Integrated dataset of Physico-chemical properties and Isolation of metal resistant bacteria from the Penna river, Chennur Mandal, Kadapa district, Andhra Pradesh.

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Abstract:

Penna river is one of the key of the state of Andhra Pradesh. This data was attempted to develop water quality index and to isolate microbes from the river water samples. The present work purposes to analyze the physico-chemical parameters and isolation of metal resistant bacteria from sediment of the penna river. The development of water quality index was monitored with parameters includes p^H ,Temperature, Electronic Conductivity, Alkalinity, Total Dissolved Solids (TDS), Salinity, Total Hardness, Calcium and Magnesium Hardness, Chlorides, Fluorides, Sulphates , Phosphates, Nitrates , Biological Oxygen Demand (BOD), COD. In this study, sediment samples were tested to isolation of metal resistant bacteria by using serial dilution method, staining &bio-chemical tests. Respectively, MIC and Antibiotic resistance was carried out by Well diffusion method, Disc diffusion method. Heavy metals which are present in the water samples were analysed by Inductively Coupled Plasma Spectroscopy (ICP/OES). The obtained results showed that the average values of Lead in water samples were more than the normal drinking water.

Key words: Penna river, Physico Chemical parameter, Metal resistant bacteria.

Introduction :

Water is considered to be the main requirement for the biosphere. Increase in population and industrialization, has caused the demand of the freshwater to increase in the last couple of decades. This demand fulfilled by the rivers which provide the water for human life and agriculture purposes. Due to the waste discharged from the human and industrial activities, the quality of river water has deteriorated. This affects human as well as aquatic life. According to WHO,CPCB, BIS, ICMR, the water quality of about 70%

river water was contaminated due to pollutants in India and some of the river water was too poor for human consumption (Rama krishnaiah et al., 2009; Jindal and Sharma, 2010). Assessment of quality of river water using various parameters (physico-chemical and biological) and the different ways and techniques to protect the river water have been reported in the literature (Santosh et al., 2008; Yisa andJimoh, 2010; Shah et al., 2015). Water Quality Index (WQI), found to be an efficient and useful method for assessing the water quality. This method gives an idea about the overall quality of water to the concern policy makers (Asadi et al., 2007). The WQI incorporates the different physical, chemical and biological parameters for the determination of water quality indices using the several mathematical equations (Yogendra and Puttaiah, 2008). The use of a WQI was initially proposed by Horton (1965) and Brown et al. (1970). Since then, many different methods for the calculation of WQI's have been developed. The procedure for the calculation of WQI was differently proposed by the scientists (Zagatto et al., 1998; Stambuk Giljanovic, 1999). The different WQI used worldwide are US National Sanitation Foundation Water Quality Index (NSFWQI), Canadian Council of Ministers of the Environment Water Quality Index (CCMEWQI), British Columbia Water Quality Index (BCWQI), Oregon Water Quality Index (OWQI), Weighted Arithmetic Water Quality Index (WAWQI) (Abbasi, 2002; Kannel et al., 2007; Lumb et al., 2006; Sharifi, 1990). Studies on the effect of the various physico-chemical and biological parameters on the quality of water of the Narmada River has been performed and discussed the suitability of the water for human consumption based on WQI. The methods used for calculation of WQI are Weighted Arithmetic Water Quality Index (WAWQI), National Sanitation Foundation WaterQuality Index (NSFWQI) and Canadian Council of Ministers of the Environment Water Quality Index (CCMEWQI) in this study.

Air, water and land which are the essential elements of life are contaminated constantly due to increasing population, rapid urbanization and industrialization (Chhikara and Dhankhar 2008). At present, the bioaccumulation of heavy metals in environment is a major warning to human life (Yigit and Ahmet, 2006; Hooda, 2007). Water pollution caused by industrial wastage, is frequent (Ogedengbe and Akinbile, 2004) by toxic sludge, heavy metals, and solvents as they fall into natural water sources and agricultural environment. Heavy metals containing industrial effluent cause health hazards to plants, animals, aquatic life and humans increasing pressures on the flora and fauna (Robin et al., 2012). Among industrial usage of heavy metals, tannery industries use a significant part of it. Tannery effluent is highly polluted because it contains imbalance suspended solids, nitrogen, conductivity, sulfate, sulfide and chromium, copper, cadmium and manganese, biological oxygen demand (BOD) and chemical oxygen demand (COD) (Mondal et al., 2005; Zahid et al., 2006). In Bangladesh unprocessed tannery effluents are released into water sources (Favazzi, 2002; Verheijen et al., 1996). Consequently, the elevated concentrations of some heavy metals are found in agricultural soils located in surrounding areas to the tannery industries which exceed the tolerable limit. Lead and cadmium which are major contaminants found in the environment, are extremely poisonous to human(s), animals, plants and microbes which can damage cell membranes, alter particularity of enzymes, and destroy the structure of DNA. This toxicity is generated by the displacement of essential metals from their native binding sites or ligand interactions (Olaniran et al., 2013). Chrome powder and chrome liquor are applied in tanning industry, and are highly toxic heavy metals (Cr^{6+}) that cause water pollution (Sing, 1994), where A lot of (>170000 tons) chromium wastes are released to the surroundings (Kamaludeen et al., 2003). It causes health hazards since it can easily enter biological cell membranes (Chaudhary et al., 2003). Tanned skin-cut wastes (SCW) which are used to produce feeds and fertilizers, are the direct phenomenon of chromium toxicity (Rafiqullah et al., 2008). Hexavalent Chromium (Cr^{6+}) is 100–1000 times more poisonous compared to trivalent (Cr^{3+}) form (Gauglhofer and Bianchi, 1991). So, conversion of Cr^{6+} is one of the significant mechanisms for microorganisms that can be used for detoxification of chromium. Lacking a single waste-water treatment facility, a notorious and substantial ruin of the environment is another fate of concern from such a pivotal industry to sustain a billion-dollar business. An excess of such chemicals in the water and soils is harmful for the health of the people crammed into the area (Sunder et al., 2010). The breakthrough toward the sustainable mitigation of this overwhelming problem is nothing but the installation of an appropriate effluent treatment plant in every industry in terms of efficiency, cost effectiveness, simplicity and more importantly it should be environment friendly. Due to lack of treatment plants and environment management schemes in most of the tanneries in our country, raw wastes are simply discharged into the environment, causing severe environmental and public health troubles in particular areas. Appropriate environmental management is needed (Hasnat et al., 2013) to overcome this hazardous issue and tannery productivity.

Several microorganisms have gradually developed detoxification and respiration mechanisms from heavy metals and thus become resistant to it (Ezaka and Anyanwa, 2011). The isolation and characterization of heavy metal resistant bacteria is significant for its metal accumulation capability along with its resistance capacity. In our study the sampling sites are the three nearby surroundings of Madina tannery which is the largest tannery located at Jalalabad area, near Oxygen point in Chittagong, Bangladesh. It was established in 1983, and is renowned for manufacturing all kinds of export quality crust and refined leather. In the surroundings of Madina tannery, large municipal areas have been observed. So, the present study aims to investigate the ability of natural inhabitant bacteria of tannery effluent in reducing and detoxifying of heavy metals (Pb, Cr and Cd) at privileged conditions, where objectives include – isolation of naturally occurring bacteria from tannery effluent, screening of top three isolates as the reducer of Pb, Cr and Cd, characterization of heavy metal resistance, identification of those bacteria up to genus and profiling of their plasmids as a fundamental research to ensure the basis of their resistance in order to use them for detoxification in an incorporated bioremediation scheme.

Materials and methods:

Sampling area and Sample collection:

The river water samples were collected from Chennur mandal, Kadapa district, India. The samples were collected from the three different sites in the same river and sediment sample at the depth of 10cm.Samples were collected in sterile bottles and sterile polythene bags and transported to the laboratory for measuring the physico - chemical properties and isolate the metal resistant bacteria respectively from the river water and sediment.

The water samples from the river were collected from the selected six locations (S1–S6) (Ref). Observations were regularly recorded for the analysis of physic-chemical parameters viz., temperature, pH, dissolved oxygen, turbidity etc. This analysis is to assess the nature and degree of pollution in the study area. Other parameters such as Total Dissolved Solid (TDS), Phosphate (PO_4^{3-}), nitrate (NO_3^{-}) and Bio-chemical Oxygen Demand were analyzed (APHA, 2012). Each analysis was done in triplicate and the mean value was considered.

Water quality parameters

A water quality management policy of surface waters, in general, should maintain the existing pollution parameters below certain threshold levels and ensure minimum dissolved oxygen concentrations for survival of aquatic life. The main pollution parameters that have to be considered for surface water quality management, in general, include water temperature, pH, dissolved and suspended solids, turbidity, dissolved oxygen, compounds of phosphorus and nitrogen,biochemical oxygen demand and chemical oxygen demand.

pH

According to the different standards proposed by WHO, ICMR, CPCB, BIS (listed in Table 1), the range of pH lies between 6.5 to 8.5. If the pH is less than 6.5, it discontinues the making of vitamins and minerals in the human body. If the pH is ≥ 8.5 it cause the taste of water more salty and causes eye irritation and skin disorder for pH of more than 11. The rainwater which has no minerals useful for human body has a pH of 5.5–6 and not harmful on used as drinking purpose. pH in the range 3.5–4.5 affects the aquatic life (Adarsh and Mahantesh, 2006; Leo and Dekkar,2000).

Dissolved Oxygen (DO)

The dissolved oxygen reveals the changes occur in the biological parameters due to aerobic or anaerobic phenomenon and signifies the condition of the river/stream water for the Biosphere (Chang, 2005).The aquatic life is disturbed due to low values of () DO (Cox, 2003). The range of 5–14.5 mg O_2 $L^{-1}$was found to be suitable for the natural waters depending on turbulence, temperature, salinity, and altitude. As per the standards proposed by US EPA (1986) and CPCB and BIS (Table 1), the range of DO lies between 4 to 6 mg L−1ensures better aquatic lifein the water body (Leo and Dekkar, 2000; Burden et al., 2002; De, 2003).

Bio-chemical Oxygen Demand (BOD)

BOD is used for determination of requirement of oxygen for stabilizing household and industrial wastes (De, 2003). The domestic and industrial effluents and industrial effluents contaminate the quality of the water which can be assessed by BOD determination (Sawyer et al., 1994). According to WHO drinking water standard, BOD should not exceed 6 mg L^{-1} (De, 2003). 3 mg $L^{-1}$is the maximum BOD for fisheries (Salmonid type) (EEC, 1978).

Total Dissolved Solids (TDS)

TDS is determined for measuring the amount of solid materials dissolved in the water (surface, ground). High TDS values causes harmful effect to the public health such as the central nervous system, provoking paralysis of tongue, lips, face, irritability, and dizziness. The presence of synthetic organic chemicals even in small concentrations imparts objectionable and offensive tastes, odors and colors to fish and aquatic plants (Chang, 2005). The range of TDS falls between 500–1500 mg $L^{-1}$are prescribed by the US EPA (1997), and ICMR, WHO and BIS (Table 1) (Sawyer et al.,1994; Leo and Dekkar, 2000).

Nitrate-Nitrogen (NO3-N)

Different agricultural activities yield in the increase of nitrate concentration in ground and surface water (Nas and Berktay, 2006). Increase in the amounts of Nitrate-Nitrogen in surface water causes different problems such as level of oxygen in the water decreased results in effects on the aquatic life, plants and algae (Davie, 2003). Blue baby syndrome disease in human body occurred due to reaction of nitrite and iron in with red blood cell create methemoglobin which stops oxygen level. The children under age of 1 year suffered most due to consumption water contaminated with nitrate. The range of Nitrate-Nitrogen prescribed by ICMR, WHO, BIS are 20, 45, 45 mg L^{-1}respectively (Nyamangara et al.,2013).

Turbidity

The increase of turbidity of water results in interference of the penetration of light. This will damage the aquatic life and also deteriorate the quality of surface water. In the season of monsoon heavy soil erosion and suspended solids from sewage increased the turbidity which has an effect on the river and aquatic life (Verma et al., 1984). High values of turbidity minimize the filter runs which cause pathogenic organisms to be more hazardous to the human life. Due to this reason the WHO, ICMR and BIS (Table 1) proposed a maximum range of 2.5, 5 and 5 NTU respectively depending upon the processes used for treatment of waste water (Sawyer et al., 1994; Burden et al., 2002; De, 2003).

Phosphate (PO_4^{3-})

Industrial and sewage waste create the pollution due to the presence of phosphates which caused growth of nuisance for micro-organisms. The maximum use of fertilizer is the main source of phosphate which comes from agricultural or residential cultivated land into surface waters with storm runoff. High phosphate level causes muscle damage, problem with breathing and kidney failure (Nyamangara et al., 2013). The increase in phosphorus concentrations in the rivers leads to eutrophication and depletion of dissolved oxygen concentrations (Davie, 2003). The limit for phosphate phosphorus is 0.1 mg L^{-1} (US EPA, 1986).

Heavy metal analysis:

For the selective screening of heavy metal resistant bacteria, 300 lg/mL of heavy metal (Lead) incorporated LB (Luria Bertani) agar plates (Peptone 10.00 g/L, yeast extract, 5.00 g/L, NaCl 5.00 g/L, dextrose anhydrate 10.00 g/L and agar 30.00 g/L: pH -7.00) were used and screened by standard pour plate method observed at 37 ºC. After 24 h of incubation the plates were observed for any kind of development on the culture medium. After preliminary screening of effluent samples containing heavy metal degrading isolates, serial dilution was done as Azad et al. (2013) to isolate desired bacteria. Streak plate technique was followed during isolation. Control plates also prepared with LB media without including any heavy metal to make comparison. Colonies differing in morphological characteristics were selected, picked, purified and then preserved on different plates for further studies.

Determination of minimum inhibitory concentration (MIC)

To asses MIC, heavy metal resistant selected isolates were grown on heavy metal incorporated media against respective heavy metal. It was identified by gently inclining the concentration of the heavy metals (Pb, Cd and Cr) on LB agar plates until the isolates failed to give colonies on the petri plate. The starting concentration of the heavy metals was 50 lg/mL and the culture growing on the final concentration was transferred to the higher concentration each time by streaking on the agar plate. When the isolates failed to grow on petri plate, MIC was assessed according to standard protocol of European food safety authority (EFSA , Parma, Italy, 2012.

Relative effects of heavy metal consumption on microbial growth

The optimal growth conditions with reference to different amounts of three heavy metals were determined. The isolates were grown in a rotary shaker (Wise cube, Korea) at 150 rpm and pH 7.0, while the temperature was 37 ºC in LB broth medium supplemented with different types of heavy metals (gradually increasing 100 lg/mL at every time, until it reaches to 1000 lg/mL) separately. The optical density (OD) was measured (at k =600 nm) using UV spectrophotometer

(Shimadzu, Japan). After 6–8 h of incubation the effect of heavy metal concentration on their growth was assessed.

Phenotypic and biochemical characterization of bacterial isolates:

The bacterial isolates were characterized based on biochemical, cultural and morphological and biochemical parameters (Barrow and Feltham, 1993). For the activities of oxidase, catalase, methyl red, indole production, citrate utilization and carbohydrate (Glucose, Sucrose, Maltose, Xylose and Lactose) utilization, isolates were biochemically analyzed (Barrow and Feltham, 1993). According to Bergey's Manual of systemic Bacteriology the isolates were provisionally identified up to genus level (Claus and Berkeley, 1986).

Results and Discussion:

RESULTS & DISCUSSION:

Physico-chemical properties :

The physico-chemical properties of Penna river water samples of kadapa district were given in (Table-1). In the present study, water colour was clear in site- 1 when compared with sites- 2 & 3. Site- 2 & 3 showed light brown colour and there was no odour at all sites. The pH of water in study sites showed a narrow range of variation, showing the slightly acidic character i.e. 5.57-6.42. It is not in desired range according to Bureau of Indian standards(BIS), standardisation the pH for drinking water should be within 6.5-8.5. However, the quality of PPL and IFFCO effluent water are categorized under desirable class water. Since the pH of three sites were <7, it dissolves many metals resulting in their toxicity. The observed water temperature among the three study sites showed little variation(28.7° C-30 ° C),supports the finding of Mishra et al. who reported the same trend of water temperature in the mangrove forest of Bhitarkanika, Odisha.

The Electrical conductivity of water samples at different study sites was found in the range of 1.24-1.39 mS/cm. Thereafter, conductivity showed gradual increase upto site- 2,

BIS has recommended a drinking water conductivity limit of 750 µS/cm at 25°C which can be extended to a conductivity about 3000 µS/cm at 25°C in case of no alternate source. The higher value of conductivity registered during the study period indicating the pollution level in the water. The value of TDS (Total Dissolved Oxygen) content in all the sites except site- 2 remains almost same. TDS is generally associated with inorganic salt and there is a close parallelism between TDS and conductivity. Though there is no generally valid exact quantitative relationship between TDS and conductivity but high conductivity indicates high TDS. BIS standard value for TDS is 500-1000 mg/l. In the present investigation the TDS are found in the range of 4510 – 11900 mg/l indicating the pollution of the water which are mostly due to the mixing of sea water with the river water. In water, total dissolved solids are composed mainly of carbonates, bicarbonates, chlorides, phosphates and nitrates of calcium, magnesium, sodium, potassium and manganese, organic matter, salt and other particles. Total hardness is in desired range according to APHA standards are 0-60mg/lit- soft hardness, 60-120mg/lit-medium hardness,120-180mg/lit->180mg/lit-very hardness. In this study is soft, which means that means the resultant value of Total hardness, calcium hardness and magnesium hardness at all three sites is <60 or 10-40mg/L.

Alkalinity composed primarily of carbonate and bicarbonate ions and it acts as a stabilizer for pH. In this study it reveals the highest value of alkalinity is 50 at site- 2 and minimum value of alkalinity is 35. The maximum value of chloride is due to increase of bicarbonates concentration in water sample. Total hardness values were fluctuates from 10mg/lit to 40mg/lit. The maximum value (40mg/lit) was recorded in the site-3 and minimum value (10mg/lit) was recorded in the site-2 and the Chloride content was observed minimum(74.4mg/lit) at site- 1& 3. Among the three study sites, site-2 showed nearly same chloride content (79.4mg/lit). According to the tolerance limit standardized by State Pollution Control Board the standard limit of chloride for drinking purpose is (250 mg/L) and can be extended up 1000 mg/L. In the present work the observed Chloride contents at all the sites are beyond permissible limits.

There is a significant variation of phosphate content at all three sites. It showed gradual increase at site- 2 then decreased slowly at site-3. The phosphate content at three sites is from 1.02mg/1it, 1.40mg/lit, 1.30mg/lit. Phosphate occurs naturally in waters in low amounts many aquatic plants absorb and store phosphorous as many times than actual immediate needs. The BOD reveals the ranged from the maximum value at the site-3 is 13, and the minimum value at site-1 is 11. The acceptable BOD level in the normal water meant for treatment is 3 mg/lit while more than 2mg/lit BOD indicated the non suitability of river water for domestic use as per Indian standards. The observed COD value ranged from the maximum value at site-2 is 158.4 and the minimum value at site-1 is 155.2 (units)

Table:1. Physico- chemical parameters of the River Penna

Parameters/ Site	**Site1**	**Site2**	**Site3**
Colour	Clear	Light brown	Light brown
Odour	-	-	-
p^H	5.57	6.16	6.42
Temperature(^{0}c)	30	29	28.7
Conductivity(µs)	1.24	1.39	1.27
TDS(ppt or ppm)	0.73	0.81	0.74
Salinity(ppt)	0.86	0.06	0.88
Alkalinity(mg/lit)	35	50	45
Total hardness(mg/lit)	30	10	40
Calcium hardness(mg/lit)	12.62	16.82	16.82
Magnesium hardness(mg/lit)	4.22	-	5.63
Chlorides(mg/lit)	74.4	79.4	74.4
Phosphates(mg/lit)	1.025	1.402	1.312
COD(ppm)	155.2	158.4	156.8
BOD(ppm)	8	11	13

Metal Analysis:

Metal Analysis was carried out by ICP-OES in zuari cement private limited M.R palli at Tirupati.The results were obtained as mentioned below.

Table2:Metal analysis

S.no	Concentration
Chromium	0.027
Cadmium	0.466
Lead	0.077
Arsenic	Nil
Zinc	Nil
Nickel	Nil
Copper	Nill
Iron	Nil

Enumeration of Metal resistant bacteria:

Total Metal resistant bacteria ranged from for site-1 is 0.065, for site-2 0.233 for site-3 0.325

Table:3. Isolation of metal resistant bacteria at site-1

Site	Sample	Pb concentration	Dilution	No. of Metal resistant bacteriax10^5/ml
1.	Water	1mM	10^{-5}	0.3664
		2mM	10^{-5}	0.3456
		3mM	10^{-5}	0.3180
		4mM	10^{-5}	0.3120
		5mM	10^{-5}	0.2864

Table-4:Isolation of metal resistant bacteria at site-2.

Site	Sample	Pb concentration	Dilution	No.of Metal resistant bacteria x10^5/ml
2.	Water	1mM	10^{-5}	0.2760
		2mM	10^{-5}	0.2392
		3mM	10^{-5}	0.2200
		4mM	10^{-5}	0.2192
		5mM	10^{-5}	0.2116

Table-5: Isolation of metal resistriant bacteria at site-3.

Site	Sample	Pb cocentration	Dilution	No.of Metal resistant bacteriax10^5/ml
3.	Water	1mM	10^{-5}	0.3604
		2mM	10^{-5}	0.3424
		3mM	10^{-5}	0.3216
		4mM	10^{-5}	0.3128
		5mM	10^{-5}	0.2892

Table-6: Colony Morphology

STRAINS	Isolated Colony Morphology
Organism 1	Yellow,medium sized,rounded,entire.
Organism2	Milky white,small,rounded,entire
Organism3	Creamy,small,rounded,entire

Minimum Inhibitory Concentration (MIC):

Minimum Inhibitory Concentration refers to the minimum concentration of lead at which he growth can be inhibited.After the MIC test it was concluded that out of the three isolates from 3 different sites the strains were inhibited when grown on Nutrient Agar media supplemented with different concentration (1mM to

5mM and 10 to 50mM) of Lead acetate. They showed a zone of inhibition of 6.7cm,7.5cm, 8.5cm, 9cm respectively (Table-7)

Table-7: Minimum Inhibition Concentration (MIC):

Concentrations of Metal (mM)	Zone of inhibition Organism1(mm)	Zone of inhibition organism2(mm)	Zone of inhibition organism3(mm)
Control	-	-	-
1Mm	-	-	-
2mM	-	-	-
3mM	-	-	-
4mM	-	-	-
5mM	-	-	-
10mM	6.7	6	8
20mM	7.5	8	10
30mM	8.5	9	12
40mM	9	10	13
50mM	10	11	13.5

Morphology of isolated strains:

All the three strains isolated were gram negative and 2 among them were round shaped and 1 is rod shaped.

Table-8:Colony morphology, Cell morphology, Gram stain reaction

Strains	**Cell Morphology**			**Gram's Test**
	Shape	**colour**	**Margin**	
Organism1	Round	yellow	Entire	-ve
Organism2	Round	White	Entire	-ve
Organism3	Rod	creamy	Entire	-ve

Antibiotic Sensitivity Test:

Antibiotic sensitivity Test was performed by well diffusion method. By this test it was concluded that the Organism-1 has resistance to Amoxillin and high sensitivity to Ciprofloxacin. Organism-2 has high sensitivity to Cefodroxil and resistance to Cefolac and Ciprofloxacin. Organism-3 has resistance to cefolac and cefodroxil and also has high sensitivity to cifran.

Table-9:Antibiotic Sensitivity

S.no	Antibiotics	INHIBITION ZONE(mm)		
		Organism1	Organism2	Organism3
1.	Amoxillin	-	12	3
2.	Cefolac	12	9	-
3.	Ciprofloxacin	16	9	17
4.	Cefadroxil	2	14	-
5.	Gentamycin	13	10	11
6.	Cifran	15	10	20

Biochemical Tests:

After performing the below mentioned tests, it was deciphered that the strains organisms 1,2 and 3 showed negative test for indole, oxidase and organism 1&3 are positive for Citrate and Catalase and respectively organism 2 was negative .Organism 1&3 are negative and organism2 was positive for Urease test.

Table 10: Biochemical test

Sources	Organism1	Organism2	Organism3
Catalase	+ve	-ve	+ve
Oxidase	-ve	-ve	-ve
Indole	-ve	-ve	-ve
Citrate	+ve	-ve	+ve
Urease	-ve	+ve	-ve
MR test	+ve	-ve	-ve

Catalase Test:

Catalse positive: organism 1& 3 are positive that means may be Staphylococcus species are present.

Catalase negative: organism of 2 is negative, may be Streptococcus species is present.

Oxidase Test:

Oxidase negative: All 3 organisms are negative, may be E-coli, Klebsilla or Salmonella.

Indole Test:

Indole negative: All 3 organisms are also negative for indole test that means these organisms may be Klebsilla, Salmonella, Shigella species.

Citrate Test:

Positive: organism1&3 are positive, may be Klebsiella species.

Negative: organism 2 is negative for this test, may be E-coli is present.

Urease Test:

Positive: Organism2 is positive, that means may be Proteus species, Morganella morganii. Negative: Organism 1&3 are negative, means may be E;coli is present.

Methyl Red Test:

Positive: Only organism 1 is positive, may be E-coli is present.

Negative: Organism 2& 3 are negative for this test, means may be Enterobacter aerogenes are present.

Table:11 Effect of Metal on Bacterial growth:

Concentration	OD at 600nm
Blank	0
1mM	0.673
2mM	0.582
3mM	0.409
4mM	0.051

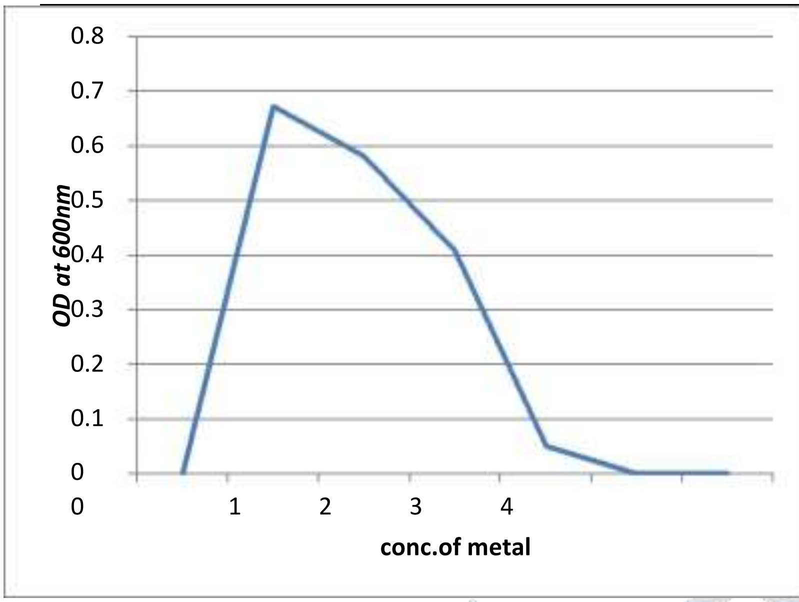

Fig-13: Effect of metal on bacterial growth

Bacterial growth was decreased with the increasing concentrations of metal. It cannot penetrate into cell wall.

Conclusion:

The study of physical and chemical parameters provided information about the water quality in penna river water of Kadapa district. A comparative study of three sites of river was carried out by taking certain important parameters like colour, odour, pH, temperature, total dissolved solids, alkalinity, total hardness, chlorides, phosphates, and total metal resistant bacteria.

In this study it was found that values are maximum and were not according to the standards of drinking water. The parameters like pH is below the permissible limits i.e. containing acidic nature, BOD was observed above the permissible limits. This indicates that the river water lightly polluted and unsafe for human use. Hence, this need conventional treatment including disinfection.

REFERENCES:

1. Achal, V., X. Pan, D. Zhang and Q.L. Fu, 2012. Biomineralization based remediation of As(III) contaminated soil by *Sporosarcinaginsengisoli*. *J. Hazard. Mater*., 201-202: 178–184.

2. Aftab, Begum, S. Y, Noorjahan, C. M., Dawood, Sharif, S (2005), Physico-chemical and fungal analysis of a fertilizer factory effluent, *Nature Environment & Pollution Technology*, 4(4), 529-531.

3. Basavaraja, Simpi, S. M., Hiremath, K. N. S. Murthy, K. N. Chandrashekarappa, Anil N. Patel, E. T. Puttiah, (2011), Analysis of Water Quality Using Physico-Chemical Parameters Hosahalli Tank in Shimoga District, Karnataka, India, *Global Journal of Science Frontier*, Research, 1(3), pp 31-34.

4. Bhattacharya T., Chakraborty S. and Tuck Neha.,(2012) Physico-chemical Characterization of ground water of Anand district, Gujarat, India, *I. Res. J. Environment Sci.,* **1(1)**, 28-33.

5. Brim. H, H. Hener, E. Kroyerrecklenfort, and K. Smalla (1999). Characterization of the bacterial community of a zinc – polluted soil. *Can. J. Microbial* 45: 326-338.

6. Bruins, M.R, S. Kapal, WF, Oehme,2000, *Ecotoxicology and Environmental Safety.*, 45, 198-207.

7. Cervants,C.,J.Campos-Garcia,S.Derars,F.Gutierrez-Corona,H.Loza-Ta and R. Moreno Sanchez.(2001)Interaction of chromium with microorganism 25:335-347.

8. Chaalal O, Zekri AY, Islam R (2005). Uptake of heavy metals by microorganisms: an experimental approach. *Energy Sources*. 27(1-2): 87-100**.**

9. Chen, C.Y. and Lin, T.H. (1998): Nickel toxicity tohuman term placenta: in vitro study on lipid per oxidation. *Journal of Toxicology and Environmental Health* Part A **54**, 37- 47.

10. Correa, M., Roig-Navarro, A.F., Aragon, C.M.G., 2004. Motor behavior and brain enzymatic changes after acute lead intoxication on different strains of mice. *Life Sci*. 74, 2009–2021.

11. Costa, M. and Klein, C.B. (1999): Nickel carcinogenesis, mutation, epigenetics, or selection. *Environmental Health Perspectives* Part A **107**, 438-439.

12. Ekwenye U.K. and C.A oji, (2008)"Quality of water from boreholes in Umualia, Nigeria," *Environment and Ecology*, vol.26,.543-545.

13. Fichet, D., Boucher,G.,Radenac, G., Miramand, P., 1999.Concentration and mobilisation of Cd, Cu, Pb and Zn by meiofauna populations living in harbour sediment: their role in the heavy metal flux from sediment to food web. *Sci. Total Environ*. 243/244, 263–272.

14. Gochfeld. M, *Ecotoxicology and Environmental Safety.*, **2003**, 56, 174

15. Haq R, SK Zaidi, IR Shakoori, *Word J Microbiol Biotechnol*, **1999**, 15: 283-290.

16. Hari, O. S.,Nepal, M. S. Aryo, and N. Singh. (1994), Combined effect of waste of distillery and sugar mill on seed germination, seeding growth and biomass of okra. *Journal of Environmental Biology*, 3(15), pp 171-175.

17. Howell, R., 1982. Levels of heavy metal pollutants in two species of marine nematodes. *Mar. Pollut. Bull*. 13, 396–398.

18. Howell, R., 1983. Heavy metals in marine nematodes: uptake, tissue distribution and loss of copper and zinc. *Mar. Pollut. Bull*. 14, 263–268.

19. IARC monographs on arsenic in drinking water; (2004) IARC, Lyons, France, IARC (International Agency for Research on Cancer*). Monographs*. Vol 84**.**

20. Jayakumar .P, N. Jothivel, A. Thimmappaand, and V.I Paul,(2009) "Physico-chemical characterization of a lentic water body from Tamilnadu with special reference to its pollution status*," The Ecoscan* , vol.3, no.1-2, pp.59-64.

21. Ji, G., and Silver, S. (1995). Bacterial resistance mechanisms for heavy metals of environmental concern. *Journal of Indian Microbiology*, 14:61–75.

22. Kamal.D, A.N. Khan, M.A. Rahman and F. Ahamed, 2007. Study on the Physico Chemical Properties of Water of Mouri River, Khulna,Bangladesh . *Pakistan Journal of Biological Sciences, 10: 710717.*

23. Koplan JP, (1999) Toxicological profile for cadmium, Published by ATSDR, *U.S. Department of Health and Human Services*, **1999**, 439P.

24. Kerketta P, Baxla SL, Gora RH, Kumari S and Roushan RK(2013) Analysis of physico-chemical

25. properties and heavy metals in drinking water from different sources in and around Ranchi, Jharkhand, India, 6(7):370-375.

26. Kulkarni, G. J., (1997), Water supply and sanitary engineering. 10th Ed. Farooq Kitabs Ghar. Karachi, 497.

27. Levinson, H.S., Mahler, I., Blackwelder, P. and Hood, T. (1996). Lead resistance and sensitivity in *Staphylococcus aureus*. *FEMS Microbiol Lett.*, 145:421-425.

28. Lopez -Errasquin, E. and C. Vazquez, 2003. Tolerance and uptake of heavymetals by Trichoderma atroviride isolated from sludge.*Chemosphere*, 50: 137–143.

29. Low, K.S., Lee, C.K. and Liew, S.C. (2000) Sorption of cadmium and lead from aqueous solution by spent grain. *Process Biochemistry*, **36**, 59-64.

30. Archives 201(4):1008-10112.

31. Malik A (2004). Metal biormediation through growing cell, *Environ. Int.* 30(2), 261-278.

32. Mandal BK, Suzuki KT (2002). Arsenic round the world*: A Rev. Talanta*. 58(1), 201-235.

33. Manjare, S. A., S. A. Vhanalakar and D. V. Muley, (2010), Analysis of water Quality using Physico-Chemical parameters Tamdalge Tank in Kolhapur District, Maharashtra, *International Journal of Advanced Biotechnology and Research*, 1(2), 115-119.

34. Meghraj HOOKOOM et al 2013 Isolation and Identification of Heavy Metals Tolerant Bacteria from Industrial and Agricultural Areas in Mauritius *Current Research in Microbiology and Biotechnology,* 1,119-123.

35. Mire, C.E., Tourjee, J.A., O'Brien, W.F., Ramanujachary,K.V. and Hecht, G.B. (2004). Lead precipitation by *Vibrio harveyi*: evidence for novel quorum-sensing interactions. *Appl Environ Microbiol.,* 70:855-864.

36. Munoz, R.A., Munoz, M.T., Terrazas, E., Guieysse, B. and Mattisasson, B. (2006) Sequential removal of heavy metals ions and organic pollutants using an algal-bacterial consortium. *Chemosphere*, **63**, 903-991.

37. Nascimento, A.M.A. and E.C. Souza, 2003. Operon mer: bacterial resistance to mercury and potential for bioremediation of contaminated environments. *J. Genet. Mol. Res.*, 2: 92-101.

38. Nies, D.H., 2003. Efflux- mediated heavy metal resistance in prokaryotes. *FEMS Microbiol. Rev.*, 27: 313–339.

39. Nitin Gupta et al 2011, “physico-chemical assessment of water quality of river chambal in kota city area of rajasthan state” *Rasayan J chem,* 4, 686-692.

40. Njoku HO, Ogbulie JN, Nnubia (1990), Microbial Ecology of Traditional Fermentation of African Oil bean seed for Ugba production *Journal of Food and Microbiology* 3:18-28.

41. Pandit R.J.1,et al Isolation, characterization and molecular identification of heavy metal resistant bacteria from industrial effluents, Amala-khadi- Ankleshwar, *Gujarat international journal of environmental sciences* 3(5): 2013.

42. Parungao, M.M., Tacata P.S., Tanayan, C. and Trinidad, L. (2007). Biosorption of copper, cadmium and lead by copper-resistant bacteria isolated from Mogpog River, Marinduque. *Philippine Journal of Science*, 136(2):155-165.

43. Prasenjit, B. and Sumathi, S. (2005) Uptake of chromium by *Aspergillus foetidus*. *Journal of Material Cycles and Waste Management*, **7**: 88-92.

44. Premlata, Vikal, (2009), Multivariant analysis of drinking water quality parameters of lake Pichhola in Udaipur, India. Biological Forum, Biological Forum- *An International Journal*, 1(2): 97-102.

45. Rajendran, P., Muthukrishnan and Gunasekaran, P. (2003). Microbes in heavy metal remediation. *Indian Journal of Experimental Biology,* 41:935-944.

46. Saravanakumar, K. and R. Ranjith, Kumar, (2011), Analysis of water quality parameters of groundwater near Ambattur industrial area, Tamil Nadu, India, *IndianJournal of Science and Technology*, 4(5), pp 1732-1736.

47. Satarug S, MR Moore, *Environmen Health Perspect*, **2004**, 112(10): 1099-1103.

48. Sharma PK, DL Balkwill, A Frenkel,(2000) MA Vairavamurthy, *Appl Environ Microbiol*, **2000**, 66(7): 3083-3087.

49. Sivasubramanian, V. (2006). Phycoremediation - Issues and challenges. *Indian Hydrobiology*, 9(1):13-22.

50. Silver, S. and L.T. Phung, 1996. Bacterial heavy metal resistance: new surprises. *Ann. Rev. Microbiol*., 50: 753–789.

51. Singh D.K. and I.Singh, (2008)"Interrelationship of certain physic chemical parameters with plankton community of Motipus Ox-bow lakes (Muzaffarpur,Bihar)," *Environment and Ecology*, vol.26,no.2,pp.800-803.

52. Titus JA(1984), RM Pfister, *Arch Environ Contam Toxicol* 13: 271-277.

53. *Ugwu et al* 2012 a study of seasonal physicochemical parameters in river usma. *American Journal of Environmental Science*, 2012, 8 (5), 569-576 Van Damme, D., Heip, C.,Willems, K.A., 1984. Influence of pollution on the harpacticoid copepods of two north sea estuaries. *Hydrobiologia* 112, 143–160.

54. Vandysh O.I.(2004),"Zooplankton as an indicator of the state of lake ecosystems polluted with mining waste water in the Kola Peninsula," *Russian journal of Ecology*, vol.35,no.2,pp.110-116.

55. Venkateswara Rao B., **(2011)** Physico-chemical analysis of selected groundwater samples of Vijayawada rural and urban in Krishna district, Andhra Pradesh, India, *International Journal Environmental Sciences*, **2(2)**, 710-714 .

56. Vieira, R. and Volesky, B. (2000) Biosorption: A solution to pollution? *International Journal of Food Microbialogy*, **3**,17-24.

57. Wagner Dobler, I., H. Von Canstein, K.N. Timmis, Y. Li and W.D. Deckwer, 2000. Removal of mercury from chemical wastewater by microorganisms in technical scale. *Environ. Sci. Tecnhnol*., 34: 4628-4634.

58. Waisberg, M., Joseph, P., Hale, B. and Beyersmann, D. (2003**)** Molecular mechanism of cadmium carcinogenesis. *Toxicology*, **192**, 95-117.

59. Wasi, S., Tabrez, S. and Ahmad, M. (2011a). Suitability of immobilized Pseudomonas fluorescens SM1 strain for remediation of phenols, heavy metals and pesticides from water. *Water, Air, and Soil Pollution*, 220(1-4): 89-99.

60. Wasi. S., Tabrez, S. and Ahmad, M. (2011b). Detoxification potential of Pseudomonas fluorescens SM1 strain forremediation of major toxicants in Indian water bodies. *Water, Air, and Soil Pollution,* 222(1-4):39-51.

61. Wright D And Welbourne P (2002) *Environmental Toxicology*. *Factors affecting Toxicology*. Cambridge Environmental Chemistry Series11. Cambridge University Press, Cambridge.

62. Zhang, Q., V. Achal , W.N. Xiang and D. Wang , 2014. Identification of heavy metal resistant bacteria isolated from yangtze river, China. *Int. J. Agric. Biol*., 16: 619–623