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Original Article

Effect of Altitudinal and Seasonal Factors on Diatomological Mapping of Waterbodies: Implication in Drowning Investigations

تأثير العوامل الموسمية والارتفاع عن سطح البحر على رسم خرائط طيف الهائمات المائية: تطبيقات التحقيق في قضايا الغرق

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Abstract

Diatoms are an essential part of forensic investigation in drowning cases. They are used to differentiate between ante-mortem and post-mortem drowning and to ascertain the site of drowning. However, to do so it is imperative to perform the diatomological mapping of water sources in different regions. The issue of localizing a drowning site may be resolved with the help of diatomological monitoring and mapping of the water sources, which generates substantial references for meeting the 'criterion of concordance'. The present study has generated a database of diatom diversity in relation to different seasons and altitudes.

Water sampling was made from low, mid and high altitudes during summer and autumn seasons. Important information was found after a microscopic examination of diatoms in water samples from different water bodies at different geographical altitudes.

Twenty-seven diatom genera have been observed in the water samples from the selected localities. The findings of the current study have exposed the significant effect of seasonal changes and varied altitudes on occurrence of diatoms. A variety of commonly occurring site restricted diatoms were identified in the water samples.

This useful information can be significant while investigating drowning cases from this particular region when the drowning site is either questioned or unknown.

Keywords: *Forensic Sciences, Diatoms, Drowning, Altitude, Seasons.*

المستخلص

كانت الهائمات المائية (أحياء دقيقة مائية) وما تزال جزءًا أساسيًا في التحقيق الجنائي لحالات الغرق. وتستخدم للتمييز بين الغرق ما قبل الوفاة والغرق ما بعد الوفاة والتأكد من موقع الغرق. ومع ذلك، للقيام بذلك، لا بد من إجراء ورسم خرائط لطيف الهائمات المائية لمصادر الماء في مناطق مختلفة. ويمكن حل مسألة تحديد موقع الغرق بمساعدة مراقبة الهائمات المائية ورسم خرائط خاصة بها للمسطحات المائية ما ينتج عنه قواعد بيانات ومراجع كبيرة لتلبية «معيار التوافق». وقد عملت هذه الدراسة على بناء قاعدة بيانات متنوعة من طيف الهائمات المائية وذلك في فصول سنة وارتفاعات مختلفة عن سطح البحر. تم أخذ عينات المياه من الارتفاعات المنخفضة والمتوسطة والعالية خلال مواسم الصيف والخريف. وتم الحصول على معلومات هامة من الفحص المجهري للهائمات المائية في عينات المياه من مناطق مختلفة. وقد لوحظ وجود ٢٧ نوع من الهائمات المائية في عينات المياه من المناطق المختارة. وقد كشفت نتائج الدراسة الحالية تأثيراً كبيراً للتغيرات الموسمية والارتفاعات المتنوعة على تواجد وتنوع الهائمات. كما حددت مجموعة متنوعة من الهائمات الشائعة والمقيدة المواقع في عينات المياه. يمكن أن تكون هذه المعلومات المفيدة مهمة أثناء حل قضايا الغرق في مناطق محددة عندما يكون موضع الغرق إما موضع شك أو غير معروف.

الكلمات المفتاحية: علوم الأدلة الجنائية، الهائمات المائية، الغرق، الارتفاع، فصول السنة.

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1. Introduction

Diatoms are microscopic, single-celled algae that have intricate glass-like outer cell walls partially composed of silicon. Different species of diatom can be identified based upon the structure of these walls. Many diatom species are planktonic, suspended in the water column moving according to water currents. Diatoms have been extensively used over the years to understand ecology, climate and other aspects related to water bodies. There is also an important use of diatoms to answer queries related to drowning cases i.e. to determine the putative site of drowning and also to differentiate between ante-mortem and post mortem drowning. In Ante-mortem drowning, a person is still alive and immersed in water. In such cases, some water enters the lung cavity with force and exerts pressure on the lung walls. Owing to this pressure, lung alveoli get ruptured and water reaches the blood stream. Till the last heartbeat, water keeps on mixing with the blood and during this course some diatoms (if present in the water), particularly the smaller ones, are also pushed into the blood circulation from where they reach the vital organs like the liver, spleen, kidneys, brain and bone marrow. It is the respiratory pressure which helps diatoms to reach these distant organs. On the other hand, the chance of diatoms entering in distant vital organs remains negligible, if the person is already dead and is thrown into the water (post-mortem immersion). In certain instances, however, the diatoms can be directed in the lungs even in the case of post-mortem drowning when, due to the passive absorption of water, diatoms can reach the lung cavity without entering further into the body [2].

The diatomological profile of a particular water body may be taken as reference to compare the diatoms recovered from the drowned body trapped in apprehensive circumstances of drowning [3-5]. The diagnosis of drowning by diatom test should be considered positive only when the number of diatoms is above the minimal established limit i.e. 20 diatoms/ 100μl of pellet obtained by enzymatic digestion of 10 gm of lung sample, and 5 diatoms from other distant organs. Furthermore, a positive comparison of diatoms extracted from a bone marrow sample and reference water body sample should be achieved to draw a positive conclusion towards ante-mortem drowning or post-mortem drowning. This criterion is called 'Criterion of Concordance' [3-4]. The diatom test can further be strengthened by corroborating it with other circumstantial evidences. Beside the positive aspects of a diatom test, the reliability and applicability of diatom test is in dispute. Some scientists have found diatoms in the tissues and bone marrow of living people [25]. This observation needs more research as few forensic scientists have contributed to this particular issue [3-5].

Drowning can be defined as death due to full or partial submersion in a fluid. It is a medicolegal inquiry where both the medical practitioner and forensic expert are involved. During autopsy, the medical practitioner examines the classical findings present in the freshly drowned bodies i.e. the signs of drowning such as large amounts of froth present around nostrils and mouth, and inflated and water logged lungs etc., which may help to diagnose the case of drowning. The forensic expert uses this unicellular microorganism in diatom test to confirm drowning as a cause of death [1-2]. Use of diatoms has also been reported in the localization of a putative site of drowning [8]. Qualitative and quantitative diatomological research from different localities can make it possible. Continuous river monitoring and diatomological mapping (D-maps) of water bodies has been a great success [3-4]. These methods of diatom research characterize water bodies on the basis of diatom profiles generated following parameters such as

seasonal changes and water quality. Diatom communities exist almost everywhere. The communities that live in water usually consist of more species than those communities found in aerial habitats or in soil [9-10]. These factors are essential for the forensic investigator to solve the suspected drowning cases [11]. Each lake or pond can develop a distinctive floral community comprised of specific populations of species. Water bodies with similar physical and chemical characteristics may develop particular algal communities by which they may be distinguished. Diatoms grow in practically all bodies of water, but a few may restrict and survive in only particular habitats [10]. It is scientifically proven that the growth of diatoms may be highly diversified and specific depending upon seasonal (light and temperature) and other conditions such as salinity, pH and nutrient contents of water [22]. Beside this, the intra water bodies studies were conducted to find the exact drowning site [12]. In conclusion, the distributional patterns of diatoms can respond to a multitude of different factors, ranging from bio-geographical to biogeochemical and human influenced [13-17]. Another problem which might arise while matching diatomological profiles is due to seasonal variation. This problem arises when the profile is generated during a different season than that in which the body was found. In such cases, it is more useful to compare the diatom species found in various body parts to the diatomological profile of water bodies in the same season.

Himachal Pradesh is one of the northern states of India known for tourism. This hilly state has a channel of different types of water bodies like lakes, rivers and dams running at different altitudes. This topographic diversity and natural beauty attract number of tourists. Some water bodies are used for transportation and for recreation. Therefore, these places become more prone to accidental drowning during boat capsizing. Cases of suicides and homicides also take place in these types of water bodies. An important study confirmed 514 cases of drowning from Himachal Pradesh from 2006 to 2010 [19]. The literature survey has not produced any study on diatom flora of water bodies from Himachal Pradesh, especially in connection to forensic science. Hence, here we present a comprehensive and prominent study of diatom distributions from lakes in Himachal Pradesh. An important and interesting aspect of this study was to screen diatoms from the water bodies flowing at different altitudes.

2. Materials and Methods

2.1 Study Areas and Collection of Samples

Eight water bodies namely Renuka lake, Macchial lake, Gobind Sagar lake, Rewalsar lake, Manikaran, Pondoh dam, Prashar lake and Nako lake were chosen from known heights above sea level to test the variable of of low, mid and high altitudes. Localities along with relevant details have been given in Table-1 & Figure-1. Altitude difference is clear from Table-1. Altitude ranges were fixed as low altitude (up to 1000 meters), mid altitude (between 1000 to 2000 meters) and high altitude (above 2000 meters) above sea level.

Figure 1- *Study sites of Himachal Pradesh.*

Table-1: *Relevant details of the selected eight water bodies.*

Altitude Ranges	Name of Water body with self-assigned Code	Type of Water-body	District	Geographical Coordinates	Altitude from Sea Level (meters)	Environment Temperature (max.) at the Time of Sampling (°C)		Water Temperature (max.) at the Time of Sampling (°C)	
						S	A	S	A
Low range	GobindSagar lake (GBS)	freshwater	Una and Bilaspiur	31°24'39'N 76°26'0' E	560	36	30	30	26
	Renuka lake (RNL)	freshwater	Sirmaur	30°36'36'N 77°27'30' E	672	25	20	22	19
	Macchial Lake (MCL)	freshwater	Mandi	31°56'17'N 76°47'49' E	950	26	25	26	22
Mid range	Pondoh Dam (PD)	freshwater	Mandi	31°40'17'N 77°04'01' E	1350	20	17	17	15
	Rewalsar lake (RSL)	freshwater	Mandi	31.63389°N 76.83333' E	1360	26	22	30	20
	Manikaran-Lake (MNK)	freshwater	Kullu	32.0279°N 77.3480° E	1760	22	19	75	70
High range	Prashar lake (PL)	freshwater	Mandi	31.75426°N 77.10141°E	2730	17	15	10	10
	Nako Lake (NL)	freshwater	Kinnaur	31°52'53.47'N 78°37'38.87'E	3662	19	15	10	12

S; summer, A; Autumn.

2.2 Collection and Analysis of Water Samples

500 ml water samples were collected from the surface of each water body during summer and autumn seasons in the month of June and September, respectively, in 2014. Sampling was done from at least three different locations from the bank (distance 1m, 5m, 10m) of a water body for standardization and to reduce sampling error. The water and environment temperatures (max.) were also noted down using a digital thermometer during daytime. Five permanent slides were made for every water sample collected using DPX as mounting medium to check reproducibility in results. The final pellet was taken onto the microscopic slide, dried at room temperature and covered with coverslip. The later was fixed with mountant DPX, after treating a 250 ml water sample with concentrated nitric acid and later centrifuged at 5000 rpm for 15 minutes. Photomicrographs of the diatoms were taken with the help of photo capturing device (Nikon- DS-FI2) and a compound microscope (with digital camera). Diatoms were identified on the basis of descriptions in the manual given by Hartley et al. 1996 [20]. The water samples were analyzed twice under the same set of experimental conditions to check the reproducibility of results and their concordance with each other. A diatomological database was compiled according to methods available in the literature [8].

3. Results

Seasonal and altitude factors are reflected in the results of this study. It is important to note that both these factors have contributed to notable changes in qualitative (types of species) and quantitative (quantum of species) findings. These findings have helped to characterize some of the selected water bodies. Microscopic analysis identified 27 genera of diatoms in the water bodies from all locations. The photomicrographs of diatoms were given in figures 1-20 (Figure-2). Of this number, three genera namely Navicula (fig. 20), Nitzschia (fig.23) and Pinnularia (fig.19) were present in water bodies from all sites; therefore they were marked as commonly occurring diatoms. It was found that at high level altitude, less number of diatoms were found because of the adverse environmental conditions (low temperatures). Most of the diatoms genera belonged to order pennales as compared to order centrales.

4. Discussion

4.1 Diatoms at Different Altitudes

It is evident from Table-2 that there is a slight but important disparity in occurrence of diatoms at different altitudes. Some of the diatoms were specific to altitude. Diatoms such as Bacillaria (fig.20), Cyclotella (fig.3), Meloseria (fig.17), Pleurosigma (fig. 10), Sellophora (fig.19) and Synedra (fig.21) were exclusively identified at low altitude levels (up to 1000 meters), while middle range altitude (between 1000-2000 meters) produced Aulacoseria (fig.23), Caloneis (fig.1), Cymatopleura (fig.9), Encyonema (fig.15), Mastogloia, Nedium and Stauroneis diatom species. Some species of Amphora, Epithermia (fig. 12) and Surirella (fig.7) were seen at high altitude (above 3000 meters) water samples. Diatoms genera, namely Cocconies (fig.6), Cymbella (fig.4), Gyrosigma (fig.8), Hantzschia (fig.2), Gomphoneis (fig.26) and Rhoicosphenia (fig. 16), were common at more than one altitude range, while Navicula (fig.5), Nitzschia and Pinnularia (fig.14) diatom species were common and abundantly observed in water bodies from all altitude ranges (Table-2). The present observations are in agreement with Nautiyal [23], who studied the diatom bio-diversity of some Himalayan lotic systems in the Mandakini region at different altitudes. They found 200 diatom taxa in the mandakini basin and found that the species richness and diversity increase gradually

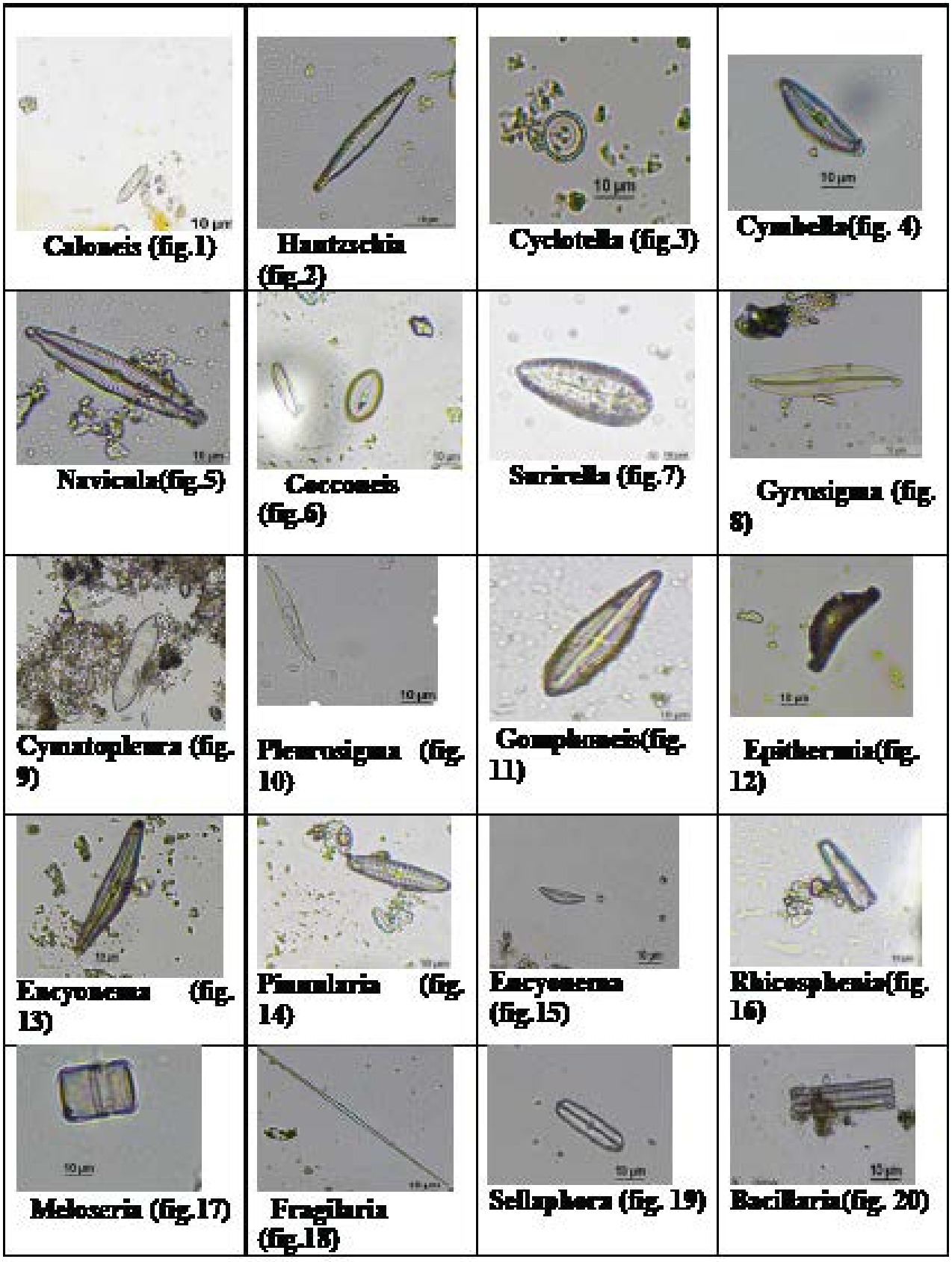

Figure 2 - *Photomicrographs of diatoms at 400X.*

with a decrease in altitude.

4.2 Diatoms at Different Seasons

The seasonal effects on diatom distribution are evident from Table-3. There is noteworthy effect of seasonal changes on diatom growth in water bodies. Notable observations pertaining to temperature difference and occurrence of diatoms have been recorded. Summer and autumn are two distinct seasons where change in temperature can be observed. Table-1 clearly shows that maximum temperature observed at the time of sampling in summer (June) was 36 °C, while the lowest was 15 °C in autumn (September). A short rainy season between these two seasons also brought changes in the diatom diversity in different water bodies. Details of diatoms existing in different seasons and temperatures in selected water bodies can be extracted from Table-3. Renuka lake is the low altitude water body located in the Sirmaur district. This is a religious sacred place having pure water. Diatoms such as Cymbella (fig.25), Synedra (fig.29), Cyclotella (fig. 3), Rhicosphenia (fig.17), Cocconeis (fig.6), Pleurosigma (fig.11) and Meloseria were observed in both seasons. Autumn produced highly diverse populations of diatoms in comparison to summer. Macchial lake is another low altitude water body situated in the Mandi district. Due to fishing, its water was found to be contaminated. Both the seasons produced verities of diatom species comparatively more than other localities. Fragilaria (fig.18) and Gyrosigma (fig.8) were distinguishing diatoms of this water body. Gobind Sagar lake is the largest of all water bodies and is situated at the lowest altitude in the Bilaspur and Una districts. Water was slightly contaminated due to anthropogenic activities here. Bacillaria (fig.26), Cymbella (fig.4) and Fragilaria (fig.18) were also found here. Rewalsar lake is the mid altitude water body located in a mountainous

Table-2: *Effect of altitude on Diatoms composition.*

Altitude range	Site-restricted Diatoms at Individual Altitude	Diatoms Common at more than one Altitude	Diatoms Common at all Altitudes
low altitude (Up to 1000 meters)	Bacillaria Cyclotella Meloseria Pleurosigma Sellophora Synedra	Cocconeis Fragilaria Cymbella Gyrosigma Hantzschia Rhicosphenia	
Mid altitude (Between 1000-2000 meters)	Aulacoseria Caloneis Cymatopleura Encyonema Mastogloia Nedium Stauroneis	Cocconeis Cymbella Fragilaria Gomphoneis Tabularia	Navicula Nitzschia Pinnularia
High altitude (Above 2000 meters)	Amphora Epithermia Surirella	Gomphoneis Gyrosigma Hantzschia Rhicosphenia	

Table-3: *Distribution of diatoms in selected water bodies during both seasons.*

Diatoms Genera	Low Altitude						Mid Altitude						High Altitude			
	Renuka Lake		Macchail Lake		Gobind-Sagar		Rewalsar lake		Mani-karan Gu-rudwara		Pondoh Dam		Prashar Lake		Nako Lake	
	S	A	S	A	S	A	S	A	S	A	S	A	S	A	S	A
Pinnularia	-	+	-	+	-	+	-	+	+	-	-	+		+	-	+
Meloseria	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitzschia	-	+	-	+	-	+	-	+	-	+	-	+	-	+	+	+
Rhoicosphenia	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Cocconeis	-	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-
Pleurosigma	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fragilaria	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-
Gyrosigma	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
Navicula	-	+	-	+	+	+	+	+	+	-	+	+	+	+	-	+
Surirella	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
Hantzschia	-	-	-	+	-	-	-	-	-	-	-	-	-	+		-
Synedra	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Sellaphora	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Cyclotella	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
Bacillaria	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Cymbella	+	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-
Aulacoseria	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Encyonema	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Tabularia	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-
Gomphoneis	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+
Cymatopleura	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Mastogloia	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Stauroneis	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Nedium	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Amphora	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Epithemia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Caloneis	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-

+ (Present) - (Absent) S=Summer A= Autumn

area in the Mandi district. This water body was found polluted due to external sources. Aulacoseria (fig.30) was noted as the site-specific diatom found in both seasons. Manikaran is mid altitude water body located in the north east of the Bhuntar district in the Kullu district. The water of this water body is extremely hot due to high temperatures. Navicula (fig. 35), Pinnularia (fig.19), Cocconeis (fig.21) and Gomphoneis (fig. 24) were seen here. Pondoh dam is mid altitude water body located on the Beas river in the Mandi district. This dam is used for hydroelectric power generation. Most of the pennate diatoms i.e. Caloneis (fig.1) were the site-specific diatom in this water body. Prashar lake is the high-altitude water body located north of the Mandi district. Amphora was the characteristic diatom of this water body. Nako lake is another high-altitude water body located in Pooh, a sub division of Kinnaur district. Rhicosphenia (fig.32) was found in summer, while diatoms i.e. Epithermia, Gomphoneis (fig.12) and Nitzschia (fig.23) were seen in autumn. Epithermia (fig.13) was seen in this water. Prashar lake and Nako lake are high altitude water bodies in which the smallest number of diatoms were observed due to low temperatures.

4.3 Frequency of Occurrence of Diatoms

As usual, the highest abundance of diatoms was recorded in autumn due to favourable climatic conditions, whereas declination in diatom occurrence was observed during hostile summer conditions. The highest diatoms frequency i.e. was observed in Macchail lake (11) followed by Pandoh Dam (10) and Renuka lake (10). Diatom population was lowest at Prashar lake. It is evident from Figure-3 that the frequency of Nitzschia diatom species was highest in the total population, followed by Pinnularia (fig.14) Other diatoms such as Navicula (fig.5), Cocconeis (fig. 6) and

Table-4: *Morphometric measurements of diatoms.*

Diatom Genera	Length (μm)	Width (μm)	Diatom Genera	Length (μm)	Width (μm)
Aulacoseria	45.60	5.02	**Meloseria**	27.44	13.14
Amphora	24.80	19.08	**Mastogloia**	26.40	7.48
Bacillaria	62.43	11.29	**Navicula**	30.91	10.05
Cyclotella	16.60	-	**Nitzschia**	36.71	3.48
Cymbella	186.16	67.90	**Nedium**	36.06	8.30
Cocconeis	14.92	11.21	**Pinnularia**	32.84	10.43
Caloneis	98.33	18.65	**Pleurosigma**	39.88	5.02
Cymatopleura	86.14	18.78	**Rhoicosphenia**	25.89	10.43
Epithemia	35.30	9.88	**Surirella**	47.42	16.99
Encyonema	37.14	9.19	**Synedra**	895.04	23.54
Fragilaria	32.72	23.33	**Sellaphora**	33.56	8.30
Gyrosigma	57.40	11.41	**Stauroneis**	8.67	2.41
Gomphoneis	59.07	16.94	**Tabularia**	343.13	34.00
Hantzschia	19.79	3.89			

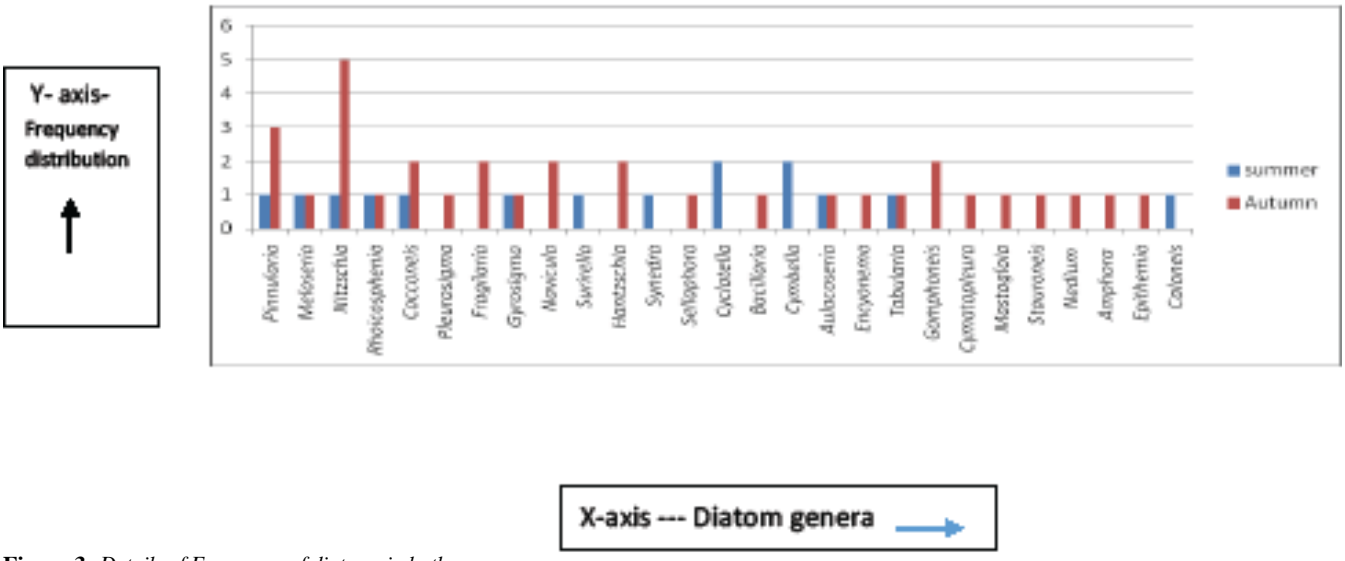

Figure 3- *Details of Frequency of diatoms in both seasons.*

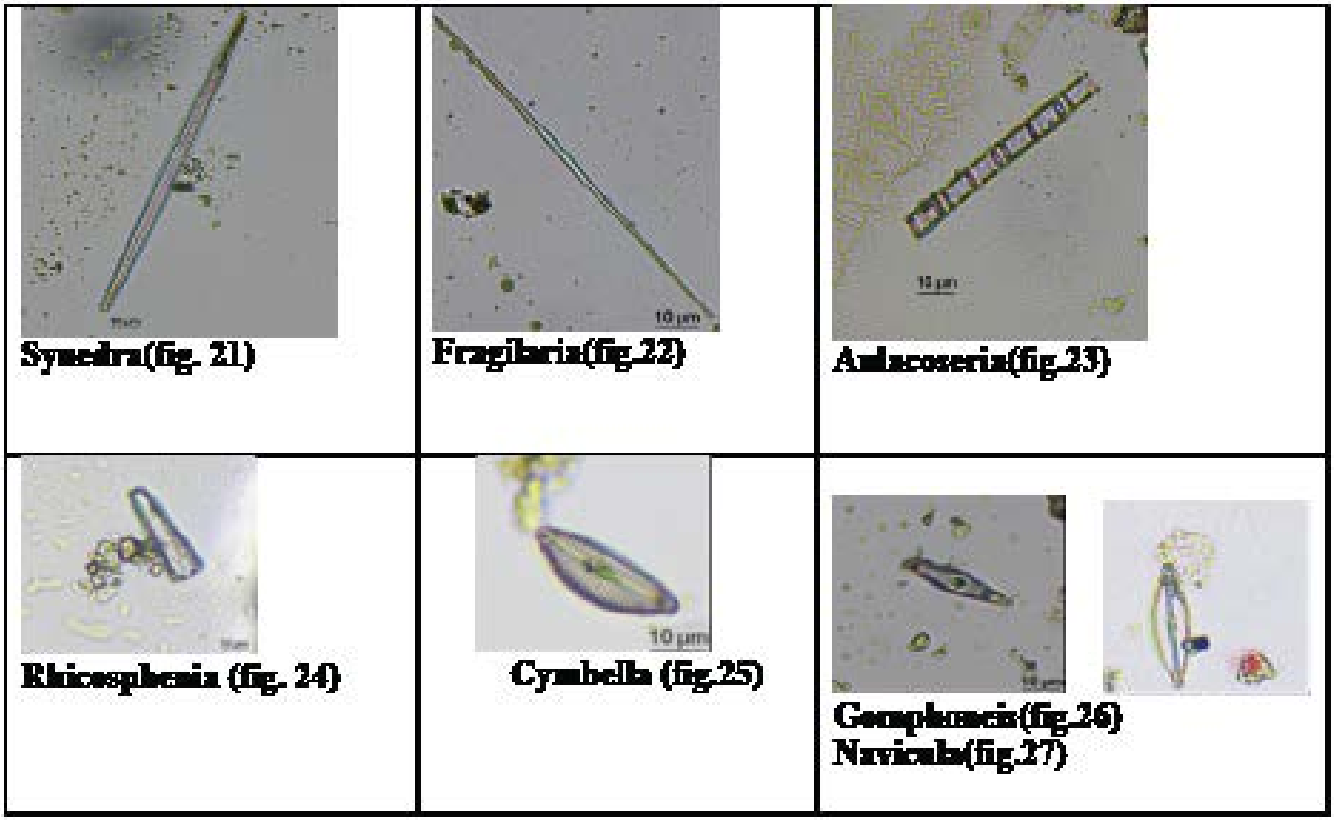

Figure 4 - *Photomicrographs of large (fig. 21-23) and small (fig. 24-27) size diatoms at 400 X.*

Cyclotella (fig.3) were also major contributors of the population. There were also single species of Caloneis (fig.1), Amphora, Nedium, Epithemia (fig.12) and Surirella (fig.7) diatoms.

4.4 Morphological and Morphometric Analysis

Morphological analysis of diatoms revealed a noticeable shape variation. The shape and size of diatoms also plays a role in drowning. When a diatom is elongated in shape and small in size, it is more likely to penetrate

into lung tissues and enter into the blood stream [24]. The shapes of most of the diatoms found in the selected water bodies were elliptical, rod like and oval in shape. Synedra (fig.21) and Melosira (fig.17) species were centric diatoms, whereas Cocconeis (fig.6) was oval shape. Cymatopleura (fig.9) and Gyrosigma (fig.8) had irregular shapes. Details of photomicrographs of large and small size diatom are given as figures at 400x magnification. Some important morphometric features recorded with the help of a microscope equipped with a micrometer have been given in Table-4. Distinguishing dimensions were seen amongst diatom. Both large such as Synedra (fig.21) and Fragilaria (fig.22) and small i.e. Cymbella (fig. 25) and Rhoicosphenia (fig.24) etc., were seen distributed in the water bodies (Figure-4).

5. Conclusion

The findings of the present study revealed the effect of altitude and seasons on the occurrence of diatoms genera. The diatoms genera richness increases gradually with decrease in altitude; more diversity in diatoms species were observed in lower altitude waterbodies as compared to high altitude waterbodies in autumn than the summer. Therefore, based on these findings it can be safely concluded that altitude along with season have an impact on diatom composition. This aspect of variation in diatom composition must be taken into consideration while using the "diatom test" for investigating drowning cases to avoid false results. It is apparent from the literature that the influence of altitude factor on diatom composition in a water body is poorly understood. Therefore, similar diatomological studies should be carried out on water bodies at different altitudes across the globe. The outcome of these studies will not only be helpful to forensic experts but the interdisplinary nature of such studies can also be helpful for researchers from the fields of botany and environmental sciences.

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Conflict of Interset

None Declared.

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