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Forensic limnology: Diversity of diatom population in relation to environmental factors in Gujarat region, India

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ABSTRACT

In drowning cases, diatoms provide an insight into ante-mortem and post-mortem evidence as well as aid in determining the geographical location. Diatoms are unicellular aquatic organisms that represent a major taxonomic division of the phytoplankton. The diversity of these diatoms depends on the geographical and environmental conditions as well as on the physico-chemical properties of the habitat. Hence, studying the diversity of diatoms in different water bodies in the Gujarat region will generate a diatomological map of the water bodies, which would assist in forensic analysis. In the present study, 100 water samples were collected from various water bodies, viz. rivers, lakes, ponds, etc., across 33 districts of Gujarat state, divided into 5 geographical regions (Saurashtra, Kutch, North, Central, and South). Identification of the diatoms was carried out by trinocular microscopic techniques. Physico-chemical parameters like pH, temperature, total dissolved solids (TDS), and electrical conductivity (EC) were also analyzed. Heavy metal concentrations were determined using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). The diversity analysis reveals that each body of water in a different region has its own characteristics of diatoms. The physico-chemical parameters affect the diversity of diatoms by increasing their growth, but higher loads of heavy metals may affect the diatoms by reducing their growth and biochemical compositions. In conclusion, the present study provides an insight into understanding the regional patterns of diatom diversity and formulates reference conditions in these and other water bodies of this region.

Selection and peer-review under responsibility of the scientific committee of the International Conference on Nanomaterials in Biology.

1. Introduction

Diatoms are eukaryotic unicellular creatures that range in size from 5 to 1000 μm and are one of the most frequent forms of phytoplankton [1]. Because of their widespread distribution in aquatic habitats, they contribute 20–25 percent of global oxygen release and carbon fixation, as well as roughly half of global primary output [2]. The term "diatom" means "cut in half" and is derived from the Greek: (dia) = "through" + (temnein) = "to cut," i.e., "cut in half." Diatom cells are distinguished by the presence of frustules, which are silica (hydrated silicon dioxide) cell walls [3]. There are two orders of diatoms; the Centrales, which are now called the Biddulphiales, have valve striae that are arranged radially around a point, an annulus, or a central areola. The Pennales, which are now called the Bacillariales, have valve striae that are arranged bilaterally around a line. [4]. Because diatoms are so common and have a wide range of species, specialised habitats, and the ability to stay alive, they can be used in forensic geosciences to help solve crimes [5].

Drowning is the world's third-most common cause of accidental mortality, accounting for 7 % of all injury-related deaths. According to the World Health Organization, India ranks 51st in the world in drowning events, with a rate of 3.76 percent. India reported 37,238 drowning cases in 2020, a 2.2 percent increase from 32,671 drowning cases in 2019 [5]. Deaths from drowning are reliably identified by the presence of diatoms, which are used as distinctive forensic markers. Drowning can be accidental, suicidal, or even homicidal [6]. When a forensic expert analyses a body recovered from the water, it is difficult to determine the cause of death, particularly separating drowning from other causes [7]. A recently drowned body would typically have several distinguishing features, such as whitish skin and foam in the lips and nostrils, among other

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things. However, when a person is submerged in water for an extended period of time, these postmortem symptoms disappear, making it much more difficult to determine the cause of death [8]. This is because the body is no longer exposed to the air. Aspiration of liquid into the lungs, followed by its entry into the bloodstream via the alveoli, is a characteristic feature of drowning. Some of the water's sediments, bacteria, and pollen are deposited in the capillaries of various organs as the water travels through the body [7,9]. Because their silica shells are acid-resistant, diatoms can be easily extracted from organ tissues by employing acid-digestive extractions. Even after chemical digestion, the morphologies and patterns of diatom species can be distinguished by forensic pathologists. Because they are difficult to hide, species that are unique to a given environment can be used as evidence to verify the location of a crime.

Gujarat is an Indian state on the west coast. It has the longest coastline in the country, about 1,600 km, and most of it is on the Kathiawar peninsula. Its latitude is 22.309425 and its longitude coordinates are 72.136230. Winters in Gujarat are mild, pleasant, and dry, with average daytime temperatures of around 83 °F (29 °C) and nighttime temperatures of around 53 °F (12 °C). Summers are extremely hot and dry, with daytime temperatures approaching 105°F (41 °C) and nighttime temperatures dropping to 85°F (29 °C). The average rainfall in Gujarat ranges from 33 to 152 cms. Because most diatom species live in distinct aquatic environments, this isn't the case in the vast majority of drownings. However, diatoms are particularly susceptible to variations in ecological conditions, such as pH, TDS, temperature, and other physico-chemical qualities; consequently, the number of diatom species varies from one body of water to another. It is possible to identify where a victim fell into the water by comparing the patterns of diatom biodiversity in tissues and suspect water samples [10–12].

In this paper, we have examined the morphological diversity of diatom species across 33 districts of Gujarat (India) that are distributed into 5 regions (Saurashtra, Kutch, North, Central, and South Gujarat) (Fig. 1) along with physico-chemical parameters like pH, TDS, EC, and temperature that affect the diversity of diatoms in different geographical areas.

2. Materials and methods

2.1. Study area

The study was conducted on the diatom diversity of rivers, lakes, ponds, and streams draining into the Gujarat region in India (Asia). The rivers that flow through Gujarat are the Narmada River, the Mahi River, the Sabarmati River, and the Tapi River. During the study of different types of diatom habitat found in the Gujarat region, we divided it into 5 regions (Saurashtra, Kutch, North, Central, and South) for a total of 33 districts. Within these districts, water samples were collected from water bodies. Gujarat has diverse climatic conditions, with certain regions experiencing higher humidity levels in coastal areas than in the interior, where the climate is very different. The study was conducted from February to May 2022, when the average annual temperature ranged from 26 to 45 °C.

2.2. Sample collection

Water samples were collected in amber bottles (1000 ml), which were then properly sealed with caps and labeled with the sampling site's location, as well as the time, date, and month. The temperature was also noted at the time of sample collection. Samples were obtained at least 2 feet below the surface from the bank, along and across the river, lake, and ponds. The collected samples were taken to the laboratory for testing [13,14].

2.3. Diatom examination

An acid oxidation method was used for the cleaning as well as visualisation of diatom frustules. The standard acid digestion method (conc. nitric acid) was followed in this study to extract diatoms from collected water samples [15]. In brief, water samples were treated with conc. HNO3 in a 10:1 ratio. The 100 ml of water sample from the sample container was taken in a small beaker (after properly shaking it for 1–2 min) and then 10 ml of conc. HNO3 was added to it [10,16].The concentrated acid digests the organic content in the water, leaving only the diatoms' siliceous skeletons (frustules). The beaker was then wrapped in aluminium foil and left overnight to facilitate digestion. After 24 h, the settled contents of the sample were transferred to falcon tubes (15 ml) and centrifuged at 4000 rpm for roughly 10 min. To obtain the pellet, the supernatant was discarded. The above processes are repeated 2–3 times more by adding distilled water to the pellet to eliminate any leftover acid content. For 1–2 min, the pellets were dried at 30–40 °C on a hot plate after being dropped onto glass slides and mounted. In the end, a single drop of DPX (fixer) was placed on the slides, and the cover slip was placed over it to ensure proper fixation and air drying. [5,17,18]. A slide was examined under the microscope for identification of diatoms with a trinocular microscope (Nikon Eclipse Ei) at 40 X and 100 X (oil immersion) magnifications and images were captured with a fitted camera (GT 12 – 12 Mega Pixel). All samples were processed through these same steps [19].

2.4. Diatom identification

The diatoms were identified using their morphological characteristics, which included shape, symmetry, raphe, and striae, among other things, and were categorised into Centric, Araphid, Eunotioid, Symmetric Biraphid, Monoraphid, Asymmetric Biraphid, Epithemioid, Nitzschioid, and Surirelloid [20–21]. The identification of diatoms was done by using online diatom databases such as Diatoms of North America and Diatom Image Database of India (DIDI) [22,23]. Their identification was based as follows: (1) Recognize diatom frustules, if present. (2) differentiating diatom physical characteristics such as diameter and raphe (centric, excentric). (3) Compare the number of punctuated rows, the length of the valve, the strait diameter, and the breadth. (4) validate symmetry (bilateral, radial) after the identification of diatoms.

2.5. Determination of physico-chemical parameters

The physico-chemical factors (such as pH, conductivity, TDS, and temperature) were measured for all the samples using standard methods and protocols. The temperature was also noted at the time of sample collection in degrees Celsius (°C). The pH was measured using a digital pH meter (Eutech Instruments pH 2700 m), and the total dissolved solids (TDS) and electrical conductivity (EC) were calculated using a multi-probe meter (NEXQUA TDS/EC METER) [24–25].

2.6. Heavy metal analysis

For heavy metal analysis, collected water samples were processed for ICP MS. In brief, a total of 10 elements (Cr, Mn, Fe, Co, Ni, Cu, Zn, Sr, Cd, and Pb) were determined in all water samples. A 5-point calibration curve (10 ppb, 50 ppb, 100 ppb, 500 ppb, 1000 ppb) assay was performed with a multi-element standard (1000 ppm) with Bismuth (Bi) as an internal standard (20 ppb).

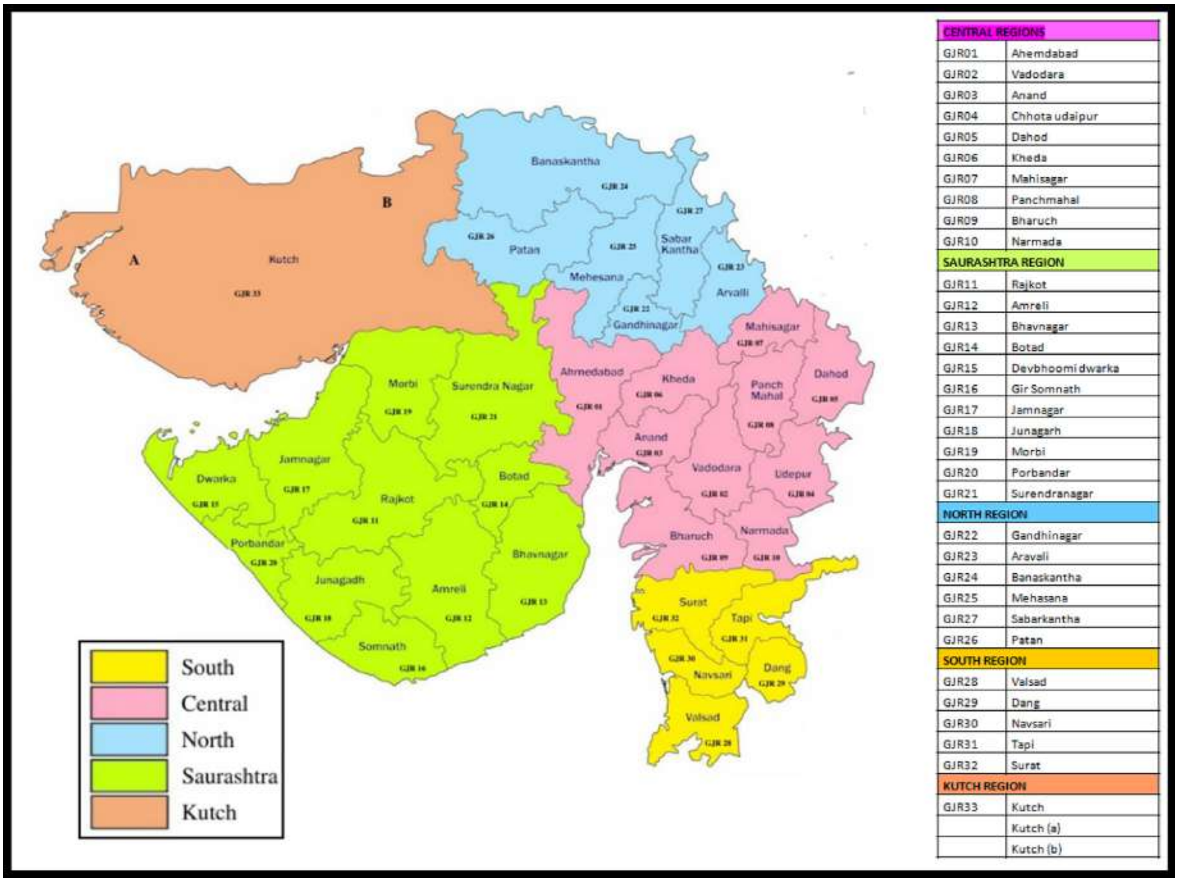

Fig. 1. Illustration of sampling sites across Gujarat state (Central, Saurashtra, North, South, Kutch) of India.

About 10 ml of each water sample was filtered using a 0.45-μm syringe filter and collected in a falcon tube, and 1 % nitric acid (HNO3) was added to all the samples. All samples were spiked with an internal standard solution [26].

Heavy metal analysis was carried out using ICP-MS (Thermo Fisher Scientific iCAPRQ, Waltham, MA, USA) equipped with a nebulizer, a Teflon spray chamber, a nickel (Ni) sampling cone, and a platinum skimmer cone. The Peristaltic pumps and an auto sampler ASX560 were used to transfer the solutions from the tubes. After 20–30 min of stabilisation, the ICP-working MS's capacity was optimised daily by tuning the torch's horizontal and vertical position, extraction lens, CCT (collision cell technology) focus lens, and radio frequency power at 1550 W to reduce interference effects and increase signal strength. Auxiliary flow was maintained at 0.8 ml/min, nebulizer flow at 1.0 ml/min, and nebulizer nebulizer flow at 5.3 ml/min were used for the carrier gases.All elements were analyzed using the kinetic energy discrimination (KED) mode with pure helium gas as the collision [27–30].

2.7. Statisical analysis

Statistical analysis was performed to determine the impact of environmental factors on biodiversity using SPSS 20.0 statistical software for Windows (SPSS Inc., Chicago, Illinois, USA). Descriptive statistics were expressed as mean ± SD. The significance of the data was determined using the ANOVA, and the correlation between diatom diversity and environmental factors was calculated using the Pearson correlation coefficient. A p-value of<0.05 was used to indicate statistical significance across all tests.

3. Result

In the present study, diatom diversity and their distribution show a distinctive variation among the different water bodies across the Gujarat region. Diatom species are reported to have sensitivity against changes in pH, TDS, EC, and temperature. Variations in water quality allow them to function as environmental and forensic indicators. Water samples for diatom analysis were collected within the months of February 2022 to May 2022 with an average temperature range of 26 °C–45 °C from different water bodies (rivers, lakes, ponds) of Gujarat.

In the present study, more than 250 species belonging to 66 genera were identified from all sampling sites (Figs. 2, 3). Hence, a large number of diatoms were identified on the basis of their morphology includes CENTRIC (Melosira, Mellosiravarians, Guinardia, Aulacoseira, Cyclotella, Cyclostephanos, lindavia, Pilocaenicus, Stephanocyclus, Ulacoseria, Urosolenia) the most commonly found diatoms throughout the region; ARAPHID(Pseudostaurosira, Synedra, Tabularia, Fragilaria, Stauroforma, Fragilariophyceae, Ulnaria, Asterionella, Diprora, Fragilariforma, Fragilaria biceps, Distrinella; SYMMETRIC BIRAPHID (Frustulia, Navicula, Craspedostauros, Diplonesis, Diadesmis, Pinnularia, Proschkinia, Staurophora, Frickea, Scoliopleura, Kobayasiella, Placoneis, Aneumastus, Diatomella, Lacustriella, Geissleria, Haslea, Krasskella, Fistulifera, Gyrosigma, Naviculagregaria; MONORAPHID (Stauroneis, Envekadea, Mastogloia, Achnanthidium, Rossithidium); ASYMMETRIC BIRAPHID (Halamphora, Rhiocosphenia, Afrocymbella, Gomphosinica, Cymbella, Navicymbula, Encyonopsis, Cymbopleura, Gomphonema, Encyonrma); NITZSCHIOID (Cylindrotheca, Simonsenia, Bacillaria, Tryblionella, Nitzschia, Hantzschia) and SURIRELLOID (Stenopterobia) least commonly found genera of diatoms (Table 1).

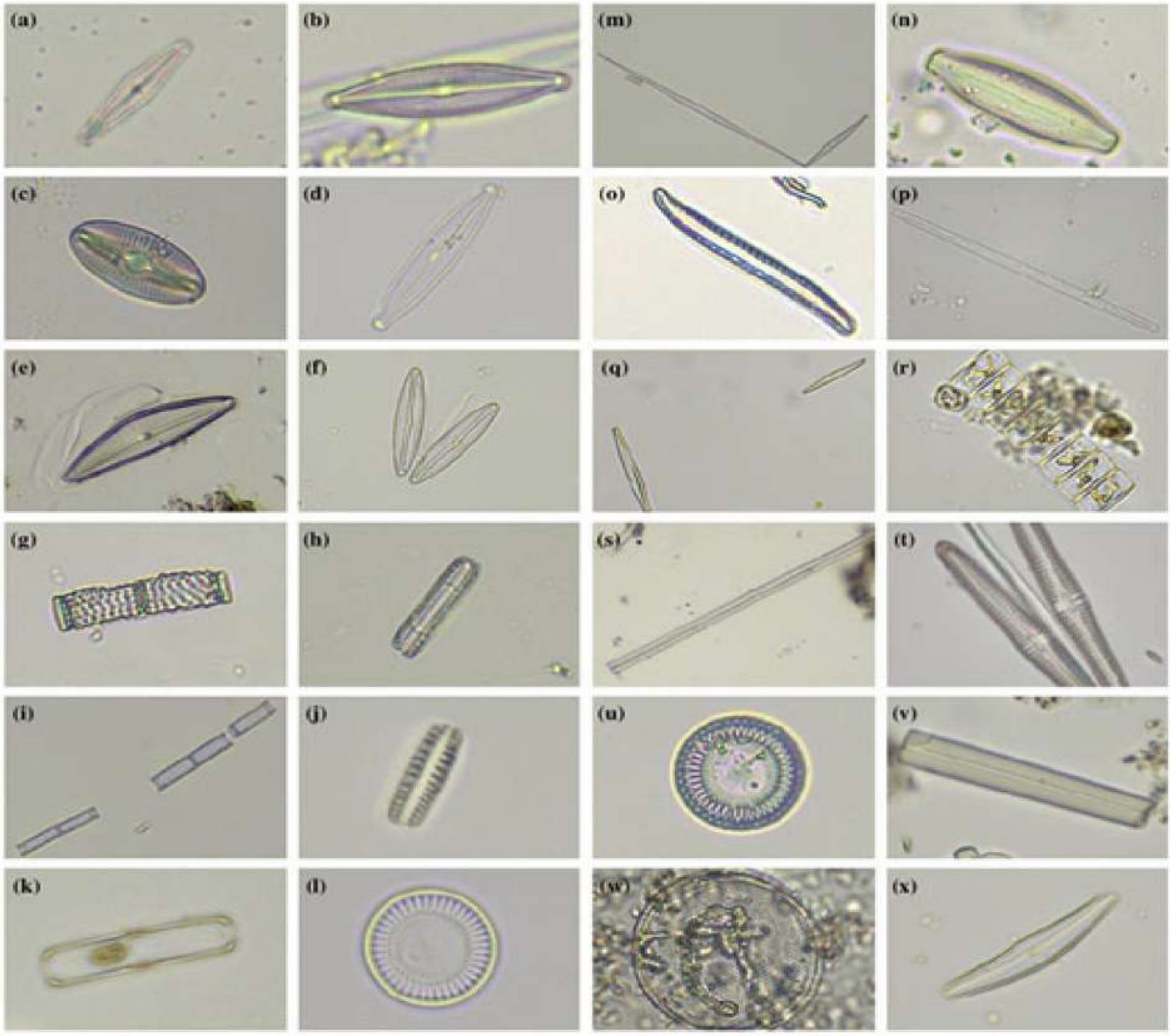

Fig. 2. Morphology of some diatom found in regions of Gujarat; India. a) Frustulia [SMB]; b) Proschkinia [SMB]; c) Diplonesis [SMB]; d) Staurophora [SMB]; e) Afrocymbella [ASMB]; f) Envekadea[SMB]; g)Guinardia [CNT]; h) Scolioplura[SMB]; i) Aulacoseria [CNT] j) Rhiocosphenia [ASMB]; k) Navicula virdis [SMB]; l) Cyclostephanos [CNT]; m) Haslea [SMB]; n) Adlafia [SMB]; o) Gyrosigma [SMB]; p) Bacillaria [NTZ]; q) Tabularia [ARP]; r) Diprora [ARP]; s) Synedra [ARP]; t) Encyonopsis [ASMB]; u) Cyclotella [CNT]; v) Thallasionema Nitschzoids [NTZ]; w) Coscinodiscus[CNT] and x) Cymbella [ASMB]. **Note:-** CNT: Centric; SMB: Symmetric Biraphid; ASMB: Asymmetric Biraphid; ARP: Araphid; NTZ: Nitzschioid.

In Central Region: The most commonly occurring diatoms genera in the central region belongs to Araphid, Symmetric Biraphid, Centric, Asymmetric Biraphid, and the least abundant were Nitzschioid and Monoraphid. The Cyclostephanos, Asterionella, Stauroforma, Diprora, Districnella, Humidophila, Sellaphora, Rossithidium, Bacillaria were found at GJR01; Aulacoseira, Pseudostaurosira, Diatomella, Halamphora were found at GJR02; Diatomella, Genkalia, Navicymbula, and Simonsenia were found at GJR03; Synedra, Halamphora, Rhiocosphenia, Afrocymbella, Gomphosinica, Cymbella were found at GJR04; Melosira, Tabularia, Frustulia, Cylindrotheca were found at GJR05; Pseudostaurosira, Synedra, Navicula, Craspedostauros, Diplonesis, Navicymbula, Cymbella, Encyonopsis were found at GJR06; Asterionella, Districonella, Syndera were found at GJR07; Cyclotella, Guinardia Fragilaria were found at GJR08; Guinardia, Ctenophore, Sciopleura, Adlafia were found at GJR09 and Melosira, Diadesmis and Rhiocosphenia were found at GJR10.

In Saurashtra Region: The most commonly found diatoms genera in saurashtra region were centric, araphid, symmetric biraphid, and least frequently were asymmetric biraphid, monoraphid, and nitzschioid morphology. Site specific diatoms at GJR11 site were Cyclotella, Cyclostephanos, Lindavia, Melosira, Pilocaenicus, Aulacoseria, Synedra, Fragilariophyceae, Proschkinia, Staurophora, Diadesmis, Navicymbula; at GJR12- Fragilaria, Craspedostauros, Frickea, Sciopleura; at GJR13 Fragileria, Ulnaria, Asterionella; at GJR14 Cyclostephanos, Guinardia, Kobayasiella, Placoneis, Navicula, Proschkinia; at GJR15 Ulnaria, Navicula; At GJR16 Pilocaenicus, Cyclostephanos, Stephanocyclus, Cyclotella, Aneumastus; at GJR17 Staurophora, Tryblionella, Nitzschia; at GJR18 Tabularia, Diatomella, Nitzschia; At GJR19 Fragileria, Diprora; at GJR20 Cyclotella, Orthoseira, Pinnularia, Gomphosinica, Nitzschia, Stenopterobia; at GJR21 Achnanthidium, Rhoicosphenia and Navicymbula were found.

In North Region: The commonly occurring diatoms genera in the north region were symmetric biraphid, centric, araphid, and the least abundant were asymmetric biraphid and nitzschioid. At GJR22, Cyclotella, Sciopleura, Krasskella, Lacustriella, Geissleria, Envekadea, and Mastogloia were found; at GJR 23, tabularia, Ulnaria, Halamphora; at GJR 24, Aulacoseira, Diphora, geissleria, stauroneis, envekadea, mastogloia; at GJR25, Melosira, Cylindrotheca,

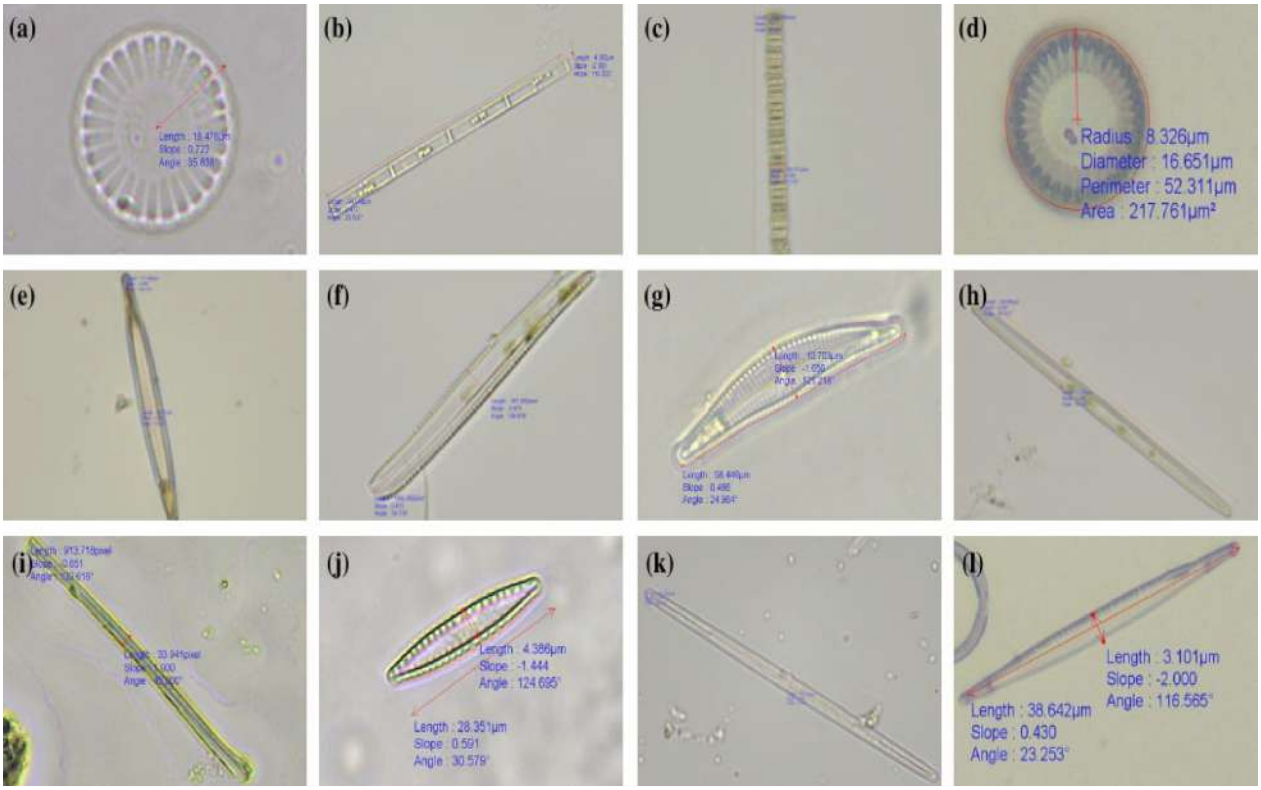

Fig. 3. Morphology of diatom (graduated) across Gujarat regions. a) Stephanocyclus [CNT]; b) Guinardia [CNT; c) Skeletonema [CNT]; d) Puncticulata [CNT]; e) Navicula gracilis [SMB]; f) Pinnularia [SMB]; g) Afrocymbella [ASMB]; h) Ulnaria [ARP]; i) Bacillaria [NTZ]; j) Tabularia [ARP]; k) Navicula viridula [SMB] and l) Craspedostauros [SMB]. **Note:-** CNT: Centric; SMB: Symmetric Biraphid; ASMB: Asymmetric Biraphid; ARP: Araphid; NTZ: Nitzschioid.

Table 1
Morphological categories of diatom genera and their Characteristics.

S. No.	Morphological Categories	Genera	Characteristics
1.	**Centric**	*Aulacoseira, Actinocyclus, Coscinodiscus, Melosira, Cyclostephanos, Cyclotella, Discostella, Guinardia, Lindavia, Orthoseira, Pliocaenicus, Skeletonema, Stephanocyclus, Thalassiosira*	• Radially symmetrical valves (symmetric about a point) Cells lack raphe and motility Cells may possess fultoportulae (strutted processes) and rimoportulae (labiate processes)
2.	**Araphid**	*Asterionella, Ctenophora, Diatoma, Diprora, Distrionella, Fragilaria, Fragilariforma, Pseudostaurosira, Stauroforma, Tabellaria, Pseudostaurosiropsis, Synedra, Tabularia, Ulnaria, Tibetiella*	• Bilaterally symmetrical valves (symmetric about a line) Cells lack a raphe and motility Rimoportulae (labiate process) may be present
3.	**Symmetric Biraphid**	*Adlafia, Amphipleura, Aneumastus, Caloneis, Anomoeoneis, Brachysira, Caloneis, Craticula, Cavinula, Craspedostauros, Diadesmis, Diatomella, Diploneis, Fistulifera, Frustulia, Gyrosigma, Kobayasiella, Krasskella, Navicula, Muelleria, Placoneis, Pinnularia, Pleurosigma, Proschkinia, Scoliopleura, Sellaphora, Staurophora*	• Bilaterally symmetrical valves Symmetric apical/transapical valves Well developed raphe system with highly motile cells
4.	**Monoraphid**	*Achnanthes, Achnanthidium, Cocconeis, Lemnicola, Gogorevia, Rossithidium, Platessa*	• Bilaterally symmetrical valves Raphe system only on one valve (raphe valve) and absent on the other valve (rapheless valve) Heterovalvar ornamentation
5.	**Asymmetric Biraphid**	*Afrocymbella, Amphora, Brebissonia, Cymbella, Cymbopleura, Delicata, Encyonema, Encyonopsis, Omphonema, Halamphora, Oricymba, Navicymbula, Seminavis*	• Asymmetric valves to apical axis or transapical axis, or both Well developed raphe system Some genera possess apical porefields that secrete mucilaginous stalks and others secrete mucilagous tubes
6.	**Nitzschioid**	*Bacillaria, Cylindrotheca, Cymbellonitzschia, Denticula, Simonsenia, Nitzschia, Tryblionella, Grunowia*	• Bilaterally symmetrical valves Apical and transapical symmetrical valves Well developed raphe system and enclosed within a canal which is positioned near one valve margin
7.	**Surirelloid**	*Campylodiscus, Cymatopleura, Entomoneis, Iconella, Stenopterobia, Surirella*	• Bilaterally symmetrical valves Well developed raphe system and enclosed within a canal Raphe often circumferential on the valve margin and raised onto a keel

Hantzschia, and Nitzschia were observed; at GJR 26, Afrocymbella, cylindrotheca, hantzschia, Nitzschia and at GJR27 Cyclotella, Guinardia, Navicula, Encyonopsis and Cymbopleura were observed.

In South Region: The commonly occurring diatoms in the south region were centric, symmetric biraphid, and least frequently were araphid, asymmetric biraphid, and nitzschioid. Site-specific diatoms were discovered at GJR28 Lindavia, Aulacoseria, Urosolenia, and Haslea; at GJR29 Pinnularia, Halamphora, Encyonema, Nitzschia; at GJR30 Cyclostephanos, Cyclotella, Krasskella; at GJR31 Cyclotella, Melosira, Aulacoseria, Synedra; at GJR32 Cyclostephanos and Fragilariforma were found.

In Kutch Region: The commonly occurring diatoms in the kutch region were symmetric biraphid, nitzschioid, and asymmetric biraphid, and the least occurring were centric and araphid. Site specific diatoms; at GJR33: Fistulifera, Geissleria, Gyrosigma, Halamphora, gomphonema at site A; Cyclotella, Distrionella and at site B; Navicula gregaria, Nitzschia, and Cylindrotheca were found (Table 2).

3.1. Physico-chemical characteristics of water samples

The physico-chemical properties of water were recorded for pH, TDS (ppm), EC (μS/cm), temperature (°C), across the central, saurasthra, north, south, and kutch regions of gujarat (Fig. 4). The mean values of pH, TDS, EC, and temperature in the Central Region were 7.64 ± 0.38, 252 ± 55.83, 503 ± 117.85, 37.30 ± 2.31, respectively. The mean pH, TDS, EC, and temperature values in the Saurashtra Region were 7.39 ± 0.60, 631 ± 282.62, 1260 ± 570.81, 36.30 ± 3.35, respectively. The mean values of pH, TDS, EC, and temperature in the North Region were recorded as 7.67 ± 0.39, 556.17 ± 223.07, 1098.83 ± 430.13, 36.50 ± 4.81. The mean values of pH, TDS, EC, and temperature in the South Region were recorded as 7.44 ± 0.18, 708.40 ± 419.29, 1392.60 ± 836.03, 35.60 ± 1.52. The mean pH, TDS, EC, and temperature values in the Kutch Region were 7.61 ± 0.44, 711 ± 327.34, 1384 ± 653.33, 38.67 ± 0.58 respectively. The study indicated that pH, temperature, total dissolved solids (TDS), and electrical conductivity (EC) varied among the central, saurasthra, north, south, and kutch regions.

3.2. Heavy metal analysis

Heavy metals concentrations in water samples were examined through Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) technique using Thermo Scientific iCAP RQ system and were determinate the concentrations of Cr, Mn, Fe, Co, Ni, Cu, Zn, Sr, Cd, and Pb in ppb (parts per billion). The mean concentration values of Chromium were 0.231 ± 0.209, 0.139 ± 0.130, 0.236 ± 0.216, 0.378 ± 0.127, 0.126 ± 0.059. The mean values of manganese were 1.702 ± 1.483, 1.029 ± 1.002, 0.771 ± 0.587, 0.988 ± 0.627, 2.424 ± 2.047. The mean concentration of Iron was 27.506 ± 22.491, 31.844 ± 26.201, 28.321 ± 24.522, 35.293 ± 12.006, 34.199 ± 16.279. The mean value of Cobalt was 0.031 ± 0.013, 0.103 ± 0.101, 0.99 ± 0.062, 0.065 ± 0.058, 0.059 ± 0.040. The mean concentration of Nickel was 1.037 ± 0.0556, 1.293 ± 0.995, 1.557 ± 0.936, 2.439 ± 2.152, 1.133 ± 0.890. The mean concentration of Copper was 1.109 ± 0.551, 1.438 ± 1.053, 1.300 ± 0.677, 4.243 ± 3.335, 1.192 ± 1.058. The mean concentration of Zinc were 2.370 ± 1.368, 4.876 ± 2.004, 2.814 ± 2.298, 18.137 ± 10.589, 3.038 ± 2.050.The mean concentration of Strontium were 230.803 ± 145.007, 299.644 ± 230.487, 301.582 ± 271.382, 495.180 ± 357.195, 463.851 ± 158.36. The mean value of Cadmium was 0.023 ± 0.021, 0.117 ± 0.103, 0.021 ± 0.015, 0.159 ± 0.117, 0.015 ± 0.009. The mean value of Lead were 0.

Table 2
Morphological diversity of diatom found among five different regions of Gujarat, India.

	Sample no.	CENTRIC	ARAPHID	SYMMETRIC BIRAPHID	MONORAPHID	ASYMMETRIC BIRAPHID	NITZSCHIOID	SURIRELLOID
CENTRAL REGION	GJR01	✓	✓	✓	✓		✓	
	GJR02	✓	✓	✓		✓		
	GJR03			✓		✓	✓	
	GJR04		✓			✓		
	GJR05	✓	✓	✓			✓	
	GJR06		✓	✓		✓		
	GJR07		✓					
	GJR08	✓	✓					
	GJR09	✓	✓	✓				
	GJR10	✓		✓		✓		
SAURASHTRA REGION	GJR11	✓	✓	✓		✓		
	GJR12		✓	✓				
	GJR13		✓					
	GJR14	✓		✓				
	GJR15		✓	✓				
	GJR16	✓		✓				
	GJR17			✓			✓	
	GJR18		✓	✓			✓	
	GJR19		✓					
	GJR20	✓		✓		✓	✓	✓
	GJR21				✓	✓		
NORTH REGION	GJR22	✓		✓				
	GJR23		✓			✓		
	GJR24	✓	✓	✓				
	GJR25	✓	✓	✓				
	GJR26					✓	✓	
	GJR27	✓		✓		✓		
SOUTH REGION	GJR28	✓		✓				
	GJR29			✓		✓	✓	
	GJR30	✓		✓				
	GJR31	✓	✓					
	GJR32	✓	✓					
KUTCH REGION	GJR33			✓		✓		
	Kutch A	✓	✓					
	Kutch B			✓			✓	

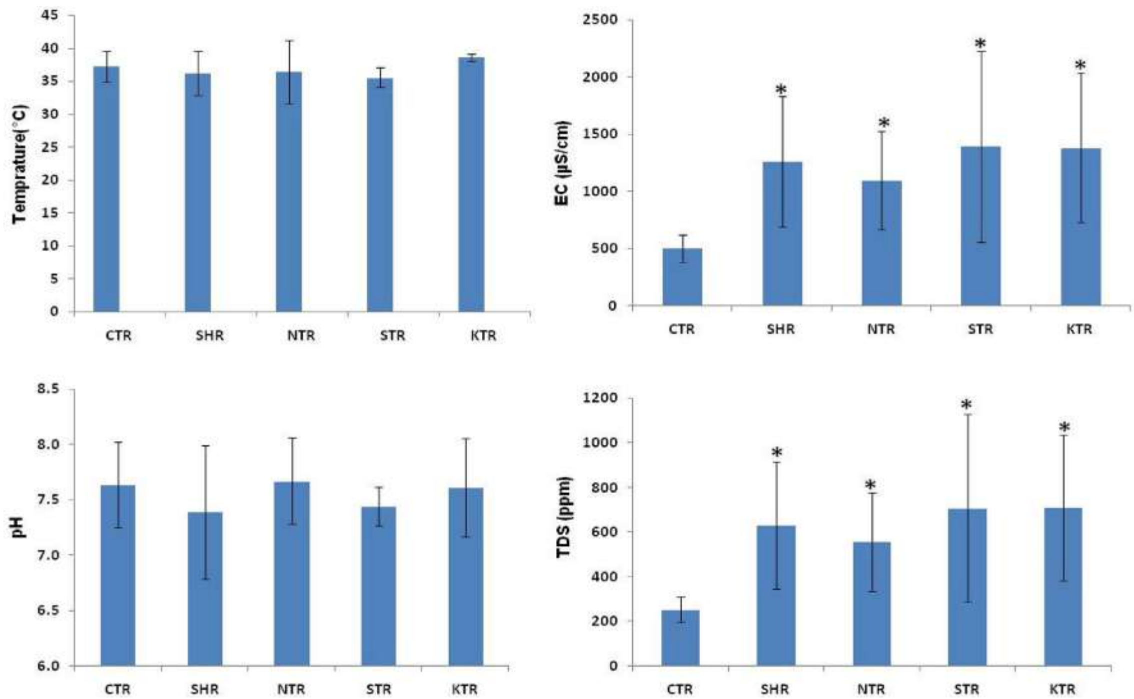

Fig. 4. Graphical representation of Physico chemical parameter of Gujarat region. **Note:** CTR-Central region, SHR-Saurashtra region, NTR- North region, STR- South region, KTR- Kutch region.

002 ± 0.001, 0.304 ± 0.285, 0.003 ± 0.002, 0.611 ± 0.521, 0.006 ± 0.004 in central, saurashtra, north, south and kutch regions respectively.

4. Discussion

The diversity of diatoms with reference to variation in physico-chemical parameters and heavy metal concentration has been studied in detail. In the present studies, we have observed that variation in geographical location and environmental conditions causes a difference in the distribution of diatoms (Fig. 2). Diatoms are able to survive and reproduce in a variety of water ecosystems based on their adaptability and versatility, resulting in substantial variances in the distribution of diatom species [31]. Therefore, diatomological maps of various diatom taxa at various bodies of water would be of great assistance in further forensic investigation and research analysis [32]. The most common method used for isolating and examining the morphological characteristics of diatoms is the acid digestion method. However, diatom testing based on morphological assessment is often closely related to the knowledge of the examiner during the investigation [20,33]. We have observed that the size of diatoms also shifts depending on environmental factors. Diatoms of smaller sizes were found in lentic water bodies like lakes and ponds, and diatoms of larger sizes were found in lotic water systems like rivers and streams. [34]. The distribution pattern and variance of several forms of diatoms in bodies of water were classified as commonly occurring and least commonly occurring diatom genera. About 70 % of diatom genera are centric, araphid, symmetric biraphid, and asymmetric biraphid; about 25 % are nitzschioid and monoraphid; and 5 % are surirelloid genera found at a specific site (GJR20) in the Gujarat region [35–38].The commonly observed genera are Lindavia, Cyclostephanos, Haslea, Krasskella, Halamphora, Distrionella, Diphora, Tabularia, Diatomella, Guinardia, and others. The least occurring genera are Synedra, Rossithidium, Cylindrotheca, Rhiocosphenia, Frustulia, Diadesmis, and Nitzschia.

The environmental variables (pH, TDS, EC) are the most important measures that are associated with the distribution of diatom assemblages from water, varying from region to region (Fig. 4). Throughout the analysis, the pH ranged between 7.39 and 7.67. In places with relatively high pH levels, the water is dominated by diatoms with Cyclotella, Melosira, Aulacoseira, Diphora, Fragilaria, and Navicula. Afrocymbella, Scoliopleura, Diatomella, Tryblionella, Aneumastus, and Mastogloia are the least common genuses.Additionally, an earlier study has demonstrated that pH has an effect on the range of diatom species. In accordance with the findings of prior studies, the acidity or alkalinity of the water was found to be a key factor for diatom growth. Therefore, pH has a significant impact on the growth of diatoms, either directly through the induction of physiological stress or indirectly through the influence it has on other physico-chemical variables [39]. The TDS value from our study ranged between 556.17 and 711.00 ppm. The finding demonstrates that with an increase in TDS values, the diversity of diatoms also increases. Electrical conductivity (EC) is considered an enrichment indicator parameter and is an important determinant factor of diatom distribution [40]. EC reflects ions that may influence nutrient availability and uptake by diatoms during primary production. The value of EC ranged between 503.00 and 1392.60 S/cm in the Gujarat region [41].

Heavy metal pollution of the natural habitats is consistently increasing due to the release of vast amounts of metal ions through industrial wastes, sedimentation of rocks, agriculture runoff, and mining activities. The source of metallic elements such as copper, chromium, iron, nickel, and zinc in the effluent is due to corrosion of materials from construction. All these metal ions tend to accumulate in biological systems, nature and are toxic at elevated concentrations [42–43]. The heavy metal analysis shows that the south and Kutch regions had higher concentrations of heavy metals as compared to the central, Saurasthra, and north regions (Fig. 5). The water samples having lower amounts of Cr, Pb, Cd, Cu, Ni, Co, and Mn had more diatom species, whereas the samples having more values of strontium, iron, and zinc showed diatom genus def-

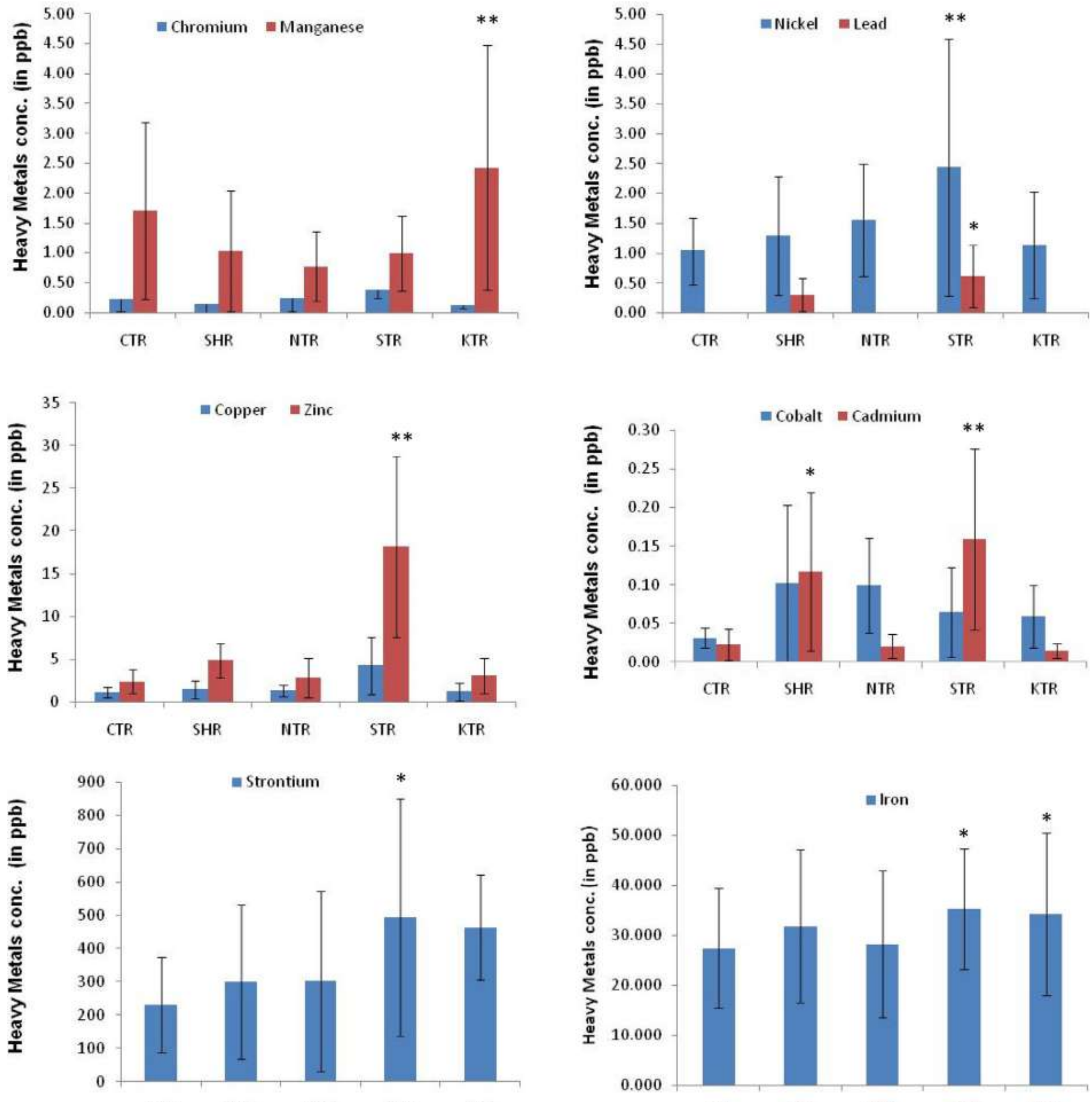

Fig. 5. Graphical representation showing heavy metal concentration (in ppb) across Gujarat regions. **Note:** CTR-Central region, SHR-Saurashtra region, NTR- North region, STR- South region, KTR- Kutch region. **Note:** * pvalue < 0.05 considered significant and ** p value < 0.01considered highly significant.

icit (Table 3). Cymbella, and Tabelaria diatom are very susceptible to heavy metal concentration, the potential effect of heavy metal stress on primary productivity of the ecosystem. Therefore, the discharge of metal toxicants in water is serious concern, affecting the aquatic environment. Fragilaria intermedia and Synedra ulna are most resistant to heavy metal pollution [44–45]. Exposure to heavy metals has been reported to be toxic to diatoms, they impairs the cellular metabolic processes (primary productivity, respiration, nutrient and oligo element fluxes) as well as functional components at several sites along the passage of the toxicant over the membrane and into the cell. High metal accumulation in diatoms also reported to impairs phosphorus metabolism, photosynthesis (either by producing an increase in reactive oxygen species or by affecting the function of the xanthophyll cycle), and homeostasis. Therefore, high heavy metal concentration in water bodies may reduce diatom biomass by delaying or preventing their growth [46].

This research data demonstrates the importance of diatom biodiversity in environmental and forensic investigations, as well as future biotechnological developments. Because diatoms may describe pre and post mortem drowning investigations, this diatomic database makes analysts' tasks easier and more useful [10,19]. Diatoms are gaining popularity in applications such as biology and nanotechnology. Because of its oil-secreting ability, diatom milking is beneficial in the manufacturing of gasoline. Diatoms are also being used to make solar panels, cosmetics, medicines, soaps, and biofuels [47–50].

5. Conclusion

Forensic diatomology plays a vital role in resolving drowning cases' mysteries. It helps determine death cause and drowning location. Having enough diatoms in vital organs can confirm ante-mortem drowning. The scattering of diatoms in any body of

Table 3
Pearson correlation coefficient (r) for diatom diversity with reference to environment factor in different region of Gujarat.

Regions	Diatom Diversity	pH	TDS	EC	Temp.	Cr	Mn	Fe	Co	Ni	Cu	Zn	Sr	Cd	Pb
Central	CENTRIC	0.473	-0.156	-0.168	-0.261	0.281	-0.465	-0.153	-0.608	-0.461	-0.298	0.076	0.070	0.004	–
	ARAPHID	0.375	0.457	0.416	-0.502	0.338	-0.106	-0.525	-0.341	-0.018	-0.220	0.434	0.008	0.130	–
	SYMMETRIC BIRAPHID	178	0.310	0.283	-0.507	251	-0.835**	0.397	-0.093	-0.065	-0.271	0.157	0.248	-0.013	–
	MONORAPHID	-0.218	-0.128	-0.256	-0.502	0.866**	-0.318	-0.112	-0.396	-0.102	0.116	0.203	-0.268	-0.266	–
	ASYMMETRIC BIRAPHID	0.41	0.023	0.048	0.410	-0.085	-0.087	0.136	0.308	0.068	-0.110	-0.219	-0.114	-0.205	–
	NITZSCHIOID	-0.441	0.069	-0.008	-0.488	0.317	-0.165	0.463	0.383	0.096	0.197	0.161	-0.222	-0.500	–
	SURIRELLOID	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Saurashtra	CENTRIC	-0.509	0.021	0.015	−242	0.070	-0.001	0.054	-0.243	0.145	-0.168	0.006	0.305	-0.191	-0.263
	ARAPHID	0.288	-0.193	−189	-0.094	0.299	0.340	0.011	-0.097	0.181	0.239	0.023	-0.149	-0.321	-0.050
	SYMMETRIC BIRAPHID	-0.668*	0.195	0.200	-0.012	0.043	0.421	0.475	0.395	0.474	0.144	0.163	0.363	0.244	0.332
	MONORAPHID	0.443	-0.537	-0.531	0.072	-0.142	-0.183	-0.300	−238	-0.287	-0.083	0.138	-0.373	-0.139	-0.183
	ASYMMETRIC BIRAPHID	0.357	-0.386	-0.373	-0.372	-0.061	0.249	-0.103	-0.188	0.092	-0.066	0.520	0.252	-0.140	-0.166
	NITZSCHIOID	-0.046	0.589	0.597	0.012	-0.207	0.500	0.685	0.719	0.388	-0.100	0.225	0.592	0.518	0.518
	SURIRELLOID	0.188	0.201	0.216	-0.126	-0.148	0.102	0.045	-0.024	-0.035	0.008	0.060	0.499	-0.053	-0.053
North	CENTRIC	0.766	-0.088	-0.041	-0.483	0.530	0.740	0.246	0.519	0.741	0.588	0.925**	0.18	0.597	0.316
	ARAPHID	-0.061	-0.040	-0.006	0.266	-0.050	-0.280	0.687	0.467	0.162	0.228	0.170	0.843*	0.710	-0.447
	SYMMETRIC BIRAPHID	0.766	-0.088	-0.041	-0.483	0.530	0.740	0.246	0.519	0.741	0.588	0.925**	0.18	0.597	0.316
	MONORAPHID	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	ASYMMETRIC BIRAPHID	-0.238	-0.394	-0.444	0.418	-0.793	-0.096	-0.480	-0.738	-0.858*	-0.304	-0.862*	-0.362	-0.710	0.447
	NITZSCHIOID	-0.754	-0.754	0.430	0.561	-0.427	-0.549	-0.399	-0.376	-0.709	-0.810	-0.600	-0.518	-0.558	-0.200
	SURIRELLOID	–	–	–	–	–	–	–	–	–	–	–	–	–	–
South	CENTRIC	0.296	-0.095	-0.024	-0.885*	-0.552	-0.888*	-0.482	-0.543	-0.440	-0.656	-0.566	-0.846	-0.665	-0.781
	ARAPHID	-0.278	-0.186	-0.155	-0.060	-0.322	-0.627	0.325	-0.741	-0.695	-0.694	-0.656	-0.257	-0.677	-0.648
	SYMMETRIC BIRAPHID	0.278	0.186	0.155	0.060	0.322	0.627	-0.325	0.741	0.695	0.694	0.656	0.257	0.677	0.648
	MONORAPHID	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	ASYMMETRIC BIRAPHID	-0.296	0.095	0.024	0.885*	0.552	0.888*	0.482	0.543	0.440	0.656	0.566	0.846	0.665	0.781
	NITZSCHIOID	-0.296	0.095	0.024	0.885*	0.552	0.888*	0.482	0.543	0.440	0.656	0.566	0.846	0.665	0.781
	SURIRELLOID	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Kutch	CENTRIC	-0.809	0.828	0.881	−1.000**	0.607	-0.117	0.216	-0.025	0.517	0.698	0.229	0.445	0.590	1.000**
	ARAPHID	-0.809	0.828	0.881	−1.000**	0.607	-0.117	0.216	-0.025	0.517	0.698	0.229	0.445	0.590	1.000**
	SYMMETRIC BIRAPHID	0.809	-0.828	-0.881	1.000**	-0.607	0.117	-0.216	0.025	-0.517	-0.698	-0.229	-0.445	-0.590	−1.000**
	MONORAPHID	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	ASYMMETRIC BIRAPHID	0.913	0.071	-0.032	0.500	-0.992	-0.801	0.738	-0.853	−1.000*	-0.969	-0.957	0.553	-0.994	-0.500
	NITZSCHIOID	-0.104	-0.900	-0.850	0.500	0.385	0.919	-0.954	0.878	0.483	0.271	0.729	-0.998*	0.404	-0.500
	SURIRELLOID	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Note: *a Correlation is significant at the pvalue < 0.05 and ** highly significant at p value < 0.01.

water and their interrelation with those from the drowned body can help determine the site of drowning. Diatom findings from the water as well as biological samples provide a lot of information regarding the cause of death, locality and probable season. Many attempts to prepare diatomological data have been made in many parts of India, including Haryana, Punjab, Delhi, Mumbai, Orissa, Mizoram, and Indore. The Gujarat region has less diatomological data. This diatomological data provides a qualitative distribution of diatoms across various water bodies in the region. In this study, Diatom diversity has been shown to be unique to locations important in the forensic investigation of drowning cases. Observations also show variance in diatoms in relation to physico-chemical parameters and other environmental factors like heavy metals. Diatom species were substantially more abundant in areas with low micro-elemental composition. Thus, physico-chemical and heavy metal studies such as Ph, TDS, EC, and metal content in water have a significant impact on the diatomic community.

CRediT authorship contribution statement

Satish Kumar: Validation, Supervision. **Malay A Shukla:** Writing - review & editing. **Chandra Shekhar Yadav:** Validation, Conceptualization, Supervision.

Data availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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