

Research Article

Diversity of Freshwater Algae of Khotokha Ramsar Wetland, Wangduephodrang District, Bhutan

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ABSTRACT

This study on the algal diversity at Khotokha Ramsar Wetland in the Wangdue Phodrang district sought to count the species of algae, ascertain the impact of aquatic environment's physicochemical factors, and compare the diversity of algae among various habitats. Algal samples were collected from rivers and streams using a systematic sampling method, and ponds were sampled purposefully. Fifteen of the thirty-nine algae were identified to the species level, ten of which are new to Bhutan. Additionally, the study found that the greatest diversity of algae is found in ponds (32 species), followed by streams (25 species) and rivers (21 species). With 16 distinct species (37%) under the Ochrophyta genus, the Khotokha Ramsar wetland has the highest species richness of these, followed by Cyanobacteria (10 species, 23%), Charophyta (8 species, 19%), and Chlorophyta (6 species, 14%). Heterokonta had the lowest species richness. According to the study, ponds with extensive algal diversity had higher water quality than rivers and streams.

Key words: Diversity, Freshwater, Physiochemical, Parameters, Systematic

INTRODUCTION

Algae are simple organisms without appropriate roots, stems, leaves and sexual organs are not enclosed in a protective covering (Sahoo & Seckbach, 2015). The size of algae varies from unicellular microscopic to giant sea kelp which is visible to naked eyes (Bellinger, 2015). Algae are widely distributed as substrate associated organisms such as rocks, soil, ice, woods, plants and animals (Bellinger, 2015). Aquatic algae are found in freshwater lakes, streams, ponds and wetlands (Wehr, 2003). The growth of algae depends on numerous factors such as light intensity, photoperiod, temperature, salinity, pH, depth of water bodies and amount of nitrogen (Gani *et al.*, 2019). Algae being photosynthetic organisms play a major role in maintaining the ecosystem through transferring of energy, oxygenating the water ecosystem and serve as food for herbivores of aquatic ecosystem (Sahoo & Seckbach, 2015). As photosynthetic organisms, they are responsible for absorbing carbon from atmosphere and putting back oxygen acting as the sink of carbon sequestration (Jyothi *et al.*, 2016).

Bhutan is one of the global biological hotspots however, organisms such as algae are less studied (Banerjee & Bandopadhyay, 2016). Besides, identifying algal species in a field is a challenge due to technical constraint and taxonomic difficulty (Taylor *et al.*, 2007). The neighboring countries Nepal and India have reported a large number of algae while Bhutan has recorded only 109 algae (Ghalley *et al.*, 2023).

Algae are also ecosystem bio-indicators since they are responsive to environmental changes and can be used as a benchmark to gauge the ecosystem's health (Wehr, 2011). Algae can be used to measure the amount

and severity of pollution in an ecosystem, which prompts the need for human action to stop pollution-causing activities (Jyothi *et al.*, 2016).

Algae are another factor in algal blooms because when they predominate a certain ecosystem on the surface, sunlight cannot reach the benthic level, which kills aquatic life (Environmental & Agency, 2002). Less emphasis is placed on algae, despite the fact that they play a significant role in ecosystems and form the foundation of the food chain.

Algae as a primary producer of wetland aquatic ecosystem, it maintains the diversity of aquatic flora and fauna by serving as food source for smaller faunal organisms such as macro-invertebrates.

Therefore, this study of enumerating algal diversity at Khotokha wetland is to identify algae species present in the area, compare diversities of algae among different habitats and determine the effect of physiochemical parameters on diversity of algae.

MATERIALS AND METHODS

Study Area

Khotokha wetland is located under Bjena Geog in Wangdue district. The area had been designated as Ramsar Wetland of international importance on 7th May, 2012 with Ramsar Site Number 2033 (Tshering *et al.*, 2021) and located at 27° 25' 54.92" N and 89° 59' 33.27" E. The elevation of the area ranges between the minimum of 2500 masl to 2700 masl measuring a total area about 113.5 ha (Anderson & Davis, 2013). The average maximum annual temperature is 15.5°C and the average minimum temperature is 3.50°C. The average minimum and maximum rainfall of the area are 2361 mm and 94 mm respectively.

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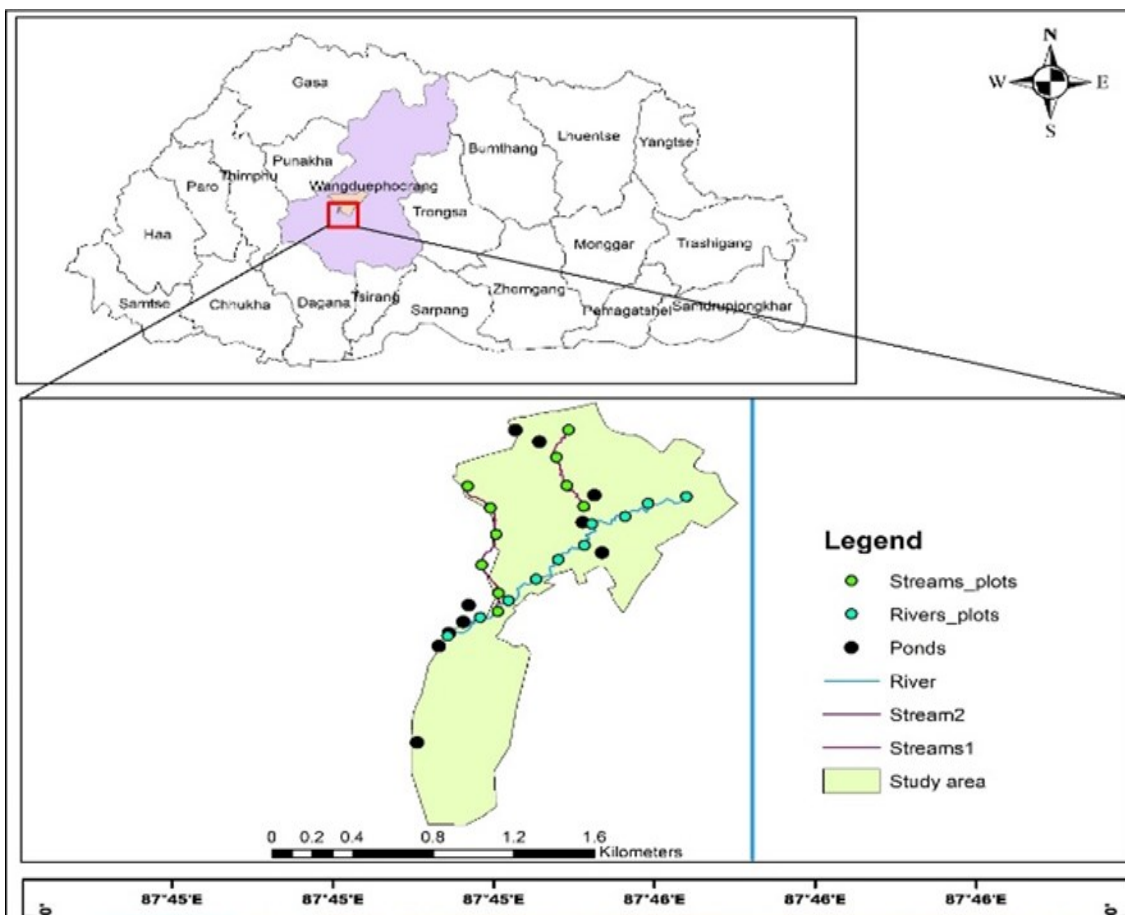


Figure 1. The map showing plots of the study area

One river Pangza and two streams Goenchhu and Lechhu located in the wetland were selected for the sampling, and the length of river and stream to be included was determined by boundary of the wetland. The river and stream length falling outside the wetland was excluded from the study. In order to acquire maximum diversity of algae, the study site also includes 30 plots combining all possible freshwater bodies. 10 plots from river and 10 plots from streams (lentic ecosystem) was determined by systematic sampling using Google Earth Pro and GIS whereas, 10 plots from ponds (lotic) was surveyed through purposive sampling since most part of the wetland was dry in winter (Figure 1).

Sampling Design

In this study, a transect line had been laid along the stream with uniform plots using ArcGis and Google Earth prior to the field work. The size of the plot was laid 3 m × 3 m and the distance between the two adjacent plots was kept 200 m and for the navigation from one plot to another, Softwel (SW) Map was used. As the time for sample collection was in winter, purposive sampling was used for ponds and wetlands since most part of the wetland was dry.

Sample Collection and Preservation

The survey was carried out in April, 2022 in Khotokha Ramsar wetland, Wangdue Phodrang district. Samples were collected where there were signs of algae growth such as slimy appearance, deposition of green material and visible body of algae called thallus. Besides algae

sample collection, 1 L of water sample was collected from every plot for assessing physiochemical properties of water. For shallow water body such as streams, scraping and scooping methods are easier to use (Poikane *et al.*, 2016). For these methods, instruments such as algae scrapers and scoopers were used for the sample collection (Majaneva *et al.*, 2009). For the two methods, toothbrush has been used to extract algae from the substrate since it served the similar function to algae scrapers and scoopers which are rarely available in Bhutan.

The instant identification of samples collected from the field was not possible as identification required a laboratory set-up (Hallegraeff, 1977). Therefore, samples were preserved in their ideal conditions as to prevent disintegration of structure and composition of their cells (Quality & Division, 2013). When freshwater algae are removed from their natural habitat, their cell wall ruptures and the cellular structure will be destroyed thus, identification will become difficult (Bhattarai *et al.*, 2007). The algae samples were preserved in airtight bottles using Formalin-acetic acid (FAA) solution (Iturriaga & Sullivan, 2015).

Collection of information on Site Factors

The data for site factors which contribute to the diversity of algae such as temperature, salinity and pH, PCSTestr 35 was used as it was portable and user friendly. The depth of every plot was measured using a metre stick. The geographic location of all the plots was recorded using the Garmin GPS etrex 30x. The preserved samples were brought to the laboratory of College of Natural Resources (CNR) for identification and analysis.

Physical and Chemical Test for water Samples

The physical and chemical parameters tested in the laboratory were pH, Conductivity, TDS, salinity, Total Hardness, Dissolved Oxygen, Ammonia and Chloride. Although, pH, conductivity, TDS and salinity were determined in the field using PCSTestr 35, Microprocessor COND-TDS-SAL Meter was used to confirm the values. The pH was further confirmed using Microprocessor pH Meter. Total Hardness was determined using, EDTA Titration Method, Chlorine with Iodometric Method and Ammonia by reacting with sulphuric acid (H₂SO₄).

Sample Preparation and Species Identification

The slide was prepared from one drop of each sample using a dropper and for visible algae, the algae was mounted using the forceps. The algae sample was covered by a coverslip and excess water was soaked using paper towels. Different types of algae were observed under a compound microscope using objective lens of 10x, 40x, and 100 oil emersion. For the measurement of algae length and width, the microscope was calibrated involving eyepiece graticule and stage micrometer. Calibration was carried to obtain the same result under different magnifications and subsequently, length and width of algae were noted. After the size of

algae samples were determined, photos were taken using a camera by attaching an adapter connecting to microscope. Algae were identified following Prescott (1951), Desikachary (1959), Jüttner *et al.* (2000), McGregor (2018), and Rai and Dhakal (2020).

Data Analysis

Data collected were stored in Microsoft Excel Sheet recording relevant information such as plot ID, sample ID, location, date of collection, name of the collector, and data on site factors. SPSS (Statistical Package for Social Science) was used for testing data normality, correlational test, ANOVA and regression to determine the relationship between species richness and physiochemical parameters of water in the study area and further, these analyses were confirmed using R software.

RESULTS AND DISCUSSION**Enumeration of Algae**

The present study reported a total of 39 algae belonging to 21 genera, 20 families and 6 classes (Table 1). Out of 39 algae, only 15 were identified up to species levels which also include 10 species new to Bhutan (Table 1). Rivers, ponds and streams harbored 21, 32 and 25 species, respectively. Ponds harbored the maximum algal species richness ($S = 32$ species).

Table 1. List of algae with occurrence in different habitats.

Class	Family	Algae	Occurrence		
			R	P	S
Cyanophyceae	Notocaceae	1. <i>Anabaena sp. 1</i>	-	+	-
		2. <i>Anabaena sp. 2</i>	-	+	-
	Merismopediaceae	3. <i>Aphanocapsa sp.</i>	-	+	-
	Chroococcaceae	4. <i>Chroococcus sp.</i>	-	+	-
	Aphanizomenonaceae	5. <i>Gloetrichia echinulata*</i> Richter	-	+	-
	Oscillatoriaceae	6. <i>Oscillatoria princeps*</i> Vaucher ex Gomon	+	+	-
		7. <i>Oscillatoria sp. 1</i>	+	+	+
Chlorophyceae	Haematococcaceae	8. <i>Haematococcus sp. 1</i>	-	-	+
		9. <i>Haematococcus sp. 2</i>	-	+	-
	Oedogoniaceae	10. <i>Oedogonium boscii*</i> Wittrock ex Hirn	-	+	+
		11. <i>Oedogonium sp. 1</i>	+	-	+
		12. <i>Oedogonium sp. 2</i>	+	-	-
		Scenedesmaceae	13. <i>Scenedesmus sp.</i>	-	+
Ulvophyceae	Ulotrichaceae	14. <i>Ulothrix sp.</i>	+	+	+

Zygnematophyceae	Desmidiaceae	15. <i>Cosmarium javanicum</i> * Nordstedt	-	+	+
	Zygnemataceae	16. <i>Mougeotia sp. 1</i>	+	+	+
		17. <i>Mougeotia sp. 2</i>	+	+	-
	Spirogyraceae	18. <i>Spirogyra majuscula</i> * Kützing	+	-	+
		19. <i>Spirogyra pseudomaxima</i> * Kadlubowska	-	+	+
		20. <i>Spirogyra rectangularis</i> * Transeau	-	+	+
		21. <i>Spirogyra scrobiculata</i> * (Stockmayer) Czurda	-	+	-
Coccinodiscophyceae	Melosiraceae	22. <i>Melosira varians</i> Agardh	-	+	-
Bacillariophyceae	Cymbellaceae	23. <i>Cymbella sp. 1</i>	+	+	+
		24. <i>Cymbella sp. 2</i>	+	+	+
		25. <i>Encyonema sp.</i>	-	+	+
	Rhopalodiaceae	26. <i>Epithemia adnata</i> * (Kützing) Brébisson	+	-	-
		27. <i>Epithemia sorex</i> Kützing	+	+	+
	Naviculaceae	28. <i>Navicula radiosa</i> Kützing	+	+	-
		29. <i>Navicula sp. 1</i>	+	-	-
		30. <i>Navicula sp. 2</i>	-	-	+
	Bacillariaceae	31. <i>Nitzschia linearis</i> * Smith	+	+	+
		32. <i>Nitzschia sp. 1</i>	+	+	+
		33. <i>Nitzschia sp. 2</i>	+	+	+
	Pinnulariaceae	34. <i>Pinnularia viridis</i> (Nitzsch) Ehrenberg	-	+	+
		35. <i>Pinnularia sp.</i>	+	+	+
	Fragilariaceae	36. <i>Synedra sp. 1</i>	+	+	+
		37. <i>Synedra sp. 2</i>	+	+	+
		38. <i>Synedra sp. 3</i>	+	+	+
	Ulnariaceae	39. <i>Ulnaria ulna</i> (Nitzsch) Compère	+	+	+

* New report to Bhutan, R = River, P = Pond, S = Stream, + Presence, - Absent,

Physiochemical parameters

The summary of physico-chemical parameters of different habitats was analyzed (Table 2). Among three habitats, ponds had total hardness (max = 960 mg/L, $M = 618$ mg/L), chloride (max = 69.48 mg/L, $M = 51.86$ mg/L), ammonia (max = .05 mg/L, $M = .04$ mg/L), TDS (max = 5.33 ppm, $M = 2.86$ ppm) and conductivity (max = 8.20 μ S/cm, $M = 4.31$ μ S/cm), stream had maximum DO (max = 9.29 ppm, $M = 8.36$ ppm, however, river had maximum salinity (max = 4.74 ppm, $M = 2.43$ ppm) and pH (max = 6.67, $M = 6.37$).

The maximum total hardness recorded from ponds could be from the maximum deposition of cations from various sources whereas lesser total hardness in

rivers and streams could be due to low rate of accumulation of cations as rivers and streams keep flowing Akram and Rehman (2018). The maximum concentration of chloride in ponds at study site could be a contribution from agriculture and wastes produced by humans, thus depositing on the surface of wetlands. Ammonia is used in fertilizers, animal feed production and produced by human faeces, on dissolution in water, Ammonia forms ammonium cation along with hydroxyl ions and the degree of ionization depends on temperature, pH higher salinity areas by flooding and irrigation mismanagement. Rivers in Khotokha were found to be the source of irrigation for agriculture, thus, irrigation mismanagement could have been the contributing factor

in higher salinity. pH also depends on several factors such as release of CO₂ and concentration of other dissolved salts (WHO, 1996). The higher concentration of dissolved Ammonia in ponds could have been caused by accumulation of fertilizers from agriculture fields and human wastes produced in the vicinity of the wetlands.

TDS comprise inorganic salts (calcium, chloride, magnesium, potassium, sodium, bicarbonate and sulphates) and organic matters dissolved in water according to Zhang et. al (2017). Although natural water bodies contain certain level of TDS, could be increased by human activities such as agriculture, water use and urbanization as depicted by the accumulated concentration of TDS in ponds as compared to streams and rivers (Peng, et. al., 2019). It could be also due to higher concentration of chloride as shown in the Table 2.

Electrical Conductivity measures the concentration of total dissolved ions in the water, therefore, higher the concentration of total dissolved ions and salt in water, greater is the electrical conductivity (Carmel *et al.*, 2011). It depends on numerous factors which are both natural (geology and size of waterway) and anthropogenic activities (wastewater and agriculture) (Carmel *et al.*, 2011). The greater conductivity of ponds in relation to streams and rivers is because of higher total hardness (greater magnesium and calcium ions), chloride, ammonia and TDS accumulated with the still water in

contrast to flowing water bodies where the accumulation rate is lower (Behar, 1996).

However, the Dissolved Oxygen (DO) was found to be in higher concentration in streams as compared to rivers and ponds. According to Wetzel (1983), DO of a water body depends on several factors such as whether water is flowing or not, presence of rocks, intensity of plants growing in the water, depth and rate of pollution. The study also mentioned that a moderate population of plants growing around the water body (which produce oxygen instead of using DO for photosynthesis), increases the amount of DO, when depth increases, the concentration of DO decreases and when the rate of pollution is higher, DO decreases. With greater depth and lesser population of plants in big rivers, its DO was measured less than streams. On the other hand, the higher rate of pollution in ponds has contributed to lower DO. Consequently, the DO was comparatively higher in streams.

Salinity was observed higher in river as compared to ponds and streams. According to El-Swaify (1983), salinity depends on several factors such as geochemical weathering of rocks, directly derived from higher salinity areas by flooding and irrigation mismanagement. Rivers in Khotokha were found to be the source of irrigation for agriculture, thus, irrigation mismanagement could have been the contributing factor in higher salinity.

Table 2. Physiochemical properties of habitats.

Parameters	Pond				River				Stream			
	Max	Min	Mean	SD	Max	Min	Mean	SD	Max	Min	Mean	SD
Total Hardness (mg/L)	960	370	618	177.3	470	175	283	95.92	740	135	494	188.26
Chloride (mg/L)	69.48	32.26	51.86	12.13	16.13	11.17	13.28	1.55	14.89	9.93	13.03	1.78
Ammonia (mg/L)	0.05	0.03	.04	0.01	0.04	0.01	0.03	0.01	0.03	0.01	0.02	0.00
pH	7.2	5.75	6.28	0.4	6.67	6.04	6.37	.21	6.75	5.85	6.3	0.30
Conductivity (µs/cm)	8.20	1.82	4.31	1.94	6.50	1.38	3.19	1.41	2.58	1.35	1.72	0.35
TDS (ppm)	5.33	1.18	2.86	1.5	4.22	0.89	2.13	0.94	1.20	0.87	1.05	0.11
Salinity (ppm)	4.45	1.33	2.92	1.27	4.74	1.31	2.43	1.01	2.29	0.98	1.29	0.37
DO (ppm)	7.44	2.95	5.44	1.83	9.15	4.10	7.33	1.74	9.29	7.66	8.36	0.43

Where, TDS = total dissolved solids, DO = dissolved oxygen, ppm = parts per million.

pH also depends on several factors such as release of CO₂ by anthropogenic activities and atmospheric increase of CO₂ due to climate change (Beaune et al., 2018). The greater pH in rivers could have been due to both human activities and climate change. During the time of study, it was observed that people were washing their vehicles and dumping wastes along the banks of rivers.

Relationship between species richness and water physiochemical parameters

Ponds

There was significant relationship between all the physiochemical parameters and species richness of ponds since the $p < .05$. Unlike river and streams, ponds have no factors such as water current, therefore, individual physiochemical properties of ponds significantly predicts the species richness in ponds (Tessy & Sree Kumar, 2008). Total hardness, pH, conductivity and TDS have positive correlation with species richness whereas, chloride, ammonia, salinity and DO have negative correlation with species richness. The study by Fonge (2012) at Ndop wetland plain in Cameroon also showed that increase in total hardness, acidity, conductivity and TDS increases the species richness. However, the study argues that filamentous algae have their tolerance level to physiochemical properties of water. In general, the species richness of algae might increase with increase in some physiochemical properties but, after the optimum level, the species richness will begin to decline.

The species richness declines with increase in alkalinity and physiochemical properties such as chloride, ammonia and salinity contribute to increase in alkalinity consequently, the rate of photosynthesis decreases as available carbon dioxide decreases (Zareidarki, 2009). Most filamentous algae species such as *Oedogonium boscii*, *Oedogonium* sp. 1 and 2, *Oscillatoria princeps* and *Oscillatoria* sp., *Mougetia* sp. 1, *Spirogyra pseudomaxima*, *Mougetia* sp. 2, *Spirogyra retangularis* and *Spirogyra scrobiculata* were reported from the ponds.

Streams

There was no significant relationship between species richness and physiochemical parameters of streams since $p > .05$ for all the parameters. Different physiochemical properties of water act differently for different habitats (Popper et al., 2014). Although physiochemical parameters had significant differences in ponds, there was no significant correlation between species richness and physiochemical properties of water. This could be explained by the velocity of streams which decreases the rate of accumulation of chemicals, thus, making less contribution to algae diversity (Yasmin, 2018). It was also observed that the sizes of streams were small with no specific substrates for algae to establish its population. However, few species of filamentous algae were recorded as they are collected from few substrates such as rocks and logs encountered during the survey. However, stream habitats were dominated by diatoms such as *Nitzschia*, *Cymbella*, *Pinnularia* and *Ulnaria* species. Some of the filamentous algae recorded from streams were *Oedogonium boscii*, *Spirogyra pseudomaxima*, *Spirogyra retangularis*, *Spirogyra majuscula* and *Mougetia*.

Rivers

There was no significant relationship between species

richness and physiochemical parameters of rivers since $p > .05$ for all the parameters. Similar to the streams, rivers in Bhutan are fast flowing, therefore, physiochemical properties have less impact on algae distribution. The velocity of rivers is further increased by the steep slopes in mountainous region. Although, there are enough substrates to support algae establishment, velocity of rivers makes unfavourable habitat for filamentous algae growth (Cas, 2012). Rivers are also the destinations where sewage and irrigation channels drain their waste water thereby, increasing the turbidity (Das & Adhikary, 2012). The increasing turbidity decreases the amount of light (decreases the rate of photosynthesis) that is penetrated into the river habitat making algae establishment less favourable (Bhakta & Das, 2020).

Consequently, the river ecosystem in fast flowing rivers are dominated by diatoms and non-filamentous algae such as *Nitzschia* sp., *Fragilaria* sp., *Pinnularia* sp., *Cymbella* sp. and *Navicula* sp. Few substrate associated (mostly rocks) filamentous algae were also recorded from rivers such as *Oedogonium* sp. 1, *Oedogonium* sp. 2, *Oscillatoria princeps*, *Oscillatoria* sp. 1, *Mougetia* sp. 1, *Mougetia* sp. 2 and *Pinnularia viridis*.

Comparing species richness and physiochemical parameters between and within groups

Species richness and physiochemical parameters were compared between habitats by oneway ANOVA. There was a significant difference in species richness of three habitats of total hardness, chloride, ammonia, conductivity, TDS, salinity, and DO, however, there was no significant difference in pH among three habitats. After confirming the significance differences between and within groups, pair wise comparison was conducted by Bonferroni correction (Figure 2).

Bonferroni test was performed to determine the relationship of species richness and physical parameters among habitats. There was significant difference in species richness of ponds ($M = 7$, $SD \pm 1.63$) and streams ($M = 4.7$, $SD \pm 1.70$), $p = 0.01$, rivers ($M = 3.1$, $SD \pm 0.99$) and ponds ($M = 7$, $SD \pm 1.63$), $p = .00$, total hardness of streams ($M = 494$, $SD \pm 1.88.26$) and rivers ($M = 283$, $SD \pm 95.92$), $p = .02$, rivers ($M = 283$, $SD \pm 95.92$) and ponds ($M = 618$, $SD \pm 177.29$), $p = 0.00$, chloride of ponds ($M = 51.86$, $SD \pm 12.12$) and streams ($M = 13.02$, $SD \pm 1.79$), $p = 0.00$, river ($M = 13.28$, $SD \pm 1.55$) and ponds ($M = 51.86$, $SD \pm 12.12$), $p = 0.00$, ammonia of ponds ($M = 0.35$, $SD \pm .007$) and streams ($M = 0.017$, $SD \pm .005$), $p = 0.00$, stream ($M = 0.017$, $SD \pm 0.005$) and river ($M = .028$, $SD \pm .009$), $p = .01$, conductivity of ponds ($M = 4.31$, $SD \pm 1.94$) and streams ($M = 1.72$, $SD \pm 0.35$), $p = 0.00$, TDS of ponds ($M = 2.86$, $SD \pm 1.50$) and streams ($M = 1.04$, $SD \pm 0.113$), $p = 0.00$, salinity of ponds ($M = 2.92$, $SD \pm 1.27$) and streams ($M = 1.29$, $SD \pm 0.37$), $p = 0.00$, streams ($M = 1.29$, $SD \pm 0.37$) and rivers ($M = 2.43$, $SD \pm 1.01$), $p = 0.04$, DO of ponds ($M = 5.44$, $SD \pm 1.83$) and streams ($M = 8.36$, $SD \pm .43$), $p = 0.00$, ponds ($M = 5.44$, $SD \pm 1.83$) and rivers ($M = 7.33$, $SD \pm 1.74$), $p = 0.02$.

Out of 39 different species, 32 was found in ponds accounting to 82.05% of species richness, 25 species was observed in streams (64.1%) and 21 species was recorded from rivers (53.8%). The algal biomass in running water (rivers and streams) is typically lower than standing water habitats such as ponds, lakes and bogs since suspended algae are transported by water

current and the development of established population is prevented (Sohani, 2015). Therefore, fast growing algae (especially diatoms) are mostly recorded from rivers and streams. The other factor in algae development is depth of habitats, when depth increases, the diversity of algae decreases as sunlight penetration decreases with increase in depth (Singh *et al.*, 2014). The lowest species richness in rivers is due to depth and water current.

Significance differences in total hardness, ammonia, and conductivity, TDS, chloride and DO between the three habitats are dependent on both natural and anthropogenic factors (Seng *et al.*, 2018). The natural factors are temperature, altitude, natural weathering of rocks, sizes of habitats, velocity and the slope of the area (Misra *et al.*, 2005). As proven by the regression analysis of rivers and streams, there was no significance correlation between physiochemical parameters and species richness of these two habitats.

The principal factors affecting species richness could be temperature, altitude, velocity and slope of the area. The optimal temperature favouring the growth of algae is between 20°C and 30°C (Schnurr & Allen, 2015). During the survey, the recorded range of temperature was between 6°C and 8°C which indicated that Khotokha Ramsar wetland was not favourable for establishment of algae population in winter. The increase in altitude also negatively affect species richness as the temperature of the area decreases (Hui *et al.*, 2022) and Khotokha Ramsar wetland lies above 2500 masl. The velocity of rivers and streams negatively affect the establishment of algae by transporting downstream and rendering short duration for establishment (Das, 2014).

The distribution of algae according to phylum

The distribution of algae according to phylum (Figure 3) showed that Khotokha Ramsar wetland had the

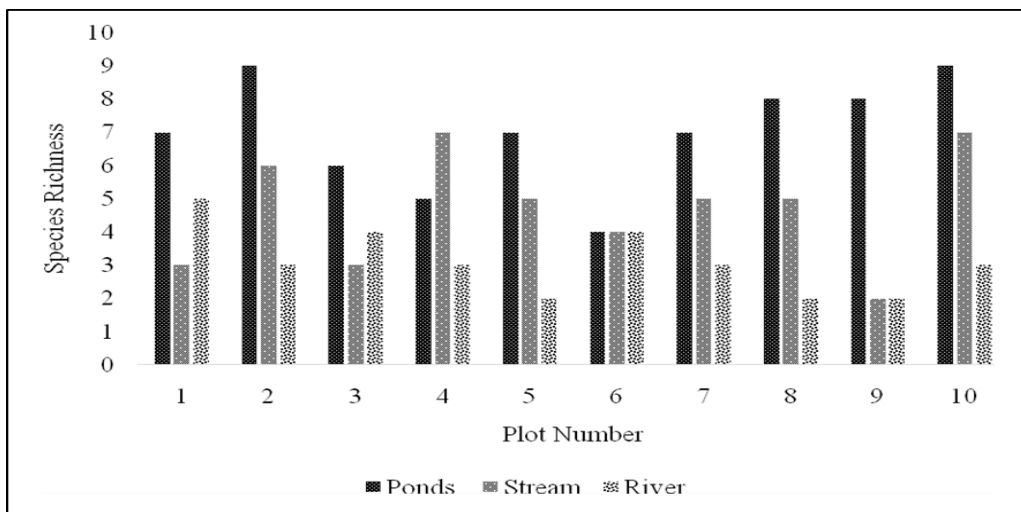


Figure 2. Species richness of ponds, rivers and streams

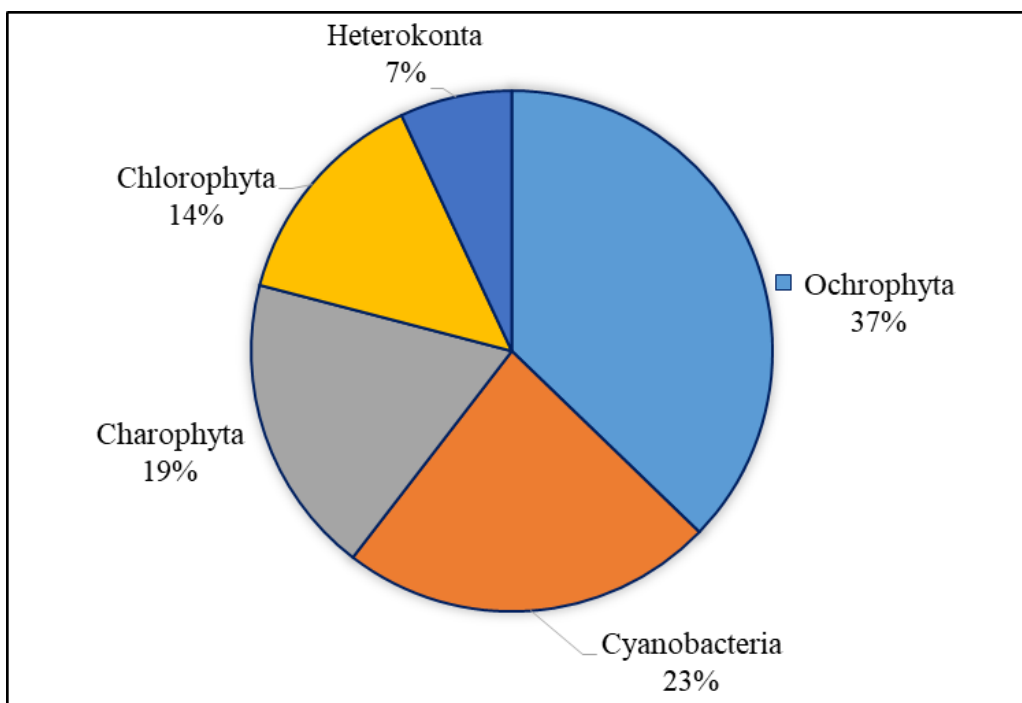


Figure 3. Chart representing algae phylum distribution in Khotokha Ramsar wetland.

highest species richness under Ochrophyta with 16 different species (37%) followed by Cyanobacteria with 10 species (23%), Charophyta with 8 species (19%), Chlorophyta with 6 species (14%) and the least species richness was Heterokonta with 3 species.

CONCLUSION

The diversity of freshwater algae and their relationship with physiochemical parameters of water in Khotokha Ramsar Wetland, Wangdue Phodrang was conducted. The study reported a total of 39 algae belonging to 20 families of which 10 species were new to Bhutan. Among the habitats, maximum algal species were found in the ponds than the river and streams. The current study showed that lentic water ecosystem such as ponds provide suitable habitat for algal growth and propagation whereas in lotic water ecosystem, even the parameters for growth are suitable, the water current makes it difficult for algae to distribute abundantly. The study also concludes that there are differences in physiochemical parameters among habitats of algae and contribute differently in different habitats. The study also revealed that Khotokha Ramsar wetland had the highest species richness under Ochrophyta with 16 different species (37%) followed by Cyanobacteria with 10 species (23%), Charophyta with 8 species (19%), Chlorophyta with 6 species (14%) and the least species richness was Heterokonta with 3 species. Further, the study proved that physiochemical parameters (pH, salinity, TDS, conductivity, total hardness, ammonia, chloride and DO) were significantly affecting the diversity of algae in ponds as these properties were altered both by natural and anthropogenic factors. On the other hand, these parameters had no significance in flowing water bodies (habitats) since the velocity of water flowing downstream dilutes the chemicals and the diversity was also observed to be less than ponds as suspended algae were transported by the velocity of water. The study also concludes that different algae species have different habitat preferences but largely affected by the altitude and temperature of the area.

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