Microbial analysis for assessment of water quality from selected wells of Mapusa city

A Dissertation for

MIC-651 Discipline Specific Dissertation

16 credits

Submitted in partial fulfillment of Master's Degree

M.Sc. in Microbiology

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I here by declare that the data presented in this Dissertation report entitled, "Microbial analysis for assessment of water quality from selected wells of Mapusa" is based on the results of investigations carried out by me in the Microbiology Programme at the School of Biological Sciences and Biotechnology, Goa University under the supervision of Dr. Trupti Asolkar and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will not be responsible for the correctness of observations / experimental or other findings given the dissertation.

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This is to certify that the dissertation report, "**Microbial analysis for assessment of water quality from selected wells of Mapusa**" is a bonafide work carried out by Ms. Apurva Avinash Vaingankar under my supervision in partial fulfillment of the requirements for the award of the degree of **MSc in Microbiology** in the Microbiology Programme at the School of Biological Sciences and Biotechnology, Goa University.

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PREFACE

Access to potable water is vital for human life, yet only a small fraction of Earth's water is freshwater. Contaminated groundwater poses serious health risks, with various pathogens remaining stable for extended periods. Malfunctioning septic systems and leaking sewer lines are primary sources of contamination. The global population's growth, urbanization, and industrialization exacerbate water pollution issues. Industrial effluents introduce heavy metals into ecosystems, threatening public health. Antibiotic-resistant bacteria from human and animal sources further contaminate water environments. Conversely, industrial antibiotics disrupt microbial ecosystems. This study in Mapusa, Goa, evaluates well water microbial quality and sensitivity to antibiotics and metals. Understanding microbial diversity and sensitivity aids in mitigating health risks and managing water resources effectively.

ACKNOWLEDGEMENT

Behind every milestone lies a story of support, and this thesis is no exception. It is with heartfelt appreciation that I acknowledge the pivotal role played by several individuals whose unwavering contributions have brought this work to fruition. First and foremost, I am deeply grateful to my guide Dr. Trupti Asolkar whose constant support, encouragement, and guidance throughout the study have been instrumental in shaping this thesis.

I am grateful to the program director, Dr. Lakshangy Charya for providing the necessities required for this project work. I am thankful to all the teaching staff of the Microbiology Program for their cooperation and help.

To the wonderful non-teaching staff your presence and assistance made the lab work a little less stressful than it could have been. Especially Sir Surendra in whose absence not one thing in the lab could be done.

I am sincerely thankful to Ms. Sonal Chari, from the School of Earth Ocean and Atmospheric Sciences (SEOAS), Goa University for her help in the sampling process.

To all my classmates, each of your help no matter how small it might have been greatly appreciated. To Bhavana, Nikita, and Rachel it's finally over and we can breathe a sigh of relief. Special thanks to Eden who made these last two years bearable and helped me in more ways than one.

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ABBREVIATIONS USED

Entity	**Abbreviation**
Most Probable Number	MPN
Multi-Drug Resistance	MDR
Quantitative reverse transcription polymerase chain reaction	qRT-PCR
Real-time polymerase chain reaction	PCR
Biological Oxygen Demand	BOD
Dissolved Oxygen	DO
Colony forming unit per milliliter	Cfu/ml
World Health Organization	WHO
Milligrams per liter	mg/l
Maximum Contaminant Level	MCL
Antibiotic Resistance Genes	ARGs
milliliter	ml
Sulphuric acid	H_2SO_4
Normal	N
Nutrient Agar	NA
Degrees Celsius	˚C
Nutrient Broth	NB
Potato Dextrose Agar	PDA
Hours	hrs
Eosin Methylene Blue	EMB

Thiosulfate citrate bile-salt sucrose	TCBS
Salmonella Shigella	SS
Indole, Methyl Red, Voges Proskauer& Citrate	IMViC
Methyl Red - Voges Proskauer	MR - VP
Methyl Red	MR
Voges Proskauer	VP
Triple sugar iron	TSI
Practical salinity unit	psu
Hydrogen sulfide	H_2S
Millimolar	mM
Micrograms	μm
Micrograms per milliliter	μg/ml

ABSTRACT

This study focuses on the microbial contamination of well water samples in the Mapusa area and pathogens were enumerated on their specific media. Water samples were analyzed for total viable count of fungi and bacteria. A total of 53 isolates were obtained after the viable count method. These isolates were screened for metal and antibiotic sensitivity. The presence of pathogenic bacterial species was analyzed by preliminary analysis using pathogen specific medias.

The presence of coliforms was tested by Most Probable Number (MPN), the isolates were identified at the genus level using biochemical analysis.

Keywords: Contaminated water, anthropogenic activity, mercury, manganese, ampicillin, streptomycin

CHAPTER 1:

INTRODUCTION

1.1 BACKGROUND

Potable water is key to life. The lack of access to a sufficient quantity of water or access to non-potable water can cause serious and irreparable harm to people. (Oliveira 2017)

Groundwater is used as drinking water, and its contamination significantly increases the risk to public health. Outbreaks of various diseases have been linked to groundwater pathogen contamination. Multiple studies have shown pathogens present in groundwater for up to 400 days. (Pandey et al, 2014)

A significant number of microbial pathogens, including over 100 viruses and several bacterial pathogens of fecal origin, can contaminate groundwater. Illnesses resulting from infection range in severity, with the most common being acute gastrointestinal illness. Contamination can occur through various routes, with malfunctioning septic systems and leaking sewer lines being primary concerns. Transport to groundwater is influenced by hydrogeological setting and climate. (Sinclaire et al, 2009)

The global population is rapidly expanding, urbanization, industrialization, and agricultural production are increasing, and the economy is growing. As a result, we are now confronted with the issue of the adverse effects of human-made contaminants. (Li et al, 2021)

Industrial effluents are the primary cause of leaching metals into the environment. Metals being soluble in water gives them an entry into living organisms. (Shrestha et al, 2021).

Antibiotic-resistant bacteria from human and animal sources make their way into water environments. These bacteria can transfer their genes to water-dwelling microbes, which already possess resistance genes. Conversely, numerous antibiotics of industrial origin flow through water environments, potentially disrupting microbial ecosystems. (Baquero et al, 2008)

1.2 AIM AND OBJECTIVE

Given the above, a study was conducted to assess the microbial quality of water using selected wells from the city of Mapusa, Goa. The study aims to evaluate the current status of well water concerning the presence and abundance of different microorganisms. The objectives of the study are as follows:

- Evaluating the partial microbial diversity of well water samples
- Screening of bacterial isolates for sensitivity to various antibiotics and metals

1.3 HYPOTHESIS

An increase in anthropogenic activities has led to the contamination of water bodies, and the degree of contamination of natural resources has been on the rise in recent years. Since the well water is considered to be pure and potable by local communities there is a need for scientific analysis of these water bodies. The presence of pathogenic microorganisms can cause health hazards to the community consuming that water. Microbiological analysis of well water will provide an overview of the status of water and portability

1.4 SCOPE

The study will help in identifying the diversity of organisms in well water. Additionally, it will also provide data regarding the presence of pathogenic microorganisms, consumption of which may lead to health hazards to the local communities. This work will serve as a baseline to develop preventive measures to protect the locals.

CHAPTER 2: LITERATURE REVIEW

2.1 GLOBAL CONCERNS ABOUT WATER QUALITY

Water quality and water resources play a crucial role in development and the ecological environment, particularly in regions facing severe water shortages. Recent studies show microbes and metals in the water. These issues also occur in developed nations. (Xiao et al, 2019; Islam et al, 2001)

2.1.1 CONTAMINATION DUE TO ANTHROPOGENIC ACTIVITY

The water samples in Pakistan were tested for various parameters to check their usability for irrigation. Based on results it was seen that the water was alkaline. The anthropogenic activities increased arsenic. (Rasool et al, 2017)

Fourteen communities in Wisconsin were subjected to a single sampling event, from which a total of thirty-three wells were tested for traces of wastewater and viruses. Of these wells, four tested positive for traces of wastewater while five were found to contain viruses through the qRT-PCR method. It was discovered pumping enabled the fast transport of infectious viruses, showing that drinking well water remains susceptible to contamination from even small amounts of virus-laden water. (Hunt et al, 2010)

Water from seven wells in Pennsylvania, which were sampled at the beginning of urbanization in 1953 and 1956 and again in 1979, had an increase in the concentration of most dissolved constituents. Some wells are now unsuitable for public supply due to groundwater contamination. The concentration of lead was higher than the national median. The quality of low streamflow has been degraded due to effluent from sewage treatment plants. (Sloto and Davis, 1982)

In March 2003, 12 samples were gathered from an estate in Peshawar including seven from industrial effluents, one from a main drain, and four from tube or dug wells. The samples were analyzed for physico-chemical parameters. The results showed that there was a threat to

underground water. BOD levels and various metals were above acceptable amounts. (Tariq et al, 2006)

Groundwater system in Andhra Pradesh was assessed to determine the impact of multiple industrial-pollutant sources. The groundwater was polluted due to effluents entering various water sources. Samples of water were collected from different water bodies and high metal concentrations were seen in them. (Subrahmanyam and Yadaiah, 2001)

2.3 HEALTH IMPLICATION OF GROUNDWATER CONTAMINATION

A study conducted using 30 samples collected from the industrial area of Bangalore revealed the presence of heavy contamination rendering 77% of water sources non-potable. Discussions revealed severe health problems in the community due to the use of this water, showing a clear correlation between ill health and groundwater contamination. (Shankar et al, 2008) Naik and Phadke, 2022, reported the high nitrate, low DO, and high coliform load in the water samples studied from Candolim. The coliform index was also very high. (Naik & Phadke, 2022)

2.4 MICROBIAL CONTAMINATION IN WELL WATER

In Karnataka fecal indicator organisms were found, and most of the water sources were contaminated with coliforms. A total of 170 bacterial isolates were collected and a significant number of the isolates exhibited MDR. (Mukhopadhyay et al, 2012)

The highest microbial load of 1650 cfu/ml was observed in one sample after 24 hours of incubation, while the minimum load of 128 cfu/ml was found in a different sample. *E. coli* was detected in two wells, and *Enterobacter* was present in many wells. The MPN indexing of the water samples indicated a wide variation, ranging up to 2400. According to WHO recommendations, almost all water samples were deemed unfit for drinking purposes. Coliform bacterium serves as the primary bacterial indicator for fecal pollution in water. (Parihar et al, 2012)

During the pre-monsoon period, nitrate concentration exceeded 45 mg/l in seven samples from Moradabad, and in four samples during the post-monsoon period, indicating human influences. The microbiological analysis revealed increased levels of heterotrophic bacterial count in the water samples. Additionally, the water samples tested positive for total and fecal coliform, which should ideally be absent according to the World Health Organization (WHO) guidelines, thus indicating that the water is unsuitable for drinking and domestic use. (Saba et al, 2015)

In Bangladesh study on biophysiochemical parameters from well water found zooplankton and bacteria present. Pseudopodans were identified in the water samples along with coliforms (Islam et al, 2001)

The water quality of wells in two northeastern Ohio counties was evaluated. A total of 45% showed contamination with total coliforms. *E. coli* was found which was confirmed by PCR (Won et al, 2013)

Various industrial activities release heavy metals into the groundwater systems through runoff, leaching, etc. (Mukherjee et al, 2021)

The groundwater in a middle-class neighborhood in Lagos was evaluated for heavy metal contamination. Although none of the samples contained Aluminum above the Maximum Contaminant Level (MCL), 93.88% showed its presence. 38% contained Cadmium, and 32.65% had concentrations above the MCL. Almost 60% had detectable levels of Lead, with 36.73% exceeding the MCL. Heavy metals were found suggesting risk due to the toxicity of these metals. (Momudu et al, 2010)

Mining operations produce significant quantities of mine tailings and acid mine drainage, both of which contain various heavy metals. These heavy metals are influential in the co-selection of bacterial antibiotic resistance. (Zou et al, 2021)

The water of Maozhou River contained 141 antibiotic resistance genes (ARGs), while the sediments contained 150 antibiotic resistance genes (ARGs). Among the water samples, a total of 116 antibiotic resistance genes (ARGs) were identified. (Wu et al, 2020)

Bacteria naturally develop resistance over time. The presence of antibiotics in the environment has led to the emergence of antibiotic-resistant bacteria and the spread of antibiotic-resistant genes (ARGs). This can be attributed to the rising use of antibiotics and their incomplete removal. Traditional water treatment methods are ineffective in completely removing antibiotics and bacteria, particularly in eliminating antibiotic-resistance genes (ARGs). (Baquero et al, 2008)

CHAPTER 3: METHODOLOGY

OBJECTIVE 1: MATERIALS AND METHOD

3.1 SAMPLE COLLECTION

The data collection involved the sampling of various wells located throughout Mapusa. Selection criteria included accessibility, location, the distance between wells, and obtaining permission from private owners and locals. A total of 15 wells were chosen, with 10 designated for microbial analysis. Sampling took place during January and February 2024.

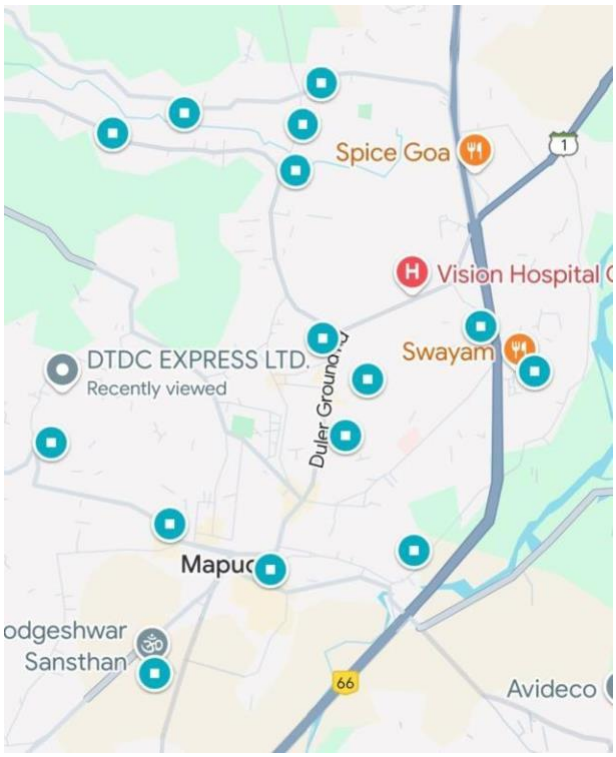

Fig 3.1: Sampling area

Well water was collected in a 100ml sterile sampling bottle for microbial analysis. A different 100ml sterile sampling bottle was used to collect water for MPN and physicochemical parameters. Water was also collected in a sterile 300ml glass stopper bottle without air bubbles and immediately fixed with Winklers A and Winklers B solution to further use it to check DO.

3.2 ANALYSIS OF PHYSICOCHEMICAL PARAMETERS

3.2.1 Temperature

Temperature was measured using a thermometer at the sampling site as soon as the water was collected.

3.2.2 Salinity

The salinity of the water was checked using a refractometer in the lab.

3.2.3 pH analysis

The pH was studied using a digital pH meter in the lab.

3.2.4 Dissolved oxygen

Dissolved oxygen was determined using Wrinkler's modified Azide method (Barnett and Hurwitz, 1939). Water was fixed by the addition of Wrinkler's A and Wrinkler's B solution at the sampling site. To this 1ml of concentrated sulfuric acid (H_2SO_4) was added to dissolve they formed precipitate. It was then titrated against 0.0125 N sodium thiosulphate and starch was used as the indicator.

3.3 MICROBIAL ANALYSIS

3.3.1 Total Viable Count

The abundance of microorganisms was enumerated by spread plating on Nutrient Agar (NA). The water sample was diluted to 10^{-3} using sterile distilled water and 0.1ml of aliquot from 10^{-2} to 10^{-3} were spread plated in triplicates and incubated for 24 hrs. at 37 Ċ The number of colonies were counted and noted. (Clark, 1965)

A) Purification

The isolates obtained after spread plating were streaked on NA plates to get pure colonies and then colonies were restreaked.

B) Glycerol Stocks

The Purified cultures were stored in the form of Glycerol stocks. 70% Glycerol was prepared. 0.6 ml of this glycerol was added to 0.4 ml of NB to which each isolate was added and stored at -20°C

3.3.2 Total fungal count

Total Fungi were enumerated by spread plating 10^0 and 10^{-1} on Potato Dextrose Agar (PDA) plates in triplicates. The plates were incubated at 37°C for 48 hrs. The fungi colonies were counted and noted. (Praveen et al, 2011)

3.3.3 Total viable pathogenic count

The abundance of different pathogens was calculated using MPN and spread plating on different selective media in triplicates. The media used were Eosin Methylene Blue (EMB) agar, Thiosodium Citrate Bile Salts Agar (TCBS), Aero Pseudo agar, and Salmonella Shigella (SS) agar.

I) Most Probable Number (MPN)

MPN is used to identify the number of organisms in the sample. (Some et al, 2021)

A] Preliminary test

MacConkey broth tubes were prepared into five tubes of 2x concentration and ten tubes of 1x concentration with inverted Durham's tubes. Sample water was inoculated in volumes 10ml, 1ml, and 0.1ml respectively, and incubated at 37°C for 24 hours. The positive tubes had color change and gas production.

B] Complete test

A loopful of samples from any two selected positive tubes was spread-plated on EMB agar, incubated at 37℃ for 24 hours, and checked for metallic sheen colonies, pink mucoid colonies, and others.

C] Confirmatory test

Cultures were purified on NA plates and biochemical analysis of isolates was performed to check for the organisms present.

Biochemical Analysis

A) IMViC Test (Mercuri and Cox, 1979)

Indole Test: To check the isolate's ability to utilize tryptone to break it down to Indole. The bacterial culture was inoculated in tryptone broth. Kovacs's reagent was added to check the red color change on the upper layer of broth.

MR-VP Test: The same broth medium containing glucose is used. The bacterial culture is inoculated in Glucose Phosphate Broth and incubated for 24 hours at 37℃. For MR-VP positive test is indicated by the red coloration of the media.

Citrate test: To check the ability of the isolates to utilize citrate as its carbon and energy source. Simmon citrate agar is used to prepare slants in sterile test tubes and the slants are streaked upon using the bacterial isolate. A positive test is indicated by the citrate slant turning blue.

B) TSI Test

Triple sugar Iron test to check isolates' ability to ferment three different sugars and to produce hydrogen sulfide and gas.

C) Motility Test

Nutrient agar containing 0.5% agar was stab inoculated to check for bacterial isolates' motility by causing diffusion near stabbed agar.

3.3.5 Thiosulphate citrate bile salt sucrose agar

It is a selective and differential enrichment media for *Vibrio* species isolation. It contains thiosulphate and sodium citrate which inhibits Enterobacteriaceae and thiosulphate acts as a source of Sulphur to detect hydrogen sulfide production. Sucrose is fermented by *Vibrio* spp. Gram-positive species are inhibited by bile salts and an alkaline medium pH. Bromothymol blue and thymol blue are pH indicators. 10^0 and 10^{-1} samples were spread onto TCBS plates in triplicates, and incubated for 24 hours 37℃.

Expected Results:

Vibrio cholerae like organisms Flat yellow colonies, less than 2mm

Vibrio alginolyticus like organism's Large yellow colonies

Vibrio fluvialis, Vibrio vulnificus like organisms yellow or translucent colonies

Vibrio parahaemolyticus like organism's colorless colonies with green center

3.3.6. Aero Pseudo agar

It is a selective and differential media for the isolation of *Aeromonas* and *Pseudomonas* spp. It contains sodium glutamate and starch as sources of carbon. *Aeromonas* utilizes starch to produce acid causing phenol red to change color from red to yellow. While *Pseudomonas* doesn't metabolize starch. 10^0 and 10^{-1} dilution of water sample was spread plated on agar plates and incubated at 37℃ for 24 hours.

Expected results:

Pseudomonas aeruginosa like organisms' red-violet surrounded by a red violet zone

Aeromonas hydrophila like organism's yellow surrounded by a yellow zone

Aeromonas caviae like organism's yellow surrounded by a yellow zone

3.3.7 Salmonella and shigella agar (SS agar)

SS agar is a differential and selective media for the isolation of *Salmonella* and *Shigella* spp. It is selective as it contains bile salts, brilliant green, and sodium citrate which inhibits Gram-positive isolates. Peptone and beef extracts as sources of nitrogen. Lactose is a fermentable source of carbon and thiosulphate is a source of Sulphur and H_2S production. 10^0 and 10^{-1}samplewas spread plated on agar and incubated at 37℃ for 24 hours.

Expected Results:

Salmonella choleraesuis like organisms colorless with a black center

Salmonella typhi like organisms colorless with a black center

Shigella flexneri like organisms colorless

Salmonella typhimurium like organisms colorless with a black center

RESULT

The Physicochemical analysis

Table 3.1: Physicochemical parameters of well water sample

Sample	Well 1	Well 2	Well 4	Well 5	Well 7	Well 8	Well 9	Well 10	Well 12	Well 13
Temperature	27.8°C	27.5°C	27.5°C	26.1°C	26°C	24.8°C	25.5°C	25.7°C	27.3°C	24°C
Salinity	2 psu	2 psu	2 psu	2 psu	2 psu	2 psu	2 psu	2 psu	2 psu	2 psu
pH	6.55	5.15	4.66	6.30	5.89	5.49	6.37	6.23	5.71	5.68
DO	6.9 mg/L	7.1 mg/L	6.3 mg/L	5.4 mg/L	7.2 mg/L	5.8 mg/L	7.4 mg/L	6.5 mg/L	6.3 mg/L	7.7 mg/L

Temperature:

The sampling was done in the morning between 8 to 9 and the temperature of the water sample was taken at the site and ranged from 24 to 27°C.

Salinity:

Salinity was measured in the lab using a refractometer. Salinity was noted to be 2 psu

pH:

The pH of the water sample was analyzed using a pH meter. The pH ranges from 4.66 to 6.55

DO:

DO was checked by Winkler's modified azide method. The DO ranges from 5 to 8 mg/L

Total viable count

Total plate count of bacteria was done using NA. The cfu/ml results are noted in the table below.

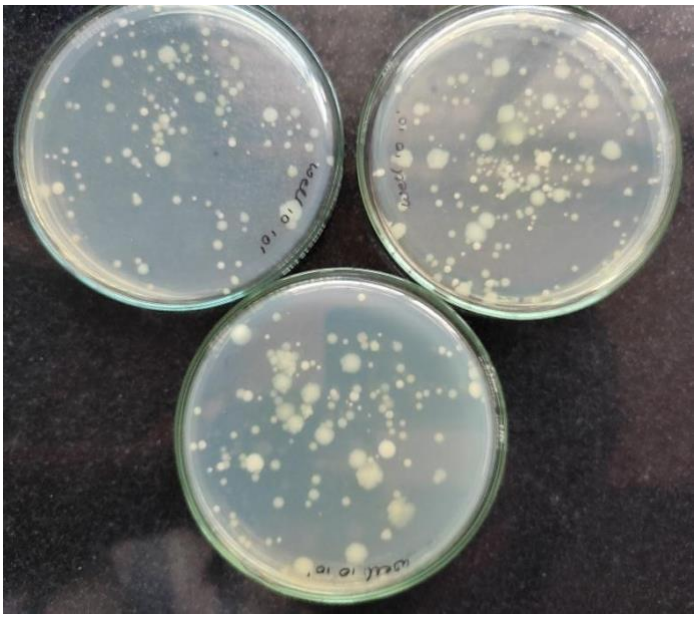

Fig 3.2: Total Viable count on NA plate

Table 3.2: Total Viable Count of Well water in cfu/ml

NA	Well 1	Well 2	Well 4	Well5	Well 7	Well 8	Well 9	Well 10	Well 12	Well 13
10^{-2}	51.3 x 10^3	9.3x 10^3	82.6 x 10^3	13.3 x 10^3	19.5 x 10^3	21 x 10^3	13 x10^3	23.6 x 10^3	1.6 x 10^3	21.3 x 10^3

Preparation of Glycerol stocks

A total of 53 bacterial colonies were obtained from the Viable count plates.

These were selected based on differences in colony characteristics within each well. Each of the isolates of purified and sub-cultured. Each of the tubes was labeled based on the well and sample isolate that is W1S1 (Well 1 Sample 2) and so on.

Total Fungal count

The total fungal count was taken on PDA plates. The fungal count was found to be very low.

Table 3.3: Total fungal count

PDA	Well 1	Well 2	Well 4	Well 5	Well 7	Well 8	Well 9	Well 10	Well 12	Well 13
10^0	1	0	0	0	0	1	0	0	1	1

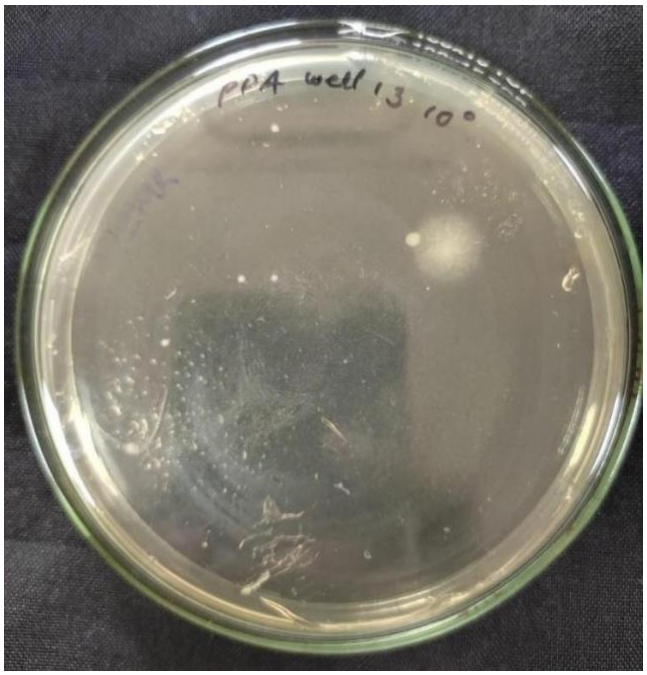

Fig 3.3: Total Fungal count on PDA plate

Total Pathogen Count

A) MPN preliminary test: The MPN index was measured by McCrady's Table. Maximum number of tubes showed color change and gas production. The MPN index for majority of the sample was between 1600 to ≧ 1600 per 100ml except for one well whose MPN index was 0

Table 3.4: MPN index of well water sample

Media volume	10ml (2X strength)	10ml (1X strength)	10ml (1X strength)	
Sample volume	10ml	1ml	0.1ml	MPN (per 100ml)
Dilution factor	1 in 1	1 in 10	1 in 100	
Well 1	0	0	0	0
Well 2	5	5	5	≥1600
Well 4	5	5	4	1600
Well 5	5	5	4	1600
Well 7	5	5	4	1600
Well 8	5	5	4	1600
Well 9	5	5	4	1600
Well 10	5	5	5	≥1600
Well 12	5	5	5	≥1600
Well 13	5	5	5	≥1600

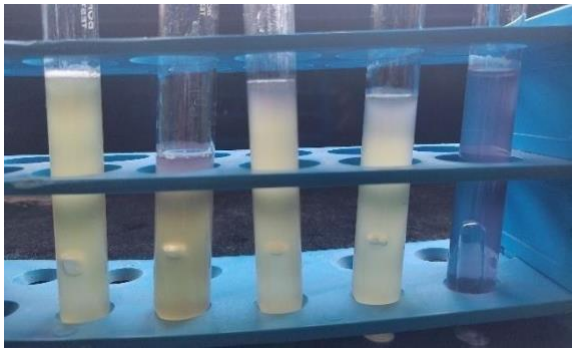

Fig 3.4: MPN 1x tube shows test tubes 1 to 4 giving positive results and tube 5 shows negative results

B) Complete test: Two selected tubes from the positive tubes from each well sample were streaked on EMB and NA plates. On EMB plates there was a mixture of mucoidal colonies, mucoidal colonies with pink center, dark mucoidal colonies and colonies with metallic sheen seen. NA plates showed *Pseudomonas* spp. Along with other colonies seen.

C) Confirmatory test: Biochemical tests were done.

Table 3.5: Biochemical tests of isolate

	Indole	MR	VP	Citrate	TSI	Motility	Tentative identification
Isolate 1	+	-	-	+	-	-	*Citrobacter spp*
Isolate 2	+	-	-	-	-	-	*E. coli*
Isolate 3	-	+	-	+	-	-	*Klebsiella spp*

Isolate 4: *Pseudomonas spp* was confirmed visually by greenish-coloured pigment production on NA plate

Table 3.6: Well wise pathogen distribution

Well number	Pathogen identified
Well 1	Nil
Well 2	*Citrobacter spp* *E. coli* *Pseudomonas spp*
Well 4	*Citrobacter spp*
Well 5	*Citrobacter spp* *Klebsiella spp* *Pseudomonas spp*
Well 7	*Citrobacter spp* *Klebsiella spp* *Pseudomonas spp*
Well 8	*E. coli* *Citrobacter spp*
Well 9	*E. coli* *Citrobacter spp* *Pseudomonas spp*
Well 10	*E. coli* *Citrobacter spp*

Well 12	*E. coli* *Pseudomonas spp*
Well 13	*E. coli* *Citrobacter spp*

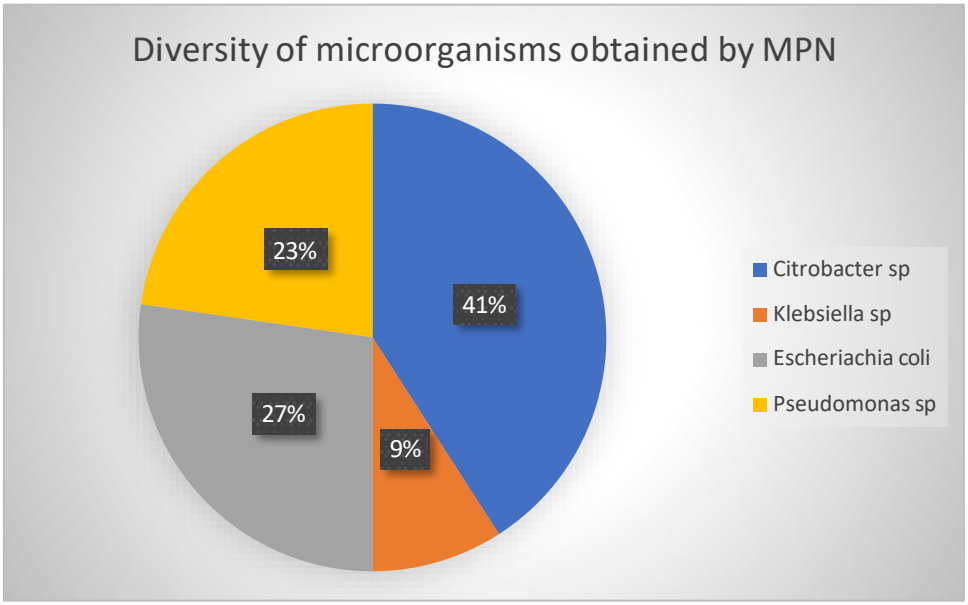

Fig 3.5: Pie chart showing the diversity of microorganisms obtained by MPN

From the diversity of organisms observed *Citrobacter spp* was 41% of the isolates and *E. coli* was seen 27%. *Pseudomonas spp* was 23% isolates obtained. *Klebsiella spp* was the least, only 9% isolates.

TCBS: TCBS plates were prepared and spread-plated in triplicates. After 24 hours incubation different types of *Vibrio spp* were absorbed. *Vibrio cholerae, Vibrio vulficiens, Vibrio alginolyticus* and *Vibrio mimicus* were observed on the plates.

Table 3.7: TCBS pathogen count

		Well 1	Well 2	Well 4	Well 5	Well 7	Well 8	Well 9	Well 10	Well 12	Well 13
V. cholerae like organisms	10^0	0	1	0	1	1	2	0	17	0	3
V. vulficiens like organisms	10^0	0	0	0	2	0	0	1	0	0	34
V. alginolyticus like organisms	10^0	0	0	0	2	0	0	0	0	0	0
V. mimicus like organisms	10^0	0	0	0	0	0	2	0	0	0	0

SS Agar: *Shigella spp* was observed in almost all the wells analyzed. One well also had *Shigella flexneri* like species.

Table 3.8: Salmonella Shigella pathogen count

		Well 1	Well 2	Well 4	Well 5	Well 7	Well 8	Well 9	Well 10	Well 12	Well 13
Shigella like species	10^0	0	3	0	1	1	0	3	5	7	2
Shigella flexneri like species	10^0	0	0	0	0	0	0	0	0	0	0

Aero Pseudo Agar: Both *Aeromonas* like species and *Pseudomonas* like species were seen in large numbers in all of the wells.

Table 3.9: Aero Pseuo pathogen count in cfu/ml

		Well 1	Well 2	Well 4	Well 5	Well 7	Well 8	Well 9	Well 10	Well 12	Well 13
Aeromonas like species	10^0	6.6 x 10^0	23.6 x 10^0	5.3 x 10^0	133.6 x 10^0	11.3 x 10^0	6 x 10^0	0	2.6 x 10^0	32 x 10^0	48 x 10^0
Pseudomonas like species	10^0	9.6 x 10^0	9.3 x 10^0	28.3 x 10^0	18.6 x 10^0	3.3 x 10^0	6.3 x 10^0	0	6.6 x 10^0	25.6 x 10^0	27.6 x 10^0

Well water of 10 selected wells of Mapusa was analyzed to check their microbial diversity. The well waters were slightly saline. The pH was seen to be on the lower end of the expected pH range. Well 4 had an acidic pH of 4.66 and well 5 showed a considerably lower DO of 5.4 both of which were lower than expected. The viable count gave 53 different isolates which were used for antibiotic and metal sensitivity testing.

The samples from the majority of the sites had an MPN index ranging from 1600 to ≧ 1600 per 100ml except for one well. This implies that each water sample had an estimated approx. 1600 coliforms per 100mL. It was observed that the water quality was unsatisfactory at all sites due to the high presence of coliforms. This strongly suggests that the sampled wells' water quality is poor and may be contaminated with pathogens and fecal matter. (Ting et al, 2021)

From the diversity of organisms observed *Citrobacter spp* was 41% of the isolates and *E. coli* was seen 27%. *Pseudomonas spp* was 23% isolates obtained. *Klebsiella spp* was the least, with only 9% isolates. Similar results were obtained for Somaratne and Hallas where *Citrobacter spp* was seen 65%, *Klebsiella spp* 34% and *Pseudomonas spp* 10% (Somaratne and Hallas, 2015)

Species like *V. cholerae, V. vulficens, Aeromonas* and *Pseudomonas* were seen in large numbers on the specific media plates.

OBJECTIVE 2 MATERIALS AND METHOD

3.4 Heavy Metal tolerance of bacterial isolates

All the bacterial isolates from the wells were tested for their tolerance to manganese and mercury. The mercury tolerance test was conducted using nutrient agar containing 0.05mM, 0.02mM, 0.04mM, 0.06mM, and 1mM mercuric chloride. Manganese tolerance was determined using nutrient agar plates containing 2 mM, 3 mM, 4 mM, 6 mM, and 10 mM manganese.

The bacterial isolates were inoculated as a patch on the plates. The growth of bacteria on plates meant that they were tolerant of the given metal.

3.5 Antibiotic resistance of bacterial isolates

Ampicillin and Streptomycin were filter sterilized through 0.22μm syringe filters. Mueller Hinton agar was incorporated with a final antibiotic concentration of ampicillin (50μg/ml) and streptomycin (25μg/ml). All the isolates were spot-inoculated on media and they were checked for resistance after 24 hours incubation of the plates.

RESULTS

Heavy metal tolerance

67% of the isolates were resistant to 0.02mM Mercuric chloride. This number reduced to 32% for 0.04mM. 80% of the isolates were sensitive to 0.06mM Mercuric chloride.

Table 3.10: Mercuric chloride tolerance of the isolates

Isolates	Mercury		
	0.02mM	0.04mM	0.06mM
W1 S1	++	++	++
W1 S2	-	-	-
W1 S3	+	+	-
W1 S4	+	+	-
W1 S5	++	++	++
W2 S1	++	++	-
W2 S2	++	+	-
W2 S3	++	-	-
W2 S4	++	++	-
W2 S5	++	+	-
W4 S1	-	-	-
W4 S2	+	+	-
W4 S3	-	-	-
W4 S4	++	+	-
W5 S1	++	++	-
W5 S2	++	++	++
W5 S3	++	++	-
W7 S1	++	-	-
W7 S2	++	-	-
W7 S3	++	++	++
W7 S4	++	+	-
W7 S5	++	+	-
W7 S6	++	++	++
W8 S1	++	++	-
W8 S2	++	+	-
W8 S3	++	-	-
W8 S4	++	-	-
W9 S1	++	-	-
W9 S2	-	-	-
W9 S3	-	-	-
W9 S4	++	+	-
W9 S5	++	-	-
W9 S6	++	+	-
W10 S1	++	+	-
W10 S2	++	++	++

W10 S3	+	-	-
W10 S4	++	++	++
W10 S5	++	-	-
W10 S6	++	++	++
W10 S7	-	-	-
W10 S8	++	-	-
W12 S1	++	+	-
W12 S2	+	+	-
W12 S3	+	+	-
W12 S4	++	++	++
W12 S5	++	++	++
W12 S6	++	+	-
W12 S7	++	++	++
W12 S8	+	-	-
W13 S1	-	-	-
W13 S2	++	-	-
W13 S3	++	-	-
W13 S4	+	-	-

Key: - No growth

\+ Slight growth

++ Proper growth

All of the isolates grew on 2mM and 3mM Manganese chloride. 90% and 73% isolates were resistant to 4mM and 6mM Manganese chloride respectively. For 10mM concentration 67% of the isolates were sensitive after incubation.

Table 3.11: Manganese chloride tolerance of the isolates

Isolates	Manganese				
	2mM	3mM	4mM	6mM	10mM
W1 S1	++	++	++	-	-
W1 S2	++	++	++	+	-
W1 S3	++	++	++	++	++
W1 S4	++	++	++	++	++
W1 S5	++	++	++	++	-
W2 S1	++	++	++	++	-
W2 S2	++	++	-	-	-
W2 S3	++	++	++	++	-
W2 S4	++	++	++	++	-

W2 S5	++	++	++	++	++
W4 S1	++	++	++	-	-
W4 S2	++	++	++	++	++
W4 S3	++	++	++	++	++
W4 S4	++	++	++	++	++
W5 S1	++	++	++	++	-
W5 S2	++	++	++	+	-
W5 S3	++	++	++	++	++
W7 S1	++	++	++	++	-
W7 S2	++	++	++	++	-
W7 S3	++	++	++	+	-
W7 S4	++	++	++	-	-
W7 S5	++	++	++	++	-
W7 S6	++	++	-	-	-
W8 S1	++	++	+	-	-
W8 S2	++	++	++	++	-
W8 S3	++	++	-	-	-
W8 S4	++	++	++	++	-
W9 S1	++	++	++	++	-
W9 S2	++	++	-	-	-
W9 S3	++	++	++	++	-
W9 S4	++	++	++	++	++
W9 S5	++	++	++	++	-
W9 S6	++	++	-	-	-
W10 S1	++	++	++	++	++
W10 S2	++	++	++	+	-
W10 S3	++	++	++	++	-
W10 S4	++	++	++	++	++
W10 S5	++	++	++	++	++
W10 S6	++	++	++	++	++
W10 S7	++	++	++	++	-
W10 S8	++	++	++	++	-
W12 S1	++	++	++	++	++
W12 S2	++	++	++	++	++
W12 S3	++	++	++	++	++
W12 S4	++	++	++	++	-
W12 S5	++	++	++	++	++
W12 S6	++	++	++	++	++
W12 S7	++	++	++	++	-
W12 S8	++	++	++	+	-
W13 S1	++	++	++	+	-
W13 S2	++	++	++	++	-
W13 S3	++	++	++	++	-
W13 S4	++	++	++	++	++

Key: - No growth

\+ Slight growth

++ Proper growth

Antibiotic tolerance

Of all the isolates tested 53% were sensitive to Ampicillin while 62% of the isolates were sensitive to Streptomycin.

Table 3.12: Antibiotic tolerance of the isolates

Isolates	Ampicillin	Streptomycin
W1 S1	-	+
W1 S2	-	-
W1 S3	++	++
W1 S4	++	-
W1 S5	++	-
W2 S1	++	+
W2 S2	-	-
W2 S3	-	-
W2 S4	++	+
W2 S5	++	++
W4 S1	-	-
W4 S2	-	-
W4 S3	-	-
W4 S4	-	-
W5 S1	++	+
W5 S2	++	++
W5 S3	++	-
W7 S1	-	-
W7 S2	-	-
W7 S3	-	-
W7 S4	-	-
W7 S5	-	+
W7 S6	++	++
W8 S1	-	-
W8 S2	++	++
W8 S3	-	-
W8 S4	-	-
W9 S1	-	-

W9 S2	-	-
W9 S3	++	++
W9 S4	-	+
W9 S5	++	+
W9 S6	-	-
W10 S1	++	++
W10 S2	++	++
W10 S3	+	+
W10 S4	++	+
W10 S5	++	-
W10 S6	++	+
W10 S7	-	-
W10 S8	+	+
W12 S1	++	++
W12 S2	-	-
W12 S3	-	-
W12 S4	++	-
W12 S5	++	-
W12 S6	-	-
W12 S7	++	++
W12 S8	-	+
W13 S1	++	-
W13 S2	-	-
W13 S3	-	-
W13 S4	-	-

Key: - No growth

\+ Slight growth

++ Proper growth

Mining activities have been well known for heavy metal leaching into the environment (Singh and Kamal, 2015). The isolates showed resistance to low levels of Manganese but were sensitive to high concentrations, likely due to abundant manganese ore in Goa (Parvez et al, 2013). Bacterial resistance to Mercury is common, likely due to industrial mercury emissions. Isolates have shown sensitivity to higher concentrations of mercury indicating toxicity exhibited by mercury. (Zheng et al, 2022)

The antibiotics tested exhibited resistance in over 50% of the isolates, possibly attributable to prevalent antibiotic resistance genes (ARGs) in the environment. Co-selection between heavy metal and antibiotic resistance genes is evident, as microbes with metal resistance may promote the proliferation of ARGs. This is likely due to metals persisting longer in the environment compared to antibiotics. (Zou et al, 2021)

CHAPTER 4: ANALYSIS AND CONCLUSION

The analysis of well water samples from Mapusa reveals concerning findings regarding microbial diversity, water quality, and environmental contamination. High levels of coliforms in the samples indicate poor water quality and potential contamination with pathogens and fecal matter, posing significant risks to human health and necessitating immediate remediation measures. The dominance of *Citrobacter spp*, *E. coli*, *Pseudomonas spp*, and *Klebsiella spp*. in the isolates suggests a microbial community composition indicative of pollution and environmental stress, further highlighting the need for intervention to safeguard water quality.

Resistance to manganese and mercury among isolates indicates heavy metal pollution likely stemming from mining activities in the region. Co-selection between heavy metal and antibiotic resistance genes emphasizes the interconnectedness of environmental pollutants and the spread of antibiotic resistance, underscoring the importance of integrated approaches to address both issues simultaneously.

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APPENDIX I

Nutrient agar (HiMedia)

Ingredients	Gms / L
Peptone	5.000
Sodium chloride	5.000
HM peptone	1.500
Yeast extract	1.500
Agar	15.000
Final pH (at 25°C)	7.4±0.2

Potato Dextrose agar (HiMedia)

Ingredients	Gms/L
Potato infusion	200
Dextrose	20
Agar	15
pH at 25°C	5.6±0.2

MacConkey broth(HiMedia)

Ingredients	Gms/L
Gelatin peptone	20.000
Lactose monohydrate	10.00

Dehydrated bile	5.00
Bromo cresol purple	0.010
pH after sterilization (at 25℃)	7.3±0.2

EMB (HiMedia)

Ingredients	**Gms / L**
Peptone	10.00
Dipotassium hydrogen phosphate	2.000
Lactose	5.000
Saccharose (Sucrose)	5.000
Eosin – Y	0.400
Methylene blue	0.065
Agar	13.500
Final pH (at 25°C)	7.2±0.2

TCBS (HiMedia)

Ingredients	**Gms / L**
Protease peptone	10.00
Yeast extract	5.00
Sodium thiosulphate	10.00
Sodium citrate	10.00

Bile	8.00
Sucrose	20.00
Sodium chloride	10.00
Ferric citrate	1.00
Bromo thymol blue	0.040
Thymol blue	0.040
Agar	15.00
Final pH (at 25°C)	8.6±0.2

Aero Pseudo selective agar (HiMedia)

Ingredients	Gms / L
Sodium glutamate	10.00
Starch, soluble	20.00
Potassium dihydrogen phosphate	2.000
Magnesium sulfate	0.500
Phenol red	0.360
Agar	12.000
Final pH (at 25°C)	7.2 ± 0.2

SS agar (HiMedia)

Ingredients	Gms / L

Peptone	5.000
HM peptone B #	5.000
Lactose	10.000
Bile salts mixture	8.500
Sodium citrate	10.000
Sodium thiosulphate	8.500
Ferric citrate	1.000
Brilliant green	0.00033
Neutral red	0.025
Agar	15.000
Final pH (at 25°C)	7.0±0.2

Nutrient broth (HiMedia)

Ingredients	Gms/L
Peptones	10
HM Peptone	1
Yeast extract	2
Sodium chloride	5
pH final	6.8 ± 0.2

Muller Hinton agar (HiMedia)

Ingredients	Gms/L
HM infusion B	300.000
Casein acid hydrolysate	17.500
Starch	1.500
Agar	17.000
Final pH (at 25°C)	7.3±0.1

Glucose peptone broth

Ingredients	Gms/L
Peptone	10
Glucose	10
Sodium Chloride	8.5
Final pH (at 25°C)	7±0.2

Tryptone Broth

Ingredients	Gms/L
Tryptone	10
Sodium chloride	5

Simons Citrate agar (HiMedia)

Ingredients	Gms/L
Sodium Chloride	5.0
Sodium Citrate (dehydrate)	2.0
Ammonium Dihydrogen Phosphate	1.0
Dipotassium Phosphate	1.0
Magnesium Sulfate (heptahydrate)	0.2
Bromothymol Blue	0.08
Agar	15.0

Triple Sugar Iron Agar (HiMedia)

Ingredients	Gms/L
Peptone	10.000
Tryptone	10.000
Yeast extract	3.000
HM Peptone B#	3.000
Lactose	10.000
Sucrose	10.000
Dextrose (Glucose)	1.000
Sodium chloride	5.000
Ferrous sulphate	0.200
Sodium thiosulphate	0.300
Phenol red	0.024

Agar	12.000
Final pH (at 25°C)	7.4 ± 0.2

APPENDIX II

Glycerol 70%

Ingredients	Gms/100ml
Glycerol	70ml
Distilled water	30ml

Winkler's A

Ingredients	Gms/100ml
Manganese chloride	40

Winkler's B

Ingredients	Gms/100ml
Potassium iodide	60
Potassium hydroxide	30

Manganese metal stock (100mM)

Ingredients	Gms/100ml
Manganese Chloride	1.979

Mercury metal stock (50mM)

Ingredients	Gms/100ml
Mercury chloride	1.3575

This was syringe filtered using a 0.22 micro m filter and stored at 4°C

Ampicillin sodium salt

Ingredients	Gms/50ml
Ampicillin sodium salt	1

This was syringe filtered using a 0.22 micro m filter and stored at 4°C

Streptomycin

Ingredients	Gms/50ml
Streptomycin	0.5

This was syringe filtered using a 0.22 micro m filter and stored at 4°C

Methyl red reagent

Ingredients	Gms/40ml
Methyl red	0.2
Ethyl alcohol	60

O'Meara Reagent

Ingredients	Gms/100ml
Potassium hydroxide	40.0
Creatine	0.30

Kovacs' reagent

Ingredients	Gms/100ml
p-dimethyl amino benzaldehyde	5.0
Amyl alcohol	75.0
Hydrochloric Acid	25.0