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Review

Pulp and paper mill wastewater and coliform as health hazards: A review

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ABSTRACT

This review article depicts the presence of coliform bacteria in the pulp and paper mill wastewater and also emphasizes their effect in health hazards. Pulp and paper-mill wastewater discharged into freshwater, estuarine and marine ecosystems alter aquatic habitats, affect aquatic life and adversely impact on human health.The wastewater contains many organic and inorganic compounds including large number of coliforms that are severely hazardous to living being. In which *Klebsiella* is predominately present in pulp and paper mill wastewater while *Escherichia coli*, *Citrobacter*, *Enterobacter*, etc. are in less number. The fecal *Streptococci* (*Enterococci)* alternative indicators of fecal health hazards are commonly present in all mills wastewater in the absence of fecal matter. Total coliforms, fecal coliforms and *Enterococci* or *E. coli* counts as indicators of fecal contamination, which causes health hazard in human and animals. This review is focused on various aspects such as nature and characteristics of organic and inorganic pollutants, coliform population, detection techniques, diseases caused by coliforms and persistent organic pollutant (POP) in industrial waste.

Keywords: Coliform, fecal contamination, health hazards, POPs, wastewater.

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INTRODUCTION

In past, up to five decades, it was observed that coliforms can survive in fecal contaminated environment such as in mammalian intestine and are significant indicator of fecal contamination in wastewater (Clark, 1995). The present study has shown that coliforms such as *Klebsiella*, *E. coli* etc., can flourish in carbohydrate rich environment e.g. cellulose, hemicellulose and lignin, which are predominantly found in the wastewater of pulp and paper, sugarcane and textile mills (Ivnitski et al., 1999; Donnison, 1992; Gauthier et al., 2000; Singh et al., 2013). Research on pulp and paper mills waste water shows that the coliform bacteria also inhabited in pulp and paper mill effluent, which indicates the occurrence of pathogenic microbes. Coliforms belong to two groups: Total coliforms (TC) and fecal coliforms (FC) (Archibald, 2000; Gauthier and Archibald 2001; Giri et al., 2014). They exist in the colon of human and animal intestine; multiple copies of coliforms discharge with excreta from human intestine and spread in surrounding environment such as river, lakes, ponds, etc. and cause hazardous effect, such as diarrhea, abdominal pain, nausea, vomiting and fever after consuming contaminated water (Mobius, 1991; Donnison, 1992; Clark, 1995). They have lactose fermenting enzyme machinery that ferments the lactose sugar in acid and gas at 37°C. Most of the strains including facultative intestinal bacteria are non-pathogenic to human being but some species such as *Camphylobacter, Salmonella, E. coli* (O157:H7), *Shigella* and *Klebsiella* etc. causes human and animal diseases (Gapes et al., 1999; Dey and Goswami, 2011). Seasonal variations in population of coliforms can be seen in mill water system, for instance summer, monsoon and winter. There was variable numbers of coliforms in wastewater seen as a result of high temperatures, high carbohydrate levels, low dissolved oxygen levels and low fixed nitrogen levels, and also seen ratio between human and animal origin in contrast of pulp and paper mill waste water (Gauthier et al., 2000; Gauthier and Archibald 2000; Beauchamp et al., 2006; Chandra et al., 2006).

Worldwide, the total pulp and paper mills are 7745 and

paper demand is 402 million per annum. However, in India, 759 pulp and paper mills are exist and their approximate production ranges up to 10.11 million tons of paper, which is about 2.52% of the total world production. The present consumption of paper and paper board is about 11.15 million tons per year (Archibald, 2000; Kulkarni, 2013). Bishnoi et al. (2006) and Kumar et al. (2015) reported that pulp and paper wastewater contain many toxic elements and compound such as Nickel, Copper, Chromium, Lead, Trichlorophenol, Trichloroguicol, Tetrachloroguicol, Dichlorophenol, Dichoroguicol and Pentachlorophenol, etc. These are major contaminants present in the wastewater of pulp and paper mills may cause toxicity to human and animals. This article describes the distribution of coliforms and chemicals present in pulp and paper mills wastewater and their impacts on living beings.

PULP AND PAPER MILL WASTEWATER

Pulp and paper mills generate substantial quantities of wastewater, highly toxic against living beings in water bodies. The pulp and paper mill wastewater produced from different process of paper making such as digestion, pulping and bleaching contains harmful chemicals and causes successive biomagnifications in the food chains (Thompson et al., 2001). Pulp wastewater contains dissolved wood derived contaminants which are extracted from the wood during pulping and bleaching processes. The pulping process generates a large amount of wastewater (approximately 200 m^3/tones), containing highly toxic compounds (Thompson et al., 2001; Ince et al., 2011). Therefore, there is need to remove the harmful chemicals from the wastewater and make it usable form (Ince et al., 2011) (Figure 1).

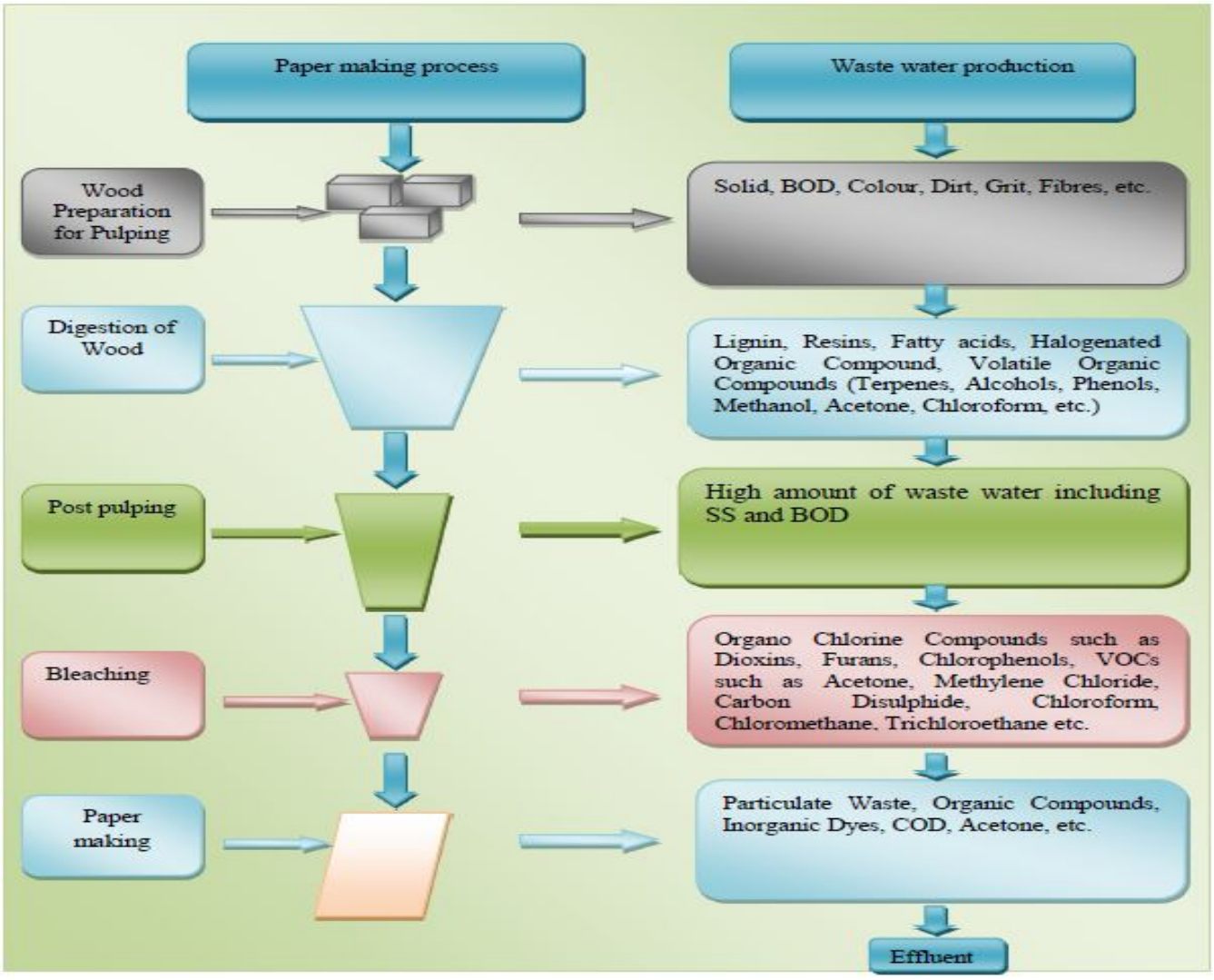

Figure 1. The various processes involved in production of paper products and generation of wastewater from pulp and paper mills. Modified from Ince et al. (2011).

Table 1. Important physico-chemical characteristics of pulp paper mill wastewater.

SNo.	Parameter	Kuzhali et al. (2012)	Chandra and Sankhwar (2011)	Saikia and Lohar (2012)	Medhi et al. (2008)
1.	Temperature (°C)	30.33 + 1.52	-	-	-
2.	Color	Light white	Dark brown	-	Dark Brown
3.	pH	8.56 + 0.33	9.2 ± 1.72	8.5 ± 0.346 ve	8.4 + 0.707
4.	EC (d Sm^{-1}),	1300 + 26.0	-	1.34 ± 0.010	4.27+ 0.086
5.	COD (mg/l)	1830 + 81.85	213136 ± 583.59	553 ± 7.57	590 + 17.57
6.	BOD (mg/l)	380 + 36.55	72143 ± 164. 81	396.33 ± 2.56	73 + 4.219
7.	TDS (mg/l)	2950 + 38.0	13402 ± 96.32	1311 ± 28.431	1370 + 1.643
8.	Ca (mg/l)	299.66 + 19.09	-		164 + 7.08
9.	TSS (mg/l)	-	-	3.91 ± 0.021	430 + 1.414
10.	TS (mg/l)	-	-	1761 ± 52.82	1800 + 1.843
11.	D.O (mg/l)	-	0.78 ± 1.13	-	0.9 + 0.1
12.	Alkalinity (mg/l)	-	-	322 ± 6.65	-
13.	Sulphate (mg/l)	-	17224 ± 141.89	-	-
14.	$PO4^{-3}$ (mg/l)	-	-	1.02 ± 0.02	1.871 + 0.157
15.	Total nitrogen (mg/l)	-	-	4.68 ± 0.02	-
16.	Mg (mg/l	184.2 + 12.48	-	-	42 + 7.123
17.	Na(mg/l)	260.99 + 7.06	1248 ± 81.73	-	85.32 + 0.974
18.	Potassium (mg/l)	-	135 ± 7.47	-	12.06 + 0653
19.	Chloride (mg/l)	-	41362 ± 11.24	-	518 + 15.36

NATURE AND CHARACTERISTICS OF PULP AND PAPER MILLS WASTEWATER

It is well known that wastewater generated from pulp and paper mill industry causes adverse effect on the environment. In order to assess its environmental effect on soil, water and crop plants, a significant number of studies have been done on national level for the physico-chemical characterization of wastewater for Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Total Dissolve Solid (TDS), Dissolve Oxygen (DO), Suspended Solid (SS) and pH, from paper industries (Kesalkar et al., 2012). Moreover, other characteristics of pulp and paper mill effluent reported by different authors are given in Table 1.

Lignin and lignin derivatives from pulp and paper wastewater cause more toxicity in the aquatic system. Today it is a big problem which if not solved results in strong black brown colour of liquid due to the contribution of lignin and lignin derivatives during pulping, bleaching and chemical recovery stages. Its decreases the photosynthesis and increases water temperature after mixing in water bodies and also decreases the concentration of dissolved oxygen. Toxicity of wastewater depends on the involvement of lignin and lignin derivatives (Kumar et al., 2015; Bajpai, 2015). In pulping, bleaching and chemical recovery sections, huge quantities of contaminated water are discharged, which contained lignin and lignin derivative. The high chlorine content of bleached plant reacts with lignin and its derivatives, to form highly toxic and recalcitrant compounds which are responsible for high biological and chemical oxygen demand. Trichlorophenol, Trichloroguicol, Tetrachloroguicol, Dichlorophenol, Dichoroguicol, Pentachlorophenol, Furan and Dioxin (Figure 2) are major contaminates found in wastewater of pulp and paper mills (Kumar et al., 2015).

The large amount of wastewater constitutes one of the major sources of aquatic pollution. The bleaching process produces the largest volume of organic and inorganic pollutants, which generates several chlorinated compounds via chlorination and others toxic organic and phenolic compounds such as lignin, highly toxic materials are formed from lignin and its derivatives, while recalcitrant compounds are responsible for the high BOD, COD, and Total Suspended Solid (TSS). Therefore, large percentages of the toxic compounds are released into the environment through pulp and paper mill wastewater and some persistent organic compound are also added which persist in the environment for a long time and are highly toxic to living beings (Chandra et al., 2008; Barneto et al., 2012). After concluding verity of review literature, all the Persistent Organic Pollutant (POP) in pulp and paper mill waste water are still not known; hence there is need for further study on this aspect.

PULP AND PAPER MILL WASTE WATER AS ENVIRONMENTAL HEALTH HAZARDS

Many of the chlorinated organic compounds randomly synthesized during pulp bleaching has been

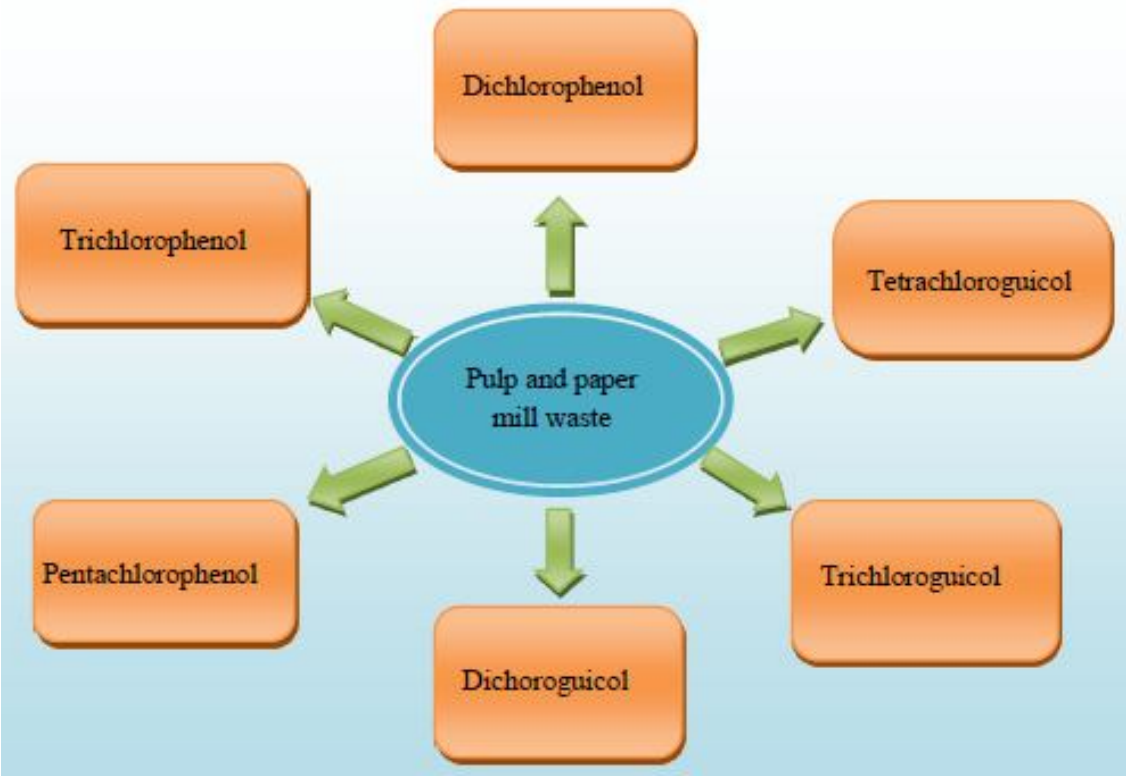

Figure 2. Highly toxic chlorinated compounds reported in pulp and paper mills waste water.

characterized as xenobiotic compounds (Dioxin, Furan, Trichlorophenol, Trichloroguicol, Tetrachloroguicol, Tichlorophenol, Dichoroguicol, Pentachlorophenol, etc) and persist in the environment for long period of time (Claxton et al., 1998; Kalmbach et al., 1997; Kumar et al., 2015). Therefore, the hazardous effects of these toxic compounds on human population are of great concerns. All these toxic compounds accumulate in human body through consumption of water and fish and cause several diseases. The detection of pulp and paper mill wastewater for mutagenic potential generally follows three approaches: assessment of entire effluent streams, fractionated effluent streams, and pure compounds recognized as effluent components. However, chloroacetones has been designated as substantial mutagenic and do not pose a serious human health risk as these compounds are quite unstable and tend to degrade rapidly (Kumar et al., 2015). On the other hand, chlorinated aliphatic hydrocarbons, such as trichloro- and tetra-chloroethylene, and their breakdown products are known mammalian carcinogens (Gauthier et al., 2000; Stevens et al., 2003; Chandra and Sankhwar, 2011). Dioxins increase the risk of soft-tissue sarcomas in heavily exposed industrial workers and acute dioxin exposure is known to have caused nausea and a relatively long-lasting (2 to 3 years) skin condition called chloracne. Although dioxin and furan do not appear to be highly toxic to human beings, they are extremely toxic to certain species of animals (Gauthier et al., 2000).

COLIFORMS IN PULP AND PAPER MILL WASTEWATER

Total coliforms

Total coliforms are a group of coliforms that are widespread in nature. Most of the members of total coliform groups occur in human feces, but some are also present in animal manure, soil, submerged wood, living wood, sugarcane juice and pulp and paper and textile industry wastewater (Parveen et al., 1997; Clark et al., 1997; Gauthier and Archibald, 2001; Abhirosh and Hatha, 2005). Thus, the usefulness of total coliforms as an indicator of fecal contamination depends on the extent to which the bacterial species found are of fecal and human origin, but some strains of *E. coli* cause disease in human beings (Clark, 1995). Public health agencies have used total coliforms and fecal coliforms as indicators since 1920. For recreational waters, total coliforms are no longer recommended as an indicator. For drinking water, total coliforms are still the standard test because their presence indicates contamination of a water supply by an outside source (Sengupta and Saha, 2013; Ince etal., 2011; Borrego and Figueras 1997). The total coliform group of bacteria was originally used as a surrogate for

E. coli, which in turn was considered to show fecal pollution due to *Escherichia, Enterobacter, Klebsiella, Citrobacter,* etc.

Fecal coliforms

Fecal coliforms are a sub-group of total coliforms. They are found in the intestines and feces of mammalian (Borrego and Figueras 1997; Parveen et al., 1997; Sengupta and Saha, 2013). Fecal coliform in a drinking water sample often indicates fecal contamination, a greater risk for animals. Because, the origins of fecal coliforms are more specific than the origins of the more general total coliforms group of bacteria; fecal coliforms are considered a more accurate indication of animal or human waste than total coliforms. However, even this group contains a genus *Klebsiella* which are not fecal origin (Parveen et al., 1999; Clark, 1995). Earlier researchers discovered that if feces contaminated bacteria are present in water, the indication is that the water is not safe for drinking.

Escherich in 1885 observed a microorganisms present in faeces, one of which he named *Bacillus coli* which is now called *Escherichia coli* and the concept that the presence of *B. coli* implied pollution of water was readily adopted (Barolia et al., 2011; Parveen et al., 1997). Comparison among total coliforms in fecal matter (1 g) and paper mill effluent (1 ml), succeeded in concluding a variety of research/review paper, the total population of coliforms represents in human faeces nearly 109 bacteria per gram and the paper mill effluent stream more or less (5×10^4 CFU of TC/ 1 ml) (Parveen et al., 1997; Stevens et al., 2003). Variable numbers of coliform, mainly the four genera *E. coli, Klebsiella* sp*. Enterobacter* and *Citrobacter* sp. have been detected in wastewater (Ince et al., 2011; Stevens et al*.,* 2003; Dufresne et al., 2001).

COLIFORM BACTERIA AS HEALTH HAZARDS

It is observed that coliforms originate in the mammalian intestine after two months of childbirth and show the association in the human intestine. They also can survive on carbohydrate rich environment such as cellulose hemicelluloses and lignin (Mobius, 1991; Nataro and Kaper, 1998; Hatha et al., 2004). Multiple copies of coliforms reach the environment through human-animal excreta and pulp and paper mill wastewater, and cause many health risks in the human and animals. The less frequent pathogenic bacteria in wastewater such as *Salmonella, Campylobacter jejuni, Mycobacterium ulcerans, Vibrio* sp*.* and *Legionella* sp. or protozoan *Cryptosporidium* cysts and *Giardia* cysts are now detectable at low levels, lactose fermentation in the presence of bile salts or contain other detergents or dyes inhibiting the growth of most bacteria (Nataro and Kaper, 1998; Beauchamp et al., 2006; Hatha et al., 2006; Ince et al., 2011; Giri et al., 2014). The coliforms are distinguished from the other bacterial groups in the *Enterobacteriaceae* by the possession of the enzyme galactosidase, enabling them to produce acid and gas (CO_2) from lactose. Although *Salmonella* and *Shigella*, belonging to *Enterobacteriaceae* are closely related to the coliform group but not fermented lactose (Nataro and Kaper, 1998; Almadidy et al., 2002; Ince et al., 2011). It is generally accepted that coliform bacteria exist in human and animal fecal matter but another huge sources of coliforms is pulp and paper mill wastewater and other carbohydrate rich environment such as sugarcane and textile mills wastewater (Dufresne et al., 2001; Mark, 2003; Ince et al., 2011). Pulp and paper wastewater contained mostly *Klebsiella* sp. approximately eighty percent and twenty percent of others such as *E. coli* and *Citrobacter*, etc. All these microorganism causes contamination in river, lake and sea waters, resulting in health hazards in humans and animals.

Numerous health hazards are caused after water consumption from mill wastewater are discharged in the river. Large numbers of coliforms are contained in the paper mill discharging site; coliform population after reaching the enteric part of human and animals' intestines cause diseases in them. Figures 3 and 4 show how pulp and paper mills wastewater discharge in huge quantities from industry, and then mixed into water bodies such as pond, river etc. where cattle, human, aquatic life and agriculture crop have been affected. In the world, waterborne disease is thought to account for nearly 6,000 deaths per day, mostly in children (Beauchamp et al., 2006; Clark, 1995). The health effects of exposure to disease-causing coliform bacteria in the water area vary. The most common symptoms of waterborne illness include nausea, vomiting, and diarrhea. Infants, the elderly, and those with compromised immune systems may suffer more severe effects. In extreme cases, some pathogens may infect the lungs, skin, eyes, nervous system, kidney or liver and the effects may be more severe, chronic, or even fatal. It is assumed that coliforms distribution in the aquatic environment through human and animals are based on level of fecal matter, however, pulp mill wastewater are also responsible for the same (Durhan et al., 2002).

THE COLIFORMS GROUP INDICATORS BASED ON PHYSIOLOGY

Currently, enzymatic and molecular based methods are being used in microbiology. The last two decades in microbiology have seen a move away from selective growth media based recovery methods for faecal bacteria (Stevens et al., 2003).

Acid and gas producing coliform from lactose

These coliforms are characterized as thermotolerant

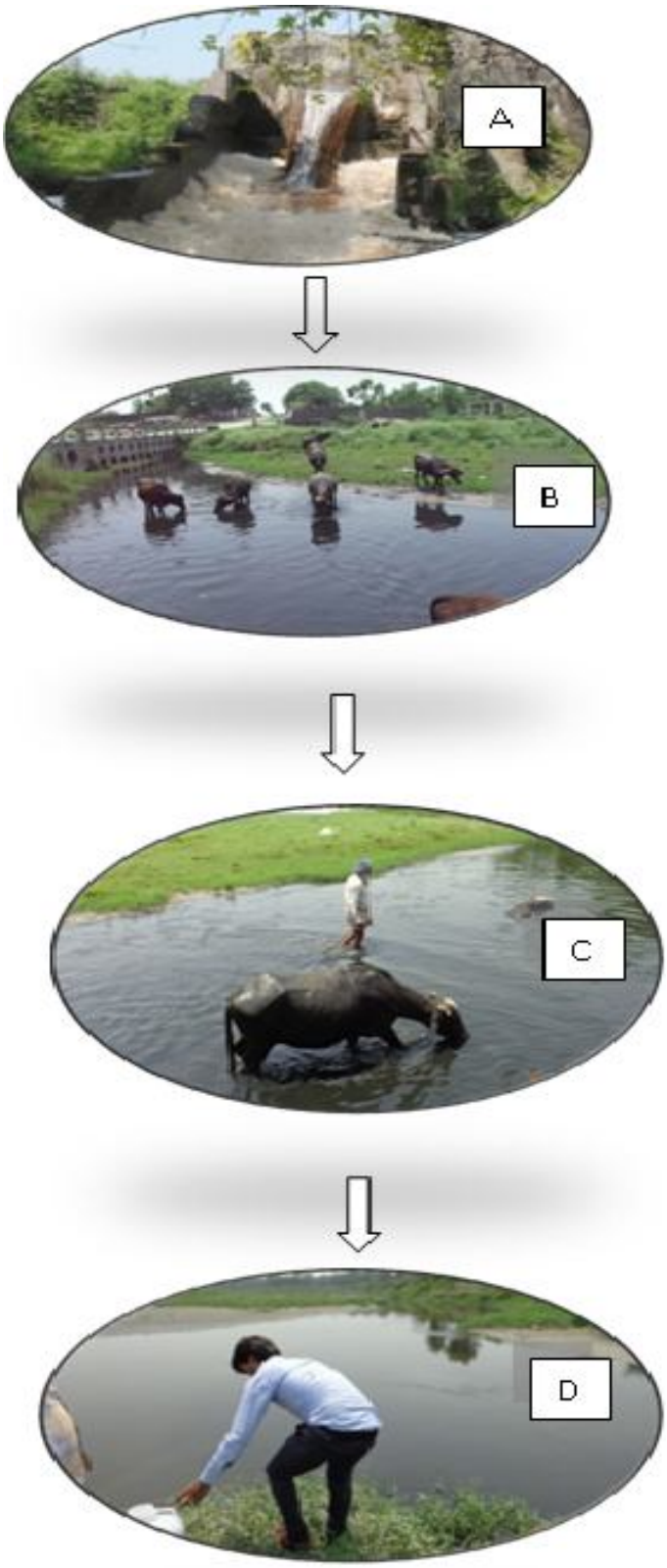

Figure 3. (A) Discharging of pulp paper mill effluent into the Golla River Lalkua Uttrakhand; (B) Effluent discharging from paper mill; (C) indicating the effluent drinking by cattle is the direct indication of health hazards; (D) effluent mixing into the river also an indication for health hazards for aquatic life.

because of their ability to produce indole from tryptophan and acid-gas production e.g. *Escherichia, Klebsiella, Enterobacter* and *Citrobacter*.

Acid producing coliform from lactose

These groups of coliforms produce acid from lactose e.g. *Escherichia, Klebsiella, Enterobacter, Citrobacter, Yersinia, Sarratia, Hafnia, Pantoea* and *Kluybera.*

Enzymes producing coliform from lactose

The lactose fermentation is one of the key process mediated by coliform due to the presence of a specific enzyme ß-galactosidase. The ß-galactosidase enzyme producing coliforms are: *Escherichia, Klebsiella, Enterobacter, Citrobacter, Yersinia, Sarratia, Hafnia, Pantoea, Kluyvera, Cedecea, Ewingella, Moellerella, Leclercia, Rahnella,* and *Yokenella.*

METHODS FOR DETECTION OF COLIFORM BACTERIA

Cultivation of colifoms by different method for the detection or confirmation and presence of total coliforms in wastewater / pulp and paper mill wastewater is necessary. Media cultivation can be categorized into two types: (1) enzyme-based media - this method uses the chromogenic substrate for the detection and confirmation of total coliforms in a single step such as defined substrate method; and (2) presumptive coliform detection media - there are required additional steps to confirm the presence of total coliforms for instance most probable number. The detection, confirmation and the presence of total coliforms in a single step are based on the presence of the enzyme ß-galactosidase (Amann et al., 1995), and all groups of coliforms enclosing an enzyme ß-galactosidase (APHA, 1998). The enzyme ß-galactosidase is used to hydrolyze a chromogenic substrate for instance, ortho-nitrophenyl-ß-d-galactopyranoside in the media to release a colored compound e.g., ortho-nitrophenol yellow, which changes the color of the media. The change in medium color indicates the presence of total coliforms (Clark, 1995); both the presence-absence and quantitative results are possible, depending on the enzyme based method being used. Some enzyme based methods are proposed in such manner that they also hamper non-coliform bacterial growth, thus non-coliform bacteria do not interfere with the recovery of coliforms (Lechevallier et al., 1990). Other methods for the confirmation of total coliforms recovered are membrane filter (MF) technique and defined substrate methods have been described. Although multiple types of tests can be used, and all the methods

Figure 4. Toxicity of pulp paper mill effluent on agriculture crops.

show variability for the confirmation of total coliforms or detection of coliforms. It has been observed that the non-coliform bacteria present in the sample can also affect the result coliforms (Barolia et al., 2011; Ince et al., 2011). It is also important to use validated or standardized methods to make correct and timely public health decisions.

MOST PROBABLE NUMBER (MPN) METHOD

In 1914, the first US Public Health Service Drinking Water Standard adopted a bacteriological standard that was applicable to confirm the quality of water supply by an interstate common carrier (Bajpai, 2015; Brenner et al., 1996; APHA, 1998). The MPN technique is a statistical method of examining the turbidity of bacteria in a test sample. This method not provides the actual information about test organisms in the sample but specify the information about approximate number of microbial population in a sample (Almadidy et al., 2002). MPN method is completed in three steps which are described below:

Presumptive test

In the presumptive test, a series of 10 ml lactose broth tubes are inoculated with measured amounts of the water sample such as 0.01, 0.1 and 1 ml approximate, the series of tubes may consist of three or four groups of three, five or more tubes. After 24 h at 37 to 38°C if gas production occurs, this indicates the presence of coliforms in test sample and the MPN of coliforms/100 ml of the water sample can be estimated by the number of positive tubes and all positive tubes set out for confirmation test.

Confirmed test

If any of inoculated tubes are showing gas production with the test sample, the water is presumed to be unsafe. However, it is possible that the formation of gas may not be due to the presence of coliforms as some non-coliform bacteria also produce gas. The detection or confirmation of the presence of coliforms in test sample, is necessary to inoculate Eosine Methylene Blue (EMB) agar plates from a positive presumptive tube. The methylene blue in EMB agar inhibits gram positive organisms and allows the gram negative to grow. *E. coli* colonies are small and have a green metallic sheen (Figure 5A), whereas *Enterobacter aerogenes* forms large pinkish colonies (Figure 5B).

Completed test

After picking the cells from EMB agar plate, they are transferred to an agar slant and again transferred to lactose broth. If acid and gas are again seen now it is clear indication about the presence of coliforms in the sample and if Gram-negative rods shape bacteria are found in the slant culture, then the identification of coliform is considered positive.

Membrane filter (MF) procedure

Commonly MF procedure was introduced to bacteriological analysis of water in 1951, later on its capacity to produce results equivalent to those obtained by the MF procedure was demonstrated (APHA, 1998; ISO, 2000; Gauthier and Archibald, 2001). It is a quantitative procedure that uses membrane filters with pore sizes sufficiently small to retain the microorganisms. The water sample is filtered through the membrane, which is then transferred to an appropriate growth medium for identification and quantization. Both enzyme-based methods and presumptive coliform media can be used. This procedure is able to examine larger volumes of water than can be examined with MF, is more sensitive and reliable, and requires significantly reduced time,

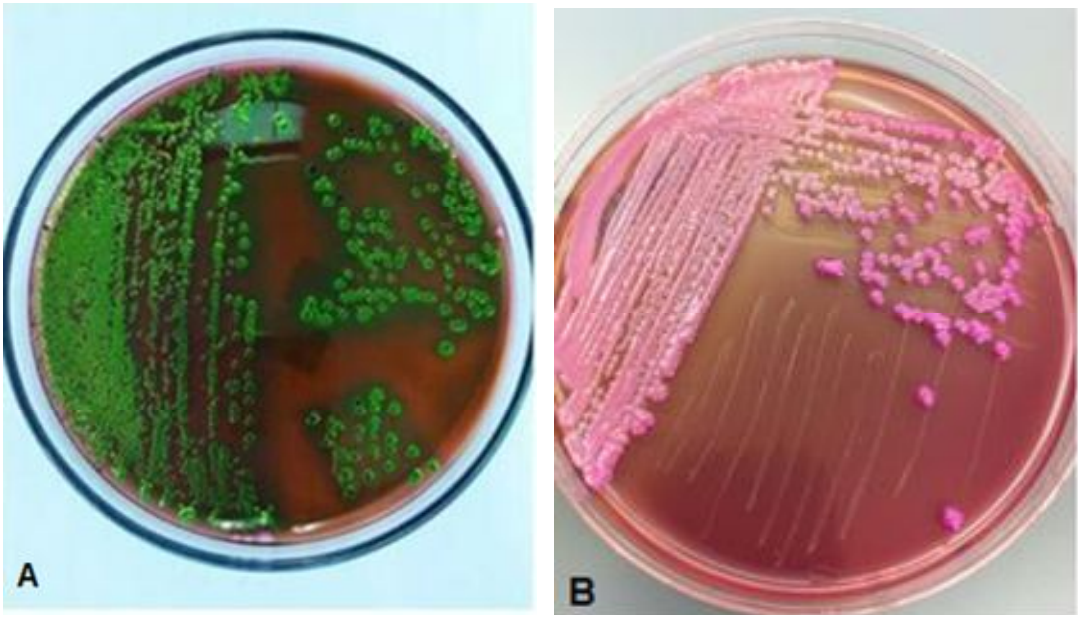

Figure 5. (A) *E. coli* (metallic green colony); (B) *Enterobacter aerogenes* (pinkish colony).

labor, equipment, space and materials. These qualities have made the MF technique the method of choice in some jurisdictions for the routine enumeration of coliforms in drinking water (Lechevallier, 1990). However, this method may underestimate the number of viable coliforms in a sample. Standard methods for the examination of water and wastewater do provide 95% confidence limits for MF results (APHA, 1998).

Defined substrate methods

In this method, detection and confirmation of coliforms media without harsh selective agents but specific enzyme substrates allow significant improvements in recoveries and identification of target bacteria. In the case of *E. coli*, such so called defined substrate methods (Edberg et al., 1991; Amann et al., 1995; Fricker et al., 1997; Eckner, 1998) appear to pick up traditionally non-culturable coliforms. These developments have led to further changes in definitions of coliforms and *E. coli* in the UK; for example, total coliforms are members of total genera or species within the family Enterobacteriaceae, capable of growth at 37°C, which possess β-galactosidase. In an international calibration of methods, *E. coli* was enzymatically distinguished by the lack of urease and presence of β-glucuronidase (Fricker et al., 1997; Eckner, 1998).

BIOSENSOR TECHNIQUES USED FOR THE DETECTION OF COLIFORMS PATHOGENCITY

Biosensors used for the detection of bacteria usually involve a biological recognition constituent such as receptors, nucleic acids or antibodies in intimate contact with an appropriate transducer (Almadidy et al., 2002; Mozaza et al., 2005; Mozaza et al., 2005; Sassolas et al., 2012). In recent years, attempts have been made for the improvement of biosensor technology for its portable, rapid and sensitive application so that they can be performed virtually anywhere *viz* clinical diagnostics, food analysis, bioprocess engineering, environmental monitoring, etc. but environmental monitoring of coliforms is very interesting; various contaminated sources such as mills wastewater are polluting the environment (Iles and Kallichurn, 2012; Arora et al., 2011; Gapes et al., 1999). The significance of biosensors results from their high specificity and sensitivity, which allows the detection of frequent pathogenic bacterial communities in the environment. Bacterial biosensors can be divided into sensors operating in batch (intermittent) and continuous (monitoring) mode. Presently, some bacterial biosensors are used frequently for coliform analysis as shown in Table 2.

ASSESSMENT OF *E. COLI* VIRULENCE PATHOGENECITY

Various genetic probes are available for assessing the pathogencity of *E. coli* (Table 3). DNA probes are used for detection of target gene sequence, which is responsible for the pathogencity (Parveen et al., 1997). The pathogencity of a given strain is mainly determined by specific virulence factors, which includes shiga toxins, adherence factors/fimbriae, heat labile toxins, colonization factors, heat stable toxins, invasion factors,

Table 2. Biosensor based technique for the detection of coliforms.

Biosensor	Application	References
Direct detection of coliforms		
Optical biosensors	Optical biosensor used for direct detection of Bacteria in sample e.g. *Escherichia coli* O157:H7, and *Salmonella typhimurium.*	Ivnitski et al. (1999), Connelly and Baeumner (2012), Gauthier et al., (2000), Archibald (2000)
Bioluminescent sensors	The bioluminescence approach is a new approach; The and its inherent ability to distinguish viable from non non viable cells e.g. *Listeria* spp.	Ivnitski et al. (1999)
Piezoelectric biosensors	A piezoelectric biosensors crystal immunosensor has been developed for the detection of enterobacteria in drinking water using antibodies against the enterobacterial antigen e.g. *Salmonella, Escherichia coli.*	Ivnitski et al. (1999)
Electrical impedance biosensors	This technique has been used for estimating microbial biomass for detecting microbial metabolism and for detecting theconcentration and physiological state of bacteria for instance *Staphylococcus aureus, Proteus vulgaris.*	Ivnitski et al. (1999)
Indirect detection of coliforms		
Fluorescence labeled biosensors	Using this technique it was possible to detect *E. coli* O157:H7 in the range of 105 to 109 CFU/ml with an assay time of 4 h. This technique was also used for detecting *S. typhimurium and Klebsiella pneumonia.*	Ivnitski et al. (1999)
Microbial metabolism based biosensors	This type of biosensor can only be used for well-defined samples because of the possible presence of enzymes from sources other than the bacteria of inters e.g. *E. coli, S. aureus* and *Enterococcus* spp.	Ivnitski et al. (1999)
Electrochemical immune detection of bacteria	This new immunoassay approach can be easily extended to the detection of other bacterial cells and may be a basis for creating new, highly sensitive and rapid immunosensors e.g. *Salmonella*, *Listeria*.	Ivnitski et al. (1999)
Flow immunosensors	This technique can be easily automated, the analyses performed quickly and continuously and the renewal of the sensing surface of immunosensor was easily accomplished e.g. *Shigella* spp., *E. coli.*	Ivnitski et al. (1999)
Genosensors	This technique uses the heat-stable DNA polymerase of *Thermus aquaticus*, and allows short lengths of a double-stranded target DNA (template) to be copied in vitro thousands or millions of times, very quickly e.g. *Cryptosporidium* spp. *E. coli.*	Ivnitski et al. (1999)
The electronic nose	The method currently used for determining the status of meat, with respect to spoilage, is analysis of the total bacterial count e.g. *Proteus, Haemophilus influenza.*	Ivnitski et al. (1999)
Commercial instrumental systems	These devices have been used for detection of microbial growth in environment e.g. *E. coli, S. aureus* and *Enterococcus serolicida.*	Ivnitski et al. (1999)

hemolysins, iron transport systems, cytotoxic necrotizing factors, etc. These all virulence factors are used for the

Table 3. Probes used for detection of *E. coli* virulence and target genes. Adapted from Kuhnert et al. (2000).

Probe	Name	Target gene
A1[a]	Ferrichrome-iron receptor (Fhu)	*fhuA*
A2[a]	Type 1 fimbriae (Fim)	*fimA*
A3	P fimbriae (Pap)	*PapA*
A4	S-fimbriae (Sfa)	*sfaA*
A5	S-fimbriae (Sfa)	*sfaS*
A6	F1C fimbriae	*Foc*
A7	Bundel forming pilus (Bfp)	*bfpA*
A8	Colonization fimbriae (CFA/I)	*cfa/I*
B1	Colonization fimbriae (CS3)	*cfa/II*
B2	Aggregative adherence fimbriae (AAF/I)	*aaf/I*
B3	Intimin (Eae)	*Eae*
B4	Invasion-plasmid antigen (Ipa)	*ipaH*
B5	Aerobactin	*iucC*
B6	K1 capsule antigen	*neuA+ neuC*
B7	K5 capsule antigen	*kfiB*
B8	Heat-stable toxins (ST)	*stIa/stIb*
C1	Shiga Toxin I (Stx1)	*stxI*
C2	Shiga Toxin II (Stx2)	*stxII*
C3	Heat-labile toxin I (LT-I)	*eltI*
C4	Heat-labile toxin II (LT-II)	*eltIIa*
C5	Alpha-hemolysin (HlyA)	*hlyA*
C6	Enterohemolysin (ElyA)	*elyA*
C7	Cytotoxic necrotizing factor (CNF1)	*cnf-1*
C8	Low MW heat-stable toxin (EAST1)	*astA*

detection of infection in the host cell (Parveen et al., 1997; Kuhnert et al., 2000).

The primers used for probe amplification were either chosen from previous studies on virulence gene detection or designed from available gene sequences. Target gene is a particular segment of DNA, which is responsible for the production of virulence factor against a host cell such as toxins, capsule and fimbriae, virulence factors are responsible for causing pathogencity in the host cell for instance toxins, inhibit protein synthesis during a DNA replication. Second virulence factor capsule contain lipopolysaccharide, which protect bacteria from extrinsic factors (Parveen et al., 1997; Zeyaullah et al., 2010).

PREVENTION AND CONTROLS MEASURES RELATED TO WASTEWATER PROBLEMS

Pulp and paper mills discharge contains numbers of chlorine-based organic compounds and pathogenic microbes that may create various types of diseases are described in Table 4. In processes that do not use chlorine from the pulping process are recycled for re-use and energy generation. Some of the control measures related to waste water health hazards as described by WBG (1998) and Ince et al. (2011) are:

1. Integrated solid waste management of pulp and paper mills can be made via anaerobic digestion, composting, land applications, thermal processes such as incineration/combustion, pyrolysis, steam reforming and wet oxidation. End-of-pipe treatment tools and techniques may be used before the disposal.
2. Biopulping can be used to reduce the chemical and energy utilization in the pulping process because it improves the properties of pulp and reduces the toxic compounds. In biopulping technology microbes or their enzymes such as xylanases, pectinases, cellulases, hemicellulases, ligninases are used in the pulping process to minimize the problems of lethal materials release in pulp and paper mills discharges.
3. Use of total chlorine-free (TCF) processes or at minimum doses during bleaching systems can reduce the release of pollutants related to chlorine derivatives. The extended cooking and oxygen delignification can also minimize the production of toxicants related to chlorine.
4. Compared to traditional chemical treatment process, the biological treatment systems, such as activated sludge, aerated lagoons, and anaerobic fermentation, can reduce BOD, COD and other problems related to

Table 4. Diseases caused by coliform bacteria in human and animals and their sources.

S. no.	Pathogenic coliforms	Sources	Diseases
1.	*Escherichia coli*	Fecal matter and sugar mill, pulp and paper and textile mills	Kidney failure, anemia, nose bleed, colitis, Diarrhea, sepsis, bladder infection, fatigue and gastroenteritis
2.	*Klebsiella* spp.	Sugar mill, pulp and paper and textile mills	Urinary tract, nosocomial pneumonia and intra abdominal Infection
3.	*Salmonella* spp.	Fecal matter, food, pulp and paper mills.	Swelling of the abdomen, heart beat slows down, abdominal pain, typhoid fever
4.	*Citrobacter* spp.	Pulp and paper mills	Water diarrhea
5.	*Shigella* spp.	Pulp and paper, food industries	Caused plague
6.	*Enterobacter* spp.	Pulp and paper mills	Cerebral abscess, pneumonia, meningitis, Septicemia, and wound urinary tract

waste water.

CONCLUSIONS AND RECOMMENDATIONS

The pulp and paper mill wastewater promotes the growth of pathogenic bacterial population in water, therefore the toxicity and pathogenic microbes that cause diseases (for example urinary tract infection, nosocomial pneumonia, intra abdominal, diarrheal diseases, etc) in human and animals. The industrial wastewater mixing in rivers and lakes are consumed by living beings and are infected by the pathogens (coliforms). It is believed that coliform bacteria can survive only fecal contaminated environment but it was later found that coliform community can also survive in pulp and paper mill wastewater and in carbohydrate rich environments such as cellulose and hemicelluloses; therefore sugar mills, textile mills and paper mill effluent has enormous coliform population because they have cellulose and hemicellulose degrading machinery. These industrial wastewater discharges into aquatic environment before its adequate treatment which causes eutrophication in aquatic system and health hazards in human and animal; and some unknown Persistent Organic Pollutant (POP) also found in pulp and paper mill wastewater are highly hazardous to living being. Hence there is need for detail study in respect of persistent organic pollutant.

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