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Diatom Analysis in Thermophilic water bodies: A Study on Hot Water Springs of Madhya Pradesh

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ABSTRACT

Introduction: This study was done with the objective to study the presence of various species of diatoms present in the thermophilic water bodies of Madhya Pradesh (Anhoni and ChavalPani). And to determine the correlation of physiological variables such as temperature and pH to the diatom density.

Methodology: For the detection of diatom species in the water samples acid digestion method was used.50 ml of sample was taken and 10 ml of concentrated nitric acid (HNO3) was added. The sample is then kept undisturbed for 24 hours. the sample was then centrifuged for 10 minutes at 3000rpm 3 times. the palettes formed were suspended in distilled water and centrifuged at 3000 rpm to remove acid content. The palettes are then transferred onto a clean and dry slide and are dried by keeping them on a hot plate at 30-40 C for 4-5 minutes. This slide is then observed under a phase contrast microscope and pictures are taken of varied diatom species using the attached camera.

Observations and Results: The analysis in our research concluded that there are various diatoms present in these thermal water springs belonging to the class Bacillariophyceae, Mediophyceae and Fragiariophyceae. The diatoms that were viewed and captured under Phase contrast microscope were, Nitzschiapalea, Nitzschialinearis, Discostellastelligera, Achnanthidium Sp., Tabularia Sp., Anomoeoneissphaerophora, Amphipleura Sp., Tryblionella Sp., Fragilariacrotonensis., and Nitzschiafiliformis The research lead us to the results that hot water springs that had lower temperatures had more percentage of diatom density as compared to the hot water springs with higher temperatures. According to our research 60% of the diatoms found were from the geothermal spring of Anhoni, Madhya Pardesh which had a lower temperature while the geothermal spring at Chawalpani, Madhya Pradesh was found to have lower diatom density due to its high temperatures and low nutrient index. When the pH of these water samples was studied against the diatom density, no significant

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difference was found due to the nominal range difference in pH. Yet geothermal springs had a 10% more diatom density at a low pH level which is 5 in this case study. This research leads to certain crucial conclusions that gave us strong evidence for co-relation amidst temperature, pH and diatoms.

Conclusion: Geothermal springs are a habitat to many diatom species. These diatom species are specific to a particular site and are used in cases of drowning death for leads. On extraction and analysis of these diatoms we can detect and compare them to the standard diatoms present in the water sources of that area. This helps Forensic Scientist finds leads in any case of death due to drowning. the analysis in our research concludes the presence of diatom species in the geothermal springs of Madhya Pradesh, namely Anhoni and Chawalpani. These diatoms include species belonging to class of Bacillariophyceae, Mediophyceae and Fragilariophyceae. it also establishes the correlation between the temperature, pH and diatom density of these geothermal springs. It can be concluded that the geothermal springs that have lower temperature and pH are prove to have higher diatom density and diversity as compared to the ones with higher temperature.

Keywords: Diatom; Geothermal; Physiological variables; Phase contrast microscope; Diatom density.

INTRODUCTION

Diatoms are microscopic algae with silica walls of varied structures. These are unicellular photosynthetic autotrophs that thrive in freshwater environments.[1] These species need nutrients to survive and hence cannot survive in domestic water sources. You can find varied diatom species in lakes, ponds, rivers, oceans and geothermal water springs. Diatoms generally fall in the 2–200 micron size profile and belong to 2 groups, one is centric with lateral symmetry and the other is pinnate which exhibits bilateral symmetry.[2] They help find lead in the cases of drowning or disposal of body into any water source. Varied types of diatoms have been recorded over the years. There are at present 8000 known species of diatoms. Diatoms serve as a vital evidence in cases of death due to drowning. deaths by drowning, accidental, suicidal or homicidal have been an issue of concern. Deaths due to drowning have alarming numbers. There have been 23, 600 million annual deaths by drowning worldwide in the year 2020 as stated in a report by world health organization (WHO). In a report by National crime bureau, India accidental deaths and suicides in the year 2020 have been 7.5% of all unnatural deaths which makes up 29,456 deaths by drowning. The large part of these numbers has been from the child deaths by injury due to drowning. 83 people die every day due to drowning, which is sad and alarming at the same time.

Forensic scientists have used diatom analysis to detect the presence of various diatom species specific to a particular water body. Years of research has leaded us to effective means and methods to get leads in the drowning deaths through diatom analysis.[3] Other than fresh water sources, hot water springs found in nature also have heat-resistant diatom species. Geothermal springs are formed due to tectonic movements of the earth or the volcanic eruptions in the area leading to the release of hot water into to various water bodies. These geothermal springs house for a diversity of species meant to survive and thrive in such conditions. These hot water springs have varied diatom species which benefit the work of forensic scientists and help cases in forensic science.[4] Multiple studies have been conducted on these species in order to make out the presence of a kind of diatom species in a particular water body. Death by drowning can be explained as death due to submersion of a body in water causing hypoxemia and irreversible cerebral anoxia. During this process diatoms in water bodies tend to flush organs. Diatom analysis involves the recovery of these diatoms from various parts of the body like lung, liver, spleen, kidney and brain etc.[2] The diatom species found in the tissues of the drowning person are then matched to these standard recorded diatoms to confer the source of water.[5] these diatoms species have a co-relationl with the pH and temperature of water. The diatom density depends on these both physiological factors.[6] Many researchers have carried out study to find the various diatom species in various water bodies. Our research aims at providing proofs of presence of diatom species in the geothermal springs of Madhya Pradesh. the physiological variables of any water source also has correlation with the diatom species and the diatom density

at that site. Our research focuses on showing the correlation between the temperature, pH, diatom density and presence of specific diatoms in the geothermal springs.

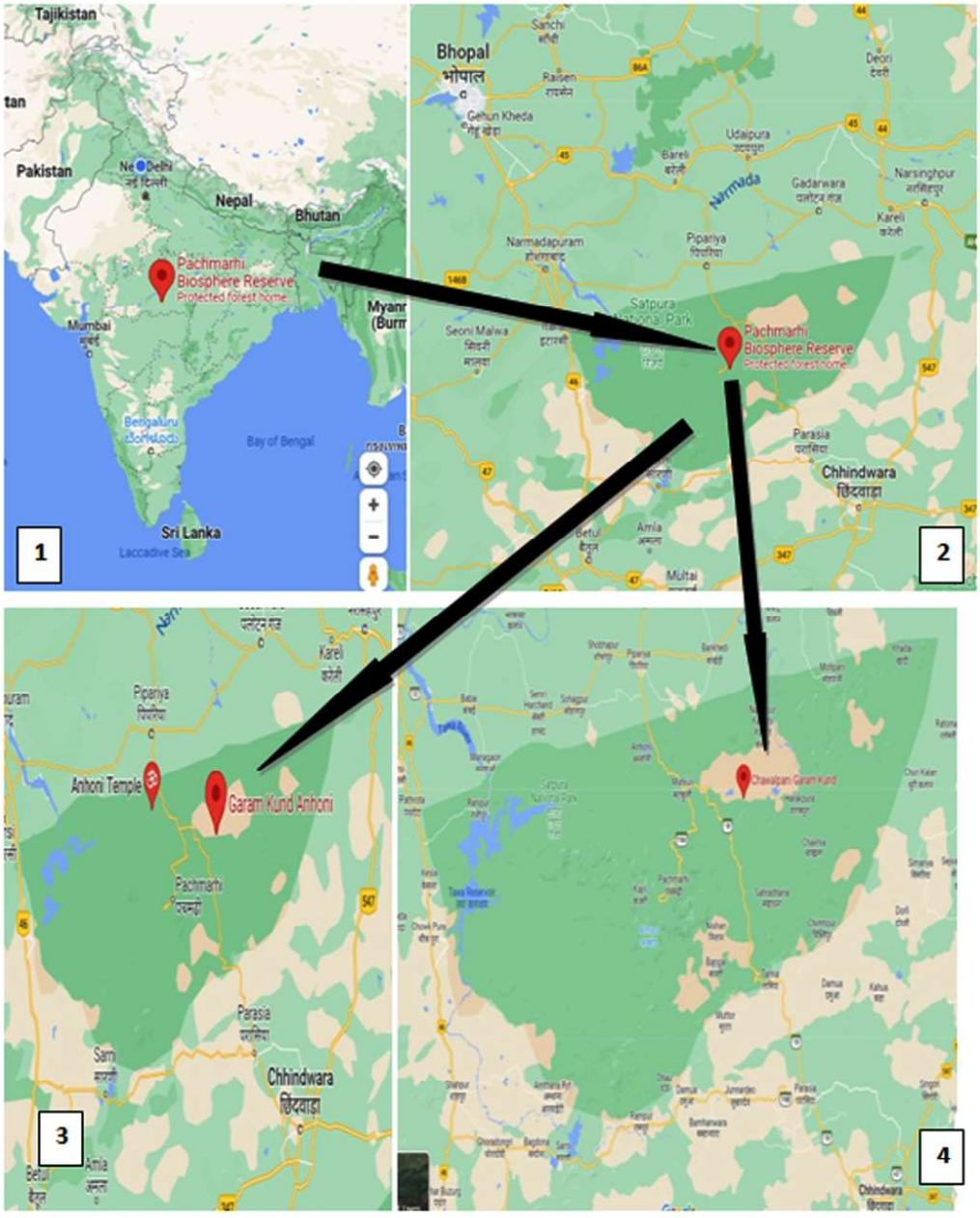


Fig. 1: Site map showing the study areas. The red location symbol represents the areas of study. Image 1 & 2: depict the Pachmarhi biosphere reserve.
Image 3: Anhoni Garam kund, Pachmarhi biosphere reserve, Madhya Pradesh. Image 4: Chawalpani Garam kund, Madhya Pradesh. **Image Source:** Google maps.

Brief History and Significance of Diatom Testing in Forensic Science

The detection and discovery of diatom analysis dates back to the 1900's. Hofmann first detected diatoms in lung fluid. Post which many different researches and theories came up regarding diatom analysis. The first time diatom analysis was carried out in the field of forensic was in the year 1960's and 1970's by Timperman, who in his research stated the presence of diatoms in bone marrow, liver, spleen, lung, kidney and brain tissue in case of death due to drowning.[2] Many researches came up year by year improvising the method of diatom analysis.[7] Pollanen improvised the traditional method and gave the method of acid digestion using nitric acid for the detection of diatoms in the sample. This method is proven to be the best

method and is used till date. Diatom analysis has become and inevitable part of forensic science in cases for death due to drowning.

Diagnosis of drowning deaths which focuses on detecting the presence of certain diatoms in freshwater ecology and there after its relation to the crime scene is studied under the branch of Forensic Limnology.[8] The victims suspect or the other crime scene evidences are tested for the presence of diatom species and then matched to the standard data to find the common source.[9] Diatom analysis has helped solve many drowning cases. Forensic spans a great use of these diatoms when it comes to detection of cause of death at the time of ante and post-mortem. At present there are many researches being carried out to detect diatom species in water samples and also new ways are being conferred to carry out diatom analysis. Many researches are in process to invent advanced technologies for detection of diatom species using molecular biology, nuclear magnetic resonance and also certain tools for automatic detection of diatom species.

Analysis and Detection of Diatoms from Geothermal water samples

1. Studied area

Our sites of interest were two hot springs of Madhya Pradesh in the Pachmarhi hill station. This beautiful hill station is located in the valley of the Satpura range in the Narmadapuram district of Madhya Pradesh. Pachmarhi is a popular tourist spot in Madhya Pradesh. The two hot springs that we studied are located in the 'Pachmarhi biosphere reserve'. First hot water spring is Anhoni in Pachmarhi biosphere reserve located at 22°42´42N 78°11 E longitude and latitude. This is a hot water spring located in the district of Chhindwara, Madhya Pradesh. Anhoni is a remote forest village known for its boiling water kund. This hot water spring holds spiritual sentiments and has a temple made around it where you can view devotees who come to pray. The second hot water spring is Chavalpani. Chavalpani hot spring is also located in the district of Chhindwara, this hot water spring is located near Pachmarhi hills in the plateau of Mahadeo hills, Madhya Pradesh. The hot water spring is 2 miles away from the Jhirpa village on the Chhindwara to Piparia road. Both these hot water springs are known for their magical waters that supposedly cure all body pains and ailments.

2. Samples collections

The samples were collected from four different locations at both the hot water springs. The samples were collected from the inner pond of Anhoni hot spring and the second sample was collected from the outer pond of hot water spring. The next sample was collected from the hot water spring of Chavalpani from its main stream pond. And the last sample was collected from the outer outlet of the Chavalpani hot water spring. For the collection purpose the water surface was first disturbed to move the settled mud and algae. The a plastic bottle of 1L was filled with the muddy water along with the algal growth from its walls which was removed while collection. Post collection the water was treated with ethanol and lugol iodine to prevent from contamination. The thermal conductivity and pH of waters using pH strips was measured at the spot alone. The algal samples from these researches were preserved in 4% formalin solution and carried to the lab.

Fig. 2: Images captured at site of collection 1: Pachmarhi Biosphere reserve gate 2: Anhoni hot water spring, the water from the inner pond is collected to be used in holy rituals, whereas the water from the outer pond is used for bathing to cure ailments. 3: Temple at the banks of the hot water spring.

3. Processing of samples

For the detection of diatom species in the given sample acid digestion method was used. In the lab 50 ml of water sample was taken into a clean and dry beaker. To this 10 ml of concentrated nitric acid (HNO_3) was added. The sample is then kept undisturbed for 24 hours. Now the sample is moved into a centrifugation tube and centrifuged for 10 minutes at 3000rpm. The centrifugation is carried out 3 times by taking 5ml of sample each time to increase the concentration. The supernatant is removed and the palettes formed are suspended into distilled water and centrifuged at 3000 rpm to remove acid content. The palettes are then transferred onto a clean and dry slide and are dried by keeping on a hot plate at 30-40 C for 4-5 minutes. A drop of DPX was added over the slide and covered with cover slip. This slide is then observed under a phase contrast microscope and pictures are taken of varied diatom species using the attached camera.

RESULTS AND DISCUSSIONS

The water samples collected from the geothermal springs were studied for the presence of diatoms using the acid digestion method and also the physiological features of the samples were studied to draw the correlation amidst the physiological variables of the geothermal spring and the diatom species. The physiological variables that were recorded were pH, temperature and thermal conductivity. The pH was detected using the pH meter by simply dipping the strip into water source and them matching it to the standard pH scale. The temperature was recorded using a standard thermal thermometer at the spot alone. The results are recorded were recorded in the table below.

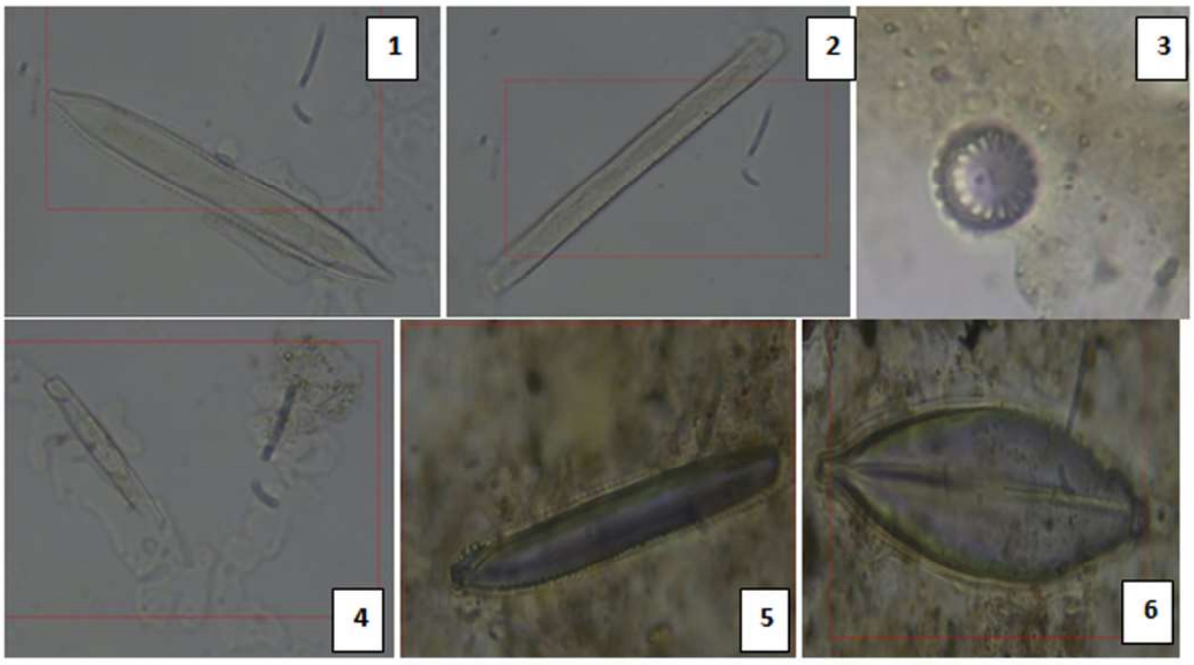
Table 1: Physiological variables as recorded for the geothermal springs during the time of collection.

S. No	Site of collection	pH	Average Temperature (in degree Celsius)
1.	Inner Pond at Hot water Spring of Anhoni, Pachmadi Biosphere Reserve, Madhya Pradesh	5	47
2.	Outer Pond at Hot Water Spring of Anhoni, Pachmadi Biosphere Reserve, Madhya Pradesh	5	45
3.	Main Stream of Hot Water Spring of Chaval Pani, Pachmadi Biosphere Reserve, Madhya Pradesh	6	50
4.	Outlet of Water at Hot Water Spring of Chaval Pani, Pachmadi Biosphere Reserve, Madhya Pradesh	7	49

The diversity of diatoms that was recovered post Nitric acid digestion was studied under a microscope and pictures were recorded using a camera attached to microscope. Post observing the slides under the Phase contrast microscope various diatom species was detected in the samples. The nitric acid digestion had helped isolate the diatom species present in the water samples. When these isolated samples were studied under the Phase contrast microscope there were clear diatom structures found. These structures were studied for conducting taxonomical classification of these detected species. Taxonomical identification of these diatoms were conducted by comparing structures of the taxon with monographs of *Hustedt* (*1930*)[10], *Foged* (*1981*)[11], *Gandhi* (*1999*)[12] and *Karthick et al.* (*2013*).[13] The results are crossed checked by comparing to the diatom and algal database of research papers and journals. Arrangement of the concluded diatoms is as per *Hendey* (*1964*)[14] used by researchers. The table below enlists all the diatoms found in the sample of hot water spring of Anhoni and chawalpani.

Table 2: Taxonomical classification of diatom species found in the hot water springs of Anhoni and Chawalpani, Madhya Pradesh

S. No.	Class	Order	Family	Genera	Species
1.	Bacillariophyceae	Bacillariales	bacillariaceae	Nitzschia	Nitzschia palea
2.	Bacillariophyceae	Bacillariales	bacillariaceae	Nitzschia	Nitzschia linearis
3.	Mediophyceae	Stephanodiscales	stephanodiscaceae	Discotella	Discostella stelligera
4.	Bacillariophyceae	achnanthales	achnanthidiaceae	Achnanthidium	Achnanthidium Sp.
5.	Fragilariophyceae	Licmophorales	Ulnariaceae	Tabularia	Tabularia Sp.
6.	Bacillariophyceae	Cymbellales	Anomoeoneidaceae	Anomoeoneis	Anomoeoneis sphaerophora
7.	Bacillariophyceae	Naviculales	Amphipleuraceae	Amphipleura	Amphipleura Sp.
8.	Bacillariophyceae	Bacillariales	Bacillariaceae	Tryblionella	Tryblionella Sp.
9.	Fragilariophyceae	Fragilariales	Fragilariaceae	Fragilaria	Fragilaria crotonensis
10.	Bacillariophyceae	Bacillariales	bacillariaceae	Nitzschia	Nitzschia filiformis



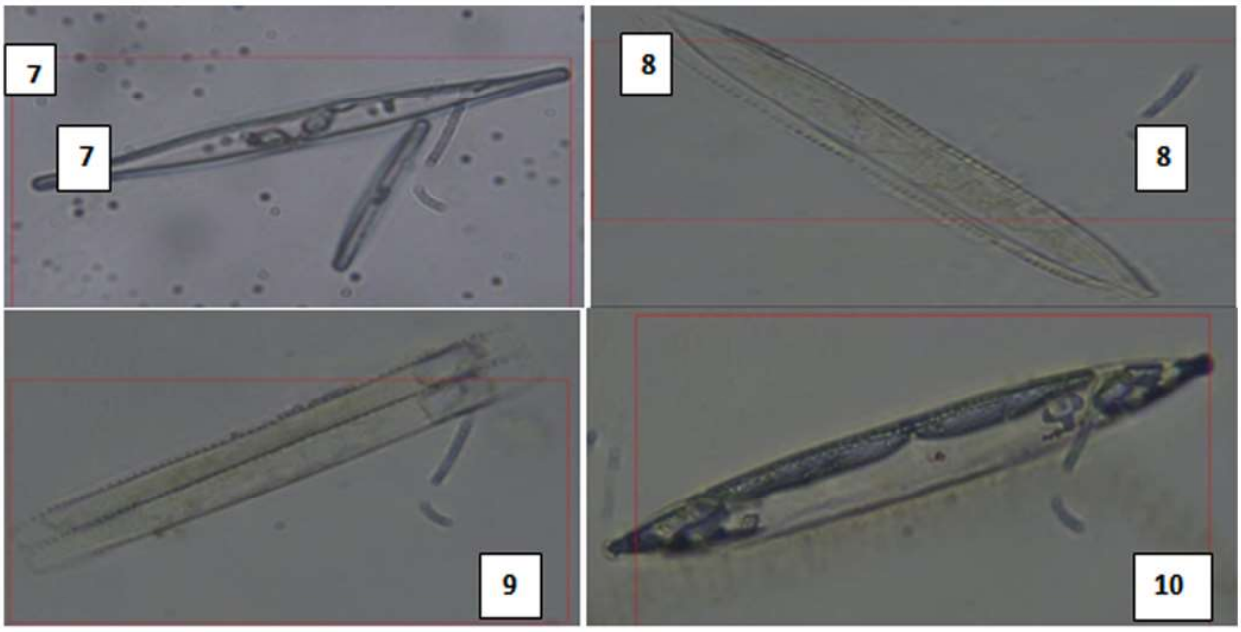


Fig. 3: Diatoms as viewed and captured under Phase contrast microscope (1) Nitzschia palea (2) Nitzschia linearis (3) Discostella stelligera (4) Achnanthidium Sp. (5) Tabularia Sp. (6) Anomoeoneis sphaerophora (7) Amphipleura Sp. (8) Tryblionella Sp. (9) Fragilaria crotonensis. (10) Nitzschia filiformis

The majority species ound in the geothermal water springs of Anhoni and Chawalpani, Madhya Pradesh belong to the class of Bacillariophyceae. These are a class of unicellular algae that are filamentous in structure. They have a frustules that is indicative of their presence. Other than these diatoms of Mediophyceaeand Fragilariophyceae were found. the species found in these hot water springs were:

1. Nitzschia palea

Nitzschia palea are a diatom species belonging to the family of Bacillariaceae. These fresh water species have a length of 12 - 42 µm. they have tapering ends that terminate with subcapitate apices. These species have valves with lanceolate on the side of the body that run parallel. This is a species of benthic diatom that is moderately motile.

2. Nitzschia linearis

Nitzschia linearis is another benthic diatom speies that thrives in natural fresh water bodies. There size ranges from 1001 – 10000 µm. these moderately motile species thrive in solitary colonies and have prostate attachments. Nitzschia linearis have valves that posses linear lanceolate which run along the parallel sides. It has a concave middle and its fibulae vary in size.

3. Discostella stelligera

Discostella stelligera is a diatom species that are found in lakes, large rivers, on the surface of hot water springs etc. these are immotile species that range in size from 101 – 1000 µm. they have a planktonic habitat and live in solitary colconies. These species have valves with distinct central area and well defined marginal rings. They are spherical in shape with a concave or convex center.

4. Achnanthidium Sp.

Achnanthidium Sp. Belongs to the family of achnanthidiaceae. These belong to the category of monoraphid's and is 3.5 – 4.5 µm in width and 11.5 – 18.2 µm in length. They posses linear elliptical valve with subcapitate ends. Striae radiate from the middle of these species and moves towards the end becoming parallel.

5. Tabularia Sp.

Tabularia Sp. is a diatom species that is present in tufted colonies with oval plastids on vaucheria. These species have linear elliptical valve with a convex valve plane. These vary in a size range of 1.00 – 1.87 µm. these species posses wider striae and a narrow lanceolate sternum.

6. Anomoeoneis sphaerophora

Anomoeoneis sphaerophora belong to the category of symmetric biraphid. These have a length of 43 – 83 µm and are 16.4 – 22.0 µm wide. This species is found on prairie stream, fens or shallow lakes or waters with alkaline pH. This species has elliptic lanceolate with sub-rostrate apices. The middle region of the specie shas ghost striae. The proximal raphe on this species are inflated and bent towards a particular side.

7. Amphipleura Sp.

Amphipleura species are benthic diatom species of symmetric biraphid that are 7.0 µm in width. These are species have a length of 68.0 – 105.0 µm. these linear species have linear lanceolate valves with tapering narrow ends with round apices. These species have a media rib that raphe branches coming out on both sides.

8. Tryblionella Sp.

Tryblionella belongs to the family of bacillariaceae. There size ranges from 1001 -10000 µm and are moderately motile. These species are narrow and linear and slightly constricted in the middle. They have flattened ends to create a panduriform shape for the valve. The raphe is eccentric and a central nodule is also present.

9. Fragilaria crotonensis

Fragilariacrotonensis is a category of araphid diatom species. These species are present in the temperate, mesotrophic lakes. These species join together to form large ribbon like structure. There size ranges from 101 – 1000 µm. these are non-motile species of diatom that posses lanceolate valves with capitates ends. The middle portion of this species is wider and a bit swollen than its rest of the body. It has dimorphic spines that are present along its valve.

10. Nitzschia filiformis

Nitzschiafiliformis are a species of benthic diatoms. These diatoms are 101 - 1000 µm in size and are moderately motile. They have linear lanceolate valves with acutely round apices. The raphe of this specie is placed on its margin it has a central nodule with fine striae.

Table 3: Showing the distribution of diatom species along the sites of hot water springs.

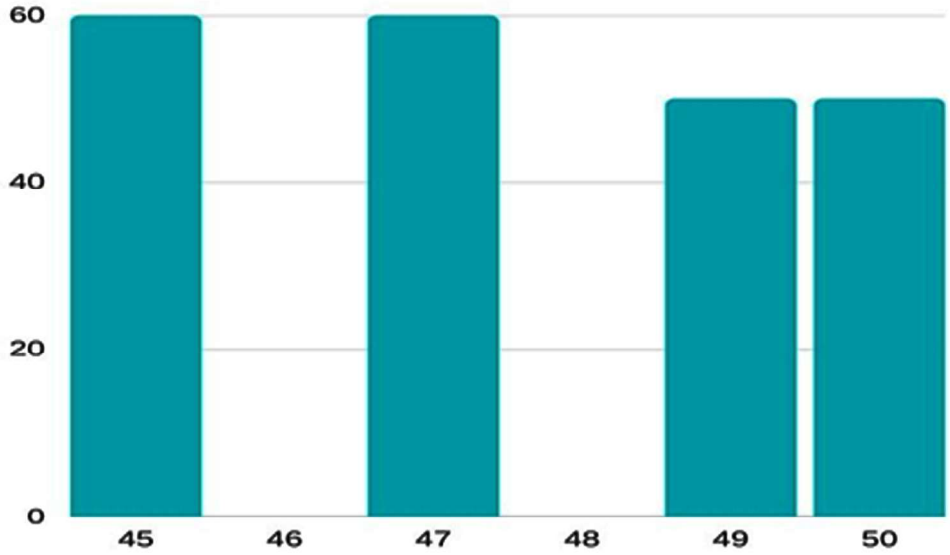
S. No	Site	Diatom Species
1.	Inner Pond At Hot Water Spring Of Anhoni, Pachmadi Biosphere Reserve, Madhya Pradesh	Nitzschia palea, Nitzschia linearis, Anomoeoneis sphaerophora, Amphipleura Sp., Tryblionella Sp., Fragilaria crotonensis.
2.	Outer Pond At Hot Water Spring Of Anhoni, Pachmadi Biosphere Reserve, Madhya Pradesh	Nitzschia palea, Nitzschia linearis, Anomoeoneis sphaerophora, Amphipleura Sp., Tryblionella Sp., Fragilaria crotonensis.
3.	Main Stream Of Hot Water Spring Of Chaval Pani, Pachmadi Biosphere Reserve, Madhya Pradesh	Discostella stelligera, Achnanthidium Sp., Tryblionella Sp., Fragilaria crotonensis., Nitzschia filiformis
4.	Outlet Of Water At Hot Water Spring Of Chaval Pani, Pachmadi Biosphere Reserve, Madhya Pradesh	Nitzschia palea, Tabularia Sp., Tryblionella Sp., Fragilaria crotonensis., Nitzschia filiformis

Co-relation amidst temperature, pH and diatoms:

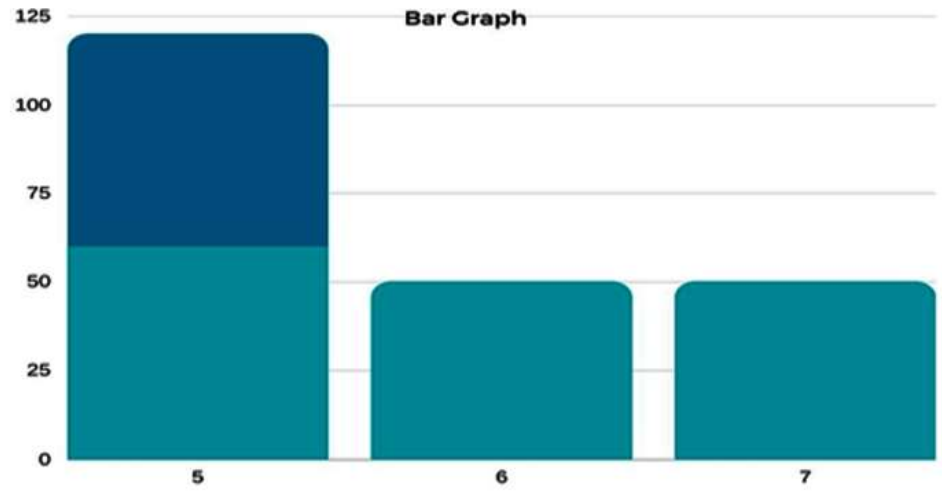
Varied researches suggest a co-relation amidst water temperature and diatom density. It can be concluded from this research that temperature and pH play a major role in controlling diatom community composition. Certain species are present in particular water bodies with a particular nutrient composition. The 2 study sites of Anhoni and Chawalpani were visted to record the average temperature of these natural hot water springs and the pH of these hot water springs. Then the presence of diatom and the density in which they were present was conferred to study the co-relation amidst these three factors. On the basis of the recorded values a study was conducted:

The research lead us to the results that hot water springs that had lower temperatures had more percentage of diatom density as compared to the hot water springs with higher temperatures. Certain diatom species are heat resistant and can sustain survival at higher temperatures. Often

water bodies with lower water temperatures are found to have more diatoms density. According to our research 60% of the diatoms found where from the geothermal spring of Anhoni, Madhya



Graph 1: Showing the percentage of diatom density with respect of temperature. The water sources with lower temperature were found to have the maximum density of diatoms. The x-axis displays the percentage of diatom density while the y-axis has temperatures in degree Celsius.



Graph 2: Showing the presence of diatom density with respect to the pH of water samples. More diatom density was found at sites with water samples of lower pH.

Pardesh which had lower temperature while the geothermal spring at Chawalpani, Madhya Pradesh was found to have lower diatom density due to its high temperatures and low nutrient index. When the pH of these water samples was studied against the diatom density, no significant difference was found due to nominal range difference in pH. Yet geothermal spring had a 10% more diatom density at low pH level which is 5 in this case study. This research leads to certain crucial conclusions that gave us strong evidence for co-relation amidst temperature, pH and diatoms.

Conclusion

Geothermal springs are a habitat to many diatom species. These diatom species are specific to a particular site and are used in cases of drowning

death for leads. a body that drowns in deep waters is subjected to high flush of water that results in settling of these diatom species in the organs. On extraction and analysis of these diatoms we can detect and compare them to the standard diatoms present in the water sources of that area. This helps Forensic Scientist finds leads in any case of death due to drowning. the analysis in our research concludes the presence of diatom species in the geothermal springs of Madhya Pradesh, namely Anhoni and Chawalpani. These diatoms include species belonging to class of Bacillariophyceae, Mediophyceaeand Fragilariophyceae. it also establishes the correlation between the temperature, pH and diatom density of these geothermal springs. It can be concluded that the geothermal springs that have lower temperature and pH are prove to have higher diatom density and diversity as compared to the ones with higher temperature. The physiological variables of geothermal water bodies and there correlation to diatom density has been studied by many biologists and ecologists. It has been found that diatom species are of great significance in detecting and monitoring ecosystem changes as their biomass has been found variable in different nutrient pollutions in water bodies, hence these species are useful in detecting environmental condition and water pollution.

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