



RESEARCH ARTICLE

BACTERIOLOGICAL INVESTIGATION OF SIX DIFFERENT PONDS WITHIN
THE PATNA URBAN AREA OF BIHAR, INDIA

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Glossary of Abbreviations

P1 to P6-Sample sequence
MR-Methyl Red Test
VP-Voges-Proskauer Test
Ci-Simmon's Citrate Test
In-Indole Test
MPN-Most Probable Number
APHA-American Public Health Association.

ABSTRACT

Background: Microorganisms like bacteria, viruses are common water contaminants and they lead to waterborne disease and epidemics. The aim of this study was to assess the quality of pond water. In this respect we evaluate Coliforms and other bacteria present in the pond water environment. Six samples of pond water were collected from six major ponds of the Patna urban area which is used for pisciculture. **Methods:** Samples were processed in isolation of different microbes by using MacConkey agar, EMB agar and etc. Microbes were identified by different biochemical test related to bacterial metabolism. All the experimental work was carried out between the periods of December, 2017 to February, 2018 at the Department of Biotechnology, A. N. College, Patna. **Results:** The result indicates that all the six ponds contain Coliforms for above the APHA limits. They also show the presence of *Pseudomonas aeruginosa*, *Klebsiella*, *Shizella*, *Enterobacter* etc., which could further endanger for both fishes and consumers. **Conclusion:** Results suggest that of monitoring the water quality from time to time. It will help to highlight the route of contamination.

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INTRODUCTION

With more than 60% of the earth's surface covered by water and our body consists of about 75%, strongly advocates the necessity of the water for life on earth. The pond is also a source of water. Generally two types of pond- made and natural; which holds water for at least one quarter of the year [Biggs et al., 2005]. Due to the anthropogenic effect, domestic work, chemicals, ponds are getting polluted. Physicochemical and microbial analysis detects the quality of this pond water. It also reflects biotic and abiotic status of the ecosystem [IAAB, 1998; Kulshrestha, 2006]. Type of domestic waste, neighbor population and their activities and location are some major factors which affect the microbial property of pond water [Rai et al., 1993].

The present investigation emphasizes on the microbial status of six major fishing ponds of the Patna urban area. In ponds various microorganisms are found like bacteria, fungi, algae, etc. Among these bacteria are more important microbes in ponds. They may be beneficial type or harmful type. Beneficial bacteria are naturally occurring in ponds and maintain a pond environment and ecosystem [Fredrickson et al., 2004]. The presence of coliform is determined by MPN test, Coliforms are lactose fermenting bacteria [Singh et al., 2017], total and faecal Coliforms are indicator organisms [Pelzer et al., 1993]. Indicator organisms are those whose presence in water shows that water is polluted with faecal material. Coliforms include members of the family *Enterobacteriaceae* [Nwachukwu, 2006; Singh et al., 2017]. This study assessed the water quality by analysis of waterborne pathogens, including coli forms of the six fish ponds.

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MATERIALS AND METHODS

A total six water sample was collected and represented in Table 1. All analytical works were carried out in the laboratory of the Department of Biotechnology, A. N. College, Patna.

Study site: Patna is situated between 24° 97' - 25° 27' North latitude and 84° 44' - 86° 57' East longitude at an elevation of about 129 meter above mean sea level. It is about 20 km long (from east to west) and 4 km width (from north to south) [Kanth et al., 2017]. Samples were collected at morning time in sterile containers with a capacity of 70 ml from Dec, 2017 to Feb, 2018 [Kanth et al., 2018].

All samples were analyzed for MPN and biochemical test (IMViC test) following the guidance of APHA [American Public Health Association, 1998]. MPN (Most Probable Number) was used to estimate total and faecal coli form. Various types of biochemical test were performed for identification of isolated bacteria these are:-

Indole Test: Using Kovac's reagent, it identifies those bacteria which are capable to degrade tryptophan into indole. Positive results show red or red violet colour while negative appears yellow on the surface of a broth medium [MacFaddin, 1980].

Simmon's Citrate Test: Citrate is an important factor in the Krebs cycle. Bacteria those utilized the citrate were indicated by a change of colour of medium from green to deep blue.

Table 1. Results represent the sampling site of different pond and MPN value

| S.No | Sample Code | Name of Pond | MPN | MPN Index |
|------|-------------|-------------------|-------|-----------|
| 1 | P1 | Mithapur Talab | 5-5-5 | >1,600 |
| 2 | P2 | Manikchand Talab | 5-5-5 | >1,600 |
| 3 | P3 | Kachhi Talab | 5-5-5 | >1,600 |
| 4 | P4 | Adalateganj Talab | 5-5-5 | >1,600 |
| 5 | P5 | Mangal Talab | 5-5-5 | >1,600 |
| 6 | P6 | Ranipur Talab | 5-5-5 | >1,600 |

Table 2. Bacterial isolates of different pond water samples

| Samples | Ci | In | MR | VP | Possible bacterium |
|----------------|-----|-----|-----|-----|----------------------|
| P2, P3, P4, P5 | -ve | +ve | +ve | -ve | E. coli |
| P1, P2 | +ve | -ve | -ve | +ve | Klebsiella aerogenes |
| P1, P2 | +ve | -ve | +ve | -ve | Klebsiella pneumonia |
| P6 | D | +ve | +ve | +ve | Proteus vulgaris |
| P1 | +ve | -ve | -ve | -ve | Proteus mirabilis |
| P1, P6 | -ve | +ve | +ve | -ve | Vibrio cholera |
| P1 | -ve | -ve | +ve | -ve | Salmonala species |
| P1 | -ve | D | +ve | -ve | Shigella Species |
| P6 | +ve | -ve | -ve | -ve | Pseudomonas Species |
| P4, P5 | +ve | -ve | -ve | +ve | Enterobacter species |

Note- Here (D) denotes a different strain of bacteria gives different results.

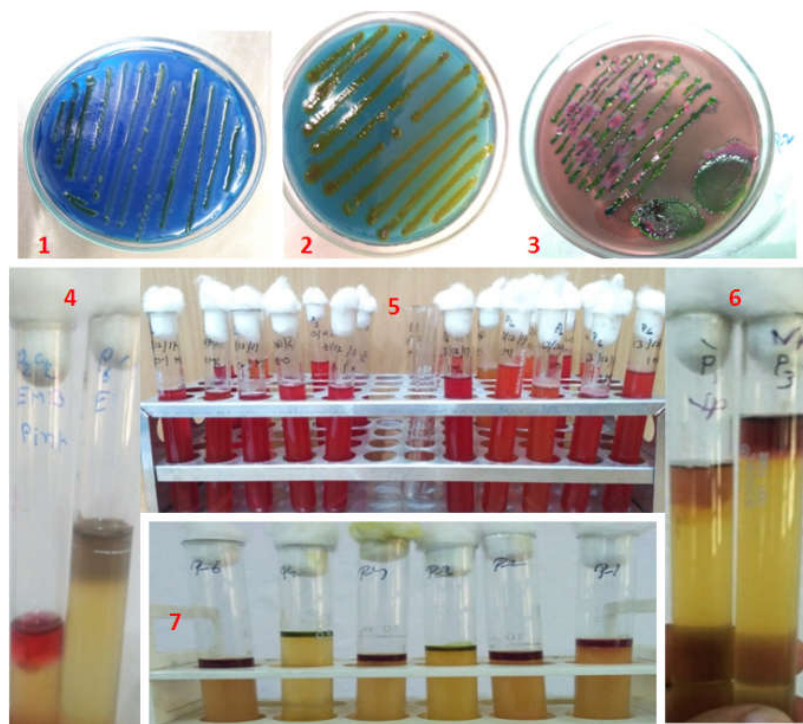


Fig. 1. In this figure 1,2 Positive and negative results of Simmon's Citrate test, respectively. 3. Mixed cultures of bacteria on EMB agar. 4. Positive and negative result of MR test. 5. Positive and negative result of MPN test. 6. Positive and negative results of VP test. 7. Positive and negative result of Indole test.

Methyl Red Test: This test determines whether the bacteria could oxidize glucose and produce acetate or lactate. Red colour indicates positive and yellow colour shows negative.

Voges-Proskauer Test: This test confirms the presence of Acetoin is an end product of glucose metabolism. If culture turns pink to cherry red colour, it is positive while if culture turns yellow to copper colour it is negative.

Images of above biochemical test are shown in Figure 1.

RESULT AND DISCUSSION

Present investigation helps to determine the microbial properties of fish pond water source from the area are listed in table 1. In table 2 represented biochemical test results of the sample and possible bacterium findings are listed. Sample P1 has a heavy load of bacteria. Results indicate that ponds are highly contaminated by different bacteria. Results also show that *E.coli* bacteria are the major groups found in four out of six ponds.

Conclusion

Microbial analysis confirms the presence of *Klebsiella*, *Proteus*, *Salmonella*, *Shigella*, *E.coli*, *Enterobacter spp.* in pond water. Contamination with above pathogenic bacteria could be harmful to fish health and those who intake under cooked fish from these ponds. It is suggested that the surrounding environment of these fish pond should be pollution free and weeds free because these can promote pathogenic microorganism growth. Time to time monitoring and examination microbial quality in the laboratory gives insight to the presence of particular microorganism. These organisms can reduce the production of fish, cause diseases and economic loss.

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