

Micro-biological and physico-chemical quality of potable water in Borra, agency area, Andhra Pradesh

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SUMMARY

The present study was undertaken to evaluate the water quality of the Borra Panchayat of Ananthagiri mandal in Visakhapatnam district with affable means. The physicochemical and the microbial studies are most important regions by which we are able to test the portability of water. The isolation and characterisation of the pathogenic microorganism from the water sample collected were the main emphasized area of the study. In this study drinking water samples were collected from a hand bore, a tap and a stream for a period of two years *i.e.*, from April 2011 to March 2013. The various constituents monitored include the physicochemical characters, the bacterial parameters like total plate count (TPC), most probable number (MPN) and isolation and identification of pathogenic bacteria. The physicochemical characters of all the three drinking water samples were within the recommended permissible level of WHO. 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 and September. The bacteria isolated were *E. coli*, *Salmonella*, *Shigella*, *Staphylococcus*, *Group D Streptococcus*, *Vibrio cholera* and *V. parahaemolyticus* and *Pseudomonas*. The samples were inoculated and were incubated at 37°C for 24 hrs or 48hrs. for appropriate bacterial growths. Thus this study can be used for the assessment of the water and to resolve the hygienic problems of the water.

Key Words : Drinking water, Quality assessment, Pathogenic bacteria, Borra Panchayat

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Water plays an important role in human life. Water is the most common solvent for many substances and it rarely occurs in its pure nature (Caccio, 1973). It is the most basic and vital resource of our planet. Water can be obtained from a number of sources, among which are streams, lakes, rivers, ponds, rain, springs and taps (Okonko *et al.*, 2008). Water of good drinking quality is of basic importance to human physiology and man's continued existence depends very much on its availability (Lamikanra,

1999; FAO, 1997). In recent years, because of continuous growth in population, rapid industrialization and the accompanying technologies involving waste disposals, the rate of discharge of the pollutants into the environment is far higher than the rates of their purification. Before water can be described as potable, it has to comply with certain physical, chemical and microbiological standards, which are designed to ensure that the water is potable and safe for drinking (Tebutt, 1983). Potable water is defined as water that is free from disease producing microorganisms and chemical substances deleterious to health (Ihekoronye and Ngoddy, 1985). The ensuring of good quality drinking water is a basic factor in guaranteeing public health, the protection of the environment and sustainable development (Ranjini *et al.*, 2010). The provision of potable water to rural and urban population is necessary to prevent health hazards associated with poor drinking water (Nikoladze and Alastal 1989; Lemo, 2002). A

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significant proportion of the world's population use potable water for drinking, cooking, personal and home hygiene (WHO, 2004).

MATERIAL AND METHODS

Study area:

Ananthagiri (18°17'14"N, 83°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 Panchayats in Ananthagiri mandal, Borra Panchayat with 30sq.km area was selected for the present study. The total population present in this Panchayat is around 4,500 and includes 2,700 literates. The different tribal types present in this panchayat are "Konda Dora, Kotea, Nooka Dora, Parena Karja, Petege, Bagatha, Valmiki and Gadaba" and most of them depend on agriculture. The mean temperature is 36°C and receives 1171.0mm normal annual rainfall. Based on their economic status they live in different types of houses such as sheet houses, tiled houses and slab houses. Drinking water sources include taps each one per village; 5 taps and a small stream. The stream is the main source of drinking water.

In the present study, water samples were collected from three sources *i.e.*, a bore, a tap and pond once in a month for a period of 24 month from April 2011 to March 2013, 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 temperature in the box was maintained at 4°C by using ice packs.

Plating for microbial isolation:

The collected samples were serially diluted tenfold in order to reduce the number of microbes in the water samples. The bacteria were isolated by pour plate and spread plate methods using 10^{-3} and 10^{-4} dilutions.

In pour plate method 1ml of the sample was taken from both 10^{-3} and 10^{-4} dilutions separately and transferred into two Petri dishes. The nutrient agar was autoclaved and then poured in the Petri dish. The agar was allowed to solidify and incubated at 37°C for 24-48 hrs. In spread plate method sterile Petri dishes were taken and sterilized nutrient agar was poured into them. On the solidified agar surface, 0.1 ml of the sample (diluted sample *i.e.*, 10^{-3} and 10^{-4} dilutions) were poured and spread evenly using an L-shaped bent glass rod (spreader). The plates were incubated at 37°C for 24-48 hrs.

Microbial analysis and identification of bacteria:

Total plate count was determined by pour plate method. After 48 hrs of incubation colonies were counted by using colony counter and results were expressed as CFU/ml. Coliforms in the water samples were determined by most

probable number (MPN) method (FAO, 1992). Water analysis was carried out by multiple tube method. In this method double strength and single strength Mac conkey broth was prepared. Measured volumes of water to be tested were added to tubes containing medium and incubated. Most probable number (MPN) coliforms per 100ml of water sample were calculated from the relevant MPN table.

For identification of bacterial staining, colony characteristics, cultural characteristics, biochemical tests and characteristics of bacteria were used. In staining of bacteria Gram staining, endospore staining, capsule staining and motility test were done. In order to study the morphology of bacteria, cells were heat killed and fixed on the slide. The fixed bacteria were stained and observed for size, shape, arrangement, spore formation and capsulation etc. Hanging drop method was performed to study motility of bacteria. The colony characteristics such as size, shape, margin and elevation were observed on nutrient agar medium. Haemolytic behavior was observed on blood agar. The cultural characteristics of isolates were observed on selective media. The media used were Eosin Methylene Blue (EMB), Salmonella – Shigella agar (SSA), Mac-conkey agar, Manitol salt agar, TCBS agar and Bile esilin agar. Biochemical behavior of bacteria for utilization of specific substrate and enzymatic activity were studied by carbohydrate fermentation, catalase test, gelatin hydrolysis, IMViC test and urease test.

Analysis of water for physicochemical characters:

The pH of the water samples was measured by using the electrometric methods and other physicochemical parameters such as total dissolved solids and fluoride content were analysed by standard methods given in APHA(1989).

RESULTS AND DISCUSSION

Water samples collected from Borra Panchayat for a period of two years *i.e.*, during April 2011 to March 2013 were analysed 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 bacterial staining, biochemical and growth on selective media were performed.

The mean pH value of stream water was 7. In bore water it was in the range of 7.1-7.29 with the mean pH value 7.16. In tap water it was in the range of 6.82-7.19 with mean P^H value 7.015. The pH value in the three water samples is in the safe limit as recommended by WHO (2004).

The amount of total dissolved solids of the stream water was on the average 107.84mg/l and fluoride content on the average was 0.1mg/l. The amount of total dissolved solids of the bore water on the average was 273.25mg/l and fluoride

content on the average was 0.104mg/l. The amount of total dissolved solids of the tap water on the average was 175.08mg/l and fluoride content on the average was 0.109mg/l. Both the values in the three samples were in the permissible limits as recommended by WHO (2004).

The total plate counts of bacteria in the three water samples are given in figure1. In stream water the total plate count fell in the range of 35-69 CFU's/ml. The water sample showed the maximum number of CFU's (69CFU's/ml) in August 2012 and minimum number was noted in May 2013 (35 CFU's/ml). In bore water the total plate count fell in the range of 39-79 CFU's/ml. The water sample showed the maximum number of CFU's(79CFU's/ml) in October 2012 and minimum number was noted in June 2011 and March 2012 (39 CFU's/ml). In tap water the total plate count fell in the range of 54-145 CFU's/ml. The water sample showed the maximum number of CFU's(145CFU's/ml) in August 2012 and minimum number was noted in March 2013 (54 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 (2014) guideline values(<10CFU's/ml). The total plate count in all the three water samples was highest during the rainy season *i.e.*, August – October and was due to the contribution of all the pathogenic bacteria. However, the water samples of tap showed relatively higher plate count throughout the year. This may be due to the presence of sewage surrounding the tap which continuously seeps into the tap water. This study is in conformation with the result of Zaky *et al.* (2006) who reported increased bacterial content in the water of Manzala Lake, Egypt which is polluted by drainage and sewage.

The MPN values for coliforms present in all the water samples are presented in Fig. 2. In stream water the MPN index ranged from 3-15/100ml. The maximum MPN index was recorded in (15/100ml) August 2011, October 2011, August 2012 and October 2012. The minimum MPN index was recorded in (3/100ml) April 2011, May 2011 and May 2012. In bore water the MPN index ranged from 3-21/100ml. The maximum MPN index was recorded in (21/100ml) September 2011 and August 2012. The minimum MPN index was recorded in (3/100ml) May 2012. In tap water the MPN index ranged from 28-9/100ml. The maximum MPN index was recorded in (28/100ml) August 2011, August 2012 and September 2012. The minimum MPN index was recorded in (9/100ml) January 2012, March 2012, May 2012 and March 2013. The coliforms also showed their increased presence during August and September in stream and bore well while in tap water the increase was noticed during June, July and August.

During the study period all the three water samples (*i.e.* stream, bore and tap) showed the presence of the nine pathogenic bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*,

Shigella dysenteriae, *Staphylococcus aureus*, Group D *Streptococcus*, *Vibrio cholerae* and *V. parahaemolyticus*.

Escherichia coli is a Gram negative rod. It forms circular, low convex mucoid, opaque colonies with entire marginal growth on nutrient agar. Green metallic sheen colonies were observed on EMB agar. These are the most widely adopted indicator of faecal pollution and they can also be isolated and identified simply, with their numbers usually being given in the form of faecal coliforms/100 ml of wastewater (De Boer and Heuvelink, 2000) Outbreaks of these diseases can occur as a result of, drinking water from taps polluted by a combination of different wastewater microorganism species, eating contaminated fish, or indulging in recreational activities in polluted water bodies containing water borne pathogen. *E. coli* cause urinary tract infection and diarrhea (Fine *et al.*, 1996).

Group D *Streptococcus* is a 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* causes urinary tract infections, meningitis, neonatal sepsis, spontaneous bacterial peritonitis, septic arthritis, and vertebral osteomyelitis diseases.

Vibrio cholera is a Gram negative curved rod. It forms abundant, thick, mucous white coloured colonies on nutrient agar and yellow coloured colonies on TCBS agar. *Vibrio cholerae* is responsible for the occurrence of cholera.

Vibrio parahaemolyticus is a Gram negative curved rod. It forms abundant, thick, mucous white coloured colonies on nutrient agar and green coloured colonies on TCBS agar. *V. parahaemolyticus* is responsible for gastrointestinal illness in humans.

Klebsiella pneumonia is a Gram negative rod. It forms slimy, white somewhat translucent, raised growth on nutrient agar and dark pink coloured colonies on mac - conkey agar. *Klebsiella pneumonia* is responsible for pneumonia, thrombophlebitis, urinary tract infection (UTI), cholecystitis, diarrhoea, upper respiratory tract infection, wound infection, osteomyelitis, meningitis, and bacteremia and septicemia.

Pseudomonas aeruginosa is a common bacterium which can cause disease in animals and humans (Balcht Aldona, 1994). *Pseudomonas aeruginosa* can cause a range of infections but rarely causes serious illness in healthy individuals without some predisposing factor. It predominantly colonizes damaged sites such as burn and surgical wounds, the respiratory tract of people with underlying disease and physically damaged eyes. From these sites, it may invade the body, causing destructive lesions or septicemia and meningitis. Cystic fibrosis and immune compromised patients are prone to colonization with *P. aeruginosa*, which may lead to serious progressive pulmonary infections (WHO, 2004).

Staphylococcus aureus is a 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. It is the most common cause of staph infections. It is a spherical bacterium, frequently found in the nose and skin of a person. *S. aureus* can cause a range of illnesses from minor skin infections, such as pimples, impetigo, boils, cellulitis folliculitis, furuncles, carbuncles, scalded skin syndrome and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, and septicaemia. Its incidence is from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections (Fine *et al.*, 1996).

Shigella is a Gram negative rod. It forms grayish growth on nutrient agar and colourless colonies on SS agar. It can cause serious intestinal diseases, including bacillary dysentery. Abdominal cramps, fever and watery diarrhoea occur early in the disease. All species can produce severe disease, but in the case of *S. dysenteriae*, clinical manifestations may proceed to an ulceration process, with bloody diarrhoea and high concentrations of neutrofiles in the stool. The production of Shiga toxin by the pathogen plays an important role in this outcome. *Shigella* spp. seems to be better adapted to cause human disease than most other enteric bacterial pathogens (WHO, 2004).

Salmonella infections typically cause four clinical manifestations: gastroenteritis, bacteraemia or septicaemia, typhoid fever/enteric fever and a carrier state in persons with previous infections. In regard to enteric illness, *Salmonella* spp. can be divided into two fairly distinct groups: the typhoidal species/serovars *i.e.* *Salmonella typhi* and *S. paratyphi* and the remaining non-typhoidal species/serovars (WHO 2004).

The faecal coliforms *E.coli* and *Klebsiella pneumoniae* were recorded in all the water samples in the present study. High level of contamination of ground water with faecal coliforms were found in urban areas of Karachi (Zubair and Rippy, 2000; Khan *et al.*, 2000) found that more than 50% water samples of Peshawar, Nowshera and Charsada were highly contaminated with pathogenic microorganisms and were considered unfit for human consumption. These faecal coliforms were also reported from Umian lake water (Rajurkar

et al., 2003) and from different water samples at Sivakasi (Radha Krishnan *et al.*, 2007). The presence of *E.coli* is an indication of faecal contamination. It can cause urinary tract infections. Certain strains of *E.coli* produce enterotoxins that cause traveler's diarrhoea and occasionally cause very serious food borne diseases (Tatora *et al.*, 2009).

The faecal streptococci group comprises of *Streptococcus faecalis*, *S.bovis*, *S.equinus* and *S.avium*. In the present study all the water samples were contaminated with *S.avium*. It was positively correlated with the faecal streptococci group in Ooranis and tap water samples at Ramanathapuram district, in the range of 0.0 to 2.8 x 10 FS/100ml (Joshi *et al.*, 2002). This group was also recorded from drinking, bore well and sewage water samples of Thiruthangal and were not found in all water samples of S.N Street and N.N Street of Sivakasi (Radha Krishnan *et al.*, 2007). The reason for the high number of faecal streptococci might be due to addition of human and warm blooded animal's excreta.

Human and animal wastes are the primary source of different bacteria in water. The sources of bacterial contamination include run off from feedlots, pastures, dog runs and other land areas where animal wastes are deposited. Bacteria from these sources can enter in taps that are either open at the land surface, or don't have water tight casing or caps, or don't have seal in the annular space (the space between the wall of the drilled tap and the outside of the tap casing). Insects, rodents and animals entering the tap are other sources of contamination. Another way through which bacteria can enter the water supply is through inundation or infiltration by flood waters or by surface runoff. Flood water commonly contains high level of bacteria. Small depressions filled with flood water provide excellent breeding ground for bacteria (Ley and Samant, 2003). In the present study area the places surrounding the drinking water sources are not hygienic. The open taps are surrounded by drainage, throughout the year. The daily house hold activities like washing clothes and cleaning utensils are being carried out at the hand bores. The stream water gets polluted in multiple ways. Cleaning the domestic animals and washing clothes in the stream and throwing domestic wastes into the stream contaminate the water throughout the year.

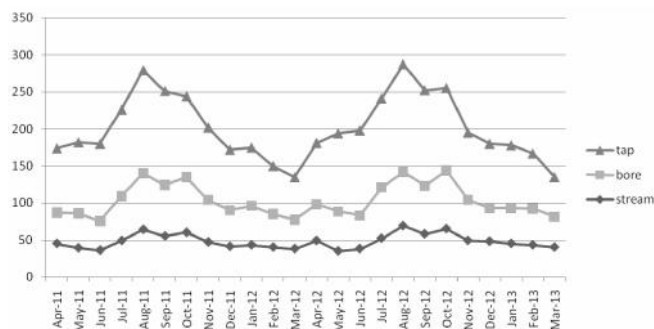


Fig. 1: Total plate count (CFU/ml) of bacteria in three water samples

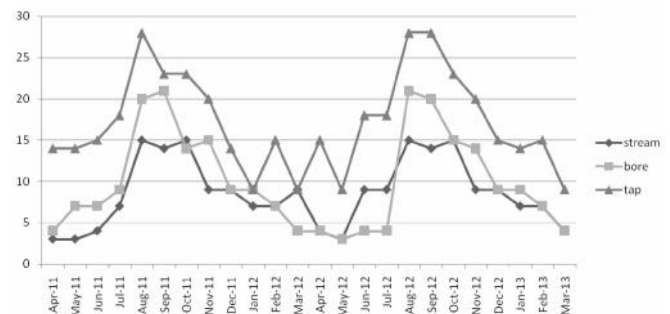


Fig. 2: Most probable number (/100ml) of coliforms in three water samples

Chan *et al.* (2007) isolated pathogenic bacteria such as *E. coli*, *Streptococcus faecalis* and *Pseudomonas aeruginosa* from the water samples. Ajibade *et al.* (2008) confirmed the presence of the coliforms. They isolated different pathogenic bacteria viz., *Pseudomonas sp.*, *Escherichia coli*, *Acetobacter sp.*, *Maroxalla sp.*, *Bacillus sp.*, and *Klebsiella sp.* from the river water samples. As a result they concluded that the water of the four rivers in the park is not potable during the wet seasons. Omezuruike *et al.* (2008). Isolated different bacterial pathogens and these pathogens were identified to be *Staphylococcus aureus*, *Salmonella species*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Bacillus species*, *Proteus species*, *Klebsiella species*, *Flavobacterium species* and *Acinetobacter species*. Usharani *et al.* (2010) in their bacteriological study showed that the total heterotrophic bacteria, total coliforms, faecal coliforms, faecal Streptococci and FC/FS ratio in the river water samples were found to be greater than the standard WHO limits. The generic distribution in the samples revealed that the presence of *Escherichia coli*, *Staphylococcus*, *Enterobacter*, *Streptococci*, *Bacillus* and *Micrococcus* were predominant in river water samples.

Conclusion:

The study provided information about the water quality status of the Borra Panchayat of Ananthagiri mandal in Visakhapatnam. The physico-chemical parameters were 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.

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