

An aerial photograph of a mangrove wetland. A winding, turquoise-colored river flows through a dense forest of green mangrove trees. The water has a milky, opalescent appearance. A small, red and white boat is visible on the river, moving towards the right. The surrounding land is a mix of green foliage and light-colored, sandy or silty soil.

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Alreem Island Mangrove, UAE photo by Hooreya Al Mufflahi

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Analysis of water quality and heavy metal content of chromium in water, sediment, and flesh of tilapia (*Oreochromis niloticus*) in the Premulung River, Surakarta, Central Java, Indonesia

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Abstract. Pitasari IS, Setyono P, Wiryanto. 2022. Analysis of water quality and heavy metal content of chromium in water, sediment, and flesh of tilapia (*Oreochromis niloticus*) in the Premulung River, Surakarta, Central Java, Indonesia. *Intl J Bonorowo Wetlands* 12: 56-62. The river is a lotic ecosystem that plays an essential role in living things' lives, including as a habitat for aquatic organisms, drainage channels, and water sources for human needs. The Premulung River crosses the city of Surakarta, Central Java, Indonesia. The area along the Premulung River is surrounded by settlements, markets, textile industries, batik, screen printing, and hospitals, which produce waste that has the potential to pollute and degrade water quality. This study is aimed to determine the water quality and content of heavy chromium metal (Cr) in water, sediment, and tilapia meat (*Oreochromis niloticus* Linnaeus, 1758) in the Premulung River, Surakarta City, as well as the relationship between chromium content in water, in sediment and fish meat. This research was conducted by field observation at three stations of the Premulung River flows, namely under the Kleco Bridge, Griyan, and Jongke. The parameter data of temperature, TDS, pH, DO, BOD, COD, and Cr water compared to PPRI No. 82 of 2001 (class 2 water); chromium sediment compared to ANZECC Year 2000; Fish meat chromium compared to CFSA 2012. The Premulung River water quality was analyzed using the STORET method, while the Pearson correlation analyzed the relationship between chromium in water, sediment, and fish meat. The results showed that the water quality of the Premulung River in Surakarta City was moderately polluted. The heavy metal content of Premulung River chromium water (ttt to 0.0344) mg/L and chromium sediment (1.307-4.948) mg/kg still meets the standard quality, while the tilapia meat chromium (1.958-3.535) mg/kg exceeds the standard quality. The relationship between chromium water content was very strong and directly proportional ($r= 0.997$) with chromium fish meat, while chromium sediment with chromium fish meat was inversely proportional ($r= -0.470$).

Keywords: Chromium, Cr, *Oreochromis niloticus*, Premulung River, water quality

INTRODUCTION

Rivers have an essential role in the life of living things, including humans. Apart from being a drainage channel, the river can be used as a source of drinking water. However, due to human activities and rapid industrial development, domestic and industrial waste affects the river water environment, thus disrupting its designation (Krupnova et al. 2018; Primadiani et al. 2018; Yousif et al. 2021; Samudra et al. 2022).

According to Sudarmaji et al. (2006), Kapahi and Sachdeva (2019), and Sari et al. (2019), large natural waters can be contaminated by heavy metals resulting from human activities, such as domestic and industrial waste. Heavy metals that are often found in industrial waste are lead (Pb), mercury (Hg), cadmium (Cd), arsenic (As), and chromium (Cr) (Ida 2012; Palar 2012). Heavy metals will undergo a bioaccumulation process in water, sediment, and food chain flows (Yi and Zhang 2012; Bui et al. 2016).

The Premulung River crosses Surakarta City, flows from the Kartasura area of Sukoharjo Regency, and continues eastward through Kleco, Pajang, Sondakan, Tipis Villages, and finally empties into Bengawan Solo.

The width of this river is between 5-12 m, with a depth of 0.7-12 m (Martini 2001). The area along the Premulung River has many residential areas and textile and batik industries. The flow of this river water is not bright and clear and often looks colored and has odors. According to Astirin et al. (2002), the Premulung River is household and industrial waste disposal. This waste potentially contains pollutants such as heavy metals. Chromium is a heavy metal pollutant due to fabric coloring activities in the textile, batik, tanning leather, and metal coating industries (Ackerley et al. 2004).

Fish accumulate heavy metals in their organs and tissues through different pathways, namely respiration, ingestion, and biological membranes (Abdulali et al. 2011; Dhanakumar et al. 2015; Abarshi et al. 2017; Ouma et al. 2019). Therefore, many studies that examine the level of metal pollution in fish consumption are related to the selection of fish as an indicator of pollution in an aquatic ecosystem despite a source of protein and omega 3 (Bhuvaneshwari et al. 2012; Laibu et al. 2018; Rajeshkumar and Li 2018).

Communities around the Premulung River watershed have fishing habits for the river fish, one of which is

tilapia, to be processed into daily food. However, the consumption of fish contaminated with heavy chromium metal can cause ulcers on the nose and skin, skin hyperpigmentation, skin cancer, and renal tubular necrosis indications (Puspita et al. 2011). Based on those facts, it is necessary to research to determine water quality and heavy chromium (Cr) metal content in water, sediment, and meat of tilapia (*O. niloticus*) caught in the Premulung River, Surakarta City.

MATERIALS AND METHODS

Research time and place

The study was conducted in June 2016. The sampling of river water, sediment, and tilapia, as well as the measurement of parameters such as pH, temperature, DO, and TDS, were carried out in three sections of the Premulung River, namely Kleco Bridge (station 1), Griyan (station 2), and Jongke (station 3). In addition, BOD, COD, and heavy chromium (Cr) metal parameters were tested in the Center for Environmental Health Engineering and Disease Control (BBTKLPP) laboratory in Yogyakarta, Indonesia.

Research tools and materials

For this study, the tools are; fishing rods, buckets, transparent plastic, markers, ice boxes, and water samplers. Also, DO meters, pH meters, TDS meters, thermometers, hygrometers, Eickman grabs, bottles, Polarized Zeeman Atomic Absorption Spectrophotometry (AAS) HITACHI type Z-2000, MARS microwave digester. Other tools include a vessel, fume hood, oven, Erlenmeyer, beaker, water bath, polyethylene bottle, measuring cup, filter paper, stopwatch, funnel, micropipette, analytical balance, flask bottle, knife, porcelain cup, and grinder.

The materials used in this study included: samples of river water, sediment, tilapia, 1,000 mg/L Cr base liquor, concentrated nitric acid (HNO_3), aquabides, aquades, and Whatman filter paper no. 42, digester solution (high and low concentrations within), sulfuric acid reagent (H_2SO_4), potassium hydrogen phthalate, standard solution, and microbial seed suspension solution.

Procedure

River water sampling

The integrated sampling method conducted the river water sampling manually, taking water samples instantaneously from different places. Water samples were taken from each station with three replications on the left edge, right, and center. Water samples were taken using a water sampler. The water sample is then put in the bottles.

Sediment sampling

Sediment sampling using the Eickman Grab was conducted by determining two plots at each station with an area of 15 cm x 15 cm and a depth of 3-5 cm (Sulistyo 2014). The sampling method is by lowering the Eickman Grab in an open and straight position, slowly falling until it feels like it has touched the riverbed. Next, the Eickman

Grab was shaken to cover his mouth, then pulled back up. Finally, the Eickman grab is opened. The sediment sample is poured into a container, put in a plastic bag, and labeled according to the location code for the laboratory analysis.

Tilapia fish sampling

Tilapia samples were taken using a fishing rod. The sample of tilapia's body length was 10 to 20 cm selected. Fish samples were put in an ice box to maintain the freshness level.

Temperature measurement

The water river temperature parameters were measured directly in the field using a thermometer with Celsius ($^{\circ}\text{C}$) units, while the air temperature was measured using a hygrometer. The thermometer is immediately immersed in the test sample and left for 2 to 5 minutes until it shows a stable value. The reading of the thermometer scale is recorded without lifting the thermometer from the water (SNI 2005).

pH measurement

The pH parameter is measured directly using a pH meter. The electrode is first calibrated. Then, the instrument is turned on, and the electrode is inserted into the sample. The numbers on the pH-meter display indicate the magnitude of the pH value (SNI 2004).

TDS measurement

The TDS parameter is measured directly using a TDS meter. The detector is first calibrated. Then, the instrument is turned on, and the detector is inserted into the sample. The number on the TDS-meter screen shows the magnitude of the TDS value in mg/L.

DO measurement

The DO parameter is measured directly using a DO meter. The detector is first calibrated. Then, the instrument is turned on, and the detector is inserted into the sample. The number on the DO-meter screen shows the DO value in mg/L.

BOD measurement

Two DO bottles were prepared, each marked with the notation A1 and A2. The sample solution was put into each bottle of DO A1 and A2 until it overflowed, then the bottle was closed carefully to avoid air bubbles forming. Shaking was carried out several times, then mineral-free water was added around the closed DO bottle mouth. Bottle A2 was stored in an incubator at $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for five days. On the other hand, dissolved oxygen was measured in the solution in bottle A1 with a calibrated DO meter. The measurement result is the value of dissolved oxygen opening (zero) day (A1). Dissolved oxygen measurements at opening days should be made no later than 30 minutes after dilution. The above process was repeated for A2 bottles, which had been incubated for five days \pm 6 hours. The measurements obtained are dissolved oxygen values for five days (A2).

The sample solution preparation methods mentioned above were repeated to determine blanks using a diluent

solution without a sample. The measurement results obtained are the value of dissolved oxygen for opening (zero) days (B1) and the value of dissolved oxygen for five days (B2). The same sample solution preparation was performed again to determine standard controls using a glucose-glutamic acid solution. The measurement results obtained are the value of dissolved oxygen for opening (zero) days (C1) and the value of dissolved oxygen for five days (C2). The BOD value is calculated by the BOD5 formula (SNI 2009a).

COD measurement

according to the character of the sample test, a total of 2.5 mL of the sample was diluted. Next, those samples were put into a borosilicate tube, then 1.5 mL of high or low-digesting solution and 3.5 mL of acid reagent were added until the color of the solution became orange/orange-greenish. The tube was closed and shaken slowly until homogeneous, then placed in the COD reactor, heated at a temperature of 150°C, refluxed for 2 hours, and then cooled at room temperature.

The refluxed sample was slowly cooled to room temperature to prevent the formation of a precipitate. If necessary, the cover sample is occasionally opened during the cooling process to contain gas pressure. The suspension is allowed to settle and ensure that the measured part is completely clear. The absorption sample was measured at a predetermined wavelength (600 nm). The COD value is calculated based on the linear equation of the calibration curve (SNI 2009b).

Measurement of chromium (Cr) in water

The heavy metal chromium in river water was analyzed by adding 50 mL of water sample (sample test) into a 100 mL beaker or 100 mL Erlenmeyer and 5 mL of concentrated HNO₃. Those solutions were covered with a watch glass and then heated slowly until the remaining volume was 15 mL, conducted on a fume hood. Next, while the digestion is not complete (not clear), 5 mL of concentrated HNO₃ is added, covered with a watch glass, and then heated again (not boiling). This process is repeated until all the metal dissolves, known from the color of the sample test precipitate, and becomes clear (slightly white). Afterward, the watch glass was rinsed, and the rinsed water was put into the beaker. Then the sample test was transferred to a 50 mL volumetric flask (filtered if necessary), and distilled water was added until the mark was homogenized. Afterward, the absorption was read at 359.3 nm using the Atomic Absorption Spectrophotometry (AAS) instrument.

Measurement of chromium (Cr) in sediment

The sediment sample was oven-dried for 24 hours to reduce its water content, then mashed using a porcelain dish and a grinder to form a fine dry sample. A total of 0.5 g of dry sample was put in a vessel, 10 mL of concentrated nitric acid (HNO₃) was added, and the vessel was tightly closed and put in a MARS microwave digester for digestion. Furthermore, the sample was filtered to obtain 100 mL of liquid sediment sample; then, the absorbance

was read at λ 359.3 nm using Flame Atomic Absorption Spectrophotometry (FAAS) flames.

Measurement of chromium (Cr) in fish meat

The fish samples were cleaned for scales; the fish meat was taken and washed with water. Next, fish meat samples were baked in an oven for 24 hours; then, the samples were mashed using a porcelain dish and a grinder to form dried fish meat samples. A total of 0.5 g of dry sample was put in a vessel, added 10 mL of nitric acid (HNO₃) concentration tightly closed, and put in a MARS microwave digester for the destruction process. Next, the sample was filtered to obtain 10 mL of liquid tilapia meat sample, diluted 50x. The absorbance was read at λ 359.3 using a carbon furnace Graphite Furnace Atomic Absorption Spectrophotometry (AAS) instrument.

Data analysis

The data from the measurement of temperature, pH, DO, TDS, BOD, COD, and Chromium heavy metal for each sample were compared with PPRI No. 82 of 2001 (2001). Concerning Water Quality Management and Water Pollution Control (second-class water quality standard), the PPRI stated that chromium (Cr) water standard quality is 0.05 mg/L. While the concentration of standard chromium heavy metal in the sediment compared to ANZECC (2000) was 80 mg/kg. Then the concentration of Chromium heavy metal in tilapia (*O. niloticus*) was compared with CFSA GB 2762-2012 the Year 2012 (2012). The maximum limit of Chromium heavy metal contamination in fish and its processed products is 2.0 mg/kg. Furthermore, Pearson correlation analysis was performed using the SPSS 16 program to determine the relationship between environmental parameters and chromium concentrations in water, sediment, and tilapia meat.

The determination of water quality status using the STORET method is based on the Decree of the Minister of Environment No. 115 of 2003 concerning Guidelines for Determining the Status of Water Quality. That is done by comparing the data from the measurement of water quality parameters with the quality standard according to the PPRI No. 1 water class. 82 of 2001 (2001). Score 0 if the measurement results meet the standard water quality value (measurement result \leq standard quality). Suppose the measurement result does not meet the standard water quality (measurement result $>$ standard quality). The overall score is shown in Table 1, presented below.

Table 1. Value system parameters to determine the water quality status

| Sample amount | Score | Parameters | | |
|---------------|---------|------------|-----------|---------|
| | | Physics | Chemistry | Biology |
| <10 | Max | -1 | -2 | -3 |
| | Min | -1 | -2 | -3 |
| | Average | -3 | -6 | -9 |
| \geq 10 | Max | -2 | -4 | -6 |
| | Min | -2 | -4 | -6 |
| | Average | -6 | -12 | -18 |

Note: The scoring system used is the US-EPA value system which classifies water quality classes into four: Class A: very good,

score = 0 (meets standard quality) (i), Class B: good, score = -1 to -10 (lightly polluted) (ii), Class C: moderate, score = -11 to -30

RESULTS AND DISCUSSIONS

Water quality of Premulung River, Surakarta City

Based on the research obtained, compared with PPRI No. 82 of 2001 (2001), environmental parameter data at the sampling station are presented in Table 2.

Furthermore, the STORET method is used to determine water quality status. The results of calculating the water quality status at the three research stations according to the STORET method value are presented in Table 3.

Table 3 shows that the Premulung River water in Surakarta City is of moderately polluted water quality, with a score value of -11 to -30. There was an increase in scores from station 1, station 2, to station 3. That indicates that further downstream, there is an increase in pollution by community waste from around the Premulung River, domestic, household, hospital, and textile industry, and even batik and screen-printing wastes. This river flow often looks dirty by plastic and household organic waste such as food and vegetable residue. Some parts of the river flow look purple to black, reducing the water's aesthetics.

The heavy metal chromium (Cr) content in water, sediment, and tilapia meat

This study measured the total content of heavy metal chromium (Cr) in water, sediment, and tilapia meat in the Premulung River, Surakarta City. The average total heavy metal chromium (Cr) concentration in water, sediment, and flesh of tilapia (*O. niloticus*) is presented in Table 4.

Content of heavy metal chromium in water

Table 4 shows that heavy metal chromium content in the three stations has not exceeded the maximum chromium water threshold according to PPRI No. 82 of 2001 (2001), which is equal to 0.05 mg/L. The dilution process of rainwater influenced the chromium concentration measured in water because the day before sampling, it was raining at the research location. The amount of rainwater in the river causes the volume of the river water to increase so that the measured concentration of heavy metals is lower due to the dilution process.

(moderately polluted) (iii), Class D: poor, score \geq -31 (severely polluted) (iv)

The average chromium concentration in the water at station 1 usually was higher than in station 2 and station 3. This phenomenon was influenced by the Premulung River's presence, which flows from Sukoharjo Regency to Surakarta City. At station 1, the water flow was polluted by waste from residential and industrial activities, which was carried into Surakarta City. Also, there is a dam that collects water at station 1. As a result, much water-dissolved chromium is estimated to be accumulated in station 1. As a result, less water-dissolved chromium will flow to station 2 and station 3.

The heavy metal content of chromium in sediment

Table 4 shows that the chromium content in the sediment at the three stations is still far below the maximum chromium in sediment according to ANZECC (2000), which is 80 mg/kg. However, the average chromium concentration in sediment at station 3 was higher than in station 1 and station 2. That is because the waters around station 3 contain high organic matter, indicated by high levels of BOD and COD and low DO (Table 2). In the Premulung River water, which is in the weak-basal (alkaline) condition, the chromium (III) ions will form complex ingredients with organic materials and are deposited on the bottom of the river, according to Palar (2012).

The sediment at station 3 is estimated to contain high organic materials in finer particle size than station 1 and station 2, so it is strongly adsorbed to heavy metals. According to Rahardjo et al. (2014), the smaller/finer sediment particle size has a higher metal accumulation ability than the coarser particles.

Table 3. Premulung River water quality status in Surakarta City, Central Java, Indonesia, according to the STORET method

| Premulung-River section | Score | Water quality |
|-------------------------|-------|---------------------|
| Station 1 | -26 | Moderately polluted |
| Station 2 | -27 | Moderately polluted |
| Station 3 | -28 | Moderately polluted |

Table 2. The average measurement of environmental parameters results in the Premulung River, Surakarta City, Central Java, Indonesia

| Parameters | Premulung River sections | | | Quality standard |
|------------------------|--------------------------|---------------|---------------|------------------|
| | Station 1 | Station 2 | Station 3 | |
| Air temperature (°C) | 33.6 | 33.9 | 32.9 | - |
| Water temperature (°C) | 30.47 | 29.23 | 30.47 | ± 3 |
| TDS (mg/L) | 223.67 | 264.67 | 270 | 1000 |
| pH | 7.48 | 7.78 | 7.82 | 6-9 |
| DO (mg/L) | 2.63 | 3.46 | 2.3 | 4 |
| BOD (mg/L) | 6.33 | 5.13 | 9.97 | 3 |
| COD (mg/L) | 23.83 | 24.33 | 28.53 | 25 |
| Cr (mg/L) | 0.0344 | <0.0213 (ttt) | <0.0213 (ttt) | 0.05 |

Note: * PPRI No. 82 of 2001 (2001) (Class 2 water quality)

The content of heavy metal chromium in tilapia meat

Table 4 shows the chromium content in tilapia meat at station 1 was higher than in station 2 and station 3. Tilapia caught at station 1 was primarily found in the dam area, with a large body size of an average body length of 20 cm, so it is estimated that the tilapia are mature and have lived in these waters for a long time. Therefore, those tilapias could have been exposed to chromium metal longer than stations 2 and 3, which are at juvenile stages. These juvenile tilapias are smaller, with an average body length of 14 cm and 12 cm.

According to Yi and Zhang's (2012) research, there is a positive correlation between fish body size and length with the accumulation of chromium in fish meat. Therefore, the larger fish are more tolerant of chromium than the small fish. In addition, tilapia aged 120 days were more tolerant of chromium and could accumulate 60.92 mg/kg compared to tilapia aged 60 days which only could accumulate chromium of 36.86 mg/kg (Javed and Shaukat 2013).

The content of heavy metal chromium in tilapia meat that exceeded the standard quality from the three research stations was caught at station 1 and station 2 with chromium concentrations of 3.535 mg/kg and 2.089 mg/kg. These results have not exceeded the maximum tolerance limit for tilapia. Based on research by Javed and Shaukat (2013), the lethal concentration (LC₅₀) of chromium in tilapia is 141.06 mg/L. Tilapia accumulated chromium in its body up to 66.28 mg/kg concentration.

The concentration of chromium in tilapia meat in this study has exceeded the limit for Cr metal contamination in the food, according to the China National Center for Food Safety Risk Assessment (CFSA), which is 2.0 mg/kg for fish meat. Based on this, it is necessary to consider the feasibility of tilapia caught in the Premulung River for human consumption because it was detected to contain chromium exceeding the metal contamination standard limit. That correlates with the intake of total chromium in food recommended for consumption of 30-100 g/day (Eisler 1986). Furthermore, consumption of high

concentrations of fish contaminated with heavy metal chromium could cause skin ulcers, skin hyperpigmentation, skin cancer, and an indication of renal tubular necrosis (Puspita et al. 2011).

Correlation between abiotic parameters and chromium content in water, sediment, and tilapia meat

Pearson correlations between environmental parameters and chromium concentrations in water, sediment, and tilapia meat (*O. niloticus*) are presented in Table 5. This table shows a positive correlation between temperature and sediment of chromium concentration ($r= 0.591$) and temperature with chromium in tilapia meat ($r= 0.434$). The higher the temperature would lead to the higher accumulation of chromium in the sediment and fish meat. According to Mahida (1984), a 10°C temperature will increase twice the water's chemical and biological reactions. Thus, the increase in temperature causes an increase in the accumulation of heavy metals in the tissues. Temperature affects chemical reactions and metabolism in the organism's body (Odum and Barrett 2004).

TDS with concentrations of chromium in water and fish meat had a very strong negative correlation ($r= -0.815$) and a perfect negative correlation ($r= -1.00$), while chromium in sediments had a moderate positive correlation ($r= 0.499$). The higher the dissolved solids content in the water, the accumulation of chromium by the sediment will also increase.

According to Sulisty (2014), the leading cause of TDS is inorganic ions in the water. Some of the chromium metal in water is a form of dissolved inorganic chromium ions, so when the TDS is higher, it is estimated that the dissolved chromium metal is higher too. The results showed a negative correlation between TDS and chromium in water. A negative correlation means a non-unidirectional relationship; when the TDS level is high, the concentration of chromium in the water will decrease, and less chromium will be absorbed in the fish's body.

Table 4. The average heavy metal chromium (Cr) concentration in water, sediment, and flesh of tilapia (*Oreochromis niloticus*)

| Component | Premulung River Section | | | Quality Standard |
|-------------------------|-------------------------|---------------|---------------|------------------|
| | Station 1 | Station 1 | Station 1 | |
| Cr water (mg/L) | 0.0344 | <0.0213 (ttt) | <0.0213 (ttt) | 0.05* |
| Cr sediment (mg/kg) | 1.740 | 1.307 | 4.948 | 80** |
| Cr tilapia meat (mg/kg) | 3.535 | 2.089 | 1.958 | 2.0*** |

Note:* PPRI No. 82 of 2001 (2001) (class 2 water); **ANZECC (2000); *** CFSA (2012)

Not detected (ttt) for very low concentrations <0.0213 mg/L

Table 5. Pearson correlation between environmental parameters and Cr concentration in water, sediment, tilapia meat

| Parameters | Pearson correlation coefficient | | |
|-------------|---------------------------------|-------------|---------|
| | Cr water | Cr sediment | Cr meat |
| Temperature | -0.029 | 0.591 | 0.434 |
| TDS | -0.815** | 0.497 | -1.000* |
| pH | -0.387 | 0.499 | -0.999 |
| DO | 0.085 | -0.791 | -0.168 |
| BOD | -0.139 | 0.991 | -0.351 |
| COD | -0.645 | 0.979 | -0.641 |

Note:* significant at 0.05 level; **significant at the 0.01 level

Dissolved solids consist of organic and inorganic compounds soluble in water, minerals, and salts. Inorganic compounds that often exist in water are ions (Fardiaz 1992). According to Eisler (1986), chromium (III) is slightly soluble in water and tends to form complex compounds with negatively charged organic compounds or inorganic compounds in colloids or particles and then settle to the bottom of the water.

The chromium pH parameter in water and fish meat had a weak negative correlation ($r = -0,387$) and a very strong negative correlation ($r = -0,999$). A negative correlation means that the higher the pH value, the higher the chromium concentration in the water will decrease. Because the increasing water pH will change the stability of the carbonate to form the hydroxide, which forms metal bonds with particles in the water river to settle, and mud is formed. Increasing the water pH reduces chromium concentration in the water and fish meat.

The correlation between pH and chromium in sediments has a unidirectional correlation of 0.499, which means the higher the pH in the water, the higher concentration of chromium in the sediment will also increase. Therefore, in basal (alkaline) water conditions, chromium (III) ions will be deposited at the bottom of the river (Palar 2012).

The DO parameter strongly correlates with chromium sediment ($r = -0.791$), which means in waters with low dissolved oxygen levels, indicating that pollution occurs with high organic matter and lower solubility of chromium metal. The chromium metal with these organic compounds would form complex compounds with a more considerable molecular weight that will settle down to the bottom of the river.

The BOD parameter has a very strong positive correlation with sediment chromium ($r = 0.991$), which means that the higher the BOD level in the water, the higher the chromium concentration in the sediment. Chromium (III) forms complex compounds with organic materials that are readily adsorbed to the surface of sediment particles. BOD indicates the amount of dissolved oxygen required for the oxidation of organic matter through the degradation process of microorganisms (Fardiaz 1992). When the BOD level is high, it is indicated that the organic matter content in the waters is also high

The COD and chromium parameters in the sediment have a very strong positive correlation ($r = 0.979$). A positive correlation means that the relationship is unidirectional, i.e., when the COD level is high, the concentration of chromium metal in the sediment is also high. COD with chromium in water and fish meat has a strong correlation, but the value is negative/not directly ($r = -0.645$) and ($r = -0.641$). Therefore, when the COD value in the waters increases, chromium concentration will decrease, leading to less chromium being absorbed by fish.

Correlation between chromium content in water and sediment with fish meat

The Pearson correlation between chromium content in water and sediment with chromium in tilapia meat (*O. niloticus*) is presented in Table 6.

Table 6. Pearson correlation between chromium content in water and sediment with chromium in tilapia meat

| Parameters | Pearson correlation |
|----------------------|---------------------|
| | Cr tilapia meat |
| Chromium in water | 0.997* |
| Chromium in sediment | -0.470 |

Note:*significant level at 0.05

Table 6 shows that chromium content in water and tilapia fish meat has a very strong positive correlation, which means that increasing the concentration of chromium dissolved in water will significantly increase chromium concentration in fish meat. On the other hand, chromium content in the sediment and tilapia meat has a moderate negative correlation. Therefore, increasing the chromium concentration in the sediment will decrease the chromium concentration in tilapia meat.

According to Javed and Shauket's (2013) research, increasing the chromium concentration in the waters would increase the chromium concentration absorbed by fish, thereby increasing the accumulation of chromium in fish meat. That is affected by the tilapia behavior that lives floating in water rivers directly in contact with chromium dissolved in water. The fish's high mobility, especially in chromium-polluted waters, could cause an increase in the accumulation of chromium in their body tissues.

The above research could be concluded that: (i) The water quality of the Premulung River in Surakarta City is of the moderately polluted quality standard according to the STORET method. (ii) The chromium (Cr) heavy metal content in the waters of the Premulung River in Surakarta City of (ttt to 0.0344) mg/L meets the standard quality (0.05 mg/L) of PPRI No. 82 of 2001 (2001) water class 2. The sediment of (1,307- 4,948) mg/kg also meets the standard quality (80 mg/kg) of ANZECC (2000). While the tilapia meat (*O. niloticus*) of (1.958-3.535) mg/kg exceeds the standard quality (2.0 mg/kg) to CFSA (2012). (iii) The relationship between chromium content in water is very strong and directly proportional ($r = 0.997$) with chromium in tilapia meat (*O. niloticus*). In contrast, the relationship between chromium in sediments and the tilapia meat (*O. niloticus*) was inversely proportional ($r = -0.470$).

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The bacterial flora of *Oreochromis niloticus* and *Clarias gariepinus* from earthen ponds in Sagana and Masinga, Kenya

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Abstract. Karimi RD, Ngeranwa JJN, Njagi ENM, Kariuki S. 2022. The bacterial flora of *Oreochromis niloticus* and *Clarias gariepinus* from earthen ponds in Sagana and Masinga, Kenya. *Intl J Bonorowo Wetlands* 12: 63-73. Food-borne diseases traced to fish consumption have been reported globally, including in Kenya. The aspect of food quality as far as fish consumption is concerned is underestimated in Kenya though aquaculture has been promoted. The bacterial flora of Tilapia (*Oreochromis niloticus* Linnaeus, 1758) and Catfish (*Clarias gariepinus* Burchell, 1822) from Masinga Dam and earthen ponds at Sagana fish farm was determined in this study to determine the anti-microbial response of the pathogenic bacteria. Tilapia fish and Catfish samples were collected from Masinga Dam and Sagana farm in the dry and rainy seasons. The fish were skinned, and gut content was taken for laboratory tests. The water and water sediment samples from these two study sites were also collected. Those samples were processed and cultured in MacConkey agar, and the selective media were subcultured in the colonies and then subjected to morphological examination from cultures. Then, the biochemical tests were carried out using commercially available API kits. The study showed the presence of bacterial species belonging to *Enterobacter* spp. (n=34), *Pseudomonas* spp. (n=6), *Aeromonas* spp. (n=5), *Vibrio* spp. (n=3) and *Acinetobacter* spp. (n=2) isolates during the dry season, while bacterial species belonging to *Enterobacter* spp. (n=31), *Pseudomonas* spp. (n=6), *Aeromonas* spp. (n=4) isolates during the dry season. The anti-microbial susceptibility analysis showed that the highest resistance rates were found against Ampicillin (Amp) (61.5% of isolates), Amoxicillin (AmL) (65.9% of isolates), Tetracycline (Te) (31.8% of isolates), and Chloramphenicol (C) (27.5% of isolates) while the lowest was Nalidixic acid (Na), Cefuroxime (Cxm) and Streptomycin (S) at (4.4% of isolates) each. All isolates were sensitive to Gentamycin (Gen), Ciprofloxacin (Cip), and Cefotaxime (CTX). The presence of the above organisms, some potentially pathogenic to humans, indicates that improperly handled, undercooked, or consumed raw fish may cause disease in susceptible individuals. At the same time, some isolates' anti-microbial resistance indicates that the use of antibiotics in aquaculture to promote growth should be studied further with a view to policy formulation.

Keywords: Bacteria, consumption, fish, microorganisms

INTRODUCTION

Fish is an important component of diets worldwide, and an estimated 1 billion people rely on fish as their main source of animal protein (FAO 2007; Novoslavskij et al. 2016; Novoslavskij et al. 2016; Priatni et al. 2018; Aboagye et al. 2020). Fish production is estimated globally to be 148.5 million tonnes per year, which capture fisheries accounting for 88.6 million tonnes and aquaculture 59.9 million tonnes annually (FAO 2012). Fish at affordable prices provide much-needed protein to people, especially in developing countries. It sustains many through employment in fish and fish products-related services and feeds millions daily. In addition, their nutritional attributes are highly praised as it has high-quality vitamins, rich in essential amino acids, and their fatty acid fraction has well-established health benefits (with their anti-thrombotic activity) (Rahmanifarah et al. 2014; Partasmita et al. 2015; Suvitha et al. 2015; Sayuti et al. 2022). Therefore, its availability in many developing countries should enable fish to contribute significantly to a balanced and healthy diet. Moreover, it is estimated that fish supplied around 60% of the population in many developing countries

derives over 30% of their animal protein. In comparison, in most developed countries, almost 80% of the population obtains their animal protein supplies from fish, in less than 20% (FAO 2000).

The risks of food-borne disease associated with products from aquaculture are related to coastal or inland ecosystems, and the potential for environmental contamination is greater than in capture fisheries (Costa 2013). Most of the food safety hazards associated with products from aquaculture could be controlled by appropriate consumer education and good fish farm management practices concerning such risks as eating raw or partially cooked products that could contain pathogenic bacteria (Reilly and Kaferstein 1998). In developing countries, the estimated mortality of food and water-borne infectious diseases annually amounts to high death rates for children and infants. Moreover, microbiological food-borne illnesses affect up to 30% of the population in industrialized countries. It is estimated that each year 20 out of a million inhabitants die from food-borne diseases. Unwholesome fishery products and fish cause up to 30% of food-borne illnesses (WHO 1999).

In Asia, around 40 million people are affected by fish and water-borne parasitic diseases, especially trematodes. These parasitic diseases are widespread mainly in Viet Nam, Thailand, China, and Laos, where they encourage the consumption of raw fish as their habit. In addition, fish-borne illnesses can have costly health adverse effects on the economic losses incurred because of fish spoilage, medical expenses, the loss of productivity, and adverse publicity to the companies. Additional costs in international trade include the cost of rejections, recalls, product detections, and the resulting adverse publicity to the industry and even to the fish's country of origin (Lahsen 2003). Moreover, the financial implications of food-borne disease outbreaks could have grave consequences. An outbreak of cholera in Peru in 1991 cost 770 million dollars, for example, and a similar outbreak in Tanzania in 1998, cost 36 million dollars. However, effective surveillance systems and simple preventive measures, which would cost less, might have prevented these outbreaks and definitely reduced the impact (Lahsen 2003). For example, due to an outbreak of *Salmonella* in 1996 and an outbreak of cholera in 1997 in Kenya, there was a ban on fish and fishery exports to the EU (Ministry of Fisheries Development 1999).

Current knowledge of the impact of antibiotics used in aquaculture on the health and environment is poor, particularly in developing countries. In addition, drug residues may remain in fish used for human consumption; therefore, the antibiotics released into the environment can lead to antibiotic-resistant bacteria development in the food chain (Cabello 2006). Resistance to antibacterial agents is a global public health problem and one that is increasing as the antibacterial continues to lose effectiveness (Akinbowale et al. 2006).

This study aims to determine the bacterial flora of Tilapia, Catfish, water, and water sediments and their anti-microbial response from Masinga Dam and earthen ponds at Sagana fish farm during dry and wet seasons. Then, the specific objectives of this study are (i) To isolate and identify bacterial flora species during the dry and wet seasons at Masinga Dam and Sagana fish ponds, (ii) To identify whether there is a significant difference in the number of bacteria flora species isolated in fish specimen types from dams and ponds, during the dry and wet season, (iii) To determine the anti-microbial response of the pathogenic bacteria isolates from Masinga Dams and Sagana fish farm.

MATERIALS AND METHODS

Study area

The study was conducted at Masinga Dam and Sagana Aquaculture Centre, situated in the Tana River and Kirinyaga Districts, respectively. Masinga Dam is situated on the Tana River, and the main catches are Catfish and Tilapia. Dam fisheries account for about Kshs.34 million annually, with Nairobi City being the most consumed. Sagana Aquaculture Centre (Figure 1) is a Department of Fisheries breeding farm in Sagana town, Kirinyaga District (100 km North East of Nairobi, latitude 0°39'S and longitude 37°12'E, and altitude 1,230 m), with an area covering approximately 50 hectares, with ponds covering 18 hectares; each pond covers an area on average about 40 by 20 m² with a depth of 1 m. Ragati river water was diverted and delivered through a canal by gravity. It acts as a training center for fish farmers in aquaculture and one of the fisheries department's two main national fish hatcheries and acts as a training center for fish farmers in aquaculture.

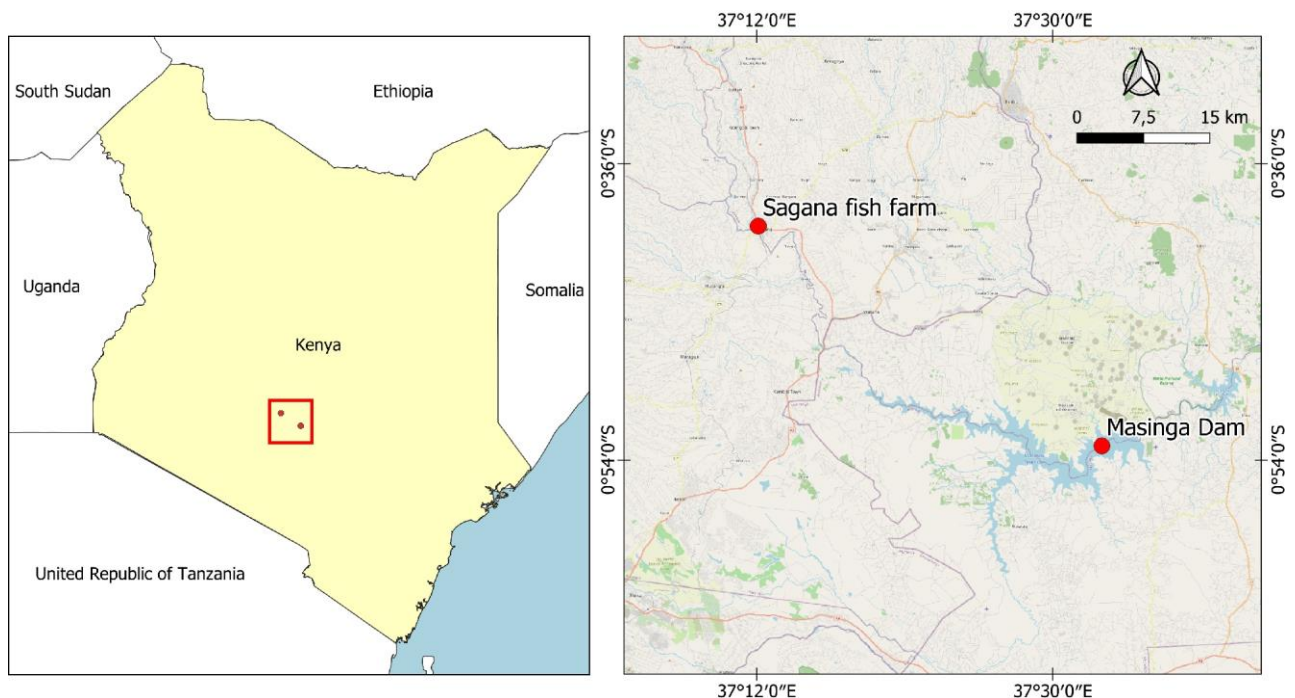


Figure 1. Map of Kenya showing Sagana fish farm and Masinga Dam, Kenya

The farm is involved in the culture of Catfish and Tilapia as the main species. In addition, the farm provides quality fish feeds to farmers, demonstrates the economic viability of integrated fish farming, and conducts research. Fish farming is conducted on still-water earthen ponds with semi-intensive systems. The ponds are fertilized using organic manure from cattle, chicken, and kitchen wastes and artificial fertilizers to enhance the growth of the phytoplankton. The other alternative feeds are wheat germ, rice bran, and maize bran. The experimental period started during the dry season in August 2007 and continued to December 2007 rainy season.

Sample size

The sample size was determined using the ICMSF sampling standard, which relates the sampling plan stringency to the food's degree of hazard. When the health hazard is low, the A 3-class plan is used. In this plan, $n = 5$ and $c = 3$, n is the number of samples drawn, and the maximum allowable number of positive results is c (3).

Sampling procedure

Collection of fish samples

The study design adopted was a purposive study. Fish was sampled from the Sagana fish farm of 20 organically fertilized ponds; the ponds were selected at an interval of 4 ponds, and 5 pieces of table size Tilapia were harvested using a scoop net and taken in a cool box to the laboratory. The fish were aseptically skinned in the laboratory to get the skin sample. First, up to 25 g of fish skin was weighed and mixed with 225 mL of buffered peptone water in a sterile blender. Then, it was blended, and a part of the pure mix was inoculated into the MacConkey agar culture media. Next, to have distinguishable colonies, diluted subcultures were made from the initial culture after overnight incubation at 37°C at dilutions of 1:10, 1:100, and 1:1,000 using peptone water and incubated overnight at 37°C. Growth of distinct colonies was achieved at the 1:100 dilutions. Next, the colonies were sub-cultured in various selective media Hektoen Enteric (HE) agar, Xylose-Lysine Deoxycholate (XLD) agar, and *Salmonella-Shigella* (SS) agar and incubated. The colonies were morphologically identified and, for further identification, subjected to biochemical tests. Final identification was done using the API 20E method.

The fish was cleaned using 70% alcohol for the gut sample, an incision was made over the peritoneal cavity, and dissected to get the gut contents. The gut contents were combined and weighed, similarly to the skin, and the same procedure was followed, and again, the growth of distinct colonies was at 1:100 dilutions. These same procedures were conducted for the dam fish during the two seasons.

Collection of water samples

Water was collected from one end of the pond and the center, about 20 cm beneath the surface, in sterile bottles of 100 mL. The water sample was incubated at 37°C overnight and enriched using peptone water; this was inoculated to MacConkey culture media and incubated at 37°C overnight. The growth of distinct colonies was

obtained at the 1: 10 dilution. Then, they were sub-cultured in selective media and incubated. The colonies were morphologically identified and then subjected to biochemical tests for further identification. The final identification was using the API 20E method.

The Dam water was collected in the early morning and taken within 2 hours to the laboratory. The sample was collected using sterile bottles away from the bank at a one-foot depth below the surface, and the bottle mouth was directed towards the current, filled, and covered immediately. The dam water was stored in a cooler box during transportation. The same procedure was used for the pond water applied to the dam water.

Collection of water sediments

Sediment samples were collected from the bottom of the dam and pond using an Ekman grab in all procedures, with the sediments collected at the edge and the center of the ponds. The sediments were then taken to the laboratory, mixed, and enriched using peptone water cultured in MacConkey and incubated at 37°C overnight. Next, the colonies were sub-cultured in selective media. The colonies were morphologically identified and then subjected to biochemical tests, with the final identification using the API 20E method.

The Analytical Profile Index (API 20E)

All test chambers were rehydrated by inoculation with a saline suspension of a pure culture of the bacterial strain subjected to the identification. After incubation in a humidity chamber at 37°C for 18 to 24 hours, the color reactions were read. The test reaction results were converted to a seven-digit code. Then, the code was looked up in the database book for the genus and species identification of the test microorganism.

Antibiotic susceptibility testing

Antibiotic sensitivity profile

The antibiotic sensitivity testing against commonly used anti-microbial agents' was performed on all the isolates (Table 1). The Kirby – Bauer disk diffusion test was the standard recommended by the National Committee for Clinical Laboratory Standards (NCCLS 2009). After incubation at 37°C for 24 hours, the diameters of inhibition zones were measured and compared with the control organism *Escherichia coli* the ATCC 25922.

Table 1. Anti-microbial agents used for sensitivity testing

| Anti-microbial agent | Concentration |
|----------------------|---------------|
| Ampicillin (Amp) | 10 µg/mL |
| Chloramphenicol (C) | 30 µg/mL |
| Streptomycin (S) | 10 µg/mL |
| Tetracycline (Te) | 30 µg/mL |
| Nalidixic acid (Na) | 30 µg/mL |
| Ciprofloxacin (Cip) | 5 µg/mL |
| Gentamycin (Gen) | 10 µg/mL |
| Cefuroxime (Cxm) | 30 µg/mL |
| Amoxicillin (Aml) | 5 µg/mL |
| Cefotaxime (CTX) | 30 µg/mL |

RESULTS AND DISCUSSION

Bacteria isolates per specimen type

The sum of 91 bacteria were isolates from six specimen types: Tilapia Gut, Tilapia skin, Catfish skin, Catfish Gut, and water and water sediments. The Tilapia gut had the highest proportion (20; 22%) of bacteria isolates than the rest. Tilapia skin and catfish skin isolates at 16 (17.6%) bacteria. The water and catfish guts had the same number of bacteria isolates at 15 (16.5%). The water sediments had the lowest proportion (9; 9.9%) of bacteria isolates compared to the rest of the specimens (Figure 2).

Types of bacteria isolates from Catfish, Tilapia, water, and water sediments

A total of 91 (100%) bacterial isolates were identified, of which *Citrobacter freundii* were 16 (17.6%) than the rest of the bacteria isolates. The *E. coli* and *C. freundii* are the only bacteria that occurred in five specimen types except in water sediments and Tilapia skin, respectively (Table 2).

In Catfish skin, the bacterial isolates were 16 (17.6%) same as the bacterial isolates in Tilapia skin. The bacteria isolates that occurred both in Tilapia Skin and Catfish skin were: *Aeromonas sobia*, *C. freundii*, *E. coli*, *Edwardsiella tarda*, *Enterobacter cloacae*, *Enterobacter sakazakii*, *Proteus mirabilis* and *Pseudomonas aeruginosa* (Table 2). In Tilapia Gut, 20 (22.0%) bacterial isolates were identified. On the contrary, 15 (16.5%) bacteria were isolates from Catfish Gut. Bacterial isolates that occurred in both Tilapia gut and Catfish skin were; *A. sobia*, *C. freundii*, *E. tarda*, *E. sakazakii*, *E. coli*, and *Pseudomonas*

fluorescens (Table 2). In water, 15 (16.5%) bacteria were isolates, then 9 (9.9%) bacterial isolates from water sediments. Bacterial isolates in water and water sediments were; *Enterobacter fergusonii*, *E. coli*, *P. fluorescens*, and *Salmonella* spp. (Table 2).

Types of bacteria isolates from Sagana Pond and Masinga Dam, Kenya

The bacteria isolates from Sagana Ponds were 54 (59.3%), while from Masinga Dam were 37 (40.7%) isolates. The *C. freundii* found 16 (17.6%), of which 10 (11.0%) were isolates from Sagana Pond, while from Masinga Dam, were 6 (6.6%). All bacteria isolates include *A. sobia*, *C. freundii*, *E. coli*, *E. tarda*, *P. aeruginosa*, *P. fluorescens*, and *P. mirabilis*, occurred in both Masinga Dam and Sagana Pond (Table 3).

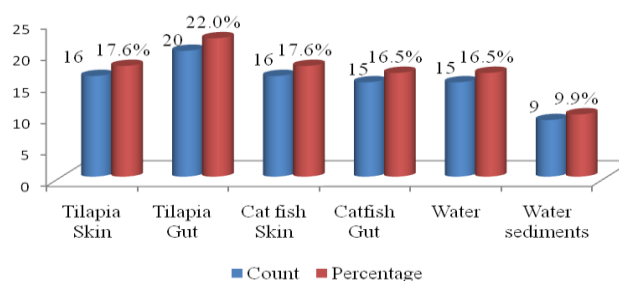


Figure 2. Bacteria isolates from various collected samples

Table 2. Bacteria isolates in Tilapia, Catfish, water, and water sediments

| Isolates | Tilapia skin | Catfish skin | Tilapia gut | Catfish gut | Water | Water sediments | Total |
|----------------------------------|--------------|--------------|-------------|-------------|------------|-----------------|-------------|
| <i>Acinetobacter</i> spp | - | - | - | - | 1 | - | 1 (1.1%) |
| <i>Aeromonas sobia</i> | 2 | 2 | 1 | 1 | - | - | 6 (6.6%) |
| <i>Chromobacterium violaceum</i> | - | - | 1 | - | - | - | 1 (1.1%) |
| <i>Citrobacter freundii</i> | 4 | 2 | 5 | 3 | 2 | - | 16 (17.6%) |
| <i>Escherichia coli</i> | - | 1 | 2 | 2 | 5 | 4 | 14 (15.4%) |
| <i>Edwardsiella tarda</i> | 1 | 1 | 2 | 2 | - | - | 6 (6.6%) |
| <i>Enterobacter agglomerans</i> | - | 1 | - | - | - | 1 | 2 (2.2%) |
| <i>Enterobacter amnigenus</i> | - | - | 1 | - | - | - | 1 (1.1%) |
| <i>Enterobacter cloacae</i> | 1 | 1 | - | - | 1 | - | 3 (3.3%) |
| <i>Enterobacter fergusonii</i> | - | - | - | - | 1 | 1 | 2 (2.2%) |
| <i>Enterobacter sakazakii</i> | 1 | 1 | 1 | 3 | - | - | 6 (6.6%) |
| <i>Klebsiella onithnolytica</i> | 1 | - | - | - | - | - | 1 (1.1%) |
| <i>Klebsiella pneumonia</i> | 1 | - | 1 | - | - | - | 2 (2.2%) |
| <i>Plesiomonas shigelloides</i> | - | - | - | - | 1 | - | 1 (1.1%) |
| <i>Proteus mirabilis</i> | 1 | 1 | - | - | - | 1 | 3 (3.3%) |
| <i>Providentia stuartii</i> | - | 3 | - | 2 | - | - | 5 (5.5%) |
| <i>Pseudomonas aeruginosa</i> | 2 | 3 | 3 | - | 1 | - | 9 (9.9%) |
| <i>Pseudomonas fluorescens</i> | - | - | 1 | 1 | 1 | 1 | 4 (4.4%) |
| <i>Salmonella</i> spp | 1 | - | 1 | - | 1 | 1 | 4 (4.4%) |
| <i>Shigella boydii</i> | - | - | - | 1 | - | - | 1 (1.1%) |
| <i>Vibrio mechnikovii</i> | 1 | - | - | - | 1 | - | 2 (2.2%) |
| <i>Vibrio vulnificus</i> | - | - | 1 | - | - | - | 1 (1.1%) |
| Total | 16 (17.6%) | 16 (17.6%) | 20 (22.0%) | 15 (16.5%) | 15 (16.5%) | 9 (9.9%) | 91 (100.0%) |

Table 3. Types of bacteria isolated from Sagana Pond and Masinga Dam, Kenya

| Bacteria isolated | Sagana Pond | Masinga Dam | Total |
|----------------------------------|-------------|-------------|-----------|
| | n (%) | n (%) | n (%) |
| <i>Acinetobacter</i> spp | 1 (1.1) | 0 (0.0) | 1 (1.1) |
| <i>Aeromonas sobia</i> * | 4 (4.4) | 2 (2.2) | 6 (6.6) |
| <i>Chromobacterium violaceum</i> | 1(1.1) | 0 (0.0) | 1 (1.1) |
| <i>Citrobacter freundii</i> * | 10 (11.0) | 6 (6.6) | 16 (17.6) |
| <i>Escherichia coli</i> * | 6 (6.6) | 8 (8.8) | 14 (15.4) |
| <i>Edwardsiella tarda</i> * | 4 (4.4) | 2 (2.2) | 6 (6.6) |
| <i>Enterobacter agglomerans</i> | 0 (0.0) | 2 (2.2) | 2 (2.2) |
| <i>Enterobacter amnigenus</i> | 1(1.1) | 0 (0.0) | 1 (1.1) |
| <i>Enterobacter fergusonii</i> | 2 (2.2) | 0 (0.0) | 2 (2.2) |
| <i>Enterobacter sakazakii</i> | 0 (0.0) | 6 (6.6) | 6 (6.6) |
| <i>Enterobacter cloacae</i> | 0 (0.0) | 3 (3.3) | 3 (3.3) |
| <i>Klebsiella onitnolytica</i> | 1 (1.1) | 0 (0.0) | 1 (1.1) |
| <i>Klebsiella pneumonia</i> | 2 (2.2) | 0 (0.0) | 2 (2.2) |
| <i>Plesiomonas shigelloides</i> | 0 (0.0) | 1 (1.1) | 1 (1.1) |
| <i>Proteus mirabilis</i> * | 1 (1.1) | 2 (2.2) | 3 (3.3) |
| <i>Providencia stuartii</i> | 5 (5.5) | 0 (0.0) | 5 (5.5) |
| <i>Pseudomonas aeruginosa</i> * | 7 (7.7) | 2 (2.2) | 9 (9.9) |
| <i>Pseudomonas fluorescens</i> * | 2 (2.2) | 2 (2.2) | 4 (4.4) |
| <i>Salmonella</i> spp | 4 (4.4) | 0 (0.0) | 4 (4.4) |
| <i>Shigella boydii</i> | 1 (1.1) | 0 (0.0) | 1 (1.1) |
| <i>Vibrio mechnikovii</i> | 2 (2.2) | 0 (0.0) | 2 (2.2) |
| <i>Vibrio vulnificus</i> | 0 (0.0) | 1 (1.1) | 1 (1.1) |
| Total | 54 (59.3) | 37 (40.7) | 91(100) |

Note: +/- Species occurred/ did not occur, * Bacteria isolated both in Sagana and Masinga Dam, Kenya

Bacteria isolates from Sagana Pond and Masinga Dam by specimen type

Tilapia gut had a total of 20 (22.0%) bacteria isolates, of which 12 (13.2%) were from Sagana Ponds and Masinga were 8 (8.8%) bacteria isolates. Water sediments had the lowest proportion (9; 9.9%) from Sagana Ponds (4; 4.4%) and Masinga Dam (5; 5.5%) of bacteria isolates, respectively. When the results were subjected to chi-square, there was no significant difference between a pond and dam water in bacteria flora species isolates, $\chi^2 = 3.853$, $df=5$, $P=0.571$) (Table 4).

Bacteria isolates from Tilapia, Catfish, water, and water sediments in dry and wet seasons

The tilapia gut bacteria isolates were (20; 22%), of which 11 (12.1%) were isolates during dry seasons, while in the wet season were 9 (9.9%) bacteria isolates. In all specimen types, there were more bacteria isolates during the dry season than the wet season, except the tilapia skin specimen, where (6; 6.6%) bacteria were isolates in the dry

and 10 (11.0%) in the wet season, respectively. The reason adduced for a higher number of bacteria isolates during the dry season than the wet season, according to Wemedo (2002), is that lower temperatures inhibited microbial activity during the wet season. Another reason for this phenomenon is that the soil saturation by rain reduces aeration by limiting activity (Marshall and Devinny 1998). (Figure 3).

Types of bacteria isolate from the dry and wet season

During the dry season, 50 (54.9%) bacteria were isolates, while 41 (45.1%) bacteria were isolates in the wet season. The number of *C. freundii* isolates from both dry and wet season were 16 (17.6%), of which 9 (9.9%) were isolates during the dry season while 7 (7.7%) were isolates in the wet season, *A. sobia*, *Acinetobacter* spp., *C. freundii*, *E. sakazakii*, *E. tarda*, *E. coli*, *E. cloacae*, *P. aeruginosa*, and *Providencia stuartii* were isolates in both seasons (Table 5).

Bacteria isolates during the wet and dry seasons by specimen type

The total of all bacterial isolates was 91, with tilapia fish gut having 20 (22.0%), of which 11 (12.1%) were during the dry season while 9 (9.9%) were during the wet season. There was no significant difference between the number of bacteria isolates from the Tilapia fish gut and the two seasons ($p>0.05$). That was the same for other specimen types despite more bacteria isolates during the dry season (Table 6), including Tilapia fish skin which had 6 (6.6%) and 10 (11.0%) bacteria isolates from both dry and wet seasons, respectively, There was no significant difference to show any association of bacteria isolates and the source ($\chi^2 = 3.006$, $df=5$, $P=0.699$).

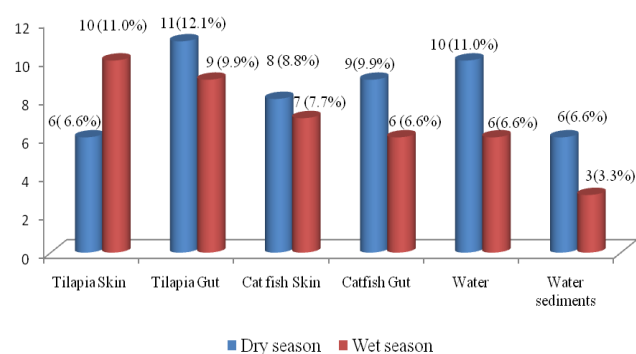


Figure 3. Bacteria isolates in the dry and wet seasons by specimen type

Table 4. Number of bacteria isolated from Sagana farm and Masinga Dam, Kenya

| Specimen type | Sagana Farm | Masinga Dam | Total | χ^2 | Df | P-Value |
|-----------------|-------------|-------------|------------|----------|----|---------|
| | n (%) | n (%) | n (%) | | | |
| Tilapia Skin | 12 (23.2%) | 4 (4.4%) | 16 (17.6%) | 4.00 | 1 | 0.050 |
| Tilapia Gut | 12 (23.2%) | 8 (8.8%) | 20 (22.0%) | 0.800 | 1 | 0.371 |
| Catfish Skin | 9 (9.9%) | 7 (7.7%) | 16 (17.6%) | 0.250 | 1 | 0.617 |
| Catfish Gut | 7 (7.7%) | 8 (8.8%) | 15 (16.5%) | 0.670 | 1 | 0.796 |
| Water | 10 (11.0%) | 5 (5.5%) | 15 (16.5%) | 1.667 | 1 | 0.197 |
| Water sediments | 4 (4.4%) | 5 (5.5%) | 9 (9.9%) | 0.111 | 1 | 0.739 |
| Total | 54 (59.3%) | 37 (40.7%) | 91 (100%) | 3.853 | 5 | 0.571 |

Note: *:Significant at 0.05

Table 5. Bacteria isolates from the dry and wet season

| Bacteria isolates | Dry season | Wet season | Total n |
|----------------------------------|------------|------------|-----------|
| | n (%) | n (%) | (%) |
| <i>Acinetobacter</i> spp.* | 1 (1.1) | 1 (1.1) | 1 (1.1) |
| <i>Aeromonas sobia</i> * | 4 (4.4) | 2 (2.2) | 6 (6.6) |
| <i>Chromobacterium violaceum</i> | 0 (0.0) | 1 (1.1) | 1 (1.1) |
| <i>Citrobacter freundii</i> * | 9 (9.9) | 7 (7.7) | 16 (17.6) |
| <i>Escherichia coli</i> * | 6 (6.6) | 8 (8.8) | 14 (15.4) |
| <i>Edwardsiella tarda</i> * | 3 (3.3) | 3 (3.3) | 6 (6.6) |
| <i>Enterobacter agglomerans</i> | 2 (2.2) | 0 (0.0) | 2 (2.2) |
| <i>Enterobacter amnigenus</i> | 1 (1.1) | 0 (0.0) | 1 (1.1) |
| <i>Enterobacter fergusonii</i> | 2 (2.2) | 0 (0.0) | 2 (2.2) |
| <i>Enterobacter sakazakii</i> * | 4 (4.4) | 2 (2.2) | 6 (6.6) |
| <i>Enterobacter cloacae</i> * | 2 (2.2) | 1 (1.1) | 3 (3.3) |
| <i>Klebsiella onithnolytica</i> | 0 (0.0) | 1 (1.1) | 1 (1.1) |
| <i>Klebsiella pneumonia</i> | 0 (0.0) | 2 (2.2) | 2 (2.2) |
| <i>Plesiomonas shigelloides</i> | 1 (1.1) | 0 (0.0) | 1 (1.1) |
| <i>Proteus mirabilis</i> | 0 (0.0) | 3 (3.3) | 3 (3.3) |
| <i>Providencia stuartii</i> * | 3 (3.3) | 2 (2.2) | 5 (5.5) |
| <i>Pseudomonas aeruginosa</i> * | 4 (4.4) | 5 (5.5) | 9 (9.9) |
| <i>Pseudomonas fluorescens</i> | 0 (0.0) | 4 (4.4) | 4 (4.4) |
| <i>Salmonella</i> spp. | 4 (4.4) | 0 (0.0) | 4 (4.4) |
| <i>Shigella boydii</i> | 1(1.1) | 0 (0.0) | 1(1.1) |
| <i>Vibrio mechikovii</i> | 2 (2.2) | 0 (0.0) | 2 (2.2) |
| <i>Vibrio vulnificus</i> | 1(1.1) | 0 (0.0) | 1(1.1) |
| Total | 50 (54.9) | 41 (45.1) | 91(100) |

Note: +: Species occurred, -: Species did not occur, *: Bacteria isolated in both dry and wet season

Table 6. Bacteria isolates in specimen type by season

| Specimen type | Dry Season | Wet Season | Total | X ² | df | P-value |
|----------------|------------|------------|-----------|----------------|----|---------|
| Tilapia Skin | 6 (6.6%) | 10 (11.0%) | 16(17.6%) | 1.000 | 1 | 0.317 |
| Tilapia Gut | 11(12.1%) | 9(9.9%) | 20(22.0%) | 0.200 | 1 | 0.655 |
| Catfish Skin | 8 (8.8%) | 7(7.7%) | 15(16.5%) | 0.067 | 1 | 0.796 |
| Catfish Gut | 9 (9.9%) | 6(6.6%) | 15(16.5%) | 0.600 | 1 | 0.439 |
| Water | 10 (11.0%) | 6(6.6%) | 16(17.6%) | 1.000 | 1 | 0.317 |
| Water sediment | 6 (6.6%) | 3(3.3%) | 9(9.9%) | 1.000 | 1 | 0.317 |
| Total | 50 (54.9%) | 41(45.05%) | 91(100%) | 3.006 | 5 | 0.699 |

Correlation between bacteria isolates from specimen types, sites, and season

There was a strong positive correlation between bacteria isolates from the specimen and the site during the dry and wet seasons (Ponds and Dams). Furthermore, from the two sites, the more bacteria isolates, the higher the significant difference between the bacteria isolated during dry and wet seasons ($r=0.734$, $P=0.000$) (Table 7). On the other hand, Pearson’s correlation analysis did not indicate a significant correlation between the bacteria isolates from the specimen and the site. The correlation between the two variables was weak and negatively correlated ($r=0.734$, $P=0.000$) (Table 7). On the other hand, between the bacteria isolates from specimen types and the site, there was a weak positive correlation(Sagana Ponds and Masinga Dam) ($r=0.136$, $P=0.197$) (Table 7).

Overall antibacterial response of isolates

All bacteria isolates were examined to commonly used anti-microbial agents for susceptibility. The inhibition

zones were read after incubation, compared against measurement standards, and recorded as resistant, intermediate, or sensitive. The CIP antibiotic was susceptible to all (100%) bacterial isolates; on the contrary, none of the bacterial isolates registered resistance to GEN, CXT, and CIP antibiotics. The drug with the highest resistance was AML, with 60 (65.9%) bacteria isolates registering resistance, then by AMP at 56 (61.5%) bacterial isolates. On average, 64 (70.3%) of bacteria isolated registered susceptibility, 18 (20%) registered resistance to drugs, and 9 (9.6%) registered intermediate (Table 8 and Figure 4).

Antibacterial response of isolates from Sagana Ponds

The total Sagana Ponds isolates were 54, with 38 (70.4%) registered resistance to 7 anti-microbial agents. Bacterial isolates from Sagana Ponds registered high resistance to AML and AMP drugs, 38 (70%) and 36 (67%), respectively. The rest of the isolates’ resistance registered to antibiotics was; 16 (30%) were resistant to TE, 15 (28%) to C, and 5 (10%) to NA and S, respectively. No resistance was registered to CIP, CXT, and GEN for bacterial isolates from Sagana Ponds (Figure 5)

Concerning bacteria type isolates from Sagana Pond, *Salmonella* spp. showed resistance to AMP, AML, and CXM. Most bacterial isolates from Sagana showed resistance to an average of 2 to 3 drugs except for klebsiella pneumonia, which registered resistance to five antibiotics: AML, AMP, TE, S, and C. There was variation in the antibacterial response of isolates from the Sagana Pond ($F=8.4$, $P=0.000$) (Table 9).

Table 7. correlation between specimen, site, and season

| | | Season | Site |
|----------|---------------------|--------------|-------|
| Site | Pearson Correlation | 0.734** | 1 |
| | Sig. (2-tailed) | 0.000 | |
| | N | 91 | 91 |
| Specimen | Pearson Correlation | -0.162 | 0.136 |
| | Sig. (2-tailed) | 0.124 | 0.197 |
| | N | | 91 |

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

Table 8. Antimicrobial response of isolates to various antibiotics

| Antibiotic | Resistant | | Sensitive | | Intermediate | | Total | |
|------------|-----------|------|-----------|------|--------------|------|-------|-----|
| | N | % | n | % | n | % | N | % |
| AML | 60 | 65.9 | 27 | 29.6 | 4 | 5.5 | 91 | 100 |
| CXT | 0 | 0 | 83 | 91.2 | 8 | 7.14 | 91 | 100 |
| CIP | 0 | 0 | 91 | 100 | 0 | 0 | 91 | 100 |
| S | 4 | 4.4 | 69 | 75.8 | 18 | 19.7 | 91 | 100 |
| AMP | 56 | 61.5 | 29 | 31.8 | 6 | 6.6 | 91 | 100 |
| GEN | 0 | 0 | 90 | 98.9 | 1 | 1.1 | 91 | 100 |
| TE | 29 | 31.8 | 40 | 43.9 | 22 | 24.2 | 91 | 100 |
| C | 25 | 27.5 | 47 | 51.6 | 19 | 20.9 | 91 | 100 |
| NA | 4 | 4.4 | 78 | 85.7 | 9 | 9.8 | 91 | 100 |
| CXM | 4 | 4.4 | 86 | 94.5 | 1 | 1.1 | 91 | 100 |
| Average | 18 | 20.0 | 64.0 | 70.3 | 9 | 9.6 | 91 | 100 |

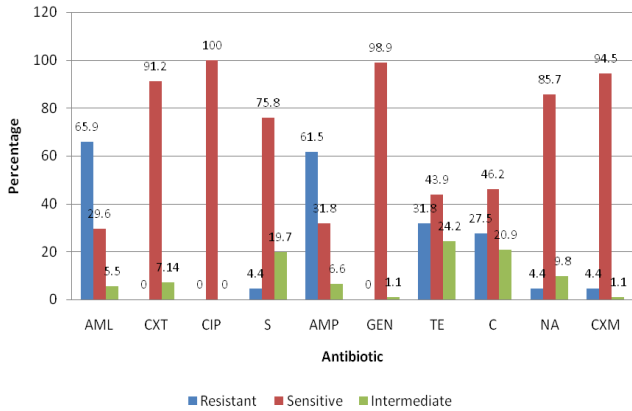


Figure 4. Percentage distribution of anti-microbial response of isolates to various antibiotics

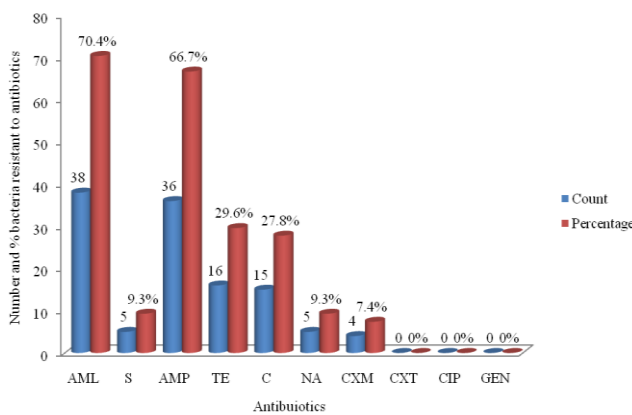


Figure 5. Number of Isolates resistant to antibiotics from Sagana, Kenya

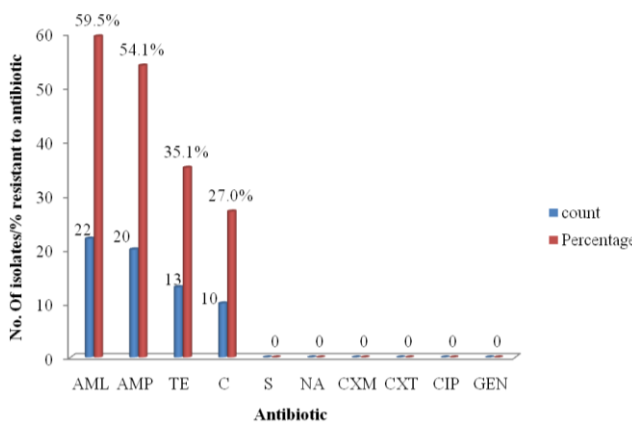


Figure 6. Number of bacterial isolates from Masinga Dam, Kenya, resistant to antibiotics

Antibacterial response of Isolates from Masinga Dam

Bacterial isolates from Masinga Dam registered resistance to four antibiotics: AML, AMP, TE, and C. There were 37 isolates, with 22 (60%) isolates registering displayed high resistance in AML, followed by 20 (54%) bacteria that displayed resistance to AMP. Of the rest, 13 (35%) registered resistance to Te, and 10 (27%) displayed resistance to C (Figure 6).

The bacterial isolates from Masinga Dam registered resistance to at least one antibiotic, *Pleisiomonas shigelloides* registered resistance to AML and AMP, while *E. coli*, to AML, AMP, and TE. Like the isolates from the Sagana Pond, the antibacterial response of isolates from the Masinga Dam was more resistant to AML and AMP than the rest of the antibiotics. However, for bacterial isolates from the Masinga, there was no significant difference in an antibacterial response for bacterial isolates from the dam (F=1.84, P=0.14) (Table 10).

Discussion

Members of Enterobacteriaceae are part of the gut flora found in the intestines of humans and other animals. In contrast, others are found in soil, water, or parasites on various animals and plants. Although in this study, there is no exception for fish, most of the bacteria isolates were from the family Enterobacteriaceae; *C. freundii*, *E. sakazakii*, *E. cloacae*, *E. tarda*, *Enterobacter amnigenus*, *Enterobacter agglomerans*, *E. coli*, *Klebsiella ornithinolytica*, *Klebsiella pneumoniae*, *P. mirabilis*, *P. stuartii*, *Shigella boydii*, *Salmonella* spp., and *P. shigelloides*. That aligns with the findings of Ogbondeminu and Olayemi (1993), who reported that 50% of fish and water of the microorganisms recovered from an earthen pond fertilized with animal fecal waste were members of the family Enterobacteriaceae.

The other bacteria isolated include; *A. sobia*, *Chromobacterium violaceum*, *P. aeruginosa*, *P. fluorescens*, *Vibrio mechnikovii*, and *Acinetobacter* spp.. They are widely distributed in the marine environment and the soil and have been implicated in causing human diseases as opportunistic pathogens. In other studies by Nganou et al. (2011), these bacteria have been isolates from tilapia fish, which isolate *Aeromonas* spp., *Vibrio* spp., *Pleisomonas* spp., *Acinetobacter* spp., Enterobacteriaceae, *Pseudomonas* spp., collected from four lakes in Cameroon. Other studies (Naim et al. 2012) recovered *A. hydrophila*, *Edwardsiella* spp., *Streptococcus* spp., *S. putrefaciens*, *Staphylococcus* sp., and *Vibrio* spp. In addition, *Aeromonas* spp., *C. freundii*, *C. violaceum*, *E. coli*, and *P. shigelloides* were isolates in the gastrointestinal regions of semi-intensively cultured Tilapia, *Oreochromis niloticus* (Linnaeus, 1758). Although these bacteria are not often associated with fish or enteric diseases in human beings, the health implications should not be ignored on introducing these organisms into natural water via the fish feces in aquaculture wastewaters (Naim et al. 2012).

During the two seasons, more bacterial species isolates from the ponds than from the dams. Furthermore, there was a correlation between the sites where the bacteria were isolates, which could be attributed that the ponds being fertilized with animal manure to enhance alga bloom and the accumulation of fish feces and leftover feed in the earthen pond (Davis and Goulder 1993; Makosora and Jazek 1994). In addition, according to Wemedo (2002), higher temperatures inhibit micro-bacterial activity, which supports more bacteria isolates during the dry season compared to the wet season. Another reason for this phenomenon is that soil saturation by rain limits activity by reducing aeration (Marshall and Devinny 1998).

Table 9. Antimicrobial response of isolates from Sagana, Kenya

| Isolates | No | AML | | | CTX | | | CIP | | | S | | | AMP | | | GEN | | | TE | | | C | | | NA | | | CXM | | |
|----------------------------------|-----|-----|----|---|-----|----|---|-----|-----|---|---|----|----|-----|----|---|-----|----|---|----|----|----|----|----|----|----|----|---|-----|----|---|
| | | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I | | | |
| <i>Citrobacter freundii</i> | 10 | 9 | 1 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 5 | 5 | 9 | 1 | 0 | 0 | 10 | 0 | 2 | 4 | 4 | 3 | 4 | 3 | 0 | 10 | 0 | 0 | 10 | 0 |
| <i>Aeromonas sobia</i> | 4 | 4 | 3 | 0 | 0 | 7 | 0 | 0 | 7 | 0 | 0 | 6 | 1 | 6 | 1 | 0 | 0 | 7 | 0 | 0 | 7 | 0 | 0 | 7 | 0 | 0 | 7 | 0 | 0 | 7 | 0 |
| <i>Vibrio mechnikovii</i> | 2 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 1 | 1 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 1 | 1 | 0 | 0 | 2 | 0 | 0 | 2 | 0 |
| <i>Salmonella</i> spp. | 4 | 1 | 1 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 2 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 |
| <i>Edwardsiella tarda</i> | 4 | 0 | 1 | 1 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 1 | 1 | 0 | 2 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 2 | 0 | 0 | 2 | 0 |
| <i>Shigella boydii</i> | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| <i>Escherichia coli</i> | 6 | 0 | 5 | 1 | 0 | 6 | 0 | 0 | 6 | 0 | 0 | 6 | 0 | 0 | 4 | 1 | 0 | 6 | 0 | 3 | 2 | 1 | 2 | 2 | 2 | 0 | 4 | 2 | 0 | 6 | 0 |
| <i>Enterobacter amnigenus</i> | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 |
| <i>Enterobacter fergusonii</i> | 2 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| <i>Acinetobacter</i> spp. | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| <i>Pseudomonas aeruginosa</i> | 7 | 7 | 0 | 0 | 0 | 6 | 1 | 0 | 7 | 0 | 1 | 4 | 2 | 5 | 1 | 1 | 0 | 6 | 1 | 3 | 0 | 4 | 5 | 0 | 2 | 4 | 3 | 0 | 3 | 4 | 0 |
| <i>Klebsiella onithnolytica</i> | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| <i>Chromobacterium violaceum</i> | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| <i>Proteus mirabilis</i> | 1 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 1 | 0 | 1 | 0 | 2 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 2 | 0 | 0 | 2 | 0 |
| <i>Pseudomonas fluorescences</i> | 2 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 1 | 2 | 0 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 1 | 1 | 0 | 2 | 0 | 1 | 1 |
| <i>Klebsiella pneumonia</i> | 2 | 2 | 2 | 0 | 0 | 4 | 0 | 0 | 4 | 0 | 1 | 3 | 0 | 2 | 2 | 0 | 0 | 4 | 0 | 2 | 1 | 1 | 1 | 3 | 0 | 0 | 4 | 0 | 0 | 4 | 0 |
| <i>Providencia stuartii</i> | 5 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 1 | 2 | 0 | 1 | 2 | 0 | 0 | 3 | 0 | 0 | 3 | 0 |
| Total | 54 | 34 | 15 | 2 | 0 | 52 | 2 | 0 | 54 | 0 | 2 | 40 | 11 | 34 | 16 | 4 | 0 | 51 | 2 | 14 | 26 | 14 | 16 | 26 | 9 | 6 | 44 | 5 | 3 | 48 | 2 |
| % | 100 | 63 | 28 | 4 | 0 | 97 | 4 | 0 | 100 | 0 | 4 | 74 | 20 | 63 | 30 | 7 | 0 | 94 | 4 | 26 | 48 | 26 | 30 | 48 | 17 | 11 | 82 | 9 | 6 | 89 | 4 |

Note: F=8.4, df=127, P-value=0.000

Table 10. Antibacterial response of isolates from Masinga Dam, Kenya

| Isolates | No | AML | | | CTX | | | CIP | | | S | | | AMP | | | GEN | | | TE | | | C | | | NA | | | CXM | | |
|---------------------------------|-----|-----|----|---|-----|----|----|-----|----|----|---|----|----|-----|----|----|-----|----|----|----|----|----|----|----|----|----|----|----|-----|-----|---|
| | | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I | R | S | I | | | |
| <i>Citrobacter freundii</i> | 6 | 5 | 1 | 0 | 0 | 6 | 0 | 0 | 6 | 0 | 0 | 3 | 3 | 5 | 1 | 0 | 0 | 6 | 0 | 1 | 3 | 2 | 3 | 3 | 0 | 0 | 6 | 0 | 0 | 6 | 0 |
| <i>Aeromonas sobia</i> | 2 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 1 | 1 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 |
| <i>Vibrio vulnificus</i> | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| <i>Edwardsiella tarda</i> | 2 | 0 | 1 | 1 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 0 |
| <i>Escherichia coli</i> | 8 | 2 | 6 | 0 | 0 | 6 | 2 | 0 | 5 | 3 | 0 | 6 | 2 | 2 | 3 | 3 | 0 | 6 | 2 | 2 | 4 | 2 | 0 | 4 | 4 | 0 | 4 | 4 | 0 | 8 | 0 |
| <i>Enterobacter sakazakazii</i> | 6 | 3 | 3 | 0 | 0 | 6 | 0 | 0 | 3 | 3 | 0 | 6 | 0 | 2 | 4 | 0 | 0 | 3 | 3 | 2 | 3 | 1 | 1 | 4 | 1 | 0 | 4 | 2 | 0 | 4 | 2 |
| <i>Enterobacter cloace</i> | 3 | 1 | 2 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 2 | 1 | 0 | 0 | 3 | 0 | 1 | 2 | 0 | 1 | 2 | 0 | 0 | 3 | 0 | 0 | 3 | 0 |
| <i>Pseudomonas fluorescens</i> | 2 | 2 | 0 | 0 | 0 | 2 | 1 | 0 | 2 | 0 | 0 | 2 | 0 | 3 | 0 | 1 | 0 | 6 | 1 | 3 | 0 | 4 | 4 | 1 | 2 | 0 | 3 | 4 | 0 | 4 | 0 |
| <i>Proteus mirabilis</i> | 2 | 2 | 2 | 0 | 0 | 4 | 0 | 0 | 4 | 0 | 0 | 4 | 4 | 0 | 0 | 0 | 0 | 4 | 0 | 2 | 2 | 1 | 1 | 3 | 0 | 0 | 4 | 0 | 0 | 4 | 0 |
| <i>Enterobacter agglomerans</i> | 2 | 2 | 0 | 0 | 0 | 1 | 1 | 0 | 2 | 0 | 0 | 1 | 1 | 2 | 0 | 0 | 0 | 1 | 1 | 0 | 2 | 0 | 0 | 1 | 1 | 0 | 2 | 0 | 0 | 1 | 1 |
| <i>Plesiomonas shigelloides</i> | 2 | 2 | 1 | 0 | 0 | 2 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 2 | 0 | 0 | 0 | 1 | 1 | 0 | 2 | 0 | 0 | 1 | 1 | 0 | 2 | 0 | 0 | 2 | 0 |
| <i>Pseudomonas aureginosa</i> | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| Total | 37 | 22 | 16 | 1 | 0 | 35 | 5 | 0 | 31 | 8 | 0 | 32 | 11 | 20 | 13 | 4 | 0 | 35 | 9 | 13 | 22 | 10 | 10 | 25 | 9 | 0 | 34 | 10 | 0 | 38 | 3 |
| % | 100 | 59 | 43 | 3 | 0 | 95 | 14 | 0 | 84 | 22 | 0 | 86 | 30 | 54 | 35 | 11 | 0 | 95 | 24 | 35 | 59 | 27 | 27 | 68 | 24 | 0 | 92 | 27 | 0 | 103 | 8 |

This study also revealed that all the specimens sampled sediment had the lowest number of bacteria isolates; this aligns with an earlier study by Niemi and Taipalinen (1982), who reported a lack of sunlight could play an important role in the bacteria growth that could be attributed to a very low count in sediment (Ferguson et al. 1996). In this study, there were more bacteria in all specimens during the dry season; this could be attributed to bacteria multiplying more in high temperatures. (Chowdhury et al. 2000) observed similar results in Tilapia's intestinal Tilapia bacterial load. Research by Sugita et al. (1985) and Markosova and Jezek (1994) reported that during summer months, populations of indicator bacteria increased with increasing water temperature as the temperature became favorable for the growth of bacteria.

The findings from the dams and the ponds revealed that different members of Enterobacteriaceae were isolates, some of which are pathogenic and others non-pathogenic. In other studies, there are reports of isolation of different members of Enterobacteriaceae as potential fish and human pathogens from naturally manured carps, Tilapia, eel, striped bass, and its earthen culture environment (Nair and Nair 1988; Nedoluha and Westhoff 1997; Muratori et al. 2000). This pathogenic Enterobacteriaceae was more in the Sagana Ponds than in the Masinga Dam, which could be due to the animal manure in the ponds (Ogbondeinu and Olayemi 1993). In the present study, *Salmonella* members of Enterobacteriaceae and *Shigella* have recovered from ponds at Sagana fish ponds, at fish, water, and water sediments, where they use integrated fish culture systems. That indication was observed in naturally manured earthen ponds by Nedoluha and Westhoff (1997) of an inherent risk of contamination by pathogens in the environment. The presence of *Salmonella* spp. indicates fecal contamination of water from which the fishes were harvested.

Aeromonas sobria was one of the bacteria isolates from both sources during the two seasons. It is a well-known human pathogen (Mateos et al. 1993; Thune et al. 1993, Austin and Adams 1996) and therefore poses a risk to consumers of fish-borne *Aeromonas* gastroenteritis if not properly cooked. Furthermore, the finding of *Vibrio* spp. during the dry season aligns with the studies conducted by (Al-Harbi and Uddin 2003), who found more bacterial counts during the summer than in winter.

The *P. aeruginosa*, a potential human pathogen that can persist even after processing, was isolates during the two seasons, posing a health hazard to consumers. Furthermore, Lyhs et al. (1998) reported that *Pseudomonas* was the organism important in food spoilage, with economic losses responsible for 15.3% of spoilage of preserved fish products. Therefore, the fish from both the Sagana Ponds and the Masinga Dam should be processed and stored properly to eliminate contamination.

The *E. coli* has been recognized traditionally as an indicator organism of fecal contamination of seafood and water (Geldreich 1997). The *E. coli* are inhabitants normally of the intestinal tracts of all warm-blooded animals. In this study, *E. coli* was recovered in all fish and water samples indicating poor sanitary condition and

hygiene in Sagana Ponds and Masinga Dams. Similar to many landing beaches in Kenya, the lack of proper sanitation facilities at the Masinga Dam landing sites could explain the presence of *E. coli* in all specimens. According to Chandraval et al. (2010), other studies found that fish and water samples were contaminated with fecal coliforms like *E. coli* from the Nadia District of West Bengal in India.

The *E. tarda* isolates from fish samples in both seasons are considered a serious problem in subtropical or tropical areas. Infections associated with this species include wound infections, gastroenteritis, and systemic diseases such as meningitis, cholecystitis, septicemia, and osteomyelitis (Janda and Abbott 1993). In addition, *E. tarda* has been isolates in fish from retail markets and freshwater aquaculture environments in India (Pankajkumar 2009).

The *P. mirabilis* and *C. freundii* were isolates in the Masinga Dam samples and have also been isolates in other studies (Niemi and Taipalinen 1982; Apun et al. 1999).

The *C. violaceum* is a Gram-negative rod isolated from soil and water in subtropical and tropical regions, while in this study, it was isolates in Sagana Ponds only. Even though infections caused by *C. violaceum* are rare among mammals, Apun et al. (1999) reported two cases of human infection caused by both pigmented and non-pigmented strains of *C. violaceum*. The *P. shigelloides* is a common pathogen in tropical regions associated with occasional opportunistic human infections and diarrhea. This study was isolates from the Masinga Dam during the dry season.

The bacterial isolates were highly sensitive to ciprofloxacin (100%) and gentamycin (98.9%), which aligns with the findings of (Jawahar 2011), whose findings were similar to human bacterial pathogens highly sensitive to ciprofloxacin (91%), chloramphenicol (88%) and gentamycin (85%). The relatively high resistance to ampicillin of 61.5% to most of the isolates is in partial follows with the findings by (Barat et al. 2002), who found the resistance of gram-negative bacteria isolates from fish to ampicillin prevalence of 93.4%, also Newaj-fyzul et al. (2006) findings of predominance resistance to ampicillin of 90.2%. That could be due to the limited use of antibiotics in aquaculture in Kenya. The finding of 31.8% of isolates resistant to tetracycline is comparable with 47% reported by Castro-Escarpulli et al. (2003) for isolates recovered from Tilapia (*O. niloticus*) in Mexico intended for human consumption.

Cow dung manure serves as a potential carrier of pathogenic bacteria as a result of contact with the manure, which is capable of transmitting zoonotic diseases to humans; when this untreated manure is used to fertilize fish ponds, it may serve as a potential source of food-borne infections for the fish consumers and lead to an increase in bacterial infections in the fish. However, resistance to the anti-microbial agents may be because of the widespread, indiscriminate, and lengthy use of chloramphenicol, tetracycline, and gentamicin in cow infection treatments (Omojowo and Omojasola 2013). Sagana fish ponds are fertilized with cow dung manure, which could explain why bacterial isolates from the Masanga dams showed resistance to more antibiotics. Another study concluded

that integrated fish farming favors antimicrobial-resistant bacteria in the pond environment by Andreas et al. (2002). Another study by Anja et al. (2000) found that high levels of individual and multiple anti-microbial resistances within the collected *Flavobacteria* and *Aeromonads* were demonstrated, which indicated a substantial impact on several groups of bacteria associated with aquaculture environments of fish farming.

In conclusions, (i) Fish from both the Masinga Dam and Sagana Ponds dam harbor bacteria and pathogenic bacteria, which naturally inhabit the animal gut flora. In Sagana, *Salmonella*, *S. boydii*, and *E. coli* were isolates; in Masinga Dam, the isolates were *P. shigelloides* and *E. coli*. (ii) No significant difference of bacteria flora species isolated in the two seasons or the sites. Some bacteria isolates in both seasons were *E. coli*, *S. boydii*, *C. freundii*, *Salmonella spp*, and others. The study shows that bacteria species found in the gut and on fish skin are similar to the bacteria found in cultured fish environments. (iii) The study showed that there was relatively high antibiotic resistance in the isolates to ampicillin of 61.5% and sensitivity to ciprofloxacin (100%) and gentamycin (98.9%). (iv) There was various antibacterial response of isolates from Sagana Ponds but not significantly different in the Masinga Dam.

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Mangrove conservation efforts with the ecotourism development in the Cengkong Mangrove Ecotourism, Trenggalek District, East Java, Indonesia

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Abstract. Kurniawati B, Sulistyningrum N, Nugroho GD, Sunarto, Kusumaningrum L, Rahawarin YY, Flores AB, Yap CK, Setyawan AD. 2022. Mangrove conservation efforts with the ecotourism development in the Cengkong Mangrove Ecotourism, Trenggalek District, East Java, Indonesia. *Intl J Bonorowo Wetlands* 12: 75-81. For mangrove conservation efforts, it is necessary to develop ecotourism so that people can also take advantage of the existence of mangrove forests. Analysis of the role mangrove ecosystem is carried out to develop a mangrove conservation strategy and the concept of sustainable development. This research examined conservation and ecotourism efforts by the community around the Cengkong Mangrove Ecotourism and social perceptions of ecotourism based on mangrove development. The research was conducted in June 2022 at Mangrove Cengkong Ecotourism, Karanggandu Village, Watulimo Sub-district, Trenggalek District, East Java, Indonesia, by conducting interviews with 50 respondents. Data was collected through field observations and interviews with ecotourism administrators and residents around the location. The results of this study indicate that the conservation efforts carried out are protecting, conserving, and utilizing mangroves by seeding, planting, managing, and monitoring mangroves. The POKMASWAS or the Community Monitoring Group manages the Cengkong Mangrove Ecotourism area. However, the utilization of mangrove forest resources that have economic value by the community around the Cengkong Mangrove Ecotourism area is still low because it is only limited to the cultivation of crabs, shells, making mangrove syrup, cakes and chips. In addition, the place is quite crowded with tourists. Indeed, area development still lacks preservation, utilization, and conservation for the management, surrounding communities, and tourists. Limited facilities and infrastructure are a challenge for the sustainable development of mangrove ecotourism. In addition, increasing community involvement and collaboration with institutions and the government in mangrove management must also be improved so that all the surrounding communities can also benefit. With the increasing economic benefits of mangrove ecotourism management, the community around the mangrove forest will participate in maintaining and preserving the mangrove forest ecosystem.

Keywords: Cengkong, community, conservation, development, ecotourism, mangrove

INTRODUCTION

Mangrove forests are typically found along the coast, or river estuaries affected by tides, located in tropical and subtropical biosphere areas, and have economic and social benefits (Kainuma et al. 2013; Junk et al. 2014). In addition, mangroves have a high productivity role compared to other ecosystems, thus making mangrove ecosystems necessary for the life of living things (Li et al. 2015). A mangrove forest is a tropical coastal vegetation community dominated by several types of mangrove trees that can grow and develop from the influence of seawater (Rahmila and Halim 2018). Therefore, only a few species of plants can usually adapt to the conditions.

The natural resources found in mangrove forests are very abundant. Mangrove forests provide various environmental functions, such as providing surfaces and

shelters for land and water animals and general improvement of coastal ecosystems (Hakim et al. 2017). Mangrove forests also provide environmental services in carbon storage that positively impact the environment and humans (Pricillia et al. 2018). Most of the mangrove forests are found in developing countries, which have recently experienced several problems that can eventually lead to the extinction of the mangrove ecosystem (Purwaningrum 2020). Sustainable management of mangrove forests will guarantee many benefits for the environment and surrounding communities. Forest management and protection challenges in Indonesia often come from forest communities (Setiawan et al. 2017).

The low utilization of mangroves can be a threat as Qurniati et al. (2017) revealed that the community around the forest has limited capital, so it can reduce conservation efforts by the community. Therefore, mangrove

conservation efforts must be balanced with sustainable use by developing ecotourism that focuses on mangroves. Ecotourism activities in mangrove areas, in principle, are the use of mangrove areas while maintaining the biological/ecological functions of mangrove areas that have social and economic value for the local community (Irwansyah et al. 2021). Mangrove forest has the potential to be used for ecotourism because it is a very good place even though it is on the beach (Rahmila and Halim 2018). Mangrove forests that are used for ecotourism will increase conservation and reduce things that damage mangrove areas, such as (i) felling of mangrove trees, (ii) conversion into fish and shrimp ponds, clearing land for settlements or agricultural areas, and (iii) final disposal or toxic waste. The use of mangrove forests for the concept of tourism (ecotourism) is in line with the shift in tourist interest from old tourism, namely tourists who only come to do tours without any education and conservation elements, to new tourism, namely tourists who come to do tours in which there are elements of education and conservation (Umam et al. 2015).

The Cengkong mangrove area is one of the ecotourism located in Karanggandu Village, Watulimo Sub-district, Trenggalek District, East Java, Indonesia. As an ecotourism area, the Cengkong mangrove area has an important role, not only in the natural balance of the coastal area but also in the economy of the people living around the mangrove area (Faizal et al. 2017). Ecotourism is one of the uses of mangrove forests from an economic point of view for the welfare of the community by paying attention to environmental factors and preserving the surrounding nature. This can effectively overcome environmental problems in the mangrove forest ecosystem, such as the

level of overexploitation by the community, by creating an economical alternative for the community.

The Cengkong Mangrove Ecotourism is located approximately 500 meters from the coast of the Cengkong area and faces east towards the waters of Prigi Bay which goes directly to the ocean off the Indian Ocean. The Cengkong mangrove area, in developing its territory as an ecotourism place, still has various shortcomings. The surrounding community's participation is considered minimal in managing, conserving, and utilizing mangrove areas. Therefore, This research examined conservation and ecotourism efforts by the community around the Cengkong Mangrove Ecotourism and social perceptions of ecotourism based on mangrove development.

MATERIALS AND METHODS

Study area

The study was conducted in June 2022 at the Cengkong Mangrove Ecotourism located in Karanggandu Village, Watulimo Sub-district, Trenggalek District, East Java, Indonesia ($8^{\circ}29'778''-111^{\circ}70'2692''$) (Figure 1). It is located on the Kalisongo River and empties directly into the Cengkong beach and faces east towards the waters of Prigi Bay which directly leads to the ocean off the Indian Ocean. Some of the panoramas and ecotourism activities at Cengkong Mangrove Ecotourism can be seen in Figure 2. This location is chosen because the Cengkong Mangrove Ecotourism is always crowded with tourists, especially on weekends. However, in terms of its development, it still lacks preservation, utilization, and conservation for the management, surrounding communities, and tourists.

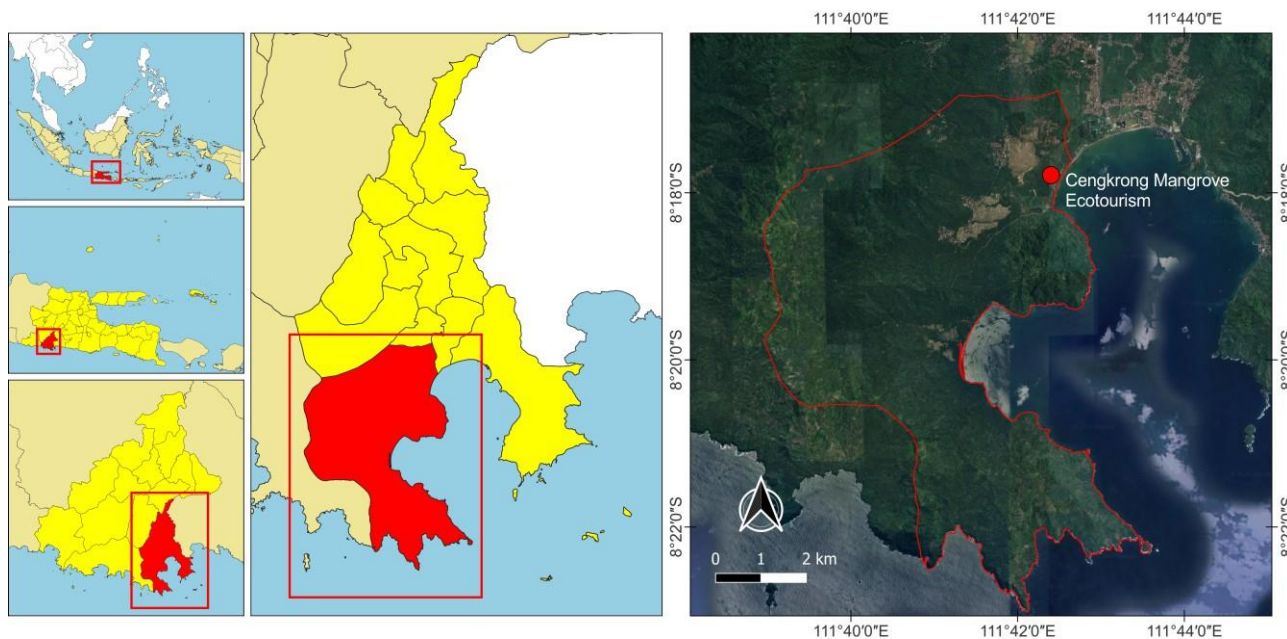


Figure 1. Map of the research location in Cengkong Mangrove Ecotourism, Karanggandu Village, Watulimo Sub-district, Trenggalek District, East Java, Indonesia

Data collection and analysis

Data was collected using field observations, structured and in-depth interviews, and literature studies. Observations and in-depth interviews were used to collect data on community conservation efforts. Interviews using questionnaires were conducted to determine the perception of the people of Karanggandu Village regarding the development of ecotourism in the Cengkong Mangrove Forest. The literature study was used to collect data about the general description of Karanggandu Village and the mangrove forests in the Cengkong Mangrove Ecotourism. The snowball sampling technique determines six key aspects of the sample in conservation efforts (Setiawan et al. 2017). Sampling with the snowball sampling technique is a non-probability sampling technique that starts with a small population of known individuals and then expands the range of the sample by asking the initial participants to identify other people who should participate in the study. Respondents for ecotourism development are 50 respondents who live around the Cengkong Mangrove Ecotourism. Respondent profiles can be seen in Table 1. Conservation efforts were then analyzed descriptively. Public perception of ecotourism development is carried out using a screening method to identify the level of community perception in the development of mangrove-based ecotourism. The conservation efforts were analyzed descriptively based on Indonesian Government Regulation No. 5 of 1990 concerning Conservation Efforts. The public perception of ecotourism development is categorized by the scoring method to determine the level of public perception of the development of mangrove-based ecotourism.

Table 1. Respondent profile (n = 50)

| Profile | Category | Number | Percentage (%) |
|------------|----------------------------|--------|----------------|
| Gender | Male | 21 | 42 |
| | Female | 29 | 58 |
| | Amount | 50 | 100 |
| Age | 20-30 | 5 | 10 |
| | 31-40 | 11 | 22 |
| | 41-50 | 16 | 32 |
| | 51-60 | 14 | 28 |
| | 61-70 | 4 | 8 |
| | Amount | 50 | 100 |
| Education | Elementary School or below | 32 | 64 |
| | Junior High School | 10 | 20 |
| | Senior High School | 8 | 16 |
| | Amount | 50 | 100 |
| Occupation | Trader | 37 | 72 |
| | Housewife | 3 | 6 |
| | Fisherman | 6 | 12 |
| | POKMASWAS staff | 4 | 8 |
| | Amount | 50 | 100 |

RESULTS AND DISCUSSION

Based on the Government Regulation of the Republic of Indonesia No. 5 of 1990 and research by Setiawan et al. (2017), conservation efforts manage and utilize natural resources wisely to ensure their current and future existence. It includes three main activities: protection, preservation, and sustainable utilization of natural resources.

Protecting mangrove forests in Cengkong Mangrove Ecotourism

One of the efforts to protect mangroves is carried out by the people of Karanggandu Village, namely prohibiting residents from entering the mangrove core area, cutting down mangrove trees, and littering with garbage and waste. The protection of mangrove forests in the Cengkong Mangrove Forest is carried out by POKMASWAS, also known as the Community Monitoring Group. The Community Monitoring Group comprises the Karanggandu Village community around the Cengkong Mangrove Forest. POKMASWAS is in charge of the caretaker of the Cengkong Mangrove Forest and charge of the protection and management of the area. The POKMASWAS consists of about 19 people. Reports regarding damage to the mangrove area must go through the POKMASWAS management, and then they will be forwarded to the PSDKP of Trenggalek District. The *Pengawasan Sumber Daya Kelautan dan Perikanan* (PSDKP), or Supervision of Marine and Fishery Resources, is one of the priority programs/activities of the Ministry of Maritime Affairs and Fisheries as one of the steps to support the realization of sustainable management of marine and fisheries resources for the welfare of the community. Sidik et al. (2018) said that the mangrove forest ecosystem benefits the ecosystem and society. Therefore, the community around the area must not damage the existing mangroves and must follow the existing rules so that mangrove conservation activities run properly.

Preservation of mangrove forest resources in Cengkong Mangrove Ecotourism

At first, around 1998, the Cengkong mangrove area was damaged. Then in 2006-2007, the Department of Fisheries and Marine Affairs of Trenggalek District took the initiative to hold a restoration or conservation program in the tidal sea area by replanting damaged mangrove plants to be returned to their previous condition. The area of the Cengkong mangrove forest is about 165 hectares. In the past, the damage that occurred was mostly done by the surrounding community to meet their daily needs. In the past, communities around mangrove forests cut down mangrove trees to be traded or used for community animal feed. In addition, the community's buffaloes also entered the mangrove forest area and consumed the mangrove plants which caused the decline of the mangrove forest in size. Then with the mangrove conservation movement, management, and supervision by POKMASWAS and related agencies, the surrounding community has never again cut down the mangrove trees there.

The growth of mangrove plants that tend to take a long time has made the conservation program run for quite a long time until now. Mangrove plants every year can only grow about 30-40 cm. According to Alwidakdo et al. (2014), several environmental factors affect the growth of mangroves in a location, including coastal physiography (topography), tides (length, duration, range), waves and currents, climate (light, rainfall, temperature, wind), salinity, oxygen dissolved, soil, and nutrients. The Department of Fisheries and Marine Affairs of Trenggalek District provided free lessons and seeds to residents who wanted to participate in conservation activities at that time. The conservation program has been running until now along with its protection and preservation efforts. Communities that previously participated in mangrove planting in 2006-2007 became members of the POKMASWAS management. However, not all POKMASWAS members are actively participating at this time.

Currently, the Cengkong mangrove forest area is also developing nurseries and planting mangroves. The price of seeds offered is 35 million for 1000 seeds. Cengkong Mangrove Forest is currently also able to supply mangrove plant seeds to other areas in East Java. Usually, the order for mangrove seeds is made 1 week before planting activities are carried out. In the Cengkong Mangrove Forest, an event is sometimes held regarding planting mangrove plants that all levels of society can attend. In addition to planting, cleaning activities are also carried out around the mangrove forest area. According to Setiawan et al. (2017), one of the factors driving the willingness to plant and develop mangrove nurseries is the economic factor.

Utilization of mangrove forests in Cengrong Mangrove Ecotourism

The utilization of mangrove forest resources by the community around the Cengkong Mangrove Ecotourism area is limited to cultivating crabs, shellfish, mangrove syrup, cakes, and chips. The manager carries out crab and shellfish cultivation activities as one activity carried out to increase the economy and income in the Cengkong mangrove ecotourism area. The results from cultivating mangrove crabs and shellfish can be sold to visitors as souvenirs. Currently, the local community can no longer produce mangrove syrup, cakes and chips because there are rarely interested buyers. One example of successfully utilizing mangrove fruit from the *Avicenna marina* species to make cakes is in the Baros area, Bantul, Yogyakarta, Indonesia (Ikawati and Primasari 2019). Meanwhile, the cultivation of crabs and shellfish is the thing that people are most interested in. Crab and shellfish cultivated can be made into dishes to be eaten by visitors at tourist attractions or used as souvenirs. In addition to cultivation, the community often carries out activities for catching crabs and shellfish in mangrove areas. People can hunt for crabs or shellfish if they do not damage the mangrove forest area

or take it excessively. The price of mud crab (*Scylla* spp.) can range from IDR 50,000 to IDR 158,000 per kg.

The surrounding community also uses mangrove plants as medicine for itching, ulcers, and healing wounds. The parts of the mangrove plant used as medicine by the community are young roots, bark, leaves, and fruit. According to Das et al. (2016) and Nugroho et al. (2020), mangrove species do have chemical compounds that have the potential to become plants that cure various diseases experienced by humans.

Not all communities in Karanggandu Village participate in the management of the Cengkong Mangrove ecotourism area. Only people who are members of the Community Monitoring Group (POKMASWAS) participate in the management and supervision of the Cengkong mangrove area. Ecotourism development in the Cengkong Mangrove Forest is still not going well. Many facilities are inadequate, the tenants of the sellers are not well organized, and the cleanliness of the area is still very lacking. Damaged facilities also make it difficult and endanger the safety of tourists who want to visit. Lack of maintenance is one of the factors that damage the available facilities. As has been explained, Cengkong Mangrove Ecotourism is still developing, so in various aspects, it is still not optimal (Figure 2).

Community perception of ecotourism development

Tourism is the activity of traveling to a place to relax. Tourism or vacation can also refer to a vacation activity and visits to places of interest and want to visit. Ecotourism is a special tourism category concerned with nature conservation and maintaining the well-being of local people (Bandara and Vlosky 2016). Tourism activities that are governed by a conservation strategy are called ecotourism. Suppose conservation is an effort to maintain the continuity of using natural resources for the present and the future. In that case, ecotourism is the management of nature and community culture that prioritizes sustainability and profit (Mustaqim 2018). Almost 100% of the people of Karanggandu do not understand the concept of ecotourism. They think that ecotourism is the same as tourism in general. Community members who understand the concept of ecotourism are people who participate in ecotourism management, know about ecotourism from extension programs and are active in community group activities. This finding is in line with Setiawan et al. (2017), who found that the people of Margasari consider ecotourism to be ordinary tourism.

According to Setiawan et al. (2017), community views regarding ecotourism development are divided into several aspects, which include aspects of community knowledge about ecotourism; community willingness to participate in ecotourism development; government intervention in ecotourism development; the economic benefits felt by the community by the existence of ecotourism; damage to ecotourism caused by visitors; and sustainable development and ecotourism development in the Cengkong Mangrove Forest. These perceptions are listed in Table 2.



Figure 2. Several panoramas and ecotourism activities in Cengkong Mangrove Ecotourism, Trenggalek District, East Java, Indonesia

Table 2. Community perception of ecotourism development (n = 50)

| Behavior | Agree/ willing (%) | Not agree/ willing (%) |
|--|-----------------------|---------------------------|
| <i>Willingness to participate in ecotourism development</i> | | |
| Planning activities | 38 | 62 |
| Tour guide activities | 38 | 62 |
| Souvenir activities | 82 | 18 |
| Providing homestay | 36 | 64 |
| <i>Community perception of ecotourism group</i> | | |
| Manager | 42 | 54 |
| Businessmen | 80 | 20 |
| <i>Government intervention in Cengkong Mangrove Ecotourism</i> | | |
| Partial intervene | 10 | 90 |
| Full intervene | 90 | 10 |
| <i>Ecotourism impact on economic benefits</i> | | |
| Increase economic benefit for community | 72 | 28 |
| Economic benefit only for some participants | 28 | 72 |
| <i>Visitors effect the environment damage</i> | | |
| Garbage in forest area | 0 | 100 |
| Garbage in village | 0 | 100 |
| Damaging the mangrove trees | 0 | 100 |
| Damaging the ecotourism facilities | 0 | 100 |
| <i>Willingness to develop sustainable mangrove ecotourism</i> | 100 | 0 |

The first aspect is the willingness of the community to participate in the management of the Cengkong Mangrove Ecotourism. There are 4 sub-aspects: activity planners, activity tour guides, making souvenirs and providing homestays. Based on structured interviews with 50 respondents, the sub-aspects of activity planners and tour guides have the same percentage, which is 38% of the community willing or agreeing to plan and tour guide activities in the Cengkong Mangrove Ecotourism. The management of natural tourism objects is influenced by the service quality of officers (Hartati et al. 2021). The existence of planning and a tour guide will greatly help visitors and the community how important the mangrove forest is. Moreover, it is known that many visitors are still unfamiliar with mangroves and their benefits. Furthermore, the percentage of community participation in making souvenirs and souvenirs typical of the Cengkong Mangrove Ecotourism is 84%. The use of the Cengkong Mangrove Forest has been at the stage of making souvenirs or souvenirs made by the community. The typical souvenirs of Cengkong Mangrove Forest are mangrove fruit syrup, cake and chips. While the people who want to make lodging as much as 36%. Most people are still unsure about making lodging because the mangrove forest and Cengkong Beach, which are right in front of the mangrove forest, often have high tides, which causes residents' houses and stalls to be often submerged by the tide. For this reason, making lodging around the Cengkong Mangrove Ecotourism area is still too difficult to realize.

An ecotourism group is an institution established by villagers around the ecotourism area who have a concern and responsibility for managing tourism and the environment. The second aspect is people's perception of ecotourism groups. In Cengkong Mangrove Ecotourism area, there is already an ecotourism group responsible for

the management and protection of the Cengkong Mangrove forest area, commonly called POKMASWAS. The Community Monitoring Group or POKMASWAS consists of about 19 people. There are 2 sub-aspects consisting of the willingness of the community as the manager of the Cengkong Mangrove Ecotourism and the availability of the community to carry out ecotourism support activities as business actors. The availability of the community in carrying out mangrove management has a percentage of 42%. At the same time, the availability of the community to support ecotourism activities as business actors have a percentage of 80%. Most people have education between an elementary and high school which causes them to lack the confidence to participate as managers. So most people choose to support ecotourism as a business actor.

The third aspect is the public's perception of government intervention or intervention in managing the Cengkong Mangrove. Based on the data obtained, most people agree that the management of the Cengkong Mangrove Ecotourism requires a governmental intervention with percentage of 90%. The community recognizes that management with government intervention is more profitable than without government intervention. Mangrove management is more structured and controlled, making it easier for the community to carry out their activities as business actors. In addition, the management of the Cengkong Mangrove Ecotourism area is only carried out by POKMASWAS, whose members consist of several participating communities. However, there are still many members who are not active. So, community participation is considered still less than optimal. However, most people agree more with government intervention in its management so that all levels of society can feel the benefits. Idajati et al. (2016) also discussed the management of mangrove forest ecotourism in Wonorejo and explained that management was carried out with the principle of "Deleged Power" so that certain representative groups only carried out management. Collaboration is a key principle in community-based tourism approaches in most developing countries. Stone (2015) points out that participation in ecotourism brings mixed results on biodiversity conservation and community livelihoods due to the involvement of multiple stakeholders in the design, planning, and implementation of ecotourism projects.

The fourth aspect is the impact of ecotourism on the community's economy. The impact of ecotourism brings economic benefits to the surrounding community. There are 36 respondents with a percentage of 68% who agree that ecotourism activities can improve the economy for the whole community. For example, with the Cengkong Mangrove Forest, people from various regions can participate in trading around the ecotourism area. The fishermen can also sell the fish they catch in the mangrove ecotourism area. In addition, not far from there, there are several ponds owned by residents that contain crabs and shrimp that can be sold to visitors. These results align with the ecotourism goal that ecotourism develops community welfare, providing additional livelihoods and increasing family income (Faizal et al. 2017).

The development of mangrove ecotourism needs to consider the possibility of disturbance from visitors. For this reason, the fifth aspect is the environmental damage caused by visitors to the Cengkong Mangrove Ecotourism. There are 4 sub-aspects consisting of the presence of waste in the ecotourism area, the presence of garbage in the area, damage to mangrove trees, and damage to existing facilities in the Cengkong Mangrove Ecotourism area. Currently, disturbances caused by visitors, such as environmental damage to mangrove forest ecosystems, ecotourism facilities, or waste in ecotourism and village environments, are low. However, the garbage in the ecotourism area occurs due to the lack of trash cans, so many visitors throw garbage carelessly. This makes the aesthetic value or beauty of the Cengkong Mangrove Ecotourism decline. Visitors who throw garbage in the village or its surroundings are also not justified because it can cause inconvenience to the surrounding community. For damage to mangrove trees themselves, as long as they do not cause mangrove trees to die or decrease, the manager will impose no sanctions on visitors. Meanwhile, damage to facilities rarely occurs in the Cengkong Mangrove Ecotourism area. However, if this happens, there will be sanctions that must be carried out by visitors who have caused damage. These sub-aspects have the same result with a percentage of 100%. This means that both the community and the manager of the Cengkong Mangrove Ecotourism agree that visitors should not cause damage or waste in the Cengkong mangrove area. The loss of mangrove ecosystems means the loss of vast ecological niches for foraging, breeding, and hatching fish, marine creatures, and migratory species (Hakim et al. 2017). The loss of mangroves also contributes significantly to the negative impact on the coastal economy, where many coastal populations depend on marine resources and the fishing industry.

The desire of the community to manage mangrove ecotourism areas sustainably is very large. The utilization of mangrove areas to be developed into an ecotourism area is an alternative that is very rational to be applied in coastal areas because it can provide economic benefits and environmental services without exploiting mangroves. Ecotourism brings many benefits from economic, socio-cultural, and environmental aspects. The economic benefits felt by the community are that it can improve the economy by providing employment opportunities in the form of managers and business actors (Umam et al. 2015). Ecotourism has many advantages as a place for fun and nature tourism as well as study and development material that can be used for educational and cultural support activities (Mustaqim 2018). Socio-cultural aspects include cleaning activities for the Cengkong Mangrove Ecotourism area, which are routinely carried out once a week and preserving marine anchoring activities as a form of gratitude to the Almighty. Ecotourism is an activity that provides opportunities for tourists to gain experience in nature and culture to learn and understand how important the conservation of biodiversity and local culture is (Fahrian et al. 2015). While the ecological benefits are the existence of a mangrove forest that functions as a breakwater so that at high tide, the waves are not felt too

big; places where animals shelter and breed, conduct nurseries to expand mangrove forest areas, and much more (Li et al. 2016; Harahab and Setiawan 2017; Irwansyah et al. 2021; Wiraatmaja et al. 2022).

The conclusion of this study is that as one of the protection efforts, the POKMASWAS group as the manager has the policy to preserve the mangrove ecosystem. Mangrove restoration activities have been carried out since 2006 until now by POKMASWAS. Protection efforts in the form of a ban on entering the mangrove area are considered effective in preserving mangroves and reducing environmental damage. However, the prohibition has limited efforts used by the community. Institutional protection should provide greater space for communities to utilize forest resources (Hidayat 2017). Currently, the utilization of mangrove forest resources by the community around the Cengkong Mangrove Ecotourism area is very low, even though this place is quite a place visited by tourists. However, in terms of area development, it still lacks preservation, utilization, and conservation for the management and tourists. Limited facilities and infrastructure are a challenge for the sustainable development of mangrove ecotourism. In addition, increasing community involvement and collaboration with institutions and government in mangrove management must also be increased so that all communities around the area also benefit. With the increasing economic benefits of mangrove ecotourism management, the community around the mangrove forest will participate in maintaining and preserving the mangrove forest ecosystem.

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Sustainability level of mangrove forest management based on RAP-MForest approach in Pancer Cengkong, Trenggalek District, Indonesia

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Abstract. Sabrina AD, Ramadhandi AR, Nur AAI, Liza N, Sutarno, Yap CK, Indrawan M, Setyawan AD. 2022. Sustainability level of mangrove forest management based on RAP-MForest approach in Pancer Cengkong, Trenggalek District, Indonesia. *Intl J Bonorowo Wetlands 12*: 82-88. Mangrove forest area is a coastal resource that benefits the community's welfare. Protection and preservation of the mangrove ecosystem need to be carried out by taking into account various aspects to achieve sustainability. This study aims to analyze the level of sustainability of mangrove forest management and the contributing factors in the Pancer Cengkong Mangrove Forest area in Karanggandu Village, Trenggalek District, East Java, Indonesia. This research was conducted in June 2022 through field interviews and literature studies. Three dimensions (ecological, economic, and social) were selected, along with their attributes. Each attribute was scored to indicate the status of sustainability. Then, the data obtained were processed by Multi-Dimensional Scaling (MDS) analysis using the Rapid Appraisal for Mangrove Forest (RAP-MForest) method. Furthermore, leverage analysis determines the sensitive attributes in each dimension. The results depicted that management in Pancer Cengkong Mangrove Forest is less sustainable in the economic dimension and fairly sustainable in the two remaining dimensions. The social dimension achieved the highest sustainability index value at 72.92. Furthermore, knowledge of the sustainability index and sensitive factors can help provide recommendations for management in the Pancer Cengkong mangrove area to achieve a sustainable level. In this case, the economic dimension should be more concerned with promoting environmentally sustainable development.

Keywords: Mangrove management, MDS, Pancer Cengkong, RAP-MForest

INTRODUCTION

Mangroves are a distinctive community of tropical and subtropical coastal vegetation that grows and develops in tidal areas protected by mud or sandy mud substrates (Prihadi et al. 2018). In tropical and subtropical areas, mangrove forests grow in saline sediments in coastal and estuarine environments (Malik et al. 2017). Mangrove forest areas are known as coastal resources that play an important role in life. That is because the mangrove forest area has the potential to improve community welfare in 3 aspects, consisting economic, social, and environmental (Hamzah et al. 2020).

Mangrove forests have various benefits, including as a habitat for fish and crustaceans, effective sediment traps, and a role in nutrient recycling (Datta et al. 2012). Mangrove forests also play a role in protecting coastlines, supporting coastal fisheries, producing useful forest products, and as a place for a thriving ecotourism industry (Kusmana 2015). Another benefit that can be obtained from mangrove forests is that they can provide environmental services where mangrove forests can absorb more carbon than other plants on earth (Dinilhuda et al.

2018). That is useful in reducing the amount of carbon in the atmosphere, which shows the important role of mangrove areas in global warming mitigation (Senoaji and Hidayat 2016).

However, like other ecosystems, mangrove forests can also experience natural disturbances from nature and human anthropogenic activities (Alongi 2008). According to Abino et al. (2014), the mangrove area is one of the most threatened areas in the world. It can be experienced loss by a natural disaster, such as the tsunami that occurred in 1996 that affected more than 200 Ha mangrove degraded in Biak Island, Indonesia (Baigo et al. 2019). In addition, the location of the mangrove ecosystem, which is close to residential communities, makes the high level of utilization of mangrove forest products such as overfishing, extraction of wood for fuel, and other detrimental activities (Pattimahu et al. 2017). In Indonesia, it was reported that sand mining led to the decrease of carbon stock in the mangrove area (Slamet et al. 2020), and land use change to brackish water ponds and timber exploitation has been the main factor of the mangrove area degradation over the centuries in outside of Java Island (Ilman et al. 2016).

In managing mangrove forests, the economic, social, and ecological aspects must be maintained to create harmony and have no negative impact on other aspects. However, according to Mughofar et al. (2018), the community's knowledge about the Pancer Cengkong mangrove forest area regarding the function of the mangrove forest is still very limited. That is evidenced by the decreasing area and community of mangrove forests caused by the exploitation of mangrove forests for fuel and household furniture. In the Pancer Cengkong mangrove forest area, there are about 32 hectares in lightly damaged condition and about 5 hectares in heavily damaged condition (Dinas Kelautan Perikanan, 2016 in Paringsih et al. 2018). Therefore, this study aims to analyze the level of sustainability of mangrove forest management and the contributing factors in the Pancer Cengkong Mangrove Forest area in Karanggandu Village, Watulimo Sub-district, Trenggalek District, East Java, Indonesia. This location was chosen because of the limited information about the analysis of the sustainability of mangrove forest management in this area. In contrast, the area has a profound potential to improve community welfare and promote ecological function. This study supposes that the condition of the mangrove forest in Karanggandu Village can be maintained and can support the welfare of the people who live around it.

MATERIALS AND METHODS

Study area

The research was conducted in the Pancer Cengkong Mangrove Forest, situated in Karanggandu Village, Watulimo Sub-district, Trenggalek District, East Java, Indonesia (Figure 1), which is about 43 km from

Trenggalek capital city. The Karanggandu Village is geographically located at coordinates $8^{\circ}17'52''\text{S}$ $111^{\circ}42'25''\text{E}$. This research was done in June 2022.

Procedures

Data collection

The sustainability of Pancer Cengkong Mangrove Forest in Karanggandu Village was examined based on three dimensions, consisting of ecological, economical, and social factors. A combination of literature study and interviews will gain data regarding ecological factors. At the same time, the remaining dimensions were investigated through interviews with the targeted informants. During the interview, respondents were asked about 22 attributes, including eight ecological, six economical, and eight social (Table 1). In this study, the attributes of the sustainability of the mangrove forest ecosystem refer to the previous research conducted by Melo et al. (2020) and Yuliasamaya (2021). Each attribute in each dimension is given a score indicating the sustainability of that dimension. Scores are given on an ordinal scale ranging from 0-2, which are interpreted as good to bad or low to high, depending on the sustainability criteria of each dimension. A bad value indicates an unsustainable condition, while a good value indicates a sustainable condition could be achieved. The scoring system refers to the previous research by Melo et al. (2020).

A purposive sampling method was used to choose the respondents who deeply correlated with the mangrove forest (Yuliasamaya 2021). As a result, researchers interviewed 40 informants: mangrove area managers, fishermen, traders, inhabitants surrounding the mangrove forest, and the Karanggandu Village's authorities. The respondents were 20-80 years old, with 20 men and 20 women.

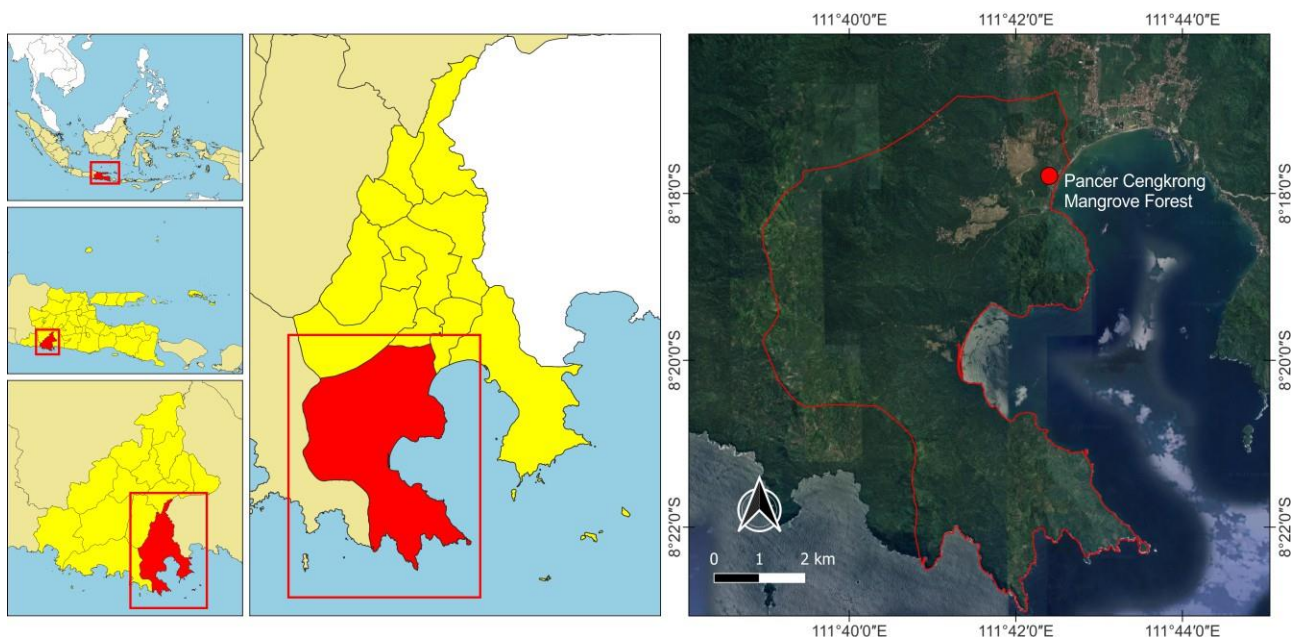


Figure 1. Research area in Pancer Cengkong Mangrove Forest in Karanggandu Village, Trenggalek District, East Java, Indonesia

Table 1. Dimensions and attributes of the sustainability value index of Pancer Cengkong Mangrove Forest management in Karanggandu Village, Watulimo Sub-district, Trenggalek District, East Java, Indonesia

| Ecological dimension | Economical dimension | Social dimension |
|-----------------------------------|-----------------------------------|---|
| Mangrove density | Income to minimum wage | Participation in mangrove management |
| Mangrove cover | Land use zoning | Public awareness of the importance of mangroves |
| Mangrove land pressure | Mangrove usage by people | Local wisdom |
| Mangrove diversity | Direct economic value of mangrove | Community knowledge about mangroves |
| Mangrove ecosystem rehabilitation | Stakeholder engagement | Community education level |
| Mangrove fauna diversity | Mangrove utilization inventory | Community access to mangrove forests |
| Salinity level | | Mangrove damage by the community |
| Coastline changes | | Conflict over mangrove use |

Table 2. Category of sustainability status of mangrove forest management (Sahputra et al. 2022)

| Index value (%) | Category |
|-----------------|--------------------|
| 0.00-25.00 | Unsustainable |
| 25.01-50.00 | Less sustainable |
| 50.01-75.00 | Fairly sustainable |
| 75.01-100.00 | Sustainable |

Table 3. The sustainability index from each dimension

| Dimension | MDS result | Category |
|-----------|------------|--------------------|
| Ecology | 56.50 | Fairly sustainable |
| Economy | 27.7 | Less sustainable |
| Social | 72.92 | Fairly sustainable |

Data analysis

The analysis used in this study was carried out by Multi-Dimensional Scaling (MDS) with the Rapid Appraisal for Mangrove Forest (RAP-MForest) method. This method enables us to see the sustainability level of the ecosystem by evaluating essential dimensions. Furthermore, it could show the important factors that play prominent roles in improving ecosystem conditions (Pitcher and Preikshot 2001).

After obtaining the scoring data, each was analyzed using Multi-Dimensional Scaling (MDS) to determine the relative position of mangrove forest management. The index scale for the sustainability of mangrove forest management ranges from 0-100% (Table 2). After that, the sustainability index value of each dimension is visualized in the form of a kite diagram, which is shaped as a triangle with symmetry determined by the index of each dimension. The angle with the greatest value indicates the most sensitive attribute. The last step is conducting the leverage analysis to determine the influencing aspects of each of the analyzed indicators (Melo et al. 2020).

needs more attention to achieve sustainability. From the ecological perspective, it shows two main sensitive attributes with the highest value, namely the diversity of mangrove fauna with a value of 4.08 and the pressure of mangrove land with a value of 3.75 (Figure 3). The sustainability index value of the ecological dimension was 56.50 (fairly sustainable) (Table 3). In the economic dimension, there is the highest sensitive attribute, namely the inventory of mangrove utilization valued at 9.42. Another important sensitive attribute is the community's use of mangroves by 5.84 (Figure 4). The sustainability index value of the economic dimension was 27.7 (less sustainable) (Table 3). Lastly, the main sensitive attribute in the social dimension is local wisdom, with a score of 8.14, and community knowledge about mangroves scored 6.19 (Figure 5). Finally, the sustainability index value of social culture was 72.92 (fairly sustainable) (Table 3). From the given results, more consideration needs to be given to those significant attributes from every dimension to achieve the ecosystem's sustainability.

RESULTS AND DISCUSSION

Results

This study managed to evaluate the sustainability condition of the Mangrove Forest in Karanggandu Village, from ecological, economical, and social dimensions. The assessment was done using Rapid Appraisal of Mangrove Forest (Rap-MForest) by examining 22 indicators from three dimensions. The study results in Figure 2 shows that the mangrove ecosystem management in Karanggandu Village tends to be more dominant in two aspects, namely the social and ecological aspects. Thus, the economic factor

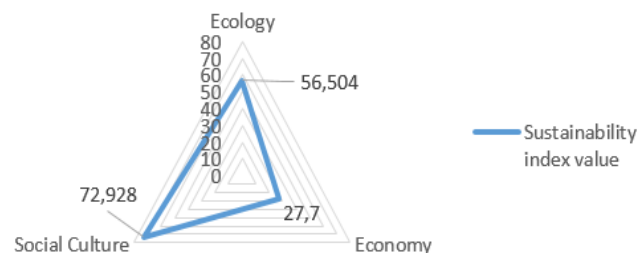


Figure 2. Kite diagram of mangrove management sustainability index values in Pancer Cengkong Mangrove Forest, Indonesia

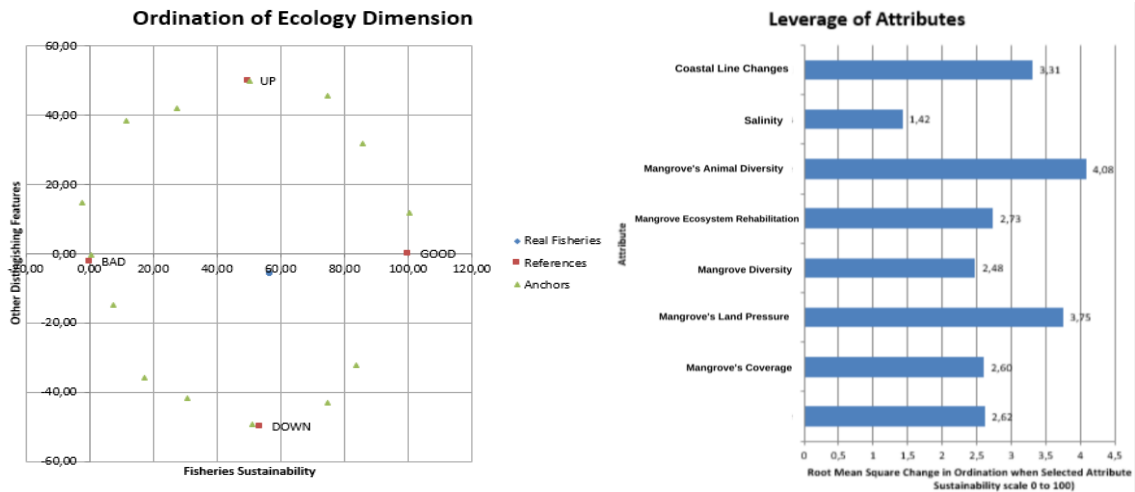


Figure 3. Sustainability index and attribute leverage in the ecological dimension

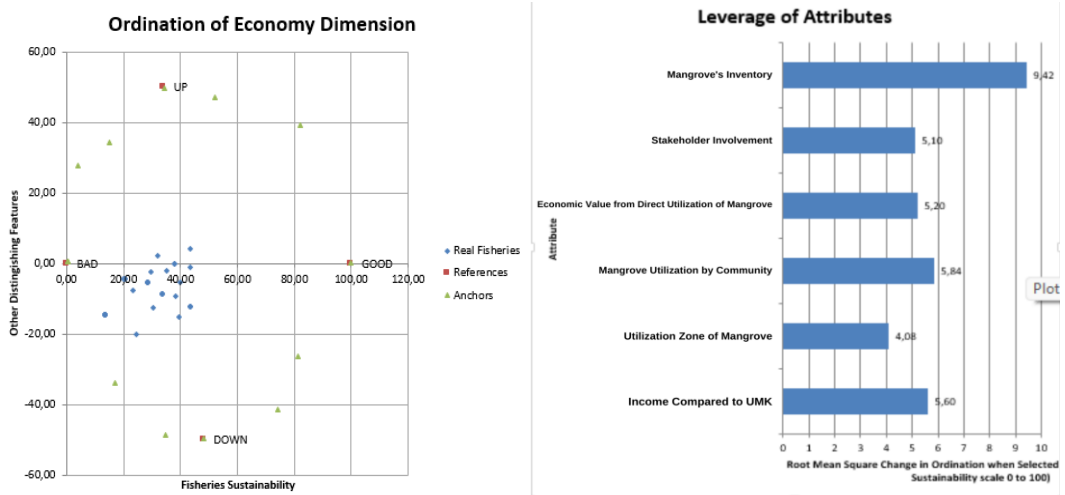


Figure 4. Sustainability index and attribute leverage in the economic dimension

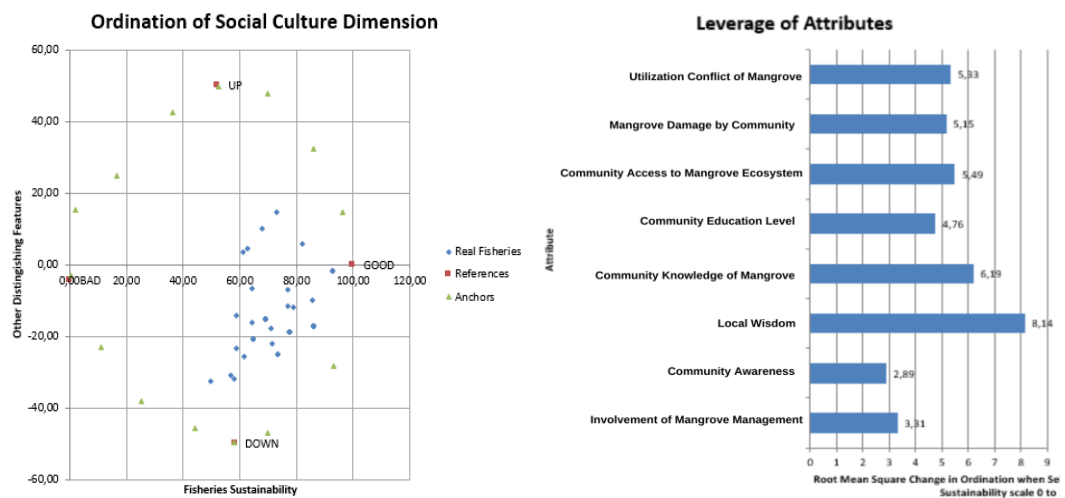


Figure 5. Sustainability index and attribute leverage in the social dimension

Discussion

Sustainable development has the principle of being able to meet human needs without impacting the environment. Using the RAP MForest method, the assessment of sustainability status produces a sustainability index value for each dimension. Therefore, at least three main dimensions will support it, namely the ecological, economic, and socio-cultural dimensions (Rahmayanti and Ananda 2017).

Mangrove ecosystem management status

The results of the RAP MForest analysis show that the mangrove ecosystem management in Karanggandu Village, Trenggalek District, is classified as less sustainable. That happened because the management of the mangrove ecosystem was not balanced yet in three sustainability aspects. Mangrove area management must proportionally consider the sustainability of three aspects; if there is a discrepancy in one of these aspects, it will cause an imbalance, leading to negative impacts on the others. The maintenance of the mangrove ecosystem in Karanggandu Village is routinely carried out by planting and repairing facilities to support ecotourism activities. Additionally, the local community has already reached a level of understanding about the importance of protecting the mangrove ecosystem. However, the existence of the Kadilangu mangrove cannot yet support the surrounding community's economic conditions. In addition, the pandemic conditions caused tourists to decline, so it impacted the economic conditions of parties related to the mangrove ecosystem. Based on the results in Figure 5, the social and ecological dimensions are considered quite sustainable, and the economic dimensions are considered less sustainable.

The highest sustainability index value in this study is the social dimension. Local involvement is considered the key factor in ecosystem management. Ouwor et al. (2019) said that communities have an essential view on this matter; thus, their participation in regulating is necessary. Raising their awareness to involve in conservation practices is a crucial aspect. Some determinant factor is needed to engage their willingness to participate in this activity (Roy 2014). Stone et al. (2008) study showed that the authority needs to provide the community with ecosystem services to promote conservation activity. In addition, the economic dimension with the lowest index needs to be considered. It requires cooperation between stakeholders in the development of economic activities such as ecotourism and entrepreneurship around the site to support economic sustainability.

Attributes from each dimension

The multi-dimensional analysis (ecology, economy, and social) of the Pancer Cengkong Mangrove Forest ecosystem shows the sustainability level of various attributes as the assessment factors. Leverage analysis examined which attributes most influence sustainability management (Pitcher and Preikshot 2001). A great value showed the sensitive indicator in leverage analysis. Furthermore, management sustainability could be achieved

by giving more attention to those important indicators (Melo et al. 2020).

The analysis of the ecological dimension leverage (Figure 3) shows the two highest influential attributes, namely the diversity of mangrove fauna with a value of 4.08 and mangrove pressure at 3.75. Regarding fauna diversity, Sawitri et al. (2019) reported that the fauna found in the Pancer Cengkong mangrove Forest were 18 families with 30 species, spreading in mangrove and residential areas. This condition is in the moderate category, with *Faunus ater* as the highest Important Value Index species. The higher the diversity index, the better the level of mangrove health (Sari et al. 2019). It is because mangrove areas provide suitable habitats for fauna to thrive, such as breeding grounds and foraging areas (Zakaria and Rajpar 2015). For instance, canopy cover depletion could negatively impact the crab diversity in a mangrove forest in southern Kenya (Barbanera et al. 2022). Another major sensitive attribute is mangrove land pressure. Because the coastal communities in Pancer Cengkong Mangrove Forest have been knowledgeable about the importance of this area to their livelihood, any damage from anthropogenic activities, for instance, land pressure, is uncommon. Another human activity that usually drives mangrove ecosystems in danger is the expansion of aquaculture and residential areas (Moschetto et al. 2021; Phan and Stive 2022). Land pressure profoundly contributes to threatening forest areas by increasing the critical land area (Kurnia et al. 2020).

Subsequently, the important factors in achieving sustainability from an economical dimension are mangrove utilization inventory and mangrove usage by people. The local authorities guide what kind of activities are allowed and prohibited in the mangrove area. That is reinforced by the application of fines and even imprisonment for violators of the rules. In addition, the locals of Karanggandu Village have achieved a level of understanding about protecting the mangrove ecosystem; thus, there is a minimal illegal activity in the mangrove area. The previous research proposed a similar result by Damastuti et al. (2022) in Central Java, Indonesia, which mentioned that the highest result of conservation effort in mangrove areas was achieved by applying a community-based mangrove management approach. Thus, the synergy between locals and the governments makes strong protection of the mangrove ecosystem, leading to a balance between human needs and nature.

Nearby communities around the Pancer Cengkong Mangrove Forest benefit from the ecotourism it provides. The location of ecotourism in the mangrove area provides job opportunities for local residents, such as traders, managers, and fishermen. In addition, coastal inhabitants also gain a huge advantage from the fisheries sector. Therefore, the community realizes the importance of preserving the mangrove forest, so they gain more advantages provided by the mangrove, both economically and ecologically. Such as a finding in Mexico, more than 80% of coastal societies willingly participate in mangrove protection efforts because they assume that mangrove

ecosystem is essential and provides advantageous services to communities (Jadin and Rousseau 2022).

Lastly, the keystone attribute from the social dimension is local wisdom at 8,14. Local culture in the nearby Pancer Cengkong is called "*labuh laut*," which is held annually. This activity is usually carried out at Prigi Beach, about 3.7 km from Pancer Cengkong, by fisherman communities. The traditional ritual is conducted as a gratitude for the marine products provided by nature and a wish to be blessed. Before the traditional ceremony, the community makes traditional food (*Lodho*) in large quantities. The event's highlight was floating a giant *tumpeng* into the middle of the sea, which was participated by local people and tourists.

Community knowledge about mangroves became the second highest sensitive attribute, valued at 6,19. The people of Karanggandu are well aware of the benefits and applicable regulations related to the mangrove ecosystem. The use of mangroves as a support for economic activities such as ecotourism and protecting coastal areas as an ecological function of mangroves is one of the driving factors for conservation and increasing public knowledge about the mangrove ecosystem (Ritohardoyo and Ardi 2014). Therefore, community awareness towards conservation and sustainability of mangrove ecosystems because the community plays an important role in maintaining the stability of the mangrove ecosystem both in the ecological and social dimensions.

In conclusion, ecosystem management in the Pancer Cengkong Mangrove Forest is fairly sustainable. Of the three aspects, namely economic, social and ecological, the economic aspect is the category with the lowest sustainability value. At the same time, the other two aspects are classified as fairly sustainable. The sustainability index is needed to determine the level of sustainability of mangrove forest management to preserve and support local communities welfare. Knowing the important factors in each dimension is also important to know the priority steps that must be taken to achieve sustainable management. Because every aspect in each dimension represents the factor in reaching the full potential and benefit of Pancer Cengkong Mangrove Forest, based on the research that has been done, it can be seen that the economic factor still needs more attention to achieve the level of sustainability. The mangrove ecosystem management in Karanggandu Village tends to be more dominant in social and ecological dimensions. Thus, the policymakers and the locals should put a bigger effort into improving the economy without adverse impacts on environmental management. Local governments can release policies regarding the sustainable use of mangroves, and the communities can use mangroves to help their economic needs, such as for fish nurseries, manufacture of food products, and products made from mangrove trees.

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Effect of water physicochemistry on amphibian abundance in Sub-tropical Kupinde Lake of the Nepal Himalaya

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Abstract. Sunar CB, Pandey N, Chand B, Upadhyaya LP, Thapa B, Pant RR, Khanal L. 2022. Effect of water physicochemistry on amphibian abundance in Sub-tropical Kupinde Lake of the Nepal Himalaya. *Intl J Bonorowo Wetlands* 12: 89-95. Amphibians are the key bioindicators of ecosystem health. Their diversity and abundance are affected by the physicochemical factors of the environment. Wetland ecosystems of the Himalaya are under the threat of human activities and current climate change. However, hydrochemical status and faunal diversity are poorly documented in the wetlands of western Nepal. This study characterized the physicochemical parameters of water in Kupinde Lake in the Salyan District of Karnali Province, Nepal, and established the association between amphibian abundance and those parameters. A total of 24 samples of surface water were collected at the lake's edge during October 2021, and 18 physical and chemical parameters were analyzed. Visual encounter surveys were conducted at each water sampling site to count amphibians within a circle of two meters in diameter. The lake water was slightly alkaline (mean pH = 8.16±0.29), and the major dominating cation and anion were Ca²⁺ and HCO₃⁻, respectively. Amphibian abundance was positively correlated with pH, HCO₃⁻, and NH₄⁺ and negatively correlated with EC, TDS, CO₂, Cl⁻, and Na⁺. The polynomial regression analysis revealed amphibian abundance has a consistent positive association with water pH ($r^2 = 0.497$, $p < 0.05$) and unimodal relation with the temperature ($r^2 = 0.188$, $p < 0.05$). Low amphibian diversity and dominance of a single amphibian species, the Indian skipper frog (*Euphlyctis cyanophlyctis* Schneider, 1799), indicate poor ecosystem health of the lake. The findings of this study provide baseline information for monitoring and managing the Kupinde Lake.

Keywords: Bioindicators, *Euphlyctis cyanophlyctis*, herpetofauna, hydrochemistry, Karnali Province

INTRODUCTION

Amphibians are unique among vertebrates because they use aquatic and terrestrial habitats at different stages of their lives (Compton et al. 2007; Rittenhouse and Semlitsch 2007). They utilize a variety of terrestrial and aquatic habitats for breeding, larval development, and overwintering (Shah and Tiwari 2004). They play important roles in the trophic levels of the ecosystems (Mifsud 2014; Thakuri and Pokhrel 2017; Ali et al. 2018). Amphibians are excellent indicators of wetland ecosystem health because they rely heavily on water (Hecnar 2004; Amankwaa et al. 2020; Riyanto and Rahmadi 2021; Paudel et al. 2023) and play a key role in forecasting environmental quality in their habitats (Mifsud 2014; Amarasinghe et al. 2021). They provide various ecosystem services, such as religious and cultural services, food, and medicine (Shah and Tiwari 2004; Paulding and Randhir 2021). In addition to regulatory functions, they also disperse seeds and control biological pests (Paulding and Randhir 2021).

Amphibian populations are declining due to reduced habitat and degraded microhabitat quality, climate change, pollution, anthropogenic activities, diseases, etc. (Rastegar-Pouyani et al. 2015; Blaustein et al. 2018; Muths et al. 2020; Paulding and Randhir 2021). In wetlands, factors

such as permanent water, the spatial configuration of wetlands and upland habitats, the characteristics of local habitats, and water chemistry and quality have an extensive effect on the composition of amphibian communities and the abundance of individual species (Hecnar 2004). Dissolved oxygen, temperature, pH, salinity and water conductivity, organic carbons, and pollutants are important factors in their habitats that can affect survival, growth, maturation, and physical development (Sparling 2010). These wetland characteristics are influenced by hydroperiod, resulting in changes in the abundance, density, reproduction, and growth rates of amphibians (Brannelly et al. 2019; Boelter et al. 2020). In addition, fertilizer and pesticides are commonly applied to increase agriculture production, negatively affecting the amphibians and reptiles that inhabit agricultural fields and downstream water bodies (Ghosh and Basu 2020). Various agricultural chemicals, such as pesticides, herbicides, fungicides, and fertilizers, contribute to water pollution (Mann et al. 2009). Polluted water can affect amphibians through their permeable skin (Boyer and Grue 1995), and they become prone to infections, limb deformities and a decrease in their numbers (Linzey et al. 2003). In order to survive, reproduce and develop, amphibians, particularly anuran species, are highly dependent on suitable water quality (Pollet and Bendell-Young 2000; Calderon et al. 2019).

Wetlands with poor water quality may not sustain future generations of such sensitive amphibians.

Water is essential to the earth's uniqueness and provides all forms of life (Gorde and Jadhav 2013). Amphibians that inhabit the water have been affected by increasing salinization, nitrification, hydrocarbons, and pesticide contamination (Salman 2019). There has been evidence that amphibian development, growth, reproduction, and survivability are hampered by high levels of electrical conductance, nitrates, nitrites, total phosphates, chloride, and unionized ammonium, and low concentrations of dissolved oxygen (Serrano et al. 2016; Babini et al. 2018). In addition to these effects, pesticides are also destroying amphibians' fitness and survival as a result of their massive use (Mann et al. 2009). Aquatic communities in wetlands are potentially exposed to chemical stressors as well as road salt (Trombulak and Frissell 2000). There is a possibility that road salt can negatively impact ecosystem health, biological diversity, and the functioning of lake ecosystems due to its deposition (Lewis et al. 2021; Szklarek et al. 2022).

Wetlands of the Himalayan region are threatened due to anthropogenic pressure and climate change (Paudel et al. 2023). Lakes in the mid-hill region of the Nepal Himalayas are important ecosystems providing multifaceted services. For example, Kupinde Lake, a sub-tropical lake in western Nepal, is an important tourist destination of religious importance. Currently, the lake area is being developed with the construction of roads, making it easier accessibility to visitors. Consequently, road salt contamination and the spread of invasive species in lake ecosystems are possible results of this anthropogenic

activity (Lewis et al. 2021; Ren et al. 2021; Szklarek et al. 2022). In order to understand the ecosystem health and provide baseline information for future management of the lake, it is crucial to characterize the physicochemical parameters and establish their association with the lifeform being supported by the lake. Therefore, this study aimed to i) characterize the physicochemical parameters of the Kupinde Lake water, ii) explore the amphibian community inhabiting the lake and, iii) establish an association between amphibian abundance and water quality parameters.

MATERIALS AND METHODS

Study area

The Kupinde Lake is in the Salyan District of Karnali Province, Nepal (Figure 1). It lies 28°24.701' N and 82°03.608' E, at an 1137 m asl and 15 km from the district headquarters, Khalanga. Salyan District is a hilly area covering an area of 1462 km² with a population of 241,716 (CBS 2011). The district is bounded by Rolpa to the east, Surkhet and Bardiya to the west, Rukum and Jajarkot to the north, and Dang and Banke districts to the south. The district has a sub-tropical to temperate climate with maximum temperatures of 31°C, minimums of 3°C and annual rainfall of 1100 mm. Kupinde Lake is one of the most popular tourist destinations in Bangad Kupinde Municipality of Salyan District. It occupies an area of 0.24 km² which is surrounded by sparse forest.

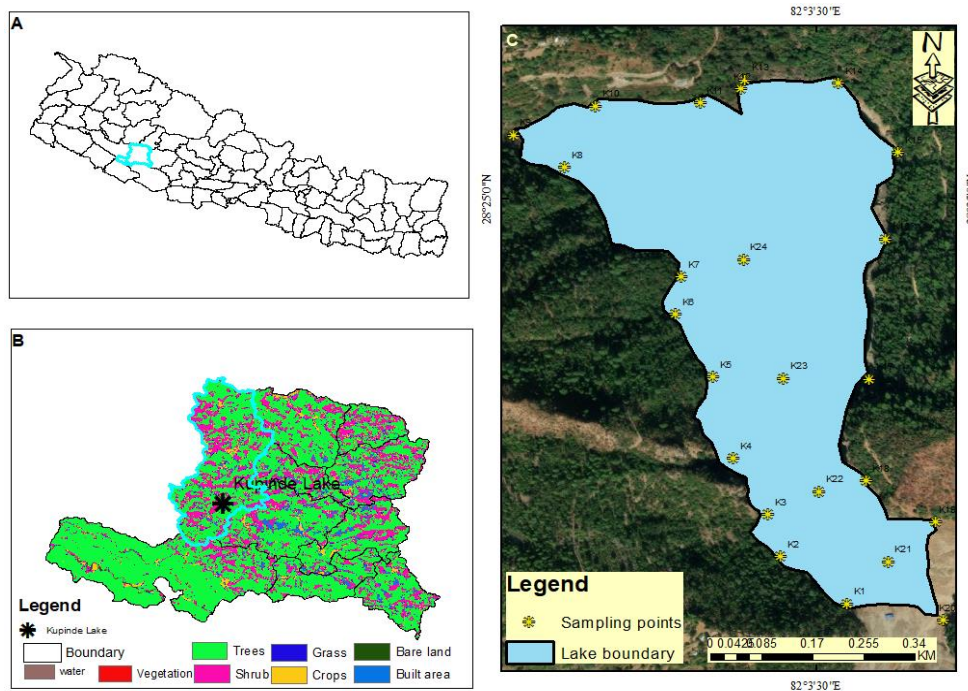


Figure 1. Map of the study area: A. Map of Nepal showing Salyan District, B. Map of Salyan District showing the Kupinde Lake, C. Map of Kupinde Lake showing sampling points

Water sampling and physicochemical parameters measurement

A total of 24 water samples were collected in October 2021 from the periphery and middle of the lake using a boat. The location of the water sample was recorded with Garmin e-trex10 GPS unit (Garmin, Chicago, IL). From each sampling site one liter water sample was collected in High Density Polyethylene (HDPE) bottle. Out of 24 samples, 20 were taken from the periphery and four were from the inner area of the lake such that it could represent the entire lake area (Figure 1C). Water samples were analyzed for eighteen important physicochemical parameters. The physical parameters like pH, temperature, (pH meter (Model No. pH55) Total Dissolved Solids (TDS) and Electric Conductivity (EC) (Multipurpose meter (SN: 11003290111) were measured on-site. On-site measurements were repeated until a consistent reading was obtained. Chemical parameters like Total Hardness (TH), Calcium Hardness (CaH), Magnesium Hardness (MgH), alkalinity (HCO_3^-), and chloride (Cl^-) were measured by titrimetric method. In addition, chemical parameters like ammonium (NH_4^+), nitrate (NO_3^-), and phosphate (PO_4^{3-}) were analyzed using an UV-visible spectrophotometer. Potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), and sodium (Na^+) were analyzed using a Flame Photometer in the laboratory of the Central Department of Environmental Science, under the Institute of Science and Technology, Tribhuvan University (CDES-TU), Kathmandu, Nepal.

Amphibian survey

Species richness of amphibian fauna was observed by visual encounter survey during October 2021. The survey was conducted from 5:00 to 10:00 hours in the morning, 2:00 to 5:00 afternoon and 7:00 to 9:00 evening. At each sampling point, 2-3 people visited twice each day for a total of 10 minutes each. A survey was conducted along the lake's perimeter, looking for individuals at its edge. The sites (1, 2, 9, 10, 11, 12, 13, 14, 15, 18, 19, 20 and 21) were visited 10 times during the study. The sampling sites (3, 4, 5, 6, 7, 8, 16, 17, 22 23 and 24) were visited four times. Because some sites were less accessible, survey efforts were not the same for all sites. Amphibian counts were performed within a circle of 2-meter diameter of each water sampling site prior to sampling and average value from repeated counts was used as amphibian abundance in downstream analysis. Photographs and samples of specimens were taken for both identification and evidence. The collected specimens were preserved in a plastic bottle with 70% ethanol for further identification. The collected specimens have been stored at the Central Department of Zoology, Tribhuvan University, Kathmandu.

Data analysis

Spearman's rank correlation analysis was performed to establish the relations between the physicochemical parameters of the lake water and amphibian abundance. The Shapiro-Wilk test was run to determine the collinearity of all the variables. Only one variable was selected for the Generalized Linear Modeling between the variables having pairwise correlation coefficient $r > |0.7|$. The GLM analysis

(function 'glm' in R) with Poisson distribution and log-link function employed in the R software (R-Studio 2022) was used to analyze the relations between the selected physicochemical parameters of water and amphibian abundance. Polynomial regression (third order) was performed between the abundance of amphibians and major physicochemical parameters (pH and temperature). Third-order polynomial regression was selected based on the least AIC value.

RESULTS AND DISCUSSION

Physicochemical parameters of water in Kupinde Lake

The mean temperature of Kupinde Lake water was $22.58^\circ\text{C} \pm 0.69$, and the pH was 8.16 ± 0.29 (Figure 2). Temperature is one of the important regulators of the physicochemical and biological activities of the aquatic ecosystem. The average lake water temperature varies with seasons and geographical position of the lake. Several factors affect lake surface temperature, including the location of the lakes, morphometric parameters, wind speed, transparency, and human activities (Ptak et al. 2018; Yang et al. 2019). The Kupinde Lake water was slightly alkaline. A similar pH range was also reported from Lake Rara (Gurung et al. 2018), one of the high-altitude lakes in the Nepal Himalayas. Alkalinity is a common phenomenon in most freshwater ecosystems and that might be attributed to the presence of limestone rocks in the lake catchments (Ormerod et al. 1990).

The mean EC was $136.54 \pm 3.04 \mu\text{S}/\text{cm}$, and TDS was $68.25 \pm 1.67 \text{ mg}/\text{L}$. Electrical conductivity effectively indicates human-induced changes in ion concentration in aquatic ecosystems (Wu et al. 2020). TDS measures the concentration of ionic constituents in water. It is believed that TDS and EC are related to the composition of water ions and the concentration of dissolved solids (Taylor et al. 2018). The lesser Himalayan lake in Pokhara valley had a conductivity of $120.48 \pm 2.52 \mu\text{S}/\text{cm}$ and TDS of 97.73 ± 2.97 (Khadka and Ramanathan 2021), and the high-altitude lake in Rara had EC $193.85 \mu\text{S}/\text{cm}$ and TDS $96.85 \text{ mg}/\text{L}$ (Kaphle et al. 2021) and the previous study by Pant et al. (2020) reported the EC of Ghodaghodi lake was $142 \mu\text{S}/\text{cm}$ and TDS $77 \text{ mg}/\text{L}$. Kupinde Lake had lower electrical conductivity and TDS levels than other lakes, except Phewa Lake, which indicates that it is less polluted. The most dominant cation and anion in Kupinde Lake were Ca^{2+} ($27.97 \pm 4.33 \text{ mg}/\text{L}$) and HCO_3^- ($157.71 \pm 69.09 \text{ mg}/\text{L}$), respectively. This finding is consistent with previous study Phewa lake of lesser Himalayan (Khadka and Ramanathan 2021), Rara Lake (Kaphle et al. 2021), and Ghodaghodi lake (Pant et al. 2020). The major ion chemistry of lake water provides valuable insight into the sources of dissolved ions, weathering, and hydrogeochemical processes (Singh et al. 2016). Mostly, major cations and anion levels are affected by carbonate weathering (Pant et al. 2020; Kaphle et al. 2021; Khadka and Ramanathan 2021), and anthropogenic activities (Zhao et al. 2021).

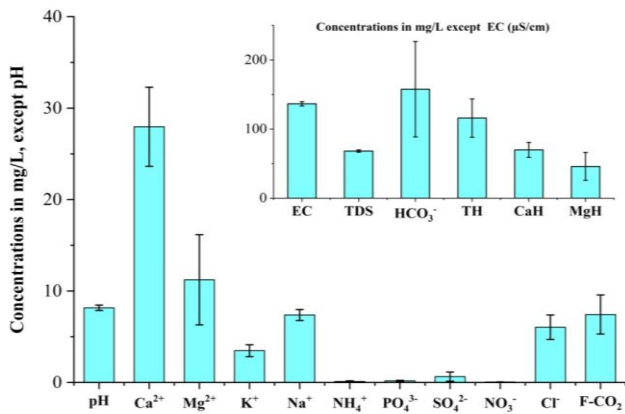


Figure 2. Concentration (mean values with standard deviation) of physicochemical parameters of water in Kupinde Lake, Nepal

Amphibian community structure in Kupinde Lake

Two species of Anuran amphibians- Indian skipper frog (*Euphlyctis cyanophlyctis* Schneider, 1799) and Syhadra frog (*Fejervarya syhadrensis* Annandale, 1919) were recorded from the Kupinde Lake during the study period. The *E. cyanophlyctis* was the most abundant amphibian species. Among the 24 study sites, *E. cyanophlyctis* was detected from the 20 sampling points. The highest average abundance of species was found at site 21 (n = 120), followed by site 20 (n = 115). Amphibians were not observed in the site six and center of the lake (i.e., Site 22,

23, and 24). A permanent water body would provide a suitable habitat for *E. cyanophlyctis*, since it prefers the littoral zone over deep water (Chowdhury et al. 2021). In this study, *E. cyanophlyctis* was the most abundant near the lake's periphery. There may be a reason for this, since plant leaves provide them with a shelter where they can lay eggs and food resources are nearby. In the study area, ecological conditions were problematic for amphibians because of the geographical position and the presence of rocky cliffs on the lake's periphery. This may contribute to the low amphibian diversity in the area.

Relationship between physicochemical parameters and amphibian abundance

A positive correlation was observed between amphibian abundance and pH, HCO₃⁻, and NH₄⁺ (Figure 3). In Kupinde Lake water, the chemical parameter HCO₃⁻, and NH₄⁺ have a strong positive correlation (Figure 3), which influences the pH. This positive association may be explained by the physicochemical parameter in the lake catchment interacting with limiting conditions. Alternatively, the amphibian abundance correlated negatively with EC, TDS, CO₂, Cl⁻, and Na²⁺. In these parameters, there was a positive correlation with (EC-TDS) and (CO₂ - Cl⁻) (Figure 3). However, these parameters showed a negative correlation with amphibian abundance, possibly due to the relatively low concentration in lake water.

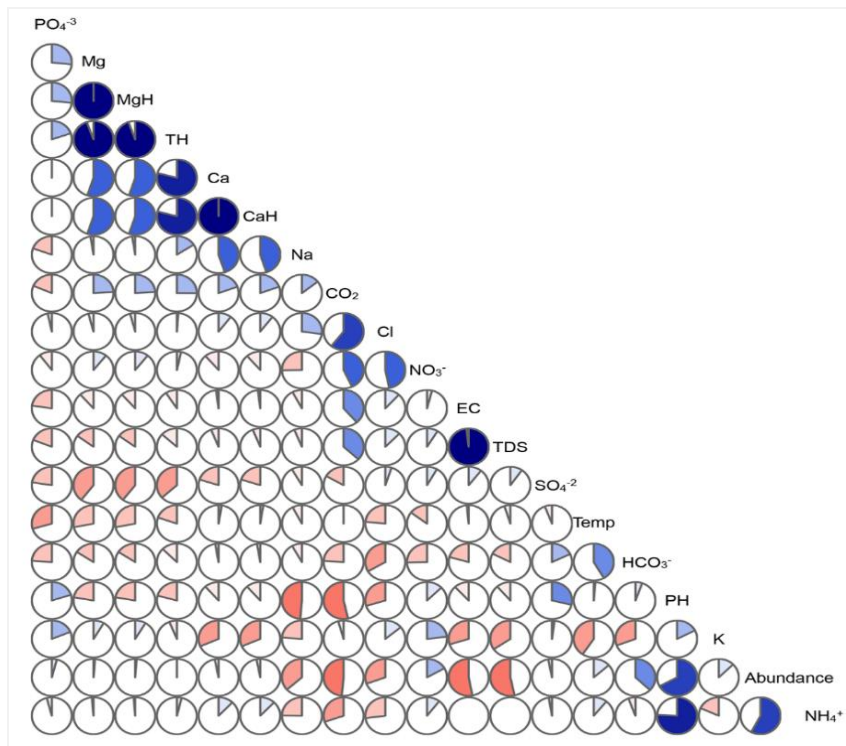


Figure 3. Correlogram of the pairwise correlation (r) between physicochemical parameters and amphibian abundance. Shaded blue and red colors within circles depict positive and negative correlations, respectively. Empty circle represents r = 0, full blue circle represents r = 1 and full red circle represents r = -1

Based on the physicochemical parameter testing, the Generalized Linear Model (GLM) (Table 1) indicated amphibian abundance was significantly affected by temperature, pH, TDS alkalinity, calcium, sulfate, and sodium. According to the test parameters, amphibian species abundance decreased with increasing temperature, TDS, sulfate, and sodium. Similarly, species abundance is positively related to pH, alkalinity, and calcium concentration.

The regression analysis between the abundance of amphibians and major physicochemical parameters i.e., temperature and pH based on the AIC values were better suited to the third order ($p < 0.05$) (Table 2). The polynomial regression analysis between several individuals and pH revealed a consistent positive association ($r^2 = 0.497$, $p < 0.05$). On the other hand, the association between amphibian abundance and temperature was unimodal ($r^2 = 0.188$, $p < 0.05$).

The results indicate that temperature, pH, TDS, alkalinity, calcium, sulfate, and sodium significantly associate with anuran abundance in Kupinde Lake. Amphibian abundance had a negative association with temperature, TDS, sulfate, and sodium; whereas a positive association existed with calcium, alkalinity, and pH (Figure 4). The lake micro-environment is influenced by these physicochemical factors that, in-turn, affect the species inhabiting the lake. A negative relationship was observed between amphibian abundance and temperatures. It might be due to the lake's water's relatively low temperature. Amphibian gonadal structures and sex ratio are generally affected by extremely low and high temperatures (Phuge 2017). The temperature of the water is influenced by the temperature of the air and the intensity of solar radiation (Oli et al. 2013). Temperature changes can affect breeding activities and early growth (Wheeler et al. 2015; Catenazzi and Kupferberg 2017). A rise in water temperature influences larval development and survival until metamorphosis (Skelly et al. 2002).

The hydrogen ion concentration (pH) levels were consistently normal at all sampling points (mean pH 8.16). The pH and calcium have a weak negative correlation, indicating that pH has consistently been within the normal range, while relatively high calcium concentrations enhance the pH values (Brown 1983). This is consistent with scientific studies on maintaining a balance of hydrogen ions in amphibian habitats within a pH range of

6.5 to 8.5. Water with low pH can affect amphibians' reproductive directly by killing embryos and larvae and disrupting trophic relationships between them and other aquatic animals (Serrano et al. 2016). Hence, the abundance of amphibians is negatively correlated with low and high pH values, which may affect the size of populations (Skei and Dolmen 2006). The species abundance was high, with a minimum TDS of 64 ppm. Amphibians need a TDS value between 50 and 250 ppm to survive and anything below this range will be detrimental (Shaikh et al. 2014). A negative association was found between TDS and amphibians. TDS solution contains several ions, including sodium, chloride, potassium, magnesium, sulfate, chloride, and bicarbonate (Chapman et al. 2000). different ions might achieve a similar effect at a similar concentration, however, it depends on their identity and concentration. The volume of TDS was extremely high compared to normal, which could lead to amphibian mortality due to excessive organic and inorganic components (Shaikh et al. 2014).

Table 1. Generalized Linear Model (GLM) with Poisson distribution and log link function test showing the relations between amphibian abundance and physical-chemical parameters of water quality in Kupinde Lake, Nepal

| Parameter | Estimate | SE | z value | Pr (> z) |
|------------|----------|------|---------|-----------|
| Intercept | 28.82 | 8.83 | 3.26 | 0.001* |
| Temp | -0.68 | 0.21 | -3.23 | 0.001** |
| pH | 1.80 | 0.91 | 1.97 | 0.047* |
| TDS | -0.35 | 0.07 | -5.03 | 0.000*** |
| Alkalinity | 0.00 | 0.00 | 3.05 | 0.002** |
| Ca | 0.06 | 0.01 | 3.54 | 0.000*** |
| Sulphate | -0.62 | 0.21 | -2.90 | 0.003** |
| Na | -0.60 | 0.17 | -3.40 | 0.000*** |

Table 2. Polynomial regression of abundance of amphibians with temperature and pH

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

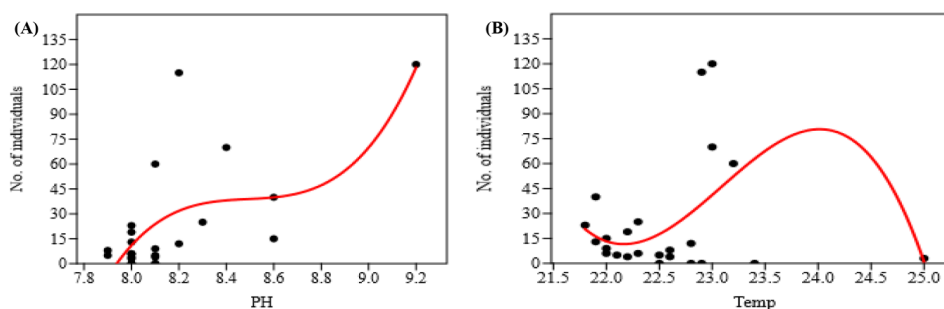


Figure 4. Relationship between amphibian abundance and major physicochemical parameters; A. Number of amphibian individuals and pH, and B. Number of amphibian individuals and temperature

An intriguing finding of this study was that Electrical Conductivity (EC) was negatively correlated with species abundance. The reason could be that the lake has low EC (mean 136) while aquatic organisms require ECs between 150 and 500 (Sparling 2010). In contrast, a positive association has been shown between the EC and amphibian abundance (Calderon et al. 2019). Evidence shows that a significant increase in conductivity negatively impacts amphibian behavior, growth, and development (Chambers 2011; Klaver et al. 2013). It appears that bicarbonate is the most dominant anion among the major anions. The results indicate that it has a significant effect on amphibian abundance. Generally, bicarbonate is derived from calcium carbonate rocks (CaCO₃) and calcium magnesium carbonate rocks (dolomite) (Mallick 2017). A significant impact of amphibian abundance was found in the current study. In frog blood, the calcium concentration is similar to that of vertebrates. Calcium metabolism in amphibians seems to involve endocrine and humoral factors such as parathyroid hormone, calcitonin, vitamin D, and prolactin. Calcium amounts vary with the season, increasing in spring and summer and decreasing in winter (Stiffler 1993).

Amphibian abundance is negatively correlated with sulfate and sodium levels in the lake due to its extremely low levels. Water quality is considered the main factor influencing health and disease in all aspects of a biotic system. However, hydrochemistry is little studied concerning *E. cyanophlyctis*. In Kupinde Lake, the physiochemical characteristics of water, such as temperature, pH, alkalinity, etc., showed a strong influence over the anuran survival, growth and reproduction, which influences how appropriate the lake environment is for the amphibians. For example, temperature or pH ranges are more conducive to anuran growth and reproduction whereas some levels of TDS, alkalinity, calcium, sulfate, and sodium may be necessary for proper physiological functioning. Therefore, a further detailed study is required to determine how these factors interact with each other. This should be acknowledged that we explored amphibian abundance by visual encounter method during the post-monsoon season. Although we tried to observe the amphibians carefully, we could have missed some species or individuals from the survey. Therefore, future studies during the monsoon season with a combination of different sampling methods could yield better results.

In conclusion, the Kupinde Lake in the sub-tropical climate of the Nepal Himalaya has slightly alkaline water dominated by Ca²⁺ and HCO₃⁻. Amphibian abundance was negatively affected by the temperature, TDS, sulfate, and sodium, while significant changes in pH, alkalinity, and calcium. Low amphibian diversity and dominance of a single species (*E. cyanophlyctis*) indicate poor ecosystem health of the lake. The findings of this study could be important for monitoring and managing the Kupinde Lake.

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