patnawomenscollege.in/journal

Botany
Explore, 2010, Page No. 57-62
Vol. II No. 2

Physico-chemical & Biological Analysis of unprotected water

Madhuranjana Gargi*, Jyoti*, Pinky Prasad**
*B.Sc.-II (2008-2011), Department of Botany, Patna Women's College, Patna University
**Lecturer (Guest Faculty), Department of Botany, Patna Women's College, Patna University

Water is the basic & primary need of all vital life processes. Major part of earth surface (about ¾ th) is covered by water mainly by ocean and to some extend by lakes, rivers, streams, ponds. Unfortunately, clean, pure and safe water only exists briefly in nature and is immediately polluted by prevailing environmental factors and human activities. The quality of water depends on various chemical constituents and their concentration, most of which are derived from the geological layout of the particular region.

The analysis of water would show its effect on human health, aquatic plants, animals and marine life as well. thus there is a need to look some useful indicator both chemical and physical which can be used to monitor the quality of water.

The present investigation on physicochemical and biological analysis of unprotected water was done by collecting water samples from different water bodies around Patna. Chemical analysis such as Dissolved Oxygen, Biological Oxygen Demand, Chemical Oxygen Demand, Chlorides, Nitrate, Phosphate contents of the samples were determined by the standard methods. Biological analysis included the isolation of bacteria from water samples. The water samples were also analyzed physically by measuring pH and temperature at the time of collection. A good knowledge of the chemical and biological qualities of raw water is necessary so as to guide its suitability for use. Thus, regular physicochemical analysis of water at source must be carried out to determine or check the effectiveness of treatment process.

Key words: *Water quality, Chemical analysis, Biological analysis, Physical analysis.*

Introduction: Water is the elixir of life. It is an essential part of the protoplasm and create a state for metabolic activities to occur smoothly. Therefore no life can exist without water. With increasing industrialization and population growth, water sources available for various purpose, such as drinking, recreation, aqua, agriculture, have been contaminated and polluted with industrial as well as animal and human wastes. There are thousands of micro organisms which live in water and transported through it. This, in turn, make the water habitat favourable for some micro organisms while unfavourable for others. (Dubey & Maheswari, 2004)

Water receive micro organisms from air, soil, sewages organic wastes, dead plants and animals. It is obvious that at times any organisms may be found in water, those finding unfavourable conditions would die and the others finding favourable conditions grow and multiply to increase in population. However, in stored water the micro organisms are affected by several factors such as sedimentation, U.V. light, temperature, osmotic effects, food supply and activities of other micro organisms as well. Typical organisms found in different types of water belong to fungi, protozoa, algae, bacteria actinomycetes and viruses, for e.g. the causative agents of dysentery (*Entamoeba histolytica*), Typhoid fever (*Salmonella typhi*), Cholera (*Vibrio cholera*), Policmycetes virus and infectious Hepatitis virus (Hepatitis A virus). According to a study by WHO (1980), at least 30 thousand people die in every developing country of the world because of unsanitary water supply. (Aneja, 1993)

Before water can be described as potable, it has to comply with certain physical, chemical and microbiology standards, which are designed to ensure that water is

palatable. This work, therefore, is an attempt to examine the unprotected water and compare with standard table water for conformity to microbiological and physicochemical standards.

Methodology:

Samples of water collected from three different sites of Patna, viz., Mithapur, Bypass near Bus Stand, Mithapur and from Adalat Ganj, Budha Marg, were taken to the laboratory and analyzed physico-chemically and microbiologically using standard methods.

Observations:

Physical analysis

The temperature of the water samples were recorded at the site of collection along with the date and time of collection. The pH of the samples was recorded in the laboratory with the help of the pH-meter. The readings are tabulated in Table-1.

Table-1 showing temperature and pH of the water samples

Water Samples	Date of collection	Time of collection	Tempe-rature	pH
1.	26th July 2009	11:35 a.m.	94.3 F	7.0
2.	26th July 2009	12:05 a.m.	93.8F	7.0
3.	26th July 2009	12:45 a.m.	94.5F	7.0

Chemical analysis

1) **Determination of dissolved oxygen (do) water sample :** Dissolved oxygen of the water samples was measured by titrimetric method. The theory behind this method is that the dissolved oxygen combines with magnous hydroxide which in turn liberates iodine (equivalent to that oxygen fixed) after acidification with sulphuric acid. The iodine can be titrated with sodium thiosulphate solution by using starch indicator. (Dubey & Maheswari, 2004). The observation of the three water samples are tabulated in Table-2 to 4.

Table-2 Determination of Dissolved Oxygen (DO) of water sample-1

Obse-rvation No.	Volume of Water sample	Burette reading (ml)			Diffe-rence	Concu-rrent reading
		Initial	middle	Final		
1.	50 ml	0	20.2	23.3	23.3	
2.	50 ml	23.3	43.8	46.6	23.3	
3.	50 ml	0	21.2	24.1	24.1	23.3
4.	50 ml	24.1	44.3	47.4	23.3	

Table-3 Determination of Dissolved Oxygen (DO) of water sample-2

Obse-rvation No.	Volume of Water sample	Burette reading (ml)			Diffe-rence	Concu-rrent reading
		Initial	middle	Final		
1.	50 ml	0	17.3	19.4	19.4	
2.	50 ml	19.4	36.7	38.8	19.4	
3.	50 ml	0	17.4	19.5	19.5	19.4 ml.
4.	50 ml	19.5	36.8	38.9	19.4	

Table-4 Determination of Dissolved Oxygen (DO) of water sample-3

Obse-rvation No.	Volume of Water sample	Burette reading (ml)			Diffe-rence	Concu-rrent reading
		Initial	middle	Final		
1.	50 ml	0	20.2	23.4	23.4	
2.	50 ml	23.4	43.2	45.6	22.2	23.4 ml.
3.	50 ml	0	20.2	23.4	23.4	
4.	50 ml	23.4	43.2	46.8	23.4	

Calculation : DO for SAMPLE – 1 = 93.2 mg/1

DO for SAMPLE – 2 = 77.6 mg/1

DO for SAMPLE – 3 = 93.6 mg/1

2) **Determination of biological oxygen demand (bod) of water samples:** For measuring the Biological Oxygen Demand of the water samples, the samples were incubated at 20°C for 5 days in dark under aerobic condition and the Biological Oxygen Demand for each sample was estimated. The observations are tabulated in Table-5 to Table-7.

Table-5 Determination of Biological Oxygen Demand (BOD) of water sample-1

Obse-rvation No.	Volume of Water sample	Burette reading (ml)			Diffe-rence	Concu-rrent reading
		Initial	middle	Final		
1.	50 ml	0	21.3	22.6	22.6	
2.	50 ml	22.8	44.1	45.4	22.6	22.6 ml.
3.	50 ml	0	21.1	23.0	23.0	
4.	50 ml	0	19.8	22.6	22.6	

Table-6 Determination of Biological Oxygen Demand (BOD) of water sample-2

Obse-rvation No.	Volume of Water sample	Burette reading (ml)			Diffe-rence	Concu-rrent reading
		Initial	middle	Final		
1.	50 ml	0	16.6	18.9	18.9	
2.	50 ml	0	18.1	20.6	20.6	18.9 ml.
3.	50 ml	25.6	46.7	48.2	18.9	
4.	50 ml	24.1	39.2	42	18.9	

Table-7 Determination of Biological Oxygen Demand (BOD) of water sample-3

Obse-rvation No.	Volume of Water sample	Burette reading (ml)			Diffe-rence	Concu-rrent reading
		Initial	Middle	Final		
1.	50 ml	0	16.3	18.3	18.3	
2.	50 ml	0	16.1	18.3	18.3	18.3 ml.
3.	50 ml	18.3	33.5	35.2	18.3	
4.	50 ml	0	16.5	18.3	18.3	

Calculation: BOD for SAMPLE-1 = 2.8 mg/1
BOD for SAMPLE-1 = 2.0 mg/1
BOD for SAMPLE-1 = 20.4 mg/1

3) **Determination of chemical oxygen demand (COD) in water samples:** For measuring the Chemical Oxygen Demand of the water samples, the chemical oxidant potassium dichromate was used to measure the oxidisability of the organic matter of water samples where the oxidant oxidized the constituents. Then potassium iodide was added. The excess amount of oxygen reacted with KI and liberated iodine. By using starch indicator, iodine was treated with sodium thiosulphate and the amount for the samples and the blank was estimated. The observations are tabulated in Table-8 to Table-11.

Table-8 Determination of Chemical Oxygen Demand (COD) of water sample-1

Obse-rvation No.	Volume of Water sample	Burette reading (ml)			Diffe-rence	Concu-rrent reading
		Initial	Middle	Final		
1.	50 ml	0	11.5	13.2	13.2	
2.	50 ml	0	15.5	17.6	17.6	17.6 ml.
3.	50 ml	17.6	32.1	35.2	17.6	
4.	50 ml	24.1	39.6	41.7	17.6	

Table-9 Determination of Chemical Oxygen Demand (COD) of water sample-2

Obse-rvation No.	Volume of Water sample	Burette reading (ml)			Diffe-rence	Concu-rrent reading
		Initial	Middle	Final		
1.	50 ml	0	15.1	17.3	17.3	
2.	50 ml	19.4	35.8	37.7	17.3	17.3 ml.
3.	50 ml	0	16.8	19.5	9.5	
4.	50 ml	17.4	15.4	34.7	17.3	

Table-10 Determination of Chemical Oxygen Demand (COD) of water sample-3

Obse-rvation No.	Volume of Water sample	Burette reading (ml)			Diffe-rence	Concu-rrent reading
		Initial	Middle	Final		
1.	50 ml	0	16.8	19.3	19.3	
2.	50 ml	20.1	37.6	39.4	19.3	19.3 ml.
3.	50 ml	0	17.1	19.6	19.6	
4.	50 ml	19.8	37.4	39.1	19.3	

Table-11 Determination of Chemical Oxygen Demand (COD) of Blank

Obse-rvation No.	Volume of Water sample	Burette reading (ml)			Diffe-rence	Concu-rrent reading
		Initial	Middle	Final		
1.	50 ml	0	12.4	15.1	15.1	
2.	50 ml	15.5	28.1	30.4	14.9	15.1 ml.
3.	50 ml	0	12.3	15.1	15.1	
4.	50 ml	20	32.3	35.1	15.1	

Calculation : COD for SAMPLE-1 = 0.04 mg/1
COD for SAMPLE-1 = 0.0352mg/1
COD for SAMPLE-1 = 0.0672 mg/1

4) **Estimation of chlorine in water samples:** Potassium dichromate solution was added to water

samples in the flask and titrated with Silver nitrate solution taken in burette until orangish colour appeared. The observations are tabulated in Table-12 to Table-15.

Table-12 Determination of Chlorine in water sample-1

Observation No.	Volume of Water sample	Burette reading (ml)			Concurrent reading
		Initial	Final	Difference	
1.	50 ml	0	13.2	13.2	
2.	50 ml	13.2	26.4	13.2	13.2 ml.
3.	50 ml	26.4	40	13.6	
4.	50 ml	40.0	53.2	13.2	

Table-13 Determination of Chlorine in water sample-2

Observation No.	Volume of Water sample	Burette reading (ml)			Concurrent reading
		Initial	Final	Difference	
1.	50 ml	0	11.4	11.4	
2.	50 ml	11.4	22.8	11.4	11.4 ml.
3.	50 ml	22.8	34.5	11.7	
4.	50 ml	34.5	45.9	11.4	

Table-14 Determination of Chlorine in water sample-3

Observation No.	Volume of Water sample	Burette reading (ml)			Concurrent reading
		Initial	Final	Difference	
1.	50 ml	0	13.0	13.0	
2.	50 ml	13.0	26.0	13.0	13.0 ml.
3.	50 ml	26.0	39.9	13.9	
4.	50 ml	39.9	52.9	13.9	

Table-15 Determination of Chlorine in Blank

Observation No.	Volume of Water sample	Burette reading (ml)			Concurrent reading
		Initial	Final	Difference	
1.	50 ml	0	1.7	1.7	
2.	50 ml	1.7	3.4	1.7	11.4 ml.
3.	50 ml	3.4	5.3	1.9	
4.	50 ml	5.3	7.0	1.7	

Calculation: Chloride for SAMPLE 1 = 1.149 mg/1
Chloride for SAMPLE 1 = 0.969 mg/1
Chloride for SAMPLE 1 = 1.129 mg/1

5) **Estimation of phosphate content in water samples:** The Optical Density of the water samples was noted down at 690 nm with the help of spectrophotometer and the amount of phosphate content in the water samples was estimated from the standard graph of the phosphate solution of concentration 1µg/ml-10 µg/ml as shown in Table-16. The phosphate content of the water samples are tabulated in Table-17.

Table-16 Standard curve of phosphate (1µg/ml-10 µg/ml)

Observation No.	Concentration (1µg/ml)	Working standard Stock + D.W.	Optical Density (690 nm)
1.	1	10 + 90	0.172
2.	2	20 + 80	0.307
3.	3	30 + 70	0.574
4.	4	40 + 60	0.956
5.	5	50 + 50	0.667
6.	6	60 + 40	0.955
7.	7	70 + 30	0.706
8.	8	80 + 20	0.673
9.	9	90 + 10	0.966
10.	10	100 + 0	0.910

Table-17 estimation of phosphate content in water samples

Water samples	Optical Density at 690 nm (Average of three)	Concentration
1.	1.167	10.32 µg/ml
2.	Negligible	Negligible
3.	Negligible	Negligible

6) **Estimation of nitrate in water samples:** The Optical Density of the water sample was noted down at 220 nm with the help of spectrophotometer and the amount of nitrate content in the water samples was estimated from the standard graph of the nitrate solution of concentration 0.1 µg/ml-1µg/ml and 1 µg/ml- 10µg/ml as shown in Table-18 to Table-19. The nitrate content of the water samples are tabulated in Table-20.

Table-18 Standard curve of nitrate (0.1µg/ml-1 µg/ml)

Observation No.	Concentration (µg/ml)	Working standard Stock + D.W. (50 ml.)	Optical Density (220 nm)
1.	0.1	5 + 45	0.112
2.	0.2	10 + 40	0.096
3.	0.3	15 + 35	0.133
4.	0.4	20 + 30	0.167
5.	0.5	25 + 25	0.191
6.	0.6	30 + 20	0.218
7.	0.7	35 + 15	0.262
8.	0.8	40 + 10	0.303
9.	0.9	45 + 5	0.312
10.	1	50 + 0	0.349

Table-19 Standard curve of nitrate (1µg/ml-10 µg/ml)

Observation No.	Concentration (µg/ml)	Working standard Stock + D.W. (50 ml.)	Optical Density (220 nm)
1.	1.	5 + 45	0.262
2.	2.	10 + 40	0.528
3.	3.	15 + 35	0.888
4.	4.	20 + 30	0.818
5.	5.	25 + 25	1.433
6.	6.	30 + 20	1.284
7.	7.	35 + 15	1.869
8.	8.	40 + 10	0.831
9.	9.	45 + 5	0.843
10.	10.	50 + 0	1.053

Table-20 Estimation of nitrate in water samples

Water samples	Optical Density at 220 nm (Average of three)	Concentration
1.	1.167	0.43 µg/ml
2.	0.658	0.25 µg/ml
3.	0.861	0.35 µg/ml

Biological analysis: The Morphological characteristics of the isolate obtained from the water samples on Nutrient Agar (NA) are shown in Table-21 to Table-23.

Table-21 showing the colony characteristics and the Gram's reaction of the bacterial strains obtained from water sample-1

Colony Characteristics	Colony-1	Colony-2	Colony-3	Colony-4
Colour	Off-white	Off-white	Off-white	Off-white
Texture	Dry	Dry	Slimy	Dry
Margin	Smooth	Irregular	Smooth	Irregular
Elevation	Flat	Flat	Flat	Flat
Gram's reaction	(-)ve bacillus	(-)ve Coccus	(-)ve Coccus	(-)ve bacillus

Table-22 showing the colony characteristics and the Gram's reaction of the bacterial strains obtained from water sample-2

Colony Characteristics	Colony-1	Colony-2	Colony-3	Colony-4
Colour	Off-white	Off-white	Off-white	Off-white
Texture	Dry	Dry	Slimy	Dry
Margin	Irregular	Irregular	Irregular	Irregular
Elevation	Flat	Flat	Flat	Flat
Gram's reaction	(-)ve Coccus	(-)ve Coccus	(-)ve Coccus	(-)ve bacillus

Table-23 showing the colony characteristics and the Gram's reaction of the bacterial strains obtained from water sample-3

Colony Characteristics	Colony-1	Colony-2	Colony-3	Colony-4
Colour	Off-white	white	Off-white	Off-white
Texture	Dry	Slimy	Slimy	Slimy
Margin	Irregular	Irregular	Irregular	Irregular
Elevation	Flat	Flat	Flat	Flat
Gram's reaction	(-)ve bacillus	(-)ve bacillus	(-)ve Coccus	(-)ve bacillus

Results and Discussion: Water of good drinking quality is of basic importance to human physiology and man's continued existence depends very much on its availability. The provision of portable water to the rural and urban population is necessary to prevent health hazards. Potable water is defined as water that is free from diseases producing microorganisms and chemical substances deleterious to health (AJB, 2008). Unfortunately, clean, pure and safe water only exists briefly in nature and is immediately polluted by prevailing environmental factors and human activities. Water from most sources is, therefore, unfit for immediate consumption without some sort of treatment. In many developing countries, availability of potable water has become a critical and urgent problem and it is a matter of great concern to families and communities depending on non-public water supply system. This work is, therefore, an attempt to examine the different

sources of drinking water in Patna, compared with standard table water for conformity to microbiological and physicochemical standards for treated water samples. The result of Dissolved Oxygen show that the oxygen level is greater in water sample 1 & 3 as compared to the water sample 2. The source of dissolved oxygen in water are the autotrophic aquatic plant which as a result of photosynthesis evolve oxygen, and air where from oxygen is dissolved in water depending on salinity, temperature and water movement. More growth of microorganisms, plants and animal depletes oxygen.

Biological Oxygen Demand (BOD) is index of water pollution. Higher the amount of BOD show, the higher amount of water pollution and vice-versa. So according to the results, BOD in sample 3 is highest as compared to sample 1 & 2. The sample 3 is more polluted and is a good index of organic pollution. BOD shows the amount of organic compounds in water which is digested by the bacteria that results in the depleting oxygen level and cause the death of fish. Therefore, in sample 3, the aquatic life will be hazardous compared to sample 1 & 2.

Chemical Oxygen Demand (COD) shows the parameter of increasing pollution. The chemically oxidisable organic substance discharged in water depletes the amount of oxygen. As shown in results, COD of water sample 3 is greater than that of sample 1 & 2. Thus sample 3 shows more sewage wastage which shows the presence of harmful microorganisms causing diseases. Chlorine is widely used in the disinfection of water. So its presence in water, checked the purity. However, its high content in water brings out a hazardous problem as it reacts with organic matter in the water to from trihalomethane, a suspected carcinogen. As the result obtained, water samples 1 & 3 have higher chloride content compared to water sample 2. Water sample 2 has fair amount of chlorine in then which can be act as a disinfectant.

Phosphate is an essential nutrient for growth. It can initiate growth of algal bloom. As shown in Table-17, sample 1 shows the presence of phosphate, it can reduce the growth of aquatic plant and also its reproductive ability. While water sample 2 & 3 has negligible amount of phosphate and is said to be phosphate free.

Nitrate composition in water shows the impurity of it. It can be increased due to organic matter, wastes, bacteria, etc. It chemically indicates when the water has become potentially harmful. According to the result as shown in Table-20, sample 1 shows higher concentration followed by the samples 2 & 3. So water 1 is more harmful. Its higher content can stimulate unwanted algae growth.

The microbiological analysis of the water samples indicates that the samples have the microbial load. Its presence can bring about many harmful diseases.

Thus, regular physicochemical and biological analysis of water at source must be carried out to determine or check the quality of water bodies.

References:

1. *Aneja, K.R. (1993). Experiments in Microbiology, Plant pathology & Tissue culture Vishwa Prakashan.*
2. *Dubey, R.C.; Maheswari, D.K. (2004). Practical Microbiology, S. Chand & Company Ltd., New Delhi.*
3. *Eds. Arnold, E. Greenberg, Lenore S. Clesceri, Andrew D. Eaton. (1992). Standard Methods for the examination of water & waste water, 18th edition, American Health Association.*
4. *James G, Cappuccina, Natalie Sherman. (2005). Microbiology A laboratory manual, 6th Edition, Pearion Education Pvt. Ltd.*
5. *http://www.academicjournals.org/african African Journal of Biotechnology Vol. 7 (5), pp. 617-621, 4 March, 2008.*