of a phosphate fertilizer plant on the contamination of edible aquatic resources by heavy metals. The accumulation of U, Cr, V, Mn, Ni, Co, Cu, As, Cd, and Pb was investigated in the gill, liver, and kidney of five common fish species, in crabs, mussels, and patella samples, in terms of total metal concentration and molecular mass fractionation.

Materials and methods

Sample collection and study area

Five commonly consumed fish species *Diplodus sargus*, *Oblada melanura*, *Pagellus erythrinus*, *Siganus rivulatus*, and Redcoat squirrelfish (*Nylon*); crabs (*Portunus trituberculatus*); mussels (*Mytilus edulis sp.*); and patella (*Patella vulgata*) were collected from two areas of the Lebanese coastal zone Selaata (34° 16′ 54.9″ N; 35° 38′ 19.37″ E), an area subjected to the impact of a phosphate fertilizer plant and Jbeil (34° 07′ 50.0″ N; 35° 36′ 28.78″ E), away from the direct industrial impact. The collected samples were transferred to the laboratory in an ice box, rinsed with deionized water, and kept frozen until dissection. All fish samples were dissected with sterile scissors to separate gills, liver, and kidney; muscles of crabs, mussels, and patella were removed for analysis. Tissues from individuals of the same species were pooled together, lyophilized, and stored (at 4 °C) until analysis.

Reagents

All the solutions were prepared with high-purity deionized water (resistivity≤18.2 Ω cm) obtained from a Milli-Q Elix 3 water purification system (Millipore, Molsheim, France). All chemicals and reagents were of the highest grade available from each supplier. The hydrogen peroxide, H_2O_2 (30 %), used was AnalaR NORMAPUR, HNO_3 (70 %, INSTRA-ANALYZED)—from Baker (Deventer, The Netherlands)—and the protein standards were purchased from Sigma-Aldrich (St. Quentin Fallavier, France). A SPEX CertiPrep (Metuchen, NJ) PlasmaCAL mono-element standards of U, Th, Cr, V, Mn, Ni, Co, Cu, As, Cd, and Pb were used to prepare standard working solutions by diluting the stock solution of 1000 μg.g^{-1} of each element with 2 % nitric acid. DORM-2: Dogfish muscle certified reference material from the National Research Council of Canada was used.

Multielemental analysis

Digestions were performed using a DigiPREP device (SCP Science, Courtaboeuf, France). One milliliter of hydrogen peroxide and 3 mL of conc. nitric acid were added to the weighed sample (50 mg) in a PE tube of 50 m; the cap was tightened and the tube placed in the instrument at 65 °C for 4 h. Digests were diluted to obtain a final concentration of 2 % HNO_3 prior to total determination. Elemental analysis was conducted using an Agilent ICP-MS 7500ce (Agilent, Tokyo, Japan), fitted with an octopole reaction-collision cell. The operating conditions are given in Table 1. Single-element standard stock solutions PlasmaCAL (1000 ppm) were used for the calibration. The method has been validated by the analysis of a DORM-2 material.

Fractionation analysis

The cytosols were prepared from 50 mg of the gill, liver, and kidney tissues of the *S. rivulatus* species, diluted in 2 mL of 100 mM ammonium acetate buffer (pH 7.4). The samples were sonicated with an ultrasonic probe (Branson Ultrasonics Corporation, Danbury, USA). Metal determination was performed in the supernatant (cytosolic fraction) after centrifugation (Thermo, CR3i multifunction centrifuge) at 32,000g for 45 min at 4 °C. An Agilent 1100 (Agilent, Wilmington, DE) liquid chromatography equipped with a binary HPLC pump, an autosampler, and a diode array detector were used. Detection was performed by ICP-MS with an Agilent ICP-MS 7500ce (Agilent, Tokyo, Japan). A Superdex 200 HR size-exclusion column (GE Healthcare, Orsay, France) with a mass separation range between 10 and 600 kDa was used at the operating conditions shown in Table 1. The column was calibrated with commercial solutions of thyroglobulin

Table 1 Operating conditions of the ICP-MS and HPLC-ICP-MS analysis

Plasma conditions	
RF power	1500 V
Make up gas flow rate	0.15 L.min^{-1}
Carrier gas flow rate	1 L.min^{-1}
Nebulization temperature	2 °C
Data acquisition parameters for total analysis	
Monitored masses	V51, Cr52, Mn55, Ni58, Co59, Cu63, Zn64, As75, Cd109, Cd111, Pb208, Pb210, Pb212, Pb214, U238
Integration time	0.1 s
Points per peak	3
Collision cell	4 mL.min^{-1} H$_2$
Data acquisition parameters for SEC analysis (time resolved analysis mode)	
Monitored masses	V51, Cr52, Mn55, Ni58, Co59, Cu63, Zn64, As75, Cd109, Cd111, Pb208, Pb210, Pb212, Pb214, U238
Integration time	0.1 s
Points per peak	1
Collision cell	4 mL.min^{-1} H$_2$

(670 kDa), ferritin (474 kDa), metallothionein (6–7 kDa), carbonic anhydrase (29 kDa), albumin (66 kDa), and Vit E/cobalamin (1.3 kDa). One hundred microliters of fish cytosol was injected and fractionated isocratically at 0.7 mL.min.$^{-1}$ of 100 mM ammonium acetate (pH 7.4). UV absorbance was monitored at 280 nm.

Results and discussion

The Selaata phosphate fertilizer plant is located at the coast 46 km north of Beirut. The fertilizers are derived from phosphate ores which—in addition to phosphate minerals—contain a wide range of metals such as Hg, Cd, As, Pb, Cu, Ni, Cr, and Zn (Al-Masri et al. 2004; Aoun et al. 2010). During the production process, part of the contaminants is transferred from ores to finished products: phosphoric acid and fertilizers. However, most of the contaminants are found in the effluents and mainly in the solid waste of the phosphate fertilizer industry, known as phosphogypsum (Al-Masri et al. 2004). Due to weathering processes, piles of phosphogypsum disposed without any treatment may lead to chemical contamination of the surrounding environment. In this study, fish belonging to five commonly consumed species and patella were caught all around the industrial area while crabs and mussels were collected from both sides (north and south) of the factory.

Metal contamination in marine organisms

Trace metal in marine environment can be divided into two classes: (i) those essential for effective function of biochemical processes (e.g., cobalt, copper, zinc,

manganese) but toxic in excess and (2) those with no established biological functions and considered as toxic (e.g., cadmium, chromium, lead) (Kennish 2001). The concentration levels of Pb, Ni, Cu, V, Cr, Cd, As, Zn, Mn, Co, and U in the liver, gill, and kidney of the investigated species were shown in Fig. 1. Taking into consideration all the collected fish species from the two stations, the mean metal concentrations in the gill, liver, and kidney were, respectively, 2.53, 1.52, and 0.87 mg.kg^{-1} for V; 0.74, 0.88, and 1.04 mg.kg^{-1} for Cr; 14.76, 5.36, and 4.86 mg.kg^{-1} for Mn; 1.47, 2.76, and 1.77 mg.kg^{-1} for Ni; 0.88, 1.03, and 0.58 mg.kg^{-1} for Co; 3.32, 35.96, and 4.24 mg.kg^{-1} for Cu; 60.18, 164.15, and 109.67 mg.kg^{-1} for Zn; 4.85, 8.76, and 7.75 mg.kg^{-1} for As; 3.18, 21.43, and 5.50 mg.kg^{-1} for Cd; 3.51, 1.44, and 1.08 mg.kg^{-1} for Pb; and 0.34, 0.31, and 0.27 mg.kg^{-1} for U. The overall metal accumulation capability of the studied fish organs decreases in the order liver>gill>kidney. Several previous studies approved that the liver is a target organ for metal accumulation and detoxification and can be considered as an important sample for the water pollution monitoring (Kljakovic et al. 2002; Canli and Atli 2003).

The mean concentrations of essential elements in fish organs follow the sequence: Zn>Cu>Mn>>Co and Zn>Cu−Mn>Co> in Selaata and Jbeil, respectively. The concentrations of the toxic elements Cd, As, Pb, Ni, Cr, and U varied in a broad range between 0.3 mg.kg^{-1} for U and 92.5 mg.kg^{-1} for Cd in the fish organs. Concentrations of Ni and Cr are higher in fish collected from Selaata than from Jbeil showing a contamination of the area by the fertilizer plant.

Results obtained for the molluscs are presented in Fig. 2. As for fish, the concentrations of the three essential elements Zn, Cu, and, Mn in the analyzed crabs, mussels, and patella
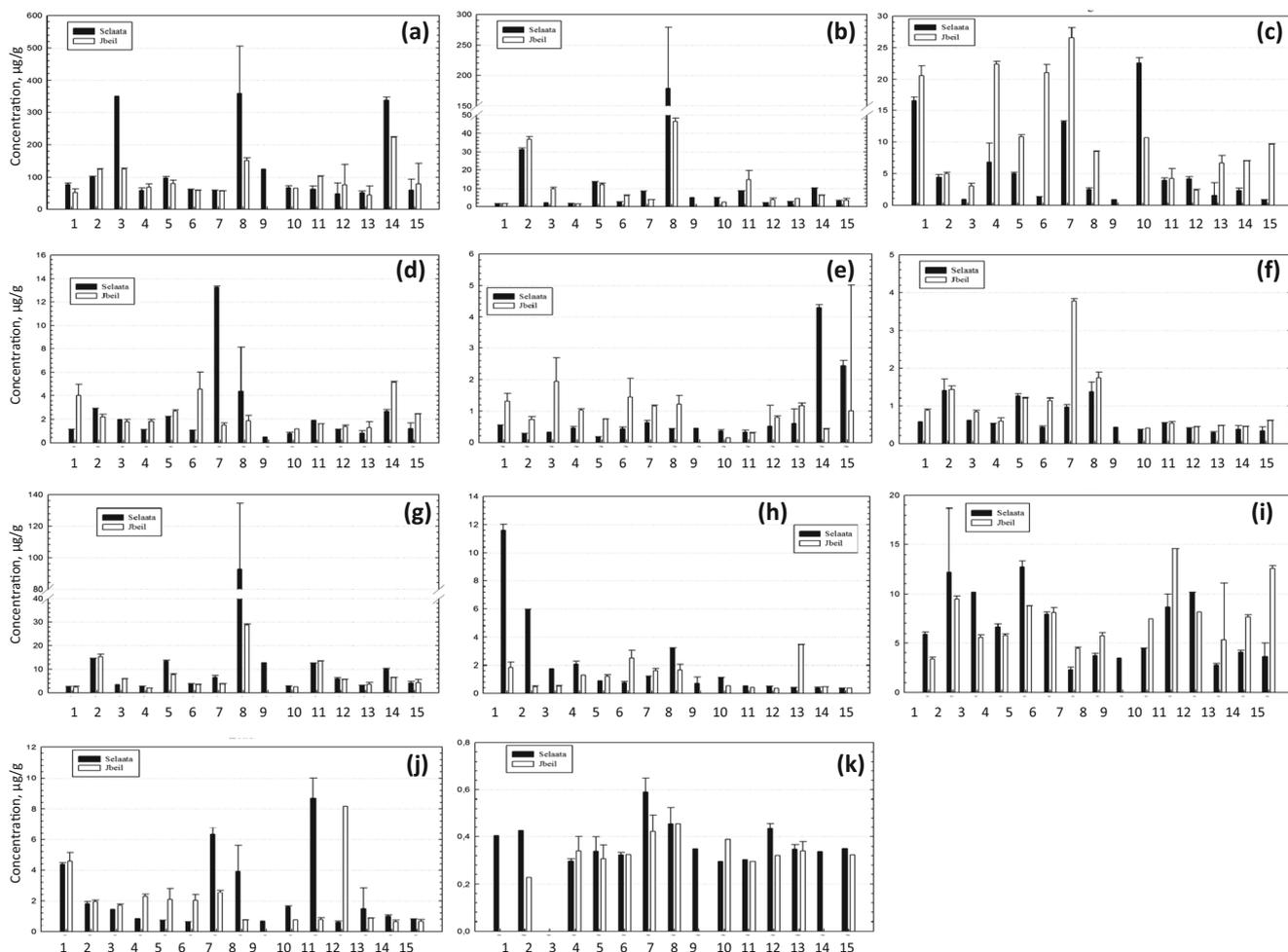
Fig. 1 Accumulation levels of **a** Zn, **b** Cu, **c** Mn, **d** Ni, **e** Cr, **f** Co, **g** Cd, **h** V, **i** As, **j** Pb, and **k** U in *Diplodus sargus* (*1* indicates the gill, *2* the liver, and *3* the kidney), *Pagellus erythrinus* (*4* indicates the gill, *5* the liver, and *6* the kidney), *Siganus rivulatus* (*7* indicates the gill, *8* the liver, and *9* the kidney), *Oblada melanura* (*10* indicates the gill, *11* the liver, and *12* the kidney), and *Nylon* (*13* indicates the gill, *14* the liver, and *15* the kidney)

samples were the highest among those in the studied elements. There are higher levels of Ni and Cr in the molluscs than in fish organs that were observed, especially in mussel samples (Fig. 2). Related to other toxic elements, the highest levels of Cd obtained in mussels, crabs, and patella were 3.2, 9.7, and 5.3 mg.kg^{-1} in Selaata. Similarly, as for fish organs, high accumulation of V and As was observed in both regions while the high level of lead 13.5 mg.kg^{-1} was determined in mussels from Jbeil; this region away from the direct impact of the plant is surrounded by several other industries.

Zinc It is an essential trace element which becomes toxic in excessive amounts. Its concentrations in the studied fish organs varied between 44 and 360 mg.kg^{-1} dry. In several studies, high accumulations of Zn were observed in fish organs, which is in agreement with our results as Zn was the most abundant metal in the studied organs of the fish. The lowest levels of Zn were observed in the gills even if for fish seawater is the main source of Zn, and some researchers indicated that

gills are an important organ of its accumulation (Fernandes et al. 2007; Yilmaz and Yilmaz 2007). The levels found in this study in gills of *D. sargus* and *S. rivulatus* were several times lower than in the previous report of Uysal and Emre 2011 (Tables 2 and 3), and high levels were observed in the liver organ; in fact, it was also reported that when the levels of Zn exceeded the requirement in the gills, the excess was excreted by the liver (Birungi et al. 2007), which is considered a detoxification organ. In this study, high accumulation of Zn was observed in the liver of *S. rivulatus* and *Nylon* and in the kidney of *D. sargus* collected from Selaata industrial area compared to that from the area of Jbeil highlighting the impact of the phosphate industry. No effect of the sampling location was observed for organs of *O. melanura* and *P. erythrinus*, although the concentrations found in the last species were slightly higher than in Turkish coastal water (Uluturhan and Kucuksezgin 2007; Dural et al. 2010). In fact, in addition to the water pollution level, metal concentrations are strongly dependent on fish species and other environmental factors

Fig. 2 Accumulation levels of Cu, Mn, Ni, Cr, Co, Cd, V, As, Pb, and U in molluscs; **a** crabs, **b** mussels, **c** patella
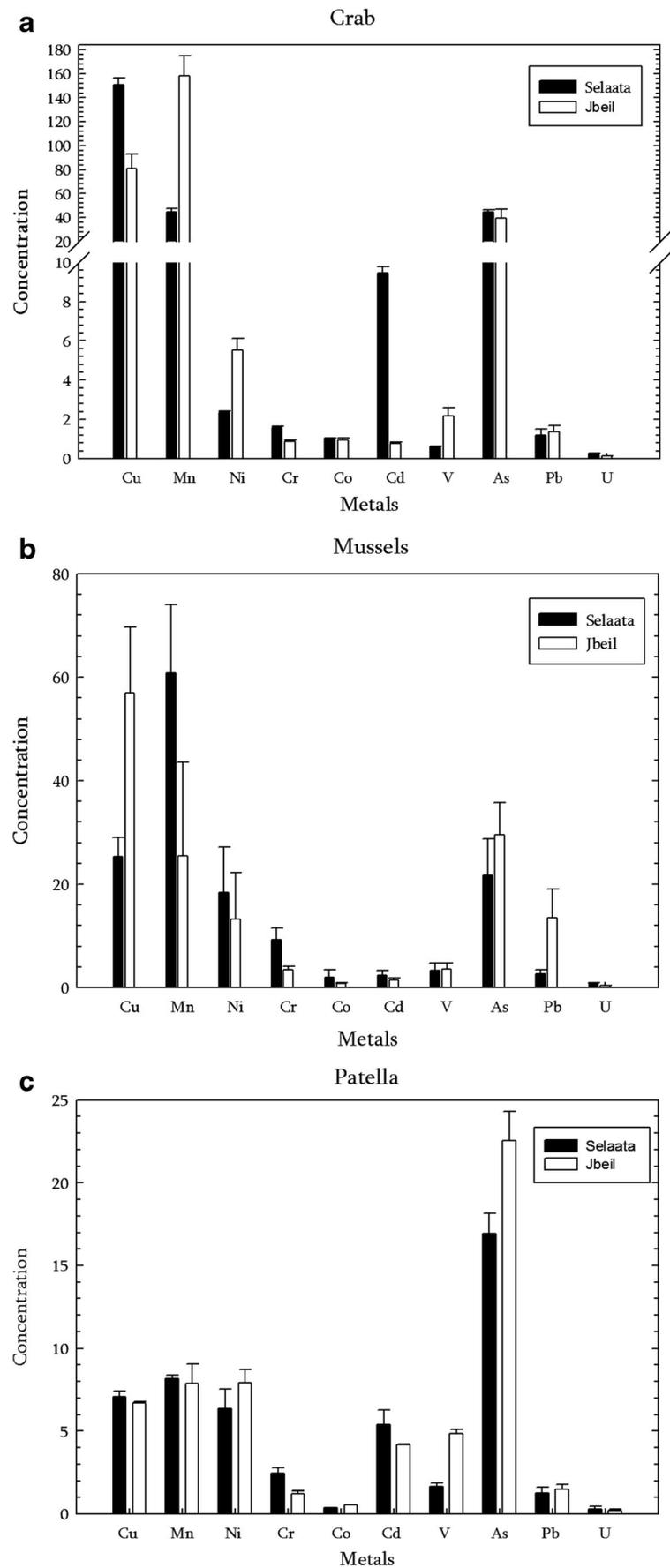
Table 2 Heavy metal concentrations (mg.kg^{-1}) found in the liver of *Pagellus erythrinus*

	Cd	Cr	Cu	Mn	Pb	Zn	Reference
Turkey	1.1		10.1		1.36	29.46	(Uluturhan and Kucuksezgin 2007)
	0.4	0.69	5.79	8.2	2.9	59.71	(Dural et al. 2010)
Jbeil (Lebanon)	7.73	0.74	12.06	10.8	2.09	80.45	Present study
Selaata (Lebanon)	13.26	0.16	13.48	5.06	0.75	98.27	Present study

(Canli and Atli 2003). Concentrations of Zn measured here in crabs, mussels, and patella were higher than those found in other areas (Table 4); Zn in patella samples were higher in our study even than in the contaminated area in Cravo studies (Table 4), which is an indicative of environmental pollution from industrial sources.

Copper This essential element assuring healthy metabolism and growth of marine organisms becomes highly toxic at concentrations higher than the levels required for growth (Eisler 2000). In this study, the concentrations of Cu in liver tissues were significantly higher than in the gill and kidney; data obtained by previous studies show that even at low levels, Cu show a distinct affinity to the liver organ (Jezierska and Witeska 2006). However, the enrichment related to possible industrial contamination was observed for one species only: *S. rivulatus* with Cu values of 179 and 46 mg.kg^{-1} for Selaata and Jbeil, respectively. The values reported for *P. erythrinus* liver were in the same range as those cited by (Uluturhan and Kucuksezgin 2007; Dural et al. 2010) for the species sampled in Turkish coastal water. The levels of copper found in the gill were the same or slightly higher than those reported by (Uysal and Emre 2011) (Table 3) for *D. sargus* and *S. rivulatus*, respectively. Cu in crabs and mussels from Selaata were higher than those found in other areas such as Kuwait bay, Adriatic Sea, and Venice lagoon (Table 4), which is an indicative of environmental pollution from industrial sources.

Manganese The toxicity of this element is considered to be low (Kennish 2001). The fish organs contained between 0.7 and 26.6 mg.kg^{-1} in the kidney of *S. rivulatus* in Selaata and in the gill of *S. rivulatus* in Jbeil, respectively. The privileged organ for Mn accumulation in fish was the gill. The Mn in crabs and mussels were significantly higher reaching for some locations more than 100 mg.kg^{-1}. The concentration of Mn found in the gill of *S. rivulatus* and *D. sargus* were slightly higher than those reported by Uysal and Emre 2011 (Table 3). The values reported for *P. erythrinus* liver were in the same range as those found for the species sampled in Turkish coastal water (Uluturhan and Kucuksezgin 2007; Dural et al. 2010), which can be due to the fact that manganese levels in phosphogypsum are between 3.5 and 20 mg.kg^{-1}, low compared to other metal concentrations (Davister 1998).

Nickel Chronic Ni toxicity to marine organisms was recently evaluated and found to be strongly species dependent (DeForest and Schlekat 2013). Levels of Ni in fish organs were relatively low in comparison to those of other metals studied and ranged from 0.5 to 4.4 in Selaata and 1.1 to 5.1 mg.kg^{-1} in Selaata and Jbeil, respectively. Similar levels were observed for crabs, patella, and mussels with the exception of mussels sampled in the south from the Selaata plant where the concentration as high as 18.4 mg.kg^{-1} was found.

Chromium Toxicological impact can result both from the action of Cr (VI) itself as an oxidizing agent and from Cr (III) which is capable to form complexes with various organic compounds and thus may inhibit several metalloenzyme systems. In the studied fish organs, concentrations of Cr were at the level of a few mg.kg^{-1}; the highest value (4.2 mg.kg^{-1}) was obtained except for the liver of the *Nylon* sampled in Selaata. The same levels were present in crabs, mussels, and patella with the highest concentrations observed in the north of the Selaata plant. The content obtained in *P. erythrinus* liver was in agreement with that observed (Dural et al. 2010) in Turkish coastal water.

Cobalt It is generally considered to present no or very low toxicity. In this study, no significant differences were observed for Co levels in the Selaata and Jbeil sampling sites; the

Table 3 Heavy metal concentrations (mg.kg^{-1}) found in the gill of *Siganus rivulatus* and *Diplodus sargus*

	Siganus Rivulatus			*Diplodus sargus*			Reference
	Cu	Mn	Zn	Cu	Mn	Zn	
Turkey	1.2	8	320	1.4	9	300	(Uysal and Emre 2011)
Jbeil (Lebanon)	3.85	26.6	56.64	1.58	20.6	51.69	Present study
Selaata (Lebanon)	8.59	13.3	59.63	1.44	16.5	77.15	Present study

Table 4 Heavy metal concentrations (mg.kg^{-1}) in marine organisms from different countries

Species	Zn	Cd	Cu	Pb	Co	Cr	Ni	Region of occurrence	Reference
Crabs	206		124	1.9				Kuwait Bay–Persian Gulf	(Al-Mohanna and Subrahmanyam 2001)
	2715[a]	0.7[a]	80.9[a]	1.36[a]	0.9[a]	0.9[a]	5.5[a]	Lebanon–Mediterranean Sea	Present study
	2700[b]	9.7[b]	167[b]	1.44[b]	1.6[b]	2.3[b]	3.7[b]		
Mussel	82.45	0.91	4.13	0.85				Adriatic Sea	(Fattorini et al. 2008)
	249.5	2.01	7.54	2.36				Venice lagoon	(Nesto et al. 2007)
	2384[a]	1.46[a]	57.0[a]	13.5[a]	0.78[a]	3.4[a]	13.2[a]	Lebanon–Mediterranean Sea	Present study
	1238[b]	3.24[b]	27.4[b]	1.6[b]	0.57[b]	10.5[b]	7.3[b]		
Patella	3.5–14.6		1.2–2.4		0.2–0.5			Favignana, Italy	(Campanella et al. 2001)
	62.3[c]	0.6[c]	6.1[c]		4.3[c]			Portugal	(Cravo and Bebianno 2005)
	129[d]	1.6[d]	8.1[d]		4.1[d]				
	841[a]	4.2[a]	6.7[a]	1.5[a]	0.54[a]	1.22[a]	7.9[a]	Lebanon–Mediterranean Sea	Present study
	752[b]	5.4[b]	7.1[b]	1.3[b]	0.37[b]	2.46[b]	8.2[b]		

[a] Jbeil

[b] Selaata

[c] Clean area

[d] Contaminated area

highest concentrations in fish organs and molluscs were at the same level: 3.7 mg.kg^{-1} in the gill of *S. rivulatus* of Jbeil and 3.4 mg.kg^{-1} in mussel from Selaata.

Cadmium It is capable to produce chronic effects even at low levels (Taylor 1983). In the tissues, studied concentrations of Cd were generally below 10 mg.kg^{-1} for both fish and molluscs with the exceptionally high values observed for *S. rivulatus* liver in Selaata (92.5 mg.kg^{-1}) and in Jbeil (29 mg.kg^{-1}). It was shown that the liver is the primary target for Cd accumulation; the levels in Selaata were generally higher than in Jbeil. Cadmium levels observed in the liver of *P. erythrinus* were significantly higher than those reported in previous works on the same species sampled in Turkish coastal areas (Uluturhan and Kucuksezgin 2007; Dural et al. 2010); it was reported in previous studies that cadmium is taken by to the gills and distributed throughout the body and accumulates in the liver for detoxification (Uluturhan and Kucuksezgin 2007) which may explain this difference, showing a contamination of the area by the plant. Cd, in patella, were higher in our study than in others studies, even than in contaminated area in Cravo studies (Cravo and Bebianno 2005).

Vanadium The pH is an important modulator of V toxicity. No clear link was established between the sampling location and vanadium levels with the exception of the organs of *D. sargus* where the concentrations in Selaata were significantly higher than in Jbeil. On the basis of the obtained data, the accumulation of vanadium seems to be strongly species dependent for both fish and molluscs.

Arsenic Toxicity of this trace element is highly species dependent; however, in marine biota, most of arsenic is known to be present in the form of harmless organic species such as arsenobetaine. Similar to vanadium, the accumulation of As was found strongly species dependent with no clear correlation with the sampling location. Out of the fish organs studied, the livers contained the highest levels of As up to 14.6 mg.kg^{-1} in the liver of *O. melanura* from Jbeil. The concentrations found in molluscs were significantly higher reaching 44.8 mg.kg^{-1} for crabs.

Lead It is a non-essential element which causes toxic effects (Garcia-Leston et al. 2010). Distribution of Pb was similar between the different organs of both *P. erythrinus* and *O. melanura* species, and a similar accumulation was observed in *D. sargus* species from both regions. The highest levels were generally observed in the gills, with the highest concentration of 10.2 mg.kg^{-1} found in the gill of *Nylon* from Jbeil. Lead levels observed in the liver of *P. erythrinus* were at the same level as those reported in the work of Uluturhan and Kucuksezgin 2007 and Dural et al. 2010 for the same species sampled in Turkish coastal areas. Previous studies have reported low levels of Pb in seawater, and fish bioaccumulates Pb proportionally to its concentrations in seawater, or Pb concentrations in phosphogypsum are low compared to the levels of other metals (0.5–17 mg.kg^{-1}) (Davister 1998), which may explain in our study the low levels of Pb compared to those of other metals in fish organs.

Uranium Uranium is a non-essential element, with documented chemical and radiological toxicity (Al Kaddissi et al. 2011). The levels observed in both fish and molluscs were
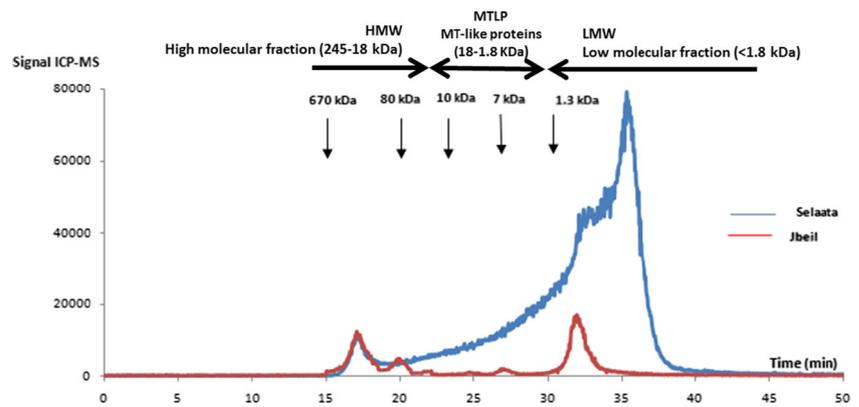
generally low (with a maximum of 0.6 mg.kg^{-1} in the gill of *S. rivulatus* from Selaata) with no significant differences in the levels for the different organs and species studied. Levels of U in marine organisms from Selaata were higher than from Jbeil.

Difference of accumulation between species

When compared with other studies, the observed difference in distribution of the studied elements between organs of the different species in this study is in agreement with that in the previous studies on other fish species (Kljakovic et al. 2002; Yilmaz and Yilmaz 2007). Metal concentrations were strongly dependent on fish species which is reported to may be related to the differences in ecological needs, swimming behaviors, and the metabolic activities of the different species (Canli and Atli 2003). Few studies exist on metal levels in the chosen fish species; based on the obtained data, the *S. rivulatus* species has almost the highest levels of Co, Cu, Zn, Cd, Pb, and U; *D. sargus* had the highest levels of V and Ni; *Nylon* had the highest levels of Cr; and the highest levels of As and Mn were observed in the *O. melanura* and *P. erythrinus* species, respectively. The concentrations were higher in crabs: levels of 2715, 167, 158, and 52 mg.kg^{-1} were observed for Zn, Cu, Mn, and As, respectively. Accumulation in patella samples was lower; the highest concentration found was 841.5 mg.kg^{-1} for Zn while U had the lowest level of 0.2 mg.kg^{-1}.

Difference of accumulations between locations

The comparison of data obtained for fish showed that metal concentrations varied between the sampling locations. For instance, significant Mn concentration was found in Jbeil, while those of Zn, Cu, and Cd were in Selaata. The highest metal concentrations in molluscs were generally found in Selaata, except for V, Ni, and Mn which were concentrated in Jbeil samples. The levels of heavy metals in patella collected from Jbeil and Selaata were comparable.

In general, the concentrations of metals in fish organs in Selaata were higher than those in Jbeil, which may

be due to the impact of the phosphate fertilizer industry in this area. Phosphate loading activities and phosphogypsum storage are considered as the main sources of Zn, Cu, and Cd which can be leached to the surrounding environment (Al-Masri et al. 2004; Aoun et al. 2010). The high levels of some elements found in the species from Jbeil, such as Cd and Mn concentrations, may have anthropogenic origin. This area is highly populated with considerable agricultural and metallurgic industry activities.

Pearson test was performed to find the intercorrelation between the organs of the collected fish samples. The linear correlation between each pairs of organs based on the Pearson correlation factor (two-tailed sigma, $p=0.05$) (Weiss 2008) showed significant linear relations between the gill, liver, and kidney for V and Cd in Selaata sample ($p<0.05$), also between the liver and kidney for Cu and Cr ($p=0.023$, $p=0.0001$) and between gill–liver for U, As, and Pb ($p=0.04$, $p=0.00$, $p=0.012$). Few significant correlations between organs were observed in the fish species collected from Jbeil, mainly between the gill, liver, and kidney for As; gill–kidney for U; and liver–kidney for Pb (Annexe 2).

The linear correlation between each pairs of elements based on the Pearson correlation factor (two-tailed sigma, $p=0.05$) showed strong correlations between many elements in the three organs of fish samples collected from Selaata, especially in the liver (Annexe 2). For instance, Zn correlates positively with Ni, Cu, Cd, and U with Pb while strong correlations were observed between Ni, Cu, Co, and Cd. These correlations are absent in the case of Jbeil which allows to attribute the origin of these metals to the phosphate plant.

Speciation studies

For the assessment of toxicity, mobility, and bioavailability of the studied metals, their molecular size fractionation was investigated further to their total determination. The experiments were focused on the cytosolic fraction of the liver, gill, and kidney of the *S. rivulatus*, which contains the highest

levels of metals. The species eluting from the size-exclusion column were divided into three metal–ligand pools: (i) high molecular weight fraction (HMW) (245–18 kDa), from 12 to 22 min; (ii) metallothionein-like protein fraction (MTLP) (18–1.8 kDa) in the range of 22–30 min; and (iii) lower molecular fraction (LMW) (<1.8 kDa) after 30 min as shown in Fig. 3. Most of the metals were bound to several species, e.g., Cd binds to different protein fractions, mainly to MTLP in the liver and gill and low molecular weight species in the kidney (Annexe 3). Surprisingly, only a small fraction of the cytosolic Cu was bound to MTLP in the liver, kidney, and gill of the *Siganus rivulatus* from both Jbeil and Selaata. As showed in Fig. 3, Cu was preferably bound to the LMW fraction, which increased with the concentration of Cu in the liver samples of *Siganus rivulatus* from Selaata. This highlights a high contamination and healthy effect. Indeed, ecotoxicological studies have shown that MTLP are detoxifier proteins whereas HMW and LMW would be biomarkers of harmful effects (Giguère et al. 2003).

Arsenic, cobalt, and lead were found predominantly in the low molecular fraction, whereas a significant fraction of nickel (up to 72 % depending on the organ) was bound to high molecular proteins.

Conclusion

Data have shown a heavy contamination of marine biota directly related to the phosphate plant activity in Selaata, especially for Zn, Cu, and Cd which are contained in the coproduct and industrial waste of the factory. The contamination is species dependent. The highest metal concentrations were found in the livers (organ of detoxification) of the studied fish species. The concentrations of Zn in all organs were the highest. The metals were relatively homogenously distributed among the pools of biological ligands which suggest that the levels of contamination exceed those at which molecular mechanisms are still active.

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