



Detection and monitoring of algal toxins and causative harmful algae in Abu Dhabi waters-A pilot study

Anbiah Rajan¹, Steve L Morton², Rajasekhar Thankamony¹, Azza Al Raisi¹, Ponpandi Perumal¹, Shaikha Al Hosani¹

¹ Environment Quality Sector, Environment Agency Abu Dhabi, Abu Dhabi, United Arab Emirates

² NOAA/NOS Marine Biotoxin Program, Ft. Johnson, Charleston, United States

Abstract

Harmful algal blooms along the coastline of the Arabian Gulf have been common for many years and increasing over the past few decades. The microalgal species including diatoms and dinoflagellates produce a variety of marine biotoxins (algal toxins) that may be harmful to marine organisms and people. The detection and monitoring techniques for algal toxins and the causative harmful algal blooms (HABs) are critical for the protection of marine organisms, potable water quality, and public health. In response to this growing concern, the Environment Agency - Abu Dhabi (EAD) initiated a monitoring programme in 2006 and identified several toxin-producing species, including domoic acid-producing diatom (*Pseudo-nitzschia* sp), saxitoxin-producing dinoflagellate (*Alexandrium* sp), and okadaic acid-producing dinoflagellate *Dinophysis* sp). Since algal toxin-producing algae were detected, the scientific understanding and detection of the algal toxin in Abu Dhabi waters have become essential. Hence, EAD started a pilot project to study the feasibility of using the Solid Phase Adsorption Toxin Tracking (SPATT) technology in the detection of three algal toxins, particularly near desalination plants in Abu Dhabi waters. Results of the eight-week pilot project include weekly phytoplankton cell enumeration with toxin determination on phytoplankton samples and SPATT bags deployment in Abu Dhabi waters. The SPATT signalled that the presence of domoic acid from Abu Dhabi waters correlated with high concentrations of *Pseudo-nitzschia pseudodelicatissima*. This study is the first to evaluate SPATT deployments in Abu Dhabi waters as well as demonstrate the applicability of SPATT towards the detection of hydrophilic phycotoxins in the United Arab Emirates.

Keywords: algal toxins, harmful algae, waters

Introduction

Harmful algal blooms (HABs) are now a recurrent and serious problem in many areas of the world, and evidence suggests that the frequency and distribution of HABs are increasing (Heisler *et al.*, 2008^[5]; Andersen, 1996^[1]). Deleterious impacts of HABs have increased public awareness and the demand for intervention to reduce or eliminate bloom impacts on coastal resources and local economies, and threats to public health. Globally, the phytoplankton communities of many coastal ecosystems have transformed in recent decades, becoming increasingly dominated by HABs. In the Arabian Gulf, there is a lack of information regarding the potential for the production of known biotoxins associated with human health concerns, particularly in Abu Dhabi. However, previous phytoplankton surveys (Rajan and Al Abdessalaam, 2008)^[9] have identified 34 bloom-forming species and 11 toxin-producing species, including the domoic acid-producing diatom *Pseudo-nitzschia* sp., the saxitoxin-producing dinoflagellate *Alexandrium* sp., and the okadaic acid-producing dinoflagellate *Dinophysis* sp. In addition, they reported that the HAB incidents in Abu Dhabi territorial waters are increasing every year. Furthermore, there were records of dense blooms of toxic HAB species in the vicinity of desalination plants e.g., Umm Al Nar desalination plant in Abu Dhabi. Since there is a growing risk to public health and plant operations, scientific understanding and engineering challenges are vital to mitigate the impact of harmful algal blooms on desalination plants. Hence, EAD carried out a pilot project to find out the occurrence and distribution of harmful algal toxins such as domoic acid, saxitoxin, and okadaic in Abu Dhabi waters and investigate the feasibility of using the Solid Phase Adsorption Toxin Tracking (SPATT) technology (new technology) to estimate the toxins near desalination plants.

Materials and Methods

Abu Dhabi is located in the Arabian Gulf and is characterised by an arid subtropical climate with an average of <5 cm rainfall along the coast. Four sites were selected for the current investigation: among these, three sites such as Musaffah south channel (MS) and Eastern corniche (EC1& EC2) were eutrophic confined areas where EAD recorded frequent algal blooms. The last one was Bateen Beach (BB), which is located in an open channel. All the sites were continuously monitored through MWQ automation Buoys (Figure 1). The hydrographic data

were collected from both the surface and bottom of the channel using Hydrolab-DS5, whereas the nutrients were measured only from the surface by using standard methods. Phytoplankton samples were collected for quantitative analysis using the Niskin water bottle. For qualitative analysis, the phytoplankton was trawled horizontally during the early morning hours using nets with mesh sizes of 20 μ m. The samples were collected weekly from 19 October 2015 up to 28 December 2015. The plankton samples were fixed immediately using Lugol's solution and stored in dark bottles. In the laboratory, the phytoplankton species were identified, and their diversity was recorded. The present pilot project was designed to determine the occurrence of three toxins, (domoic acid, saxitoxin, and okadaic acid) in Abu Dhabi coastal waters. Further, the study proposed to combine phytoplankton cell enumeration with toxin determination on plankton samples and SPATT bags (MacKenzie *et al.*, 2004) [8]. At each site, a known volume of water (usually between 200 -500 mL) was filtered onto a GF/B filter and frozen for toxin analysis. Moreover, SPATT bags were deployed at each site for one week. At sites BB, MS, and EC1, the existing EAD water quality buoys were used to deploy each SPATT bag. SPATT was also deployed using the EAD boat dock at site EC2 (Figure 1). Qualitative phytoplankton analysis was performed on each sample using a Swift M10-DT light microscopy (Hamilton *et al.*, 2001[4]; Trainer *et al.*, 2014) [13] and NEOSCI-ruled microscope slide to estimate the relative abundance in the samples collected. These samples were agitated gently. Using a 3-mL transfer pipette (Fisher Scientific, Pittsburgh, PA), 2 drops of the sample were placed at the centre of the grid. After placing a coverslip on the slide, every square was viewed and the percent coverage of the slide was estimated (Shuler *et al.*, 2012)[12]. Qualitative enumerations of phytoplankton samples were carried out using a Nikon inverted microscope (TS100) with bright field illumination magnification 200 and 400x using a Sedwick Rafter counting chamber (Hamilton *et al.*, 2001) [4]. Hydrographic parameters (temperature, salinity, pH level, dissolved oxygen,) were measured using a Hydrolab Surveyor (Model MS5). Nutrients were analysed by the method APHA 4500. Sample preparation for SEM (JEOL 5600LV) imaging was achieved through sample dehydration in an ethanol series (30 -100%), with final dehydration in hexamethyldisilazane (HMDS). At each site, 200 to 500 ml of water was filtered through a Whatman GF/B glass fibre filter. These filtered samples were extracted depending on which species dominated in the qualitative analysis. If *Pseudo-nitzschia* sp. was the dominant species, the filters were extracted using 10% methanol: 90% water and sonicated for 10 minutes. If *Alexandrium* sp., *Pyrodinium* sp., or *Gymnodinium* sp. was the dominant species, the filters were extracted using 10% HCL and sonicated for 10 minutes. The resulting extract would be analysed for saxitoxin using the Abraxis ELISA kit for Saxitoxin. Finally, if *Dinophysis* sp. was the dominant species, the filters would be extracted using 80% methanol: 20% water and sonicated for 10 minutes. The resulting extract would be analysed for okadaic acid using the Abraxis ELISA kit for okadaic acid. The SPATT bags were extracted by soaking them in 500 ml of water twice, for 5 min each time, to remove salts, and vigorously shaking them free of most water before removing the resin contents and soaking them for 2 h in 80 ml 100% methanol. The extract was washed and filtered through glass wool and an anhydrous NaSO₄ column (2ml), with an additional 80 ml of methanol. The sample was rotary evaporated to dryness, resuspended in 15ml methanol, transferred to a glass vial, and again reduced to dryness on a heating block at 40°C. The sample was re-suspended in 5 ml 80% methanol, filtered (0.2 mm) and an aliquot dispensed into vials for analysis using the Abraxis ELISA.

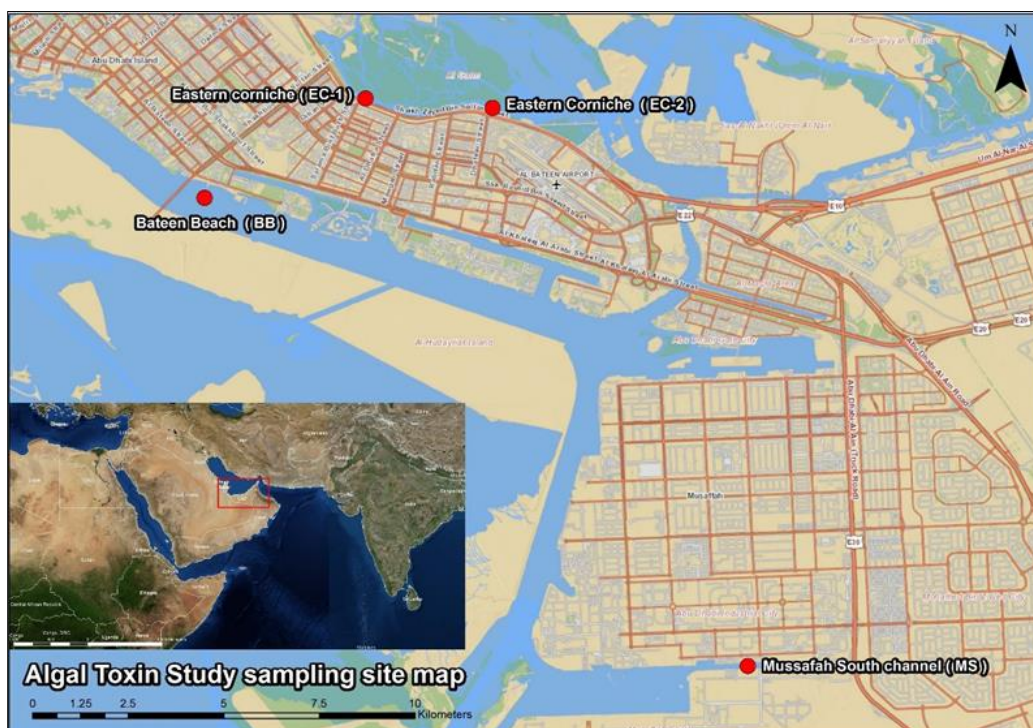


Fig 1: Sampling sites

Result and Discussion

Environmental Parameters

The surface water temperature at Bateen beach (BB) ranged from 22.09°C to 31.34°C. A gradual decrease was noted as the study progressed. The salinity, ranging from 44.89 psu to 47.95 psu, did not vary significantly during the study period. The temperature in the Musaffah South Channel (MS) decreased slightly during the study period, ranging from a maximum of 32.55°C to a minimum of 24.2°C. The salinity did vary significantly during the same time, with a maximum of 42.72 psu and a minimum of 34.58 psu. Both sites at Eastern Corniche displayed similar trends during the study. The temperature ranged from 22.28°C to 31.49°C at both EC1 and EC2. Salinities did not significantly change during the study, ranging from 38.4 psu to 48.02 psu. The nutrient concentration observed in various sampling sites varied significantly. The nitrite values at BB varied between 0 and 100 µg/l, with a mean of 10 µg/l. In MS, these values fluctuated between 70 and 1280 µg/l, with a mean value of 451 µg/l. EC1 and EC2 showed fluctuations between 0-390 µg/l and 0 to 130 µg/l, respectively. The nitrate values fluctuated from 40 to 580 µg/l in BB, 20070 to 42600 µg/l at MS, and 350 to 20200.4 µg/l at EC1 and 710 to 18600.4 µg/l at EC2. The phosphate values at BB varied from 0 to 340 µg/l. MS showed very high phosphate values, ranging between 1630 and 3160 µg/l. At EC1 and EC2, these values oscillated between 90 and 2030 µg/l, and 0 and 1720 µg/l, respectively. The ammonia values at BB, MS, EC1 and EC2 varied between 0 and 160 µg/l, 50 and 510 µg/l, 20 and 220 µg/l, and 10 and 170 µg/l, respectively. The silicate concentration fluctuated between 240 and 1000 µg/l at BB station, 2810 and 5750 µg/l at MS, 1200 and 7410 µg/l at EC1, and from 1600 and 4190 µg/l at EC2 (Table 1).

Moreover, the chlorophyll concentration also varied very much between the sampling sites. At BB, the chlorophyll concentration in the surface water varied between 2.24 and 4.83 µg/l. In MS, these values fluctuated between 9.21 and 47.5 µg/l. In both EC 1 & 2, chlorophyll concentration values showed fluctuations between 1.64 and 53.95 µg/l, and 1.32 and 69.93 µg/l, respectively.

Various water quality parameters showed abnormal values, particularly in the case of nutrients, and were many folds higher than normal seawater. Among the sampling sites, both Mussafah South channel and Eastern Corniche were eutrophic, especially the Mussafah South Channel. The pH values (7.99-8.71) surface and 6.04-8.31 bottom) and dissolved oxygen values (2.85-11.27 mg/L at the surface and 0.07- 0.98 mg/L bottom) also varied from normal seawater. The low values of dissolved oxygen and change in pH values could be due to the eutrophic condition of the area, photosynthetic activity, and other related activities of the algal bloom present in the water (Livingston, 2001)^[7]. Nutrient enrichment was probably created by the continuous release of nutrient rich discharges in the sampling sites, especially treated sewage (400000 m³ per day) in the Mussafah South Channel. The eutrophic condition of the sampling sites produced frequent algal blooms and subsequent killings of fish. The present results were in agreement with the findings of Redfield (1948)^[10], Rutner (1963)^[11], Keefe (1972)^[6], and Boto and Bunt (1981)^[2].

Table 1: Minimum, maximum, and mean values of different water quality parameters

	Eastern Cornich-1			Eastern Corniche -2			Bateen Beach			Mussafah South Channel		
	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
Temperature °C	22.28	31.4	26.71	22.94	31.49	26.572	22.09	31.34	26.30	24.2	32.55	27.96
Salinity (psu)	38.4	46.89	44.3	42.15	48.02	45.95	44.89	47.95	46.64	34.58	42.72	38.63
pH	7.66	8.48	8.08	7.85	8.43	8.12	8.08	8.19	8.15	7.99	8.71	8.33
D. Oxygen (mg/L)	4.35	8.25	5.52	4.33	8.79	5.64	4.36	5.54	5.14	2.85	11.27	7.47
Chlorophyll-a (µg/L)	1.64	53.9	11.86	1.32	69.93	11.89	2.24	4.83	3.68	9.21	47.5	27.19
Nitrite-N (µg/L)	0	390	114	0	130	66	0	100	10	70	1280	451
Nitrate- N (µg/L)	350	20200	7933	710	18600	4031	40	580	216	20070	42600	27157
Phosphate -P (µg/L)	90	2030	904	0	1720	516	0	340	134	1630	3160	2670
Silicate-Si (µg/L)	1200	7410	3560	1600	4190	2886	240	1000	529	2810	5750	4156
Ammonia -N (µg/L)	20	220	82	10	170	54	0	160	34	50	510	277

Distribution of Phytoplankton in Bateen Beach Site (BB)

In all the examined samples, dinoflagellates dominated the phytoplankton population. Dominant species included *Ceratium furca*, *Scrippsiella trochoidea*, *Protoperidinium divergens*, *P. depressum*, and *P. steinii*. *Pyrophacus horologium* was also observed. During the 11/23/15 sample, a low abundance of *Prorocentrum micans* was observed. Diatoms were also observed to be in very low abundance, with *Coscinodiscus* sp., *Melosira* sp., and *Pleurosigma* sp. being the dominant species. A large number of Barnacle Nauplius and Copepods were also present in the site. Furthermore, *Ceratium furca* and *Prorocentrum micans* are of note since these species have been associated with red water events and fish kills worldwide. Total phytoplankton concentrations at the site ranged between 148,752 cells/L to 7,025,000 cells/L. *Oscillatoria* sp. was the dominant species driving an increase in the total phytoplankton population, except for the 12/14/15 sample date. At this time, *Pseudo-nitzschia* sp. (12/14/15) was the dominant species, with over 1,500,000 cells/L. During the pilot project, two distinct populations of *Pseudo-nitzschia* sp. were observed, the first on 10/25/2016 and the second on 12/14/2015. Utilizing Scanning Electron Microscopy, it was determined that the first peak was *Pseudo-nitzschia pungens*, while the second was *Pseudo-nitzschia pseudodelicatissima*. Both species are known producers of the toxin, domoic acid.

Mussafah South Channel (MS)

This site was dominated by the cyanobacteria, *Oscillatoria* sp., and the dinoflagellates, *Prorocentrum micans* and *P. triestinum*. Large abundances of heterotrophic dinoflagellates, including *Protoperidinium divergens*, *P. depressum*, and *P. steinii* were observed. Moreover, a large number of the dinoflagellate, *Heterocapsa* sp., was also observed. Further, high abundances of the heterotrophic dinoflagellate *Polykrikos schwartzii* and *Kofoidium velleloides* were observed in the samples collected on 10/19/15 and 11/23/15. Dominant diatoms include *Coscinodiscus* sp. and *Pleurosigma* sp. In addition, a large number of tintinnids and Nauplius were also observed on the abovementioned sample dates. Large numbers of Salps were observed in the sample collected on 11/02/15. On 11/23/15, the dinoflagellate, *Akashiwo sanguinea*, was observed in low abundance. This site contained very high total phytoplankton concentrations, ranging from 2,100,000 cells/L to 40,000,000 cells/L. *Oscillatoria* sp. was the dominant species found at this site for most of the pilot study, ranging from 1,550,000 cells/L to 33,330,000 cells/L. Extremely high cell counts like these found at this site are indicative of a highly eutrophic and impacted system. During the pilot study, many planktonic *Prorocentrum* sp. were observed, including *P. gracile*, *P. micans*, *P. minimum*, *P. sigmoides*, and *P. triestinum*. These species were masked in the total phytoplankton concentrations due to the extreme cell concentrations of *Oscillatoria* sp. Moreover, very high cell concentrations of *P. triestinum* (7,500,000 cells/L) were observed on 12/19/2015. A very high concentration of *Pseudo-nitzschia* sp. was also observed on 12/14/2015. At this time, over 2,600,000 cells/L, corresponding to large concentrations of *Pseudo-nitzschia* sp., were observed at the Bateen Beach (BB) site. Again, the species was *P. pseudodelicatissima*. Similar to the BB site, a small increase in *Pseudo-nitzschia pungens* was observed on 11/2/2015, at 240,000 cells/L.

Eastern Corniche-1 (EC1)

The dinoflagellates, including *Scrippsiella trochoidea*, *Ceratium furca*, *Prorocentrum micans*, and *Protoperidinium divergens*, dominated the plankton community at the Eastern Corniche site. Dominant diatoms included *Thalassiosira* sp., *Leptocylindrus danicus*, *Pleurosigma* sp., *Rhizosolenia* sp., and *Coscinodiscus* sp. During the sample collection on 11/9/15, a low abundance of *Pseudo-nitzschia* sp. was observed. On this date, the dinoflagellate *Peridinium quinquecorne* was also observed. These two species were not observed in any subsequent samples. In the sample collected on 11/23/15, the ciliate, *Mesodinium rubrum* was observed. A very low phytoplankton population but extremely high zooplankton was observed in the 11/30/15 sample. Moreover, a high abundance of *Pseudo-nitzschia* sp. was observed in the 12/11/15 sample. Eastern Corniche 1 has a total phytoplankton community ranging from approximately 500,000 cells/L to 37,050,000 cells/L. The extremely high cell concentration was due to a bloom of the dinoflagellate, *Peridinium quinquecorne*. This species dominated the total phytoplankton numbers throughout the pilot study. A very high concentration of *Pseudo-nitzschia* sp. was observed during 12/14/2015. At this time, over 2,600,000 cells/L were observed, corresponding to large concentrations of *Pseudo-nitzschia* sp. at the Bateen Beach (BB) site. Again, the species was *P. pseudodelicatissima*. Similar to the BB site, a small increase in *Pseudo-nitzschia pungens* was observed on 11/2/2015, at 240,000 cells/L.

Eastern Corniche-2 (EC2)

This site displayed a similar species diversity as site EC 1. In the sample collected on 11/9/15, a large number of the diatom, *Hemidiscus cuneiformis*, and *Pseudo-nitzschia* sp. were observed. Moreover, at this time, large abundances of *Protoperidinium* sp., *Scrippsiella* sp., and *Heterocapsa* sp. were observed. Similar to the EC1 site, the sample collected on 11/30/15 contained very little phytoplankton. Low concentrations of *Dinophysis acuminata* was observed in the sample collected on 11/23/15. A bloom of *Pseudo-nitzschia* sp. and *Cylindrotheca closterium* (12/14/15 sample) was followed by a high abundance of *Skeletonema costatum* (12/14/15 sample). EC2 displayed a similar phytoplankton trend as the other Eastern Corniche site (EC1), except for being delayed by a number of days. The highest phytoplankton concentration of over 6,000,000 occurred on 11/9/2015 or 14 days after a similar peak cell concentration on site EC1. Again, the dinoflagellate, *Peridinium*

quinquecorne, dominated the phytoplankton community. A high concentration of *Pseudo-nitzschia* sp., similar to site BB and EC1, was observed at this site, with over 1,050,000 cells/L. However, a high concentration of *Pseudo-nitzschia pseudodelicatissima* was observed on 12/22/2015 or 20 days after the high concentrations at sites BB and EC1.

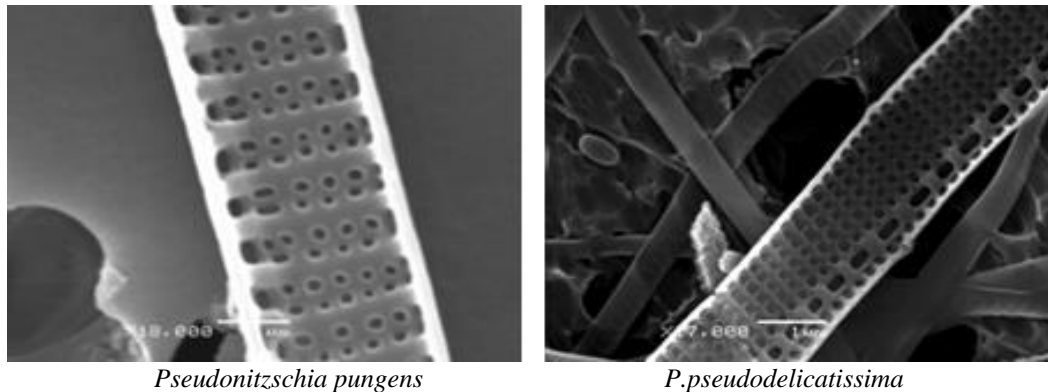


Fig 2

Toxin Analysis

Filter and SPATT bag samples from Eastern Corniche (EC1 and EC2) with observed *Pseudo-nitzschia* sp. were extracted using 10% Methanol: 90% water per the instruction for the ELISA kits. These extracts were extracted using the Abraxis domoic acid and ELISA test kits. All samples were negative except the filter sample from site EC1, collected on 12/14/2015. This sample displayed a weakly positive response on the ELISA kit. Since the response was lower than the detection limit of the assay, the concentration was lower than 0.17 ng/ml. This concentration of domoic acid was much lower than the action limit of this marine toxin, which was currently set at 20mg/ml. Since species capable of producing saxitoxin (*Alexandrium* sp., *Pyrodinium* sp., and *Gymnodinium* sp.) or okadaic acid (*Dinophysis* sp.) were not observed, extracts for these toxins were not performed. This is the first report of domoic acid from the Abu Dhabi waters correlating with high concentrations of *Pseudo-nitzschia pseudodelicatissima*. The amount of domoic acid was very low, approximately 3 orders of magnitude less than the concentrates required for human health concerns. A similar concentration of domoic acid was found in southeastern United States, where blooms of *P. pseudodelicatissima* (3,500,000 cells/L) were found in plankton samples at trace concentrations (lower than the level of quantification, but higher than the detection limit) (Shuler *et al.*, 2012) [12]. Since *Pseudo-nitzschia* are an open ocean species, its populations are most likely transported into coastal waters around Abu Dhabi city by wind and currents. Like the southeastern United States, offshore populations are moved into nutrient rich coastal waters where blooms and subsequent toxic events may occur (Shuler *et al.*, 2012) [12] and have an effect on coastal marine resources (Fire *et al.*, 2009) [3]. However, the second species of *Pseudo-nitzschia* sp. identified in this pilot program, *P. pungens*, is known to produce higher levels of domoic acid than *P. pseudodelicatissima*. If Abu Dhabi experiences a bloom of *P. pungens* at these very high cell concentrations, there is a greater risk of domoic acid exposure. Domoic acid accumulates in shellfish, sardines, and anchovies, which are then eaten by sea lions, otters, cetaceans, and humans, among others. Exposure to this biotoxin affects the brain, causing intoxicated individuals or marine mammals to become lethargic, disoriented, and have seizures that sometimes result in death. A human can be exposed to domoic acid through the ingestion of contaminated finfish or shellfish, leading to Amnesic Shellfish Poisoning (ASP). Based on this pilot study, additional samples throughout the year would be warranted to determine the temporal and spatial trends of potentially toxic species along the coast of Abu Dhabi. The current samples used in the pilot study are suitable for a yearlong study to determine the temporal and spatial patterns in potential toxicogenic harmful algae. Continued phytoplankton counts would help detect the best time to deploy SPATT bags. Based on Andersen (1996) [1] it is advised that toxin testing be initiated when populations of *Pseudo-nitzschia* sp. are above 1,000,000 cells/L, *Pyrodinium bahamense* are above 200 cells/L, *Dinophysis* sp. are above 500 cells/L, and *Alexandrium* sp. are above 1000 cells/L. These potential toxins in Abu Dhabi would have effects on food resources and potentially water resources if they remain active after the desalination process.

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