HYDRO-GEOCHEMISTRY AND AMPHIBIAN ASSEMBLAGE OF KUPINDE LAKE, SALYAN DISTRICT, NEPAL

Chandra Bahadur Sunar

T.U. Registration No.: 5-3-28-194-2018

T.U. Examination Roll No.: Zoo 709/075

Batch: 2075/2076

A thesis submitted in partial fulfilment of the requirements for the award of the degree of Master of Science in Zoology with special paper Ecology and Environment.

Submitted to

Central Department of Zoology
Institute of Science and Technology
Tribhuvan University
Kathmandu Nepal

August, 2022

DECLARATION

I hereby declare that the work presented in this thesis entitled "**Hydro-Geochemistry and Amphibian Assemblage of Kupinde Lake, Salyan District, Nepal**" has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author(s) or institution(s).

Date: August-26-2022

Sunar
....

Chandra Bahadur Sunar

त्रिभुवन विश्वविद्यालय
TRIBHUVAN UNIVERSITY
प्राणी शास्त्र केन्द्रीय विभाग
CENTRAL DEPARTMENT OF ZOOLOGY
कीर्तिपुर, काठमाडौं, नेपाल।
Kirtipur, Kathmandu, Nepal.
०१-४३३१८९६
01-4331896
Email: info@cdztu.edu.np
URL: www.cdztu.edu.np

पत्र संख्या :-
च.नं. Ref.No.:-

RECOMMENDATION

This is to recommend that the thesis entitled **"Hydro-Geochemistry and Amphibian Assemblage of Kupinde Lake, Salyan District, Nepal"** has been carried out by Mr. Chandra Bahadur Sunar for the partial fulfillment Master's Degree of Science in Zoology with special paper of Ecology and Environment. This is his original work and has been carried out under our joint supervision. To the best of our knowledge, this thesis work has not been submitted for any other degree in any institutions.

Dr. Laxman Khanal	Dr. Ramesh Raj Pant
Associate Professor	Assistant Professor
Supervisor	Co-supervisor
Central Department of Zoology	Central Department of Environmental Science
Tribhuvan, University	Tribhuvan, University
Kathmandu, Nepal	Kathmandu, Nepal

Date: August -26- 2022

त्रिभुवन विश्वविद्यालय
TRIBHUVAN UNIVERSITY
प्राणी शास्त्र केन्द्रीय विभाग
CENTRAL DEPARTMENT OF ZOOLOGY
कीर्तिपुर, काठमाडौं, नेपाल।
Kirtipur, Kathmandu, Nepal.

०१-४३३१८९६
01-4331896
Email: info@cdztu.edu.np
URL: www.cdztu.edu.np

पत्र संख्या :-
च.नं. Ref.No.:-

LETTER OF APPROVAL

On the recommendation of supervisor Dr. Laxman Khanal, Associate Professor, Central Department of Zoology, and Co-supervisor Dr. Ramesh Raj Pant, Assistant Professor Central Department of Environmental Science, Tribhuvan University, this thesis submitted by Mr. Chandra Bahadur Sunar entitled **"Hydro-Geochemistry and Amphibian Assemblage of Kupinde Lake, Salyan District, Nepal"** is approved for the examination and submitted to the Tribhuvan University in partial fulfillment of the requirements for Master's Degree of Science in Zoology with special paper Ecology and Environment.

....

Prof. Dr. Tej Bahadur Thapa

Head of Department

Central Department of Zoology,

Tribhuvan University

Kirtipur, Kathmandu, Nepal

Date: August-26-2022

त्रिभुवन विश्वविद्यालय
TRIBHUVAN UNIVERSITY
प्राणी शास्त्र केन्द्रीय विभाग
CENTRAL DEPARTMENT OF ZOOLOGY
कीर्तिपुर, काठमाडौं, नेपाल ।
Kirtipur, Kathmandu, Nepal.

०१-४३३१८९६
01-4331896
Email: info@cdztu.edu.np
URL: www.cdztu.edu.np

पत्र संख्या :-
च.नं. Ref.No.:-

CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Mr. Chandra Bahadur Sunar entitled **"Hydro-Geochemistry and Amphibian Assemblage of Kupinde Lake, Salyan District, Nepal"** has been accepted as a partial fulfillment for the requirement of Master's Degree of Science in Zoology with special paper Ecology and Environment.

EVALUATION COMMITTEE

....

Dr. Laxman Khanal
Supervisor, Associate Professor
Central Department of Zoology
Tribhuvan University
Kirtipur, Kathmandu, Nepal

....

Prof. Dr. Tej Bahadur Thapa
Head of Department
Central Department of Zoology
Tribhuvan University
Kirtipur, Kathmandu, Nepal

....

External Examiner

Dr. Narayan Pd. Koju

....

Internal Examiner

Dr. Bishnu Pd. Bhattarai

Date of **Examination: September 7, 2022**

ACKNOWLEDGEMENTS

First and foremost, I am grateful to my academic supervisor Associate Prof. Dr. Laxman Khanal, Central Department of Zoology, Tribhuvan University for providing me a platform to conduct this research and also for his enthusiastic supervision. I am equally grateful to my Co- supervisor Assistant Prof. Dr. Ramesh Raj Pant, Central Department of Environmental Science for his continuous supervision, support and advice to complete this work.

I am thankful to Prof. Dr. Tej Bahadur Thapa, Head of Central Department of Zoology for providing such an opportunity to carry out this dissertation work.

I would like to express my gratitude to all my teachers and all staffs of Central Department of Zoology for their continuous aspiration and motivation. My special thanks goes to Mr. Naresh Pandey for his support in data analysis.

I am very thankful to my friends Mr. Ramesh Budhathoki, Mr. Bijaya Chand and friend Ms. Bina Thapa for their help and support throughout my thesis work.

I am grateful to my family members for their kind support and encouragement which lead to the successful completion of the research work.

Chandra Bahadur Sunar

Examination Roll No.: 709/075

Batch No: 075/076

chandansuanr27@gmail.com

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

µS/cm	Micro Siemen Per Centimeter
AIC	Akaike information criterion
CA	Cluster Analysis
CaH	Calcium Hardness
CBS	Central Bureau of statistics
CROSS	Cation Ratio of Soil Structural Stability
EC	Electrical Conductivity
GLM	Generalized Linear Model
GPS	Global Positioning System
HDPE	High Density Polyethylene
K	Kupinde Lake
KMO	Kaiser-Meyer-Olkin
KR	Kelly Ratio
MaH	Magnesium Hardness
MASL	Meter Above Sea Level
MH	Magnesium Hazard
Na %	Sodium Percentage
PCA	Principal Component Analysis
pH	Hydrogen Ion Concentration
PI	Permeability Index
SAR	Sodium Absorption Ratio
SD	Standard Deviation
SE	Standard Error
TDS	Total Dissolved Solids
TH	Total Hardness
WHO	World Health Organization

ABSTRACT

Hydrochemical assessment of the freshwater lakes provides significant insights into the sources of dissolved ions, geochemical processes, and anthropogenic activities taking place in the aquatic environment. Anuran amphibians spend a large portion of their lives in aquatic environments. The destruction of habitats and degradation of water quality are the major causes of amphibian population declines. The objective of this study was to assess the hydro-geochemistry and determine the distribution of amphibians in and around Kupinde Lake and to find the association between amphibian abundance and water quality parameters in Kupinde Lake, Karnali Province, Nepal. The study was conducted during October 2021. Surface water samples were collected from 24 different locations of the lake and 18 different physicochemical parameters were analyzed for understanding hydrochemistry and the water quality of the lake. The abundance of amphibians was estimated based on repeated visual encounter surveys conducted prior to the water sampling. The pH, temperature, EC and TDS were measured on-site, and concentration of major ions (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, NH_4^{+}, HCO_3^{-}, Cl^{-}, SO_4^{2-}, PO_4^{3-}, NO_3^{-}, TH, CaH, MaH, and free-CO_2) were measured in the laboratory. Based on the analytical results, lake water quality in the study area is slightly alkaline. The abundance of the major ions was in the following order: $Ca^{2+} > Mg^{2+} > Na^{+} > K^{+} > NH_4^{+}$ and $HCO_3^{-} > Cl^{-} > SO_4^{2-} > PO_4^{3-} > NO_3^{-}$ for the cations and anions, respectively. The lake water is dominated by Ca-HCO_3, indicating calcium carbonate lithology in the area. Gibbs and Piper diagrams illustrated rock weathering among the most dominant processes in controlling hydrochemistry of the Kupinde Lake. The suitability of water for drinking and irrigation determined using geochemical indices and WHO drinking water standards indicated that the lake water was suitable for drinking purposes and irrigation. The temperature, TDS, sulphate, and sodium negatively affected amphibian abundance, while pH, alkalinity, and calcium positively affected it. This study provides information on anthropogenic activities that may negatively impact aquatic biodiversity and provides strategies to minimize the impacts of those activities.

1. INTRODUCTION

1.1 Background

Wetlands are natural or man-made landscapes like swamps, marsh, fen, lakes, and streams (Rijal et al. 2021). These areas are located between the terrestrial and aquatic systems and serve as a habitat for birds, amphibian, fish, and reptiles (Bhandari 2009). In addition, wetland ecosystems provide a variety of ecosystem goods and services, which fulfill human needs economically as well as environmentally (Rijal et al. 2021). In addition to being able to absorb and store various environmental pollutants, wetlands can also bio-geochemically change. For instance, they can regulate the nitrogen cycle, which can negatively affect aquatic ecosystems and human health. (Hey et al. 2012). The use of fertilizers and pesticides has degraded the quality of water. Also, due to climate change and urbanization, the water supply is becoming more vulnerable to contamination (Sapkota et al. 2021) . Lake ecosystems are facing the most serious problem worldwide, and such impacts on water quality can be recognized and monitored using physical and chemical parameters (Amankwaa et al. 2020).

Wetlands are unique ecosystems, and they provide variety of ecosystem services to both plant and animals. These including climate regulation, carbon storage, water reservoirs, runoff containment and flood risk reduction (Cimon-Morin & Poulin 2018, Rijal et al. 2021). Besides these, heavy metals can be removed by wetlands through chemical, physical and biological processes (Bhowmik 2020). Additionally, wetlands play a significant role in supporting sustainable quality of life through ecotourism, recreation, and educational activities (Pedersen et al. 2019). As one of the most diverse ecosystem services, wetlands contribute to many aspects of cultural and recreational values, as well as future, historical, aesthetic, educational, symbolic, and therapeutic values (Zhou et al. 2020). Wetlands also support a high amount of biodiversity compared to their space by providing a habitat for a diverse range of flora and fauna (Kingsford et al. 2016). Globally, wetlands are disappearing at alarming rates, with 71 percent of wetlands converted to other land-cover types since 1900 (McCarthy et al. 2018).

Water is an essential component of the earth's uniqueness and provides all forms of life on the earth (Gorde & Jadhav 2013). In order to be suitable for a particular use, water must meet a variety of biological, chemical, and physical characteristics (Pfister et al. 2011, Gorde & Jadhav 2013). Globally, the hydrological, chemical, and physical characteristics of water are affected by rapid urbanization and infrastructure development, water extraction, contamination, land use for agriculture and livestock, and pesticides (Gorde & Jadhav 2013, Calderon et al. 2019, Newton et al. 2020). Aquatic biodiversity is affected by these anthropogenic activities, which change the wetlands ecosystem (Foti et al. 2012). In order to survive, reproduce and develop, amphibians, particularly anuran species, are highly dependent on aquatic ecosystems (Calderon et al. 2019). It is possible that wetlands with poor water quality will not sustain future generations, and amphibians can migrate from wetlands of low productivity to wetlands of high productivity (Pollet & Bendell-Young 2000).

Amphibians are unique among vertebrates in the fact that they use aquatic and terrestrial habitats at different stages of their lives (Compton et al. 2007, Rittenhouse & Semlitsch 2007) and they are key bio-indicators playing a crucial role in ecosystems (Mifsud 2014, Thakuri & Pokhrel 2017). Amphibian provide a variety of ecosystem services, such as religious and cultural services, food and medicine (Shah & Tiwari 2004, Paulding & Randhir 2021). They are supporting farmers as a regulator, seed dispersal, and biological pest control (Paulding & Randhir 2021). Amphibians utilize a variety of terrestrial and aquatic habitats for breeding, larval development, and over wintering (Shah & Tiwari 2004). Their population is declining due to habitat loss, degradation, fragmentation, climate change and pollution (Paulding & Randhir 2021). In addition to rivers, lakes, reservoirs, wetlands, irrigated rice fields, and ponds (5% of Nepal's total area), there is a vast amount of freshwater ecosystems supporting diverse flora and fauna in Nepal (Sharma 2008). These freshwater ecosystem plays an extremely crucial role in amphibian conservation projects because it is necessary to complete their life cycles. It is also necessary to ensure that their populations remain viable and grow (Rittenhouse & Semlitsch 2007). There is little information on hydrochemistry of wetlands in western Nepal, despite the region's abundant freshwater resources.

1.2 Significance of the study

Kupinde Lake is located in the Salyan District of western Nepal, and it is one of the most popular tourist areas of the district. The lake's most significant features are its religious and social values, as well as its recreational importance. There is ongoing construction and improvement of roads in the lake area, as well as the development of a large tourist complex. In the lake area, domestic tourists have increased over the past few years due to the improvement of the access roads. As a result, the lake could experience increased anthropogenic impacts, threatening its ecosystem services and unique biodiversity. There is no scientific study conducted on the physiochemical characteristics and amphibian diversity in and around the lake. In order to understand water quality and amphibian diversity, a detailed hydrochemistry and amphibian study is needed. This study will provide vital information on chemical composition, amphibian diversity and their assemblage in the Kupinde Lake. It will be helpful to planners, policymakers, developers and conservationists for sustainable management of the Kupinde Lake.

1.3 Research objectives

The general objective of this study was to explore the hydrogeochemistry and amphibian assemblage of Kupinde Lake in Salyan District of western Nepal.

The specific objectives were

1. To measure physico-chemical parameters of water in Kupinde Lake
2. To find the association between amphibian abundance and the water quality parameters in the Kupinde Lake

2. LITERATURE REVIEW

2.1 Hydrochemistry of lake water

The major ion chemistry of lake water provides valuable insight into the sources of dissolved ions, weathering, and hydrogeochemical processes (Singh et al. 2016). Kaphle et al. (2021) explored the ionic concentration of water samples in Rara Lake and reported that the concentrations of the foremost cations had been within the order of $Ca^{2+} > Mg^{2+} > K^{+} > Na^{+}$ and for anions $HCO_3^{-} > SO_4^{2-} > Cl^{-} > NO_3^{-}$. Among the cations and anion levels in the lake water, Ca^{2+} and HCO_3^{-}, represent 48.14% and 71.8%, respectively. Similarly, Mayanglambam & Neelam (2020) an analysis of physicochemical parameters as well as possible toxic elements in the water of Loktak Lake during different seasons, found that cation concentrations followed an order of $Ca^{2+} > Mg^{2+} > Na^{+} > K^{+}$, while anions followed an order of $HCO_3^{-} > Cl^{-} > NO_3^{-} > PO_4^{3-} > SO_4^{2-}$. It was found that the majority of samples contained a level of Pb above the acceptable threshold for consumption. Pre-monsoon samples was mainly in the poor category, due to waters from river inlets, the national park region, and the lake's northeastern portion, compared to post-monsoon samples that are in the good category. Pal et al. (2021) studied the ionic concentration of water samples in Jhilmila Lake and determined that $Ca^{2+} > Na^{+} > Mg^{2+} > K^{+} > NH_4^{+}$ and $HCO_3^{-} > Cl^{-} > SO_4^{2-} > NO_3^{-} > PO_4^{3-}$, were the most abundant major cations and anions. In this lake, Ca^{2+} and NH_4^{+} demonstrate the highest and lowest concentrations among the major cations, with the contributions ~40% and 1.47%, respectively. In terms of anion abundance, HCO_3^{-} composed 88 % of the total anionic contribution, followed by Cl^{-} and SO_4^{2-}. In the lake, the pH of the water was 7.35, which indicates that it was slightly alkaline. There was less pollution in Jhilmila Lake based on physico-chemical parameters and it falls under the permissible limits. Egbueri (2019) the hydrogeochemistry of the Ogbaru district, Ion exchange, and water quality statistics of the area concluded that the Niger River in the southeastern part of Nigeria was dominated by the cations $Ca^{2+} > Mg^{2+} > Na^{+} > K^{+}$ and anions $HCO_3^{-} > Cl^{-} > NO_3^{-} > SO_4^{2-} > PO_4^{3-} > NO_2$, respectively. Nearly 47.4% of the total sample was made up of the Mg-Ca-HCO_3 water type. There were approximately 16% of the samples containing Ca-Mg-HCO_3^{-}, 10.5% containing Mg-Ca-Na and Ca-Mg-Na-HCO_3, and 5.2% containing Ca-Mg-Cl, Mg-Ca-NO_3, and Ca-Mg type. Nearly 47.4% of the total sample was made up of the

Mg-Ca-HCO3- water type. As a result of the study, most groundwater samples were of high quality based on their physical and chemical properties, however, the presence of coliforms indicates poor quality and infers poor quality. Kaushik et al. (2021) examined the water chemistry in Lato Lake, which was slightly alkaline with pH ranging from 7.6 to 8.1. Its hydrochemistry was controlled by the rock lithology because of Ca^{2+} and HCO_3^- dominated. In order of dominance $HCO_3^- > SO_4^{2-} > NO_3^- > Cl^- > F^-$ and $Ca^{2+} > Mg^{2+} > Na^+ > K^+$ are the major ions. There are several factors that influence the chemistry of lakes. These include rocks and soils in the catchment area, atmospheric gases and aerosols, basin morphology, climate, prevailing fauna and flora, and human activities (Gaury et al. 2018). The pollution of a number of Himalayan lakes is the result of both rock weathering and human activities in the catchment (Das & Kaur 2001, Gaury et al. 2018, Kaphle et al. 2021). Major ions in Himalayan lakes are derived from carbonate weathering in their catchment areas (Mayanglambam & Neelam 2020, Kaphle et al. 2021).

2.2 Amphibian diversity as indicators of wetland habitats

Anuran amphibians are highly dependent on aquatic ecosystems. They can be terrestrial, aquatic, or even tree dwelling in habit (Shah & Tiwari 2004). These are bio-indicator, which determine the environmental stress in ecosystem and play key role in trophic levels (Ali et al. 2018). For aquatic and terrestrial changes, frogs and toads are helpful indicators, but lizards indicate a changing forest ecosystem (Amankwaa et al. 2020). Amphibians are excellent indicators of ecosystem health due to the fact that they rely heavily on water (Hecnar 2004). Polluted water can affect amphibians because their skin is permeable (Boyer & Grue 1995). Beside these, its population had decline due to reduced habitat and microhabitat quality, pollution, climate change and anthropogenic activities (Rastegar-Pouyani et al. 2015). In wetlands, factors such as the existence of permanent water, the spatial configuration of wetlands and upland habitat, the characteristics of local habitats, and water chemistry and quality are able to have an extensive effect on the composition of amphibian communities and the abundance of individuals (Hecnar 2004). In addition, fertilizer and pesticides are most commonly applied to increase agriculture production, which negatively affects the amphibians and reptiles that inhabit agriculture fields (Ghosh & Basu 2020). A variety of agricultural chemicals, such as pesticides, herbicides, fungicides, and fertilizers, contribute to pollution (Mann et al. 2009). They become prone to infections, limb deformities and a decrease in amphibian numbers (Linzey et al. 2003). Various amphibian and reptile species live in the wetland ecosystems. Amphibian that inhabit the water have been affected by increasing salinization, nitrification, hydrocarbons and pesticide contamination (Salman 2019). There has been evidence that amphibian development, growth, reproduction, and survivability are hampered by high levels of electrical conductance, nitrates, nitrites, total phosphates, chloride, and unionized ammonium, and low concentrations of dissolved oxygen (Serrano et al. 2016, Babini et al. 2018). Pesticides are destroying amphibians' fitness and survival as a result of their massive use (Mann et al. 2009).

3. MATERIALS AND METHODS

3.1 Study area

The Kupinde Lake is in Salyan District of Kanali Province, Nepal (Fig.1). It lies 28º 24.701'N and 082º 03.608' E and at an elevation about 1137 m asl and 15 km away from the district headquarter, Khalanga. Salyan District is a hilly area covering an area of 1462 square kilometers with a population of 241,716 (CBS 2011). The district is bounded by Rolpa to the east, Surkhet and Bardiya to the west, Rukum and Jajarkot to the north and Dang and Bank to the south. The district has a sub-tropical to temperate climate with maximum temperatures of 31°C and minimums of 3°C and annual rainfall of 1100 mm. Kupinde Lake is one of the most popular lakes in the district. It is situated in Ward No.8 of Bangad Kupinde Municipality. The lake is one of the most famous tourism areas and natural water resources of the district. It occupies 0.24 km^2 which is surrounded by sparse forest.

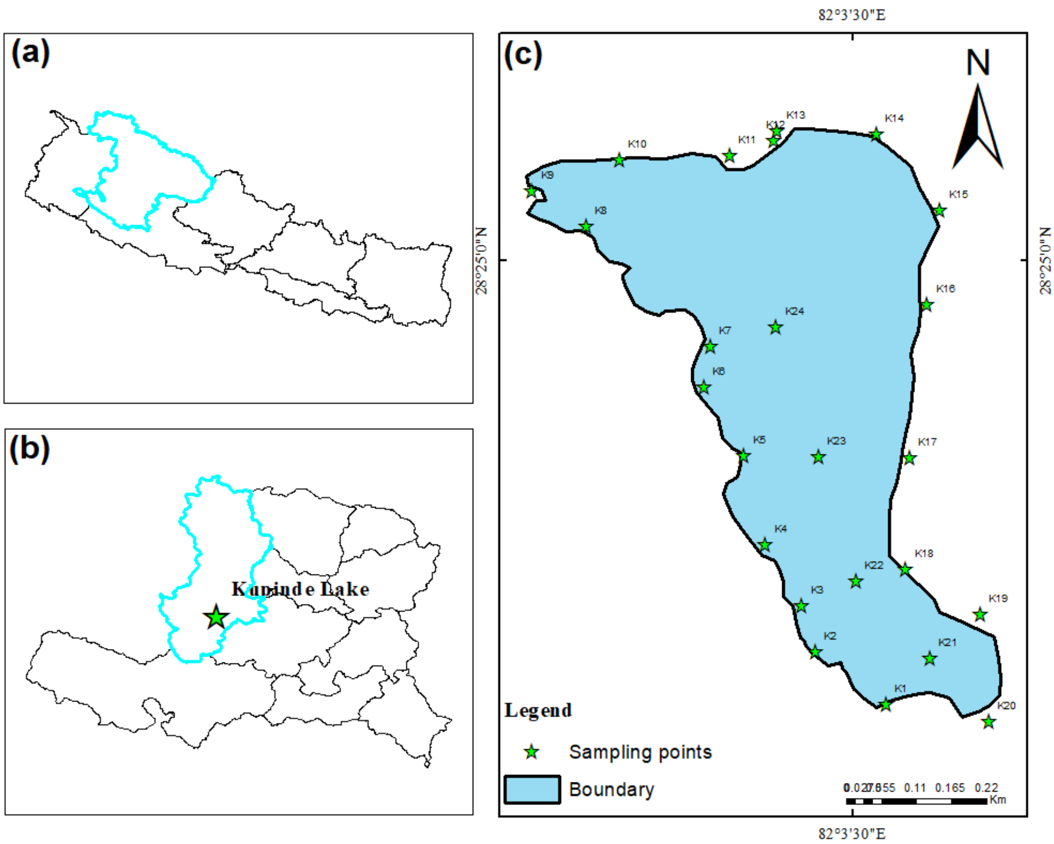

Figure 1. (a) Map of Nepal showing Karnali Province (b) Map of Salyan District showing study area (b) Map of Kupinde Lake showing sampling points.

3.2 Sampling and laboratory analysis

A total of 24 sampling points were selected from the lake for the spatial analysis of water quality in October 2021. The water samples were collected systematically from the periphery and middle of the lake using a boat. The location of the water sample was recorded with Garmin e-trex10 GPS unit (Garmin, Chicago, IL) with accuracy of < 5 m. Water samples were collected in HDPE (high density polyethylene) bottles and the samples were carefully transported and stored under freezing conditions before further analysis. Out of 24 samples, 20 were taken from the periphery and four were from the inner area of the lake. The physical parameters like pH, temperature, total dissolve solids (TDS) and electric conductivity (EC) were measured onsite and chemical parameters like total hardness (TH), calcium hardness (CaH), magnesium hardness (MgH), alkalinity (HCO_3^-), and chloride (Cl^-) were measured by titrimetric method. In addition, chemical parameters like ammonium (NH_4^+), nitrate (NO_3^-), and phosphate (PO_4^{3-}) were analyzed using UV-visible spectrophotometer. Potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), and sodium (Na^+) were analyzed using a Flame Photometer in the laboratory of the Central Department of Environmental Science, under the Institute of Science and Technology, Tribhuvan University (CDES-TU), Kathmandu, Nepal.

3.3 Amphibian survey

Species richness of amphibian fauna was observed by visual encounter survey. The survey was conducted from 5:00 to 10:00 hours in the morning, 2:00 to 5:00 afternoon and 7:00 to 9:00 evening. The sites (1, 2, 9, 10, 11, 12, 13, 14, 15, 18, 19, 20 and 21) were visited most frequently (10 times) during study. The sample sites (3, 4, 5, 6, 7, 8, 16, and 17) were visited four times. Amphibian counts were performed within a circle of 2-meter diameter of each water sample site prior to sampling and average value from repeated counts was used in downstream analysis. Photographs and samples of specimens were taken for both identification and evidence. The species was released in the same habitat after identification. The collected specimens were preserved in a plastic bottle with 70% ethanol for further identification. The collected species were stored at the Central Department of Zoology, Tribhuvan University.

3.4 Data analysis

For analyzing the data, the overall hydrochemistry, source identification, and grouping the similar points, descriptive statistics (maximum, minimum, mean, and standard deviation), correlation matrices, principal component analysis (PCA), and cluster analysis (CA) were applied. Shapiro-Wilk test was performed for the linearity of the variables. In addition, the sample adequacy was examined by Kaiser-Meyer-Olkin (KMO) and the Bartlett Sphericity and the outcomes were found satisfactory. The degree of relationship between the physicochemical parameters was described using Spearman's correlation matrix. In addition, the Piper plot, Gibbs plot and the Mixing diagram were used for the further understanding of hydrochemical characteristics. Furthermore, the irrigation suitability of the lake water was determined by using the following equations:

1. Sodium absorption ratio (SAR) = $\frac{Na^{+}}{\sqrt{(Ca^{2+}+Mg^{2+})/2}}$ (Richards 1954)
2. Sodium percentage (Na %) = $\frac{(Na^{+}+K^{+})*100}{(Ca^{2+}+Mg^{2+}+Na^{+}+K^{+})}$ (Richards 1954)
3. Kelly's ratio (KR) = $\frac{Na^{+}}{(Ca^{2+}+Mg^{2+})}$ (Kelly, 1940)
4. Permeability index (PI) = $\frac{(Na^{+}+\sqrt{HCO_3{}^{-}})*100}{Ca^{2+}+Mg^{2+}+Na^{+}}$ (Doneen 1964)
5. Magnesium Hazard (MH)= $\frac{Mg^{2+}*100}{(Ca^{2+}+Mg^{2+})}$ (Paliwali, 1972)

The generalized linear model before performing the GLM, the Shapiro-Wilk test was run to determine the linearity of the variables. The Generalized Liner Model (GLM; function 'glm' in R) with Poisson distribution and log link function employed in the R software (R-Core-Team 2022) was used to analyze the relations between physico-chemical parameters of water and amphibian abundance.

Polynomial regression (third order) was performed between the abundance of amphibians and physicochemical parameters (pH, temperature). Third order polynomial regression was performed based on the least AIC value.

4. RESULTS

4.1 Hydrogeochemistry of Kupinde Lake

The general hydrochemistry and its statistical evaluation of Kupinde Lake water are presented in Table 1 and Fig. 2. The mean temperature of Kupinde Lake water was 22.58 °C, with range from 21.8 to 25 °C. The pH of Kupinde Lake water was 8.16, ranging from 7.9 to 9.2. The highest pH (9.2) was measured at the initial sample points (K1), while the lowest pH (7.9) was measured at two points K4 and K13. The mean EC was found to be 136.54 µS/cm with range from 129 to 143 µS/cm and TDS was 68.25 mg/L with range from 64 to 72 mg/L. The highest and lowest EC and TDS values were found in the sample points L18 and L20. The most dominant cation and anion in Kupinde Lake had been Ca^{2+} and HCO_3^-, respectively.

Table 1. Descriptive statistics of the physiochemical parameters of the Kupinde Lake

Parameter	Min	Max	Mean	SD	WHO
Temp	21.8	25	22.58	0.69	-
pH	7.9	9.2	8.16	0.29	6 - 8.5
EC	129	143	136.54	3.04	1500
TDS	64	72	68.25	1.67	1000
F-CO_2	4.4	11	7.43	2.13	-
Cl^-	4.26	8.52	6.04	1.34	250
HCO_3^-	40	300	157.71	69.09	600
TH	86	174	115.92	27.67	-
CaH	56	90	69.92	10.82	-
MgH	18	84	46	20.21	-
Ca^{2+}	22.4	36	27.97	4.33	100
Mg^{2+}	4.39	20.5	11.23	4.93	50
PO_4^{3-}	0.11	0.29	0.19	0.05	1
NO_3^-	0.05	0.06	0.06	0	50
NH_4^+	0.09	0.44	0.11	0.07	0.5
SO_4^{2-}	0.13	2.28	0.64	0.5	250
K^+	2.4	4.9	3.47	0.65	100
Na^+	6.5	8.5	7.38	0.6	200

Unit: concentration in mg/L except pH, Temp and EC in µS/cm

The highest concentration of HCO_3^- was detected at sample points K21 while the lowest concentration was measured in site K9. The dominancy order of the major cations and anions was $Ca^{2+} > Mg^{2+} > Na^+ > K^+ > NH_4^+$ and $HCO_3^- > Cl^- > SO_4^{2-} >$

PO_4^{3-} > NO_3^-, respectively (Table 1). Sample points K1 had higher concentrations of TH, CaH, and MgH. Concentrations of cations such as Ca^{2+}, Mg^{2+}, Na^+, K^+, and NH_4^+ was most abundant at sampling points K11, K13, K14, and K1. Cl^- and SO_4^{2-} were concentrated at sampling points K6 and K2 whereas, PO_4^{2-} and NO_3^- were evenly distributed in lake surface water. The concentration of free-CO_2 was highest at the collection points (K13, K4, K5, and K6).

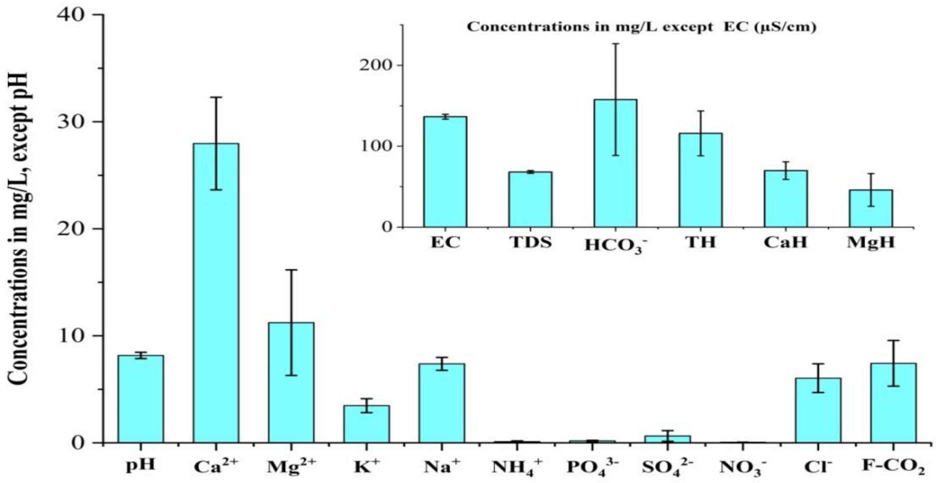

Figure 2. Concentration physicochemical parameters of Kupinde Lake

4.1.1 Hydrogeochemical facies

Piper plot was used for the characterization and source identification of major ions (Fig. 3). In the cation triangle, most of the sample lies in the lower left corner, indicating a high concentration of calcium and a plot of anion triangle water sample lies near the HCO_3^- concentration in the lower left corner. The alkali earth metal Ca^{2+} and Mg^{2+} dominate the alkali metals (Na^+ and K^+) and weak acids (HCO_3^-) dominate the strong acids (Cl^- and SO_4^{2-}).

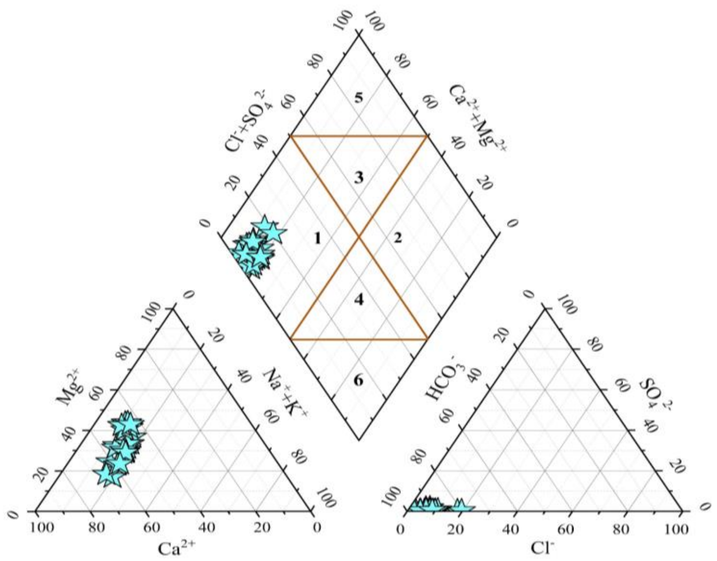

Figure 3. Characterization of hydrochemical facies of the Kupinde Lake based on the Piper Trillinear diagram

4.1.2 Controlling mechanism of the hydrochemistry

A Gibbs diagram can be used to determine the major chemistry-controlling mechanisms of the water bodies. The concentration of TDS and ratios of Na^+/ (Na^+ + Ca^{2+}) and Cl^- / (Cl^- + HCO_3^-) was plotted in the diagram (Fig. 4). The majority of the samples exhibited moderate TDS and low ratios of Na^+/ (Na^++ Ca^{2+}) and Cl^- / Cl^- + HCO_3^-) showing a dominant influence of rock weathering in the Lake water. It appears that all the sites are mainly affected by rock weathering, the carbonate type.

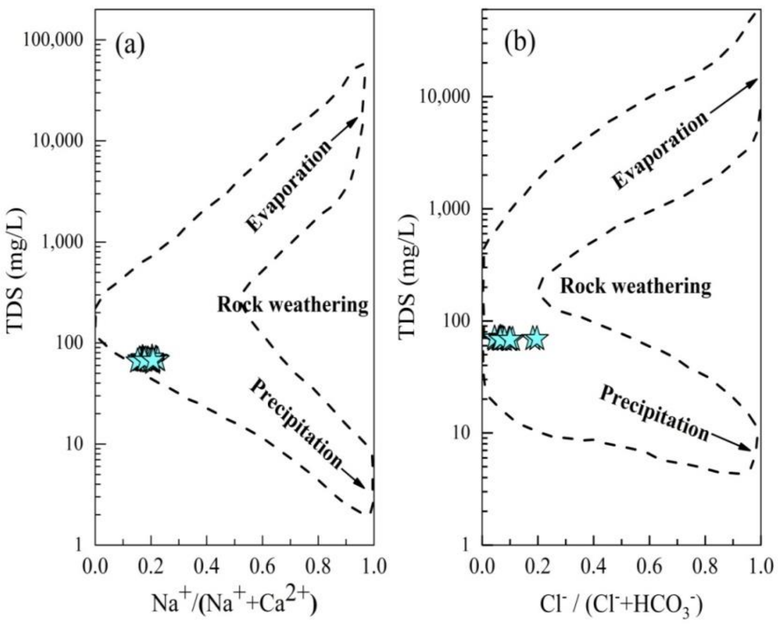

Figure 4. Gibbs diagram showing the major ions source of Kupinde Lake

The results revealed Na^+-normalized hydrochemistry of the Kupinde Lake (Fig. 5). In the Lake, most samples were found near the carbonate end-members, whereas few samples contribute to weathering silicates, indicating the Lake has carbonate types of rock weathering.

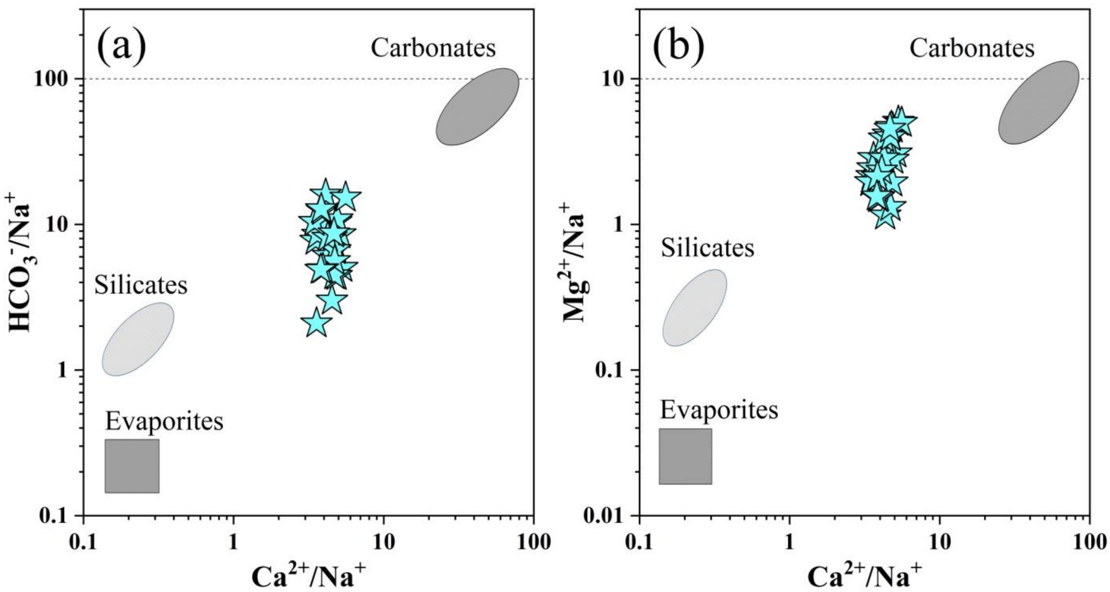

Figure 5. Mixing diagram (a) Ca^{2+}/Na^+ versus HCO_3^-/ Na^+ and (b) Ca^{2+}/ Na^+ versus Mg^{2+}/Na^+) in the Kupinde Lake.

4.1.3 Associations among physico-chemical parameters

The correlation between temperature and alkalinity (HCO_3^-) ($p < 0.05$) is moderately positive and between temperature and K^+ is moderately negative ($p < 0.05$) (Table 2). The hydrogen ion concentration (pH) has moderately negative correlation with both free-CO_2 ($p< 0.01$) and Na^+, while NH_4^+ has strongly positive correlation ($p < 0.01$) with pH. The correlation between EC and TDS is 0.98 ($p< 0.01$), indicating a strongly positive correlation between the variables. In terms of free-CO_2 and Cl^-, the correlation between variable is 0.60 ($p < 0.01$) and between free-CO_2 and NO_3^- is 0.43 ($p< 0.05$). The correlation between these variables is moderately positive. The Spearman correlation between Cl^- and NO_3^- has moderately positive ($p < 0.05$). In this study, it is interesting to note that TH has a very strong correlation with CaH, MgH, Ca^{2+} and Mg^{2+} ($p <0.01$). Similarly, CaH has very strong correlation with Ca^{2+} and moderate correlation with MgH ($p < 0.01$), Mg^{2+} ($p < 0.01$) and Na^+ ($p < 0.05$) (Table 2). Furthermore, MgH has very strong correlation with Mg^{2+} and moderate correlation with Ca^{2+}. In addition, Ca^{2+} has moderate correlation with Mg^{2+} ($p < 0.01$) and Na^+ ($p < 0.05$). In contrast, Mg^{2+}, PO_4^{3-}, NO_3^-, NH_4^+, SO_4^{2-}, K^+, and Na^+ cannot show good correlation with other variables (Table 2).

The Hierarchical agglomerative clustering approach was applied to find intuitive similarity relationships between sampling sites which is illustrated by a dendrogram (Fig. 6). Cluster 1 formed by sampling sites (4, 5, 6, 17, 18, & 20). Similarly, Cluster 2 formed by collecting data from sites 19 and 21. Furthermore, cluster 3 is composed of sites (1, 2, 3, 7, 13, 14, & 23) and cluster 4 is composed of collection sites 9 and 10. Cluster 5 was formed by the inlet and center point of sampling sites (8, 11, 12, 15, 16, 22, and 24).

Table 2. Spearman correlation matrix of physico-chemical parameters of Kupinde Lake

Parameter	**Temp**	**pH**	**EC**	**TDS**	**F- CO_2**	**Cl^-**	**HCO_3^-**	**TH**	**CaH**	**MgH**	**Ca^{2+}**	**Mg^{2+}**	**PO_4^{3-}**	**NO_3^-**	**NH_4^+**	**SO_4^{2-}**	**K^+**	**Na**
Temp	1																	
pH	0.013	1																
EC	-0.015	-0.119	1															
TDS	-0.048	-0.114	**.981****	1														
F-CO_2	0.006	**-.535****	0.386	0.368	1													
Cl^-	-0.234	-0.298	0.118	0.124	**.606****	1												
HCO_3^-	**.416***	0.055	-0.206	-0.175	-0.240	-0.334	1											
TH	-0.196	-0.207	-0.092	-0.133	0.251	0.011	-0.125	1										
CaH	0.024	-0.115	-0.020	-0.061	0.194	0.104	-0.026	**.792****	1									
MgH	-0.281	-0.222	-0.115	-0.149	0.240	-0.041	-0.158	**.945****	**.550****	1								
Ca^{2+}	0.024	-0.115	-0.020	-0.061	0.194	0.104	-0.026	**.792****	**1.000****	**.550****	1							
Mg^{2+}	-0.281	-0.222	-0.115	-0.149	0.240	-0.041	-0.157	**.945****	**.550****	**1.000****	**.550****	1						
PO_4^{3-}	-0.295	0.200	-0.226	-0.189	-0.186	-0.033	-0.239	0.197	0.006	0.266	0.006	0.266	1					
NO_3^-	-0.147	0.127	0.040	0.088	**.431***	**.469***	-0.254	0.034	-0.114	0.107	-0.114	0.107	-0.102	1				
NH_4^+	0.108	**.757****	-0.002	0.002	-0.303	-0.271	-0.056	0.038	0.121	-0.013	0.121	-0.013	-0.041	0.104	1			
SO_4^{2-}	-0.059	0.288	0.105	0.097	-0.167	0.050	0.179	-0.367	-0.200	-0.395	-0.200	-0.395	-0.225	0.082	-0.027	1		
K^+	**-.406***	0.174	-0.298	-0.344	-0.044	0.140	-0.308	-0.061	-0.315	0.085	-0.315	0.085	0.187	0.230	-0.182	0.019	1	
Na^+	-0.077	**-.486***	-0.078	-0.063	0.143	0.272	-0.081	0.159	**.450***	-0.024	**.450***	-0.024	-0.195	-0.253	-0.248	-0.084	-0.23	1

***. Correlation is significant at the 0.05 level (2-tailed).**

****. Correlation is significant at the 0.01 level (2-tailed).**

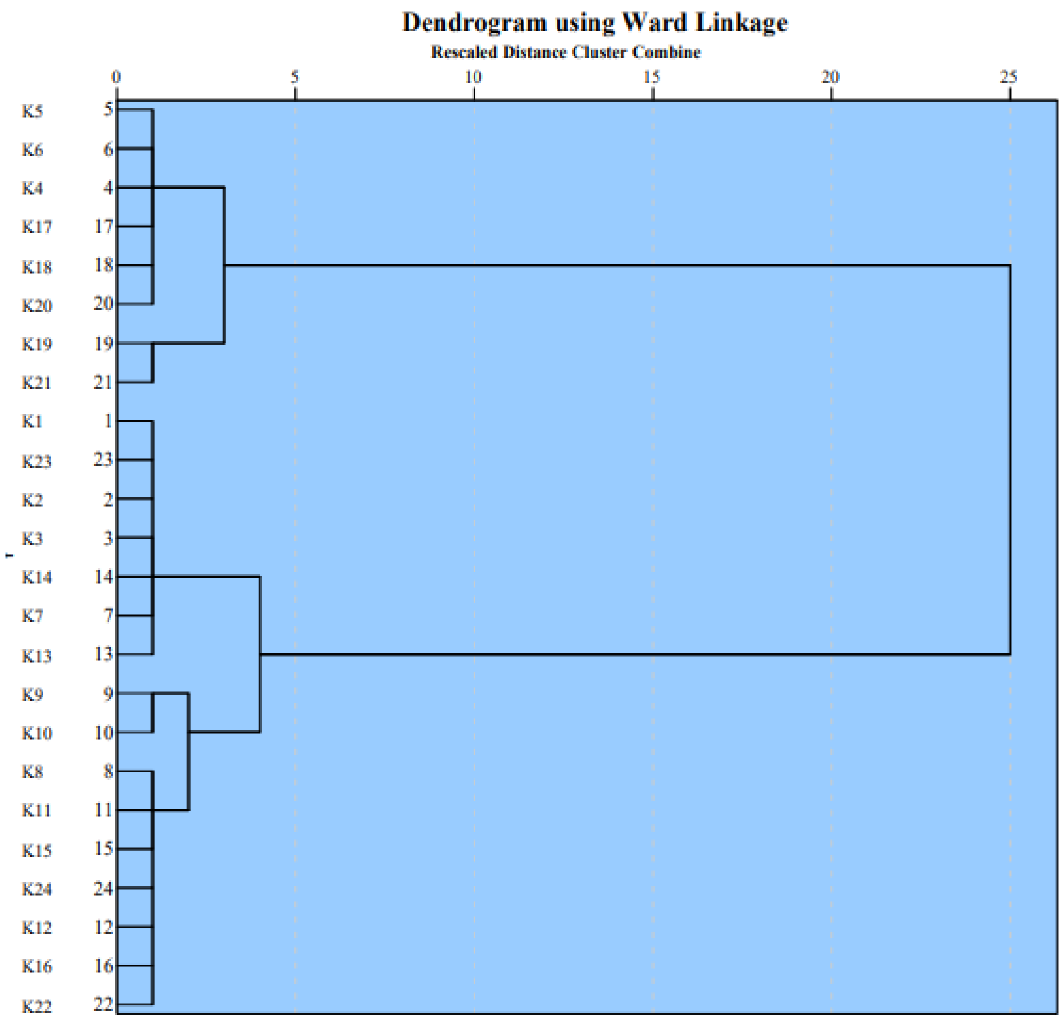

Figure 6. Dendrogram of the hydrochemical variables in the Kupinde Lake based hierarchical cluster of sampling points.

A total of five principal components have been extracted (Table 3). In the data set, the first five parameters account for 76.05 % of the total variance. The first principal component (PC1) explains 24.78 % of the variance in the data, and CaH, MgH, Mg^{2+}, and Ca^{2+} are strongly positively loaded. PC2 shows strong positive loadings with Cl^- and NO_3^- whereas HCO_3^- displays moderately negative loadings, which explains about 14.43 % of total variance in the results. In PC3, PO_4^{3-} has a moderately positive loading and SO_4^{2-} has a moderately negative loading, which accounts for 13.08 % of the variance in the result Similarly, it is observed that NH_4^+ is moderately loaded in PC4 and Na^+ is strongly negative loaded, which accounts for 12.09 % of the variance. The TDS is strongly negative loaded and the K^+ is moderately positive loaded, explaining 11.66 % of the total variance in PC5.

Table 3. Varimax rotated component matrix of Kupinde Lake

Parameter	**Component**				
	1	**2**	**3**	**4**	**5**
TDS	-0.15	0.23	0.03	0.08	**-0.79**
Cl^-	0.05	**0.76**	-0.09	-0.45	-0.06
HCO_3^-	0.01	**-0.60**	-0.55	0.09	0.21
CaH	**0.93**	-0.05	-0.03	-0.19	-0.15
MgH	**0.77**	0.12	0.41	0.20	0.27
Ca^{2+}	**0.93**	-0.05	-0.03	-0.19	-0.15
Mg^{2+}	**0.77**	0.12	0.41	0.20	0.27
PO_4^{3-}	-0.01	-0.06	**0.73**	0.04	0.20
NO_3^-	0.03	**0.82**	-0.19	0.33	0.09
NH_4^+	0.16	-0.08	-0.10	**0.68**	-0.31
SO_4^{2-}	-0.28	0.10	**-0.64**	0.03	0.06
K^+	-0.26	0.39	0.23	0.01	**0.68**
Na^+	0.33	-0.07	-0.12	**-0.80**	-0.19
% of Variance	**24.78**	**14.43**	**13.08**	**12.09**	**11.66**
Cumulative %	**24.78**	**39.21**	**52.30**	**64.39**	**76.05**

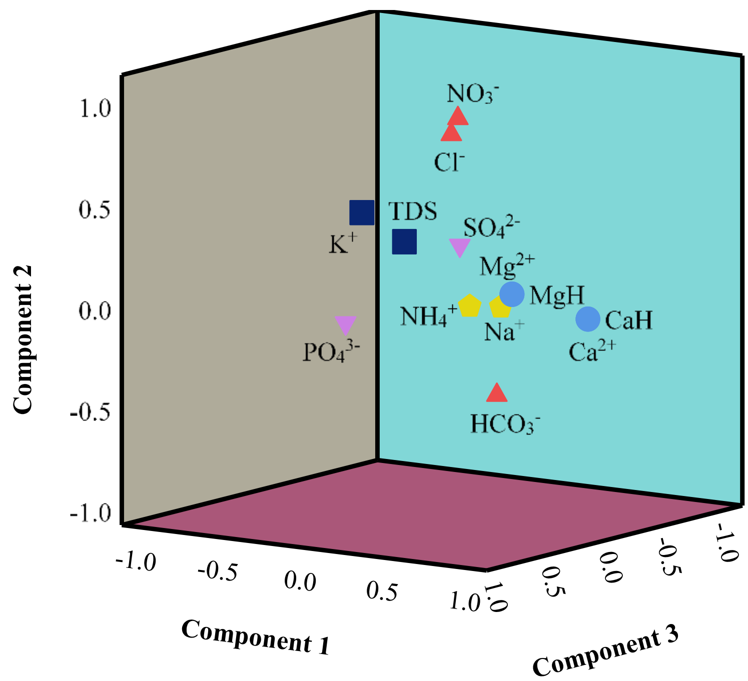

Figure 7. Factor loading plot of the principal component analysis

4.1.4 Water quality status

Table 4 presents the results of the evaluation of Kupinde Lake's irrigation suitability based on different methods. The average value of EC, Na%, and SAR was 136.54, 15.53, and 0.30, falling into the category of excellent. According to the Wilcox diagram (Fig. 8), water samples from Kupinde Lake were plotted into C1S1 section, indicating excellent irrigation properties. Additionally, the MH and KR values of the lake water are within the safe range for irrigation. The average PI value indicated that the water was of acceptable quality. According to this study, the CROSS values of lake water are excellent.

Table 4. Classification of water samples for irrigation based on various parameter

Parameter	**Average value**	**Category**
EC	136.54	Excellent
Na%	15.53	Excellent
SAR	0.30	Excellent
MH	38.38	Suitable
KR	0.14	Safe
PI	74.74	Class I

CROSS	0.38	Excellent

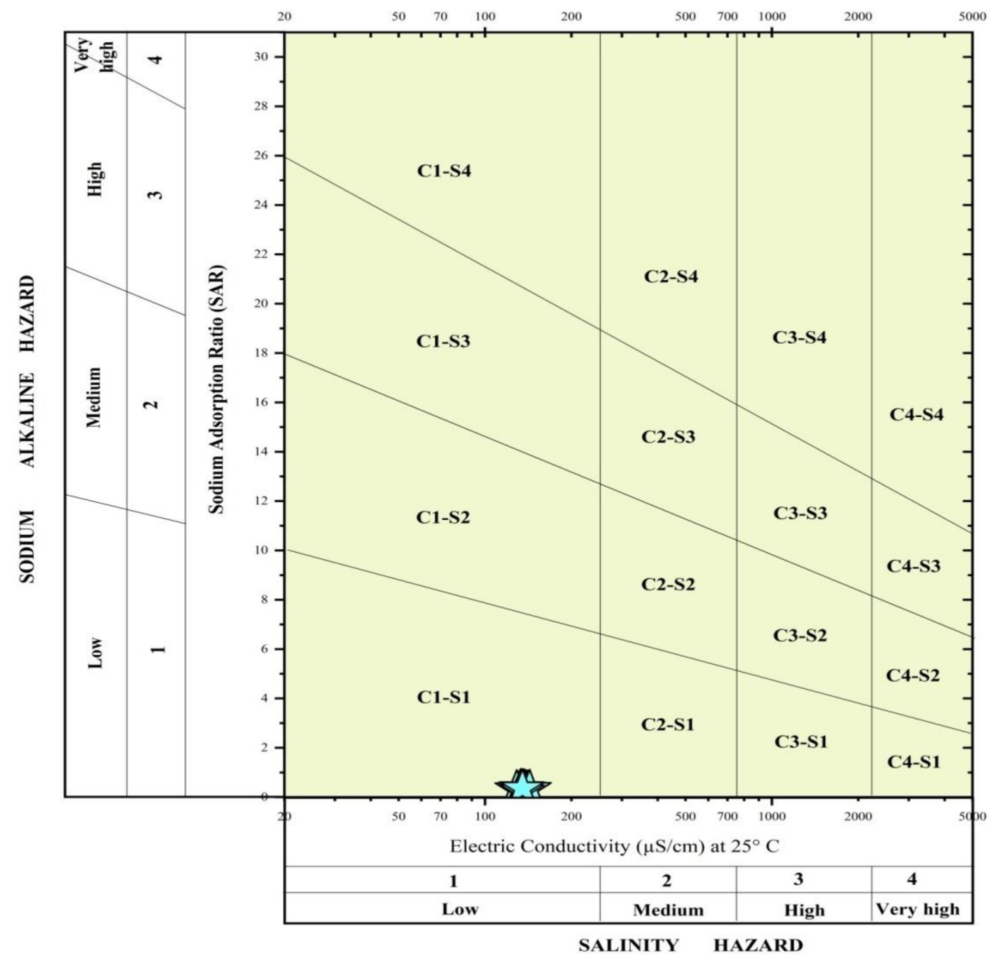

Figure 8. Wilcox diagram showing the suitability of water for irrigation purpose.

4.2 Amphibian community structure and determinants

Only two species of Anurans: *Euphlyctis cyanophlyctis* and *Fejervarya syhadrensis* were recorded during the study period from the Kupinde Lake. *Euphlyctis cyanophlyctis* was the most abundant amphibian species. Among the 24 study sites, *Euphlytis cyanophlyctis* was detected in the 20 sampling points. The highest abundance of species was found at site 1 (n = 120), followed by site 20 (n = 115). Species was not observed in the Site 6 and center of the lake (i.e., Site 22, 23, and 24).

Based on the physico-chemical parameter testing, the generalized liner model (GLM) indicated amphibian abundance was significantly affected by temperature, pH, TDS alkalinity, calcium, sulphate and sodium (Table 5). According to the test parameters, amphibian species abundance decreased with increasing temperature, TDS, sulphate, and sodium. Similarly, the abundance of species is positively related to pH, alkalinity, and calcium concentration.

Table 5. Generalized linear model (GLM) with Poisson distribution and log link function test showing the relations between amphibian abundance and physical-chemical parameters of water quality in Kupinde Lake.

Parameter	**Estimate**	**SE**	**z value**	**Pr (>|z|**
Intercept	28.82	8.83	3.26	0.001108**
Temp	**-0.68**	0.21	-3.23	0.001209**
pH	1.80	0.91	1.97	0.047912*
TDS	**-0.35**	0.07	-5.03	0.000000488***
Alkalinity	0.00	0.00	3.05	0.002287**
Ca	0.06	0.01	3.54	0.000389***
Sulphate	**-0.62**	0.21	-2.90	0.003691**
Na	**-0.60**	0.17	-3.40	0.000658***

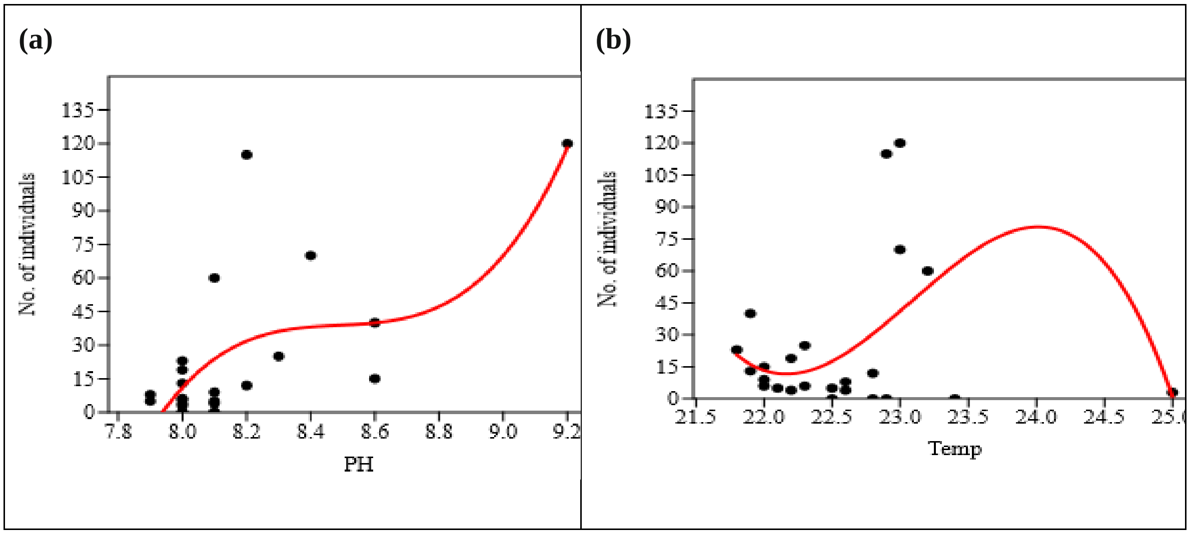

Figure 9. Relationship between (a) Number of individuals and pH and (b) Number of individuals and temperature

A polynomial regression analysis of number of individuals, with temperature, and pH is shown in table 7 and figure 9 A and B. Temperature and pH were better suited to the third order ($p < 0.05$).

Table 6. Polynomial regression of abundance of amphibians with temperature and pH

Regression	**Temperature**	**pH**
First order r^2	0.018	0.449
AIC	26440	14829
Second order r^2	0.116	0.452
AIC	23789	14743
Third order r^2	0.188	0.497
AIC	21867	13541

5. DISCUSSION

5.1 Hydrogeochemistry of Kupinde Lake

The mean temperature of Kupinde Lake water was 22.58 ^{O}C and varies over a range of spatial and temporal scales. The pH of Kupinde Lake water was 8.16, ranging from 7.9 to 9.2, indicating the lake is slightly alkaline. A variety of factors affect the pH of freshwater, including regional geomorphology, hydrology, climate, anthropogenic factors, rainfall, and soil type (Feng et al. 2017, Zhu et al. 2022). The pH of lake water was found to be alkaline for all the sampling sites and shown to be within the permissible limit of the World Health Organization (WHO). The pH values in this study are consistent with the previous studies (Singh et al. 2016, Gurung et al. 2018, Rout & Vasudevan 2022), and (Pant et al. 2021). The mean EC was found to be 136.54 μS/cm with range from 129 to 143 μS/cm and TDS was 68.25 mg/L with range from 64 to 72 mg/L. The EC of Chandra Tal (195.95) and Rara lake (193.85) are comparatively higher (Kaphle et al. 2021, Rout & Vasudevan 2022), whereas Ramarosan (67.85) and Rajarani (54.05) are comparatively lower (Adhikari et al. 2020, Dangol et al. 2022). The EC is the parameter which indicates the mobility of the major ions and is directly related to the total dissolved solids (TDS). Minerals, salts, metals, ions, and organic matter dissolved in water constitute TDS. EC and TDS were substantially associated among hydrochemical parameters. As a result the highest and lowest EC and TDS values were found in the sample locations K18 and K20. The highest concentration of EC and TDS are most likely owing to road construction, whereas the lowest concentrations are most likely due to Kailubraha Temple at the sampling points. Both EC and TDS were found to be below WHO acceptable limits. Hence, the water of the lake is suitable for drinking as well as for sustaining aquatic ecosystems.

The surface water contains naturally occurring calcium (Ca^{2+}) and magnesium (Mg^{2+}). In this study, Ca^{+2} are the dominant cation, with a mean concentration of 27.97 mg/L, and ranges from 22.4 to 36 mg/L. The mean concentration of Mg^{2+} is 11.23 mg/L with a range from 4.39 to 20.5 mg/L. There are many factors that influence the concentrations of calcium and magnesium in surface water. These factors include geological contents, soil type, plant cover, weather conditions, land relief, and type and intensity of water supply (Potasznik & Szymczyk 2015). Ca^{2+} and Mg^{2+} levels were found to be less than WHO recommendations. This could be due to the less weathering of the underlying rocks and also relatively less anthropogenic interferences. Furthermore, calcium and magnesium are the major ions responsible for the total hardness. Hardness of the water ranged from 86 to 174 mg/L with the mean value at 115.92 mg/L. Total hardness can be classified as follows: 1) soft (0-30), 2) moderately soft (30-60), 3) moderately hard (60-120), 4) hard (120-180), and 5) very hard (>180) (WHO 2011). In this study, most of the samples are classified as moderately hard. The main causes of moderate water hardness could be due to the reason that the weathering of major ions in the region is relatively less intense (Mallick 2017). Total calcium hardness ranges from 56 to 90 mg/L with mean 69.92 mg/L. Magnesium hardness varies from 18 to 84 mg/L with mean 46 mg/L. Na^+ concentrations range from 6.5 to 8.5 mg/L with a mean value of 7.38 mg/L. K^+ varies between 2.4 and 4.9 mg/L with a mean value of 3.47 mg/L. NH_4^+ is in the range of 0.09 to 0.44 mg/L with a mean value of 0.11 mg/L. The dominancy order of the major cations were $Ca^{2+} > Mg^{2+} > Na^+ > K^+ > NH_4^+$. This finding is consistent with measured by Rara Lake (Kaphle et al. 2021), Lato lake (Kaushik et al. 2021) and Chandra Tal (Rout & Vasudevan 2022).

The bicarbonate (HCO_3^-) concentration in lake water ranged from 40 to 300 mg/L with a mean of 157.71 mg/L. It is most dominant among anion, and is formed primarily from the weathering and decomposition of organic matter in the catchment (Jha et al. 2009). Additionally, bicarbonates are major constituents of carbonate rocks containing calcite ($CaCO_3$) and dolomite ($CaMg(CO_3)^2$) (Mallick 2017). In the present study, the concentrations of Cl^- range from 4.26 to 8.52, with a mean of 6.04 indicating that atmospheric inputs and anthropogenic sources have a minor influence on chloride input to the lake (Pant et al. 2021). Furthermore, SO_4^{2-} varies from 0.13 to 2.28 mg/L with mean 0.64 mg/L. Moreover, PO_4^{3-}varies from 0.11 to 0.29 mg/L with mean value 0.19 mg/L. Limited amounts of NO_3^- are present in the present study varying from 0.05 to 0.06 mg/L with mean value 0.06 mg/L. Among the dissolved ions, Cl^-, NO_3^-, and SO_4^{2-} mainly come from anthropogenic sources, such as fertilizers applied to fields, industrial effluents, and atmospheric emissions (Sun et al. 2010). However, the area surrounding the Lake shows less anthropogenic disturbance, indicating that it is less polluted. The dominancy order of the major anions were HCO_3^- > Cl^- >SO_4^{2-} > PO_4^{3-} > NO_3^-. Previous investigations in the Rajarani Lake (Adhikari et al. 2020), Jhilmila Lake (Pal et al. 2021), Ramarosan Lake (Dangol et al. 2022) and Ghodadhi Lake (Pant et al. 2021) indicated similar superiority of major anions.

5.1.1 Hydrogeochemical facies

Piper plot (Piper 1944) was used for the characterization and source identification of major ions. The milli-equivalent percentage of major ions was plotted in the trilinear diagram. A water type is classified and named based on the previous studies (Pant et al. 2021) . On the Piper diagram, both cations and anion fall into the Ca-HCO_3 type in the central diamond field. As a consequence of these findings, carbonate dominated lithology is considered the dominant mechanism that regulates the hydrogeochemistry of Kupinde Lake. The finding of this study is to similar with the Phewa lake lesser Himalayan (Khadka & Ramanathan 2021) , Ghodaghodi Lake (Pant et al. 2020) and Rara Lake (Kaphle et al. 2021).

5.1.2 Controlling mechanism of the Hydrochemistry

A Gibbs diagram can be used to determine the major chemistry-controlling mechanisms of the water bodies (Gibbs 1970). The effects of hydrochemistry on the surface water environment include precipitation, rock weathering, and evaporation (Pant et al. 2018). The lower concentration of TDS and higher ratios of Na^+/ (Na^+ + Ca^{2+}) and Cl^-/ (Cl^- + HCO_3^-) suggest precipitation influences which lies in the lower right corner. Generally, rock weathering occur when the TDS concentration is moderate and the ratios of Na^+/ (Na^+ + Ca^{2+}) and Cl^-/ (Cl^- + HCO_3^-) are lower on the left. Likewise, the upper right corner of the diagram represents dominance of evaporation which influence the concentrations of TDS with high of Na^+/ (Na^+ + Ca^{2+}) and Cl^-/ (Cl^- + HCO_3^-) ratios (Pant et al. 2018) . In the present study, carbonate weathering was shown to have a dominant influence on rock weathering. This is evidenced by the increase concentration of major cations Ca^{2+}, Na^+ and anions HCO_3^- and Cl^- in the lake catchment. This finding is similar to those of previous studies, which have shown that rock weathering significantly impacts Himalayan lakes (Kaphle et al. 2021, Kaushik et al. 2021). In another study (Adhikari et al. 2020), they found similar results that supported the present findings.

The Kupinde Lake water chemistry is originated from the chemical weathering of rock. Generally, carbonates, silicates, and evaporates are the dominant sources of rock weathering and Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, HCO_3^{-}, SO_4^{2-}, and Cl^{-} influenced to chemical rock weathering in hydrochemical composition (Pant et al. 2018). The findings of this study indicate that carbonate are the most common types of rock weathering. The weathering process for carbonate rock is more rapid than that for silicate rocks (Fan et al. 2014). Carbonates and silicates are formed based on the amount of TDS and CO_2 in water (Fan et al. 2014). Based on the results of this study, the dominant tendency of TDS has affected the formation of carbonates. In contrast, the lowest trend of CO_2 has affected the formation of silicates.

5.1.3 Clustering of physiochemical parameters

In hydrogeochemistry, the correlation matrix is widely used to determine the degree of dependence between two variables by establishing their relationship (Singh et al. 2016) . Na^{+}-Ca^{2+}, Mg^{2}-Ca^{2+}, Ca^{2+}-MgH, and Ca^{2+}-CaH have positive correlation coefficients, indicating a common origin due to intense weathering of carbonate rock (Singh et al. 2016). In the same way, the correlation coefficient of Cl^{-} - NO_3^{-}, CO_2- NO_3^{-}, CO_2- NO_3^{-} , and CO_2 - Cl^{-} are positive, indicating that these materials have a common origin. There is a positive correlation between EC -TDS, temperature - HCO_3^{-}, and NH_4^{+} - pH. In addition, the relation between K^{+} - temperature, Na^{+} - pH shows negative. Furthermore, there is a negative correlation between K^{+} and temperature, as well as Na^{+} and pH.

In this study, the major ions (HCO_3^{-}, Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Cl^{-}, SO_4^{2-} , NO_3^{-} , PO_4^{3-} and NH_4^{+}) was used to perform cluster analysis. All the 24 sampling points were grouped into 5 main clusters with low distance criterion from 0 to 5. Cluster 1 formed by sampling points (4, 5, 6, 17, 18, & 20). Cluster 1 contains fewer disturbances because samples 4, 5, and 6 were collected in large rocky mountain areas. Sample 17 and 18 are located in dense forest, and sampling 20 lies near the Kailubrah Temple. In addition, Cluster 2 (sampling points 19 and 21) is the most disturbed area due to anthropogenic activity, as it is near the road and lobby. Both cluster 3 and 4 are situated on the periphery of the lake and are connected by a road and small path. Cluster 3 is composed of sampling points (1, 2, 3, 7, 13, 14, & 23) and cluster 4 is composed of sampling points 9 and 10. However, cluster 5 was formed by the inlet and center point of sampling points (8, 11, 12, 15, 16, 22, & 24).

A PCA is a powerful tool for hydrochemical studies that reduces the dimensionality of large datasets by explaining variance with a smaller set of independent variables. In this study the PCA was applied to TDS and the major cations and anions. In the definition of factor loadings, a factor loading is graded as strong, moderate, or weak when it is greater than 0.75, between 0.75 and 0.50, and between 0.50 and 0.30 (Pant et al. 2020) . The first principal component (PC1) explains 24.78 % of the variance in the data, and CaH, MgH, Mg^{2+}, and Ca^{2+} are strongly positively loaded, which might be due to common sources of ions. PC2 shows strong positive loadings with Cl^- and NO_3^- whereas HCO_3^- displays moderately negative loadings, which explains about 14.43 % of total variance in the results. The influences of rainfall and anthropogenic inputs on lakes are discussed here. In PC3, PO_4^{3-} has a moderately positive loading and SO_4^{2-} has a moderately negative loading, which accounts for 13.08 % of the variance in the result indicating less polluted in lake water. Similarly, it is observed that NH_4^+ is moderately loaded in PC4 and Na^+ is strongly negative loaded, which accounts for 12.09 % of the variance. The TDS is strongly negative loaded and the K^+ is moderately positive loaded, explaining 11.66 % of the total variance in PC5.

5.1.4 Water quality status

All sampling points were found excellent in the EC's categorization, and the Kupinde lake water was deemed suitable for irrigation. Irrigation water can be classified into five types in terms of Na^+ (Acharya et al. 2020). Similarly, average values of Na% is 15.53, categories as excellent with compare standard value. The sodium absorption ratio (SAR) is 0.30, which is divided into three categories depending on SAR content, with lowest SAR content indicating ideal for irrigation. Wilcox diagram exhibit, water samples of Kupinde Lake was plotted into C1S1 section, indicating excellent category for irrigation.

In addition KR values are within the safe category for irrigation, the lake water is suitable for irrigation. Irrigation water was divided into three categories based on the PI values. The average PI value indicated that the water was of good quality. In this investigation, the CROSS values of the lake water are excellent category.

5.2 Relationships between amphibian community and water quality parameters

The results indicate that temperature, pH, TDS, alkalinity, calcium, sulphate, and sodium have significant impacts on anuran abundance in Kupinde Lake. The abundance of amphibians and the temperature were negatively correlated. The temperature of the water is influenced by the temperature of the air and the intensity of solar radiation (Oli et al. 2013). Changes in temperature can affect breeding activities and early growth (Wheeler et al. 2015, Catenazzi & Kupferberg 2017). A rise in water temperature influences larval development and survival until metamorphosis (Skelly et al. 2002). The pH levels were consistently normal at all sampling points (mean pH 8.16). This is consistent with scientific studies on maintaining a balance of hydrogen ions in amphibian habitats within a pH range of 6.5 to 8.5. Water with low pH can affect amphibians' reproductive directly by killing embryos and larvae, and disrupt trophic relationships between them and other aquatic animals (Serrano et al. 2016). The species abundance was high with a minimum TDS of 64 ppm. Amphibians need a TDS value between 50 and 250 ppm to survive and anything below this range will be detrimental (Shaikh et al. 2014). A negative association was found between TDS and amphibians. TDS solution contains several ions, including sodium, chloride, potassium, magnesium, sulfate, chloride and bicarbonate (Chapman et al. 2000). There might be a similar effect achieved by different ions at a similar concentration, however, it depends on their identity and concentration. The volume of TDS was extremely high compared to normal, which could lead to amphibian mortality due to excessive organic and inorganic components (Shaikh et al. 2014). It appears that bicarbonate is the most dominant anion among the major anions. The results indicate that it has a significant effect on amphibian abundance. Generally, bicarbonate is derived from calcium carbonate rocks ($CaCO_3$) and calcium magnesium carbonate rocks (dolomite) (Mallick 2017). A significant impact of amphibian abundance was found in the current study. In frog blood, the calcium concentration is similar to that of vertebrates. Calcium metabolism in amphibians seems to involve endocrine and humoral factors such as parathyroid hormone, calcitonin, vitamin D, and prolactin. The amount of calcium varies with the season, increasing in spring and summer and decreasing in winter (Stiffler 1993). Amphibian abundance is negatively correlated with sulphate and sodium levels in the lake due to its extremely low levels. Water quality is considered the main factor influencing health and disease in all aspects of a biotic system. Hydrochemistry is little studied in relation to *Euphlytis cyanophlyctis.* Further research is required to determine how these factors interact with each other.

6. CONCLUSION AND RECOMMENDATION

The hydrochemistry and the water quality the Kupinde Lake were assessed. The water has a pH of slightly alkaline and moderately hard in nature, while its EC and TDS are within WHO guidelines. Moreover, the chemical analysis results indicate that major cations are most abundant in the order $Ca^{2+} > Mg^{2+} > Na^{+} > K^{+} > NH_4^{+}$, while anions are most abundant in the order $HCO_3^{-} > Cl^{-} > SO_4^{2-} > PO_4^{3-} > NO_3^{-}$. Based on the results of this study, all physicochemical parameters were within the WHO permissible limits. In a piper trilinear plot, Ca^{2+} and HCO_3^{-} increase in cation and anion triangles that fall into the Ca-HCO_3 type, indicating the dominant hydrochemistry of Lake water is carbonate weathering. In multivariate statistical analysis, it was found that lake water's hydrochemistry is primarily influenced by carbonate rock weathering with minor contribution of the silicates. Based on the irrigation parameters of Kupinde Lake water, all samples fell within the excellent category, thus the lake water is an excellent irrigation resource. Amphibian abundance was negatively affected by the concentration of temperature, TDS, sulphate, and sodium while significant changes in pH, alkalinity, and calcium. This study provides a set of reference values for assessing the potential impacts of development around the lake.

Recommendation

This study recommends that hydrochemistry and anuran amphibian association should be studied in the Himalayan lakes. The specific recommendations are as follows:

- For a comprehensive understanding of hydrochemistry, a seasonal and depth-wise study is necessary.
- In order to understand whether *Euphlytis cyanophlyctis* and hydrochemistry are related, it is necessary to conduct physiological and ecological studies.

REFERENCES

Acharya, A., Pant, R. R., Pokhrel, B. N., Neupane, S., Adhikari, B., Chaudhari, S. K., et al. 2020. Chemical characteristics of the Karmanasha River water and its appropriateness for irrigational usage. Journal of Nepal Chemical Society **41**(1):94–102. https://doi.org/10.3126/jncs.v41i1.30494.

Adhikari, B., Pant, R. R., Baral, U., Shrestha, S., Neupane, S., Khanal, B., et al. 2020. Geochemical and multivariate assessment of water quality in the Rajarani Lake, Dhankuta, Nepal. Journal of Nepal Geological Society **60**:37–49. https://doi.org/10.3126/jngs.v60i0.31264.

Ali, W., Javid, A., Hussain, A. and Bukhari, S. M. 2018. Diversity and habitat preferences of amphibians and reptiles in Pakistan: a review. Journal of Asia-Pacific Biodiversity **11**(2):173–187. https://doi.org/10.1016/j.japb.2018.01.009.

Amankwaa, G., Yin, X., Zhang, L., Huang, W., Cao, Y. and Ni, X. 2020. Hydrochemistry and multivariate statistical analysis of the quality of water from Lake Bosomtwe for agricultural and human consumption. Journal of Water Supply: Research and Technology-Aqua **69**(7):704–719. https://doi.org/10.2166/aqua.2020.061.

Babini, M. S., de Lourdes Bionda, C., Salinas, Z. A., Salas, N. E. and Martino, A. L. 2018. Reproductive endpoints of Rhinella arenarum (Anura, Bufonidae): Populations that persist in agroecosystems and their use for the environmental health assessment. Ecotoxicology and Environmenal Safety **154**:294–301. https://doi.org/10.1016/j.ecoenv.2018.02.050.

Bhandari, B. B. 2009. Wise use of Wetlands in Nepal. Banko Janakari **19**(3):10–17. https://doi.org/10.3126/banko.v19i3.2206.

Bhowmik, S. 2020. Ecological and economic importance of wetlands and their vulnerability. In: P. K. Rathoure and P. B. Chahaun (Eds) Current State and Future Impacts of Climate Change on Biodiversity. Shriram Institute for Industrial Research, Delhi. pp 95–112.

Boyer, R. and Grue, C. E. 1995. The need for water quality criteria for frogs. Environ Health Perspect **103**(4):352–357. https://doi.org/10.1289/ehp.95103352.

Calderon, M. R., Almeida, C. A., González, P. and Jofré, M. B. 2019. Influence of water quality and habitat conditions on amphibian community metrics in rivers affected by urban activity. Urban Ecosystems **22**(4):743–755. https://doi.org/10.1007/s11252-019-00862-w.

Catenazzi, A. and Kupferberg, S. J. 2017. Variation in thermal niche of a declining river-breeding frog: From counter-gradient responses to population distribution patterns. Freshwater Biology **62**(7):1255–1265. https://doi.org/10.1111/fwb.12942.

CBS 2011. Government of Nepal Central bureau of statistics

Chapman, P. M., Bailey, H. and Canaria, E. 2000. Toxicity of total dissolved solids associated with two mine effluents to chironomid larvae and early life stages of rainbow trout. Environmental Toxicology and Chemistry **19**(1):210-214. https://doi.org/10.1002/etc.5620190125.

Cimon-Morin, J. and Poulin, M. 2018. Setting conservation priorities in cities: approaches, targets and planning units adapted to wetland biodiversity and ecosystem services. Landscape Ecology **33**(11):1975–1995. https://doi.org/10.1007/s10980-018-0707-z.

Compton, B. W., McGarigal, K., Cushman, S. A. and Gamble, L. R. 2007. A resistant-kernel model of connectivity for amphibians that breed in vernal pools. Conservation Biology **21**(3):788-799. https://doi.org/10.1111/j.1523-1739.2007.00674.x.

Dangol, A., Pant, R. R., Chalaune, T. B., Sharma, J., Thapa, B., Thapa, L. B., et al. 2022. Hydrochemical Characterization of Lentic and Lotic Environments of Ramaroshan Area, Sudurpaschim Province, Nepal. Geographical Journal of Nepal:83–102. https://doi.org/10.3126/gjn.v15i01.42888.

Das, B. and Kaur, P. 2001. Major ion chemistry of Renuka Lake and weathering processes, Sirmaur District, Himachal Pradesh, India. Environmental Geology **40**(7):908–917. https://doi.org/10.1007/s002540100268.

Doneen, L. D. 1964. *Notes on water quality in agriculture*. Department of Water Science and Engineering, University of California, Davis. p.

Egbueri, J. C. 2019. Evaluation and characterization of the groundwater quality and hydrogeochemistry of Ogbaru farming district in southeastern Nigeria. SN Applied Sciences **1**(8). https://doi.org/10.1007/s42452-019-0853-1.

Fan, B.-L., Zhao, Z.-Q., Tao, F.-X., Liu, B.-J., Tao, Z.-H., Gao, S., et al. 2014. Characteristics of carbonate, evaporite and silicate weathering in Huanghe River basin: A comparison among the upstream, midstream and downstream. Journal of Asian Earth Sciences **96**:17–26. https://doi.org/10.1016/j.jseaes.2014.09.005.

Feng, Z., Su, B., Xiao, D.-D. and Ye, L.-Y. 2017. Study on pH value and its variation characteristics of the main rivers into Dianchi lake under the anthropogenic and natural processes, Yunnan, China. Journal of Information and Optimization Sciences **38**(7):1197-1210. https://doi.org/10.1080/02522667.2017.1367501.

Foti, R., del Jesus, M., Rinaldo, A. and Rodriguez-Iturbe, I. 2012. Hydroperiod regime controls the organization of plant species in wetlands. Proceedings of the National Academy of Sciences **109**(48):19596–19600. https://doi.org/10.1073/pnas.1218056109.

Gaury, P. K., Meena, N. K. and Mahajan, A. K. 2018. Hydrochemistry and water quality of Rewalsar Lake of Lesser Himalaya, Himachal Pradesh, India. Environment Monitoring Assessment **190**(2):84. https://doi.org/10.1007/s10661-017-6451-z.

Ghosh, D. and Basu, P. 2020. Factors influencing herpetofauna abundance and diversity in a tropical agricultural landscape mosaic. Biotropica **52**(5):927–937. https://doi.org/10.1111/btp.12799.

Gibbs, R. J. 1970. Mechanisms controlling world water chemistry. Science **170**(3962):1088-1090.

Gorde, S. P. and Jadhav, M. V. 2013. Assessment of Water quality parameters: A Review. International Journal of Engineering Research and Applications **3**(6):2029–2035.

Gurung, S., Gurung, A., Sharma, C. M., Jüttner, I., Tripathee, L., Bajracharya, R. M., et al. 2018. Hydrochemistry of Lake Rara: A high mountain lake in western Nepal. Lakes & Reservoirs: Science, Policy and Management for Sustainable Use **23**(2):87–97. https://doi.org/10.1111/lre.12218.

Hecnar, S. J. 2004. Great Lakes wetlands as amphibian habitats: A review. Aquatic Ecosystem Health & Management **7**(2):289–303. https://doi.org/10.1080/14634980490461542.

Hey, D. L., Kostel, J. A., Crumpton, W. G., Mitsch, W. J. and Scott, B. 2012. The roles and benefits of wetlands in managing reactive nitrogen. Journal of Soil and Water Conservation **67**(2):47A–53A. https://doi.org/10.2489/jswc.67.2.47A.

Jha, P. K., Tiwari, J., Singh, U. K., Kumar, M. and Subramanian, V. 2009. Chemical weathering and associated CO2 consumption in the Godavari river basin, India. Chemical Geology **264**(1-4):364-374. https://doi.org/10.1016/j.chemgeo.2009.03.025.

Kaphle, B., Wang, J.-b., Kai, J.-l., Lyu, X.-m., Paudayal, K. N. and Adhikari, S. 2021. Hydrochemistry of Rara Lake: A Ramsar lake from the southern slope of the central Himalayas, Nepal. Journal of Mountain Science **18**(1):141–158. https://doi.org/10.1007/s11629-019-5910-0.

Kaushik, H., Ramanathan, A. L., Soheb, M., Shamurailatpam, M. S., Biswal, K., Mandal, A., et al. 2021. Climate change-induced high-altitude lake: Hydrochemistry and area changes of a moraine-dammed lake in Leh-Ladakh. Acta Geophysica **69**(6):2377–2391. https://doi.org/10.1007/s11600-021-00670-x.

Khadka, U. R. and Ramanathan, A. L. 2021. Hydrogeochemical analysis of Phewa Lake: A lesser Himalayan Lake in the Pokhara Valley, Nepal. Environment and Natural Resources Journal **19**(1):68–83. https://doi.org/10.32526/ennrj/19/2020083.

Kingsford, R. T., Basset, A. and Jackson, L. 2016. Wetlands: conservation's poor cousins. Aquatic Conservation: Marine and Freshwater Ecosystems **26**(5):892–916. https://doi.org/10.1002/aqc.2709.

Linzey, D., Burroughs, J., Hudson, L., Marini, M., Robertson, J., Bacon, J., et al. 2003. Role of environmental pollutants on immune functions, parasitic infections and limb malformations in marine toads and whistling frogs from Bermuda. International Journal Environmental Health Research **13**(2):125–148. https://doi.org/10.1080/0960312031000098053.

Mallick, J. 2017. Hydrogeochemical characteristics and assessment of water quality in the Al-Saad Lake, Abha Saudi Arabia. Applied Water Science **7**(6):2869–2882. https://doi.org/10.1007/s13201-017-0553-1.

Mann, R. M., Hyne, R. V., Choung, C. B. and Wilson, S. P. 2009. Amphibians and agricultural chemicals: review of the risks in a complex environment. Environmental pollution **157**(11):2903–2927. https://doi.org/10.1016/j.envpol.2009.05.015.

Mayanglambam, B. and Neelam, S. S. 2020. Physicochemistry and water quality of Loktak Lake water, Manipur, India. International Journal of Environmental Analytical Chemistry **102**(7):1638–1661. https://doi.org/10.1080/03067319.2020.1742888.

McCarthy, M. J., Radabaugh, K. R., Moyer, R. P. and Muller-Karger, F. E. 2018. Enabling efficient, large-scale high-spatial resolution wetland mapping using satellites. Remote Sensing of Environment **208**:189–201. https://doi.org/10.1016/j.rse.2018.02.021.

Mifsud, D. A. 2014. A status assessment and review of the herpetofauna within the Saginaw Bay of Lake Huron. Journal of Great Lakes Research **40**:183–191. https://doi.org/10.1016/j.jglr.2013.09.017.

Newton, A., Icely, J., Cristina, S., Perillo, G. M. E., Turner, R. E., Ashan, D., et al. 2020. Anthropogenic, Direct Pressures on Coastal Wetlands. Frontiers in Ecology and Evolution **8**. https://doi.org/10.3389/fevo.2020.00144.

Oli, B. B., Jha, D. K., Aryal, P. C., Shrestha, M. K., Dangol, D. R. and Gautam, B. 2013. Seasonal variation in water quality and fish diversity of Rampur Ghol, a wetland in Chitwan, Central Nepal. Nepalese Journal of Biosciences **3**(1):9–17. https://doi.org/10.3126/njbs.v3i1.41420.

Pal, K. B., Bishwakarma, K., Chalaune, T. B., Upadhaya, D., Joshi, T. R., Thapa, L. B., et al. 2021. Hydrochemical assessment Of Jhilmila Lake, Kanchanpur, Nepal. Scientific World **14**(14):124–131. https://doi.org/10.3126/sw.v14i14.35023.

Pant, R. R., Pal, K. B., Bishwakarma, K., Thapa, L. B., Dangol, A., Dawadi, B., et al. 2020. Application of Multivariate Approaches to the Hydro-chemical Assessment of the Ghodaghodi Lake, Sudurpaschim Province, Nepal. **19**((2)):46–54. https://doi.org/10.3126/njst.v20i1.39390.

Pant, R. R., Pathak, G., Pal, K. B., Bishwakarma, K., Thapa, L. B., Dhital, Y. P., et al. 2021. Water quality and health risk assessment of trace elements contamination in Ghodaghodi Lake, Sudurpaschim Province, Nepal. Nepal Journal of Environmental Science **9**(1):29–40. https://doi.org/10.3126/njes.v9i1.36603.

Pant, R. R., Qaiser, F. U. R., Wang, G., Adhikari, S., Bishwakarma, K., Baral, U., et al. 2021. Hydrochemical appraisal and solute acquisitions in Seti River Basin, Central Himalaya, Nepal. Environmental monitoring and assessment **193**(10):656. https://doi.org/10.1007/s10661-021-09437-9.

Pant, R. R., Zhang, F., Rehman, F. U., Wang, G., Ye, M., Zeng, C., et al. 2018. Spatiotemporal variations of hydrogeochemistry and its controlling factors in the Gandaki River Basin, Central Himalaya Nepal. Science of the Total Environment **622-623**:770–782. https://doi.org/10.1016/j.scitotenv.2017.12.063.

Paulding, C. and Randhir, T. O. 2021. An ecohydrological assessment of potential impacts of climate change on the herpetofauna. Sustainability and Climate Change **14**(1):22–34. https://doi.org/10.1089/scc.2020.0027.

Pedersen, E., Weisner, S. E. B. and Johansson, M. 2019. Wetland areas' direct contributions to residents' well-being entitle them to high cultural ecosystem values. Science of the Total Environment **646**:1315–1326. https://doi.org/10.1016/j.scitotenv.2018.07.236.

Pfister, S., Bayer, P., Koehler, A. and Hellweg, S. 2011. Projected water consumption in future global agriculture: scenarios and related impacts. Science of the Total Environment **409**(20):4206–4216. https://doi.org/10.1016/j.scitotenv.2011.07.019.

Piper, A. M. 1944. A graphic procedure in the geochemical interpretation of water-analyses. **25**(6):914–928. https://doi.org/10.1029/TR025i006p00914.

Pollet, I. and Bendell-Young, L. I. 2000. Amphibians as indicators of wetland quality in wetlands formed from oil sands effluent. Environmental Toxicology and Chemistry **19**(10):2589–2597. https://doi.org/10.1002/etc.5620191027.

Potasznik, A. and Szymczyk, S. 2015. Magnesium and calcium concentrations in the surface water and bottom deposits of a river-lake system. Journal of Elementology **20**(3):677–692. https://doi.org/10.5601/jelem.2015.20.1.788.

R-Core-Team 2022. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Rastegar-Pouyani, N., Gholamifard, A., Karamiani, R., Bahmani, Z., Mobaraki, A., Abtin, E., et al. 2015. Sustainable management of the Herpetofauna of the Iranian plateau and coastal Iran. Amphibian and Reptile Conservation **9**:1–15.

Richards, L. A. 1954. *Diagnosis and improvement of saline and alkali soils*. LWW. p.

Rijal, S., Rauniyar, A., Thapa, S., Paudel, U. and Gautam, D. 2021. Ecosystem services of Wetlands and threats in context of Nepal. International Journal of Forest, Soil and Erosion **11**(2).

Rittenhouse, T. A. G. and Semlitsch, R. D. 2007. Distribution of amphibians in terrestrial habitat surrounding wetlands. Wetlands **27**(1):153-161. https://doi.org/10.1672/0277-5212(2007)27[153:Doaith]2.0.Co;2.

Rout, S. P. and Vasudevan, S. 2022. Hydrogeochemical delineation of the Chandra Tal: a high-altitude lake in Chandra Valley, Lahul and Spiti, Himachal Pradesh, India.

Arabian Journal of Geosciences **15**(10). https://doi.org/10.1007/s12517-022-10198-z.

Salman, N. A. 2019. A Review of Southern Iraq Herpetofauna. Biological and Applied Envrironmental Research **3**(1):61–71.

Sapkota, M., Pant, R. R., Pathak, L., Khanal, B., Shrestha, S., Poudel, B., et al. 2021. Assessment of water quality using multivariate statistical approaches in Jagadishpur Reservoir, Lumbini Province, Nepal. **7**(5):1-12.

Serrano, L., Diaz-Paniagua, C., Gomez-Rodriguez, C., Florencio, M., Marchand, M. A., Roelofs, J. G., et al. 2016. Susceptibility to acidification of groundwater-dependent wetlands affected by water level declines, and potential risk to an early-breeding amphibian species. Science of the Total Environment **571**:1253-1261. https://doi.org/10.1016/j.scitotenv.2016.07.156.

Shah, K. B. and Tiwari, S. 2004. *Herpetofauna of Nepal: A conservation companion*. IUCN Nepal. p 237.

Shaikh, K., Gachal, G. S., Memon, S. Q., Ahmed, N., Sodho, M. and Yusuf, S. 2014. Assessment of amphibian environment through physicochemical analysis in Pakistan. Journal of Biodiversity and Environmental Sciences (JBES) **5**((3)):255–261. https://doi.org/10.1.1.657.1086.

Sharma, C. M. 2008. Freshwater fishes, fisheries, and habitat prospects of Nepal. Aquatic Ecosystem Health & Management **11**(3):289–297. https://doi.org/10.1080/14634980802317329.

Singh, V. B., Ramanathan, A. L. and Mandal, A. 2016. Hydrogeochemistry of high-altitude lake: a case study of the Chandra Tal, Western Himalaya, India. Arabian Journal of Geosciences **9**(4). https://doi.org/10.1007/s12517-016-2358-1.

Skelly, D. K., Freidenburg, L. K. and Kiesecker, J. M. 2002. Forest canopy and the performance of larval amphibians. Ecology **83**(4):983–992. https://doi.org/10.1890/0012-9658(2002)083[0983:Fcatpo]2.0.Co;2.

Stiffler, D. F. 1993. Amphibian calcium metabolism. journal of Experimental Biology **184**(1):47-61. https://doi.org/10.1242/jeb.184.1.47.

Sun, H., Han, J., Li, D., Zhang, S. and Lu, X. 2010. Chemical weathering inferred from riverine water chemistry in the lower Xijiang basin, South China. Science of the Total Environment **408**(20):4749–4760. https://doi.org/10.1016/j.scitotenv.2010.06.007.

Thakuri, S. and Pokhrel, G. K. 2017. Herpetofaunal diversity in Manaslu Conservation Area, Nepal. Our nature **14**(1):99–106. https://doi.org/10.3126/on.v14i1.16448.

Wheeler, C. A., Bettaso, J. B., Ashton, D. T. and Welsh, H. H. 2015. Effects of water temperature on breeding phenology, growth, and metamorphosis of foothill Yellow-Legged Frogs (Rana boylii): A case study of the regulated mainstem and unregulated tributaries of California's trinity River River Research and Applications **31**(10):1276–1286. https://doi.org/10.1002/rra.2820.

WHO 2011. Guidelines for drinking-water quality. WHO chronicle **38**(4):104-108.

Zhou, L., Guan, D., Huang, X., Yuan, X. and Zhang, M. 2020. Evaluation of the cultural ecosystem services of wetland park. Ecological Indicators **114**. https://doi.org/10.1016/j.ecolind.2020.106286.

Zhu, D., Cheng, X., Li, W., Niu, F. and Wen, J. 2022. Temporal and spatial variation characteristics of water quality in the middle and lower reaches of the Lijiang River, China and their responses to environmental factors. International Journal of Environmental Research and Public Health **19**(13). https://doi.org/10.3390/ijerph19138089.