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Primary Study of the Non-Marine Epilithic Diatom Communities of Malta and Gozo

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ABSTRACT

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Non-marine epilithic diatom communities of two major Maltese islands, Malta & Gozo, were investigated for the first time. Fourty four samples were collected from eleven stations, which represented different springs and spring-fed streams distributed over the two islands. Extraction of diatoms and preparation of clean slides for microscipic investigation was carried out. A total number of 51 taxa belonging to 23 genera were identified. The most represented genera were *Nitzschia*, *Amphora*, *Surirella*, *Luticola* & *Navicula*. The most represented species were *Gomphonema parvulum*, *Hanztschia amphysioxy*, *Nitzschia palea* & *Amphora ovalis*. Most of the identified taxa show moderate to strong association with pollution, and show potential as indicators for forensic science and environmental assessment.

Introduction

Diatoms are a very important large group of algae with at least 200,000 species (Mann and Droop, 1996). They are abundant in several aquatic environments responding quickly to the environmental conditions, maybe due to their relatively short life span. So they consider good bioindicators for environmental changes. Diatoms can be applied for general aquatic bioassessment, that use species composition and abundance to assess environmental changes and human impact on aquatic environments (Smol and Stoermer, 2010). Epilithic diatom assemblages have been used before for understanding the biology and environment of oligotrophic lakes at high mountains and also some springs and streams at several islands (Sanchez-Castillo *et al.*, 2008; Zalat and Al-Wosabi, 2011; Delgado *et al.*, 2013).Those diatom assemblages could obtain silica and other nutrients from the rocky substrate that they live on (Douglas & Smol, 1995). The non-marine epilithic diatom assemblages of Malta have never been studied before.

Malta and Gozo are characterized by a subtropical-Mediterranean climate and the presence of a number of springs and spring-fed streams (Schembri, 1993). The environment of islands is controlled by a complex interaction of geological, climatic and anthropic factors. The rainfall occurs mainly during winter, percolating through the porous limestone rock and accumulating in aquifers from where it seeps out to form a number of springs in the valleys. The rain water does not escape from the upper coralline limestone layer due to the underlying layer of impermeable blue clay, while in summer the limestone becomes completely dry (Schembri, 1993; Galdies, 2013). Diatom assemblages are adapted to the local environmental conditions in relation to changes in water residence time and to the variations in the ionic content that is due to variations in flow volume (Aboal *et al*., 1996).

The main goal of this study is to survey the non-marine diatom epilithic assemblages of different spring-fed streams in Malta and Gozo.

Materials and Methods

Study area

The Maltese Islands are a group of small islands located at the center of the Mediterranean Sea (latitude: 35' 48' 28" – 36° 05' 00" North and longitude: 14° 11' 04" – 14° 34' 37" East). The land area of Malta is 245.7 km^2, while Gozo is 67.1 km^2 (Schembri, 1993). Eleven stations of springs and spring-fed streams, belonging to eleven different valleys distributed over Malta and Gozo islands as shown in Figure 1, were selected to monitor the non-marine epilithic diatom assemblages over the islands. The coordinates of the eleven stations are presented in Table 1.

Sample collection

Fourty four samples were collected from the eleven mentioned stations. Sample collection was carried out at the begining and at the end of the wet period (October and February). Two samples at 20 metre distance from each other represented each station at each of two collections. Three to five rocks covered with epilithic diatoms, were usually picked up from the deepest part of the stream, usually the ideal site as the main current flows through it. Then epilithic diatoms were scraped away from rock surface, using a toothbrush, and collected in clear vials (McLaughlin, 1995; Stevenson, 2007). The pH, conductivity and temperature were measured at the station using the Thermo Scientific Orion Star A215 Benchtop and Star A325 Portable pH / conductivity meter. Samples were placed in a refrigerator at 4ºC until analysed.

Sample cleaning and identification

For cleaning diatoms, the hot hydrogen peroxide with hydrochloric acid method was used according to Zalat & Al-Wosabi 2011. Permanent slides were prepared and then examined under the optical microscope, Olympus BA410, containing software named Motic Image Plus 2.0ML, with a 600x magnification. A grid was placed on a clean empty slide to measure a particular species size by taking a diatom photo and placing it on the grid. Diatom identification was based on microscopic morphology, with reference to images in published literature (Wojtal, 2009; Round *et al*., 1990).

Results and Discussion

Environment Parameters

The pH values ranged from slightly alkaline to alkaline (Table 2). The alkalinity of the water may be due to the geochemical nature of the island rocks, most being composed of limestone of Oligo-Miocene age (Schembri, 1993). The partial solubility of calcium carbonate content of such rocks increases the pH level of the rainfall freshwater. Temperature values were in agreement with the values present in the literature (Chetcuti *et al*., 1992; Galdies, 2011). Electrical conductivity (EC) was influenced by the amount of salt dissolved in the water, and its values were in correlation with the values of the Total Dissolved Solids (TDS) (Thirumalini & Joseph, 2009).

The highest EC value was recorded at Wied Mistra (*MST*) station that probably means it had the highest TDS value, while the lowest EC value was recorded at Wied Għollieqa (*GHO*), which means that it had the lowest TDS values.

Diatom Analysis

A total number of fifty one taxa were identified, belonging to 23 genera (Table 3). The most represented genera; *Nitzschia* was represented by 8 taxa, followed by *Amphora* and *Surirella* that were both represented by 5 taxa, and finally *Luticola* and *Navicula*; each were represented by 4 taxa. The highest taxa diversity was recorded at MFN station (21 taxa) followed by QLE (19) and then QAN (17). The lowest taxa diversity was recorded at LUQ and GHO stations (4 taxa). Most of the identified taxa were alkaliophilous oligohalobious with tolerance to relative-higher salinity freshwater and also tolerant to moderate and strong levels of nutrients and organic pollutants such as pesticides, solvents and industrial chemicals. Only four mesohalobous taxa were identified: *Fallacia pygmaea, Nitzschia sigma, Trybionella apiculata* and *Trybionella hungarica,* and one polyhalobous taxon, *Seminavis* sp. Moreover all the identified taxa were pennate, except *Cyclotella meneghinana* and *Cyclotella* sp. present at SWD, QAN and MFN.

The high concentration of *Fallacia pygmeae* (mesohalobous) and the presence of *Cocconeis placentula* (oligohalobous), which show tolerance to higher salinity levels, correlates with water salinity. Moreover, the conductivity values were evidence to the different habitat with higher salinity at the MST station.

Fallacia pygmeae here is an example for excellent taxa for forensic applications, as its presence in significant amounts means that one expects to identify the taxa easily in the investigation of crimes at the MST valley region. The commonest forensic application is identification of diatoms in lungs, bone marrow and other body organs in cases of suspected drowning. Others should use diatom assemblage from skin and clothes to correlate victims to crime scenes.

Primary ecological indication for each station

Most stations were high polluted. The presence of *Hantzschia amphioxys*, and *Luticola* spp. and their high concentrations may be related to the shortage of water and the tolerance of such taxa for terresterial habitats and soils.

GHS & LUQ

Polluted stations indicated by the presence of taxa that have high tolerance for pollution, such as *Gomphonema parvulum* in a considerable concentration (Sen *et al*., 2013).

QLE

The species richness that appeared here may be related to the increase in nutrient concentration and decrease in the current flow.

Luticola mutica is present in considerable concentration during october because it is terresterial taxa and can tolerate the lack of water during october.

High pollution evidence especially during the october indicated by the presence of taxa that have high tolerance for pollution, such as *Gomphonema parvulum, Amphora ovalis and Nitzschia palea* in considerable concentration. (Sen *et al.*, 2013)

MSC

May be the station has moderate pollution due to the presence of *Nitzschia linearis*, but generally less polluted than the previous sations.

SWD

Heavily polluted station, specially during october, indicated by the presence of high tolerance pollution taxa including *Nitzschia palea, Gomphonema parvulum, Cyclotella meneghinana and surirella ovalis* in considerable concentration. (Sen *et al.*, 2013)

The concentration of nutrients may be diluted during febreuary by rain waters, and pollution level may increase.

GHO

Polluted station due to the presence of the high pollution tolerant taxa *Nitzschia palea* in considerable concentration.

Table.1 Samples location coordinates.

Coordinates / Station name *(symbol)*	Latitude	Longitude
Wied il-Għasel (*GHS*)	35° 54' 58.23" N	14° 25' 37.11" E
Wied il-Luq (*LUQ*)	35° 51' 31.28" N	14° 24' 3.21" E
Wied il-Qlejgħa (*QLE*)	35° 53' 36.69" N	14° 23' 30.69" E
Wied ta' Marsascala (*MSC*)	35° 52' 8.69" N	14° 33' 34.80" E
Wied is-Sewda (*SWD*)	35° 53' 1.80" N	14° 27' 42.10" E
Wied Għollieqa (*GHO*)	35° 54' 15.99" N	14° 28' 57.41" E
Wied il-Lunzjata (*LUN*)	36° 2' 27.15" N	14° 13' 57.52" E
Wied ta' Marsalforn (*MFN*)	36° 4' 9.45" N	14° 15' 37.40" E
Wied Mistra (*MST*)	35° 57' 27.23" N	14° 23' 20.77" E
Wied Qannotta (*QAN*)	35° 55' 57.18" N	14° 25' 11.42" E
Għadira ta' San Raflu *(GSR)*	36° 2' 11.93"N	14° 11' 56.85"E

Table.2 Average values of pH, Temperature & Conductivity for ten stations.

Parameters / Stations	Average pH	Average Temperature (°C)	Average conductivity (µS/Cm)
GHS	7.875 ± 0.325	13.35 ± 0.35	1868 ± 300
LUQ	7.545 ± 0.275	16.55 ± 0.25	1540 ± 36
QLE	7.995 ± 0.295	12.95 ± 0.35	2243 ± 13
MSC	7.87 ± 0.24	13.55 ± 0.45	1329 ± 968
SWD	8.605 ± 0.165	13.65 ± 0.15	576.5 ± 17.5
GHO	7.645 ± 0.065	13.65 ± 0.15	425.5 ± 117.5
LUN	8.27 ± 0.2	13.4 ± 0.8	469 ± 216
MFN	8.41 ± 0.19	12.25 ± 0.45	1463 ± 3
MST	8.595 ± 0.495	16.75 ± 0.35	5365 ± 2175
QAN	8.705 ± 0.055	16.75 ± 0.35	1456 ± 904.5

Fig.1 Eleven stations distributed over both islands of Malta & Gozo.

Plate.1 Some of the identified taxa. *A. Gomphonema parvulvum, B. Cocconeis placentula, C. Surirella minuta, D. Gomphonema parvulvum, E. Amphora sp., F. Cyclotella meneghinana, G - H. Amphora sp.1, I. Navicula sp., J. Luticola sp., K. Navicula radiosa, L. Nitzschia paleace, M. Tabularia fasciculate, N. Nitzschia linearis, O - P. Hantzschia amphioxys, Q. Tryblionella apiculata.*

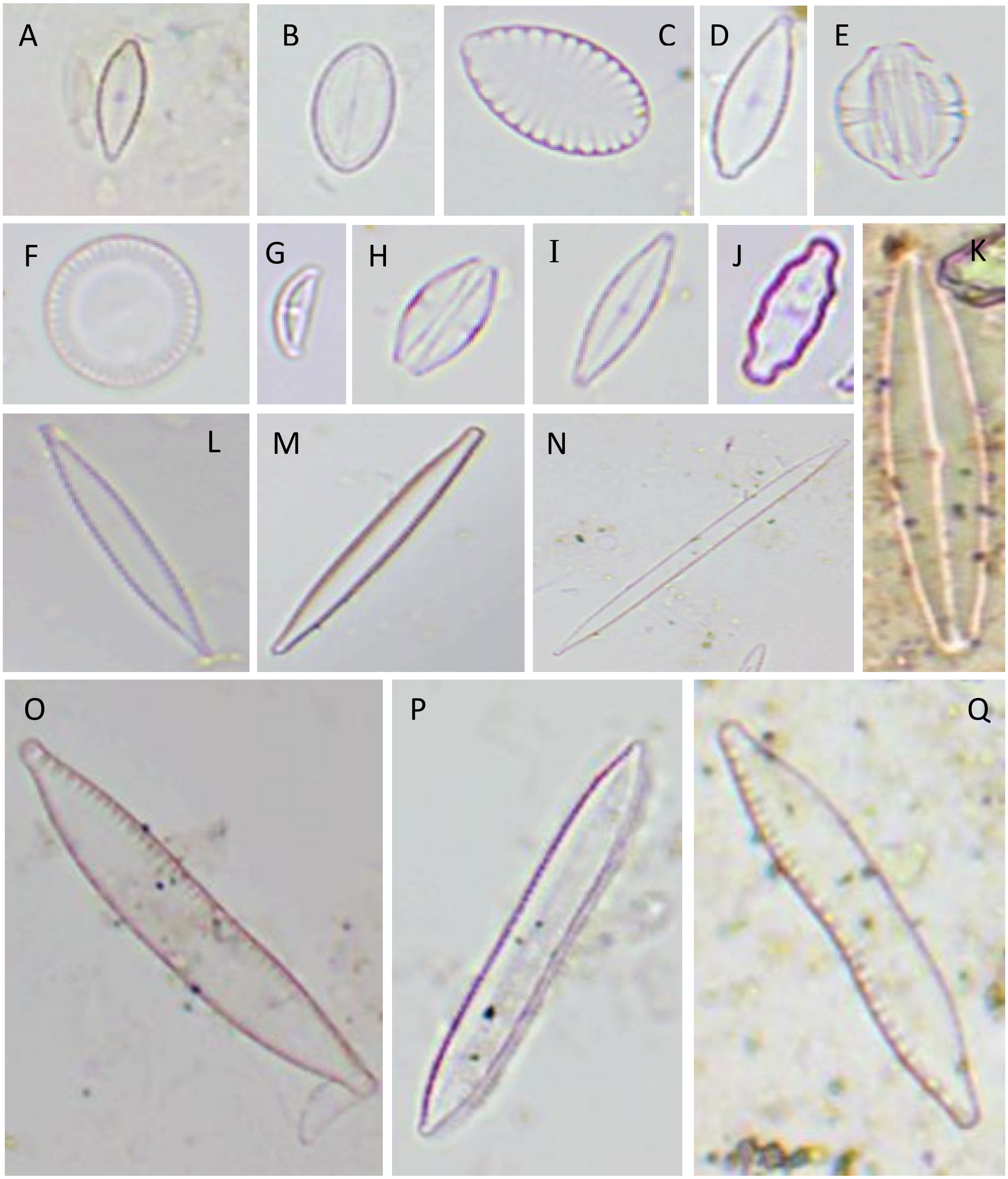

Table.3 List of all microscopically identified diatom taxa from the studied stations during october (1) and febreuary (2). Taxa were arranged in alphabetical order. (-) no evidence of the taxon, (+) the taxon present in the sample, (O = oligohalobous, M = mesohalobous, P = polyhalobous).

Station / Taxa	GHS		LUQ		QLE		MSC		SWD		GHO		LUN		MFN		MST		QAN		GSR		salinity
Month	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	
***Achnanthidium* sp.**	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**O**
Amphora ovalis	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	**O**
***Amphora* sp.1**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-
***Amphora* sp.2**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-
***Amphora* sp.3**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
***Amphora* sp.4**	+	-	-	-	+	+	+	+	-	-	-	+	+	+	-	-	-	-	-	+	-	-	-
Cocconeis placentula	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	**O**
***Cocconeis* sp.**	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cyclotella meneghinana	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	**O**
***Cyclotella* sp.**	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
***Cymbella* sp.**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Cymbella lanceolata	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**O**
Encyonema cf. brevicapitatum	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
***Eunotia* sp.**	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fallacia pygmaea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	**M**
***Fallacia* sp.**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-
***Frustulia* sp.**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Gomphonema gracile	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	+	-	-	-	-	-	-	**O**
Gomphonema parvulum	+	-	-	+	+	+	-	-	+	+	-	-	+	+	-	+	-	-	+	-	-	+	**O**
Hantzschia amphioxys	+	-	+	-	+	-	-	+	+	+	-	-	-	-	+	-	-	-	+	+	-	-	**O**
***Luticola* sp.1**	-	-	-	-	+	+	-	-	-	+	+	+	-	-	+	-	-	-	+	+	-	-	-
***Luticola* sp.2**	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
***Luticola* sp.3**	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Luticola mutica	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**O**
***Navicula radiosa* Kützing**	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	**O**
***Navicula* sp. 1**	+	+	-	-	-	-	-	-	+	+	-	+	+	+	-	-	-	-	+	+	-	+	-
***Navicula* sp.2**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-
Navicula cryptocephala var. veneta	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	**O**
Neidium cf. affine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	**O**
Nitzschia dubia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	**O**
Nitzschia frustulum	+	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	**O**
Nitzschia gracilis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	**O**

Nitzschia linearis	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	+	-	-	-	-	-	-	**O**
Nitzschia palea	-	-	-	-	+	-	-	-	+	+	+	+	-	-	+	+	+	-	+	+	-	-	**O**
Nitzschia paleace	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**O**
Nitzschia sigma	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	**M**
***Nitzschia* sp.**	-	+	-	+	-	+	-	-	-	-	-	-	-	+	-	+	-	+	-	-	+	+	**-**
***Placoneis* sp.**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	**-**
Planothidium delicatulum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	**O**
***Planothidium* sp.**	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Rhoicosphaenia abbreviata	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**O**
***Seminavis* sp.**	+	-	+	-	+	-	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	**P**
Surirella angusta	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	**O**
Surirella brebissonii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	**O**
Surirella minuta	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	**O**
Surirella ovalis	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	**O**
***Surirella* sp.**	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
***Tabularia* sp.**	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	**-**
Trybionella hungarica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	**M**
Tryblionella apiculata	-	-	-	-	+	+	-	-	-	-	-	-	+	-	+	+	-	-	+	+	-	+	**M**
***Ulnaria* sp.**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	**O**

LUN

Polluted station due to the presence of the heavily polluted tolerant taxa, *Gomphonema parvulum and Navicula radiosa* in considerable concentration.(Sen *et al*., 2013)

May be the level of pollution increases during febreuary.

MFN

The species richness that appeared here may be related to the increase in nutrient concentration and decrease in the flow current.

Heavily polluted station indicated from the presence of the heavily pollution tolerant taxa, *Navicula cryptocephala var.veneta, Nitzschia palea, , Gomphonema parvulum and Cyclotella meneghinana* in considerable concentration. (Sen *et al*., 2013).

MST

Heavily polluted brackish habitat is indicated from the significant amount of *Fallacia pygmeae*, which is mesohalobous, and the presence of *Cocconeis placentula*, which is oligohalobous, but tolerant to higher salinity levels, besides the conductivity value.

QAN

The species richness that appeared here may be related to the increase in nutrient concentration and decrease in the flow current.

Heavly Polluted station due to the presence of the heavy pollution tolerant taxa, *Gomphonema parvulum, Nitzschia palea, Nitzschia sigma, Cyclotella meneghinana, Nitzschia gracilis* in considerable concentration. (Sen *et al*., 2013)

GSR

Polluted station due to the presence of *Gomphonema parvulum* and *Amphora ovalis* in considerable concentration. (Sen *et al*., 2013)

Conclusion & Further Research

Apart from the limitations that we faced to conduct this study, good preliminary results were obtained and all taxa were recorded for the first time on these islands. The epilithic diatom assemblages changed in a response to environmental parameters. Most species were alkaliphilous with moderate to strong tolerance to pollution t produced from intensive human activities; such as agriculture, industries, and sewage direct discharge. For forensic applications, the presence of certain taxa, such as *Fallacia pygmeae*, may help to obtain the information of crime location. More research should be carried out on both coastal and non-marine diatom assemblages for professional environmental assesment and to prepare a chick list and a spatial map for forensic usage. One should also use better optical micrscopes with higher magnification power. Scanning and Transmission electron microscopes should be used for correct identification of taxa, due to the dominance of very small taxa of *Nitzschia* spp., *Luticola* spp. and *Navicula* spp.

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