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Analysis of heavy metals and coliform in samples of drinking water collected from municipal ward offices of western suburbs of Mumbai, India

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Abstract

14 drinking water samples were collected from Vile Parle to Dadar of Suburban's of (P-South ward offices of B.M.C (Bombay Municipal Corporation) Mumbai in the month from June to December 2012. The samples of drinking-water were analyzed for Cu, Zn, Mn, Fe, As, Cr, Ni, Pb, Cd and Hg. From the results so obtained, the contamination due to heavy metals – Zn (3.115 ppm and 7.816 ppm), Mn (3.115 ppm and 7.426 ppm), Fe (1.124 ppm and 2.872 ppm), As (0.011 ppm to 0.091 ppm), Cr (0.188 ppm and 0.998 ppm), Pb (1.587 ppm and 4.56 ppm) and Cd (0.011 ppm and 0.051 ppm) was found to be high whereas the contamination due to Cu (0.012 ppm and 0.313 ppm), Ni (0.126 ppm and 0.774 ppm), were found below the acceptable limits and no Hg was detected in the samples of drinking-water. In the present work, MacConkey Broth was used as a differential medium for detection and enumeration of coliforms from a wide variety water samples. The presence of positive doubtful presumptive test immediately suggests that the water is non potable (i.e., both acid and gas develops in a tube after 48 hours incubation). Confirmation suggests that there is fecal contamination in the water under investigation and hence it is non potable. All the fourteen samples (streaked from positive Ma cConkey broth tubes) were found to be contaminated with *E. coli,* which was further confirmed by the presence of colonies with green metallic sheen observed under a microscope confirmed that the said samples were contaminated with *E. coli* - the major indicator of fecal contamination.

Keywords: *Coliform, drinking water, E. coli, heavy metals, Mac Conkey broth, municipal ward*

Introduction

Water-related diseases are responsible for 80% of all illnesses/deaths in developing countries, and they kill more than 5 million people every year (WHO, 1995) (UNESCO, 2007). Water, the precious gift of nature to human being is being polluted day by day with increasing urbanization. Although three-fourth part of earth is surrounded by water but a very small portion of it can be used for drinking purposes. Ground water is an important source of drinking water for humankind. It contains over 90% of the fresh water resources and it is an important reserve of good quality water. Ground water, like any other water resource, is not just concerned with public health but is also of economic value (Armon and Kitty 1994). Water is one of the essentials that supports all forms of plant and animal life (Vanloon and Duffy, 2005) and it is generally obtained from two principal natural sources: Surface water such as fresh water from lakes, rivers, streams, etc. and Ground water such borehole water and well water (McMurry and Fay, 2004; Mendie, 2005). Water is not pure as it acquires contaminants from its surrounding and those arising from humans and animals as well as other biological activities (Mendie, 2005). The water pollution by heavy metals has become a question of considerable public and scientific concern in the light of the evidence of their toxicity to human health and biological systems (Anazawa *et al.,* 2004). Heavy metals receive particular concern considering their strong toxicity even at low concentrations (Marcovecchio *et al.,* 2007). They exist in water in colloidal, particulate and dissolved phases (Adepoju - Bello and Alabi, 2009) with their occurrence in water bodies being either of natural origin (e.g. eroded minerals within sediments, leaching of ore deposits and volcanism extruded products) or of anthropogenic origin (i.e. solid waste disposal , industrial or domestic effluents) (Marcovecchio *et al.*, 2007).

The ever increasing human population and industrialization has resulted in tremendous build up of organics in many forms in water. Many unexpected organics are reported to be found even

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in remote parts like hills of Himalayas, Alaska and North Pole due to man-made devastation of our environment. The environmental education has aroused much awareness about the toxicity of traces of environmental pollutants in general and organics in particular.Internationally several organizations namely Food and Agriculture Organization FAO (1983), APHA: (1992), US Environmental Protection Agency (EPA) (2000), US Public Health Services (USPHS) (1991). The National Academy of Sciences (NAS) (1999), USA, etc. have worked on toxicity levels that can influence the human beings on short and long term basis and correlated corresponding symptoms chronic effects and diseases observed. The contaminants contributed in water, have also been reported along with toxicity tests. At present the population of Mumbai is severally suffering from lots of disorders particularly respiratory and digestive, due to air and drinking waters. Most of these causes have been identified and remedial measures have been taken up. However, toxic effect due to contamination of drinking waters, of the population of Mumbai is not primarily addressed and completely neglected. In fact the relevant toxic effect may be already prevalent in the society and most probably they may become severe in due course of time. Hence, the stage has already reached to address the problem in detail and to dig the thought under the problem.It is therefore necessary to determine the extent of contaminants in the water so that the warning signals can be given to the society in case the threshold limits have reached. Even otherwise it becomes necessary to educate the society of the social evils of pollution. The study can also provide the information on possible causes of pollution. So that mitigation measures to minimize provide the information on possible causes of pollution. So that mitigation measures to minimize the pollution can be taken in time.

Material and methods

a) Sample Collection

14 drinking water samples were collected from (Vile Parle west and east; Santacruz west and east; Khar west and east; Bandra west and east; Mahim west and east; Matunga west and east and Dadar west and east) P-*South ward* offices of B.M.C (Bombay Municipal Corporation) from Suburban's of Mumbai in the month from June to December 2012. The water samples were collected in propylene bottles and were stored at – 20 ^{0}C in freezer in the Department of Zoology, S.S & L.S. Patkar College, Goregaon (West) Mumbai for further treatment.

b) Sample Treatment

The water samples were well mixed with 2ml concentrated HNO_3 per liter sample and capped tightly until they were ready for analysis as proposed by (Ehi-Eromosele and Okiei, 2012)

c) Preparation of MacConkey Broth:

Ma cConkey Broth was used as a differential medium for detection and enumeration of coliforms from a wide variety water samples.

d) Preparation of standard metal ion solutions:

Stock solutions (1µg / ml) of each of the metal ions were prepared using appropriate metal salt of AR grade quality in dilute hydrochloric acid. The working standards of these solutions were prepared by appropriate dilutions in distilled water.

e) Instrumentation:

The samples were analyzed on Inductively Coupled Plasma Atomic Emissions Spectroscopy (ICP-AES, Model ARCOS from M/s. Spectro, Germany) at the Sophisticated Analytical Instrument Facility (RSIC), Indian Institute of Technology (IIT) Powai, Mumbai-400076, India.

d) Chemicals and Reagents:

All the chemicals and reagents were procured from S.D. Fine Chemicals and Himedia were of AR grade quality

Results and discussion

Contamination of drinking water with high level of copper may lead to chronic anemia (Acharya et al., 2008). Studies have shown that ingesting copper may also be implicated in coronary heart diseases and high blood pressure, although coronary heart diseases have also been linked to copper deficiency. High levels of copper in drinking water can cause vomiting, abdominal pain, nausea, diarrhea. Darthmouth, (2001) has reported that copper leached into drinking water from copper pipes and described that just as little copper is essential for good health (0.01 mg/l), too much can be harmful. Ingesting large amounts of copper compounds (such as copper sulphate) can cause death by nervous system, liver and kidney failure.

Table 1. Concentration of metal ions in drinking drinking water samples obtained from ward offices of B.M.C (Bombay Municipal Corporation) from Vile Parle to Dadar of Suburban's of Mumbai.

S.NO	Sample	Cu Ppm N=3	Zn Ppm N=3	Mn Ppm N=3	Fe Ppm N=3	As ppm N=3	Cr ppm N=3	Ni ppm N=3	Pb ppm N=3	Cd ppm N=3	Hg ppm N=3
1	Vile Parle (W)	0.230	6.221	5.344	1.186	0.022	0.971	0.153	1.606	0.011	ND
2	Vile Parle (E)	0.216	3.237	5.136	1.277	0.034	0.188	0.126	2.588	0.029	ND
3	Santacruz (W)	0.207	5.512	5.214	1.322	0.054	0.598	0.234	1.626	0.038	ND
4	Santacruz (E)	0.311	4.155	6.145	1.798	0.074	0.527	0.276	3.565	0.024	ND
5	Khar (W)	0.243	7.816	7.426	1.489	0.064	0.664	0.498	2.584	0.029	ND
6	Khar(E)	0.205	7.264	3.115	1.284	0.087	0.719	0.187	1.618	0.031	ND
7	Bandra(W)	0.199	6.143	6.352	1.453	0.032	0.624	0.711	1.585	0.024	ND
8	Bandra(E)	0.207	4.186	5.139	2.872	0.023	0.892	0.676	3.667	0.021	ND
9	Mahim(W)	0.243	4.515	6.115	1.376	0.053	0.979	0.487	4.56	0.018	ND
10	Mahim(E)	0.207	3.434	5.131	1.552	0.062	0.675	0.143	2.525	0.031	ND
11	Matunga(W)	0.311	2.231	4.309	1.787	0.091	0.973	0.213	2.604	0.029	ND
12	Matunga(E)	0.216	5.354	4.214	1.628	0.012	0.321	0.432	3.514	0.039	ND
13	Dadar(W)	0.313	7.155	5.127	1.124	0.028	0.324	0.621	2.628	0.017	ND
14	Dadar(E)	0.112	6.312	7.338	1.259	0.011	0.998	0.774	1.587	0.051	ND

Total reading taken = N (Average reading=3) ND= less than 0.001ppm

Table 2. Determination of MPN (Coliform) in drinking drinking water samples obtained from ward offices of B.M.C (Bombay Municipal Corporation) from Vile Parle to Dadar of Suburban's of Mumbai.

S. No	Water Sample		No. of tubes showing Positive test (10ml) N=3	No. of tubes showing Positive test (1ml) N=3	No. of tubes showing Positive test (0.1ml) N=3	MPN /100 ml
1	Vile Parle	1W	03	02	01	17
		2E	03	02	00	14
2	Santacruz	3W	02	01	01	09
		4E	02	01	01	09
3	Khar	5W	01	01	00	06
		6E	01	01	00	06
4	Bandra	7W	04	04	00	34
		8E	04	04	00	34
5	Mahim	9W	02	03	00	12
		10E	03	02	00	14
6	Matunga	11W	02	00	01	07
		12E	04	03	00	27
7	Dadar	13W	04	04	00	34
		14E	04	04	00	34

(Note: With presumptive test both acid and gas formation is taken as positive)

Average readings = N=3
W= west
E= east
MPN= Most probable number of Coli form
W.H.O (World Health Organization).1993
Satisfactory quality = 05 bacteria / L of water
Drinking water = 03 or less bacteria / L ofwater
Polluted = 100 bacteria / L of water
Slightly polluted = 10 bacteria / L of water
Heavily polluted = 10000 bacteria / L of water

The effect of heavy metal concentration which was higher in untreated sewage water of Musi river near Hydrabad, India was studied by Raj *et al* (2006). The distribution and characterization of heavy metals in water in Jeedimetla industrial area in Andhra Pradesh showed concentration of Cu (4.2 – 13.7ppb), the concentration of this element was found to be far above the permissible level in water (Govil, 2001). The estimated mean concentration of

Cu was 0.500 ppm, in regions around a fertilizer factory in Punjab (Dey *et al,* 1997) was found below the permissible limits. Water sample in industrially polluted areas in Bangalore, Karnataka, India had higher concentration of Cu 33.63ppm (Gowda *et al.*, 2003). Monitoring and assessing the heavy metal like Cu contents in the industrial effluents from Ambanath area in Maharashtra state India, revealed that Cu concentration varied from 80 to 10.2 ppm which is above the specified maximum acceptable concentration (1.0 mg/l) (Lokhande & Sathe, 2001). Tuzen and Soylak, (2006), evaluated the lowest and highest values of copper in Resadiye and Erbaa. In the present work, the lowest and highest mean concentrations of copper are 0.012 ppm and 0.313 ppm respectively. None of the water samples contained copper above the specified maximum acceptable concentration as prescribed by (1.0 ppm) WHO (1998). The main industrial uses of zinc are galvanization and preparation of alloys. It is believed that galvanized pipes, through which water is supplied to various places, is the source of zinc in natural waters including drinking water. Gowda *et al.* (2003) found that the levels of zinc in the water samples obtained from industrially polluted areas in Bangalore, India, were higher (41.09 ppm) than the limit prescribed by WHO, (1998). The mean level of zinc in drinking water samples in drinking water from Xian, China has been reported as 21.84 µg/l (Guo *et al.*, 2004) and that from Tehran, Iran, has been reported as 220 ug/l (Yamini *et al.*, 2004). It has been reported that the concentrations of zinc ranged from 0.086 ppm to 0.163 ppm in samples of drinking water obtained from the surface water level of lower lake in Bhopal, India. These values are lower than the value prescribed by WHO, (1998) (Gupta *et al.*, 2005).In the present work, the lowest and highest mean concentrations of zinc are 3.115 ppm and 7.816 ppm respectively. Thus, the highest mean concentration of zinc is found to be higher than the (5.0 ppm) WHO, (1998) standard.Manganese is a mineral that is required in small amounts in the human body, and it is present in enzymes like oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases (Oga, 2008; Goldberg 1975) which are necessary for several biological functions. Manganese is involved in the function of numerous organ systems and is needed for normal immune function, regulation of blood sugars production of cellular energy, reproduction, digestion, and bone growth. Manganese works with vitamin K to support clotting of the blood and is considered necessary for various biological functions (Goldberg 1975).The effect of heavy metal concentration which was higher in untreated sewage water of Musi river near Hydrabad, India was studied by Raj *et al.*, (2006). Sewage water collected all along the Musi river at different sites was contaminated with Ni, with mean concentration of 0.21 ppm respectively. Almost all the manganese concentration fell within the standard limit (0.05 ppm), which varied from 0.05 ppm to 0.00 ppm from all areas except one sample from Heliopolice with 0.1 ppm (Hanaa *et al.*, 2000).In the present work, the lowest and highest mean concentrations of Mn are 3.115 ppm and 7.426 ppm respectively. Thus, the lowest and highest mean concentration of Mn is found to be above the (0.05 ppm) WHO 1998 standard.Iron in drinking water is present as Fe^2+ and Fe^3+ in suspended form (Sonawane, 2003). Drinking water containing iron above 1.00 mg/l is not considered to be suitable for drinking purposes.

As per the standards set by WHO, the permissible level of iron is 0.3ppm. Above 1.00 ppm of iron in drinking water is not considered to be suitable for drinking purposes WHO. 1993. In North-East region of India, the amount of iron is relatively high and almost all states of India contain iron above the permissible limit. While the lowest iron level was found in water samples obtained from Niksar (14.5±0.1ppm), the highest iron concentration was found in water samples obtained from Resadiye (34.6±0.3ppm). WHO has proposed a guideline value of 0.3 ppm for drinking-water (Singh, 2006).

In the present work the lowest and highest mean concentration of iron are 1.124 ppm and 2.872 ppm respectively, the upper tolerable limit of iron being above the permissible limit as prescribed by WHO.

The main forms of human exposure to arsenic are ingestion and inhalation. The main source of contamination of drinking-water is the naturally occurring arsenic in groundwater. Some of the countries that have such extensive naturally occurring arsenic in groundwater and hence, potentially in drinking water include - India (especially in Bengal), Bangladesh, Nepal, Thailand, China, Mongolia, Tibet, Vietnam, Laos, Cambodia, Myanmar, various South American countries and areas in North America and Western Australia (Michael *et al.,* 2001).The arsenic concentration was found to be a major threat in northeast states of India. It has been reported that arsenic concentration in groundwater exceeds the permissible level (50 µg/l) in Assam, Arunachal Pradesh, Nagaland, Manipur and Tripura states, Arunachal Pradesh.Arsenic concentration was found to be higher in the area adjacent to foothills bounded by Himalayan mountains. The concentration of arsenic was relatively high in shallow tubewell (15- 40 m deep) as compared to deep tube well and rings-well (Singh, 2004). In the present work, the values of the mean minimum and maximum concentrations of arsenic in the drinking-water samples are found to be 0.011 ppm to 0.091 ppm respectively. These values are above the limit prescribed WHO 1993.Chromium is essential to animals and human. Chromium in excess amounts can be toxic especially the hexavalent form. Chromium is used in metal alloys and pigments for paints, cement, paper, rubber, and other materials. Electroplating can release chromic acid spray and air-borne Cr-trioxide, both can result in direct damage to skin and lungs (Grounse, *et al.,* 1983) as well as chromium dust has been considered as a potential cause of lung cancer (Hyodo, *et al.,* 1980). Sub chronic and chronic exposure to chromic acid can cause dermatitis and ulceration of the skin (U.S.EPA, 1999). Long-term exposure can cause kidney and liver damage, and damage too circulatory and nerve tissue. Chromium often accumulates in aquatic life, adding also to the danger eating fish that may have been exposed to high levels of chromium.The lowest and highest concentration of Cr was obtained by Roberto *et al.,* (2008) in drinking water i.e. was 0.06 mg l^{-1}, to 0.08 mg l^{-1}.In the present work, the values of the mean minimum and maximum concentrations of Cr in the drinking water samples are found to be 0.188 ppm and 0.998 ppm respectively. These values are above the limit prescribed WHO 1993. Nickel is used as alloys product, nickel-plating for anticorrosion and in the manufacture of batteries. It is regarded as an essential trace metal but toxic in large amount to human health. It is considered as carcinogenic to human. Ambrose *et al.* (1976) reported that high-dose of nickel in rats and dogs were significantly decreasing their body weights.Hanaa *et al.,* (2000) reported highest nickel concentrations from El-Salam and the lowest from El-Mataria areas and El-Marg which were 1.42 ppm, 1.42 ppm and 0.05 ppm respectively In the present work, the values of the mean minimum and maximum concentrations of lead in the drinking water samples are found to be 0.126 ppm and 0.774 ppm respectively. These values are below the limit prescribed WHO 1993. Lead is a commulative poison and a possible human carcinogen (Bakare, 2005). It is a neurotoxin and is responsible for the most common type of human metal toxicosis (Berman, 1980). Also, studies have linked lead exposures even at low levels with an increase in blood pressure (Zietz *et al*., 2007) as well as with reduced intelligence quotient in children (Needleman, 1993) and with attention disorders (Yule and Rutter, 1985). The possible long term effects of chronic exposure to lead present in drinking water are subject to considerable public concern (Zietz *et al*., 2007). Some of the common sources of lead poisoning are from lead paints, utensils made of lead containing alloys and tetraethyl lead, used as an anti-knock agent in motor fuel. The lead compounds carried into the air as fumes of lead oxide and lead bromide (from motor exhaust) ultimately settle in the field and through food and drinks enter human body (Banerjea, 1995). Adepoju and Alabi (2005), carried out the analysis of 10 samples of well-water and 19 samples of borehole-water and found maximum contaminant level (0.01mg/l) with the maximum concentration detected being 0.024 mg/l.These results are of concern as lead has been recognized for centuries as a cumulative general metabolic poison. Bakir *et al.,* (2003) found that lead contents of the water samples investigated from Tokat city were below 5 µg/l and this level of lead is below the limit prescribed 0.01 mg/l by WHO (1993). In the present work, the values of the mean minimum and maximum concentrations of lead in the drinking water samples are found to be 1.587 ppm and 4.56 ppm respectively. These values are above the limit prescribed WHO 1993. Cadmium is extremely toxic even in low concentrations, and will bio-accumulate in organisms and eco-systems and it has a long biological half-life in the human body, ranging from 10 to 33 years (Es' haghi et *al.,* 2011). Cadmium is considered as one of the priority pollutants from monitoring in most countries and international organizations. Cadmium can produce coughing, headaches, and vomiting. In larger doses, cadmium can accumulate in the liver and kidneys, and can replace calcium in bones, leading to painful bone disorders and to a renal failure Celik and Oehlenschlager (2007). The maximum level of Cd was observed in March with an average of 0.014 mg l^{-1} while the concentration for the rest of the months was quite homogeneous Roberto *et al.*, 2008. Momodu and Anyakora (2010). The effect of heavy metal concentration which was higher in untreated sewage water of Musi river near Hydrabad, India was studied by Raj *et al* (2006). The estimated mean concentration of Cd was 0.006 ppm, in regions around a fertilizer factory in Punjab (Dey *et al*, 1997). The cadmium water samples analyzed by Gowada *et al.,* (2003) were found to be 0.05ppm Gupta *et al.*, (2005) found 0.014 to 0.41 ppm and Hyodo, (2005) found 0.0045 to 0.013 ppm in drinking waters.In the present work, the values of the mean minimum and maximum concentrations of cadmium in the drinking water samples are found to be 0.011 ppm and 0.051 ppm respectively. These values are above the limit prescribed WHO 1993.Naturally occurring mercury has been widely distributed by natural processes such as volcanic activity. Mercury finds use in industrial processes, in electrical appliances (lamps, mercury cells), in industrial and control instruments (thermometers, barometers), in laboratory apparatus and as a raw material for various mercury compounds. The latter are used as fungicides, antiseptics, preservatives, pharmaceuticals, electrodes and reagents. Mercury has also been widely used in dental amalgams. A less well characterized use is in ethnic and folk remedies, some of which can give rise to significant exposure of individuals (IPCS, 2003). Levels of mercury in rainwater are in the range 5–100 ng/litre, but mean levels as low as 1 ng/litre has been reported (IPCS, 2003). Naturally occurring levels of mercury in groundwater and surface water are less than 0.5 µg/ litre. Ware, (1989) has reported that a small number of ground waters and shallow wells in the USA were shown to have mercury levels that exceeded the maximum contaminant level of 2 µg /litre set by the US Environmental Protection Agency for drinking-water (2000). An increase in the mercury concentration up to 5.5 µg / litre was reported for wells in Izu Oshima Island (Japan), where volcanic activity is frequent (Magara et al., 1989). In a contaminated lake system in Canada, methyl mercury was found to constitute a varying proportion of total mercury, depending on the lake (IPCS, 2003). There have been no reports of methyl mercury being found in drinking-water.In the present work, no mercury was detected in the drinking-water samples under investigation. It can therefore be concluded that, if at all mercury was present in the drinking-water samples, its content was less than 0.001 ppm and hence not detected.

MPN (Most probable Number)

MacConkey Broth was used as a differential medium for detection and enumeration of coliforms from a wide variety water samples. The presence of positive doubtful presumptive test immediately suggests that the water is non potable (i.e., both acid and gas develops in a tube after 48 hours incubation). Confirmation of these results is the presumptive tests of result of coli form origin that is recognized as indicators of fecal contamination. With confirmed test all the fourteen samples (streaked from a positive Ma cConkey broth tubes from the presumptive test) were found to be contaminated with *E. coli* the major indicator of fecal contamination (presence of colonies with green metallic sheen).The presence of *E. coli* in the sample of drinking-water is a major health concern and call for remedial attention. The presence of this pathogen in the samples is an indication of the likely presence of other enteric pathogens suggesting that the samples of drinking-water are heavily contaminated with faecal matter (Petridis *et al.,* 2002).The water supplied to the Mumbai city from the different lakes might not be free from contamination. The location of water-pipelines close to potential dumps containing wastes such as expired drugs, batteries, waste oils, synthetic detergents, disinfectants, human and animal wastes, etc. could lead to ground water pollution. Detergents and other chemicals released in water due to activities such as bathing, washing of clothes; etc. in the vicinity of the water body can pollute the groundwater with complex organic and inorganic chemicals. The location of water-pipelines close to drain-pipelines can cause faecal contamination. These contaminants may infiltrate into the lakes thereby constituting a serious health threat. Diseases such as diarrhea, meningitis, acute renal failure, urinary tract infections, and haemolytic anaemia have been known to result from consumption of such contaminated waters as described by (Petridis *et al.*, 2002). In the present work, all the fourteen samples (streaked from positive MacConkey broth tubes) were found to be contaminated with *E. coli*, which was further confirmed by the presence of colonies with green metallic sheen observed under a microscope.

Conclusion

The 14 samples of drinking-water were randomly collected from Vile Parle to Dadar. From the results obtained, the contamination due to heavy metals - Zn, Mn, Fe, As, Cr, Pb and Cd was found to be high whereas the contamination due to Cu, Ni; was found below the acceptable limits and no Hg was detected in the samples of drinking-water. Therefore, it can be concluded that if excess of the elemental toxicants enter the human / animal body through drinking water, they can pose health hazards because of their cumulative effect in the body. In view of these findings, the lakes which supply the drinking water to the Mumbai city should be monitored periodically to avoid excessive intake of trace metals by human, and to monitor the pollution of aquatic environment. Further, strict method of waste disposal control should be adopted to ensure the safety of the environment and to safeguard our aquatic life. As there is an indication of the presence of E. coli in all the samples, it has been concluded that the samples are contaminated and are not free from fecal contamination. The present reports however may eliminate the possibility of opportunistic pathogens in the water samples. There may be a possibility of contamination of ground water also and care should be taken to avoid the contact of water bodies with sewage drainage of water sources. The intermittent bacteriological analysis is needed to rule out the presence of opportunistic pathogens and recent fecal contamination if any.

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