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DIATOMS AS BIOINDICATORS OF LITTORAL ZONE: A CASE STUDY

NERMIN ADEL EL SEMARY*

Department of Botany and Microbiology, Faculty of Science,
Helwan University, Cairo, Egypt

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Abstract

Diversity of diatoms was examined from littoral zone water, Suez Governorate, Egypt having fossil fuel-operated boats around. HPLC analysis of the water showed presence of some polycyclic aromatic hydrocarbons with the carcinogen benz[*a*]anthracene being highest in concentration. Pennate diatoms dominated the diatom community. Interestingly, a number of genera that were originally thought to be freshwater species also appeared in the littoral zone. There was a large number of Foraminifera and testate amoeba that can be used as co-indicators of pollution. *Navicula salinarum, Toxarium heenedyanum, Synedra famelica, Nitzschia* sp. and *Ardissonea crystallina* may be used as indicators of polyaromatic hydrocarbon pollution. The role of these diatoms in the bioremediation and counteracting organic pollution of coastal water can be explored in the future.

Introduction

The coastal and littoral regions of Ain Sokhna, Suez Governorate, Egypt are rapidly changing due to increased massive urbanization, anthropogenic activities and pollution associated with those activities. For example, oil and fossil fuel combustion products, i.e. polycyclic aromatic hydrocarbons are taken up by marine organisms such as plankton and fish causing their poisoning and disruption of food web (Neff and Anderson 1975, Ortmann *et al*. 2012). Sources of this type of organic pollution may result from combustion of fossil fuel, oil spillage and wastes. The use of microalgae as indicators of water quality has long been the focus of several studies. For example, Eplley and Weiler (1979) highlighted the association between the flagellated nannoplankton especially *Chrysochromulina* and petroleum oil pollution, even in small quantities, in marine environments. They also highlighted the association between pennate diatoms such as *Nitzschia* and *Navicula* with marine polluted environments. Indeed, diatoms are used as indicators of environmental conditions in freshwater systems especially benthic forms where diatoms like *Acanthes*, *Navicula* and *Gomphonema* were abundant in different eutrophic streams in Africa (Bellinger *et al*. 2006). The reasons behind the use of diatoms as bioindicators include: (i) They are primary producers at the base of the food web and are directly affected by environmental factors; (ii) The accumulated knowledge about species specific needs and range of tolerance allowed the development of different environmental indices (Descy 1979, Kelly and Whitton 1995); (iii) The possibility of paleo-reconstruction using sediment frustules (Round 1991). An example of the indices developed for freshwater bodies is the Trophic Diatom Index (TDI) (Kelly and Whitton 1995). With regard to organic pollution, the relative abundance of motile diatom species was used to indicate such pollution only in freshwater bodies (Kelly and Whitton 1998). However, indices devised for freshwater systems cannot be applicable for marine environments as freshwater diatom communities differ from marine ones. Indeed, marine diatom taxa have been shown to be less silicified than freshwater diatoms because of the lower availability of silicic acid

*Present address: Biological Sciences department, College of Science, King Faisal University, Kingdom of Saudi Arabia. <nerminel_semary@yahoo.co.uk>.

as salinity interferes with silica fixation (Conley *et al*. 1989). Obviously, marine water bodies await intensive studies for devising more specific indices. In that regard, the littoral zones are rarely studied and no diatoms-based indices, to the best of our knowledge, are in use. Nevertheless, the littoral zone pollution has been indicated by other biological forms such as abundant foraminifera tests and testable amoeba (Alves 1995). The site of the present study is a littoral zone, Red Sea near a Moore used for fossil fuel-operated boats, 140 kilometers from Cairo. It has both economic and recreational value. The main objective of the study was to investigate diatom diversity in this zone that is polluted by fuel combustion products and was never studied. We analysed the polycyclic aromatic hydrocarbons to verify this pollution. The polycyclic aromatic hydrocarbons are lipophilic organic compounds formed of multiple aromatic rings (between 2 - 7 rings). They arise as products of fossil fuel combustion (Yan *et al*. 2004). Different fuels have different combustion products and some of those compounds are carcinogenic (Fetzer 2000). The Environment protection Agency of the United States identified 16 PAH compounds as priority pollutants that should be tested for in environmental samples. Those 16 compounds are naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*] pyrene, dibenz(ah)anthracene, benzo[*ghi*]perylene, and indeno[1,2,3-*cd*]pyrene (Yang *at al*. 2004). The diversity and abundance of diatom taxa encountered are recorded by microscopic examination to investigate which diatom taxa are present and which species are most dominant. The research also aims at selecting the most tolerant diatom species for organic pollution and highlighting their possible role in bioremediation.

Materials and Methods

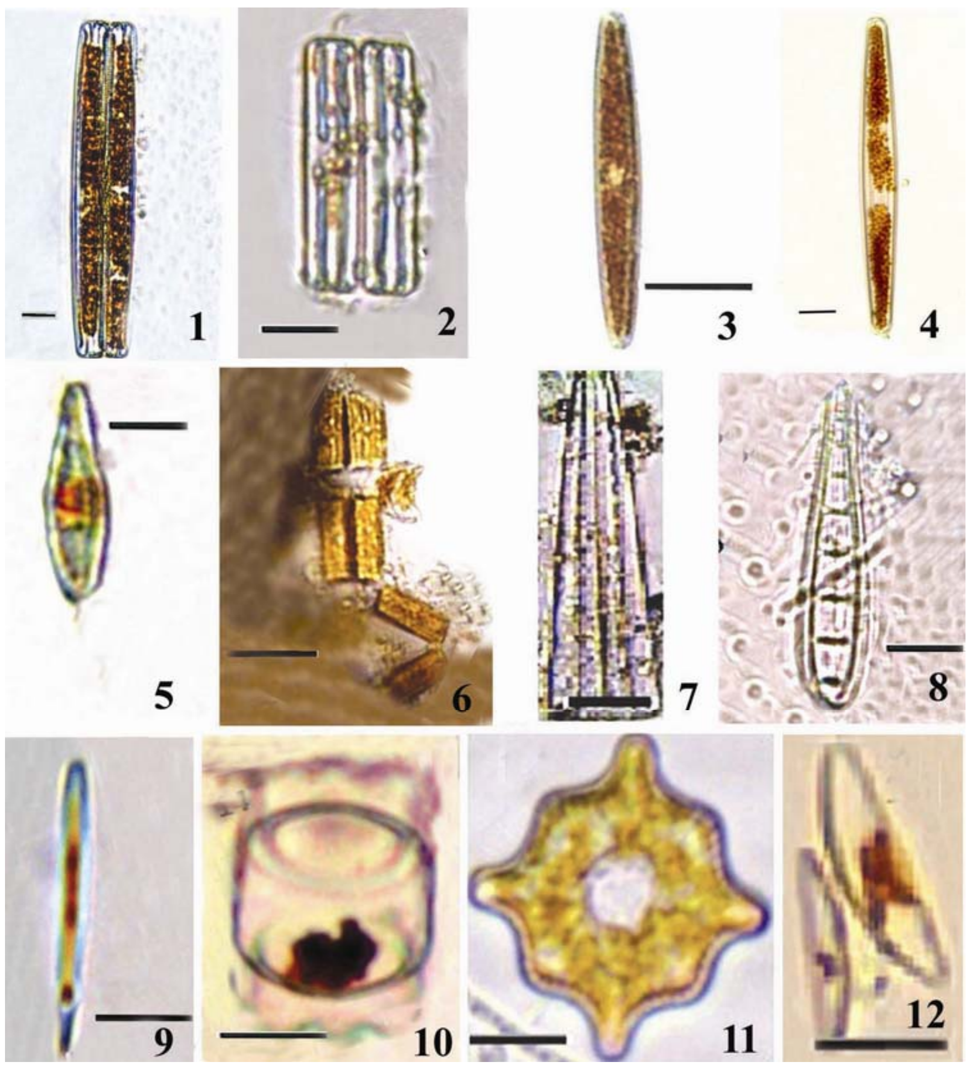
Light microscopic examination was performed on bottle samples that were collected vertically from the littoral zone, Ain Sokhna, Suez Governorate, Egypt. The movement of several diatoms species was particularly noted. The identification of taxa was based on Round *et al*. (1990), website for diatoms identification from USA (http://westerndiatoms.colorado.edu) and Sato (2008).

Littoral Red sea water sample (250 ml) was filtered through microfilter to remove any particulate matter or microorganisms. The sample was analysed for the presence of 16 types of polycyclic aromatic hydrocarbons resulting from fossil fuel combustion which are listed in US-EPA as priority pollutants. Those aromatic hydrocarbons (16 PAHs) were determined using HPLC, Agilent (model 1200 series, USA) with PDA detector (model 1260 infinity) set at 254 nm according to standard test method listed in Environmental Protection Agency (EPA-method 550). Calibration curve was done by reference standard mix of EPA 16 PAHs of known concentration**.** Analytical concentration of those compounds was expressed as mg/l.

Results and Discussion

Different *Navicula* spp. were amazingly active over a long period of time. Interestingly, the araphid elongated diatom *Toxarium hennedyanum*, *Tabularia flocculsa* and *Ardissonea crystallina* were also moving at a much slower rate than *Navicula* spp. with some minute trails of mucilage occasionally observed. The diatoms taxa identified and their abundance were recorded. The pennate diatoms dominated the diatom community (Tables 1, 2 and 3 and Figs 1 - 38). Several auxospores belonging to different species were observed with the distinctive silicified perizonium wall and chlorplasts inside. In addition, resting stages of pennate diatoms were also recorded where several cells are included within common gelatious sheath and attached to sand grains. Massive numbers of sponge spicules were also observed (Fig. 39) as well as testate amoeba. Live foraminifera and empty foraminifera shells were also observed as well as other algae such as cyanobacteria and euglenoids.

With regard to the polycyclic aromatic hydrocarbons, the HPLC analysis showed the presence of two ring-, one of the three ring-, one of the four ring-, all the five ring-, and one of the six-ring- aromatic hydrocarbon compounds derived from fuel (Table 4). The highest compound in concentration was benzo(a)anthracene with concentration of 115.59 mg/l. The highly carcinogenic/mutagenic compound benzo(a)pyrene was fourth at a concentration of 0.09 mg/l.



Figs 1 - 12. 1. *Toxarium hennedyanum* (Gregory) Pelletan, 2. *Tabellaria fenestrata* Kützing, 3-4. *Fragilaria virescens* Ralfs, 5. *Gomphonma lanceolatum* C. Agardh, 6. *Diatoma vulgare* Bory, 7-8. *Meridion circulare* (Greville) C. Agardh, 9. *Nitzschia* sp., 10. *Biddulphia roperiana* var. *obtusa* (Kützing), 11. *Trinacria exsculpta* (Heiberg) Hustedt and 12. *Cymbella* sp.

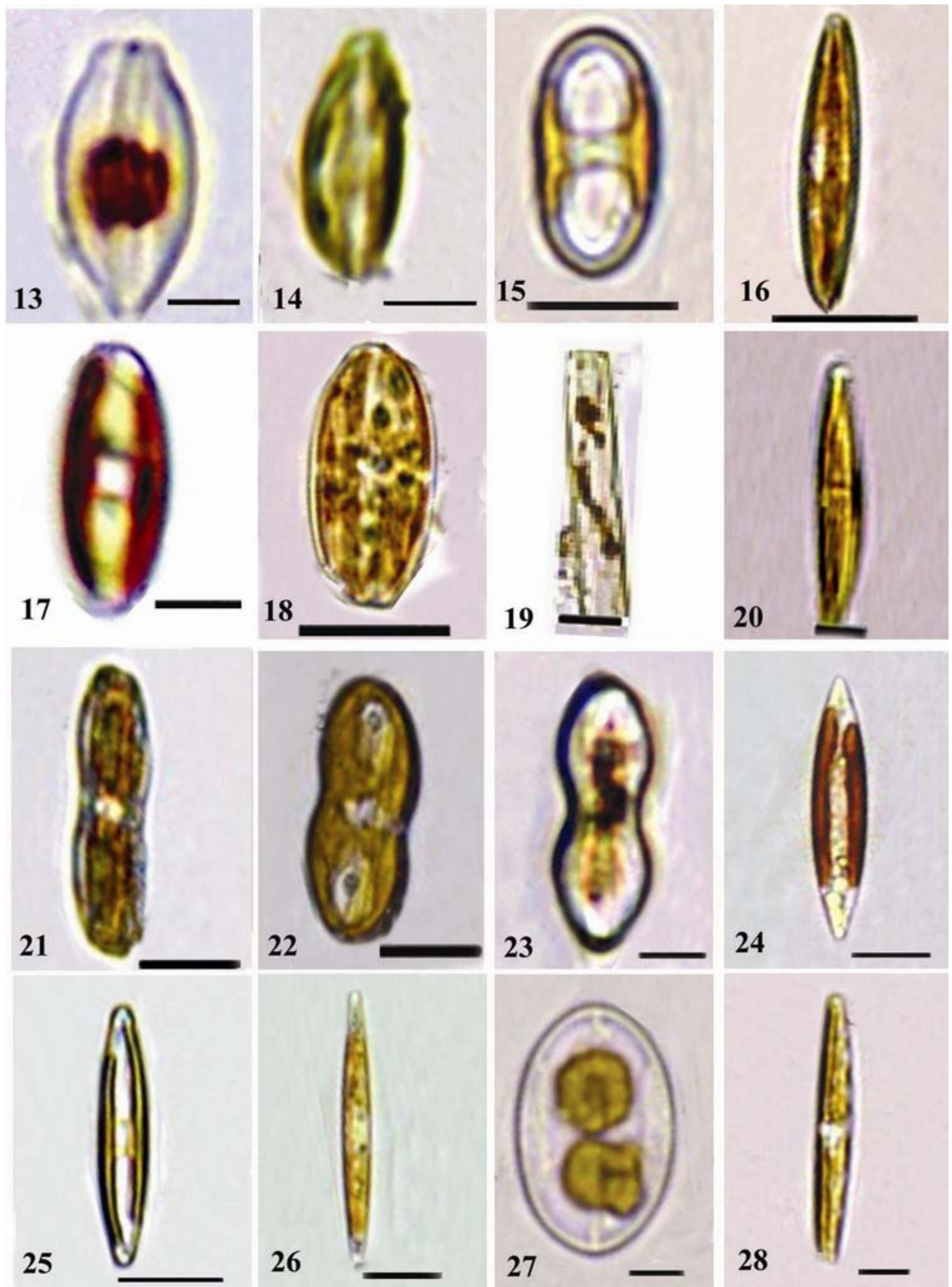
The movement of raphid diatoms species is quite expected. The raphe is a slit found on both valves in biraphid diatoms and only on one valve in monoraphids and on special structures such as Keel or canal in other raphid diatoms (http://westerndiatoms.colorado.edu). The presence of raphe facilitates the smooth movement of raphid diatoms in water. Therefore, it was rather unsurprising that we observed active movement in *Navicula* spp. and *Nitzschia* spp., both are raphid genera, whose motility is facilitated by the presence of raphe. On the other hand, araphid diatoms lack this raphe and consequently most of them were thought to lack motility. However, Sato (2008)

Table 1. Centric, araphid and monoraphid diatom species.

Species	Dimensions (μm)	Character	Abundance
Biddulphia roperiana Greville var. *obtusa* Kützing	L:30-50,W:20-40	C	+[1]
Achnanthes minutissima Kützing	L:9-20,W:2-4	M	++
Acnanthidium atomus Monnier, Lange-Bertalot and Ector	L:10-21,W:3-5	M	++
Anorthoneis dulcis Hein 1991	L:14-22, W: 6-12	M	+++
Cocconeis scutellum Ehrenberg	L:20-40, W: 15-25	M	++
C. placentula Ehrenberg	L.:19-55, W: 12-55	M	++++
Amphora minutissima Smith	L:12-20, W.: 3-5	AS	++++
Cymbella affinis Kützing	L.:18-30,W: 5-7	AS	+++
Ardissonea crystallina Grunow	L:180-420,W: 20-50	A	++++++++++++++
Synedra famelica Kützing	L:40-100,W: 4-12	A	++++++++++++
Tabellaria fenestrata (Lyngbye) Kützing	L: 30-80,W: 4-10	A	++++
Grammatophora marina (Lyngbye) Kützing	L:20-50,W: 10-35	A	+++++++
G. oceanica Ehr. var. Bailey	L:16-52,W: 4-22	A	+++++++
T. flocculosa Kützing	L: 30-90,W: 3-12	A	++++++++
Fragilaria virescens Ralfs	L.:60-80,W.:4-6	A	+++++++
Diatoma vulgaris Bory	L: 20-60,W:8-12	A	++++++
Meridion circulare (Greville) Agardh	L.:20-80,W.:3-4	A	++++++++++++++
Climacosphenia spp. Ehrenberg	L:190-410, W:90-220	A	++++++++++++++

[1]Plus sign (+) symbolises the frequency of species presence in samples examined microscopically. One or 2 plus signs: rarely-found, 3-6: moderately-found, 6 or more plus signs: frequently-found.

reported that araphid diatoms which do not possess raphe are sometimes capable of movement by special mechanisms. He recorded the movement of *licomophora hyalina* and attributed this movement to gliding possibly through its labiate processes. He also cited Hopkins (1969) who reported the movement of *Tabularia tabulata* (Agardh) Snoeijis (as *Synedra tabulata*). Consistently, we also observed motility in several araphid species including *Ardissonea crystallina* and *Toxarium hennedyanum*. Similarly, Pickett-Heaps *et al*. (1991) observed a gliding movement in *Ardissonea crystallina* whereas Kooistra *et al.* (2003) observed this movement in *Toxarium undulatum* Bailey. They reported that movement was facilitated by secretion of mucilage. However, those elongated pinnate-like diatoms lack labiate processes, unlike *Licmophora hylina* which may imply different mechanism of mucilage secretion. They also lack median sterna (midribs), which subtend a system of parallel transapical ribs (costae) and striae (rows of pores) (Kooistra *et al.* 2003). Therefore, we can only speculate that similarity in anatomical features may extend to include similarity in mechanism of movement. The presence of



Figs 13 - 28. 13-14. *Amphora* spp., 15. *Diploneis puella* (Schumann) Cleve, 16. *Navicula* sp., 17. *Caloneis* sp., 18. *Amphora* sp., 19. *Fragilaria* sp., 20. *Nitzschia* sp., 21-23. *Diploneis interrupta* (Kuetz.) Cleve, 24. *Navicula salinarum* Grunow, 25. *Navicula libonensis* Schoeman, 26. *Nitzschia* sp., 27. *Cavinula lacustris* (Gregory) Mann & Stickle and 28. *Nitzschia longissima* Brébisson.

moving araphid species was not the only surprise. It was even more surprising to record some freshwater taxa in that marine site. Several species, previously thought to be freshwater taxa were recorded in the samples such as *Anorthoneis ductalis*, *Navicula reinhardtii*, *luticola mutica*, *Pinnularia* spp. In this regard, Marinês and Marciana (2012) reported the presence of usually freshwater diatom *Anorthoneis ductalis* in coastal area and attributed this to the expansion of the autecological tolerance of that taxon that enabled it to inhabit freshwater and marine niches. However, the limited abundance of those diatoms does not support this explanation. It has been suggested that during winter time during the heavy rain fall on mountains, freshwater accumulates

Table 2. Biraphid diatoms species.

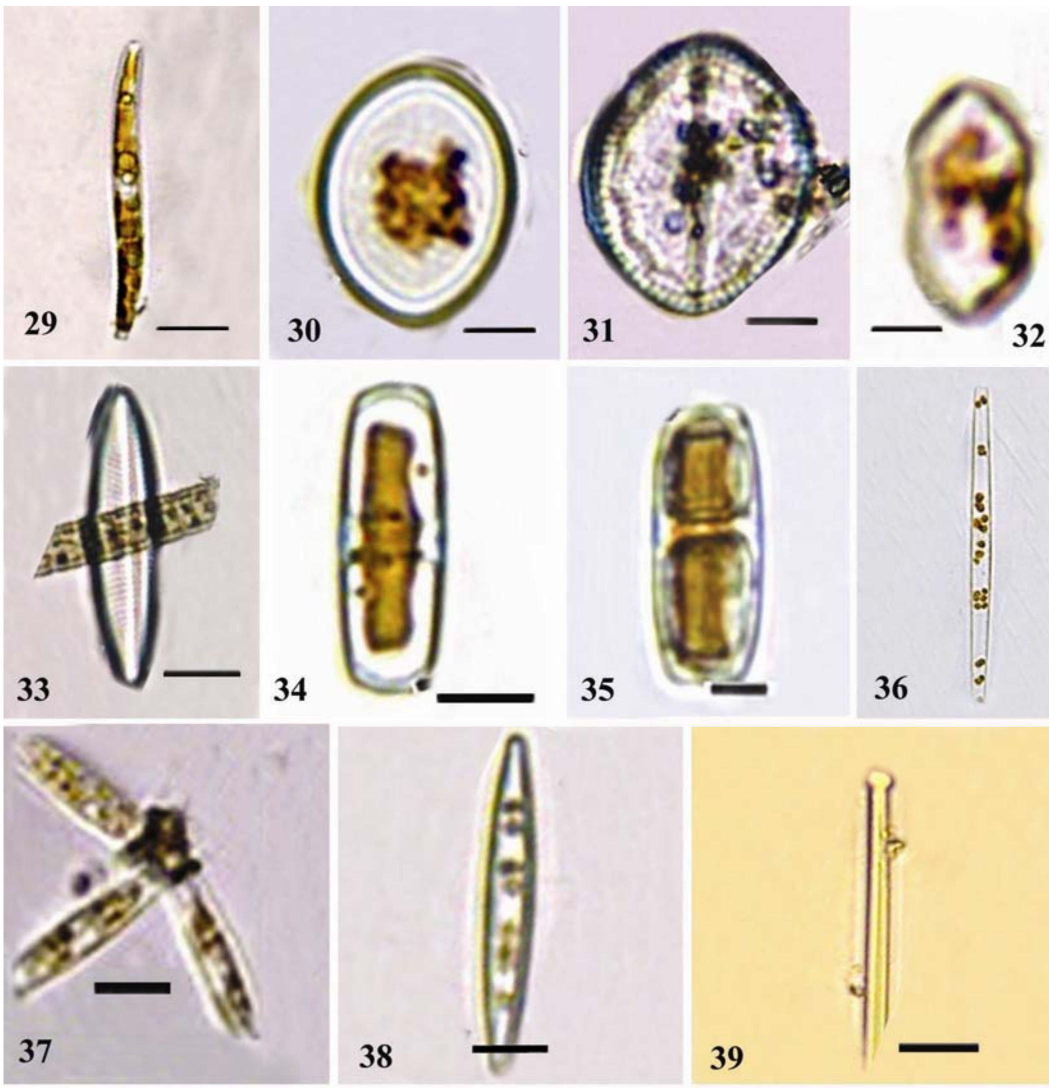
Species	Dimensions (μm)	Character	Abundance
Diploneis puella Cleve	L.: 10-20,W.**:** 3-5	B	+++
Navicula sp.	L.:10-14,W.: 4-6	B	+++
Caloneis sp.	L.: 50-100,W.: 12-20	B	+++
Nitzschia sigmoidae Smith	L.:30-60,W.: 20-50	B	++
Pinnularia brebissonii Rabenhorst	L.:20-50,W.: 10-15	B	++
Diploneis interrupta Cleve	L.: 50-70,W.:10-15	B	+++
Navicula radiosa Kützing	L.: 50-70,W.: 30-50	B	++
N. reinhardtii Grunow	L.:34-40,W.: 14-17	B	+++
N. salinarum Grunow	L.: 10-20,W.**:** 3-5	B	+++++++++
Nitzschia longissima Brébisson	L.:50-100,W.: 12-20	B	++++

Table 3. The HPLC analysis for 16 polycyclic aramoatic hydrocarbons.

No. of PAHs rings	16 PAHs name	16 PAHs abbreviations	Concentration (mg/l)
2-PAHs rings	Naphthalene	NAP	BDL
3-PAHs rings	Acenaphthylene	ACE	"
	Acenaphthene	ACP	"
	Fluorene	FLU	"
	Phenanthrene	PHE	"
	Anthracene	ANT	0.0219[1]
4-PAHs rings	Fluoranthene	FLA	BDL
	Pyrene	PYR	"
	Benzo(a)anthracene	B(a)ANT	115.585
	Chrysene	CHR	BDL
5-PAHs rings	Benzo(b)fluoranthene	B(b)FLA	0.0294
	Benzo(k)fluoranthene	B(k)FLA	0.0301
	Benzo(a)pyrene	B(a)PYR	0.0862
	Dibenzo(a,h)anthracene	DB(a,h)ANT	2.199
6-PAHs rings	Benzo(g,h,i)perylene	B(g,h,i)PER	0.4231
	Indeno(1,2,3-D)pyrene	INDP	BDL
	Total US-EPA 16 PAHs		118.375

BDL= Below detection limit, [1]Eight compounds out of 17 were detected.

within small ponds within the mountains and may after a period of accumulation, overflow causing torrential floods towards sea sweeping some diatoms into sea. The survival of those diatoms is determined by their tolerance range. Another explanation may be the discharge of ballast water from ships thereby introducing species that were not native to the niche before. Also the role of birds and biofouling algae on boats in transferring some diatoms species cannot be ruled out. Similarly, the misinterpretation of the original description of these taxa as freshwater as



Figs 29 - 39. 29. *Nitzschia fasciculata* (Grun.) Grunow., 30. *Cocconeis placentula*., 31. *Cocconeis* sp. 32. *Nitzschia tryblionella* Hantzsch., 33. *Pinnularia brebissonii* (Kuetz.) Raben., 34-35. *Navicula pupula* Kuetz., 36. *Synedra* sp., 37. *Synedra actinestroides* Lemm., 38. *Nitzschia romana* Grun., 39. *Synedra* (?).

no worldwide screening programmes were thoroughly made to verify this description cannot be ruled out. There were also three distinctive morphotypes of *Climacosphenia* which is araphid pennate-like centric diatom growing in the tropical and subtropical areas as an epiphyte on algae (Round 1982) that appeared frequently during microscopic examination. It differed from the two described *Climacosphenia* species (C. *moniligera* and *C. elongata*) in the presence of a distinct axial area (sternum-like which is absent in those two species) or a modified annulus that subtends ribs internally as well as externally (Round 1982). This morphotype shares the character of *C. elongata* of being abruptly narrowed part-way down the valve. It also shares the parallel striation with *C. moniligera.* So we cannot consider it to be a variant of the former or the latter but we suggest it to be rather distinct type, possibly an underreported/unreported species of the genus *Climacosphenia.*

The abundance of rather elongated diatoms such as *Climacosphenia* spp., *Toxarium hennedyanum* and *Ardissonea crystallina* is particularly interesting. Medlin *et al*. (2008) gave a molecular evidence to the genetic closeness of these elongated diatom taxa and showed that they should be considered as centric diatoms though pennate-like in overall morphology. They showed that they lack the anatomical features characteristic of pennate diatoms and molecular analyses confirmed their closeness. In line with this finding, it might be possible that there is a related genomic adaptability of these taxa to the polluted conditions as indicated by their abundance. More research should explore this possibility further. The presence of many observable resting stages within sample may indicate the presence of unfavourable conditions. More interestingly was the abundance of *Synedra* spp. and *Synerda*-like spp. This genus was believed to be exclusively freshwater and lots of its members were transferred to other genera, such as *Toxarium*, according to Round *et al*. (1990) and *Ardissonea crystallina* which was formerly known as *Synedra crystallina*. Nevertheless, other taxonomically-valid *Synedra* species were also abundant. This overall abundance is possibly related to their ability to withstand and biodegrade polycyclic aromatic hydrocarbons (Cerniglia 1992) which in turn may have selected for the abundance of certain diatoms at the expense of others. With regard to pollution, the EPA has identified seven PAH compounds as carcinogenic compounds: benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*b*] fluoranthene, benzo[*k*]fluoranthene, chrysene, dibenz(a,h)anthracene, and indeno(1,2,3-cd) pyrene. In our sample, five of these compounds were identified with benz[*a*]anthracene being highest in concentration. This finding only emphasizes on the danger this pollution imposes on both the environment and living organisms. Also, it must be said that there was a time gap between sampling and the analysis of PAHs. Therefore, it might be possible that some PAHs were exposed to biodegradation and that is why we cannot rule out the presence of higher concentrations of these PAHs in the original niche which is even more alarming. Finally, we could not overlook the presence of massive amounts of empty sponge spicules, foraminifera as well as testate amoeba. Alves (1995) used the latter biological forms to indicate the pollution of littoral zones whereas the former may result from destruction of sponge reef as a result of unlawful fishing, biological destruction as well as hydrocarbon pollution (Canadian Parks and Wilderness Society 2004). Here the pollution with some polycyclic aromatic hydrocarbons was established and it can possibly be linked to the destruction of the glass sponge, one of the major valuable components of sponge reef, yet providing another bioindicator for pollution. Therefore, it might be possible to use the previously-mentioned forms in conjunction with diatoms as bioindicators in more frequent screening programmes to monitor the environmental status of those coastal littoral zones. Prevention of pollution can protect the economic and recreational value of those niches and maintain the ecological balance and natural biodiversity. Also the possible role of these diatoms in biodegradation of polycyclic aromatic hydrocarbons can be further investigated.

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