Environmental Science and Pollution Research (2022) 29:16103–16112
https://doi.org/10.1007/s11356-021-16893-w

RESEARCH ARTICLE

Seasonal heavy metal accumulations in the bivalve *Barbatia decussate* and their relationships with water quality and the metal-induced biochemical biomarkers

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Received: 14 November 2020 / Accepted: 1 October 2021 / Published online: 13 October 2021

Abstract
Seasonal tissue concentrations ofheavy metals, antioxidant enzymes, immunological components, and water quality parameters were investigated during1 year in the ark clam, *Barbatia decussate*, from the coast of Lengeh port, locatedin the north of the Persian Gulf, Iran. The tissue accumulation of the heavy metals (Cd, Pb, Hg) significantlyincreased accumulations in late autumn and winter ($P<0.01$). Theconcentrations of Ni andCr nearly remained unchanged throughout the 1 year sampling period ($P>0.01$).Seasonal changes were also observed in metal-induced biochemical components. In this regard, the malondialdehyde (MDA)levels and the activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) elevatedthroughout spring and summer and then declined during autumn and winter ($P<0.01$). Phagocytosis activity significantly decreased from December to February and then increased from March toSeptember ($P<0.01$). Total hemocyte counts decreased from October to March andthen elevated until April ($P<0.01$). Significant relationships were foundbetween tissue heavy metal concentrations, water quality parameters, and biochemical components ($P<0.01$). The negative correlations were O_2 vs. antioxidant enzymes,phagocytosis, and total counts of the hemocytes (THCs); pH vs. SOD; salinity vs. Cr; and temperature vs. GPx and Ni.Positive correlations were O_2 vs. Cd, Pb, Hg, and Ni; temperature vs. phagocytosis and THCs;and turbidity vs. phagocytosis, THCs, CAT, and GPx. The results of the present study showed a seasonal pattern in theaccumulation of heavy metals, with maximum levels in winter for the ark clam, *B. decussate*. Furthermore, antioxidant defense and immunity of *B. decussate* arereduced during winter, which may make *B. decussate* susceptible to diseases.

Keywords Pollution · Marine · Bivalve · Heavy metal · Season

Introduction

Bivalves are a branch of marine mollusks, which are known as bioindicators of marine pollution due to their filter-feeding behavior (Sarkar et al. 1994; Boening 1999; Yusof et al. 2004; Nilin et al. 2012; Zuykov et al. 2013). Owing to this fact, bivalves are able to accumulate various types of pollutants such as aromatic hydrocarbons, crude oil, pesticides, and heavy metals (Sbriz et al. 1998; Boening 1999; Damásio et al. 2010; Zuykov et al. 2013). Metals due to some properties, especially toxicity, extensive resources, stability, and bioaccumulation, are considered as a serious problem in the marine environments (Ansari et al. 2004; Boran and Altınok 2010; Wang et al. 2013; Mostofi 2018). Industrial wastewater, agricultural drainage, transportation, combustion of fossil fuels, weathering, and erosion of rocks and soil are among the sources of heavy metals (HEMs), entering into the aqueous bodies (Windom 1992; Jayaraju et al. 2009; Al Naggar et al. 2018; Bat and Özkan 2019, Dmytro 2020). Several studies have reported the adverse impacts of heavy metals on sea life, in particular, bivalves (Naimo 1995; Jakimska et al. 2011). It is well recognized that the toxicity induced by heavy metals could impair immunity (Sheir 2010; Rault et al. 2013; Ivanina et al. 2016), hematology (Cheng and Sullivan 1984; Bindya Bhargavan and Mohammed Salih 2008), feeding (Anandraj et al. 2002; Liu et al. 2014), metabolism (Devi 1996; Faubelet al. 2008), and osmoregulation (Gregory et al. 1999) of bivalves.

There are some studies reporting the seasonal variations in the accumulation rate of HEMs in the bivalves (Lakshmanan and Nambisan 1983; Cain and Luoma 1986; Beldi

Responsible Editor: V.V.S.S. Sarma

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et al. 2006; Góngora-Gómez et al. 2018). Salinity and temperature changes have been introduced as the main factors affecting the seasonal-dependent variations in metal bioaccumulations. The relationships between the seasonal accumulation of HEMs and the immune system of bivalve have been the focus of several studies (Solé et al. 1995; Sheehan and Power 1999; Wilhelm Filho et al. 2001; Lau et al. 2004; Manduzio et al. 2004; Bocchetti and Regoli 2006; Nahrgang et al. 2013). The Persian Gulf is one of the vital and high-traffic waterways in the world for oil transit (Din 1990). Massive oil spills from tankers during the Persian Gulf War, extraction and transportation of oil, shipping, the existence of industries and coastal facilities, etc. have made this waterway one of the most polluted marine areas (Mafi-Gholami et al. 2019). In this regard, the northern parts of the gulf are also more affected by pollutants due to shallow depth, limited water circulation, salinity, and high temperatures.

The ark clam, *Barbatia decussate*, is one of the filter-feeder sessile bivalves of the Persian Gulf that lives on the rocky beds of intertidal zones. In the present work, the seasonal changes of heavy metal accumulations and immunity components (antioxidant enzymes, hemocyte counts, and phagocytosis) in the ark clam, *Barbatia decussate*, as well as water quality parameters were investigated to identify the heavy metal–induced biomarkers and their changes with heavy metal accumulation rate and environmental conditions. For the first time, the results of this study can provide physiologically and ecologically useful information about the seasonal change pattern of heavy metal accumulation and environmental conditions with biochemical changes in the ark clam.

Materials and methods

Collection of clam specimens

The ark clam (*B. decussate*) specimens were collected monthly from tidal regions of the 4 stations along the coast of Lengeh port, located in the north of the Persian Gulf, for 1 year. The sampling stations were station 1 (Kong) (26° 36′ 12″ N, 54° 56′ 46″ E), station 2 (Lengeh) (26° 33′ 29″ N, 54° 53′ 12″ E), station 3 (Shenas) (26° 31′ 27″ N, 54° 47′ 37″ E), and station 4 (Bustaneh) (26° 30′ 30″ N, 54° 39′ 42″ E) (Fig. 1). From each station, the sampling was conducted according to the method described by Zeinalipour et al. (2015). In total, 240 ark clams (mean shell length 26 ± 5.5 mm) were collected from each station with a monthly average of 20 clams per month. The clams were transferred to the lab in plastic bags labeled with the date and place of collection. In the lab, the clams were placed in seawater for 24 h to fully evacuate their digestive tract and to remove wastes from the shells as much as possible. Then, the biometric characteristics (length and weight) were measured, and the specimens were dissected to remove the gill, soft tissue, and digestive gland. The tissues were kept at − 20 °C till chemical analysis.

In addition, the water quality parameters were monthly measured in each sampling station as follows: pH and temperature by a pH meter (Model 6 APX15/C, WTW-330i), salinity by a salinometer (DEMETRA model TM-30D, Japan), dissolved oxygen by an oxygen meter (Model Oxy-Gouard, Water Management Technologies, Inc., USA), and turbidity by a Secchi disk.

Heavy metal assays

The metal concentration was assayed in soft tissue samples of the clams. The assay was conducted according to the method of Vinodhini and Narayanan (2008) with little modifications. In this regard, 1-g tissue samples were chemically digested in ultrapure concentrated nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) (1:1 v/v) within 25-mL flasks, heated to 130 °C, cooled for 2 h, diluted by distilled water, and finally filtered by a 0.45-µm nitrocellulose membrane filter. All metals (Cd, Pb, Ni, Hg, Cr) were assayed by atomic absorption spectrophotometer (Shimadzu AA 6200). All reagents were provided by *Sigma-Aldrich* Company (USA), and the assay procedures were conducted according to the manufacturer's instructions.

Biochemical assays

The antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were assayed in the digestive gland of the clams. Also, the gill tissues were sampled to determine the malondialdehyde(MDA) levels.

For antioxidant enzyme assays, the digestive glands were pre-prepared, as described by Niyogi et al. (2001) with some modifications. Briefly, the tissue samples (2 g) were immediately homogenized in 4 volumes of ice-cold 10 mM Tris_HCl buffer (pH 7.6) and centrifuged (13,000 *g* for 25 min at 4 °C) to collect the supernatants. The supernatants were filtered (Buchner funnel), the resulting liquids were centrifuged (10,000 *g* for 5 min) again, and the supernatants were finally stored at − 20 °C till enzyme assays.

The SOD was determined through reacting xanthine with WST-1 [(2–(4-iodophenyl)-3–(4-nitrophenyl)-5–(2,4-disulfophenyl)-2Htetrazolium, monosodium salt)] and subsequent reduction of superoxide anion (O_2^-). The red formazan dye generated during the reaction was measured at 505 nm, and the one unit of SOD activity was estimated as the amount of enzyme that caused a 50% decrease in the rate of WST-1 reduction (Sigma, St. Louis, MO, USA).

The GPX levels were assayed through the oxidation of NADPH in the presence of glutathione reductase and

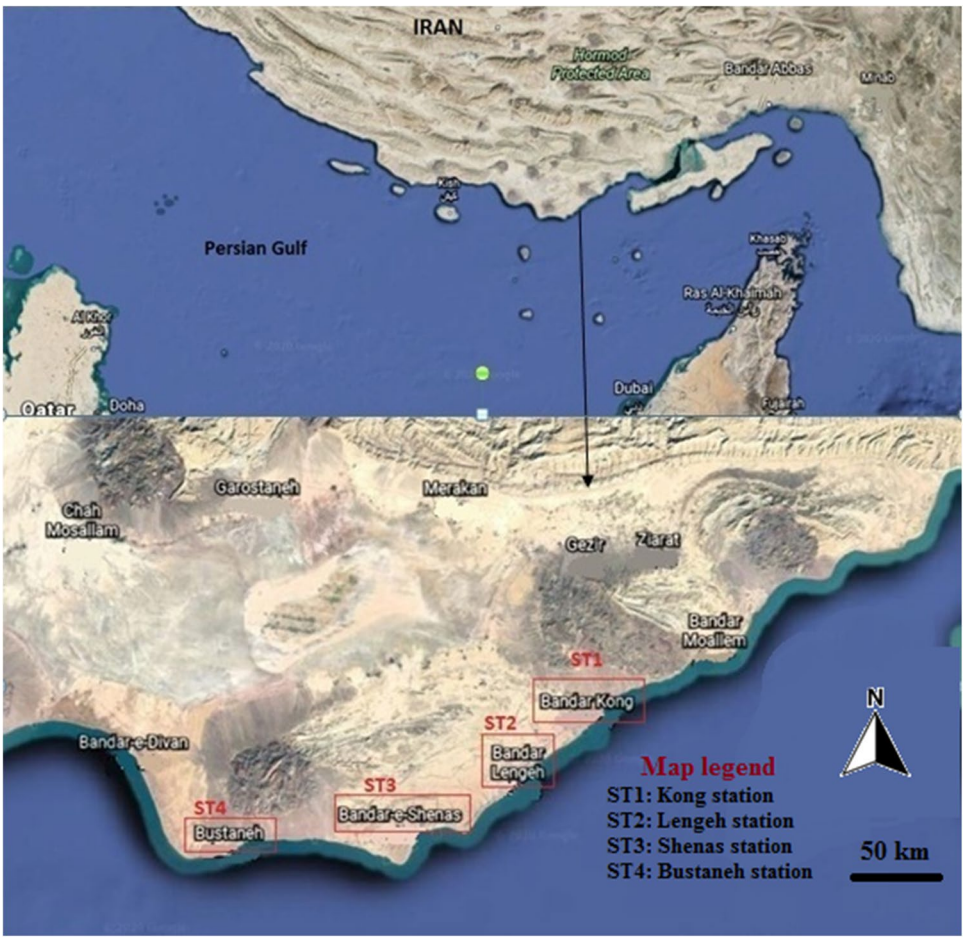

Fig. 1 Sampling area and stations. ST1 (Kong): 26° 36′ 12″ N, 54° 56′ 46″ E; ST2 (Lengeh): 26° 33′ 29″ N, 54° 53′ 12″ E; ST3 (Shenas): 26° 31′ 27″ N, 54° 47′ 37″ E; and ST4 (Bustaneh): 26° 30′ 30″ N, 54° 39′ 42″ E

cumene hydroperoxide (as a substrate). The decrease of absorbance at 340 nm was attributed to the reduction of oxidized glutathione by NADPH, H^+. One unit GPx activity corresponded to the oxidation of 1 µmol of NADPH per min.

The activity of CAT was determined by estimating the degradation rate of H_2O_2 at 240 nm; 1 mmol of H_2O_2 degraded per minute was considered as one unit of catalase activity.

A thiobarbituric acid method was used to determine the levels of MDA, an indicator of lipid peroxidation. In this method, gill tissue samples were lyophilized in liquid nitrogen, homogenized (1:4 v/v) in 50 mM potassium phosphate buffer (pH 7.0 at 20 °C), sonicated for 6 s, and centrifuged for 4 min at 13,000 × *g* at 4 °C to separate the supernatants (SPNs). Thiobarbituric acid (0.375%) and butylated hydroxytoluene (2%) were added to SPNs and all reagents (blanks and standards). Then, the solutions were heated for 15 min at 100 °C and centrifuged at 13,000 × *g* for 5 min at room temperature. The reaction between MDA and thiobarbituric acid created a pink chromagen, which was read spectrophotometrically at 532 nm. The MDA levels were expressed in µmol g^{-1} wet weight.

The immunological parameters, including the total counts of the hemocytes (THCs) and the phagocytosis activity of the hemocytes, were assayed in hemolymph samples extracted from the posterior adductor muscle of the clams. The hemolymph was sampled using a sterile 1-mL syringe, filtered using a 1-mm^2 mesh sterile gauze to remove the debris, and pooled in 50-mL Falcon tubes at 4 °C.

THCs were estimated based on the method of Zhu et al. (2011) and Mackenzie et al. (2014). Briefly, 100 mL hemolymph was diluted in 800 mL phosphate-buffered saline(PBS) and fixed by adding 100 mL 2.5% glutaraldehyde. A wet mount of the fixed hemolymph was examined with a Neubauer hemocytometer (XB-K-25, Anxin Optical Instrument) and observed under a Nikon Eclipse E600 microscopy (magnification × 1000).

Phagocytosis activity of the hemocytes was evaluated according to the methods described by Su et al. (2017). For this purpose, 20-µL yeast suspension (7 mg yeast in 1000 mL 0.1 M PBS) was added to 100 µL hemolymph mixed in 100 µL Alsever's solution (ALS, Noble Ryder). The mixture was left for 30 min at room temperature and then incubated in a cool water bath at 4 °C for 60 min. Finally, the phagocytosis activity was ceased using 2.5% glutaraldehyde, and the

blood smears were prepared and stained with Wright's stain. The phagocytic activity was examined under a microscope (Nikon Eclipse E600 light microscope, × 1000).

Data analysis

Analysis of data was performed using the SPSS software. The normality of data was examined before the analysis of variance using the *Kolmogorov–Smirnov test*. After a one-way analysis of variance, the means were compared using Tukey's test. In addition, the bivariate Pearson correlation was used to determine the relationships between the parameters.

Results

Water quality parameters

The physico-chemical parameters of water showed seasonal patterns during 1 year sampling period. In this regard, the water temperature showed significant decreases from

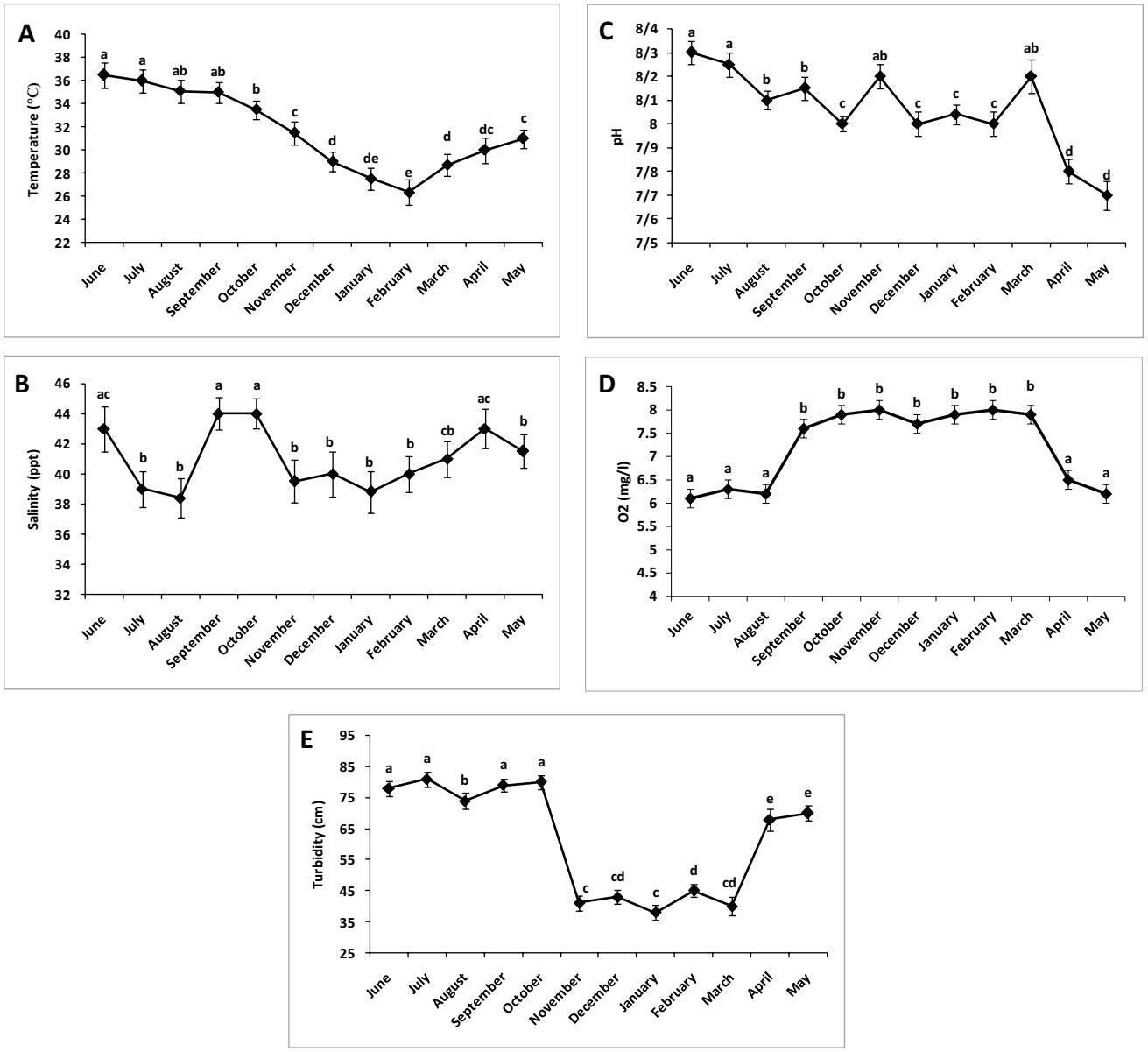

Fig. 2 **A**–**E** Monthly variations of water quality, from Lengeh port. The results are expressed as mean ± *SD*. For each parameter, different letters indicate significant differences (Tukey's test, $P < 0.01$)

October to February and then increased until May (Fig. 2A, $P < 0.01$). Water salinity decreased in June and August, return to initial values in September and October, decreased again in November, and nearly remained unchanged until May (Fig. 2B, $P < 0.01$). Except for an increase in November and March, water pH showed a declining trend throughout the sampling period (Fig. 2C, $P < 0.01$). The oxygen content of the water significantly increased during August, remained unchanged until March, and then decreased during April and May (Fig. 2D, $P < 0.01$). The water turbidity significantly decreased in November, nearly remained unchanged until March, and apparently increased in April and May (Fig. 2E, $P < 0.01$).

Heavy metal accumulations

The accumulation of the heavy metals including Cd, Pb, and Hg showed seasonal changes with maximum accumulations in late autumn and winter (Table 1, $P < 0.01$). During spring and summer, the levels of these metals significantly decreased (Table 1, $P < 0.01$). The concentrations of Ni and Cr nearly remained unchanged throughout the experiment period (Table 1, $P < 0.01$).

Metal-induced biochemical biomarkers

Seasonal changes were observed in metal-induced biochemical components including antioxidant enzymes (SOD, CAT, GPx) and immunological components (phagocytosis activity, THCs) (Fig. 3, $P < 0.01$). In this regard, the MDA levels (Fig. 3A, $P < 0.01$) and the activity of SOD (Fig. 3B, $P < 0.01$), CAT (Fig. 3C, $P < 0.01$), and GPx (Fig. 3D, $P < 0.01$) increased throughout April to August and then decreased during September to February ($P < 0.01$). The Phagocytosis activity (Fig. 3E, $P < 0.01$) showed significant decreases from December to February, while it increased from March to September ($P < 0.01$). THCs showed significant decreases from October to March and then elevated gently until April (Fig. 3F, $P < 0.01$).

Correlations

According to the analysis of Pearson, significant correlations were observed between tissue heavy metal concentrations, water quality parameters, and biochemical components (Table 2, $P < 0.01$). Negative correlations were O_2 vs. antioxidant enzymes, phagocytosis, and THCs; pH vs. SOD; salinity vs. Cr; and temperature vs. GPx and Ni. Positive correlations were O_2 vs. Cd, Pb, Hg, and Ni; temperature vs. phagocytosis and THCs; turbidity vs. phagocytosis, THCs, CAT, and GPx (Tables 3 and 4).

Discussion

In the present study, seasonal variations were observed in the accumulation of heavy metals in the ark clam, *Barbatia decussate*, as reported previously in many studies with bivalves. In wedge clam, *Donax trunculus*, the highest accumulation of heavy metals in summer was attributed to the lower marine currents and also reproduction of the bivalve in this season (Beldi et al. 2006). In *Mytilus galloprovincialis*, the higher accumulations occurred in winter for Zn, Cu, and Cd, while Pb showed higher accumulation in summer (Rouane-Hacene et al. 2015). These results might be due to the seasonal variations in water quality parameters, animal metabolism rate, food availability, and entrance of land and air-based pollutions to the sea (Rouane-Hacene et al. 2015). In the study of Góngora-Gómez et al. (2018), a seasonality was observed in concentrations of heavy metals in soft tissue and muscle of the pen shell *Atrina Maura* in the dry season

Table 1 Monthly variations of heavy metals in bivalve *Barbatia decussate* from Lengeh port. The results are expressed as mean ± *SD*. For each metal, different letters indicate significant differences (Tukey's test, $P < 0.01$)

	Heavy metals (µg/g dry tissue)				
	Cd	Pb	Hg	Cr	Ni
January	23.2 ± 7.5^{a}	90 ± 22.5^{a}	1.6 ± 0.4^{ac}	3.6 ± 1.1	6.2 ± 3.3
February	27.3 ± 9.1^{a}	88 ± 20.3^{a}	2.1 ± 0.2^{a}	3.4 ± 0.8	8.1 ± 4.2
March	10.1 ± 2.1^{b}	35 ± 15.3^{b}	1.1 ± 0.3^{bc}	2.1 ± 1.5	7.2 ± 3.1
April	11.5 ± 4.2^{cb}	41 ± 17.6^{b}	1.2 ± 0.7^{bc}	2.8 ± 1.7	6.6 ± 3.1
May	7.5 ± 3.4^{b}	32 ± 12.8^{b}	0.9 ± 0.2^{b}	2.5 ± 1.2	5.8 ± 3.4
June	5.6 ± 2.7^{b}	50.8 ± 14.2^{bc}	0.8 ± 0.2^{b}	2.1 ± 1.3	6.2 ± 3.6
July	6.5 ± 4.5^{b}	29 ± 18.7^{b}	0.9 ± 0.1^{b}	1.9 ± 1.1	7.3 ± 3.1
August	7.2 ± 3.3^{b}	33 ± 19.3^{b}	0.7 ± 0.3^{b}	2.4 ± 0.9	9.1 ± 4.9
September	19.4 ± 5.5^{ac}	77.1 ± 15.1^{ac}	1.7 ± 0.5^{a}	2.7 ± 0.8	5.7 ± 3.2
October	22.8 ± 4.5^{a}	80.3 ± 21.4^{a}	1.9 ± 0.6^{a}	3.1 ± 0.5	6.1 ± 3.4
November	24.2 ± 10.2^{a}	83 ± 17.9^{a}	2.2 ± 0.4^{a}	3 ± 1.1	8.1 ± 3.1
December	23.1 ± 8.5^{a}	80 ± 14.7^{a}	2.1 ± 0.2^{a}	3.3 ± 1.3	7.7 ± 3.2

(winter and spring), which was related to the upwelling currents and anthropogenic activities such as agriculture and aquaculture. In *C. glaucum*, the highest metal accumulations occurred during the winter and autumn seasons, while less accumulation was found during spring and summer. Similar to the above studies, seasonal variations were observed in tissue concentration of heavy metals of *B. decussate*. The maximum accumulations were found in late autumn and winter, which may be due to the decreased metabolism of the *B. decussate* and consequently its weak ability to excrete the metals to medium. In addition, the water quality parameters may affect the metal accumulations, because these parameters showed some significant correlations with tissue metal content. In this regard, water temperature and salinity had negative correlations with tissue concentrations of Ni and Cr, respectively. In contrast, water dissolved oxygen showed positive correlations with tissue concentrations of Cd, Pb, Hg, and Ni.

In the present study, seasonality was also observed in the values of immunological and antioxidant components, as previously reported for other bivalves (Sheehan and Power 1999). A El-Saidy et al. (2020) found seasonality in metal accumulations, antioxidant enzymes (CAT, GPx), and MDA levels in the bivalve *C. glaucum*, where the activity of these

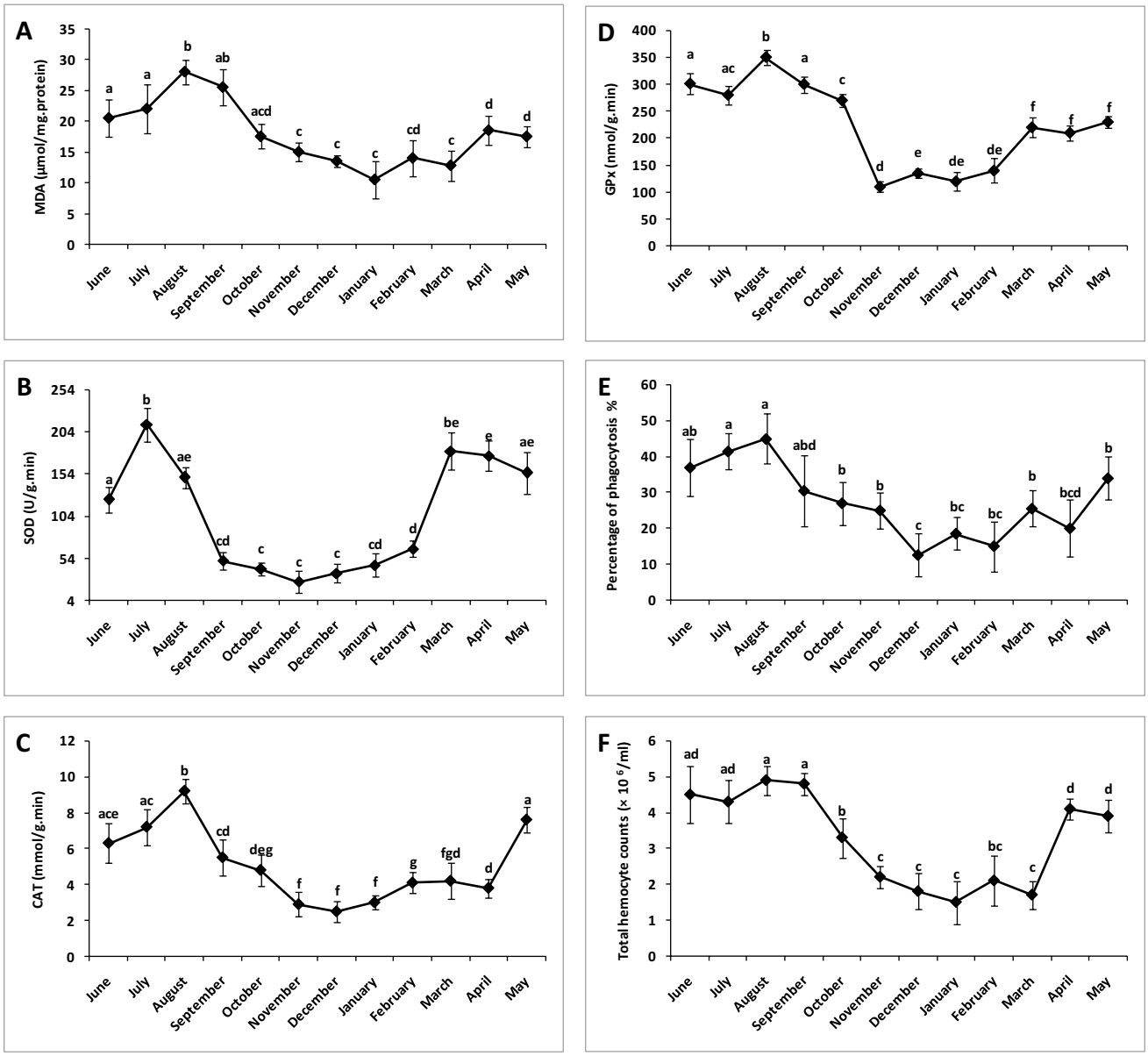

Fig. 3 **A**–**F** Monthly variations of MDA levels, antioxidant enzymes, and immunological parameters in bivalve *Barbatia decussate* from Lengeh port. The results are expressed as mean ± *SD*. For each metal, different letters indicate significant differences (Tukey's test, $P < 0.01$)

enzymes was lower during autumn and winter compared to other seasons. However, in the same study, the MDA levels were higher during autumn and winter, as observed during winter in the present study. Seasonal variations in

Table 2 The relationships between water quality parameters in ark clam, *Barbatia decussate*, from Lengeh port. The significant correlations presented as superscripted asterisks

		O_2	pH	Salin	Temp	Turb
O_2	Pearson correlation	1	.109	−.023	−.592*	−.670*
	Sig. (2-tailed)		.735	.943	.043	.017
pH	Pearson correlation	.109	1	−.164	.454	.016
	Sig. (2-tailed)	.735		.610	.138	.961
Salinity	Pearson correlation	−.023	−.164	1	.272	.500
	Sig. (2-tailed)	.943	.610		.392	.098
Temperature	Pearson correlation	−.592*	.454	.272	1	.842**
	Sig. (2-tailed)	.043	.138	.392		.001
Turbidity	Pearson correlation	−.670*	.016	.500	.842**	1
	Sig. (2-tailed)	.017	.961	.098	.001	

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

Table 3 The relationships between water quality parameters and metal-induced biochemicals in ark clam, *Barbatia decussate*, from Lengeh port. The significant correlations presented as superscripted asterisks

		SOD	CAT	GPx	Phago	THCs
O_2	Pearson correlation	−.718**	−.772**	−.638*	−.719**	−.776**
	Sig. (2-tailed)	.009	.003	.026	.008	.003
pH	Pearson correlation	−.026	.040	.225	.333	.029
	Sig. (2-tailed)	.935	.901	.482	.291	.930
Salinity	Pearson correlation	−.083	−.042	.345	−.033	.371
	Sig. (2-tailed)	.798	.897	.271	.918	.235
Temperature	Pearson correlation	.267	.674*	.825**	.846**	.830**
	Sig. (2-tailed)	.402	.016	.001	.001	.001
Turbidity	Pearson correlation	.372	.726**	.867**	.722**	.927**
	Sig. (2-tailed)	.233	.008	.000	.008	.000

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

Table 4 The relationships between water quality parameters and heavy metal accumulations in ark clam, *Barbatia decussate*, from Lengeh port. The significant correlations presented as superscripted asterisks

		Cd	Pb	Hg	Cr	NI
O_2	Pearson correlation	.874**	.792**	.854**	.054	.668*
	Sig. (2-tailed)	.000	.002	.000	.869	.018
pH	Pearson correlation	−.089	.049	−.051	.241	−.390
	Sig. (2-tailed)	.784	.880	.874	.450	.211
Salinity	Pearson correlation	−.043	.045	.026	−.714**	−.101
	Sig. (2-tailed)	.894	.889	.937	.009	.756
Temperature	Pearson correlation	−.560	−.402	−.492	−.123	−.661*
	Sig. (2-tailed)	.058	.195	.104	.704	.019
Turbidity	Pearson correlation	−.551	−.452	−.523	−.336	−.541
	Sig. (2-tailed)	.064	.140	.081	.285	.069

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

antioxidant enzymes were also observed in *M. galloprovincialis*, which its pattern was similar to tissue concentrations of PAHs, PCBs, DDTs, and lindane (Solé et al. 1995). The activity of antioxidant enzyme (SOD, GPx) decreased during winter in *M. edulis*, which was attributed to decreased antioxidant defense, as a result of lowered temperature and food availability (Manduzio et al. 2004). As a biomarker of oxidative stress (Hajirezaee et al. 2019), the MDA levels and also the activity of SOD, CAT, and GPx increased throughout the spring, summer, and mid-autumn and then decreased during late autumn and whole winter. It was recognized that temperature and food availability induce oxygen consumption and cellular oxyradical generation, which are compensated by enhancing antioxidant defenses (Sheehan and Power 1999; Manduzio et al. 2004). Therefore, the increased activity of ANEs during spring and summer may be a result of increases in temperature and food availability during these seasons. In addition, we found negative relationships between metal accumulations and activity of the antioxidant enzymes, which may be due to the suppressing effects of metals toxicity on the antioxidant defense of *B. decussate*. THCs and phagocytosis activity are known as the main components of immunity in Mollusca. There are some studies regarding the seasonal alternations in the immune-related components of bivalves. In the study of Hong et al. (2020), the decrease in the THCs was attributed to the reduced immunity of the giant honeycomb oyster, *Hyotissa hyotis*, after reproduction in spring. Flye-Sainte-Marie et al. (2009) showed that seasonal fluctuations in temperature control the THC in *Ruditapes philippinarum*.

In the present study, the phagocytosis activity and THCs decreased during December to February and November to March, respectively. In addition, negative correlations were found between immunological parameters and tissue concentration of heavy metals. These results may indicate the immunosuppressive effects of these metals on the ark clam immunity, which may make this species susceptible to diseases during winter.

Conclusion

The results of the present study revealed a close relationship between heavy metal accumulations in ark clam, water quality parameters, and the clam's immune components. In this regard, decreased water oxygen levels and temperature significantly reduced the immune-related components including antioxidant enzymes, phagocytosis, and THCs. In contrast, an increase in temperature and turbidity was accompanied by a considerable elevation in phagocytosis, THCs, CAT, and GPx activity. In general, the above results show that the temperature and oxygen level of water adversely affects the immunity of the ark clam. However, more studies, especially laboratory studies, are needed in the future to examine the relationships more closely.

Acknowledgements The authors would like to thank all those who contributed to this research.

Author contribution AJ as the only author of this article has made the testing, collected the data, analyzed the results, and wrote the article.

Funding There is no government or organizational fund for this work.

Data availability The datasets used during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval In this study, all stages of sampling and manipulation of animals have been performed in accordance with ethical standards.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The author declares no competing interests.

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