

# Physico-chemical and Microbiological Characterization of Recycled, Open and Eutrophicated Lake Water Grabs and Assessment of its Effects on A Vertebrate System Zebra Fish (*Danio rerio*): A Case Study of Mallathahalli Lake, Bangalore, India

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Bangalore is a metropolitan and capital city of the state of Karnataka in India, it is considered to be the fastest growing Information technology and Biotechnology hub of the world. In contrast with the same the city is subjected to continuous development through anthropogenic activities where in it has lost most of its wetlands like lakes and the existing water bodies are exploited to the highest order the resultant of which is seen through the spread of diseases and loss of existing natural aquatic bodies and loss of ecosystem. In the present study grab water samples were collected from three distinct sampling stations of Mallathahalli Lake, Bangalore, India and assessed for various physical and chemical parameters betwixt December 2014 and February 2015; Microbiological evaluation was also extrapolated for the lake betwixt the same period. The average values recorded for the physical parameters like pH and temperature for Recycled, Open and Eutrophicated lake water was found to be within the limit. The average values recorded for the chemical parameters from the three sampling stations were found to be within the standards set by World Health Organization (WHO) for drinking water quality. The DO, BOD and COD values were found to be varying in the three different sampling stations implicating organic pollution. Bacteriological examination of water indicated high microbial activity and proved water to be unfit for human consumption. The effect of the water quality was supported by heart beat count and acetyl choline esterase activity which was established in zebra fish a vertebrate model. Zebra fish analysis concluded recycled water to be of better quality when compared to eutrophicated and open water system.

**Key words:** Mallathahalli Lake, Zebra fish, *Danio rerio*, Acetyl choline esterase activity, heartbeat count.

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Wetlands play an important role in survival of life forms on planet earth and they are a pivotal source of water. Water is one of the basic amenities for survival of fauna and flora; lakes, estuaries, rivers etc form an integral part of wetland

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ecosystem. Lentic ecosystems especially lakes are the most productive ecosystems of the biosphere and they are constantly deteriorated by chemical pollution and anthropogenic activities; this has lead to loss of ecosystem and reduced socio-economic development (Constanza 1997, Rapport *et al.*, 1998). The lakes occupied about 4.8% of geographical area of Bangalore and served as the major water source for drinking and irrigation

(Ramachandra *et al.*, 2001), The wetlands account for drinking water, fish culture, replenish ground water and also reduce natural calamities (Gurunathan 2006), Human activities has led to loss of wetlands the resultant of which is discontinuation of drainage networks in the city of Bangalore (Deepa *et al.*, 1998) and the surviving lakes are reduced to cesspools due to continuous exploitation and dumping of waste (Ramachandra 2007). High pH, TDS, Sulphates and Phosphates has altered the nutrient concentration of the lakes in Bangalore and reduced the dissolved oxygen levels in the lakes leading to fish mortality (Zutshi *et al.*, 2008). Fish death and sewage disposal has increased the microbial population resulting in diseases and infections. According to APHA (1992) pathogenic organisms transmitted through water are Salmonella, Klebsiella, *E.coli*, Shigella etc. Microbial population cause spoilage of fish after its death and also results in its decomposition (Nandini 1995). Water clogging inside water distribution system results in formation of bio-films and may result in spread of disease (Lee *et al.*, 2010). The chemical pollutants and microbial population has largely deteriorated the water quality which in turn has influenced the composition, distribution and abundance of macro invertebrates and vertebrates (Zutshi *et al.*, 2007), unplanned development of the city, industrial and domestic wastes, autochthonous wastes mainly due to plants have a direct effect on the water quality, influencing the light penetration and efficacy of the lakes (Ramachandra 2009). Ecological succession and growth of water weeds is the result of eutrophication observed in ibalur lake, Bangalore (Raj *et al.*, 2014). Mallathahalli lake water was hard water type and can be used for irrigation (Ravikumar *et al.*, 2013). Wetland ecosystems like lakes in Bangalore are largely degraded by pollutants and urbanization, with this ideology the objective of the study was to assess Mallathahalli Lake for physico-chemical characters and microbial characters and to evaluate its effects on zebra fish (*Danio rerio*) a vertebrate model.

## MATERIALS AND METHODS

### Sampling station- Mallathahalli Lake

Mallathahalli Lake is a natural fresh water lake which is positioned at latitude of 12.9603 and

a longitude of 77.4967, it is situated about 14 kilometres from the Kempegowda bus terminus and categorised under the south ward of urban Bangalore, India. The lake has a periphery of about 3.5 kilometres with a catchment area of 625 hectares; the lake was recouped in recent years for recreational purpose by bunds and fencing. Million litres per Day – Sewage Treatment Plant (MLD-STP) was set up to treat sewage water. The lake is bordered by a crematorium to the south, areca plantation to the west, a sewage treatment plant to the north and it is encroached by houses to the east. Three sampling stations were identified to collect water samples. Sample A- Recycler was a point near MLD-STP, at this point sewage water was recycled and ingested into the lake, Sample B- Boat camp was the second sampling station and this area was of the open water system thereby was well aerated and received good sunlight. Sample C- Plant was the site where in plants was found growing along with algal forms indicating high degree of eutrophication.

Sampling method:

### Physico chemical and Microbial characterization

BOD bottles and well cleaned plastic bottles were used to collect water samples from the sampling stations betwixt December 2014 and February 2015. Dissolved oxygen was fixed at the site, pH and temperature was determined at the sampling stations. Standard procedures prescribed in NEERI lab manual by Trivedi and Goel (1986) and APHA (1998) were used to judge the parameters like DO, BOD, COD, Sulphates, Calcium, Alkalinity, Ammonical nitrogen, Turbidity, Sodium and Potassium. Standard methods prescribed in lab manual of Aneja (2003) was followed to determine microbial parameters like most probable number (MPN) and total microbial counts.

### Zebra fish (*Danio rerio*) examination

Fully developed breeding stock of zebra fish were procured from local aquarium vendors and grown for a week in small glass tanks fitted with a sieve at the bottom. The tanks were well aerated and lit. The fishes were fed with flakes twice a day and were allowed to fertilize. Spawning happened early in the morning and the eggs were trapped in the sieve which was further transferred to petriplates containing reverse osmosis water and the lake water samples to establish morphological changes and heart beat count of

the embryos to correlate their growth and development.

**Assay for Acetyl choline esterase activity**

Titrimetric method for acetylcholine esterase activity (Guruprasad *et al.*, 2014) was followed. Adult male fishes which were grown in the lake water samples was subjected to euthanization to dissect out the brain cells, the brain cells were homogenized with phosphate buffer in a pre-chilled mortar using the pestle. The homogenate was centrifuged with phosphate buffer at 4° C and the supernatant served to be the enzyme source. The supernatant was diluted to 20X strength for further assay. The diluted enzyme solution was mixed with acetylcholine iodide and titrated with sodium hydroxide with phenolphthalein as the indicator.

**RESULTS AND DISCUSSION**

The lake water was analysed in three different sampling stations betwixt December 2014 and February 2015. The data for various physical and chemical parameters measured are tabulated. The data for Sample A – Recycler over the three sampling seasons is recorded in table 1. Similarly

the data for the sample B- Boat camp and sample C- Plant are recorded in table 2 and 3 respectively. The microbial parameters which were examined are also recorded and indicated in table 5 and 6 respectively. The zebra fish analysis which was carried out to determine the effects on a biotic system are tabulated in table 7 and 8.

The pH of the lake water was found to be 7.31, the average pH value of the Recycler, Boat camp and Plant was found to be 7.53, 7.26 and 7.13 respectively. High pH imparts a bitter taste to the water and may also corrode water distribution system. A pH range of 6-9 is expected for survival of aquatic life failing which there is slow deterioration of life forms (Alabaster *et al.*, 1980). Temperature of lake water is dependent on the dissolved oxygen (Salah 2014); higher temperature will result in chemical reactions. The mean values of temperature were found to be 22.33 samples A and B and 22.66 for sample C respectively. Dissolved oxygen is supposed to be 5mg/L for ecological sustenance (Ramachandra *et al.*, 2001) below which it may lead to stress in survival (Sisodiya *et al.*, 2006). The DO values for day 1 and day 5 observed is depicted in figure 5. Sample C- Plant showed a very low DO level. Highest DO

**Table 1.** Indicating the Physico-Chemical parameters for the grab sample A - Recycler for the month of December 2014, January and February 2015

S No	Physico-chemical Parameter	World Health Organization (WHO) Standards	Grab 1- Recycler Dec- 2014	Grab 1- Recycler Jan - 2015	Grab 1- Recycler Feb-2015	Average Values for Recycler	Standard deviation For Recycler
1	pH	6.5-8.5	7.6	7.6	7.4	7.533	0.11547
2	Temperature	-	22	21	24	22.333	1.527525
3	DO	5.0	5.8,5.2	4.8,3.2	3.2,2.4		
4	BOD	28.30	0.6	1.6	0.8	1	0.52915
5	COD	10	68	73.0	73.6	71.533	3.0746
6	Calcium	75-200	64	62	66	64	2
7	Sulphates	42-45	4	1.2	0.65	1.95	1.796524
8	Total alkalinity	200	140	95	95	110	25.98076
a.	Hydroxide alkalinity	0	0	0	0	0	0
b.	Carbonate alkalinity	-	16	10	10	12	3.464102
c.	Bicarbonate alkalinity	-	140	85	85	103.333	31.75426
9	Turbidity	5 NTU	1.5	1.5	1.5	1.5	0
10	Sodium	200	48	46	48.5	47.5	1.322876
11	Potassium	-	5	6	5	5.3333	0.57735
12	Ammonical nitrogen	50	<1	<1	<1	<1	

recorded was 2.3 and the lowest was 1.6. These DO values indicated are below the WHO standards for the sample C which also proves stress conditions for survival of aquatic life. BOD is the oxygen requirement for breakdown of organic compounds, low BOD indicates good water quality

**Table 2.** indicating the Physico-Chemical parameters for the grab sample B - Boat Camp for the month of December 2014, January and February 2015

S No	Physico-chemical Parameter	World Health Organization (WHO) Standards	Grab 2- Recycler Dec-2014	Grab 2- Recycler Jan -2015	Grab 2- Recycler Feb-2015	Average Values for Recycler	Standard deviation For Recycler
1	pH	6.5-8.5	7.2	7.3	7.3	7.266667	0.057735
2	Temperature	-	21	22	24	22.333333	1.527525
3	DO	5.0	5.2,4.9	4.7,2.9	4.8,1.6		
4	BOD	28.30	0.3	1.8	3.2	1.766667	1.450287
5	COD	10	435	430	441.6	435.5333	5.818362
6	Calcium	75-200	82	84	86	84	2
7	Sulphates	42-45	40	34	35	36.333333	3.21455
8	Total alkalinity	200	152	155	155	154	1.732051
a.	Hydroxide alkalinity	0	0	0	0	0	0
b.	Carbonate alkalinity	-	14	10	10	11.333333	2.309401
c.	Bicarbonate alkalinity	-	152	145	145	147.3333	4.041452
9	Turbidity	5 NTU	31.0	31.0	31.0	31	0
10	Sodium	200	70	73	70.1	71.03333	1.703917
11	Potassium	-	9	8	8	8.333333	0.57735
12	Ammonical nitrogen	50	<1	<1	<1		

**Table 3.** Indicating the Physico-Chemical parameters for the grab sample C - Plant for the month of December 2014, January and February 2015

S No	Physico-chemical Parameter	World Health Organization (WHO) Standards	Grab 3- Recycler Dec-2014	Grab 3- Recycler Jan -2015	Grab 3- Recycler Feb-2015	Average Values for Recycler	Standard deviation For Recycler
1	pH	6.5-8.5	7.2	7.1	7.1	7.133333	0.057735
2	Temperature	-	21	22	25	22.66667	2.081666
3	DO	5.0	2.3,2.1	2.5,2.2	1.6,1.6		
4	BOD	28.30	0.2	0.3	0	0.166667	0.152753
5	COD	10	142	145	140	142.3333	2.516611
6	Calcium	75-200	74	79	74	75.66667	2.886751
7	Sulphates	42-45	12	12	13.4	12.46667	0.80829
8	Total alkalinity	200	150	145	145	146.6667	2.886751
a.	Hydroxide alkalinity	0	0	0	0	0	0
b.	Carbonate alkalinity	-	16	10	10	12	3.464102
c.	Bicarbonate alkalinity	-	150	135	135	140	8.660254
9	Turbidity	5 NTU	6	6	6	6	0
10	Sodium	200	92	96	100	96	4
11	Potassium	-	9	12	9	10	1.732051
12	Ammonical nitrogen	50	<1	<1	<1		

and high BOD indicates water quality to be poor and affects the survival of aquatic life (Shelton 1991). If BOD is less than 3 mg/L then water is considered to be good. 1, 1.76 and 0.16 are the BOD values observed for the three sampling zones which indicate Mallathahalli lake water to be good. COD is a parameter which is reliable to measure the organic pollution of wetlands (Amirkolaie 2008). Table 1, 2 and 3 indicates the values of COD for the three sampling points. 216.46 is the average value of COD observed which is indicated in table 4. The

values indicated for COD in the above tables exceeded the WHO standards and imply that the lake water has a very high organic pollution affecting the survival and metabolism of aquatic life. Calcium is an essential nutrient required by water dwelling organisms; 74.55 mg/L was found to be the average value of calcium in the lake which is found to be within the WHO standard. 1.95, 36.333 and 12.46 mg/L were the mean values of sulphate for the three samples respectively with 16.91 mg/L to be the overall concentration in the

**Table 4.** Indicating the average values of Physico-Chemical parameters for the grab samples A- Recycler, B- Boat Camp and C- Plant the month of December 2014, January and February 2015

Sl No	Physico-chemical Parameter	World Health Organization (WHO) Standards	Average of Grab 1 - Recycler	Average Grab 2- Boat Camp	Average of Grab 3 - Plant	Average Values	Standard deviation
a	pH	6.5-8.5	7.533	7.2666	7.13333	7.310977	0.203497
2	Temperature	-	22.333	22.333	22.6666	22.4442	0.192604
3	DO	5.0					
4	BOD	28.30	1	1.7666	0.16666	0.977753	0.800202
5	COD	10	71.533	435.53	142.333	216.4653	192.99
6	Calcium	75-200	64	84	75.6666	74.55553	10.04619
7	Sulphates	42-45	1.95	36.333	12.4666	16.91653	17.61815
8	Total alkalinity	200	110	154	146.666	136.8887	23.57323
a.	Hydroxide alkalinity	0	0	0	0	0	0
b.	Carbonate alkalinity	-	12	11.333	12	11.77767	0.385093
c.	Bicarbonate alkalinity	-	103.333	147.33	140	130.22	23.57406
9	Turbidity	5 NTU	1.5	31	6	12.83333	15.89287
10	Sodium	200	47.5	71.033	96	71.511	24.25353
11	Potassium	-	5.3333	8.3333	10	7.888767	2.365043
12	Ammonical nitrogen	50	<1	<1	<1	1	0

**Table 5.** Indicating the Most Probable Number results for the grab samples (Recycler, Boat Camp and Plant) for the month of December 2014, January and February 2015

Sampling site	Month	Number of tubes showing positive results			MPN index per 100 ml
		3 out of 10 ml	3 out of 1ml	3 out of 0.1 ml	
Sample A- Recycler	December 2014	3	3	3	2400
	January 2015	3	3	3	2400
	February 2015	3	3	3	2400
Sample B- Boat camp	December 2014	3	3	3	2400
	January 2015	3	3	3	2400
	February 2015	3	3	3	2400
Sample C- Plant	December 2014	3	3	3	2400
	January 2015	3	3	3	2400
	February 2015	3	3	3	2400

**Table 6.** Indicating the colony characters and the gram characteristics of isolated microorganisms

Sample	Colony	Size	Outline	Colour	Shape	Elevation	Texture	Transparency	Gram character
<b>Sample A- Recycler</b>	Colony 1	1.5mm	Irregular	Off White	Circular	Flat	Mucoid	Opaque	G+ Rods
	Colony 2	<1mm	Regular	Off White	Circular	Low convex	Butyrous	Opaque	G+ Cocci
	Colony 3	1mm	Irregular	Off White	Circular	Flat	Mucoid	Opaque	G+ Rods with Endospores
	Colony 4	<1mm Punctiform	Entire	Off White	Circular	Raised	Smooth Mucoid	Translucent	G- Rods
<b>Sample B- Boat camp</b>	Colony 1	<1mm	Regular	Orange	Circular	Low convex	Butyrous	Opaque	G+ Cocci
	Colony 2	1mm	Irregular	Off White	Circular	Flat	Mucoid	Opaque	G+ Rods with Endospores
	Colony 3	<1mm Punctiform	Entire	Off White	Circular	Raised	Smooth Mucoid	Translucent	G- Rods
<b>Sample C- Plant</b>	Colony 1	<1mm	Regular	Orange	Circular	Low convex	Butyrous	Opaque	G+ Cocci in clusters
	Colony 2	<1mm Punctiform	Entire	Off White	Circular	Raised	Smooth Mucoid	Translucent	G- Rods
	Colony 3	2mm	Entire	Yellow	Circular	Convex	Butyrous	Opaque	G+ Cocci in chains

lake which was found to be within the standard. Alkalinity is said to increase with pH and chemical pollutants, the lake water showed a value of 136.88 mg/L. The average values of the sampling zones are indicated in table 1, 2 and 3 respectively. The lake water was found to be within the acceptable range and proved to be good. Clay, silt, organic pollution, phytoplankton may lead to turbidity of water (kishore *et al.*, 2005). 1.5 NTU, 31 NTU, and 6 NTU are the values of the three sampling stations as indicated in table 4. This indicates Sample B to

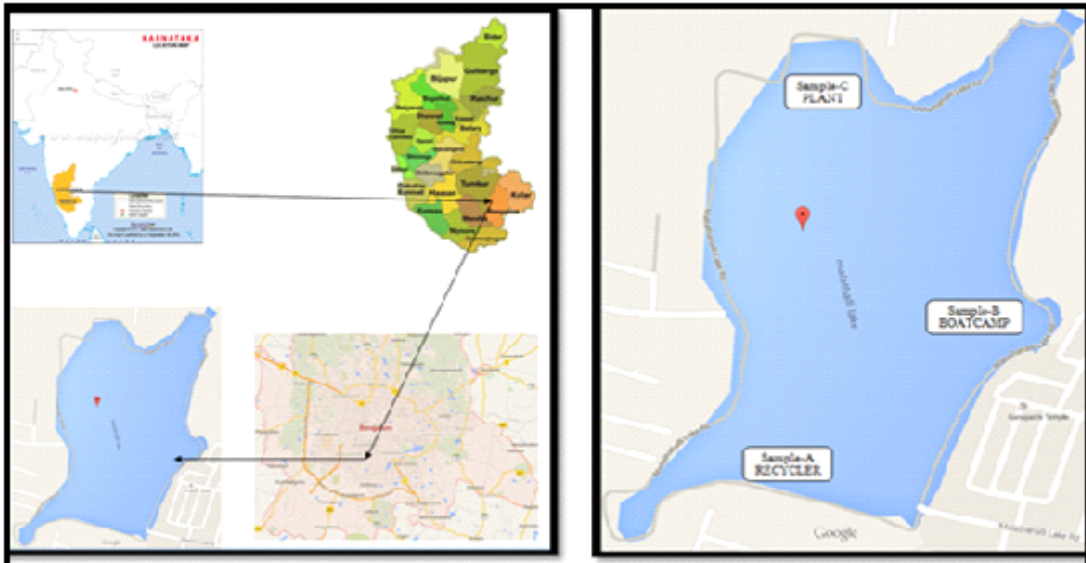
be highly turbid. Ground water contains high sodium depositions when compared to surface waters (WHO 1979). 47.5mg/l, 71.03 mg/l and 96 mg/l were the observed values of sodium in the sampling points Recycler, Boat camp and Plant respectively, these values clearly depict that the

**Table 7.** Indicating the Heart Beat count recorded in Zebra fish (*Danio rerio*) for the samples Recycler, Boat Camp and Plant

S. No	Analyte	Heart Beat Recorded
1.	Control	145/minute
2.	Test 1- Recycler	148/minute
3.	Test 2- Boat Camp	110/minute
4.	Test 3- Plant	70/minute

**Table 8.** Indicating the Acetyl choline esterase activity recorded in Zebra fish (*Danio rerio*) for the samples Recycler, Boat Camp and Plant

S. No	Analyte	Acetyl choline esterase activity
1.	Control	1.7934 micromoles / minute
2.	Test 1- Recycler	1.6943 micromoles / minute
3.	Test 2- Boat Camp	1.9878 micromoles / minute
4.	Test 3- Plant	2.8246 micromoles / minute

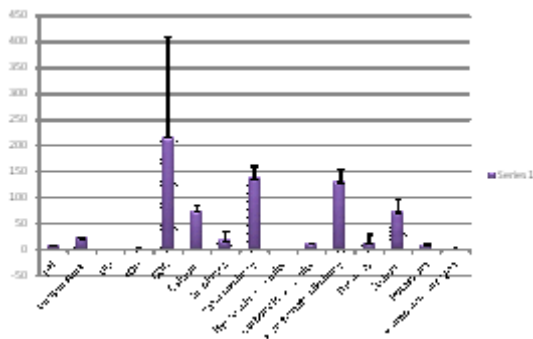


**Fig. 1.** Showing sampling location Mallathahalli like in Bangalore, India

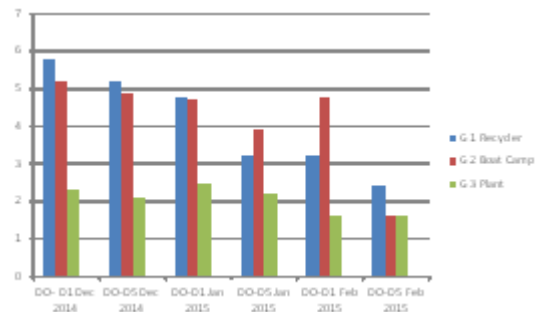
**Fig. 1.** Showing the sampling stations in Mallathahalli Indore



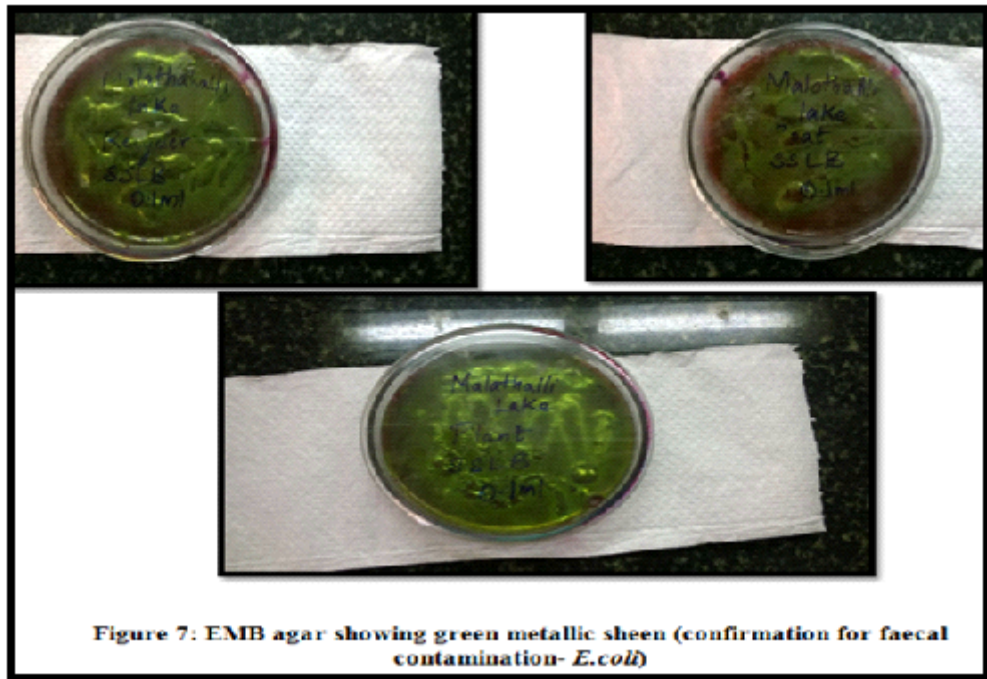
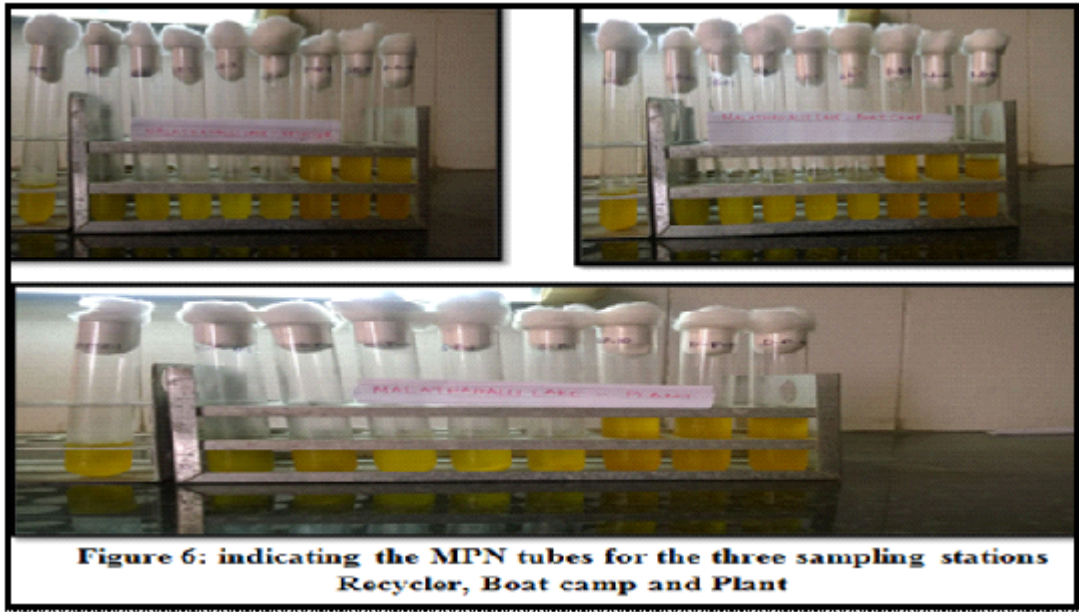
**Fig. 1.** Showing the photographs of the sampling station (A) Recycler (B) Boat Camp (c) Plant



**Fig. 4.** Indicating the average values and the standard deviation of Physico-Chemical parameters



**Fig. 5.** indicating the values of Dissolved oxygen (Expressed in mg/L) for the grab samples Recycler, Boat Camp and Plant for the month of December 2014, January 2015 and February 2015



sample B and sample C have crossed the permissible limit. Migratory ability of potassium is inadequate because of which it found to be in very low concentration in surface waters. 7.88mg/L is the average value of potassium observed in the lake. The individual values the three sampling wings are tabulated in table 1, 2, 3 and 4. Ammonical

nitrogen observed in the lake is negligible. Based on the physico-chemical parameters Mallathahalli Lake water is found to be of good quality for drinking and irrigation. Sampling point A- recycler where in the MLD-STP is stationed was found to be better when compared to sample B- Boat camp that is the open water system and sample C-Plant.



Sample C was found to poor due to organic waste and eutrophication.

The MPN analysis carried out from the three sampling stations showed a very high microbial population, the MPN tubes showed acid and gas formation in all the lactose fermentation tubes which is depicted in figure 6; 2400 microorganisms were found to be present in 1ml of

The water sample. Water samples from the positive tubes were inoculated onto EMB agar and this confirmed the presence of faecal contaminants especially *E.coli* which was confirmed through green metallic sheen on the EMB media. This is depicted in figure 7.

The colony characteristics were determined and the same is recorded in table 6. Gram positive rods, gram positive cocci, gram positive rods with endospores and gram negative rods were observed in the three sampling wings. Gram positive cocci in clusters and gram positive cocci in chains were noticed in sampling point B and C. The microbial examination of the three sampling stations implies that the lake water is poor and unfit for drinking.

The effects of the lake water on a biotic system carried out on zebra fish embryos and adult zebra fish showed variations with respect to heart beat count and external morphology of the embryos. The heart beat count recorded is tabulated in table 7; these values indicate that the heart beat recorded in sample A is proximate to the control set up stipulating good water quality; Whereas the sample B showed deviation from the control indicating poor water quality. Sample C- Plant showed 70 heart beats per minute which is almost half of the control value connoting water quality to be bad.

A very high enzyme activity was observed for sample C-Plant when compared to the control set up. This implies that the neurotransmitter acetylcholine was degraded at a faster rate by the enzyme making the survival of life forms stressful.

### CONCLUSION

The physico-chemical analysis of Mallathahalli Lake holds good for the adjudged parameters except DO and COD. Based on the same water can be used for human activities, the

microbial examination clearly indicates water sample from the lake is completely unfit for human consumption. The evidence of heart beat count and acetylcholine esterase activity indicated that water quality is poor for survival of aquatic life because of stressed environment noticed in sampling station B and C. The recycled lake water (Sample A) was found to be good for biotic life and also gives us clarity that lake water can be monitored by stationing MLD-STP. Proper maintenance of the lake will help us in conservation of the lake, which in turn helps to replenish ground water and also preserves an ecosystem. Similar studies will create awareness about the wetlands especially lakes amidst the society. Educating the society on the same will help in long term existence of the lakes and also helps in homeostatic mechanism of planet earth for the better survival of the human race.

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