Evaluation of water quality and dam for sustaining the fish population dynamics

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Received: 25 November 2020 / Accepted: 24 June 2022 / Published online: 24 August 2022
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Abstract
Bhagirathi is a Himalayan River, which flows in Uttarakashi district in Uttarakhand state. It is one of the most important streams of the Ganga River. This river is famous for dam construction and fisheries development. The fishes of commercial importance in cold water are Mahaseer, Snow Trout. The fish production depends on the physical, chemical as well as biological qualities of water. In the present paper, water quality evaluation and dam in the sustaining fish population dynamics have been reviewed to make aware the fish culturist and environmentalist about the water quality factors which influence health of a pond and to increase the fish yields to meet the growing demands of present time scenario of the our country. The construction of dams causes many problems. Water quality and discharge of water from the dam wall systems also indirectly affect the fish populations. In this study, we also review some of the important impacts associated with dams and their recommended mitigation measures. There is a negative impact on fish population dynamics, especially for downstream habits due to dams. Water quality is measured like temperature, turbidity, carbon dioxide, pH, alkalinity, hardness, BOD, TDS, Turbidity. The maximum oxygen content of water was recorded in January 13.02 ± 0.166 mg l⁻¹ and minimum 9.4 ± 0.05 mg l⁻¹ in July in site I phase I (before the reservoir dam wall). While in site II and phase I (After the dam), the maximum DO was observed in the month of January of 12.1 mg l⁻¹ and minimum in July of 8.93 mg l⁻¹. This is because water level is minimized in site II almost dried in winter. The CO₂ of the Maneri Bhali phase I -Site I (before dam) was recorded maximum in April 3.10 ± 0.012 mg l⁻¹. In Maneri Bhali phase I -Site II (After dam), the CO₂ was recorded maximum 3.01 ± 0.003 mg l⁻¹ in the month July. The river of Garhwal Himalaya harbors a rich aquatic diversity, the most common endemic fish species that inhabit this fresh water is the Schizothorax species. The maximum no. of fish were observed in winter and minimum in monsoon. At present, the production of these fishes from the streams is very poor and is not well managed from recreational and conservation point of view. If such is developed properly, it can have revenue potential for our state government. Sports, fisheries, tourism should be promoted.

Keywords Hydropower project · Fish population dynamics · Fluctuations of water level · Water quality parameters

Introduction

Uttarakhand is formed as a 27th state of India on November 9, 2000. It encompasses thirteen districts, i.e., Uttarkashi, Tehri Garhwal, Rudraprayag, Chamoli, Dehradun, Puari Garhwal, Pithoragarh, Champawat, Almora, Bageshwar, Nainital, Udham Singh Nagar and Haridwar. Water is a natural resource and is fundamentally important for our lives. Which is used for food production and sanitation. It is a key driver of economic and social development and is necessary for life, livelihood, agriculture and sustainable development of the civilization of the Uttarakhand people. The Bhagirathi River is originated from Gaumukh at an elevation of 3892 m about 12,769 feet above sea level, at the foot of Gangotri and Khatling glaciers in the Garhwal Himalaya. Thus, it is joined by various tributaries, Kedar Ganga at Gangotri, Jadh Ganga at Bhaironghati, Kakora Gad and Jalandhari Gad near Harsil, Siyan Gad near Jhala, Asi Ganga near Uttarkashi and Bhilangna River near Old Tehri.
Water quality and dam are the most important factors affecting fish health and performance in aquaculture production system. Due to the Maneri Bhalı Phase I and II dams built across the Bhagirathi River in Uttarkashi, the water level of the Bhagirathi River in Uttarkashi district changes dramatically. It is natural to find changes in water quality and populations of fish. The hyroelectric projects often have major effects on fish and other aquatic life. Sometimes, reservoirs positively affect certain fish species and fisheries by increasing the area of available as aquatic habitat. However, the impacts are negative because the dam blocks the up and downward fish migrations. Water quality is also affected, by dam because dams tend to collect pollutants along with sediment, which must be released and controlled in some way. Especially in the case of deep dams, thermal stratification develops because there is no current to mix the water. As a result, the deeper water is very cold and has poor oxygen circulation, while the surface water is warmer than usual. When colder deep water is released from dams, it can cause serious problems downstream for fish and other aquatic life due to its poor oxygenation and the toxins it may carry. Dams are constructed for a variety of reasons like water storage, flood prevention, irrigation, navigation and recreation (Franciso 2004). Hydroelectric power production is flexible as the production can be increased or decreased very quickly as per energy demand. Construction of larger dam in river which interrupts the flow of river and can harm local ecosystem, displace people and wildlife (World watch Institute 2012). An integrated assessment of the effects of river dams on aquatic organisms was carried out at the Sabar River Dam. This dam created two reservoirs: primary and secondary. Changes in water quality were significant due to dam construction (Santos et al. 2017). Dam construction can cause damage to the river basin, including changes in downstream flow, changes in river species and other fauna, and material sedimentation. Certain measures were taken to reduce the negative impacts on the rivers. They are reservoir ecological regulation, ecohydraulic engineering construction and comprehensive water pollution treatment (Qicai 2011; Qi and Ruan 2005). Dams are constructed on the Bhagirathi River to meet the various requirements of the common people such as power, irrigation, drinking water and flood control. Sikkim, a small Indian state, initiated a program of hydropower project construction and community perceptions of the environmental and socioeconomic impacts of these projects in three rural areas to study and further discuss the implications for future livelihoods. These economic development projects have brought benefits to the rural community like employment (Chandy et al. 2012). In Bhagirathi river, Maneri Dam I is located at Maneri from 8.5 km east of Uttarkashi, Uttrakhand, India. The purpose of this dam is to divert water into a tunnel which feeds the 90 megawatts run-of-the-river Tiloth Power Plan ("Power from Maneri Bhalı by year-end"). Maneri Dam II Power Project is located at Joshiyara, Uttarkashi, Uttrakhand, India. This hydro power plant capacity is 304 megawatts. Both are operated by Uttarkhand Jal Vidyut Nigam Ltd. (UJVNL [^ "Dharasu HEP (4×76 = 304 MW)"]. Dams impact ecosystems in a number of ways: altering the natural cycle of flow, transforming the biological and physical characteristics of river channels and floodplains and fragmenting the continuity of rivers (Petts 1984). Along with the demand for agricultural water in the Portuguese side of the Douro River, hydroelectricity production is also increasing. Due to the transverse barrier in the river, the river basin is affected by the fragmentation. Multi-criteria decision analysis is used to prioritize the removal of dams or barriers. Removal of the dam increases the possibility of fish species spreading across the river (Terêncio 2021). Fire in forests can also cause other serious problems in aquatic system. The decrease in infiltration and increase in loose sediment after a forest fire are the results from the devastation of vegetation and changes in physical and hydrologic properties of the soil. These changes can increase the amount of sediment, nutrients and other constituents delivered to reservoirs and streams throughout the watershed (Terêncio et al. 2020). In the construction of hydropower project, the schemes do not produce direct waste and have a considerably lower output level of carbon dioxide (CO₂) than fossil fuel powered energy plants (Renewable Global Status Report 2011). Large dams and the reservoir created by them cause changes in water temperature, water chemistry and blockage of migration in aquatic life forms (McAllister et al. 2001). Advancement of rivers system for dams has emerged as one of the most significant and valuable tools for the managing of water resources (Mul et al. 2018). Dams are promoted to social and economical development of the state and country. They are also provided to generation of electricity, water supplies and flood control of a common masses (Hossain et al. 2012). Dams are an integral component of economic and social development in the USA. But its construction and operation have been a controversial—several main dams in the USA have been seen as a public infrastructure failures in terms of social equality and environmental impacts (Ho et al. 2017). Construction of dam is one of the most primary drivers which change the environmental conditions of river ecosystems and may thus affect water quality and water self-purification (Wei et al. 2009). Dams act as physical barriers to migrants preventing passage of fish to their usual breeding, rearing, and feeding ground (Agarwal et al. 2018). Definitely this study will suggest a method for well managing river Bhagirathi. Focusing specifically on changing of parameters and fish populations after dam construction in Bhagirathi River (1) Establish study sites where maximum change in water level of river is observed. (2) Record changes in the physicochemical
parameters of water in study site. (3) Difference of population of fishes before and after the dam.

**Study area**

First location of the study is at near the Maneri dam I, where it forms a reservoir near village Maneri, district, Uttarkashi (30° 44’ 16” N 78° 32’ 22” E). It is located 8.5 km upstream from Uttarkashi. At this location, two study sites were selected, study site 1 was approximately 200 m before the dam wall and study Site 2 was 200 m after the dam reservoir wall. Second location was near the Maneri dam II Joshiyara, Uttarkashi (30° 42’ 36” N 78° 24’ 7” E). At this location also, two study sites were selected, study Site I was 200 m from before dam wall and study Site II was 200 m after the dam reservoir wall. Network of rivers in Garhwal Himalaya, Uttarakhand. All the major tributaries supporting the Ganges are marked in the figure along with study sites of Meneri dam shown in Fig. 1.

**Geology of the study area**

The land in this study area is mainly covered by deciduous forest, agricultural fallow lands, clear forests and clear lands, grazing lands, water bodies (mainly rivers and reservoirs) and settlements. There are a number of settlements within a 5 km radius of the Maneri bhali I and II. There are different types of rock formations, i.e., white and cream colored quartzite grading into talc-chlorite schist’s along the thrust and migmatities, augen gneisses, garnetiferous mica-schist’s and amphibolite’. It is generally accepted that the rock types in the area are quartzite. The quartzite rock is inter-bedded with silts, metal-volcanoes and gases interactive.

**Materials and methods**

Water samples were collected from four sampling sites viz. two sampling sites in Maneri bhali phase I—Site 1, Site 2, other two sites in Maneri bhali phase II—Site 1, Site 2. The samples were collected into plastic canes and brought to the laboratory with precautions. The study was carried from January 2018 to December 2018. Various physicochemical parameters of the water samples were analyzed by following the standard methods of Trivedi and Goyal (1986), APHA (2005) and Dutta and Munshi (1995). Readings were usually taken in triplicates. A set of ten most commonly used water quality parameters are as follows: air tem and depth tem, pH, electrical conductivity (EC), total dissolved solid (TDS), turbidity (NTU), alkalinity, CO₂, total hardness (TH), dissolved oxygen (DO), biochemical oxygen demand (BOD). Method of Zippins et al. was used for estimation of fish population.
(Zippin 1958). Sampling was a problem during peak summer and monsoon as the volume of water increases tremendously and only few vantage points are available for casing net. The sampling data during these months are not absolute but indicative.

Results and discussion

The Bhagirathi water contains maximum dissolved oxygen in colder month followed by gradual decreases to its lowest value during summer months. The colder months are due to low water temperature low turbidity and photosynthetic activities of green algae. The maximum oxygen content of water was recorded in January 13.02 ± 0.166 mg l−1 and minimum 9.4 ± 0.05 mg l−1 in the month of July in site I -phase I (before the dam). In the site II after dam wall, maximum DO was recorded 12.1 ± 0.2 mg l−1 in the month of January and minimum DO was recorded 8.93 ± 0.08 mg l−1 in the month of July (Fig. 2). Maximum DO was recorded 14.07 ± 0.2 mg l−1 in the month of January and minimum DO was recorded 8.37 ± 0.03 mg l−1 in the month of May in Phase II and site I(before the dam wall). Maximum DO was recorded 12.27 ± 0.14 mg l−1 in the month of February and minimum DO was recorded 9.1 ± 0.1 mg l−1 in the month of April and July in Phase II and site II (after the dam) (Fig. 2). The level of DO was very less in summer April and July. This is because of the low solubility of gases at high temperature (Hynes 1978); further in summer, the amount of water also gets decreased and becomes more concentrated with pollutants; this also consumes more O2 for the oxidation of organic contents (Sharma et al. 1981). The increase in DO during winter, i.e., January months might be due to higher solubility of O2 at lower temperature (Raj et al. 1984). It has been showed that the value of DO decreases with an increase in the temperature after the Dam wall. Maximum air temperature recorded at site before dam wall at Maneri Bhali phase I was 30 °C in the month of August 2018 and minimum 11 °C in the month of January, 2018. At same site, water temperature (also referred to as depth temperature) was maximum 17 °C in the month of August 2018 and minimum 5 °C in the month of January, 2018 (Fig. 3). At Maneri Bhali Phase I, after dam wall, maximum air temperature recorded was 31 °C in the month of July, 2018 and minimum 12 °C in the month of January 2018, while water temperature was maximum 18 °C in the month of August 2018 and minimum 6 °C in the month of January 2018. Maximum air temperature recorded at site before dam wall at Maneri Bhali phase II was 32 °C in the month of July 2018 and minimum 12 °C in the month of January, 2018. At same site, water temperature (also referred to as depth temperature) was maximum 17 °C in the month of July and August 2018 and minimum 5 °C in the month of January, 2018 (Fig. 4) At Maneri Bhali Phase II, after dam wall, maximum air temperature recorded was 32 °C in the month of May 2018 and minimum 13 °C in the month of January 2018, while water temperature was maximum 16 °C in the month of July 2018 and minimum 6 °C in the month of January 2018. In this study area, higher value of water and air temperature was observed in monsoon and lower in winter (Welch 1952). The lower water temperature recorded during winter months was due to rarely rainfall and cold, alter higher temperature during, i.e., April and May was associated with longer photo-period bright sunshine any wind and other condition. A slight increase in water temperature at Site 2 was noticed. It may due to minimization of the water level and warm up of the gradients (NTPC report 2011). The CO2 of the Maneri Bhali phase I (before dam) Site I was recorded maximum in April 3.10 ± 0.012 mg l−1 and minimum in 1.60 ± 0.003 mg l−1 in the month of January (Fig. 5) and Maneri Bhali phase I (After dam) Site II, the CO2 was recorded maximum 3.01 ± 0.003 mg l−1 in the month July and minimum 1.69 ± 0.003 mg l−1 in the month of January. The CO2 of the Maneri Bhali phase II (before dam) Site I

Fig. 2 Monthly recorded DO (mg l−1) of Maneri phase I and phase II—site I & II Bhagirathi river in the year 2018
was recorded maximum in May 3.3 ± 0.005 mg l−1 and minimum in 1.47 ± 0.003 mg l−1 in the month of January. In phase II (After dam) Site II, the CO2 was recorded maximum 3.08 ± 0.011 mg l−1 in the month July and minimum 1.69 ± 0.008 mg l−1 in the month of January (Fig. 5). In the study of Maneri Bhali Phase I & II, the CO2 was found slightly greater after the dam as compared before the dam, this is due to low level of free CO2 is mainly due to photosynthetic activity with using free CO2. Free CO2 showed a negative correlation with dissolved oxygen. Alkalinity of Maneri dam phase I (Site I) showed maximum mean value 30.50 ± 0.06 mg l−1 in the month of March and minimum
mean value in the month of August (16.33 ± 0.013 mg l\(^{-1}\)). In site II and phase I, the maximum mean value was recorded 33.5 ± 0.0058 mg l\(^{-1}\) in March and minimum 17 ± 0.0208 mg l\(^{-1}\) in the month of August (Fig. 6). Alkalinity of Maneri dam phase II (Site I) showed maximum mean value 26.25 ± 0.015 mg l\(^{-1}\) in the month of April and minimum mean value in August (18.33 ± 0.10 mg l\(^{-1}\)). In site II of Phase II, the maximum mean value was recorded 26.33 ± 0.006 mg l\(^{-1}\) in April and minimum 18.67 ± 0.0145 mg l\(^{-1}\) in the month of August (Fig. 6). In the present study, the alkalinity was found to be maximum in summer and minimum during Monsoon months in all study site. NTPC have also reported high alkalinity during summer period and low during Monsoon. The pH of the Bhagirathi river ranges from 7.1 to 7.9 in Maneri Bhali phase I and Phase II. Hardness of water is mainly due to calcium and magnesium ions present in it, hardness is generally measured as concentration of only calcium and magnesium as carbonate. Hardness of Maneri Bhali phase I (before dam) Site I was observed maximum mean value 100 ± 0.058 mg l\(^{-1}\) in February and minimum mean value 46.00 ± 0.57 mg l\(^{-1}\) in the month of August. In Site II Maneri Bhali phase I (after dam), the maximum value was observed 109.33 ± 0.088 mg l\(^{-1}\) in the month of February and minimum value was observed 60.0 ± 0.33 mg l\(^{-1}\) in July (Fig. 7) Hardness of Maneri Bhali phase II (before dam) Site I was observed maximum mean value 98 ± 0.058 mg l\(^{-1}\) in February and minimum mean value 48.00 ± 0.58 mg l\(^{-1}\) in the month of June. In site II Maneri Bhali phase II (after dam), the maximum value was observed 95.33 ± 0.185 mg l\(^{-1}\) in the month of February and minimum value was observed 54.67 ± 0.088 mg l\(^{-1}\) in June (Fig. 8). Total hardness of water was found to be maximum in winter and minimum in monsoon season (Ayoada et al. 2009). In the Maneri Bhali Phase I & II, the total hardness was found greater after the dam wall. Same result was found NEERI (2011) upstream and downstream of river Bhagirathi. The conductivity recorded at site I, i.e., Maneri Bhali phase I (before dam) maximum in April 143.6 ± 0.06 μS cm\(^{-1}\) and minimum in 99.18 ± 0.49 μS cm\(^{-1}\) in the month of August. In site II Maneri Bhali Phase I (after dam), the conductivity was recorded maximum 148 ± 0.3 μS cm\(^{-1}\) in the month January
and minimum 101.73 ± 0.53 µ S cm⁻¹ in the month of August (Fig. 9). The conductivity recorded at site I, i.e., Maneri Bhali phase II (before dam) was maximum in April 140.33 ± 0.33 µ S cm⁻¹ and minimum in 83.30 ± 0.12 µ S cm⁻¹ in the month of August. In site II Maneri Bhali Phase II (after dam), the conductivity was recorded maximum 148 ± 0.06 µ S cm⁻¹ in the month of May and minimum 85.27 ± 0.03 µ S cm⁻¹ in the month of August (Fig. 9). Higher conductivity values were recorded in all sampling site in winter and lower conductivity in summer. This may be due to low volume of water in the dry season. This may be due to low volume of water which brought about concentration effect. The conductivity, of NO₃-N, PO₄-P and Na⁺ were higher in during low water level (high concentration of Ions). Due to higher concentration of Ions. In the study of Maneri Bhali Phase I & II, the conductivity was found slightly greater after the dam as compared to before the dam. Same result was obtained by NEERI (2011). The TDS of the Maneri Bhali phase II (before dam) Site I was recorded maximum in July 85.10 ± 0.497 mg l⁻¹ and minimum in 59.87 ± 0.593 mg l⁻¹ in the month of Feb. In Maneri Bhali phase II (After dam) Site II, the TDS was recorded maximum 86.30 ± 0.173 mg l⁻¹ in the month of July and minimum 61.37 ± 0.273 mg l⁻¹ in the month of February (Fig. 10). Higher total dissolve solid values were recorded in all sampling site in Monsoon and lower in winter. The value of TDS depend on rainfall and variation of water level body in dam. Water with a high TDS indicates more ionic concentration, which is of inferior palatability and induces an unfavorable physicochemical reaction in the consumers. In the study of Maneri Bhali Phase I & II, the TDS of was found slightly greater after the dams as comparison to before the dam. Same result was obtained by NEERI (2011). The turbidity recorded at site I, i.e., Maneri Bhali phase I (before dam) was maximum in August 160.3 ± 0.67 NTU and minimum in 0.9 ± 0.03 NTU in the month of Feb. In site II Maneri Bhali phase I (After dam) Site II, the turbidity was recorded maximum 162.7 ± 0.33 NTU in the month August and
minimum 1.7 ± 0.09 NTU in the month of February (Fig. 11). The turbidity recorded at site I, i.e., Maneri Bhali phase II (before dam) was maximum in August 151.0 ± 0.58 NTU and minimum in 0.9 ± 0.06 NTU in the month of Jan. In site II Maneri Bhali Phase II (after dam), the turbidity was recorded maximum 152.0 ± 0.58 NTU in the month August and minimum 1.3 ± 0.03 NTU in the month of February (Fig. 11). In the present study, the turbidity was found its be maximum in monsoon and minimum during winter months in all study site. Many workers have also reported high turbidity during monsoon period and low during summer and winter (Badola 2009). In the study of Maneri Bhali phase I & II, the turbidity was found slightly greater after the dams as compared before the dam, but not significant. Higher turbidity was due to the construction activities and mixing of muck into the water. Same result was recorded by the report NTPC 2011 and NEERI (2011) in the same river. The increase in turbidity during monsoon is mainly influenced by slit, sand, clay and various type of organic and inorganic matter in bhagirathi river. Which are washed off during heavy rainfall. As a result the turbidity increases in monsoon seasons. Same trends were found by Schmitz W (1961). The maximum BOD content of water was recorded in August 4.33 ± 0.15 mg l⁻¹ and minimum 0.70 ± 0.03 mg l⁻¹ in January site I (phase I) before the dam. In site II after dam, maximum BOD was recorded 4.62 ± 0.01 mg l⁻¹ in the month of August and minimum BOD was recorded 0.86 ± 0.01 mg l⁻¹ in the month of January (Fig. 12). The maximum BOD content of water was recorded in August 4.71 ± 0.01 mg l⁻¹ and minimum 0.62 ± 0.02 mg l⁻¹ in January site I (phase II) before the dam. In the site II after dam, maximum BOD was recorded 4.83 ± 0.07 mg l⁻¹ in the month of August and minimum BOD was 1.23±0.03 mg l⁻¹ in the month of January (Fig. 12). BOD showed higher value during monsoon and comparatively low during winter, respectively, (Sati et al. 2011). In the study Maneri Phase I & II, the BOD is higher after dam compared to before dam. Same results were observed from NTPC report 2011 upstream and downstream of Maneri Phase I, while Phase II showed no more variation in BOD according NTPC report 2011. The maximum fish population observed at Phase I before dam site during 2018 was 100 in the month of March, while the minimum number

Fig. 10 Monthly recorded TDS (mg l⁻¹) of Maneri Phase I and II -site I & II of Bhagirathi river in the year 2018

Fig. 11 Monthly recorded Turbidity (NTU) of maneri Phase I and II -site I & II of Bhagirathi river in the year 2018
of fish were nil in the month of August. The maximum fish population observed at Phase I after dam site during 2018 was 50 in the month of March, while the minimum number of fish were nil in the month of August (Fig. 13). The fish number of Schizothorax sp. increased from January to March and then decreases from April to August and increases from September to November. The maximum fish population observed at Phase II before dam site during 2018 was 110 in the month of March, while the minimum number of fish were 15 in the month of July. The maximum fish population observed at Phase II after dam site during 2018 was 85 in the month of March, while the minimum number of fish were 12 in the month of July (Fig. 14). The maximum number of fish were found during the month of March and minimum number of fish population was found in the month of August or July at both phases of I & II sites. Fish numbers/population of before dam is much compared after dam at both site Maneri Bhali Phase I &II. Dams cut off
migration passage, fragment habitat, alter flow patterns, and change the connectivity between river and lake. As a result, some fish species are threatened, some are disappeared indeed, the proposition of migration fish decreases. Our data on the measure of fish population—the number of fishes—report lesser number of fishes when the water levels are higher. This variation in data is due to fact that during summers and monsoon, the river is very fast flowing and the volume of water is enormous and there is no method by which the sampling area can be cordonned off. Sampling is relatively easier in winter months where the water level is low and the data set is more reliable and accurate. However, our data during the higher water volume are a good indicator of numbers of fishes at our sites despite shortage of vantage points for throwing cast net and the data are relatively indicative rather than absolute values.

Conclusion

From the above study, it is concluded that the building of a dam has a major impact on fish populations migrations and other fish movements. Fishes suffered major damage during their transit through hydraulic turbines or over spillways. Bhagirathi river is one of the biggest sources of fresh water and fulfills the requirements of the local people of Uttarkashi. We were observed good number of fishes in spring season (February–April) due to maintaining the restoring the quality of water with dam water level. Fish population was nil causing by heavy rainfall and high water level in rainy seasons due to fluctuation of water quality. Flow regulation has been found to affect fishes inhabiting shallow water areas more than those in deep water. This has been attributed to a reduction in species adapted to fluvial conditions. It is concluded from the present study that an integrated effort should be made for the conservation and management of aquatic biodiversity of Bagirathi for maintaining the proper functioning of fluvial system. A significant level of water discharge should be maintained after the dam wall in the main river of Bhagirathi so as to maintain the aquatic biodiversity.

Acknowledgements The authors thankfully acknowledge the department of chemistry and Zoology of Our College. The authors are also thankful to Principal of R.C.U. P.G College Uttarkashi for providing infrastructural facilities for accomplishing this work.

Author contributions PT contributed to conceptualization. MPT contributed to methodology. MPT contributed to formal analysis and investigation. PT contributed to writing—original draft preparation. PT contributed to writing—review and editing. MPT contributed to resources. MPT contributed to supervision.

Funding No funding was received to assist with the preparation of this manuscript. No funding was received for conducting this study. Authors received support equipments and chemicals from the organization which already in the laboratory of chemistry and zoology. Financial interests: The authors declare they have no financial interests.

Declarations

Conflict of interest Authors are not feel there is any conflict, disclosure of relationships and interests provides a more complete and transparent process, leading to an accurate and objective assessment of the work. Awareness of a real or perceived conflicts of interest is a perspective to which the readers are entitled. This is not meant to imply that a financial relationship with an organization. Authors declare that they have no conflict of interest.

Human and animal rights All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional. “For this type of study, formal consent is not required.” The welfare of animals used for research must be respected. When reporting experiments on animals, authors should indicate whether the international, national, and/or institutional guidelines for the care and use of animals have been followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.”

Informed consent Informed consent was obtained from all individual participants included in the study.

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