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Research Paper

PHYSICO-CHEMICAL AND MICROBIAL ANALYSIS OF TWO POND SYSTEMS OF JAGDALPUR CITY, C.G. INDIA

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Abstract

Physico-chemical and microbial analysis of Dalpat sagar and Gangamunda pond of Jagdalpur was carried out on 1st August 2014. After analysis, it is concluded that Gangamunda is more polluted than Dalpat sagar at both physico-chemical and microbial level. In physico-chemical analysis although the parameters like pH, turbidity and TDS, COD, total alkalinity of both the ponds were found within the standard range recommended by ISI (Indian Standard Institute) but on comparison among the two ponds, these parameters were higher in Gangamunda. DO, BOD level of Gangamunda exceeded the standard values while that of Dalpat sagar was found within the standard range. Chloride level of both the ponds exceeded the standard range but chloride level of Gangamunda was comparatively higher. Microbial analysis showed that more bacteria are present in Gangamunda i.e.141x104 CFU/ml higher than that of Dalpat sagar i.e. 46 x104 CFU/ml. Bacillus cereus, Proteus vulgaris, Pseudomonas aeruginosa, E. coli were identified in Dalpat sagar and Corynebacterium diptheriae, Bacillus cereus, E. coli, Bordetella pertusis were identified in Gangamunda. Coliphage detection after analysis showed that the population of coliphages is more in Dalpat sagar than Gangamunda. Hence water of Gangamunda is unfit for drinking and other domestic purposes.

Key words: Physico-chemical analysis, Gangamunda, Dalpat sagar, coliphages, microbial analysis.

INTRODUCTION

Water is one of the most precious natural resource and acts as an essential component of life for all living beings of the world. Rapidly increasing human population, urbanization and industrialization has polluted and contaminated the water bodies at several places. For drinking purpose water requires various purification procedures and must be free from many toxic and bacterial infections.

Pond system is generally referred to as a man-made or natural water body which is between 1 m² and 2 ha in areas, which holds or retain water for four months of the year or more [1]. Pond waters as well as other water bodies are being getting polluted due to discharge of industrial effluents, domestic wastes, chemicals and fertilizers from chemical factories. Quality of such polluted pond water could be detected through physicochemical and microbial analysis so that people could be aware of its uses for different purposes. Moreover physico-chemical and

microbiological analysis of pond system reflects on abiotic and biotic status of the ecosystem [2,3].

The physico-chemical and biological properties of water depend upon its location, type of sewage and discharge of domestic waste, surrounding human population and their activities [4]. Due to all these factors when a water body comes in contact with pollutant, its physico-chemical properties shows several types of changes which results in a complete change in the biota [5].

Jagdalpur city is located in Bastar district of Chhattisgarh state well known for its greenery, deep valleys, dense forests, streams, waterfalls and its tribal culture. Earlier Jagdalpur was surrounded by many ponds and lakes but at present time only two pond system are there ie, Ganga Munda and Dalpat Sagar which are in good condition, others are dry up. Ganga Munda and Dalpat Sagar are great attraction for local people as well as tourists coming to Jagdalpur. Due to industrialization, urbanization, automobiles, domestic waste and industrial effluent discharges, water quality of both the pond system is disturbed completely.

Therefore the aim of this present investigation is to analyze the physico-chemical characteristics and microbial status of both the ponds in a comparative manner so, that their pollution level could be detected out to some extent.

MATERIAL AND METHODS

Study area

Analysis of the physicochemical characteristics and microbial study of the two pond system was carried out at Dalpat Sagar located at Dalpat sagar ward and Ganga Munda pond situated at Naya Munda ward of Jagdalpur on 1st August 2014.

Sample Collection

At 10:30 AM water samples were collected from Dalpat Sagar and Ganga Munda pond in two different bottles. Bottle containing water sample collected from Dalpat sagar was marked as "D" and the other bottle containing water sample from Ganga Munda was marked as "N" which were used for further analysis.

Physico chemical analysis

Different parameters like odor, color, pH, Temperature, Dissolved oxygen (DO), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), TDS, total alkalinity, chlorine content were analyzed using standard procedures. (APHA, 1998) [6]

Microbial analysis

Microbial analysis like isolation of bacteria, calculation of bacterial colonies in CFU/ml, isolation of coli phage (bacteriophage), morphology of bacterial colonies as well as identification of bacterial population was performed.

Isolation of bacteria and enumeration of bacterial colonies

For isolation of bacteria from the samples, serial dilutions were made separately for both the samples. For calculation of no of bacterial colonies according to standard plate count method, approximately 1ml of solutions from appropriate dilutions (10⁻⁴) of both the samples were inoculated on nutrient agar plates. Plates were kept for incubation at 37°C inside the incubator for 24-28 hrs. After incubation, no of bacterial colonies appeared on each plates of both the samples were counted with the help of colony counter and colony forming units (CFU/ml) of the samples were calculated.

Morphology and Identification of bacteria

Pure cultures of bacteria were made separately from mixed populations of both the samples through streak plate method in four plates which were marked as plate no.1, 2, 3, 4. Plates were streaked and incubated at 37°C for 24-48 hrs after which the colonies were examined for their morphology and staining. For identification purpose, biochemical tests were performed and the bacteria were identified after comparing their morphological and biochemical results according to Bergey's Manual for Determinative Bacteriology.

Isolation of coli phages

24 hr broth cultures of *E.coli*, 100 ml of Tryptone hard agar and Tryptone soft agar, 500 ml of bacteriophage nutrient broth were prepared. 5ml of bacteriophage nutrient broth and 5 ml of

E.coli broth culture were aseptically added to 45ml of both the samples in two different flasks which were incubated for 24 hrs at 37°C. After incubation phage infected culture was poured into 100 ml of centrifuge tubes and was centrifuged at 2500 rpm for 20 min. After centrifugation the supernatant was poured through a sterile membrane filter apparatus to collect the bacteria free phage containing filtrate into another flask. Five tryptone hard agar plates and five molten soft agar tubes were labeled as 1, 2, 3, 4, 5 for both the samples. 0.1 ml of the E.coli broth culture was added to all the molten soft agar tubes. 1, 2, 3, 4 and 5 drops of the filtrate was added separately the respectively labeled molten soft- agar tubes. After mixing each tubes of soft agar were poured into its appropriately labeled agar plate. Which were further incubated in an inverted position for 24 hrs at 37°C. After incubation all the plates were observed for plaque forming units (PFU) that develops as clear zones on the lawn of bacteria. The no of plaques were counted in all the plates and tabulated.

RESULTS

Physico-chemical analysis

Results of various physicochemical characteristics of two pond systems of Jagdalpur area are given in table no.1 and 2.

Table no.1 Physico-chemical characteristics of Dalpat sagar pond system

| Table 10.1 Physico-chemical characteristics of Darpat Sagar point system | | | | | | | | | | |
|--|------------------|-------|------------|----------------------------|--|--|--|--|--|--|
| S.No. | Parameters | Unit | Result | Standards | | | | | | |
| | | | | | | | | | | |
| | 0.1 | | 0.11 | | | | | | | |
| 1. | Odour | - | Odourless | - | | | | | | |
| 2. | Colour | - | Light pale | - | | | | | | |
| | | | yellow | | | | | | | |
| 3. | рН | - | 7 | 6.5-8.5 (ISI- acceptable | | | | | | |
| | | | | limit) | | | | | | |
| 4. | Turbidity | NTU | 0.26 | 5 (ISI- acceptable limit) | | | | | | |
| 5. | TDS | mg/lt | 300 | 500 (ISI-acceptable limit) | | | | | | |
| 6. | DO | mg/lt | 9.2 | 4 (ISI- tolerance limit) | | | | | | |
| 7. | COD | mg/lt | 0.32 | 10 | | | | | | |
| 8. | BOD | mg/lt | 3 | 5 | | | | | | |
| 9. | Total alkalinity | mg/lt | 18 | 200 (ISI- desirable limit) | | | | | | |
| 10. | Chlorides | mg/lt | 1171 | 250 (ISI- desirable limit) | | | | | | |
| | | | | | | | | | | |

ISI- Indian Standard Institute

Table no. 2. Physico-chemical characteristics of Ganga Munda pond system

| Table | Table no. 2. I hysico-chemical characteristics of danga Munua pond system | | | | | | | | | |
|--------|---|-------|-------------|---------------------------------|--|--|--|--|--|--|
| S. No. | Parameters | Unit | Result | Standards | | | | | | |
| | | | | | | | | | | |
| 1. | Odour | - | Odorless | - | | | | | | |
| 2. | Colour | - | pale yellow | - | | | | | | |
| 3. | рН | - | 8 | 6.5-8.5 (ISI- acceptable limit) | | | | | | |
| 4. | Turbidity | NTU | 0.14 | 5 (ISI- acceptable limit) | | | | | | |
| 5. | TDS | mg/lt | 700 | 500 (ISI-acceptable limit) | | | | | | |
| 6. | DO | mg/lt | 11.6 | 4 (ISI- tolerance limit) | | | | | | |
| 7. | COD | mg/lt | 0.64 | 10 | | | | | | |
| 8. | BOD | mg/lt | 7.4 | 5 | | | | | | |
| 9. | Total alkalinity | mg/lt | 42 | 200 (ISI- desirable limit) | | | | | | |
| 10. | Chlorides | mg/lt | 1544 | 250 (ISI- acceptable limit) | | | | | | |
| | | | | | | | | | | |

Microbial analysis

Results for microbial analysis are given under following tables as described below.

Standard plate count

Bacterial colonies isolated from Dalpat sagar pond system and Ganga Munda Pond systems through standard plate count method in the form of CFU/ml are shown in table no. 3.

Table 3. Tabulation for results of standard plate count of bacterial colonies from Dalpat Sagar Pond System and Ganga Munda pond system.

| S.No. | Sample | CFU (at dilution 10 ⁻⁴) (CFU/ml) |
|-------|-------------------|--|
| 1. | Dalpat sagar pond | 46x10 ⁴ |
| 2. | Ganga Munda pond | 141x10 ⁴ |

Identification and characterization of bacteria

Water samples of both the ponds collected were examined for their microbial analysis through various microscopic examinations such as staining, motility test, colony morphology and colony characteristics, results of which are tabulated in table no.4 and 5. Biochemical characters for the samples are shown in table no. 6 and 7.

Table 4. Tabulation for results of staining; motility test and colony morphology of both the pond system

| S.No. | Samples | Microscopic examinations | | | | | | |
|-------|--------------|--------------------------|------------|------------|--|--|--|--|
| | | Gram staining | Motility | morphology | | | | |
| | Dalpat sagar | | | | | | | |
| 1. | Plate no.1 | G+ve | Motile | Rod | | | | |
| 2. | Plate no.2 | G-ve | Motile | Rod | | | | |
| 3. | Plate no.3 | G-ve | Motile | Rod | | | | |
| 4. | Plate no. | G-ve | Motile | Rod | | | | |
| | Ganga Munda | | | | | | | |
| 5. | Plate no.1 | G+ve | Non motile | rod | | | | |
| 6. | Plate no.2 | G+ve | Motile | Rod | | | | |
| 7. | Plate no.3 | G-ve | Motile | rod | | | | |
| 8. | Plate no. 4 | G-ve | Non motile | Rod* | | | | |

Rod*- Coccobacillus

Table 5. Results of colony characteristics of bacteria isolated from water samples of both the ponds.

| S.No | Samples | Colony characteristics | | | | | | | | | | |
|------|--------------|------------------------|------------------|-------------|-----------------|----------|-----------|--|--|--|--|--|
| | | Colony surface | Colony colour | Density | colony shape | Margin | elevation | | | | | |
| | Dalpat Sagar | | | | | | | | | | | |
| 1. | Plate no.1 | rough | Dull | Opaque | irregula | undulate | flat | | | | | |
| 2. | Plate no.2 | Smooth surface | Creamy yellow | Transparent | Circular | Even | Raised | | | | | |
| 3. | Plate no.3 | rough | Pale yellow | Opaque | Circular | Undulate | raised | | | | | |
| 4. | Plate no. 4 | smooth | whitish | Translucent | circular | entire | convex | | | | | |
| | Ganga Munda | | | | | | | | | | | |
| 5. | Plate no.1 | rough | grayish | Translucent | circular | wavy | flat | | | | | |
| 6. | Plate no.2 | rough | Dull | Opaque | irregula | undulate | flat | | | | | |
| 7. | Plate no.3 | smooth | whitish | Translucent | circular | entire | Convex | | | | | |

| 8. | Plate no. 4 | smooth | Whitish | Transparent | circular | domed | convex |
|----|-------------|--------|---------|-------------|----------|-------|--------|
| | | | grey | | | | |

Table 6. Biochemical characteristics of the isolated organisms from samples obtained from Dalpat sagar pond system.

| S. No. | Samples | Indo le | MR | VP | Citrate utilizat ion | amyl ase | catal ase | H2S Product ion | Fermentation | | Identified organisms | |
|-----------|-----------------|------------|----|----|----------------------------|-------------|--------------|-----------------------|--------------|-------------|-------------------------|-------------------------------|
| | | | | | | | | | gluc ose | Sucr ose | Lact ose | |
| | DalpatSag ar | | | | | | | | | | | |
| 1 | Plate no.1 | - | - | + | + | + | + | - | A | A | - | Bacillus cereus |
| 2 | Plate no.2 | + | + | - | - | - | + | + | AG | AG | - | Proteus vulgaris |
| 3 | Plate no.3 | - | - | - | + | - | + | - | - | - | - | Pseudomo nas aeruginosa |
| 4 | Plate no.4 | + | + | - | - | - | + | - | AG | A | AG | E.coli |

Table 7. Biochemical characteristics of the isolated organisms from water samples of Ganga Munda pond system

| S. No. | Samples | Ind ole | MR | VP | Citrate utiliza tion | amyl ase | catal ase | H2S produc tion | Fermentation | | | Identified organisms |
|-----------|----------------|------------|----|----|----------------------------|-------------|--------------|-----------------------|--------------|-------------|----------|-------------------------------|
| | | | | | | | | | Gluc ose | sucr ose | Lact ose | |
| | Ganga Munda | | | | | | | | | | | |
| 1. | Plate no.1 | - | - | - | - | + | + | + | Α | - | - | Corynebacterium diptheriae |
| 2 | Plate no.2 | - | - | + | + | + | + | - | A | A | - | Bacillus cereus |
| 3 | Plateno. | + | + | - | - | - | + | - | AG | A | AG | E.coli |
| 4 | Plate no4 | - | - | - | - | - | + | - | - | - | - | Bordetella pertusis |

A-acid production

AG- Acid and gas production

Detection of Coli phage

Result for detecting the presence and absence of coli phage in Dalpat Sagar and Ganga Munda pond is tabulated under table no.8 and table no.9.

Table no 8. Tabulation indicating the presence (+) or absence (-) of plaques in each of the cultures of Dalpat Sagar pond in the chart.

| Drops of phage | 1 | 2 | 3 | 4 | 5 |
|---------------------------|---|---|----------|-----------|-----------|
| filtrate Plaque formation | - | - | +, 8 PFU | +, 50 PFU | +, 80 PFU |

Table no. 9. Tabulation indicating the presence (+) or absence (-) of plaques in each of the cultures of Ganga munda pond in the chart.

| Drops of | 1 | 2 | 3 | 4 | 5 |
|-------------------|---|---|---|---|---------|
| phage filtrate | | | | | |
| Plaque | - | - | - | - | +,4 PFU |
| formation | | | | | |

DISCUSSION

Comparative analysis of overall physico-chemical parameters of water samples collected from Dalpat Sagar pond system and Ganga Munda pond system on 1st August 2014 have been interpretated graphically in the figure. No. 1, 2, 3, 4, 5, 6, 7, 8, 11, 24.

After analyzing the smelling sense of the water samples of both the ponds, they were found odorless. Slight difference was found in the colour of water samples as water of Dalpat sagar was found to be light yellow and Ganga Munda was found to be quite pale yellow.

The pH values of the water samples of both the ponds analyzed using pH meter was found to be pH 7 for Dalpat sagar and pH 8 for Ganga Munda pond system. On comparison it was found that pH value of Ganga Munda is more than the Dalpat sagar, which prove out to be slightly alkaline as explained graphically in fig 2. Although the pH values of both the pond system was found within the permissible range of 6 to 8 by WHO [7]. Medera *et al.* [8] reported that the pH of most natural water ranges from 6.5-8.5 while deviation from the neutral 7.0 is caused due to CO2/ bicarbonate/ carbonate equilibrium.

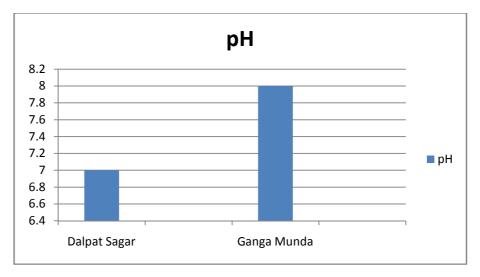


Fig 1. Graph showing pH values of the ponds.

The turbidity value of Dalpat sagar resulted out was 0.14 NTU lower than that of Ganga Munda i.e. 0.26 NTU as shown in fig 2. Turbidity of both the pond systems are lower than the standard acceptable value of ISI i.e. 5 NTU. It means clarity of both the ponds are quite high but on

comparing it was found out that the water of Dalpat sagar posses more water clarity than Ganga Munda because lack of turbidity results in clarity and clearness. Ramesh *et al.* [9] reported the turbidity value of ground water at Erode from min to max range as 0 to 16 NTU with an average value of 0.86 NTU which was well within the acceptable limit given by ISI.

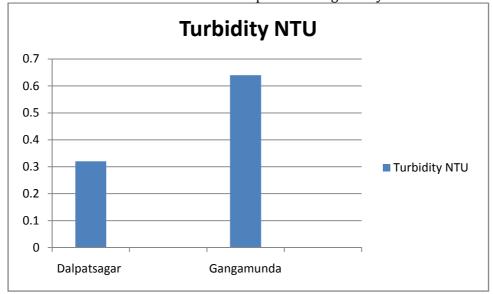


Fig 2. Graph showing turbidity values of both the ponds in NTU.

The Total dissolved solids (TDS) of Dalpat sagar was found out to be 200 mg/lt while that of Ganga Munda pond system was found out to be 300 mg/lt as shown graphically in fig 3. The acceptable limit given as standard value for TDS by ISI is 500 mg/lt. The high values of TDS are unsuitable for drinking and irrigation [9]. On comparing the TDS of both the ponds it could be interpretive that Dalpat sagar pond is more suitable for drinking purpose than Ganga Munda pond.

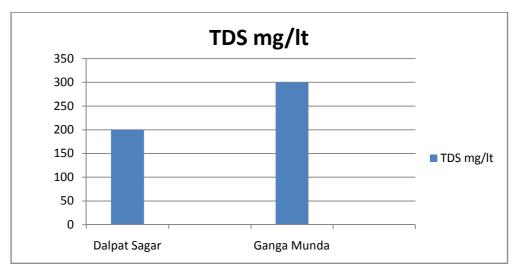


Fig 3.Graphical representation of TDS values of Dalpat Sagar and Ganga Munda pond in mg/lt

Dissolved oxygen can be referred to as an important indicator of healthy aquatic ecosystem as oxygen is necessary for aquatic animals **[5]**. According to ISI (1982), 4 mg/lt is the tolerance limit for DO for inland surface water subjected to pollution **[5]**. For good water quality the optimum value for DO is 4-6 mg/lt, which ensures healthy aquatic life in a water body **[10]**. DO values resulted out for Dalpat Sagar was 9.2 mg/lt and that of Ganga Munda was 11.6 mg/lt given in fig 4. DO values of both ponds exceed the standard values so, it is concluded that aquatic ecosystems of both the ponds are disturbed and both of them doesn't ensures healthy

aquatic life in a water body. If we compare the DO values of both the ponds it is concluded that aquatic ecosystem of Ganga Munda is more disturbed and unhealthy than the Dalpat sagar aquatic ecosystem.

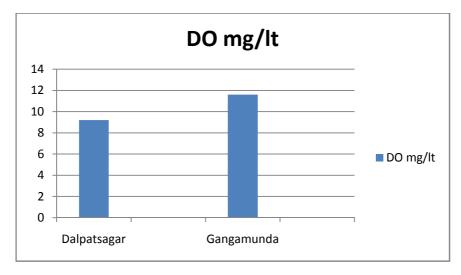


Fig 4.Graphical representation of DO values of Dalpat Sagar and Ganga Munda pond in mg/lt.

BOD values resulted out for Dalpat sagar and Ganga Munda pond were 3 mg/lt and 7.4 mg/lt as shown graphically in fig no.5. Those water bodies not meeting the desired water quality standards and also those having their BOD level 6mg/lt are considered as polluted water bodies/stretches [11]. Therefore again on comparing BOD values of both the ponds, Ganga Munda pond is found to be more polluted than Dalpat sagar pond, because it exceeds the standard values. Similarly Raman *et al* [12] reported the BOD values of the canal water above the tolerance limits for inland surface water quality standards subject to pollution.

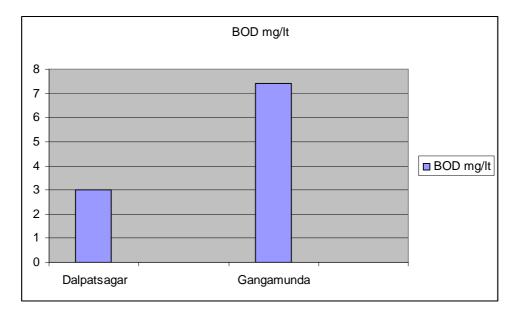


Fig 5.Graphical representation of BDO values of Dalpat Sagar and Ganga Munda pond in mg/lt.

Estimation of BOD alone could not give the exact idea of pollutants present in water, therefore, COD is estimated. Due to rapidly increasing industrialization near the ponds and lakes chemically oxidisable organic substances discharged in water depletes the amount of oxygen. COD refers to the oxygen consumed by the oxidisable organic substances. COD detected for Dalpat sagar pond was 0.32 mg/lt and Ganga Munda pond was 0.64 mg/lt represented

graphically in fig no.6, which are quite lower than the standard value of COD i.e. 10mg/lt. It means chemically they are less polluted but on a survey it was found out that some industrialization is there near the Ganga Munda pond which might be the reason for its greater COD value than the Dalpat sagar pond.

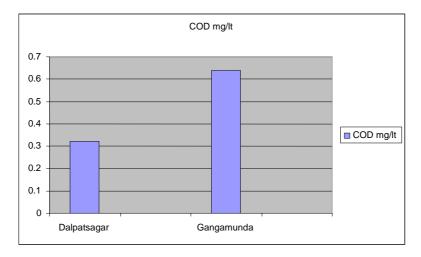


Fig 6.Graphical representation of COD values of Dalpat Sagar and Ganga Munda pond in mg/lt.

Alkalinity of water represents the presence of hydroxyl ions (-OH) in water; hence it is capacity of water to neutralize a strong acid. After detection it was found out that the total alkalinity of water of Dalpat sagar pond is 18 mg/lt and that of Ganga Munda is 42 mg/lt lower than that of standard value recommended by ISI i.e. 200 mg/lt. but still on comparing the two ponds, total alkalinity of Ganga Munda found to be greater than that of Dalpat sagar pond as shown graphically in fig .7

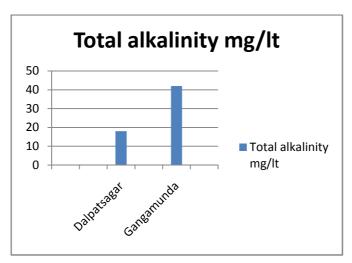


Fig 7.Graphical representation of total alkalinity values of Dalpat Sagar and Ganga Munda pond in mg/lt.

Chloride is also one of the most important parameter in assessing the level of water quality [5]. If the concentration of chloride is high in water, it is considered to be an indicator of pollution due to discharge and accumulation of animal wastes and industrial effluents [13]. It is the indicator of contamination with human and animal waste [14]. According to Cholonky [15] high value of chloride is not good for irrigation and also harmful to aquatic life. Here the chloride value detected out from water of Dalpat sagar pond is 1171 mg/lt and that of Ganga Munda is 1544 mg/lt which are higher than that of the standard value i.e. 250 mg/lt. It means that both ponds are unsuitable for irrigation purpose and moreover both the ponds are highly

contaminated with human and animal waste. But on comparison it was found that Ganga Munda pond was more highly contaminated with animal and human waste because it contains more chloride than Dalpat sagar pond as shown graphically in fig 8.

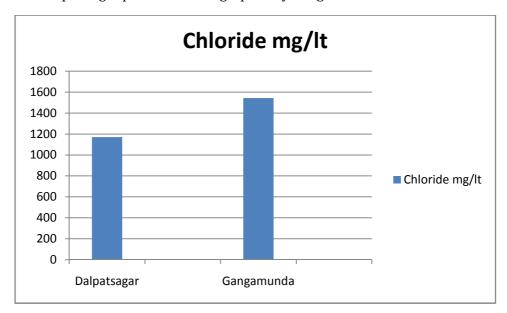


Fig 8.Graphical representation of chloride values of Dalpat Sagar and Ganga Munda pond in mg/lt.

Standard plate count method which was carried out to count the no. of bacterial colonies in CFU/ml present in both the pond systems detected out to be were $141x10^4$ CFU/ml for Ganga Munda pond and $46 \ x10^4$ CFU/ml for Dalpat sagar pond. After calculation it was clear that Ganga Munda pond system contain more bacterial population than Dalpat sagar found as shown in fig 9and 10 and also shown graphically in fig 11



Fig. 9.Bacterial colonies found in Dalpat sagar. Fig 10.Bacterial colonies found in Ganga munda.

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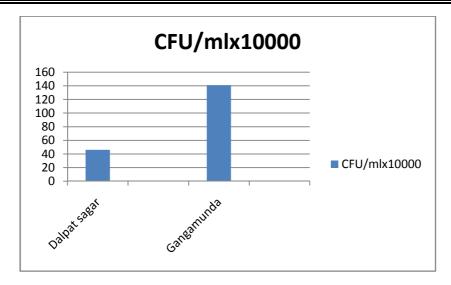


Fig 11.Graphical representation of bacterial populationin CFU/ml of Dalpat Sagar and Ganga Munda pond.

On the basis of staining, motility test, colony morphology, colony characteristics and after performing biochemical activities specific bacterial populations were identified in both the ponds. *Bacillus cereus* (plate no.1), *Proteus vulgaris* (plate no.2), *Pseudomonas aeruginosa* (plate no.3), *E. coli* (plate no.4) were identified in Dalpat sagar pond and *Corynebacterium diptheriae* (plate no.1), *Bacillus cereus* (plate no. 2), *E. coli* (plate no.3), *Bordetella pertusis* (plate no.4) were identified in Ganga Munda pond.

Bacillus cereus (plate no.1 of Dalpat sagar pond and plate no.2 of Ganga Munda pond)) is a gram positive motile rod shaped bacteria as shown in fig no.12 and 13, was identified as common bacteria in both Dalpat sagar and Ganga Munda pond. This pathogenic bacterium is responsible for causing nausea, vomiting, diarrhea [16].



Fig. 12: gram positive rod shaped Bacillus cereus (plate 1) of Dalpata sagar pond.

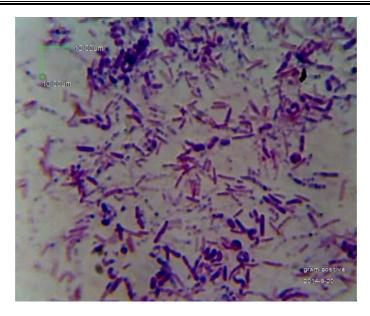


Fig 13: Gram positive, rod shaped *Bacillus cereus* (plate 2) isolated fronm Ganga Munda pond.

Proteus vulgaris (plate no.2) was detected out from Dalpat Sagar pond is a gram –ve motile rod shaped bacteria, as shown in fig no 14, an opportunistic pathogen of humans generally found in soil, water, and fecal matter and known to cause urinary tract infections and wound infections.

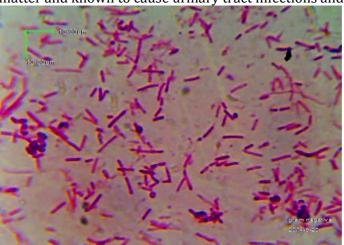


Fig 14: gram negative rod shaped *Proteus vulgaris* (plate 2) isolated from Dalpat sagar pond

Pseudomonas aeruginosa (plate no. 3) detected out in Dalpat sagar pond are gram negative motile rod shaped bacteria as shown in fig no. 15, is a common bacterium that can cause disease in animals and humans generally found in soil, water, and manmade environments. It infects the urinary tract, burns, and wounds and also causes other blood infections **[17]**.



Fig 15: gram negative, rod shaped *Pseudomonas aeruginosa* (plate 3) isolated fron Dalpat sagar pond.

E.coli (plate no. 4 of Dalpat sagar and plate no.3 of Ganga Munda pond) was also found to be common bacteria detected out from both the ponds which is a gram negative rod shaped motile bacterium as given in fig no. 16 and 17 which mostly do not cause disease but some virulent strains can cause gastroenteritis, urinary tract infections, neonatal meningitis, septicemia, pneumonia [18].



Fig 16: gram negative, rod shaped E. coli (plate 4) isolated from Dalpat sagar pond.

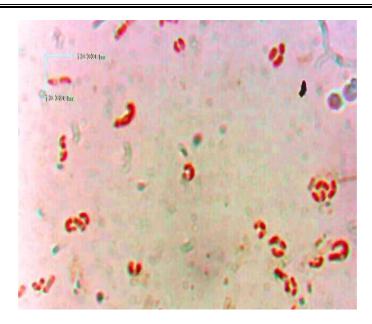


Fig 17: gram negative, rod shaped E. coli (plate 3) isolated from Ganga Munda pond.

Corynebacterium diptheriae, a gram positive rod shaped non motile bacteria as shown in fig no.18 detected out from Gangamunda pond is a causative agent of diphtheria [19]. Generally found in the mouth, normal skin flora of humans and animals, bodily secretions, soil, water, food products and gets spread through direct contact [20, 21].

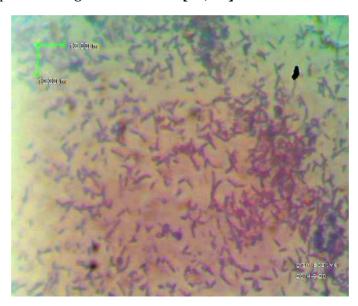


Fig 18: gram positive rod shaped *Corynebacterium diptheriae* isolated from Ganga Munda pond.

Bordetella pertusis obtained from Ganga Munda pond is a gram negative coccobacillus (intermediate between cocci and bacilli) as shown in fig no.19, causative agent of pertusis or whooping cough **[22]** and prove itself to be pathogenic bacteria.

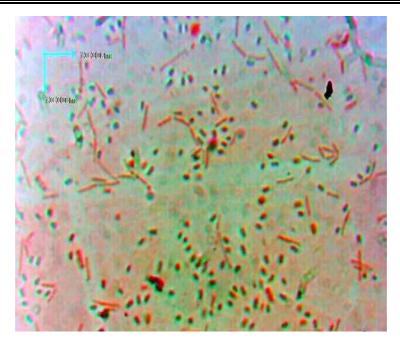


Fig 19: gram negative coccobacilli, *Bordetella pertusis* (plate 4) isolated from Ganga Munda.

Coli phage is a type of bacteria that infects E.coli. As plaque formation is indicative of presence of coli phage in the culture therefore, for Coli phage detection as shown in table no. 8 and 9, the relationship between the number of plaques observed and the number of drops of filtrate in each culture is examined. On the basis of this relationship it is concluded that both of them contain fewer no of coli phages but on comparison it was found out that Dalpat Sagar pond contain more no. of coli phages than Ganga Munda pond because in Dalpat sagar at 3rd drop 8, 4th drop50 and at 5th drop 80 plaques formations were observed indicated with (+) sign which when compared with Ganga Munda pond it was observed that plaque formation was observed lastly at 5th drop i.e. 4 PFU, as shown in fig no. 20,21,22,23. It clears that at lower concentration of only three drops 8 plaque was seen in Dalpat sagar pond but in Ganga Munda at higher concentration of 5 drops only 4 plaque was seen, which again clears the picture that more coli phages are present in Dalpat sagar pond detected out in 3rd drop only but in Ganga Munda at higher concentration of 5 drop it was detected out which proves that it contains lower no of coli phages as shown graphically in fig 24.



Fig 20: Culture of drop 3 showing plaques isolated from Dalpat sagar pond.



Fig 21: Culture of drop 4 showing plaques isolated from Dalpat sagar pond.

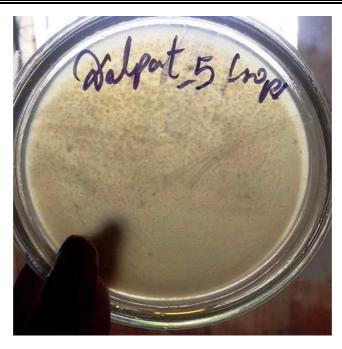


Fig 22: Culture of drop 5 showing plaques isolated from Dalpat sagar pond.

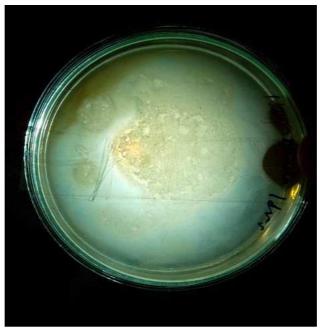


Fig 23: Culture of 5th drop showing plaques isolated from Ganga Munda pond.

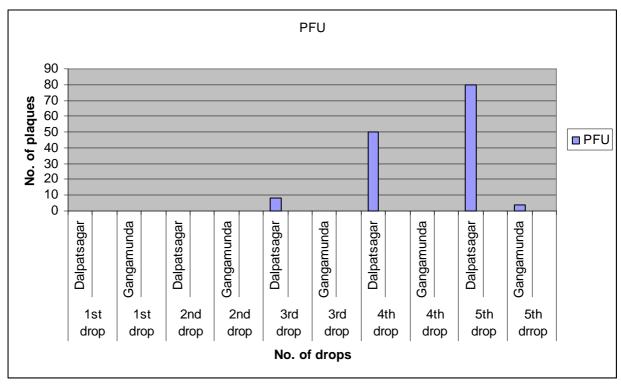


Fig 24: graphical representation of the no of coliphages obtained dropwise from both the ponds.

CONCLUSION

After complete physico-chemical and microbial; analysis of both the ponds in a comparatively manner lastly it is interpreted that Ganga Munda pond is more polluted than Dalpat sagar pond at both physic- chemical and microbial analysis point of view. Physico- chemical analysis showed that although the turbidity and TDS values were found within the acceptable limit recommended by ISI but on comparison turbidity and TDS values of Ganga Munda was higher than Dalpat sagar. DO of both the ponds exceed the standard values and Ganga Munda was found out to have higher DO value. BOD level of Dalpat sagar pond was found out within the standard value but Ganga Munda pond exceeded the value which clears that it is highly polluted with bacteria. COD values of both the ponds were found within the range of standard values but still on comparison COD of Ganga Munda is greater than Dalpat Sagar that means it is highly polluted than Dalpat sagar. Total; alkalinity level of both the ponds were also found within the standard range but again on comparison Ganga Munda's total alkalinity value was higher. Chloride level of both the ponds exceeded the desired limit recommended by ISI, and lastly it was again found out that chloride value of Ganga Munda was higher than Dalpat Sagar.

Microbial analysis proved that Ganga Munda possessed more bacterial population than Dalpat sagar and pathogenic bacteria like *Bacillus cereus, Proteus vulgaris, Pseudomonas aeruginosa, E. coli* were identified in Dalpat sagar pond and more pathogenic bacteria like *Corynebacterium diptheriae, Bacillus cereus, E. coli, Bordetella pertusis* were identified in Ganga Munda pond. On the basis of coli phage analysis it was also interpreted that Dalpat sagar pond possess more coli phages than Ganga Munda pond. Hence, on the basis of overall physicochemical and microbial analysis we can say that water of Ganga Munda pond is unsafe for drinking and other domestic purposes.

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