



1 **Holistic monitoring of increased pollutant loading and its impact on the environmental**
2 **condition of a coastal lagoon with *Ammonia* as a proxy for impact on biodiversity**

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23 Abstract

24 Eutrophication poses a serious threat to the ecological functioning of marginal marine habitats in
 25 the era of Anthropocene. Coastal lagoons are particularly vulnerable to nutrient enrichment and
 26 associated changes in environmental condition due to their limited marine connection and longer
 27 water residence time. Benthic organisms are more susceptible to the impacts of nutrient
 28 enrichment as organic carbon produced in water column production gets sequestered in the
 29 sediment compartment leading to increased bacterial degradation that may cause hypoxia. Apart
 30 from nutrient enrichment, addition of different heavy metals as Potential Toxic Elements (PTE)
 31 from industrial sources also impacts the biota. In the present study, the concentrations of
 32 different nutrients and PTEs have been measured from the water profile of the World's second
 33 largest coastal lagoon, Chilika. Alongside characterization of the sedimentary organic carbon
 34 was also carried out. The globally present coastal benthic foraminiferal genera *Ammonia* was
 35 also tested for its applicability as a biotic indicator of pollution in this habitat. The study was
 36 conducted for a period of twelve months. The investigation revealed that concentration of
 37 dissolved nitrate in the water column was extremely high along with increased values of
 38 sedimentary organic carbon deposit, both of which are characteristics of coastal eutrophication.
 39 Intermittent hypoxia within the pore space was also recorded. Characterization of stable isotopes
 40 from the sedimentary carbon revealed the origin of it to be autochthonous in nature, thus
 41 supporting the idea of nutrient driven increased primary production. Concentrations of PTEs
 42 were in most cases below bio-available values, however occasional high values were also
 43 observed. The number of specimens belonging to *Ammonia* spp. also appeared to be a potent
 44 biotic proxy of eutrophication as it displayed significant correlation with both nitrate and
 45 concentration of organic carbon.

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48 Key Words: Coastal eutrophication, lagoon, dissolved nitrate, dissolved phosphate, dissolved
 49 silicate, dissolved ammonium, TOC, $\delta^{13}\text{C}\%$, hypoxia, *Ammonia*.



50 1 Introduction

51 The understanding of humans as the greatest force of global change expands through all forms of
52 ecological settings. With approximately sixty to seventy percent of the global human population
53 inhabiting the world's coastlines, the realization that eutrophication can impact coastal
54 environments is however relatively recent (Nixon, 1995). Conceptualized as an increased rate of
55 succession observed in freshwater environments, Nixon (1995) was the first to implement the
56 idea of eutrophication to coastal marine environments. Since then the impacts of coastal
57 eutrophication has emerged as a major concern globally (McGlathery et al., 2007). Increased
58 loading of dissolved nutrients in near shore environments through land clearing, uninhibited
59 usage of fertilizers, discharge of waste materials and burning of fossil fuels has markedly
60 increased since the middle of the 20th century (Cloern, 2001). In addition, recent changes in
61 global climatic condition have also acted as a catalyst along with coastal eutrophication (Lloret et
62 al., 2008). In coastal aquatic environments increased loading of nitrogen (N) has been identified
63 as one of the greatest consequences of recent anthropogenic impacts (Vitousek et al., 1997;
64 Boesch, 2002; Scavia et al., 2002). Phosphorus has also been considered by many investigators
65 as a major contributor to coastal eutrophication (Hecky and Kilham, 1988; Hecky, 1998). Apart
66 from nutrient loading from external sources the phenomenon of internal loading i.e. release of
67 nutrients from bottom sediments into water column (Sondergaard et al., 2003) can act as a major
68 factor leading to eutrophication. Contributions from internal loading have been observed to be in
69 the same magnitude as external loading (Donazzolo et al., 1989, Markou et al., 2007). Nitrogen
70 is known to be released from sedimentary organic matter in the form of ammonium and nitrate
71 (Kim et al., 2003) while phosphorus is present in the water column in predominantly
72 orthophosphate form. Another major nutrient in aquatic ecosystems is silicate. Silicate is of
73 prime importance to the formation of exoskeletons in diatoms, a major contributor to surface
74 productivity. The historically steady concentration of silicate loading (Gilpin et al., 2004) as
75 opposed to increased loads of N and P in coastal zones is particularly of interest while comparing
76 between the different nutrient concentrations.

77 Coastal lagoons that make up thirteen percent of world's coastlines (Barnes, 1980) are more
78 vulnerable to eutrophication due to their limited nature of marine connection. Performing as
79 potential zones of CO₂ efflux, coastal lagoons act as major sites for organic carbon



80 mineralization (Jansson et al., 2000) which can be accelerated by increased nutrient mobilization
81 into these shallow environments (Cloern, 2001). Rapidly evolving concepts have attributed
82 increased sedimentary organic carbon loading directly to eutrophication in shallow coastal
83 regimes (Cloern, 2001) and thus sedimentary environment and benthic communities are
84 becoming extremely vulnerable to the effects of eutrophication (Jørgensen, 1996). Higher
85 concentrations of organic carbon in sediments derived from breakdown of more complex organic
86 matter has been also linked with grain size composition of sediments; specifically higher content
87 of fine grained particles has been reported to be rich in organic matter (Buchanan and
88 Longbottom, 1970; Mayer, 1994 a,b; Tyson, 1995). Higher content of fine grained particles (silt,
89 clay, mud) coupled with increased concentration of available organic carbon may subsequently
90 lead to lower penetration of oxygen within the sediment pore space and resulting in generation of
91 toxic byproducts such as ammonia (Florek and Rowe, 1983; Santschi et al., 1990). Accumulation
92 of such products eventually results in the lowering of benthic biodiversity by selectively
93 allowing the growth of opportunistic species (Como et al., 2007).

94 Benthic foraminifera are one of the most dominant microscopic organisms that characterize the
95 benthic diversity of shallow marginal marine environments and are widely used as bioproxy for
96 environmental monitoring of lagoons (Samir, 2000; Martins et al., 2013). Lagoons characterized
97 by high nutrient input and longer water residence time are often dominated by stress tolerant
98 foraminiferal taxa (Hallock, 2012). One of the best examples regarding the impact of
99 eutrophication on benthic communities in coastal lagoons comes from the phylum foraminifera.
100 Donicci et al. (1997) reported seasonal peaks in the occurrence of *Ammonia beccarii* coinciding
101 with increased phytoplankton numbers in surface water in Venice lagoon, Italy. The
102 foraminiferal assemblage from Venice lagoon was further investigated by Albani et al. (2007),
103 where they compared the benthic foraminiferal assemblages between 1983 and 2001. The
104 investigators did observe changes in the community composition in certain regions of the lagoon
105 following the establishment of a water treatment plant that reduced nutrient loading and found
106 the presence of surface phytoplankton to be the major factor driving benthic foraminiferal
107 production. Martins et al. (2013) studied the living foraminiferal assemblage from Ria de Aveiro
108 lagoon, Portugal and also reported 61 species of foraminifera amongst which *Ammonia tepida* to
109 be dominant in the interior parts of the lagoon.



110 Considered to be one of the most common foraminifera worldwide, *Ammonia* are known to
111 inhabit sheltered, shallow marine to brackish water environments (Hayward et al., 2004).
112 Globally members of the genus *Ammonia* are considered to be tolerant to environmental stresses
113 such as changes in pH, hypoxia and the presence of rare earth elements (RRE) (Le Cadre and
114 Debenay, 2006; Martinez-Colon and Hallock, 2010). Members belonging to this genus have
115 been extensively studied with respect to taxonomy (Holzmann and Pawlowski, 1997; Holzmann
116 et al., 1998; Holzmann, 2000; Hayward et al., 2004) and as a proxy for physiological changes
117 related to oceanic pH (Glas et al., 2012; Keul et al., 2013). Very few studies have however
118 looked into the environmental factors contributing to its dominance in shallow water zones.
119 Members of the genus are known to be tolerant to hypoxic ($< 2 \text{ mgL}^{-1}$) and dysoxic ($< 1 \text{ mgL}^{-1}$)
120 conditions originating due to increased nutrient loading in surface waters (Kitazato, 1994; Platon
121 and Sen Gupta, 2001). Based on its tolerance to low oxygen conditions Sen Gupta et al. (1996)
122 introduced the *Ammonia* – *Elphidium* index for characterizing coastal environmental monitoring.
123 Proper implementation of such an index however requires a thorough understanding of the
124 spatial and temporal preferences of the concerned organism.

125 The objective of the present work is to investigate the effects of nutrient loading on the
126 sedimentary organic carbon and the genus *Ammonia* from a subtropical coastal lagoon for a
127 period of twelve months. The investigation mainly focuses on the relationship of both living and
128 dead assemblages of *Ammonia* with increased nutrient influx in coastal zones. Apart from
129 investigating the impact of nutrient loading on biota, concentration of Potentially Toxic Elements
130 (PTEs) i.e. Chromium (Cr), Cobalt (Co), Zinc (Zn), Lead (Pb), Copper (Cu), Iron (Fe),
131 Manganese (Mn), Nickel (Ni) were also determined from surface water to identify any potential
132 industrial pollution in the environment. The work has been carried out from Chilika lagoon
133 (latitude $19^{\circ}28' - 19^{\circ}54' \text{N}$, longitude $85^{\circ} 06' - 85^{\circ} 35' \text{E}$), India; on the north-west coast of Bay
134 of Bengal. The lagoon represents an ideal shallow water coastal zone with an average depth < 2
135 m and can be classified as a choked lagoon (Kjerfve, 1986). Chilika lagoon lost its natural
136 connection with the Bay of Bengal in 2001 due to siltation, which persists to be a threat to the
137 lagoon's survival as it receives approximately $1.5 \text{ million MT year}^{-1}$ of silt from the distributaries
138 of Mahanadi river basin that drain into the lagoon (Ghosh et al., 2006). The lagoon also receives
139 approximately $550 \text{ million L day}^{-1}$ of untreated sewage discharge from the neighboring city of
140 Bhubaneswar along with untreated domestic water seepage from 141 villages that surround the



lagoon (Panigrahi et al., 2009). High rate of silt deposition coupled with decreased marine water inflow and increased loading of pollutants has resulted in extensive macrophyte growth in the lagoon bed which roughly cover 523 km² (Ghosh et al., 2006) of the watershed (704 km² in pre-monsoon to 1020 km² in monsoon, Gupta et al., 2008).

2 Materials and methods

2.1 Sampling stations

The study monitored six stations across the lagoon, selected based on their location with respect to potential sources of pollution (Fig. 1). Out of the six stations CS1, CS3, CS4 were far away from any human habituated zone of the lagoon. CS1 was located in the southern most part of the lagoon bearing greater depth of water than the other stations; CS3 was located adjacent to the declared bird sanctuary of Nalabana Island within Chilika while CS4 was located in close vicinity to the presently blocked opening of the lagoon into the Bay of Bengal. Other three stations were located more close to sources of nutrient influx into the lagoon. The station CS2 was located in the immediate vicinity of densely populated town of Balugaon; CS5 was located near the opening of Kusumi river, flowing through the western catchment of the lagoon. CS6 was located in the most north-eastern part of the lagoon that receives inflow from the Mahanadi basin. All the six stations were monitored on a monthly basis for a period of twelve months starting from March 2014 till February 2015.

2.2 Measurement of *in situ* environmental parameters

Parameters reflecting the prevailing environmental conditions at the time of sample collection were recorded using onboard equipments. Air temperature and temperature of the surface water were recorded using a digital thermometer. Salinity was determined using a handheld refractometer and pH was determined using a pH meter (Eutech Instruments Pte. Ltd., Singapore) from the surface water while measurements from the bottom water overlaying the sediment was undertaken after collecting the water using a Niskin sampler (General Oceanics, Florida, USA). Dissolved oxygen (DO) concentrations from the surface water, sediment water interface and from the top layer of the sediment were measured *in situ* by inserting the galvanometric probe of a microprocessor based DO meter (Eutech Instruments Pte. Ltd., Singapore) to respective zones.



170 2.3 Sample collection

171 Sample collection from the Chilika lagoon was carried out on a monthly basis for 12 months
172 from March 2014 till February 2015 . Water samples were collected from the surface layer and
173 from the sediment water interface to estimates the concentration of dissolved nutrients while
174 samples for extracting PTEs were only collected from the surface waters. Water samples from
175 the sediment water inter face were collected by deploying the Niskin sampler. All water samples
176 collected for the purpose of dissolved nutrients estimation were collected and transported to
177 laboratory following published protocol (Choudhury et al., 2015). Samples collected for the
178 estimation of PTEs were immediately filtered through 0.22 μm nylon filters and reduced by
179 addition of nitric acid to a final concentration of 5%.

180 Sediment samples were collected using a Ponar grab (Wild Co., Florida, USA) of 0.025 m^2 area.
181 Sediment sub-samples were cored in triplicates from the collected sediment using a push corer
182 having a length of 10 cm and inner diameter of 3.5 cm from which the topmost 0-2 cm was
183 collected for foraminiferal analysis and were immediately stained with rose Bengal (2gm L^{-1})
184 and fixed with 4% pH neutral formaldehyde solution. Distinction between live and dead
185 collected specimens was done based on rose Bengal staining of the protoplasm. Fixed sample
186 fractions were stored under dark conditions for a minimum of thirty days before undertaking
187 further analyses. Additional replicates of the surface 0-2 cm fraction were also collected for
188 extraction of pore water and determination of total organic carbon (TOC) from sediment.

189 2.4 Extraction of pore water:

190 Pore water from the surface 2 cm of sediment was extracted from replicate fractions immediately
191 upon return to laboratory for the period of nine months from June 2014 till February 2015.
192 Approximately 500 cc of sediment sample was centrifuged at 5000 rpm for 20 minutes and the
193 resultant supernatant water was collected as pore water. Salinity and pH of the extracted water
194 was immediately measured as following protocols described previously.

195 2.5 Measurement of dissolved nutrient concentrations

196 Concentrations of dissolved nutrients i.e. nitrate (NO_3^-), ortho-phosphate (PO_4^{3-}), ammonium
197 (NH_4^+) and silicate (SiO_4^-) were measured from collected surface water samples and bottom



198 water samples along with extracted pore water. The samples were passed through 0.45 μm nylon
 199 filter (Merck-Millipore, Darmstadt, Germany) in order to remove suspended particulate matters
 200 and the concentrations of dissolved nutrients were determined spectrophotometrically (U-
 201 2900UV/VIS Spectrophotometer, Hitachi, Tokyo, Japan). Dissolved NO_3^- concentrations were
 202 subsequently measured following published method (Finch et al., 1998). Likewise, dissolved
 203 PO_4^{3-} and SiO_4^- levels were measured spectrophotometrically by acid-molybdate (Strickland &
 204 Parsons, 1972) and ammonium molybdate (Turner et al., 1998) methods respectively.
 205 Concentrations of dissolved NH_4^+ were also determined following potassium ferrocyanide
 206 method (Liddicoat et al., 1975).

207

208 2.6 Measurement of PTEs

209 Concentrations of eight different potentially toxic elements (PTE) i.e. Co, Cr, Cu, Fe, Mn, Ni, Pb
 210 and Zn; were measured from the surface waters of studied stations by using Ionization Coupled
 211 Plasma – Mass Spectroscopy (ICP-MS) (ThermoFisher-Scientific X-series 2, Massachusetts,
 212 USA). The water samples were ran with respect to previously known concentrations of the
 213 elements to determine their respective values. Measurement of samples were carried using
 214 standard curves having regression values greater than 0.99.

215 2.7 Determination of sediment composition

216 Composition of the surface sediment was determined from unfixed fractions collected during
 217 sampling. Sediment samples were initially treated with 10 % hydrogen peroxide (H_2O_2) for 24
 218 hours to remove all organic components. The sediment samples were then washed vigorously
 219 under deionized water and dried for 24 hours at 60 °C to remove all moisture content. Following
 220 which 10 gm (dry weight) of the sediment was vigorously stirred for 2 hours in a solution of 0.01
 221 % sodium hexa-metaphosphate ($(\text{NaPO}_3)_6$) to separate the sediment particles. The resulting
 222 mixture was then wet sieved through the mesh sizes of 500 μm , 250 μm , 125 μm and 63 μm .
 223 The resultant fractions were dried and weighed to determine the sediment composition and were
 224 defined according to the Wentworth size classes (Buchanan, 1984).

225 2.8 Characterization of sedimentary organic carbon



Approximately 1 cm³ of surface (up to 1 cm depth) sediment samples were initially acidified with 5 % HCl in order to dissolve all carbonates and were followed by rinsing with a minimum of 1000 ml of deionized water. Subsequently, sediment was dried at 60 °C for 24-48 hours. Dried samples were grinded to finely powdered form using mortar and pestle. Samples for Carbon stable isotope analysis were combusted in FLASH 2000 Elemental Analyzer (ThermoFisher-Scientific, Massachusetts, USA). Stable isotopes ratios were determined on a MAT-253 Mass Spectrometer (ThermoFisher-Scientific, Massachusetts, USA) with respect to tank CO₂ and are expressed relative to Vienna Pee Dee Belemnite (VPDB) as δ values, defined as:

$$\delta^{13}\text{C}_{\text{‰}} = \frac{X_{\text{sample}} - X_{\text{standard}}}{X_{\text{standard}}} \times 10^3 [\text{‰}]$$

where $X = {}^{13}\text{C}/{}^{12}\text{C}$. Reproducibility of values was calibrated to a precision of 0.1 ‰. Determination of Total Organic Carbon (TOC) was performed by calibrating the tank CO₂ with respect to IAEA-CH₃ (cellulose) used as internal reference materials, measured after every four samples to maintain the quality of the estimation.

2.9 Identification and estimation of *Ammonia* spp. from sediments

Identification of foraminiferal specimens as belonging to *Ammonia* spp. was done following the genus description established by Hayward et al. (2004). Counting of specimens belonging to *Ammonia* spp. were carried out from the stained and fixed fractions of surface 2 cm of sediment collected from the lagoon bed. Sediment of 10 c.c. from each fraction was wet sieved under a jet of freshwater through mesh sizes of 500 μm followed by 63 μm. The collected residue in ≥ 63 μm mesh sizes were observed under binocular microscope (Zeiss Stemi DV4, Carl Zeiss AG, Oberkochen, Germany) by wet splitting. Specimens bearing stained cytoplasm up to the penultimate chamber were considered to be live while unstained specimens having all the chambers finely preserved upto the prolocular chamber were considered as being dead at the time of collection. Specimens displaying visible primary organic sheet were considered to be taphonomically altered along with specimens having test breakage and were not considered for the study.

2.10 Statistical analyses



254 Relationships within the different environmental factors and with observed numbers of live and
255 total (live + dead) foraminifera were tested by calculating Pearson's correlation co-efficient and
256 considering p values ≥ 0.05 as significant. Presence of seasonal or spatial variation within the
257 *Ammonia* assemblage was tested by performing a cluster analysis. All abundance data were Log
258 $(X + 1)$ transformed prior to analysis and the analysis was performed using Bray-Curtis
259 similarity measure. All statistical analyses were performed in PaST version 3.09 (Hammer et al.,
260 2001).

261 3 Results

262 3.1 *In situ* hydrological parameters

263 Temperature at the surface of the lagoon closely reflected the prevailing air temperature at the
264 time of sample collection (Fig. 2). The temperature reflected the subtropical climate of the region
265 with increased values during the months of March till May 2014 which corresponds to summer
266 in the region. The temperature was lowered following the monsoon period of July to October, in
267 the months of November 2014 till February 2015 which roughly equates to winter in the north-
268 west coast of Bay of Bengal.

269 Salinity profile was grossly influenced by the advent of monsoon in the region which is
270 traditionally considered to arrive in late June and continue up to the end of October (Fig. 3).
271 During the pre-monsoon months of March till June, the surface water salinity displayed
272 variability (Max. 30.3, Min. 0.7) within the sampling stations. The highest value was recorded at
273 CS4 in the month of April, while the lowest was recorded at CS5. Salinity of the water
274 overlaying the sediment also reflected this variability within stations (Max. 30.3, Min. 0.7). Pore
275 water salinity for this period was only determined for one month (June 2014) which also had
276 highest value (26.7) at CS4 and lowest (6.0) at CS5. During monsoon all the stations displayed a
277 progressive decrease in their salinity profile with respect to time. The impact of monsoon was
278 more pronounced on surface and bottom water values of five stations (barring CS1) which were
279 entirely devoid of salinity in September. Pore water salinity values for the period ranged between
280 25.7 (CS4 July) and 0 (CS5 September). Following the cessation of monsoon an immediate
281 increase in salinity profile was not observed in November, during which the surface values
282 continued to be 0 in four out of six stations. During the post-monsoon (November-February)



gradual increase was observed in the salinity profile. Salinity values across depth profile displayed significant ($p \leq 0.05$) correlation among themselves (Table 1).

Values of pH followed a similar trend across surface and bottom waters displaying almost no variation among them (Fig. 3). Surface water pH values (Max. 9.8, Min. 6.5) varied widely between sampling times, a trend mirrored by bottom water overlaying the sediment (Max. 9.8, Min. 6.8). Increased values of pH in surface waters were observed in the month of October (Max. 9.7, Min. 8.2), as well as in the bottom water (Max. 9.5, Min. 8.2). Lowering of pH was observed in November for both surface (Max. 7.7, Min. 6.5) and bottom (Max. 8.2, Min. 6.8) water. Significant correlation thus existed between the surface values of pH and values recorded from bottom water (Table 1). Pore water pH displayed a more acidic condition (Max. 8.6, Min. 7.1) compared to its overlaying counterparts. Most of the pH values determined from the pore space had values < 8.0 .

Concentration of dissolved oxygen (DO) in the water column and pore space displayed an overall stability during pre-monsoon and monsoon months (Fig. 3). Post-monsoon months showed a continuous increase in the DO values. Lowest range of DO values in surface waters were observed in August (Max. 4.19 mgL^{-1} , Min. 3.60 mgL^{-1}) while the highest was observed in February (Max. 8.88 mgL^{-1} , Min. 6.67 mgL^{-1}). Spatially, however the highest DO value was recorded at CS6 (10.74 mgL^{-1}) in November. The lowest value from surface water was also reported from CS6 (3.60 mgL^{-1}) in the month of August. The DO values from sediment water interface reflected general trends observed in the surface water although having lower range of values. The lowest value (2.87 mgL^{-1}) was recorded from CS2 in March while the highest (10.98 mgL^{-1}) came from CS6 in November. DO values from the top 2 cm of the sediment displayed comparatively lower range (Max. 6.14 mgL^{-1} , Min. 1.22 mgL^{-1}). CS2 displayed lower DO concentrations (Max. 5.65 mgL^{-1} , Min. 1.22 mgL^{-1}) in its sediments compared to other stations. Significant correlations within the profiles were also observed the studied profiles, a trend similar to salinity (Table 1).

3.2 Concentration of dissolved nutrients

Dissolved nitrate (NO_3^-) concentration followed a strong seasonal pattern in surface and bottom waters of the lagoon (Fig. 4). During the pre-monsoon months NO_3^- concentrations were



312 relatively high in both surface (Max. 86.25 μM , Min. 19.5 μM) and bottom (Max. 90.17 μM ,
313 Min. 26.75 μM) waters. During this period CS5 had higher values (surface: Max. 86.25 μM ,
314 Min. 54 μM ; bottom: Max. 90.17 μM , Min. 45.12 μM) of nutrients in both the profiles compared
315 to other stations. Pore water concentrations of NO_3^- were significantly higher (Max. 131.67 μM ,
316 Min. 68.33 μM) in the pre-monsoon month of June compared to the other layers studied with the
317 highest value recorded from CS5. During the monsoon there was considerable lowering of NO_3^-
318 values in surface (Max. 47.2 μM , Min. 19.73 μM) and bottom water (Max. 46.07 μM , Min. 18.8
319 μM) values as compared to concentrations in the pore water (Max. 162.47 μM , Min. 27.78 μM).
320 There was significant correlation between the surface water values and bottom water values
321 while no such strong relationship was observed with their pore water counterpart (Table 1). Post-
322 monsoon observed a gradual return to pre-monsoon values in the concentration of dissolved
323 NO_3^- in both surface (Max. 83.67 μM , Min. 18.33 μM) and bottom waters (Max. 95.33 μM , Min.
324 16 μM). The pore water concentration however lowered (Max. 105.8 μM , Min. 9 μM) during
325 this period.

326 Orthophosphate (PO_4^{3-}) concentrations (Fig. 4) in surface waters showed greater variability
327 (Max. 8.57 μM , Min. beyond detection limit) among the stations during the pre-monsoon months
328 while lower range of values was observed in bottom water (Max. 3.92 μM , Min. 0.09 μM).
329 Values from pore water were mostly below detection during June. The concentration of PO_4^{3-}
330 lowered during the monsoon months in both surface (Max. 4.0 μM , Min. beyond detection limit)
331 and bottom waters (Max. 3.22 μM , Min. beyond detection limit). In pore waters the
332 concentration of PO_4^{3-} relatively higher compared to June 2014 (Max. 5.33 μM , Min. beyond
333 detection limit). During post-monsoon surface water concentrations of PO_4^{3-} were mostly low
334 (Max. 2.98 μM , Min. beyond detection limit) barring one sample (CS4 November 2014, 5.62
335 μM). Similarly bottom water concentrations were also lowered (Max. 1.93 μM , Min. 0.18 μM)
336 along with pore water concentrations (Max. 2.18 μM , Min. beyond detection limit). Within the
337 profiles significant correlation existed between the surface water and bottom water
338 concentrations, a relation not present with pore water pH values.

339 Concentrations of dissolved silicate (SiO_4^-) displayed a strong seasonal pattern across the studied
340 stations (Fig. 4). Mostly low values of SiO_4^- were observed during the pre-monsoon period
341 (surface Max. 90 μM , Min., 2.33 μM ; bottom Max. 279 μM , Min. 5 μM ; pore water Max. 36.17



342 μM , Min. $2.33 \mu\text{M}$). Rapid increase in values were observed during monsoon (surface Max. 351
 343 μM , Min. $24 \mu\text{M}$; bottom Max. $377.5 \mu\text{M}$ Min. $71.5 \mu\text{M}$, pore water Max. $268 \mu\text{M}$ Min. 13.7
 344 μM) with CS5 and CS6 having comparatively higher values than other stations. Following
 345 monsoon, a drop in concentrations was observed starting November. Surface water values
 346 reflected a reversal to pre-monsoon values (Max. $340.67 \mu\text{M}$, Min. $53 \mu\text{M}$) along with bottom
 347 water (Max. $310.5 \mu\text{M}$, Min. $59.5 \mu\text{M}$) except for CS6 which showed higher values till
 348 December. Pore water SiO_4^- concentrations though lower (Max. $165 \mu\text{M}$, Min. $33 \mu\text{M}$) than
 349 monsoon values was still higher compared to June. Amongst the nutrient concentrations studied
 350 SiO_4^- displayed the strongest relationship within depth profiles as significant correlations existed
 351 between all the studied compartments (Table 1).

352 Dissolved ammonium (NH_4^+) concentrations were mostly lower than detection limit during the
 353 first three months in the surface layer followed by low concentrations in June (Fig. 4). Bottom
 354 water concentrations of the nutrient were also low during the pre-monsoon period (Max. $6.5 \mu\text{M}$,
 355 Min. beyond detection limit). Pore water values (Max. $6.5 \mu\text{M}$, Min. beyond detection limit)
 356 were also low in June. Surface water concentrations did not vary much during monsoon (Max.
 357 $13.0 \mu\text{M}$, Min. beyond detection limit) and post-monsoon (Max. $12.33 \mu\text{M}$, Min. $0.67 \mu\text{M}$).
 358 Similar ranges of values were also recorded from bottom water samples (monsoon Max. 12.67
 359 μM , Min. beyond detection limit, post-monsoon Max $10.33 \mu\text{M}$, Min. beyond detection limit).
 360 Increased values of NH_4^+ were detected from pore water during the monsoon months of August
 361 till October (Max. $91.0 \mu\text{M}$, Min. $0.05 \mu\text{M}$) and in November (Max. $73.5 \mu\text{M}$, Min. $15 \mu\text{M}$).

362 3.3 Concentration of PTEs

363 Among the eight elements studied, four (Cu, Fe, Ni, Zn) displayed variations in their mean
 364 values with respect to the month of sample collection while the remaining (Cr, Co, Pb, Mn)
 365 displayed a more stable value throughout the study period. Cu concentrations were relatively
 366 higher during the pre-monsoon months (Max. $17.31 \mu\text{g L}^{-1}$, Min. $3.28 \mu\text{g L}^{-1}$) and in July (Max.
 367 $9.21 \mu\text{g L}^{-1}$, Min. $4.40 \mu\text{g L}^{-1}$) as compared to the months stretching from August till January
 368 (Max. $6.89 \mu\text{g L}^{-1}$, Min. $1.57 \mu\text{g L}^{-1}$), following which increased values were observed in
 369 February (Max. $23.88 \mu\text{g L}^{-1}$, Min. $14.09 \mu\text{g L}^{-1}$). Fe concentrations in the surface water
 370 displayed a continuous lowering of values along the months studied having highest range of
 371 values in March (Max. $780.2 \mu\text{g L}^{-1}$, Min. $168.1 \mu\text{g L}^{-1}$) to the lowest range of values in February



(Max. 363.4 $\mu\text{g L}^{-1}$, Min. 60.66 $\mu\text{g L}^{-1}$). A similar trend was present in case of Ni, which initially increased in concentration during the pre-monsoon months (March Max 7.46 $\mu\text{g L}^{-1}$, Min. 3.29 $\mu\text{g L}^{-1}$ – June Max. 8.79 $\mu\text{g L}^{-1}$, Min. 4.33 $\mu\text{g L}^{-1}$) but then continued to decrease in concentration (July Max 6.07 $\mu\text{g L}^{-1}$, Min. 1.68 $\mu\text{g L}^{-1}$ – February Max. 3.17 $\mu\text{g L}^{-1}$, Min. 2.53 $\mu\text{g L}^{-1}$). Values of Zn decreased markedly during the monsoon below the level of detection in most samples, following which the concentrations gradually increased in the later part of post-monsoon (January Max 11.15 $\mu\text{g L}^{-1}$, Min. 5.01 $\mu\text{g L}^{-1}$ – February Max. 32.78 $\mu\text{g L}^{-1}$, Min. 5.90 $\mu\text{g L}^{-1}$).

3.4 Sediment composition

Sediment composition for the six stations (Fig. 5) displayed little variation with time, among which four stations i.e. CS1, CS2, CS3 and CS6 was entirely dominated by silt-clay (< 63 μm) fraction (silt-clay content Max: 99.69 %, Min. 25.14 %). CS4 was the only station with a higher fraction of sand (> 63 μm) particles. But on the other hand, CS5 station displayed more variation in its sediment composition compared to other stations. The variation in silt-clay content (Max. 86.24 %, Min. 33.94 %) was considerably large among the samples collected from CS5.

3.5 Sedimentary organic carbon

Stable isotopic ratios of carbon ($\delta^{13}\text{C}_{\text{‰}}$) from the sediments varied within a narrow range of values (Max. -20.7, Min. -24.2) and did not exhibit any seasonal pattern (Fig. 7). Total Organic Carbon (TOC) content however displayed some oscillations at CS5 with increased values observed during the pre-monsoon months of March till May (Max. 5.66 %, Min. 2.87 %) and the post-monsoon months of December-February (Max. 3.88 %, Min. 2.66 %). Values of TOC in other stations varied within a narrow margin (Max. 3.71 %, Min. 0.31 %) and changed little with respect to seasons.

3.6 Characterization of *Ammonia* spp. population

Number of benthic foraminiferal specimens identified as *Ammonia* spp. displayed great variation from sample to sample with respect to the presence of both live and dead specimens (Fig. 8). Out of the total 72 sediment samples studied, 46 samples bore stained individuals who were considered live at the time of collection while 53 samples had dead specimens alongside live



ones. The stations varied greatly amongst themselves with respect to both the number of samples bearing *Ammonia* spp. as well as the number of live and dead specimens present. The lowest number of samples bearing live (4) and dead (5) specimens were observed at CS1. The number of specimens belonging to each category was also relatively low at CS1 as the highest number of live specimens (8 / 10 c.c.) was observed in the sample of July 2014, while the highest number of dead specimens (30 / 10 c.c.) were recorded in September 2014. In comparison, CS2 had the highest number of dead specimens recorded from across the lagoon with the highest values being 819 / 10 c.c. (June 2014) and also having only 2 samples where no dead specimens were observed. The highest number of live specimens at CS2 was observed in November 2014 (36 / 10 c.c.). Station CS3, during most part of the study period was characterized by low number of live and dead specimens of *Ammonia* spp. except in September 2014 sample, where the number of live specimens were 54 / 10 c.c. However, no dead specimens were present in that particular sample. The highest number of dead specimens observed at CS3 was recorded in the sample collected in December 2014 (24 / 10 c.c.) while no dead specimens were recorded in 3 occasions. The population at CS4 was also mostly characterized by low number of dead specimens alongside fewer live individuals except in the months of March and April 2014, when the number of dead specimens were relatively high (129 / 10 c.c. and 501 / 10 c.c. respectively). The number of live specimens was however extremely low in this station (Max. 14 / 10 c.c.). Occasional increase in the number of live specimens was observed at CS5 with highest values (126 / 10 c.c.) being present in May 2014 sample which was also the highest value recorded across the study period for the entirety of the lagoon. The number of dead specimens at CS5 displayed great variability similar to the number of live specimens, with relatively higher values observed during the months of May 2014 and February 2015 (336 / 10 c.c. and 255 / 10 c.c. respectively). The station CS6 was also mostly characterized by a low number of live and dead specimens for the majority of the sampling period, except in the month of June 2014 when increased numbers of live (106 / 10 c.c.) and dead (111 / 10 c.c.) specimens were recorded. Overall, a significant correlation ($r = 0.4$, $p = 0.0005$) was observed among the number of dead and live specimens for the entire study period across the lagoon.

A cluster analysis was performed in order to test for spatial and seasonal grouping of samples (Fig. 9). The analysis generated six clusters that were formed based on the abundance and contribution of live and dead specimens present in each sample. Cluster I was comprised of 5



431 samples characterized by the absence of live specimens and served as an outgroup with the rest
 432 of the dendrogram. The numbers of dead specimens in samples belonging to this cluster were
 433 also extremely low. Cluster II was also characterized mostly by the absence of live individuals
 434 except one sample (CS2Jun14) where the number of live specimens was negligible compared to
 435 its dead counterpart. In this cluster however the number of dead specimens was comparatively
 436 greater than its predecessor. Nine samples spread across almost the entirety of the lagoon, having
 437 low numbers of live and dead specimens formed cluster III. Cluster IV was represented by 12
 438 samples having comparatively higher number than the rest of the samples and having greater or
 439 significant proportion of live specimens being present in each sample. Similar proportions were
 440 also present in the 18 samples that formed cluster V; however the number of observed specimens
 441 was much lower as compared to cluster IV. The final cluster VI was comprised of samples
 442 having very little contribution of live specimens as well as having large numbers of dead
 443 specimens pertaining to the genus *Ammonia*.

444 Separately a correlation analysis was performed to investigate the relationships between the
 445 number of live specimens and total (live + dead) specimens with the studied environmental
 446 factors. Significant correlation ($r = 0.23$, $p = 0.04$) was observed between the surface water
 447 concentrations of dissolved NO_3^- and the number of live *Ammonia* specimens. Sedimentary TOC
 448 values also displayed significant correlation with both live ($r = 0.31$, $p = 0.009$) and total ($r =$
 449 0.29 , $p = 0.01$) number of specimens.

450

451

452 4 Discussion

453 The present study investigated the environmental quality of Chilika lagoon, a coastal water body
 454 which has been reported to receive huge influx of untreated waste water and has been threatened
 455 with eutrophication. The study was carried for a period of twelve months and concentration of
 456 various water borne pollutants in the lagoon was studied along with potential impacts of such
 457 pollution throughout different compartments of the lagoon's water column. Further attempts
 458 were made to characterize the organic carbon load in the sediment which can be considered as a
 459 direct effect of eutrophication in the lagoon. *Ammonia* spp., a benthic foraminiferal species



460 globally reported as stress tolerant taxa was also investigated with regards to its utility as a
461 potential indicator of pollution in this shallow marginal marine habitat.

462 Chilika lagoon, the largest coastal lagoon in Asia and the second largest in the world, is a
463 designated Ramsar site since 1981. The lagoon was placed in the Montreux records for being a
464 wetland under threat in 1993, from which it was removed in 2002 following successful
465 management practices including dredging and opening of an artificial connection to the Bay of
466 Bengal which increased the salinity profile of the lagoon. During the present study period
467 increased values of salinity were observed during the pre-monsoon months of March till June, a
468 period that corresponds to summer in the north-western coast of Bay of Bengal. Salinity values
469 plummeted soon after as the monsoon precipitations began in late June and early July of 2014.
470 The amount of total precipitation in the region increased from 297.8 cm in the month of June to
471 1505.9 cm in July, values similar to which lasted till the passing away of monsoon in November
472 during which total precipitation decreased to 5.4 cm. Apart from the localized freshwater input
473 due to the monsoon, the lagoon receives an enormous volume (approx. $5.09 \times 10^9 \text{ m}^3$) of
474 freshwater from 52 rivers and rivulets that drain into it (Panda and Mohanty, 2008). The impact
475 of freshwater influx on the salinity profile of the lagoon is prominent through all the studied
476 layers. Salinity on certain stations continued to persist albeit in lower concentrations, mostly due
477 to localized inflow of sea-water from point sources nearby. Jeong et al. (2008) applied a self-
478 organizing map approach in characterizing the lagoon's hydrology and found strong dependence
479 of the lagoons salinity on climatic factors. Based on the present findings monsoonal precipitation
480 can be a major driver in shaping the lagoons salinity regime. The lowering of salinity during this
481 period leads to the increased growth of freshwater invasive species like *Azolla*, *Eichhornia*,
482 *Pistia* and emerging species like *Ipomea* (Panigrahi et al., 2009). The presence of such large
483 scale photosynthetic plants may also be the causative factor behind increased values of surface
484 water pH as observed in the post-monsoon months of January-February 2015. An earlier
485 investigation by Nayak et al. (2004) have also linked increased pH values observed in the
486 northern and central parts of the lagoon with the presence of aquatic weeds. Apart from the
487 increased values in the surface waters in January-February 2015, an increase was observed in
488 October 2014, which was restricted to the central and northern stations. This increase was
489 immediately followed by a lowering of pH values in the month of November, a trend mirrored in
490 the bottom water values. Acidification of shallow water zones can stem from the upward



491 migration of H_2S generated from anaerobic degradation of organic matter in sediments (Koretsky
492 et al., 2005). The upward migration of H_2S in the oxic layers of water column lead to the
493 generation of sulphuric acid (Curtis, 1987; Martin, 1999a), which may explain sudden drops in
494 surface and bottom water pH. The pore water pH values however displayed lesser variation. The
495 Central Pollution Control Board under the Government of India mandates a pH range of 6.5 – 8.5
496 for ecologically sensitive coastal waterbodies. The pH values from Chilika lagoon broadly falls
497 within this range except for the increased values mentioned previously.

498 The lagoons health however appears to be under severe threat from increased nutrient loading
499 from its catchment basin. Investigations regarding eutrophication of coastal water shed zones,
500 majorly revolves around the changes in the ratios of N, P and Si and their impacts of primary
501 production. The N:P ratio of 16:1 has been historically set as an benchmark for differentiating
502 between N-limitation and P-limitation in oceanic waters (Falkowski, 1997; Tyrrell, 1999; Lenton
503 and Watson, 2000). Changes in the N:P ratio have been associated with changes in primary
504 producers like phytoplankton species composition. Strong evidence of how human induced
505 changes in the N:P ratio can lead to discrete community shifts comes from more than two
506 decades long study undertaken in the Wadden Sea (Philippart et al., 2000). During the present
507 investigation the observed N:P ratio was markedly above the 16:1 mark for maximum number of
508 samples (Fig. 10), irrespective of the collection profile indicating increased N loading to be the
509 major source of eutrophication in the lagoon. Surface water values of nitrate (NO_3^-) displayed a
510 strong negative relationship with seasonal precipitation as proven by a significant positive
511 correlation with surface water salinity. The increased influx of fresh water during the monsoon
512 actually lowered the N:P ratio towards 16:1 as NO_3^- concentrations diluted. Concentrations of
513 ammonium (NH_4^+), another component of the Dissolved Inorganic Nitrogen (DIN) pool was
514 negligible and often beyond the lower detection limit in the lagoon surface a similarity shared
515 with orthophosphate (PO_4^{3-}), which is another component of the Redfield ratio. Very few
516 samples from surface water fraction displayed values characteristic of P limitation and majority
517 of these samples were collected during the monsoon period. N:P ratios measured from the
518 bottom water compartments strongly reflected the surface water conditions as significant
519 correlations existed between surface water and bottom water values of NO_3^- and PO_4^{3-} . Pore
520 water values of N:P is however particularly interesting, as within the interstitial space the major
521 component of DIN is not NO_3^- , but NH_4^+ . Increased values of NH_4^+ were present mostly during



522 the monsoon period, which coincided with lowered values of NO_3^- concentration. The observed
523 shift can be accounted by increased generation of NH_4^+ through the degradation of sedimentary
524 organic load. The absence of any significant correlation of pore water concentrations of NH_4^+
525 with its other counterparts is suggestive of a sedimentary origin and absence of internal loading
526 to overlying water column.

527 Apart from N:P ratio, the ratio between N and Si is of particular interest with regards to
528 eutrophication because anthropogenic loading has not been a factor in case of Si concentrations
529 in coastal zones as majority of it originates from natural sources (Cloern, 2001). Diatoms, a
530 major contributor of surface water primary productivity, require N and Si at the molar ratio of
531 approximately 1 (Redfield et al., 1963; Dortch and Whitledge, 1992). A shift towards N:Si > 1 is
532 normally associated with increased loading of N, followed by an environment selectively
533 supporting non-diatom taxa that require less Si (Conley et al., 1993). Increased loading of N as
534 compared to Si have been often associated with non-diatom blooms (Bodeanu, 1993). In Chilika
535 lagoon, the N:Si values were mostly < 1 in case of surface and bottom water environment, a
536 condition favorable for diatom growth (Fig. 10). Srichandran et al. (2015a) studied the
537 phytoplankton community from the lagoon for a period of twelve months and found
538 Bacillariophyta to be the most abundant group. Lowered values of N:Si may be due to the high
539 values of dissolved SiO_4^- that is brought into the lagoon during the monsoon freshwater flow, as
540 evidenced from the significant negative correlation observed with salinity. The relationship
541 between the two nutrients (i.e. SiO_4^- and NO_3^-) also displayed negative correlation between them
542 as they have opposite relationships with monsoonal precipitation. The trend exists in bottom
543 water compartment too resulting in similar N:Si ratios, while in pore water most of the values are
544 way beyond extremes most likely due to increased concentration of NH_4^+ evolving from
545 breakdown of sedimentary organic matter.

546 Sedimentary composition of the lagoon bottom has been described as a mixture of fine to
547 medium sized sand fractions (Mahapatro et al., 2010; Ansari et al., 2015). In the present study
548 four out of the six stations displayed > 90% silt-clay (< 63 μm) content in the sediment which
549 can be a resultant of the increased siltation of the lagoon. Ghosh et al. (2006) have reported the
550 silt carried by the distributaries of Mahanadi basin to be 1.5 million MT year⁻¹ (approx) resulting
551 in decreased depth profiles in the northern and central parts of the lagoon (< 1 m). Previous



investigation has revealed the positive relationship between higher content of finer sediment fractions with increased organic matter content (Tyson, 1995). An area having low hydrodynamic energy tends to allow enhanced settlement of silt-clay particles which in turn leads to a higher content of organic matter due to increased surface area. In the present work TOC content from the surface 2 cm of the sediment as a proxy for sediment organic matter. Globally coastal lagoon sediments are characterized by a higher TOC content as compared to other coastal marine environments (Tyson, 1995). The TOC values of Chilika lagoon as presented in the current study had a comparatively lower median value (1.44%) as compared to the well studied Mediterranean coastal lagoons. Cabras lagoon, off the west coast of Sardinia is reported to have the highest median value of surface sediment TOC at 3.41% (De Falco et al., 2004). Values similar to the present study have been recorded from S'ena Arrubia lagoon (< 2 %) in west Mediterranean (De Falco and Guerzoni, 1995) and also from Etang de Vendres lagoon (~ 0.2 - 7.0 %) in Southern France (Aloisi and Gadel, 1992). Values such as these are characteristics of OM enrichment in sediment compartments (Lardicci et al., 2001; Frascari et al., 2002). In Chilika, however values of even greater magnitude were observed at a station (CS5) located near the outfall of a river, where the values of TOC ranged between 0.6 % and 5.66 %, with 66.7 % of the values recorded from this station being greater than the median value of the entire lagoon. The station however was characterized by a lower mean of silt-clay content which explains the absence of any significance correlation between sediment silt-clay content and TOC. The TOC values from this station however did display strong seasonal trends very similar to observed nitrate concentration in the water columns, which may be indicative of autochthonous deposition of TOC due to increased surface primary production following monsoon.

The ratio of stable carbon isotopes ($\delta^{13}\text{C}\text{‰}$) was studied to identify the source of sedimentary TOC. Values of $\delta^{13}\text{C}\text{‰}$ provide a mean to distinguish between sediment organic matter derived from freshwater and marine source. Vascular land plants and freshwater algae utilizing the C_3 Calvin pathway have $\delta^{13}\text{C}\text{‰}$ range between -26 and -28‰ approximately while marine particulate organic matter comprising of particulate organic carbon (POC), algal and bacterial cells range approximately -19 to -22‰. Organic matter derived from C_4 Hatch-Slack pathway range approximately at -12 to -16‰ (Meyers, 1994). The values of $\delta^{13}\text{C}\text{‰}$ observed in the



582 present study displayed ranges that are mostly characteristic of a marine POC generated using
583 dissolved HCO_3^- , which indicates towards an autochthonous origin of observed TOC values. As
584 mentioned earlier, members of the group Bacillariophyta have been reported to dominate the
585 surface water primary producer community in the Chilika lagoon. Higher values were observed
586 in the station neighboring the river opening (Fig. 11) and having seasonal variation in TOC
587 content as compared to the other studied sites. This may indicate the influence of localized
588 nutrient loading in that particular area of the lagoon resulting in seasonal increases in algal
589 production that inhabits the brackish water conditions. Lowered values of $\delta^{13}\text{C}\text{‰}$ were present in
590 low TOC bearing samples from near the lagoon's marine opening (CS4), which was
591 characterized by higher sand content. The proximity to a marine source in this scenario may lead
592 to increased flushing of the bottom sediment, thus presenting an altered value of TOC and
593 $\delta^{13}\text{C}\text{‰}$. Vizzini et al. (2005) studied the Manguio lagoon in southern France in order to identify
594 the trophic pathways with respect to the sources of organic matter in the sediment using stable
595 isotopic ratios of carbon and nitrogen. The study found spatial zonation with respect to the
596 $\delta^{13}\text{C}\text{‰}$ values recorded from the sediment. Depleted values of $\delta^{13}\text{C}\text{‰}$ were observed near the
597 outfall of freshwater bearing rivers, thus exhibiting the utility of stable isotopes in deciphering
598 the respective sources of organic carbon in sediments.

599 One direct impact of eutrophication on the biotic component stems from the availability of
600 oxygen. Higher silt-clay content coupled with higher values of TOC may lead to lesser
601 permeability of oxygen and a higher microbial oxygen demand, a phenomenon particularly
602 noticeable in the interstitial compartment of sediment and thus have maximum impacts on
603 benthic organisms. Hypoxia in coastal waters has been reported to show an exponential rate of
604 growth with respect to newer areas being characterized as hypoxic (Vaquer-Sunyer and Duarte,
605 2008). The theoretical limit at which oxygen becomes limiting for survival of coastal biota has
606 ranged broadly from 0.28 mg L^{-1} (Fiadeiro and Strickla, 1968) to 4 mg L^{-1} (Paerl, 2006), with
607 most published data considering a value of 2 mg L^{-1} (Diaz and Rosenberg, 1995; Turner et al.,
608 2005). The Central Pollution Control Board under the Government of India also recognizes the
609 lowest limit to be 3.5 mg L^{-1} for the safe propagation of biodiversity in coastal zones. Values of
610 DO concentration from the surface and bottom water column recorded in the present study is
611 well above the level of concern with respect to hypoxia. However, hypoxic values ($< 2 \text{ mg L}^{-1}$)



612 are observed from the interstitial space of the sediment in two cases. Interestingly in both cases
613 the values have been recorded from a region that is in close proximity of human habitation.
614 Relationship may exist between observed cases of hypoxia and potential release of untreated
615 sewage from the neighboring human habitats, as the particulate organic matter (POM) and
616 dissolved organic matter (DOM) present in untreated waste water may also cause hypoxia in the
617 lagoon (Ganguly et al., 2015). Further detailed studies are required to understand the occurrence
618 of such sudden hypoxic conditions within the sedimentary profile.

619 The impact of eutrophication generated hypoxia on the benthic biotic community has been
620 extensively reviewed by Diaz and Rosenberg (1995) and later by Gray et al. (2002). The vast
621 interest of palaeo-oceanographers along with data from oxygen minimum zones and hypoxic
622 basins has lead to the existence of vast amount of research on foraminiferal taxa as indicators
623 (Bernhard and Sen Gupta, 1999; Gooday et al., 2009), however debates still exists regarding
624 whether to attribute observed changes to depleted oxygen values or increased organic matter load
625 (Levin et al., 2009). The utility of live versus total foraminiferal assemblages in tracking
626 environmental change is also a highly debated issue. While Schönfeld et al. (2012) has mandated
627 only the use of live specimens in ecological studies, but have also mentioned the utility of
628 incorporating dead tests, as they can provide information about environmental and biological
629 changes on a decadal scale. In the present investigation, the utility of both live and total specime
630 benthic foraminiferal genera *Ammonia* were tested for their suitability as biotic proxies for
631 coastal eutrophication. The live foraminiferal assemblage of the lagoon has already been
632 characterized as dominated by *Ammonia* spp. (Sen and Bhadury, 2016). Cluster analysis did not
633 reveal the presence of distinct seasonal or spatial trends present in the assemblage. The
634 investigation however revealed significant correlation between observed number of live
635 specimens and dead specimens, thus in the present condition the total assemblage of foraminifera
636 can also provide valuable information regarding environmental change. Significant correlation
637 was observed with surface water concentrations of dissolved NO_3^- with both live and dead tests
638 of *Ammonia*. Similarly significant correlation existed in case of sediment TOC content. In both
639 cases however the level of significance was greater when considering live specimens.
640 Concentration of dissolved oxygen did not appear to impact the live assemblage of *Ammonia*.
641 *Ammonia* along with *Elphidium* is the most abundant foraminiferal genera globally (Murray,
642 2006) and is mostly known to inhabit shallow, brackish, coastal zones (Murray, 1991). Members



643 of the genus *Ammonia* are known to display greater tolerance to hypoxia than *Elphidium* spp.
644 from field based observations (Kitazato, 1994; Platon and Sengupta, 2001) while very few
645 laboratory based experiments exists testing sensitivity of the genus to varying concentrations of
646 oxygen. Moodley and Hess (1992) observed that *Ammonia* can survive for at least 24 hours even
647 in anoxic (O_2 conc. 0 mg L^{-1}). Members of the genus have been generally considered to be stress
648 tolerant in nature (Carnahan et al., 2009). The present findings strongly agree with the statement
649 of Hallock (2012) that *Ammonia* generally dominates the sediments where intermittent hypoxia
650 may be present along with abundant source of food. The observed relationship between live
651 individuals with surface concentrations of nitrate and sediment TOC supports the utilization of
652 the genus as an indicator of coastal eutrophication, rightly considered to be an opportunistic taxa
653 in the FORAM index (Hallock et al., 2003) for monitoring of coastal habitats.

654 Benthic foraminifera are also widely used for tracing the impact of industry generated pollution
655 in coastal waters (Samir, 2000; Martínez-Colón et al., 2009). Potentially Toxic Elements (PTE)
656 acts as indicators of point source pollutions from industrial effluents and sewage water run-offs.
657 Incorporation of such PTEs during early development of foraminiferal tests leads to the
658 formation of morphologically abnormal tests that can be utilized for environmental monitoring
659 (Yanko et al., 1998; Martins et al., 2015). In the present study the number of such abnormal tests
660 were negligible ($<0.01\%$) which is congruent with the values of PTEs present in surface water.
661 Concentration of lead (Pb) was mostly below the 1 PPB permissible limit as mandated by the
662 CPCB, Govt. of India. Increased values were however observed during February (Max. 9.64
663 PPB, Min. 1.65 PPB), thus the occurrence of occasional loading of pollutants in the lagoon
664 cannot be ruled out. Presence of Pb is mostly associated with pollution originating from battery
665 run offs (Martínez-Colón et al., 2009) which may be realized by motorized boats that have
666 increased activity in the months of December-February stemming from tourism. *Ammonia* has
667 been reported to be particularly sensitive to increased concentrations of copper (Cu), showing the
668 development of abnormal chambers after being exposed for twenty days at 10 PPB and major
669 cessation of growth at 200 PPB (Le Cadre and Debenay, 2006). Cu concentrations in Chilika
670 lagoon can thus be considered bioavailable as for foraminifera as values > 10 PPB were observed
671 sporadically in the first eleven months and entirely in the last month of sampling. A major source
672 of Cu in coastal waters can originate from agricultural and anti-fouling agents used in painting
673 boats. Comparison of the present study with other studies from similar settings is however



difficult as data from surface water compartments are not considered in most published works as sedimentary concentrations are estimated more widely (Samir 2000, Martins et al. 2015, Schintu et al., 2015). A more detailed study regarding the concentrations of PTEs are required from the present setting to develop a more concrete understanding of the impacts of industrial pollution.

5 Conclusions

The present study investigated the environmental condition of a coastal lagoon reported to receive increased quantity of pollutants. The study was carried for a period of twelve months and also studied the utility of the globally present foraminiferal genera *Ammonia* as a potential indicator of increased pollutant loading. Findings of the present study indicate the ecological status of the lagoon to be eutrophic based on observed values of Redfield ratio. The inflow of freshwater during the monsoon appeared to be a major factor in controlling the nutrient load. Increased loading of dissolved nitrate present throughout the study period appears to be the major driving factor behind the eutrophication of the lagoon. Dissolved silicate, carried into the system via monsoonal flow is also present in large quantities and can potentially be the major reason for the primary production to be diatom dependent, as evident from characterizing the sedimentary TOC. Concentration of PTEs in the surface water was also indicated to be influenced by human activity, however further detailed investigation into the role of industrial pollution is warranted for a more composite view. The biotic proxy utilized revealed that live specimens of *Ammonia* provided greater significant variation with respect to surface water concentrations of nitrate and sedimentary TOC content as compared to total (live + dead) number of observed specimens. The observed assemblage of *Ammonia* in the lagoon did not display seasonal variation; however spatial variation did exist with respect to total number of specimens, thus indicating the utility of total foraminifer counts in establishing long term understandings of environments. The findings from the present study can be applied to better the understanding of ecological indicators that utilize the stress tolerant nature of *Ammonia* in coastal habitats. The findings can also be utilized in estimating the globally recognized problem of coastal eutrophication and its impact on coastal biota.

Author contribution



702 AS carried out execution of field collection, sample analysis, data analysis and manuscript
703 writing. PB designed the sampling strategy, analysis strategy and manuscript writing.

704 Competing interests

705 The authors declare that no competing interests exist for this study.

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966 List of Tables and Figures

967 Table 1. Values of Pearson's correlation co-efficient coefficient between the environmental and
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 969 bottom water fractions have been calculated by considering a sample size of $n = 72$, while values
 970 from pore water fractions have been calculated by using, $n = 54$. Significant values ($p \leq 0.05$)
 971 have been emboldened.

972 Table 2. Values of Pearson's correlation co-efficient between observed number of *Ammonia*
 973 specimens and studied parameters. Values from surface and bottom water fractions have been
 974 calculated by considering a sample size of $n = 72$, while values from pore water fractions have
 975 been calculated by using, $n = 54$. Significant values ($p \leq 0.05$) have been emboldened.

976 Figure 1. (a) Location of the study area with respect to the Mahanadi basin which is the major
 977 source of freshwater influx in the Chilika lagoon; (b) Location of the sampling stations with
 978 respect to the different rivers that flow into the lagoon that can act as potential point sources of
 979 pollutions.

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 985 sampling period.

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 989 classes of sediment particles have been grouped following Wentworth scale.

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 992 station during the sampling period.



993 Figure 8. Number of observed live and dead specimens of *Ammonia* spp. in 10 c.c. of surface (0-
994 2 cm) sediment.

995 Figure 9. Cluster analysis of *Ammonia* spp. assemblage using a Bray-Curtis similarity measure.
996 The numerical abundance of live and dead foraminifera were Log (X+1) transformed prior to the
997 analysis.

998 Figure 10. A comparison of N:P and N:Si ratios from all the studied compartments across the
999 sampling stations. DIN was calculated by combining the values of dissolved NO_3^- and dissolved
1000 NH_4^+ . Ratios from surface and bottom water fractions have been calculated by considering a
1001 sample size of $n = 72$, while values from pore water fractions have been calculated by using, $n =$
1002 54.

1003 Figure 11. A comparison of observed TOC values and $\delta^{13}\text{C}\text{‰}$ estimated from the surface (0-2
1004 cm) sediment column in order to generate an idea of spatial patterning of carbon in the lagoon
1005 bottom.

1006



Table 1. Values of Pearson’s correlation co-efficient between the environmental and nutrient parameters observed in the lagoon for the studied period. Values from surface and bottom water fractions have been calculated by considering a sample size of n = 72, while values from pore water fractions have been calculated by using, n = 54. Significant values ($p \leq 0.05$) have been emboldened.

	Salinity			pH			dis. O ₂			dis. NO ₃			dis. PO ₄ ³⁻			dis. SiO ₄			dis. NH ₄ ⁺		
	Surface Water	Bottom Water	Pore water	Surface Water	Bottom Water	Pore water	Surface Water	Bottom Water	Pore water	Surface Water	Bottom Water	Pore water	Surface Water	Bottom Water	Pore water	Surface Water	Bottom Water	Pore water	Surface Water	Bottom Water	Pore water
Salinity																					
Surface Water		0.81	0.72	0.77																	
Bottom Water																					
Pore water																					
Surface Water		0.05	-0.04	-0.01																	
Bottom Water		0.11	-0.01	0.05	0.83																
Pore water		-0.35	-0.25	-0.18	0.16	0.15															
Surface Water		0.10	0.04	0.06	0.01	0.01	-0.30														
Bottom Water		-0.01	-0.05	-0.03	-0.05	-0.07	-0.24	0.86													
Pore water		-0.22	-0.27	-0.20	0.08	0.02	-0.26	0.40	0.43												
Surface Water		0.17	0.16	0.13	0.22	0.28	0.02	0.04	0.10	0.07											
Bottom Water		0.09	0.07	-0.06	0.27	0.30	0.10	0.02	0.14	0.11	0.81										
Pore water		0.13	0.22	0.19	0.05	-0.04	0.10	-0.28	-0.23	-0.26	0.25	0.10									
Surface Water		0.11	0.32	0.18	-0.41	-0.36	0.19	-0.10	-0.21	-0.16	0.03	-0.09	0.03								
Bottom Water		0.01	0.05	0.14	-0.03	0.01	0.20	-0.04	-0.18	-0.13	-0.10	0.02	0.12	0.25							
Pore water		-0.16	-0.13	0.08	-0.16	-0.04	0.19	-0.29	-0.45	-0.38	0.05	0.05	0.16	0.24	0.24						
Surface Water		-0.56	-0.49	-0.65	-0.18	-0.34	0.35	-0.06	0.13	0.11	-0.39	-0.26	-0.07	-0.13	-0.23	-0.02					
Bottom Water		-0.49	-0.48	-0.57	-0.14	-0.28	0.22	0.04	0.17	0.30	-0.24	-0.18	-0.16	-0.03	-0.15	-0.08	0.78				
Pore water		-0.43	-0.48	-0.55	0.27	0.20	-0.11	0.03	0.06	0.51	-0.14	0.01	-0.28	-0.32	-0.25	-0.20	0.35	0.35			
Surface Water		-0.14	-0.12	0.11	0.02	0.12	0.16	0.32	0.27	0.03	-0.14	-0.15	-0.12	-0.07	-0.06	0.11	0.14	0.16	-0.01		
Bottom Water		-0.04	0.02	0.06	0.19	0.28	0.13	0.32	0.29	0.09	0.21	0.15	-0.07	-0.06	-0.18	-0.07	0.01	-0.05	0.18	0.34	
Pore water		-0.42	-0.43	-0.37	0.12	-0.14	0.20	-0.28	-0.22	-0.01	-0.24	-0.13	-0.08	0.01	-0.17	-0.02	0.62	0.01	0.40	-0.18	0.01

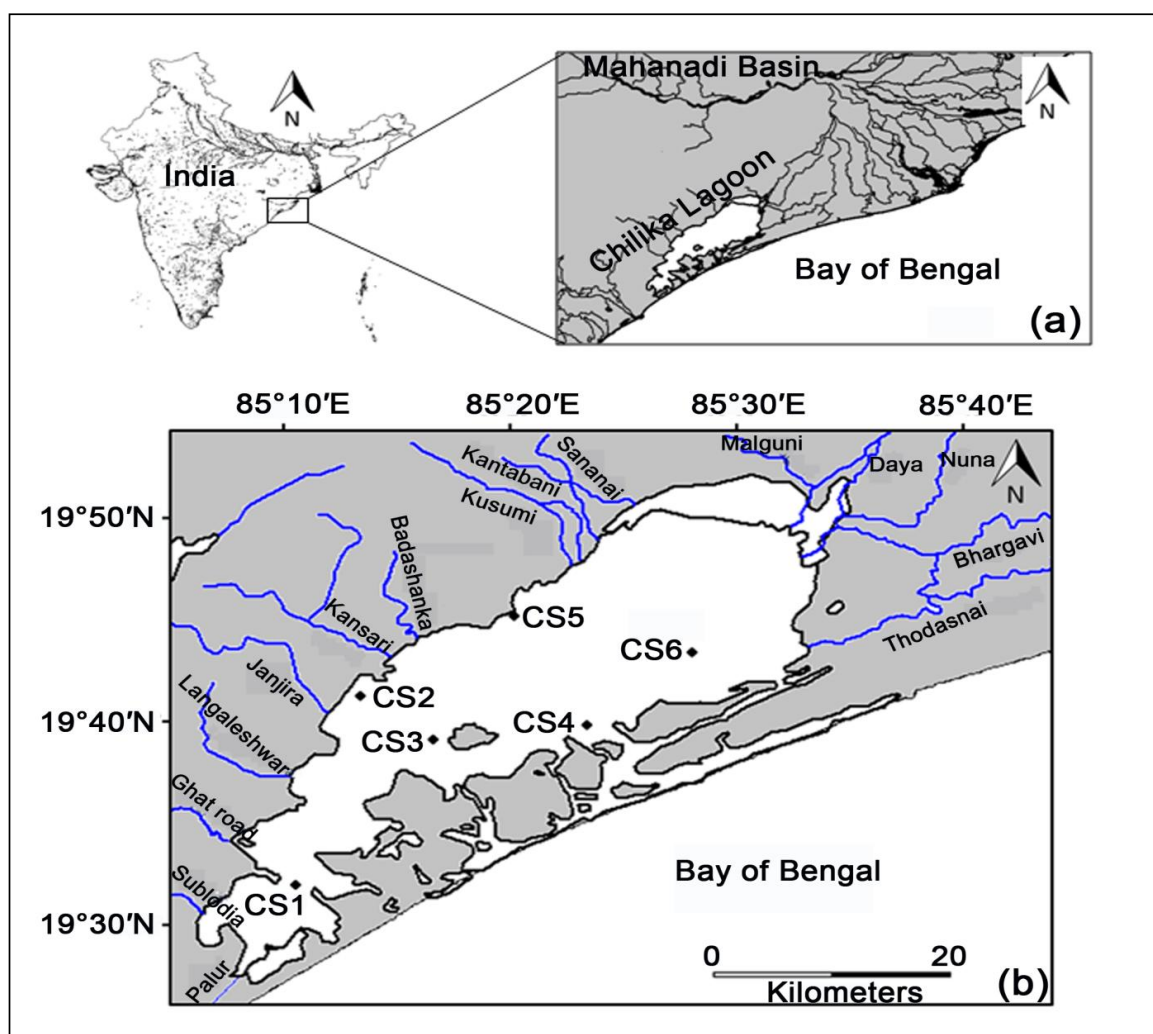


Table 2. Values of Pearson's correlation co-efficient between observed number of *Ammonia* specimens and the studied parameters. Values from surface and bottom water fractions have been calculated by considering a sample size of $n = 72$, while values from pore water fractions have been calculated by using, $n = 54$. Significant values ($p \leq 0.05$) have been emboldened.

		Live <i>Ammonia</i> spp.	Total <i>Ammonia</i> spp.
Salinity	Surface Water	-0.09	0.18
	Bottom Water	0.07	0.13
	Pore water	0.08	0.03
pH	Surface Water	-0.08	-0.11
	Bottom Water	-0.14	-0.11
	Pore water	0.10	-0.16
dis. O ₂	Surface Water	-0.18	-0.06
	Bottom Water	0.06	-0.04
	Pore water	-0.03	-0.39
dis. NO ₃ ⁻	Surface Water	0.23	0.21
	Bottom Water	0.29	0.21
	Pore water	0.20	0.04
dis. PO ₄ ³⁻	Surface Water	-0.04	0.02
	Bottom Water	-0.06	-0.02
	Pore water	-0.02	0.13
dis. SiO ₄ ⁻	Surface Water	0.10	-0.11
	Bottom Water	-0.07	-0.20
	Pore water	0.02	-0.17
dis. NH ₄ ⁺	Surface Water	-0.11	-0.14
	Bottom Water	-0.02	-0.02
	Pore water	0.24	-0.04
TOC		0.31	0.29



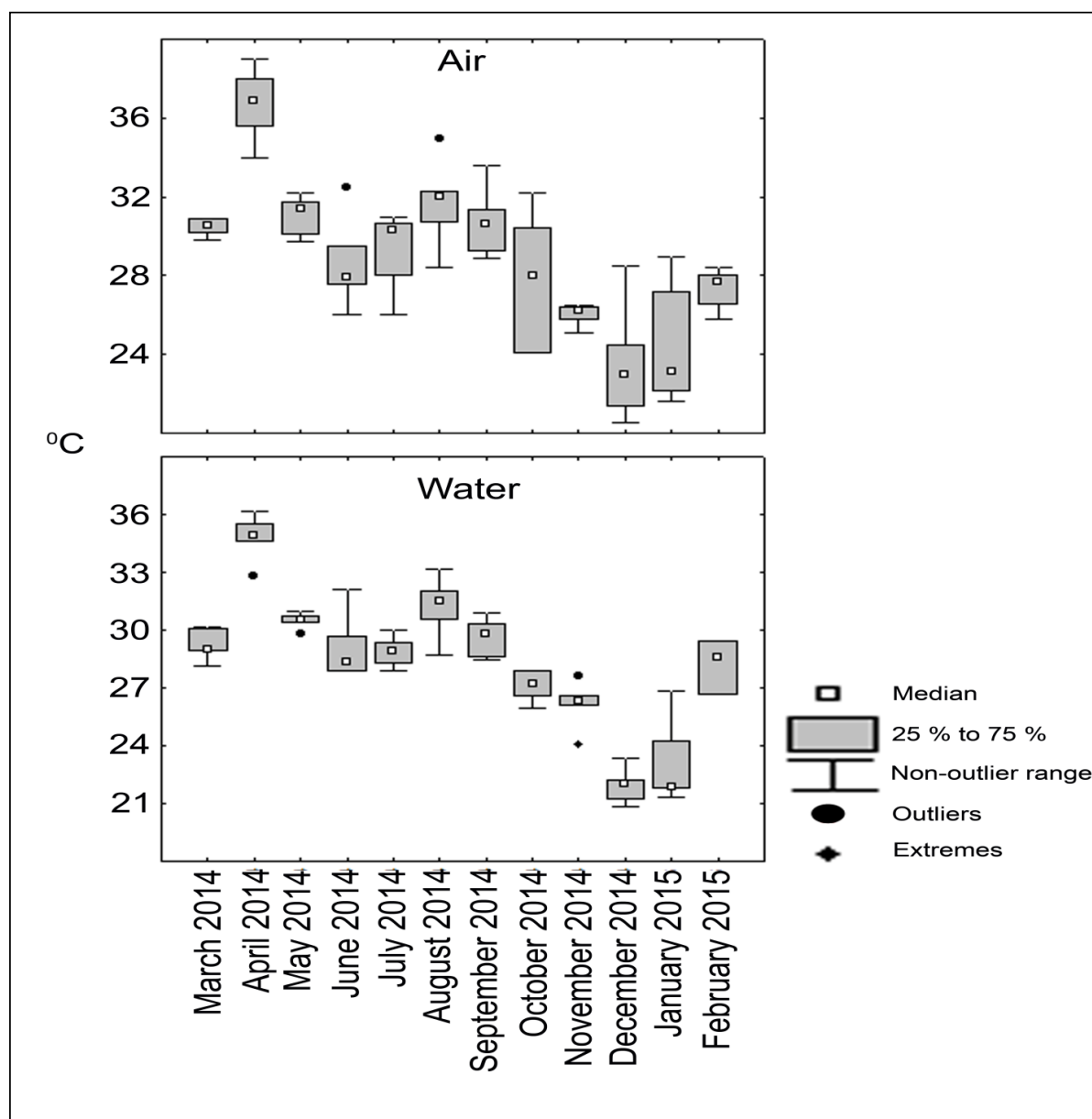
1017 Figure 1. (a) Location of the study area with respect to the Mahanadi basin which is the major source of
 1018 freshwater influx in the Chilika lagoon; (b) Location of the sampling stations with respect to the
 1019 different rivers that flow into the lagoon that can act as potential point sources of pollutions.



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1021 Figure 2. Box and whiskers plot depicting the variation of temperature (°C) in the lagoon observed
 1022 during the sampling period.



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Figure 3. Values of environmental parameters measured from the sampling stations during the sampling period.

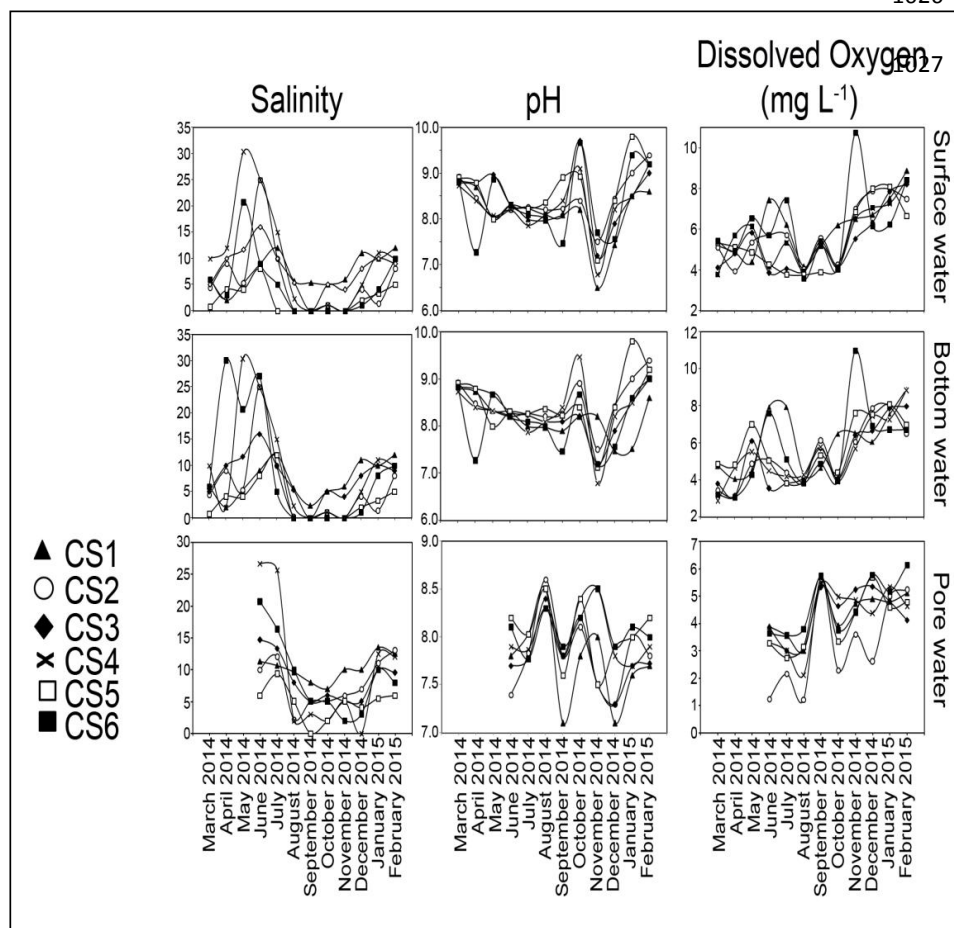




Figure 4. Concentrations of dissolved nutrients estimated from samples collected during the sampling period.

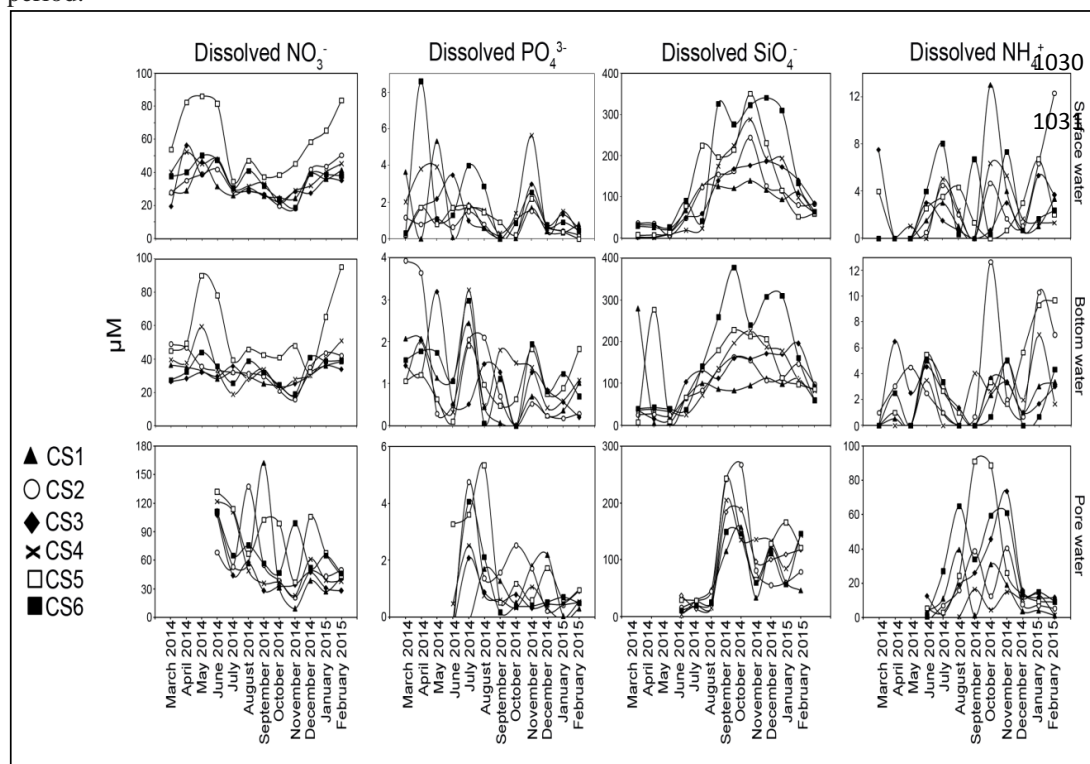




Figure 5. Box and whiskers plot depicting the variation in the concentration of the measured PTEs

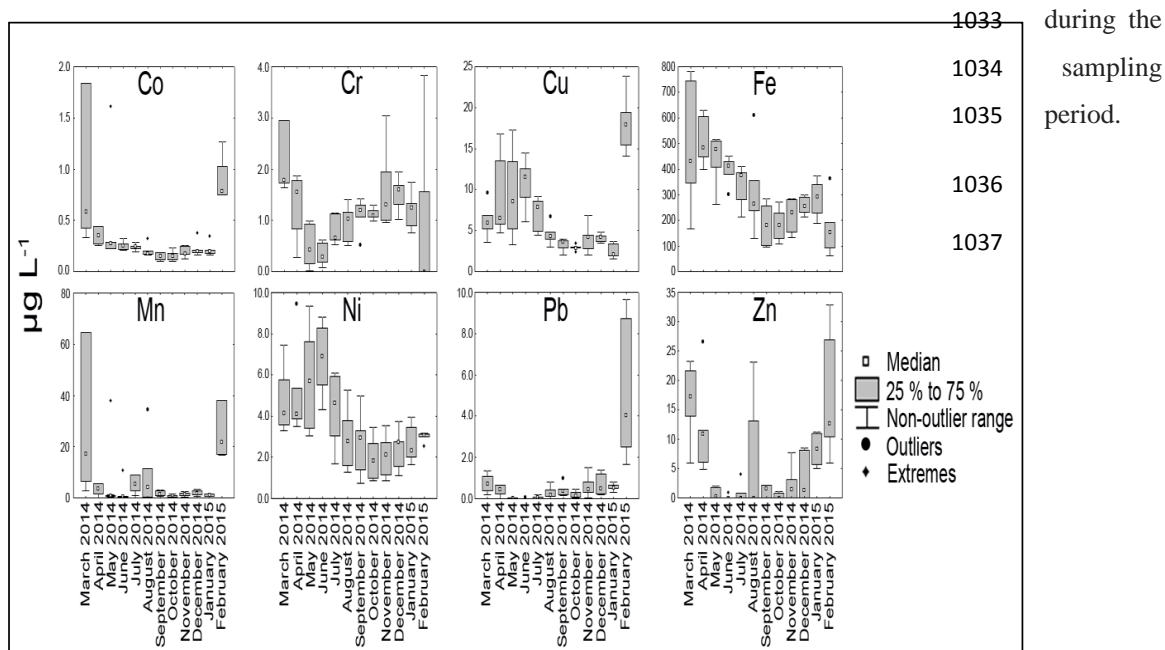




Figure 6. Sediment composition across the sampling stations in Chilika lagoon. Observed size classes of sediment particles have been grouped following Wentworth scale.

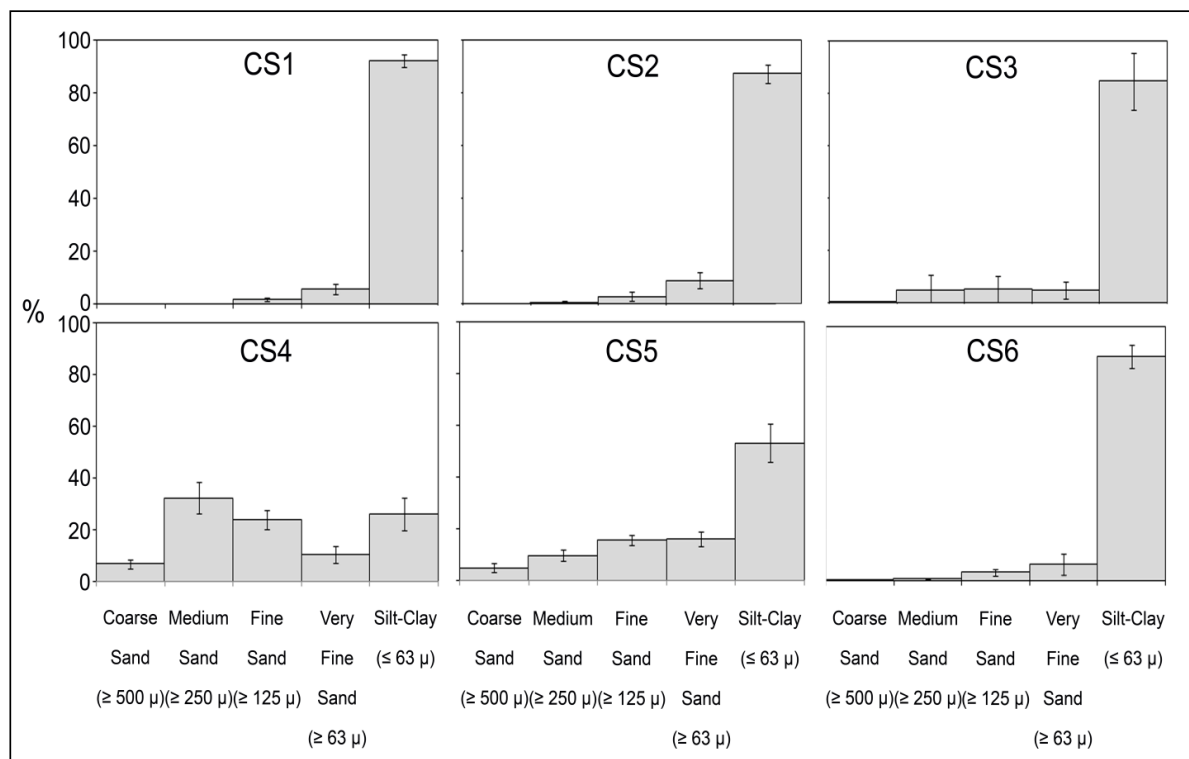
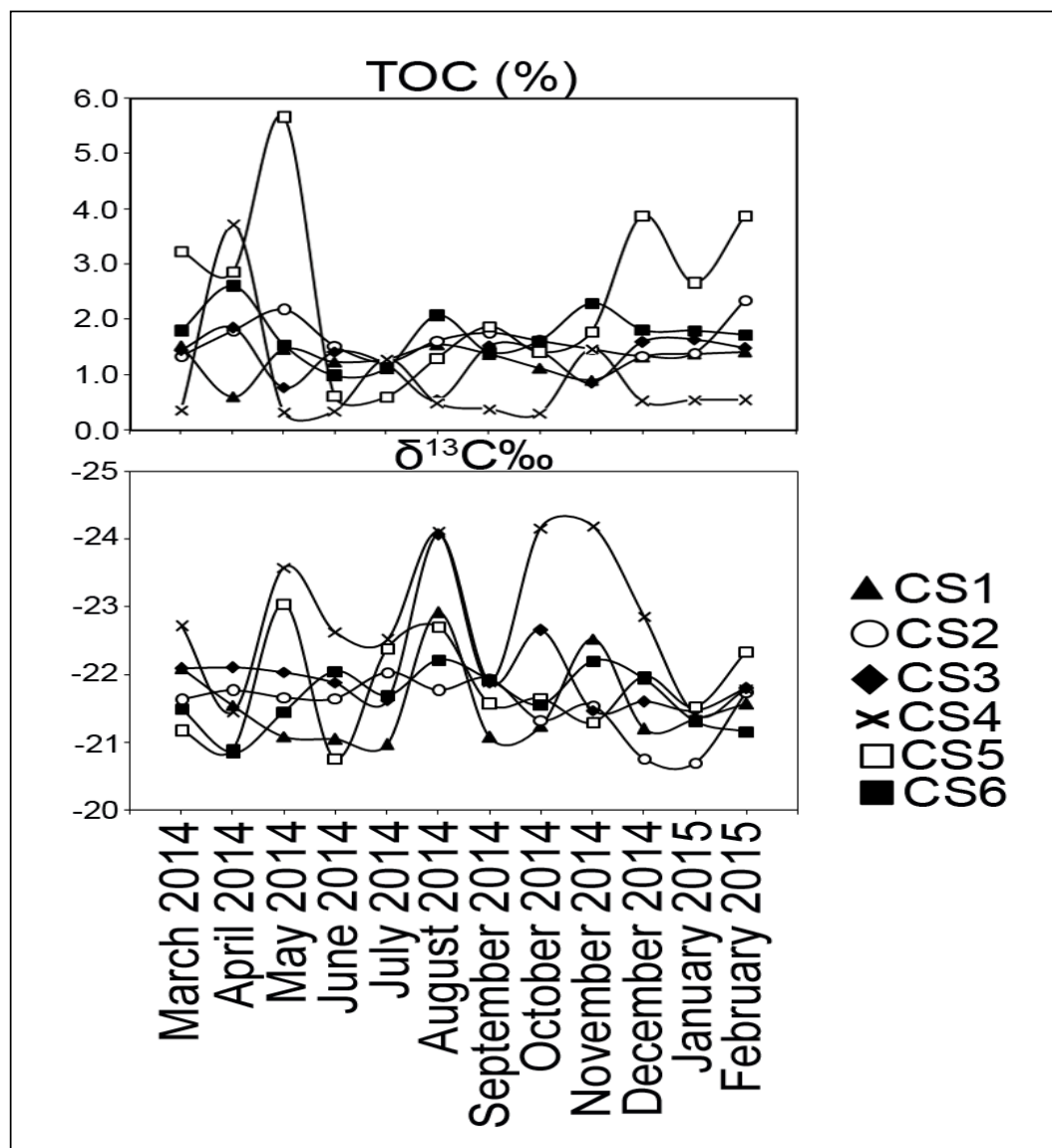




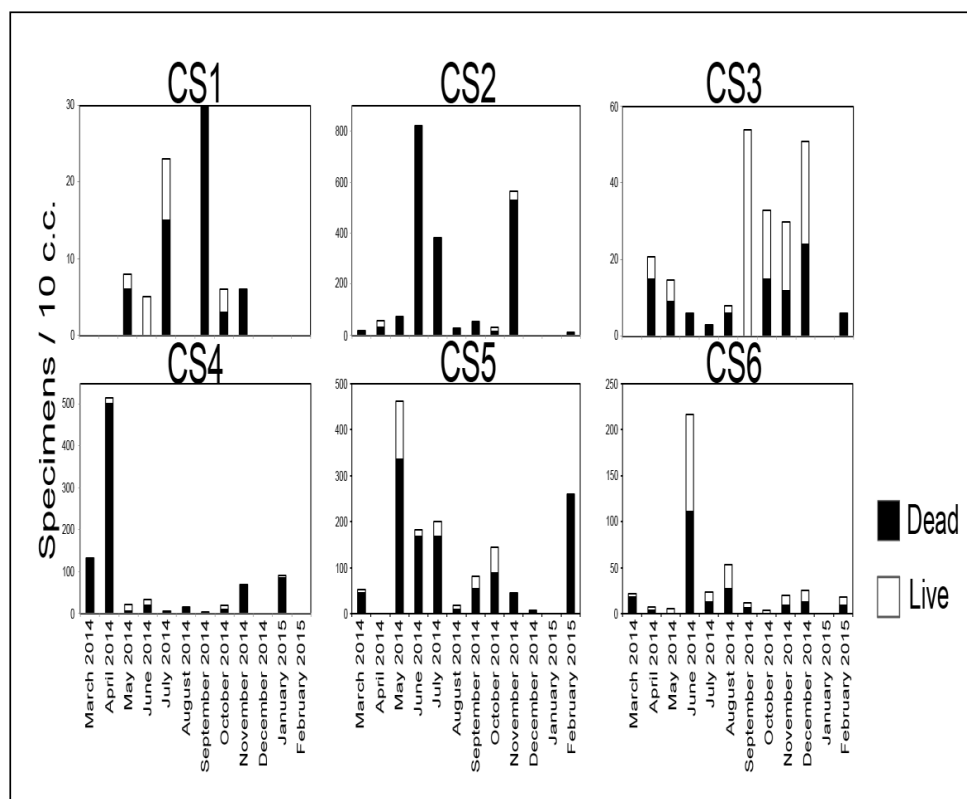
Figure 7. Characterization of sedimentary organic carbon in Chilika lagoon. Values of TOC and $\delta^{13}\text{C}$ ‰ estimated from the surface (0-2 cm) sediment column collected from each sampling station during the



sampling period.

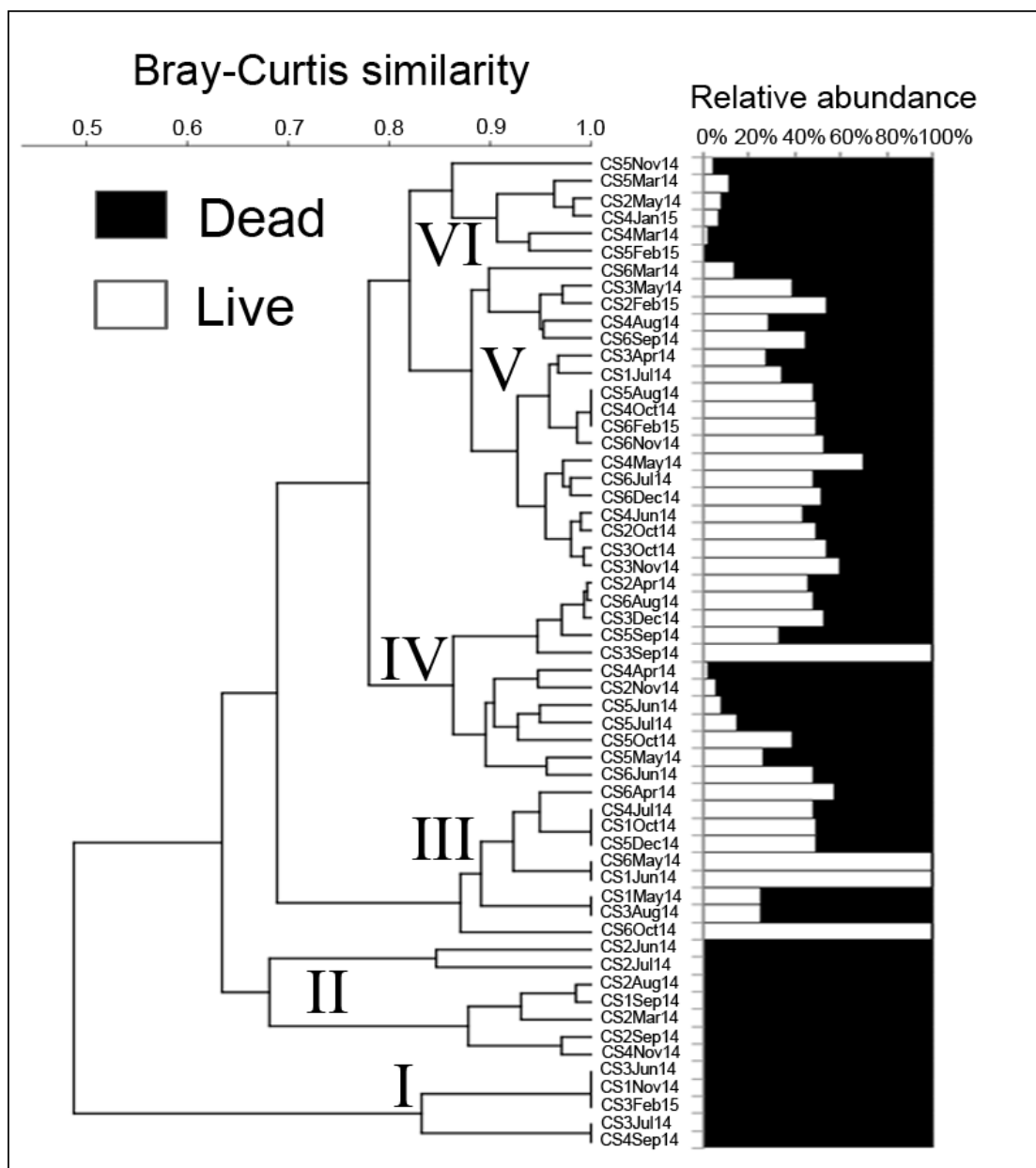


Figure 8. Number of observed live and dead specimens of *Ammonia* spp. in 10 c.c. of surface (0-2 cm) sediment.





1052 Figure 9. Cluster analysis of *Ammonia* spp. assemblage using a Bray-Curtis similarity measure. The
 1053 numerical abundance of live and dead foraminifera were Log (X+1) transformed prior to the analysis.

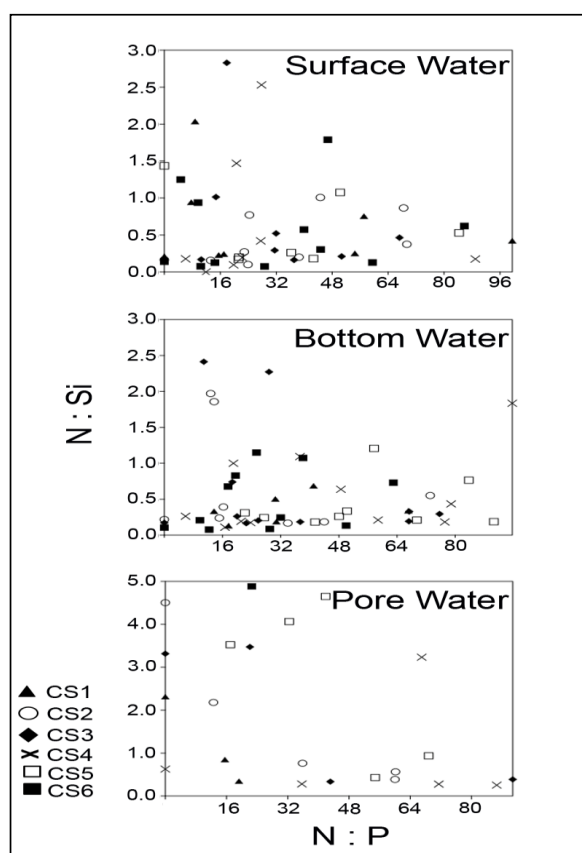


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Figure 10. A comparison of N:P and N:Si ratios from all the studied compartments across the sampling stations. DIN was calculated by combining the values of dissolved NO_3^- and dissolved NH_4^+ . Ratios from surface and bottom water fractions have been calculated by considering a sample size of $n = 72$, while values from pore water fractions have been calculated by using, $n = 54$.





1077 Figure 11. A comparison of observed TOC values and $\delta^{13}\text{C}\text{‰}$ estimated from the surface (0-2 cm)
 1078 sediment column in order to generate an idea of spatial patterning of carbon in the lagoon bottom.

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