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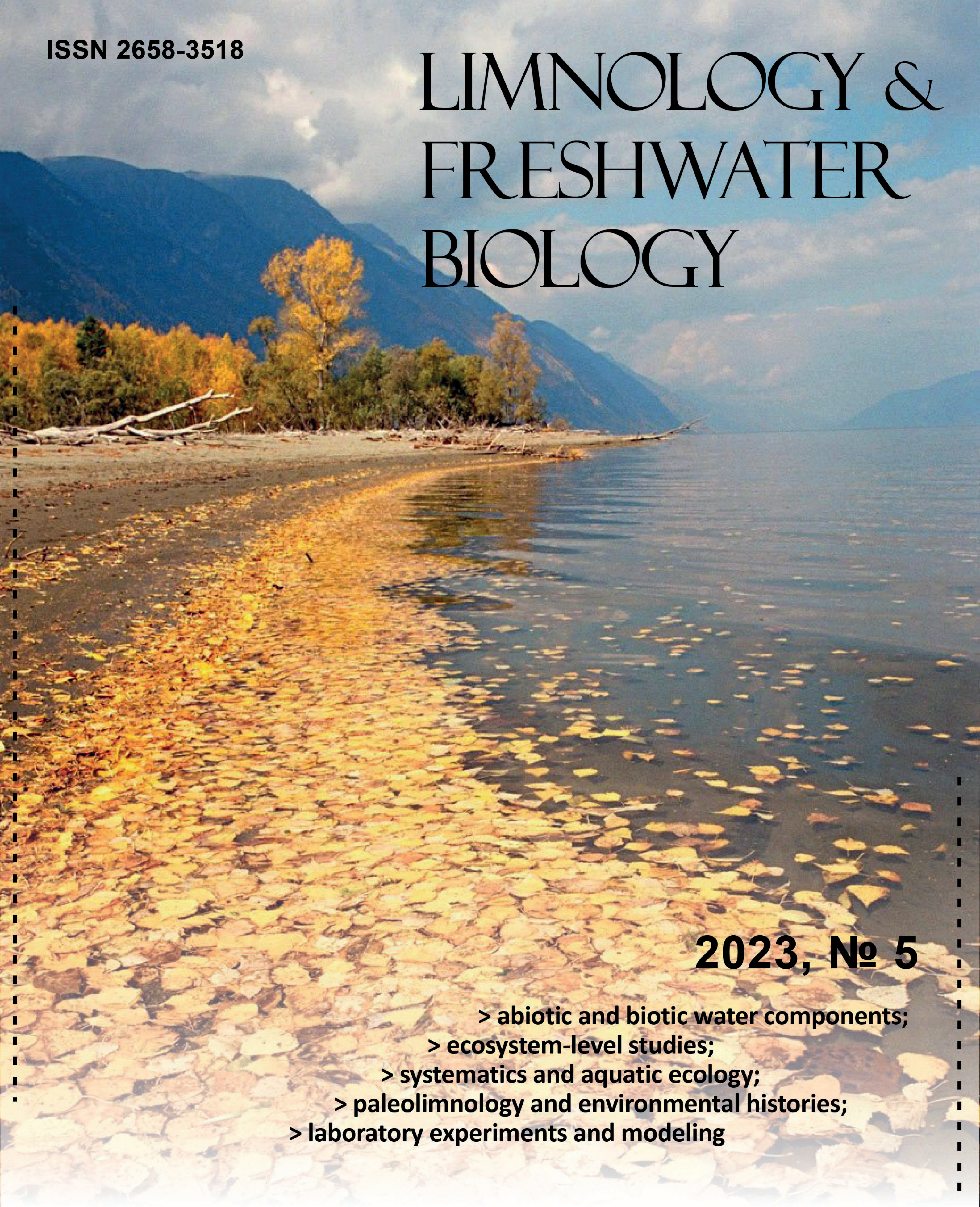

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Assessing the potential of a surface water body for multiple uses. Case study: the Anambra River Basin, south-eastern Nigeria

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ABSTRACT. Anthropogenic activities causing deterioration in water quality and a decrease in meeting the ecosystem service potentials of surface water are considered one of the major basic environmental challenges. Water samples were collected from three sampling stations (Ogurugu, Otuocha, and Otu-Nsugbe) in the Anambra River Basin and analyzed following standard protocols and procedures for nine months. The cosystem services potentials of the Anambra River Basin were evaluated using physico-chemical parameters and some indices: Comprehensive pollution index (CPI), nutrient pollution index (NPI), salinity potential, and soluble sodium percentage (SSP). All the water quality parameters were within the standard level for drinking water, aquaculture practice, recreation (swimming/bathing) and agricultural purposes, with the exception of pH and phosphate, whereas dissolved oxygen met aquaculture criteria only in station II. Comprehensive pollution index (0.150 – 0.449), nutrient pollution index (0.07 – 0.46), and soluble sodium percentage (6.0% -26.92%) were within the category of non-polluted or excellent to sub-clean. Potential salinity (39.39 - 97.19) was high, indicating water from the Anambra River Basin contained crystallized solutes. Conclusion: the Anambra River Basin is not at risk of eutrophication but may not support irrigation program due to the high potential salinity. However, the river needs to be monitored, and anthropogenic activities need to be regulated.

Keywords: Pollution, surface-water, physico-chemical, irrigation, indices

1. Introduction

The deterioration of water quality of surface water bodies as a result of human activities such urbanization and agriculture to meet basic needs of man has become a global problem (Mateo-Sagasta et al., 2017; Wang et al., 2020). Rivers are facing multi-dimensional stressors such as flow disturbance, water pollution, climate influences, habitat fragmentation leading to degradation and biodiversity erosion (Matta et al., 2020).

Saving and controlling surface water from pollution and having solid data on surface water quality for proper management have become very necessary. Considering the dynamic nature of the surface water ecosystem and the influence of various watershed activities, rapid interpretation of water quality is required (Effendi, 2016). However, the interpretation of data from a large number of physicochemical parameters and drawing proper conclusions can be very challenging (Popović et al., 2016). Therefore, it is necessary to apply appropriate water quality indices (Anyanwu and Umeham, 2020; Isiuku and Enyoh, 2020; Anyanwu et al., 2022). Regular evaluation of the surface water quality using useful and reproducible indices is required to maintain and control the surface water from degradation (Barakat et al., 2016; Shukla et al., 2017; Matta et al., 2020).

Water quality indices (WQIs) are accepted and useful tools for evaluating water quality, and different indices use different sets of variables (Bharti and Katyal, 2011). They are useful communication tools for presenting the health status of waterbodies to the public in a reliable way by policymakers, environmentalists, conservationists and different governmental agencies (Sadiq et al., 2010). The use of water quality indices has become a prevalent and reliable approach to water quality assessment and monitoring and has been extensively used by researchers (Adimalla et al., 2020;

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Anyanwu and Umeham, 2020; de Andrade Costa et al., 2020; Sharma et al., 2021; Anyanwu et al., 2022). This study was carried out to evaluate the potential of the Anambra River Basin water in south-eastern Nigeria for multiple uses with some selected water quality indices.

2. Material and methods

2.1. The studied area

The Anambra River Basin is situated in south-east of Nigeria, it is approximately 207.4 km to 210 km long (Odo, 2004) and rises from the Ankpa hills (ca. 305-610m above sea level) and flows into the River Niger at Onitsha (Odo, 2004). It is spatially located between latitudes 6°00'N and 6°30'N and longitudes 6°45'E and 7°15'E. A total of 14014 km² are drained by the river basin (Odo, 2004) (Fig.1).

2.2. Description

The Ogurugu fishing site (station I)

The Ogurugu fishing site is upstream with GPS coordinates (N 6°47'28" and E 6°56'48"). It is located in the Uzo-uwani local Government Area, Enugu State. A number of anthropogenic activities were observed, such as laundry, swimming, fishing, extraction of drinking water, manual sand mining, and farming (rice, cassava), at flood plain, transportation of different goods and human beings, and lumbering. The surface was partially canopied with aquatic plants, and the flow velocity was moderate.

The Otuocha Fishing site (station II)

The Otuocha Fishing site is about 210 km downstream of Ogurugu (N 6°20'30", E 6°50'33"), with a daily market along its bank. Notable human activities within the site are water transportation by canoe, washing, fishing, farming along banks, and extraction of water for irrigation.

The Otu-Nsugbe Fishing site (station III)

The Otu-Nsugbe is about 17 km downstream of the Otuocha fishing site (N 6°16'71", E 6°48'73"). It is located in Nsugbe at the Anambra River floodplain with notable anthropogenic activities, such as farming activities, mechanical dredging, fishing, and water transportation

Samples collection and analyses

Water samples were collected monthly for nine months (February - October 2022). Samples were collected with a clean 1 litre water sampler, after collection transferred and stored in 1litre plastic bottles and then transported to the laboratory in an ice chest for analysis. The physicochemical parameters were analyzed using standard methods. Three parameters - electrical conductivity, pH, and dissolved oxygen (DO) – were determined in situ with a multi-parameter meter (HQ40d). In the laboratory, nitrate nitrogen (NO_3-N) and phosphate (PO_4-P) were determined using the HACH-DR 6000 UV-Vis spectrophotometer, total dissolved solids (TDS)-gravimetric; Cl^- - titrimetric; K^+, Na^+-flame photometric (APHA, 1992); Ca^{2+} , Mg^{2+}- spectrophotometric (ISO 11885, 2007); SO_4^{2-} -

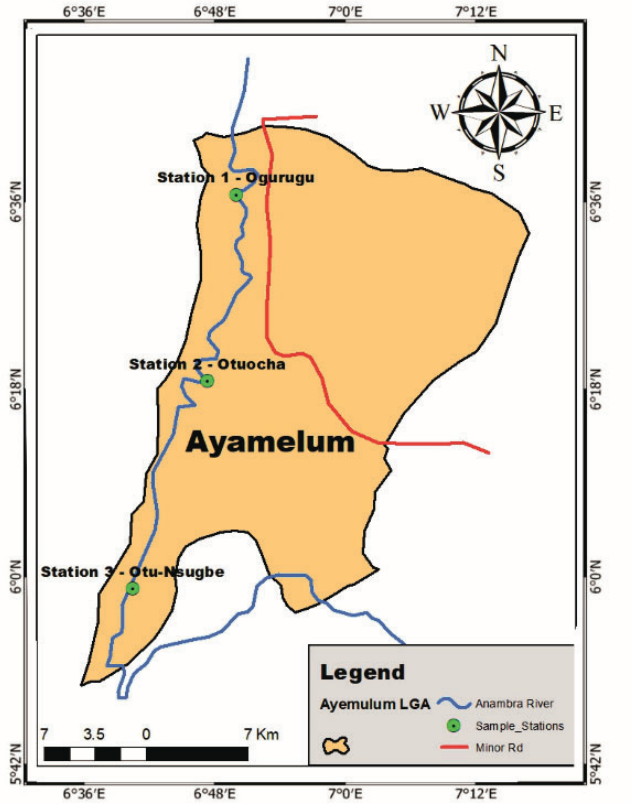

Fig.1. Map of studied area

turbidimetric method (APHA, 2005), while biochemical oxygen demand was determined after five days using the multi-parameter (HQ40d).

Comprehensive pollution index (CPI)

The comprehensive pollution index method offers useful information for the management and control of the pollution in a watershed (Son et al., 2020; Anyanwu et al., 2022). The equation for computing *CPI* is as follows:

$$PI_i = \frac{C_i}{S_i} \quad (1)$$

where C_i - measured concentration of parameter in water; S_i - permitted standard of parameter according to environmental standard (FMEnv., 2011).

$$CPI = \frac{1}{n}\sum_{i=0}^{n} PI_i \quad (2)$$

where *CPI* = Comprehensive Pollution Index; *n* = number of parameters investigated; PI_i = single factor pollution index number *i*. The *CPI* was computed using 13 water parameters: dissolved oxygen, biochemical oxygen demand, electrical conductivity, pH, turbidity, total dissolved solid, total suspended solid, chemical demand oxygen, chloride, phosphate, nitrate, sulphate, sodium, potassium, calcium, and magnesium.

Nutrient pollution index (NPI)

NPI was computed using the expression in Eq.

$$NPI = \frac{c_n}{\max_c} + \frac{c_p}{\max_p} \quad (3)$$

where $NPI = C_n$ an C_p are the monthly mean concentration of nitrate and phosphate in water samples, and are maximum allowable concentration of 50 mg/l and

5 mg/l for nitrate and phosphate in surface water respectively (FMEnv., 2011). The classification for *NPI* is categorized as *NPI* of < 1 (non-polluted), *NPI* of 3 ≤ 6 (considerable polluted) and NPI of > 6 (very high polluted) (Isiuku and Enyoh, 2020).

Potential salinity (PS)

This index indicates the suitability of water on the basis of concentration of insoluble salt (Meena and Bisht, 2020). It was computed using equation below

$$\text{Potential salinity } (PS) = Chloride + \frac{Sulphate}{2} \quad (4).$$

Soluble Sodium Percentage (SSP)

Soluble Sodium percentage is another important parameter used in evaluating sodium hazard and water quality for agricultural purposes (Udom et al., 2019). It is calculated using the following formula:

$$\text{Soluble Sodium Percentage } (SSP) = \frac{Na^{+} + K^{+}}{Ca^{2+} + Mg^{2+} + Na^{+} + K^{+}} \cdot 100 \quad (5)$$

3. Results and discussion

3.1. Physicochemical parameters

The summary of physico-chemical parameters determined in the Anambra River is shown in Table 1.

Water Temperature

Temperature is a principal water indicator that changes with the variation of climatic conditions of an area. Higher water temperature increases the rate of biochemical activity of aquatic organisms (Bhatnagar and Devi, 2013), thus increasing oxygen demand. The solubility of oxygen and the level of ammonia in aquatic ecosystems depend on water temperature. The spatio-temporal variations of water temperature ranged between 26.87°C and 30.12°C, with mean values of 29.24 ±2.04°C (station I), 28.76±3.12°C (station II), and 28.10±2.11°C (station III). The lowest value (26.87°C) was recorded at station 1 (September 2022), and the highest (30.12°C) was recorded also at station 1 (February, 2022). All the water temperature values were within the permissible range (40°C) and 20 – 32°C for drinking water (WHO, 2010) and for aquaculture (Kasmir and Rosmiati, 2014; Mutea et al., 2021). However, the temporal-spatial values registered during the sampling period were lower than the recommended level (22–26°C) for swimming and recreational aquatic environments (WHO, 2007).

Water pH and Dissolved Oxygen

The pH is measured as the negative logarithm of hydrogen ions concentration. The pH of natural aquatic ecosystems is greatly influenced by the concentration of carbon dioxide, which is an acidic gas (Boyd, 1979). It is very important to maintain the aquatic resource within a pH range of 6.5–8.5, as any alteration may lead to the destruction of aquatic organisms (Mutea, et al., 2021). Water with a low pH promotes corrosion of metal pipes and fittings, which can cause a sour taste (WHO, 2011). Swimming in surface water with a low pH can indeed result in the corrosive nature of water, skin and eye irritation, loss of chlorine, and skin stains in swimmers (Hoseinzadeh et al., 2013).

The spatio-temporal variations of water pH ranged between 5.0 and 6.9, with mean values of 5.53±0.30 (station I), 5.42±1.01 (station II), and 5.5±0.19 (station III). The lowest value (5.0) was recorded at station 1 (April and May, 2022) and the highest was also recorded at station 1 (February, 2022). The pH values were within the acidic range with mean values below permissible pH limits (6.5 to 9) for aquaculture (Ekubo and Abowei2011; Bhatnagar and Devi, 2019). The mean values exceeded also the range for water drinking, swimming/bathing and agriculture

Table 1. Summary of the mean values of the water quality parameters at each sampling station and the standard recommended for multi-uses

Param.	Min	Max	Station I	Station II	Station III	Aquaculture benchmark (VEPA, 2015)	Drinking water (WHO, 2010)	Recreation (WHO,2007)	Agriculture (FAO,1994)
Tem. (°C)	26.87	30.12	29.24±2.04[a]	28.76±3.12[a]	28.10±2.11[a]	20-32	40	22-26	-
pH	5.0	6.9	5.53±0.30[a]	5.42±1.01[a]	5.50±0.19[a]	6.5-8.85	6.5-8.5	7.0-8.4	6.5-8.5
DO (mg/l)	1.9	10	3.85±0.24[a]	6.11±1.00[b]	4.60±0.13[c]	5-15	> 5	9 -10	-
BOD (mg/l)	1.10	4.4	1.50±0.10[a]	2.16±0.08[a]	1.67±0.11[a]	01-02	-	1-2	-
TDS (mg/l)	0.40	15.50	2.75±0.10[a]	4.25±1.10[b]	4.10±0.84[b]	-	500	500	3000
TSS (mg/l)	0.90	3.10	1.92±0.18[a]	1.86±0.09[a]	1.47±0.20[b]	25-150	50	50	0.25
Cl (mg/l)	34.90	97.10	45.90±7.11[a]	64.76±12.03[a]	55.69±10.14[c]	50	250	100	1065
PO_4^{3-} (mg/l)	0.21	1.43	0.46±0.11[a]	0.56±0.03[b]	0.49±0.13[c]	0.05-0.5	11	0.2	-
SO_4^{2-}	0.04	0.35	0.10±0.00[a]	0.14±0.04[b]	0.15±0.07[c]	-	200	1000	-
NO_3^-	0.11	0.51	0.20±0.05[a]	0.37±0.14[a]	0.27±0.10[c]	0.1-4.50	50	20	10
CPI	0.15	0.31	0.22±0.05	0.27±0.11	0.22±0.05				
NPI	0.07	0.46	0.15±0.11	0.20±0.06	0.17±0.14				
PS	34.93	97.19	45.95±9.49	65.94±15.22	56.54±9.05				
SSP	11.58	22.92	17.10±3.91	17.35±3.4	18.87±4.63				

purpose (Klamt et al., 2021).

The results of this study agreed with those noted by Odo et al. (2022) that recorded a range of 4.6 to 6.6 mg/l in the Akor River in Ikwuano. The pH value has a direct relationship to the quality of water suitable for human consumption. Water with a high carbon dioxide content, low total alkalinity and low pH is considered aggressive (Klamt et al., 2021).

Dissolved oxygen affects the growth, survival, distribution, behaviour and physiology of aquatic organisms (Solis, 1988). Spatio-temporal variations of dissolved oxygen (DO) ranged from 1.9 to 10 mg/l, with a mean value of 3.85 ± 0.24 mg/l (station I), 6.11 ± 1.00 (station II), and 4.6 ± 0.13 (station III). The lowest value was recorded at station I (February, 2022), while the highest was recorded at station II (June, 2022). Spatio-temporal values of dissolved oxygen met standards for aquaculture, swimming, and agriculture between April and June 2022 at all the stations, while the mean values for stations I and III were lower than the recommended value (5–15 mg/l) for aquaculture and other aquatic biota (Boyd, 2014). Concentrations below 5 mg/l may adversely affect the functioning and survival of biological communities, and concentrations below 2 mg/l may lead to the death of most fish (Chapman, 1996). Dissolved oxygen is the quantity of oxygen dissolved in water, and it is essential to determine whether the water under study can support aquatic life (Nalder and Islam, 2015). A higher concentration of dissolved oxygen is associated with better water quality and taste (Omer, 2019). The survival and physiological activities of aquatic organisms at station I and station III, may be adversely affected. Dissolved oxygen depletion in water causes poor feeding of fish, starvation, reduced growth, and more fish mortality, either directly or indirectly (Bhatnagar and Garg, 2000). Aquatic ecosystem with a dissolved oxygen level of 3.0-5.0 mg/l is unproductive, and for average or good production, it should be above 5.0 mg/l (Bhatnagar and Devi, 2013). Very high concentration (> 14) of dissolved oxygen sometimes becomes lethal to fish fry in nursery ponds (Bhatnagar et al., 2004).

BOD

Bhatnagar and Dev (2013) pointed out that the desired ranges of BOD level for aquaculture should be between 1 mg/l and 2 mg/l. Spatio-temporal variations of biochemical oxygen demand (BOD) ranged between 1.10 and 4.40 mg/l, with a mean value of 1.50 ± 0.10 mg/l (station I), 2.16 ± 0.08 mg/l (station II), and 1.67 ± 0.11 mg/l (station III) (Table 1). The lowest value was recorded in station I (March, 2022), while the highest was in station II (May, 2022). The high BOD concentrations in this study may be ascribed to high levels of organic contamination from the agricultural farms and other anthropogenic activities that support micro-bacteria growth (Crim, 2007). All the values of BOD recorded were within the values (1 – 2 mg/l) recommended for aquaculture (VEPA, 2015) and water body for swimming/ recreation purposes (WHO, 2007). A high mean value at station II and wet season may cause sufficient oxygen for respiring aquatic organisms in the river. Individuals involved in water-based activities (swimming, sporting, and other recreational) are likely to be most sensitive to eutrophic conditions linked to high BOD, and the demand for all recreational activities is likely to be impacted due to impediment of activities, discomfort, and visual unpleasantness (Breen et al., 2018). Aquatic ecosystem with BOD levels between 1.0 and 2.0 mg/l is considered clean, 3.0 mg/l is fairly clean, 5.0 mg/l is considered doubtful and 10.0 mg/l is definitely bad and polluted (Ekubo and Abowei, 2011).

Total dissolved solid (TDS) and Total suspended solid (TSS)

Total dissolved solids influence the aesthetic value of the water through altering the turbidity and limit water body from performing its ecosystem functions as a drinking water source and irrigation supply (Titilawo et al., 2022). Total dissolved solid (TDS) ranged from 0.4 mg/l at station I (April, 2022) to 15.5 mg/l at station II (August, 2022), with mean values of 2.75 ± 0.10 (station I), 4.25 ± 1.10 (station II), and 4.10 ± 0.84 (station III).

Total suspended solid (TSS) ranged between 0.9 mg/l at station III (February, 2022) and 3.10 mg/l at station II (August, 2022), with mean values of 1.92 ± 0.18 (station I), 1.86 ± 0.09 (station II), and 1.47 ± 0.20 (station III). All the values of TSS were within the range (25-150 mg/l) recommended for aquaculture, and 500 mg/l for drinking water and swimming/ recreation and (WHO, 2007), 0.25 mg/l for agricultural purposes (Table 1). A large accumulation of suspended solids will reduce light penetration; thereby suppress photosynthetic activity of phytoplankton, algae and macrophytes. TSS > 80 mg/l may cause injure fish gills (Teodorowicz et al., 2006). The results of this study are quite below 450 mg/l set by FAO (2004), as cited by Misstear et al., (2017) for irrigation agriculture, and 600 mg/l set by WHO (2012) for drinking water. The results were in line with those of Odo et al., (2022) that recorded a range between 0.6 mg/l and 4.2 mg/l in the Akor River. A total suspended solids concentration below 20 mg/l appears clear, while levels over 40 mg/l may begin to appear cloudy (Fondriest Environmental, 2014).

Chloride (Cl^-)

Chloride is a common component of most aquatic ecosystem and is useful in maintaining their osmotic balance of aquatic organisms. When the level of chloride in the aquatic environment exceeds 100 mg/l, it causes burns on the gills and other parts. Chloride ranged from 34.9 mg/l at station I – 97.1 mg/l at station II (August, 2022), with mean values; 45.90 ± 7.11(station I), 64.76 ± 12.03(station II) and 55.69 ± 10.14 (station III).

All Cl values fall within the level desirable of 250 mg/l for drinking water, 100 mg/l for recreational activities, and 1065 mg/l for agricultural purposes (Table 1). However, few (45%) of the water samples had a concentration of chlorine (Cl) above the permissible value (50 mg/l) for aquaculture production (Table 1). High concentrations of chloride can make waters unpalatable and, therefore, unfit for drinking or livestock watering (Chapman, 1996), as well as inhibit plant growth, impair reproduction, and reduce the diversity of organisms in streams (United States Geological Survey, 2009). The free residual chlorine < 3 mg/l in the water pool was indicative of unsatisfactory management of the water disinfection and filtration process, because free residual chlorine may be unable to oxidize the organic compounds and kill the microorganisms that had enhanced the water while passing through the filters (Fadaei and Sadeghi, 2014).

Phosphate (PO_4^{3-})

It is an essential plant nutrient and stimulates plant (algae) growth in aquatic ecosystems. Phosphate is often in limited supply and its role for increasing the aquatic productivity is well recognized (Bhatnagar and Devi, 2013). Phosphate ranged from 0.21mg/l at station II (April, 2022) – 1.43 mg/l at station 1 (July), with mean values of 0.46±0.11(station I), 0.56±0.03(station II), and 0.49±0.13 (station III). More than half (65.24%) of sampled water had values above 0.05 - 0.5 mg/l recommended for aquaculture and 0.2 mg/l for swimming/recreation water (Table 1). However, all the values recorded were quite below the level (11 mg/l) for drinking water. The values recorded in this recent study were within/slightly higher than the recommended level (0.05 to 0.5 mg) for aquaculture (Bhatnagar et al., 2004; Stone and Thomforde, 2004; Bhatnagar and Devi, 2013).

Sulphate (SO_4^{2-})

The presence of sulphate in aquatic ecosystem can alter taste of drinking water from the system, and very high levels can cause a laxative effect in unaccustomed consumers (Klamt et al., 2021). Sulphate ranged from 0.04 mg/l at station I (April, 2022) to 0.35 mg/l at station III (July, 2022), with mean values of 0.10±0.002 (station I), 0.14±0.04 (station II) and 0.15±0.07 (station III). All the values recorded were within the adverse level (200 mg/l) good for drinking water (WHO, 2010), the desirable level (1000 mg/l) for swimming/recreational activities, and the level (0-96 mg/l) for agricultural purpose (FAO, 1994). High concentrations of sulphate above 400 mg/l may make water unpleasant to drink (Chapman, 1996). Gastrointestinal related diseases have been linked to drinking water with a high level of SO42- (Klamt et al., 2021). It is recommended that health authorities should be notified of drinking water sources with SO42- concentrations above 500 mg/l (WHO, 2017).

Nitrate (NO_3^-)

Nitrate ranged from 0.11 mg/l at station I (April) to 0.51 mg/l at station III (July), with mean values of 0.20±0.05 (station I), 0.37±0.14 mg/l (station II), and 0.27±0.10 mg/l (station III). All the values recorded in the sampled water during the sampling period were within the level (0.1 to 4.5 mg/l) good for aquaculture (Bhatnagar and Devi, 2013). Nitrate is relatively nontoxic to fish and causes no health hazard except at exceedingly high levels (above 90 mg/l) (Stone and Thomforde, 2004; Santhosh and Singh, 2007). Nitrates have immense significance as major nutrients for the succession and productivity of phytoplankton and aquatic macrophytes (Mishra and Patro, 2015).

Generally, the nitrate concentration was within the maximum permissible limit of 50 mg/l for drinking water, as recommended by the Standard Organization of Nigeria (2015). Excess nitrate ion in drinking water is worrying as it causes blue baby syndrome in newborns and may cause stomach cancer in adults as well as increasing the likelihood of breast cancer in women (Baird and Cann, 2011) and other health disorders (USEPA, 2017). Contamination of fresh water with nitrates leads to significant environmental problems and health risks when water is used for drinking (WHO, 2007).

3.2. Drinking water quality indices

Comprehensive Pollution Index (CPI)

Spatio-temporal variations of CPI ranged from 0.150 at station I (March, 2022) to 0.449 at station II (May, 2022). All the CP1 values were within the status of non-polluted (< 0.4). However, station II in May 2022 exceeded the non-polluted level (<0.4). The results were within the level of CPI recorded by Anyanwu et al. (2022) in the Ikwu River, Umuahia. However, Matta et al. (2018) and Imneisi and Aydin (2018) reported high level (0.54 -2.47) in the Ganga River at Rishikesh, India, and (0.60-0.88) in the Elmali and Karacomak streams, Turkey respectively.

Nutrient Pollution Index (NPI)

Spatio-temporal variations of the nutrient pollution index (NPI) ranged from 0.07 at station I (April, 2022) to 0.46 at station I (July, 2022). All the NPI values were within the category of non-polluted (< 1) throughout the sampling period. Season has a great effect on the nutrient pollution index, although the seasonal activities have not had negative impact on water quality.

Pollution of food and water sources with nitrate is of concern due to its mental retardation as a result of methemoglonaemia (Isiuku and Enyoh, 2020). This indicated that throughout the sampling duration, the Anambra River Basin was not enriched with phosphate and nitrate; thus, both human and aquatic animals that depend on the river for socio-economic activities and survival, respectively, are not at risk of eutrophication. According to Isiuku and Enyoh (2020), surface water bodies are moderately polluted in the dry season and highly polluted in therainy season in south-eastern Nigeria.

3.3. Irrigation water quality indices

Potential Salinity (PS)

Potential Salinity indicates the suitability of water on the basis of the concentration of insoluble salt. Potential salinity (PS) in water from th Anambra River Basin ranged from 34.93 mg/l at station I (February, 2022) to 97.19 mg/l at station II (August, 2022). All the water from the three stations exhibited high potential salinity. The results indicated that water from the Anambra River Basin is not good for irrigation purposes. The results were within the high potential salinity recorded in Chaksu tehsil, Jaipur District, Rajasthan, India (Meena and Bisht, 2020). However, the result was quite higher than < 3.0 recorded by Pivic et al. (2022). Majority (91.63%) of their water samples had a potential salinity (PS) of more than 70%; thus falling out of good water for irrigation.

Soluble Sodium Percentage (SSP)

High sodium level in water for irrigation adversely affect the soil permeability, water infiltration, and total salinity leading to insoluble salt crystals and alkaline soil, which negatively affect some vulnerable crops (Megahed, 2020). Soluble sodium percentage (SSP) ranged between 11.58 % (September, 2022) at station I and 22.92 % (April, 2022) at station I. Wilcox (1950) classified SSP as <20% (excellent), 20 – 40% (good), 40 – 80% (fair), and >80% (poor/unsuitable).

SSP is one of the vital parameters widely used for assessing sodium hazard and water quality for irrigation purposes (Anyanwu et al., 2022). The SSP values were within the excellent irrigation category (<20%) at station I, with exception of 26.92% and 25.27% recorded in April and May 2022, respectively; which are within the good water category. At station II, all the values were within the excellent category (< 20%), except 21.95% recorded in August 2022. All the values recorded at station III were within the good irrigation water category, except for those recorded in July-September 2022. The SSP values were within the excellent irrigation. The SSP values were lower than those previously recorded in some Nigerian rivers; (28.16-34.69%) recorded by Udom et al. (2019) in the Abak River were within the good irrigation water category, and Eruola et al. (2020) recorded values (51.8 – 54.0%) that was within the fair/permissible category in the Owiwi River, Abeokuta, Ogun State. However, Anyanwu et al. (2022) and Omofunmi et al. (2019) recorded values within the excellent category in the Ikwu River, Umuahia, Abia State, and Ero dam, Ikun –Ekiti, Ekiti State, all in Nigeria.

4. Conclusion

This assessment revealed that all the parameters evaluated were within acceptable limits for drinking, fish production, recreation, and agriculture purposes, except pH (May - October 2022) and dissolved oxygen (February - March, July - October 2022). All the indices were also favourable and within their respective acceptable limits. The indices applied effectively examined the water quality of Anambra for multiple purposes and revealed that the water quality of the Anambra River Basin was suitable for the designated purposes.

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Conflict of interests

The authors hereby state that this research work and manuscript production complied with ethical standards, and none of the authors have any potential conflict of interests. We further declare that this research was not funded by any agency.

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Sanitary-microbiological characteristics of water in the area of the Zmeinyi thermal spring (Northern Baikal, Russia, 2022)

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ABSTRACT. The analysis of sanitary-significant microorganisms in two baths of the thermal spring Zmeinyi, the streams of the baths and at a distance of 5 and 20 meters from the shore was carried out during the day before and the day after tourists' bathing. Exceedance of permissible values of sanitary-microbiological indicators in accordance with SanPiN 1.2.3685-21 were detected in the baths, streams of outflow and in the water edge at a distance of up to 5 meters. The indicator of total (generalized) coliform bacteria (TCB) in all studied samples did not decrease throughout the day, the highest values of the number of opportunistic microorganisms were observed in the baths of the thermal spring and in the streams of outflow, where the water temperature was ~ 32 - 33°C. The obtained self-cleaning coefficients in the littoral zone up to 20 m had low values (from 0.0008 to 2.53).

Keywords: Zmeinyi sulfide spring, organotrophic bacteria, coliform bacteria, enterococci

1. Introduction

There are about 60 hydrotherms with different temperature characteristics in the Baikal Rift Zone (BRZ) (Lomonosov, 1974; Borisenko and Zamana, 1978; Zamana and Askarov, 2010). Basically, waters of hydrotherms have sodium composition, low mineralization (from 0.1 g/l to 2.0 g/l), high concentration of silicon in the form of orthosilicic acid H_4SiO_4 (40 - 120 mg/dm^3) and alkaline environment (pH 8.5 - 10.0).

Studies of sanitary condition of hydrotherms are episodically carried out. In alkaline mineral springs of the Barguzin valley high TCB indices were revealed, as well as the presence of opportunistic bacteria of the genera *Enterobacter*, *Klebsiella*, *Escherichia*, *Citrobacter* in frequently visited springs (Kuchiger, Seyuya, Goryachinsk, Kumyska), which indicated a large anthropogenic load (Darmaeva, 2007; Barkhutova, Darmayeva and Namsarayev, 2012).

The Zmeinyi spring located in the Chivyrkuisky bay is very popular place, and a significant number of tourists visit it during the summer period. Its waters are hydrocarbonate-sulphate, contain hydrogen sulphide (H_2S) at a concentration of 23.2 mg/l, and are used for treatment and prevention of radiculitis and disorders of the musculoskeletal system (Namsaraev et al., 2007).

When examining the diversity of microbial communities by sequencing the V2-V3 region of the 16S rRNA gene on the MiSeq platform (Illumina), representatives of the families that included opportunistic species were identified in different parts of the Zmeinyi Spring in 2019 (Chernitsyna et al., 2023). Significant exceedances of sanitary-bacteriological indicators (SanPiN 1.2.3685-21) were also observed in Zmeinaya Bay in 2022. The number of TCB in this area exceeded the normative indicators 2 times, enterococci 44 times (Suslova et al., 2022). All previous studies were based on single sampling, data on the dynamics of sanitary-significant microorganisms during a day have not been presented before.

The aim of the study is to assess the number of sanitary-significant microorganisms in the thermal spring Zmeiny and the coastal zone during the day before and the day after tourists` bathing, as well as determining the self-purifying capacity of coastal waters in the places of springs outflow.

2. Materials and methods

Surface water samples were taken according to GOST 31942-2012 in the small and large baths (SB, LB), in the streams of the small and large bath (sSB, sLB), near the pier (P), as well as at a distance of 5 and 20 meters from the shore (5 m and 20 m) (Fig.1). Sampling was conducted on July 11, 2022 at 23:00 after daytime tourists` bathing, on July 12 in the early morning after 6-hour absence of bathers and at 9:00 immediately

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after bathing of several groups of tourists. A total of 16 water samples were collected and 320 analyses were conducted in accordance with MUK 4.2.1884-04 and GOST 24849-2014.

At all sites, water quality was assessed by basic sanitary-microbiological indicators in accordance with SanPiN 1.2.3685-21. The following parameters were determined in each sample in accordance with the methodological instructions (MUK 4.2.1884-04, GOST 24849-2014): TCB, *E. coli*, enterococci, the number of which should not exceed 500 CFU/100 cm^3, 100 CFU/100 cm^3, 10 CFU/100 cm^3, respectively.

The total microbial population (TMP) was conducted by direct microscopic counting of microorganisms on 0.22 µm pore size membrane filters (REATREK-Filter) using DAPI dye (4,6-diamino-2-phenylindole) (Porter, 1980). Microscopy was performed with an epifluorescence microscope AxioImager.M1 ("Carl Zeiss", Germany). At least 20 fields of vision were viewed. Cell counting in the photographs was carried out using the ImageTest program. Calculation of the TMP value was done by the formula:

$$X = \frac{a \cdot b \cdot 10^6}{c \cdot d \cdot e}$$

where a - is the area of filter (mm^2); b - number of bacteria counted; c - micrometer area (μm^2); d - volume of applied preparation (ml); e - number of counted fields of vision (Gerhardt, 1981).

To determine the self-cleansing coefficient, we used MUK.4.2.1884-04. The total microbial count (TMC) was calculated, reflecting the total content of mesophilic aerobic and facultatively anaerobic microorganisms capable of forming colonies on meat-peptone nutrient agar (MPA) at 37°C for 24 h (MPA 37°C) and at 22°C for 72 h (MPA 22°C). Also, to determine the abundance of organotrophic bacteria, a medium with fish-peptone agar (FPA:10) was used, the cultivation temperature was 37°C (Gorbenko, 1961).

Physicochemical characteristics of water (pH, Eh, T) were measured with an instrument pH 3310 (WTW, Germany).

3. Results

3.1. Physicochemical characteristics of the spring

The Zmeinyi spring is characterized by relatively high temperature compared to other springs of the Barguzin Basin (Namsaraev et al., 2007). The water temperature in the large bath was 43°C, and in the small one was 38.6°C, pH was 9.6. The redox potential (ROP) in the large bath was -434 mV and in the small bath -427 mV, indicating reduced conditions in the spring. At the water's edge, the water temperature was 17°C, pH was 8.8, ROP was -239 mV.

Fig.1. Geographical map of the study area and sampling locations in Zmeinaya Bay (Lake Baikal, Russia). **LB** – large bath, **SB** – small bath, **sLB** – stream of the large bath, **sSB** – stream of the small bath, P – pier, **5 m** - 5 meters, **20 m** – 20 meters. Software Earth 7.1.8.3036 Pro https://www.google.com/intl/ru/earth/versions/#earth-pro (accessed on February 20, 2023).

3.2. Number of sanitary-significant microorganisms

On July 11 at 23:00 p.m. after mass tourists bathing in the area of the thermal spring outlet (SB, LB, sSB, sLB), the high TMP values were recorded when cultured on MPA at 37°C, their values ranged from 2013 to 12426 CFU/cm^3 (Fig.2).

This indicator was low at the water's edge near the pier and at a distance of 5 - 20 meters from the shore (10-36 CFU/cm^3). High count of bacteria growing at 22°C (MPA) and 37°C (FPA:10) was recorded in the stream of small bath (up to 5760 and 3613 CFU/cm^3, respectively). The TMP value was relatively high in that time in the streams of large and small baths and was 8.36±0.34 and 8.28±0.38 million cells/ml, respectively (Table 1).

High number of TCB was recorded in the large and small baths, in streams and in the littoral zone near the pier (Fig. 3). At a distance of 5 meters from the shore this indicator was also high (up to 626 CFU/100 cm^3). The stream sample from the large bath showed

Table 1. Total microbial count (million cells/mL)

Sampling site / Sampling time	LB	SB	sLB	sSB	P	5 m	20 m
23:00	6.87±0.32	6.83±0.14	8.36±0.34	8.28±0.38	2.05±0.07	1.97±0.05	2.14±0.08
5:00	*	8.02±0.37	5.09±0.22	7.96±0.21	2.27±0.06	2.20±0.07	3.00±0.15
9:00	4.69±0.11	3.01±0.22	-	-	-	-	-

Note: "–" no study was conducted; "*" - number exceeding the maximum value for counting on filters.

continuous growth, while in the littoral zone of the lake this indicator was already within normal limits at 20 meters from the stream (180 CFU/100 cm^3). High levels of *E.coli* bacteria and recent fecal contamination recorded only in the baths and streams. Spores of sulphite-reducing clostridia were found in the large bath and (2 and 1 CFU/20 cm^3, respectively).

At 5:00 a.m. on July 12, after a 6-hour break, the count of mesophilic aerobic and facultatively anaerobic microorganisms growing on MPA at 37°C and 22°C decreased. Their count ranged from 563 to 7386 CFU/cm^3 and from 23 to 826 CFU/cm^3, respectively (Fig. 2). In the littoral zone at a distance of 20 m from the shore, the TMC value (MPA 37°C) increased to 3440 CFU/cm^3. At this time, an increase in the number of organotrophic bacteria growing on FPA:10 (2330 and 2963 CFU/cm^3, respectively) was recorded in the small and large baths). A high TMP value was recorded in a large bath when filtering 1 ml of diluted sample, cells formed conglomerates, making counting difficult, which did not allow obtaining reliable values (Table 1). Low TMP values were recorded near the pier and at 5 and 20 m from the shore. Number of TCB in the baths and streams increased approximately 2 times compared to evening values (Fig. 3). At the same time, in the coastal water zone at a distance of 5 and 20 meters from the shore, this indicator decreased by about 30 - 40%. There was also a decrease in the number of *E. coli* and enterococci by 60 - 70 %. Spores of sulfite-reducing clostridia were found only in the stream of the large bath (4 CFU/20 cm^3) and were not detected in other samples.

At 9:00 a.m. on July 12, after several groups of people bathed, in the large and small baths, the TMC counted on the MPA at 37°C decreased to 903 and 1,050 CFU/cm^3, respectively, compared to the morning and evening values. The TMP values in the large and small baths were relatively low (4.69 ± 0.11 and 3.01 ± 0.22 million cells per ml, respectively). The number of TCB in the large bath remained high as it was at 5:00 a.m., while in the small bath this index decreased to 3246 CFU/100 cm^3 (see Fig. 3). The number of spores of sulfite-reducing clostridia was maximum at this time and reached 15 and 12 CFU/20 cm^3.

Relatively high self-cleaning coefficient was observed in the coastal zone: near the pier (2.53) and at a distance of 5 meters from the shore at 23:00 and 5:00 (1 and 2.16, respectively) (Table 2).

4. Discussion

The healing properties of geothermal springs have been known for a long time. As shown in the work of Buslov S.P. (Buslov, 1990), mineral water from BRZ (Baikal Rift Zone) springs has a beneficial effect on various organ systems: nervous, respiratory, motor, genitourinary, etc., as well as improves skin condition and promotes wound healing. Therefore, the springs are very popular among the population and are open to the public (Namsaraev et al., 2007). At the same time, the geothermal springs of BRZ exceeded repeatedly the normative values of sanitary-significant

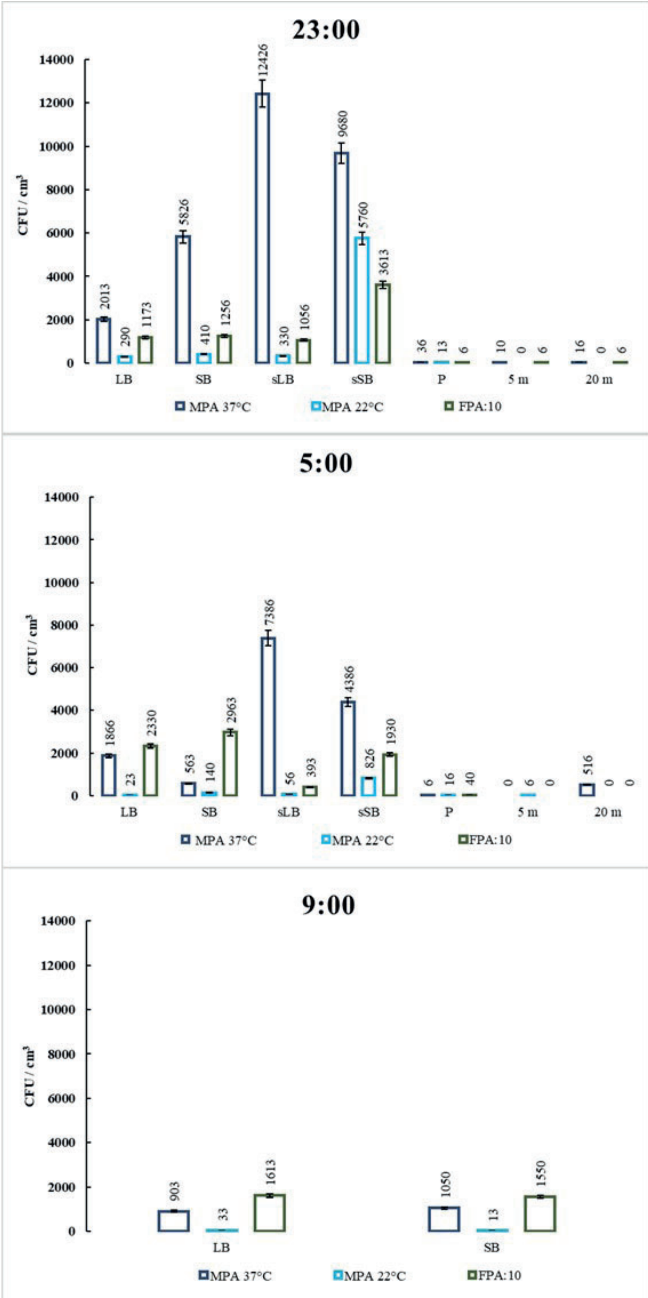

Fig.2. Number of organotrophic mesophilic aerobic and facultatively anaerobic microorganisms in the studied water zone during a day

Table 2. Self-cleaning coefficient in the water area of Zmeinaya Bay

Sampling site / Sampling time	P	5 m	20 m
23:00	0.27	1	0
5:00	2.53	2.16	0.0008
9:00	-	-	-

Note: "-" – no studies were conducted microorganisms. Thus, in 2012, opportunistic bacteria of the *E. coli* group and bacteria of genus *Enterococcus* were revealed in mineral waters of the Shumak River valley (Suslova et al., 2013). In cold and hot springs of the Baikal region (Kumyska, Serebryany, Goryachinsk), opportunistic microorganisms belonging to the genera *Enterobacter*, *Klebsiella*, *Escherichia*, *Citrobacter* and the pathogenic species *Clostridium perfringens*, which is a causative agent of human food poisoning and one of the causative agents of gas gangrene, have also been detected (Barkhutova et al., 2012). As we mentioned above, exceedances of values of sanitary-significant bacteria in the summer period were repeatedly noted in the Zmeinyi spring. Although according to the chemical analysis data, the water of the Zmeinyi spring is constant in time, its composition does not change and corresponds to the previously obtained data (Namsaraev et al., 2007; Plyusnin et al., 2013; Kalmychkov et al., 2020; Chernitsyna et al., 2020). As our studies have shown, an increased content of sanitary-significant microorganisms in the thermal spring of Zmeinaya Bay was observed at different time of the day after the visit of tourists. The highest values exceeding the normative values according to SanPiN 1.2.3685-21 were observed in the evening time of a day (23:00). Early in the morning, despite the 6-hour absence of bathers, the values of TCB, *E. coli*, and enterococci also exceeded the permissible values. Incomplete self-purification processes or insufficient dilution of thermal waters with Baikal waters is evidenced by the values of self-purification coefficient (2.16 - 2.53) near the pier and in the lake littoral zone at a distance of 5 m from the shore.

One of the reasons for constant contamination of the spring with opportunistic microorganisms may be high water temperature (40 - 42°C), which is close to the optimum (37°C) for their growth. For example, *E. coli* has a growth optimum of 37°C, although some strains are able to grow from 40 to 49°C (Ingledew and Poole, 1984). Preservation of bacterial viability, including sanitary bacteria, may be facilitated by low flow of the spring and restored environmental conditions. In streams where water is saturated with O_2 and water temperature is ~ 32 - 33°C, microbial abundance also remains high. The values of TCB, *E.coli*, and enterococci abundance meet the norms only in the littoral zone at a distance of 5 - 20 meters from the shore, where water temperatures are 17°C. It is obvious that a combination of such factors as temperature, water renewal rate in the baths and the number of bathers affect the development and distribution of opportunistic microorganisms in the investigated spring and coastal water area. According to our research results, the waters in the large and small baths of the thermal spring Zmeinyi do not meet the requirements of SanPiN 1.2.3685-21 and are not safe for balneological purposes without special measures. Possible measures to increase the flow of waters of this spring is adding Baikal water at night. Dilution of thermal waters and temporary reduction of temperature in them can reduce the survival rate of opportunistic microorganisms and improve the quality of water in the baths of the spring.

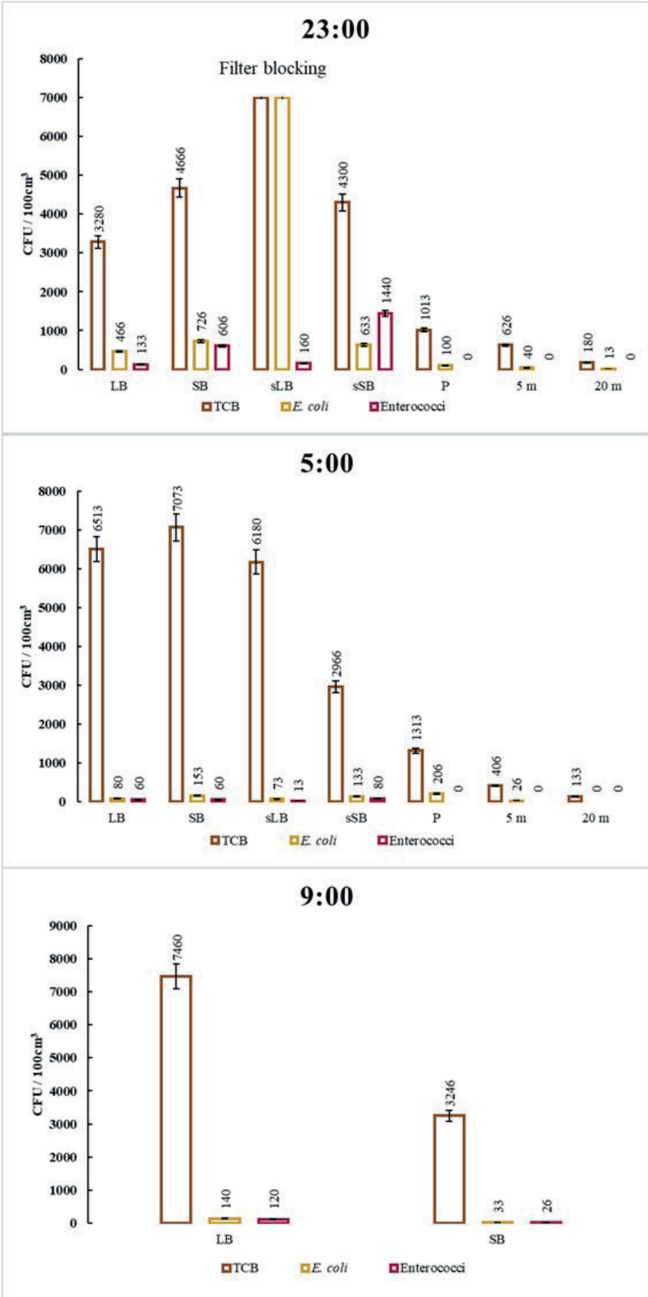

Fig.3. Number of sanitary-significant microorganisms in the studied water area during a day

5. Conclusion

According to SanPiN 1.2.3685-21, at different time of the day in the Zmeinyi spring exceedance in the norms of sanitary-microbiological indicators (TCB, *E. coli*, enterococci) were observed. The highest content was observed after tourists bathing, their number remained high and after a 6-hour break. In the littoral zone of the lake (20 meters from the shore) the number of the studied groups corresponded mainly to the norms. It is obvious that a combination of such factors as temperature, water renewal rate in baths and the number of bathers affect the development and distribution of opportunistic microorganisms in the studied spring and coastal water area.

Healing springs, as objects of permanent recreational visit, should be protected from the transfer of opportunistic bacteria in the places of mineral water outlet using special measures. There is also an obvious need to develop regulations for tourists visiting this spring.

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Conflict of interests

The authors declare no conflicts of interest.

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Санитарно-микробиологическая характеристика воды в районе термального источника Змеиный (Северный Байкал, Россия, 2022)

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АННОТАЦИЯ. Проведен анализ санитарно-значимых микроорганизмов в двух ваннах термального источника Змеиный, их ручьях и на расстоянии 5 и 20 метров от берега в течении суток до и после купания туристов. Выявлены превышения допустимых значений санитарно-микробиологических показателей в соответствии с СанПиН 1.2.3685-21 в ваннах, ручьях излива и в урезе воды на расстоянии до 5 м. Показатель общих (обобщенных) колиформных бактерий (ОКБ) во всех исследованных пробах не снижался на протяжении всего дня, наиболее высокие значения численности условно-патогенных микроорганизмов отмечены в ваннах термального источника и в ручьях излива, где температура воды составляет ~ 32 - 33°С. Полученные коэффициенты самоочищения в литорали на расстоянии до 20 м имели низкие значения (от 0.0008 до 2.53).

Ключевые слова: сульфидный источник Змеиный, органотрофные бактерии, колиформные бактерии, энтерококки

1. Введение

В Байкальской рифтовой зоне (БРЗ) насчитывается около 60 гидротерм с разными температурными характеристиками (Ломоносов, 1974; Борисенко и Замана, 1978; Замана и Аскаров, 2010). В основном, воды гидротерм имеют натриевый состав, низкую минерализацию (от 0.1 г/л – 2.0 г/л), высокую концентрацию кремния в виде ортокремниевой кислоты H_4SiO_4 (40 - 120 мг/дм^3) и щелочную среду (pH 8.5 – 10.0).

Исследования санитарного состояния гидротерм проводятся эпизодически. В щелочных минеральных источниках Баргузинской долины выявлены высокие показатели ОКБ, а также наличие условно-патогенных бактерий родов *Enterobacter*, *Klebsiella*, *Escherichia*, *Citrobacter* в часто посещаемых источниках (Кучигер, Сеюя, Горячинск, Кумыска), что свидетельствует о большой антропогенной нагрузке (Дармаева, 2007; Бархутова и др., 2012).

Источник Змеиный, расположенный в Чивыркуйском заливе, пользуется большой популярностью, в летний период его посещает значительное количество туристов. Его воды относятся к гидрокарбонатно-сульфатным, содержат сероводород (H_2S) в концентрации 23.2 мг/л, и используются для лечения и профилактики радикулита и заболеваний опорно-двигательной системы (Намсараев и др., 2007).

При исследовании разнообразия микробных сообществ методом секвенирования региона V2–V3 гена 16S рРНК на платформе MiSeq (Illumina), в разных частях источника Змеиный в 2019 году были выявлены представители семейств, которые включают условно-патогенные виды (Chernitsyna et al., 2023). Значительные превышения санитарно-бактериологических показателей (СанПиН 1.2.3685-21) в бухте Змеиная отмечались и в 2022 г. Количество ОКБ в этом районе превышало нормативные показатели в 2 раза, энтерококков в 44 раза (Suslova et al., 2022). Все предыдущие исследования основывались на разовых отборах образцов, данные о динамике санитарно-значимых микроорганизмов в течение суток ранее не приводились.

Целью работы являлась оценка численности санитарно-значимых микроорганизмов в термальном источнике Змеиный и прибрежной зоне в течение суток до и после купания туристов, а также определение самоочищающей способности прибрежных вод в местах излива источников.

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2. Материалы и методы

Пробы поверхностной воды отбирали в соответствии с ГОСТ 31942-2012 в малой и большой ванне (БВ, МВ), в ручьях малой и большой ванны (рБВ, рМВ), у пирса (П), а также на расстоянии 5 и 20 метрах от берега (5 м и 20 м) (Рис.1). Отбор проб проводили 11 июля 2022 года в 23:00 после дневного купания туристов, 12 июля рано утром после 6-часового отсутствия купающихся и в 9:00 сразу же после купания нескольких групп туристов. Всего отобрано 16 образцов воды и проведено 320 анализов в соответствии с МУК 4.2.1884-04 и ГОСТ 24849-2014.

На всех участках качество воды оценивали по основным санитарно-микробиологическим показателям в соответствии с СанПиН 1.2.3685-21. В каждом образце в соответствии с методическими указаниями (МУК 4.2.1884-04, ГОСТ 24849-2014) определяли следующие показатели: ОКБ, *E. coli*, энтерококки, количество которых не должно превышать 500 КОЕ/100 см^3, 100 КОЕ/100 см^3, 10 КОЕ/100 см^3, соответственно.

Подсчет общей численности микроорганизмов (ОЧМ) проводили по методике прямого микроскопического подсчета микроорганизмов на мембранных фильтрах с размером пор 0.22 мкм (РЕАТРЕК-Фильтр) с использованием красителя ДАФИ (4.6-диамино-2-фенилиндол) (Porter, 1980). Микроскопию проводили на эпифлуоресцентном микроскопе AxioImager.M1 (“Carl Zeiss”, Германия). Просмотрено не менее 20 полей зрения. Подсчет клеток на фотографиях проводили с помощью программы ImageTest. Вычисление ОЧМ проводили по формуле:

$$X=\frac{a \cdot b \cdot 10^6}{c \cdot d \cdot e}$$

где a – площадь фильтра (мм^2); b – число подсчитанных бактерий; c –площадь микрометра (мкм^2); d – объем наносимого препарата (мл); e – число просчитанных полей зрения (Gerhardt, 1981).

Для определения коэффициента самоочищения использовали МУК.4.2.1884-04. Подсчитано общее микробное число (ОМЧ), отражающее общее содержание мезофильных аэробных и факультативно анаэробных микроорганизмов способных образовывать на мясо-пептонном питательном агаре (МПА) колонии при температуре 37°C в течение 24 ч (МПА 37°C) и при температуре 22°C в течение 72 ч (МПА 22°C). Также для определения численности органотрофных бактерий использовали среду с рыбо-пептонным агаром (РПА:10), температура культивирования составляла 37°C (Горбенко, 1961).

Физико-химические характеристики воды (pH, Eh, T) измерены с помощью прибора pH 3310 (WTW, Германия).

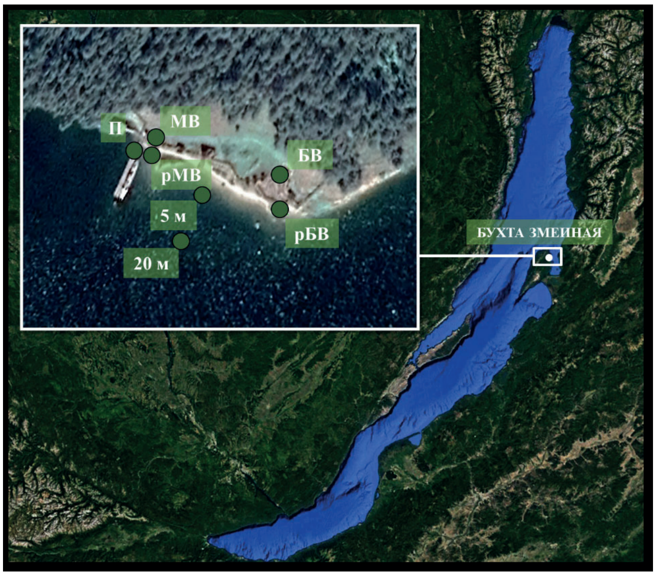

Рис.1. Географическая карта района исследования и мест отбора проб в бухте Змеиная (озеро Байкал, Россия). **БВ** – большая ванна, **МВ** - малая ванна, **рБВ** – ручей большой ванны, **рМВ** – ручей малой ванны, **П** – пирс, **5 м** - 5 метров, **20 м** – 20 метров. Программное обеспечение Earth 7.1.8.3036 Pro https://www.google.com/intl/ru/earth/versions/#earth-pro (дата обращения: 20 февраля 2023 года).

3. Результаты

3.1. Физико-химические характеристики источника

Источник Змеиный характеризуется относительно высокой температурой по сравнению с другими источниками Баргузинской котловины (Намсараев и др., 2007). Температура воды в большой ванне составляла 43°C, в малой – 38.6°C, pH 9.6. Окислительно-восстановительный потенциал (ОВП) в большой ванне был -434 мВ, в малой -427 мВ, что свидетельствует о восстановленных условиях в источнике. В урезе воды температура воды составляла 17°C, pH 8.8, ОВП -239 мВ.

3.2. Численность санитарно-значимых микроорганизмов

Вечером 11 июля в 23:00 после массового купания туристов в районе выхода термального источника (МВ, БВ, рМВ, рБВ) зафиксированы высокие показатели ОМЧ при культивировании на МПА при 37°C, их значения варьировали от 2013 до 12426 КОЕ/см^3 (Рис. 2). В урезе воды у пирса и на расстоянии 5-20 метров от берега данный показатель был низким (10-36 КОЕ/см^3). Большое количество бактерий, растущих при температуре 22°C (МПА) и 37°C (РПА:10), зарегистрировано в ручье малой ванны (до 5760 и 3613 КОЕ/см^3, соответственно). Показатель ОЧМ в это время был относительно высоким в ручьях большой и малой ванн и составлял 8.36±0.34 и 8.28±0.38 млн. клеток на мл, соответственно (Табл. 1).

Таблица 1. Общая численность микроорганизмов (млн. кл/мл)

Район отбора / Время отбора	БВ	МВ	рБВ	рМВ	П	5 м	20 м
23:00	6.87 ± 0.32	6.83 ± 0.14	8.36 ± 0.34	8.28 ± 0.38	2.05 ± 0.07	1.97 ± 0.05	2.14 ± 0.08
5:00	*	8.02 ± 0.37	5.09 ± 0.22	7.96 ± 0.21	2.27 ± 0.06	2.20 ± 0.07	3.00 ± 0.15
9:00	4.69 ± 0.11	3.01 ± 0.22	-	-	-	-	-

Примечание: «-» - исследования не проводились; «*» - число превышающее максимальное значение для подсчета на фильтрах.

Высокая численность ОКБ зарегистрирована в большой и малой ваннах, в ручьях и в литорали у пирса (Рис. 3). На расстоянии 5 метров от берега данный показатель также был высок (до 626 КОЕ/100 см^3). В образце ручья из большой ванны отмечен сплошной рост, а в литорали озера, в 20 метрах от ручья, данный показатель был уже в пределах нормы (180 КОЕ/100 см^3). Высокие уровни бактерий кишечной палочки и энтерококков, свидетельствующие о свежем фекальном загрязнении, зарегистрированы только в ваннах и ручьях. Споры сульфитредуцирующих клостридий обнаружены в большой ванне и в ручье излива (2 и 1 КОЕ/20 см^3, соответственно).

В 5:00 утра 12 июля после 6-часового перерыва, численность мезофильных аэробных и факультативно анаэробных микроорганизмов растущих на МПА при температуре 37°С и 22°С, снизилась. Их количество варьировало от 563 до 7386 КОЕ/см^3 и от 23 до 826 КОЕ/см^3, соответственно (Рис. 2). В литорали на расстоянии 20 м от берега значение ОМЧ (МПА 37°С) возросло до 3440 КОЕ/см^3. В это время в малой и большой ваннах зафиксировано повышение численности органотрофных бактерий, растущих на РПА:10 (2330 и 2963 КОЕ/см^3, соответственно). Высокое значение ОЧМ было зарегистрировано в большой ванне: при фильтровании 1 мл разведенного образца, клетки образовывали конгломераты, затрудняя подсчет, что не позволило получить достоверных значений (табл.1). Низкие значения ОЧМ отмечены у пирса и на расстоянии 5 и 20 м от берега. Количество ОКБ в ваннах и ручьях увеличилось примерно в 2 раза, по сравнению с вечерними значениями (Рис. 3). Вместе с тем, в прибрежной акватории на расстоянии 5 и 20 метров от берега данный показатель снизился примерно на 30 - 40 %. Также отмечено снижение численности *E. coli* и энтерококков на 60 - 70 %. Споры сульфитредуцирующих клостридий обнаружены только в ручье большой ванны (4 КОЕ/20 см^3) и не выявлены в других образцах.

В 9:00 утра 12 июля после купания нескольких групп людей, в большой и малой ваннах ОМЧ, учтенное на МПА при 37°С, снизилось до 903 и 1050 КОЕ/см^3, соответственно, в сравнении с утренними и вечерними показателями. Значения ОЧМ в большой и малой ваннах было относительно низким (4.69 ± 0.11 и 3.01 ± 0.22 млн. клеток на мл, соответственно). Численность ОКБ в большой ванне оставалась высокой, как и в 5:00 утра, в малой ванне данный показатель снизился до 3246 КОЕ/100 см^3

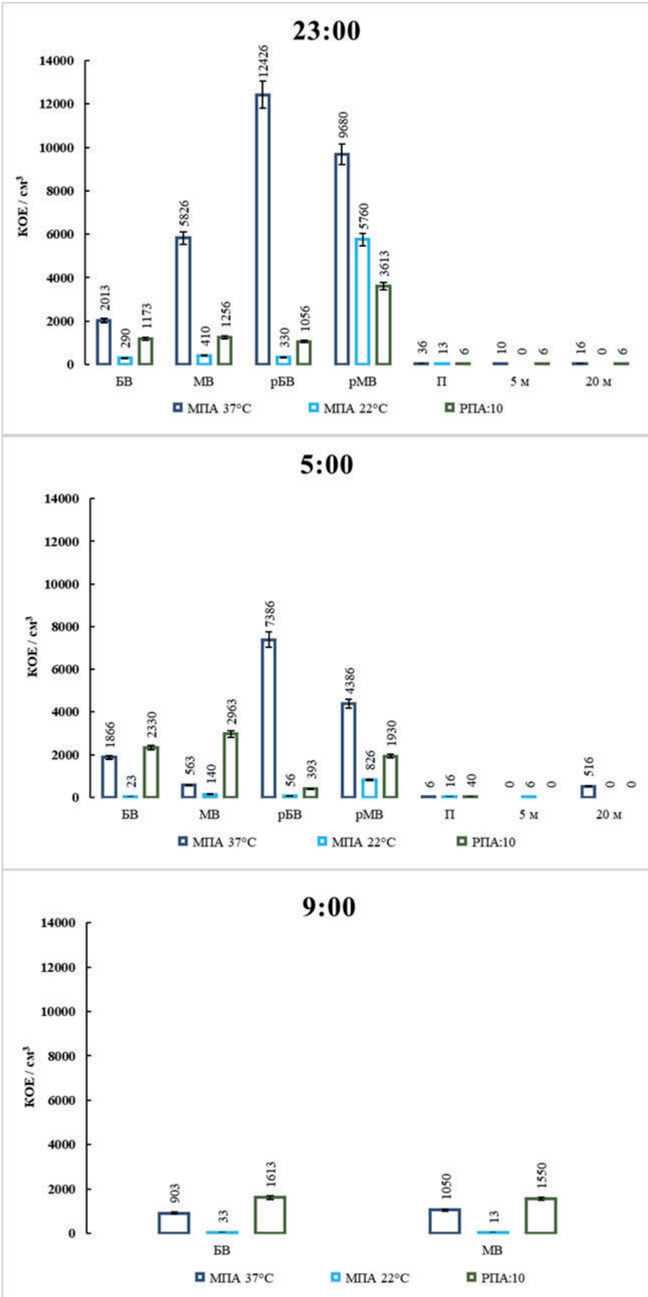

Рис.2. Численность органотрофных мезофильных аэробных и факультативно анаэробных микроорганизмов в исследуемой акватории в течении суток

(см. рис. 3). Количество спор сульфитредуцирующих клостридий в это время было максимальным и достигало 15 и 12 КОЕ/20 см^3.

Относительно высокий коэффициент самоочищения отмечен в прибрежной зоне: у пирса (2.53) и на расстоянии 5 метров от берега в 23:00 и 5:00 (1 и 2.16 соответственно) (табл. 2).

4. Обсуждение

О целебных свойствах геотермальных источников известно давно. Как показано в работе Буслова С.П. (Буслов, 1990), минеральная вода источников БРЗ, оказывает благотворное влияние на разные системы органов: нервную, дыхательную, двигательную, мочеполовую и др., а также улучшает состояние кожи и способствует заживлению ран. Поэтому источники очень популярны среди населения и открыты для посещения (Намсараев и др., 2007). Вместе с тем, в геотермальных источниках БРЗ неоднократно отмечалось превышение нормативных значений санитарно-значимых микроорганизмов. Так, в 2012 году в минеральных водах долины реки Шумак, выявлены условно-патогенные бактерии группы кишечной палочки и бактерии рода *Enterococcus* (Суслова и др., 2013). В холодных и горячих источниках Прибайкалья (Кумыска, Серебряный, Горячинск), также были обнаружены условно-патогенные микроорганизмы, относящиеся к родам *Enterobacter*, *Klebsiella*, *Escherichia*, *Citrobacter* и патогенный вид *Clostridium perfringens*, который является возбудителем пищевых отравлений человека и одним из возбудителей газовой гангрены (Бархутова, Дармаева и Намсараев, 2012). Как мы упоминали выше, превышения значений санитарно-значимых бактерий в летний период неоднократно отмечались и в источнике Змеином. Хотя по данным химического анализа вода источника Змеиный постоянна во времени, ее состав не изменяется и соответствует ранее полученным данным (Намсараев и др., 2007; Плюснин и др., 2013; Калмычков и др., 2020; Chernitsyna et al., 2020). Как показали наши исследования, повышенное содержание санитарно-значимых микроорганизмов в термальном источнике в бухте Змеиная отмечено в разное время суток после посещения туристов. Наиболее высокие значения, превышающие нормативные по СанПиН 1.2.3685-21, наблюдались в вечернее время суток (23:00). Рано утром, несмотря на 6-часовое отсутствие купающихся, значения показателей ОКБ, *E. coli*, энтерококков также превышали допустимые значения. О незавершенных процессах самоочищения или недостаточном разбавлении термальных вод байкальскими свидетельствует значения коэффициента самоочищения (2.16-2.53) у пирса и в литорали озера на расстоянии 5 м от берега.

Одной из причин постоянной обсеменённости источника условно-патогенными микроорганизмами может быть высокая температура

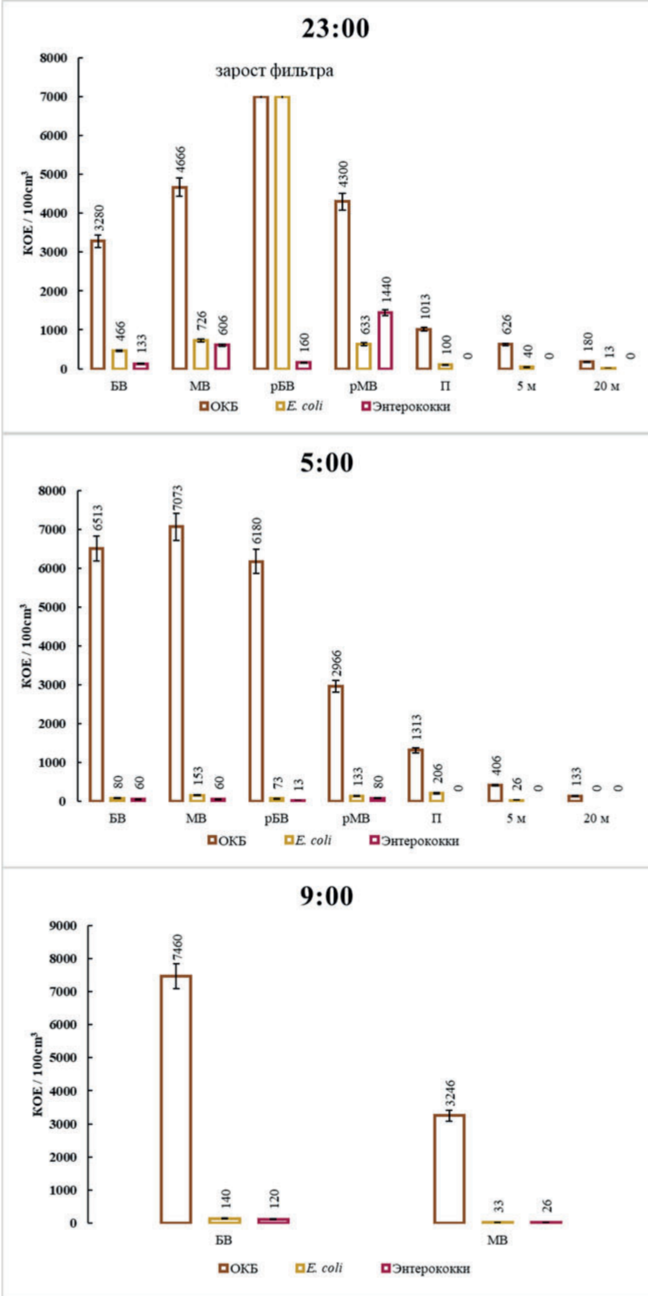

Рис.3. Численность санитарно-значимых микроорганизмов в исследуемой акватории в течении суток

Таблица 2. Коэффициент самоочищения в акватории бухты Змеиной

Район отбора / Время отбора	П	5 м	20 м
23:00	0.27	1	0
5:00	2.53	2.16	0.0008
9:00	-	-	-

Примечание: «-» - исследования не проводились воды в нем (40-42°С), близкая к оптимальной (37°С) для их роста. Например, *E. coli* имеет оптимум роста 37°С, хотя некоторые штаммы способны расти от 40 до 49°С (Ingledew and Poole, 1984). Сохранению жизнеспособности бактерий, в том числе санитарно-значимых, может способствовать низкая проточность источника и восстановленные условия среды. В ручьях, где происходит насыщение воды O_2, и температура воды составляет ~ 32 - 33°С, численность микроорганизмов также остается высокой. Значения численности ОКБ, *E.coli*, энтерококков соответствовали нормативам лишь в литорали на расстоянии 5-20 метров от берега, где температуры воды составляла 17°С. Очевидно, что совокупность таких факторов, как температура, скорость обновления вод в ваннах и количество купающихся влияют на развитие и распространение условно-патогенных микроорганизмов в исследованном источнике и прибрежной акватории. По результатам наших исследований, воды в большой и малой ваннах термального источника Змеиный не соответствуют требованиям СанПиН 1.2.3685-21 и не безопасны для бальнеологических целей без проведения специальных мероприятий. В качестве таких мероприятий можно увеличить проточность вод этого источника, добавлением байкальской воды в ночное время. Разбавление термальных вод и временное снижение температуры в них может снизить выживаемость условно-патогенных микроорганизмов и улучшить качество воды в ваннах источника.

5. Выводы

В различное время суток в источнике Змеиный наблюдались превышения нормативов санитарно-микробиологических показателей (ОКБ, *E. coli*, энтерококков) в соответствии с СанПиН 1.2.3685-21. Наиболее высокое содержание отмечалось после купания туристов, их количество оставалось высоким и после 6-часового перерыва. В литорали озера (20 метров от берега) численность исследованных групп в основном соответствовала нормативам. Очевидно, что совокупность таких факторов, как температура, скорость обновления вод в ваннах и количество купающихся влияют на развитие и распространение условно-патогенных микроорганизмов в исследованном источнике и прибрежной акватории.

Целебные источники, как объекты постоянного рекреационного посещения, должны быть защищены от попадания условно-патогенных бактерий в местах выхода минеральных вод посредством проведения специальных мероприятий. Также очевидна необходимость разработки регламента посещения туристами данного источника.

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Water Bears in the Land of Diversity: A Comprehensive Review of Tardigrades in India

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ABSTRACT. Tardigrades, also known as water bears, are a group of microscopic invertebrates with four pairs of stout legs. They are known to be found in freshwater, terrestrial and marine environments. An exceptional feature of the tardigrades is their ability to withstand extreme low temperatures, desiccation and other such severe environmental conditions. Globally, 70.8% of the earth is covered with the oceans and seas, while the Indian Ocean accounts for 29% of the global ocean area and is known to be a region of high biodiversity, where India is one of the countries. Tardigrades are known to form a habitat with mosses, lichens, freshwater ecosystems, oceans, and Himalayas in India. One of the main areas of research on tardigrades in India is the diversity and distribution of tardigrade species in different regions of the country, however, very few studies have been conducted on this phylum. Tardigrades have become the subject of increasing interest due to their potential economic importance like their significance in environmental monitoring and space research. Lastly, this phylum needs to be explored in terms of the species distribution and their economic importance, making them a valuable resource for India.

Keywords: Water Bears, Tardigrades, India, Biodiversity, Cryptobiosis, Biogeographic patterns

1. Introduction

Tardigrades are microscopic invertebrates, roughly cylindrical with four pairs of brief stubby legs terminating in claws. Their physical size varies from 50 μm in just hatched individuals to a maximum of 1200 μm in a few exceptionally large species. For fully mature adults, average sizes range typically from 200 to 500 μm. These animals are called “water bears” in many instances due to their bear-like appearance (legs with claws), and sluggish lumbering gait (Nelson et al., 2016). Tardigrades can be found in freshwater, terrestrial, and marine environments. The most well-known tardigrades are those found in terrestrial environments, where they live in the thin film of water on mosses, lichens, algae, other plants, leaf litter, and soil, and are active when there is at least a thin film of water on the substrate. Tardigrades frequently coexist with bdelloid rotifers, nematodes, protozoans, and other animals. Aquatic freshwater tardigrades live on submerged plants or in sediment but they are not water column dwellers. Some tardigrade species can live in both freshwater and aquatic environments.

Over the last couple of decades, the number of studies conducted within the integrative taxonomy has increased, leading to genetically confirmed documentation of tardigrade species from multiple locations for the first time in the history of biogeography. Although the majority of such examples are drawn from a geographically limited sample, i.e. a single continent or a part of it (Michalczyk et al., 2022). Studies show that at least some tardigrades (most with a parthenogenetic mode of reproduction) exhibit wide geographic ranges, but due to the low number of such examples it is not clear whether they reflect the “Everything is everywhere, but environment selects” hypothesis (Baas Becking, 1934) or are a result of anthropogenic dispersal (Gąsiorek et al., 2019; Morek et al., 2021). Thus, tardigrade species distribution patterns are complex, and we need integrative data on more genera and species before drawing sound general conclusions about tardigrade`s biogeography (Michalczyk et al., 2022).

The aim of this study is to examine the variety and occurrence of tardigrade species in various parts of India, and to identify their possible economic significance for environmental assessment and other possible applications in different fields such as space research and

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biotechnology. This study will provide a comprehensive survey of tardigrade diversity and distribution in India, and will highlight the unique features and applications of these resilient microorganisms, which can withstand extreme low temperatures, desiccation and other severe environmental conditions.

2. Classification

According to the recent checklist of tardigrades species as per the studies published by Degma and Guidetti in 2023, there are 1464 species, 159 genera and 33 families present currently (Degma et al., 2019; Degma & Guidetti, 2023). The Tardigrada phylum accepted two classes and four orders. Apochela and Parachela make up Eutardigrada, while Echiniscoidea and Arthrotardigrada make up Heterotardigrada (Jørgensen et al., 2018). Tardigrades are mainly classified into three classes: Heterotardigrada, Eutardigrada and Mesotardigrada. The majority of known species belong to the class Heterotardigrada (Guidetti & Bertolani, 2005). However, the class Mesotardigrada should be considered *nomen dubium* since no such physical evidence is reported for the species namely *Thermozodium esakii* which was known to be placed under class Mesotardigrada evoking the formation of a new class as per the study conducted by Grothman et al., (2017).

The Heterotardigrada class is defined by their possession of plates on their cuticles, while the Eutardigrada class is defined by the lack of such plates (Guidetti and Bertolani, 2005). This classification helps to distinguish tardigrades based on the presence or absence of certain morphological characteristics. The plates on the cuticles of Heterotardigrada species provide them with protection from predators and help them to conserve moisture. Other physical characteristics which are considered for the identification of these two taxa are: the presence of a separate gonopore and anus in Heterotardigrada and the presence of sclerified structures within pharynx which has a different shape and nature as compared to the Eutardigrada taxa. Moreover, the Eutardigrada taxa differs from Heterotardigrada by showing the presence of a common cloaca and "Malpighian tubules". Classification of tardigrades is important for understanding their diversity and evolution. The three classes of tardigrades are differentiated by their morphological characteristics, such as the presence or absence of plates on their cuticles, their leg structures, and their existence. Further research is necessary to better understand the characteristics and evolution of each class of tardigrades.

3. Characteristics

Tardigrades belong to the phylum Tardigrada and are considered to be among the most resilient micro-animals in the world, as they can withstand extreme environmental conditions such as drought, high pressure, and extreme temperatures (Guidetti & Bertolani, 2005). One of the most notable characteristics of tardigrades is their ability to enter a state of suspended animation, known as cryptobiosis, when faced with harsh environmental conditions. In this state, tardigrades can survive without water or nutrients for extended periods of time (Boothby et al., 2015). Naturalists have long been fascinated by tardigrades ability to 'resurrect' after desiccation, when water becomes available. This phenomenon of cryptobiosis, or anabiosis, has assisted tardigrades in surviving 'normal' as well as 'experimental' adverse conditions such as temperatures ranging from - 272 to > 340°C; gases such as CO_2 and H_2S; strong acids, including osmic acid; and alcohol, as well as radiation under ultraviolet light (McInnes, 1994). This ability to enter cryptobiosis is one of the key characteristics that sets tardigrades apart from other micro-animals. Another key characteristic of tardigrades is their hard exoskeleton, known as a cuticle, which provides them with protection from predators and helps them to conserve moisture (Nelson, 2002). The cuticle of tardigrades is made of a material called chitin, which is also found in the exoskeletons of insects and crustaceans. Tardigrades are also known for their unique leg structure, which consists of four pair of legs that are equipped with claws (Nelson, 2002). These legs provide them with the ability to move quickly and efficiently through their environments. The unique leg structure of tardigrades is a characteristic that sets them apart from other micro-animals. Tardigrades are fascinating micro-animals that are known for their ability to enter cryptobiosis, their hard exoskeleton, and their unique leg structure. These characteristics have helped tardigrades to survive and adapt to a wide range of environmental conditions, making them among the most resilient micro-animals in the world.

4. Aquatic and Terrestrial Habitats

Tardigrades are commonly found in freshwater, saltwater, and brackish water environments. They are able to survive in these aquatic habitats due to their ability to regulate their body fluids and osmotic pressure. Tardigrades that live in freshwater habitats, for example, must be able to deal with hypotonic conditions, which means that there is a lower concentration of solutes outside their bodies than inside. They are able to do this by regulating the amount of water in their bodies and by producing certain solutes to maintain osmotic balance. Marine tardigrades, on the other hand, live in a more isotonic environment, which means that the concentration of solutes is the same inside and outside their bodies. However, they still face challenges in the form of high salinity and pressure, as well as changes in temperature and pH. Tardigrades have adapted to these conditions by developing protective mechanisms, such as the production of antioxidants and the ability to repair DNA damage caused by exposure to ultraviolet radiation (Erdmann and Kaczmarek, 2017). Tardigrades have also been found in brackish water habitats, such as estuaries, where they must be able to tolerate fluctuations in salinity and temperature (Zawierucha et al., 2016). Studies have shown that tardigrades in brackish water habitats have a higher tolerance for exposure to low oxygen levels and can even survive periods of anoxia (Jönsson et al., 2008).

Tardigrades are also found in a variety of terrestrial habitats, including soil, moss, lichens, and leaf litter. These habitats pose different challenges than aquatic environments, such as fluctuations in temperature and humidity, as well as exposure to desiccation (drying out). Tardigrades have evolved a number of strategies to deal with these challenges, including the ability to enter a state of suspended animation known as cryptobiosis. During cryptobiosis, tardigrades are able to shut down most of their metabolic processes and become almost completely dehydrated, reducing their water content to as little as 1% of their normal level. This allows them to survive for extended periods of time in harsh conditions (Rebecchi et al., 2007). When conditions improve, tardigrades are able to rehydrate and resume normal activity. Tardigrades in terrestrial habitats also face exposure to radiation from the sun, which can cause DNA damage. To protect against this, tardigrades have developed the ability to produce special proteins that protect against radiation damage and repair DNA damage caused by exposure to high levels of ultraviolet radiation (Jönsson et al., 2008).

5. Global Distribution of Tardigrades

According to a study conducted on Global distribution of tardigrades in freshwater by (Garey et al., 2008), out of 910 species, only 62 species representing 13 genera were found to be the only ones in aquatic habitats. Five genera namely *Carphania*, *Dactylobiotus*, *Macroversum*, *Pseudobiotus*, and *Thermozodium* were found to be exclusively aquatic while other genera like *Hypsibius*, *Isohypsibius*, *Amphibolus*, *Mixibius* had some species that were aquatic as per the study. According to the literature studies, there are over 50 Antarctic and Neotropic species present in freshwater habitats, which primarily are Eutardigrades belonging to 2 orders and 8 families as per (Degma et al., 2009; Nelson et al., 2016). Due to difficulty in access and fewer researchers, the total number of species reported in the regions of Antarctica and Neotropic are much less than other zoographic regions, except for the regions namely Argentina, Chile, Costa Rica and the Antarctic Penisula.

As for previous literature by (Nelson et al., 2020), there are two endemic genera: *Ramajendas* (currently belonging to family Isohypsibiidae), which are found in freshwater sediment, mosses and lichens of the Antarctica and Sub-Antarctica islands. However, in the recent study proposed by (Tumanov, 2022), through the molecular phylogenetic analysis and morphological description of the Antarctic Tardigrade of genus Ramajendas, evoked the formation of a new family named Ramajendidae to place these Ramajendas species. The second monospecific genera are Acutuncus, which previously belonged to family Hypsibiidae but currently the study conducted by Vecchi et al. in 2023 revealed the extension of Acutunus species into a new family named Acutuncidae based on the phylogenetic analyses and morphological studies (Vecchi et al., 2023).

Acutuncus antarcticus is a pan-Antarctic species, most abundant and common tardigrade in Antarctica with a habitat in freshwater ecosystems and terrestrial soils, mosses, algae and lichens in non-glacial areas. Whilst, the Neotropical region contributes to a very diverse freshwater habitats like the tardigrades are found in rivers, streams and lakes in tropical lowlands (in Central America and Northern South America) up to high mountain lakes, ponds and glaciers in the Andean Mountains. The freshwater tardigrades are known to be found in very cold ponds, rivers, lakes and in cryoconite holes on glaciers in the southern end of South America. Meanwhile, the central region provides freshwater habitats in temporary dry or very hot climate zones of South America. Also, the Neotropic regions are known to provide some unusual freshwater habitats for tardigrades, which include tree holes and bromeliads (Nelson et al., 2020).

6. Indian Scenario

Globally, 70.8% of the earth is covered by the oceans and seas, with a global coastline of 1.6 million km. The Marine and Coastal Ecosystem occur in 123 countries worldwide. These ecosystems generally include sand dune areas (where freshwater and sea water mix), nearshore coastal areas and open ocean marine areas. The Indian Ocean constitutes 29% of the global ocean area and is known to be a region of high biodiversity, where India is one of the countries in the region. The land of biodiversity, India is known to be a part of the list of 12 mega-biodiversity countries and 25 hotspots of the richest and highly endangered eco-regions of the world. It is known to have a coastline of about 8000 km. It is also the only country amongst the Asian countries, which has a long record and complete list of coastal and marine biodiversity dating back at least two centuries old (Venkataraman and Wafar, 2005)

In India, Tardigrades happen to be found as meiofauna on the sandy beaches up to 2-3 meters from the sea's edge. Out of the three classes of the phylum Tardigrada, the Heterotardigrada is known to prevail in the marine, freshwater and high-altitude mountain regions of the country. As for the reports, only 10 species under 2 families and 3 genera occur as the meiofauna of the marine regions in India (Venkataraman et al., 2020). Tardigrades are also known to form a habitat with mosses, lichens, freshwater ecosystems, oceans, and Himalayas (Chandra et al., 2018)

Scientific studies on tardigrades have known to be increased throughout the world in the 20th century, yet this phylum remains unexplored and little-known to the scientific community. With regards to India, very few studies have been conducted on this phylum. To know more about this phylum and understand its diversity, detailed studies should be conducted (Dey and Mandal, 2018).

7. Species Diversity in India

Initial studies on tardigrades found in India was conducted as early as in the beginning of 20th century (Murray, 1907) followed by (Iharos, 1969) in the second part of the century. Though, very little was known about the terrestrial tardigrades of India. As a part of the Zoological Survey of India, a review study was conducted, which depicted that 41 species of Tardigrades were known to India, out of which 23 species were found in the Indian Himalayas (Chandra et al., 2018). The above study also referred to the presence of *M. hufelandi* in India, showing the progress in our understanding of the taxonomy of this genus, which is known to be a different species than the last recorded by Murray in 1907 (Murray, 1907). Apart from the larger studies conducted in India, there were also some smaller islands around the subcontinent, like different species of tardigrades found in Andaman and Nicobar Islands by McInnes in 1994 (McInnes, 1994). Geographic representation on a map (Figure 1) followed by a list (Table 1) of Tardigrades species found in various regions of India is given below.

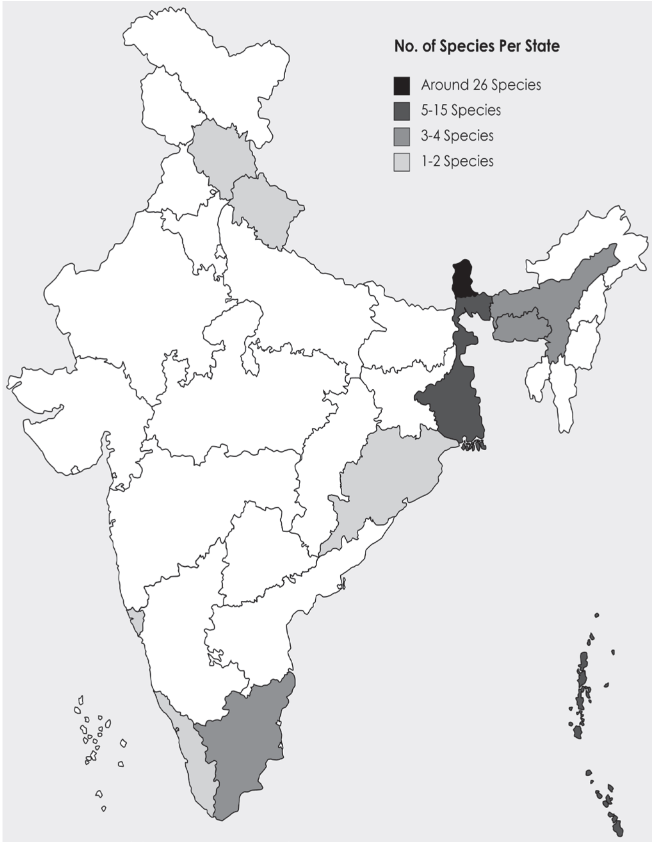

Fig.1. Geographic distribution of Tardigrade species found in different regions of India (1. Sikkim 2. Andaman Islands, West Bengal 3. Assam, Meghalaya, Tamil Nadu 4. Goa, Himachal Pradesh, Kerala, Odisha, Uttarakhand)

8. Research Scenario of Tardigrades in India & it's Ecological Importance

Aquatic meiofauna and terrestrial invertebrate species can be used as a biomonitoring tool to indicate environmental quality. Tardigrade being a meiofauna has also been studied and used as an indicator of pollution in different freshwater habitats (Nelson et al., 2010). A recent study was conducted to inspect the meiofaunal biodiversity of the Dahisar River, Mumbai, the urban development and its impacts in the environmental management by (Salian et al., 2022). This study concluded the presence of tardigrades species in Sanjay Gandhi National Park and Borivali, which were found to be more polluted as compared to other areas, which indicated that tardigrades could survive in polluted areas, thus, acting as an important pollution indicator. This has important implications for industries that are reliant on the health of ecosystems, such as agriculture and forestry, as well as for the development of new technologies for environmental monitoring (Jönsson et al., 2008)

The understanding of ecophysiology of tardigrades and their responses to different environmental conditions becomes a pre-requisite to use them as a tool for biomonitoring of environmental pollution or as an indicator of environmental change (Massa et al., 2023). For instance, a study on the prevalence and distribution of different tardigrades species in some tropical areas of Tamil Nadu and their different temperature tolerance capacity was conducted by (Abirami et al., 2021). This study showed that most of the isolated species belonged to the genus *Milnesium sp.*, others were found to be *Murrayon sp.*, and *Macrobiotus sp.* It was also observed that *Milnesium tardigradum* exhibited higher tolerance to all different temperature conditions as compared to *Macrobiotus sapiens*. This research has important implications for the conservation and management of ecosystems in India. There has been a growing interest in the study of tardigrades in India in recent years. Research on tardigrade diversity and distribution, ecophysiology, and potential as bioindicators has contributed to our understanding of these unique micro-animals and their significance in the Indian environment (delBarco-Trillo, 2019).

Tardigrades have become the subject of increasing interest due to their potential economic importance. Despite their small size, tardigrades play a significant role in several key industries and have the potential to be valuable resources in the future. One area where tardigrades have economic importance is biotechnology. Tardigrades are known for their ability to withstand extreme environmental conditions, including high temperatures, radiation, and desiccation. This resilience has led to their use in the development of new technologies, such as cryopreservation methods for the storage of cells and tissues (Bertolani et al., 2004). In addition, tardigrade genes have been used in the development of new methods for the production of proteins, such as the production of vaccines (Boothby et al., 2017). Apart from this, the mechanism behind the physiological adaptations made by tardigrades to survive extreme environmental conditions drives the scientific community to develop products having application in various fields. For instance, a recent study published in 2023, conducted by (Mredha et al.,

Table 1. List of Tardigrade species found in India

No.	Species	Location	References
1	*Adropion scoticum*	Sikkim	(Chandra et al., 2018)
2	*Batillipes carnonensis*	Odisha	(Rao, 1971)
3	*Bryodelphax ortholineatus*	Andaman Island	(McInnes, 1994)
4	**Calcarobiotus gildae*	Andaman Island	(McInnes, 1994)
5	**Claxtonia wendti*	Sikkim	(Chandra et al., 2018)
6	*Cornechiniscus madagascariensis*	Himachal Pradesh	(Abe and Takeda, 2000)
7	*Dactylobiotus macronyx*	Sikkim	(Chandra et al., 2018)
8	**Dianea acuminata*	Sikkim Tamil Nadu	(Chandra et al., 2018) (Degma and Guidetti, 2023)
9	**Dianea sattleri*	Sikkim	(Chandra et al., 2018)
10	*Diphascon chilenense*	Sikkim	(Murray, 1907) (Chandra et al., 2018)
11	*Diphascon pingue*	Sikkim	(Chandra et al., 2018)
12	*Echiniscus arctomys*	Sikkim	(Murray, 1907) (Chandra et al., 2018)
13	**Pseudechiniscus suillus*	Sikkim	(Murray, 1907) (Degma and Guidetti, 2023)
14	*Echiniscus quadrispinosus*	Sikkim West Bengal	(Chandra et al., 2018) (Murray, 1907)
15	*Echiniscus testudo*	Assam Meghalaya West Bengal	(Degma and Guidetti, 2023) (Murray, 1907)
16	*Hypsibius convergens*	Andaman Islands Sikkim	(Degma and Guidetti, 2023) (Chandra et al., 2018)
17	**Kristenseniscus kofordi*	Andaman Island	(McInnes, 1994)
18	*Macrobiotus echinogenitus*	Sikkim West Bengal	(Chandra et al., 2018) (Murray, 1907)
19	*Macrobiotus gemmatus*	Sikkim	(Chandra et al., 2018)
20	*Macrobiotus hufelandi*	Assam Meghalaya Sikkim West Bengal	(Degma and Guidetti, 2023) (Shil, 2001) (Chandra et al., 2018) (Murray, 1907)
21	*Macrobiotus kamilae*	Uttarakhand	(Coughlan and Stec, 2019)
22	*Macrobiotus polyopus*	Andaman Island	(McInnes, 1994)
23	*Macrobiotus rubens*	Sikkim	(Chandra et al., 2018)
24	*Macrobiotus sapiens*	Tamil Nadu	(Abirami et al., 2021)
25	*Macrobiotus topali*	Sikkim	(Chandra et al., 2018)
26	**Mesobiotus coronatus*	Andaman Island	(McInnes, 1994)
27	**Mesobiotus furciger*	Andaman Island	(McInnes, 1994)
28	**Mesobiotus harmsworthi*	Andaman Island	(McInnes, 1994)
29	**Mesobiotus mauccii*	Andaman Island	(McInnes, 1994)
30	*Milnesium tardigradum tardigradum*	Andaman Island Sikkim Tamil Nadu West Bengal	(McInnes, 1994) (Chandra et al., 2018) (Abirami et al., 2021) (Murray, 1907)
31	**Minibiotus aculeatus*	Andaman Island Sikkim West Bengal	(McInnes, 1994) (Chandra et al., 2018) (Murray, 1907)
32	**Minibiotus furcatus*	Assam Meghalaya West Bengal	(Degma and Guidetti, 2023) (Shil, 2001) (Murray, 1907)

No.	Species	Location	References
33	*Minibiotus intermedius*	Andaman Island Sikkim	(McInnes, 1994) (Chandra et al., 2018)
34	*Nebularmis indicus*	Goa	(Gąsiorek, Vončina, Ciosek et al., 2021)
35	**Nebularmis reticulatus*	Sikkim	(Murray, 1907) (Chandra et al., 2018)
36	**Paramacrobiotus areolatus*	Sikkim	(Murray, 1907)
37	**Paramacrobiotus chieregoi*	Andaman Island	(McInnes, 1994)
38	**Paramacrobiotus richtersi*	Andaman Island Sikkim Tamil Nadu	(McInnes, 1994) (Chandra et al., 2018) (Abirami et al., 2021)
39	**Pseudechiniscus (Meridioniscus) juanitae*	Sikkim	(Chandra et al., 2018) (Gąsiorek, Vončina, Zając et al., 2021)
40	*Ramazzottius oberhaeuseri*	Sikkim	(Chandra et al., 2018)
41	*Stygarctus keralensis*	Kerala	(Vishnudattan et al., 2021)
42	**Stygarctus bradypus*	Odisha	(Rao, 1971) (Schulz, 1951)
43	**Testechiniscus macronyx*	Sikkim	(Murray, 1907)
44	**Ursulinius mihelcici*	Sikkim	(Chandra et al., 2018)

Note: * – indicates changed taxa as for updated checklist by (Degma and Guidetti, 2023)

2023) depicted the development of an extremotolerant glycerogels by studying the tun formation process in tardigrades and taking inspiration from this process. These gels have varied applications in the field of biomedicine, energy storage devices, sensors, and soft robotics.

Another remarkable study showing the natural occurrence of a fluorescence against ultraviolet radiation in the Eutardigrade *Paramacrobiotus* species conducted by (Suma et al., 2020). The study provided experimental evidence that the *Paramacrobiotus* species produces a protective fluorescent shield that absorbs ultraviolet radiation and thus, this can be used to protect UV-sensitive tardigrades and nematode like *Caenorhabditis elegans* from UV radiation used as a germicide.

Another interesting area where tardigrades have economic importance is space research. Tardigrades have been found to survive in the harsh conditions of space, including extreme temperatures and high levels of radiation (Jönsson et al., 2008). This has led to their use in space research and the development of new technologies for space exploration. Tardigrades have significant economic importance in several key industries, including biotechnology, environmental monitoring, and space research. As research of these micro-animals continues, it is likely that their importance will only increase, making them valuable resources for the future.

9. Conclusion

Little or very few studies have been conducted and there are still areas, which remain unexplored in terms of the species diversity and distribution pattern in India. Increasing research on tardigrade diversity and distribution, ecophysiology, and potential as bioindicators has contributed to our understanding of these unique micro-animals and their significance in the Indian environment. Tardigrades have also become a subject of interest due to their potential economic importance in fields like Biotechnology, environmental monitoring, and space research. The rise in research of these microscopic invertebrates will increase their significance and make them a valuable resource for future applications.

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Conflict of Interest

The authors declare no conflicts of interest.

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