Physico-chemical and microbial analysis of water samples
in Anakapalli Municipal Corporation

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Abstract

An investigation of microbiological and physico-chemical properties of drinking water samples from five different locations in Anakapalli municipal corporation. The water samples are subject to physico-chemical analysis by standard methods. The microbial isolation was done by a streak plate method on nutrient agar and on selective media for their identification. The final identification was done according to the Bergey's Manual. The physico-chemical characters of all the five drinking water samples were within the recommended permissible level of WHO. The total plate count was above the WHO guideline values (<10CFU's/ml) in the five water samples. The highest count was noticed in S_5 sample (54 CFU's/ml). The highest coliform count was observed in S3 samples (11/100 ml). Six isolates of bacteria namely: *E.coli, Enterobacter aerogenes, Klebsiella pneumonia, Salmonella, Shigella,* and *Staphylococcus* were isolated, which are highly pathogenic, the result of the study indicates that the water in Andhra University pump houses is highly contaminated and not safe for drinking and utility purposes. The study therefore, stresses on the need to control the fecal pollution of water bodies.

Introduction

In our country, almost 70% of the water has become polluted due to the discharge of domestic sewage and industrial effluents into natural water sources. Health of human and other organisms directly related with safe water. That is why safe water resources are very important. In most of the developing areas, it is normally observed that due to lack of safe drinking and utility water people become ill. Water in nature is seldom totally pure. Increase in human population and urbanization in recent years resulted in gradual deterioration of water quality. The uniqueness of the water body have deteriorated as it is getting enriched with pollutants. It is estimated that each year 10 million people die from drinking contaminated water. According to WHO (2006) organization about 80% of all diseases in human is caused by water. Atlas and Bartha (1993) considered that bacteria play an important role in global ecosystems which are major factors in controlling the quality of water and are fated determinants' of pollution released into the environment. Clark and Pagel (1977) considered bacterial as are reliable indicators of contamination. Water, which looks and taste good, may not necessarily be safe to drink it may be polluted with harmful bacteria, parasites and viruses. These microbes can exist in surface and ground water supplies, and can cause immediate sickness in humans if not properly treated (PFRA, 2003). Quality standards for treating drinking water vary from place to place. The objective of most

treatment schemes is largely to reduce the possibility of the spread of waterborne disease to barest minimum in addition to consideration for its wholesomeness and palatability in all respects (Edema et al., 2001).

Material and Methods

Study area

Anakapalli municipality is a revenue division part of greater Visakhapatnam Municipal Corporation in the Visakhapatnam district of Andhra Pradesh. The Anakapalli municipality had an area 23.28 Sq. Kms with the population of 86,612 as per 2011 census.This anakapalli municipality is located on the banks of river Sarada at 17.68^o N, 83.02^oE it has an average elevation of 26 mts.The present study was carried out at some selected sampling sites within the municipality.

In the present study, water samples were collected from three sources i.e., a well, a hand pump and stream once in a month for a period of 12 months 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 temperature in the box was maintained at 4^oC by using ice packs. The microbial isolation was done by a streak plate method on nutrient agar and on selective media for their identification (Sherman Cappuccino 2009). The final identification of resulted isolates was done with the biochemical tests in accordance with the Bergey's Manual.

Result and Discussion

The P^H values of the water samples ranged from 7.10-7.4. The P^H value of S_1 was 7.2, S_2 was 7.1, S_3 was 7.3, S_4 was 7.4 and S_5 was 7.1. The P^H of the five water samples is in the safe limit as recommended by WHO (2006). The P^H in most of the natural water ranges from 6.5-8.5 while deviation from the neutral 7.0 is as a result of the bicarbonate or carbonate equilibrium (Medera and Allen, 1982). The P^H values are set for domestic use as prescribed by APHA (1998). According to US Environmental Protection Agency the higher the mineral contents in the water more total suspended solids will be formed. Total Dissolved Solids in the water consist of ammonia, nitrate, nitrite, phosphate, alkalis, some acids, sulphates, metallic ions etc. The Total Dissolved Solids values is not desirable for utility water because a high content of dissolved solids elevates the density of water, influence osmoregulation of fresh water, reduction of solubility of gases (oxygen) and utility of water for drinking purposes. In the present investigation an average S_1 has 324.0, S2 has 378.1, S_3 has 143.1, S4 has 568.0 and S_5 has 547.3 mg/L. Hence the values of the five samples were in the permissible limits as recommended by WHO (2006).

Fluoride occurs naturally in most groundwater wells and can help to prevent dental cavities. As fluoride levels increases, there is an increase in the tendency to cause tooth mottling. Fluoride levels less than 2 mg/L are not considered a problem for livestock. On an average the Fluoride content should be 0.105 mg/L and coming to the present investigation S_1 contains 0.12 mg/L, S_2 contains 0.16 mg/L, S_3 contains 0.18 mg/L, S4 contains 0.11 mg/L and S_5contains 0.1 mg/L. Hence the values are in permissible limits as recommended by WHO (2006).

The result obtained from the TPC is given in figure 1. On an average from S_1 contains 95.5 CFU's/ml, S_2 contains 89.6 CFU's/ml, S_3 contains 60 CFU's/ml, S_4 contains 95 CFU's/ml, S_5 contains 93.5 CFU's/ml. The

**Fig. 1.** Total Plate Count (CFU/ml) of Bacteria in five water samples
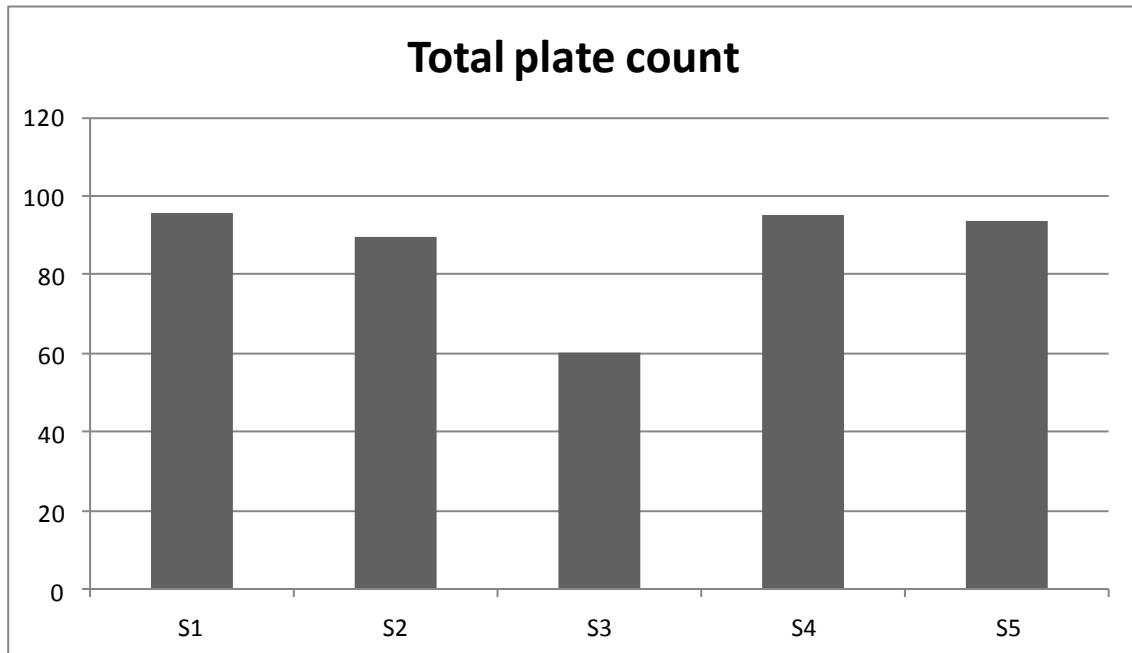
above all five samples showed the maximum no. of CFU's in June, July, August followed September 2013. The TPC results showed in higher values in the month of June, July, August 2013 i.e. rainy season and also showed that bacteria in the samples are above the WHO guidelines values (<10 CFU's/ml). In S_5 on an average higher TPC value is observed. This may be due to unmanaged construction of bores from where the water continuously seeps into the pump houses of Andhra University. This study is in conformation with the result of (Zaky, 2006) who reported increased bacterial content in the water of Manzala Lake, Egypt which is polluted by drainage and sewage. The recommended standard MPN for water is less than 2 MPN for 100 ml (FAO, 1997). In the present analysis, the most probable number in the sample S_1 is 4.6 /100 ml, S_2 is 5.5 /100 ml, S_3 is 10.6 /100 ml, S_4 is 2.8 /100 ml, S_5 is 3.3/100 ml (Fig. 2). The presence of coliform group in water sample generally suggests that a certain selection of water

may have been contaminated with faeces either of human or animal origin. The other more dangerous microorganism could be present in the water sample (Sapkota Rajendra et al., 2012). The present results obtained for Total plate count and Most probable number were similar to the results obtained by Okonoko et al. (2008); Oluyege Jacob Olaoluwa et al. (2010).

During the study period all the five water samples 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. parahaemolytics. 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 et al., 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).

Fig. 2. Most Probable Number (100 ml) of Coliforms in five water samples
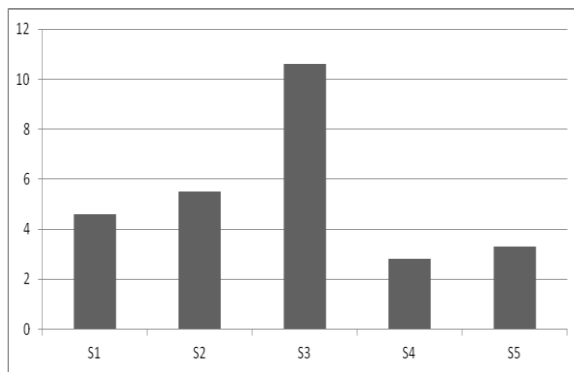
S_1 = Perugu Bazar; S_2 = Nukalamma temples; S_3= Near Railway station; S_4 = Womens college (Gavara veedi); S_5 = Sarada nagar

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 parahaemolytics* 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. parahaemolytics* 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, diarrohea, 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 septicaemia 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 colored 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 septicemia. 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 a grayish growth on nutrient agar and colorless 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 neutrofils in the stool. The production of Shiga toxin by the pathogen plays an important role in this outcome. *Shigella* sp. Seems to be better adapted to cause human disease than most other enteric bacterial pathogens (WHO, 2004).

Salmonella infections typically cause for clinical manifestations: gastroenteritis, bacterium or septicemia, typhoid fever/enteric fever and a carrier state in persons with previous infections. In regard to enteric illness, *Salmonella* sp. 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 fecal 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 fecal 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.*

color is an indication of fecal contamination. It can cause urinary tract infections. Certain strains of *E. coli* produce enterotoxins that cause traveler's diarrhea and occasionally cause very serious food borne diseases (Tatora et al., 2009).

The fecal streptococcus 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 fecal streptococcus group in Ooranis and tap water samples at Ramanathapuram district, in the range of 0.0 to 2.8 x 10 FS/100 ml (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 fecal streptococci might be due to addition of human and warm blooded animal excreta. Human and animal wastes are the primary source of different bacteria in water. The sources of bacterial contamination include runoff 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 a 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 or 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 an excellent breeding ground for bacteria (Ley and Samant, 2003). In the present investigation, area the places surrounding the

drinking water sources are not hygienic. The open taps are surrounded by drainage, throughout the year. The daily household 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. 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 results of the present study indicated that water in Anakapalli municipality is contaminated with various pathogenic bacteria and unfit for drinking. Open defecation, water-logging environment, poor drainage facilities and unscrupulous dumping of domestic wastes resulted in the deterioration of water quality in the study area. The study also revealed that large-scale waterborne diseases in this area are prevalent. Since quality of water is critical in disease prevalence, the water sources used for drinking should be monitored from time to time for reducing disease epidemics.

References

Atlas, RM and Bartha, R (1993), Microbial ecology: Fundamentals and Applications, 3rd Edition, Redwood city, Benjamin.

Clark, IA and Pagel, JE (1977). Pollution indicator bacterial associated with Municipal raw and drinking water supplies. Canadian journal, 28 pp 465-470.

Edema MO, Omemu AM, Fapetu OM (2001). Microbiological and physicochemical analysis of different sources of drinking water, Nigeria Journal of Microbioloy 15: 57-61.

Prairie Farm Rehabilitation Administration (PFRA), Agriculture and Agri-Food Canada. 2003.

Medera, V., Allen, HE., Minear. 1982 RC Non-metallic constituents; Examination of Water Pollution Control. A reference handbook. Physical Chemical and Radiological Examination, 169-357

Ajibade W. A., Ayodele I. A., Agbede S.A. (2008); Microbiological characteristics of waters in the major Rivers in Kainji Lake National Park, African Journal of Environmental Science and Technology 2 (8): 208-216.

Balcht Aldona (1994); Pseudomonas aeruginosa: Infections and Treatment. Informa Health Care, 21: 83–84

Chan C.L., Zalifah M.K., Norrakiah A.S. (2007); Microbiological and Physicochemical Quality of Drinking Water, the Malaysian Journal of Analytical Sciences, 11(2): 414 – 420

De Boer E, Heuvelink AE (2000); Methods for the detection and isolation of Shiga toxin-producing Escherichia coli. Symp Ser Soc Appl Microbiol, (29): 133–143.

Ekwenye, U.N. and Ehilegbu, C.U 2008. Microbial analysis of bottled water samples sold commerciallyin umuahia meteropolis in Abia state, Nigeria. J.Pure.Appl.Micro.2(1): 97- 102.

Fine MJ, Smith MA, Carson CA (1996); Prognosis and outcomes of patients with community acquired pneumonia. A meta-analysis JAMA, 275(2): 134-141.

Food and Agriculture Organization (FAO) (1997a). Chemical analysis manual for food and water. J. Agric Food Chemical, 1(2):20-26.

Food and Agriculture Organization (FAO) (1997b). Annual report on food Quality Control. J.Agric FoodChemical, 1(6):11-13

Gajendran, C. and Thamarai, P. 2008. Study on statistical relationship between ground water quality parameters in Nambiyar river basin, Tamil Nadu, India. Poll. Res.27 (4): 679 – 683.

Hasan, N. Zulfiqar, A. M. and Seema , i. 2010. Bacterial Indicators of Risk of Disease from Drinking water. Balwois. 2010.Ohrid. Republic. Macedonia: 1- 5.

Ihekoronye A.I, Ngoddy P.O (1985). Integrated food science and technology for tropics. Oxford: Macmillan Press, pp. 95-195.

Joshi VA, Manivel V, Ravindra Rao R and Kelkar, PS. Water quality assessment in Ramanathapuram district. Ind. J. Environ. Protec., 2002; 22(9): 970-977.

Khan M, Ihsanullah TS, Fazal M and Abdus S. Ocurence of pathogenic microorganisms in food and water supplies in different areas of Peshawar, Nowshera and Charsada. *Pak. J. Food Sci.*, 2000; 10: 37-40.

Karunakaran K, Thamilarasu P, Sharmila R (2009). Statistical study on Physicochemical characteristics of Groundwater in and around Namakkal, Tamilnadu. India E-Journal of Chem., 6(3): 909-914.

Lamikanra, A. (1999). Essential Microbiology for students and practitioner of pharmacy: Medicine and Microbiology, 2nded. Lagos: Amkra books, p.406.

Lemo, O.O. (2002). Bacteriology Determination of water with long term storage (B.Sc. Thesis), Abeokuta: UNAA, p.40.

Ley V and Samant J. Bacteriological analysis of drinking water of Kolhapur City, Maharashtra, India. Department of Environmental Sciences, Sub Centre, Mumbai University, Mirjole, M.I.D.C. Ratnagiri 2003; 415 639, India, 24: 689-694.

Nikoladze G.D.M Alastal S.(1989). Water Treatment For Public and Industrial Supply. Moscoul: MIR Publisher , p.163.

Okonko,I.O; Adejoje, O.D; Ogunnusi, T.A; Fajobi, E; Shittu, O.B (2008) "Microbiological and physicochemical analysis of different water samples used for domestic purposes in Abeokuta and Ojota, Lagos Nigeria. African Jounal of Biolechnology, 7(5) 617-6721.

Omezuruike Okonko Iheanyi, Damilola Adejoye Oluseyi, Adeola Ogunnusi Tolulope, Fajobi, Enobong A., Shittu Olufunke B. (2008); Microbiological and

physicochemical analysis of different water samples used for domestic purposes in Abeokuta and Ojota, Lagos State, Nigeria, African Journal of Biotechnology 7(5): 617-621.

Radha Krishnan R, Dharmaraj K and Ranjitha Kumari BD.: A comparative study on the physicochemical and bacterial analysis of drinking, boretap and sewage water in the three different places of Sivakasi. Ind. J. Environmental. Biology., 2007; 28(1): 105-108.

Rajini K; Roland P; John C; Vincent R. (2010). Microbiological and physicochemical analysis of drinking water in George town. Nature and Science, 8 (8)261-265.

Rajurkar NS, Nongbri B and Patwardhan AM.: Physicochemical and microbial analysis of Umian (Brapani) lake water. Ind. J. Environ. Protec., 2003; 23(6): 633-639.

Tatora GJ, Funke BR and Case CL. Microbiology: An Introduction, Pearson Education, Inc. India, 2009.

Tebutt T.H.Y. (1983). Principles of Quality Control. England: Pergamon, p.232.

Usharani K, Umarani K, Ayyasamy P M, Shanthi K, Lakshmanaperumalsamy P (2010), PhysicoChemical and Bacteriological Characteristics of Noyyal River and Ground Water Quality of Perur, India, J. Appl. Sci. Environ. Manage. 14 (2): 29 – 35

WHO (2004); Guidelines for Drinking-water Quality, Vol. 1: 3rd ed. World Health Organization Geneva.

World Health Organization,(2004). Guidelines for drinking water quality. 3rd edition, Switzerland: WHO press pp.16,89

Zaky MM. Environmental factors influencing multi drug resistant and harbouring plasmid DNA Aeromonas Hyrdophila isolated from polluted waters of Lake

30.Zuabair A and Rippy B. Evaluation of shallows ground water quality in urban areas of Karachi (Pakistan)-Inorganic nutrients and bacterial contamination. *Pak. J. Sci. Ind. Res.,* 2000; 43: 221-225.