Unialgal culture of *Pseudonitzschia* (Bacillariophyceae) species a Domoic Acid (AD) toxin producer, local of Oman Sea

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Abstract

Diatom *pseudonitzchia* is a poisonous genus of family Bacillariaceae causing harmful algal bloom in coastal waters in many parts of the world. At the time of bloom, these diatoms produce domoic acid and neurotoxin that cause Amnesic Shellfish Poisoning (ASP) and can change ecosystems equilibrium following mortality of important groups like marine mammals and seabirds. In addition, it can threaten human health via food chains. Several cases of neurological disorder observed in humans 48 hours after consuming shellfishes. During the sampling of coastal waters of Chabahar Bay in February 2019, *Pseudonitzchia sp.* was observed. In this study, seawater samples were collected for identification and isolation of *Pseudonitzchia* like phytoplanktons. Then, the single cell diatoms species were isolated and transferred to petri dish including f2 media and maintained in the phycolab at 12L:12D period at 25°C. Morphological observations showed the purified sample was most similar to *Pseudonitzchia australia*. Due to economic damages of HAB phytoplanktons to the aquaculture industry and threatening health of environment and humans, it is necessary to identify their morphology and phylogeny accurately.

Keywords: Isolation, Phytoplankton, Chabahar Bay, *Pseudonitzschia*

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Introduction

Pseudo-nitzschia H.Peragallo is a genus of marine diatoms known as Nitzschia until 1994 but due to chain formation and other minor morphological differences, it was changed to *Pseudo-nitzschia* and recognized (Halse and Rytter, 1995). *Pseudo-nitzschia* species can be found in coastal areas around the world. They have been identified along the Pacific coast from Canada to California, the northeastern Atlantic, the Gulf of Mexico, along the coast of the Indian Ocean, as well as in the Gulf in general, including the coasts of Canada, Portugal, Italy, Greece, Croatia, and Australia (Dhar Bidhan *et al*., 2015). Some species of *Pseudo-nitzchia* are capable of producing neurotoxins and domoic acid toxins (AD).

Domoic acid can lead to neurological disorders caused by consuming Amnesic Shellfish Poisoning (ASP) (Maldonado *et al*., 2002). During harmful alge blooms, oysters become poisonous by feeding on *Pseudonitzchia* and can be transmitted to humans if consumed (Dhar and Roberto, 2015).

Currently, 26 species of *Pseudonitzchia* have the potential to produce domic acid (DA).

The algae bloom *Pseudonitzchia*, which occurred in 1987 on Prince Edward Island Bay in Canada, poisoned more than 100 people, killing three people (Hong *et al*., 2012). This incident refuted hypotheses that only dinoflagellates were capable of producing harmful algal blooms (HABs) (Maldonado *et al*., 2002). After that, many blooms of this genus were observed in coastal waters around the world, which research has shown to be related to increasing the concentration of nutrients (Halse and Syvertsen, 1997; Horner, 2002, Parsons and Dortch,. 2002; Fehling *et al*., 2004; Howard *et al*., 2007).

P. australis is a known species of the genus *Pseudo-nitzschia* found in temperate and subtropical seas. Domic acid production also causes harmful algal blooms and can lead to disease and death in many marine organisms as well as humans (Garrison *et al*., 1992). The needle shape of this diatom has led to physical damage to the skin and gills of fish and as well as increasing photosynthetic activity as a result of this bloom causes oxygen saturation of the environment (Huang *et al*., 2019). In this article, an attempt has been made to isolation and identify *Pseudo-nitzschia* through morphological observations and report its presence in the waters of the Oman sea also the method of laboratory culture and preparation of unialgal sample because of the importance of this genus of microalgae due to the ability to produce harmful blooms (HABs) and the effects thereby on a variety of marine and farmed species as well as humans and ecosystems. The importance of correct and early detection of algal blooms of *Pseudo nitzschia australis* in the environment and sustainable monitoring of harmful algal species in order to reduce environmental hazards as well as reduce damage to the local fisheries and fishing industry is very important. Having enough

information helps to implement management strategies to reduce the effects of such an event.

Materials and methods

This research was carried out by sampling the coastal waters of Chabahar bay located in the Oman Sea with a longitude of (25º59°16") and a latitude of (60º67°27") in Februry 2019 Water was sampled from the surface to a depth of 50 cm by a completely sterile bottle and immediately transferred to the laboratory to identify phytoblankton species (Fig. 1). In the laboratory, the species were immediately examined under a stereomicroscope model TF100 NIKON for initial study of species morphology and cell density was done by counting on Sedgwick-Rfter slide. Unialgal culture provided by single cell isolation as presented by Attaran-Fariman (2007). The single cells were then placed in a sterile petri dish containing 30 ml of F2 medium. The plates were transferd to the cultivation room with 12 hours of light and 12 hours of darkness and 25°C temperature and 25% humidity with a light amount of 1800 lux were placed. After two weeks, the sample was imaged under the Nikon 50I light microscope and KE Keview version 3.7 imaging software to accurately identify the morphology as well as the species response to the culture method. The specimen was identified by magnifying 100x to the species level

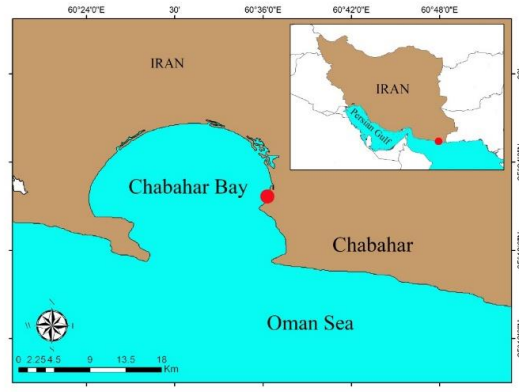

Figure 1: Sampling location.

Results

Pseudo-nitzschia sp. is a microalgae in the phylum Bacillariophyta, class Bacillariophyceae of the order Bacillariales and family Bacillariaceae (Kuwata Dvid, H, 2015). This genus of diatoms has the potential to produce harmful algal blooms and produce DA toxin (Bates *et al.*, 2018). During this study, the identified *Pseudo nitzschia* was most similar to *Pseudo-nitzschia australis.* Water samples were collected from the station for algae counting and cultivation in the 2019. *Pseudo nitzschia* sp. was the first dominant group with a density of 800 cells per liter and *Nitzschia* sp. was the second group with a density of 470 cells per liter. Other genera observed in the collected sample from Chabahar bay station include diatoms *Navicula* sp. 400 cells /L, *Thalassionema* sp. 50 cells per liter, *Pleurosigma* sp. 16 cells/L, *Cylindrotheca* sp 270/L *Chaetoceros* sp. 50 cells per liter, *Skeletonema* sp. 160 cells per liter Dinoflagellates including *Prorocentrum spp.* with a density of 160 cells per liter, *Amphidinium* sp. 200 cells

per liter, *karenia seliformis.* 103 cells per liter, *Gymnodinium* sp. 110 cells per liter, *Alexandrium* sp. 50 cells per liter were observed simultaneously. Cell counts were performed in 5 replications for each genus and species. The environmental parameters that measured at the site of the sample collection station were as follows: water temperature 29°C, salinity 43% and 7.8 pH. Cell morphological examinations were observed under a microscope. Morphological examination of *Pseudo-nitzschia australis* was observed under a microscope which is a slender, needle-shaped and boat-shaped species that is completely similar to other studyies in terms of shape and size (Fehling *et al*., 2004). It has two plate-like chloroplasts on each side symmetrically and It has a protective cell wall layer of silica. The color of the cell was yellowish brown and the cell without flagella was observed. The cells completely overlapped and formed a chain In this case, the cells overlap between one-third to one-quarter the length of each cell. The length of each cell measured is about 75 to 144 micrometers and their width is between 6 to 8 micrometers (Fig. 2). Measurement of cells by observing the cell under a 50I model light microscope and photographing by KE software Keview version 3.7 and then comparing the data with samples from other studies (Hasle and Syvertsen, 1997; Lundholm and Moestrup, 2002; Katsanevakis *et al*., 2014) .

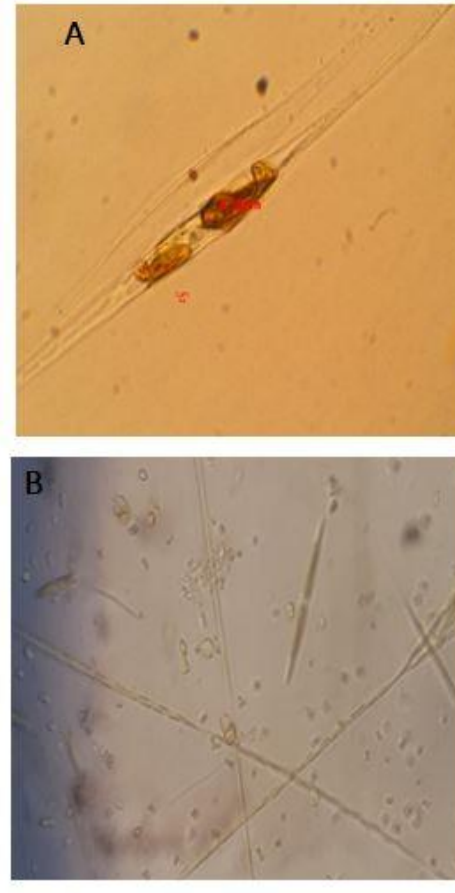

Figure 2: Isolated *Pseudo nitzschia* cells from Iran Southeast coast.

Discussion

Pseudo nitzschia is a genus of marine diatoms of which more than 50 species have been identified (DiatomBase, 2019). Of these species identified in the world, 26 species produce the neurotoxin AD toxin (Bates *et al*., 2018). Toxin produced by *Pseudo-nitzschia* microalgae in harmful algal blooms (HABs) pose a serious threat to ecosystems and human health worldwide through biotoxin contaminated food chain (Gilbert Burford, 2017). In March 2018, an unprecedented diatom algal bloom was reported in the Berow River in the Southern Hemisphere (Ajani

et al., 2018). The largest recorded *Pseudo nitzschia* bloom occurred in September 2004 off the northwest coast of the United States. In this harmful algal bloom, the maximum cell density was reported to be 1.3×10^6 cells per liter (Auro Maureen and Cochlan, 2013). A total of 20 species of *Pseudo-nitzschia* have been reported in Australian coastal waters that 4 species of which produce DA toxin (Lapworth *et al*., 2001; Ajani *et al.*, 2013). It was also found that increasing or decreasing DA production in laboratory and field conditions can be influenced by various physical factors such as temperature, light intensity, salinity, pH, mineral and other micronutrients, trace elements and biological agents such as bacteria, predators and secreted chemicals (Bates *et al*., 2018; Lelong *et al.*, 2012). In the genus *Pseudo nitzschia,* it appears that by increasing the density and reducing the distance between cells, it facilitates contact and receiving intercellular chemical signals and the diatom enters the sexual reproductive phase, which corresponds perfectly to the conditions suitable for growth by forming a chain in the laboratory culture medium. Thus, chain formation increases the density of the species and occurs with increasing cell concentration of sexual reproduction (Rhodes *et al*., 1998). The researchers also found that there was a close relationship between increased coastal nutrient deposition and increased *Pseudo nitzschia* bloom. The researchers also found that there was a close relationship between increased coastal nutrient deposition and increased *Pseudo nitzschia* bloom (Parsons and Dortch, 2002). *P.austeralis* is a poisonous species with the ability to cause harmful blooms, which is found in shallow and coastal parts of the oceans from a depth of 0 to 170 meters. They live in temperate and subtropical waters with temperatures ranging from 1 to 29°C (Fehling *et al*., 2004). This species can reproduce both sexually and asexually, that sexual reproduction is associated with higher levels of Domoic acid production (Du *et al*., 2016). Many factors are involved in promoting DA production, including adequate light and increasing and decreasing pH, as well as nutritional limitations (Bates *et al*., 2018). *Pseudo nitzschia* has also shown a dramatic response to changes in the concentrations of heavy metals such as iron and copper. With the decrease of iron in the environment, the amount of AD secretion increases from 6 to 25 times, and this increase in them leads to more iron absorption in the environment with stressful conditions, which itself has destructive effects (Maldonado *et al*.,, 2002). The blooms of *Pseudo nitzschia* has a negative impact on fisheries and the economy.(Ryan *et al*., 2017). In New Zealand, for example, fishing was halted for four years due to rising damoic acid levels (Rhodes *et al*., 2011). For this reason, the monitoring and management system is very important for recreational and commercial fishing (Álvarez-González *et al*., 2009; Haschek and Rousseeaux,

2013). Therefore, according to the observations confirming the presence of *Pseudo-nitzschia* along with genera of diatoms and dinoflagellates with the ability to form harmful algal blooms, which were islated from the Oman Sea According to the reports and researches, it is necessary to conduct regular investigations for heavy metal contamination and changes in the concentration of elements and changes in water factors, each of which is an effective factor in the occurrence of harmful algal blooms of *Pseudo nitzschia* in the Oman sea.

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