Hot spring diatoms are linked to extreme cold conditions: A new perspective for astrobiological implication from the sinter deposit of Puga hot spring, Ladakh, India

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<u>This preprint is submitted to ESSOAr preprints portal. This is a working manuscript.</u>

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Abstract

The hot springs are known to host a variety of organisms, such as Cyanobacteria, Archaea, and Eukaryotes. The growth and survival of these life forms in extreme environments, where spring water temperature and associated minerals play a significant role, provide analogous conditions like Mars and thus attract researchers to find the possible existence of life beyond the Earth. Many studies have therefore been conducted from the hot springs to understand the controlling factors for these organisms' survival, mainly Cyanobacteria, which are believed to be true thermophiles. However, little is known about diatoms, especially from the hot springs of India, as most of the studies have concentrated on the diversity and distribution of Cyanobacteria. Here, we present a study of diatoms using a geothermal vent sinter from the Puga hot spring of Ladakh, India. Our results suggest that the diatoms preserved in the geothermal vent sinter are less abundant with low diversity and, therefore, represented by a few species only. By correlating the ecological preferences of diatom species with the sinter's morphological characteristics and geochemical analyses, we conclude that these diatom species could be manifested through a secondary deposit on the geothermal vent sinter from the adjacent cold waters of the Puga hot spring. We propose that the temperature gradient could be a key parameter for the occurrence and survival of diatoms in the Puga geothermal vent sinter rather than the gushed hot water. Consequently, the mere presence of diatoms around the hot spring vent cannot be directly linked to their survival in extreme, i.e., hot water conditions. Therefore, eukaryotic forms like diatoms from the hot springs should be used with caution to elucidate the existence of life in extreme (hot water) conditions. In contrast, cold conditions around the hot spring may be the primary drivers for diatoms' survival, which can be used to infer astrobiological implications.

Keywords: Extreme environment, terrestrial analogue, diatom abundance, physicochemical analysis.

1. Introduction

Hot springs receive heated deep water source where lithology, flow rates, depth of penetration of water into the crust, and the availability of heat source control the temperature and ionic composition of water (Ashton and Schoeman, 1984; Nicholson, 1993). The hot springs were known to host the discovery of a third domain of life, the Archaea (Barns et al., 1994, 1996). Some algae and microbes are thermophilous in nature and can survive in the water temperatures >50°C (Glazier, 2012). Beside this, hyperthermophiles (some algae and microbes) can survive in water temperatures >80°C (Stetter, 1999).

Diatoms are unicellular eukaryotic golden brown algae, which usually survive in the temperature range of 10°C to 45°C (Round et al., 1990). However, diatoms are found to survive in both extremes i.e., "hot waters" (temperature >50°C - Beowulf Spring, Yellowstone National Park, U.S.A., Hobbs et al., 2009) and "cold waters" (temperature <0°C - Polar regions, Armand et al., 2005; Martin and Mcminn, 2018). Such a great adaptability makes diatoms a useful tool to understand life in extreme conditions (both hot and cold conditions). Consequently, diatoms have been studied from the hot springs worldwide in terms of their occurrence; abundance, species richness, and adaptability (**see Table 1**). However, questions remained open that whether diatoms could actually flourish in such hot waters or colder diatom species can adapt in hot spring environment (Nikulina and Kociolek, 2011)? Moreover, the role of diatoms for astrobiological implication has been proposed from the Sabkha Oum Dba, Western Sahara, Morocco, where mat-forming benthic diatoms and cyanobacteria formed microterracing geomorphic features with the help of saline water and relatively dry season with no importance of temperature (Barbieri and Cavalazzi, 2018). Likewise, diatoms have been recorded from a temperate salt-pan site, Cervia salterns, Italy where salinity played an important role for the occurrence of diatoms (Barbieri and Cavalazzi, 2022).

The hot springs of the Ladakh region are of great interest for understanding the life in extreme conditions as these springs are boiled at a much lower temperature compared to other locations on the Earth (Phartiyal et al., 2021). These hot springs are known to host a variety of organisms including thermophilic bacteria and some eukaryotic organisms like diatoms. The Puga hot spring of Ladakh is an ideal site to understand the signatures of life forms surviving in extreme conditions where life forms have been reported to survive in the vicinity of terrestrial hot springs (Sarkar et al., 2022 and references therein). However, records of diatoms are sparse from these important sites in India, where some taxonomy and diversity related studies of diatoms have only been carried out from some hot springs (Pardhi et al., 2023). We present here diatom data preserved in the sinter sample of Puga hot spring, Ladakh along with the morphological and geochemical characterization of the sinter deposit. Our study aims to answer some key questions: 1) Can diatoms flourish in the hot waters of Puga hot spring? 2) What are the controlling factor(s) for their growth and abundance? 3) Whether Ladakh's diatoms can be used for astrobiological implications or not?

2. Study area

Ladakh is the most remote region of Jammu & Kashmir State, which has a dry cold climate with minimum winter temperatures of −40°C. Most of the area of Ladakh is situated at >3500 m above sea level. The Puga Valley of Ladakh is known for its geothermal power generation and is located in the Indus Valley in the eastern Ladakh region of the NorthWest Himalayas. Puga is situated in the Tso Morari area (also spelt Tso Moriri), south of the Indus Suture Zone, having hot springs, mud pools, and sulfur and borax deposits covering an area of ca.15 km2 (Craig et al., 2013; Dutta et al., 2023; Fig. 1). The water temperature of the Puga hot spring at the geothermal site is 84°C, whereas 5°C in the cold regions (Craig et al., 2013). The host lithology of the basement rock is comprised of crystalline Limestone, Marble, carbonaceous shale, Green chert, Mafic to ultramafic rocks etc. (Dutta et al, 2023).

Materials and methods

A geothermal sinter sample was collected by one of the authors (Binita Phartiyal) from the Puga hot spring of the Ladakh, India (33°13′39.38″N, 78°18′22.98″E, 4414 m.a.s.l., Fig. 1). The thin sections (30 µm thick) of the sinter sample were prepared in the section cutting laboratory of the Birbal Sahni Institute of Palaeosciences (BSIP) for studying the morphological features. Such morphological features were studied through digital scanning and petrography. The thin sections were digitally scanned utilizing an automated slide scanner (Model: Grundium Ocus130 MGU-00001) at Vertebrate Palaeontology and Preparation Laboratory, BSIP.

This is a working manuscript, under preparation for submission to a peer-reviewed journal.

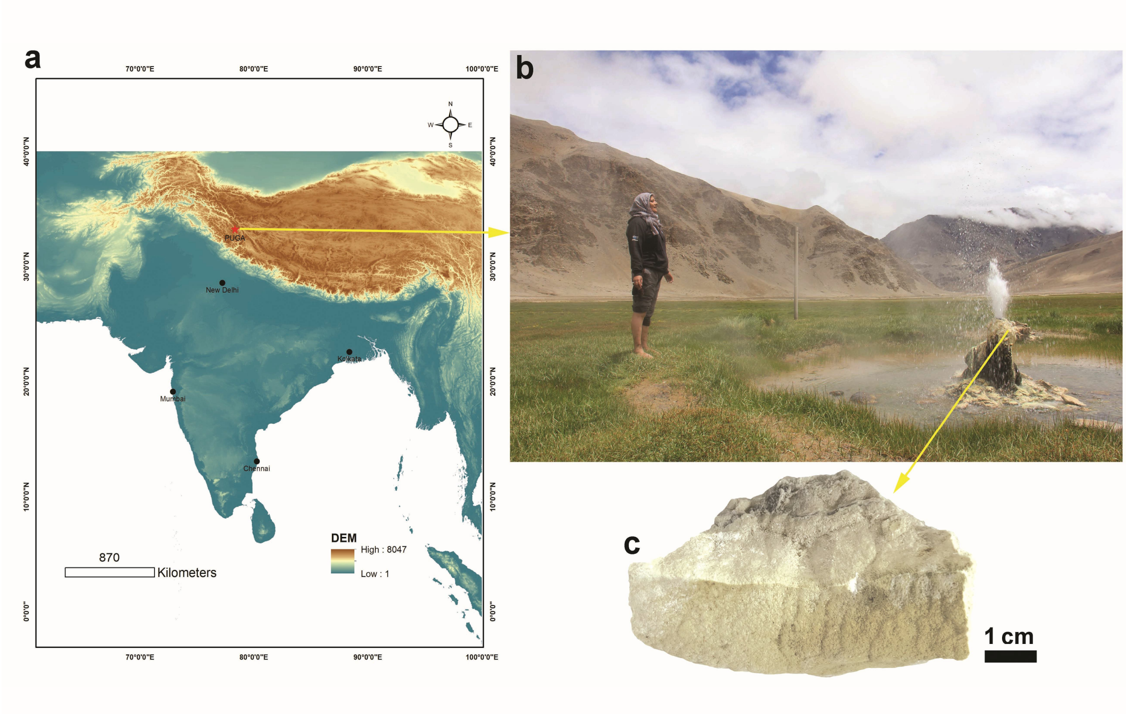

Fig. 1. DEM map showing the location of the investigated hot spring site Puga in the Ladakh region of northern India (a), Panoramic view of the sample collection site at Puga showing the hot spring vent (b), and the digital photograph of the investigated sinter sample (c).

A JEOL FESEM 7610F electron microscope was used to analyze the surface morphology and elemental composition of the sinter sample. To investigate the morphological traits, specimens were examined at various magnifications with a secondary electron detector at 5 kV and 15 kV acceleration voltages. TEAM software was used to capture EDS spectra from an EDAX Octane Plus detector at 15 kV accelerating voltage.

To study the diatoms in detail, we extracted the diatoms from the sinter sample. The weighed sinter sample (2-3 g) was processed for the removal of organic material and carbonates (if any) following the methods of Battarbee et al. (2001) and Crosta et al. (2020). The diatom counting was performed following the standard procedure described by Crosta and Koç (2007) using an Olympus light microscope.

The elemental compositions of the sinter sample were studied in X-ray fluorescence (XRF) laboratory at BSIP, Lucknow. For glass bead preparation, 10 gm Lithium tetraborate and 1 gm, (74 µm size) sample was correctly mixed in the agate motor pestle and then fused at 1100^{o}C in the platinum crucible at 15 minutes (Watanabe, 2015; Chaddha et al., 2022). The molten material was then allowed to cool on the platinum holder and then analyzed on the Wroxy application by the XRF machine at BSIP, Lucknow.

The Accelerator Mass Spectrometry (AMS) radiocarbon ages of the sinter sample were obtained using the methods detailed in Bhushan et al. (2019a, 2019b), and calibrated using Calib8.2; IntCal20 (Reimer et al., 2020). The average radiocarbon age of the sinter sample represent ~37538 cal yr BP. Such ages of the sinter sample represent the ages of the basement rock (mainly limestone) from where the water gushed from the vent and eventually deposited in the form of sinter.

3. Results and discussion

3.1. Digital scanning of the thin sections

The digital scanning of the thin sections of the sinter sample from Puga hot spring, Ladakh revealed branching spicules (having both laminated and featureless cores surrounded by cortex) of opal silica (Fig. 2). In general, the sinter samples from the hot spring across the globe are known to showcase 'Spicules' and 'Spicule-columns' as internal structures with both branching and non-branching spicules composed of opal silica displaying a 'Cortex' encasing a laminated or unlaminated 'Core' (refer to Fig. 4 in Jones and Renaut, 2003). Although, the published literature generally agrees that the 'Spicules' are smaller (in diameter) compared to 'Columns' (Campbell et al., 2015 and references therein), varied definitions of these structures have been proposed based on the dimensional dataset (Walter, 1979; Braunstein and Lowe, 2001; Jones and Renaut, 2003). Interestingly, the diameter of the observed spicules is generally <250 microns (Fig. 2) i.e., at least 50% less as compared to the spicules previously recorded in the sinter samples from the Yellowstone National Park, Wyoming, North America (Walter, 1979; Braunstein and Lowe, 2001) and the Whakarewarewa-Orakeikorako geothermal areas, North Island, New Zealand (Jones and Renaut, 2003). This is plausibly due to less availability of silica at Puga hot spring in comparison to the above-mentioned sites.

Fig. 2. Digital photograph of the thin section of the sinter sample showcasing the typical closely packed upward expanding and branching spicules of opal silica.

3.2. Petrography

Petrographic investigation was performed for the sinter sample by using thin sections. Overall, the texture of the petrographic thin section resembles with multi-oriented random set of elongated flares/crystals of calcite along with overgrowth (Fig. 3A). Oriented 2-set of cleavages within the calcite were rarely observed (Fig. 3A and 3C). Secondary overgrowth was prominently seen in the absence of set of cleavages (Fig. 3A and 3C). Patches of drusy calcite were also observed over primary calcification (Fig. 3A). Very meager amount of amorphous silica/silica gel encountered within the gap between elongated flares/crystal of calcite, which suggest the presence of amorphous silica in the system (Fig. 3B). Bulbous shaped incremental growth can be seen within the primary growth of calcite (Fig. 3C), which complement the physical feature of the sinter sample collected.

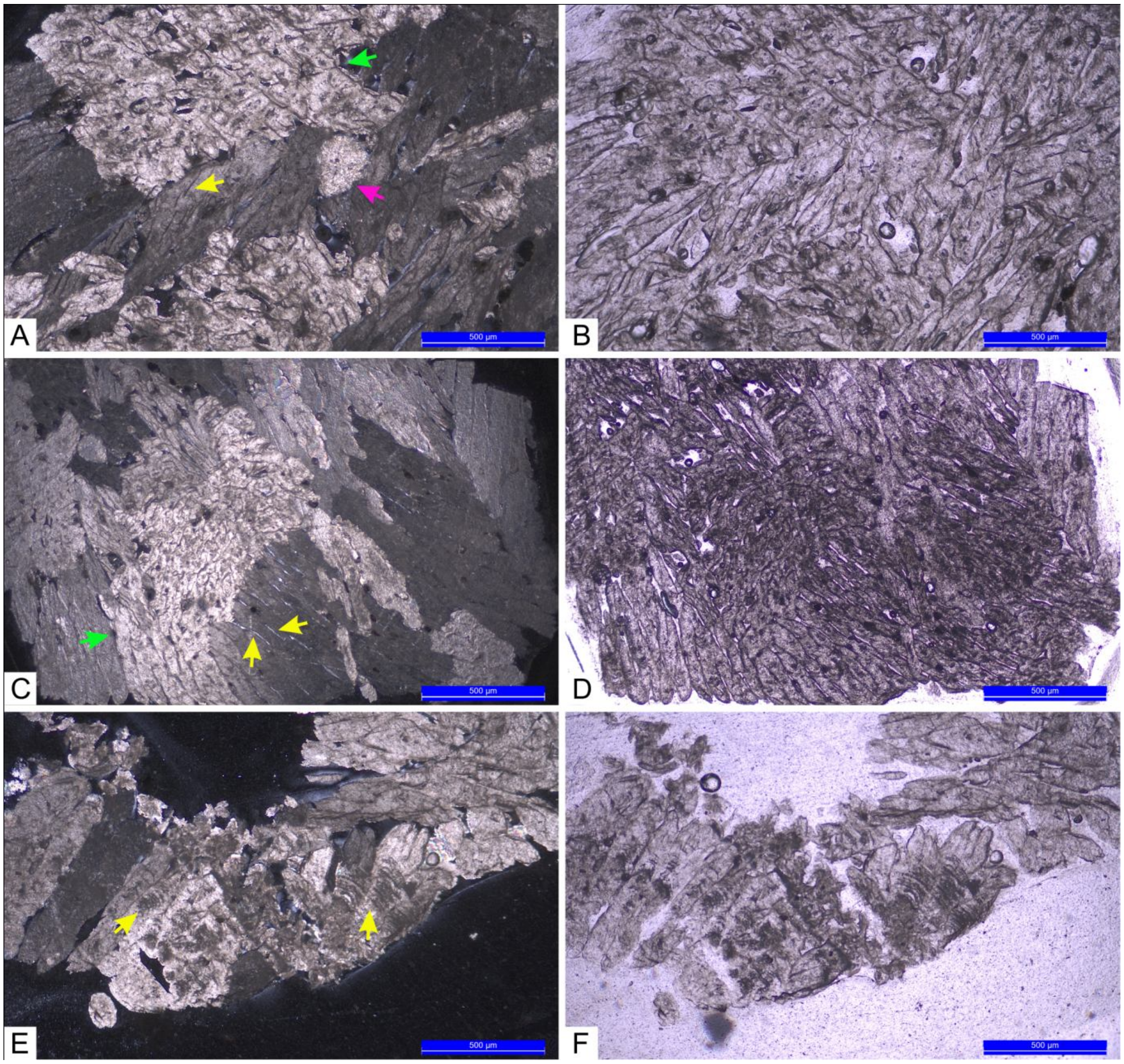

Fig. 3. Photomicrograph of Sinter: Photomicrograph of primary calcite in form of elongated flares (yellow arrow) and secondary overgrowth (green arrow) over it, Drusy calcite (pink arrow) (Under cross Nicol) **(A)**; Photomicrograph of 'A' (under plane polarized light) (**B**); Elongated space between two primary flare/crystal of calcite filled with amorphous silica (see yellow arrow) (**C)**; Photomicrograph of 'B' (under plane polarized light) **D)**; Photomicrograph showing bulbous shaped incremental growth within the primary calcite crystal (in yellow arrow) **(E)**; Photomicrograph of 'E' (under plane polarized light) (**F)**.

3.3. Surface morphology and elemental analysis

Secondary electrons were used through FESEM-EDS (Field Emission Scanning Electron Microscopy-Energy Dispersive X-Ray Spectroscopy) to study the surface morphological and elemental variations between the host (sinter sample) and diatoms present in the sinter sample (Fig. 4). There is a significant distinction between the lighter shade of diatoms immersed in the extracellular polymeric substances (EPS) biofilm type morphology (Alleon et al., 2021)

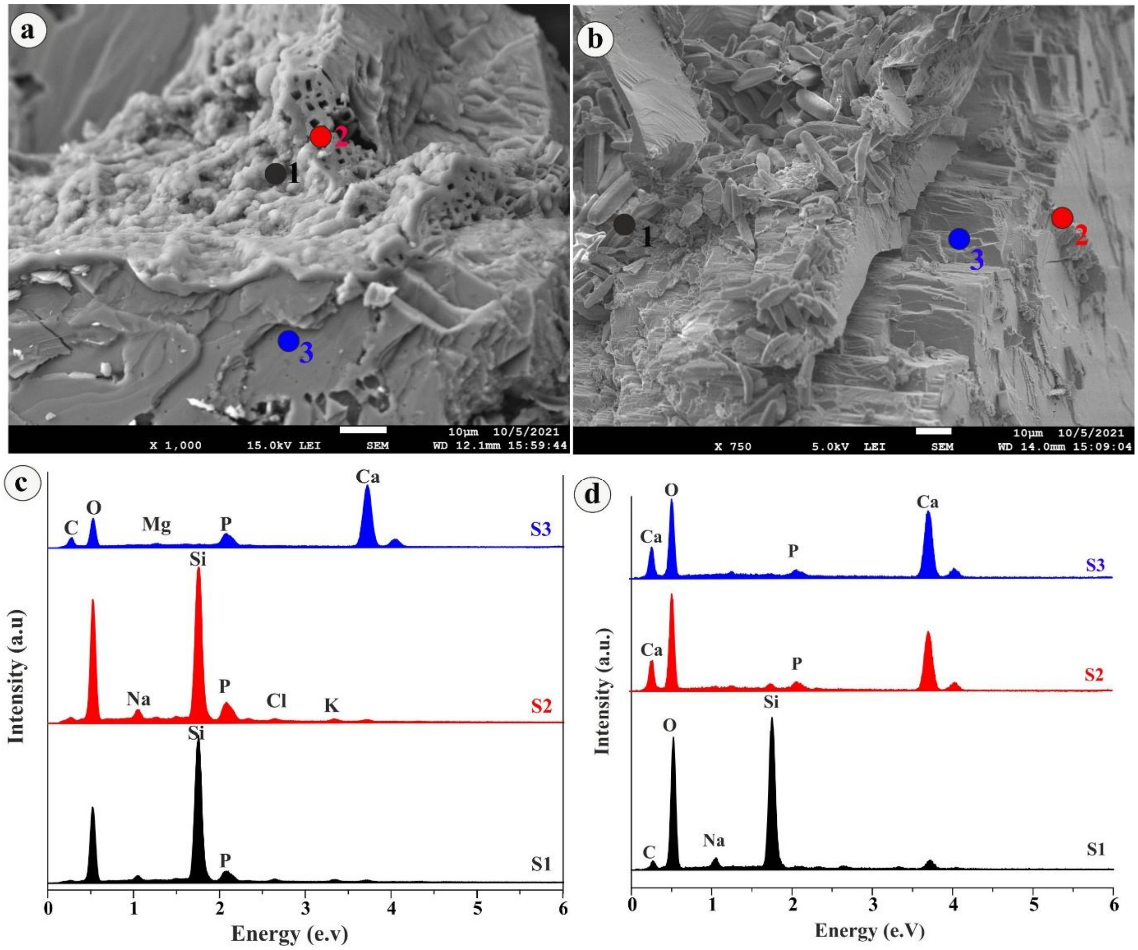

Fig. 4. Presence of Diatoms embedded in the microbial extracellular polymeric substance (EPS) demonstrating clear demarcation between calcic rich sinter substrate (a); abundance of diatoms visible on the sinter substrate (b); and multi-spot elemental analysis demonstrating the elemental contrast in the diatom rich layer and the calcic sinter (c, d).

and the darker sinter substrate hosting the diatoms. EDS analysis confirms the diatoms' and sinter's distinct texture and elemental makeup (Fig. 4c,d). Multi-spot elemental analysis demonstrates an enrichment of Si in the diatom rich layer, as well as Na and Cl, as described by spots 1 and 2 (Fig. 4c) (Fig. 4d). The presence of Na in the sinter sample might be owing to the crystallisation of Ca-rich sinter from the mother liquid, which is Puga's Na-Cl-HCO_3 rich spring water (Dutta et al., 2023). Notably, there is a difference in the presence of P in the diatom rich layer spots 1 and 2 (Fig. 4c). This P enrichment might be attributed to the presence of microbial EPS (Duan et al., 2023; Zhou et al., 2017) on the sinter. Furthermore, the presence of Mg in the sample suggests that EPS produced by bacteria include organic molecules that accelerate the incorporation of Mg in the carbonate mineral (Al Disi et al., 2019). As a result, the appearance of diatoms on the sinter could be due to a secondary process that possibly occurred when the hot water from the spring cools owing to the temperature gradient, precipitation, and crystal development of Ca-rich sinter around the hot spring vent.

3.4. Diatoms

The diatom assemblage comprised of four species, namely, *Achnanthidium minutissimum* (Kütz.) Czarn. 1994; *Nitzschia palea* (Kutz.) W. Sm. 1856; *Rhopalodia gibba* (Ehrenberg) O. Müller 1895; and *Denticula thermaloides* Van de Vijver & Cocquyt 2009 (Fig. 5). *Achnanthidium minutissimum* dominated the diatom assemblage followed by *Nitzschia palea*, *Rhopalodia gibba*, and *Denticula thermaloides*.

Achnanthidium minutissimum is a freshwater slightly motile benthic diatom species found in the inland waters of lakes and rivers (Potapova and Hamilton, 2007; Wojtal et al., 2011). *Achnanthidium minutissimum* was reported from a glacial lake Hausburg Tarn from Mount Kenya (Cocquyt 2007). Therefore, this species doesn't show any relation with the Puga hot water. *Nitzschia palea* is also a benthic diatom species which is moderately motile and ubiquitous in nature. It has been suggested that diatom genus *Nitzschia* is a pollution indicator (Palmer, 1969) and therefore represent organic pollution in the studied area where heavy metal pollution or nutrient enrichment could have favored the growth of this diatom (Chen et al., 2014; Lowe, 2003; Taylor and Coqcuyt, 2016; Singh et al., 2020). It is worth noting that the Puga area of Ladkah is an interesting site for the tourists, which could be the possible reason for such anthropogenic signatures. Therefore, Puga area of Ladakh should be protected and conserved for its geoheritage.

Rhopalodia gibba is an endosymbiontic diatom species containing cyanobacterial inclusions (Floener and Bothe, 1980; Kneip et al., 2008; Prechtl et al., 2004). The occurrence of this species in the Puga sinter sample points towards an association with the cyanobacteria which is substantiated by the nutrient enrichment indicated through diatom species *Nitzschia palea* in our sample. Owing to the endosymbiotic in nature by hosting cyanobacterial inclusions, the occurrence of *Rhopalodia gibba* in the Puga hot spring sinter sample may hint towards astrobiological implication of this diatom species. However, *Rhopalodia gibba* is an inland diatom species found usually in rivers and lakes (Patrick and Reimer, 1975) rather than being endemic to hot springs. Therefore, attribution of this species with the Puga sinter sample could be due to the secondary deposition through the adjacent environment where temperature gradient and nutrient enrichment possibly supported the growth of *Rhopalodia gibba*.

The diatom species *Denticula thermaloides* was discovered by Van de Vijver and Cocquyt (2009) from La Calera hot spring of Peru by scraping off algal material from stones. In Ladakh, this species has been recorded as an epiphyte in the stream, pool, and also from the Chumathang Hot Springs (Pardhi et al., 2023). Thus, *Denticula thermaloides* seems to show variable habitats (from stream and pool to hot springs) as well as microhabitats (from epilithic to epiphytic). The preferential temperature data for *Denticula thermaloides* is lacking and could not be measured from the type locality due to the logistical constraints (Van de Vijver and Cocquyt, 2009). If this species only occur in the hot springs then it could have been considered as a true thermophile. However, occurrence of this species in streams and pools along with the hot springs' stones and plants doesn't compassionating this species to be a true thermophilic organism. We therefore suggest that the occurrence of *Denticula thermaloides* in the sinter sample of Puga hot spring could be sourced from the adjacent epilithic/epiphytic environment where cooler waters might have favored the growth of this diatom species rather than the hot water gushed from vent.

Most of the studies on diatoms from the hot springs used the epilithic/epiphytic samples, microbial mats/algal mats, and water samples and therefore considered temperature as a controlling factor for diatoms (see Table 1). However, the location of the water sampling is crucial in terms of proximity to the hot spring vent and away from the vent at the downstream as hot spring environment has high temperatures at the source (near the vent) which is changing to lower temperatures at the downstream (Cousins et al., 2018). Our observations are in agreement with the study of Negus et al (2020), which suggested a key role of temperature gradient in defining the diatom occurrence and community structure in the hot spring. The hot spring complex in tropical north Queensland, Australia with the water temperature of 62.7°C at the hot spring vent whereas water temperature of 26°C at the downstream showed a strong anti-correlation with the richness of diatoms being no diatoms in the hot water at the vents (Negus et al., 2020), which was also observed by previous studies (Pentecost, 2005; Sterrenburg et al., 2007). The inverse relationship of warmer temperature and diatom richness have also been recorded from the hot springs of South Africa (Jonker et al., 2013); Kenya (Owen et al., 2004); Iceland, New Zealand, and Kenya (Owen et al., 2008); Kamchatka Peninsula (Nikulina et al., 2019); and Odisha, India (Bhakta et al 2016). The water temperature of 84°C in the thermal region, whereas the water temperature of 5°C in the cold region of the Puga hot spring (Craig et al., 2013), supports that cold water diatom species found in the Puga sinter sample could have resulted from secondary deposition rather than flourishing in hot water gushed from the vent.

Fig. 5. Diatom species found in the sinter sample of the Puga hot spring: *Achnanthidium minutissimum* (A-E), *Nitzschia palea* (F and G), *Rhopalodia gibba* (H), and *Denticula thermaloides* (I).

3.5. Elemental composition

To understand the elemental composition of the collected sinter deposit of the Puga hot spring, X-ray fluoroscence technique was employed, which shows that the sample mainly consists of CaO>MnO>MgO (Fig. 6) whereas other major oxides are present in the traces and therefore classified as travertine. The surface water which enters through the zildat fault, when heated by the geothermal processes under the subsurface, reacts with the existing rocks (Crystalline Limestone, Marble, carbonaceous shale, Green chert, Mafic to ultramafic rocks etc.) (Dutta et al, 2023). The brine that comes out through the Puga hot spring is rich in Na-Cl-HCO_3 (Dutta et al, 2023), precipitating as $CaCO_3$ near the hot spring on cooling, as the temperature gradient is higher in the Ladakh region. Interestingly, the presence of a highly enriched sulfur value of 3460 ppm as compared to sulfur values in Bulk continental crust (BCC), which are 404 ppm (Rudnick and Fountaion 1995) shows ~9 times enrichment in the analyzed sintered sample, pointing toward the interaction of the fluids with the minerals such as thenardite, pyrite, jarosite, respectively (Dutta et al, 2023).

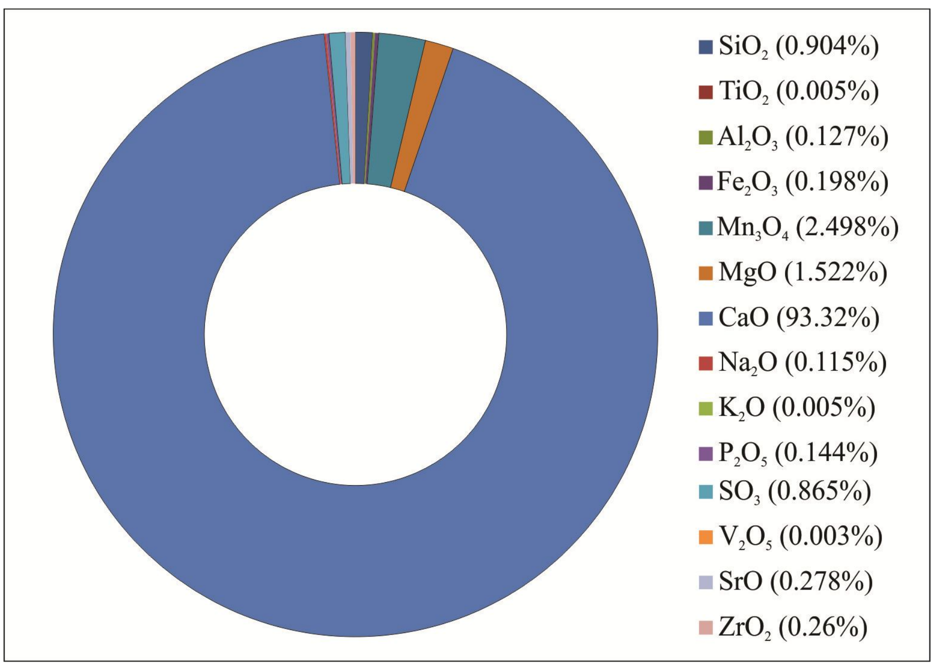

Fig. 6. Elemental composition of the sinter sample of the Puga hot spring.

3.6. Implication of hot spring diatoms governed by temperature gradient for astrobiological studies

The diatoms recovered from the sinter sample of Puga hot spring demonstrated the role of temperature gradient in defining the diatom occurrence and community structure. The occurrence and survival of diatoms in cold conditions of Antarctica suggested the potential role of diatoms for the astrobiological studies (Martin and McMinn, 2018) and therefore conditions of Ladakh having highest temperature of -27.9°C during winter and 34.8°C during summer (Chevuturi et al., 2018; Chaddha et al., 2021) can possibly present terrestrial analogues conditions to present day Mars surface conditions where diurnal temperature ranged between -80°C and -10°C (Atri et al., 2022).

4. Conclusions

The sinter sample from the Puga hot spring of Ladakh, India, utilized for the diatom analysis revealed less abundance of diatoms and low species diversity, characterized by a few species only. *Achnanthidium minutissimum* dominated the diatom assemblage followed by *Nitzschia palea*, *Rhopalodia gibba*, and *Denticula thermaloides*. The ecological preferences of these diatom species and their correlation with the previous studies suggest that these diatom species are signatures of secondary deposits on the sinter sample and substantiated with the sedimentological and geochemical data. Based on the habitat and microhabitat of diatom species found in the Puga sinter sample, we propose that the growth and abundance of diatoms in the Puga hot spring is controlled by the temperature gradient with no sign of survival and adaptation of diatoms in extreme “hot water” environment. Therefore, eukaryotic forms like diatoms from the hot springs should be used with caution for elucidating the existence of life in extreme (hot water) conditions and for the astrobiological implications.

Acknowledgements

Authors would like to thank Director, BSIP for encouraging us to perform this study and also for providing the necessary laboratory (SAIF – Sophisticated Analytical Instrumentation Facility) facilities to carry out this work.

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698 **Table 1.** Summary of important studies on hot springs diatoms carried out worldwide.

S. N.	Location	Type of sample	Water temperature range	Factors influenced diatoms' Occurrence/abundance/diversity	Reference
1.	Hot springs of Ladakh, India	Water and diatom samples	30°C to 80°C	Taxonomic survey of diatom assemblages was performed only.	Pardhi et al., (2023)
2.	Lake Shala and inflowing hotsprings of Ethiopia.	Water and diatom samples	22°C to 26°C	High phosphate, sodium (Na^+), carbonate ($CO3^{2-}$), bicarbonate ($HCO3^-$), and chloride (Cl^-) contents	Wagaw et al. (2022)
3.	La Montagne and Mariol minerals springs of Auvergne (France).	epilithic and epipelic diatom	11.50°C to 17.03°C	Environmental variables – Physical (conductivity, pH, dissolved oxygen, and temperature), and chemical (ions concentrations and pollutants ions concentrations).	Baker et al. (2022)
4.	Comanjilla geothermal zone in northern Guanajuato, Mexico.	Brown microbial mats	45°C to 92°C	Temperature, pH, dissolved solids, electrical conductivity, hardness, alkalinity, and silica concentrations.	Puy-Alquiza et al. (2021)
5.	Thermal spring in Azores Archipelago (Sáo Miguel Island, Atlantic Ocean).	Water and diatom samples	37°C to 39°C	Narrow ecological preferences.	Delgado et al. (2021)
6.	Talaroo hot springs complex, Einasleigh River catchment, North Queensland, Australia	Water and diatom samples	62.7°C at the vents and 26°C at the location furthest downstream.	Lower temperature	Negus et al. (2020)
7.	Malki, Upper Paratunka, and Dachnie thermal springs, Kamchatka	Composite wet soil samples	65.9°C (Malki), 39.5°C (Upper Paratunka), and 30-50°C (Dachnie)	High temperature, mineralization, and soil moisture.	Fazlutdinova et al. (2020)
8.	Mineral springs of Sainte Marguerite, France	Scrapped samples of diatoms from fine sediments, stones, travertine, and	4.3°C to 29.1°C	Physical and chemical characteristics and mainly due to the presence or absence of nutrients.	Beauger et al. (2020)

		metal pipes.			
9.	Hot springs of four geothermal fields (Malkinsky, Nachikinsky, Paratunsky, and Mutnovsky) in the south-eastern Kamchatka.	Algobacterial mats.	28°C to 98°C	Acidic water with the chemical type: SO4-HCO^{3}-Na-Ca and temperature higher than >55 °C resulted in reduced diatoms.	Nikulina et al. (2019)
10.	Maquinit Hot Spring in Coron, Palawan, Philippines.	Cyanobacterial mats and water samples.	38°C-41°C	Physico-chemical parameters such as alkaline pH (pH 7.6 - 7.7), high salinity (40 ppt), low thermophile water temperature (ca 41°C), and no /or low sulfur content.	Martinez-Goss et al. (2019)
11.	Thermo-mineral springs in Auvergne (France) and Sardinia (Italy).	Scrapped samples of diatoms from rock/cobbles and fine sediments.	Hot springs of Auvergne (France) – temperature range is 13.3°C to 32.6°C (Hot springs of Auvergne, France) and 11.2°C to 71.5°C (Hot springs of Sardinia, Italy).	pH, conductivity and HCO^{3-}were the most significant environmental variables.	Lai et al. (2019)
12.	Hot spring of northern Thailand.	Periphytic (epipelic and epilithic) diatom samples.	37°C to 85°C	Silicon dioxide (SiO_2), pH, conductivity, water temperature, and total hardness were the main factors for diatoms.	Pumas et al. (2018)
13.	Tha Pai Hot Spring, Mae Hong Son Province, Thailand	Diatom sample/culture sampls	39 to 45°C, culture sample can be maintained at 30°C	Alkaline pH of 9 can promote the heat tolerance of diatom *Achnanthidium exiguum*.	Pruetiworanan et al. (2018)
14.	Thermal springs of Pamir mountains, Tajikistan.	Algological samples	10°C to 86°C	Low–saline, low–alkaline, middle oxygenated clear fresh water with low organical pollution and oligo–to meso–eutrophic state.	Niyatbekov and Barinova (2018)
15.	High-altitude geothermal field	Algal samples	6.8˚C - 10˚C for rivers and	Conductivity, total phosphorous, NO^{3-},	Angel et al. (2018)

	in the Central Andean dry Puna ecoregion or southern Altiplano.		swamps, and 30˚C - 37.5˚C (for fumaroles stations.	HCO^{3-}, Mg^{2+}, temperature, dissolved oxygen, and ionic gradient.	
16.	Thermo-mineral springs in Galicia, NW Spain (3 hot springs and 2 cold springs).	Scraped diatom samples from the stones, edges, and bottom of the springs.	37˚C - 44˚C (for hot springs, namely, As Burgas, Outariz, and Cuntis), and 13˚C - 20˚C (for cold-water springs, namely Guitiriz and Augas Santas of Pantón.	Conductivity, temperature, and hydrogen sulphide concentration.	Leira et al. (2017)
17.	Thermal springs of Pamir mountains, Tajikistan.	Algological samples	10°C to 86°C	Altitude, temperature and pH.	Barinova and Niyatbekov (2017)
18.	Hot springs of Odisha, India	Epilithic and biofilm samples	35°C to 60°C	Temperature gradient	Bhakta et al. (2016)
19.	Fluvial tufas of the Mesa River, Iberian Range, Spain	Tufa surface	Water temperature at or close to resurgence points is 13-14°C in the Mesa river at site Mochales and between 20-32°C in the low-thermal springs near Jaraba.	HCO^{3-}, pCO_2, Ca^{2+}, and TDIC negatively affect diatom richness whereas abundance is positively related to the presence of mosses and algae.	Beraldi-Campesi et al. (2016)
20.	Thermal springs in Limpopo Province, South Africa	Algal mat	40°C to 67°C	Diatoms occurred at temperature <45°C.	Jonker et al. (2013)
21.	Hot springs in eastern Russia	Periphytic algae/Algal samples/water samples	24°C to 101°C	Water temperature	Nikulina and Kociolek (2011)
22.	Beowulf Spring,	Surface	67°C	Temperature and pH	Hobbs et al.

This is a working manuscript, under preparation for submission to a peer-reviewed journal.

	Yellowstone National Park, U.S.A.	sediment samples			(2009)
23.	Hot Springs of Iceland, New Zealand, and Kenya	Water samples	21°C to 99°C	Alkalinity, pH, and conductivity	Owen et al. (2008)
24.	Hot springs of Kenya Rift Valley	Surface sediments, rock scrapings, and vegetation samples	32°C to 60°C	pH, temperature, and specific conductivity, with other environmental variables such as Si and nitrate being of secondary importance.	Owen et al. (2004)
25.	Thermal spring complex at Gross Barmen near Okahandja in South West Africa/Namibia	Water samples and diatom samples	25.6°C to 65°C	Water temeprature	Schoeman and Archibald (1988)