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Environmental Emergence of Brown Tide and Water Discoloration Caused by the Dinoflagellate *Scrippsiella trochoidea* in the Eastern Harbor of Alexandria, Egypt

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ABSTRACT

Anthropogenic marine eutrophication is one of the biggest risks to the health of marine environments, causing ecosystem disruption. In recent years, eutrophication events, together with climate change, have accelerated the development of potentially toxic algal species, causing severe harmful algal blooms (HABs) to be more common in coastal areas. Dinoflagellates, which make up a significant portion of marine phytoplankton, could create hazardous algal blooms. The results revealed that the phytoplankton community in Alexandria's Eastern Harbor (EH) consisted of 26 species from 24 genera, with dinoflagellates dominating (14 species). The total phytoplankton standing crop showed an average count of 5.277 x 10^6 units L^{-1}, and biomass (Chlorophyll *a*) of 5.63 µg L^{-1}. Dinoflagellates accounted for 98.95% of the total phytoplankton abundance and were dominated by *Scrippsiella trochoidea*, followed by *Pyrophacus horologium*, *Gyrodenium fusiforme* and *Gymnodinum catenatum*. *Scrippsiella trochoidea* exhibited an extremely high count of 5.152 x 10^6 units L^{-1}, contributing for 98.66% of the total dinoflagellates, 97.62% of the total numerical standing crop. *S. trochoidea* was the causative species of brown tide blooms and water discoloration that suddenly appeared in the EH. These blooms killed a vast number of fishes, resulting in severe economic losses in the study area.

INTRODUCTION

Red tides are HABs that occur when toxic microscopic algae in marine, brackish, and freshwater environments multiply to higher-than-normal concentrations (bloom), turning the water red, brown, green, or yellow **(Jae-Wook *et al*., 2023)**. Since their frequency, duration, and dispersion have grown over the past few decades, HABs have drawn attention across the world **(Anderson *et al*., 2012)**. HABs can be produced by a wide variety of microalgae, including diatoms, dinoflagellates, raphidophytes, haptophytes, pelagophytes, and cryptophytes **(Anderson *et al*., 2015)**. Fisheries, tourism, seafood safety, and other ecosystem services are all seriously threatened by HABs, which are prevalent in coastal waters **(Bresnan *et al*., 2021)**. Fish, shellfish, birds, marine animals, and humans are all negatively impacted by the potent poisons produced by some algae that generate red tides **(Willis *et al*., 2018; Young *et al*., 2020)**. Additionally, algal blooms have the ability to drastically lower oxygen levels in natural settings, which can lead to the death of species in fresh and marine waters **(Heisler *et al*., 2008)**. During a red tide, beaches are sometimes covered in dead fish and other animals that either consumed toxins or were incapable to obtain enough oxygen; as a result, many nations restrict fishing during red tides. Both cold and tropical waters are home to the non-toxic marine dinoflagellate *Scrippsiella trochoidea*, which is known to cause "red tide" phenomena. *Scrippsiella* blooms could damage local fisheries by generating dense blooms that cause oxygen depletion or hypoxia **(Horner *et al*., 1997)**. *Scrippsiella* blooms have been reported in the southern gulf of Mexico **(Licea *et al*., 2002)**, Japan's coasts, northern Europe **(Gottschling *et al*., 2005; Spatharis *et al*., 2009)**, and China **(Yang *et al*., 2007)**. Red tide blooms and water discoloration have been monitored multiple times in EH. **Mikhail *et al.* (2020)** noted water discoloration and three red tide blooms in late summer, early autumn, and the last week of December. The centric diatom *Thalassiosira rotula*, *Heterosigma akashiwo* (Raphidophycea), and *Heterocapsa triquetra* and *Gymnodinium impudicum* (Dinophyceae) were the causal species. This investigation was based on a phenomenon observed in the surface water of the Eastern Harbor Bay of Alexandria on August 7th, 2024. Discoloration of the water and brown to reddish tide blooms were noticed, covering most of the harbor. The bloom appeared suddenly, lasted throughout the day, and then disappeared the following day, prompting us to investigate the cause of this event.

MATERIALS AND METHODS

Study area and sampling stations

The EH is a shallow semi-enclosed basin that spans an area of roughly 2.6km^2 at varying depths, with a maximum water depth of 11m and an average depth of 6.5m. It is located between latitudes 31º 12´ N and 31º 13´ N and longitudes 29º 53´ E and 29º 54´ E in the center of the Alexandria coast in Egypt (Fig. 1). The water volume in the EH, a significant commercial fishery, is 15.2 x 10^{6}m^3. Two outputs from the eastern side (El-Silsila) and the western side (EL-Boughaz) connect the harbor to the Mediterranean open sea, facilitating an active water exchange between the harbor and the Mediterranean open sea **(Abdel Ghani *et al*., 2013)**.

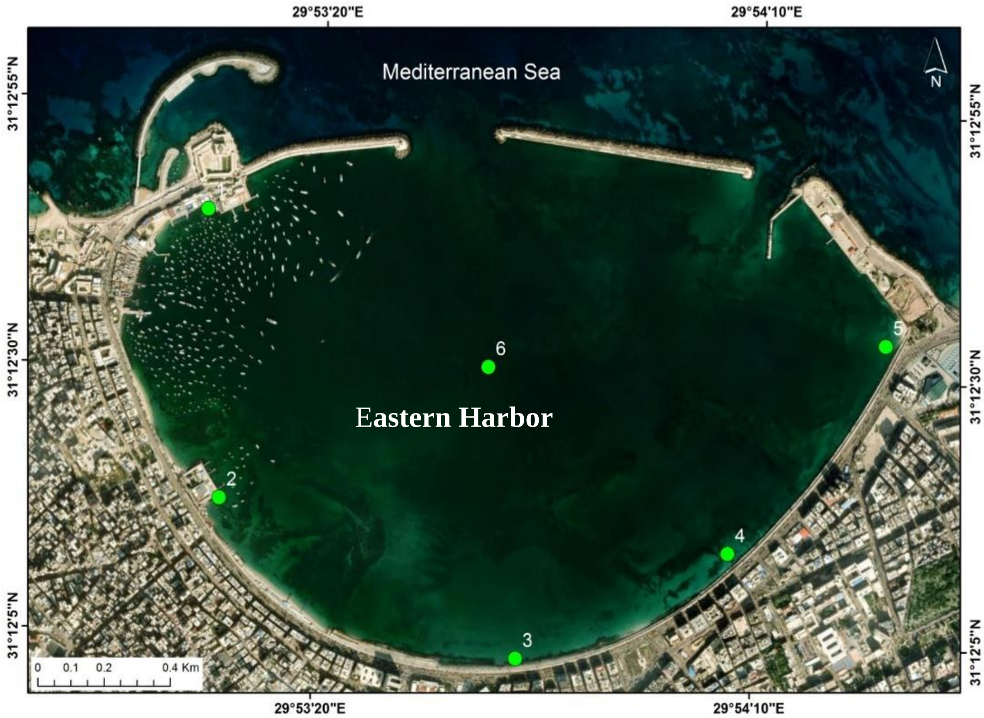

Fig. 1. Map of Eastern Harbor study area and sampling stations

Water sampling

Water sampling was carried out from the EH on the 7th of August 2024 to estimate the phytoplankton community structure and nutrient contents. Six stations (St.) were chosen to cover the different ecological areas of the harbor (St. 1 to 6) as shown in Fig. (1). According to the standard methods published by the American Public Health Association press **(APHA, 2017)**, surface water samples were collected manually through immersing a cleaned 3 liters polyethylene bottle to a depth 20cm below the surface water. One liter of samples was used for the estimation of physical parameters. For the purpose of estimating chemical parameters and conducting a chlorophyll analysis, the second liter was promptly frozen in polyethylene bottles after being filtered through Whatman GF/C filter. For phytoplankton analysis, the third liter was fixed in the field right away. Additionally, ammonia and dissolved oxygen samples were fixed in the field right away without filtration.

Water quality analysis

Physical characteristics of the water samples

A common thermometer that is sensitive to 0.1°C was used to measure the water's temperature right away throughout the sample process. The temperature ranged from 0.1 to 100°C. Using a pocket pH meter (type 201/digital pH meter), the pH value of water samples was promptly determined on the field. A Beckman salinometer (Model NO.R.S.10) was used to measure the salinity of the water. To prevent CO_2 increase or loss during the filtration process, CT and pH measurements were made on unfiltered seawater samples.

Chemical characteristics of the water samples

Dissolved oxygen (DO) samples were directly collected from the surface water using a bucket, and immediately fixed in the field. DO was calculated using the widely used Winkler method, which **Carritt and Carpenter (1966)** improved. The permanganate value test was used to measure oxidizable organic matter (OOM) **(FAO, 1975)**. Ammonia (NH_4-N), nitrites (NO_2-N), nitrates (NO_3-N), phosphates (PO_4-P), and silicates (SiO_4) were measured as examples of dissolved inorganic nutrients, along with chlorophyll a concentration. These measurements were carried out according to the methods described by **Strickland and Parsons (1972)**. Nutrient salts and chlorophyll a concentration was spectrophotometrically determined using a T60 UV-Visible spectrophotometer, and the results were expressed in $\mu M\ L^{-1}$ and $\mu g\ L^{-1}$, respectively.

Biological characteristics of the water samples

The sedimentation method for water samples was used to quantify the phytoplankton community in the EH, as described by **Utermöhl (1958)**. One liter of the water sample was collected and preserved in 5% formaldehyde solution. The different species of phytoplankton cells were counted in 3 replicates of 1ml of the concentrated samples using an optical microscope (Model 3B100/ LED Lamp / DC7.5 W/ Germany). The counting was done using a Sedgwick-Rafter cell at 20× magnification. The phytoplankton density was expressed as unit per liter (Unit L^{-1}). The identification of phytoplankton species was done using various key books including, **Lebour (1925; 1930)**, **Cupp (1943)**, **Prescott (1975)**, **Dodge (1982)**, **Sournia (1986)**, **Mizuno (1990)**, **Tomas (1997)** and **Opute (2000)**.

Examination of *Scrippsiella trochoidea*

Optical microscopy

Samples were left to settle for approximately 20 minutes, then a glass Pasteur pipette was used to draw a small amount of the sample from the bottom of the vial. The drawn sample was put on a clean microscopic slide, covered by glass cover, and then examined using the optical microscope (Model 3B100/ LED Lamp / DC7.5 W/ Germany).

Scan electron microscopy (SEM)

At the City of Scientific Research and Technological Applications (SRTA-City), New Borg El-Arab City, Alexandria, Egypt, a scanning electron microscope (SEM) (JSM-6360LA, JEOL, Japan) was used to examine the morphological characteristics of *Scrippsiella trochoidea* cells, which were clearly observed using the SEM technique. The cells were collected by centrifugation at 3000rpm for 10min, then the supernatant was removed, and the pellet cells were fixed by 2.5% glutaraldehyde at 4°C overnight. The samples were then washed three times with 0.1 M phosphate buffer solution (PBS, pH 7.4), followed by centrifugation. The cells were then fixed by 1% osmium tetroxide at 4°C for 1h and re-washed three times with PBS, followed by centrifugation. The fixed samples were then dehydrated successively through a series of alcohol solutions of 30, 50, 70, 80, 90, 95, and 100% for 20min. After dehydration, the samples were fixed with tert-butyl alcohol and then subjected to freeze-drying before final observation. The samples were placed on the sample holder and sputter-coated with gold using a sputter-coater to prepare them for examination under the scanning electron microscope. Finally, the samples were observed using the SEM instrument.

RESULTS

Environmental conditions

The values of physical and chemical characteristics of the water are illustrated in Table (1). The water temperature varied between 28.15 and 28.70°C, with an average value of 28.40 ± 0.22°C. pH value fluctuated between a minimum of 8.15 and a maximum of 8.61, with an average of 8.32 ± 0.17. The surface salinity indicated a relatively slight variation (36.21-36.60‰) in the study area, with an average value of 36.41 ± 0.16‰. However, no significant changes in the physical properties of the water were observed between the investigated stations. Dissolved oxygen and oxidizable organic matter concentrations exhibited a relatively slight variations throughout all stations, with stations 1 and 2 recording the highest values compared to others. The ranges and average values were 4.29-5.97; 4.97 ± 0.68 ml O_2 L^{-1} for dissolved oxygen and 2.74-6.35; 4.16 ±1.38 mg O_2 L^{-1} for oxidizable organic matter, respectively. The values of dissolved nutrient salts were very close at all stations, while stations 1 and 2 demonstrated the lowest values of NO_2^-, NO_3^-, NH_4^+, PO_4 and the highest one of SiO_4. The range and average values were as follows: NH_4^+ (5.53–6.99; 6.14 µM L^{-1}), NO_3^- (1.61–2.45; 2.09 µM L^{-1}), NO_2^- (0.191–0.379; 0.266 µM L^{-1}), PO_4 (0.080–0.530; 0.27 µM L^{-1}), and SiO_4 (12.19–17.35; 14.46 µM L^{-1}).

Table 1. Physical and chemical characteristics of the Eastern Harbor surface water at different sampling sites

	Physical parameter			Dissolved nutrient salts (µM L^{-1})						
Station	Temp. ^{O}C	Salinity	pH	NO_2^-	NO_3^-	NH_4^+	PO_4	SiO_4	DO (ml O_2 L^{-1})	OOM (mg O_2 L^{-1})
St. 1	28.61	36.54	8.20	0.191	1.61	5.53	0.080	17.35	5.97	6.35
St. 2	28.15	36.21	8.15	0.219	1.83	5.71	0.128	15.07	5.61	5.04
St. 3	28.34	36.46	8.61	0.236	2.20	5.98	0.220	14.28	4.65	4.31
St. 4	28.20	36.24	8.40	0.324	2.45	6.73	0.426	13.52	4.42	2.85
St. 5	28.70	36.60	8.23	0.379	2.30	6.99	0.530	12.19	4.29	2.74
St. 6	28.40	36.38	8.33	0.246	2.16	5.89	0.236	14.36	4.88	3.67
Average	28.40 ±0.22	36.41 ±0.16	8.32 ±0.17	0.266 ±0.07	2.09 ±0.31	6.14 ±0.59	0.270 ±0.17	14.46 ±1.72	4.97 ±0.68	4.16 ±1.38

OOM= Oxidizable organic matter; DO= Dissolved oxygen

Description of the bloom

The phytoplankton community structure consisted of 26 species belonging to 24 genera. It is classified into four taxonomic groups incorporating dinoflagellates (12 genera and 14 species), diatoms (9 genera and 9 species), Euglenophyceae (2 genera and 2 species), and Raphidophyceae (1 genus and 1 species). The total phytoplankton standing crop accounted for 5.277 x 10^6 units L^{-1} at all stations. Dinoflagellates were the most prominent group, representing 98.95% of the total phytoplankton abundance with an average of 5.222 x 10^6 units L^{-1} at all distributed stations. They were dominated by *Scrippsiella trochoidea*, followed by *Pyrophacus horologium*, *Gyrodenium fusiforme* and *Gymnodinum catenatum*. *Scrippsiella trochoidea* exhibited an extremely high count of 5.152 x 10^6 units L^{-1}, contributing for 98.66% of the total dinoflagellates, 97.62% of the total numerical standing crop. This species was responsible for the unexpected and suddenly appearance of brown tide blooms and water discoloration in Alexandria's Eastern Harbor, as shown in Fig. (2). Diatoms were the second order, comprising 0.34% of the total phytoplankton with an average of 17.864 x 10^3 units L^{-1}. *Rhizosolenia setigera* and *Leptocylindrus danicus* were the most abundant diatoms, accounting for 62.56 and 16.75% of the total diatoms, respectively. In addition, other phytoplankton groups like Euglenophyceae and Raphidophyceae were recorded by a limited count. *Eutreptiella* sp. and *Euglena* sp. were the represented species of Euglenophyceae, while *Chattonella marina* was the only species of Raphidophyceae. Furthermore, chlorophyll *a* content contrasted from 3.44 to 9.26µg L^{-1} with an average of 5.63µg L^{-1} at all stations. Concerning, the spatial distribution of phytoplankton community and Chlorophyll *a* in the study area, great differences at the different stations were observed. Stations 1 and 2 recorded the highest overall phytoplankton abundance and Chlorophyll *a* concentration regarding the other stations (Table 2). The morphological features of *Scrippsiella trochoidea* were examined and photographed using light and scanning electron microscopes. *S. trochoidea* cells showed a thecal plate pattern. It possessed a conical epitheca with straight sides and a prominent apical horn. Cell diameters ranged from 18-34µm in length and 17-29µm wide. The epitheca is slightly larger than the hypotheca (Figs. 3,4).

Table 2. Phytoplankton community and its frequency percentage of the Eastern Harbor surface water at different sampling sites

St.	Total phytoplankton (x10^6 Units L^{-1})	Diatoms (x10^3Units L^{-1})	Abundance %	Dinoflagellates (x10^6 Units L^{-1})	Abundance %	No. of species	Chl a (µg L^{-1})
St. 1	7.157	20.364	0.28	7.120	99.48	24	9.26
St. 2	6.840	19.668	0.29	6.767	98.94	27	7.94
St. 3	5.324	17.980	0.34	5.264	98.88	21	5.03
St. 4	3.973	16.473	0.41	3.853	96.99	18	3.54
St. 5	3.174	14.849	0.47	3.156	99.44	23	3.44
St. 6	5.195	17.850	0.34	5.168	99.49	25	4.57
All stations	**5.277**	**17.864**	**0.34**	**5.222**	**98.95**	**26**	**5.63**

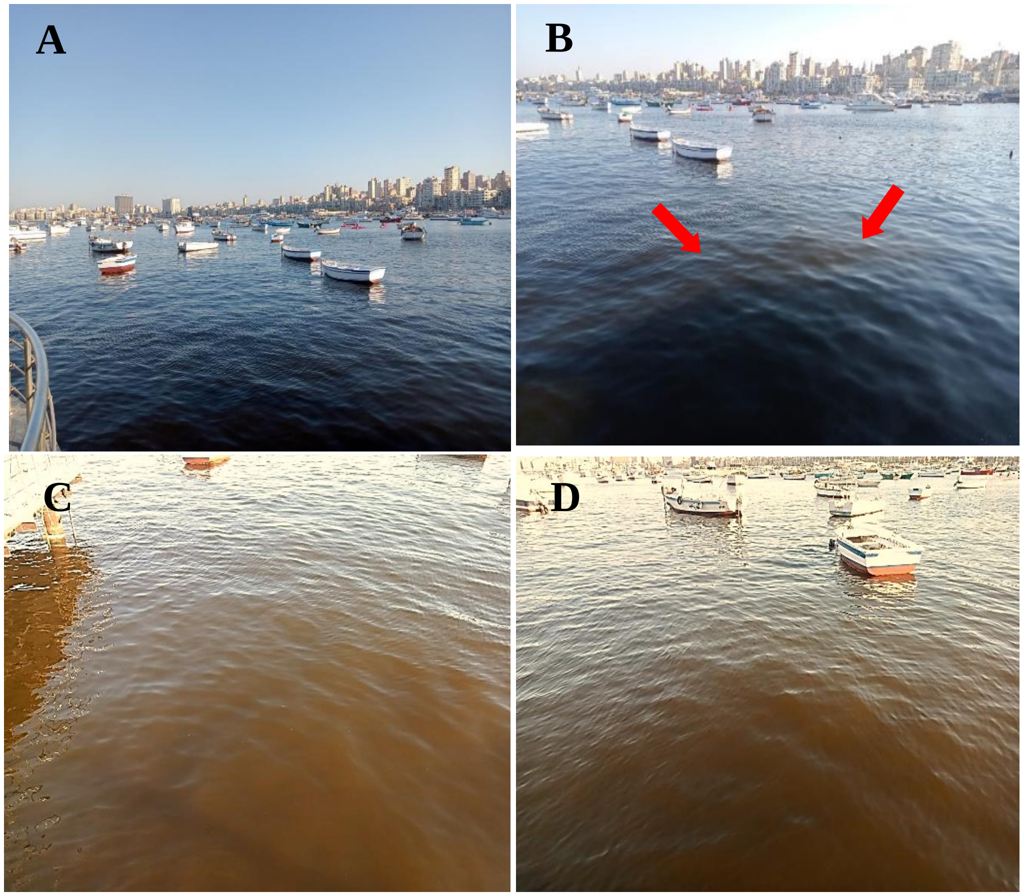

Fig. 2. Brown tide blooms caused by dinoflagellates

Scrippsiella trochoidea in the Eastern Harbor of Alexandria; **(A, B)** before algal bloom dispersion; **(C, D)** after completely dispersion of blooms

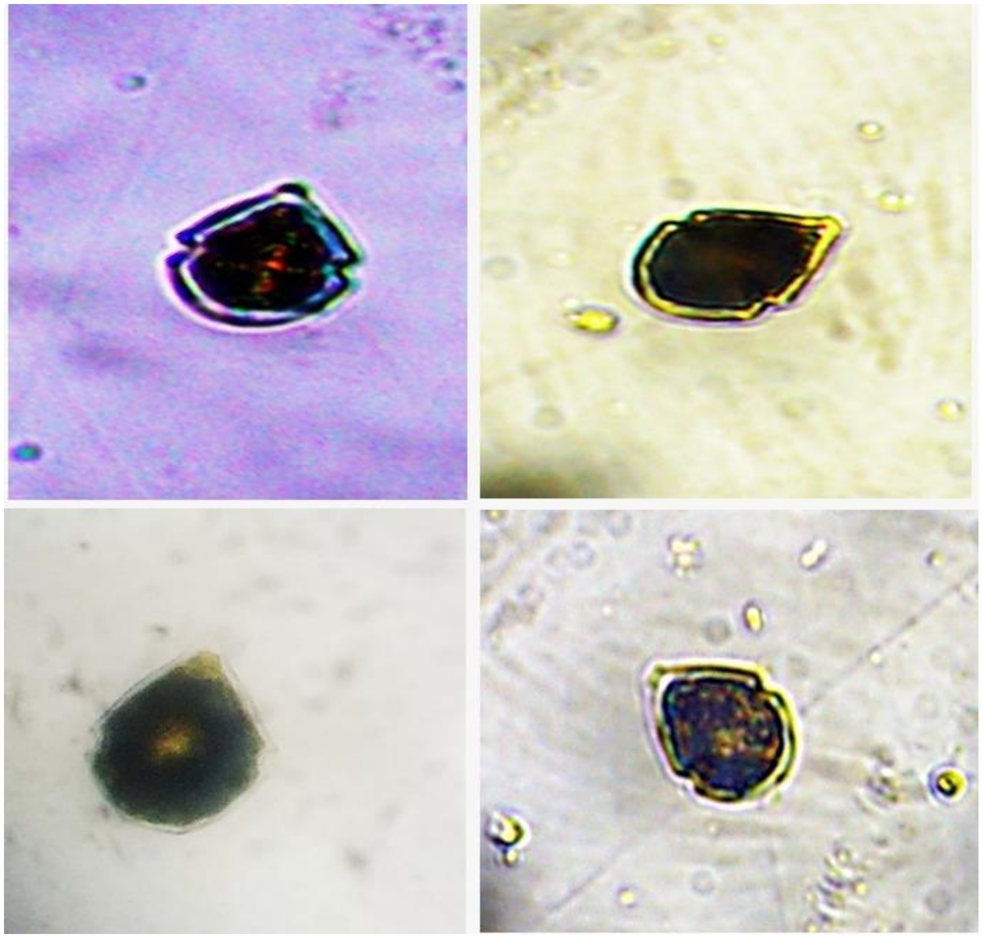

Fig. 3. Dinoflagellate *Scrippsiella trochoidea* under light microscope at magnification of 40x

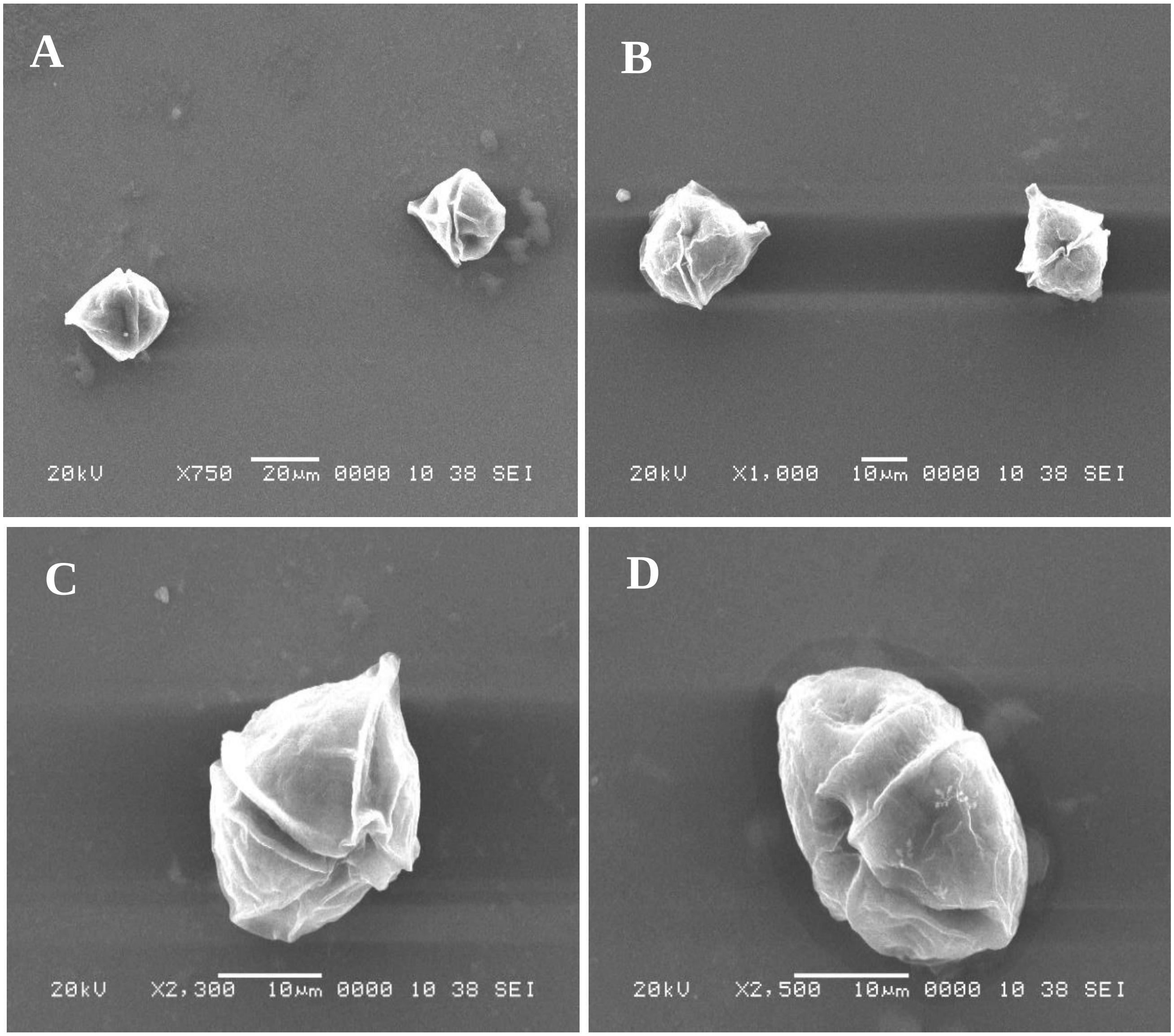

Fig. 4. SEM images of *Scrippsiella trochoidea* cells. **(A)** Dorsal view shows the intercalary plates; **(B)** Dorsal view showing conical epitheca with apical horn, and **(C, D)** Ventral view of *S. trochoidea* showing the sulcal area. All scale bars =10µm, except Fig. (4A), scale bar= 20µm

DISCUSSION

Eutrophication of coastal waters is recognized as one of the most serious threats to marine ecosystems and may be related with water discoloration and red tide problems, which were prevalent throughout the warm season **(Richlen *et al*., 2010)**. Eutrophication events are directly linked to human activities such as land runoff, river inputs, and industrial wastewater. These issues may increase the supply of nutritional inputs, particularly nitrogen and phosphorus **(Davidson *et al*., 2012; Tsikoti & Genitsaris, 2021)**. The Eastern Harbor is considered to be one of the most chronic eutrophicated areas in Alexandria's coastal zone, owing to the extensive annual discharge of industrial, agricultural, and domestic wastewater through two main sewers along the coast **(Khairy *et al*., 2014; El-Dahhar *et al*., 2021)**. The harbor is immediately influenced by sewage effluent discharged from the nearby Al Anfoushi region, as well as indirectly from El-Umoum Drain to El-Mex Bay, west of the study area **(Mikhail, 2005)**. These conditions create significant changes in the environmental conditions and dynamics of the phytoplankton population **(Labib, 2002; Madkour *et al*., 2007)**. Therefore, this area experienced repeated red tide blooms. In this study, we noticed discoloration of the eastern harbor's surface water and the appearance of a brown to reddish patch or cloud covering the surface of the water. These colored spots firstly appeared and were centered at stations 1 and 2, then spread quickly to cover the entire harbor, as shown in Fig. (2). This observation showed that the El-Mex Bay was the main source of bloom appearance.

Dissolved oxygen (DO) is an important parameter for assessing the health of marine aquatic ecosystems **(Yang *et al*., 2007)**. The results of DO exhibit a relatively higher values at station 1 and 2 than others. This might be attributed to the highest density of phytoplankton which provided a considerable amount of oxygen throughout the photosynthetic processes of aquatic plants. The decay of phytoplankton organisms may produce oxidizable organic matter (OOM) in the EH surface water. The increased concentration of OOM at station 1 and 2 might be attributed to the intensive phytoplankton counts and its higher decomposition rate, which created massive amounts of OOM compared to other stations. In addition, the results indicate that stations 1 and 2 recorded the lowest values of NO_2^-, NO_3^-, NH_4^+, PO_4 and the greatest value of SiO_4. The low nutrient salts concentration (NH_4, NO_2, NO_3, PO_4) at station 1 and 2 could be ascribed to the higher consumption rate by phytoplankton blooming, which showed the maximum density in these locations. The dominance of the dinoflagellates group, which accounts for 98.95% of total phytoplankton abundance, could explain the greater SiO_4 level at stations 1 and 2. Dinoflagellates did not require SiO_4 to grow, even though SiO_4 is considered an essential ingredient for diatom growth and skeleton formation **(Khairy *et al*., 2014)**.

Dinoflagellates constitute a significant portion of marine phytoplankton and are known to cause harmful algal blooms. Moreover, the results demonstrated that the dinoflagellate *Scrippsiella trochoidea* was the causative species of the brown tide bloom and water discoloration in the eastern harbor surface water. This species achieved an extremely high count, representing 98.66% of the total dinoflagellates and 97.62% of the total numerical standing crop. *Scrippsiella trochoidea* is a non-toxic marine dinoflagellate that is frequently found in blooms with hazardous species such as *Alexandrium minutum*. It may be found in both cold and tropical waters, and it is known to cause "red tide" phenomena **(Cooper *et al*., 2016)**. Although the blooms induced by *Scrippsiella trochoidea* in the Eastern Harbor are not toxic, the extensive blooms had dangerous effects on humans and animals. These blooms caused fish kills and the death of a large number of fishes in the surface waters of the Eastern Harbor during the bloom period by depleting dissolved oxygen in the water and reducing light penetration, resulting in severe damage to benthic organisms and significant economic losses for fishermen in the study area. These findings are consistent with those of **Manivasagan and Kim (2015)** and **Sha *et al*. (2021)**, who found that high biomass accumulation induced by HAB events may result in environmental damage such as hypoxia, anoxia, and decreased sunlight penetration to submerged vegetation, affecting the entire marine ecosystem.

In accordance with our finding, **Hallegraeff (2003)** classified water discoloration as HABs that mainly induce harmless discoloration of seawater. Under extreme conditions in restricted bays, this bloom may become so dense that it depletes oxygen, resulting in the death of fish, shellfish, and benthic invertebrates. Dinoflagellates, including *Noctiluca scintillans*, *Ceratium* spp., *Prorocentrum micans*, *Heterocapsa triquetra*, *Akashiwo sanguinea*, *Gonyaulax polygramma*, *Scrippsiella trochoidea*, and *Peridinium quinquecorne*, are the main group of algae responsible for the bloom's production. This is the first investigation to identify the dinoflagellate *Scrippsiella trochoidea* as a cause of red tide blooms in the Eastern Harbor area since 1998, according to **Labib (2000)**. *Scrippsiella trochoidea* was recorded as causative species of brown tide in the Eastern Harbor from 29 August to 3 September 1998. It accounted for 77.32- 98.17% of the total community with a population density of 5.64 x $l0^{6}$ cell. L^{-I} **(Labib, 2000)**.

CONCLUSION

In Eastern Harbor Bay's surface waters, HABs caused by the dinoflagellate *Scrippsiella trochoidea* resulted in environmental damage, healthcare issues, and economic losses. Climate change and increasing human activities along the coast are currently the main causes of these various problems. Thus, the frequent occurrence of the red tide phenomenon in the Eastern Harbor area may have detrimental economic and social effects on marine ecosystems, fisheries, tourism, and recreation (visitors avoid coastal tourism regions due to the prevalence of mucilage aggregates, foam, and water discoloration), as well as human health (due to the consumption of contaminated seafood). In the Mediterranean Sea's coastal regions, management techniques for bloom mitigation should be supported. These strategies could include preventing eutrophication by lowering nutrient inputs and wastewater discharges into the sea. Additionally, new technologies like satellite remote sensing and complex numerical models are being used for the early detection of HABs. Apart from employing physical, chemical, and biological control techniques to curb the growth of HAB and prevent its adverse effects to avoid anoxia, physical methods need to keep fish away from HABs and provide oxygen. Biological methods include using biological agents as HAB controllers by feeding (predators) or decomposing (bacteria, fungi, and viruses) HAB species; physical-chemical approaches include using flocculating materials like sand or clay to induce sedimentation of harmful microalgae; and chemical approaches include using algaecides like copper sulphate ($CuSO_4$) as a chemical control intervention in saltwater.

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CONFLICTS OF INTEREST

There is no conflict of interest, according to the authors.

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