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Influence of lignin, pentachlorophenol and heavy metal on antibiotic resistance of pathogenic bacteria isolated from pulp paper mill effluent contaminated river water

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Abstract

Pulp paper mill pollutants are the major source of aquatic contamination having metals, lignin and chlorophenols. Study was conducted to see the effect of these contaminants on antibiotic resistance pattern of isolated bacteria. Pulp paper effluents were evaluated for its physico-chemical properties i.e, BOD 72143±164. 81 to 22.32±2.48, COD 213136±583.59 to 60.40±6.34, total phenol 386±71.24 to 0.43±0.0, lignin 26312±258.59 to 73.67±31.81and microbial quality *i.e. K. pneumonae, S. typhi, S. faecalis, P. aeruginosa, E.coli, Ent. faecalis, A. hydrophila, B. subtilis, S.aureus, Y. enterolitica* and *V. vulrificus.* Antibiotic sensitivity (10-30 µg), heavy metal resistance (100-1000 µg ml^{-1}), lignin (1000-10,000 ppm) and pentachlorophenol (100-1000 ppm) tolerance of bacterial strains were assessed by seven classes of antibiotics. Eleven bacterial isolates were found multidrug resistant towards antibiotics, heavy metal, lignin and PCP. Out of 11 isolates, 90.9% were found resistant against eleven antibiotics which acquired 100% resistant in presence of heavy metal, lignin and chlorophenols. Results also revealed that concentration of lignin (50-350 ppm) and PCP (5-30 ppm) induced maximum growth (273-8050 cfu ml^{-1}) of pathogenic bacteria in river water.

Key words

Antibiotic, Bacteria, Lignin, Multidrug resistance, Pentachlorophenol (PCP), Heavy metal

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Introduction

Urban industrial activities have long been identified as a major source of environmental pollution. Waterborne infections are the most common causes of morbidity and mortality in the under developed and developing countries and 80% of the infectious diseases are waterborne in India (Tambekar *et al*., 2008). Most of the rivers in urban areas of the developing world are the end points of effluents discharged from the industries Pulp paper industries are the sixth largest effluent generating industries of the world (Ugurlu *et al.,* 2007), as these generates as low as 1.5 m^3 of effluent per tone to as high as 60 m^3 tone of paper produced (Asghar *et al.,* 2007). These effluents have been found to contain more than 200-300 different organic compounds and approximately 700 organic and inorganic compounds (Tambekar *et al.,* 2008). Organic and inorganic contents of the effluent provide ample opportunity to flourishing a variety of pathogenic microorganism (Chandra *et al*., 2006). Due to high chemical diversity of the organic pollutants in paper and pulp mill process water, a high variety of toxic effects on aquatic communities in recipient watercourses have been observed. A significant number of these substances have been classified as carcinogenic, mutagenic and clastogenic and endocrinic (Karrasch *et al.,* 2006). Although, the wastewater and drinking water are treated to eliminate pathogenic microorganisms and prevent water borne transmission, numerous studies have indicated that the conventional wastewater treatment process does not guarantee their complete elimination. Use of antibiotics to combat these infections is a common practice, but indiscriminate use of antibiotics leads to drug resistance in these microbes, which warrants the initiation of steps to prevent public health hazard (Tambekar *et al.,* 2008). Microorganisms are considered to be the best indicators of changes in environmental conditions. In general, they are very sensitive to low concentration of heavy metals, but rapidly adapt to the specific habitat conditions. Several reports are available on antibiotic and heavy metal resistant bacteria isolated from different environmental and clinical samples (Hassen *et al.,* 1997, Paniagua *et al.,* 2006). But, no report is available on the effect of different pollutants present in pulp paper effluent on the antibiotic and heavy metal resistance pattern of pathogenic bacteria. Therefore, this study was conducted to assess the impact of pulp paper mill effluent contaminants on antibiotic and heavy metal tolerance capability of bacteria isolated from pulp paper mill effluent. In present study, we have reported and correlated the antibiotic and metal tolerance pattern of bacteria isolated from pulp paper mill effluent contaminated site and influence of lignin and PCP on antibiotic and heavy metals resistance pattern over the isolated bacteria of pulp paper mill effluent.

Materials and Methods

Sampling and physico-chemical analysis of effluent samples: The pulp paper mill effluent samples were collected from *M/s* Century Pulp and Paper Mill, Lalkuan, Nainital, U.K, India. Samples were collected in sterile container from different spots of effluent discharge channel, located outside and inside of industry premises named as sample A-Before treatment, sample B-After treatment, sample C-Discharged effluent after mixing with river water and sample D-River water (Gola River, Uttrakhand). Samples were analyzed in laboratory for different physico-chemical parameters *i.e.* pH, BOD, COD, colour, phenol, sulphate etc. as per the standard methods for the analysis of water and wastewater (APHA, 2005). Lignin was estimated as described by Raj *et al.* (2007). Further, the nitrate, potassium, chloride, and sodium ions were analyzed with the help of auto ionanalyzer (Orion, model. 960). Samples were analysed for heavy metal content using atomic absorption spectroscopy (Perkin Elmer Analyst 300).

Microbial analysis: Bacterias' were isolated by using a specific and chromogenic media (HiMedia, Mumbai, India). Samples were serially diluted and spreaded onto plates, followed by incubation at 35±1°C for 24 hr. Subsequently, the colonies showing different morphology were selected and sub-cultured on same media and incubated at 32±1°C for 24 hr to obtain pure colonies. Confirmatory tests for the characterization of bacterial species were done as per Barrow and Feltham (1993).

Study on antibiotic resistance: The disc diffusion susceptibility testing was carried out based on the standard method of Bauer *et. al*. (1966). Eleven antimicrobial agents were selected as representatives of important classes of antibiotics, ampicillin (Ax) 10 µg, amoxicillin (Am) 30 µg, tetracycline (Te) 30 µg, gentamicin (G) 10 µg, ciprofloxacin (Cf) 10 µg, erythromycin (E) 15 µg, kanamycin (K) 30 µg, norfloxacin (Nf) 10 µg, neomycin (N) 10 µg, nalidixic (Na) 10 µg, and polymyxin (Pb) 10 µg. Specifically, overnight grown bacterial culture was adjusted to McFarland opacity 0.5 before inoculation onto Muller-Hinton agar plate with sterile cotton swabs (Wang *et. al*., 2006). After 10-15 min, one antibiotic disc was laid on the inoculated surface of an agar plate and incubated at 30°C for 14-16 hr. Agar plates inoculated only with bacterial test isolates without introduction of antibiotic discs served as controls. Inhibition zone diameters were measured after 24 to 48 hr according to the instructions of the manufacturer (Hi Media, Mumbai, India).

Evaluation of metal and pollutant (lignin and PCP) tolerance of bacterial isolates: Resistance to heavy metals like zinc, copper, manganese and iron of isolated bacterial strains were determined by agar dilution method (Kannan and Lee, 2008). Dilutions of the heavy metals stock solutions were incorporated into Mueller–Hinton agar plates with final concentrations ranging from 100 µg ml^{-1} to 1000 µg ml^{-1} and incubated overnight at 35°C. Plates containing media without heavy metal were inoculated in the same way as control. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of metal ion preventing bacterial growth. The minimum inhibitory concentration of pollutants (lignin and PCP) towards isolated bacterial strains was determined in same manner, concentration of lignin and PCP used, were 1000 to 10,000 and 100 to 1000 ppm, respectively.

Antibiotic resistance in presence of heavy metals, lignin and PCP: Evaluation of antibiotic resistance in presence of heavy metals and mixture of lignin and PCP up to their tolerance limit were done on Muller-Hinton agar supplemented with mixture of lignin and PCP. Antibiotics discs were placed on Muller-Hinton agar plates containing different concentration of lignin, PCP and heavy metals. Test was performed in same manner as described above in study of antibiotic resistance section.

Statistical analysis: Whole experiments were carried out in triplicates. All the data were mean (n=3) of each set. For statistical analysis, standard deviation for each experimental result was calculated using Excel Spread-sheets available in Microsoft Excel.

Results and Discussion

Physico-chemical property and microbial load in pulp and paper mill effluent and river water: A marked increase in physico-chemical parameters of river water has been observed after mixing with pulp paper mill effluent (Table 1). Difference in concentration level of lignin and total phenol can be noted in river water before and after mixing of effluent. The presence of phenols and chlorophenols in paper mill effluent is added during bleaching of pulp with chlorine, which are potentially toxic and bioaccumulated in tissues of aquatic organisms. Increase in BOD of river water is mainly due to increase in microbial load which is the result of industrial effluent contamination of river water, resulting in an increase in organic and inorganic matter which act as nutrients for microorganisms. The heavy metal concentration was noted 5-20 times (Table 1) than permissible limit for various metals as per CPCB guidelines for water quality (2008). Out of these only Cu, Fe, Mn and Zn were selected for further study as their concentration was found higher than other metals after mixing with river water.

There was a progressive increase in bacterial count (cfu ml^{-1}) from effluent discharge point to river water. Increase in microbial biomass after mixing of effluent clearly indicated the nutritive behavior

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Table - 1: Physico-chemical analysis of pulp paper effluent at different stages of disposal

Parameters	Pulp paper sample			
	A	B	C	D
pH	9.2±1.72	7.31±1.29	7.52±1. 63	6.91±1.01
TDS	13402±96.32	1213±67.92	134.12±19.03	32.42±3.65
Sulphate	17224±141.89	1238.43±2.13	11.17± 2.01	0.76±0.08
Phosphate	ND	4.16±4.23	5.64±1.52	0.61±0.05
Total Phenol	386±71.24	65.26±4.86	18.53±3.61	0.43±0.03
Pentachlorophenol	118±1.03	27.43±1.03	8±1.03	0.33±0.02
COD	213136±583.59	922.82±32.72	1120.22±45.84	60.40±6.34
BOD	72143±164. 81	365.33± 6.12	436.66±5.36	22.32±2.48
Chloride	41362±11.24	32.12±3.06	18.69±1.47	5.32±1.04
Sodium	1248±81.73	191.12±41.23	31.72±4.21	12.34±2.16
Potassium	135±7.47	105.62±5.11	5.64±1.25	2.13±0.34
Nitrate	ND	27.23±4.84	1.12±0.11	0.78±1.02
DO	0.78±1.13	2.31±0.08	4.02±1.02	6.52±1.03
Lignin	26312±258.59	317.44±29.89	73.67±31.81	Nil
Colour	336214±263.16	872.23±16.48	156.76±4.67	Nil
Heavy metals				
Cd	0.06±0.00	0.01±0.00	0.09±0.01	ND
Cr	0.25±0.04	0.07±0.01	0.06±0.01	0.02±0.00
Cu	0.15±0.00	0.04±0.00	0.05±0.00	0.02±0.00
Fe	3.92±0.15	7.66±0.43	3.55±0.12	0.31±0.00
Mn	0.16±0.01	0.14± 0.03	0.15±0.07	ND
Ni	2.84±0.02	0.08±0.02	0.03±0.00	0.01±0.00
Zn	1.52±0.02	0.06±0.02	0. 09±0.01	0.05±0.00

Values are mean of three replicates ±SD. All values are in mg l^{-1} except pH and colour (CoPt). A = Before treatment, B = After treatment, C = Discharged effluent after mixing with river water, D = River water, Nil = Not detected

Table - 2: Bacterial count (cfu ml^{-1}) in pulp paper effluent at different stages of disposal

Bacteria	Pulp paper sample			
	A	B	C	D
K. pneumonae	Nil	981±99.93	1031±98.68	11±2.12
S. typhi	-	791±29.93	8050±231.74	-
S. faecalis	-	82 ±11.13	380±14.12	21±3.13
P. aeruginosa	-	20±2.32	370±24.24	-
E.coli	-	635±21.24	7720±210.14	23±3.32
Ent. faecalis	-	94 ±8.84	3170± 129.20	13±2.24
A. hydrophila	-	35 ±3.40	730 ±64.13	-
B. subtilis	-	160±12.42	490±51.11	-
S. aureus	-	259±14.18	1020±93.06	10±1.21
Y. enterolitica	-	251±16.24	5030 ±132.16	-
V. vulrificus		24±2.64	273 ±21.50	-

Values are mean of three replicates ±SD. A= Before treatment, B= After treatment, C= Discharged effluent after mixing with river water, D= River water, Nil= no bacterial colonies were detected

of pulp paper mill pollutants after dilution. Eleven bacterial strains were isolated namely *Klebsiella pneumonae, Salmonella typhi, Streptococcus faecalis, Pesudomonas aeruginosa, Escherichia coli, Enterococcus faecalis, Aeromonas hydrophila, Bacillus subtilis, Staphylococcus aureus, Yersinia enterolitica* and *Vibrio vulrificus.* No colonies were seen in sample A due to its high pH (9.2) and organic load (lignin, PCP and their derivatives) (Table 1). Highest bacterial growth was observed in sample C followed by B and D (Table 2). The relationship between bacterial growth and concentration of lignin and PCP is also shown in Fig. 2. Maximum bacterial growth (cfu ml^{-1}) was observed in range of 50-350 ppm (lignin) and 5-30 ppm (PCP). There was noted up to 8000 fold increase in bacterial count in river water after confluence of pulp paper mill effluent as shown in Table 2. This apparently indicated that pulp paper mill effluent is a major source of microbial contamination. This might be due to mixing of treated pulp paper mill effluent containing residual recalcitrant compounds resulting in decrease in alkalinity and concentration of lignin as well as other organic and inorganic pollutants. In addition, the mixing of sewage and wastewater discharged by local inhabitants residing near industry also increases pathogenic bacteria (Chandra *et al.,* 2006).

Antibiotic resistance pattern of isolated bacteria: All the isolates showed multidrug resistant property except *B. subtilis* (Table 3). Out of 11 isolates, 90.9% were found resistant against selected antibiotics. Levels of antibiotic resistance observed in pulp paper isolates, respectively were, 91.0, 81.9, 36.3, 64.0, 73.0, 82.0, 91.0, 82.0, 82.0, 9.0 and 82.0% for ampicillin, kanamycin, gentamicin, neomycin, norfloxacin, nalidixic, polymyxin, erythromycin, tetracycline, ciprofloxacin and amoxicillin, respectively (Table 4). All isolates, except *A. hydrophila* were found sensitive against ciprofloxacin. Similar findings were also reported by

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Table - 3: Phenotypic pattern of multiple antibiotic resistances in presence of different heavy metals among isolates from pulp paper mill contaminated river water

Bacteria	Resistance to antibiotics	Resistance to antibiotics with lignin and PCP	Resistant to antibiotics with heavy metals			
			Cu	Fe	Mn	Zn
K. pneumonae	Ax, K, N, Nx, Na, Pb, E, T, Am	Ax, K, G, N, Nx, Na, Pb, E, T, Am	Ax, K, G, N, Nx, Na, Pb, E, T, Am	Ax, K, G, N, Nx, Na, Pb, E, T, Cf, Am	Ax, K, G, N, Nx, Na, Pb, E, T, Cf, Am	Ax, K, G, N, Nx, Na, Pb, E, T, Cf, Am
S. typhi	Ax, K, N, Nx, Na, Pb, E, T, Am	Ax, K, N, Nx, Na, Pb, E, T, Am, G	Ax, K, N, Nx, Na, Pb, E, T, Am	Ax, K, G, N, Na, Pb, E, T, Cf, Am	Ax, K, G, N, Nx, Na, Pb, E, T, Am	Ax, K, G, N, Nx, Na, Pb, E, T, Am
S. faecalis	Ax, K, G, N, Na, Pb, E, T, Am	Ax, K, G, N, Na, Nx Pb, E, T, Am	Ax, K, G, N, Nx, Pb, E, Am	Ax, K, G, N, Nx, Na, Pb, E, T, Am	K, N, Nx, Na, Pb, E, T, Am	Ax, K, N, Nx, Na, Pb, E, T, Am
P. aeruginosa	Ax, K, G, N, Nx, Na, Pb, E, T, Am	Ax, K, G, N, Nx, Na, Pb, E, T, Am, Cf	Ax, K, G, N, Nx, Na, Pb, E, T, Am	Ax, K, G, N, Na, Pb, E, T, Am	Ax, K, G, N, Nx, Na, Pb, E, T, Am	Ax, K, G, N, Nx, Na, Pb, E, T, Am
E.coli	Ax, K, N, Nx Na, Pb, E, T, Am	Ax, K, N, Na, Nx, Pb, E, T, Am	Ax, K, N, Nx, Na, Pb, E, T, Am Cf,	Ax, K, G, N, Nx, Na, Pb, E, T, Am	Ax, K, G, N, Nx, Na, Pb, E, T, Am	Ax, K, N, Nx, Na, Pb, E, T, Am
Ent. faecalis	Ax, K, N, Nx, Na, Pb, E, Am	Ax, K, N, G, Pb, E, T, Am	Ax, K, G, N, Na, E, T, Am	Ax, K, G, N, Nx, Na, Pb, E, T, Am	Ax, K, G, N, Nx, Na, Pb, E, T, Am	Ax, K, G, N, Nx, Na, Pb, E, T, Am
A. hydrophila	Ax, K, G, N, Nx, Na, Pb, E, T, Cf, Am	Ax, K, G, N, Nx, Na, Pb, E, T, Am	Ax, K, G, N, Nx, Na, Pb, E, T, Am	Ax, K, G, N, Nx, Na, Pb, E, T, Cf, Am	Ax, K, G, N, Nx, Na, Pb, E, T, Am	Ax, K, G, N, Nx, Na, Pb, E, T, Am
B. subtilis	Sensitive	Ax, K, G, N, Na, Pb, E, T, Am	Ax, N	K, G, N, Nx, Na, Pb, E, Am	K, G, N, Nx, Na, Pb, E, T, Am	K, G, N, Nx, Na, Pb, E, T, Am
S. aureus	Ax, K, N, Nx, Pb, T	Ax, K, G, N, Na, Nx, Pb, E, T, Am, ,	Ax, K, N, Pb, E, T, Am	Ax, K, N, Nx, Am	Ax, K, G, N, Nx, Pb, E, T, Am	Ax, K, N, Pb, E, T, Cf, Am
Y. enterolitica	Ax, N, Na, Pb, E, T, Am	Ax, K, G, N, Na, Nx, Pb, E, T, Am	Ax, K, N, Nx, Na, Pb, E, Am	Ax, K, G, N, Nx, Na, Pb, E, T, Cf, Am	Ax, K, G, N, Nx, Na, Pb, E, T, Am	Ax, K, G, N, Nx, Na, Pb, E, T, Am
V. vulrificus	Ax, K, G, N, Nx, Na, Pb, E, T, Am	Ax, K, G, N, Nx, Na, Pb, E, T, Cf, Am	Ax, K, G, N, Nx, Na, Pb, E, T, Cf, Am	Ax, K, G, N, , Na, Pb, E, T, Cf, Am	Ax, K, G, N, Nx, Na, Pb, E, T, Am	Ax, K, G, N, Nx, Na, Pb, E, T, Am

Antibiotics : Ax = Ampicillin , K = Kanamycin, G = Gentamicin, N = Neomycin, Nx = Norfloxacin, Na = Nalidixic acid, Pb = Polymyxin, E = Erythromycin, T = Tetracycline, Cf = Ciprofloxacin, Am = Amoxocillin

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Table - 4: Percentage change in resistance pattern of various bacteria towards antibiotics in presence of lignin, PCP and heavy metals

Bacteria	Antibiotics	Lignin and PCP	Heavy metals			
			Cu	Fe	Mn	Zn
K. pneumonae	81.8(9)	90.9(10)	90.9(10)	100(11)	100(11)	100(11)
S. typhi	81.8(9)	90.9(10)	81.8(9)	90.9(10)	90.9(10)	90.9(10)
S. faecalis	81.8(9)	90.9(10)	72.7(8)	90.9(10)	72.7(8)	81.8(9)
P. aeruginosa	90.9(10)	100(11)	90.9(10)	90.9(10)	90.9(10)	90.9(10)
E.coli	81.8(9)	81.8(9)	81.8(9)	90.9(10)	90.9(10)	81.8(9)
Ent. faecalis	72.7(8)	72.7(8)	72.7(8)	90.9(10)	90.9(10)	90.9(10)
A. hydrophila	81.8(9)	90.9(10)	90.9(10)	100(11)	90.9(10)	90.9(10)
B. subtilis	0(0)	81.8(9)	18.1(2)	63.6(7)	81.8(9)	81.8 (9)
S. aureus	54.5(6)	90.9(10)	63.6(7)	45.45(5)	81.8(9)	72.7(8)
Y. enterolitica	63.6(7)	90.9(10)	72.7(8)	100(11)	90.9(10)	90.9(10)
V. vulrificus	90.9(10)	100(11)	100(11)	100(11)	90.9(10)	81.8(9)

Values mentioned in parenthesis represents number of antibiotics towards which bacteria were found resistant

Table - 5: Showing groups of antibiotics and percentage resistant of isolated bacteria towards different classes of antibiotics

Class of antibiotics	% Resistant bacteria
1. β-Lactum	
a. Ampicillin	90.9
b. Amoxocillin	81.8
2. Aminoglycoside	
a. Gentamicin	36.3
b. Kanamycin	81.8
c. Neomycin	90.9
3. Quinolones	
a. Nalidixic Acid	81.8
4. Flouroquinolones	
a. Norfloxacin	72.7
b. Ciprofloxacin	09.0
5. Polyketide	
a. Tetracycline	81.8
6. Macrolide	
a. Erythromycin	81.8
7. Polymyxins	
a. Polymyxin	90.9

Akinbowale *et al*. (2007). The comparison of inhibition zone size and value of MIC suggest that exposure to pollutants changes their categorization from susceptible to resistant. The screening of bacterial resistance property towards different classes of antibiotics showed maximum resistance for aminoglycoside class of antibiotics followed by β-lactum> polymyxin> macrolide, polyketide and quinolones> flouroquinolones (Table 5). Multiple antibiotic resistances due to pollutants have been previously reported (Sabry *et al.,* 1997; Kessie *et al.,* 1998; Adelowo *et al.,* 2009). Drug and a wide variety of toxic agents provoke many biochemical changes in cells that allow them to overcome the toxic effects of either the same or other compounds (Hayes and Wolf, 1990). Plasmid conferring resistance may also play important role in liking resistance to more than one drug (Sabry *et al.,* 1997).

The MIC was noted maximum (150 μg ml^{-1}) for Mn and Fe with a minimum for Cu and Zn (115 μg ml^{-1}) as shown in Table 4. Further, the antibiotic resistance pattern of heavy metal revealed up to 100% resistance. Change in frequency of antibiotic resistance in presence of Cu were seen 100% for *V. vulrificus,* followed by 90.9% for *K. pneumonae, P. aeruginosa* and *A. hydrophila*, 81.8% for *Salmonella* and *E.coli*, 72.7% for *S. faecalis, Ent. faecalis* and *Y. enterolitica* and 63.6% for *S. aureus.* However, least conversion towards antibiotic resistance was noted for *B. subtilis* (18.1%) as shown in Table 4. Similarly, in presence of Fe the change in antibiotic resistance pattern was observed 100% for *K. pneumonae, A. hydrophila*, *Y. enterolitica* and *V. vulrificus* followed 90.9% for *S. typhi, S. faecalis, P. aeruginosa, E.coli* and *Ent. faecalis,* 63.6% for *B. subtilis* and 45.4% for *S. aureus.* Similar pattern were observed for Mn except for *S. faecalis* 72.7%, *B. subtilis and S. aureus* 81.8% and *Y. enterolitica* and *V. vulrificus* 90.9%. Resistance pattern of isolates in presence of zinc were 100% for *Vibrio*, 90.9% for *S. typhi, P. aeruginosa, Ent. faecalis, A. hydrophila* and *Y. enterolitica,* 81.8% for *S. faecalis, E.coli, B. subtilis* and *V. vulrificus* and *72.7%* for *S. aureus.* The resistance to heavy metals in both Gram-positive and Gram-negative bacteria is also reported by Thavasi *et al.* (2007). Resistant to antibiotics and heavy metal tolerance of bacteria is also seen in drinking water system by Calomiris *et al.* (1984) which clearly indicated that metal resistant isolates exhibited multiple antibiotic resistance patterns, whereas metal sensitive isolates of raw water did not., this might be due to the degrees of poly metallic pollution, type of constituents and presence of negatively charged ions like chloride in the medium may bind with metals and alters the bioavailability and toxicity of metals resulting in difference in MIC of metal (Kannan and Lee, 2008). The increase of antibiotic resistance in isolated bacterial strains were also noted in presence of lignin and PCP strains, which were sensitive to antibiotics, were also found resistance in presence of lignin and PCP. The order of resistance of different bacterial strains in presence of lignin and PCP were *V. vulrificus* and *P. aeruginosa* (100%)> *K. pneumonae S. typhi, S. faecalis, A. hydrophila, S. aureus* and *Y.*

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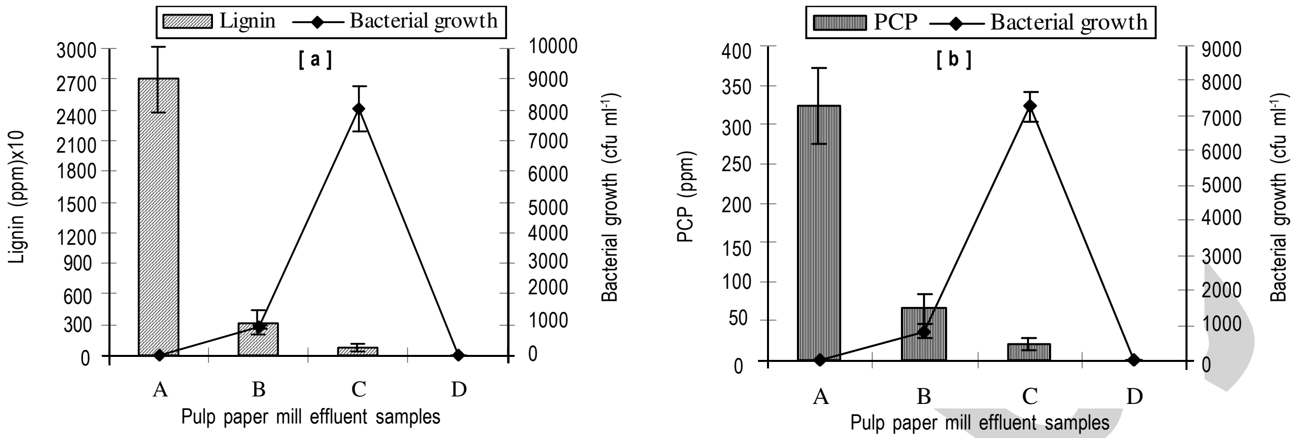

Fig. 1: Bacterial growth pattern at different concentration of lignin (a) and PCP (b) in various pulp paper effluent samples. A=Before treatment, B=After treatment, C=Discharged effluent after mixing with river water, D= River water

enterolitica (90.9%). Maximum change in resistance pattern in presence of heavy metal and lignin and PCP is seen in *B. subtilis* followed by *S. aureus* and *Y. enterolitica* (Table 3 and 4), which were not or least resistant to antibiotics. Cell-wall modification and bioprecipitation are other mechanisms employed by the bacterial cells to reduce the toxic effect (Chaudhary and Srivastava, 2001). Study of Sharma *et al.* (2006) revealed that the effect of various stress conditions resulting in the induction of outer membrane protein in *P. aeruginosa.* The resistance mechanism of Gram - negative bacteria involves the alternation of membrane permeability which resulted in the adsorption of metal which can attack the lippopolysaccharide layer of outer membrane of these bacteria. Reasons behind lignin and PCP resistance mechanism among bacteria can be proposed that high level of toxic compounds generally exerts a selective pressure on microorganism that may result in appearing variants possessing resistant property (Cerventes and Corona, 1994; Mcarthur and Tuckfield, 2000). In addition proteins also play important role in resistance mechanism towards heavy metal or any other stress environment. (Sharma *et al.,* 2006; Bar *et al.,* 2007). In present study, it is seen that effect of lignin and PCP on bacterial population is similar to the studies of Aleem *et al.,* (2003). The study is also supported by Sabry *et al.* (1997) and Sharma *et al.* (2006) that not only heavy metals but presence of pollutants either sewage, environmental or industrial may be responsible for multiple drug resistance patterns of bacteria. It is well established that there is a clear association between heavy metal and antimicrobial consumptions within a population and the frequent recovery of antibiotic resistance bacteria. However, it is apparent that a range of other agents might represent important mechanism that derives the selection of antibiotic resistance determinants (Austin *et al.,* 2006). This study has revealed for the first time the effect of lignin and PCP on antibiotic resistance pattern. Therefore, it can be concluded that pulp paper effluent waste contributed to acquire resistance among bacteria against antibiotics.

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