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Bacterial diversity, physicochemical and geothermometry of South Asian hot springs

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ABSTRACT

Extreme ecosystems with enormous arrays of physicochemical or biological physiognomies serve as an important indicator of various processes occurred and/or occurring in and on the Earth. Among extreme habitats, hot springs represent geothermal features which are complex systems with a well-defined plumbing system. Besides geological tectonic based hypsography and orology annotations, the hot springs have served as hot spots for ages where there is an amalgamation of nature, religion, faith, health, and science. Thus, there remains an escalating scope to study these hot springs all over the world. The Himalayan Geothermal Belt (HGB) banquets three densely demographic countries i.e. Pakistan, India and China, that hosts numerous hot springs. Studies on the hot springs distributed over these countries reveal Proteobacteria, Firmicutes, Bacteroidetes and Actinobacteria as the predominant bacterial phyla. The bacterial diversity shows a significant positive correlation with physico-chemical parameters like temperature, pH, Na^+, HCO_3^-, etc. Physicochemical analyses of these hot springs indicate the water mainly as Na-Cl, Na-HCO_3, SO_4-Cl, and mixed type, with temperature ranging approximately between 100-250°C as predicted by various geothermometers. Numerous studies although done, not much of a comprehensive database of the analysis are provided on the hot springs harboured by the HGB. This review aims to give a cumulative illustration on comparative facets of various characteristic features of hot springs distributed over the HGB. These are found to be of great importance with respect to the exploitation of geothermal energy and microflora in various sectors of industries and biotechnology. They are also important sources in terms of socio-economic perspective, and routes to eco-medical tourism.

1. Introduction

The earth hosts various unique, unknown and extreme niches. Among them the hot springs are the hot spots for ages where there is an amalgamation of nature, religion, faith, health, and science. Hot springs also provides the information on geological tectonic based hypsography and orology annotations. Although many of these hot springs have been studied, however there is lot to be done. A lot of data has been published and research is still going on to be pursued all around world in these areas of extreme environments. The data is however haphazardly being represented. There is neither an accumulation of data at one place nor any extensive analysis done on this precious data which may correlate many facts with the past, present and future of the microbial science. Therefore, it is important to co-integrate the data and to analyze it with respect to bacterial diversity and hydro-geochemistry to give basic ideas of geothermal areas and features which are hardly being represented and discussed in such a way.

In this review, we have extensively analyzed the data based on bacterial diversity and hydro-geochemistry of studied hot springs residing in Himalayan Geothermal areas covering India, China and Northern Pakistan. As the hot springs can be both orogenic and non-orogenic in nature, here interestingly in the HGB both these types of geothermal points can be found. Thermal springs have been in use for religious and/or balneotherapeutic purposes since ages. Bathing at hot springs helps in efficient and rapid increase in blood circulation and helps improve metabolic activities and also from various other ailments. Hence, it is significant to study the hot springs from balneotherapeutic stratagem and its chemical ecology to understand the geothermometry

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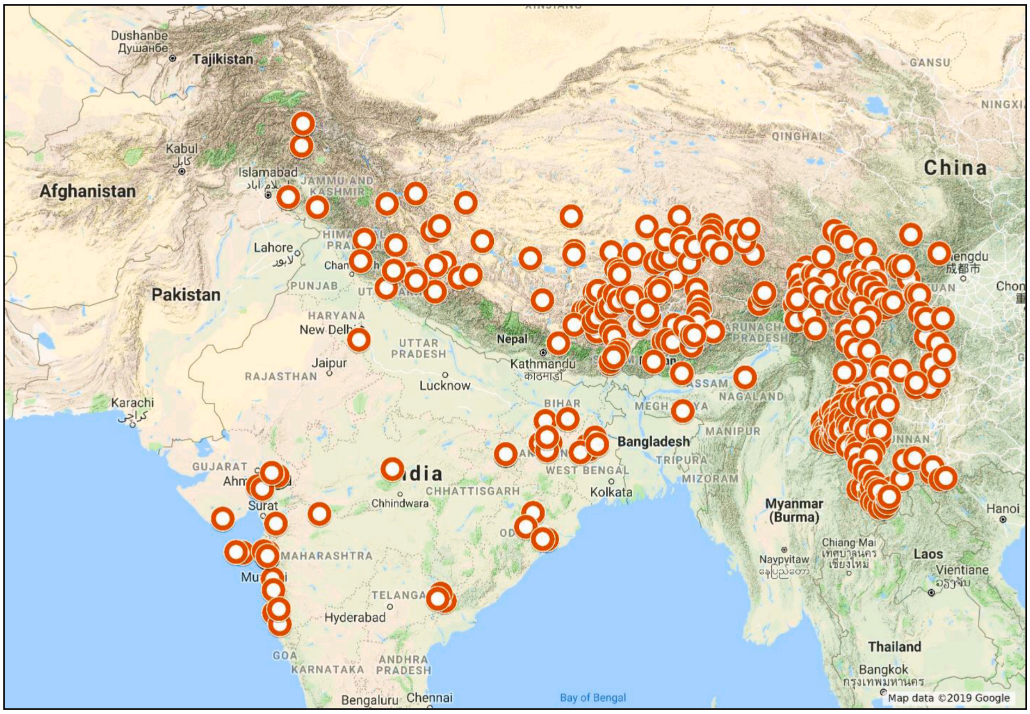

Fig. 1. The spatial arrangement of Hot Springs of Himalayan Geothermal Belt, Geothermal areas of China, Central/ Peninsular India and Northern Pakistan

of the HGB. We have also included the peninsular India in the study. We have used many statistical approaches such as Geothermometry, Piper, Durov and Giggenbach analysis for characterizing hydrogeochemistry. Whereas, Principal Component Analysis, Venn diagram, and correlation approaches using R software have been performed to describe bacterial diversity etc. Thus this review is a comprehensive description and analysis of various characteristics of geothermal features of various geothermal areas.

1.1. Hot springs

Natural thermal springs have been regarded as sociological and anthropological identities among mass civilizations (Das et al., 2012; Das et al., 2015; Das and Thakur 2020d; Das et al., 2021a). Scientific explorations pertaining to understand the possibility of life at extreme conditions manoeuvred biological sciences and cradled molecular biology (Das et al., 2020a). Thus, hot springs since time immemorial has been amalgamated with science and religion. Hot springs are geothermal (hydrothermal) points or springs on the Earth's surface which can be extended for several kilometres in a subterranean system. With temperature higher than the local groundwater (Gold, 1992), hot springs are distributed in regions with young volcanic potential and even in dormant or extinct regions where activity has ceased. The water from recharge zone or surface percolates through the cracks or fractures and feeds the groundwater. This water heated up by the hot magma chambers or deep magma flows and gets discharged outside through rock and soil crevices (Saemundsson, 2009; Walker, 1993). The water when passes through the fissures come in contact with rocks in the adjoining area and thus, there remains to high chance of mineral dissolution from rocks which then reach the surface. The thermal water carries silica along if volcanic rocks are present in its adjoining path (Saemundsson, 2009). Once the temperature cools, a silvery to whitish silica deposit forms around the spring, known as "sinter" (Campbell et al., 2015) This is inhabited by various species of micro-algae, bacteria and archaea rendering the surrounding as multi-coloured.

1.2. Physicochemical characteristics and Geothermometry

Hot spring water is usually clear, but is rich in minerals like calcium, chloride, magnesium, sodium, silica or sulfates that are dissolved from the rocks passing through, on its way to the surface (Olivier, 2011). Concentration of these minerals in hot springs is relatively higher than the non-geothermal groundwater (Zangana, 2015). Through characteristic geo-settings, diverse geothermic community microbiology has been determined worldwide (Bohorquez et al., 2012; Costa et al., 2009; Hou et al., 2013; Kubo et al., 2011; Kvist et al., 2007; Tekere 2012; Spear et al., 2005). Hot springs are by distant the most common features and befall in thermal areas as well as in confined spots. The most famous of these locations: Yellowstone (USA), North Island (New Zealand), Iceland, Kamchatka (Russia), and Japan. There are 48 countries in Asia and most of the countries hold thermal springs (Waring, 1965).

The chemical composition of geothermal groundwater depends upon the source of water and its reactions leading to mineralization and deposition through adsorption and desorption phenomena. The origin of chemical composition of geothermal groundwater is usually due to the archaic meteorological attributes or primordial sea (Ármannsson and Fridriksson, 2009). The geothermic groundwater and mineralized boulders interact among each other and may modify the geological settings. The elemental constituents responsible for the formations of such boulders are the halogen salts of aluminum, calcium, iron, magnesium, potassium, silica, sodium, manganese (Ármannsson and Fridriksson, 2009; Kristjansson et al., 1986; Smith et al., 2016). The radio-geochemistry of the geothermic fluids campaigns for geological attributes of these settings. The main geothermal indicators include dissolved gases, trace elements, isotopes, carbonate chemistry and chemical geothermometers (Cruz and França, 2006; Hobba et al., 1979; Jilali et al., 2018; Papp and Niţoi, 2006). Estimation of the reservoir's fluid temperature is measured through several chemical and isotopic geothermometers like dissolved silica, (Na^+/K^+), $(Na^+/K^+/Ca^{+2})$, $(Na^+/K^+/Ca^{+2}/Mg^{+2})$, (K^+/Mg^{+2}) or $\delta^{18}O$ (H_2O-SO), and these have been used as geochemical tools in geothermal examination since 1965 (Hobba et al., 1979; Kharaka and Mariner, 1989). The (Na/K) geothermometers are committed to temperature readings for diluted

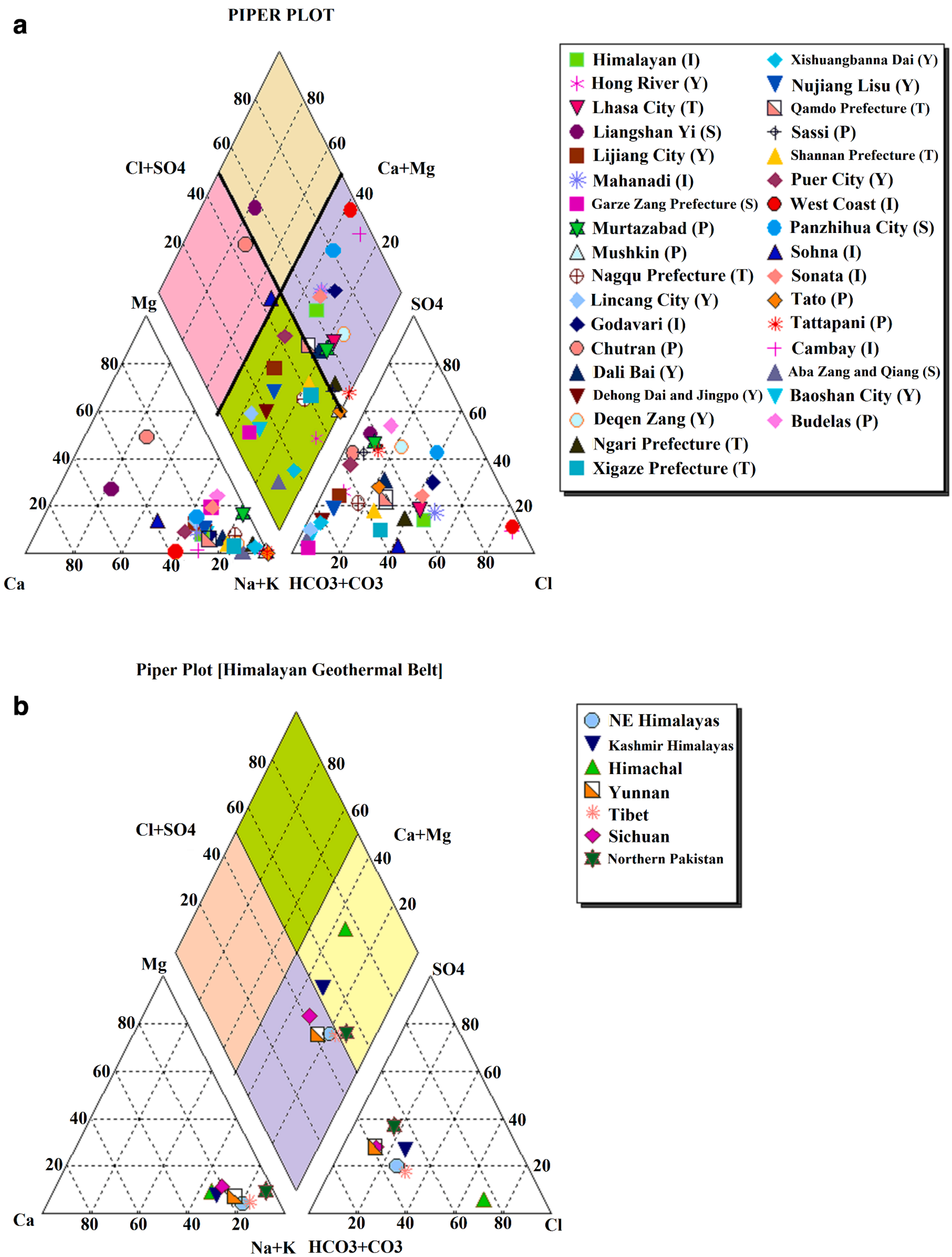

Fig. 2. Piper analysis: geothermal areas of China (left), geothermal areas of India and Northern Pakistan (right)

thermal waters from granite and volcanic regions. Similarly, sea water-derived fluid temperatures are predicted using (Na^+/K^+) and ($Na^+/K^+/Ca^{+2}$) geothermometry. Silica geothermometry proposed by Fournier (1973) (Fournier and Truesdell, 1973) showed the cistern temperature around 61-95°C whereas, the (Na^+-K^+-Ca^{+2}) geothermometry predicted it around 160-191°C. In some cases, the estimated reservoir temperature was higher than 191°C using Na^+-K^+-Ca^{+2} geothermometry. To fix this problem Fournier and Potter II (1979) applied Magnesium correction for the better estimation of cistern or reservoir or storage temperature (Fournier and Truesdell, 1973; Fournier and Potter, 1979).

The study of chemical composition of hot springs is of immense importance for its use in common household uses such as drinking and bathing, and also bears numerous aesthetic values. Studies on its chemical composition through various methodologies deduce that the dissolved minerals although possess considerable effect on various

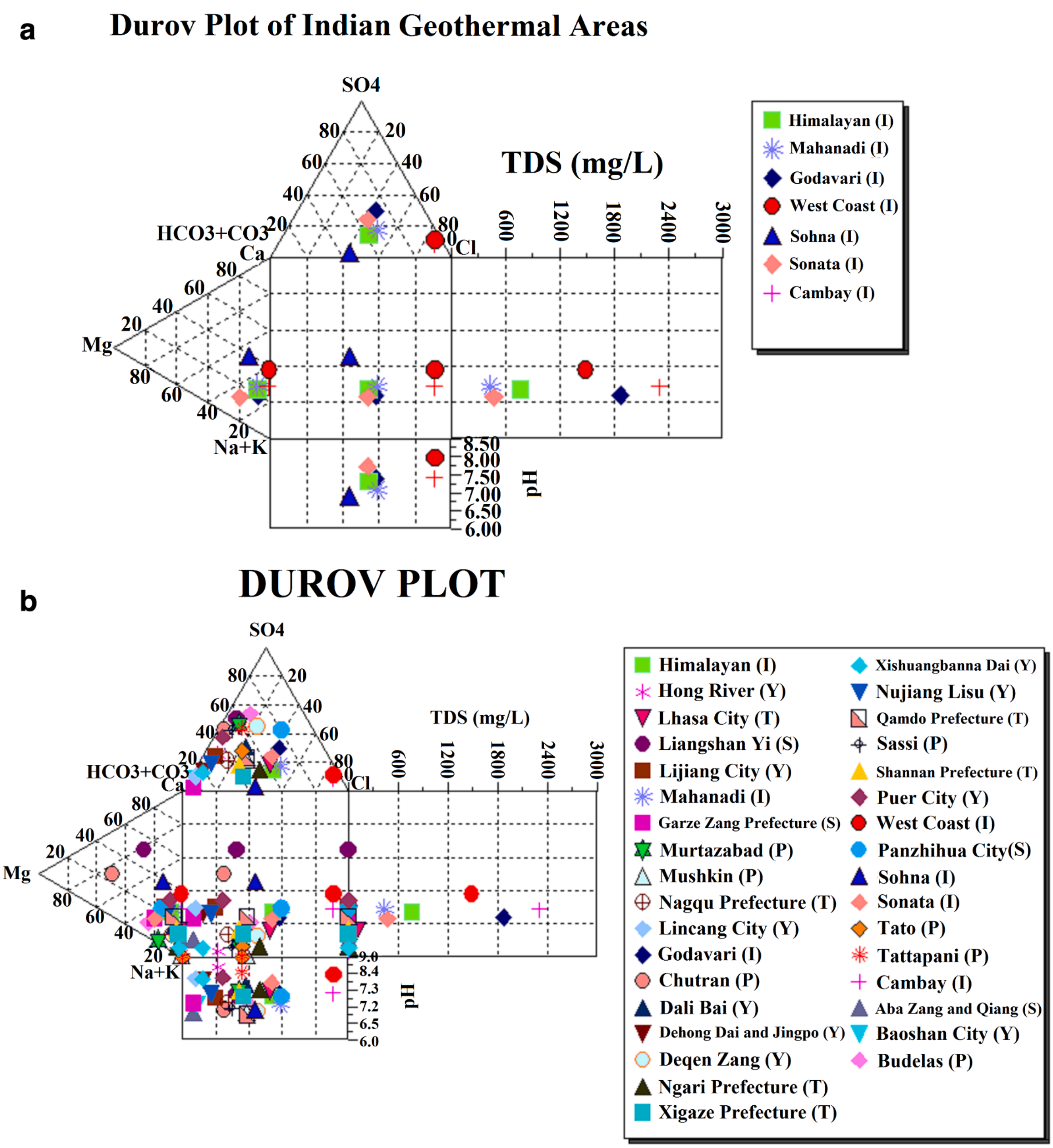

Fig. 3. Durov plot geothermal areas of India (left), geothermal areas of China and Northern Pakistan (right)

diseases, may bear harmful effects if present in higher concentrations above their threshold values (Das et al., 2020a; Gichuki and Gichumbi, 2012; Sherpa et al., 2013). This necessitates research of dissolved minerals and its concentration in hot springs. Also, a higher mineral composition in and around hot springs allows the growth of different micro and macro floral community (Mesa et al., 2017). The presence of different concentrations of heavy metals may also impart in microbes, the ability to tolerate them (De Vrij et al., 1988; Kim, 1985; Markowicz et al., 2010;, Najar et al., 2020b; Özdemir et al., 2009; Pennanen et al., 1996; Valls and De Lorenzo, 2002).

1.3. Himalayan geothermal zone

The Mediterranean–Himalayan tectonic belt stretching from Italy (Lar-Derello geothermal field) in the western side, through Iran and Pakistan, into the Tibetan–Himalaya (Yangbajing geothermal field, South Western China) and the Sanjiang area, and culminating at Indonesian (Kawa–Kamo Iiang geothermal field) constitutes one of the important geothermal zones in the world (Trifonov et al., 2012). The Himalayan geothermal zone lies in the east of Mediterranean–Himalayan tectonic belt. According to Le Fort (1975) (Fort, 1989), The Himalayan fold-thrust belt, which comprises the world's highest mountain range, formed as a result of India colliding into Eurasia at some point between 65 million years ago. The Himalayan arc formed extends over 2500 km, from Nanga Parbat (west-northwest) to Namche Barwa (east). On the northern side, it is separated by Trans-Himalayan zone by Indus-Tsangpo suture. This Himalayan arc includes major areas of Indian Himalayan province, Nepal, Bhutan followed by Tibet (China), Bangladesh and Indo-Myanmar (Fort 1989; Powell et al., 1988). The Himalayan geothermal belt (HGB) stretches from the Pamir terrain (its starting point), through Tibet and into Yunnan (Hochstein and Regenauer-Lieb, 1998). The spatial arrangement of various hot springs located in HGB and India is given in Fig. 1. In the present review hot springs of Himalayan arc involving many major Asian countries i.e., India, China, and Northern Pakistan (including central and peninsular India) have been showcased with

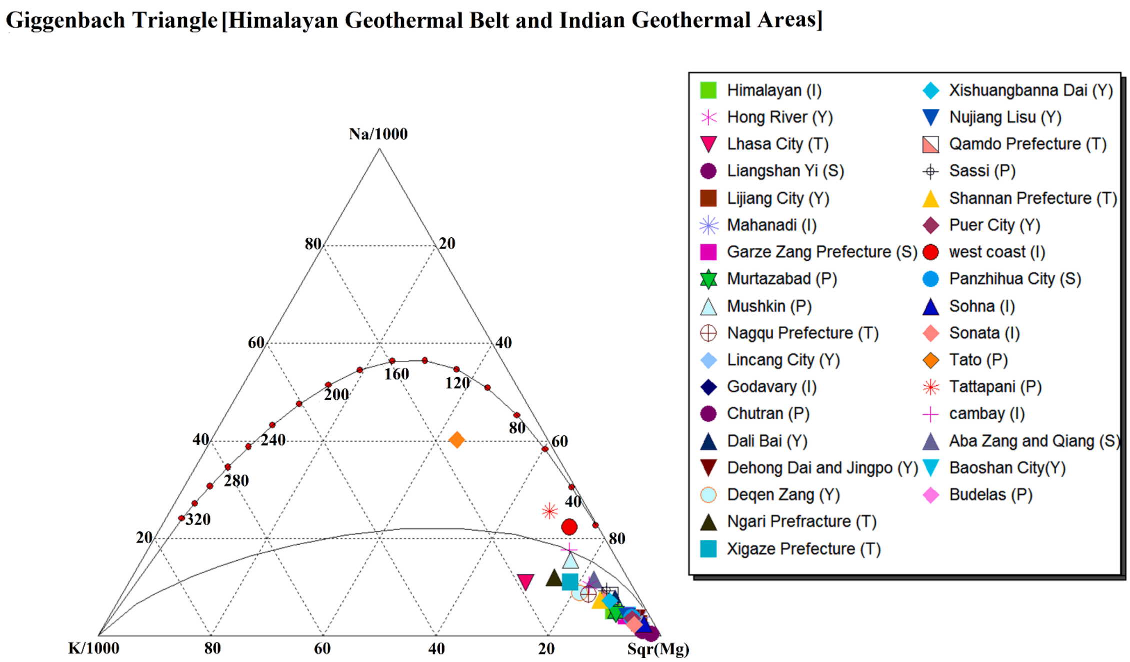

Fig. 4. Giggenbech plot, plotting geothermal areas of India, China, and Northern Pakistan.

respect to their physicochemical characteristics, geothermometry and microbiological properties.

1.4. Hydro-geochemical characterization

The physicochemical parameters of all the hot springs studied in this article were taken from various literature present. The physicochemical data (**Supplementary 2, 3**) from various representative geothermal fields were analyzed using AquaChem 2014. Piper analysis and Durov plot was also done using AquaChem 2014 (Teng et al., 2016). The frequently applied chemical geothermometers use silica geothermometry (Fournier and Truesdell, 1973), the ($Na^+/K^+/Ca^{+2}$) geothermometry (Fournier and Truesdell, 1973; Shikazono 1976), the (Na^+/K^+) geothermometry (Ellis, 1979) and the Mg-corrected ($Na^+/K^+/Ca^{+2}$) geothermometry (Fournier and Potter, 1979). The equilibrium state of hot spring water was determined by the ($Na^+/1000\text{-}K^+/100\text{-}Mg^{1/2+2}$) Giggenbach ternary diagram (Romano and Liotta, 2020). This plot relates water composition to various aspects such as the maturity of water along with estimation of the reservoir temperature. The maturity of water samples indicates the exposure of water to geothermal heat and that, it has reached equilibrium temperature. The immature zone reflects the mixing of meteoric water, lack of exposure to heat or limited time for reactions to proceed. The drawback associated with the use of (Na^+/K^+) geothermometry is that they give faulty readings to mixing and boiling processes. The reason may be because at a lower temperature, the Na^+/K^+ components in geothermic water are controlled by leaching processes rather than chemical equilibrium. Moreover, the Na^+-K^+ geothermometers tend to give higher estimation at higher calcium concentrations. Thus, to overcome these disadvantages, ($Na^+/K^+/Ca^{+2}$) geothermometers are developed (Fournier and Truesdell, 1973) that result in satisfactory readings at all conditions. Analysis using various geothermometers and the construction of Giggenbach ternary diagram was done by using AquaChem 2014. Xkms plot (left) and Xkmc plot (right) for hot springs were formed using Spreadsheets for Geothermal Water and Gas Geochemistry (Powell and Cumming, 2010).

Piper analysis was performed as shown in **Fig. 2**, and the Piper diagram showed the considerable differences between the water types of various geothermal areas studied. As per the Piper analysis, Indian geothermal areas other than the Indian Himalayan geothermal areas, Northern Pakistan and few geothermal areas of Tibet such as Lhasa city, Qamdo prefecture and Ngari prefecture possess Na^+- Cl^- water type. Sichuan geothermal areas were found to possess variable water types such as hot springs of Panzhihua City to possess Na^+-Cl^- water type, whereas hot springs of Garze Zang and Aba Zang prefectures are having mixed Ca^{+2}-Na^+-HCO_3^- or Na^+-HCO_3^- water type. However, the hot springs of the Liangshan Yi geothermal area of Sichuan represent an outlier possessing Ca^{+2}-Mg^{+2}-Cl^- type of water.

In contrast, most of the geothermal areas of Yunnan province possess similar water types. The hot springs in Yunnan geothermal areas possess mixed Ca^{+2}-Na^+-HCO_3^- water type. Similarly, Sohna geothermal field of Central India and Chutran hot springs of Northern Pakistan represents an outlier by possessing Ca^{+2}+ HCO_3^- water type.

To indicate the hydrochemical processes occurring within the hydrological systems or to represent the dissolved constituents of natural water- ***Durov diagram*** has been used in **Fig. 3** (Kozak and Tartanus, 2017). It is used to study the origin of the chemical composition of water and to determine the concentration of chemical constituents. Durov diagram has anion and cation triangles plotted based on the milli-equivalent percentage of the water samples. Data points in two triangles are projected into a square of the main field which lies perpendicular to the third axis in each triangle. The square of the Durov diagram has been divided into nine subfields that are used in deciphering the processes that control ground-water chemical properties. According to the Durov plot, most of the geothermal areas are represented in 1 and 4 subfields. Mostly the geothermal areas of Yunnan and few geothermal areas of Sichuan such as Garze Zang and Aba Zang; geothermal areas of Tibet such as Nagqu and Sassi geothermal field of Northern Pakistan mostly lie in subfield 1 as shown in **Fig. 3**, which represents the dominance of HCO_3^- and Ca^{+2}. Thus the hot springs of Yunnan indicate recharging water in limestone, sandstone and other aquifers. The subfield 4 of Durov square was mostly found to be occupied by geothermal fields of Tibet (Qamdo, Xizang, Ngari, Shannan and Lhasa), and Northern Pakistan (Murtazabad, Budelas, Tato and Tattapani). Thus this indicates the dominance of SO^{4-} and Ca^{+2} ions in the hot spring waters of these geothermal areas. It can also be indicated that there are possibilities of water mixing or water exhibiting simple dissolution. Two of the geothermal areas Liangshan Yi of Sichuan and

Table 1
The predicted reservoir temperature of the hot springs

Geothermal Areas	Temperature				Giggenbech characterization
	Quartz	Chalcedony	Na-K-Ca	Na-K-CA (Mg corrected)	
Aba Zang and Qiang (C)	118	89	146	138	Immature
Budelas (P)	-	-	197	-	Immature
Cambay (I)	134	107	140	140	Immature
Chutran (P)	-	-	223	-	Immature
Dali Bai (C)	109	79	154	62	Immature
Dehong Dai and Jingpo (C)	111	81	149	63	Immature
Deqen Zang (C)	237	227	194	91	Immature
Garze Zang Prefecture (C)	106	77	183	-	Immature
Godavary (I)	128	100	184	75	Immature
Himalayan (I)	115	86	183	80	Immature
Hong River (C)	136	109	177	82	Immature
Kashmir Himalayas (I)	126	99	186	86	Immature
Lhasa City (C)	139	112	217	152	Immature
Liangshan Yi (C)	120	92	206	49	Immature
Lijiang City (C)	130	103	165	50	Immature
Lincang City (C)	111	82	159	43	Immature
Mahanadi (I)	10	-	144	71	Immature
Murtazabad (P)	157	133	213	-	Immature
Mushkin (P)	-	-	193	65	Immature
Nagqu Prefecture (C)	123	94	193	43	Immature
Ngari Prefracture (C)	134	107	207	62	Immature
Nujiang Lisu (C)	116	87	173	53	Immature
Panzhihua City (C)	112	83	167	35	Immature
Puer City (C)	122	94	163	79	Immature
Qamdo Prefecture (C)	121	92	154	82	Immature
Sassi (P)	129	102	161	43	Immature
Shannan Prefecture (C)	121	93	178	95	Immature
Sohna (I)	76	45	147	67	Immature
Sonata (I)	138	111	196	-	Immature

Chutran of Northern Pakistan occupy subfield 2 as shown in Fig. 3, This indicates the water of these hot springs is dominated by Ca^{+2} and HCO^{3-} and as the Na^{+} concentration of these hot springs is higher than Mg^{+2}, thus there is an ion exchange occurring among them. In the case of Indian geothermal fields, the Indian Himalayas, Sonata and Godavari lie in subfield 4. However, Sohna, Cambay and West Coast geothermal fields lie in subfield 5, subfield 7 and subfield 8 respectively. Thus in Sohna geothermal water, there is not the dominance of any anion or cation, which indicates water exhibiting simple dissolution or mixing. Moreover, hot spring waters of Cambay and West Coast are rich in Na^{+} and Cl^{-} ions and thus may result from the reverse ion exchange of Na-Cl waters.

1.5. Geothermometry

Chemical geothermometry involves the use of chemistry of geothermal reservoirs to evaluate the temperature. The partial equilibrium between the fluid and host rocks controls the composition of geothermal solutions.

The Giggenbach plot (Fig. 4) shows that almost all geothermal areas of India, China, and Northern Pakistan lie to the $Mg^{1/2}$ corner in the immature zone, implicating that these areas remain far from the equilibrium and thus, the temperature of water at depth is low. This indicates that there is mixing of groundwater, rainwater, seawater and geothermal fluids, which is the main process responsible for decline in temperature in the thermal groundwater discharging to the surface. Furthermore, it can also be concluded that there remains a limited weak-rock interaction under low temperature in these geothermal areas and thus, geo-thermometry cannot be applied to these geothermal waters. However, three geothermal areas viz. hot springs of Northern Pakistan (Tato and Tattapani) and West Coast fall within the partially equilibrated water fields. Other geothermal areas such as Mushkin and Cambay lie along the curves towards the $Mg^{1/2}$ corner. The equilibrium temperature ranges between 120°C and 180°C (Fig. 4). Table 1 shows the predicted reservoir temperature of the hot springs belonging to geothermal areas falling within the partially equilibrated water fields. The temperature predicted by Na^{+}-K^{+}-Ca^{+2} geo-thermometer for Tattapani, West Coast, Cambay and Mushkin was estimated to be 157°C, 107°C, 140°C and 193°C, respectively. However, temperature in case of Tato was estimated to be 160°C as predicted by Chalcedony geo-thermometer. Analytical results were also plotted on the graph of log (K^2/Mg^{+2}) vs log (K^2/Ca^{+2}) (Fig. 5). This cross plot has been referred to as geo-indicators rather than geothermometers by Giggenbach and Goguel in 1989 (Giggenbach and Soto, 1992; Marini, 2001). It examines the temperature of both the final water-rock equilibrium and the pCO_2 of geothermic fluids. The plot reveals that some water fall below the saturation line and hence, pCO_2 is higher than those at complete equilibrium. Thus, the pre-conditions favor the transition of Ca-Al-silicates to calcite in the system, implicating that the geothermal water chemistry is regulated by the disintegration of the host rocks instead of the equilibrium between water and minerals. Moreover, through the plot, it can be inferred that temperature of the geothermal fields ranges between 50°C-140°C, with the highest temperature estimated as 130°C of Tato geothermal area. Similarly, another cross plot of the K^{+}–Mg^{+2} and quartz geo-thermometer (Giggenbach and Soto, 1992) were applied as shown in Fig. 5. Basically, this plot uses the chalcedony geo-thermometers, which are more specific to use than quartz for water from a low temperature geothermic region (Shah et al., 2019). The graph reveals similar results suggesting the reservoir temperatures of the geothermal fields between 50°C -140°C and the highest temperature of 130°C in that of Tato geothermal area.

1.6. Bacterial diversity

The Himalayan Geothermal Belt hosts numerous hot springs and they have distinct characteristics and bacterial diversity among themselves. This region predominantly consists of phylum Proteobacteria, Firmicutes, Bacteroidetes and Actinobacteria, among the main bacterial flora

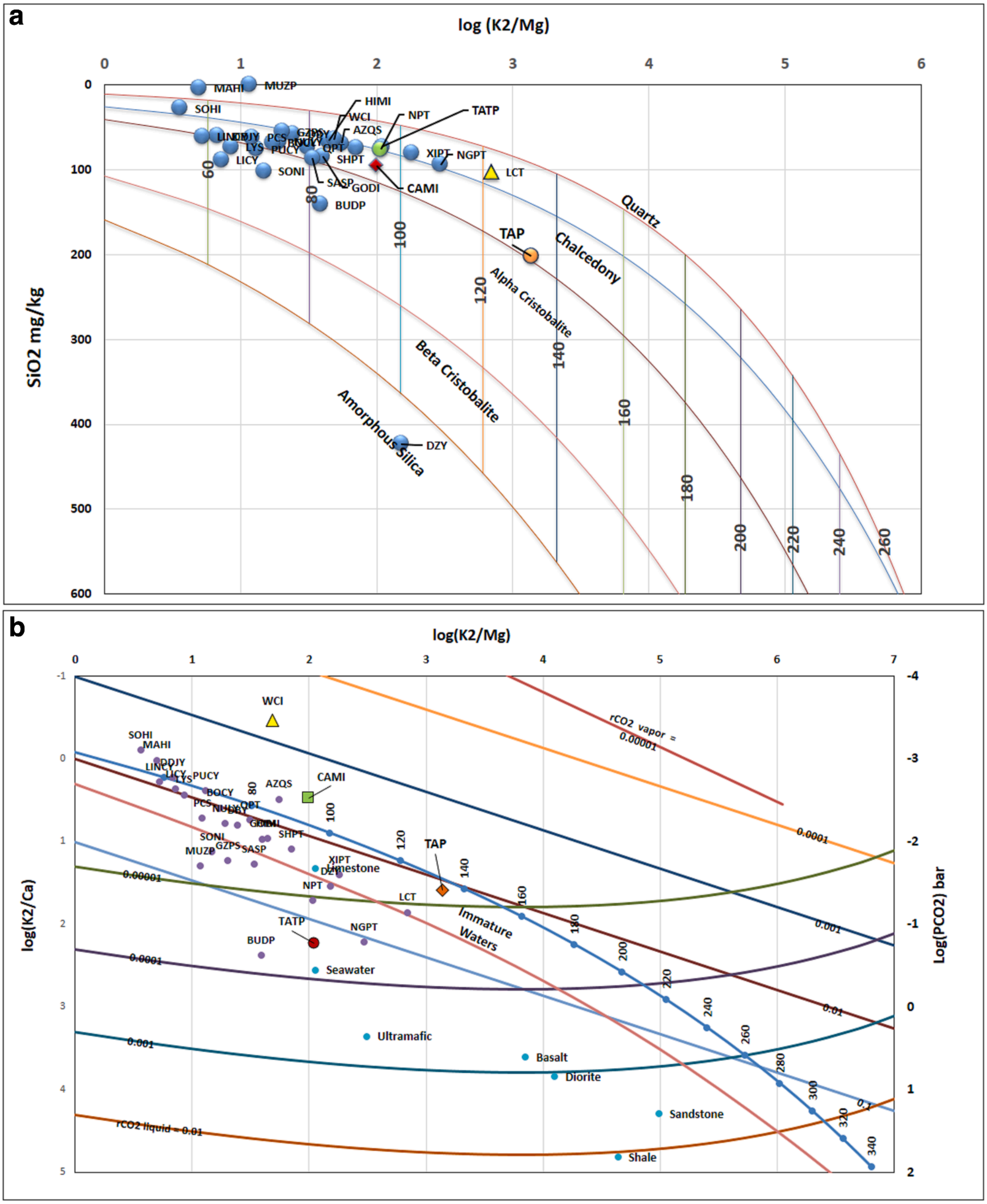

Fig. 5. Xkms plot (left) and Xkmc plot (right) for hot springs residing in geothermal areas of India, China, and Northern Pakistan.

in these ecosystem (Das and Thakur, 2020d). These microbes are generally termed as thermophiles and they have great potential biotechnological and industrial applications depending on their enzymes. Novel bacterium *Parageobacillus yumthangensis* AYN2 was discovered and also various viruses, archaea and fungi has also been studied from the various hot springs of Sikkim (Bhatia et al., 2015; Das and Thakur, 2020d; Das et al., 2020b; 2020c; Das et al., 2021b; Huang et al., 2011; Kumar et al., 2004; Najar et al., 2018a; 2018b; Najar et al., 2020a; 2020c; Pandey et al., 2014; Rawat and Joshi, 2018; Sharma et al., 2017).

The bacterial diversity data was extracted, analyzed and compiled based on up to date literatures **(Supplementary 1)**. The data was analyzed by R software (R Core Team, 2018). To compare the results Principal Component Analysis (PCA) was also done by using R software. To check the bacterial diversity pattern of various hot springs, bubble plot was constructed using R software (R Core Team, 2018). The results reveal abundance of microbiota of different phyla present in different hot springs. With the most abundant phyla being Proteobacteria, Firmicutes, Actinobacteria, Aquificae, Bacteroidetes, Chloroflexi, and Cyanobacteria, few others such as Thermi, Planctomycetes, Chlorobi, Nitrospirae and Acidobacter that showed lesser abundance were found. There is a clear distinction among various geothermal areas on the bases of this bacterial diversity. For instance, The Indian Himalayan geothermal areas, Central Indian geothermal areas such as Mahanadi

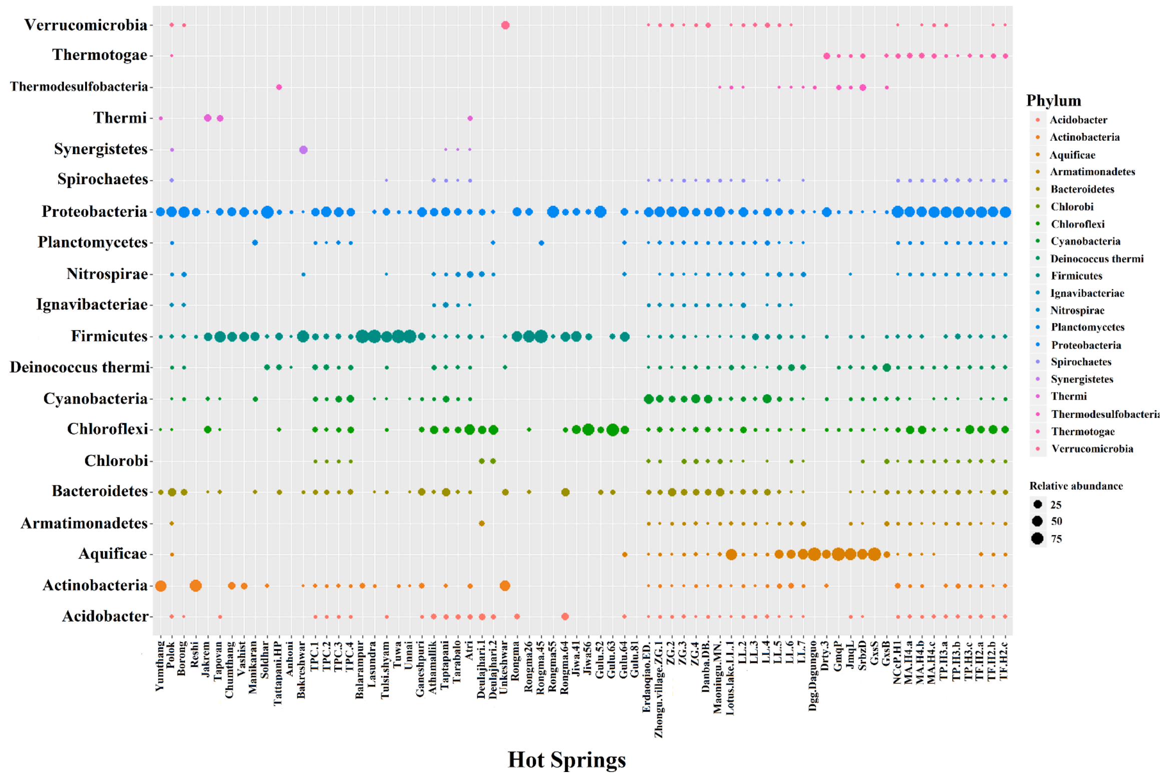

Fig. 6. Bubble plot representing the microbial diversity of various hot springs located in Indian, China, and Northern Pakistan

and Chhattisgarh, Northern Pakistan and Sichuan geothermal areas show similar kind and abundance of bacterial diversity (**Fig. 6**), while Yunnan geothermal areas attribute less diversity and a distinct phylum Aquificae. Similarly, geothermal areas of Tibet also attribute less diversity with major phyla as Proteobacteria, Firmicutes and Chloroflexi. On the other hand, other geothermal areas of India such as Sonata (Madhya Pradesh and West Bengal hot springs), Cambay and West Coast possess similar kinds of bacterial diversity with Chloroflexi as the major phylum. In order to compare the results, analysis through Principal Component Analysis (PCA) was done as shown in **Fig. 7**. The PCA gives a similar picture, where geothermal areas of China are rich in diversity followed by Indian geothermal areas. As, Northern Pakistan harbor less number of hot springs, yet a rich bacterial diversity pertains here as compared to both the Indian and Chinese hot springs. As revealed from the plot, Proteobacteria contributes as the major phylum in all the three geothermal areas, while Firmicutes dominate in geothermal areas of India and China, followed by Proteobacteria. A comparative analysis on the basis of bacterial diversity was also done by using Venn diagram as shown in **Fig. 8**.The geothermal areas were divided into four groups based on different regions such as the Indian Himalayan Geothermal Area (IHGA), other Indian Geothermal Areas (OIGA), China Himalayan Geothermal Area (CHGA) and Northern Pakistan Geothermal Areas (NPGA). The results reveal the IHGA, OIGA, CHGA and NPGA share three common phyla: Proteobacteria, Firmicutes and Bacteriodetes, all of which contribute to about 37.5% of the major bacterial diversities in these geothermal areas. The phylum Chloroflexi is common in the OIGA, CHGA and NPGA except in IHGA. On the other hand, Cyanobacteria is common among OIGA and CHGA, contributing about 12.5% of the bacterial diversity. Actinobacteria shares a 12.5 % similarity between IHGA and OIGA. There remains a clear distinction in the geothermal areas CHGA and NPGA that harbor micro-biota belonging to phylum Aquificae (12.5 %) and Thermotagae (12.5%), respectively as they are exclusively present in their respective geothermal areas.

1.7. Correlation of bacterial diversity and physicochemical parameters

Correlation between physicochemical parameters and bacterial diversity was done by Principal component analysis (PCA) using R software (R Core Team, 2018) to check the correlation between various variables such as between physicochemical parameters and various phyla. Venn diagram was constructed for comparative analysis based on bacterial diversity in groups of four geothermal areas using Venny 2.0 software (Oliveros, 2015). Combinational effect of various variables including physicochemical and various bacterial phylum were done by scattering analysis using Geoda software (Anselin et al., 2006).

In the PCA biplot, PCA1 shows the highest correlation. The plot reveals that temperature, pH, and HCO_3^- are positively correlated to phyla (having higher abundance) such as Proteobacteria, Chloroflexi, Bacteroidetes and Thermotogae. On the other hand, K, Mg, Ca, Cl and TDS show a positive correlation with other less dominant phyla such as Aquificae and Thermodesulfobacteria. However, the Firmicutes and Actinobacteria are less correlated to Na^+, K^+, Mg^{+2}, Ca^{+2}, Cl^-, and TDS as shown in **Fig. 9**, and are negatively correlated with temperature and pH. This suggests that these elements are playing a more or less significant role in the growth of these bacterial populations.

Similar results are observed as done by scattering analysis. It shows a combinational effect of various variables. For instance, the results reveal a positive correlation between various physicochemical variables (temperature, pH, HCO_3^-) and with phyla occurring in higher abundance. In case of Proteobacteria (**Fig. 10a**), while temperature attributes a significant contribution (0.336), the combinational effect of temperature-pH (0.752) and HCO_3-Na (0.601) shows the highest correlation and significantly contributes to Proteobacteria. Similarly in case of Firmicutes, the parameters temperature (0.474) and calcium (0.402) attribute a significantly higher contribution (**Fig. 10b**). Moreover, the combinational effect of temperature–pH and K^+/Ca^{+2} shows significantly a higher correlation (0.752 and 0.601, respectively) and contribution towards Firmicutes. In case of Cyanobacteria, temperature-pH, K^+/Na^+ and K^+/Mg^{+2} show the highest correlation and contribution

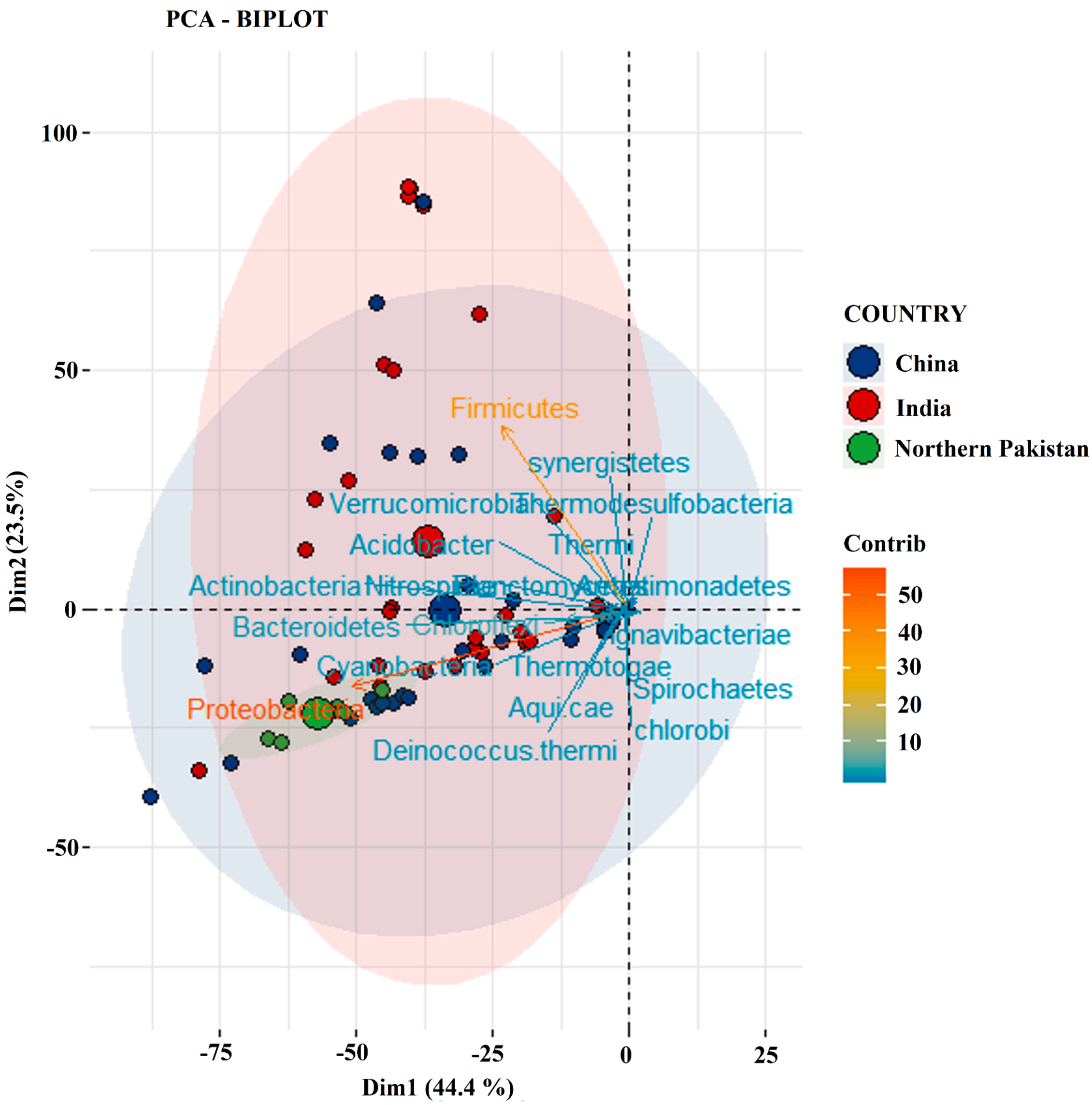

Fig. 7. Principle component analysis representing the microbial diversity and contribution of various phylum's within the hot springs of the particular country such as India (red), China (Blue) and Northern Pakistan (green)

(Fig. 10c). Highest correlation and contribution can also be seen in case of Chloroflexi (temperature-pH (0.752), temperature–HCO_3^- (0.320), and K^+/Na^+ (0.601) as shown in Fig. 10d. These results thus implicate that temperature and pH are the main important variables for the growth and development of various phyla residing in these extreme habitats. However, other chemical variables are also important in combination with temperature and pH as represented by Fig. 10a-**d.**

2. Conclusion

Hot springs are exciting geothermal features on the surface of the Earth and a great attraction to tourists, ailed patients and religious practitioners. The heat source, its chemical composition and the bacterial diversity are the interesting characteristics of hot springs that have engrossed both the geologists and microbiological scientists. Although the geothermal energy has been exploited in various regions all over the world, there is a significantly high scope for utilizing these geothermal areas as a source of geothermal energy. Using various geothermometers, the temperature of sources was predicted to be approximately between 100-250°C, which is not much high as compared to other geothermal areas around the world, suggesting that the source of thermal springs are probably the young volcanic magma beneath the Earth. The bacterial investigation revealed the presence of a huge diversity of bacterial communities in these hot springs. The major phyla found are Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria, etc. This bacterial dominance indicates the presence of various unknown, novel and industrially important microflora residing in these hot springs. In context to the Indian continent, there is a large scope with respect to investigating industrially important bacterial diversity, exploiting geothermal energy in various sectors. There is also an escalating scope of utilizing these hot springs for eco-medical tourism by maintaining these hot springs by generating well government-driven policies.

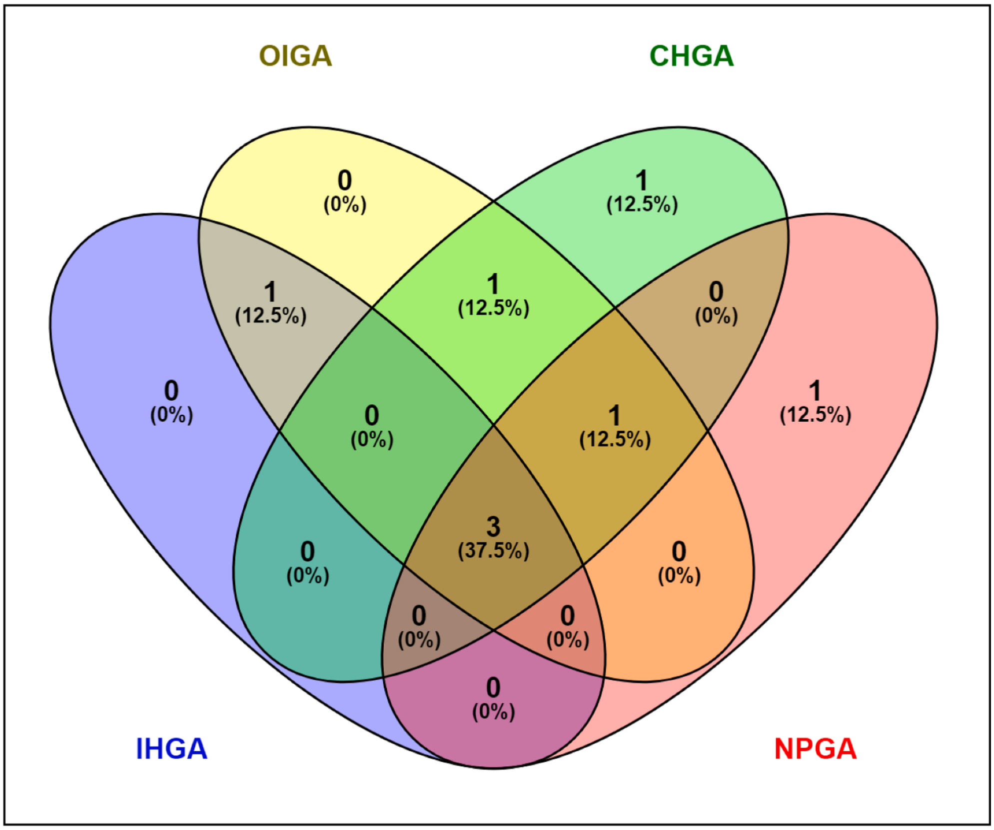

Fig. 8. ***Venn diagram:*** a comparative analysis on the basis of bacterial diversity of four randomly categorized geothermal areas viz Indian Himalayan Geothermal Area (IHGA), Other Indian Geothermal Areas (OIGA), China Himalayan Geothermal Area (CHGA) and Northern Pakistan Geothermal Areas (NPGA).

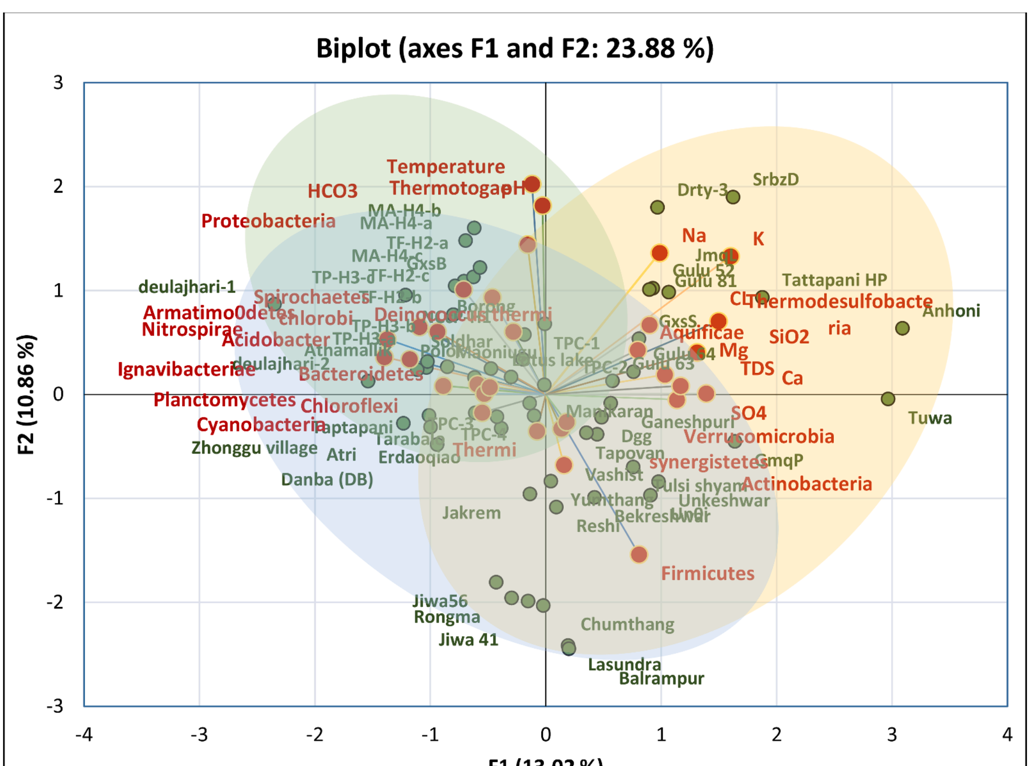

Fig. 9. Principal Component Analysis: a correlation between various variables such as between physicochemical parameters and various phyla corresponding to various hot springs studied in this review.

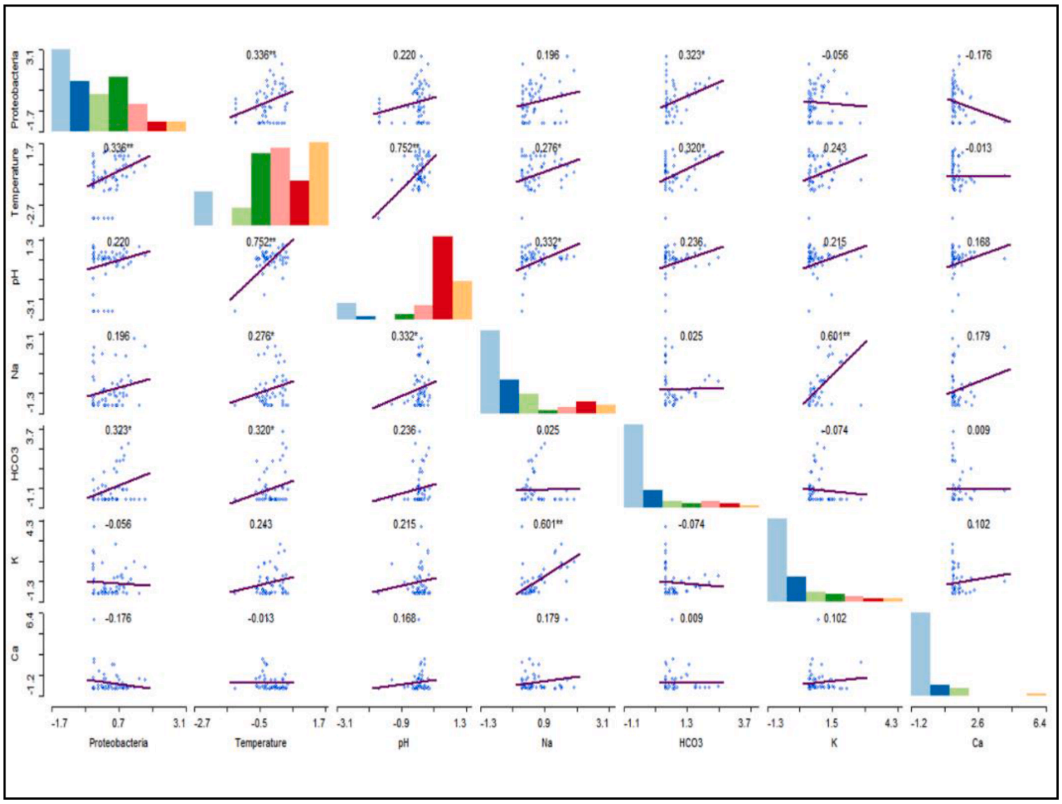

Fig. 10a. Scatter analysis between Proteobacteria and various variables such as Temperature, pH, HCO_3^-, Na^+, etc.

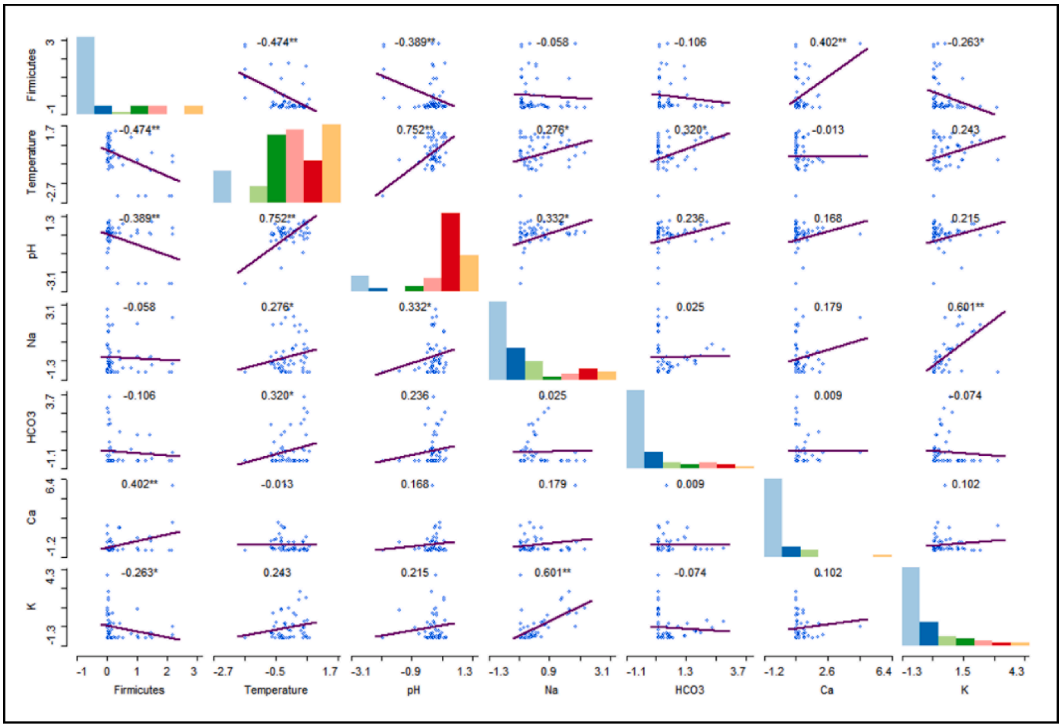

Fig. 10b. Scatter analysis between Firmicutes and various variables such as Temperature, pH, HCO_3^-, Na^+, etc.

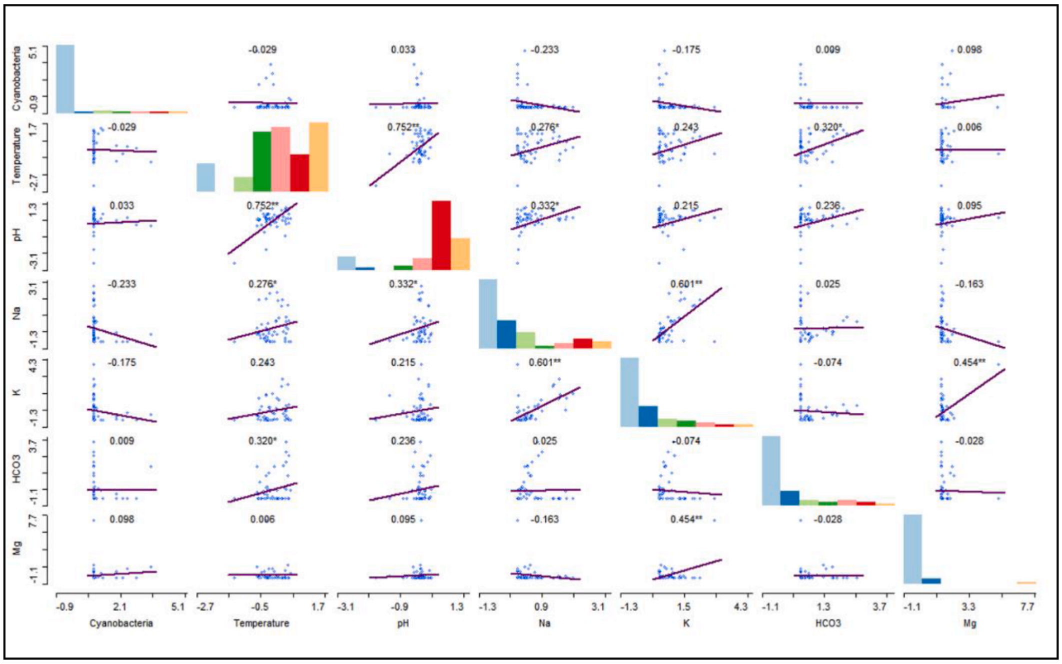

Fig. 10c. Scatter analysis between Cyanobacteria and various variables such as Temperature, pH, HCO_3^-, Na^+, etc.

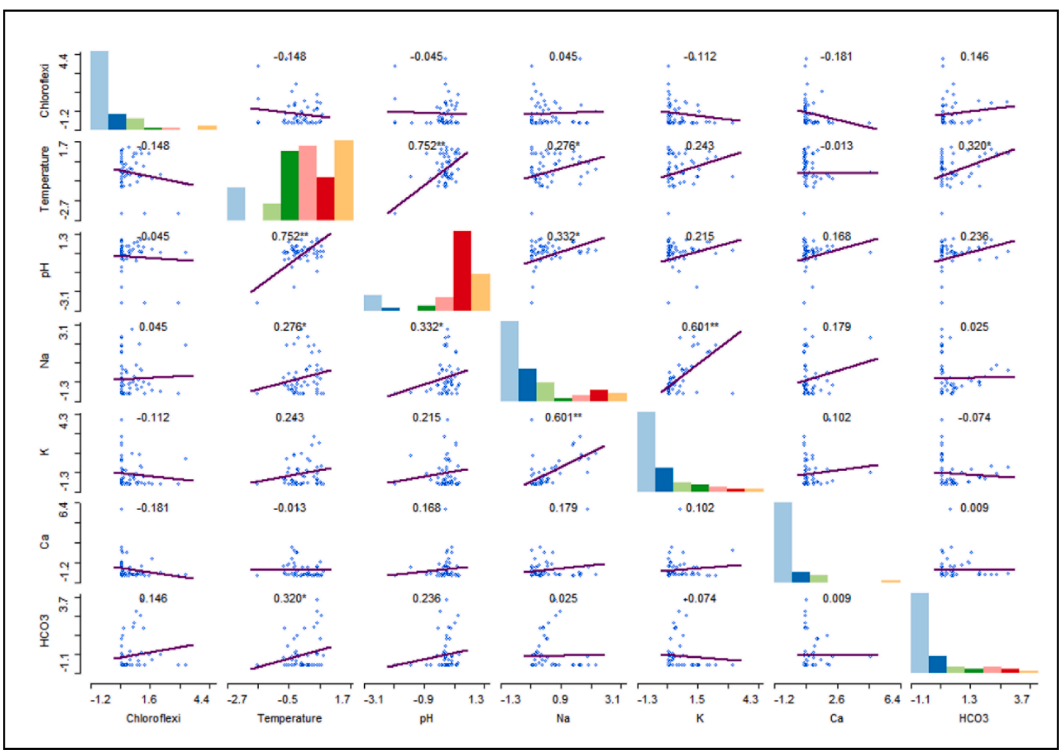

Fig. 10d. Scatter analysis between Chloroflexi and various variables such as Temperature, pH, HCO_3^-, Na^+, etc.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable.

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CRediT authorship contribution statement

Ishfaq Nabi Najar: Formal analysis, Writing – original draft. **Prayatna Sharma:** Formal analysis, Writing – original draft. **Sayak Das:** Formal analysis, Writing – original draft. **Mingma Thundu Sherpa:** Formal analysis, Writing – original draft. **Santosh Kumar:** Formal analysis, Writing – original draft. **Nagendra Thakur:** Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.crmicr.2022.100125.

REFERENCES

Anselin, L., Syabri, I., Kho, Y., 2006. GeoDa : An Introduction to Spatial Data Analysis. Geogr. Anal. 38, 5–22.

Ármannsson, H., Fridriksson, T., 2009. Application of Geochemical Methods in geothermal exploration. Short Course VII Explor. Geotherm. Resour. 1–8.

Bhatia, S., Batra, N., Pathak, A., Green, S.J., Joshi, A., Chauhan, A., 2015. Metagenomic evaluation of bacterial and archaeal diversity in the geothermal hot springs of Manikaran, India. Genome Announc. 3 (1), e01544–e015414. https://doi.org/10.1128/genomeA.01544-14.

Bohorquez, L.C., Delgado-Serrano, L., López, G., Osorio-Forero, C., Klepac-Ceraj, V., Kolter, R., Junca, H., Baena, S., Zambrano, M.M., 2012. In-depth Characterization via Complementing Culture-Independent Approaches of the Microbial Community in an Acidic Hot Spring of the Colombian Andes. Microb. Ecol. 63, 103–115. https://doi.org/10.1007/s00248-011-9943-3.

Campbell, K.A., Guido, D.M., Gautret, P., Foucher, F., Ramboz, C., Westall, F., 2015. Geyserite in hot-spring siliceous sinter: Window on Earth's hottest terrestrial (paleo) environment and its extreme life. Earth-Science Rev 148. https://doi.org/10.1016/j.earscirev.2015.05.009.

Costa, K.C., Navarro, J.B., Shock, E.L., Zhang, C.L., Soukup, D., Hedlund, B.P., 2009. Microbiology and geochemistry of great boiling and mud hot springs in the United States Great Basin. Extremophiles 13, 447–459. https://doi.org/10.1007/s00792-009-0230-x.

Cruz, J.V., França, Z., 2006. Hydrogeochemistry of thermal and mineral water springs of the Azores archipelago (Portugal). J. Volcanol. Geotherm. Res. 151, 382–398. https://doi.org/10.1016/j.jvolgeores.2005.09.001.

Das, S., Sherpa, M.T., Najar, I.N., Thakur, N., 2021a. Hot and Cold Bacteria of Sikkim: Biodiversity and Enzymology. Bioprospecting of Enzymes in Industry, Healthcare and Sustainable Environment. Springer, Singapore, pp. 269–289.

Das, S., Roy, G., Najar, I.N., Sherpa, M.T., Thakur, N., 2021b. Diversity and composition of the North Sikkim hot spring mycobiome using a culture-independent method. Folia Microbiol. (Praha) 66 (3), 457–468. https://doi.org/10.1007/s12223-021-00859-z.

Das, S., Roy, G., Najar, I.N., Sherpa, M.T., Thakur, N., 2020a. Chemical Ecology and Microbial Quality Assessment of Water of Recreational Hot Springs of Sikkim Himalayas. J. Water Environ. Technol. 18 (6), 398–414.

Das, S., Kumari, A., Sherpa, M.T., Najar, I.N., Thakur, N., 2020b. Metavirome and its functional diversity analysis through microbiome study of the Sikkim Himalayan hot spring solfataric mud sediments. Curr Res Microbial Sci 1, 18–29. https://doi.org/10.1016/j.crmicr.2020.05.002.

Das, S., Sherpa, M.T., Najar, I.N., Thakur, N., 2020c. Prevalence of methanogens in the uncultured Sikkim hot spring solfataric mud archaeal microbiome. Environ. Sustain. 3 (4), 453–469. https://doi.org/10.1007/s42398-020-00133-x.

Ed(s) Das, S., Thakur, N., 2020d. The Microbial Diversity of Hot Springs Located in Himalayan Geothermal Belts (HGB). In: Pandey, A., Sharma, A. (Eds.), Extreme Environments: Unique Ecosystems-Amazing Microbes. CRC Press. Ed(s)ISBN 9780367350161.

Ed(s) Das, S., Najar, I.N., Sherpa, M.T., Thakur, N., 2016. Hot Springs of Sikkim: Unfathomed colossal treasure of potential microbial syndicate of Northeast India - A coup d'oeil into it. In: Bag, N, Murugan, R, Bag, A (Eds.), Biotechnology in India: Initiatives and accomplishments. New India Publishing Agency, New Delhi. Ed(s) ISBN: 9789385516030.

Das, S., Sherpa, M.T., Sachdeva, S., Thakur, N., 2012. Hot springs of Sikkim (Tatopani): A socio medical conjuncture which amalgamates religion, faith, traditional belief and tourism. Asian Acad. Res. J. Social Sci. Human. 1 (4), 80–93.

De Vrij, W., Bulthuis, R.A., Konings, W.N., 1988. Comparative study of energy-transducing properties of cytoplasmic membranes from mesophilic and thermophilic bacillus species. J. Bacteriol. 170, 2359–2366.

Ellis, A.J., 1979. Chemical geothermometry in geothermal systems. Chem. Geol. 25, 219–226. https://doi.org/10.1016/0009-2541(79)90143-8.

Fort, P.L.E., 1989. The Himalayan Orogenic Segment, in: Tectonic Evolution a/the Tethyan Region. Kluwer Academic Publishers, pp. 289–386.

Fournier, R.O., Truesdell, A.H., 1973. Au empirical Na-K-Ca geothepmomete for natural waters. Geochim. Coemochimica Acta 37, 1255–1275.

Fournier, R.0, Potter, R.W., 1979. Magnesium correction to the Na-K-Ca chemical geothermometer. Geochim. Coemochimica Acta 43, 1543–1550.

Gichuki, J.G., Gichumbi, J.M., 2012. Physico-Chemical Analysis of Ground Water from Kihara Division, Kiambu County, Kenya. J. Chem., Biol. Phys. Sci. 2, 2193–2200.

Giggenbach, W.F., Soto, R.C., 1992. Isotopic and chemical composition of water and steam discharges from volcanic-magmatic-hydrothermal systems of the Guanacaste Geothermal Province. Costa Rica. Appl. Geochemistry 7, 309–332. https://doi.org/10.1016/0883-2927(92)90022-U.

Gold, T., 1992. The deep, hot biosphere. Proc. Natl. Acad. Sci. 89, 6045–6049. https://doi.org/10.1073/pnas.89.13.6045.

Hobba, W.A., Fisher, D.W., Pearson, F.J., Chemerys, J.C., 1979. Hydrology and Geochemistry of Thermal Springs of the Appalachians, Geohydrology of Geothermal Systems. United States Government Printing Office, Washington.

Hochstein, M.P., Regenauer-Lieb, K., 1998. Heat generation associated with collision of two plates: the Himalayan geothermal belt. J. Volcanol. Geotherm. Res. 83, 75–92. https://doi.org/10.1016/S0377-0273(98)00018-3.

Hou, W., Wang, S., Dong, H., Jiang, H., Briggs, B.R., Peacock, J.P., Huang, Q., Huang, L., Wu, G., Zhi, X., Li, W., Dodsworth, J.A., Hedlund, B.P., Zhang, C., Hartnett, H.E., Dijkstra, P., Hungate, B.A., 2013. A Comprehensive Census of Microbial Diversity in Hot Springs of Tengchong, Yunnan Province China Using 16S rRNA Gene Pyrosequencing. PLoS One 8, 1–15. https://doi.org/10.1371/journal.pone.0053350.

Huang, Q., Dong, C.Z., Dong, R.M., Jiang, H., Wang, S., Wang, G., et al., 2011. Archaeal and bacterial diversity in hot springs on the Tibetan Plateau, China. Extremophiles 15 (5), 549–563. https://doi.org/10.1007/s00792-011-0386-z.

Jilali, A., Chamrar, A., El Haddar, A., 2018. Hydrochemistry and geothermometry of thermal water in northeastern Morocco. Geotherm. Energy 6, 1–16. https://doi.org/10.1186/s40517-018-0095-2.

Kharaka, Y.K., Mariner, R.H., 1989. Chemical Geothermometers and Their Application to Formation Waters from Sedimentary Basins. Thermal History of Sedimentary Basins. Springer-Verlag New York Inc., pp. 99–117

Kim, S.-J., 1985. Effect of heavy metals on natural populations of bacteria from surface micro-layers and subsurface water. Mar. Ecol. Prog. Ser. 26, 203–206. https://doi.org/10.3354/meps026203.

Kozak, M., Tartanus, M., 2017. What Story Does the Durov Diagram Tell ? Colloq. Biometricum 47, 41–48.

Kristjansson, J.K., Hreggvidsson, G.O., Alfredsson, G.A., 1986. Isolation of halotolerant Thermus spp. from submarine hot springs in Iceland. Appl. Environ. Microbiol. 52, 1313–1316.

Kubo, K., Knittel, K., Amann, R., Fukui, M., Matsuura, K., 2011. Sulfur-metabolizing bacterial populations in microbial mats of the Nakabusa hot spring. Japan. Syst. Appl. Microbiol. 34, 293–302. https://doi.org/10.1016/j.syapm.2010.12.002.

Kumar, B., Trivedi, P., Mishra, A.K., Pandey, A., Palni, L.M.S., 2004. Microbial diversity of soil from two hot springs in Uttaranchal Himalaya. Microbiol. Res. 159, 141–146.

Kvist, T., Ahring, B.K., Westermann, P., 2007. Archaeal diversity in Icelandic hot springs. FEMS Microbiol. Ecol. 59, 71–80. https://doi.org/10.1111/j.1574-6941.2006.00209.x.

Marini, L., 2000. Geochemical Techniques for the Exploration and Exploitation of Geothermal Energy, in: Dipartimento per Lo Studio Del Territorio e Delle Sue Risorse. Universita degli studi di, Genova, Italy, pp. 1–82.

Markowicz, A., Plociniczak, T., Piotrowska-seget, Z., 2010. Response of Bacteria to Heavy Metals Measured as Changes in FAME Profiles. Polish J. Environ. Stud. 19, 957–965.

Tekere, Memory, 2012. An evaluation of the bacterial diversity at Tshipise, Mphephu and Sagole hot water springs, Limpopo Province, South Africa. African J. Microbiol. Res. 6, 4993–5004. https://doi.org/10.5897/AJMR12.250.

Mesa, V., Gallego, J.L.R., González-Gil, R., Lauga, B., Sánchez, J., Méndez-García, C., Peláez, A.I., 2017. Bacterial, archaeal, and eukaryotic diversity across distinct

microhabitats in an acid mine drainage. Front. Microbiol. 8, 1–17. https://doi.org/10.3389/fmicb.2017.01756.
Najar, I.N., Sherpa, M.T., Das, S., Thakur, N., 2020a. Bacterial diversity and functional metagenomics expounding the diversity of xenobiotics, stress, defense and CRISPR gene ontology providing eco-efficiency to Himalayan Hot Springs. Funct. Integr. Genomics 20, 479–496. https://doi.org/10.1007/s10142-019-00723-x.
Najar, I.N., Sherpa, M.T., Das, S., Das, S., Thakur, N., 2020b. Diversity analysis and metagenomic insights into the Antibiotic Resistance and Metal Resistances among Himalayan Hot Spring Bacteriobiome- insinuating inherent environmental baseline levels of Antibiotic and Metal tolerance. J. Glob. Antimicrob. Resist. 21, 342–352. https://doi.org/10.1016/j.jgar.2020.03.026.
Najar, I.N., Das, S., Thakur, N., 2020c. Reclassification of Geobacillus galactosidasius and Geobacillus yumthangensis as Parageobacillus galactosidasius comb. nov. and Parageobacillus yumthangensis comb. Nov. respectively. Int. J. Syst. Evol. 70 (12), 6518–6523. https://doi.org/10.1099/ijsem.0.004550.
Najar, I.N., Sherpa, M.T., Das, S., Das, S., Thakur, N., 2018a. Microbial ecology of two hot springs of Sikkim: Predominate population and geochemistry. Sci. Total Environ. 637-638 (1), 730–745. https://doi.org/10.1016/j.scitotenv.2018.05.0, 37.
Najar, I.N., Sherpa, M.T., Das, S., Verma, K., Dubey, V.K., Thakur, N., 2018b. Geobacillus yumthangensis sp. nov., a novel thermophilic bacterium isolated from a north-east Indian hot spring. Int. J. Syst. Evol. 68, 3430–3434. https://doi.org/10.1099/ijsem.0.003002.
Oliveros, J.C., 2015. VENNY. An interactive tool for comparing lists with Venn diagrams. https://bioinfoap.cnb.csic.es/tools/venny/index.html.
Olivier, J., 2011. Thermal and chemical characteristics of hot water springs in the northern part of the Limpopo Province, South Africa. Water SA. 37, 427–436.
Özdemir, S., Kilinc, E., Poli, A., Nicolaus, B., Güven, K., 2009. Biosorption of Cd, Cu, Ni, Mn and Zn from aqueous solutions by thermophilic bacteria, Geobacillus toebii sub. sp. decanicus and Geobacillus thermoleovorans sub.sp. stromboliensis: Equilibrium, kinetic and thermodynamic studies. Chem. Eng. J. 152, 195–206. https://doi.org/10.1016/j.cej.2009.04.041.
Pandey, A., Dhakar, K., Sharma, A., Priti, P., Sati, P., Kumar, B., 2014. Thermophilic bacteria, that tolerate wide temperature and pH range, colonize the Soldhar (95°C) and Ringigad (80°C) hot springs of Uttarakhand, India. Ann. Microbiol. 65 (2).
Papp, D.C., Niţoi, E., 2006. Isotopic composition and origin of mineral and geothermal waters from Tuşnad Băi Spa, Harghita Mountains, Romania. J. Geochemical Explor. 89, 314–317. https://doi.org/10.1016/j.gexplo.2005.12.008.
Pennanen, T., Frostegård, Å.S.A., Fritze, H., 1996. Phospholipid Fatty Acid Composition and Heavy Metal Tolerance of Soil Microbial Communities along Two Heavy Metal-Polluted Gradients in Coniferous Forests. Appl. Environ. Microbiol. 62, 420–428.
Powell, C.M., Roots, S.R., Veevers, J.J., 1988. Pre-breakup continental extension in East Gondwanalan and the early opening of the eastern Indian Ocean. Tectonophysics 155, 261–283.
Powell, T., Cumming, W., 2010. Spreadsheets For Geothermal Water And Gas Geochemistry, in: Proceedings, Thirty-Fifth Workshop on Geothermal Reservoir Engineering. pp. 1–10.
R Core Team, 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org.
Romano, P., Liotta, M., 2020. Using and abusing Giggenbach ternary Na-K-Mg diagram. Chem. Geol. 541, 1–6. https://doi.org/10.1016/j.chemgeo.2020.119577.
Saemundsson, K., 2009. Geothermal systems in global perspective. Geotherm. Syst. 1–11.
Shah, M., Sircar, A., Shaikh, N., Patel, K., Sharma, D., 2019. Comprehensive geochemical /hydrochemical and geo-thermometry analysis of Unai geothermal field, Gujarat, India. Acta Geochim 38, 145–158.
Sharma, A., Paul, D., Dhotre, D., Jani, K., Pandey, A., Shouche, Y.S., 2017. Deep sequencing analysis of bacterial community structure of Soldhar hot spring, India. Microbiology. 86 (1), 136–142.
Sherpa, M.T., Das, S., Thakur, N., 2013. Physicochemical analysis of hot water springs of Sikkim. Recent Res. Sci. Technol. 5, 63–67.
Shikazono, N., 1976. Thermodynamic interpretation of Na-K-Ca geothermometer in the natural water system. Geochem. J. 10, 47–50.
Smith, M., Bisiar, T., Putra, T., Blackwood, V., 2016. Geochemistry of Geothermal Fluids Rico, Colorado. Final Rep. 1–22.
Spear, J.R., Walker, J.J., McCollom, T.M., Pace, N.R., 2005. Hydrogen and bioenergetics in the Yellowstone geothermal ecosystem. Proc. Natl. Acad. Sci. U. S. A. 102, 2555–2560. https://doi.org/10.1073/pnas.0409574102.
Teng, W.C., Fong, K.L., Shenkar, D., Wilson, J.A., Foo, D.C.Y., 2016. Piper diagram–a novel visualisation tool for process design. Chem. Eng. Res. Des. 16, 1–34. https://doi.org/10.1016/j.cherd.2016.06.002.
Trifonov, V.G., Ivanova, T.P., Bachmanov, D.M., 2012. Evolution of the central Alpine-Himalayan belt in the Late Cenozoic. Russ. Geol. Geophys. 53, 221–233. https://doi.org/10.1016/j.rgg.2012.02.001.
Valls, M., De Lorenzo, V., 2002. Exploiting the genetic and biochemical capacities of bacteria for the remediation of heavy metal pollution. FEMS Microbiol. Rev. 26, 327–338. https://doi.org/10.1016/S0168-6445(02)00114-6.
From Walker, G.P.L., 1993. Basaltic-volcano systems. In: Prichard, H.M., Alabaster, T., Harris, N.B.W., Neary, C.R. (Eds.), 1993, Magmat. Process. Plate Tectonics. Geol. Soc. Spec. Publ., pp. 3–38. https://doi.org/10.1144/GSL.SP.1993.076.01.01
Waring, G., 1965. Thermal springs of the United States and other countries of the world - a summary. Geological Survey Professional Paper. U.S. Geological Survey, 604 South Pickett Street, Alexandria, VA, 22.304.
Zangana, M.H.S., 2015. Physical and Chemical Characteristics of Thermal Springs : A Case Study. Int. J. Eng. Trends Technol. 26, 200–206.