Analysis of Trace Mineral Elements and Microorganisms in Two Hot Springs in Jazan Region of Saudi Arabia

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Thermal springs have hydrothermal or volcanic activity, which causes the water to boil up and dissolve any metal that may be present there. It helps in recovery to reduce stress, promote sleep, boost blood circulation, relieve pain and heal skin problems. However, they consist of some heavy and trace metals and some non-metals, such as Mg, Mo, Na, As, Hg, Cd, Ni, Fe, Cu, Mn, Zn, Co, Ti, Cr and Pb are present. These hot springs are also known to exhibit natural microbiota, which is helpful in maintaining the public health and preventing consumer outbreaks with the advancement of research, microbiological monitoring of hot spring water can be done by utilizing high throughput sequencing. Taking these facts in concern, the present work, hot spring samples were collected from different locations of Al-Harth and Alarda hot springs, Jazan region, Saudi Arabia. The samples collected were analyzed for trace minerals and microbiological activity. These samples were found to exhibit the temperature range from 60 to 70 °C and an average pH of 6.5 to 8.5. Almost all the samples had shown the presence of trace elements with electrical conductivity and total dissolved solids (TDS) ranged from 0.02 to 0.045 dms and 27 to 43 mg/L, respectively. Scattergun metagenomic analysis was performed to determine the microbiological content. The preliminary study reports the microbiome (bacteria, archea and viruses) of alarath hot spring water employing whole-genome high-throughput sequencing and bioinformatics. Bacterial species detected were members of the phyla 64% Proteobacteria, 20% Actinobacteria, 6.3% unclassified, 2.2% *Deinococcus thermus*, 1.4 Firmcutes and 1.6% others. Fecal indicator bacteria, *i.e. Escherichia coli* or *enterococci*, were not detected in any samples examined in this study. Both archaeal DNA (0.4%) and viral DNA (0.009%) were found.

Keywords: Thermal spring, Trace element, Microbiome, Saudi Arabia, Jazan.

INTRODUCTION

Thermal springs occur naturally around tectonic zones or due to volcanic eruptions [1], which are both advantages and disadvantages for human and animals [2]. They act as the surface expression of original hot water systems [3] and can be used for hydrothermal energy production. The mineral water of these springs contains elements like sodium, potassium, calcium, magnesium and it's chlorides, fluorides, phosphates, bicarbonate and sulphate, which may be responsible for cure of various diseases [4]. World Health Organization has considered a new discipline named as medical hydrology as opposite medicine [5] as their hot water springs minerals present in water might be a cause of temporary relief of pain [6] and also help

in relaxing a person by raising the body temperature and rapid cooling that leads to body temperature and the rapid cooling that leads to a deeper and restful sleep [7].

Apart from this, human are known to have different skin type and other problems related to it. Nature is known to give the benefit without utilizing any chemical or medicine. Thus, hot spring bathing helps blood flow and cell oxygenation, improves our digestive system, reduce stress, relieve pain, heal skin problems and detoxify our body. Most of the hot springs contain more than 13 types of different minerals such as calcium, sodium, potassium, boron, magnesium, selenium, iron, sulfur, bicarbonate and its fluorite [8]. Each of these minerals can have a direct impact on our health and well-being, as human body is able to absorb these minerals in trace amounts [9]. Magnesium

and potassium in water promote healthy skin, boron which is usually found in pools helps to build muscle mass and nourish bones as well as increase brain activity.

Other benefits of hot water springs where the heat provide significant benefit to the body. The increase body temperature resulting from the hot water leads to improved blood circulation, which is reflected in the benefit of all organs and systems, as well as helps to relieve muscle and joint pain [10,11]. However, thermal water come out from the earth crust high related values of toxic elements, which can harmful for human consumption. Due to lack of clean water resources, some consume it for water [12,13]. Contamination of surface and groundwater through have metals is a substantial concern for environmental health scientists since this water normally is required for human and animal use. Research has shown that trace elements can be derived in the groundwater from natural and anthropogenic sources [14]. Trace elements can cause health issues, even in low concentrations [15-19]. Moreover, some researches have proposed that hot spring water may also contain toxic elements such as arsenic and mercury, hence care should be exercised regarding appropriate and precise use of hot springs [20,21].

Hot springs are also known to be present in the southern region of Saudi Arabia, which are beneficial for human health. The primary objective of this study is to identify the various kinds of minerals found in these thermal springs. ICP-OES method was considered as useful for the determination of trace elements. The results showed that the majority of water samples are rich in salts, especially sodium, magnesium and iron. Heavy metal concentrations were low and didn't exceed the standards established by the planet Health Organization for water [22], which makes the utilization of the waters of those springs safe and doesn't cause health problems for visitors. The concentration of lead within the water of these hot springs exceeds the permissible limit as per World Health Organization, which is 0.1 mg/L. The concentration limit is between 0.01 and 0.03 mg/L, which serves as a signal to consumers that the water they are using is not safe for consumption. The WHO [23] reports that the traditional bather (an hour in water) is unaffected by levels ten times the uttermost (*i.e.* up to 0.1 mg/L).

Metagenomic analysis, which makes use of high throughput sequencing in addition to bioinformatics, has recently come under scrutiny because of its use in detecting, identifying, and characterizing all microorganisms present in water [24]. Identifying genes inferring the infectious potential of found microbial types using their genetic codes for metabolic and pathogenic aspects [25,26]. Thus, detecting, identifying and characterizing bacteria, fungi and protists have become easier at the subspecies level. Undoubtedly, this is an enormous benefit to detect microorganisms in the water, which were pulsated before time or were not determined through culture-dependent techniques [27]. Others assured through their metagenomic surveys that ingested diet-borne items can have long- and short-term impacts on the human microbiota [28,29]. High-throughput sequencing is limitingly used to analyze hot spring water.

Furthermore, the complete microbiome is widely under consideration. This advance inspection is the initial to apply highly sensible shotgun metagenomic high-throughput sequence

analysis to distinguish items of microbial groups to characterize the Al-Harth (latitude: 42.96397; longitude: 17.05736) and Alarda (latitude: 42.9858; longitude: 17.09548) hot spring water's microbiome. Metagenomic analysis of hot spring water samples collected from two hot-springs was used. To carry out a major metagenomic examination of microbial, containing microorganisms in viable but uncultivable condition, the relative abundance of viruses, archaea and bacteria were determined.

EXPERIMENTAL

The geographical location and sampling point were recorded by a global positioning system (GPS) and were plotted within the map (Fig. 1) by employing WGS1984 geographic organization. Further, autumn (October-December 2021), water samples were collected from every sites in to a 1 L sterile glass bottles for the analysis and preserved the samples at 4 ºC.
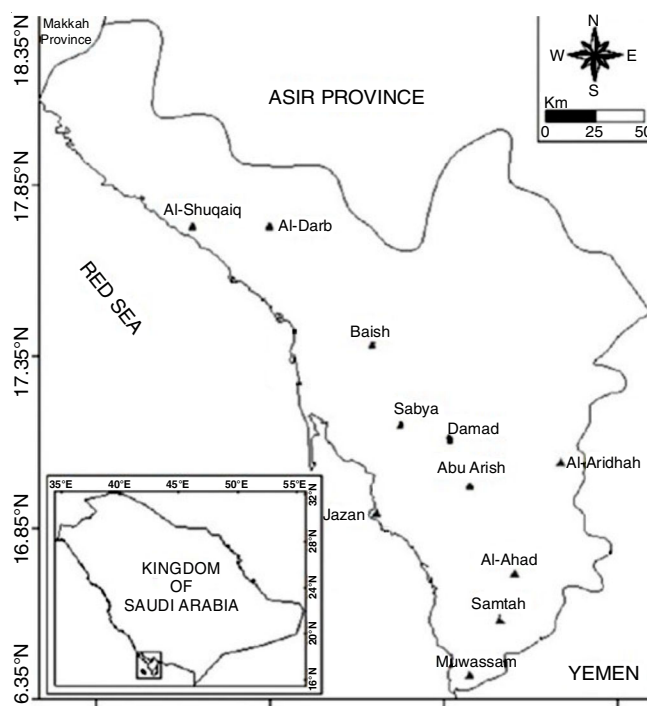

Fig. 1.   Placement of Al-Ayoun Al-Hara (arrow) near the villages of Al-Harath, Al-Khuba and Al Beni Malek, south of Jazan region, Saudi Arabia

Physico-chemical parameters: The physico-chemical parameters such as pH, electrical conductivity (EC) and TDS water sample were analyzed according to the standard procedures [30].

ICP-OES analysis: The chemical analyses of the water samples were carried out using ICP-OES (ThermoSciex Elan DRC II). All standard solutions containing 1000 mg/L were prepared by using ultrapure for Ca, Mg and Na further dilutions of 50, 100, 150 and 200 mg/L and for Al, Cr, Co, Cd, Ni, Mo, Pb, Fe, Zn and Mn dilutions 0.05, 0.1, 0.25, 0.5 and 1.0 mg/L, As and Hg standard solutions 5, 10, 20 and 40 µg/L were prepared in 2% HNO$_3$ solution.

Sample preparation: Prepared a 2.0% (v/v) nitric acid solution and aliquot the sample into a 50 mL centrifuge tube to analyze for metals. Similarly, prepared a 1% (v/v) HCl solution

and aliquot the sample into a 50 mL centrifuge tube to analyze for mercury [31].

Metagenome shotgun sequencing sample preparation: In order to construct the library, DNA and RNA were extracted. After implementing quality control, a passed sample commenced with the library construction (Macrogen, South Korea) [32].

Library construction: Random fragmentation of the sample of DNA or *c*DNA, followed by 5′ and 3′ adapter ligation, is used to conduct the sequencing library. Otherwise, tagmentation collects the fragmentation and ligation reactions into one step that safely enhances the qualification of the library preparation method. After that, PCR amplification and gen purification were performed on the adapter-ligated fragments [33].

To generate cluster from the sample, the library was carried on an influx cell where fragments are attracted on a lawn of surface-bound oligos integral with the library adapters. Subsequently, every fragment is enlarged into clonal, distinct clusters by the process of amplification. After completing cluster creation, the templates are all set for sequencing. To expose the individual bases, which comprise up a DNA template strand, Illumina SBS technology employs a patented reversible terminator based method. Due to all four reversible, terminator bound dNTPs observed through each sequencing turning, normal competition decreases foundation bias and safely weakens raw error averages compared with other technologies. It is found to be extremely accurate base-by-base sequencing that effectively excludes sequence-context-particular errors, even inside repetitive sequence areas and homopolymers [33].

Processing: Host DNA was filtered out from raw data. The host DNA was filtered out by mapping against the host reference genome using Knead Data v0.74. Trimmomatic v0.38 was used to remove the Illumina sequencing adapter and trim low-quality end bases with the following options; ILLUMINA-CLIP: Adapter. fasta:2:30:10:8:true LEADING:15 TRAILING:15 SLIDINGWINDOW:4:15 MINLEN:36. To assess raw data quality and trimmed data, FASTQC v0.11.6 was performed [34].

Taxnomy assignment for read: Centrifuge v1.0.4 was performed for the taxonomy assignment of trimmed data with primary assignments set as 1. The count and portion of the reads assigned to each taxon number were calculated at each taxon rank (Kingdom, Phylum, Class, Order, Family, Genus, Species and Subspecies) using in-house scripts and normalized abundance of a taxon at the level of subspecies, species and genus was calculated using the following equation [35]. After calcu-

lating quantity, a relative abundance ratio compared with the total abundance sum was calculated.

$$\text{Abundance} = \frac{R_a}{R_t} \times G$$

where, R_a: read count assigned to the taxon; R_t: total read count; G: average genome size of the taxon.

RESULTS AND DISCUSSION

Physico-chemical properties of water samples: The pH of water samples ranged from 7.14 to 8.13. According to WHO for drinking water quality, the permissible limit of pH is 6.5 to 8.0. The statistical analysis of physico-chemical properties of drinking water quality are given in Table-1. The electrical conductivity (EC) of the water samples ranged from 0.0248 to 0.0427 dms. The TDS were found to be between 15.87 to 42.32ppm and turbidity 0.1 to 0.4 NTU found.

Elements analysis using ICP-OES: The concentrations of sodium (Na), magnesium (Mg), aluminium (Al), calcium (Ca), chromium (Cr), manganese (Mn), iron (Fe), nickel (Ni), cobalt (Co), copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd), barium (Ba), titanium (Ti), mercury (Hg) and lead (Pb) in hot spring water samples are presented in Table-2.

This study collected two hot-spring water samples in Jazan region, called Al-Harth and Alarda. Volumes of up to 12 L of water were analyzed. It prepared sources, descriptions and elaborations of every sample. Shotgun metagenomic sequencing generated approximately 64,618,764 reads over the raw sequence libraries employing overall DNA extracted from the two samples from hot-spring water. Viruses, archaea and bacteria determined by DNA description appeared in Krona plots, representing the relative abundance of microbial species detected in Alarth hot-springs water sample. Bacteria constitute 92% of the total root, viruses constitute 0.1% of the root and archaea constitute 0.4%. The most dominant phyla were 64% Proteobacteria, 20% Actinobacteria, 6.3% unclassified, 2.2% Deinococcus-Thermus, 1.4 Firmcutes and 1.6% others, respectively. The dominant microorganisms, in the Alraith hot-spring water, at genus level, were *Acinetobacter* sp. (20%), *Porphyrobacter cryptus* (9.5%), unclassified (6.3%), *Acinetobacter* sp. 809848 (4.8%), *Methylobacterium radioblerans* (1.1%), *Rubellimicrobium thermophilum* (1.1%), *Streptomyces* sp. (1.1%) *Meiothermus ruber* (0.9%), *Vulcanii thermophilum* (0.9%) and *Sphingobium yanoikuyae* (0.8%) (Fig. 2). The most dominant

	Ain Al-Hara in Al-Ardah			Hot spring in Khouba			Al-Ain Al-Hara Sad Park in Abu Arish	Al Beni Malek
	(A)	(B)	(C)	(D)	(E)	(F)		
Temperature (°C)	42	60	60	70	60	40	60	65
Colour	Colour less	Colour less	Colour less	Colour less	Colour less	Yellow	Colour less	Yellow
Turbidity (NTU)	0.2	0.2	0.4	0.2	0.4	2.7	0.1	0.5
Presence of particles	Minor particles	There are no particles	Minor particles	There are no particles	Particles present	Particles present	Minor particle	There are no particles
pH	7.30	7.35	7.56	7.45	7.70	8.13	7.74	7.75
Electrical conductivity (dms)	0.0435	0.0421	0.0427	0.0343	0.0347	0.0380	0.0248	0.0215
TDS (ppm)	27.84	26.94	27.32	21.95	22.20	42.32	15.87	25.86

TABLE-1
PHYSICAL ANALYSIS OF WATER SAMPLES COLLECTED FROM DIFFERENT AREAS OF SAUDI ARABIA

TABLE-2
CHEMICAL ANALYSIS OF HOT SPRING WATER SAMPLES COLLECTED FROM DIFFERENT AREAS OF SAUDI ARABIA

| Metal	Ain Al-Hara in Al-Ardah			Hot spring in Khouba			Al-Ain Al-Hara Sad Park in Abu Arish	Al Beni Malek	Maximum concentration of elements in drinking to the WHO (mg/L)
	(A)	(B)	(C)	(D)	(E)	(F)			
Mg	2.33	2.10	2.09	3.77	3.85	15.26	1.31	1.21	50
Mo	0.0026	0.000	0.000	0.016	0.031	0.018	0.021	0.0083	0.07
Mn	0.06	0.030	0.031	0.036	0.034	0.083	0.02	0.025	0.4
Na	679	673	700	454	470	529	475	548	*
Ca	125.4	123	122.2	182.7	185.7	198.4	103.5	91.83	200
Cd	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003
Ni	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.07
As	0.000	0.000	0.000	0.000	0.000	0.0008	0.0009	0.0029	0.01
Fe	0.059	0.085	0.079	0.036	0.034	0.083	0.014	0.035	*
Cu	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2
Zn	0.004	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.05*
Co	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.05
Ti	0.004	0.004	0.004	0.027	0.003	0.003	0.004	0.001	0.1
Cr	0.001	0.000	0.000	0.002	0.002	0.001	0.000	0.000	0.05
Pb	0.032	0.039	0.035	0.027	0.037	0.021	0.018	0.075	0.01
Al	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.9
Hg	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006
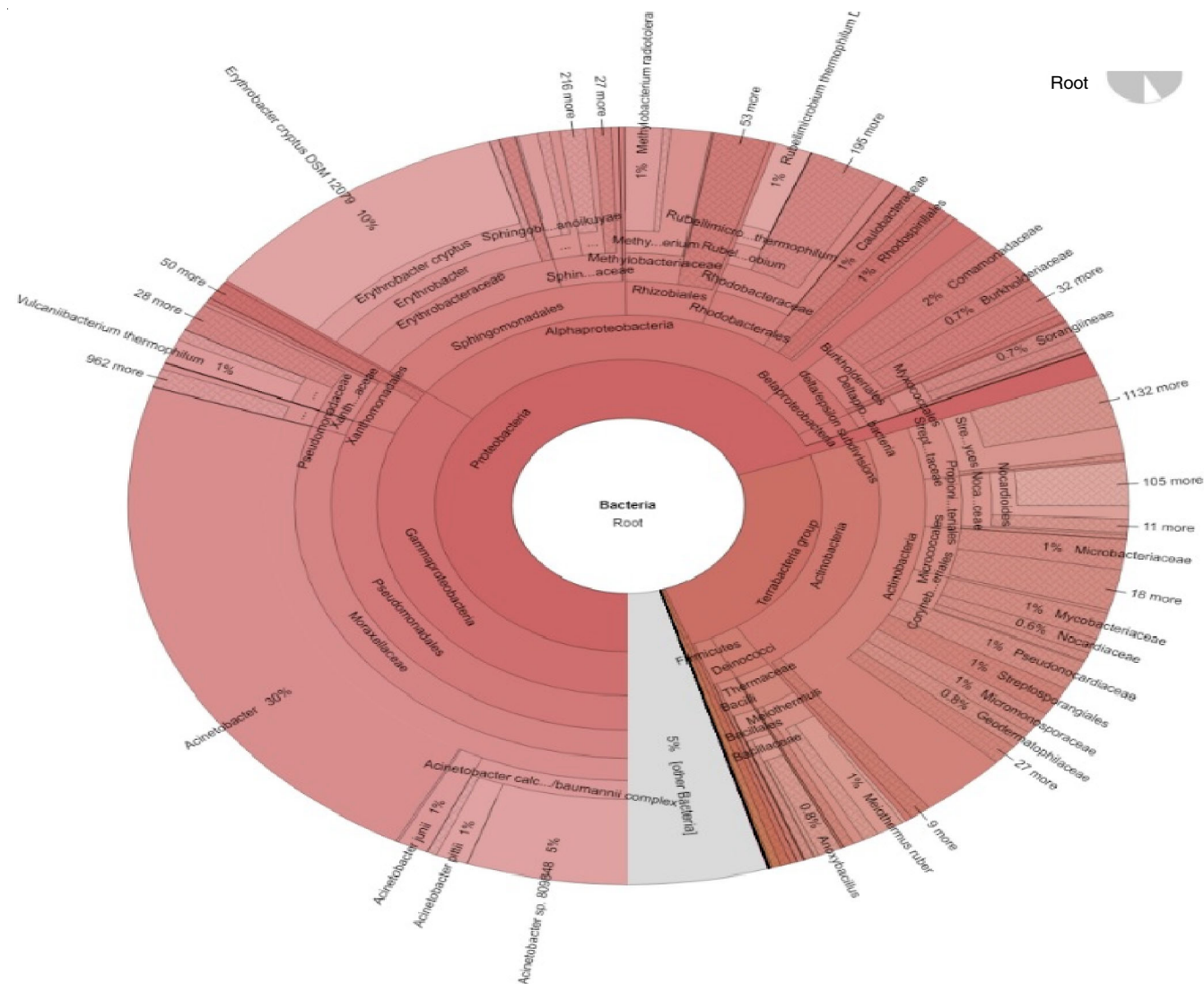
Fig. 2. Krona plot of alrith water hot-spring, bacterial species composition percentages are displayed as the normalized proportion of organism specific kmers observed relative to the total microbial species diversity detected in the sample

Archea were *Natronorubrum* sp. (2%), *Natronococcus* sp. (2%), *Halopiger* sp. (2%), *Halorthabdus* sp. (0.8%), *Halosimplex* sp. (0.8%), *Halobacterium hubeiense* (0.8%) and *Halolamina pelagica* (0.7%) (Fig. 3). Also, the most virus genus were *Halovirus* sp. (0.9%), *Mollivirus sibericum* (0.7%), *Monodnaviria* (0.4%), *Nudiviridae* (0.1%), *Hytrosaviridae* (0.07%), white spot syndrome vivuso 0.07% and finally, Pacmanvirus A23 (0.03), respectively (Fig. 4).

It is found that environmental samples are considered a huge depot of genetic variation from viruses, eukaryotes, archaea and bacteria. Standard laboratory techniques cannot cultivate approximately 99% of microorganisms. In studying environmental and genetic diversity, it is assured that metagenome sequencing is an essential method by avoiding the restriction of the cultivation-based process [36]. It has been practically proven that metagenomics sequencing process is carried out over two processes: (i) shotgun sequencing and (ii) amplicon sequencing. The present study, primers S-D-Bact-0341-b-S-17/S-D-Bact-0785-a-A-21 pair targeting the V3-V4 region of prokaryotic 16S rRNAs were employed [37]. According to *in*

Fig. 3. Krona plot of alrith water hot-spring, Archaea species composition percentages aredisplayed as the normalized proportion of organism specific kmers observed relative to thetotal microbial species diversity detected in the sample
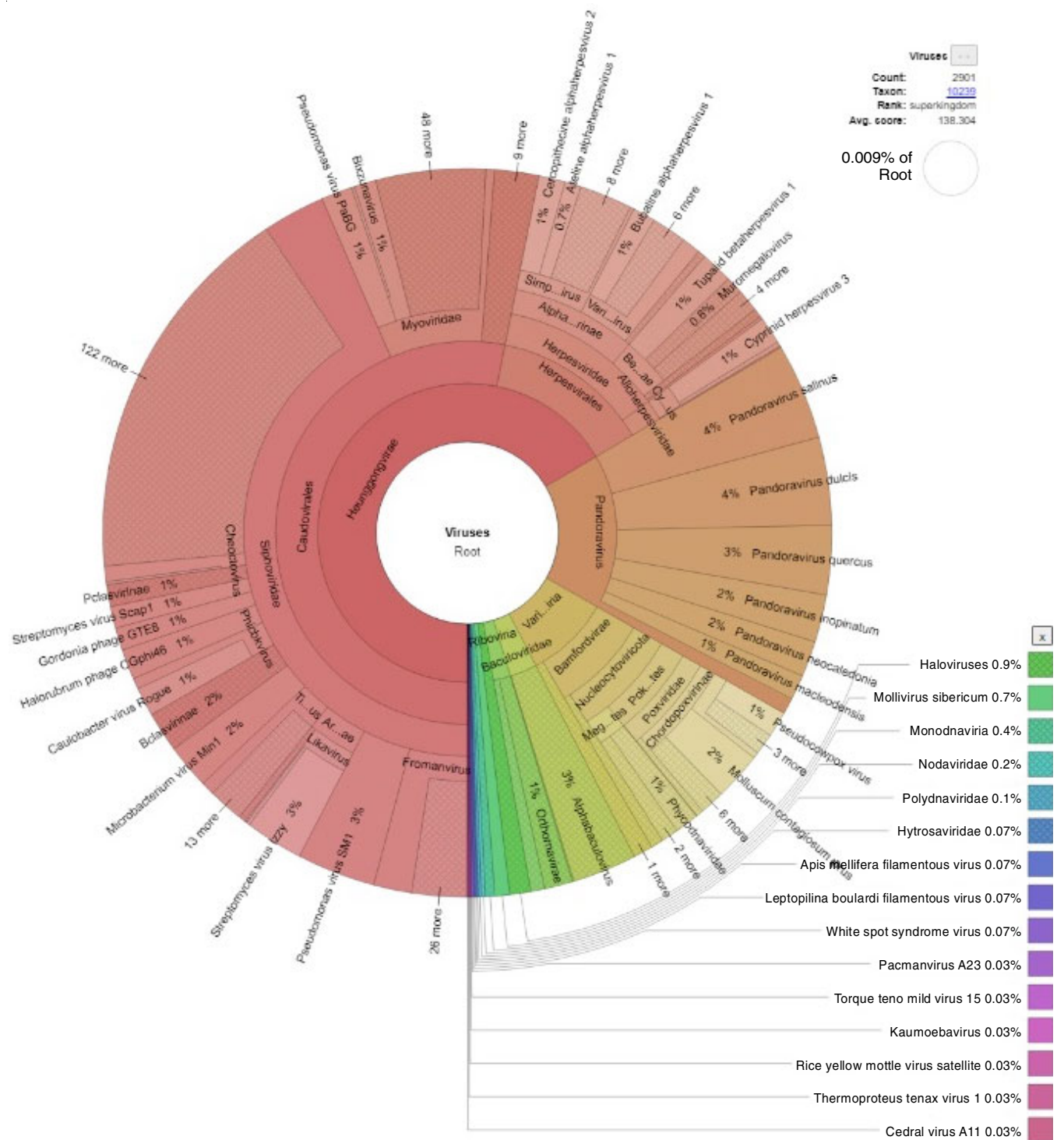
Fig. 4. Krona plot of alrith water hot-spring, Virus species composition percentages are displayed as the normalized proportion of organism specific kmers observed relative to the total microbial species diversity detected in the sample

silico evaluation analysis for not less than 370 thousand sequences, the determined primers provide comprehensive coverage of about 64.5% archaeal and 94.5% bacterial sequences reads in the database seeking if one is *in silico* nucleotide contrasting is taken into consideration [37]. Present study was able to identify 100 Phyla, 3550 genus and 25750 species from Alharth hot-spring water. In contrast, the quality control (QC) from the Alarda hot-spring sample was below the metagenomic

analysis cut of. This indicates the lower microbiome composition of the Alarda hot spring.

Almost every Saudi ambient microbiome study used 16S rRNA gene sequencing to detect microbial diversity in Saudi Arabian environmental samples. It was recorded the microbial diversity of the rhizosphere microbiome (*Avicennia marina*) from the water of Red sea. The Proteobacteria, Bacteroidetes and Firmicutes were the most predominant in their study [38].

Using 16S RNA sequencing, the soil bacterial collections from the southwestern highlands were analyzed, which is liable to the environmental variations. Acidobacteria, Actinobacteria, Verrucomicrobia and Proteobacteria were determined to be the most common of the 33 phyla discovered [39]. It was analyzed soil samples from 4 areas in the region of Mecca and found that 460 fungal species belonging to 58 families, 133 genera, 13 classes, 33 orders and four phyla. Other studies reported that dust storms in Saudi Arabian revealed a comparatively minimal abundance of Actinobacteria and a giant abundance of Proteobacteria compared with other countries' dust storms [40].

Nevertheless, the metagenomic approach was not applied in the previous studies. Thus, the bacterial diversity of soil samples taken from six Saudi Arabian hot springs was studied. According to the Culturomics-based Taxonomic Diversity method, the most common species were *Bacillus* and *Brevibacillus* [41]. Mat samples were taken from two different hot springs in the Aridhah region and analysis of the microbial communities revealed that Chloroflexus was by far the most abundant genus of bacteria [42]. Chlorinated wastewater gathered from the region of Thuwal in Saudi Arabia showed various genera corresponding to opportunistic pathogens *e.g. Legionella*, *Aeromonas*, *Streptococcus*, *Mycobacterium*, *Acinetobacter*, *Arcobacter* and *Pseudomonas*, which were identified by 16S rRNA gene-based high throughput sequencing next-generation sequencing by employing Roche 454 GS-FLX. A total of 27,356 readings were utilized for the diversity and taxonomy analyses. The average readings for each sample ranged from ±10,840–28,126.

Additionally, the taxonomic levels of three sponge species (*S. mollis*, *S. siphonella* and *P. vastifica*) were explored in order to identify bacterial communities with varied degrees of bacterial diversity. The results indicated that the metagenomic analysis is superior to the conventional method for elucidating the microbiome composition and the structure of hot spring water. Additionall, Al-Harth hot spring's microbial content is secure for the human consumption.

Conclusion

In summary, water metagenomic analysis is a valuable tool for uncovering the hidden world of microorganisms in aquatic environments. Its benefits extend to various scientific disciplines, environmental monitoring, public health, and the potential for discovering new biological resources. This present research investigation involved the collection and analysis of samples obtained from hot springs situated in different regions of Al-Harth and Alarda hotsprings located in the Jazan region of Saudi Arabia. The analyzed hot spring water samples exhibit the presence of trace minerals and microbial activity that conform to the standards set by the World Health Organization (WHO). Thus, these hot spring fluids can be utilized for animal baths and agricultural purposes.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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