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Research paper

Evaluating features of periphytic diatom communities as biomonitoring tools in fresh, brackish and marine waters

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ABSTRACT

The aims of this study were to assess the biodiversity of periphytic diatom assemblages in fresh, brackish and marine waterbodies of Korea, and to assess the effect of environmental and anthropogenic factors on parameters such as the quantity and biovolume of lipid bodies and deformations of diatoms as early warning measures of anthropogenic impact. Diatom samples were collected from 31 sites (14 freshwater, 10 brackish and 7 marine), which included less impacted (upstream) and impacted (downstream) sites in each water type. Our results showed higher abundance and biodiversity of periphytic diatoms at the less impacted sites in terms of species richness, Shannon index, cell count and biovolume of the communities than at the impacted sites for freshwater and estuarine sites, but not for marine sites. 84 diatom species were noted in freshwater, 80 in brackish water and 40 in marine waters. In comparison to diatoms of the impacted sites, those of less impacted freshwater, brackish and marine sites had less lipid bodies (also less biovolume) and a lower percentage of teratological frustules, and showed more mobile forms in the community. Principal component analysis (PCA) also showed clear segregation of impacted from less impacted sites by the extent of the presence of lipid bodies (higher both in number and biovolume) and deformities in diatom frustules. Pearson correlation analysis revealed that lipid body induction and deformities were positively correlated with metals (Cd, Co, Cr, Cu, Fe, Pb and Zn) and nutrients (total phosphorus and total nitrogen), whereas they showed negative correlation with salinity, dissolved oxygen, suspended solutes and pH. Life-forms, lipid bodies and deformities in diatoms may be an effective biomonitoring tool for assessing biological effects of pollutants in non-marine aquatic ecosystems in Korea.

1. Introduction

Potentially dangerous substances are being introduced into aquatic environments as a consequence of industrial and human activities. Without proper risk assessment of chemicals and subsequent efforts to formulate effective (enforced) protective legislation, our aquatic ecosystems will be endangered by the thousands of chemicals derived annually from industrial and municipal sources (Mallick and Rai, 2002).

Traditionally, analytical chemistry has been used to provide quantitative information on the contaminants and to determine if the status of water samples is within the range allowed by regulatory standards but this analysis cannot be used *in situ* and needs costly and sophisticated instruments, such as, atomic absorption spectroscopy (AAS), inductively coupled plasma (ICP; ICP-OES) and mass spectroscopy (MS; GC–MS and LC–MS). Furthermore, it is not possible to determine cause and effect relationships between inhabiting organisms and the causative agent using chemical methods alone (Wolska et al., 2007).

In contrast, organisms provide site-specific, integrated responses to exposure to the environmental variables, including chemicals. This is a key reason for introducing biomonitoring methods as part of a comprehensive approach to risk assessment of environmental pollution. Biomonitoring is widely employed to assess environmental threats posed by different classes of chemicals, in part because biomonitoring reduces the time and cost associated with blind chemical screening for a

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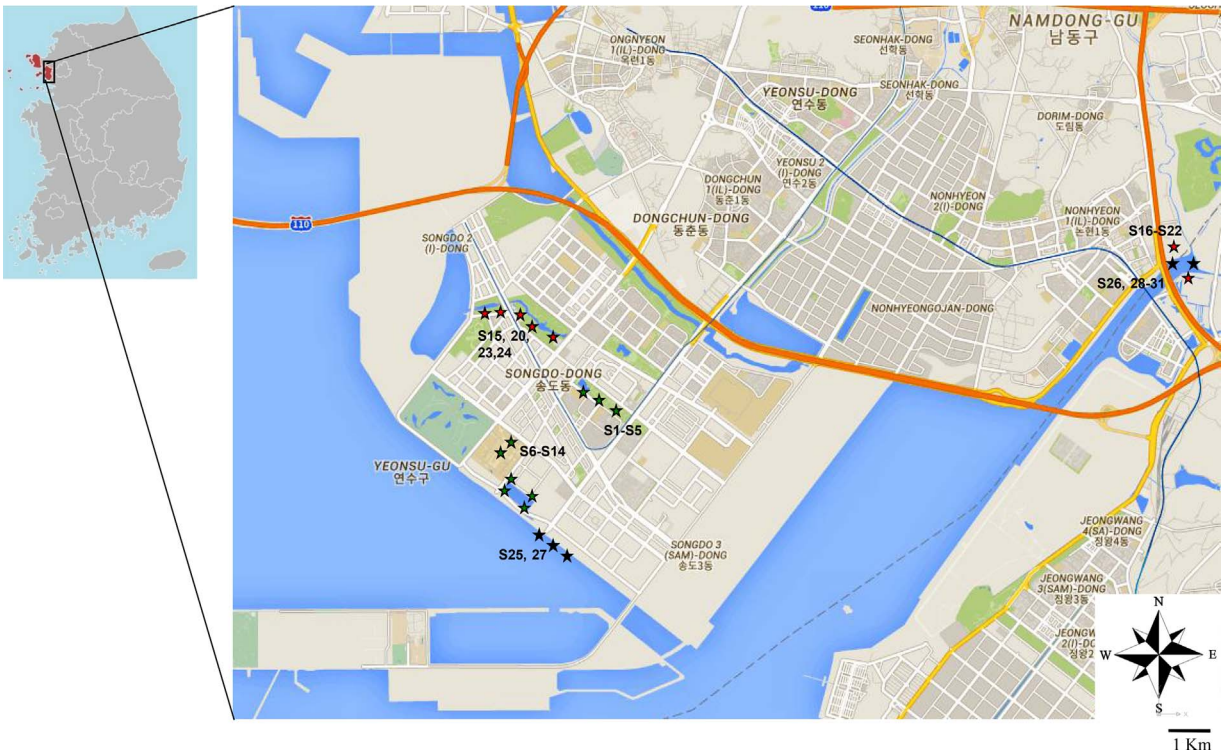

Fig. 1. Map showing the surveyed sites in Incheon, South Korea. Green, red and black colored star symbols showed fresh, brackish and marine sites. For complete information about sites see Table S1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

wide array of contaminants (Stevenson et al., 2010).

Various organisms have been used for biomonitoring practices. These organisms include protozoa, bacteria, fishes, aquatic macrophytes, algae, macroinvertebrates and zooplankton (Bettinetti et al., 2012). Algae, and diatoms in particular, have received global attention since diatoms are important primary producers of aquatic systems, playing major roles in food webs and in biogeochemical cycles, and constitute one of the most species-rich group of biological communities, making an important contribution to biodiversity and genetic resources of fluvial ecosystems (McCormick and Cairns, 1994; Stevenson et al., 2010). They inhabit almost all aquatic habitats including lakes, wetlands, oceans, estuaries, and even some ephemeral aquatic habitats. The key advantage of diatoms for use in ecotoxicology is that it is possible to examine the effects of toxicants at different levels of ecological organization, i.e., from individual cell to community levels (Debenest et al., 2013). In addition, the ease of sampling (simple scrapping of substrates), storing, observation of live features (photosynthetic apparatus and lipid bodies) and identification based on acid-cleaned frustules (mounted on permanent slides, which form a space-saving long-term record) are all features that make diatom bioassays more cost-effective than bioassays with other routinely used organisms (Pandey and Bergey, 2016; Pandey et al., 2018).

Toxicity research with diatoms has found that diatoms are more sensitive to heavy metal stress than other aquatic biota, responding characteristically through community shifts, i.e., replacement of sensitive species with more tolerant ones. For example, Hirst et al. (2002) reported that diatoms were more sensitive than macroinvertebrates for metal pollution, as indicated by a shift in diatom assemblage composition in upland streams of Wales and Cornwall. Similarly, De Jonge et al. (2008) concluded that the diatom community better explained the metal gradient (through analysis of species composition in the community) than macroinvertebrate communities in a Belgian river, the Dommel. Diatoms are also known for both rapid and chronic responses to heavy metal exposure. For example, Corcoll et al. (2012) used a translocation experiment to show that early (after 6 h and 24 h) metal toxicity was evident using chl a-fluorescence in combination with analysis of photosynthetic pigments, while chronic (3–5 weeks) toxicity of metals was better detected using cell biovolume or frustule deformity. Similarly, various researchers have reported deformities and size reduction in diatom frustules as phenomena associated with heavy metal stress (Falasco et al., 2009a; Luís et al., 2011; Pandey et al., 2014; Cantonati et al., 2014). Diatoms have short generation times and, therefore, reproduce and respond rapidly to environmental change, thus providing an early indication of biologically active heavy metal pollution.

Many environmental studies have been conducted using diatoms from separate entities of fresh, brackish and marine systems, but there is a lack of comparative studies of these three different types of waters. Furthermore, many live diatom features, such as life-forms, lipid bodies (LBs) and deformities (in live cells) have been little explored for biomonitoring purposes despite the fact that incorporation of these new live-diatom endpoints, combined with traditionally used metrics may increase the efficiency and reliability of diatom bioassays as a biomonitoring tool (Pandey and Bergey, 2016; Pandey et al., 2017).

The present study was undertaken to: (a) explore periphytic algal biodiversity from fresh, brackish and marine waterbodies of Incheon city, South Korea. (b) investigate the status of life-forms, lipid bodies and deformities in periphytic diatoms from the less impacted (upstream) and the impacted (downstream) sites and (c) recognize and evaluate the possibility to relate environmental or anthropogenic factors to these features of diatom assemblages.

2. Materials and methods

2.1. Studied area and sampling sites

The study areas were in the northwest portion of the city of Incheon, South Korea (Fig.1). The precise geographical location of sampling sites is 126°37′E, 37°28′N where fresh, brackish and marine waters adjoin (Table S1). The sampling periods were from October to December 2014.

2.2. Physicochemical parameters

Approximately 31 sites (6 samples from each site) were surveyed for water quality (Table S2). Water samples were collected in one liter plastic vessels. Variables, such as conductivity, pH and salinity, were measured in the field with a Milwaukee stainless steel probe (CD 601; MM Milwaukee meters, USA), Hanna pHep® tester (HI 98107; Hanna instruments, USA) and Atago hand refractometer (Model: JP/ATC-S/Mill-E, Atago®, Japan), respectively. Other variables, such as biological oxygen demand (BOD), total nitrogen (TN), ammonia nitrogen (NH_3-N) (ammonia electrode model 95-12, Thermo Electron Inc., USA), nitrate nitrogen (NO_3-N), silicon (Si), total phosphorus (TP), and phosphate-phosphorus (PO_4-P) were measured using the standard methods in the laboratory (APHA, 1998). Five-ml water samples from each site were used for estimating heavy metal concentration in the water fraction (Varian inductively coupled plasma-optical emission spectrophotometer [ICP-OES], Varian PRO, CA, USA). To assess dissolved organic carbon (DOC), water samples were filtered (Nylon Membrane Filters 0.2 µm, Whatman, Maidstone, UK) and analyzed with total organic carbon analyzer (TOC-500A; Shimadzu). Water samples for hardness were analyzed by titration following standard methods (APHA 1998). Suspended solids (SS) were measured by filtering 200–500 ml of water with 0.45 µm GF/C filter, light transmission by C Star transmissometer (Wet Labs, Inc). Flow rate (FR) of river was measured by measuring the distance travelled by a styrofoam float in the river water in a time period of 5 min (Pandey et al., 2014).

2.3. Periphyton sampling and community analysis

Algal samples were collected in 50 ml centrifuge tubes by scraping a fixed area (80 cm^2) of a colonized substrate (stone/cobble) using a blade, scalpel, dropper and brush. Each 40 ml sample (in 50 ml tubes) was divided into four equal parts of 10 ml, which were used for:

(a) Community analyses with 5 ml preserved in formalin (4%) or Lugol's solution for cell density and biovolume estimations while another half was used for identification of different members of algae in the community. Periphytic diatoms were microscopically investigated in the fresh (living) state as well as permanent slide preparations at 40× and 100x magnification (Nikon 450, company and country).

(b) Examining lipid bodies (LBs) in living periphytic diatoms. Lipid bodies (LBs) were investigated in live diatom cells following the protocols given by Pandey et al. (2015). The size and number of LBs in individual species were measured using a camera and computer attached to a calibrated microscope (Nikon 450), using 400 and 1000 x magnifications. Biovolume of individual LBs was calculated by considering that the LBs were more or less spherical and thus the mathematical formula for a sphere could be applied, $V = 4/3\ \pi r^3$, where V is the volume of an LB and 'r' is the measured radius of the LB. The biovolume of individual diatom species was calculated following geometrical formulae given by Hillebrand et al. (1999). The percent biovolume of all LBs inside individual diatom cells were calculated by summing the volume of each LB (total contribution of all lipid bodies) divided by the cell biovolume.

(c) Determining the proportion of different life–forms of periphytic diatoms. Periphytic diatoms can be classified into 9 life forms, following Rimet and Bouchez (2011). In the present study, diatoms sampled from the less impacted and impacted sites were classified into 6 of these life-forms (benthic, planktonic, mobile, colonial, tube-dwellers and pioneer).

(d) Qualitative and quantitative assessment of deformities in diatom frustules. Deformities of the frustule (DF's) in diatoms were determined using permanent slide preparations of diatoms following the protocols given by Biggs and Kilroy (2000). Diatom samples were treated with concentrated HNO_3 and H_2O_2 (30%) and left for 24 h in a fume hood. Acid-treated samples were washed with double distilled water at least 10 times to remove all acid residues. Treated samples were diluted as needed to produce a usable (not overcrowded) diatom frustule density, and a subsample was spread on cover slips and allowed to air dry. Dried samples were mounted on the glass slides using Pleurax mounting media (RI = 1.73). During the mounting process, slides were heated on hot plate to remove the alcohol component of the Pleurax and to permanently fix the slides. DF's in diatom frustules were categorized into four types: (1) deformities in the valve outline (D_1), (2) deformities in the striae (D_2), (3) deformities in the raphe (D_3) and (4) mixed deformities (D4) (more than one type of deformity in a single frustule) (Falasco et al., 2009a; Pandey et al., 2014, 2015).

2.4. Statistical analyses

Data were statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD test for comparing various means. PCA (Principal component analysis) and Pearson's correlation analysis were carried out by XLSTAT software (Microsoft corporation, USA). Shannon index analysis was carried out with the help of software "PAST" (Natural History Museum, University of Oslo, Norway).

3. Results

Investigated waters showed clear differences in the seven examined physico-chemical characteristics (Table S3). Differences were also apparent between the less impacted (upstream) and the impacted (downstream) sites. Approximately 90 periphytic diatom species were identified in the collected samples from the three different types of waters (Table S4).

Periphytic algal diversity was investigated using species richness and the Shannon index (Fig. 2). Among the less impacted sites, species richness did not differ between fresh and brackish waters but it was significantly lower in the marine system. In contrast to less impacted sites, species richness for the impacted sites did not differ among habitats. As compared with less impacted sites, impacted sites had significantly ($p < 0.05$) lower species richness for fresh and brackish waters but there was no significant difference in richness ($p < 0.05$) between less impacted and the impacted sites in the marine system.

The Shannon index showed a similar pattern to that of species richness. At the less impacted sites, the Shannon index had higher

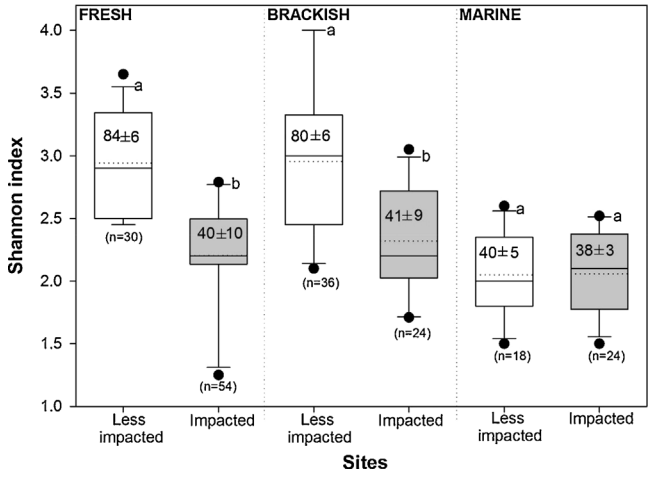

Fig. 2. Boxplot showing Shannon index and species richness (numerals within the bars) from the different investigated waterbodies. Numeral within bracket shows the number of samples investigated. The horizontal lines across the boxes correspond to the 5% quartile, mean (dashed line), median (solid line) and 95% quartile values. The whiskers outside the box show minimum and maximum values or outliers. Bars bearing different letters are significantly different from each other ($p < 0.05$; Tukey's HSD test).

values in freshwater and brackish waters than in the marine system. Comparison of the Shannon index between the less impacted and the impacted sites showed that the index was significantly ($p < 0.05$) lower in impacted freshwater and brackish water sites than in their respective less impacted sites, whereas there was no significant difference between impacted and less impacted sites in the marine system ($p < 0.05$).

When the percent relative abundance (< 1%) of diatom species in different water systems was calculated there was a difference in species depending on the tested aquatic system. The dominance ranking of the species was *Amphora pediculus* (Kutzing Grunow) (20%) > *Nitzschia palea* (Kutzing Grunow in Cleve and Grunow) (16%) > *Luticola muticopsis* (Van Heurck DG Mann) (12%) > *Achnanthes breviceps* (Agardh) (10%) for freshwater, *Entomoneis costata* (Hustedt Reimer) (13%) > *Entomoneis ornate* (Bailey Reimer) (12%) > *Tryblionella apiculata* (W. Gregory) (9%) > *Nitzschia clausii* (Hantzsch) (8%) for brackish water and *Rhizosolenia setigera* (Brightwell) (20%) > *Bacillaria paradoxa* (Gmelin) (13%) > *Nitzschia fasiculata* (Grunow) Grunow (11%) > *Licmophora communis* (Heiberg) Grunow (10%) for marine water.

Cell density of diatoms was higher in the fresh and brackish sites than in the marine sites (Fig. 3a). On the other hand, community biovolume was higher in the marine and brackish sites than in freshwater sites (Fig. 3b). In addition, both cell density and biovolume of the diatom community were significantly lower in the impacted than in the less impacted sites for fresh and brackish specimens, but not for marine samples.

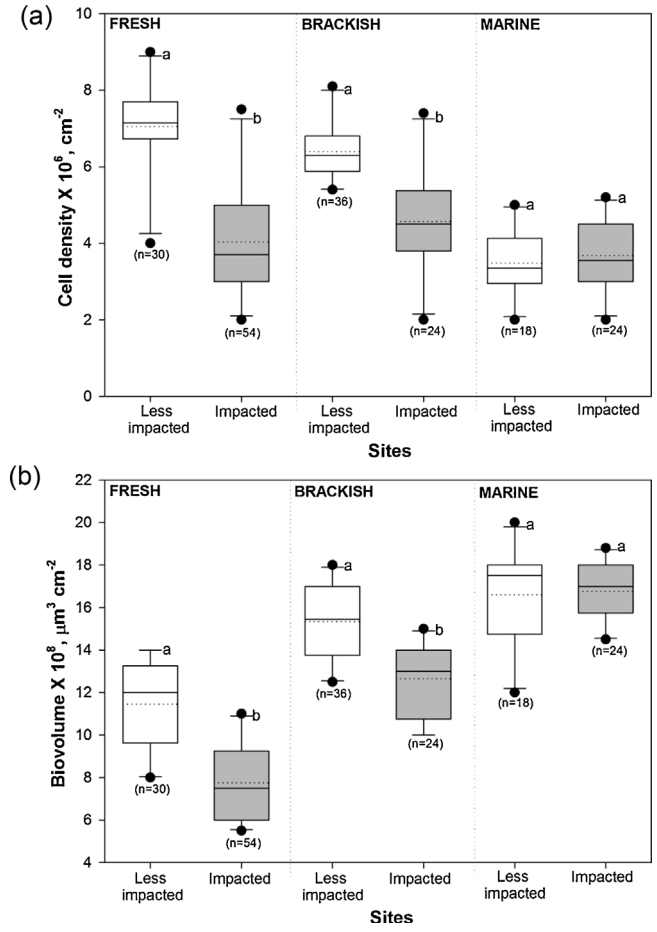

Fig. 3. Box plot showing, (a) Cell density and (b) Biovolume of periphytic diatom community from the less impacted and impacted sites of freshwater, brackish water and marine aquatic sites. The horizontal lines across the boxes correspond to the 5% quartile, mean (dashed line), median (solid line) and 95% quartile values. The whiskers outside the box show minimum and maximum values. Bars bearing different letters are significantly different from each other ($p < 0.05$; Tukey's HSD test). Numerals within brackets (n) represent the number of algal samples examined.

When the life forms of periphytic diatom communities wer compared benthic (25%) and mobile (37%) forms were prevalent in the less impacted freshwater sites while mobile (60%) forms dominated the impacted sites. In brackish water, mobile forms dominated both the less impacted (30%) and impacted (50%) sites. In comparison to the less impacted, impacted sites had an appreciable reduction in colonial (from 8 to 0% in freshwater; 12–4% in brackish water) and tube-dweller (from 15 to 4% in freshwater; 18–10% in brackish water) forms. No appreciable difference in periphytic diatom life-form diatoms were observed in the less impacted versus impacted sites of marine waters.

Lipid bodies (LBs) number and their biovolume contribution was examined in the common species from the less impacted and impacted sites of the waterbodies (Figs. 4 and 5). 9 diatom species from the freshwaters and 7 diatom species from brackish and marine waters were investigated (Tables 1a and 1b). Individual species were more numerous and had a higher percent biovolume of lipid bodies (LBs) in impacted sites than in the less impacted sites of fresh, brackish and marine waters. At all the impacted sites, inductions of LBs were more frequent in raphid diatoms than araphid ones. Furthermore, at the impacted sites larger diatoms (*Nitzschia sigmoidea* Nitzsch (W. Smith), *N. linearis* (W. Smith) and *Tryblionella apiculata* in freshwater, and *Bacillaria paradoxa* and *Tryblionella apiculata* in brackish and marine systems) had more LBs number (7–15) than smaller ones (between 2 and 5).

Morphological abnormalities were investigated in the live and acid-treated periphytic diatom frustules from less impacted and impacted sites (Figs. 6 and 7). Deformities in diatom frustules were significantly more common in impacted fresh and brackish sites than in their respective less impacted sites; this difference was not found between less impacted and impacted marine sites (Fig. 8). In the less impacted samples, three types of deformities were found; i.e., deformities in cell outline (D1), aberrations in striae pattern (D2) and mixed deformities (D4). On the other hand, impacted sites included four types of deformities in the diatom frustules (D1–D4). Deformities in raphe (D3) were exclusively observed at the impacted sites.

In Fig. 9, Axis F1 explains 65.32% of the variation while Axis F2 explains 11.74% of the variations in the data. The plot clearly segregates the less impacted and impacted sites in the right and left halves, respectively. Community parameters, such as deformities (DFs) and lipid bodies (LBs) were found closely associated with the impacted sites. LBs and DFs showed positive and statistically strong association with the heavy metals (except Ni) and nutrients (TP, nitrate and ammonia) while other environmental variables (pH, salinity SS, and DO) showed significant negative associations with DFs and LBs. In addition, the relationship between LBs and DFs was positive and statistically significant ($r^2 > 0.95$; $p = 0.05$) (Table S5).

4. Discussion

Investigated fresh, brackish and marine sites showed remarkable differences in the seven examined physico-chemical variables; differences that are consistent with salinity and nutrient differences expected in these systems (e.g., in Korea: Hwang et al., 2011). However, differences between the physico-chemical characteristics of the less impacted and impacted sites within the fresh and brackish water systems were also apparent; differences were not apparent in the less impacted and impacted marine sites.

Available literature shows that the diatom diversity index decreases with pollution (Luís et al., 2011) as was also found in the present study while others found that diversity can be either increased (Gold et al., 2003) or unaffected (Gold et al., 2002) with pollution, or that there may be differential changes in diversity depending on the type of pollution (Stevenson et al., 2010). This varying and contrasting response of diversity indices is mainly due to trade-off between various community

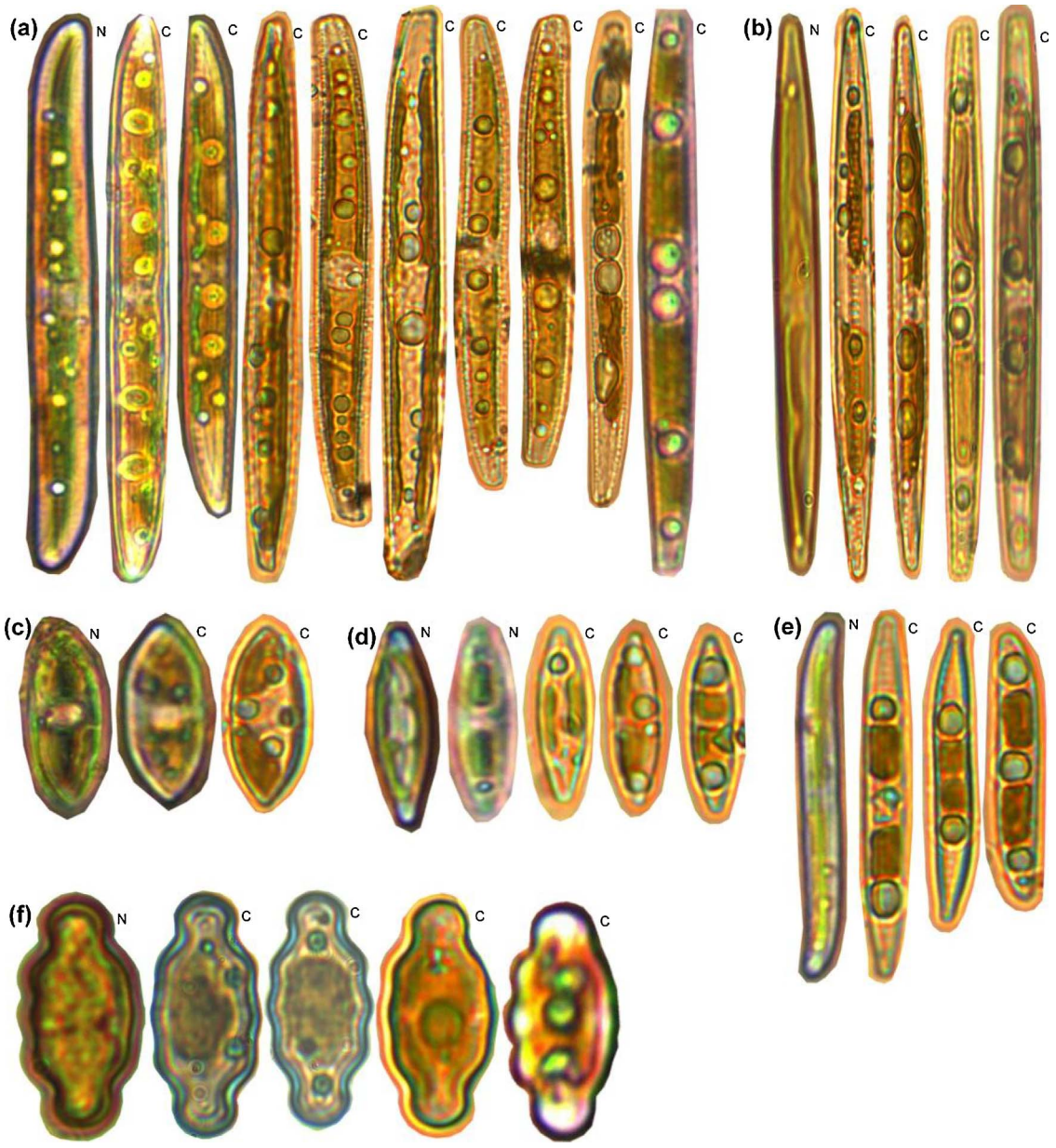

Fig. 4. Status of lipid bodies (LBs) in six live diatom frustules (a*) Nitzschia sigmodea*, (b) *Nitzschia linearis*, (c) *Tryblionella apiculata*, (d) *Navicula canalis*, (e) *Nitzschia filiformis* and (f) *Luticola muticopsis* from freshwater bodies. Subscripts 'N' show diatom frustules of specimens from less impacted sites, while subscripts 'C' show frustules of specimens of the same diatom species from impacted sites.

parameters, such as cell division rate, cell density, and species composition. By contrast, no difference was examined in the diversity indices in the impacted and less impacted sites of the marine waters. Diatoms in marine systems are more tolerant against osmotic stress (as they live in highly saline conditions i.e., salinity greater than 30 ppt) than freshwater (salinity less than 0.5 ppt) diatom species (Alverson et al., 2007). This natural tolerance for salinity may also assist marine diatoms to resist the osmotic imbalance caused by the intracellular accumulation of contaminants (particularly heavy metals in the present study) in the diatom cell. In addition, marine systems are vast and regularly diluted with tides, which neutralize the effects of contaminants.

In the present study species composition changed with changes in salinity. In the past, Admiraal and Peletier (1980) found that reduced salinity (up to 1‰) associated with inflowing freshwaters affected diatom communities in an estuarine mud flat, but that increasing salinity (20‰) did not affect diatom species composition. Similarly, Potter et al. (2006) reported changes in diatom genera along salinity gradients in the inland waters of Oklahoma, USA (Potter et al., 2006). Thus, the present study is in good agreement that salinity gradient has a strong effect on diatom community composition inhabiting different aquatic ecosystem.

The pattern of change (in physico-chemical parameters) examined in less impacted versus impacted environmental conditions was reflected in diatom density for freshwater and brackish systems, but not for the marine system. Lower diatom density was observed in the freshwater impacted sites than in the less impacted sites, a pattern well documented in freshwater (Gold et al., 2002, 2003), but which has not been reported for brackish or marine waters, as these systems are huge, not easily available and have fluctuating water conditions (due to regular tides) leading to high cost of research.

Cell densities and biovolumes of the diatom communities are regularly used for bioassessment of streams (Lavoie et al., 2006). Lower cell density of the diatom community was reported under stress

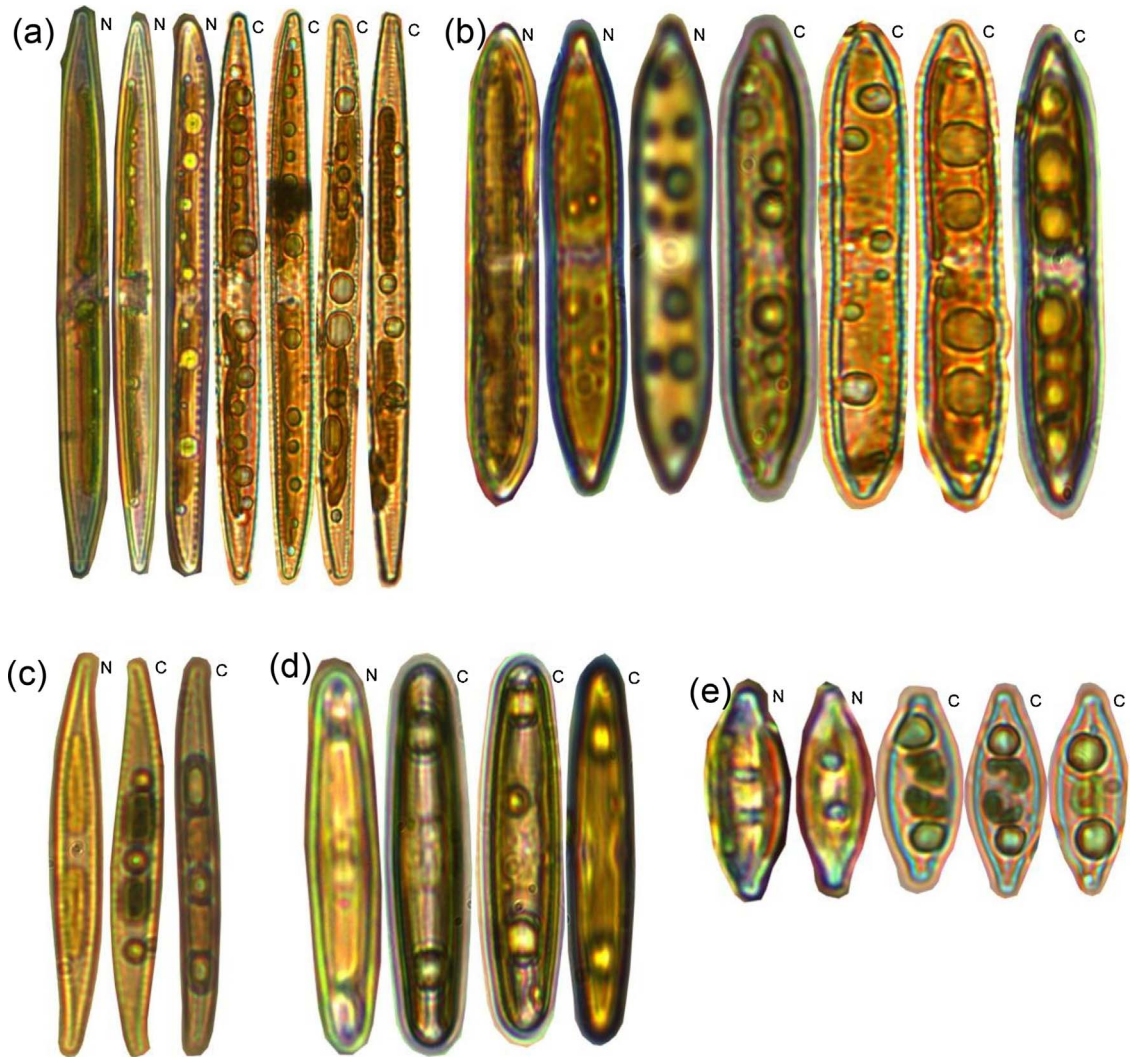

Fig. 5. Status of lipid bodies (LBs) in five live diatom frustules (a) *Bacillaria paradoxa*, (b) *Trybllionella apiculata*, (c) *Nitzschia exilis*, (d) *Pinnularia borealis* and (e) *Navicula gregaria* from brackish and marine waterbodies. Subscripts 'N' show diatom frustules of specimens from less impacted sites, while subscripts 'C' show frustules of specimens of the same diatom species from impacted sites.

Table 1a
Percentage biovolume contribution of lipid bodies (mean ± SE) to total cell volume in different diatom species found in the freshwaterbodies.

Diatom species	Percent share of lipid bodies per cell (number of oil globules)	
	Less impacted	Impacted
Amphora ovalis	8 ± 4 (2)	20 ± 5 (2)
Luticola muticopsis	5 ± 3 (1)	15 ± 6 (2)
Navicula gregaria	11 ± 1 (2)	28 ± 7 (2)
Navicula cryptocephala	7 ± 4 (2)	15 ± 4 (2)
Nitzschia sigmoidea	5 ± 3 (5)	12 ± 8 (10)
Nitzschia linearis	6 ± 3 (4)	14 ± 5 (8)
Nitzschia palea	9 ± 2 (2)	25 ± 7 (5)
Tryblionella apiculata	7 ± 4 (2)	20 ± 7 (7)
Pinnularia parvulissima	6 ± 3 (2)	12 ± 5 (2)

Table 1b
Percentage biovolume contribution of lipid bodies (mean ± SE) to total cell volume in the brackish and marine waterbodies.

Diatom species	Percent share of lipid bodies per cell (number of oil globules)	
	Less impacted	Impacted
Bacillaria paradoxa	10 ± 3 (2)	25 ± 9 (15)
Navicula gregaria	8 ± 4 (2)	17 ± 6 (2)
Navicula cryptocephala	9 ± 3 (2)	20 ± 8 (2)
Nitzschia sociabilis	8 ± 4 (2)	20 ± 7 (3)
Nitzschia palea	8 ± 4 (2)	20 ± 7 (5)
Pinnularia borealis	12 ± 3 (2)	25 ± 8 (2)
Tryblionella apiculata	10 ± 3 (3)	25 ± 8 (7)

conditions, which was also found to be in good agreement with the present study. Gold et al. (2002) reported lower diatom cell density in chronically polluted (Cd, 6.5–39.3 ppb and Zn, 395–1510 ppb) sites (Decazeville) than in the reference sites (Lot River, Cd and Zn below detection limit) of Lot and Riou-Mort Rivers of France. Under stress, lower cell density also resulted in lower biovolume of the diatom community, which was mainly attributed to the size reduction in the diatom species of the community (Arini et al., 2012). Size reduction in diatom frustules is a naturally occurring phenomenon (Laney et al., 2012) but under heavy metal stress it is more prevalent (Morin et al., 2012). For example, Barral-Fraga et al. (2016) reported a strong reduction in cell biovolume of the most abundant and other diatom species of the biofilm under short term Arsenic exposure. Similarly, Pandey and Bergey (2016) also reported a significant size reduction in the periphytic diatoms collected from sites severely impacted with Cu and Zn. Reduction of diatom community growth due to long-term effects of contaminants (especially heavy metals) may be the reason for

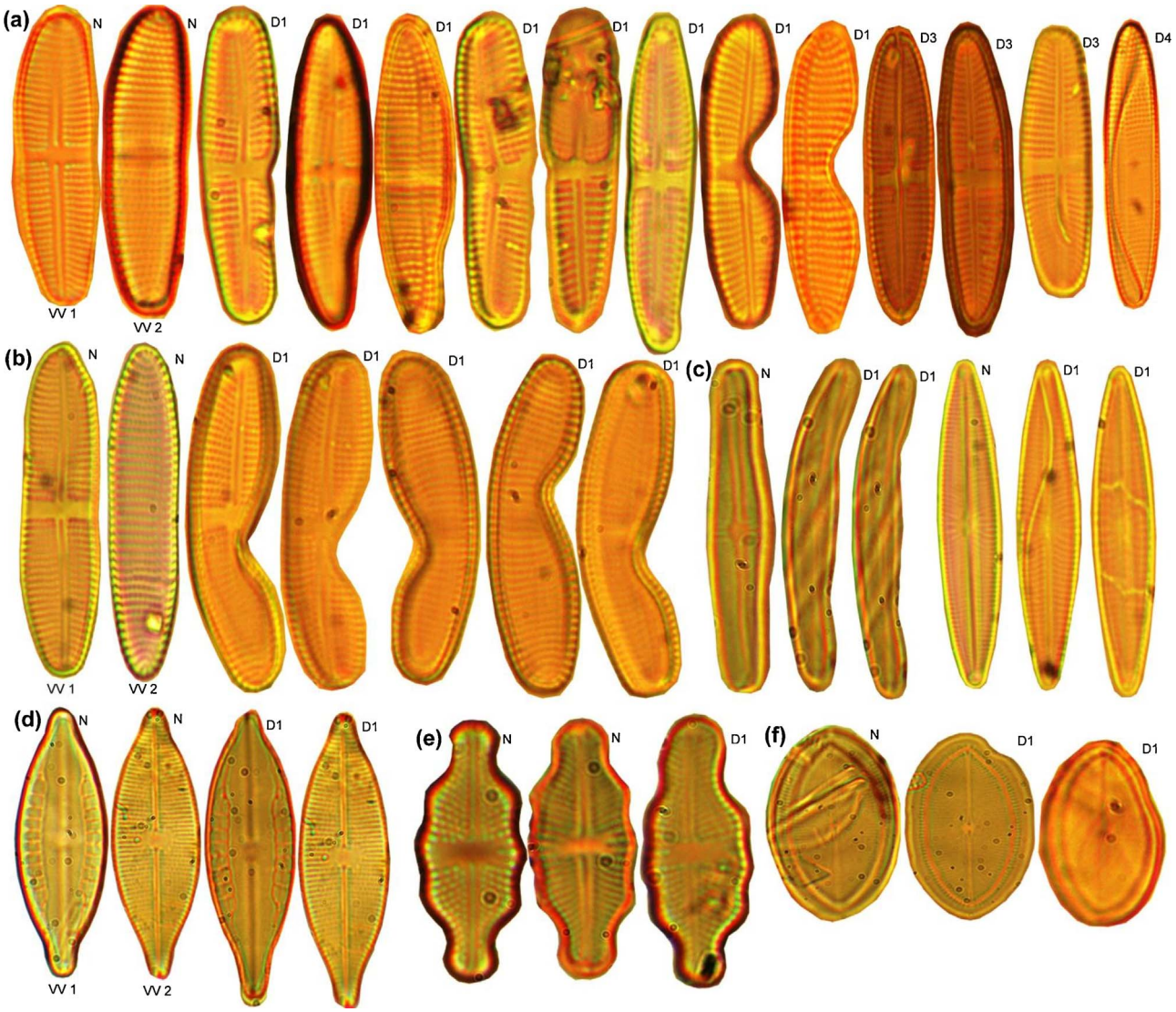

Fig. 6. Normal (N) and Deformed frustules (D1–D4) in six diatom species (a) *Achnanthidium breviceps*, (b) *Achnanthidium minutissimum*, (c) *Navicula recens*, (d) *Mastogloia smithii*, (e) *Luticola muticopsis* and (f) *Cocconeis placentula* examined in the less impacted and impacted specimens of freshwater bodies. Symbol 'VV' denotes the valve view of the diatom frustules. See section 'Materials and methods' for details.

the lower cell density (Gold et al., 2002). On the other hand, higher cell division rate in diatoms under metal-enriched conditions was reported to be the reason for lower community biovolume (Morin et al., 2012).

Seven types of life-forms (benthic, planktonic, mobile, colonial, mucous tubule, pedunculate and pioneer) are reported in the natural diatom metrics (Rimet and Bouchez, 2011). But under nutrient and organic contamination a set of particular life forms dominates in the diatom assemblage (Passy, 2007a; Rimet and Bouchez, 2011). In the present study, colonial and tube-dwelling life-forms were reduced at impacted sites, indicating greater chemical sensitivity in these groups. Rumeau and Coste (1988) also found that tube-dwelling and colonial life-forms of diatoms were pollution-sensitive. According to Passy (2007a,b), the taxa having high proportions of these life-forms are not adapted to resist water turbulence or grazing pressure, but have good ability to use dissolved nutrients in water. This likely means that they are more exposed to dissolved contaminants (heavy metals in the present case) than other life-forms, which could explain their reduced abundance in impacted sites of the examined waterbodies. In contrast, Rimet and Bouchez (2012) found that a tube-dwelling life-form was an adaptive feature for diatoms as tubes reduced pesticide contamination in a microcosm study. Larras et al. (2013) also reported that biofilm matrix controls the exposure to herbicides (atrazine, terbutryn, irgarol, diuron, isoproturon and metolachlor), and consequently their toxicity towards benthic diatoms. However, the seasons influenced both the structure and the composition of the diatom communities and consequently their response to the various contaminants, which differs significantly in different seasons (Larras et al., 2014).

The transparency of the silica frustules allows visualization of internal structures in living diatoms, such that cytoplasmic changes inside a cell can be documented. In the present study, LBs were more apparent in the impacted than the less impacted sites of the investigated waters. The impacted sites were higher in nutrients and heavy metal contaminants. Nutrient deprivation is a known factor for LB induction (Ramachandra et al., 2009). Recently, Pandey et al. (2015) reported higher LB induction in a lab-established diatom-dominated phytoplankton community under Cu and Zn stress. In the same context, other classes of algae may have increased lipid content under heavy metal and nutrient stress. For example, under Cu and Zn stress, Einicker-Lamas et al. (2002) found higher lipid content in the euglenoid alga *Euglena gracilis* and Liu et al. (2008) reported 3–7 times higher lipid content in the green alga *Chlorella vulgaris* under Fe stress, with lipid contentment increasing with metal concentration. Pillai et al. (2014) found a similar concentration-dependent formation of lipid bodies in the green algae *Chlamydomonas reinhardtii* under Ag stress in a

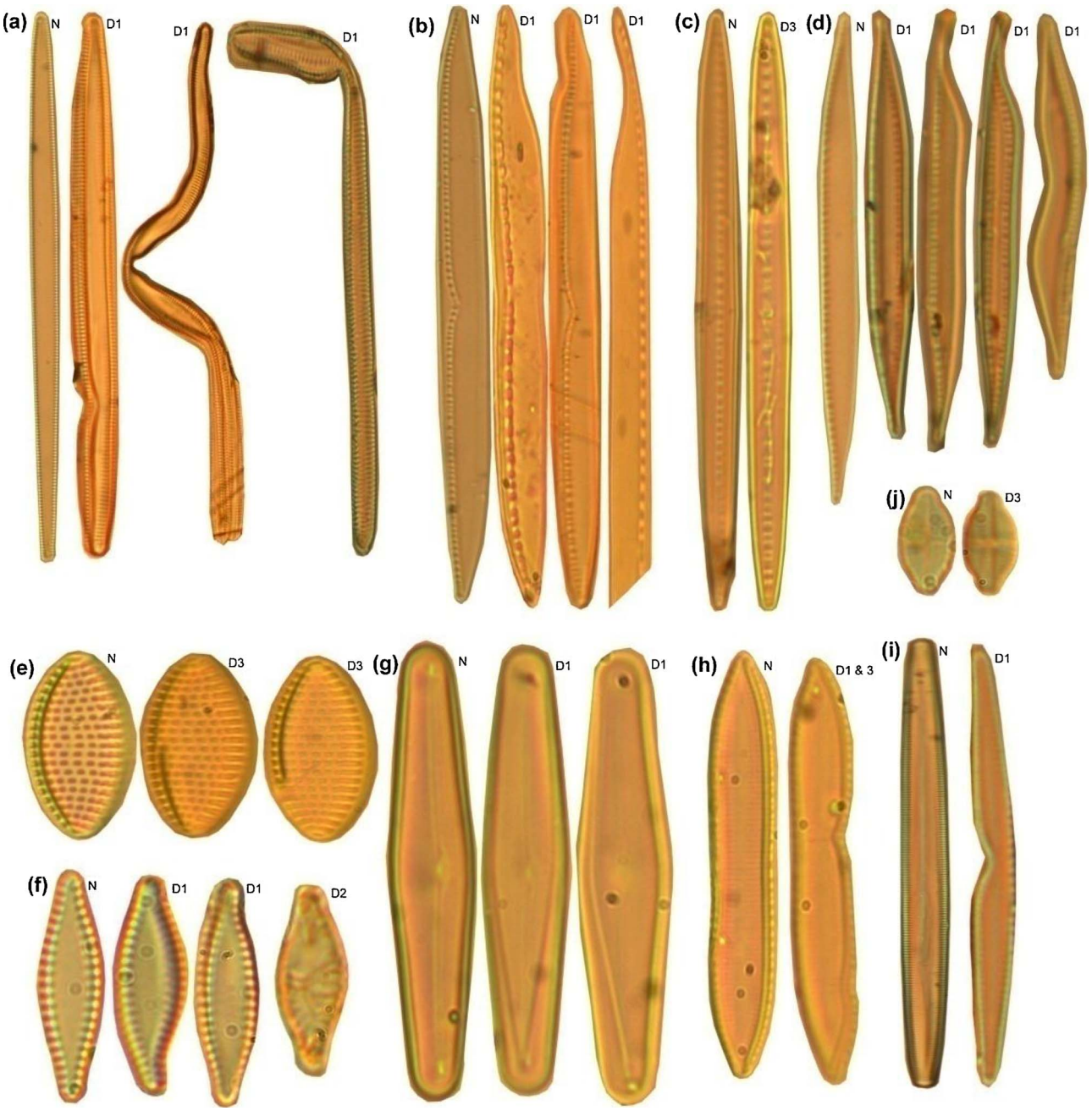

Fig. 7. Normal (N) and Deformed frustules (D1–D4) in ten diatom species (a) *Synedra ulna*, (b) *Nitzschia* sp., (c) *Bacillaria paradoxa*, (d) *Nitzschia exilis*, (e) *Nitzschia compress* and (f) *Staurosira construens*, (g) *Pinnularia borealis*, (h) *Trybllionela apiculata*, (h) *Fragilaria capucina* and (i) *Fragilaria capucina* examined in the less impacted and impacted specimens of brackish and marine waterbodies.

laboratory experiment. Nutrient stress can also induce LBs in non-diatom algae. For example, the symbiotic dinoflagellate *Symbiodinium* spp. (a zooxanthellae group) shows LB induction under nitrogen deprivation (Weng et al., 2014). On the other hand, Jones et al. (1987) found lower fatty acid and sterol content in the marine diatom *Asterionella glacialis* under organic Hg and Cd stress. The occurrence of lipid bodies in diatoms is a natural phenomenon but more frequent and prominent (larger) under different types of environmental and anthropogenic alterations (Ramachandra et al., 2009; Levitan et al., 2014). Under stress, lipid bodies in diatoms serve as a reserve food material (as energy supplement), while also providing buoyancy to the cell (Ramachandra et al., 2009; Smetacek, 2001), which can aid in their movements (Wang et al., 2013). These movements help diatoms get proper light and nutrients, and disperse away from the stress.

In addition to altered lipid bodies, morphological deformities were also apparent in diatoms at both the impacted and less impacted sites of the freshwater ecosystem. In our study, morphological anomalies were less frequent at the less impacted sites and a similarly low background frequency of abnormalities has been previously reported (Dickman, 1998; Falasco et al., 2009a; Morin et al., 2012). On the other hand, impacted sites had more deformed frustules; a result in agreement with previous studies on periphytic diatom communities (Gold et al., 2002, 2003; Dziengo-Czaja et al., 2008; Falasco et al., 2009a; Morin et al., 2012). Morphological abnormalities in diatom frustules are associated with heavy metal contamination both in nature (Pandey et al., 2014; Cantonati et al., 2014) and in the laboratory (Falasco et al., 2009b; Pandey et al., 2015), and this ecotoxicity is irrespective of the concentration of organics (Morin et al., 2008; Luís et al., 2011; Arini et al.,

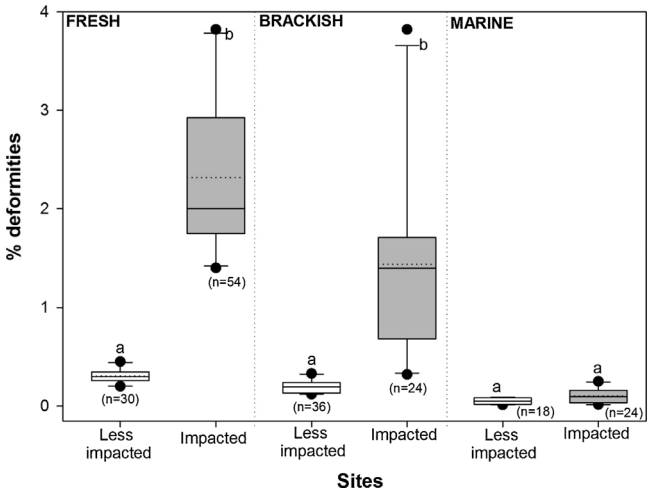

Fig. 8. Box plot showing percent deformities in diatom frustules from the less impacted and impacted freshwater, brackish water and marine sites. The horizontal lines across the boxes correspond to the 5% quartile, mean (dashed line), median (solid line) and 95% quartile values. The whiskers outside the box show minimum and maximum values. Bars bearing different letters are significantly different from each other ($p < 0.05$; Tukey's HSD test). Numerals within brackets (n) represent the number of algal samples examined.

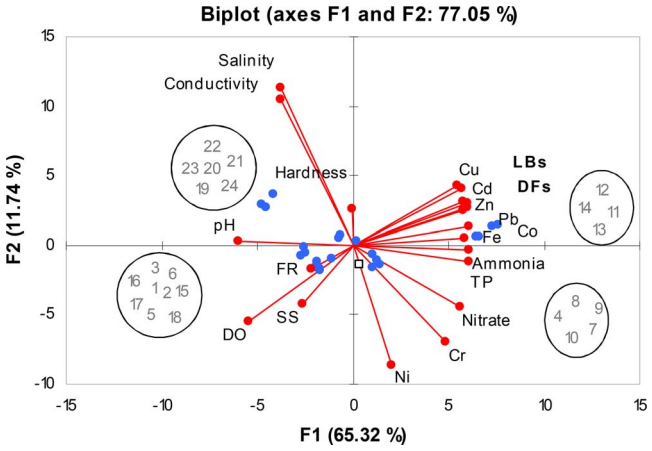

Fig. 9. Principal component analysis (PCA) based on physical and chemical parameters of freshwater, brackish water and marine sites. Blue bubbles in the figure show sites while red bubbles show various physico-chemical parameters of the three aquatic systems. DF- gives deformities in diatom frustules and LB- lipid bodies in terms of% biovolume contribution in the community. For physical and chemical parameters see Table S1–S3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2012; Lavoie et al., 2012; Cantonati et al., 2014). Thus, with regard to diatom frustule abnormalities, the ecotoxicity of heavy metals is apparently greater than the ecotoxicity of organic and xenobiotic toxicants in freshwaters (Nriagu and Pacyna, 1988).

Reports of deformities in brackish and marine systems are sporadic. Dickman (1998) reported significantly higher deformity frequency in the polluted harbor sites of Hong Kong. Specifically, he found that deformities were due to high concentration of heavy metals (above US EPA guidelines) in the sediments. Nutrient pollutants are also associated with deformities. Dziengo-Czaja et al. (2008) reported a higher percentage of deformed diatom cells (approximately 25%) from the contaminated marine sediments (organically loaded with nitrate and phosphate contamination) in Puck Bay in the southern Baltic Sea. In fresh and brackish water system, higher occurrence of deformities in diatom frustules from impacted sites than from less impacted sites is helpful in assessing the ecological health of waters. In contrast, deformities in diatoms under marine system need more efforts to define them as indicators of water quality.

In the present study, occurrence of deformities in the raphe (D3) showed a specific response of diatoms to impacted waters. Pandey et al. (2014) also reported dominance of D3 deformities under Cu treatment. Similarly, Pandey et al. (2015) and Pandey and Bergey (2016) reported dominance of D3 deformities in periphytic diatom communities exposed to Cu and Zn in the lab and in survey studies. However, available literature showed that in pennate diatoms, the first area to develop is the raphe-sternum system (Mayama and Kuriyama, 2002; Round et al., 1990), thus making raphe more susceptible to get deformed under stress. In the same context, Estes and Dute (1994) reported that raphe system is the first area to show abnormalities, while anomalies of the areola pattern and stria arrangement occur later and can be considered a secondary effect due to abnormal raphes. However, Falasco et al. (2009a) suggested that microtubules are associated with the developing raphe canal as well as with a microtuble centre; thus increased osmotic pressure leads to "microtubular poisoning" (Schmid, 1980) that may be another reason for deformities particularly in the raphe system of the diatoms.

The exact mechanisms of LB variation and frustule deformities in diatoms are still unknown. One hypothesis is that contaminants alter cell membrane polarity and cause cytoplasmic acidification, leading to disruption of cytoplasmic homeostasis (Pinto et al., 2003). In order to counter this cytoplasmic imbalance, diatom frustules start inducing the formation of LBs. In the same context, several researchers have found that under stress conditions, such as salinity, nutrient deficiency, silicon, temperature and high light stress diatoms accumulate high-energy molecules such as carotenoids and/or lipids (Ramachandra et al., 2009; Bertrand, 2010; Sharma et al., 2012; Cheng et al., 2014; Zhang et al., 2014).

Cytoplasmic imbalance in the diatom frustules also leads to microtubular changes that impact the movement of silica transporting vesicles (STV) to the silica depositing vesicles (SDV), which can result in deformities in diatom frustules. Thus, it seems that LB formation in periphytic diatoms may be the first counter response against contaminant stress, which if the stress is chronic, can result in morphological alterations or deformities. Thus, the present study showed the utility of periphytic diatom community parameters (life-forms, cell counts and biovolume of the community, Shannon index, species richness and% relative abundance) as tools for differentiating impacted sites from less impacted sites. In addition, community parameters such as LBs and deformities in periphytic diatoms showed tremendous potential to be used as a valuable warning system for assessing the ecological health of freshwaters ecosystems against heavy metal pollution.

5. Conclusions

The present study showed the effect of salinity gradient and anthropogenic disturbances (less impacted v/s impacted sites) on the periphytic diatom communities residing in the fresh, brackish and marine waters. Examined traditional diatom parameters (Shannon index, species richness, % relative abundance life-forms, cell density and biovolume of the community) was found helpful in differentiating impacted sites from less impacted sites. In addition, new community parameters such as LBs and deformities in periphytic diatoms showed higher sensitivity than traditional parameters to be used for assessing the level of anthropogenic disturbances of fresh and brackish waters ecosystems. Characteristics of diatoms in marine systems are apparently less sensitive to pollution in comparison to diatom characteristics in estuarine and freshwater systems, possibly because they are naturally endowed to tolerate osmotic stress caused due to extreme salinity (~35 ppt), which may also assist marine diatoms to counter the osmotic imbalance caused due to intracellular accumulation of contaminants (particularly heavy metals in the present study). In addition, the dispersal and chelation of pollutants by tides and currents may dilute impacts of pollutants not concentrated in the surface sediments to benthic diatoms in marine ecosystems. Further research on marine

biomonitoring using diatom characteristics is warranted.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aquatox.2017.11.003.

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