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ORIGINAL RESEARCH ARTICLE

Seasonal assessment of biological indices, bioaccumulation and bioavailability of heavy metals in mussels *Mytilus galloprovincialis* from Algerian west coast, applied to environmental monitoring☆

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Summary The aim of the present work is to broaden our knowledge on the variability of trace metals in mussel tissues, focusing on seasonal fluctuations in the three different sampling sites of Algerian west coast (Oran Harbor (S1), Ain Defla (S2) and Hadjaj (S3)). For this purpose, the bioavailability (metal indices) and bioaccumulation (metal concentrations in soft tissues) of heavy metals (Zn, Cu, Pb, and Cd), and the physiological characteristics (e.g. biological indices such as condition index (CI)) of mussels *Mytilus galloprovincialis* have been assessed and related to seasons and sites. In S1, the highest levels of metal concentrations and indices were obtained in mussels sampled in winter for Zn, Cu and Cd, but in summer for Pb. The biological indices significantly decreased in winter. In S2, the levels of concentrations and indices of all metals varied whatever the seasons, excepting in summer where the values were the lowest. In summer

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and spring, the biological indices were lower than in autumn and winter. The low growth of organisms in spring and summer might be correlated to the reproductive period and the low trophic level known in S2. S3, considered as a "pristine" area, showed low metal concentrations and indices, and high biological indices, reflecting the favorable physiological conditions for the mussel growth. This approach might be used in the monitoring of the quality of coastal waters and the present work provided a useful data set for Mediterranean monitoring network.

1. Introduction

During the last twenty years, Algeria's population has increased by 50%. About 45% of this population is concentrated on a very narrow strip of the littoral, especially in the industrial and harbor zones (Grimes et al., 2010). This coastal population is still increasing considerably and exerts a great anthropogenic pressure on the coastal marine ecosystem. Worsening conditions can be observed in large sections of the coast, particularly in the gulfs close to the Algerian largest agglomerations, such as Algiers, Oran and Annaba and near the industrial-harbor complexes (Grimes et al., 2010). Algeria participates in the implementation of the Barcelona Convention, which was intended to protect the Mediterranean Sea against pollution (UNEP, 1997). In this context, several biomonitoring studies have been conducted, during the last ten years, along the western coast of Algeria. However, most of these studies were generally limited to the use of a single approach such as dosage of pollutants, monitoring of biomarkers, or determination of biotic indices (Grimes et al., 2010; Rouane-Hacene et al., 2008; Taleb et al., 2007). These studies demonstrated that the industrial and domestic untreated wastewater effluents and run-off water contaminated by pesticides and heavy metals represented a major source of chemical contamination of this coastal area. Heavy metals which are the major anthropogenic contaminants of estuarine and coastal waters may be present in particulate or dissolved forms. Although many metals are essential biological elements, all of them have the potentiality to be toxic to organisms above certain threshold concentrations. Brown and Depledge (1998) showed that these limits should not be exceeded in aquatic environments for the protection of aquatic biota. Thus, coastal waters and sediments of Algerian coast showed high levels of heavy metals especially for Cd, Cr, Cu, Fe, Ni, Pb and Zn (Alomary and Belhadj, 2007; Soualili et al., 2008).

The mussels, in particular the marine *Mytilus* bivalves, are filter feeders widely used as spatial and temporal bio-integrators of marine pollution, for their ability to accumulate and concentrate pollutants (e.g. heavy metals) in their soft tissues at levels higher than those found in the ambient water (Pan and Wang, 2012; Sasikumar et al., 2006; Szefer et al., 2004). Several studies have shown that the variation of biological responses of these organisms to a wide range of contaminants may be caused in part by seasonal and spatial patterns (Fattorini et al., 2008; Pisanelli et al., 2009; Regoli and Orlando, 1994). Furthermore, the metal bioaccumulation may be influenced by the interactions between physiological (growth, weight loss, absorption and accumulation), chemical (metal concentration, speciation and bioavailability) and environmental (temperature and food concentration) factors (Casas and Bacherb, 2006).

In the present study, three sites (Oran Harbor, Ain Defla and Hadjaj) located on the Algerian western coast were chosen because of the distinct nature of the pollution sources present in these areas. The first sampling site was located in the large port of Oran characterized by trade, fishing and marina, receiving untreated sewage effluents from the Oran Metropolis and industrial settlements. The second was located at Ain Defla, in a rural area, near a small fishing port, receiving untreated domestic effluents and agricultural runoffs. Finally, the third was located at Hadjaj, considered as the reference site, because it was far from anthropogenic activities.

The aim of the present study was to establish a seasonal assessment of the marine environment quality and a putative contamination gradient between the three sites. For this purpose, the bioavailability and the bioaccumulation of heavy metals (Zn, Cu, Pb, and Cd), and the physiological characteristics of mussels *Mytilus galloprovincialis* were assessed and related to seasons and sites. The overall results enlighten our knowledge on the influence of seasons when monitoring the potential impact of anthropogenic activities on the water quality of Algerian west coast. The present work provides a useful data set for Mediterranean monitoring network.

2. Material and methods

2.1. Sampling sites

The studied area extends along the western coast of Algeria, from Oran to Mostaganem, as shown in Fig. 1. Three sampling sites were selected with respect to the main identified pollution sources to follow a presumed contamination gradient (Eastwards: sites 1—3): site 1 (S1: 35°42′31.07″N, 0°38′26.76″W) was located in the large port of Oran, site 2 (S2: 35°49′03.66″N, 0°28′55.89″W) at Ain Defla, and site 3 (S3: 36°06′09.59″N, 0°19′11.48″E) at Hadjaj (considered as the reference site).

2.2. Collection and preparation of samples

Coastal waters and mussel samples were collected in 2010, once each season of one annual cycle (February (winter), May (spring), August (summer), November (autumn)), from each of the three studied sites.

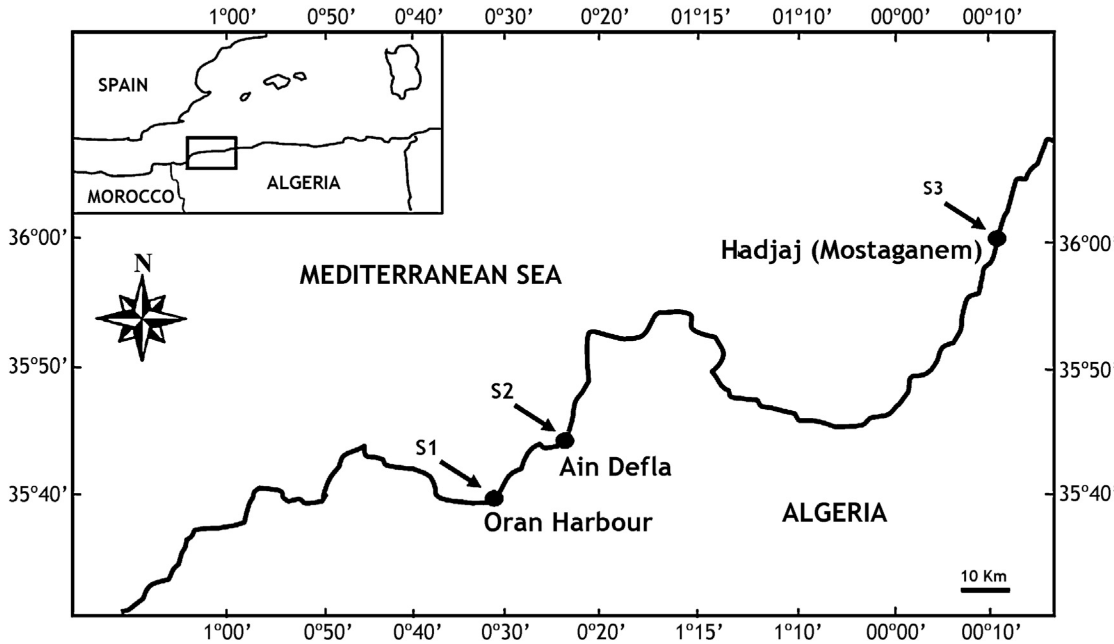

Figure 1 Geographical location of the sampling stations on the western coast of Algeria. Oran Harbor (Site 1), Ain Defla (Site 2) and Hadjaj (Site 3).

Samples of seawater were collected on the surface, using polyethylene bottles, previously washed and rinsed with distilled water. They were then transported in the cold box for storage at +4°C until analysis.

Sampling of mussels was realized according to the techniques recommended by the program QUASIMEME (1992). The specimens were immediately washed at each site with seawater to eliminate encrusted organisms and transported at +4°C in a cold box to the laboratory. Upon arrival, mussels were inspected and dead animals discarded.

Fifty individuals were used for biometric characterization, condition index determination and metal analyses. Shell length (*L*, maximum measure along the anterior-posterior axis), height (*H*, maximum dorsoventral axis), and width (*W*, maximum lateral axis) of each mussel were measured using 0.01 mm precision caliper as described by Fisher et al. (1987).

Each mussel was opened with a stainless steel knife by cutting the adductor muscle and placed with its ventral edge on filter paper to remove the internal water. Then, the individual total wet weight without internal water (TWW) and soft flesh wet weight (FWW) (up to 0.01) were then measured after dissection of each organism.

After taking biometric measurements, the whole soft body tissues of mussels were grouped in ten pools (each pool corresponds to five individuals of similar size) as replicates and dried at 70°C for 48 h for chemical analyses of trace metals.

2.3. Environmental parameters

Basic hydrological parameters of surface coastal waters were monitored simultaneously at the mollusc sampling sites to provide information on the global water quality. The three samples of seawater were collected at each site and season. Temperature, pH, salinity, dissolved oxygen and organic matter were measured using the multiparameter instrument (Multi 340i — WTW). Then samples were immediately filtered using Millipore membrane filter paper and nutrients such as silicate (SiO_4), orthophosphate (PO_4), nitrites (NO_2), nitrates (NO_3) and ammonia (NH_4) were measured by the colorimetric methods according to standard methods recommended for marine waters (Aminot and Chaussepied, 1983; Rodier, 1996).

2.4. Biometric parameters and physiological indices

The ratios height/length (*H*/*L*), width/height (*W*/*H*), width/length (*W*/*L*), and shell size factor (SSF [cm^3] = height × length × width) were calculated (Soto et al., 2000). Condition index (CI) was calculated using the formula CI = (FWW/TWW) × 100 (AFNOR, 1985).

2.5. Metal analysis

The concentration of zinc (Zn), copper (Cu), lead (Pb) and cadmium (Cd) were measured in the whole soft tissues of mussels after hot mineralization of the samples, following the method of Aminot and Chaussepied (1983). Approximately 0.2 g of dry weight was digested in 4 ml of concentrated nitric acid (Merck Suprapure) at 95°C for 1 h. The metal contents in acid solutions were determined by using a flame atomic absorption spectrophotometer equipped with a graphite furnace (Perkin Elmer AAnalyst-100 — Version 1.10). Quality assurance and quality control were assessed by processing blank samples and reference standard material (Mussel Tissue Standard Reference Material SRM 2976, National

Table 1 Certified and measured values of metal concentrations obtained for the standard sample (SRM 2976, IAEA).

	Zn	Cu	Pb	Cd
Measured value [$\mu g\ g^{-1}$ dry wt.]	128 ± 8	3.96 ± 0.08	1.15 ± 0.04	0.78 ± 0.03
Certified value [$\mu g\ g^{-1}$ dry wt.]	137 ± 13	4.02 ± 0.33	1.19 ± 0.18	0.82 ± 0.16

Results are expressed as annual mean ± SE (*n* = 10).

Institute of Standards and Technology). Metal concentrations obtained for standard reference materials were always within the 95% confidence interval of certified values (Table 1).

2.6. Calculation of metal indices

Metal/shell weight index (MSWI) was calculated as a measure of metal bioavailability according to Fischer (1984) and Soto et al. (1997) as follows:

$$\mathrm{MSWI} = \mathrm{MCSB} \times \frac{\mathrm{SFWD}}{\mathrm{SDW}},$$

where MSWI is the metal/shell weight index [μg metal g^{-1} dry shell weight], MCSB the metal concentration in soft body [μg metal g^{-1} dry flesh weight], SFWD the soft flesh dry weight [g] and SDW is the shell dry weight [g].

2.7. Statistical analysis

The data are presented as the mean ± standard error of the mean (mean ± SE). All measured biotic parameters were tested using two-way analysis of variance (ANOVA) in order to detect the site (Si) or season (Se) variability. Significant differences were established at the $p < 0.05$ level using Duncan's test for multiple range comparison between pairs of means. Statistical analysis was performed using the software STATISTICA (Statsoft STATISTICA version 6.1.478.0).

3. Results

3.1. Physico-chemical parameters of seawater

Analysis of physico-chemical parameters of seawater from each of three sites (Oran Harbor (S1), Ain Defla (S2) and Hadjaj (S3)), recorded during the four seasons, were shown in Table 2 as the annual means.

The annual means of pH, temperature, and salinity of surface water obtained for these three sites were similar to those found in the Mediterranean Sea (Borghini et al., 2014; Iorga and Lozier, 1999).

S1 was characterized by significantly higher levels of organic matter (OM), especially in spring (146.67 ± 24.2 mg L^{-1}; $p < 0.05$) and phosphate (PO_4) in winter (0.16 ± 0.01 mg L^{-1};

Table 2 Annual variations of physico-chemical parameters of coastal waters in Oran Harbor (Site 1), Ain Defla (Site 2) and Hadjaj (Site 3).

Parameter	Site 1	Site 2	Site 3
pH	7.76 ± 0.06^{a}	7.89 ± 0.07^{b}	7.91 ± 0.01^{b}
	(7.46–7.96)	(7.57–8.04)	(7.82–7.95)
T [°C]	21.40 ± 1.41^{a}	22.13 ± 1.54^{b}	21.17 ± 1.24^{a}
	(17.17–29.33)	(17.33–30.67)	(16.33–27.67)
Sal [PSU]	37.21 ± 0.19^{a}	36.96 ± 0.19^{ab}	36.75 ± 0.19^{b}
	(36.33–37.67)	(36.17–37.67)	(36.17–37.33)
O_2 [mg L^{-1}]	4.75 ± 0.42^{a}	5.81 ± 0.25^{b}	5.57 ± 0.47^{c}
	(2.33–5.77)	(4.40–6.35)	(2.90–6.58)
NO_3 [mg L^{-1}]	2.04 ± 0.26^{a}	2.25 ± 0.20^{a}	6.25 ± 1.78^{b}
	(1.17–2.67)	(1.33–2.67)	(1.67–16.33)
NO_2 [mg L^{-1}]	0.02 ± 0.01^{ab}	0.02 ± 0.01^{a}	0.03 ± 0.01^{a}
	(0.01–0.04)	(0.01–0.02)	(0.02–0.05)
NH_4 [mg L^{-1}]	0.04 ± 0.01^{a}	0.02 ± 0.01^{b}	0.03 ± 0.01^{b}
	(0.01–0.06)	(0.01–0.05)	(0.02–0.03)
PO_4 [mg L^{-1}]	0.13 ± 0.02^{a}	0.05 ± 0.01^{b}	0.08 ± 0.01^{b}
	(0.09–0.16)	(0.01–0.09)	(0.06–0.10)
SiO_4 [mg L^{-1}]	1.63 ± 0.15^{a}	1.86 ± 0.18^{a}	2.96 ± 0.79^{b}
	(1.10–2.30)	(1.07–2.53)	(1.11–6.60)
OM [mg L^{-1}]	73.79 ± 14.95^{a}	69.08 ± 3.01^{a}	69.50 ± 4.97^{a}
	(7.17–146.67)	(56.00–82.00)	(50.33–79.00)

T: temperature; Sal: salinity; O_2: dissolved oxygen; NO_3: nitrate; NO_2: nitrite; NH_4: ammonia; PO_4: phosphate; SiO_4: silicate; OM: organic matter. Results are expressed as annual mean ± SE (*n* = 12); limit values of seasonal means are in brackets (*n* = 3). For each parameter, different letters (a–c) indicate significant differences (Duncan's test, $p < 0.05$), among sites.

$p < 0.05$). Dissolved oxygen levels obtained in S1 were significantly lower than those in other sites ($p < 0.05$), with a minimum of 2.33 ± 0.11 mg L^{-1} in autumn.

Between S2 and S3, no significant annual variation of physico-chemical parameters was observed. However, the values of NO_3 and SiO_4, measured in S3 reached the highest levels compared to the other sites ($p < 0.05$), especially in winter.

3.2. Metal concentrations in tissues of mussels *Mytilus galloprovincialis*

The annual variations of heavy metal concentrations (Zn, Cu, Pb, Cd) in *Mytilus galloprovincialis*, from the three sites S1, S2 and S3, were shown in Table 3. The annual heavy metal concentrations reached the highest levels in organisms from S1. The bioaccumulation potential of metals in tissues of mussels follows a decreasing sequence of Zn > Pb ≥ Cu > Cd.

Fig. 2 shows significant seasonal variations of metal concentrations. During the winter, higher concentrations of Zn were observed in the mussels from S1 and S2 with 153.86 ± 10.77 μg g^{-1} dry weight and 108.00 ± 7.92 μg g^{-1} dry weight respectively, compared to the other seasons ($p < 0.05$; $n = 10$). No significant seasonal difference was observed for Zn concentration in mussels from S3.

The Cu concentration in the mussels from S1 reached the highest level during the winter (15.69 ± 0.62 μg g^{-1} dry weight; $p < 0.05$; $n = 10$) and the lowest during the autumn (1.15 ± 0.12 μg g^{-1} dry weight; $p < 0.05$; $n = 10$).

The highest Cu concentrations were obtained in mussels from S2 during the spring (7.74 ± 0.43 μg g^{-1} dry weight) and from S3 during the autumn (7.61 ± 0.30 μg g^{-1} dry weight). In both sites, the Cu concentrations were significantly decreased during the summer ($p < 0.05$; $n = 10$).

In the case of Pb concentration, the highest values were obtained during the summer (15.47 ± 0.50 μg g^{-1} dry weight) and autumn (12.45 ± 0.70 μg g^{-1} dry weight) in mussels from S1 and S2 respectively. In S3, Pb concentrations reached maximal values in winter (5.66 ± 0.10 μg g^{-1} dry weight) and summer (5.35 ± 0.19 μg g^{-1} dry weight).

Finally, the Cd concentrations were decreased during the summer whatever the site. However, the highest Cd concentrations were found during the winter (0.82 ± 0.08 μg g^{-1} dry weight) in mussels from S1 and during the spring in organisms from S2 (0.67 ± 0.01 μg g^{-1} dry weight) and S3 (0.74 ± 0.07 μg g^{-1} dry weight).

The annual mean concentrations of heavy metals in mussels obtained in the present study were compared with other literature data in Table 4. We observe that the concentrations of Zn and Cd found in our mussel samples were lower than those from other regions of the Mediterranean. Cu concentrations were similar to those obtained in mussels from Izmir Bay (Kucuksezgin et al., 2008) and the Balearic Islands (Deudero et al., 2009). In this present study, the Pb concentrations were much higher than those reported by other authors (Table 4).

3.3. Metal indices in mussels *Mytilus galloprovincialis*

The annual variations of metal/shell-weight indices in *Mytilus galloprovincialis*, from the three sites S1, S2 and S3, were shown in Table 5. The lowest annual metal/shell-weight indices were obtained whatever the metal in organisms from S3. Whatever the site, the decreasing sequence of indices was as follows: Zn/shell-weight > Pb/shell-weight ≥ Cu/shell-weight > Cd/shell-weight.

The significant differences were observed between seasons whatever the site (Fig. 3). The Zn/shell-weight index obtained at S1 (13.68 ± 0.96 μg g^{-1} dry shell weight) and S2 (10.00 ± 0.73 μg g^{-1} dry shell weight) were significantly higher ($p < 0.05$) during the winter than during the other seasons. In S3, the lowest level (04.34 ± 0.12 μg g^{-1} dry shell weight) was obtained in autumn.

The Cu/shell-weight index in the mussels from S1 reached the highest level during the winter (1.39 ± 0.05 μg g^{-1} dry shell weight; $p < 0.05$) and the lowest during the autumn (0.05 ± 0.01 μg g^{-1} dry shell weight; $p < 0.05$). This index decreased significantly ($p < 0.05$) at S2 (0.06 ± 0.01 μg g^{-1} dry shell weight) and S3 (0.12 ± 0.01 μg g^{-1} dry shell weight) during the summer. The highest levels were obtained in mussels from S3 in winter (0.51 ± 0.05 μg g^{-1} dry shell weight) and autumn (0.45 ± 0.02 μg g^{-1} dry shell weight), but in spring only in mussels from S2 (0.59 ± 0.03 μg g^{-1} dry shell weight).

The Pb/shell-weight index at S1 was significantly higher ($p < 0.05$) during the summer (0.60 ± 0.02 μg g^{-1} dry shell

Table 3 Annual variations of heavy metal concentrations in mussels, *Mytilus galloprovincialis*, from Oran Harbor (Site 1), Ain Defla (Site 2) and Hadjaj (Site 3).

Metal concentration	Site 1	Site 2	Site 3
Zn [μg g^{-1} dry wt.]	95.93 ± 6.39^{a}	91.14 ± 3.18^{a}	76.85 ± 1.28^{b}
	(71.61–153.86)	(81.22–108.00)	(73.53–80.10)
Cu [μg g^{-1} dry wt.]	7.21 ± 0.94^{a}	3.91 ± 0.50^{b}	4.60 ± 0.42^{c}
	(1.15–15.69)	(1.00–7.74)	(1.75–7.61)
Pb [μg g^{-1} dry wt.]	9.63 ± 0.73^{a}	8.34 ± 0.49^{b}	4.51 ± 0.21^{c}
	(5.65–15.47)	(6.26–12.45)	(3.10–5.66)
Cd [μg g^{-1} dry wt.]	0.67 ± 0.03^{a}	0.59 ± 0.02^{b}	0.60 ± 0.02^{b}
	(0.55–0.82)	(0.52–0.67)	(0.50–0.74)

Results are expressed as annual mean ± SE ($n = 40$); limit values of seasonal means are in brackets ($n = 10$). For each parameter, different letters (a–c) indicate significant differences (Duncan's test, $p < 0.05$), among sites.

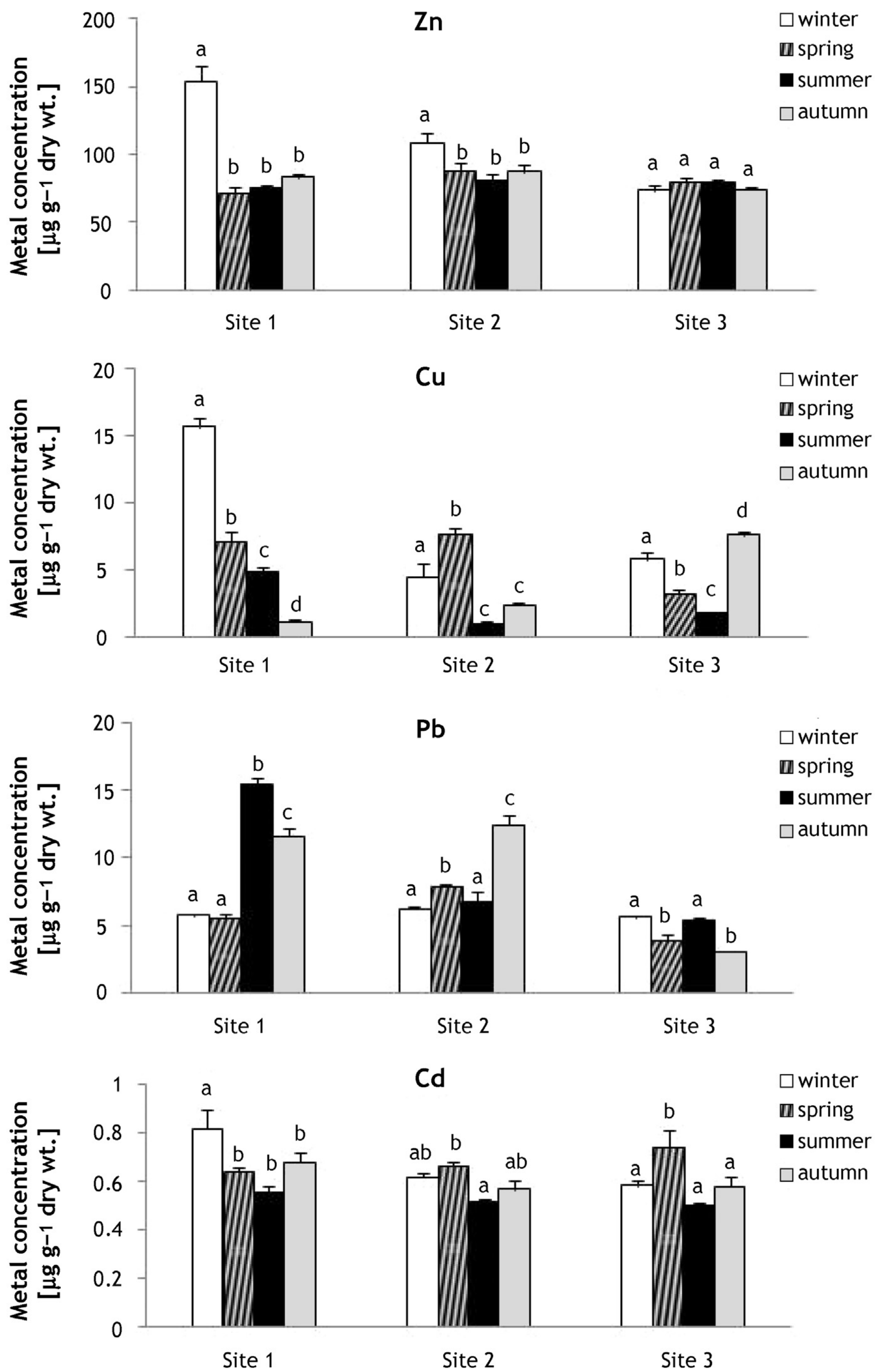

Figure 2 Seasonal variations of heavy metal concentrations in mussels, *Mytilus galloprovincialis*, from Oran Harbor (Site 1), Ain Defla (Site 2) and Hadjaj (Site 3). Results are expressed as seasonal mean ± SE (n = 10). For each parameter, different letters indicate significant differences (Duncan's test, $p < 0.05$), among seasons, for each site.

weight) than during the other seasons. This index was significantly increased ($p < 0.05$) during the autumn (0.75 ± 0.04 µg g^{-1} dry shell weight) at S2 and winter (0.49 ± 0.01 µg g^{-1} dry shell weight) at S3.

The Cd/shell-weight index reached the highest level ($p < 0.05$) during the winter and the lowest ($p < 0.05$) during the summer, whatever the site.

3.4. Biometric parameters and physiological indices of mussels *Mytilus galloprovincialis*

The seasonal variations of biometric parameters and condition index (CI) of mussels, *Mytilus galloprovincialis*, from the three sites (S1, S2 and S3) were presented in Table 6.

Table 4 Annual mean concentrations of heavy metal in mussels, *Mytilus galloprovincialis*, from different Mediterranean areas.

Location	Zn [μg g^{-1} dry wt.]	Cu [μg g^{-1} dry wt.]	Pb [μg g^{-1} dry wt.]	Cd [μg g^{-1} dry wt.]	References
Marmara Sea	264.13	7.66	1.36	1.73	Topcuoğlu et al. (2004)
Izmir Bay (Aegean Sea)	27.70	4.48	0.24	0.02	Kucuksezgin et al., 2008
Balearic Islands (Western Mediterranean)	234.16	4.76	2.48	2.83	Deudero et al. (2009)
Croatian Coasts (Adriatic Sea)	158.00	10.53	4.21	0.99	Kljaković-Gaspić et al. (2010)
Apulian Coasts (Adriatic Sea)	75.27	8.17	1.50	0.89	Spada et al., 2013
Algerian west coast	87.98	5.22	7.49	0.66	Present study[a]

[a] Each value from the present study corresponds to the annual mean concentration of heavy metal and is expressed as the annual mean of concentration of heavy metal ± SE (n = 120).

Table 5 Annual variations of metal/shell-weight indices in mussels *Mytilus galloprovincialis* from Oran Harbor (Site 1), Ain Defla (Site 2) and Hadjaj (Site 3).

Metal index	Site 1	Site 2	Site 3
Zn/shell-weight index [μg metal g^{-1} dry shell wt.]	6.10 ± 0.78^{a} (2.89–13.68)	6.82 ± 0.39^{b} (5.22–10.00)	5.54 ± 0.16^{a} (4.34–6.42)
Cu/shell-weight index [μg metal g^{-1} dry shell wt.]	0.51 ± 0.09^{a} (0.05–1.39)	0.30 ± 0.04^{b} (0.06–0.59)	0.33 ± 0.03^{b} (0.12–0.51)
Pb/shell-weight index [μg metal g^{-1} dry shell wt.]	0.49 ± 0.02^{a} (0.33–0.60)	0.59 ± 0.03^{b} (0.43–0.75)	0.33 ± 0.03^{c} (0.18–0.49)
Cd/shell-weight index [μg metal g^{-1} dry shell wt.]	0.04 ± 0.01^{a} (0.02–0.07)	0.04 ± 0.01^{a} (0.03–0.06)	0.04 ± 0.01^{a} (0.03–0.05)

Results are expressed as annual mean ± SE (n = 40); limit values of seasonal means are in brackets (n = 10). For each parameter, different letters (a–c) indicate significant differences (Duncan's test, $p < 0.05$), among sites.

The values of morphometric parameters (length, height, and width), biometric ratios (H/L, W/H and W/L) and SSF were significantly higher ($p < 0.05$) in mussels from S1 than those obtained in organisms from S2 and S3.

The mean value of CI of mussels from S1 was also higher than those of organisms from S2 and S3. However, for mussels from S1, the CI changed throughout the year depending on the season, with a significant increase in spring (39.18 ± 0.87) and decrease in winter (24.28 ± 0.75) ($p < 0.05$); the significant opposite tendency was observed for mussels from S2.

In mussels from S3, the CI decreases significantly during the summer (22.56 ± 0.79) and reached the highest level during the winter with 35.17 ± 0.63 ($p < 0.05$).

4. Discussion

Mussels are known as bioindicator organisms for monitoring chemical pollutants including trace metals, due to their ability to accumulate and concentrate pollutants in their tissues. This work presents the influence of seasons on metal bioavailability and bioaccumulation in indigenous mussels *Mytilus galloprovincialis*, issued from three distinct sites of the Algerian west coast. Besides, the variations of biometric parameters and condition index (CI), considered as integrated indicators of physiological conditions of organisms, were also measured. These biological indicators reflect the effects of pollution and the quality of the environment that organisms were exposed to throughout their development (Dame, 1996; Orban et al., 2002; Sukhotin and Maximovich, 1994). This response may be influenced by both seasons and sites.

In this context, the seasonal variations of Cu, Zn, Cd and Pb bioavailability and bioaccumulation in mussels, collected from the three sites, were assessed. The annual metal concentrations and indices in mussels, corresponding to the potential metal bioaccumulation and bioavailability respectively, were higher at S1 and S2 (Oran Coast) than at S3 (the Mostaganem coast), with the following decreasing sequence: Zn > Pb ≥ Cu > Cd. The metal bioavailability was significantly correlated with the bioaccumulation, whatever the season and site. The seasonal changes in the various metal concentration in mussel tissues could result from a combination of factors directly correlated to the weight of animals (cycle of reproduction, metabolism, development and age, food availability, temperature), and with others, more independent, such as salinity, modification of biogeochemical cycle and bioavailability of metals related to the site (Kamaruzzaman et al., 2011; Sara and Pusceddu, 2008). The physicochemical characteristics of water, which vary according to seasons and sites, modulate the bioavailability of pollutants and therefore influence both their bioaccumulation and the biological responses of organisms to these pollutants. Moreover, water contamination, varying from season to

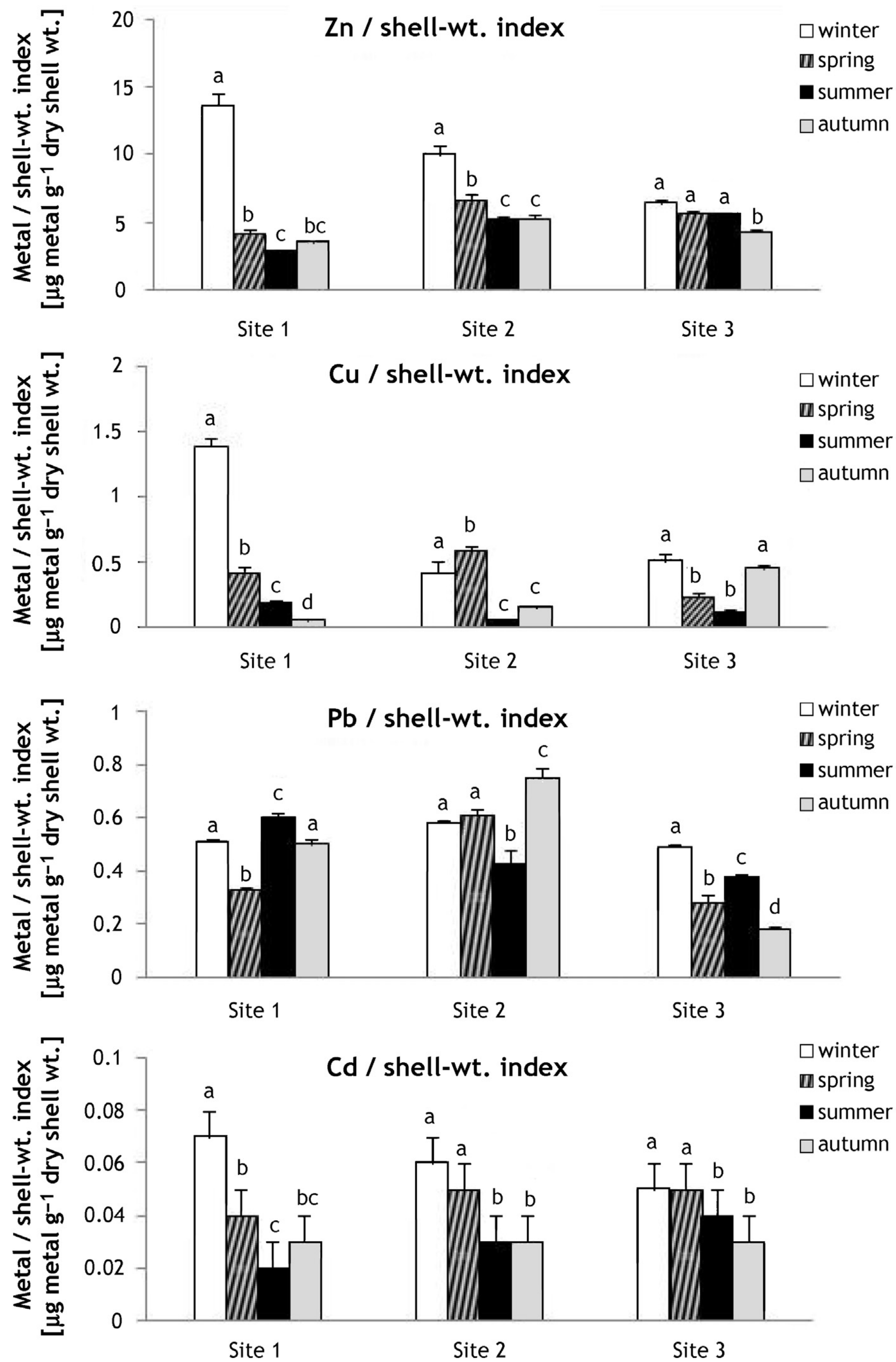

Figure 3 Seasonal variations of metal/shell-weight indices in mussels, *Mytilus galloprovincialis*, from Oran Harbor (Site 1), Ain Defla (Site 2) and Hadjaj (Site 3). Results are expressed as seasonal mean ± SE ($n = 10$). For each parameter, different letters indicate significant differences (Duncan's test, $p < 0.05$), among seasons, for each site.

season and by site, depending on contaminant type and concentration, could be considered as one possible cause of the seasonal variation of metal bioavailability and bioaccumulation in mussel tissues.

For Oran Harbor (S1), whereas the highest Zn, Cu and Cd concentrations in mussel tissues were obtained in winter, the lowest were found in spring and summer. These results are in line with few studies demonstrating the influence of seasons on metal accumulation in mussels (Langston and Spence, 1995; Mikac et al., 1996). Some authors observed in different species of filter-feeding shellfish that the peaks of metal accumulation might be reached in winter and the lowest levels in summer (Chafik et al., 2001; Goldberg et al., 1983; Odzak, 2002). The opposite tendency occurred for Pb. The strong Pb accumulation in summer could be related to an increase of urban population during this period, leading to

Table 6 Seasonal variations of biometric parameters and condition index of mussels, *Mytilus galloprovincialis*, from Oran Harbor (Site 1), Ain Defla (Site 2) and Hadjaj (Site 3).

	L [cm]	*H* [cm]	*W* [cm]	*H/L*	*W/H*	*W/L*	SSF [cm^3]	CI
Site 1								
Winter	3.73 ± 0.06^{aA}	1.90 ± 0.03^{aA}	1.18 ± 0.03^{aA}	0.51 ± 0.01^{aA}	0.62 ± 0.01^{aA}	0.32 ± 0.01^{aA}	8.60 ± 0.41^{aA}	24.28 ± 0.75^{aA}
Spring	4.29 ± 0.13^{bA}	2.13 ± 0.07^{bA}	1.43 ± 0.05^{bcA}	0.50 ± 0.01^{aA}	0.67 ± 0.01^{abA}	0.33 ± 0.01^{abA}	15.02 ± 1.50^{bA}	39.18 ± 0.87^{bA}
Summer	4.40 ± 0.11^{bA}	2.19 ± 0.06^{bA}	1.51 ± 0.05^{bA}	0.50 ± 0.01^{aA}	0.69 ± 0.01^{bA}	0.34 ± 0.01^{bA}	16.02 ± 1.26^{bA}	37.14 ± 0.62^{bA}
Autumn	4.06 ± 0.08^{cA}	1.96 ± 0.05^{aA}	1.41 ± 0.04^{cA}	0.48 ± 0.01^{bA}	0.73 ± 0.02^{bA}	0.35 ± 0.01^{bA}	11.91 ± 0.79^{aA}	34.91 ± 0.77^{cA}
Site 2								
Winter	3.22 ± 0.03^{aB}	1.41 ± 0.04^{aB}	0.99 ± 0.02^{aB}	0.44 ± 0.01^{aB}	0.73 ± 0.02^{aB}	0.31 ± 0.01^{aA}	4.51 ± 0.17^{aB}	33.10 ± 1.34^{aB}
Spring	3.61 ± 0.05^{bB}	1.72 ± 0.03^{bB}	1.03 ± 0.02^{aB}	0.48 ± 0.01^{bA}	0.60 ± 0.02^{bA}	0.28 ± 0.01^{bB}	6.63 ± 0.36^{bB}	24.57 ± 0.62^{bB}
Summer	3.78 ± 0.06^{bB}	1.84 ± 0.05^{bB}	1.12 ± 0.02^{bB}	0.49 ± 0.01^{bA}	0.62 ± 0.01^{bA}	0.30 ± 0.01^{aB}	7.98 ± 0.37^{cB}	26.26 ± 0.61^{bB}
Autumn	3.79 ± 0.06^{bB}	1.77 ± 0.06^{bB}	1.07 ± 0.02^{aB}	0.47 ± 0.01^{bA}	0.62 ± 0.01^{bB}	0.28 ± 0.01^{bB}	7.36 ± 0.39^{bcB}	29.24 ± 0.52^{cB}
Site 3								
Winter	3.59 ± 0.05^{aA}	1.60 ± 0.02^{aC}	1.01 ± 0.02^{aB}	0.45 ± 0.01^{aB}	0.63 ± 0.01^{aC}	0.28 ± 0.01^{aB}	5.91 ± 0.24^{aB}	35.17 ± 0.63^{aB}
Spring	4.02 ± 0.07^{bC}	1.69 ± 0.04^{aB}	1.14 ± 0.03^{bC}	0.42 ± 0.01^{bB}	0.69 ± 0.02^{aA}	0.28 ± 0.01^{aB}	8.10 ± 0.74^{bB}	34.17 ± 1.61^{acC}
Summer	3.90 ± 0.06^{bB}	1.59 ± 0.03^{aC}	1.12 ± 0.03^{bB}	0.41 ± 0.01^{bB}	0.70 ± 0.02^{aA}	0.29 ± 0.01^{aB}	7.16 ± 0.37^{bB}	22.56 ± 0.79^{bC}
Autumn	3.95 ± 0.06^{bAB}	1.68 ± 0.04^{aB}	1.09 ± 0.03^{aB}	0.42 ± 0.01^{abB}	0.72 ± 0.08^{aA}	0.27 ± 0.01^{aB}	7.50 ± 0.43^{bB}	32.13 ± 0.57^{cC}

Results are expressed as seasonal mean ± SE ($n = 50$). For each parameter: different small letters (a—c) indicate significant differences (Duncan's test, $p < 0.05$), among seasons, for each site; different capital letters (A—C) indicate significant differences (Duncan's test, $p < 0.05$), among sites, for each season.

higher urban wastewater discharges and atmospheric Pb levels, due to a more intense traffic. In the latter case, the contamination could be both direct by the atmospheric deposition and indirect by the leaching of roads by rainwaters (Goody et al., 1995). However, it should be noted that this increase in the bioaccumulation of Pb could be due to the increase in temperature during this summer period. Indeed, bioaccumulation of heavy metals in aquatic organisms such as mussels depends not only on environmental concentrations but also on a variety of biological and environmental factors (Mubiana and Blust, 2007). Temperature affects both metal chemistry in seawater (Byrne et al., 1988) and physiology of mussels (Dame, 1996). Temperature affects metal chemistry by changing chemical speciation, pH, solubility, reaction rates or physical kinetics (Blust et al., 1994; Byrne et al., 1988). Chemical speciation indicates that an increase in temperature generally results in an increase in the concentrations and activities of bioavailable metal forms, therefore, enhancing uptake, at least in theory.

The enrichment in metallic elements (Zn, Cu, Cd) in mussel tissues collected in winter may result from the inputs of urban and industrial sewage of Oran city, which discharge at the two main outfalls of harbor. Mussels are characterized by their ability to filter very large volumes of water (between 0.2 and 5 L h^{-1}) to satisfy their nutritional and respiratory requirements (Monfort, 2006). Thus, these bivalves are able to accumulate in their tissues high levels of contaminants such as heavy metals, present in their environment, without lethal effects (Prueli et al., 1987). In addition, the Algerian west coast remains under the direct influence of an important shipping traffic because of the presence of one of the largest Algerian harbors, the Oran Harbor (Grimes et al., 2010). In this context, various harbor and vessel maintenance activities (antifouling paints, wood preservatives, etc.) may contribute to metal contamination (Cu, Sn, As, Zn, Hg, Ni, Cd, etc.) of the surrounding waters. The large amount of Zn found in mussel tissues, in the present study, may reflect the high Zn levels found in port and marina waters, because of the leaching from the antifouling paints and anodes used to reduce corrosion of boat hulls (AERMC, 1997). Similar high Zn values in bivalve molluscs were already observed by other authors (Boutier, 1982; Chafik et al., 2001). These results may be explained by the fact that (i) mussels could be contaminated by dissolved and suspended forms of Zn present in the water (Wang et al., 1996); (ii) Zn could be involved and regulated in many biological processes in mussels (Pyatt et al., 2003). This essential metal was also shown to be toxic in *Mytilus edulis* when its concentration exceeded a certain threshold, according to Poham and D'Auria (1982).

As observed in the present work, the concentration of heavy metals in mussels from S1 reached a maximum level before the reproduction period (during the cold season, corresponding to a period of high storage of energy reserves) and a minimum after spawning (mainly in summer, after the body reserves have been depleted due to the reproduction). The same tendency was observed by Radenac et al. (1997) in *Mytilus edulis*. Other works reported that the abundance of stored energetic reserves in marine bivalves varied seasonally in accordance with reproductive cycle, development and growth (Cossa et al., 1980; Goldberg et al., 1983; Okumus and Stirling, 1998), and that the reproduction of *Mytilus galloprovincialis* occurred between the end of spring and the beginning of the summer, on the Algerian coast (Abada, 1996). The latter studies are in accordance with the seasonal variations of metal bioaccumulation found in the present work.

Morphometric parameters (length, height, width), SSF and CI in mussels from S1 increased in all seasons except in winter. The environmental conditions in spring and summer were more favorable (e.g. high level of organic matter and food availability, low metal levels in tissues) for the physiological development and growth of mussels than in cold seasons.

In winter, one possible cause of the significant reduction of mussel growth could be the reduction of the energy flow throughout the body of animals, consumed for the detoxification mechanisms, triggered by metal contamination in bivalve molluscs. This hypothesis is in accordance with studies described by other authors (Manley et al., 1984; Nielsen and Strömgren, 1991).

The highest growth observed in mussels from S1 in spring may be related to the high load of organic matter during this season, constituting an important nutrient input for the organisms. The organic matter could come from the urban and industrial wastewaters of Oran city, which are discharged untreated by the sewage outfalls located on either sides of the port. Previous biomonitoring studies in Algerian coastal waters had demonstrated that the urban and industrial wastewaters constituted the main sources of organic matter (Grimes et al., 2010). In addition, high levels of phosphates, found in the waters of S1, were below the Algerian (50 mg L^{-1}) (OJAR, 2009) and European standards (1–2 mg L^{-1}) (UE, 1995). These high levels could be due to the presence of detergents into the urban sewage discharged directly into the marine environment. Phosphate might be one of the nutrients likely promoting the growth of planktonic species according to Dufour and Merles (1972). Other studies showed that the mussel growth could depend on various biotic and abiotic factors such as substrate composition, hydrodynamic conditions, physical characteristics of habitat and food concentration (Devescovi, 2009; Galinou-Mitsoudi and Sinis, 1997). Other authors reported that the growth of filter-feeding shellfish could depend on the availability of food in the water, expressed as the amount of suspended particulate organic matter (Bayne et al., 1989; Dame, 1996; Sara and Pusceddu, 2008).

For Ain Defla (S2), the metal bioavailability was significantly correlated with the metal bioaccumulation, whatever the season. Indeed, the metal concentrations and indices varied whatever the seasons, except in summer where the values were the lowest.

The low metal bioavailability and bioaccumulation in organisms from S2 in summer might be correlated to the reproductive period as described previously in S1. According to other authors, during this period, the sensitivity of mussels to anthropogenic or natural stressors might increase, as organisms devoted more energy to reproduction than to growth and defense mechanisms (Blaise et al., 2002; Bodin et al., 2004; Guerlet et al., 2007). It was also shown that gametogenesis in mussels caused high respiration rates (Hartl et al., 2004) and therefore an increase of oxygen consumption (de Vooys, 1976), leading to the generation of reactive oxygen species (ROS) (Rajagopal et al., 2005). These changes in the mussel metabolism might lead to the increase of the organism sensitivity, and consequently to various biological perturbation and damage (Bigot et al., 2009; Verlecar et al., 2007). It is noteworthy that both biotic (e.g. reproductive cycle) and abiotic factors (e.g. temperature, chemical contaminants) could affect normal growth rates, food uptake and homeostasis of bivalve molluscs as well as their response to various environmental stressors (Cossa et al., 1979; Davies and Pirie, 1980; Phillips, 1976). Thus, in spring and summer, the lowest biological indices were obtained in mussels from S2, related both to the presence of various pollutants, high temperatures and reproductive status. In this rural area, the chemical pollution may come mainly from the discharges of domestic sewage from villages and also from the polluted runoff waters from agricultural land. Most chemicals used in agriculture, either pesticides or fertilizers, can be found in the marine waters considered as ultimate receptacle of the pollution. Moreover, the indigenous mussel population from S2, characterized by a limited growth and a reduced physiological state, could be also affected by the oligotroph waters known in this area.

In mussel tissues from S2, high levels of heavy metals, especially for Zn and Pb, were obtained in winter and autumn. The metallic indices followed the same variations. The metal accumulation patterns could differ depending on the waterborne and dietary exposure level. Moreover, Zn and Pb provided a good example of the possible increase of the metal concentration due to changes in biomass. Indeed, body metal concentrations increased when a decrease in metal assimilation was offset by a decrease in weight. More detailed studies on mussels of different length demonstrated that the concentrations of certain metals were higher in the smallest mussels within a population, size being measured as the total dry weight of the individual mussels (Boyden, 1977; Cossa et al., 1980). These authors observed that the negative correlations between metal concentrations in mussels and percentage dry weight reflected the percentage dry matter contents of these mussel populations rather than the broader effect of total weight. The highest biological indices obtained for mussels from S2, during the cold seasons, could give a valuable indication of the mussel physiological condition. These organisms might be in good condition. Indeed, mussels might be able to invest more energy in metal detoxification. The metal detoxification and maintenance of detoxification mechanisms (e.g. metallothionein synthesis, metal-rich granule formation) might be energetically expensive. In addition, biological factors such as genetic background (Knapen et al., 2004), size (Wallace et al., 2003), organism physiological conditions, or uptake route (Ng et al., 2007) might be involved in the metal detoxification capacity.

Hadjaj site (S3), considered as a "pristine" area, showed annual low metal indices and concentrations in mussel tissues and high CI levels, reflecting more favorable physiological conditions for the mussel growth, than those found in the other sites.

In S3, the CI level of mussels significantly increased between autumn and spring and reached the lowest level in summer. During summer, according to the spawning period of *Mytilus galloprovincialis*, the mussel growth fell down because of the reproductive investment. Some authors reported that in the spawning period, shellfish lost a large amount of nutrient reserves (Cossa et al., 1980). Besides, the environmental conditions such as food availability appeared particularly more favorable for the growth of bivalves during the cold period. Thus, a previous study of the Algerian coastal area showed an increase in the planktonic biomass in autumn (Lalami-Taleb, 1971). In the present work, the waters from S3 sampled at few kilometers from the estuary of Chelliff during the flood period could be enriched by nutrients coming from the estuary waters and transported along the shore currents. This hypothesis is supported by the elevated levels of NO_3 and SiO_4 obtained in the waters of S3 during the cold season. Moreover, the high load of silicates found in the marine waters (compared to the other sites) might confirm the mixture of the river freshwaters to seawater, as suggested by Casas and Bacherb (2006).

5. Conclusion highlights

This study highlights the accumulated metal concentrations measured in the tissues of indigenous mussels, collected from the three sites of Algerian west coast, vary according to the seasonal changes, with the influence of seasonal fluctuations of biotic (e.g. age, soft body weight, reproductive activity) and abiotic factors (e.g. seawater physicochemical characteristics, water contaminants, food supply), which influence baseline physiological processes in mussels and their vulnerability to various environmental stressors.

Besides, the present study also shows that the morphometric parameters and condition index, assessed in these mussels, under field conditions, were not always related to the metal concentrations accumulated in the mussel tissues.

Indeed, other factors than metal pollution might be more important in determining the physiological condition of the mussels. Schneider (1992) showed the influence of temperature and food availability, on condition index levels for zebra mussels, in the Great Lakes. Moreover, the rate at which mussels recovered a normal physiological status might depend on the differences in the food availability, physicochemical characteristics of the water, and the genetic background of mussel populations, in relation to the site and the season.

For future studies, when long-term effects of pollutants are monitored, the sampling of indigenous mussels should be preferred to that of transplanted ones. Indeed, the native population living in chronically contaminated areas may have developed adaptative mechanisms resulting in reduced susceptibility (at level of certain biological responses) to environmental stressors. Thus, any significant seasonal variation of metal concentration accumulated in mussels may be related to the level of pollution in the monitored site during the season.

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