Journal of Hazardous Materials Advances 10 (2023) 100316

Contents lists available at ScienceDirect

Journal of Hazardous Materials Advances

journal homepage: www.elsevier.com/locate/hazadv

Biogeochemical networks in the abandoned historical gold mines affecting mobilization and transport of arsenic in Kolar

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ARTICLE INFO

Keywords:
Gold mines
Arsenic
Microbial weathering
Water quality
Geogenic-anthropogenic contaminants

ABSTRACT

Enormous water-logging in ancient abandoned mining shafts of Kolar Gold Fields (KGFs), has largely induced the leaching of sulfide-rich gold minerals contaminating the aquifer system with hazardous elements. Transport of these contaminant has posed threat to the health of the urban population of Kolar township. A detailed survey of borewells, covering radius of 10 km of the KGF was carried out during pre and post-monsoon seasons and various parameters were assessed. Almost 80% of the water samples exceeded the regulatory limits of potable water criteria with excess arsenic (As; 12–127 μg/L), fluoride (F; <0.005 μg/L), dissolved salts (>500 mg/L). Water Quality Index (WQI) was used to understand the overall urban groundwater quality. At the centre of sampling circle ~~core~~, mineral dissolution was found to be the function of pH, induced by acidophilic sulfur oxidizing bacteria . Modelling of predicted microbial metabolic pathways in metagenomics libraries using PICRUSt, indicated complex functional networks. High expression of siderophore proteins (> 2 cm halo in the chrome azurol test) caused Fe-sequestration, secondary Fe-mineral formation and subsequent release of As. Sulfide bearing Au-rich minerals (Arsenopyrite, Scorodite, Jarosite) were bio-weathered leading to release of $H_3AsO_3^+$ at low pH, resulted in groundwater composition of Ca–HCO_3 type and Ca–Na–HCO_3 or Ca–Mg–Cl type.

1. Introduction

The bulk of the geogenic arsenic (As) that has been reported in contaminated aquifers across the globe, are linked to the Late Quaternary-Holocene fluvio-deltaic riverine deposits (Bhattacharya et al., 2002; Smedley and Kinniburgh, 2002; Biswas et al., 2012; Ghosh and Donselaar, 2023). Such As sources are closely linked with the oxido-reductive environment and ionic states of iron (Fe) or manganese (Mn) oxides. These contaminated regions named as As-hot spots are widely studied for their geochemistry, depositional environments, biological roles and socio-economic effects (Bhattacharya et al., 1997; 2001; Chakraborti et al., 2016). However, As is also found abundantly associated with sulfide mineral sources, majorly found in mining regions. The contaminant transport from historically-active abandoned-gold-mines, altering the fluxes of hazardous elements in the groundwater has been faced in many corners of the world (Drewniak et al., 2010). Environmental survey and regulation of mining sites has been practised for more than four decades and are been treated as industrial wastes in many countries (Ferronato and Torretta, 2019). Arsenic apart from being carcinogenic, is highly mobile in highly acidic and highly basic conditions, leading to a point of concern to the gold mines across the globe (Straskraba and Moran, 1990; Drewniak et al., 2010). The mining of sulfide ore deposits, accelerate the oxidation of As-bearing minerals resulting in pH fall. This condition is more drastic in ore deposits without any carbonate rocks. The discharge of such contaminated water from abandoned mines may lead to percolation into groundwater systems (Straskraba and Moran, 1990; Drewniak et al., 2010).

Kolar district of Karnataka is one of the well identified cratons with gold mines are located in the archean greenstone belt of Dharwar (Kunugiza et al., 1996). The Kolar Gold Fields lies in the SE extremity of the Karnataka state, located on a 2700 million year old narrow strip of schists (6 km long and 4 km wide) exposed in Kolar region (Rao and Reddy, 2006) and were active during 1900s and were highly explored by the British who colonized in India (Rice, 1994). Nearly 800 tons of gold has been mined from 50 million tons of ore averaging 16 g of gold/ tons in the past 120 years (Rao and Reddy, 2006). Such enormous quantity of earth extraction has resulted in the ~~concentration~~ accumulation of several elements (geogenic, anthropogenic, carcinogenic, and heavy metals) from the ore and gangue in and around of Kolar Gold Field (KGF). Around 32 million tons of gold tailings has been generated from the gold mining process (Rao and Reddy, 2006). Of several mine tailing dump sites, only the Kennedy's Line dump was functional before

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https://doi.org/10.1016/j.hazadv.2023.100316
Received 18 January 2023; Received in revised form 1 May 2023; Accepted 7 May 2023

closure of Kolar Gold Fields in the year 2000 (Rao and Reddy, 2006). The Kennedy's Line dump have received enormous amount of sulphide bearing tailings as slurry that contains spent ore, water bearing, and water-soluble alkali metal cyanide. Further, the pyrite minerals in these line dumps make them susceptible to acid mine drainage (AMD) (Equation 1–3; Rao and Reddy, 2006; Tabelin et al., 2019; 2020). Since there is no rehabilitation or attenuation of As-rich wastes, many incidences of As related health conditions has been reported (Chakraborti et al., 2013; Chikkanna et al., 2019).

In KGF, As is a frequently found as: arsenides, sulfides, and sulfosalts. The anoxic-reducing conditions in the 75 mining shafts with ~ 5000 ML (mega litres) of water-logged, resulted in weathering of these minerals, lowering of pH and mobilization of As and other elements (Bhattacharya and Loadh, 2018). The chemistry and presence of As in water is complex as significantly affected by the bio-chemical and geo-chemical reactions, impacting form and solubilisation of different species. These sulphides/ acidic environments are ideal places for several iron- and/or sulfur-oxidizing bacteria microbes to thrive in, breaking down Au-hosting sulfide minerals ~~and~~ while release associated Au (Hutchins et al. 1986; Gadd, 2004). ~~Their~~ Such microbial activities in these tailings can significantly influence the pH, redox ~~potential,~~ condition and the ionic strength of the soil and these tailings (Haferburg and Kothe, 2007). One such example is pyrite oxidation by the bacterial genus *Acidithiobacillus*, resulting in ~~strong decline~~ lowering of pH (Haferburg and Kothe, 2007). However, the extent to which pH can be reduced by these microbes and the magnitude of such activity is poorly understood (Haferburg and Kothe, 2007). Only a few studies related to microbial activity affecting pH is known from some parts of the world (Hogland et al., 2021). A combined effect of mining activities, water infiltration, pyrite concentration, acid drainage and microbial ~~activities~~ metabolism has contributed to accumulation of extremely high concentrations of geogenic, anthropogenic contaminants into the groundwater.

$$FeS_2(s) + 3.5\ O_2(aq) + H_2O \rightarrow Fe_2{}^{+} + 2H^{+} + 2SO_4{}^{2-} \quad (1)$$

$$Fe^{2+} + 0.25\ O_2(aq) + H^{+} \rightarrow Fe^{3+} + 0.5\ H_2O \quad (2)$$

$$FeS_2(s) + 14\ Fe^{3+} + 8H_2O \rightarrow 15\ Fe^{2+} + 16H^{+} + 2SO_4{}^{2-} \quad (3)$$

The contaminants such as cyanides, arsenites and phosphates from the mines are transported to the Kolar township (Kozhisseri, 2008). The first report of arsenicosis in this region came from Kiradalli Tanda village was July 2009, in a joint study conducted by UNICEF and Government of Karnataka (GOK 2008; GOK-UNICEF, 2010) and later several villages were also reported with well water supplies containing As above the permissible limit. The abandoned mining sites ~~activities~~ had not only affected the socio-economic condition, but also the health of the regional population. Later in a human biomarker study from this region showed a 100% prevalence of As in the human samples collected from the population of Mangalur greenstone belt (Chakraborti et al., 2013; 2016). The occurrence of dermatologic symptoms for arsenicosis is frequently observed in this belt (Chakraborti et al., 2013). However, there is a significant gap in the literature on the source of As-contamination, and mitigation steps for the problem is almost none. A large human population which is getting affected, ~~and~~ requires scientific attention to attract Government bodies and stakeholders to collaborate to provide safe drinking water. Thus the present study aims for i) mapping of contaminant source and transport dynamics across the urban areas of Kolar township; ii) developing the microbial metabolism network in the abandoned Gold mines; iii) assessing the influence of the microbial process on regional aquatic geochemistry.

1.1. Study area

The present study is conducted in the nearby regions (within 10 km radius; Fig. 1) of Kolar Gold Fields, located in the Kolar district of Andhra Pradesh. The current population size (yr. 2023) of the Kolar township is around 1750,268 (https://www.indiacensus.net/district/kolar). The township is bounded within latitudes N 12°93.1′ to N 12°99.9′ and longitudes E 78°23.7′ to E 78°29.4′, covering an area of almost 48 km^2. The Kolar Gold Field lies nearly at the core of the sampling site. The major rocks of the study area are gneisses, schists, amphibolite, and Banded Iron Formation (BIF). The gold mineralization is confined in the quartzite veins of the Kolar schist belt. A total of 23 sampling stations were identified and groundwater samples were collected over two seasons from the study area. The spatial distribution of the sampling stations is displayed in Fig. 1. The aquifers of this region are recharged through rainwater, and through network of joints and fractures (Jal Nirmal Project, 2004). The water transmittivity of the rock formations in this region ranges in between 2 and 1935 m^2/day (Jal Nirmal Project Report, 2004; DMG and CGWB, 2005). We observed a few cases of arsenicosis related skin lesion, however, the documentation of those lack any medical report to support the observation. All the water samples had a low biological oxygen demand (BOD) and most probable number (MPN) indicating low/no sewage or faecal contamination.

2. Material and methods

2.1. Water sampling and laboratory analysis

A field study was conducted within 10 km radius of Kolar Gold Fields. Water sampling was carried out during pre-monsoon (Mar-Apr 2018) and post-monsoon (Aug-Sept 2018). A total of 23 pre-installed borewells [Central KGF (n = 8); Chaitanyahalli (NE of KGF; n = 6); Kammasandra (NW of KGF n = 3); Andersonpet (S of KGF; n = 6)], were targeted covering a radius of 10 Kms from KGF (Fig. 1). Prior to sample collection, three times the volume of pipe volume water is extracted to mitigate the contamination and rusting effects of pipe on collected water sample. The water samples are collected in the polypropylene bottles, rinsed three times with de-ionized water and again rinsed two times with the sampling water to avoid any contamination. The physio-chemical parameters such as, pH, TDS, temperature (T), electrical conductivity (EC) and redox potential (Eh) were measured in situ (Hanna HI9810–6). Major anions were analysed by colorimetric analyses and the cations were quantified using inductively coupled plasma-optical emission spectrometry (ICP-OES;Avio-200, Perkin Elmer). Cyanide was quantified by colorimetric method and Biochemical Oxygen Demand (BOD) was measured directly by BOD digital incubator (Hanna HI 98,193). The Chemical Oxygen Demand (COD) was measured by Spectroquant-TR320 (Merck, Germany), and the coliform load was estimated using Most probable number test (MPN) (Oblinger et al., 1975). The ArcGIS software was used to create a spatial heatmap across the study field. The Water Quality Index (WQI) (Eq. (4)) was used to expresses overall water quality with a single digit, based on several water quality parameters (Alobaidy et al., 2010).

$$WQI = \sum_{i=1}^{I} Q_i W_i / \sum_{i=1}^{i} W_i \quad (4)$$

where, W_i is unit weight of water quality and Q_i subject index or quality rating.

2.2. Statistical analysis

The principal component analysis (PCA) was performed in R (Pinheiro et al., 2017). Each principal component is unrelated and obtained by the linear combination of the original variables. Variable loadings are defined by the orthogonal projection of the variables on each

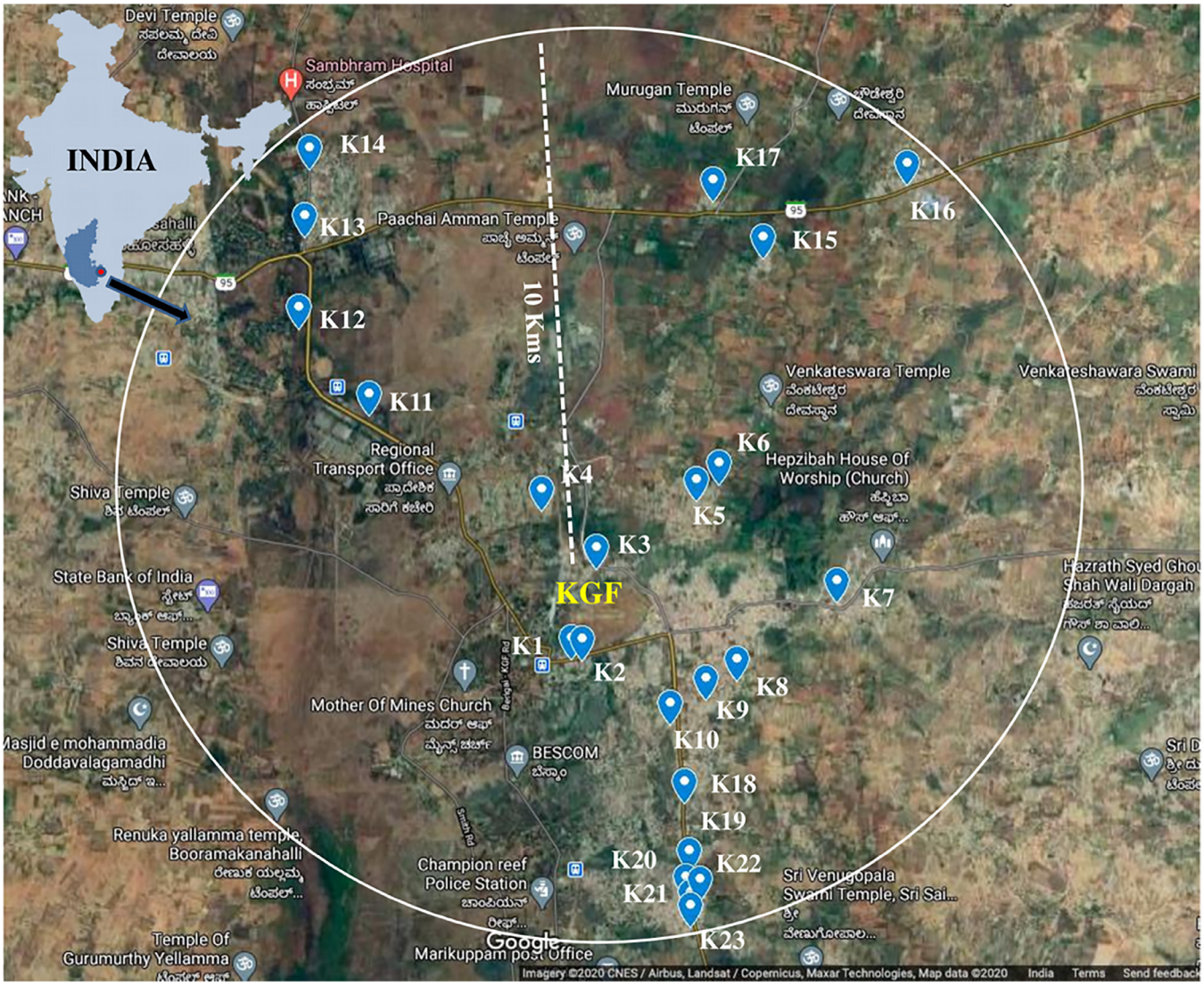

Fig. 1. Google map image of Kolar, indicating the sampling stations K1 to K23.

of the component. Each component explains certain percentage of statistical variance in the analysed data and can be explained based on the variables. The components are selected based on the eigen values (eigen value > 1) and the cumulative percentage of the variance explained.

2.3. Mineral and microbiome study for functional analysis

For the microbial community profiling, the biomass samples were collected from station K1 and K2 subdivided as mining drainage (Mdr), mining dump (Mdu), tailings (T) and biofilm on rock aggregates (BF). All the samples were stored at −20°C and 0.25 to 0.5 g of subsample was used for environmental DNA extraction using DNeasy PowerSoil Kit (Qiagen), following the manufacturer's protocol and a homogenized subsample was ground to <50 μm and used to prepare pressed powders for X-ray diffraction (XRD; Rigaku, Smart lab powder X-Ray Diffractometer) analysis following Chikkanna et al. (2021). The source used during analysis was CuKα radiation, acceleration voltage of 40 kV/30 mA with D/Tex Ultra detector. The isolated environmental DNA was quantified in a Nanodrop 2000 (Thermo Fischer) and the integrity of the sample was detected by 1% agarose gel electrophoresis. About 25 ng of the environmental DNA samples were used for amplifying the V3-V4 region of the 16S rDNA with primers - Pro341F- 5′ CCTACGGGNBGCASCAG; Pro805R- 5 GACTACNVGGGTATCTATCC [4]; V 3-V4 (464 bp). After v3-v4 amplification, the libraries are prepared using NEBNext Ultra DNA Library Prep Kit (e7370) (Gloor et al., 2010).

The NEBNext Illumina adapters used during sequencing on the HiSeq: 5´-/5Phos/GAT CGG AAG AGC ACA CGT CTG AAC TCC AGT CUA CAC TCT TTC CCT ACA CGA CGC TCT TCC GAT C*T-3´, to generate amplicons of ~530 bp. In the next round of amplification (indexing PCR) the Illumina sequencing adapters and dual indexing barcodes are added. The generated metagenomic libraries were cleaned using SPRI select beads and AMPure XP beads (Beckman Coulter) quantified and validated for quality by running an aliquot on High Sensitivity Bioanalyzer Chip (Agilent). Finally, the cleaned libraries were sequenced in Illumina HiSeq 2500 platform (Illumina, USA) at AgriGenome Labs Private Limited, Cochin, India.

The Illumina HiSeq paired-end raw reads were checked for quality using the FastQC tool (Andrews et al., 2010), and the QIIME pipeline (Version: 1.9.1; Caporaso et al., 2010) was used for the selection of 16S RNA, clustering, and OTU picking followed by taxonomic classification based on the SILVA database and statistical analysis. The de-novo chimera removal method UCHIME was implemented in the tool VSEARCH to remove chimeras. The Uclust program was used to pool and cluster the reads into Operational Taxonomic Units (OTUs) based on their sequence similarity (cutoff = 0.97). A representative sequence was identified for each OTU and aligned against the SILVA core set of

sequences using the PyNAST program (Caporaso et al., 2010). The representative sequences of the OTUs were also used to predict KEGG orthodoxy (KO) abundances using PICRUSt2 (Langille et al., 2013) and microbial pathways were inferred. The raw sequencing data will be submitted to the NCBI Sequence Read Archive.

The eight metagenomic library datasets from four different sample type (Mdr, Mdu, T, BF) were clustered based on their arithmetic mean of weighted Unifrac distance using Unweighted Pair Group Method (UPGMA). Using the 25 predominant and common bacterial 16S rRNA sequences in all libraries, an unrooted neighbor Joining (NJ) tree was build using software MEGA X version 10.1.

2.4. Characterization of arsenic metabolizing microbial groups

2.4.1. Isolation of arsenic-tolerant bacteria

Green-yellow biofilms on rock/aggregates on mining tail and on drainage from dumped tails were collected and released in sterile 50 ml normal saline (0.9% NaCl) and stored at 4 °C, then transported to the laboratory. For enrichment of As-oxidizing aerobic chemolithotrophs, MSM media with 10 mM $NaAsO_2$ (sodium meta arsenite), and for anaerobes the media was supplemented with, a) 5 mM $NaAsO_2$ and 5 mM $NaC_3H_5O_3$ (sodium lactate) and b) 5 mM NaH_2AsO_4 (sodium dihydrogen arsenate) and 5 mM $NaC_3H_5O_3$ and were placed in anaerobic chamber. The pH of the media in all setups was 6.8 and all the plates were incubated at room temperature (~25 °C) for 10 days.

2.4.2. Assay for arsenic metabolizing processes

To understand the arsenic metabolizing potential of the bacterial isolates through their respiratory processes, their arsenite oxidase and arsenate reductase enzyme activity were tested. Additional screening plates with isolated bacterial strains grown in above condition were used for the assays. After 10 days of incubation in specified conditions, the plates were flooded with 0.1 M $AgNO_3$ solution. In $NaAsO_2$ containing plates, the $AgNO_3$ reacts to form Ag_3AsO_3 (silver arsenite) in yellow, however around the colonies, a brownish precipitate represents arsenite oxidase activity. Similarly, amongst the arsenate reducers the vice versa was observed.

2.4.3. Assay for siderophore production

The siderophore expression potential of the bacterial isolates were detected using the modified chrome azurol S (CAS) agar plates (Schwyn and Neilands, 1987). The C-source used was yeast extract (0.04%) instead of glucose. The culture plates were incubated for 5 days in dark at 22 °C and the siderophore expression was detected with the formation of halos around the colonies and their respective diameter.

2.4.4. Assay for minimum inhibitory concentration (MIC)

Since the mining environment is rich in toxic chemicals and hazardous elements, the bacterial strains were presumed to tolerate these elements at elevated levels. A minimum inhibitory concentration (MIC) test was carried out to detect the tolerance bacterial strains to the elements As, Mn, Cu, Ni, Cd, Pb, Zn. The isolates were grown in a series of MSM media (see above) supplemented with elemental salts of concentrations 0 to 20 mM/L, increasing in the order of 1 mM in each triplicate set for each isolate. Since all the strains had robust As-tolerance genes, the MIC for As(III) and As(V) were tested from 0 to 50 mM and 0 to 300 mM/L respectively, with an increase of 10 mM/L. Incubation conditions are described in section 2.5.1.

2.5. Molecular characterization

2.5.1. 16S rRNA gene amplification and sequencing

For molecular characterization of the 25 mine bacterial isolates, their genomic DNA was extracted (Chikanna et al., 2021) and the 16S rRNA gene was amplified with polymerase chain reaction (PCR) using the eubacterial primers Fc27 (5′-AGA GTT TGA TCC TGG CTC AG-3′) and Rc1492 (5′-TAC GGC TAC CTT GTT ACG ACT T-3′; Lane, 1991). The reaction cocktail was prepared, and the amplified products were sequenced following Chikkanna et al., 2018. The sequences were manually checked for chimeras in Bellerophon 3.0 (Lingerfelt et al., 2017) and submitted to GenBank database (accession number MW362189-MW362213). The phylogenetic representation of the 16S rRNA gene sequences of the isolates was done with an unrooted tree using Neighbour Joining method based on Kimura 2 parameter model (Kimura, 1980) was used to construct the phylogenetic tree in MEGA version 10.0 (X) (Stecher et al., 2020).

2.5.2. Arsenic metabolizing genes

To understand the arsenic metabolism by the isolates, the presence of the functional genes encoding key subunits of arsenite oxidase and arsenate reductase were amplified and sequenced. The arsenite oxidase activity was detected using the pre-designed universal primers *aioA*F (5′ CCA CTT CTG CAT CGT GGG 3′) and *aioA*R (5′ TGT CGT TGC CCC AGA TGA 3′; Ghosh et al., 2014), to amplifying the gene *aioA* encoding the larger subunit of the enzyme arsenite oxidase. To detect arsenate reductase activity the gene encoding the small subunit of arsenate reductase *arsC* was amplified were using primers amlt-42f (5′ TCG CGT AAT ACG CTG GAG AT 3′) and amlt-376r (5′ ACT TTC TCG CCG TCT TCC TT 3′; Sun et al., 2004). The *aioA* gene of *Acidovorax* sp. stain BKP_SS9 and *arsC* gene of *Burkholderia* sp. strain BKP_SS42 (GenBank Acc. No. MW383785 and MW383818 from Ghosh et al., 2021) were used as positive controls.

3. Results and discussion

3.1. Spatial exploration of water quality parameters with local geological settings

The As heat map (Fig. 2) shows contaminant site and distribution. Increase in As load during dry season was observed, which reduces drastically during post monsoon due to dilution effect. Most of the water samples (94%) had a WQI > 75- 100, indicating a very poor quality, and 6% of the samples were between 50 and 75 indicating poor quality.

3.2. Groundwater As geochemistry

Arsenic shows wide spatio-temporal variation in natural water ranging from <30 μg/l to greater than 150 μg/l (Fig. 3; Table S2). Groundwater samples were collected from shallow borewells during pre-monsoon (Mar-Apr 2018) and post-monsoon (Aug-Sept 2018) within 10 km of KGF mines (near Kennedy Line dump). All samples collected across each season is well above the WHO recommended safety limit of 10 μg/l of As and almost 90% of the samples having higher than Indian safety standard for Arsenic (50 μg/l). This fact alone reveals the how dreadful and alarming the situation is and an urgent need to address the issue. Highest concentration of As (163 μg/l) was measured at K5 a nearby urban area (< 1 km from the tailings) during dry season. Thus, understanding the transport and distribution of Arsenic is of greater significance in assessing the impact to both human and environment.

Groundwater samples with little anthropogenic contamination from Kolar District (Bagepalli and Gudibanda Taluks) primarily belong to Ca-HCO_3 type and the rest belongs to Ca-Na-HCO_3 or Ca-Mg-Cl type (Mamatha and Rao, 2010). Additional anthropogenic inputs such as Na, Cl, Ca, and Mg can shift the inherent groundwater geochemistry to Ca-Mg-Cl/Ca-Mg-Na-Cl type (Rao et al., 2013). Higher fluoride content (0.36–3.34 mg/L) in groundwater samples with almost 75% of the samples above permissible limit for fluoride from northern regions in Kolar district (Bagepalli and Gudibanda) is attributed to enhanced fluoride dissolution favoured by calcite precipitation (Mamatha and Rao, 2010). But our samples showed lower fluoride concentration in groundwater compared to previous studies from Kolar district and no correlation was observed between fluoride and calcium hardness. As shows significant positive correlation only with pH during the dry season, indicating pH

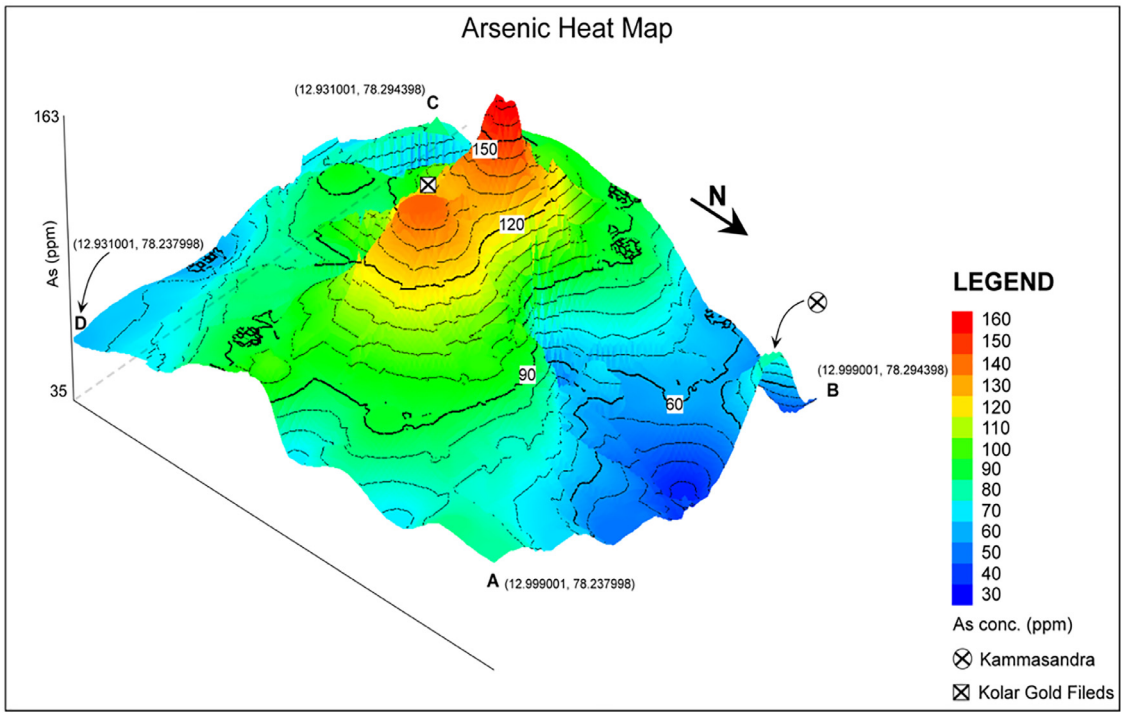

Fig. 2. The topological map showing the distribution of As in the studied region. The high As in the central mining field is transported across the township along the groundwater.

dependant (Fig. 3;) desorption of As at high pH (Chen et al., 2014; Podgorski et al., 2017). From the hydrogeochemical facies, it can be concluded that the dissolution of sulfide bearing Au-rich minerals, releasing $H_3AsO_3^+$ at low pH is transported through facilitating rock weathering, results in groundwater composition of Ca-HCO_3 type and Ca-Na-HCO_3 or Ca-Mg-Cl type (Fig. 4). Rao et al., (2013) reported significant sewage leaching into the aquifer resulting in above permissible limit of nitrate in majority of the groundwater samples from Mulbagal taluk in Kolar district. Present study indicates no nitrate contamination but shows positive correlation between nitrate and BOD during both season indicating microbial process controlling the nitrate concentration in groundwater.

3.3. Statistical analyses

The PCA results of 16 geochemical parameters for 23 groundwater collected during each season from KGF are presented in Table S2 (data shown in bold indicates high loading for each principal component). Four principal components with eigen value >1 (PCD1, PCD2, PCD3, PCD4) were found in the groundwater samples from dry season which could explain 80% of the variability in the data. Similarly, four principal components (PCM1, PCM2, PCM3, PCM4) were able to explain 25% of the variability in the post monsoon data set. The As concentration is influenced mainly by the PCD1, PCD2 during dry season and PCM2 during the post monsoon.

PCD1, which explains 33% of the total variance was mainly influenced by turbidity, BOD, DO, nitrate (NO_3^-), sulphate (SO_4^{2-}), Na and As (Table S3). This component is an indicative of organic matter decomposition and mineral weathering in aquifer. Higher turbidity indicates increased intensity of light scattering primarily caused by the presence of clay, silt, tiny inorganic and organic matter present in groundwater. The organic matter present in the aquifer is decomposed/stabilized by microbes and biodegradation of organic matter can lead to As mobilization through reduction of Fe-oxyhydroxides (Ravencroft et al., 2001). Nitrate in groundwater is anthropogenic in origin, primarily from agricultural fertilizers and untreated sewage (Rao and Reddy, 2006) resulting in high BOD of groundwater. This component indicates that in shallow aquifers of KGF during dry season, As mobilization with biodegradation of organic matter is a key process determining As concentration. The increased dissolve organic carbon (DOC) as a product of biodegradation enhances the desorption of As from the binding sites (Bauer and Blodau, 2006) On the other hand, PCM1 has high negative loadings for turbidity, alkalinity, total hardness, Mg hardness, TDS, EC, BOD, DO, nitrate (NO_3^-), sulphate (SO_4^{2-}) and Na. High loading in TDS and EC is due to dissolved ions from mineral weathering and rock water interactions such as Na, Ca, Mg, sulphate, nitrates and bicarbonates. Oxidation of the sulphide ore and the subsequent leaching can lead to sulphate in groundwater along with the dissolution of gypsum and anhydrite (Fig. 5) (Patil and Patil, 2010). The absence of As in the PCM1 and also consistently low As concentration in groundwater samples collected during post monsoon (Fig. 3b; Table S3b) indicates dilution effect but still concentration lies above the permissible limit of 10 μg/l given by WHO (Table S1). PCD2 represent 26% of the variability in data and was correlated with Alkalinity, total hardness (TH), Ca hardness, TDS, EC, and As. The component primarily indicates the mineral weathering during post monsoon. Presence of dissolved Ca enhances the sorption of As to metal oxides while bicarbonates can competitively inhibit the process (Appelo et al., 2002; Smith et al., 2003). PCM2 contributed to 15% of the total variance and had high loading for pH, alkalinity, As and F. The pH has a significant impact in sorption process, and under neutral pH and oxic conditions As is effectively immobilized by sorption/coprecipitation with metal oxides (Smedley and Kinniburgh, 2002). Weathering of granite and gneiss containing fluorite mineral controls the fluoride concentration in Karnataka groundwater (Mamatha and Rao, 2010). Unlike the previous study from the same Kolar, all the samples from current study shows very low concentration of fluoride in groundwater, however, the high fluoride concentration in Karnataka groundwater to be driven by calcite precipitation increasing the soluble fluoride. PCD3 (12% of the total variance) also had high positive loadings for Ca hardness and fluoride concentration. Fluoride in groundwater is mainly due water-rock interaction leading, possibly due to dissolution of CaF_2. Groundwater chemistry of fluoride mainly depends on fluorite and calcite saturation, and the fluoride concentra-

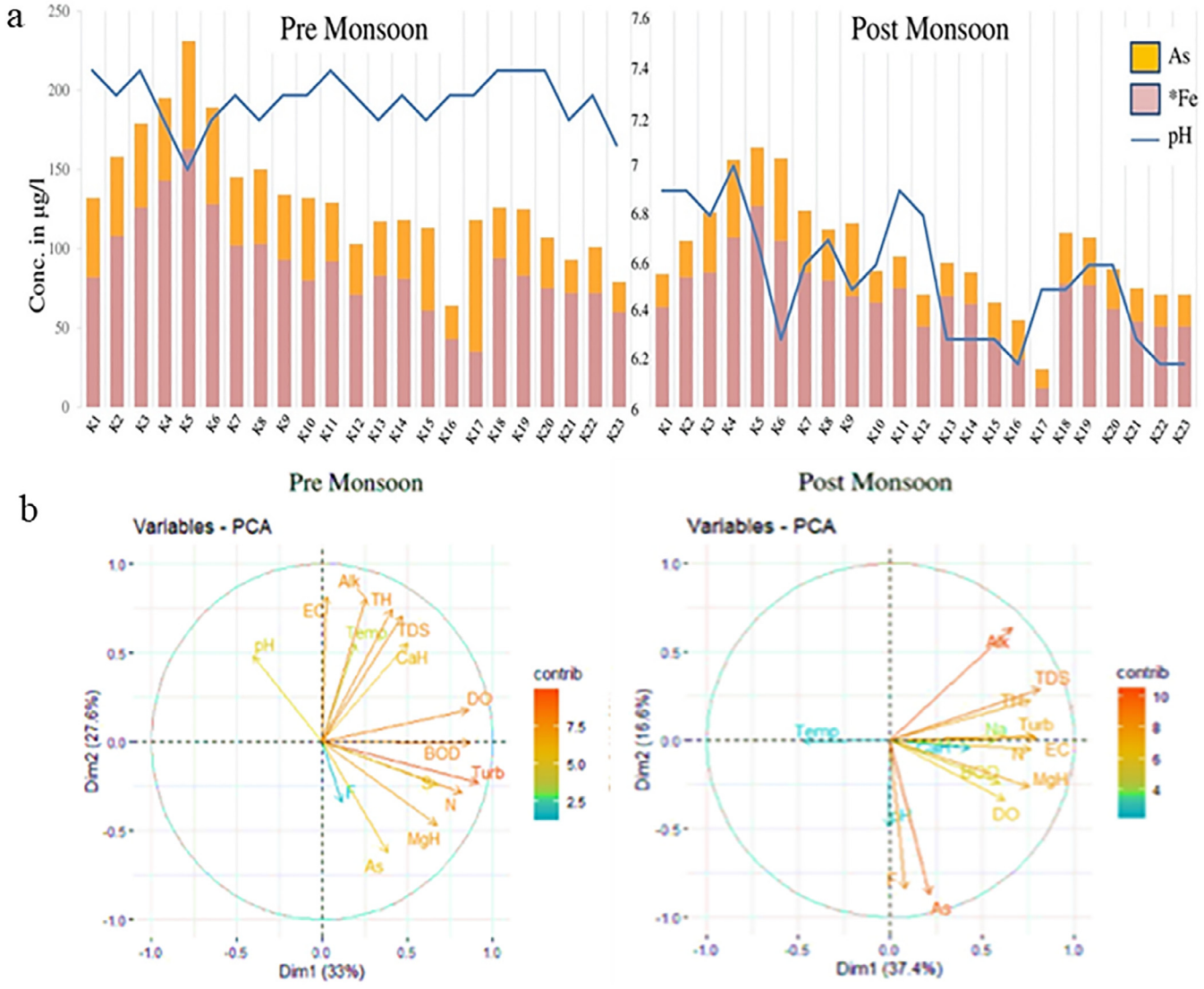

Fig. 3. (a) Alteration in As and Fe concentration of the 23 groundwater samples collected during pre-monsoon and post monsoon seasons along with change in pH [*Concentration of Fe is 10X], (b) The PCA of the correlation between physicochemical parameters and elemental fluxes during pre and post monsoon sampling seasons. Plots highlight the association of toxic elements with geo- chemical parameters such as pH.

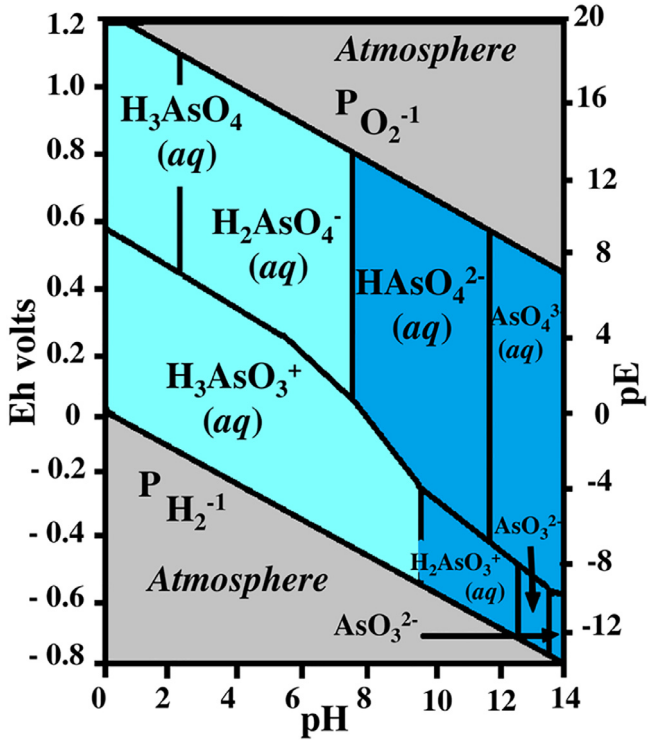

Fig. 4. The Eh-pH diagram of As speciation from ore to aqueous phase using PhreePlot. The arrow indicates possible evolution of the speciation in Kolar groundwaters.

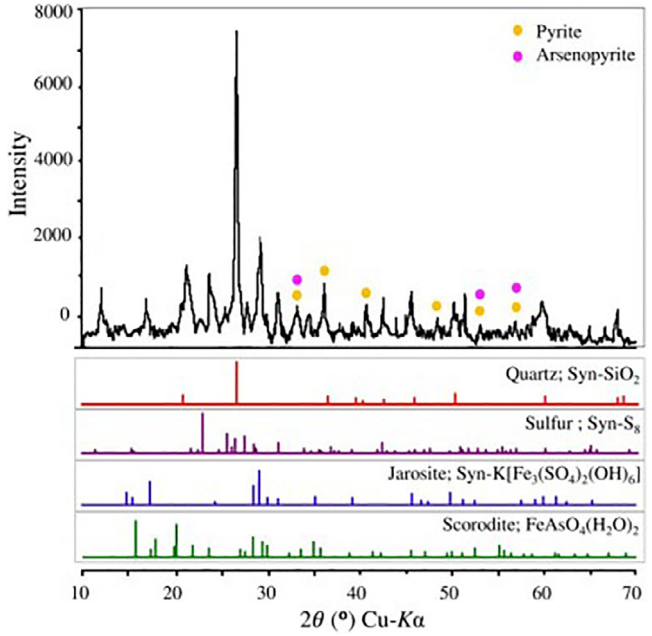

Fig. 5. X-ray diffractogram of the mining tail sample indicating primary and secondary minerals. .

tion is inversely proportional to dissolved Ca and directly proportional to the bicarbonate ion concentration (Gaciri and Davies, 1993).

3.4. Microbial metabolism of As-bearing gold mine minerals

In the above sections the pH of the water samples is suggested to be a key function controlling elemental fluxes (Fig. 4, 6). Hence based on the UPGMA clustering of the metagenomic libraries (Fig. 6a) of the source samples were divided into two clear groups: a) the highly acidic zones (HAZ) and b) the less acidic zones (LAZ) (Fig. 6b). The HAZ samples (pH 2.2 to 4.5) were collected from the channels connecting deep water logged anoxic environments, which include the samples under the nomenclature MDr and MDu, where sulfate bearing minerals such as Jarosite are abundant (Gadd, 2004; Gao et al., 2019). Based on the PICRUSt modelling of the studied libraries, the sulfur reducing bacterial (SRB) groups like *Desulfovibrio, Pseudomonas, Desulfotomaculum, Desulfomicrobium* (< 0.5%) detected were inferred to release the mineral bound S. The predominant sulfur oxidizing bacterial (SOOB) genera such as *Thiobacillus, Acidithiobacillus, Beggiatoa, Acidovorax, Sulfolobus, Thiomonas, Thiothrix, Sulfobacillus* and *Acidomonas* are involved in thiosulfate oxidation and Fe^{2+} oxidation. This results in formation of oxidised species (K^+, Fe^{3+}, SO_4^{2-}) forming Jarosites (XRD analysis; Fig. 5). The depleation of Fe^{2+} results in competition for ions and excretion of siderophore proteins in the extracellular environment. To uptake Fe^{3+} from insoluble minerals as Arsenopyrite and Scorodite (XRD analysis), the key players *Delftia, Leptospirillum, Hydrogenophaga, Chromobacterium* come into action. This metabolic process couples with the dissolution and mobilization of $As^{3+}$from these minerals (Fig. 6), subsequently leading to As adsorption by secondary minerals like Fe(oxy)hydroxides/oxides (Fig. 5).

The LAZ samples were collected from the mining tails connected to the HAZ outlets, and biofilms growing in these oxic environments, which include the samples under the nomenclature T and BF. Interestingly, these samples had higher pH (5.5 to 6.9) and a contrasting microbial community structure. The PICRUSt modelling suggest that As mobility is higher due to less acidic conditions and a higher abundance of dissimilatory arsenate reducers (DAR) such as *Rhodopseudomonas, Achromobacter, Desulforhopalus, Desulfovibrio, Bacillus* and *Pseudomonas* was observed. This lead to the formation of secondary Fe(oxy)hydroxide minerals, also indicating an abundant siderophore-derived biomineralization (XRD analysis; Fig. 5, 7). A few groups of arsenate oxidizing bacteria (AOB) such as *Hydrogenophaga, Acidovorax* are present along with dissimilatory iron oxidizing (DIO)– -siderophore-producers like *Paracoccus, Achromobacter, Paenibacillus* also caused precipitation of As as ferric arsenate, scorodite, or Fe/Al arsenate phase (Fig. 7) (Tabelin et al., 2020). The abundance of exopolysaccharide (EPS) producing groups such as *Zoogloea, Rhodopseudomonas, Rhizobium, Sinorhizobium* facilitates the formation of secondary minerals.

To confirm our observations from the metagenomic study, the isolates were screened for their As-metabolic activity and only those involved in As(V) reduction were selected for downstream analysis. Interestingly all the isolates showed variable results in triplicate experiments and altered incubating condition, and found to be both DARs and AOBs (Table S4). This was inferred by the presence of *arsC* gene (small subunit of arsenate reductase) and *aioA* gene (large subunit of arsenite oxidase enzyme) in all isolates. The As-metabolizing activity in these microorganisms subsequently leads to release of As from mining ores which contains a mixture minerals such as arsenopyrite — FeAsS, Loellingite — $FeAs_2$, traces of scorodite — $FeAsO_4{\cdot}2H_2O$ and origin ore (Fig. 8). The common EPS producing bacteria like *Pseudomonas* spp., along with the quorum sensing induced auto-aggregating bacteria like *Rhodobacter* spp. that can contribute to establish and stabilize the biofilms providing phototrophically reduced organic carbon to other heterotrophic (Puskas et al. 1997; Ayarza et al., 2014). All these indigenous mine bacterial strains show a high resistance potential against other heavy metals as well.

All the bacterial isolates expressed siderophores, indicating an efficient metabolic uptake of metals such as Fe^{2+}, As^{5+},Cu^{2+}, Ni^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+}, Hg^{2+}, Ag^{2+} and also Au^{2+}. The predominance of *Delftia* sp. amongst the studied isolates, forming the clear halo > 2 cm in the chrome azurol test plates, suggests the expression of the siderophore-delftibactin a non-ribosomal peptide (NRP) known to detoxify gold in the genus *Delftia* spp (Johnston et al., 2013).

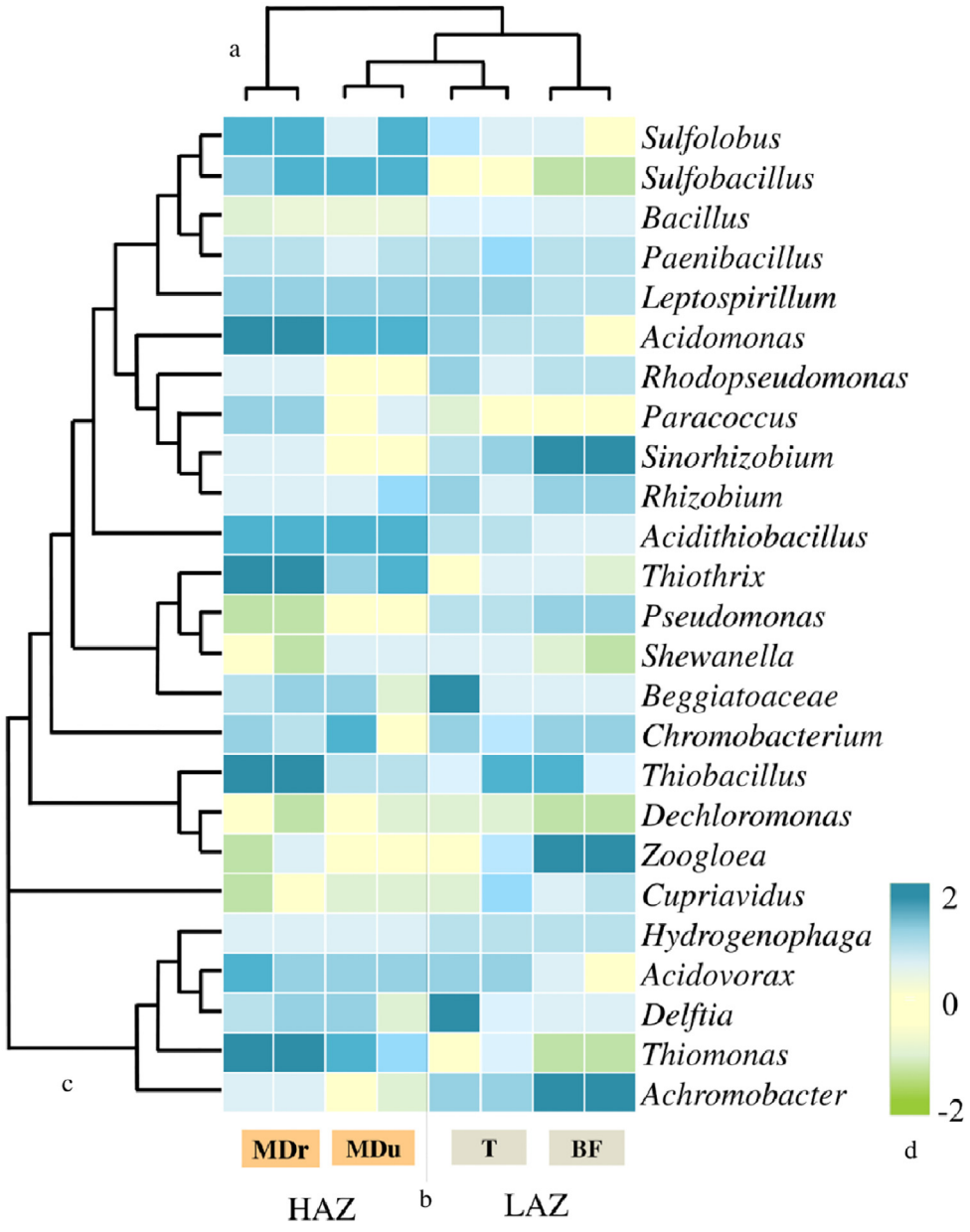

Fig. 6. (a) The samples are clustered based on their Weighted Unifrac distance calculated in a UPGMA cluster tree and (b) further based on their acidic condition into HAZ and LAZ, (c) The common predominant 25 bacterial genera in all eight metagenomic libraries were used to generate taxonomic heatmap and understand their distribution in different conditions, (d) The colour gradient indicates the distance between the raw score and the mean of the standard deviation.

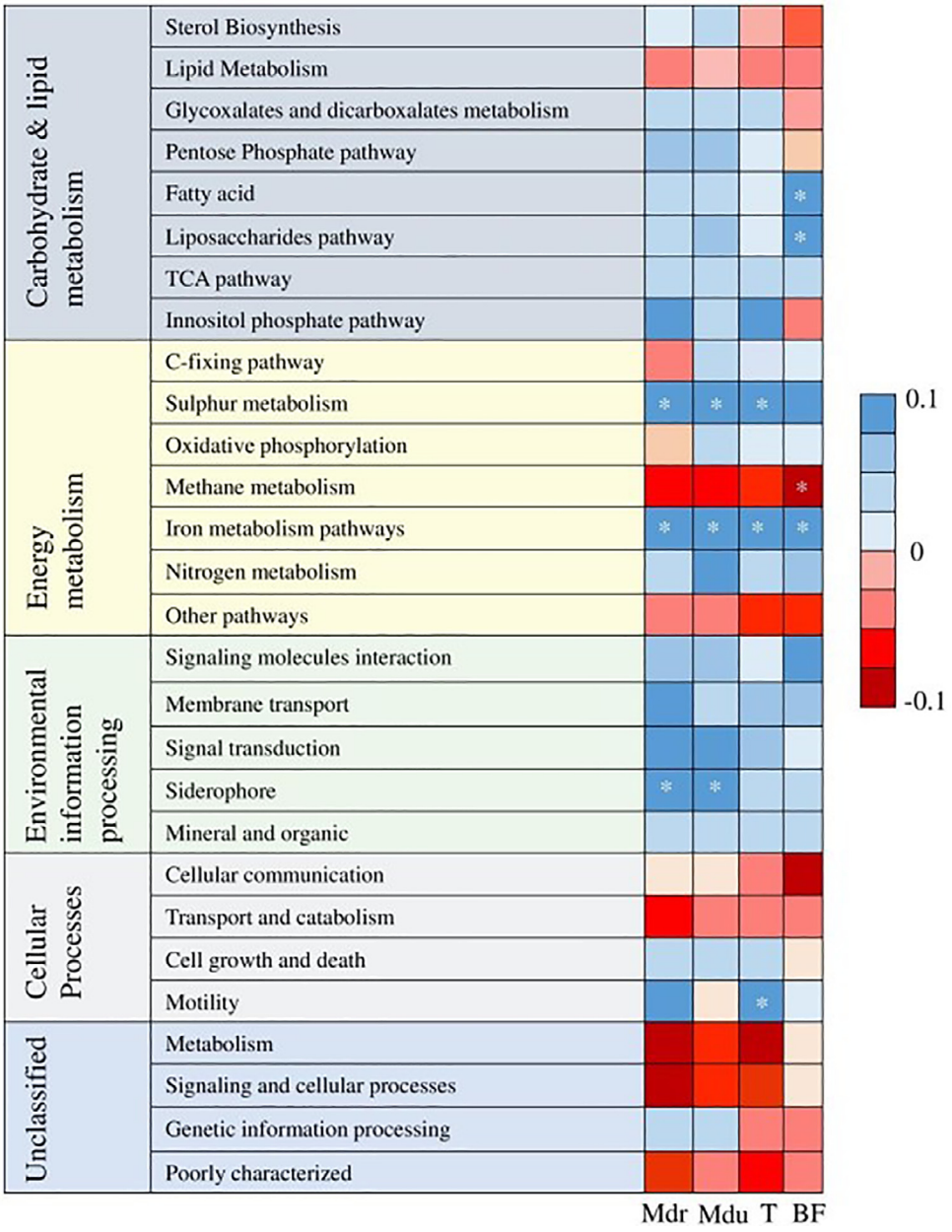

Fig. 7. Microbial PICRUSt-predicted KEGG functions relevant to metabolism in the four (in duplicates) metagenomic library datasets Mdr; Mdu; T and BF. The heatmap denotes association between each microbial PICRUSt-predicted KEGG function with iron- arsenic metabolism based on the scale given on the right. The significant associations are highlighted with '*'.

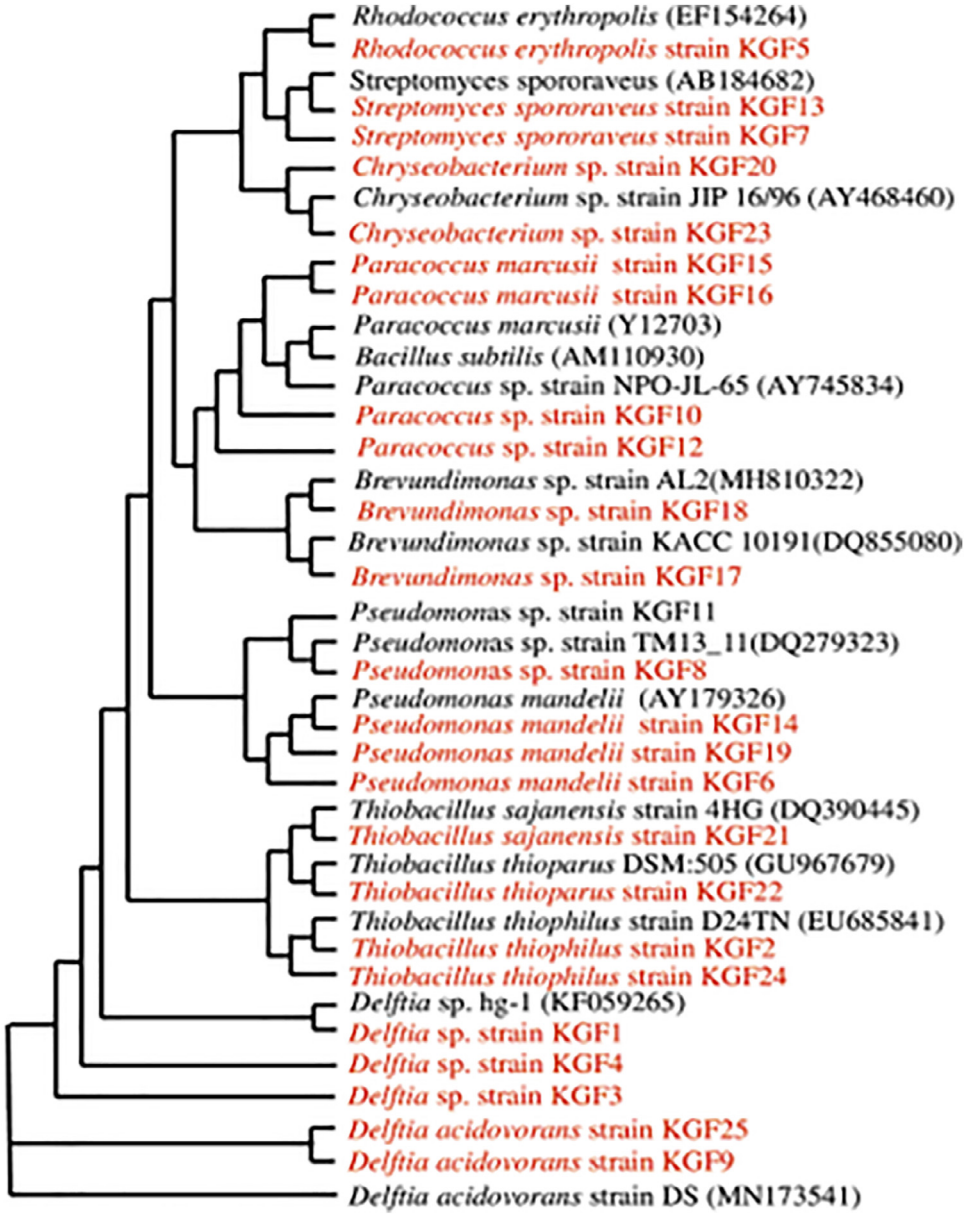

Fig. 8. Neighbour Joining phylogenetic tree of 16S rRNA gene sequences of the 25 bacterial isolates (red this study).

3.5. Mobilization of toxic elements in KGF mine tailings

Arsenopyrites are thermodynamically unstable under oxidizing condition and decomposes when in contact with rainfall, which releases As into the environment (Fig. 4). The high predominance of acidophilic bacteria such as *Thiobacillus thiophilus* (KGF2, KGF22, KGF24), *T. sajanensis* KGF21, *Delftia* sp. (KGF1, KGF3, KGF4), *D. acidovorans* (KGF9, KGF25; Fig. 8), indicates acid drainage from pyrite generally releasing ferrous ion, sulfate and acidity (H^+), and subsequently the ferrous ion is converted to ferric ion which again oxidise the pyrite adding to the acidity, an autocatalytic process, (Rimstidt and Vaughan, 2003). Iron- oxidizing bacteria (e.g., *Rhodococcus, Paracoccus, Pseudomonas, Thiobacillus, Delftia*) with most of them having sulfur oxidizing system indicates their metabolic breakdown of Au-bearing sulphide minerals by weathering of pyrite (FeS_2), chalcopyrite ($CuFeS_2$), MoS_2 (molybdenite) and sphalerite (ZnS) (Gadd, 2004).

Oxidation of sulfide minerals also releases heavy metals into the soil and aquatic environment. The drainage often contains high amounts of heavy metals such as As, Cu and Zn, whose fluxes depend on the extend of weathering of the pyrite (Blowes et al., 2003; Chen et al., 2014). The dissolution releases As(III) which are oxidized rapidly to more mobile As(V) in the vadose zone (Fig. 7). The As concentration in groundwater determined by various process such as redox reactions, adsorption/desorption process, ion exchange, solid phase precipitation and biological activity (Chen et al., 2014).

4. Conclusions

Our extensive study has featured the risks associated with abandoned gold mines of Karnataka that are complex and dynamic in multiple ways. The long avoided As contamination of potable water sources from the abandoned gold mine in Kolar has now posed a threat to the health of regional communities. The biological reductive dissolution and high mobility due to polarized pH conditions have led to percolation into groundwater systems

We suggest a supply of piped safe drinking water and/or rehabilitation of the sites is an utmost need. Our study highlights the abandoned gold mines to be acting as hot spots where indigenous microbial communities playing a role in ore dissolution by acidification and release of As and other elements. Additionally, precipitation can add nutrition and

the water-logged shafts environment and modelling of metagenomics data suggests an increase in microbial metabolic activities As release. The statistical analysis clearly indicates the low pH reducing conditions are leading to contaminant transport. The biggest concern is that it has already affected the regional community living in Kolar township. A decommission planning to barricade the exposure and drinking of contaminated water by the human, is of utmost need. This is a preliminary observation based on the small sample size, and the work is in progress to link the overall geology and mineral chemistry of the region with the reductive dissolution of As and F by biotic and abiotic factors.

Funding support

Department of Science and Technology (DST), Gov. of India grant DST/INSPIRE/04/2015/002362 awarded to DG and grant DST/EMR/2016/006662 awarded to BD.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

Acknowledgements

The authors thank the local community, school students and staff of Dr. T. Thimmaiah Institute of Technology, Kolar - for providing hospitality and kind cooperation. The authors also thank Prof. K. R. Natarajan (IISc) for his encouragement and support.

Availability of data and material

The authors confirm that the sequence data are submitted to the public database GenBank and accession numbers supporting the findings of this study are available within the article. The geochemical data that support the findings of this study can be availed from the corresponding author upon reasonable request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hazadv.2023.100316.

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