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Research Paper

A COMPARITIVE STUDY OF MICROBIAL ANALYSIS OF DRINKING WATER IN KOTHURU AND PEDABIDA PANCHAYATHHS IN ANANTHAGIRI MANDAL, VISAKHAPATNAM, ANDHRA PRADESH

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Abstract

To compare the bacteriological quality of drinking water samples of Kothuru and Pedabida panchayaths in Ananthagiri Mandal, Andhra Pradesh. In this study different water samples were collected from Kothuru and Pedabida panchayaths for bacterial assessment. This study was carried out for one year i.e., from April 2011 to March 2012. The samples were also cultured into bacteriological peptone water for enrichment. The culture in bacteriological peptone water was diluted in distilled water using serial dilution for total bacterial count. Some biochemical tests were carried out to identify the pathogens, also MPN was done for total coliform count. The microbial isolation was done by streak plate method on nutrient agar and on selective media for their identification (Sherman Cappuccino, 2009). The final identification of resulted isolates was done by the biochemical tests in accordance to the Bergey's Manual (Holt et al., 1984). The samples were inoculated and were incubated at 37^0C for 24 hrs or 48hrs.for appropriate bacterial growths. Kothuru panchayathh: The total plate count was above the WHO guidelines values (<10CFU's/ml) in the three water samples studied the highest count was during August. Increased presence of colifoms was noticed during August and October in stream; presence of coliforms was noticed in September in bore well while in well water it was during August. The resulted bacterial isolates viz. *E.coli, Salmonella, Shigella, Staphylococcus, Klebsiella pneumoniae, Group D Streptococcus, Vibrio cholera and V. parahaemolyticus* are highly pathogenic. Pedabida panchayathh: The total plate count was above the WHO guidelines values (<10CFU's/ml) in the three water samples studied and the highest count was during August. The bacteria isolated were *E. coli, Salmonella, Shigella, Staphylococcus, Group D Streptococcus, Vibrio cholera and V. parahaemolyticus and Klebisella pneumonia.* In conclusion, the presence of these pathogens in the water indicates that none of the water used for drinking purposes in these panchayathhs.

Key words: Drinking water, Pathogenic bacteria, Kothuru panchayathh, Pedabiba panchayathh.

INTRODUCTION

Over two thirds of Earth's surface is covered by water; less than a third is taken up by land. As Earth's population continues to grow, people are putting ever-increasing pressure on the planet's water resources. In a sense, our oceans, rivers, and other inland waters are being "squeezed" by human activities—not so they take up less room, but so their quality is reduced. Poorer water quality means water pollution. Clean and plentiful water provides the foundation for prosperous communities. Quality drinking water is essential for life (Samuel, Y., 2013). We rely on clean water to survive, yet right now we are heading towards a water crisis. Changing climate patterns are threatening lakes and rivers, and key sources that we tap for drinking water are being overdrawn or tainted with pollution. Many people are suffering from health problems due to consumption of the available contaminated water. The quality of water is therefore an issue of great environmental concern, according to Nouri *et al.,* (2008), water quality is determined by both natural and anthropogenic forces. The use of improved sources of drinking-water is high globally, with 87% of the world population and 84% of the people in developing regions getting their drinking-water from such sources. Even so, 884 million people in the world still do not get their drinking-water from improved sources, almost all of them in developing regions. China and India are home to more than a third of the world population. Both countries have made considerable progress. In China, 89% of the population of 1.3 billion uses drinking-water from improved sources, raised to 67% in 1990. In India, 88% of the population of 1.2 billion uses drinking-water from such sources, as compared to 72% in 1990. The majority of the infections that is associated with the lack of accessibility to potable water supply and poor environmental sanitation especially in developing countries. The following are micro-organisms associated with water; *Pseudomonas aeroginosa, Salmonella, Mycobacteria, Escherichia coli Proteus, Shigellasonnei, Klebsiella, Cyanobacteria* (Chris, 2004). Diseases that spread through the contaminated water principally in areas of poor sanitation are Hepatitis A, Hepatitis E, Typhoid fever, diarrhea and dysentery etc. (Light, 2000). Though bacteriological quality of drinking water is being monitored in urban areas and some rural areas, such monitoring was uncommon in some rural areas and especially so in tribal areas. Hence, the bacteriological quality of drinking water is important and periodical monitoring is essential for potable water. Thus this study was conducted to assess the bacteriological and physico chemical quality of sources of water used for drinking during April 2011 to March 2012 from Kothuru and Pedabida panchayathhs, Anathagiri mandal, Visakhapatnam, Andhra Pradesh, India.

MATERIAL AND METHODS:

Ananthagiri (18^{0}17’14”N, 83 ^{0}6’43”E) is about 60km away from Visakhapatnam and lies on the top of the Eastern Ghats. The area of the Ananthagiri mandal is roughly 50sq km and the entire area is inhabited by aboriginal tribes. Of the 25 panchayaths in Anantagiri mandal, Kothuru panchayath and Pedabida panchayathh was selected for the present study. Both the panchyaths contain three different water sources i.e. well, bore and a stream. The water quality of the three water samples (stream, bore and well) from the Kothuru and pedabida panchayathhs of Ananthagiri mandal, Visakhapatnam, Andhra Pradesh were studied for physicochemical and bacteriological parameters. In the present study, water samples from three sources i.e., a well, a bore and stream of the two panchayths were collected once in a month for a period of 12 month from April 2011 to March 2012, in white plastic bottles, which were previously rinsed with distilled water and sterilized with 70% alcohol. At the collection point, the containers were rinsed thrice with the sample water before being used to collect the samples. The collected samples were placed in a thermocol box. The microbial isolation was done by streak plate method on nutrient agar and on selective media for their identification (Sherman Cappuccino, 2009). The final identification of resulted isolates was done by the biochemical tests in accordance to the Bergey's Manual (Holt et al., 1984).

RESULTS

Water samples collected from Kothuru panchayath and Pedabida panchayath for a period of one year i.e., during April 2011 to March 2012 were analyzed for physical, chemical and bacteriological characteristics. The physical characteristic measured is P^H. Among the chemical characteristics Total dissolved solids (TDS) and fluoride contents were measured. For total number of viable bacteria total plate count (CFU/ml), for faecal and total coliforms most propable number (MPN/100ml) and for isolation and identification of bacteria staining, biochemical and growth on selective media were performed.

The mean P^H value of stream water in the both panchayaths was in the range 7. The mean P^H value of bore water in both Kothuru and Pedabida panchayaths was 7.09 & 7.06 respectively. The mean P^H value of well water in both panchayaths was 7.005. The P^H value in the three water samples is in the safe limit as recommended by WHO.

The amount of total dissolved solids of the stream water in both Kothuru and Pedabida panchayaths was on the average 107.84mg/l &107.64mg/l respectively and Fluoride content on the average in the both panchayaths was 0.1mg/l. The amount of total dissolved solids of the bore water in both Kothuru and Pedabida panchayaths was on the average 289.67mg/l & 273.25mg/l respectively and Fluoride content on the average was 0.12mg/l & 0.104mg/l respectively. The amount of total dissolved solids of the well water in both Kothuru and Pedabida panchayaths was on the average 191.13ma/l & 175.08mg/l respectively and Fluoride content on the average was 0.11mg/l & 0.109mg/l. Both the values in the three samples were in the permissible limits as recommended by WHO.

The total plate counts of bacteria in the water samples are given in figure1. In stream water the total plate count in both Kothuru and Pedabida panchayaths fell in the range of 36-64 CFU's/ml & 39-76 CFU's/ml respectively. The Kothuru panchayath water sample showed the maximum number of CFU's (64CFU's/ml) in August and minimum number was noted in June (36 CFU's/ml). The Pedabida panchayath water sample showed the maximum number of CFU's (76CFU's/ml) in August and minimum number was noted in March and June (39 CFU's/ml). In bore water the total plate count in both Kothuru and Pedabida panchayaths fell in the range of 39-76 CFU's/ml & 58-139 CFU's/ml respectively. The Kothuru panchayath water sample showed the maximum number of CFU's (76CFU's/ml) in August and minimum number was noted in June (39 CFU's/ml). The Pedabida panchayath water sample showed the maximum number of CFU's (139CFU's/ml) in August and minimum number was noted in March (58 CFU's/ml). In well water the total plate count in both Kothuru and Pedabida panchayaths fell in the range of 58-139 CFU's/ml & 97-236 CFU's/ml respectively. The Kothuru panchayath water sample showed the maximum number of CFU's (139CFU's/ml) in August and minimum number was noted in March (58 CFU's/ml). The Pedabida panchayth water sample showed the maximum number of CFU's (236CFU's/ml) in August and minimum number was noted in May (97 CFU's/ml). Total plate count for bacteria performed for all water samples showed that the bacteria in all the samples were above the WHO guideline values(<10CFU's/ml). The total plate count in all the three water samples was highest during the rainy season i.e., August and was due to the contribution of all the pathogenic bacteria.

The MPN values for Coliforms present in the water samples are presented in Figure 2. In stream water the MPN index in both Kothuru and Pedabida panchayaths ranged from 3-15/100ml & 4-28/100ml respectively. In Kothuru panchayath the maximum MPN index was recorded in (15/100ml) August and October and the minimum MPN index was recorded in (3/100ml) April and May. In Pedabida panchayath the maximum MPN index was recorded in (28/100ml) August and the minimum MPN index was recorded in (4/100ml) March. In bore water the MPN index in both Kothuru and Pedabida panchayaths ranged from 4-21/100ml & 9-64/100ml respectively. In Kothuru panchayath the maximum MPN index was recorded in (21/100ml) September and the minimum MPN index was recorded in (4/100ml) April and March In Pedabida panchayath the maximum MPN index was recorded in (64/100ml) August and the minimum MPN index was recorded in (8/100ml) March. In well water the MPN index in both Kothuru and Pedabida panchayaths ranged from 28-9/100ml & 20-210/100ml respectively. In Kothuru panchayath the maximum MPN index was recorded in (28/100ml) August and the minimum MPN index was recorded in (9/100ml) January and March. In Pedabida panchayath the maximum MPN index was recorded in (210/100ml) August and the minimum MPN index was recorded in (20/100ml) June.

During the study period all the three different water samples (i.e. stream, bore and well) from the two panchayathhs showed the presence of the eight pathogenic bacteria such as *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Shigella dysenteriae, Staphylococcus aureus, Group D Streptococcus, Vibrio cholerae* and *V. parahaemolytics* (Sandhya et al., 2013). (Table 1 &2)

DISCUSSION:

Water is extremely essential for survival of all living organisms and is one of the most important compounds of the ecosystem. Better quality of water described by its physical, chemical and biological characteristics. These characteristics signify the quality of water to be utilized by the people for the drinking, irrigation and also for industrial purposes. Good quality of water is the key to increase human productivity and long life (Urbansky and Magnuson, 2002). The good quality drinking water is often regarded as an important means of improve health. According to World Health Organization (WHO, 2002), there are billion cases of diarrhea and 2.2 million deaths annually due to the consumption of unsafe water has been identified. Safe drinking water is one of the most important felt needs in public health in developing countries in the 21st century (Sobsey et al., 2003). The general public today has always been found facing the problems for the availability of safe drinking water as a vast majority routinely suffers from common diseases like diarrhoea etc. and is the leading cause of mortality particularly among the children. The importance of potable drinking water is therefore obvious, emphasizing the need for its utilization (Ahmed et al., 2004). Diseases that spread through the contaminated water principally in areas of poor sanitation are Hepatitis A, Hepatitis E, Typhoid fever, diarrhea and dysentery etc. (Light, 2000).

Physical parameters such as P^{H}, TDS and fluoride content have a major influence on bacterial population growth. pH is the scale of intensity of acidity and alkalinity of water and measures the concentration of hydrogen ions. The pH of rivers and streams typically ranges from 6.5 to 8.0 standard units (Hem 1985). The pH values less than 6.5

occurred following significant storms. An increase in discharge, due to rainfall will dilute the concentration of base minerals causing a lower pH (MacDonald et al. 1991). Rainfall will also decrease pH by contributing hydrogen ions (Hem 1985). The highest pH at all sampling sites occurred during summer and fall when discharge rates were low and a greater concentration of base minerals resulted in increased p^H. P^H values ranging from 3 – 10.5 could favour both indicator and pathogenic organism's growth (Zamxaka et al., 2004). P^H provides the information about the acidity or alkalinity of water (Katyal and Satake, 1990). It also provides a means of clarifying and for collecting other characteristics or behavior such as corrosive activity (Ghandour et al., 1985). Eye irritation and exacerbation of skin disorders have been associated with P^H values greater than 11. With respect to the water samples the P^H values were in safe limit.

High TDS was commonly objectional or offensive to taste. A higher concentration of TDS usually serves no health threat to human until the values exceed 10,000mg/l (Anonymous, 1997). The TDS values of the all the water samples were within the permissible limit.

Fluoride testing in water quality analysis should be given importance because fluoride is know to cause a variety of health problems viz dental fluorosis and non skeletal manifestations when the level beyond 1.5ppm. Fluoride has come to stay as number one parameter in causing toxicological and geo-environmental problems in various countries. The fluoride content of all the water samples was within the permissible limit.

Most common and wide spread health risk associated with drinking water is the bacterial contamination caused either directly or indirectly by human or animal excreta. In the present study the heterotrophic plate count was used to estimate the total amount of bacteria in water and indicates the overall microbial status of water. The factors responsible for high microbial counts in the water samples may be due to technically ill planned sewerage network, damaged sewer lines, rust water pipe lines and poorly maintained disinfection system. Muhammad Anjum Zia et al., (2005) found that the ground water of Faisalabad city showed high bacterial counts. The pathogenic bacteria thus isolated were mostly belonging to the Enterobacteriaceae. The presence of Enterobacteriaceae members in the tested water samples indicates the faecal pollution. In general terms, the greatest microbial risks are associated with ingestion of water that is contaminated with human or animal (including bird) faeces. Faeces can be a source of pathogenic bacteria, viruses, protozoa and helminths. Faecally derived pathogens are the principal concerns in setting health-based targets for microbial safety (WHO, 2006). High level of contamination of ground water with faecal coliforms was found in urban areas of Karachi (Zubair and Rippy 2000). Khan et al., 2000 was also found that more than 50% water samples of Peswar, Nowshera and Charsada were polluted with faecal coliforms. These faecal coliforms were also reported from Umian lake water (Rajurkar et al., 2003) and also in different water samples at Sivakasi (Radha Krishnan et al., 2007). Water sources used for drinking or cleaning purpose should not contain any organism of faecal origin (Sabongari 1982, Fonseca et al., 2000). Rainfall can increase the concentration of fecal coliforms through contaminated soil runoff (Djuikom et al. 2006).

Faecal pollution was confirmed by the presence of coliforms in the water samples during the rainy seasons which may arise from animal dung carried by run-off to the rivers during the rainy season. The principal coliforms are Escherichia coli, Enterobacter aerogenes, Klebsiella spp and Citrobacter spp. E.coli is abundant found in the gastro intestinal tracts of humans, birds and animals, but rarely found in water therefore their presence in water can indicate faecal contamination. The classified indicator for water analysis is E. coli and its presence suggests enteric pathogens (Nwadiaro, 1982). There is a direct relationship between the numbers of E. coli and the extent of faecal pollution.

The results of microbial analysis of the water were presented in figure 1 and 2. The presence of pathogenic bacterial such as *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Shigella dysenteriae, Staphylococcus aureus, Group D Streptococcus, Vibrio cholerae* and *V. parahaemolytics* indicated that the water is not potable (Shittu et al.,2008).

An abscess is an abnormal cavity containing pus. Formation of abscesses in animals is very common. All the species of animals were affected with abscess in different areas of body like yoke region, liver, umbilicus, conjunctiva etc. but abscess forming in and around udder tissue are rare. Abscesses commonly develop after bite wounds, scratches, or when objects penetrate the skin and then the skin heals over. Usually cattle bit the infected area this may increase the severity and the other reason is the water borne pathogens may affect the wound area thus responsible for abscess formation. Different bacterial species like *Escherichia coli, Staphylococcus aureus, Streptococcus* were isolated as causative organism of udder abscess (G Kamalakar et al., 2014).

The study provided information about the water quality status of the Kothuru and Pedabida panchayaths of Ananthagiri mandal in Visakhapatnam. The physicochemical parameters are within the permissible standard limits. The microbial level render them unfit for human consumption though they can be used for other purposes water should meet different quality specification depending on the particular uses. Open defecation, water – logging environment, poor drainage facilities and unscrupulous dumping of domestic waste resulted in the deterioration of water quality in the study area. Water quality should be controlled in order to minimize acute problem of water related diseases which are endemic to health of man. Thus, an effective and thorough sanitary condition should be given to these water bodies in order to maintain a good water quality (Sandhya et al., 2014).

Thus, potable and domestic water should be harmless for the health of man and should have organoleptic properties and should be suitable for domestic use. Water quality should be controlled in order to minimize acute health problems of water related disease in humans.

The following three points approach is suggested for improving the quality of water supplied to the tribal communities of panchayath studied.

- ❖ Investigate the source of contamination of pipe borne water supplies to delineate the roles of the water delivery system and of household water storage system.
- ❖ Institute a system to monitor the quality of untreated water sources so that water collection can be restricted to uncontaminated sources and or water treatment advisories can be issued appropriately.
- ❖ Educate the public on appropriate water handling storage and treatment methods. It is evident that until these recommendations are implemented water supplied to the tribal communities in kothuru & pedabida panchayathh of ananthagiri mandal, Visakhapatnam district will continue to pose a health hazard to the population.

Table 1: Morphological and Cultural characteristics of Organisms

Morphological & Cultural characters	Organism	Disease caused by the organism
Gram negative rod, forms circular, low convex mucoid, opaque colonies with entire marginal growth on nutrient agar. Green metallic sheen colonies were observed on EMB agar.	*Escherichia coli*	Causal agent of gastroenteritis, urinary tract infections, and neonatal meningitis.
Gram positive coccus, non spore forming and non- motile bacteria. It forms circular, low convex with entire margin, smooth, medium opaque colony on nutrient agar. It forms yellow coloured colonies on mannitol salt agar.	*Staphylococcus aureus*	*S.aureus* incidence ranges from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It causes a range of illnesses, from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia, and sepsis. It is still one of the five most common causes of nosocomial infections and is often the cause of postsurgical wound infections.
Gram positive coccus. It forms thin, even growth on nutrient agar. Black (or) Brown coloured colonies were observed on bile esilin agar.	*Group D Streptococcus*	*Group D Streptococcus* causes urinary tract infections, meningitis, neonatal sepsis, spontaneous bacterial peritonitis, septic arthritis, and vertebral osteomyelitis diseases.
Gram negative curved rod. It forms abundant, thick, mucous white coloured colonies on nutrient agar and yellow coloured colonies on TCBS agar.	*Vibrio cholerae*	*Vibrio cholerae* is responsible for the occurrence of cholera.
Gram negative curved rod. It forms abundant, thick, mucous white coloured colonies on nutrient agar and green coloured colonies on TCBS agar.	*Vibrio parahaemolytics*	*V. parahaemolytics* is responsible for gastrointestinal illness in humans.
Gram negative rod. It forms slimy, white somewhat translucent,	*Klebsiella pneumoniae*	*Klebsiella pneumonia* is responsible for pneumonia, thrombophlebitis, urinary tract infection (UTI), cholecystitis, diarrohea, upper respiratory tract

raised growth on nutrient agar and dark pink coloured colonies on mac - conkey agar.		infection, wound infection, osteomyelitis, meningitis, and bacteremia and septicemia.
Gram negative rod. It forms thin even grayish growth on nutrient agar and dark green colonies on SS agar.	*Salmonella typhi*	*Salmonella typhi* causes typhoid.
Gram negative rod. It forms grayish growth on nutrient agar and colourless colonies on SS agar.	*Shigella dysenteriae*	*Shigella dysenteriae* is the bacillary dysentery causing bacterium.

Table 2: Biochemical Characteristics of isolates:

Test						
Catalase	+	-	+	+	+	+
Oxidase	-	-	-	+	-	-
Motility	-	-	+	+	-	
Indole	-	-	+	+	-	+
Methyl-red	-	+	+	-	+	+
Voge-Proskauer	+	-	-	+	-	-
Citrate Utilization	-	-	-	+	+	-
Urease	+	-	-	-	-	-
Hydrogen sulphide	-	-	-		+	-
Starch hydrolysis	-	-	-	-	-	-
Nitrate Utilization	-	-	+	+	+	+
Gelatin liquefication	-	-	-	+	-	-
Lactose fermentation	-	A	AG	AG	-	-
Glucose fermentation	A	A	AG	AG	AG	A
Sucrose fermentation	A	A	A(+)	AG	AG	A+/-
Organism	*Staphylococcus*	*Streptococcus*	*E. coil*	*Vibrio*	*Salmonella*	*Shigella*

A = Acid production only
AG = Acid and gas production
+/- = Variable reaction
\+ = Positive
– = Negative
(+) = Late Positive

Fig 1: Total plate count of stream waters

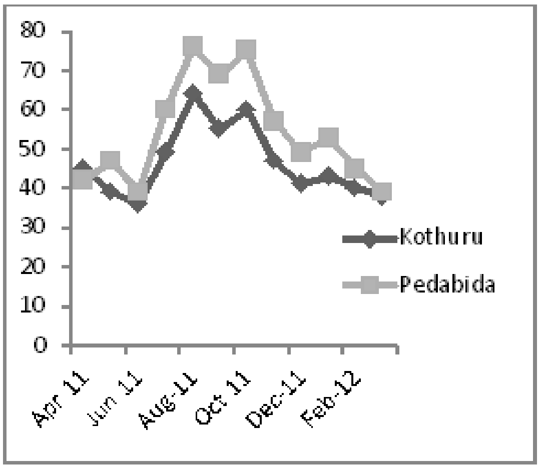


Fig 2: Most probable number of stream water

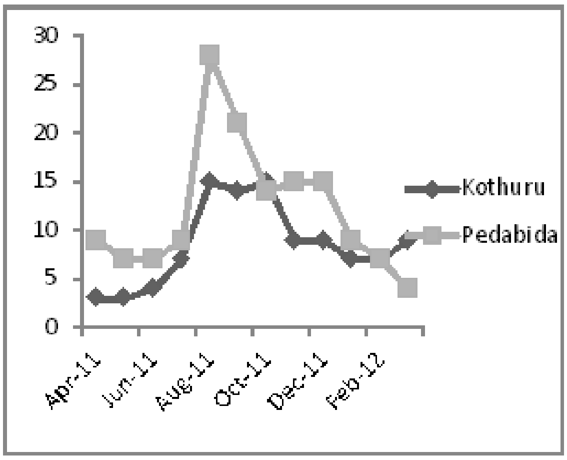


Fig 3: Total plate count of bore waters

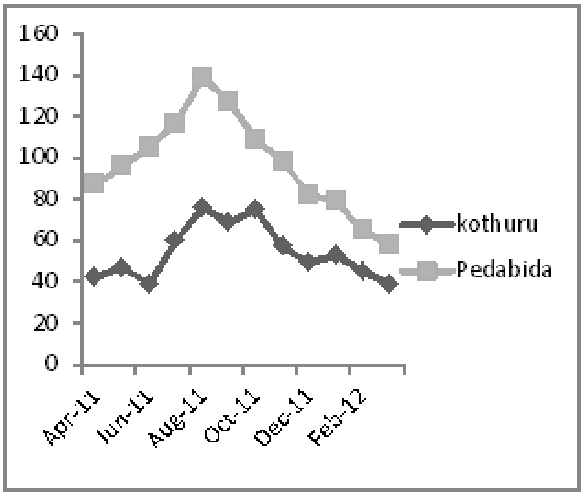


Fig 4: Most probable number of bore water

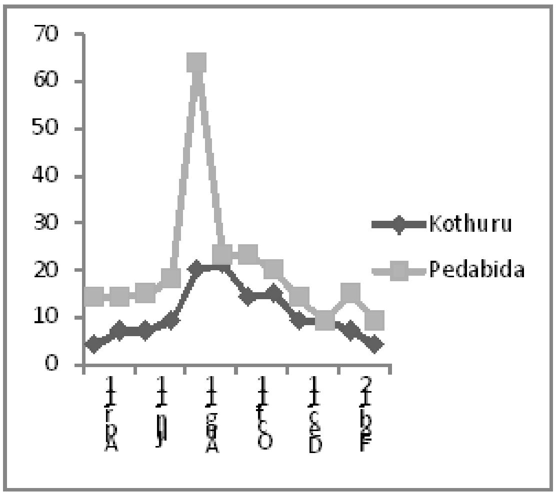


Fig 5: Total plate count of well waters

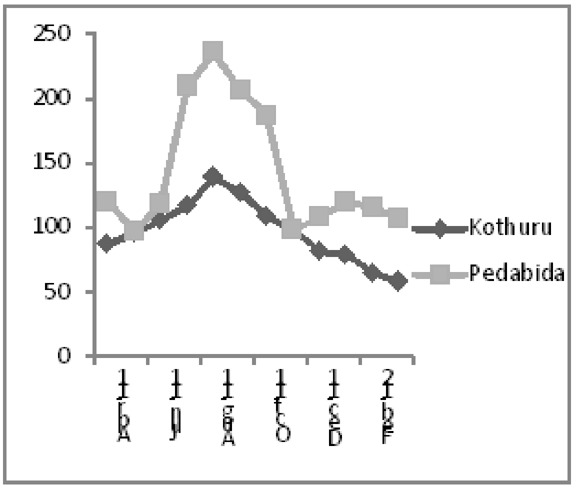
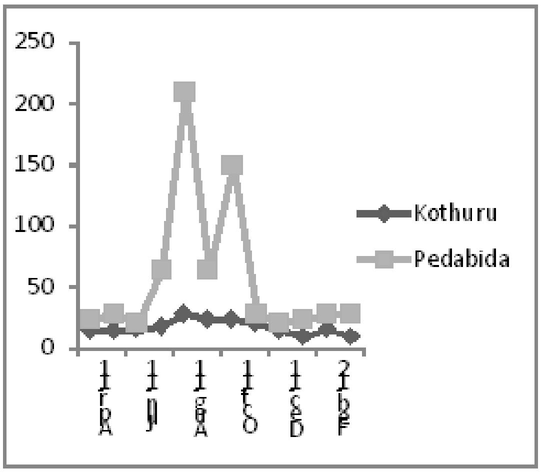


Fig 6: Most probable number of well water



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