

Research Article



**Harmful algal bloom of *Karlodinium Cf. veneficum* (Dinophyceae) and marine organism mortality from the northern coastal waters of the Oman Sea in Iran (2019)**

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**Abstract**

The present study reports widespread aquatic mortality during an unprecedented harmful algal blooms (HABs) in Chabahar Bay, the largest bay of the Oman Sea and one of the most important fishing areas of Iran, on 15 and 16 June 2019. This volume of aquatic death stopped fishing and tourist activities in this area for a short time. During this event, the microalgae *Karlodinium Cf. veneficum* with a density of  $6.8 \times 10^4$  cells mL<sup>-1</sup> was identified as the bloom former species. The toxic dinoflagellate *K. veneficum* has contributed to the aquatic mortality in many coastal areas of the world by producing karlotoxin. The phytoplankton community was studied in this bloom and 46 species of phytoplankton were identified, including 22 species of diatoms, dinoflagellates (22), Cryptophyta (1), and Chlorophyta (1). This is the first occurrence of HABs associated with the dinoflagellate *Karlodinium* bloom and the first report of the presence of two toxic and dinoflagellate species, *Amphidinium carterae* and *Ostreopsis ovata* associated with the algal bloom in the northern Oman Sea. Chabahar Bay is considered one of the most important areas of aquatic fishing grounds in the region. The occurrence of HABs regarding toxic dinoflagellates can be a serious risk to aquaculture activities, human health, and the ecosystem in the area. Water consumption of the residents of Chabahar relies on desalination plants, therefore the bloom of toxic microalgae in the Chabahar bay can disrupt the operation of the desalination plant and pose a potential threat to the water supply in this area.

**Keywords:** Dinoflagellate, Aquatic mortality, Red tide, Chabahar Bay, HABs

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## Introduction

Widespread aquatic animal mortality (Naeem and Sattar, 2007; Pinheiro *et al.*, 2010; La and Cooke, 2011; Eissa *et al.*, 2013) has been reported in many areas of the world under the influence of two major factors including hypoxia (Ram *et al.*, 2014) and harmful algal blooms (HABs) (Kangur *et al.*, 2005). HABs can cause damage in two ways; through toxin production or high biomass accumulation; in some cases, they can have both features (Polikarpov *et al.*, 2020). Phycotoxins released by toxic microalgae not only contaminate fishery products but can eventually enter the human body through the food chain via shellfish and cause poisoning or even death in some cases (Tubaro *et al.*, 2011). The toxic of HABs are spread in different ways, including contaminated seafood (Gerssen *et al.*, 2010), inhalation of suspended particles of dried algal material from the air, drinking water from desalination plants located near waters subject to toxic blooms (He *et al.*, 2016), ingestion of water, and skin contact while swimming (Werner *et al.*, 2012).

The occurrence of harmful algal blooms in the Oman Sea has spread during the last three decades, which has sometimes caused damage to the environment (AlKindi *et al.*, 2007; Thangaraja *et al.*, 2007; Richlen *et al.*, 2010; Al Gheilani *et al.*, 2011; Jalili *et al.*, 2022). Although Chabahar Bay has experienced numerous algal blooms for a long time, algal blooms related to the genus *Karlodinium* have not been reported in this area before (Attaran-

Fariman, 2010; Koochaknejad *et al.*, 2017; Ershadifar *et al.*, 2020; Dolatabadi *et al.*, 2021; Asefi and Attaran-Fariman, 2023). The present study reports a large-scale aquatic mortality event associated with a massive phytoplankton bloom that occurred in June 2019 in Chabahar Bay, the largest bay in the Oman Sea. *Karlodinium veneficum* (D. Ballantine) J. Larsen (= *K. micrum*) is a cosmopolitan dinoflagellate (Yang *et al.*, 2021) and commonly found in coastal aquatic ecosystems (Place *et al.*, 2012; Llanos-Rivera *et al.*, 2023), morphologically characterized by small size (~ 8-12  $\mu\text{m}$ ) and unarmored with a straight apical groove and distinct ventral pores (Daugbjerg *et al.*, 2000; Wang *et al.*, 2011) and one of the 8 toxin-producing species of the genus *Karodinium* (Yang *et al.*, 2021) from the family Kareniaceae belonging to the Gymnodiniales which is known as one of the important species causing HABs (Deng *et al.*, 2023) and the cause of aquatic mortality (Hallegraeff *et al.*, 2010; Dai *et al.*, 2014; Furuya *et al.*, 2018; Yang *et al.*, 2020; Tsikoti and Genitsaris, 2021; Farhat *et al.*, 2022). This microalga is a mixotrophic species and rely on photosynthesis and phagotrophy for growth (Li *et al.*, 2022). The species also produces unique polyketide toxins, called karlotoxins, which are hemolytic, cytotoxic, and ichthyotoxic (Deeds *et al.*, 2002; Farhat *et al.*, 2022). This toxin causes severe damage to the gill epithelium with its mechanism and is especially deadly for all types of fish (Li *et al.*, 2022). Blooms of *K. veneficum* appear to have increased

in recent decades, causing widespread aquatic mortality in estuaries and coastal waters worldwide, including Europe (Nielsen, 1993), China (Dai *et al.*, 2014), Australia (Adolf *et al.*, 2015), Angola (Pitcher and Louw, 2021), and the United States (Hall *et al.*, 2008; Lin *et al.*, 2018a; Wolny *et al.*, 2022).

This report also contains a description of the microalgae community present during this bloom, water parameters, and the description of marine organism mortality. The presence of two toxic and important species, *Ostreopsis ovata* and *Amphidinium carterae* in Chabahar Bay is reported for the first time in this bloom. Chabahar Bay is the most important and largest bay of the Oman Sea, and the majority of the income of its native people is provided by the fishing industry. In addition, it has a high potential for tourism and diving due to having habitat for sea turtles as well as the presence of coral reefs. Therefore, the occurrence of harmful algal bloom in this area is considered a serious and big challenge for the residents of the surrounding cities.

## Materials and methods

### *Description of the study area and red tide location*

Chabahar Bay is located in the northeast of Oman Sea and the closest waterway to the Indian Ocean with longitude 60° 30' 25" to 60° 45' 32" and latitude 25° 15' 17" to 25° 26' 08" (Fig. 1). This area is considered the largest free trade and industrial zone of Iran and has two important cities in terms of industry and population, Chabahar and Konarak. This

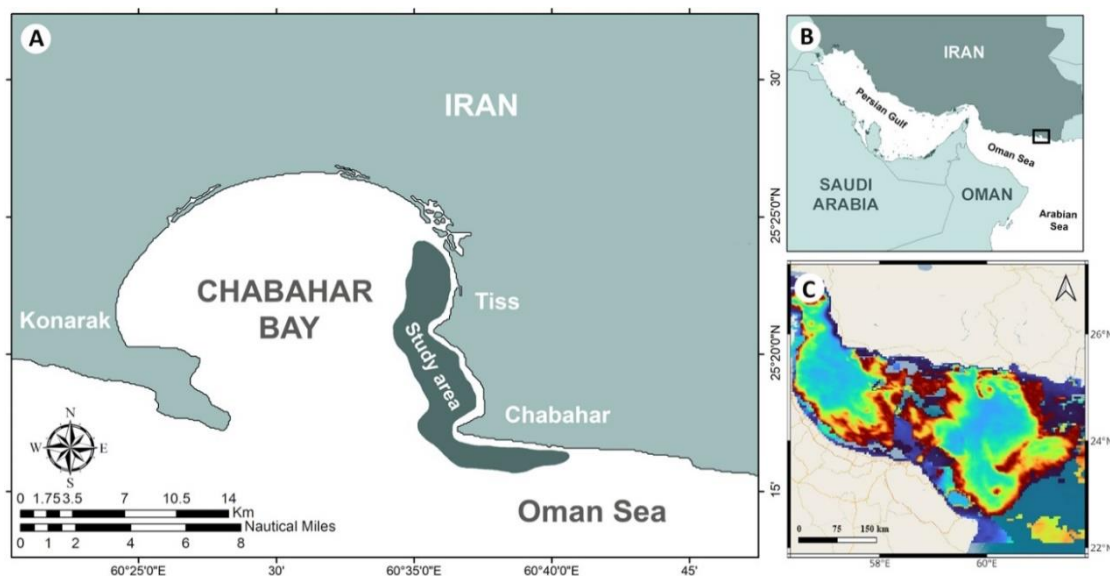
semi-enclosed bay with an area of 290 square kilometers, the width of the entrance mouth is 14 km in the east-west direction and the length is 17 km in the south-north direction, the maximum depth is about 20 m (average depth is 12 m) and it faces two monsoons, summer, and winter, which originate from the Indian subcontinent. Chabahar Bay has a temperate tropical climate and no rivers, and due to its shape ( $\Omega$ ) in geology, it is called an omega or horseshoe-shaped bay, which has limited water circulation.

### *Field sampling and culture*

Sampling was done one day after blooming on June 16, 2019, from the surface, 1 meter and 2 meters depth of seawater along the Chabahar coast was done using 1-liter sterile bottles with 3 replications. 500 ml of seawater was immediately fixed with 4% Lugol's iodine solution in the place of the bloom and transferred to the laboratory of Chabahar Maritime University (CMU) for initial identification of the microalgae causing the bloom. Temperature, salinity, and pH were measured on-site using the Lutron WA-2017SD Multi Water Quality Meter. The concentration of nutrients including ammonia, nitrate, nitrite, and phosphate in water samples was determined by standard methods (ROPME, 1999). To accurately identify the bloom-causing species and co-occurrence species in algal bloom, live cells were purified using a micropipette and each cell was washed in drops of sterile water according to Attaran-Fariman (2007) and transferred to plates containing f/2

medium. Species in a Phycolab room with a 12-12 h light: dark (L:D) program (light intensity 1800 lux) with a cool-white fluorescent lamp, temperature

$25\pm 1^\circ\text{C}$ , humidity 25%, pH 8, and salinity setting 35 ‰ were placed.



**Figure 1:** Map of Chabahar Bay, located north of the Oman Sea. A) Chabahar Bay and the area where the most mortality occurred. B) The geographical location of Chabahar Bay in the Oman Sea. C) Satellite image (MODIS) of the chlorophyll density of the Oman Sea on June 15, 2019.

### *Morphological observation and identification*

The initial identification of the species was done by transferring 1 ml of sample water to a Sedgwick-Rafter slide under a Nikon-TS100 inverted microscope with 10X and 20X magnification. For counting the cells (Woelkerling *et al.*, 1976) and accurate morphological identification, live cells of microalgae species were observed and photographed using a Nikon-ECLIPSE 50iz light microscope with 100X magnification and JmicroVision software. For epifluorescence microscopy, 1 mL of the cell culture was transferred to a 1.5 mL Microcentrifuge tube, and the cell nucleus was stained with red-fluorescent dye at  $10\ \mu\text{g mL}^{-1}$  and then incubated in

the dark at room temperature for 1 hour. Stained cells were observed and photographed using Hund Fluorescence Microscopes Wetzlar H600/12. Morphological identification of microalgae was done according to valid references (Subrahmayan, 1971; Hasle *et al.*, 1996; Tomas, 1997; Faust and Gullede, 2002; Place *et al.*, 2012).

## **Results**

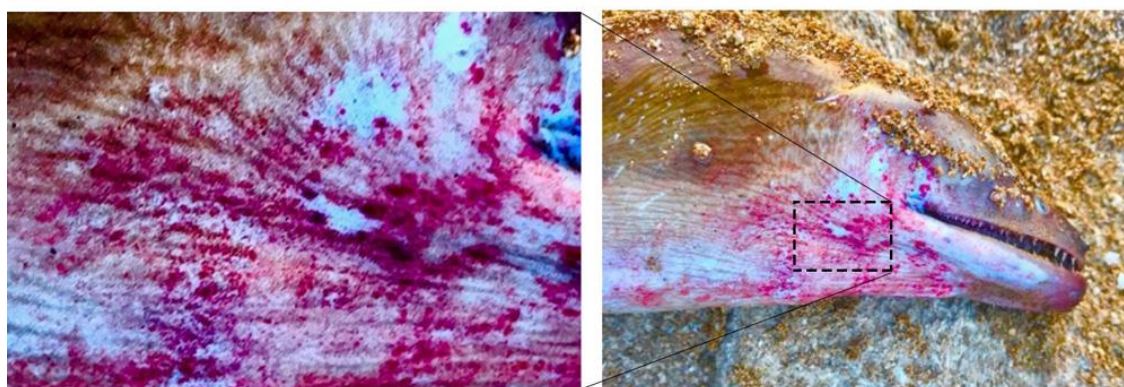
### *Description of event*

On June 15 and 16, 2019, widespread aquatic mortality occurred on the coast of Chabahar Bay. According to the census conducted by the Offshore Fisheries Research Center in Chabahar, more than 1.5 tons of aquatic animals, most of which were fish, were lost,

including Fish (Mullidae, *Siganus luridus*, *Saurida tumbil*, *Pomadasys kaakan*, *Diagramma pictum*, *Platycephalus indicus*, *Gymnura poecilura*, *Solea* sp., *Acanthopagrus* sp., *Gerres* sp., *Netuma* sp.), crab (*Portunus pelagicus*, *Charybdis annulata*), all kinds of shrimp (most of the lost shrimps were of two species, *Penaeus indicus* and *Penaeus semisulcatus*), Muraenesocidae, jellyfish (*Crambionella orsini*), sea snake (*Hydrophis schistosus*, *Hydrophis* sp.),

etc., were dead and They floated on the surface of the water.

This event coincided with a dense algal bloom that caused the water to change color and due to concerns about the pollution of the water environment in terms of toxicity and danger to residents, caused the suspension of fishing, tourism, and swimming activities in the Chabahar Bay for a week. The changes in the appearance of the skin and organs of some animals were completely evident in the mentioned bloom (Fig. 2).



**Figure 2:** Red skin lesions on eel body during *Karlodinium* cf. *veneficum* algal bloom in Chabahar Bay (15-16 June 2019) (photo by Aminrad, 2019).

The water salinity recorded during the bloom ranged from 36.4 to 37.8 ppm during the bloom. The water quality data based on the analysis was as follows:  $\text{NO}_2$  and  $\text{NO}_3$  levels were 0.014 and 2.9 ppm, respectively, and ammonia ( $\text{NH}_4^+$  and  $\text{NH}_3$ ) were less than detectable ( $<0.01 \text{ mg. L}^{-1}$ ). Phosphate was 0.23 ppm, the temperature during blooming was recorded in the range of 31.5-32.6°C, pH 8.11-8.27, and dissolved oxygen 4.08.

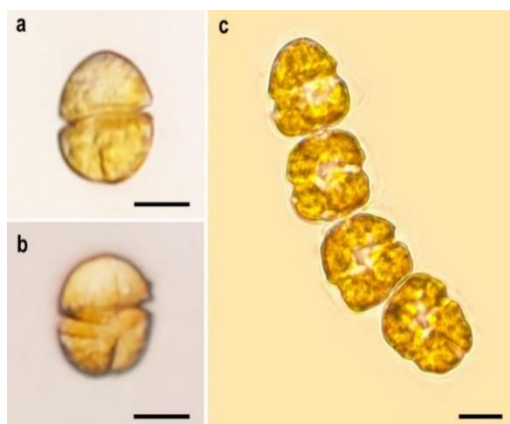
#### *Morphological analysis*

Microscopic investigation of the morphological characteristics in the water samples, including the form shape, and size of cells, showed that the change in water color is due to the blooming of the naked dinoflagellate *Karlodinium* cf. *veneficum* with a density of  $6.8 \times 10^4 \text{ cells mL}^{-1}$ . The shape of the cell was oval and the size of the epicone and hypocone was almost equal (Fig. 3a, b). The cell size was recorded as  $18.7 \pm 1.4 \mu\text{m}$  in length and  $14.4 \pm 1.2 \mu\text{m}$  in width.

During the bloom, three species of toxic dinoflagellates *Gymnodinium*

*catenatum* (Fig. 3c), *Amphidinium carterae* (Fig. 4a, b), and *Ostreopsis ovata* (Fig. 6a, b) which have potential to produce harmful algal blooms in a wide

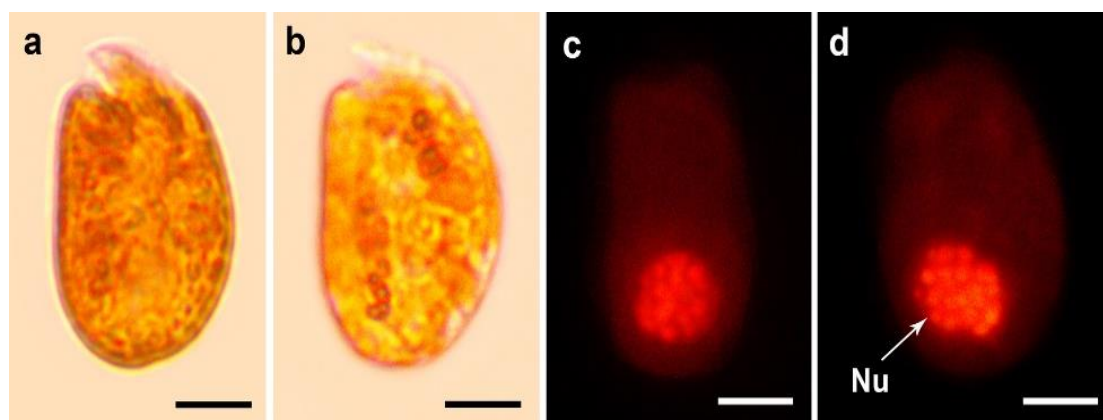
range observed and recorded as bloom co-occurrence species.



**Figure 3:** Light Microscope images of *Karlodinium cf. veneficum* (a and b), and *Gymnodinium catenatum* (c) from Chabahar Bay-northern of the Oman Sea. Scale bar=10 µm.

*A. carterae* and *O. ovata* are reported for the first time from this region. The size of *A. carterae* was recorded between 8-20 µm in length and 5-12 µm in width.

In the epifluorescence micrographs, the stained nucleus in the cell is relatively large, single, and egg-shaped in the posterior part of the hypocone (Fig. 4d, c).

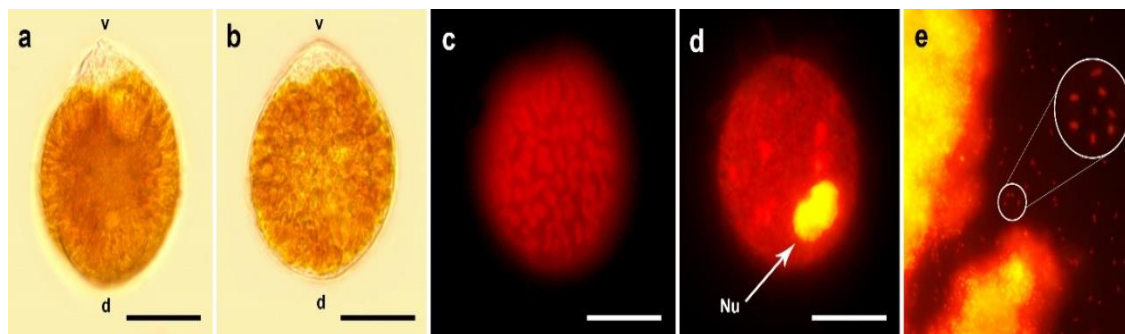


**Figure 4:** Light Microscope images of *Amphidinium carterae* (a, and b), and epifluorescence microscopy micrographs (c and d) of *Amphidinium carterae* from Chabahar Bay-northern of the Oman Sea. Scale bar=5 µm. Nu: Nucleus.

The size of *O. ovata* was also recorded, with a length between 25 and 70 (mean: 47.5) µm and a width between 17 and 55 (mean: 36) µm. The cells are oval and covered with a large number of photosynthesis golden chloroplasts (Fig. 5a-c). Between the two convex parts of

the cell, there is a large oval posterior nucleus (Fig. 5d). A mucilaginous substance surrounded the *O. ovata* microalgae, which can be easily recognized by the naked eye. Microorganisms live inside these mucilaginous substances suspended in

water, which were seen moving under the fluorescence microscope lens as reddish dots (Fig. 5e).

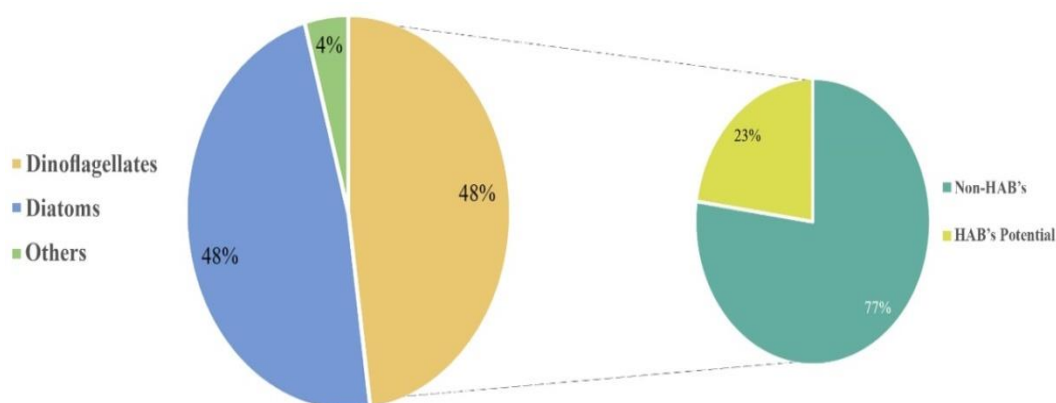


**Figure 5:** Light Microscope images of *Ostreopsis ovata* (a and b), cell chloroplast arrangement (c), and stained cell nucleus (d) under the epifluorescence microscopy, and Microorganisms of the mucilage substance (e) of *O. ovata* from Chabahar Bay-northern of the Oman Sea. Scale bar=20  $\mu\text{m}$ . Nucleus (Nu), Versal (v), Dorsal (d).

#### *The accompanying phytoplankton community*

In total, apart from the blooming species, 46 species of microalgae including 22 species of Dinophyta, 22 species of Bacillariophyta, 1 species of Chlorophyta, and 1 species of Cryptophyta were recorded (Table 1 and Figs. 6-9). In terms of diversity and types

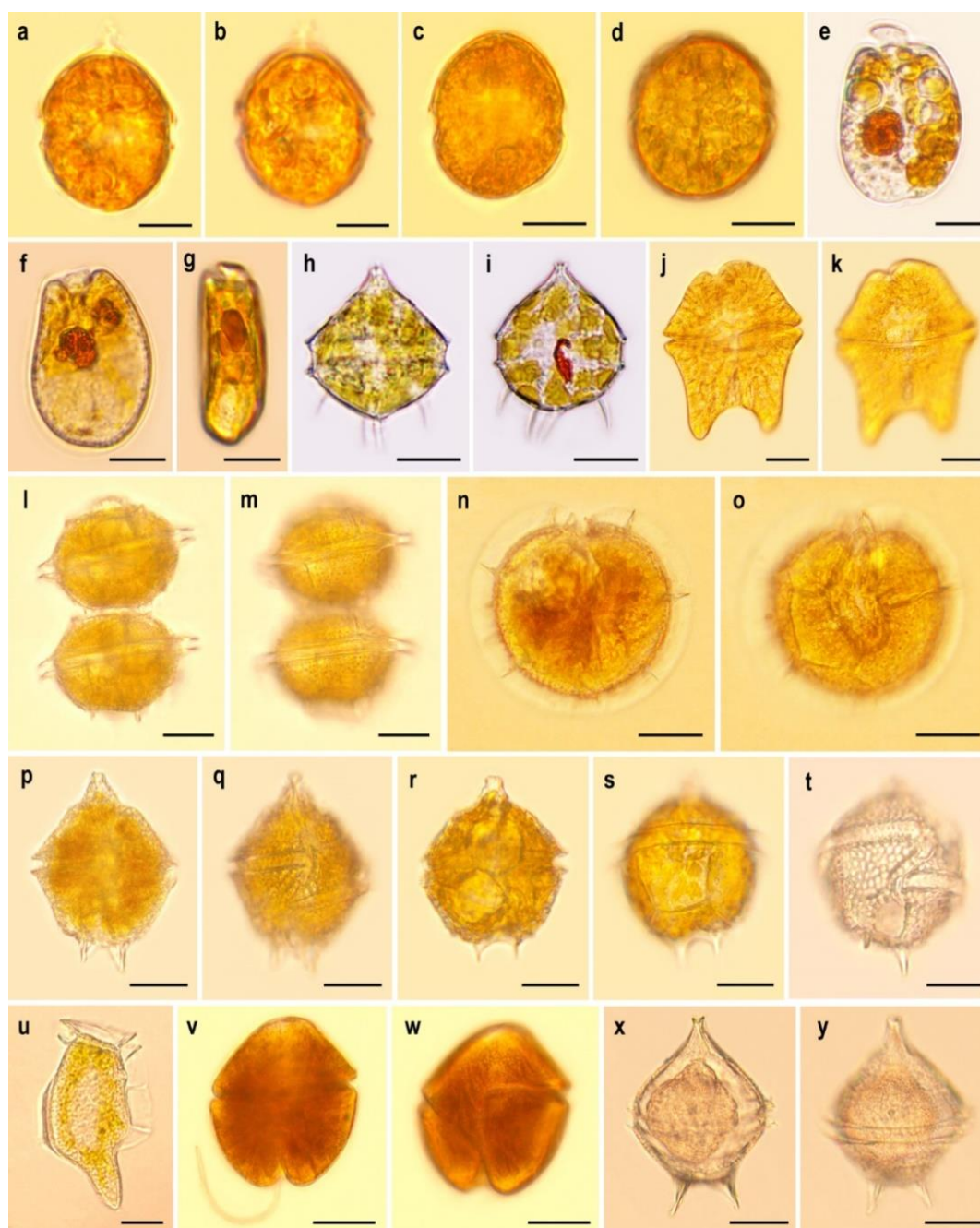
of species, diatoms (48%) were equal to dinoflagellates (48%) (Figs. 6-9). Identification and photography of co-occurrence species were done in two ways, some species were observed under the microscope for the first time on the day of blooming and some after being placed in the culture medium every 5 days.



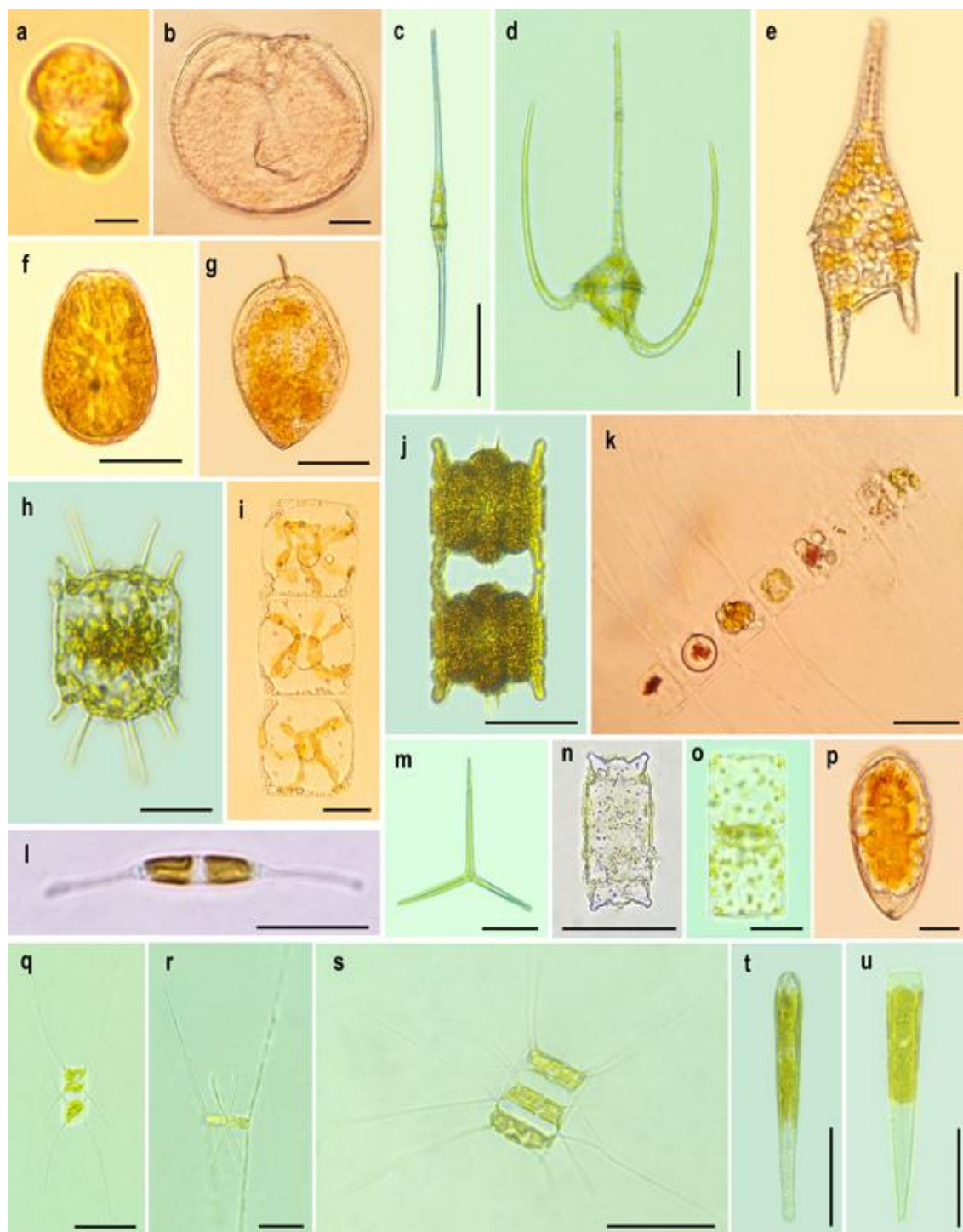
**Figure 6:** The percentage of microalgae species present in harmful algal blooms (left side) and the percentage of non-harmful and harmful dinoflagellates (right side).

**Table 1: Species present in the algal bloom of *Karlodinium cf. veneficum* in Chabahar Bay on 15 and 16 June 2019. Species that have the potential to form harmful algal blooms or contain toxins are marked with (×), non-toxic species are marked with (-), and species whose toxicity is unknown are marked with (n/a) are specified in the table.**

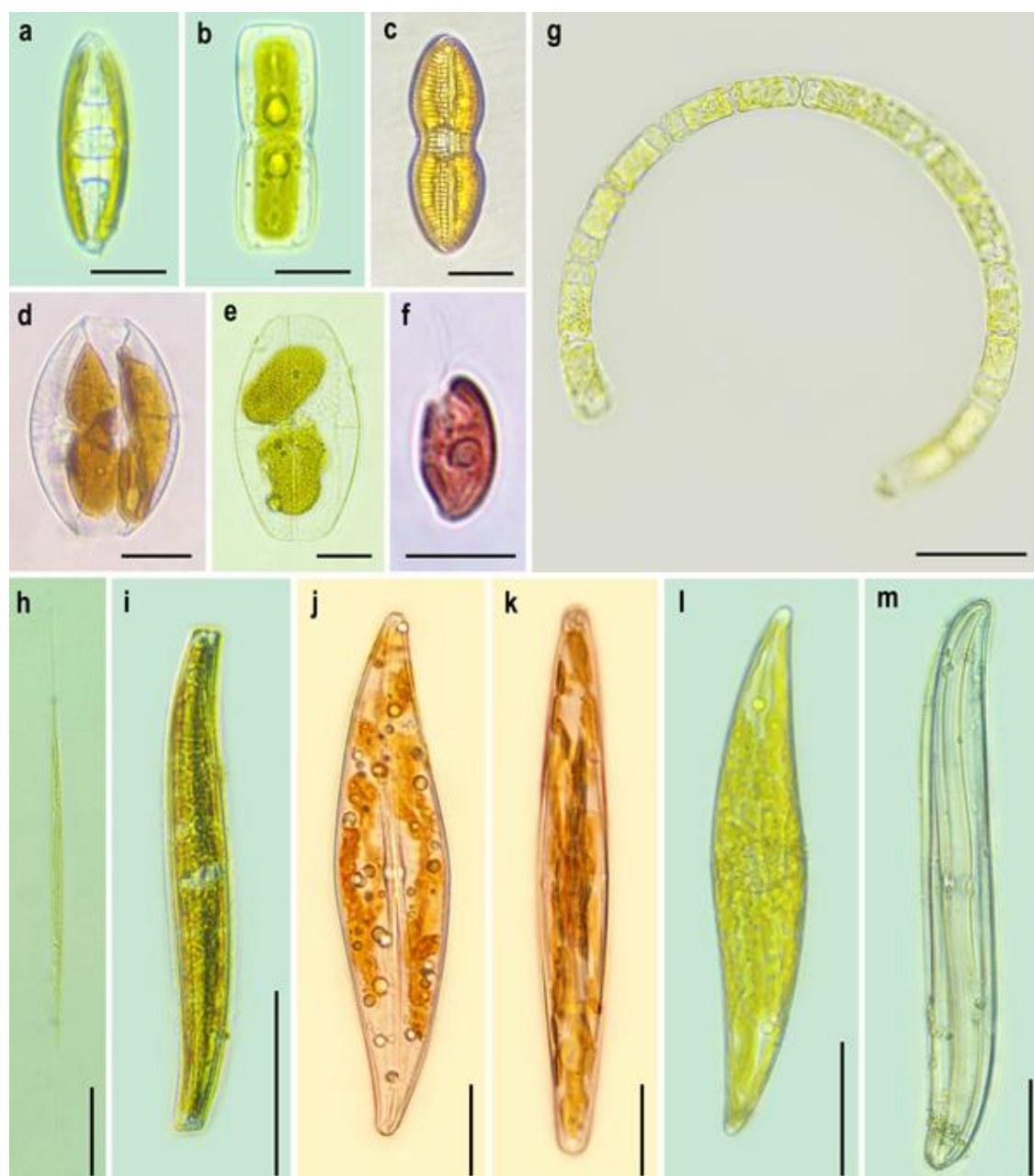
Class	Species	HAB's	Toxic	Observation Time
Dinophyceae	<i>Akashiwo sanguinea</i>	-	-	Blooming
	<i>Amphidinium cartera</i>	×	×	Blooming
	<i>Amphidinium</i> sp. 1	n/a	n/a	Culturing
	<i>Amphidinium</i> sp. 2	n/a	n/a	Culturing
	<i>Ansanella</i> sp.	n/a	n/a	Culturing
	<i>Dinophysis caudata</i>	×	×	Blooming
	<i>Diplopelta</i> sp.	-	-	Blooming
	<i>Gonyaulax polygramma</i>	-	-	Blooming
	<i>Gonyaulax</i> sp.	n/a	n/a	Culturing
	<i>Gymnodinium catenatum</i>	×	×	Blooming
	<i>Levanderina fissa</i>	-	-	Culturing
	<i>Ostreopsis ovata</i>	×	×	Blooming
	<i>Peridinium quadridentatum</i>	-	-	Blooming
	<i>Prorocentrum micans</i>	-	-	Blooming
	<i>Prorocentrum</i> sp.	n/a	n/a	Blooming
	<i>Protoperidinium</i> sp.	n/a	n/a	Culturing
	<i>Pyrodinium bahamense</i>	×	-	Culturing
	<i>Scrippsiella</i> sp.	n/a	n/a	Culturing
	<i>Scrippsiella acuminata</i>	-	-	Culturing
	Bacillariophyceae	<i>Tripos furca</i>	-	-
<i>Tripos fusus</i>		-	-	Culturing
<i>Tripos horridus</i>		-	-	Culturing
<i>Amphora</i> sp. 1		-	-	Blooming
<i>Amphora</i> sp. 2		-	-	Blooming
<i>Bacteriastrum</i> sp.		-	-	Blooming
<i>Biddulphia</i> sp.		-	-	Culturing
<i>Chaetoceros</i> sp. 1		-	-	Blooming
<i>Chaetoceros</i> sp. 2		-	-	Blooming
<i>Chaetoceros</i> sp. 3		-	-	Blooming
<i>Cylindrotheca</i> sp.		-	-	Blooming
<i>Guinardia</i> sp. 1		-	-	Culturing
<i>Guinardia</i> sp. 2		-	-	Blooming
<i>Haslea</i> sp.		-	-	Blooming
<i>Helicotheca</i> sp.		-	-	Blooming
<i>Licmophora</i> sp.		-	-	Culturing
<i>Navicula</i> sp.		-	-	Blooming
<i>Nitzschia</i> sp. 1		n/a	n/a	Blooming
<i>Nitzschia</i> sp. 2		n/a	n/a	Culturing
<i>Odontella</i> sp.		-	-	Blooming
<i>Pleurosigma</i> sp. 1	-	-	Blooming	
<i>Pleurosigma</i> sp. 2	-	-	Blooming	
<i>Pleurosigma</i> sp. 3	-	-	Blooming	
<i>Surirella</i> sp.	-	-	Blooming	
<i>Trieres mobiliensis</i>	-	-	Culturing	
Chlorophyceae	<i>Treubaria</i> sp.	-	-	Culturing
Cryptophyceae	<i>Rhodomonas</i> sp.	-	-	Blooming



**Figure 7:** Light micrographs of *Scrippsiella acuminata* (a, b), *Scrippsiella* sp. (c, d), *Amphidinium* sp.1 (e), *Amphidinium* sp. 2 (f, g), *Peridinium quadridentatum* (h, i), *Akashiwo sanguinea* (j, k), *Pyrodinium bahamense* (l-o), *Gonyaulax polygramma* (p, q), *Gonyaulax* sp. (r-t), *Dinophysis caudata* (u), *Levanderina fissa* (v, w), *Protoperidinium* sp. (x, y). Scale bars, 10  $\mu$ m (a-i); 20  $\mu$ m (j, y).



**Figure 8:** Light micrographs of *Ansanella* sp. (a), *Diplopelta* sp. (b), *Triplos fusus* (c), *Triplos horridus* (d), *Triplos furca* (e), *Prorocentrum* sp. (f), *Prorocentrum micans* (g), *Trieres mobiliensis* (h), *Helicotheca* sp. (i), *Odontella* sp. (j), *Bacteriastrium* sp. (k), *Cylindrotheca* sp. (l), *Treubaria* sp. (m), *Biddulphia* sp. (n), *Guinardia* sp. 1 (o), *Surirella* sp. (p), *Chaetoceros* sp. 1 (q), *Chaetoceros* sp. 2 (r), *Chaetoceros* sp. 3 (s), *Licmophora* sp. (t, u). Scale bars, 5  $\mu$ m (a); 20  $\mu$ m (b), 50  $\mu$ m (c-e); 20  $\mu$ m (f–u).



**Figure 9:** Light micrographs of *Navicula* sp. (a, b), *Nitzschia* sp. 1 (c), *Amphora* sp. 1 (d), *Amphora* sp. 2 (e), *Rhodomonas* sp. (f), *Guinardia* sp. 2 (g), *Haslea* sp. (h), *Nitzschia* sp. 2 (i), *Pleurosigma* sp. 1 (j, k), *Pleurosigma* sp. 2 (l), *Pleurosigma* sp. 3 (m). Scale bars, 10  $\mu\text{m}$  (a-g); 20  $\mu\text{m}$  (h-m).

### Discussion

The reproduction and blooming of dinoflagellate *K. veneficum* are strongly dependent on environmental parameters such as water salinity and pH, light intensity, temperature, and organic and mineral nutrient concentrations (Lin *et al.*, 2018b ; Huang *et al.*, 2019). This

dinoflagellate is usually observed in relatively low cell abundance ( $10^2$ - $10^3$  cells  $\text{mL}^{-1}$ ), but it can form very dense blooms ( $10^4$ - $10^5$  cells  $\text{mL}^{-1}$ ) (Deeds *et al.*, 2006; Llanos-Rivera *et al.*, 2023). Some studies show that the number of *K. veneficum* cells during blooming can increase up to  $10^6$  cells  $\text{mL}^{-1}$  (Goshorn *et*

*al.*, 2002; Hall *et al.*, 2008). The research results show that the mortality of aquatic animals due to the bloom of the species usually occurs at a density above  $10^4$  cells  $\text{mL}^{-1}$ . During a bloom in 1996 at a fish farm in Maryland, USA, an algal bloom of *K. veneficum* with a cell density of  $6 \times 10^4$  cells  $\text{mL}^{-1}$  caused extensive mortality of hybrid striped bass (Place *et al.*, 2012). *K. veneficum* is known to be the main cause of periodic fish mortality in the Chesapeake Bay in the United States (Deeds *et al.*, 2002). In 2005, many fish species were killed by *K. veneficum* cells that had reached densities ( $>10^4$  cells  $\text{mL}^{-1}$ ) in the Swan and Canning River estuaries in Perth Australia (Adolf *et al.*, 2015). In the same year, one of the largest aquatic deaths caused by this species occurred in Maryland in the upper and middle Corsica River, according to estimates, 30 to 50 thousand fish were killed in the bloom of *K. veneficum* with a cell density of more than  $56 \times 10^3$  cells  $\text{mL}^{-1}$  (Place *et al.*, 2012). A year later, a dense bloom of this ichthyotoxic dinoflagellate with a cell density ( $>200 \times 10^3$  cells  $\text{mL}^{-1}$ ) in the Neuse River Estuary, North Carolina, caused widespread aquatic mortality (Hall *et al.*, 2008). The density of *K. Veneficum* cells in the present study was  $6.8 \times 10^4$  cells  $\text{mL}^{-1}$ . However, there are reports of researchers' results that the blooming of this species in very high cell density was without mortality. For example, during a bloom in Maryland with  $100 \times 10^4$  cells  $\text{mL}^{-1}$ , no fish mortality was recorded (Place *et al.*, 2012). Therefore, the mechanism of release of toxins of this microalgae is not

properly understood. Place *et al.* (2012) hypothesize that probably shallow areas or aquaculture systems are more at risk of mortality due to *K. veneficum* bloom than other areas.

During the bloom of *K. veneficum* in Chabahar Bay, a large amount of disintegrated microalgae cells were observed, which could not be identified due to cell destruction. However, some cells, whose shape and structures were not damaged, were morphologically similar to *K. veneficum*. The destruction of microalgae cells coincided with the high mortality of marine organisms in this area. One of the main causes of fish losses in this bloom is probably suffocation caused by gill inflammation, however, the results of the researchers show that the release of intracellular toxins of this dinoflagellate during the collapse of the bloom can be the main reason for the death of aquatic animals because the organisms that died during the collapse of the *K. veneficum* bloom in other parts of the world had symptoms of poisoning. In other words, it is assumed that the aging and destruction of *K. veneficum* cells releases enough toxin to kill aquatic animals in the aquatic environment (Hall *et al.*, 2008). The toxins produced by *K. veneficum* are called karlotoxins (Deeds *et al.*, 2002), and at least 12 natural karlotoxin analogs have been identified to date (Yang *et al.*, 2021). Although these toxins are toxic to many aquatic animals, the most damage is done to fish because karlotoxin has a highly destructive power in damaging the gill-covering tissues (Place *et al.*, 2012). Also, fish exposed to *K.*

*veneficum* toxins show various symptoms including suffocation, weakness, white spots on the scales, and cloudy eyes (Furuya *et al.*, 2018). The results of the present study confirm the visible damage on the aquatic organs exposed to the bloom of this dinoflagellate (Fig. 2).

*K. veneficum* is known as a microalgae with a high ability to adapt to the surrounding environment, and for this reason, it can dominate a relatively large marine area with its cell reproduction. This dinoflagellate has a high tolerance range against environmental stresses. Also having lethal toxins against other predators and allelopathic effects on competitors are other survival strategies of *K. veneficum* for growth, survival, and expansion of its distribution range (Yang *et al.*, 2020). One of the main reasons for the survival of this microorganism and its distribution in most marine areas of the world is probably related to the type of its feeding. Despite its small size, this dinoflagellate has a feeding based on phagotrophy. The results of a study by Yang *et al.* (2020) show that *K. veneficum* is an omnivorous phagotroph feeding from dead and alive bodies and cells of fish (*Oryzias melastigma*), brine shrimp (*Artemia salina*), rotifer (*Brachionus plicatilis*) and even microalgae such as *Akashiwo sanguinea*, and It also feeds on *Margalefidinium*, *Isochrysis galbana*, and *Rhodomonas salina*. This dinoflagellate can feed on any organism, including cells of its own species. However, *K. veneficum* prefers to feed on immobile or freshly dead prey

(fish, zooplankton, or phytoplankton) (Yang *et al.*, 2020). Place *et al.* (2012) cited mixotrophy as an important strategy for *K. veneficum* bloom formation. The abundance of prey, especially nanoplankton cryptophytes, seems to be a key factor in causing toxic blooms of *K. veneficum* in eutrophic environments (Place *et al.*, 2012). Therefore, probably this important parameter can explain the success of this species in forming frequent blooms and its global distribution.

The present study reports for the first time the presence of two dinoflagellates, *Amphidinium carterae* and *Ostreopsis ovata*, which are among the most important and dangerous dinoflagellates in terms of toxicity and harmful bloom formation. These species were previously identified and recorded during the routine monitoring of phytoplankton in the waters of the Oman Sea and the Persian Gulf (Al-Yamani *et al.*, 2012; Darki and Krakhmalnyi, 2017; Saraji, 2018), however, they were not reported in Chabahar Bay until this bloom occurred. *A. carterae* is a dinoflagellate abundant in most marine areas around the world, but it was not identified in Chabahar Bay before this study. Perhaps, one of the reasons for this issue is that naked dinoflagellates are often sensitive to the type of sampling or the dose of fixer solutions and undergo deformation. For this reason, their sampling is not successful most of the time (Baig *et al.*, 2006; Okolodkov and Gárate-Lizárraga, 2006; Gárate-Lizárraga, 2012). The presence of two dinoflagellates *A. carterae* and *O.*

*ovata* are often observed together (Nascimento *et al.*, 2012), however, the cause of this relationship has not yet been investigated. So far, the presence of 14 species of the genus *Amphidinium* and 3 species of the genus *Ostreopsis* have been identified and reported in the waters of the Oman Sea and the Persian Gulf (Attaran-Fariman and Asefi, 2022), of which 3 species are *A. gibbosum*, *A. operculatum*, and *A. carterae* and 3 species, *O. lenticularis*, *O. siamensis*, and *O. ovata*, cause harmful algal blooms and are considered by UNESCO (IOC) as toxic microalgae (Lunholm *et al.*, 2009; Mandal *et al.*, 2011; Attaran-Fariman and Asefi, 2022). The unarmored dinoflagellate *A. carterae* Hulburt is known as the toxin-producing species through Ciguatera Fish Poisoning (CFP) in humans (Murray *et al.*, 2012; Karafas *et al.*, 2017). *O. ovata* species is also considered an epiphytic dinoflagellate and has the potential to produce palytoxin (PLTX) and ovatoxins (OVTXs) (Brissard *et al.*, 2014; García-Altare *et al.*, 2015). These toxins can cause severe and sometimes fatal poisoning if ingested by humans through feeding on aquatics such as fish and shellfish (Faimali *et al.*, 2012). However, the most common poisoning in humans is due to the inhalation of aerosols in the form of sprays, which include respiratory problems, skin irritations, and mild eye problems (Nascimento *et al.*, 2012; Pfannkuchen *et al.*, 2012; Gémin *et al.*, 2020).

The results of most studies show that both of these dinoflagellates tend to live in tropical waters (Gárate -Lizárraga,

2012; Seoane *et al.*, 2018; Tibiriçá *et al.*, 2019), Therefore, the warming of the earth due to climate change can probably increase their blooming in marine environments. Even though *A. carterae* has a high potential for very high reproduction in most environmental conditions, however, the report of its blooming is less observed. For example, previous records from the waters of the coastal areas of Pakistan in the Arabian Sea (Baig *et al.*, 2006), the coast of Mexico in the Gulf of California (Gárate -Lizárraga, 2012), and recently on the coast of Sydney in Australia (Murray *et al.*, 2015), which in the last case, it caused the death of a large amount of fish in the mentioned area. Although *O. ovata* is rapidly increasing its presence in many marine areas and numerous blooms are reported every year, blooms with aquatic mortality are less reported (eg. Ferreira, 2006). Although, in the past few years, Chabahar Bay has been involved in the bloom of dinoflagellates with high density, which has caused the death of marine organisms (Koochaknejad *et al.*, 2017; Ghazilou *et al.*, 2017; Asefi and Attaran-Fariman, 2023). However, the bloom of dinoflagellates *A. carterae* and *O. ovata* has not been reported from this area until now, and they have not contributed to the mortality of aquatic animals in the study area.

Most of the phytoplankton species in the Persian Gulf and the Oman Sea that have the potential to produce toxins are dinoflagellates (Attaran-Fariman and Asefi, 2022), which can not only cause health problems for humans but also kill

marine animals, including marine mammals. Also, damage to coral reefs, reduction of water quality, and economic problems, including damage to fisheries and aquaculture industries, and suspension of water desalination operations are other consequences of the blooming of toxic species in the Oman Sea. Also, these blooms forced the closure of desalination plants in the Oman Sea and the Persian Gulf (Richlen *et al.*, 2010; Villacorte *et al.*, 2015). The results of several studies indicate that harmful algal blooms (HABs) and their impacts have recently increased in the Oman Sea (Al-Azri *et al.*, 2007; Thangaraja *et al.*, 2007; Richlen *et al.*, 2010) as well as other coastal areas of the world (Glibert *et al.*, 2005). Blooms of HABs have been reported in the coastal areas of the Oman Sea since 1976 (Al Gheilani *et al.*, 2011). In general, the occurrence of HABs in the Oman Sea is more reported than in the Persian Gulf, which is probably due to the increase of Asian monsoon winds in this region, which enter from the Arabian Sea (Sedigh Marvasti *et al.*, 2016). In a similar study by Ershadifar *et al.* (2020) in Chabahar Bay, the results of the mentioned study have been confirmed. The hydrodynamics of Chabahar Bay is related to the prevailing winds blowing from southeast to northwest in this bay. These winds are so strong that they can create water currents on the seabed (Aliabad *et al.*, 2019), thus providing nutrients to the surface layer.

The fishing and fishery industry is one of the biggest and most important economic activities in Chabahar Bay.

40% of all fish caught in Iran is provided by 23 thousand local fishermen of the Chabahar Bay, which has become the biggest source of employment for its residents. This region is a rich source of economic aquatic life such as more than 30 species of shrimp, 10 species of crab, 5 species of lobster, and 70 species of commercial fish, and thus it is at a high level in terms of biological production (Jamnia *et al.*, 2015). But at the same time, it is considered a vulnerable ecosystem, as urban and industrial effluents, tourism development, increase in the number of commercial and shipping docks, and release of destroyed fishing nets by fishermen have severely exposed this area to pollution. In addition, the unprincipled burial and disposal of urban waste near the coasts of this area and its entry into the sea have polluted the water and changed the ecosystem of this area (Ershadifar *et al.*, 2020; Asefi and Attaran Fariman, 2022). Also, Chabahar Bay is exposed to various natural environmental threats, including the 120-day dust storms of Sistan and its entry into the Chabahar Bay's atmospheric regions, very low rainfall, and climate change, and the rate of flushing and limited water circulation in this area (Agah *et al.*, 2016) in addition to the above environmental hazards, the formation of harmful algal blooms as a result of increased nutrient enrichment from urban, industrial, and desalination plant effluents can damage the ecosystem make this area more vulnerable and reduce fishing activities increasingly.

Although several studies have been conducted on species causing harmful algal blooms in Chabahar Bay (Attaran-Fariman and Sharifian, 2014; Attaran-Fariman *et al.*, 2012; Dolatabadi *et al.*, 2021; Asefi and Attaran-Fariman, 2023), however, more extensive investigations are necessary to identify the presence of the above phytoplankton species in the waters of this region and to conduct detailed studies to understand the dynamics of these blooms and the factors that cause them in the ecosystem. Measures such as preventing pollution from entering the waters of this region, continuous monitoring of the sea through satellite images, more research on these species and the mechanisms leading to the death of aquatic animals, identifying the mechanisms of marine dinoflagellate toxins present in this region and informing about the exposure and diseases related to HABs to the indigenous people in the Chabahar Bay can help to reduce the risks and consequences of harmful algal blooms in this area. In general, risks cannot be completely eliminated but can be reduced to acceptable levels.

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