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TAXONOMIC INVESTIGATION OF EUPLANKTONIC DIATOM COMMUNITIES AS INDICATOR OF COPPER IN THE BANK OF THE SUBARNAREKHA RIVER, GHATSHILA, JHARKHAND, INDIA

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ABSTRACT

The aim of this study was to demonstrate and evaluate the diatom communities in the copper infected areas readily associated with the Hindustan Copper Limited (HCL) at the bank of the Subarnarekha River. This study was based on three sampling sites commonly designated as high copper (>100 $\mu g.L^{-1}$), medium copper (≤100 $\mu g.L^{-1}$) and low copper (≤50 $\mu g.L^{-1}$) contaminated area. Results indicated the detailed taxonomic description of 31 species that are dominant or less dominant over these contaminated area. Among the identified taxa, 10 were recorded as new to the Jharkhand state. Water analysis has suggested the presence of 17 species in the high copper contaminated area adjacent to HCL. Nine species was less dominant in the outlet of HCL that belonged to the medium contaminated and only 5 species were dominant over the low copper contaminated area. Physico-chemical parameters like pH, air and water temperature, salinity, conductivity, light extinction coefficient, turbidity, dissolved inorganic salts, dissolved oxygen and carbon-di-oxide, biological oxygen demand and total hardness were also estimated in the copper contaminated sites. Relatively all the species of *Cymbella* and *Navicula* were associated with high copper accumulation. Most interestingly, one harmful species *Halamphora coffeiformis,* which was recorded as most dominant species in high copper exposed area, has shown to be the best copper tolerant and copper indicator species.

Keywords: Diatom, copper, indicator, Ghatshila, HCL, taxonomy, tolerant, new record

INTRODUCTION

Biosorption is a well-known method of phytoremediation, which binds the toxic chemicals or metals and accumulates it in their biological systems especially in cellular structures. The process is widely used to encourage the remediation of heavy metals from the aquatic ecosystems. It also has the potential towards wastewater treatment. Metal toxicity in aquatic ecosystems is commonly triggered by anthropogenic activities including domestic and industrial wastewater, agricultural runoff and dumping of toxic chemicals, e-waste and others (**Satpati, 2021**). The deposition of toxic elements or trace metals in the water bodies resulting in severe environmental impacts including contamination of surface and ground water and increasing the rate of biomagnification (**Sbihi *et al*., 2014; Satpati, 2021**). Trace metals like copper (Cu) is a well known aquatic pollutant for its adverse affects on phytoplanktons, especially diatoms (**Absil, Kroon & Wolterbeek, 1994**). The heavy presence of Cu in the aquatic food chains may be hazardous to the associated living organisms and to the environment (**Nor, 1987**). Aquatic living systems may scavenge the trace metals from the water column as well as from the bottom sediments or from both. Recently, algae have served as the most potential aquatic living system or bioindicator for accumulating toxic metals (**Zeraatkar *et al*., 2016**).
Diatoms belong to the group of Bacillariophyta (**Guiry in Guiry & Guiry, 2021**, AlgaeBase), which are frequently used as bioindicators for heavy metals in aquatic bodies. They are unicellular having silicified cell wall. The cell wall consists of two valves held together by a band of girdle. Most of the studies have been done so far on taxonomic documentation. In India, there are many reports on the freshwater diatom flora with detailed taxonomic account (**Gandhi, 1959, 1967; Bhakta *et al*., 2011; Das & Adhikary, 2012; Dwivedi & Misra, 2015; Bhakta, Das & Adhikary, 2016; Bose, Bar & Pal, 2017**). Only few reports on the metal toxicity in diatoms are available. **Pandey *et al*. (2014)** have studied the morphological changes of few diatoms exposed to Cu, lead (Pb) and zinc (Zn). Modification of raphe was found more frequent in *Fragilaria capucina, Gomphonema parvulum, Nitzschia palea, Pinnularia conica* and *Ulnaria ulna*. As diatoms are planktonic, they remain in the open water systems rather in the sediment (**Cattaneo *et al*., 2011**). Diatoms are ecologically diverse from centric to pennate form and found in almost all microhabitats in the aquatic ecosystems (**Arguelles, 2019**). Diatom assemblages can be formed in the open water systems of rivers, lakes and canals (euplanktons), they may be associated with plants (epiphytic), they may be found in the sand (epipsammon), or mud (epipelon) and even in animals (epizooic) (**Dixit *et al*., 1992; Satpati *et al*., 2017; Arguelles, 2020**).
In the present research, the work has been carried out on the taxonomic investigation of some euplanktonic diatoms, which frequently dominate over the Cu mining area. Hindustan Copper Limited (HCL), situated at the bank of the Subarnarekha River of Ghatshila, is the biggest source of Cu discharge in the surrounding aquatic habitats. Cu mining wastes flow directly into the river without any treatment, resulting in significant growth of diatoms and other planktonic organisms. The sampling sites were chosen on the basis of high, medium and low Cu contamination. Diatom assemblages of these sites were identified and described in detail in relation to abundance. The dominant species from the three different sampling stations were marked on the basis of abundance. Physico-chemical parameters like nitrate, phosphate, silicate, sulphate, calcium, dissolved oxygen (DO), biological oxygen demand (BOD), conductivity, salinity and pH were also recorded in the present study.
The objective of this study was to determine the diversity of diatom flora as indicator of Cu in the adjoining water bodies of HCL and Subarnarekha River. In addition, the abundance of the diatom species in terms of low, medium and high Cu accumulation were also examined. The detailed taxonomic description suggests the proper identification of the euplanktonic diatoms as pollution indicator in aquatic ecosystems. The biochemical assessment of the water has also determined the water quality in the adjacent water bodies of HCL and Ghatshila.

MATERIAL AND METHODS

Sampling sites

For the collection of diatom and water samples, four sites were chosen: canal adjacent to HCL, outlets of HCL poured into the Subarnarekha River and the river itself commonly designated as Station 1 (**22.5954° N, 86.4519° E**), Station 2 (**22.5962° N, 86.4522° E**) and Station 3 (**21.3325° N, 87.2341° E**) respectively. All sampling stations are situated in Ghatshila, Jharkhand (Figure 1).

Figure 1 Google satellite image showing three sampling stations (https://www.google.com/maps/search/ghatshila,+hindustan+copper+limited/@22.5911511,86.4454325,4927m/data=!3m1!1e3)

Diatom collection and preservation

The copper containing sites associated to Subarnarekha River was investigated in March 2018. The diatom sample was collected through the phytoplankton net of mesh size 25 µm (**Satpati & Pal, 2017**). After collection, the turbid sample was brought to the laboratory and centrifuged at 10000 rpm for 10 minutes. The pellet thus collected was preserved in 4% (v/v) formalin for the microscopic study. All the preserved materials were assigned to Calcutta University Herbarium (CUH) voucher specimens.

Water analyses

Water samples were collected in triplicates at the depth of 0.5 m. The physico-chemical parameters were determined with the help of the filtrate obtained from the water samples. All parameters like pH, temperature, electrical conductivity, total hardness, light extinction coefficient, BOD, salinity, nitrate, phosphate, silicate, sulphate and calcium were analyzed using the standard methods of APHA (**APHA, 1998**). Salinity, pH and temperature were recorded immediately after sampling with ERMA Refractometer (ERMA, Tokyo), Beckman potentiometer zeromatic II and centigrade thermometer respectively. DO content in water sample was estimated in situ following Winkler's Iodometric titration method (**Winkler, 1888**).

Light Microscopy and Identification

For light microscopy study, slides were prepared with 20% glycerin (v/v) and photographs were taken under Carl Zeiss Axioster Plus Microscope by Cannon Power Shot 500D Camera with a coupled micrometer eyepiece (**Satpati *et al*., 2012, 2013; Satpati & Pal, 2016**). The identification of the species was done using the literature of **Hustedt (1930)**, **Hendey (1974)**, **Aboal *et al*. (2003)**, **Levkov (2009)**, **Wang *et al*. (2014)**, **Stepanek & Kociolek (2018)** etc. The classification system was based on the recent up gradation given in AlgaeBase (**Guiry in Guiry & Guiry, 2021**).

Cu accumulation study

Cu accumulated in water samples were analyzed using an ICP 2070 Spetrophotometer (Baired, USA) and AAS using a Varian Spectr AA10 apparatus with Graphite Tube Atomizer GTA-95 (Victoria, Australia). The measurement accuracy was checked by the reference of **Chmielewska & Medved (2001)**.

RESULTS

Water analyses

Water analysis report is demonstrated in table 1. During the study, all the sampling stations showed a static air temperature but slightly varied in water temperature. Water temperature was recorded minimum in station 1 with 28.35°C whereas highest in Subarnarekha River (station 3) with 30.33°C. pH ranges from slightly acidic (below 7.0) to slightly alkaline (above 7.0). The water pH of the canal adjacent to HCL (station 1) was recorded 6.8. However pH was recorded highest (7.3) in Subarnarekha River. High turbid condition of the water was noticed in station 1 followed by station 2 and 3. Electrical conductivity and light extinction coefficient was significantly decreased in the order station 3>station 2> station 1. Highest conductivity recorded in Subarnarekha River was 560.33 µ S.cm^{-1}. Total hardness varied from 260.33 in station 1 to 180.35 in station 3. Salinity was recorded highest (10 ppt) in station 1 and lowest (6 ppt) in station 3. Comparatively DO was highest in station 3 with 3.42 mg.L^{-1} and lowest in station 1 with 2.21 mg.L^{-1}. However, station 1 recorded highest amount of dissolved CO_2 and BOD instead of station 2 and 3. Interestingly nitrate, sulphate and silicate level in the water was high in station 1, which was highly polluted and found adjacent to HCL. Phosphate level in the water of Subarnarekha River was recorded highest (0.84 mg.L^{-1}) and lowest (0.57 mg.L^{-1}) in the outlet of HCL poured directly into the river. Relatively the concentration of the calcium was high in station 3 and low in station 1. Accumulation of Cu in the water body was recorded highest in station 1 (400 µg.L^{-1}) and lowest in station 3 (47.87 µg.L^{-1}).

Diatom composition

In the present study, a total number of 31 species were investigated, which belong to 16 families, 10 orders under the class Bacillariophyceae. The diatom composition has suggested the dominance of *Cymbella* with 6 species followed by 3 species each of *Nitzschia* and *Rhopalodia* in the Cu contaminated area. Two species each from the genus *Navicula*, *Pinnularia*, *Amphora* and *Synedra* were also documented from the study sites. A large number of species were documented as Cu indicator or tolerant in Station 1, closely associated canal of HCL. From Table 2 it can be obtained that, 17 species are rich in Cu in Station 1 of which *Halamphora coffeiformis* was found to be most dominant over the area. Similarly this species was absent in station 2 and 3. Interestingly all species of *Cymbella* and *Navicula* were reported as high Cu tolerant species (Table 2). In station 2, nine diatom species were dominant of which both the species of *Pinnularia*, *P. acrosphaeria* and *P. viridis* showed positive response to Cu accumulation. Two species each of *Rhopalodia* and *Nitzschia* were recommended as Cu tolerant species in Station 2. However in station 3 only 5 species dominated as Cu tolerant upto 50 µg.L^{-1}. Both the species of *Synedra*, *S. ulna* and *S. ulna* var. *amphirhynchus* were designated as Cu indicator species in Subarnarekha River. Among the identified diatom species, 10 species viz., *Halamphora coffeiformis, Rhopalodia gibberula, Mastogloia smithii* var. *lacustris, Nitzschia nana, Himantidium minus, Synedra ulna* var. *amphirhynchus, Fragilaria intermedia* var. *robusta, Grammatophora undulata, Diatoma mesodon* and *Ctenophora pulchella* were recorded as new to the Jharkhand State.

Table 1 Geospatial and physico-chemical parameters of the Cu contaminated sites

Geospatial and Physico-chemical parameters	Study sites		
	Station 1 (High Cu, (>100 µg.L^{-1})	**Station 2 (Medium Cu, (≤100 µg.L^{-1})**	**Station 3 (Low Cu, (≤50 µg.L^{-1})**
Coordinates	22.5954° N, 86.4519° E	22.5962° N, 86.4522° E	21.3325° N, 87.2341° E
pH	6.8	7.1	7.3
Air temperature (°C)	32.33	32.12	32.32
Water temperature (°C)	28.35	29.16	30.33
Turbidity (NTU)	32.22	26.33	24.21
Electrical conductivity (µ.S. cm^{-1})	485.31	520.22	560.33
Light extinction coefficient (m)	1.39	1.42	1.46
Total hardness	260.33	220.22	180.35
Salinity (ppt)	10	8	6
Dissolved oxygen (mg.L^{-1})	2.21	2.32	3.42
Dissolved CO_2 (mg.L^{-1})	10.22	8.73	7.54
Biological oxygen demand (mg.L^{-1})	8.83	7.43	4.45
Nitrate (mg.L^{-1})	1.83	1.74	0.89
Phosphate (mg.L^{-1})	0.76	0.57	0.84
Sulphate (mg.L^{-1})	62.32	54.48	45.22
Silicate (mg.L^{-1})	74.44	63.22	51.51
Calcium (mg.L^{-1})	10.42	12.55	14.75
Copper (µg.L^{-1})	400	94.62	47.87

Taxonomic description
Phylum: Bacillariophyta
Subphylum: Bacillariophytina
Class: Bacillariophyceae
Subclass: Bacillariophycidae
Order: Naviculales
Suborder: Neidiineae
Family: Diadesmidaceae
Genus: *Diadesmis*
1. *Diadesmis confervacea* Kützing [Figures 2a-b]
Aponte, Maidana & Lange-Bertalot, 2005; Slate & Stevenson, 2007; Miscoe *et al*., 2016; Li & Qi, 2018
Frustules are 2-4 times longer than broad, 10-30 µm long and 5-8 µm broad, rectangular in girdle view, frustules attached side by side to form ribbon shaped colony; striae not distinct under compound microscope.
Voucher no.: CUH/PLANK/DIA- 29/1
Family: Neidiaceae
Genus: *Neidium*
2. *Neidium affine* var. *amphirhynchus* (Ehrenberg) Cleve [Figure 2c]
Hustedt, 1930; Patrick & Reimer, 1966; Eberle, 2008
Basionym: *Navicula amphirhynchus* Ehrenberg
Valves 7-8 times longer than broad, 50-70 µm long and 7-10 µm broad, lanceolate with wide central area with rounded apices.
Voucher no.: CUH/PLANK/DIA- 29/2
Suborder: Naviculineae
Family: Naviculaceae
Genus: *Navicula*
3. *Navicula viridula* (Kützing) Ehrenberg [Figure 2d]
Hendey, 1974; Hofmann, Werum & Lange-Bertalot, 2013; John, 2018
Basionym: *Frustulia viridula* Kützing
Valves 9-10 times longer than broad, 55-65 µm long and 6-8 µm broad, linear to lanceolate with capitate ends with narrow axial area and wide central area. Striations are not clear under compound microscope.
Voucher no.: CUH/PLANK/DIA- 29/3
4. *Navicula viridula* var. *rostellata* (Kützing) Cleve [Figure 2e]
Patrick & Reimer, 1966; Hofmann, Werum & Lange-Bertalot, 2013
Basionym: *Navicula rostellata* Kützing
Valves narrow, elliptic, lanceolate with short narrowly produced rostrate ends, 4-5 times longer than broad, 38-45 µm long and 9-10 µm broad; axial area narrow and central area big, rounded; striations delicate, radial, approximately 10-12 in 10 µm area.
Voucher no.: CUH/PLANK/DIA- 29/4
Family: Amphipleuraceae
Genus: *Halamphora*
5. *Halamphora coffeiformis* (C. Agardh) Mereschkowsky [Figure 2f]
Levkov, 2009; Wang *et al*., 2014; Stepanek & Kociolek, 2018
Basionym: *Frustulia coffeiformis* C. Agradh

Table 2 List of Cu indicating diatoms in three distinct sites (+++ Most dominant, >70% of the population; ++ Dominant, 40-70% of the population; + Less dominant, <40% of the population; - Absent, No species found)

Name of the taxa	Cu contaminated area		
	Station 1-High Cu (>100 µg L^{-1})	**Station 2- Medium Cu (≤100 µg L^{-1})**	**Station 3- Low Cu (≤50 µg L^{-1})**
1. *Diadesmis confervacea* Kützing	++	+	-
2. *Neidium affine* var. *amphirhynchus* (Ehrenberg) Cleve	++	+	-
3. *Navicula viridula* (Kützing) Ehrenberg	++	-	-
4. *Navicula viridula* var. *rostellata* (Kützing) Cleve	++	+	-
5. *Halamphora coffeiformis* (C. Agardh) Mereschkowsky	+++	-	-
6. *Pinnularia acrosphaeria* W. Smith	-	++	+
7. *Pinnularia viridis* (Nitzsch) Ehrenberg	-	++	-
8. *Rhopalodia gibba* (Ehrenberg) O. Müller	++	+	+
9. *Rhopalodia gibberula* (Ehrenberg) O. Müller	-	++	-
10. *Rhopalodia operculata* (C. Agardh) Håkanasson	-	++	+
11. *Achnanthes exigua* Grunow	++	+	-
12. *Mastogloia smithii* var. *lacustris* Grunow	+	++	-
13. *Nitzschia obtusa* var. *scalpelliformis* (Grunow) Grunow	+	++	-
14. *Nitzschia nana* Grunow	+	++	-
15. *Nitzschia acicularis* (Kützing) W. Smith	-	+	++
16. *Amphora elliptica* (C. Agardh) Kützing	-	+	++
17. *Amphora ovum* Cleve	++	+	-
18. *Himantidium minus* Kützing	-	+	++
19. *Synedra ulna* (Nitzsch) Ehrenberg	-	+	++
20. *Synedra ulna* var. *amphirhynchus* (Ehrenberg) Grunow	-	+	++
21. *Fragilaria intermedia* var. *robusta* G. S. Venkataraman	+	++	-
22. *Diatoma mesodon* (Ehrenberg) Kützing	++	+	-
23. *Cymbella ehrenbergii* Kützing	++	+	-
24. *Cymbella affinis* Kützing	++	+	-
25. *Cymbella oliffii* Cholnoky	++	+	-
26. *Cymbella cistula* (Ehrenberg) O. Kirchner	++	-	+
27. *Cymbella turgidula* Grunow	++	-	-
28. *Cymbella tumida* (Brébisson) Van Heurck	++	-	+
29. *Gomphonema lanceolatum* Ehrenberg, nom. illeg.	++	-	+
30. *Grammatophora undulata* Ehrenberg	++	+	-
31. *Ctenophora pulchella* (Ralfs ex Kützing) D. M. Williams & Round	+	++	-

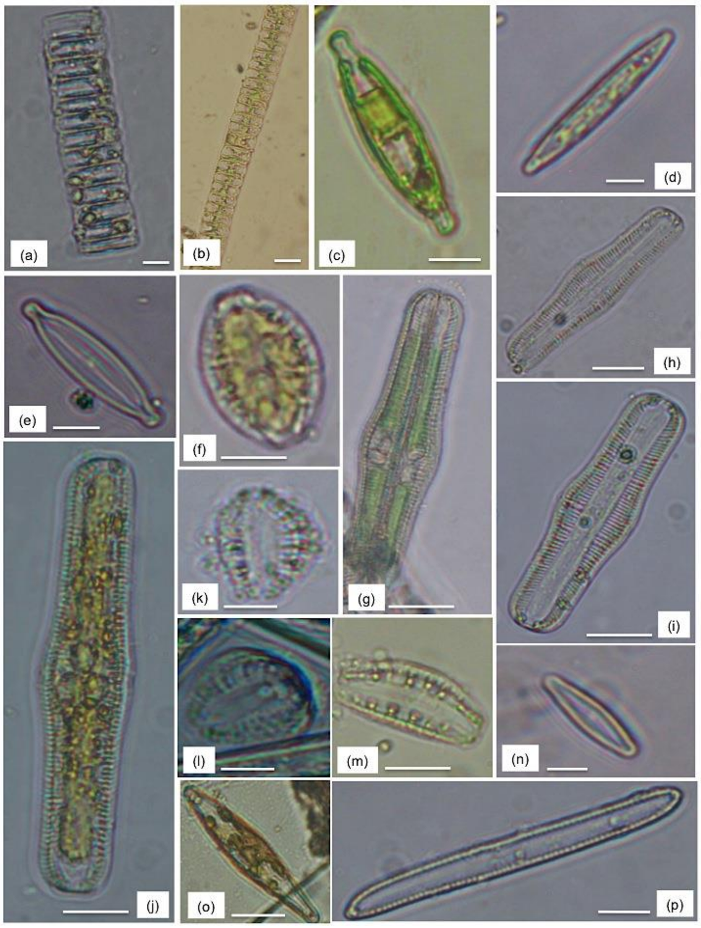

Figure 2 Light micrographs of (a-b) *Diadesmis confervacea* Kützing. (c) *Neidium affine* var. *amphirhynchus* (Ehrenberg) Cleve. (d) *Navicula viridula* (Kützing) Ehrenberg. (e) *Navicula viridula* var. *rostellata* (Kützing) Cleve. (f) *Halamphora coffeiformis* (C. Agardh) Mereschkowsky. (g) *Pinnularia acrosphaeria* W. Smith. (h) *Pinnularia viridis* (Nitzsch) Ehrenberg. (i-j) *Rhopalodia gibba* (Ehrenberg) O. Müller. (k-l) *Rhopalodia gibberula* (Ehrenberg) O. Müller. (m) *Rhopalodia operculata* (C. Agardh) Håkanasson. (n) *Achnanthes exigua* Grunow. (o) *Mastogloia smithii* var. *lacustris* Grunow. (p) *Nitzschia obtusa* var. *scalpelliformis* (Grunow) Grunow. (Scale bar a-e, g, i-l, o-p: 10 µm; f, n: 5 µm; h, m: 20 µm)

Valves semi-lanceolate, dorsal margins, ventral linear, slightly concave with rostrate or capitate apices, 4-6 times longer than broad, 30-50 µm long and 4-12 µm broad; raphe straight, excentric; striae dorsal, coarse, radiate, 8-12 in 10 µm area.

Voucher no.: CUH/PLANK/DIA- 29/5
Suborder: Sellaphorineae
Family: Pinnulariaceae
Genus: *Pinnularia*

6. *Pinnularia acrosphaeria* W. Smith [Figure 2g]
Hustedt, 1930; Proschkina-Lavrenko, 1950; Kulikovskiy *et al*., 2016
Valves linear, gibbous in the middle and at the ends, axial area broad linear and central area punctate, 4.5 to 5.5 times longer than broad, 38-62 µm long and 8-12 µm broad; striations nearly parallel and slightly radial at the ends, striae 9-12 in 10 µm area.
Voucher no.: CUH/PLANK/DIA- 29/6

7. *Pinnularia viridis* (Nitzsch) Ehrenberg [Figure 2h]
Cleve, 1895; Hu & Wei, 2006; Hofmann, Werum & Lange-Bertalot, 2013;
Synonym and Basionym: *Bacillaria viridis* Nitzsch
Valves linear with slightly convex margins and rounded ends, axial area narrow and central area is slightly widened, 5-7 times longer than broad, 90-140 µm long and 18-20 µm broad; raphe complex; striations coarse, slightly radial in the middle and convergent at the ends, striae 7-9 in 10 µm area.
Voucher no.: CUH/PLANK/DIA- 30/1
Order: Rhopalodiales
Family: Rhopalodiaceae
Genus: *Rhopalodia*

8. *Rhopalodia gibba* (Ehrenberg) O. Müller [Figures 2i-j]
Aboal *et al*., 2003; Jahn & Kusber, 2004; Cocquyt, Kusber & Jahn, 2018
Basionym: *Navicula gibba* Ehrenberg
Frustules linearly lanceolate with cuneate apices and inflated center, 7-12 times longer than broad, 45-140 µm long and 6-12 µm broad; striae distinct 12-18 in 10 µm.
Voucher no.: CUH/PLANK/DIA- 30/2

9. *Rhopalodia gibberula* (Ehrenberg) O. Müller [Figures 2k-l]
Hustedt, 1930; Proschkina-Lavrenko, 1950; John, 2018
Basionym: *Eunotia gibberula* Ehrenberg
Frustules long, elliptical with rounded ends, 1.5 times longer than broad, sometimes as long as broad, 30-42 µm long and 25-30 µm broad, dorsal side highly convex; costae 3-4 in 10 µm area.
Voucher no.: CUH/PLANK/DIA- 30/3

10. *Rhopalodia operculata* (C. Agardh) Håkanasson [Figure 2m]
Ruck *et al*., 2016; John, 2018
Basionym: *Frustulia operculata* C. Agardh
Valve linear, solitary, lanceolate or elliptic with wide rounded apices, 3 times longer than broad, 55-60 µm long and 22-24 µm broad; striae transverse, wide apart from each other.
Voucher no.: CUH/PLANK/DIA- 30/4
Order: Mastogloiales
Family: Achnanthaceae
Genus: *Achnanthes*

11. *Achnanthes exigua* Grunow [Figure 2n]
Patrick & Reimer, 1966; Krammer & Lange-Bertalot, 2004; Hofmann, Werum & Lange-Bertalot, 2013
Frustules narrow, rectangular in girdle view, forming short chains; valves narrowly lanceolate with clearly convex margins and rostrate apices; valves 5-15 µm long and 3-5 µm broad; striae is not clearly visible under compound microscope.
Voucher no.: CUH/PLANK/DIA- 30/5
Family: Mastogloiaceae
Genus: *Mastogloia*

12. *Mastogloia smithii* var. *lacustris* Grunow [Figure 2o]
Proschkina-Lavrenko, 1950; Lee *et al*., 2014
Valve linear, elliptical and constricted, two narrow ends with central broad area, 4 times longer than broad, 31-32 µm long and 7.5-8.5 µm broad; striations are not clearly visible under compound microscope.
Voucher no.: CUH/PLANK/DIA- 30/6
Order: Bacillariales
Family: Bacillariaceae
Genus: *Nitzschia*

13. *Nitzschia obtusa* var. *scalpelliformis* (Grunow) Grunow [Figure 2p]
Hustedt, 1930; Proschkina-Lavrenko, 1950
Basionym: *Nitzschia scalpelliformis* Grunow
Valves linear, 10-14 times longer than broad, 80-100 µm long and 6-10 µm broad, apices bend in opposite direction, margins parallel; striae fine 25-30 in 10 µm.
Voucher no.: CUH/PLANK/DIA- 31/1

14. *Nitzschia nana* Grunow [Figure 3a]
Hofmann, Werum & Lange-Bertalot, 2013; Miscoe *et al*., 2016; John, 2018
Valves linear, 10-15 times longer than broad, 100-200 µm long and 8-12 µm broad, apices sharp niddle like, striae is not clear under compound microscope.
Voucher no.: CUH/PLANK/DIA- 31/2

15. *Nitzschia acicularis* (Kützing) W. Smith [Figure 3b]
Hustedt, 1930; Aboal *et al*., 2003; John, 2018
Basionym: *Synedra acicularis* Kützing
Valves are spindle shaped, slightly silicified, both side of the valves are slightly tapering with sharp narrow apices; valves 15-20 times longer than broad, 35-135 µm long and 2-6 µm broad; striae is not clear under compound microscope.
Voucher no.: CUH/PLANK/DIA- 31/3
Order: Thalassiophysales
Family: Catenulaceae
Genus: *Amphora*

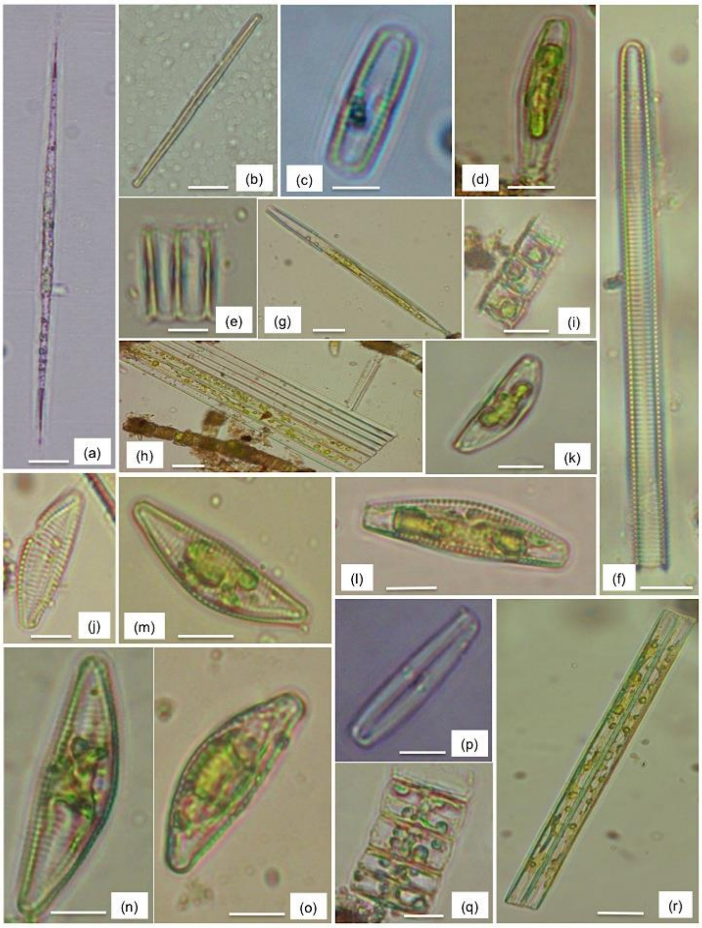

Figure 3 Light micrographs of (a) *Nitzschia nana* Grunow. (b) *Nitzschia acicularis* (Kützing) W. Smith. (c) *Amphora elliptica* (C. Agardh) Kützing. (d) *Amphora ovum* Cleve. (e) *Himantidium minus* Kützing. (f) *Synedra ulna* (Nitzsch) Ehrenberg. (g) *Synedra ulna* var. *amphirhynchus* (Ehrenberg) Grunow. (h) *Fragilaria intermedia* var. *robusta* G. S. Venkataraman. (i) *Diatoma mesodon* (Ehrenberg) Kützing. (j) *Cymbella ehrenbergii* Kützing. (k) *Cymbella affinis* Kützing. (l) *Cymbella oliffii* Cholnoky. (m) *Cymbella cistula* (Ehrenberg) O. Kirchner. (n) *Cymbella turgidula* Grunow. (o) *Cymbella tumida* (Brébisson) Van Heurck. (p) *Gomphonema lanceolatum* Ehrenberg, nom. illeg. (q) *Grammatophora undulata* Ehrenberg. (r) *Ctenophora pulchella* (Ralfs ex Kützing) D. M. Williams & Round. (Scale bar a, q: 20 µm; b-d, f-l, n, p, r: 10 µm; e, m, o: 15 µm)

16. *Amphora elliptica* (C. Agardh) Kützing [Figure 3c]
Henedy, 1974; Aboal *et al*., 2003
Basionym: *Frustulia elliptica* C. Agardh
Frustules slightly biconvex, elliptic or lanceolate with faintly attenuated apices, 3 times longer than broad, 26-42 µm long and 8-16 µm broad; striae distinct, transverse at both the sides, 6-8 in 10 µm area.
Voucher no.: CUH/PLANK/DIA- 31/4
17. *Amphora ovum* Cleve [Figure 3d]
Cleve, 1895; Kociolek *et al*., 2018
Frustules are oval or elliptical with broad rounded apices, 2-3 tomes longer than broad, 20-30 µm long and 9-11 µm broad; striae transverse, generally 5-8 in 10 µm.
Voucher no.: CUH/PLANK/DIA- 31/5
Class: Bacillariophyta classis incertae sedis
Order: Bacillariophyta ordo incertae sedis
Family: Bacillariophyta familia incertae sedis
Genus: *Himantidium*
18. *Himantidium minus* Kützing [Figure 3e]
Van Heurck, 1881; Patrick & Reimer, 1966
Frustules linear, unilateral and rectangular in girdle view, frustules attached side by side to form ribbon shaped colony, 2 times longer than broad, 30-50 µm long and 15-25 µm broad; striations are not clear under compound microscope.
Voucher no.: CUH/PLANK/DIA- 31/6
Subclass: Fragilariophycidae
Order: Fragilariales
Family: Fragilariaceae
Genus: *Synedra*
19. *Synedra ulna* (Nitzsch) Ehrenberg [Figure 3f]
Hustedt, 1930; Proschkina-Lavrenko, 1950; Aboal *et al*., 2003
Basionym: *Bacillaria ulna* Nitzsch
Frustules are linear, broadened at the ends, 25-30 times longer than broad, 80-160 µm long and 3-7 µm broad; valves linear to lanceolate and gradually tapering towards the ends; central area rounded or rectangular; striae coarse, 10-12 in 10 µm.
Voucher no.: CUH/PLANK/DIA- 32/1
20. *Synedra ulna* var. *amphirhynchus* (Ehrenberg) Grunow [Figure 3g]
Proschkina-Lavrenko, 1950; Aboal *et al*., 2003
Basionym: *Synedra amphirhynchus* Ehrenberg
Valve straight, linear and slender, narrow at the end and suddenly constricted to capitate end, 10-12 times longer than broad; 80-86 µm long and 7-8 µm broad; distinct and parallel striations present in both sides but not prominent in the middle, generally 9-15 in 10 µm area.
Voucher no.: CUH/PLANK/DIA- 32/2
Genus: *Fragilaria*
21. *Fragilaria intermedia* var. *robusta* G. S. Venkataraman [Figure 3h]
Venkataraman, 1939
Frustules are linear, rectangular in girdle view, 14-18 times longer than broad, 70-140 µm long and 5-8 µm broad; valves linear with parallel sides and gradually tapering capitate ends; striae coarse and distinct, 11-12 in 10 µm area.
Voucher no.: CUH/PLANK/DIA- 32/3
Order: Tabellariales
Family: Tabellariaceae
Genus: *Diatoma*
22. *Diatoma mesodon* (Ehrenberg) Kützing [Figure 3i]
Aboal *et al*., 2003; Hofmann, Werum & Lange-Bertalot, 2013; Lange-Bertalot *et al*., 2017
Synonym: *Odontidium mesodon* (Kützing) Kützing
Basionym: *Fragilaria mesodon* Ehrenberg
Valves are rectangular arranged in chains, 10-20 µm long and 7-10 µm broad; striae are not clearly visible under compound microscope but usually 20 in 10 µm area.
Voucher no.: CUH/PLANK/DIA- 32/4
Order: Cymbellales
Family: Cymbellaceae
Genus: *Cymbella*
23. *Cymbella ehrenbergii* Kützing [Figure 3j]
Proschkina-Lavrenko, 1950; Hu & Wei, 2006
Asymmetrical, biraphid, lanceolate frustules with obtuse end, 2.5 to 3 times longer than broad, 35-36 µm long and 12.5-13 µm broad; transverse raphe located at the middle; central nodule present; distinct transverse striations present and radial towards the center, striae 5-10 in 10 µm.
Voucher no.: CUH/PLANK/DIA- 32/5
24. *Cymbella affinis* Kützing [Figure 3k]
Aboal *et al*., 2003; Hofmann, Werum & Lange-Bertalot, 2013
Valves 5 times longer than broad, 20-30 µm long and 4-6 µm broad, strongly dorsiventral with rostrate apices; central stigma present; 8-12 striations in 10 µm area.
Voucher no.: CUH/PLANK/DIA- 32/6
25. *Cymbella oliffii* Cholnoky [Figure 3l]
Cholnoky, 1956
Frustules are oval to elliptical with large central chloroplast, 3-4 times longer than broad, 40-50 µm long and 12-14 µm broad; clear transverse striae present, 8-10 in 10 µm area.
Voucher no.: CUH/PLANK/DIA- 33/1
26. *Cymbella cistula* (Ehrenberg) O. Kirchner [Figure 3m]
Hustedt, 1930; Hu & Wei, 2006
Basionym: *Bacillaria cistula* Ehrenberg
Valve strongly dorsiventral with rounded apices, 3-5 times longer than broad, 35-80 µm long and 10-15 µm broad, ventral convex and median inflated; raphe central, proximal, straight; stigma present, 2-3; striae coarse, 8-12 in 10 µm area.
Voucher no.: CUH/PLANK/DIA- 33/2
27. *Cymbella turgidula* Grunow [Figure 3n]
Aboal *et al*., 2003; Hu & Wei, 2006; John, 2018
Valves dorsiventral, broadly lanceolate, 3 times longer than broad, 30-45 µm long and 10-15 µm broad, apices blunt, rostrate to truncate; proximal striae 10-12 and distal striae 12-15 in 10 µm area.
Voucher no.: CUH/PLANK/DIA- 33/3
28. *Cymbella tumida* (Brébisson) Van Heurck [Figure 3o]
Aboal *et al*., 2003; Hu & Wei, 2006; Hofmann, Werum & Lange-Bertalot, 2013
Valves broadly lanceolate with obtuse ends, 3-5 times longer than broad, 50-70 µm long and 10-20 µm broad; dorsal and ventral margins of the valve bent in opposite direction; striations radial, 8-10 in 10 µm area.
Voucher no.: CUH/PLANK/DIA- 33/4
Family: Gomphonemataceae
Genus: *Gomphonema*
29. *Gomphonema lanceolatum* Ehrenberg, nom. illeg. [Figure 3p]
Hustedt, 1930; Eberle, 2008
Valves lanceolate to clavate in shape with distinctly rounded apex and base, 4-4.5 time longer than broad, 50-55 µm long and 12-14 µm broad; raphe slightly thick and straight; Striae radial and lineate, 10 in 10 µm area.

Voucher no.: CUH/PLANK/DIA- 33/5
Order: Rhabdonematales
Family: Grammatophoraceae
Genus: *Grammatophora*

30. *Grammatophora undulata* Ehrenberg [Figure 3q]

Proschkina-Lavrenko, 1950; Witkowski, Lange-Bertalot & Metzeltin, 2000; Cheng & Gao, 2012

Frustules quadrangular with rounded angles, 2 times longer than broad, 40-45 µm long and 20-23 µm broad; septa slightly undulated; valves linear, oblong with capitulate ends; striations transverse, 6-8 in 10 µm area.

Voucher no.: CUH/PLANK/DIA -33/6
Order: Licmophorales
Family: Ulnariaceae
Genus: *Ctenophora*

31. *Ctenophora pulchella* (Ralfs ex Kützing) D. M. Williams & Round [Figure 3r]

Aboal *et al*., 2003; Hofmann, Werum & Lange-Bertalot, 2013

Basionym: *Exilaria pulchella* Ralfs ex Kützing

Frustules form ribbon shaped colony, 40-50 times longer than broad, 150-200 µm long and 5-7 µm broad; valves are elongate, linear to lanceolate with rounded apices; striae is not clear.

Voucher no.: CUH/PLANK/DIA-34/1

DISCUSSION

Diatoms are widely used as environmental indicators in nano technology, oil exploration and forensic examinations (**Dwivedi & Misra, 2015**). Recently the emphasis has been made on the ecological problems such as eutrophication, acidification and climate change. Freshwater diatom flora in ecologically sensitive regions of Indian continent has been discussed with very few reports. **Dwivedi & Misra (2015)** has reported 31 diatom species from the Himalayan region of which *Encyonema subalpinum* and *Gomphonema towutense* were found to be new to India. In our study we have also reported 31 species from Cu mining sites of Ghatshila of which 10 were newly recorded to the state. **Pandey *et al.* (2014)** have studied 19 periphytic diatom taxa from the river Ganges polluted with Cu, Pb and Zn.

Four types of abundance categories have been proposed in the present study: (i) most dominant, (ii) dominant, (iii) less dominant and (iv) absent, on the basis of the contaminated sites. This pattern of study is consistent with **Cattaneo *et al.* (2011)**. Diatom diversity was highest in all three sampling stations that were affected by Cu contamination. It has also been reported that the high level of Cu, Cd and Zn can stimulate the growth of diatoms and increase their diversity (**Sbihi *et al.*, 2014**). Interestingly, the present study supported the point of view of **Sbihi *et al*. (2014)**. In station 1, 17 species were found to be dominant where Cu contamination was high. Among these, *Halamphora coffeiformis* was recorded as the most dominant species and not reported in station 2 and 3. These tolerant taxa did not only survive under high Cu contamination but also showed their absolute abundance. Five species were less dominant and 9 were completely absent in station 1, which shows their low abundance in high Cu contamination (400 $\mu g.L^{-1}$). In contrast to the remarkable Cu tolerance, only 9 species dominated in the medium Cu contaminated sites of station 2. Some of these species has confirmed their presence in the high Cu contaminating sites and some of them were completely absent in the area adjacent to HCL. It has been documented that some adnate species like *Achnanthes minutissima* and *Fragilaria vaucheriae* were found to be abundant in high Cu streams (**Medley & Clements, 1998**). The growths of coastal and oceanic diatoms were highly affected by Cu. Several coastal species like *Chaetoceros decipiens*, *Thalassiosira weissflogii*, *Skeletolema costatum* and many oceanic species like *T. pseudonana*, *T. oceanica*, *S. menzellii* were found to be rich in Cu infected areas (**Annett *et al*., 2008**). These results interpret that diatom tolerance to metals obviously has limits and their diversity fluctuates with the Cu level. Ecological characteristics such as optima and tolerance along with other environmental variables suggests diatom as ecological indicator (**Dixit *et al*., 1992**). In station 3, only 5 species viz., *Nitzschia acicularis*, *Amphora elliptica*, *Himantidium minus*, *Synedra ulna* and *S. ulna* var. *amphirhynchus* showed their dominance over the less Cu contaminated area. These species were found as sensitive to high Cu (Table 2). Diatoms respond rapidly to environmental abnormalities and ecological fluctuations. Diatoms immigrate and replicate rapidly to any environmental changes. Changes in diatom assemblage in the present study sites correspond closely to shifts in other living communities such as picoplanktons, phytoplanktons, zooplanktons, fishes and hydrophytes (**Dixit *et al*., 1992**).

Limnological parameter greatly influences the assemblage of diatoms and reduces water quality (**Bigler & Hall, 2002**). It has been suggested that the diatoms respond well to hardness, alkalinity, pH, salinity and nutrients **(Greenaway *et al*., 2012)**. The appearance of low pH in water bodies indicates acidification. In our study we have observed the pH value in station 1 was 6.8, which is slightly acidic suggesting the starting point of acidification due to pollution (**Petrou *et al*., 2019**). High acidification also diminishes silica production in diatoms (**Petrou *et al*., 2019**). Minimum value of calcium in station 1 suggests the low pH. Total hardness and turbidity values in all stations suggest mineralization and eutrophic condition of the water bodies. Direct pouring of mining wastewater into the canals, outlets and in the River of Ghatshila results in significant changes to nitrate, phosphate, sulphate and silicate levels. High nitrate and sulphate values indicate the eutrophic condition of the water bodies of the sampling sites either due to anthropogenic activities or industrial pollution (**Bella *et al*., 2007**). Diatom communities play a significant role in maintaining the water quality in India. Some workers have concluded that diatoms can serve as indicator of organic and anthropogenic pollution (**Choudhury & Pal, 2010**). The photosynthetic activity of the diatoms is directly associated with DO of the water. The productivity in relation to gross primary productivity (GPP) and net primary productivity (NPP) is directly correlated with DO. Rise in DO level in water results in high GPP, whereas rise in BOD is a measure of drop in NPP in the concerned water bodies. In our study DO value sharply increased in the order: station 1 > station 2> station 3, indicating the pollution level in the water. Similarly BOD level decreased in the order: station 1 < station 2< station 3. As diatoms are primary producer they respond quickly to the ecological perturbations such as changing n physico-chemical parameters due to pollution (**Adon, Quattara & Gourene, 2012**). High value of dissolved CO_2 in station 1 adjacent to HCL indicates the competition of zooplanktons and other aquatic animals with the diatom communities for respiration. Poor water quality of station 1 due to contamination of Cu suggests diatom shift to the neighboring area of station 2 and 3. At some places diatoms can tolerate high metals whereas it drastically changes species composition in other places (**Cattaneo *et al*., 2011**). The conversion of oligotrophic to mesotrophic environment results in changing of species composition and species richness (**Cumming *et al*., 1995**). In our study it was sharply observed that the dominant species in station 3 vanished completely in station 1, which was highly polluted with Cu (Table 2). Similarly, species of highly Cu infected area were not observed in the less polluted area (station 3). Hence our study mainly focused on taxonomic implications of diatoms in Cu infected area with changing water quality.

CONCLUSION

Study of euplanktonic diatom communities in Cu mining sites is scarce. The present study was undertaken as first documentation of diatom communities in Jharkhand, India. The site is rich in Cu as it is situated adjacent to HCL on the bank of Subarnarekha River. A total number of 31 diatom species were documented from three sampling stations distinguished by high, medium and low Cu contamination. Maximum species dominated in station 1, which is closely associated to HCL. The taxonomic shifts were sharply noticed in three sampling sites. Oligotrophic to mesotrophic habitat suggests the eutrophication, acidification and mineralization of the water ecosystems. Physico-chemical parameters show the condition of the water bodies due to Cu contamination. From the study it can be concluded that the diatom assemblages of Ghatshila can serve as best bioindicator of Cu.

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