



Health Risk Assessment of Okadaic Acid and Domoic Acid in some Edible Bivalves from Hormozgan Province in the North of Persian Gulf

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Abstract

The biotoxins can enter the marine food chain, and, accordingly, seafood consumers are also at risk of ingesting toxins from contaminated aquatic animals. In the present work, the aim was to identify the okadaic acid and domoic acid producers in the coastal waters and to investigate on their accumulated concentrations in edible bivalves in order to assess the consumer's health risk. Water samples were collected during winter 2018 and summer 2019 from one control and four stations facing industrial and municipal effluents. Four species of edible bivalves were collected from coastal lines of Hormozgan province, at the lowest tide time. The concentrations of toxins were determined by indirect competitive ELISA method. *Pseudonitzschia delicatissima*, *Nitzschia pungens* and *Nitzschia seriata* in the production of domoic acid and *Dinophysis caudate*, *Prorocentrum Lima* and *Ceratium tripos* in the production of okadaic acid were identified. The okadaic acid concentrations ranged from 59.8 ± 2.38 to 121.96 ± 28.25 $\mu\text{g}/\text{kg}$, ranging from 0.85 to 83.59 ± 38.72 for Domoic acid. Among the studied bivalves, *Pinctada radiata* contained the maximum concentrations of measured toxins. For the first time at Hormozgan Province, the consumption guidelines for domoic and okadaic acid were calculated. The human health risk assessment showed that at present time, the algal consumers from Hormozgan province are not at risk of domoic and okadaic acid toxins.

Keywords: Biotoxin, Food chain, Bioaccumulation, Marine phytoplankton

INTRODUCTION

The seas are the main recipients of municipal and industrial wastewater containing organic and inorganic chemical compounds effectual on the ecosystem of the sea to host phytoplankton, which release marine toxins with health and environmental risk potential (Brown et al., 2020). Domoic acid and okadaic acid are among the marine toxins. Domoic acid (DA) is among neurotoxins and is classified in the amnesic shellfish poisoning (ASP) group. DA can cause permanent short-term memory loss. This toxin is released from the phytoplankton *Dinophysis* and *Prorocentrum* (Jeffery et al., 2004; Lefebvre & Robertson, 2010; Mattarozzi et al., 2019). Okadaic acid (OA) is one of the dinophysistoxins which is classified in the diarrhoeic shellfish poisoning (DSP) group. This toxin causes severe abdominal contractions and diarrhea, which is also known to accelerate tumors. This toxin is released from Pseudo-nitzschia phytoplankton

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(Nguyen et al., 2017). Being able to accumulate in the food chain, phytoplankton toxins can affect human health (Young et al., 2020). There are almost some reasons for the increase in phytoplankton bloom records: enhancing scientific knowledge, larger usage of coastal waters, climatic changes, and transferring toxic species through water balance (Estevez et al., 2019; Van Dolah, 2000). In addition to human health threat, there is the probability of major financial losses for marine-dependent industries such as desalination plants and aquaculture farms (Takahashi, 2020).

Unfortunately, there are few studies on qualitative and quantitative analysis of toxins in the Persian Gulf ecosystem and the Oman Sea, which are often associated with the blooms of phytoplankton in 2008 (Hamzehei et al., 2012; Mortazavi

et al., 2015). The 2008 vast red tide in Persian Gulf and Oman Sea waters led to considerable mortality in Hormozgan Province including benthic fish and other aquatics such as polychaeta, sea cucumber, mollusks (gastropod and bivalves) and crustaceans (Hormozgan Fisheries Department, 2009), after that, researchers looked into algae blooms in the Persian Gulf and Oman Sea from different perspectives (Mirza Esmaeili et al., 2020a; Mirza Esmaeili et al., 2020b). In recent decades, Hormozgan province, especially in its western parts has witnessed a high volume of establishment and activation of industries. As a result, the economic growth has been accompanied by higher urbanization rates. Thus, the waters of the Persian Gulf and the Sea of Oman receive urban, industrial and agricultural effluents, which in some cases facilitate the phenomenon of planktonic blooms by changing the stoichiometric ratio of nitrogen to phosphorus (Polikarpov et al., 2020). The coastal waters of Bandar Abbas city is the main receiving of wastewaters from industrial and urbanization activities in Hormozgan Province.

The present study aimed to determine the risk of human consumption due to phytoplankton toxins okadaic acid and domoic acid in edible bivalves of Hormozgan province. We also studied the role of urban effluents in the density of phytoplankton and identified the planktonic producing of okadaic acid and domoic acid toxins in the coastal line of Bandar Abbas city.

MATERIALS AND METHODS

Qualitative and quantitative determination of phytoplankton density was performed at four stations receiving the urban and industrial effluents, as well as the control area. The characterizes and geographical coordinates of the sampling stations are represented in Fig.1.

The water samples were collected from surface layer in two seasons of winter 2018 and summer 2019 using Nansen bottle. The samples were fixed using Lugol solution and then transferred to the laboratory for identification and counting (Al-Yamani, 2009).

1 to 3 kg of bivalve samples were manually collected from the coastal lines of Hormozgan province, including Bandar Abbas city, Bandar-e-Lengeh and Minab in the west and east of Hormozgan province, respectively, during the lowest tide time. The specifications of the collected species, including the type of species (Bosch et al., 1995; Carpenter, 1998; Hosseinzadeh et al., 2001; Abott & Dance, 2000), scientific names (WoRMS, 2020) and the collection locations, are given in Table 1.

The collected samples were immediately stored in ice powder and transferred to the laboratory and stored at -20 degrees centigrade until analysis.

After transferring the samples to the laboratory and 7-10 days of sedimentation, water was poured on the siphon and then the concentrated sample of three harvests of one milliliter was placed in Lam Sedgewick Rafter in order to identify and count phytoplankton. Cell-based density was measured in liter, and then, using invert microscope and valid identification keys, identification was made (Al-Kandari et al., 2009; Hoppenrah et al., 2009).

Preparation of bivalve samples for algal toxins analysis was performed based on the kit manual and the instrumental analysis was based on Eliza test of indirect competition type

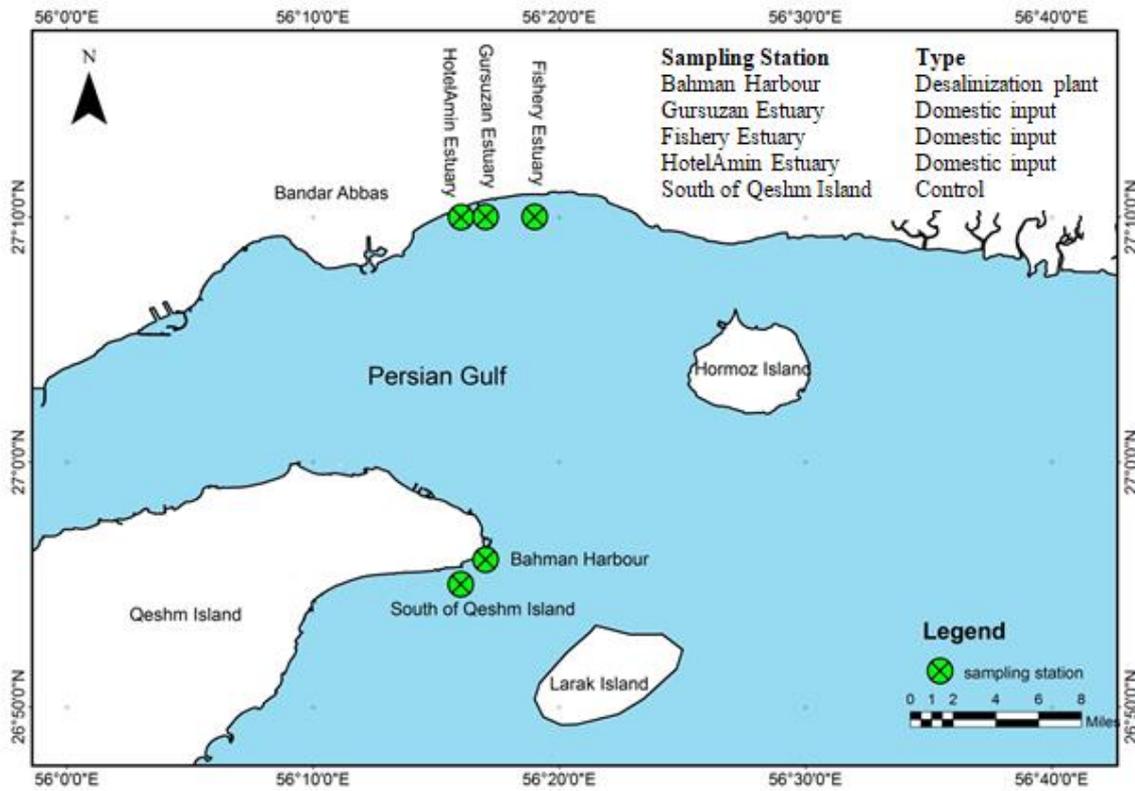


Fig. 1. Geographical coordinates and characterizes of the sampling stations

Table 1. The specifications of the collected bivalves

| Scientific name | Local name | Collection location | |
|--|-----------------|---|---|
| <i>Saccostrea cucullata</i> Family: Ostreidae | Sangi | Bandar Abbas city & Bandar-e- Lengeh |  |
| <i>I-Solen brevis</i> Family: Solenidae | Malalis / Moluk | Bandar Abbas & Minab |  |
| <i>Amiantis umbonella</i> Family: Veneridae | Kalang | Bandar Abbas & Minab |  |
| <i>Pinctada radiate</i> Family: Pteriidae | Mohar Saghir | Bandar-e- Lengeh |  |

Table 2. The used values to calculate the local guidelines

| | Clinical symptoms | LOAEL | UF |
|----|-------------------|-------|-----|
| DA | diarrhea | 40 | 100 |
| OA | vomiting | 1000 | 100 |

by using instrument BioTek-ELx 800 model. The kits used for domoic Acid with reference number 5191DOMO and for okadaic acid with reference number 5191OKA were purchased from company Europroxima of the Netherlands. The absorbance (optical density) was read at a wavelength of 450 nm.

The required information for data processing and risk assessment, such as human consumption rate of bivalves and average weight of consumers, was obtained from Hormozgan Fisheries Department. Initially, the amount of daily food exposure (DE) is obtained by Eq.1. The dietary intake of the consumer (DI) is also calculated by Eq.2 (Duy et al., 2000).

$$\text{Dietary Exposures (DE: } \mu\text{g/day)} = \text{Toxin concentrations in shellfish (} \mu\text{g/g)} \times \text{daily consumption (g/day)} \quad (1)$$

$$\text{Dietary Intakes (DI: } \mu\text{g/day.kg)} = \text{DE (} \mu\text{g/day)} / \text{mean BW(kg)} \quad (2)$$

The next step is to calculate the tolerable daily intake (TDI) based on Eq.3. The uncertainty factor includes the coefficients associated with estimating the chronic and short-term effects of toxin and other effective parameters which varies from 1 to 1000 values (Duy et al., 2000).

$$\text{TDI (Tolerable Daily Intake)} = \text{LOAEL(Lowest Observable Adverse Effect Level)} / \text{UF (Uncertainty Factor)} \quad (3)$$

Also, the guide value (GV) is determined based on Eq. 4 and using the previously calculated quantities such as TDI, BW and the daily consumption of bivalves in kilograms per day.

$$\text{GV (Guideline Value)} = (\text{TDI} * \text{BW}) + \text{P} / \text{C} \quad (4)$$

In the last step, the calculated GV and TDI for Hormozgan province are compared with the consumer's dietary intake. In the present study to calculate the local guidelines, both LOAEL and UF parameters are extracted from the articles (Takahashi, 2007), and the considered clinical symptoms are described in Table 2. The average weight of each consumer was 70 kg (Mohebbi-Nozar et al., 2014) and the consumption rate of bivalves in Hormozgan province was 117 gr/year (Hormozgan Fisheries Department, 2009).

SPSS software was used for statistical analysis and due to the non-normal distribution of data, the nonparametric Crosscalice Test of Wallis was run to compare data related to temporal and spatial distribution of toxin values. The concentration distribution of toxins among the studied species was compared by using of Anova (Tukey) Test.

RESULTS AND DISCUSSION

The results of qualitative and quantitative identification of phytoplankton in studied stations have been shown in Table 3.

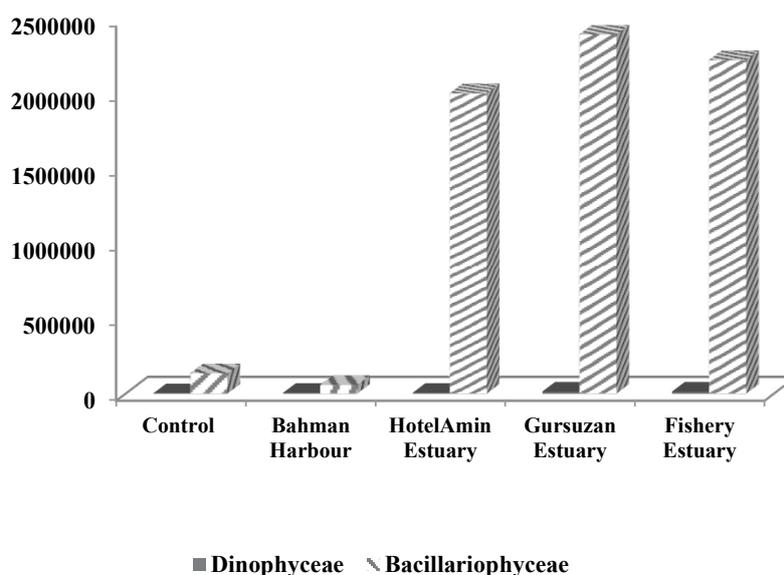
The qualitative and quantitative identification of phytoplankton in the winter of 2018 are

Table 3. The Phytoplankton identified in the present study

| | Scientific Name | winter | summer | Toxin production |
|--------------------------|---------------------------------------|--------|--------|------------------|
| Bacillariophyceae | <i>Amphiprora</i> sp. | + | - | |
| | <i>Amphora crassa</i> | + | - | |
| | <i>Asteromphelus</i> sp. | + | - | |
| | <i>Bacillaria paxillifer</i> | + | - | |
| | <i>Bellerochea malleus</i> | - | + | |
| | <i>Campylodiscus neofastuosus</i> | + | - | |
| | <i>Chaetoceros brevis</i> | + | - | |
| | <i>Chaetoceros compressus</i> | + | - | |
| | <i>Chaetoceros didymus</i> | + | + | |
| | <i>Chaetoceros Lorenzianus</i> | + | - | |
| | <i>Climacodium frauenfeldianum</i> | + | - | |
| | <i>Coscinodiscus wailesii</i> | + | - | |
| | <i>Cylindrotheca closterium</i> | + | + | |
| | <i>Cymbella</i> sp. | + | - | |
| | <i>Diploneis didyma</i> | + | - | |
| | <i>Eucampia zodiacus</i> | + | - | |
| | <i>Guinardia flaccida</i> | + | - | |
| | <i>Gyrosigma acuminatum</i> | + | - | |
| | <i>Hemiaulus indicus</i> | - | + | |
| | <i>Hemiaulus membranaceus</i> | + | - | |
| | <i>Hemiaulus sinensis</i> | + | - | |
| | <i>Lauderia annulata</i> | + | + | |
| | <i>Leptocylindrus danicus</i> | + | + | |
| | <i>Lithodesmium undulatum</i> | - | + | |
| | <i>Lithodesmium</i> sp. | + | - | |
| | <i>Meridion</i> sp. | + | - | |
| | <i>Navicula directa</i> | - | + | |
| | <i>Navicula membranacea</i> | - | + | |
| | <i>Navicula</i> sp1 | + | + | |
| | <i>Navicula</i> sp2 | + | - | |
| | <i>Nitzschia longissima</i> | + | + | |
| | <i>Nitzschia sigma</i> | + | - | |
| | <i>Odontella sinensis</i> | + | + | |
| | <i>Pleurosigma elongatum</i> | + | + | |
| | <i>Proboscia alata</i> | + | + | |
| | <i>Pseudo-nitzschia delicatissima</i> | + | - | ASP |
| | <i>Pseudonitzschia longissima</i> | + | - | |
| | <i>Pseudo-nitzschia pungens</i> | + | - | ASP |
| | <i>Pseudo-nitzschia seriata</i> | + | + | ASP |
| | <i>Rhizosolenia clevei</i> | + | - | |
| | <i>Rhizosolenia setigera</i> | + | + | |
| | <i>Skeletonema costatum</i> | + | + | |
| | <i>Streptotheca indicus</i> | + | - | |
| | <i>Thalassionema nitzschioides</i> | + | - | |
| | <i>Thalassionema frauenfeldii</i> | + | - | |
| | <i>Thalassiothrix longissima</i> | - | + | |
| | <i>Triceratium favus</i> | - | + | |

Table 3 (continued). The Phytoplankton identified in the present study

| | Scientific Name | winter | summer | Toxin production |
|--------------------------|---|--------|--------|------------------|
| Dinophyceae | <i>Akashiwo</i> sp. / <i>Akashiwo sanguinea</i> | - | + | |
| | <i>Alexandrium Catenella</i> | - | + | |
| | <i>Dinophysis caudata</i> | - | + | DSP |
| | <i>Gymnodinium catenatum</i> | + | - | |
| | <i>Gymnodinium</i> sp1 | + | - | |
| | <i>Gymnodinium</i> sp2 | + | + | |
| | <i>Peridinium quadridentatum</i> | + | - | |
| | <i>Pronoctiluca pelagica</i> | + | - | |
| | <i>Pyrophacus horologium</i> | + | - | |
| | <i>Prorocentrum gracile</i> | + | + | |
| | <i>Prorocentrum lima</i> | + | - | DSP |
| | <i>Prorocentrum micans</i> | + | + | |
| | <i>Prorocentrum sigmoides</i> | + | - | |
| | <i>Protoberidinium bipes</i> | + | - | |
| | <i>Protoberidinium divergens</i> | + | - | |
| | <i>Protoberidinium pellucidum</i> | - | + | |
| | <i>Protoberidinium quinquecorne</i> | - | + | |
| | <i>Pyrodinium bahamense</i> | + | + | |
| | <i>Scrippsiella acuminata</i> | + | + | |
| | <i>Tripos furca</i> | | + | |
| <i>Tripos fusus</i> | + | - | | |
| <i>Tripos macroceros</i> | + | - | | |
| <i>Tripos muelleri</i> | - | + | | |
| Cyanophyceae | <i>Trichodesmium erythraeum</i> | + | - | |

**Fig. 2.** Phytoplankton densities (cell/liter) in sampling stations (winter of 2018)

represented in Fig.2.

During this time, 65 species were identified and their densities determined. So that

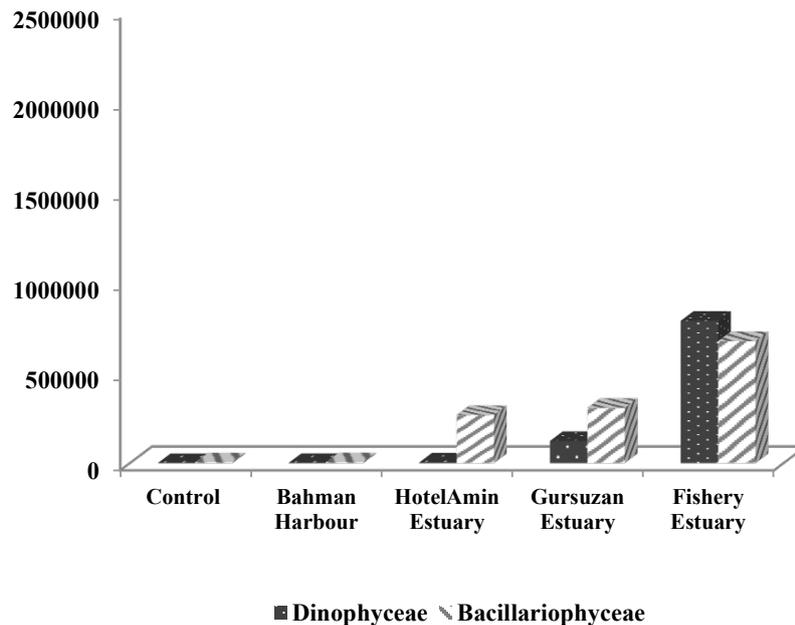


Fig.3. Phytoplankton densities (cell/liter) in sampling stations (summer of 2019)

Bacillariophyceae with 46 species, Dinophyceae with 18 species and Cyanophysis with one species were present. Among the species belonging to Bacillariophyceae, species *Coscinodiscus wailesii* with 82% had the maximum density. From Dinophyceae, *Prorocentrum micans* with 27.7, *Prorocentrum gracile* with 22.2 and *Prophacus horologium* with 10.3 percentage can be named. From Cyanophysis, *Trichodesmium earythum* was identified.

The obtained results from qualitative and quantitative study of phytoplankton in sampling stations in the summer of 2019 are displayed in Fig.3. During the study period, phytoplankton species were identified and densities were determined: Bacillariophyceae with 23 species and Dinophyceae with 13 species were recorded. Among the species belonging to Bacillaryophys, the species *Leptocylindrus danicus* with 97 percentage had a higher frequency than others but the Dinoflagelles were contained of *Protoperidinium quinquarium* with 98.9 percentage as the dominance species. The variety of identified phytoplankton species in winter was greater than that in summer.

As Fig. 1 shows Dinophyceae density had experienced changes in winter in sampling stations from minimum 2420 cell per liter in control station to maximum 13600 cell per liter in the waters facing the Gursuzan Estuary entrance. Also, the high value of 13440 cell per liter was recorded at Fishery Estuary. Moreover, Fig. 2 shows that these stations had the highest summer densities with 788963 and 123100 at Fishery and Gursuzan Estuary, respectively. The high density of Dinophyceae in the waters facing the urban wastewater is probably due to the reception of high concentration of nitrogen and phosphorus compounds in this way. Also, in Kuwait coastal waters the composition of phytoplankton blooms has been related to stoichiometric ratio of inorganic nitrogen and phosphorus (polikarpov et al., 2020).

Among the Dinophyceae in the summer, *Protoperidinium quinquarium* in the stations of Fishery and Gursuzan Estuary had the highest density and reached to 787500 and 120000 cell per liter, respectively.

In summer season, at stations facing the urban wastewater, including Fishery, HotelAmin and Gursuzan Estuaries, *Dinophysis caudate* was observed with 675, 625 and 600 cell per liter, respectively. But it had not been seen at control station, as well as at the Bahman Harbour station, which is farther from the coastline than at other stations. Thus, it can be concluded that

Table 4. Planktonic producers of DA and OA (Hallegraeff et al., 2003; Omura et al., 2012)

| DA producer | OA producer |
|---|-------------------------------|
| <i>Amphora coffeaeformis</i> | <i>Dinophysis acuminata</i> |
| <i>Nitzschia navis-varingica</i> | <i>Dinophysis acuta</i> |
| <i>Pseudo-nitzschia australis</i> | <i>Dinophysis caudata</i> |
| <i>Pseudo-nitzschia calliantha</i> | <i>Dinophysis fortii</i> |
| <i>Pseudo-nitzschia cuspidata</i> | <i>Dinophysis miles</i> |
| <i>Pseudo-nitzschia delicatissima</i> | <i>Dinophysis mitra</i> |
| <i>Pseudo-nitzschia fraudulentata</i> | <i>Dinophysis norvegica</i> |
| <i>Pseudo-nitzschia galaxiae</i> | <i>Dinophysis rapa</i> |
| <i>Pseudo-nitzschia multiseriata</i> | <i>Dinophysis rotundata</i> |
| <i>Pseudo-nitzschia multistriata</i> | <i>Dinophysis tripos</i> |
| <i>Pseudo-nitzschia pseudodelicatissima</i> | <i>Dinophysis polyedrum</i> |
| <i>Pseudo-nitzschia pungens</i> | <i>Dinophysis reticulatum</i> |
| <i>Pseudo-nitzschia seriata</i> | <i>Prorocentrum lima</i> |
| <i>Pseudo-nitzschia turgidula</i> | |

during the present study, okadaic acid has been produced by *Dinophysis caudate* in the coastal waters of Bandar Abbas city in summer. In the winter season, *Prorocentrum lima* were identified with densities 1600, 360 and 200 cell/liter at Bahman Harbour, Fishery and Gursuzan stations, respectively. It is possible to conclude that *Prorocentrum lima* is the main producer of okadaic acid in the coastal waters of Bandar Abbas in winter.

Like dinophyceae, change of Bacillariophyceae densities follows the similar pattern and in summer it had a higher total density than winter and the highest densities were observed at Fishery and Gursuzan Estuary stations. Among Bacillariophyceae in the summer, the maximum density was recorded for *Leptocylindrus danicus* with values of 675000 and 300000 cell per liter at Fishery and Gursuzan Estuary stations, respectively. In winter, Bacillariophyceae have a greater variety of species than in summer, and the dominant species was *Coscinodiscus wailesii* with the maximum density 2364000 cell per liter at Gursuzan Estuary stations.

Identified phytoplankton species had also included species capable of producing DA and OA toxins. These species included *Pseudonitzschia delicatissima*, *Nitzschia pungens* and *Nitzschia seriata* in the production of domoic acid and species *Dinophysis caudate*, *Prorocentrum Lima* and *Ceratium tripos* in the production of okadaic acid in coastal Bandar Abbas city.

Pseudonitzschia delicatissima as domoic acid producer in the coastal waters of Bandar Abbas, was recorded with 480 and 1280 cell per liter at HotelAmin and Gursuzan Estuary stations, respectively.

The similar results have been reported for OA and DA producers by other researchers (Table 4). The main producers of OA are often from *Dinophysis*, while the main producer of DA is *Pseudo-nitzschia* (Hallegraeff et al., 2003; Omura et al., 2012).

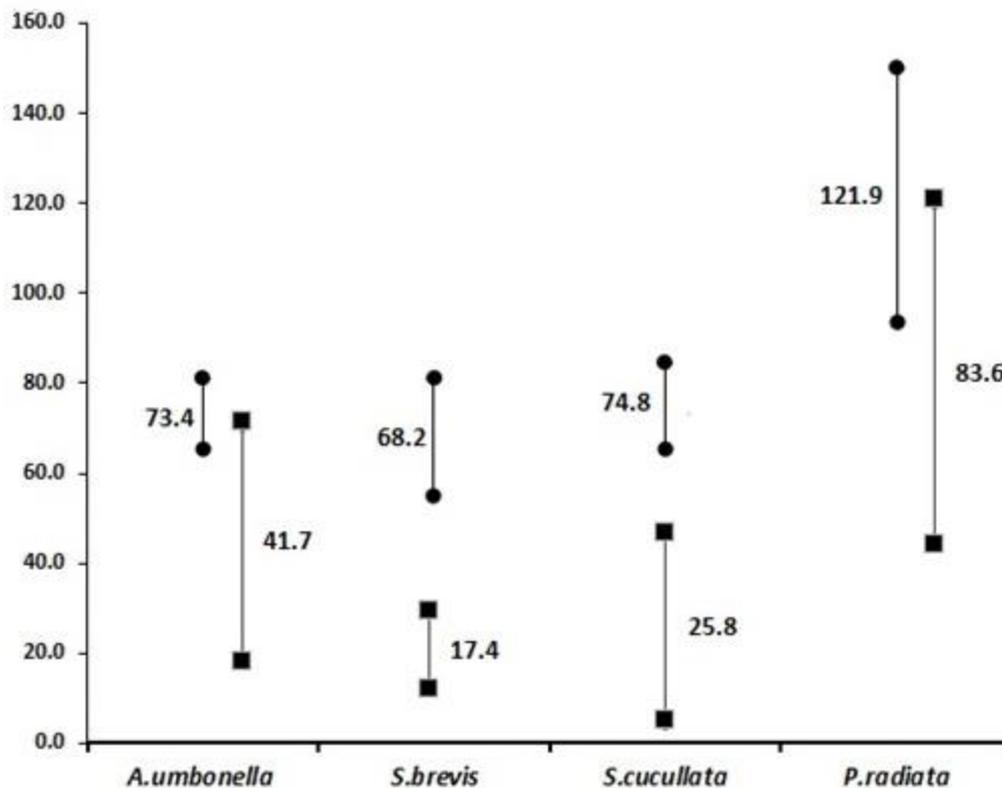
The data obtained from algal toxin measurement in the bivalves samples collected from Bandar Abbas, Bandar-e Lengeh and Minab have been illustrated in Table 5.

The highest concentration of domoic acid toxin was observed in summer in *P.radiata* collected from Bandar Lengeh area, and the lowest value was related to *S.cucullata* with the value of 0.85 µg/kg which was also from Bandar-e Lengeh area during summer. Measured concentrations of domoic acid toxin showed significant difference between summer and winter ($P < 0.050$), higher in summer with average 36.12 µg/kg. However, in terms of spatial distribution, this toxin did not have a significant difference between studied areas.

On the other hand, regardless of the area and time of sampling, the distribution of DA toxin

Table 5. Domoic acid and okadaic acid concentrations in collected bivalves

| Location | Sampling Season | Scientific Name | Domoic acid | | Okadaic acid | |
|-----------------|-----------------|--------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|
| | | | Average ($\mu\text{g}/\text{kg}$) | Maximum ($\mu\text{g}/\text{kg}$) | Average ($\mu\text{g}/\text{kg}$) | Maximum ($\mu\text{g}/\text{kg}$) |
| Bandar Abbas | Summer | <i>A.umbonella</i> | 66.84 \pm 52.94 | 104.27 | 73.24 \pm 11.13 | 80.98 |
| | | <i>S.brevis</i> | 14.30 \pm 9.21 | 19.94 | 73.77 \pm 18.64 | 94.88 |
| | Winter | <i>A.umbonella</i> | 28.52 \pm 12 | 37.04 | 73.01 \pm 7.37 | 78.25 |
| | | <i>S.brevis</i> | 16.45 \pm 7.10 | 24.42 | 64.96 \pm 9.69 | 75.97 |
| | | <i>S.cucullata</i> | 34.16 \pm 15.91 | 52.14 | 78.71 \pm 4.18 | 82.35 |
| Bandar-e Lengeh | Summer | <i>P.radiata</i> | 83.59 \pm 38.72 | 122.32 | 121.96 \pm 28.25 | 141.56 |
| | Winter | <i>S.cucullata</i> | 0.85 \pm 0 | 0.85 | 70.89 \pm 12.76 | 78.48 |
| Minab | Summer | <i>A.umbonella</i> | 38.26 \pm 6.73 | 43.68 | 73.92 \pm 29.8 | 83.49 |
| | Winter | <i>S.brevis</i> | 23.43 \pm 12.91 | 32.56 | 65.80 \pm 12.88 | 80.30 |

**Fig.4.** Domoic acid (■) and okadaic acid (●) concentrations ($\mu\text{g}/\text{kg}$) in collected bivalves

was also studied based on the bivalve species, the result of which has been shown in Fig. 4. Bivalve *Pradiata* with an average of 83.59 $\mu\text{g}/\text{kg}$ wet weight had the highest concentration of DA, while *C.callipyga* with a large difference has the lowest amount of DA toxin accumulation in its tissue. Statistical analysis showed that, after *Pradiata*, bivalve *A.umbonella* had a significantly ($P < 0.05$) higher concentration of DA toxin (Fig.4).

Also, Table 5 shows the measured concentrations of okadaic acid in the collected bivalve samples. *Pradiata* collected from Bandar-e Lengeh area showed the highest mean of 121.96 \pm 28.25 $\mu\text{g}/\text{kg}$ wet weight and also contained the highest measured concentration of OA. Other concentrations measured in bivalves collected from different locations and times did not have

Table 6. Relation of clinical symptoms with OA and DA levels in body weight of consumers

| Okadaic acid | | | Domoic acid | | |
|---------------------|-------------------|-----------------------|---------------------|---------------------------|-------------------|
| Toxin concentration | Clinical symptoms | Reference | Toxin concentration | Clinical symptoms | Reference |
| 48 µg/60kg | Mild diarrhea | Lee et al., 1987 | 1100 µg/kg | Nausea, vomiting | Perl et al., 1990 |
| 40 µg/60kg | diarrhea | Ramstad et al., 2001 | 2900 µg/kg | Confusion/disorientation | Todd, 1993 |
| 80-280 µg/kg | Severe diarrhea | Yasumoto et al., 1987 | 4200 µg/kg | Permanent neuronal damage | Todd, 1993 |
| 320 µg/kg | Profused diarrhea | Val & Sampayo, 2002 | 2.04 µg/60 kg | | Present study |
| 2.36 µg /60 kg | | Present study | | | |

a severe dispersal of toxin accumulation and fluctuated in the range of 59.80 ± 2.38 to 85.16 ± 8.27 µg/kg.

Measured concentrations of OA in both summer and winter did not show a significant difference ($P < 0.05$). In terms of spatial distribution, OA has a significant difference between Bandar Abbas and Bandar-e Lengeh ($P < 0.05$), and Bandar-e Lengeh with an average of 89.42 µg/kg had a higher concentration of this toxin.

The maximum recorded toxin concentration occurred in bivalves *P.radiata* (Fig.4); also, in terms of statistical tests, other bivalves did not show a significant difference in the accumulation of OA in their tissues ($P < 0.05$).

While the concentration of okadaic acid in both summer and winter did not show a significant difference, the difference was significantly higher for domoic acid ($P < 0.05$) in summer. A similar study on the coast of Australia has pointed to the significant role of temperature in summer on the high concentration of domoic acid toxin (Takahashi, 2007). An increase in temperature can play a role in influencing the density of phytoplankton species producing toxins. However, due to the limited data available in the present study and the lack of knowledge about the concentration of toxin in phytoplankton in the studied area, it was impossible to investigate such a relationship.

The spatial distribution of the okadaic toxin showed a significant difference between the collected locations of bivalves. The bivalves of the Bandar-e Lengeh region had higher concentrations of the okadaic acid. Since the bivalves collected from different locations inevitably varied, it seems that the significant difference between locations and even seasons depends on the type of bivalves.

Based to this view, *A.umbonella* and *P.radiata* had a significantly higher concentration of DA toxin ($P < 0.05$). For OA, the maximum recorded concentration was related to *P.radiata* and according to the results of statistical tests, other bivalves did not show a significant difference in toxin OA accumulation in their tissues ($P < 0.05$). A key factor determining the amount of toxin concentration in tissue is aquatic nutritional behavior (Contreras et al., 2012). *P.radiata* is one of the bivalves that feeds on the water column, which can help absorb more toxins and accumulate in its soft tissue which known as a good biomonitoring (Mohammad Karami et al., 2014).

In order to assessment the health risk of measured biotoxines, two methods have been used. First, the clinical guidelines are interested indicators to assess the human health risk. In this regard, for the toxins of domoic acid and okadaic acid, concentrations of toxin that cause certain clinical symptoms have been shown in Table 6.

Compared to the concentrations of toxins that cause clinical symptoms, the obtained maximum values (2.36 µg /60 kg for OA and 2.04 µg/60 kg for DA) indicate that there is no

Table 7. Human dietary intake and dietary exposures of OA and DA

| Scientific name | OA | | DA | |
|--------------------|---------------------------------------|------------------------|---------------------------------------|------------------------|
| | DI ($\mu\text{g}/\text{day.kg}$) | Calculated local GV | DI ($\mu\text{g}/\text{day.kg}$) | Calculated local GV |
| <i>A.umbonella</i> | 0.00031 | 2333333 | 0.00002 | 93333 |
| <i>S.brevis</i> | 0.00029 | | 0.00007 | |
| <i>S.cucullata</i> | 0.00032 | | 0.00011 | |
| <i>P.radiata</i> | 0.0005 | | 0.00036 | |

Table 8. The Guidance values for okadaic acid

| Toxin concentration | Country | Reference |
|--|-------------------------|----------------------|
| 0.8 $\mu\text{g}/\text{g}$ | Ireland | Carmody et al., 1996 |
| 5 MU | Most of countries | Fernandez, 2000 |
| 24 $\mu\text{g}/100\text{g}$ | Maximum tolerable level | Stabell et al.,1992 |
| 20 $\mu\text{g}/100\text{g}$ | Canada | Anderson et.al,2001 |
| 12.2 \pm 2.8 $\mu\text{g}/100\text{g}$ | Iran | Present study |
| 14.1 $\mu\text{g}/100\text{g}$ | Iran | Present study |

risk of clinical symptoms of OA and DA in Hormozgan province through the consumption of bivalves.

Second, estimating and assessing the risk of harmful chemicals from aquatics consumption depends on parameters such as consumption rate, average weight of human consumers, the type of possible disease (Takahashi, 2007). Therefore, in assessing the risk of algae toxins, it is common to determine local guideline values based on regional information. Accordingly, for the first time, these values have been calculated for consumers of Hormozgan province. The obtained values of dietary intake of measured toxins for Hormozgan people through consumption of bivalves were compared with calculated local GV's (Table 7). As shown, the daily amount of OA and DA received by Hormozgan consumers is significantly less than the local GV's and there is currently no human risk of algae toxins.

In Iran, the most researches are focused on the environmental risk assessment aspect of marine toxins (Gholami et al., 2019; Mirza Esmaili et al., 2020). But, many of countries have a protocol on how to monitor toxin concentrations in marine ecosystems to prevent poisoning by algae toxins. Monitoring of domoic acid and okadaic acid for two decades in Portugal showed that the level of domoic acid is mostly below the regulatory limit, whereas, the toxicity of okadaic acid toxin in bivalves from estuaries was considerable (Mattarozzi et al., 2019; Paredes et al., 2011; Vale et al., 2008).

The guidance values in bivalve's oral tissue for okadaic acid have been given in Table 8. These values vary from country to country, but do not exceed 200 ng/g of edible tissue (Quilliam & Wright, 1995). The detected values in present study showed that *P.radiata* has an average concentration of 12.2 \pm 2.8 μg per 100 grams of okadaic acid. The maximum observed concentration was 14.1 μg per 100 grams, which is less than the maximum tolerable amount of this toxin and also less than the allowable amount in Canada.

The average toxin in the body of patients suffering from nausea and vomiting has been measured with an HPLC device and showed a dose of 1.1 mg/kg body weight of DO. The allowable amount of this toxin for human consumption is 20 micrograms per gram of bivalve. Our results showed that *P. radiata* has an average concentration of 0.083 $\mu\text{g}/\text{g}$ of domoic acid

and the maximum concentration recorded was 0.122 µg/g which less than the allowable amount of this toxin for human consumption.

CONCLUSION

The consumption of bivalves is common in the city of Bandar Abbas and the west of Hormozgan province. Despite that, no research has ever been carried out to monitor the status of algae toxins in the province's edible bivalves with emphasis on the assessing the health of consumers. The present study showed that from the point of view of ecological health and the creation of basic data in the marine ecosystem of Hormozgan province, the concentration of domoic acid and okadaic acid toxins in the studied samples are significant. The contamination risk of these toxins for Hormozgan province is currently insignificant. However the updating the information, especially about bivalve consumption rate is necessary.

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CONFLICTS OF INTEREST

There is no any conflict of interest.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

REFERENCES

- Abott, R.T. and Dance, S. P. (2000). *Compendium of seashells*. (USA: Odyssey Publishing).
- Al-Kandari, M., Al-Yamani, F. and Al-Rifaie, K. (2009). *Marine phytoplankton atlas of Kuwait's waters*. (Kuwait: Kuwait Institute for Scientific Research).
- Al-Yamani, F. 2009. *Our Arabian Sea*. (Kuwait: Kuwait Institute for Scientific Research).
- Anderson, DM., Anderson, P. and Bricelj, VM. (2001) *Monitoring and management strategies for harmful algal blooms in coastal waters*. (France: UNESCO).
- Bosch, D., Dance, S. P., Moolenbeek, R. and Oliver, P. G, (1995), *Seashells of Eastern Arabia*. (Motive Publishing, London).
- Brown, A. R., Lilley, M., Shutler, J., Lowe, C., Artioli, Y., Torres, R., and Tyler, C. R. (2020). Assessing risks and mitigating impacts of harmful algal blooms on mariculture and marine fisheries. *Rev. Aquac.*, 12(3); 1663-1688.
- Carmody, E.P., James, K.J. and Kelly, S.S. (1996) *Dinophysistoxin-2: the predominant diarrhoetic shellfish toxin in Ireland*. *Toxicon*, 34(3); 351-359.
- Carpenter, K. E. and Niem V.H. (Eds.). (1998), *FAO species identification guide for fishery purposes; the living marine resources of the Western Central Pacific, Vol. 1.: Seaweeds, corals, bivalves and gastropods*. (ROME, FAO).
- Contreras, A. M., Marsden, I. D., and Munro, M. H. (2012). Effects of short-term exposure to paralytic shellfish toxins on clearance rates and toxin uptake in five species of New Zealand bivalve. *Mar. Freshw. Res.*, 63(2); 166-174.
- Duy, T.N., Lam, P.K.S., Shaw G.R. and Conell, D.W. (2000) *Toxicology and risk assessment of freshwater cyanobacteria (blue-green alga) toxins in water*. *Rev. Environ. Contam. Toxicol.*, 163; 113-186.
- Estevez, P., Castro, D., Pequeño-Valtierra, A., Giraldez, J. and Gago-Martinez, A. (2019). Emerging marine biotoxins in seafood from European coasts: Incidence and analytical challenges. *Foods*, 8(5);

149.

- Fernandez, M.L. (2000). Regulations for marine microalgal toxins: Towards harmonization of methods and limits. *Afr. J. Mar. Sci.*, 22; 339-346.
- Gholami, Z., Mortazavi, M. S. and Karbassi, A. (2019). Environmental risk assessment of harmful algal blooms case study: Persian Gulf and Oman Sea located at Hormozgan Province, Iran. *Hum. Ecol. Risk Assess.*, 25(1-2); 271-296.
- Hallegraeff, G., Anderson, D.M. and Cembella A.D. (2003). *Manual on harmful marine microalgae*. (France: UNESCO Publishing).
- Hamzehei, S., Bidokhti, A.A. and Mortazavi, M.S. (2012) Analysis of red tide in Strait of Hormuz in 2008-2009 using ocean satellite data. *J. Mar. Sci. Eng.*, 2(4); 225-232.
- Hoppenrath, M., Elbrachter, M. and Drebes, G. (2009) *Marine phytoplankton* (Germany: Schweizerbart Science).
- Hormozgan Fisheries Department (2009) Results of aquatic consumption survey in the urban community of Hormozgan province. Hormozgan Fisheries Department, Iran (In Persian).
- Hosseinzadeh, S., Daghoghi, H., and Rameshi, H. (2001). *Atlas of the Persian Gulf mollusks* (in Persian). (Iran, Iranian Fisheries Research Organization).
- Jeffery, B., Barlow, T., Moizer, K., Paul, S. and Boyle, C. (2004). Amnesic shellfish poison. *Food Chem. Toxicol.*, 42(4); 545-557.
- Lee, J. S., Yanagi, T., Kenma, R. and Yasumoto, T. (1987). Fluorometric determination of diarrhetic shellfish toxins by high-performance liquid chromatography. *Agric. Biol. Chem.*, 51(3); 877-881.
- Lefebvre, K. A. and Robertson, A. (2010). Domoic acid and human exposure risks: a review. *Toxicon*, 56(2); 218-230.
- Mattarozzi, M., Cavazza, A., Calfapietra, A., Cangini, M., Pigozzi, S., Bianchi, F. and Careri, M. (2019). Analytical screening of marine algal toxins for seafood safety assessment in a protected Mediterranean shallow water environment. *Food Addit. Contam. Part A*, 36(4); 612-624.
- Mirza Esmaeili, F., Mortazavi, M. S., Arjomandi, R. and Lahijanian, A. (2020a). A study on red tide risk and basic understanding of fishermen and residents in Bandar Abbas, Hormozgan Province, Iran (Persian Gulf). *Iran. J. Fish. Sci.*, 19(1); 471-487.
- Mirza Esmaeili, F., Mortazavi, M. S. and Dehghan Banadaki, A. R. (2020b). An overview of management and monitoring of harmful algal blooms in the northern part of the Persian Gulf and Oman Sea (Hormozgan Province). *Environ. Monit. Assess.*, 192(1); 1-11.
- Mohammad Karami, A., Riyahi Bakhtiari, A., Kazemi, A. and Kheirabadi, K. (2014). Assessment of Toxic Metals Concentration using Pearl Oyster, *Pinctada radiata*, as Bioindicator on the Coast of Persian Gulf, Iran. *Iran. J. Toxicol.*, 7(23); 956-961.
- Mohebbi-Nozar, S.L., Ismail, W.R. and Zakaria, M.P. (2014) Health risk of PCBs and DDTs in seafood from southern Iran. *Hum. Ecol. Risk Assess.*, 20 (5); 1164-1176.
- Mortazavi, MS., Aramideh, A. and Mohebbi, L. (2015) Investigation and determination of marine toxins in the shellfish of Persian Gulf and Oman Sea. *Iran. J. Fish. Sci.*, 24(2); 125-134.
- Nguyen, H. N., Smith, M. E., and Swoboda, H. D. (2017). *Shellfish Toxicity*. (United States: StatPearls Publishing).
- Omura T., Iwataki, M., Valeriano M B., Takayama H. and Fukuyo Y. (2012), *Marine phytoplankton of the Western Pacific*. (Japan: Kouseisha Kouseikaku Publisher).
- Paredes, I., Rietjens, I. M. C. M., Vieites, J. M., and Cabado, A. G. (2011). Update of risk assessments of main marine biotoxins in the European Union. *Toxicon*, 58(4); 336-354.
- Perl, T. M., Bedard, L., Kosatsky, T., Hockin, J. C., Todd, E. C. and Remis, R. S. (1990). An outbreak of toxic encephalopathy caused by eating mussels contaminated with domoic acid. *N Engl. J. Med.*, 322(25); 1775-1780.
- Polikarpov, I., Saburova, M. and Al-Yamani, F. (2020). Decadal changes in diversity and occurrence of microalgal blooms in the NW Arabian/Persian Gulf. *Deep Sea Res. Part II Top. Stud. Oceanogr.*, 179; 104810.
- Quilliam, M. and Wright, J. (1995) Methods for diarrhetic shellfish poisons. In: Hallegraeff G, Anderson D, and Cembella A (eds) *Manual on harmful marine microalgae*, IOC Manuals and Guides # 33 (Paris: UNESCO).
- Ramstad, H., Larsen, S. and Aune, T. (2001). The repeatability of two HPLC methods and the PP2A assay in the quantification of diarrhetic toxins in blue mussels (*Mytilus edulis*). *Toxicon*, 39(4); 515-522.
- Stabell, OB., Steffenak, I. and Aune, T. (1992) An evolution of the mouse bioassay applied to extracts of

- diarrhoeic shellfish toxins. *Food Chem. Toxicol.*, 30; 139-144.
- Takahashi, T. (2020). *Relationship between Algal Blooms and Marine- ecosystem Services. Monitoring Artificial Materials and Microbes in Marine Ecosystems: Interactions and Assessment Methods* (Singapore: Bentham Science Publishers).
- Takahashi, E. (2007). *Risk assessment of marine algal toxins on humans and dugongs* (PhD thesis), Griffith University, Australia.
- Todd, E. C. (1993). Domoic acid and amnesic shellfish poisoning-a review. *J. Food Prot.*, 56(1); 69-83.
- Vale, P., Botelho, M. J., Rodrigues, S. M., Gomes, S. S. and Sampayo, M. A. D. M. (2008). Two decades of marine biotoxin monitoring in bivalves from Portugal (1986–2006): a review of exposure assessment. *Harmful Algae*, 7(1); 11-25.
- Vale, P. and Sampayo, M.A. (2002) Evaluation of extraction methods for analysis of domoic acid in naturally contaminated shellfish from Portugal. *Harmful Algal*, 1; 127-136.
- Van Dolah, F. M. (2000). *Diversity of marine and freshwater algal toxins*. (United States: CRC Press).
- WoRMS, 2020. 15-6-2020, (<https://www.marinespecies.org/about.php>).
- Yasumoto, T., Oshima, Y. and Yamaguchi, M. (1978) Occurrence of a new type of shellfish poisoning in the Tohoku district. *Bull. Japan. Soc. Sci. Fish*, 44 (11); 129-1255.
- Young, N., Sharpe, R. A., Barciela, R., Nichols, G., Davidson, K., Berdalet, E. and Fleming, L. E. (2020). Marine harmful algal blooms and human health: A systematic scoping review. *Harmful Algae*, 98; 101901.

