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Most probable number [MPN] *Coliform* Test to detect the presence of *Coliform* from Godavari River water of Nanded city, Maharashtra, India

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Abstract

Present work deals with the study of as bacteriological study of drinking water samples from Godavari River of Nanded city, of Nanded district, Maharashtra. Samples were collected from 2 sampling locations. One from upstream and second from downstream of Godavari River from Nanded city and suggested the suitability for drinking purpose according to standards. Some of the samples were found unfit for drinking due to exceeding bacteriological limits. To avoid the ill effect of Coliform in Water, if used for drinking purposes, the following Disinfection treatment methods can be employed: Boiling Water, Chlorination, and UV treatment to avoid damage to the human body in the form of various diseases.

Drinking water should be free of substances that can adversely affect human health. Such components include minerals, organisms, and microorganisms that cause disease. The vast majority of people in developing countries suffer from health problems related to drinking water or the presence of microbiological contamination in water Van Leeuwen[14]. As with any type of industry, some form of process monitoring is required to ensure that the quality of the final product is kept to the highest possible level. In the wastewater treatment industry, laboratory testing is one of the methods used to ensure that high quality waste is maintained. Accurate and reliable laboratory analysis is absolutely necessary to monitor water leakage factors and to provide a basis for making operational changes in the treatment process itself. The protection of public health and the environment

requires safe drinking water. Modern water resources are highly polluted by the dumping of agricultural and industrial chemicals due to sewage pollution, illegal connections, leaks and rust.

Diversity of water-borne diseases and their severity are more prevalent in less developed countries especially in India, rural and urban areas and drinking water pollution has been reported as one of the major problems. Water pollution causes a wide range of diseases such as diarrhea, diarrhea, cholera, typhoid, and infectious hepatitis. A study by the World Health Organization (WHO)[15] estimates that 1.1 billion people worldwide have no access to safe drinking water. According to the WHO, biological water pollution accounts for 80% of all human diseases in developing countries. The problem has grown in our country, where studies have shown widespread pollution of drinking water in big cities and rural areas. A wide range of pathogenic microorganisms can be transmitted to humans through water contaminated with fecal material. The bacteriological level of drinking water is determined primarily by identifying organisms, their presence indicating fecal contamination. The higher the index bacteria, the higher the level of sewage and the higher the risk of infection. Coliforms especially *Escherichia coli* (Fig. 1) is a recommended indicator of a portable fluid index and a direct or indirect sewage index. It is found in large numbers of plants in the intestines of humans. Fecal coliform should not be present in 100 ml of drinking water especially Escherichia coli looks like a stick as shown in figure below.

Most Featured (MPN) is a reference method used to monitor water quality. The MPN method produces results after 3 to 4 days and disturbance by a high number of coliform-free bacteria has been shown to alter the efficiency of the analysis. Most probable number (MPN) is a method used to measure

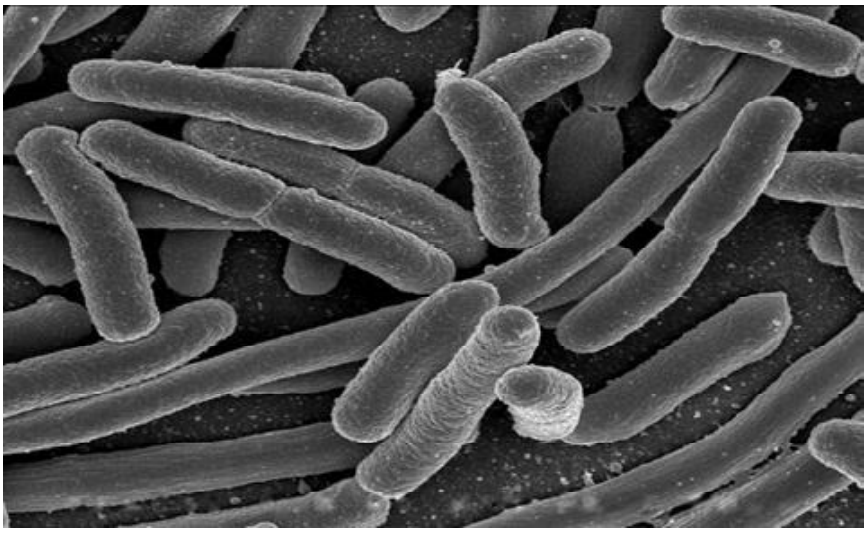

Fig. 1. Showing the observed Microscopic Structure *Escherichia coli.*

the number of micro-organisms living in a test sample. It is based on the use of opportunities in the number of positive growth responses seen in a series of general refinements. It provides practical methods for measuring the number of viruses in a test sample without any direct calculation with techniques such as microscopy. MPN is commonly used in a variety of samples such as soil, water, and agricultural products where precise cell numbers for small particles can be obtained.

This work attempts to provide a simple account of the concept of "Most Possession Number" (M.P.N.) of organisms in the form of purification. The concept is very old; the return to McCrady in 1915 has been discussed by various authors from time to time. In addition, there is some advice given on the planning of a dilution series. Purification is a method of measuring, without precise calculation, the density of organic matter in a liquid. It is mainly used to detect bacterial congestion in water and milk. This approach involves taking samples of the liquid, incubating each sample in the appropriate environment, and checking for any biological growth that has taken place. The rate of congestion is based on the prudent application of the theory of opportunity in certain concepts. For a biologist, it is more important to be clear about these concepts than to compile mathematical, complex data. Chatterjee *et al*.,[5].

Water-borne diseases are usually caused by germs that are transmitted in an orofecal way. Because they are ineffective and economical and time-consuming to detect water-borne pathogens in reservoirs, laboratory studies are performed on the basis of reference microorganisms. *Escherichia coli* is considered to be the most important indicator of water monitoring. The purpose of this study was to evaluate the Most Probable Number (MPN) method of Escherichia coli in Godavari River water.

Study Area :

In the present study the Godavari River route in the Nanded region was selected. The extension of the Godavari River from Rahati to Sangam which is about 150 km long is considered for the present investigation. To see the impact of the city and other areas on the water quality of the river this river piece has been selected. (Fig. 2&3) Nanded District is located in the Godavari region. To the northwest of Parbhani and Hingoli counties are located and to the southwest of Nanded, the Latur region is located while Yeotmal is located north of Nanded District. Andhra Pradesh County is to the east and Karnataka region to the south of Nanded County. The study area is bounded by latitude 18o15 'to 19o55' N and 77o7 'to 78o15' E longitude. Nanded District covers an area of 10528.00 sq.km. It accounts for 3.42% of the Maharashtra region area. Among the 38 districts in the province, it is ranked 14th in its ranks. Yannawar Vyankatesh[18].

Sample collection site :

We collected the water sample of Godavari River and Escherichia coli is considered to be the most important indicator of water monitoring. The purpose of this study was to evaluate the Most Probable Number (MPN) method of *Escherichia coli* in Godavari River water. We selected two points for the water sample collection of Godavari River and

collect water sample in one liter. Water cane made up of plastic, the sample were once in month collected at morning 9 to 10 AM during the period January 2020 to December 2020.

The assessing the suitability of water for various purposes like drinking, domestic, industries, and agriculture use, it is essential to determine the physical, bacteriological quality of the water. Bacteria and micro-organisms are present in water are too small to be seen by the naked eye. Some of the bacteria are harmless while some are disease-causing, known as pathogenic bacteria like coliforms. In general, the water of the Godavari River is free of pathogenic organisms unless polluted by surface drainage and anthropogonic activities by human beings in the costal or surrounding areas of river basins.

The most probable number method, otherwise known as the method of Poisson zeroes, is a method of getting quantitative data on concentrations of discrete items from positive/negative (incidence) data. There are many discrete entities that are easily detected but difficult to count. Any sort of amplification reaction or catalysis reaction obliterates easy quantification but allows presence to be detected very sensitively. Common examples include microorganism growth, enzyme action, or catalytic chemistry. The MPN method involves taking the original solution or sample, and subdividing it by orders of magnitude (frequently 10× or 2×), and assessing presence/absence in multiple subdivisions. The degree of dilution at which absence begins to appear indicates that the items have been diluted so much that there are many subsamples in which none appear. A suite of replicates at any given concentration allows finer resolution, to use the number of positive and negative samples to estimate the original concentration within the appropriate order of magnitude. Heuer and Smalla[7].

In microbiology, the cultures are incubated and assessed by eye, bypassing tedious colony counting or expensive and tedious microscopic counts. In molecular biology, a common application involves DNA templates diluted into polymerase chain reaction (PCR) reactions. Reactions only proceed when a template is present, allowing for a form of quantitative PCR, to assess the original concentration of template molecules. Another application involves diluting enzyme stocks into a solution containing a chromogenic substrate, or diluting antigens into solutions for ELISA (Enzyme-Linked Immuno Sorbent Assay) or some other antibody cascade detection reaction, to measure the original concentration of the enzyme or antigen. The major weakness of MPN methods is the need for large numbers of replicates at the appropriate dilution to narrow the confidence intervals. However, it is a very important method for counts when the appropriate order of magnitude is unknown a priori and sampling is necessarily destructive.[17].

Safety and Hygiene :

Whenever samples of wastewater are handled, it is very important that operators wash their hands before eating or smoking. While some laboratory chemicals are not dangerous, many of them are poisonous or harmful to the skin and clothing. Rubber gloves and safety glasses should be used. It is important to wash thoroughly with soap and

water after handling laboratory chemicals, especially if chemicals come into contact with the skin. Keep bench areas free of clutter and clean bench surfaces with disinfectant before and after bacteriological testing. Read the labels carefully and know what to do in case of an accidental spill. Always clean up spills quickly and in the safest possible manner using disposable rags or towels. Sterilize all inoculated tubes, filters, and culture dishes prior to disposal of bacteriological test waste materials. Always put stoppers or tops back on containers when not in use, WHO[15] and Van Leeuwen[14].

Equipment and reagents :

Whenever microbiological testing of water samples is performed, certain general considerations and techniques will be required. Since these are basically the same for every microbiological test procedure covered by this text, they will be discussed prior to the specific instructions for each test method. All reagents and media utilized in performing microbiological tests on water examples must meet the standards specified in Section 9000 of "Standard Methods for the Examination of Water and Wastewater", 18th Edition. Fermentation tubes (or test tubes) 25ml, 50 ml., Durham`s tubes, Mac Conkey Broth, Distilled water, Test Tube stands, Autoclave, Incubator, Aseptic Cotton, Safety gloves, Mask, Apron, Weighing machine, Pipettes and Refrigerator. Metcalf & Eddy[10], APHA[3] and Trivedy and Goel[12].

Procedure of MPN Coliform :

To techniques are available for the estimation of the most probable number of coliforms in water samples the multiple tube fermentation techniques and the membrane filter (MF) technique. Both the techniques are in use. The membrane filter technique involves filtering a known amount of water sample through a membrane filter of optimum pore size. This filter with trapped bacteria is kept on a Petri plate with an agar medium. These are then inoculated in suitable conditions; the colonies are counted on the bacterial density is calculated per 100 ml of water. Some types of waters, however, cannot be tested by the MF method, especially those having high turbidity or high bacterial counts. The multiple tube fermentation technique is more in use due to its applicability to almost all kinds of waters. William,[16].

The technique involves inoculating the sample and/or its several dilutions in a suitable liquid medium. After the expiry of the incubation period, the tubes are examined for gas production by the coliform organisms. This test is known as the presumptive test. Since this reaction may also produce by organisms other than the coliforms, the positive tubes from the presumptive test are subjected to a confirmatory test. For a very definite presence of coliform bacteria, the completed test is carried out. The density of bacteria is calculated on the basis of a positive and negative combination of the tubes using MPN tables. This density, however, is only the most probable number of the bacteria and is not actual bacterial density. Only multiple tube fermentation technique is described here. For the membrane filter technique, the reader is referred to APHA[3] Abid and Jamil[1] and Trivedy and Goel[12].

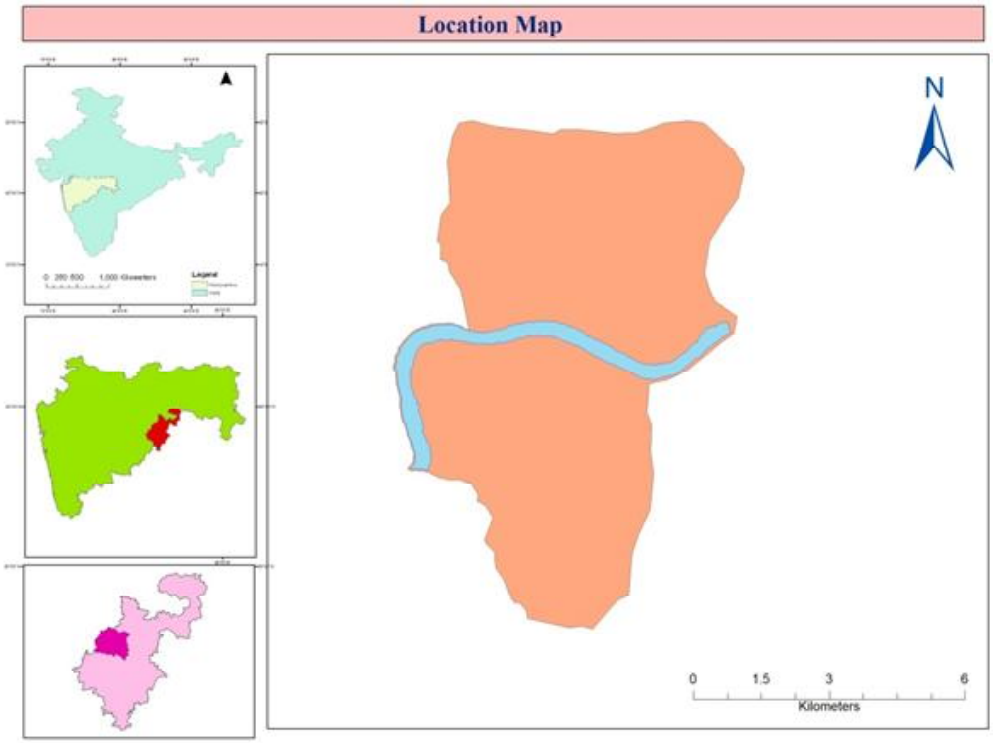

Fig. 2: Showing location map of Nanded city, sideways with Godavari River, in the city

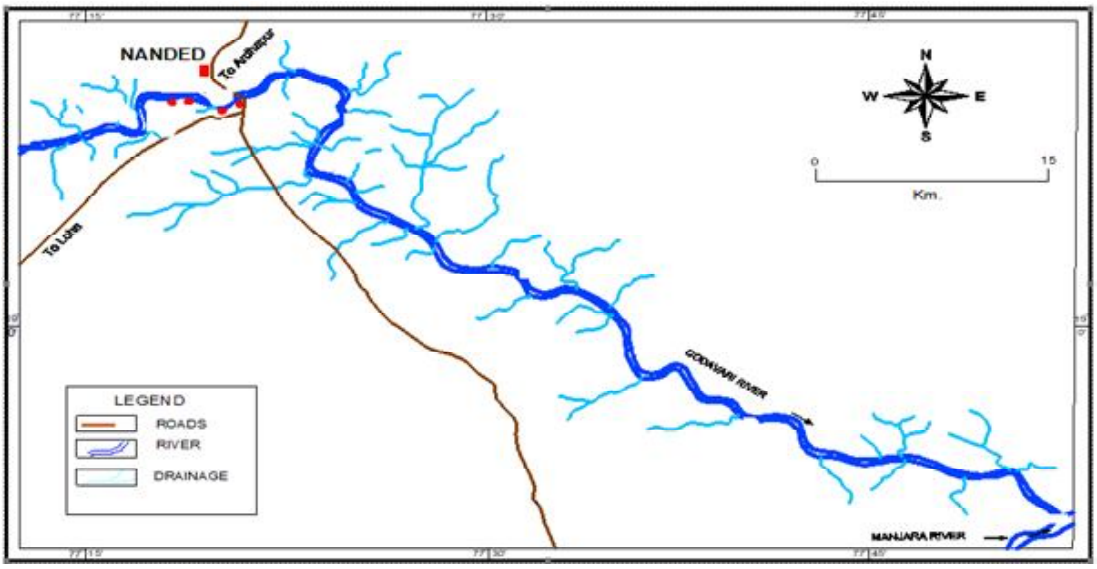

Fig.3: Showing selected sampling locations in Godavari River of Nanded City, Maharashtra

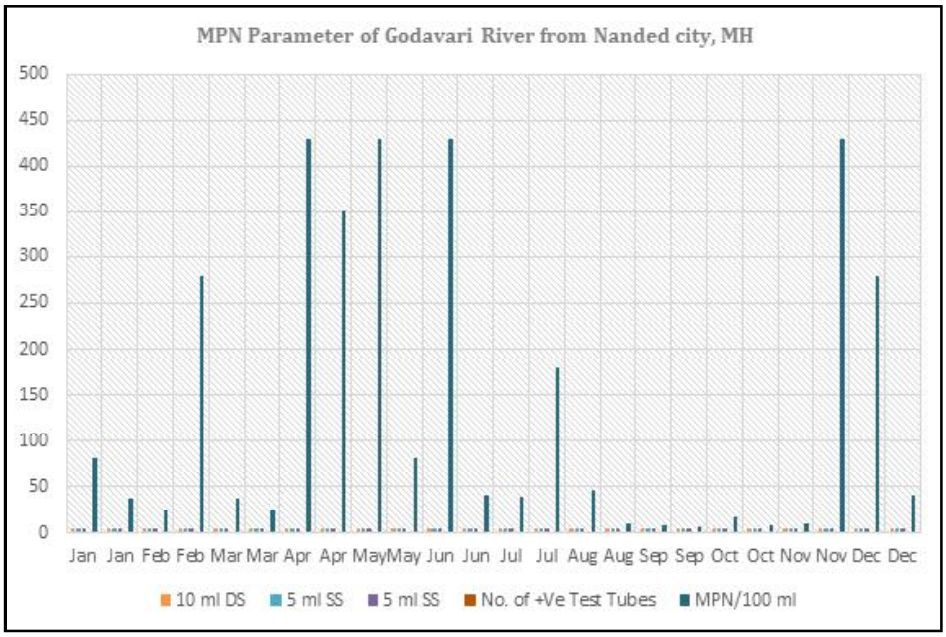

Fig. 4. Showing the MPN parameter of Godavari River from Nanded city, Maharashtra

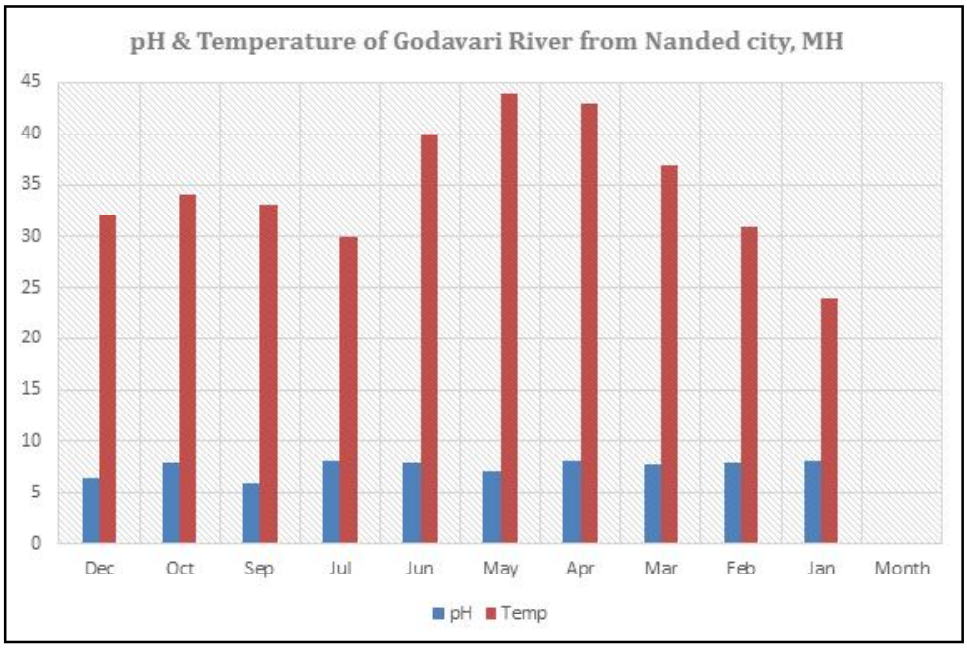

Fig. 5. Showing the pH & Temperature of Godavari River from Nanded city, Maharashtra

When three serial sample volumes (*e.g.*, dilutions) are is used in bacteriological testing of water, the resulting MPN (most probable number) values per 100 ml can be determined by using the table. The MPN values given are based on serial sample volumes10, 1and 0.1 ml. flower and higher serial sample volumes are based, the MPN values given in the table must be adjusted accordingly. For example, if sample volumes used are 100, 10, and 1 ml, the MPN values from the table are multiplied by 0.1. Similarly, if the sample volumes are 1, 0.1, and 0.01 ml, the MPN values from the table are multiplied by 10. In situations where more than three tests' dilutions have been run, the following rule is applied to select the three dilutions to be used in determining the MPN value APHA,[3]: Choose the highest dilution that gives positive results in all five portions tested (no lover dilution giving any negative results) and the two next higher dilutions. Use the results at these three volumes in computing the MPN value. In the examples given in the accompanying table, the significant dilution results are shown in boldface. The number in the numerator represents positive tubes, and in the denominator, the total tubes planted.

Multiple Tubes Fermentation Technique :

Preparation of Mac Conkey Broth media by taking 10 gm Mac Conkey Broth in 250 ml d/w is single strain and 20 gm of Mac Conkey Broth in 250 ml d/w is double strain. Then keep it to the autoclave for ½ hour. The sample was taken for MPN (same sample) we have to arrange as sample no.1, sample no. 2, sample no. 3. Arrange the test tubes of dilution 10 ml/5 test tubes,1 ml/5 test tubes 0.1 ml/5 test tubes for sample no.1, and take 10 Mac Conkey Broth in each test tube. Prepare the dilution of water sample as 1 ml water sample in 100 ml d/w which is the dilution of 10, take 1 ml from prepared dilution 10 which is the dilution of 1, take 1 ml from prepared dilution 1 which is the dilution of 0.1. Add the dilution of 10,1 and 0.1in each five test tubes for the sample. noncontinuous the same procedure for samples no.2 and 3 put the Durham's tubes inverted in every test tube. And cover the mouth of the tube with a cotton plug. Keep it to incubation for 24 hours at 37^{o}C. After 24 hours remove the test tubes and identify the positive tubes which having bubbles in. Bubbles indicate the formation of gas and if yellow color appears then there is the formation of acid.

In this study total of 2 water samples in each month's out of which 12 in pre-monsoon, and 12 in post-monsoon ware were analyzed from Godavari River, of Nanded city. A number of physical parameters like pH, temperature, and Bacteriological MPN were performed. In the present study, the data revealed that there were considerable variations in the quality with respect to their water characteristics. Most Probable Number [MPN] Coliform Test to Detect the Presence of Coliform analysis of Godavari River water from Nanded was studied in three different seasons (2021). The average value of various water quality parameters had been mentioned in the graphs represented subsequently (Figs. 4 & 5).

This graph shows the maximum MPN in the month of April, May, June and November 2021. This increase in MPN may be due to the percolation of runoff in the post-monsoon season. This method determined

E. coli O157:H7 concentrations as low as 0.1 MPN per litre, with a 95% confidence level of 0.01-0.7 MPN per litre. To avoid the ill effect of Coliform in Water, if used for drinking purposes, this method is used for drinking water quality assessment method can be employed. Brenner *et al*.,[4]. Also explored the Comparison of the Recoveries of *Escherichia coli* and Total Coliforms from Drinking Water by the MI Agar Method and the Environmental Protection Agency U.S.

Therefore, the amount of coli form of 10 per 100 ml of water means "true" value is between 2 and 23. Our new test contest validates MPN results only in four samples low level of pollution. Of the 24 samples present filed to a satisfactory level in MPN and unsatisfactory with new tests, only 3 have no visible coli forms. Even in these three instances, that is possible coliforms are suppressed by other bacteria. Hutchinson, *et al*.,[8]. And Weaver & Boiter (1951). For negative samples with new tests and positive in MPN tests, coliform counts distance between 12 and 40 at a rate of 22 ± 9 coliform per 100 ml of water. All samples with more than 40 coliforms classified as contaminated by new tests. Disagreement between tests in samples with low levels of contamination may be associated lack of accuracy in MPN approach; exactly half all points of contention fall into each of the two tests groups.

A new study of sewage detection was found to be reliable, easy to do, and necessary a few resources. Application of the pollution learning method of the Nanded urban water supply system has shown its effectiveness in a practical way emergencies. Dunling and Wanda[6] has also noticed the total coliform and *Escherichia coli* in drinking water supplies by membrane filtration techniques. Similar results are noticed in test for the detection of faecal pollution in drinking water, by Manja *et al*.,[9] and Anwar *et al*.,[2] in Qualitative assessment of bacteriological status of drinking water in Lahore. Shete and Chougule perceived some common pathogenic bacteria in water samples in the Nanded districts Naigaon (Bz.) During the period of (2012).

The purpose of this research is to detect the presence of coliform if any, in water bodies by the application of the MPN Test. For this research River water samples were collected from the affected sites where the possibility of mixing sewage in water bodies is high, and MPN tests were applied to detect the presence of coliforms which may be pathogenic in nature and are responsible for the cause of diseases like cholera, dysentery, tuberculosis, etc. in man and domestic animals, etc. To avoid the ill effect of Coliform in Water, if used for drinking purposes, the following Disinfection treatment methods can be employed: Boiling Water, Chlorination, and UV treatment to avoid damage to the human body in the form of various diseases.

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Declaration: The authors of this manuscript do not oppose the interest.

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