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Initial Report of Bloom of ichthyotoxic *Karenia mikimotoi* (Dinoflagellate sp) along the Coast of Ormara (Baluchistan) Pakistan

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Abstract

A unique HAB event of a typical ichthyotoxic dinoflagellate species *Karenia mikimotoi* was observed during routine sampling in the month of October, 2009 from coast of Ormara (Baluchistan) Pakistan (Arabian Sea). The bloom appeared as rust brown appearance stretched over approximately a km of distance. Highest cell density 2.7×10^{7} Cells/L was observed during microscopic counts, which is the highest cell concentration ever recorded. This bloom was investigated for a period of three months; however the bloom was disappeared from the area and replaced by other members of microplankton community. The observed bloom also composed of other microplanktonic groups including dinoflagellates, diatoms and ciliates with various cell abundance. Among dinoflagellates, *Triposfusus* sp and *Noctiluca scintillans* were found in high abundance of 4×10^{3} Cells/L, 1×10^{3}Cells/L, respectively. It is followed by five different species of *Prorocentrum*. Along with the bloom of *Karenia mikimotoi* 12 toxic species were also observed belonging to dinoflagellates and diatoms. The manifestation of *Karenia mikimotoi* bloom to the coastal waters of Pakistan could be an alarming situation and HAB prevention measurements should be taken to avoid the damage of ecosystem.

Keywords: *Karenia mikimotoi*; Harmful Bloom; Baluchistan; Pakistan.

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1. Introduction

Pakistan coastline is 1050km falling 250km in Sindh province and 800km in Baluchistan [16] province that is 75% of the total. It borders the productive northeast Arabian Sea famous for upwelling phenomena [20].

Ormara is a port city located in the Makran coastal region. It is located 360 kilometers west of Karachi and 230 kilometers east of Gwadar on the Arabian Sea. It is an old coastal town of Baluchistan. When going towards Gwadar through Makran coastal highway Ormara comes in midway between Karachi and Gwadar. The oceanic influence keeps the temperature lower in summer and higher in winter as compared to the inland. The uniformity of temperature is a unique characteristic of the Makran Coastal region.

The site is never explored before for the estimation of primary productivity. This is the first report of toxic bloom of ichthyotoxic dinoflagellate species *Karenia mikimotoi* and microbial community structure from Ormara coast.

Dinoflagellates bloom is a phenomenon stimulated by a combination of multiple factors, which will be physical, biological or chemical [14, 15]. These blooms generally have serious side effects depend on characteristic of microscopic organisms. This study is focused on bloom of *Karenia mikimotoi* and also on importance of other microbial communities. This specific bloom is reported from temperate to sub-tropical neritic water, from Australia, Ireland, Japan, Korea, Norvey and Scotland [23, 25]. Rust brown tide phenomena produced by *Karenia* sp are known in Europe, Australia, Japan and Korea [25]. *Karenia* sp has described as a common blooming species of Norway [4] to the coast of Spain [7] and especially in coast of Brillany [17]. The *Karenia* sp bloom is observed in each year in Jiaozhou Bay China [12].

This species is usually reported from different parts of the world in summer season [5]. It appeared in the sub-tropical region of Arabian Sea in post monsoon season (October, November and December). The main peak bloom time was third week of the October i.e. late summer time. In this study we have not only focused on bloom phenomenon but also focused other micro planktonic companions. There is a limited research done on the HAB event causes from coastal waters of Pakistan, therefore it is difficult to evaluate and predict the bloom of toxic species and their interactions to the other micro planktons and environmental hydrographics.

2. Materials and Method

Samples were collected from surface water at weekly intervals, between mid of October till December in 2009. The area is located at 6435°E and 2515°N of Northern Arabian Sea (Figure 2). The area is directly exposed to the general wind action. The bottom is rocky with the depth of 50m. Transparency was considered in minus due to thick aggregation of brown rust bloom materials, therefore samples were preferred to collect in 200ml brown bottle and preserved in 1% lugol's preservative. The technique for population analysis used was earlier described by Utermohl [26] settling method using Olympus, BX-51, Japan. The assessment was progressed as enumeration and then general morphology under the inverted microscope with ocular micrometer for sizing. Numerical abundance of all taxa was determined. Cells density was expressed as number of individuals per liter. Scanning electron microscopy (SEM) Samples for scanning electron microscopy (SEM) were cleaned by

KMNO4 oxidation of the organic material [27]. Specimen was prepared for SEM by air drying material on clean cover slips. Material was picked up onto a double sticking tape which was then mounted on a stub. Stubbs was gold coated and viewed on a SEM (JSM6380A).

3. Results

The community structure of micro plankton was consists of diatom, dinoflagellates and ciliates. The naked ciliates constitute *Strombidium* sp, *Strombidinopsis* and *Pelagostrobilidium* sp. The total species found during dinoflagellate bloom are 48 among them 25 from dinoflagellates, 12 from diatom and 11 from ciliates (Tab 1, Fig 3).

The most dominant species were *Tripos fuses* with $4.2x10^3$ Cells/L, *Noctiluca scintillans* with$1.8x10^3$ Cells /L, *Prorotoceracium* sp. $9x10^2$ Cells /L (Tab 2). The other HAB species were *Prorocentrum emerginatum*, *Prorocentrum gracile*, *Peridinium* spand *Alexandrium monilatum* were abundant with $9x10^2$ Cells /L. *Prorocentrum gracile* increases their numbers in the month of November, which increases up to $1.3x10^3$ Cells /L. Another species of *Prorocentrum micans* become abundant just after two weeks from $3x10^2$ Cells /L to $7.9x10^2$ Cells /L. *Dinophysis caudata* and *Dinophysis tripose* were also remain the part of the community till December. The persistent members were with $6x10^2$ Cells /L at the time of HABS appearance till next week.

The *Tripos fuses* reduced to $1x10^2$ Cells /L and in December become increased with $6x10^1$ Cells /L, the *Tripos furca* was decreased to $1x10^2$ Cells /L and surprisingly again reappeared as abundant species with strength of $3.6x10^2$ Cells /L, *Noctilluca scintillans* was with $1x10^2$ Cells /L in November and $1.6x10^2$ Cells /L in December. The blooming community was replaced by *Prorocentrum maxicanum*, *Gymnodinium* sp, *Peridinium* sp, *Prorocentrum dentatum* and *Prorocentrum arcuatum* after HAB event. *Dinophysis accuminata* appeared after complete removal of *Dinophysis tripos, Dinophysis caudata, Protoperidium* sp., *Tripos* sp appeared after the HAB event. *Karenia mikimotoi* total concentration was of $2.7x10^7$, which was more than usual reported counts. This HAB species was appeared as rust brown tide on the coastal water of Ormara.

The *Gymnodinium* bloom forming species has tendency to sub-surface layer to accumulate in 5-15 m down as subsurface layer. Then due to bloom generation factors they accumulate on the surface in daytime. The transparency of seawater becomes zero. The sample collection was taken exact at the time of their thick, concentrated appearance to the surface waters.

The taxonomic description in LM (light microscopy) showed that the species is photo synthetic with numerous ovals to round yellow- brown chloroplast [24]. It is a thecate species, observed as broadly oval and compressed dorso-ventraly. Cell size along with length was measured as Length 22.5µm and width calculated was 17.5 µm (Figure 1, D). The epitheca is broadly rounded and smaller than the hypotheca (Figure 1, D).Scanning electron microscopy (SEM) image showed long extended apical groove, excavated singulum, sulcus invaded in epitheca and horizontal flagellum (Figure 1 E).

4. Discussion

Karenia sp is a marine unarmed dinoflagellate genus contain 12 species and is known to form massive blooms in coastal waters mainly in summer [3] all around the world. *Karenia mikimotoi* formerly known as *Gymnodinium aureolum* and this autotrophic red tide species is known to tolerate a wide range of salinity and temperatures and proliferate millions of cells per liter [9]. This species was first observed in Irish waters linked with fish killing and also benthic fauna destruction [13]. The species bloom was found in massive fish kill incidents from China [11] and blooms also observed in regional waters from Southwest coast of India [14], Cochin waters [18]. The *Karenia mikimotoi* is a common member of dinoflagellate community from the coastal waters of Karachi Pakistan [22], but the bloom condition of this species was found for the first time from Ormara Blauchistan coast with highest cell concentrations. There is a report of bloom event of the same species at the same time period of October, 2009 was published from Southwest coast of India, Arabian Sea by [14] which showed similar discovery of intense brown colouration of water caused by *Karenia mikimotoi* bloom [18] also reported the bloom of *Karenia mikimotoi* from southeast Arabian Sea, bordering Western India, during September to November in 2004. They reported 19.37×10^4cells $L^{-1}$and 18.94×10^4 cells L^{-1} of cell abundance at near shore and offshore, respectively, while highest cell concentrations $2.7{\times}10^7$ cells $L^{-1}$was encountered in our study which is the highest recorded cell abundance till date. It suggests that proliferation of this dinoflagellate species increasing in Arabian Sea and occurs sporadically which could be harmful for the whole ecosystem. The loricate ciliate are known to predate on *Gymnodinium* sp [21, 8] and they have tendency to ingest large particles. The concentration of loricate ciliate increased within bloom condition and after the complete disappearance of bloom their abundance decreased as well (Fig 4). The conical ciliates *Strombidinopsis* sp has potentially able to feed dinoflagellate species [6]. The loricate ciliate concentration increased with the disappearance of *Gymnodinium* species where heterogonous dinoflagellates decreased rapidly. The thecate *Triposefuses* has tendency towards phagocytosis also showed dominant behavior over other dinoflagellate group. The dinoflagellates of bloom event till December shown changes in species composition, it constitute 23 species during mid of October where the next month sow similar number of species with replacement of some species by persisting their dominancy in this just after few consecutive weeks till the month of December in which dinoflagellates again dominant with 18 species (Table 1). This the time of clear sweep of diatoms, which present normal dominant trend after disappearance of bloom material. One of the important observations of this study is presence of about more than 12 toxic species along with bloom material including in family *Dinophyceae, Triposfurca***,** *Dinophysis caudata***,** *Dinophysis tripose, Gambierdiscus toxicus***,** *Noctiluca scintillans***,** *Ostreopsis accuta***,** *Ostroepsis* sp**,** *Ostreopsis lenticularis***,** *Prorocentrum concavum***,** *Prorocentrum lima* and in family *Bacillariophyceae, Pseudo-nitzscia* sp. Reference [10] Reported the harmful effects of *K. mikimotoi* bloom in which death incident due to consumption of shellfish *perna indica.* Neurotoxic shellfish poisoning (NSP) and azaspiracid shellfish poisoning (AZP) are known toxic effects of *K. mikimotoi.* This result reflects the severness of this particular Habs event and its impact on planktonic and non-planktonic organism of food web. Recently a neurophycotoxin chemical Gymnodimin A l is taken out from shellfish (South land, NZ dubbed south land town) after *Gymnodinium* sp red tide event [19]. The uniqueness of Asian monsoonal current pattern of Arabian Sea causing upwelling which brings nutrient rich waters to the surface resulting high productivity in monsoon seasons. This phenomenon also enhances the concentration of micro planktons including harmful bloom forming toxic species [1]. The process results rapid appearance of phytoplankton and then soon die down. Phytoplanktons are the primary link in the oceanic food chain so a regular check and monitoring program should be initiated regarding the safety of the ecosystem is suggested.

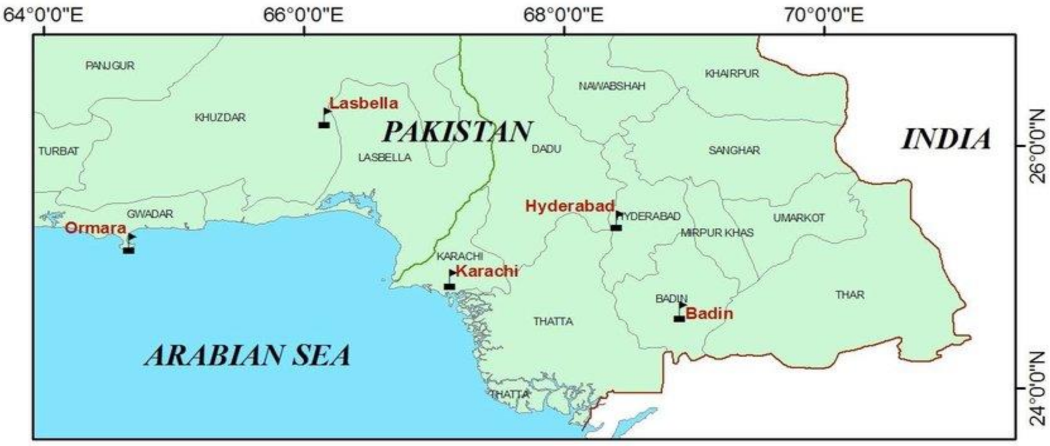

Figure 2: Map showing the sampling location.

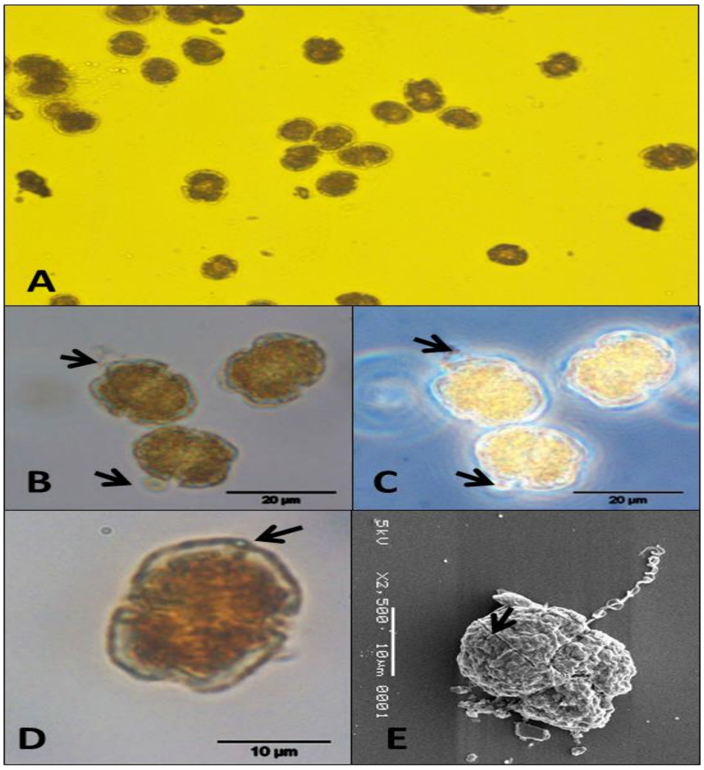

Figure 1: Microscopic images of K. mikimotoi. (A) Cell colony (B) Cells with vertical and horizontal flagellum (C) Same cells in phase contrast (D) Single cell with arrow showing clear notch of apical grove (E) SEM image with visible long extended apical grove, deeply excavated cingulum, sulcus invaded in epitheca and horizontal flagellum.

Table 2: Total abundance (Cells/l) of dinoflagellate species in bloom period (October, 2009).

Dinoflagellate species	Abundance ($x10^3$)
1.Tripos fuses	$4.2x10^3$
2.Noctiluca Scintillans	$1.8x10^3$
***3.Prorotoceracium* sp.**	$9x10^2$
4.Prorocentrum emerginatum	$9x10^2$
5.Karenia mikimotoi	$2.7x10^7$
6.Prorocentrum gracile	$9x10^2$
***7.Peridinium* sp.**	$9x10^2$
8.Alexandrium monilatum	$9x10^2$
9.Prorocentrum micans	$3x10^2$
10.Dinophysis caudata	$6x10^2$
11.Dinophysis tripos	$6x10^2$

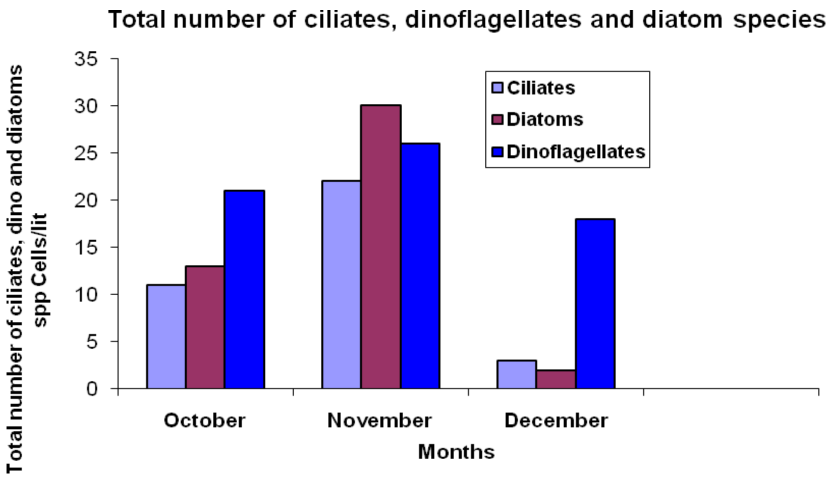

Figure 3: Total numbers of ciliates, dinoflagellates and diatoms species in Oct,Nov, Dec, 2009.

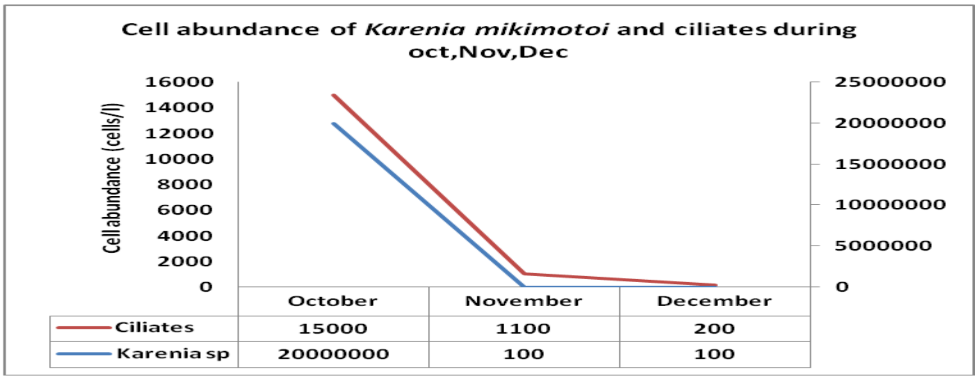

Figure 4: Cell abundance of *Karenia mikimotoi* and ciliates during Oct, Nov, Dec, 2009.

Table 1: Dinoflagellate, ciliate and diatom species list in October, 2009.

Dinoflagellate	Ciliate	Diatom
Alexandrium balticum ***Alexandrium monilatum***	*Coxiella longa*	*Achinoptychius* sp.
Alexandrium osteinfeldii	*Diddinium* sp.	*Coscinodiscus* sp.
Challcandella antiqua	*Dysteraacalate* sp.	*Cyclotella* sp.
***Tripos* sp.**		*Eupodiscus* sp.
***Triposfusus* sp.**	*Mesodinium rubrum*	*Nitzschia* sp.
***Triposfurca* sp.**	*Uronema* sp.	*Pseudo-nitzschia* sp 1.
Gambridiscus mtoxicum	*Tononia* sp.	*Pseudosolena* sp.
Dinophysis caudata ***Dinophysis tripos***	*Strombidinopsis* sp.	*Rhizosolenia calcar-avis*
Gymnodinium abbrevatium	*Pseudotontonia* sp.	*Skeletonemma* sp. *Cosrnodiscum* sp.
Noctiluca scintillans	*Strombidium wulfii*	*Skeletonemma costatum*
Ostreopsis lentacularis	*Strombidium capitatum*	*Synedra* sp.
***Ostreopsis* sp.**	*Strombium* sp.	
Ostreopsis accuta		
Peridinium balticum		
Peridinium conium		
***Protoceratium* sp.**		
***Protoperidinium* sp.**		
Prorocentrum concavum		
Prorocentrum gracile		
Prorocentrum lima		
Prorocentrum micans		
Prorocentrum* sp.** ***Prorocentrum emerginatum		

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