2. REVIEW OF LITERATURE

The harmful nature of industrial effluents in relation to plant growth and development is well recognized owing to the presence of toxic chemicals present in it. Various industries have been continuously adding lot of waste water containing nutrients, heavy metals and hazardous substances to the cultivable land (Srivastava et al., 2000). These effluents boost the level of various compounds beyond tolerance limit and cause toxicity to the crop (Mishra et al., 1999). Polluted water directly affects soil not only in industrial areas but also in agricultural fields and river beds, thereby creating secondary source of pollution (Kisku et al., 2000).

Industrial development led to the generation of industrial effluents and resulted in water, sediment and soil pollution if untreated (Fakayode, 2005). Pandey and Srivastava (2002) showed higher values of pollutants in industrial waste water than those of BSI (2009) standard discharge limits. Thus, the rapid industrialization is accompanied by both direct and indirect adverse effect on environment (Nasrullah et al., 2006).

Improper storage and waste management practices have left a legacy of contaminated soil and aquifers, frightening drinking water sources. The need to remediate these has led to the development of new technologies that stress on the detoxification and destruction of the contaminants rather than the conventional approach of disposal. Chuks Ugochukwu et al. (2008) reported that the remediation of contaminated aquifers to be a major focus of
regulatory activity and an area of scientific and technical challenge for environment professionals.

Bioremediation, the use of microorganism or microbial process to detoxify and degrade environmental contaminants is among these new technologies. Regardless of the exact nature of the treatment, all bioremediation techniques depend on having the right microbes which have the physiological and metabolic capabilities to degrade the contaminants in the right place with the right (Chandra and Bharagava, 2013).

Bioremediation, the use of microorganism or microbial process to detoxify and degrade environmental contaminants is among these new technologies. Regardless of the exact nature of the treatment, all bioremediation techniques depend on having the right microbes which have the physiological and metabolic capabilities to degrade the contaminants in the right place with the right environmental conditions for the degradation to occur. Long term irrigation with effluents increases organic carbon and heavy metals accumulation in soil and increase chance of their entrance of food and ultimately causes geoaccumulation, bioaccumulation and biomagnifications (Chopra et al., 2009; Srivastava et al., 2012). The presence of heavy metal contamination in water streams arising from the discharge of untreated metal containing effluents from industries into water bodies is one of the most important environmental issues (Dursan, 2008; Ghaedi, 2006). Heavy metals such as Cu, Zn, Ag, Cd, In, Sn, Sb, Hg, Pb, and Bi was detected in
higher concentration in environmental waste recycling facility soil samples (Ha et al., 2009).

The review of literature pertaining to the study, “Bioresmediation of heavy metals from Paper mill effluent and its application for the growth and development of blackgram has been presented.

2.1 PAPER MILL INDUSTRY

In the recent past, environmental pollution caused by different industries has become a matter of great concern world-wide. Improper management of wastes produced by industries is affecting the ecological health in a great manner. Paper industry is not an exception to this, as it uses an enormous quantity of water and chemicals during processing, which are disposed to land or water bodies as wastes. Nearly 75 to 95 percent of the water discharged by the Paper industries as effluent contains organic, inorganic pollutants and colouring materials. Presence of these chemicals may affect soil and in turn the growth and development of plants (Baruah et al., 1996, Medhi et al., 2011).

Paper industry is the third largest industrial polluter to water, air and land (Rout, 2008). India ranks the 20th position among the paper-producing countries of the world (Mahajan 1985; Bajpai and Bajpai, 1997; Malaviya and Rathore, 2011). The Indian paper industry is highly water intensive, consuming 100-250 m$^3$ of fresh water per tonne of paper, and also generates the corresponding wastewater, i.e. 75–225 m$^3$ per tonne of paper (Thompson
et al., 2001; Tewari et al., 2009). Paper mill effluent has high concentrations of phosphorous, biological oxygen demand (BOD) and chemical oxygen demand (COD) with increasing conductivity, colour and temperature (Gaete et al., 2000). The effluent water is fortified with various toxic chemicals and volatile organic compounds such as terpenes, alcohols, phenols, methanol, acetone, chloroform, methyl ethyl ketone, surfactants, dyes and pigments, acids and alkaline solutions and heavy metals like mercury, copper, iron, zinc and aluminum (Nikhileshwar, 1992; PPAH, 1998; Fortin et al., 1998; Garg et al., 2004a and b; Verma et al., 2005; Raj et al., 2005; Ali and Rahman, 2008; Suriyanarayanan et al., 2010; Medhi et al., 2011; Chopra et al., 2011). Consequently, the pulp and paper mill effluents containing these hazardous chemicals degrade the water quality and pose several negative effects on receiving aquatic ecosystems (flora and fauna) and adjacent agricultural fields, cattle, etc. (Garg et al., 2004a and b; Verma et al., 2005; Raj et al., 2005; Mishra et al., 2007; Ali and Rahman, 2008).

For the sustenance of environmental safety, regular monitoring of pollution parameters such as total suspended solids (TSS), BOD, COD, chlorophenols, dioxins and colour in these effluents has paramount importance (Raj et al., 2005; Pandey et al., 2005; Ali and Rahman, 2008).

2.2 PAPER MILL EFFLUENT

In the recent past, environmental pollution caused by different industries has become a matter of great concern world-wide. Improper management of wastes produced by industries is affecting the ecological
health in a great manner. Paper industry is not an exception to this, as it uses an enormous quantity of water and chemicals during processing, which are disposed to land or water bodies as wastes. Nearly 75 to 95 percent of the water discharged by the Paper industries as effluent contains organic, inorganic pollutants and colouring materials. Presence of these chemicals may affect soil and in turn the growth and development of plants (Baruah et al., 1996, Medhi et al., 2011).

India generates about 2270 m$^3$ of effluent containing 1484 mgL$^{-1}$ of total dissolved solids (TDS) daily. For last seventeen years, indiscriminate use of these effluents for irrigating agricultural land by the farmers in the nearby areas might cause shift in the functions and structure of native microbial communities (Hazarika et al., 2007). Thus, there is a need to study the shift in microbial diversity of such soils in relation to changes in soil physico-chemical properties, which are governed by agricultural management practices. The literature survey indicates that while there are treatment methods that remove either colour and/or phenols from effluents, there is dearth of treatment processes that effectively remove both colour and phenols from the effluent (Deilek and Bese, 2001). It is therefore, necessary to isolate, identify and conserve microorganisms which are able to detoxify the toxic components present in the industrial effluents. Information on dynamics of microbial populations in wastewater treatment systems and the relationship between population dynamics and functional stability of
treatment systems may help in reducing the toxic effects of paper mill effluents on the environment. Heavy metals are those elements with a molecular weight greater than 53, a density greater than 6 g cm$^{-3}$ and an atomic number greater than 20. They occur naturally in rocks and soils but concentrations are frequently elevated as a result of pollution. They are also called trace elements, which are toxic to living organisms at excessive concentrations but some including Zn, Cu, Mn and so on, at low but critical concentrations are micronutrients used in the redox processes, regulation of the osmotic pressure and also enzyme components which are essential for the normal healthy growth and reproduction by living organisms. At high concentrations, these micronutrients damage DNA and membrane as well as loss of functions of enzyme. However, heavy metals like Ni, Co and Pb cause oxidative stress, lipid peroxidation, carcinogenesis, mutagenesis and neurotoxicity on humans, animals and plants at low concentrations. (Joseph et al., 2012).

The paper industry typically generates large quantities of wastewater whose correct treatment prior to discharge into the environment is critical. Paper factory effluent is one of the major pollutants on the earth. The pulp and paper mill effluent is highly coloured. The on persistent dark brown colour in the released paper and pulp industrial effluent from wastewater treatment facilities is brown or black in colour due to dissolved lignin based synthetic, aromatic and chlorinated compounds derived from the blow heat
condensate, pulp decker washing, chlorine and alkali bleach waste, black liquor spillage and foul evaporator condensate (Mishra et al., 2011).

Among the various sections in paper mills, the bleaching section is considered to be the most polluted. During this stage, chlorine or chlorine dioxide is used to bleach pulp and release chlorinated and nonchlorinated compounds from lignin and wood extractives. Typically, these effluents contain high concentrations of chlorphenolic compounds, chloroacetones and chloroform, which are colored and recalcitrant. It has been reported that production of one ton of paper contributes 100 Kg of color imparting substances and 2-4 Kg of organochlorines to the bleach plant effluents. In an attempt to identify the source of heavy metals through multivariate analysis, it was concluded that Cr, Ni and Pb mainly originated from paper mill effluent (Reza et al., 2013). The high chemical diversity of these pollutants causes a variety of elastogenic, carcinogenic and mutagenic effects on fishes and other aquatic communities in recipient water bodies (Parveen Kumar et al., 2011).

2.3 DECOLOURISATION OF PAPER MILL EFFLUENT BY USING EFFICIENT MICROORGANISMS.

The industry currently constitutes one of the five major contributors to air and water pollution (Pokhrel and Viraraghavan, 2004). Pulp and paper mill effluent is highly colored that imparts brown/black appearance if discharged directly without prior treatment, to the receiving waterbody. The
color of such wastewaters primarily results from the presence of lignin and its derivatives (Duarte et al., 2003; Saravanan and Sreekrishnan, 2005) that are released from the substrate in various processing steps undertaken during paper manufacture. The dark color of receiving water body is not only esthetically unacceptable but increases the water temperature and reduces the light transmission, thereby decreasing aquatic plant photosynthesis (Joyce et al., 1984). This, in turn, reduces the dissolved oxygen content, ultimately causing the death and putrefaction of aquatic fauna (Kringstad and Lindstrom, 1984; Sahoo and Gupta, 2005).

Decolorization and detoxification of paper mill effluents is an important goal and has been the subject of several studies performed during yesteryears (Raj et al., 2007). Several physicochemical methods have been reported to be effective for reducing color in and toxicity of paper mill wastewater (Garg and Modi 1999; Garg and Tripathi, 2011). However, these methods have not been implemented at an industrial scale, mainly because they are energy intensive and cost prohibitive (Singh and Singh, 2004). The conventional biological methods employed in the industry include use of aerated lagoons and activated sludge processes. However, such treatment systems are generally less effective in removing color, chemical oxygen demand (COD), and chlorinated phenolic compounds (Raj et al., 2007; Saunamaki, 1989). Therefore, more advanced alternative biological wastewater treatment strategy will be required to meet more and new stringent discharge limits set for adsorbable organic halogens (AOX). Such
biological methods have been found to be more effective and eco-friendly since they are capable of degrading not only lignin but also chloroorganics contributing to AOX. Attempts have been made to decolorize and/or degrade toxicants in pulp and paper mill effluents with a variety of microbes, including fungi and bacteria.

Various effects of paper mill effluents, including decolorization by white-rot and other fungi, have been reviewed (Garg and Modi 1999; Garg and Tripathi, 2011; Tripathi et al. 2007). However, the main constraint in using a fungal degrading system is the requirement to maintain growth and/or enzymes (ligninases) activity at the prevailing low pH (4–5). However, at low pH, the solubility of high molecular weight fragments that are derived from lignin is reduced. Furthermore, the natural pH of pulp–paper mill effluent is alkaline (pH 8–9). Therefore, any requirement to reduce the pH to the acidic range prior to fungal augmentation would be uneconomical (Raj et al., 2007). Considering the above and other facts, bacterial treatment systems that have an optimum pH range of 7–9 may play a pivotal role in decolorizing pulp and paper mill effluents, without any prior requirement of pH adjustment. Vora et al. (1988) have reported that many bacteria, viz., *Pseudomonas*, *Flavobacterium*, *Xanthomonas*, *Nocardia*, *Aeromonas*, and *Arthrobacter* sp., are able to utilize several lignocellulosic compounds of the bleached plant effluent, including organochlorine constituents. Several bacterial species capable of metabolizing various industrial pollutants have been isolated from the natural environments, viz., *Bacillus* sp., *Bacillus subtilis*, and *Bacillus cereus* (Andretta et al., 2004;
Chandra et al., 2006 and 2009; Mishra and Thakur 2010; Niazi et al., 2001; Raj et al., 2007; Tripathi et al., 2011; Tripathi and Garg, 2010), Pseudomonas sp., Pseudomonas veronii, Pseudomonas fluorescens, and Pseudomonas aeruginosa (Chauhan and Thakur, 2002; Nam et al., 2003; Premlatha and Rajkumar, 1994; Shah and Thakur, 2003; Thakur et al., 2002), Arthrobacter chlorophenolicus A6 (Agneta et al., 2004), Serratia marcescens, Citrobacter sp., and Klebsiella pneumoniae (Chandra et al., 2011). However, a number of studies have indicated that the ligninolytic system is not inducible by lignin and that it does not serve as a growth substrate (Keyser et al., 1978). Lignin degradation is, therefore, dependent on the presence of a readily metabolizable cosubstrate such as glucose (Hammerli et al., 1986). But increased carbohydrate supply stimulates, while increased nitrogen inhibits, lignin degradation (Garg and Modi, 1999). Therefore, the C/N ratio has been considered a better predictor of lignin degradation than the absolute levels of carbohydrates and nitrogen (Reid, 1979). An important task for effluent treatment is the isolation and characterization of efficient microorganisms together with designing and optimization of process parameters to deal with specific environmental pollutants (Singh and Thakur, 2004).

2.4 TOXICITY OF PAPER MILL INDUSTRY EFFlUENT

The pulp and paper industry is a high pollution load industry ranks sixth largest polluter after oil, cement, leather, textile, and steel industries discharging a variety of gaseous, liquid, and solid wastes into the environment (Ali and Sreekrishnan, 2001). It discharge large volumes of
wastewater generated for each metric ton of paper produced, depending upon the nature of the raw material, finished product and extent of water reuse. The most significant sources of pollution in pulp and paper industry are wood preparation, pulping, pulp washing, bleaching and coating operations. Effluents of paper industry contain suspended solids (10–50 kg/t of ADP), biochemical oxygen demand (10–40 kg/t of ADP), chemical oxygen demand (20–200 kg/t of ADP), toxicity, color, AOX compounds (0–4 kg/t of ADP) and high concentration of nutrients that cause eutrophication in receiving water (Pokhrel, and Viraraghavan 2004). Wastewater from chemical pulping contains 12–20 kg of BOD/t of ADP, with values of up to 350 kg/t. The corresponding values for mechanical pulping wastewater are 15–25 kg BOD/t of ADP. For chemi-mechanical pulping, BOD discharges are 3 to 10 times higher than those for mechanical pulping. Effluent originating from the first E stage is highly colored and typically accounts for 80% of the colour, 30% of the BOD and 60% of the chemical oxygen demand (COD) of the mills total pollution load (Bajpai and Bajpai, 1994). This much high load of color, not only aesthetically unacceptable but also inhibit the natural process of photosynthesis in the stream due to absorbance of sun light. This leads to a chain of adverse effects on the aquatic ecosystem. Wastewater of pulp and paper mill also contains heavy metals such as lead, copper, nickel which comes along with wood raw material. The use of peroxide, ozone, and other chemicals in bleaching makes it necessary to use a complexing agent for heavy metals such as manganese (Choudhary et al., 2011). Mercury is used in the
production of caustic soda, which is used heavily in the pulping and bleaching processes. Mercury concentrations in caustic soda can be as high as 22 grams per tonne of caustic soda produced (Choudhary et al., 2011). Phosphorus and nitrogen are also released into wastewaters (Pokhrel and Viraraghavan 2004).

The main source of nutrients, nitrogen, and phosphorus compounds is raw material such as wood and agro-residue. Wastewater of the pulp and paper industry contains a number of toxic compounds that may cause serious environmental impacts upon direct discharge to receiving waters. Among the various sections, bleaching often accounts for the largest fraction of toxicity. More than 500 different chlorinated organic compounds have been identified including chlorate, chloroform, phenols, resin acids, chlorinated hydrocarbons, catechols, guaiacols, dioxins, furans, syringols, syringaldehydes, vanillins (Freire et al., 2003).

Toxic effects of the Paper industry effluent on the aquatic life of the receiving water bodies are very well documented. Salkinoja-Salonen and Sundman, (1980) reported that some of the compounds in the Paper mill effluent are resistant to biodegradation. Landner et. al., 1977 and Renberg et. al., 1980 showed that these compounds can bioaccumulate in the aquatic food chain. Leuenberger et. al. (1985) in their study on persistent chemicals in pulp mill effluents reported detection of tetrachlorophenol (TeCP) and pentachlorophenol (PCP) Paper mill effluent. According to Canadian
Environmental Protection Act (Environment Canada and Health and Welfare Canada, 1991) Paper mill effluent industry effluent has been defined as toxic. Berry et al. (1991) attributed acute and chronic toxicity of the effluent to the presence of chlorophenols especially polychlorinated phenols.

In wastewater, these compounds are estimated collectively as “adsorbable organic halides” (AOX). Formation of these compounds is directly proportional to consumption of chlorine or chlorine based bleaching agents (Savant et al., 2003). Some of the chlorinated compounds known to impart toxicity are: di, tri, tetra chlorophenols, chloroguaiacols, tetrachlorodibenz-p-dioxins (TCDD) and furans (TCDF) (Xie et al., 2005). Since some of the contaminants in paper industry effluents are non-biodegradable, conventional biological treatment processes are not sufficient for treatment. The extent of toxicity, total organic carbon (TOC) and colour removal by conventional biological treatment vary depending on the pulping process used. The conventional treatment for Indian pulp and paper mills include primary treatment and secondary aerobic biological system. Some of the mills have adopted anaerobic system also. It has been observed that the secondary effluent still contains color and high level of chlorinated organic compounds (AOX) that impart toxicity to the wastewater. In order to meet increasingly stringent discharge limits, pulp and paper mills are forced to adopt technologically advanced treatment systems such as constructed wetlands.
The effluent water is fortified with various toxic chemicals and volatile organic compounds such as terpenes, alcohols, phenols, methanol, acetone, chloroform, methyl ethyl ketone, surfactants, dyes and pigments, acids and alkaline solutions and heavy metals like mercury, copper, iron, zinc and aluminum (Mishra et al., 2013).

2.5 CONVENTIONAL TREATMENT METHODS:

Biotechnological processes have the potential to reduce environmental pollution through their application in processes aimed at resolving waste dumping problems (Mahjabeen Saleem et al., 2013). Chemical approaches like Precipitation (Xia and Liyuan, 2002), ion- exchange method (Addour et al., 1999), Electrochemical cells (Cossich et al., 2002), and Reverse osmosis (Xia and Liyuan, 2002) are available for remediation, but are often expensive to apply and lack the specificity required to treat the effluents. In addition, such approaches are not applicable to a cost- effective remediation of large-scale subsurface contamination in situ. As an alternative to these methods, recently, the method of removal of contaminants by means of microorganisms. Biological removal of contaminants from aquatic effluents offers great potential when the contaminants are present in trace amounts (Vinita and Radhanath, 1992). The newly discovered metal sequestering properties of certain types of microbial biomass of fungi, bacteria and algae offers considerable promise to the existing methods for metal detoxification and their recovery. The most successful biotechnological processes utilize biosorption and bio-precipitation, in addition to other processes such as binding by specific macromolecules (Volesky et al., 1993).
The conventional method of treating pulp and paper mill effluent involves the biological oxidation by bacterial action of aerobic and anaerobic conditions and aerobic lagooning method, which are less efficiency of removing COD. To overcome the drawbacks of the existing treatment process, in the present work an attempt has been made to study the electro oxidative destruction of the pulp and paper mill effluent using an electrochemical method and the effect of various parameters such as concentration of supporting electrolytes, current densities, flow rates of electrolyte and reservoir volumes of the effluent were conducted. From the experimental results it is observed that the rate of reduction of COD of the effluent increased with an increase in the supporting electrolyte (sodium chloride) concentration, current density where as it decreased with increase in the reservoir volume and the flow rate of electrolyte. The residence time distributions studies have also been conducted to study the behavior of the electrochemical reactor (Kannadasan and Sivakumar, 2011).

The incapability of conventional wastewater treatment methods to effectively remove many bio-recalcitrant pollutants leads to explore the new efficient and cost effective treatment systems for the complete degradation of these pollutants. One relevant method is oxidation, and especially the Advanced Oxidation Process (AOP) which consists of the strong action of oxidizing agents, resulting in the generation of very highly reactive hydroxyl radicals. The present study has undertaken to status of the environment
pollutants of the effluents of recycled paper and pulp mills and the strategies
to remove the organic load. The goal of the study is to evaluate the best
effective treatment which can effectively degrade the pollutants present in
waste streams.

2.6 BIOREMEDIATION

The term “bioremediation” has been used to describe the process of
using microorganisms to degrade or remove hazardous pollutants from the
environment (Volesky, 1986). Bioremediation can also be defined as any
process that uses microorganisms, green plants or their enzymes to return to
the natural environment altered by contaminants to its original condition.
Heavy metal bioremediation involves removal of heavy metal from waste
water and soil through metabolically mediated or physico-chemical
pathways (Kuyucak and Volesky, 2000a). This natural and environmental
friendly technology is cost effective, aesthetically pleasant, soil organism-
friendly, diversity enhancer, energy derivation from sunlight (Chaney et al.,
2005; Huang et al., 2004; Susarla et al., 2002) and more importantly, it is
able to retain the fertility status of the soil even after the removal of heavy
metals (Kirkham, 2006).

Bioremediation of toxic industrial effluents by microorganisms serves
as an effective method to substitute the conventional recovery and removal
process. Fungal biomasses have huge capability of treating effluents
discharged from various industries. White rot fungi are ubiquitous in nature
and their adaptability to extreme conditions makes them good biodegraders. Their enzymatic degrading activity makes them effective decolorizer; they also remove toxic metals by biosorption ultimately rendering the effluents more ecofriendly (Pagnanelli et al., 2002).

Bioremediation has been recognized as an environment friendly and less expensive method which involves the natural processes resulting in the efficient conversion of hazardous compounds into innocuous products. This technique involves suitable microbes undergoing various physical and chemical reactions in the polluted water system and during the microbial metabolism, the pollutants are degraded and removed. Recently microbial bioremediation has emerged as an alternative technique to such traditional chemical treatments. Sugar industries consume large quantities of water for various processes and discharge equally large volumes of waste waters containing variety of pollutants and coloring matter (Buvaneswari, et al., 2013)

2.6.1 Mechanisms involved in Bioremediation

The complex structure of microorganisms implies that there are many ways for the metal to be taken up by the microbial cell. The bioremediation mechanisms are various and are not fully understood. They may be classified according to various criteria (Costa et al., 2000; Gadd et al., 1998; Huang et al., 1990).
According to the dependence on cell’s metabolism, Metabolism dependent and Non-metabolism dependent, According to the location where the metal removed from solution is found extra cellular accumulation/ precipitation cell surface sorption / precipitation and Intracellular accumulation.

Transport of the metal across the cell membrane yields intracellular accumulation, which is dependent on the cell’s metabolism. This means that this kind of accumulation may take place only with viable cells. It is often associated with an active defence system of the microorganism, which reacts in the presence of toxic metal. During non- metabolism dependent process metal uptake is by physico-chemical interaction between the metal and the functional groups present on the microbial cell surface. This is based on physical adsorption, ion exchange and chemical sorption, which is not dependent on the cell’s metabolism. Cell walls of microbial biomass, mainly composed of polysaccharides, proteins and lipids have abundant metal binding groups such as carbonyl, sulphate, phosphate and amino groups. This process i.e., non- metabolism dependent is relatively rapid and can be reversible (Kuyucak and Volesky, 2000b). In the case of precipitation, the metal uptake may take place both in the solution and on the cell surface (Ercole et al., 1994). Further, it may be dependent on the cell metabolism if, in the presence of toxic metals, the microorganism produces compounds that favour the precipitation process. Precipitation may not be dependent on the
cell metabolism, if it occurs after a chemical interaction between the metal and cell surface (Thakur, 2006).

2.6.2 Microbial extra cellular polymeric substances; central elements in heavy metal bioremediation

Extracellular polymeric substances are biosynthetic polymers produced by both prokaryotic and eukaryotic microorganisms. EPS are localized at or outside the bacterial cell surface and comprised of a variety of high molecular weight organic macromolecules such as polysaccharides, proteins, nucleic acids, phospholipids along with other non-polymeric constituents of low molecular weight (Arundhati Pal and Paul, 2008).

Bacterial EPS plays an important role in cell adhesion, formation of aggregates such as biofilms, flocs, sludges and biogranaules and protect cells from hostile environment. They are involved in the degradation of particulate substances sorption of dissolved materials including heavy metals leaching minerals from sulphidic ores as well as biocorrosion (Wingender et al., 2000).

Over the last decade, studies on the use of micro-organisms for environmental restoration have primarily focused attention towards exploiting microbial potential for remediation of effluent contamination in both terrestrial and aquatic systems. Bioremediation of toxic metals and radio-nuclides from polluted sediments and waste stream employ living and non living microbial biomass/ biopolymer to form a stable, non-toxic
complex (Gadd, 2000). The electrostatic interactions between the metal ligands and negatively charged biopolymeric substances outside the cells lead to formation of stable complexes. Though availability of cheap biomass and immobilization techniques have made bioremediation process advantageous, the metal-binding capacity is known with only few functional groups potentially involved in cation-binding. In spite of all these constraints, the ease of application of purified EPS and that in biofilm, activated sludges or bio- granules in bioremediation have demonstrated efficiency in several studies which have led to a large number of reports favouring this approach (Jayabarth et al., 2009; Liu et al., 2003; Beyenal et al., 2004; Xu et al., 2004).

2.7 BIOREMEDIATION OF INDUSTRIAL EFFLUENT

Early in 1980 the capability of some microorganisms to accumulate metallic elements was witnessed. Numerous research reports have been published from toxicological points of view, but these were concerned with the accumulation due to the active metabolism of living cells, the effects of metal on the metabolic activities of the microbial cells and the consequences of accumulation on the food chain (Brown and Ahsanullah, 2000; Moore, 2000; Volesky, 2001b). However, further research has revealed that inactive/dead microbial biomass can passively bind metal ions via various physicochemical mechanisms. With these new findings, research on bioremediation became active, with numerous biosorbents of different origins being proposed for the removal of metals. Researchers have
understood and explained that bioremediation depends not only on the type or chemical composition of the biomass, but also on the external physicochemical factors.

Hanife Buyukgungor, (2000) studied the bioaccumulation of lead from aqueous solutions by immobilized cells of *Citrobacter freundii*. Broken hazelnut (*Corylus avellana*) and walnut (*Juglans regia*) shells which are agricultural wastes were used as support material and *Citrobacter freundii* cells were immobilized on the shell surface. Most of the higher organisms tend to accumulate heavy metals by ingesting metals bound to particles or sediments (Roanne and Pepper, 2000). Copper and lead are widely regarded as toxic metals for plants and native microorganisms (Czajka *et al.*, 2001).

Many hydrocarbon-contaminated environments are characterized by low or elevated temperatures, acidic or alkaline pH, high salt concentrations, or high pressure (Margesin and Schinner, 2001). Hydrocarbon-degrading microorganisms, adapted to grow and thrive in these environments played an important role in the biological treatment of polluted extreme habitats. Raina Miller, (2001) demonstrated the bioremediation of metal-contaminated wastewater. Normally whole cells or microbial exopolymers are used to concentrate or precipitate metals in the waste complex because microbial cells or large exopolymers do not move freely through the soil.

The bacterial EPS isolated from a species of *Marinobacteria* selectively bound more amount of copper per mg of EPS than lead. Both
copper and lead were sorbed more at near neutral pH than acidic pH (Bhaskar and Narayan Bhosle, 2003). Semra Ilhan et al. (2004) studied on the selective biosorption of chromium, lead and copper ions by microorganisms from industrial waste waters. Liang- Ming Whang et al. (2004) investigated the potential application of two biosurfactants, surfactin produced by Bacillus sp. and rhamolipid produced by Pseudomonas aeruginosa, for enhanced biodegradation of contaminated water and soil and reported that application of surfactin and rhamolipid in stimulating indigenous microorganisms for enhanced bioremediation confirmed their enhancing capability on both efficiency and rate of diesel biodegradation in diesel/ soil systems.

Aspergillus sp. and Rhizopus sp. isolated from agricultural field treated with sewage effluents could biosorb both metals viz., Cr and Cd and Rhizopus sp. could biosorb higher concentration of both metals as compared to Aspergillus sp. (Iqbal Ahmad et al., 2005). The bacterial isolate Aeromonas hydrophila was shown to decolorize three triarylmethane dyes tested within 24 hours with colour removal in the range of 72 to 96 percent and highlighted the potential of Aeromonas hydrophila as a biotechnological tool for remediation and detoxification of textile and other industrial wastewaters containing triarylmethane dyes (Sauidis et al., 2006).

Phytotoxicity studies carried out using Triticum aestivum, Hordeum vulgare and Lens esculentum revealed the toxic effects of triarylmethane
dyes on the plant growth parameters. However, significant reduction in toxicity was obtained after biodegradation of dyes by the Aeromonas hydrophilica thus, suggesting the treated wastewater can be used for ferti-irrigation (Saudidis et al., 2006, Faryal et al., (2006) isolated fungi viz., Aspergillus sp., Rhizopus sp., Rhodotorula sp., Dreshlera sp., and Curvularia sp. from soil which were used as a biosorbent for removal of Zn from wastewater. Ashutosh Kumar Tripathi et al. (2007) stated that water pollution through industrial discharges, which is mainly in the form of effluent or waste water, is one of the biggest problems. These effluents have strong concentrations of chemical oxygen demand (COD), phenol and its derivatives, contains metals, inorganic compounds, proteins, cyanides, chlorinated lignin and dyes.

Diana et al. (2008) tested on adsorption properties of bacterial biomass for Cd removal from liquid effluents. Comte et al. (2008) assessed the influence of pH on the metal biosorption of extracellular polymeric substances (EPS) extracted from two different activated sludges called A and B. The bacteria Alcaligenes, Bacillus and Corynebacterium isolated from sago industry effluent and effluent contaminated soil were found efficient in starch degradation and recorded 63 per cent of degradation of starch in paper industry effluent. The effluent treated by aerobic microorganisms had no negative impact on the seed germination and shoot length the root length, fresh weight, dry weight and chlorophyll content
showed an increase hence the bioremediated effluent can be effectively used for irrigation (Ayyasamy et al., 2008).

The application of bacterial extra cellular polymeric substance (EPS) in the bioremediation of heavy metals (Cd, Zn and Cu) lead to a lower concentration of the free heavy metals in solution, while a great part of them was observed in the polymeric matrix when compared to what is observed or internalized by biomass (Paula Salles et al., 2008). Dead biomass of various Pseudomonas sp. isolates were used for the biosorption experiments for removal of Copper, Cadmium and Magnesium (Korrapati Narasimhulu and Parcha Sreenivsa Rao, 2009).

Lina Velasquez and Jenny Dussan, (2009) tested Colombian Bacillus sphaericus native strains for the tolerance to As, Hg, Co, Fe and Cr, as well as the biosorption and bioaccumulation in living biomass and reported that the live and dead cells of B. sphaericus OT4b31 and B. sphaericus IV (4)10 showed biosorption of Cr and had the capacity to accumulate between 6 and 47 percent of Co, Hg, Fe and As.

Efficiency of utilizing petroleum refinery effluents under aerobic condition was demonstrated by Pseudomonas sp. and Acinetobacter sp. However, the greatest efficiency was by the consortium of both microorganism (Pseudomonas sp. and Acinetobacter sp.) (Atuanya et al., 2009). Incubation of biosurfactant producing Pantoea agglomerans and Planococcus citreus
isolated from contaminated soil, resulted in a COD reduction in the effluent and showed that these strains and the surfactants could be used in bioremediation process (Daniela Franco et al., 2009). The Extended Aeration (EA) involving microorganisms could reduce BOD, COD and TSS of sago effluent up to 84, 87.8 and 73 per cent respectively (Wahi Abd Rashid et al., 2010).

For bioremediation of polluted soil, the lower pH value and higher enzyme activity in different bioremediation processes suggested the effectiveness of combination of effective microorganisms with ryegrass. The fraction analysis indicated a priority of low molecular hydrocarbon degradation in combination of microorganisms and ryegrass, polar fraction degradation in phytoremediation and aromatic fraction in microbial degradation (Jingchun Tang, 2010). All the immobilized isolates of heavy metal resistant bacteria isolated from electroplating industrial effluent samples viz., Bacillus sp., Pseudomonas sp., and Micrococcus sp. have potential application for the removal of Cu, Cd and Pb from industrial wastewater than dead bacterial cells (Johncy Rani et al., 2010).

Deepika Lakshmipath et al. (2010) stated that the Bacillus sp. isolate with its biosurfactant production and heavy metal resistant activity could be used as potential strain and could be used as bio-remediating agent. Pseudomonas sp. is better microbial tool for bioremediation of heavy metal and can be explored to remove heavy metal load, present even in low
concentration, in waste water of pulp and paper mill effluent by using indigenous microorganisms (Arti and Smita, 2010).

Addition of surfactants, either synthetic or microbial surfactant can increase the efficiency of TPH (Total petroleum hydrocarbon) degradation of crude oil and waste motor lubricant oil (Luhur Akbar Devianto and Edwan Kardena, 2010). The *P. aeruginosa* and *Thiobacillus ferrooxidans* showed high resistant to all metals and *T. ferrooxidans* reduced/ absorbed some heavy metals from mines (Cd, Ca, Zn, Cr, Mn and Pb) and *P. aeruginosa* was effectively absorbing the most of the metals than *T. ferrooxidans* (Narayanan Mathiyazhagan and Devarajan Natarajan, 2011). All the immobilized isolates of *Aspergillus* sp., *Penicillium* sp. and *Cephalosporium* sp. have potential application for the removal of Cu, Cd and Pb from industrial wastewater than the dead fungal cells (Hemambika *et al.*, 2011). The actinomycete *Nocardia* sp. was most effective followed by bacteria (*Pseudomonas* sp. ATS-08) and fungi (*Mucor* sp. ATS-5) and could be used for treating industrial effluents having high amount of chromium (Manjunathan *et al.*, 2011). Similarly, the potential of cyanobacterial species viz., *Oscillatoria* sp., *Synechococcus* sp., *Nodularia* sp., *Nostoc* sp. and *Cyanothece* sp. are efficient agents for pollution control and degradation of industrial effluents (Sanjay Kumar Dubey *et al.*, 2011).
2.8 REUTILIZATION OF REMEDIATED INDUSTRIAL EFFLUENT

In developing countries including India, farmers are irrigating their crop plants with industrial effluents having high level of several toxic metals (Cu, Cd, Cr, Zn, Fe, Ni, Mn, Mg and Pb) due to the non-availability of alternative sources of irrigation water (Chandra et al., 2009). Untreated effluent used for irrigation is highly toxic to the plant, fish and other aquatic life. The need for economical, effective and safe methods for disposal of pollutant in effluent has resulted in the search for unconventional materials that may be helpful in reducing the pollutant in the effluent (Bako et al., 2008). Reusage of treated effluent that is normally discharged to the environment from municipal waste water treatment plants is receiving an increasing attention as a reliable water resource (Akram Tamini, 2008).

Panoras et al. (2003) studied the effects of effluent from reclaimed either by activated sludge with microorganism or by stabilization ponds on field-grown corn and reported that there is a potential risk of facing problems related to soil salinity and alkalinity if no consideration for soil reclamation is taken into account. Hulugalle et al. (2004) reported that the treated sewage effluent using Aspergillus niger contained considerable amounts of nitrogen, phosphorus and high concentrations of sodium salts. Effluent water was moderately saline, and compared with river water, had higher concentrations of Na, nitrate-N and K, and lower concentrations of Ca and Mg. Irrigation with treated sewage effluent caused large increase in
nitrate-N, small increase in exchangeable Mg, Na and K, and small decrease in heavy metals (Hulugalle et al., 2004).

Experimental effects of untreated (Raw) Paper mill effluent, discharged from a outlet unit and the post-treatment effluent from the outlet of conventional anaerobic treatment plant (Treated effluent) of the distillery unit were studied in mung bean (Vigna radiata, L.). The germination percentage, growth characters and seedling enzyme activity of Mung bean was high even in 20 percent concentration of treated effluent compared with raw effluent (Prasad, 2008).

Similarly the leaching of carbohydrates and proteins (solute efflux) were much higher in case of untreated effluent and were also dependent to the pre-soaking time. Other germination characters including percentage of germination, speed of germination index, vigour index and length of root and embryonic axis revealed significant concentration-dependent decline in untreated effluent. Evaluation of seedlings membrane transport enzymes and structural constituents (hexose, salicylic acid and phospholipids) following 6 h pre-soaking of seeds revealed concentration-dependent decline, which were much less in treated effluent as compared to the untreated effluent. Treated effluent up to 10 per cent (v/v) concentration reflected low-observed adverse effect levels (Suresh and Damodharan, 2007).

The fresh weight, dry weight, chlorophyll content and protein content were increased with increase in dilution of treated pulp and paper mill
effluent. However, the 100 percentage of treated effluent resulted in retardation of growth (Amar Nath Giri, 2008). Seed germination, shoot length, root length, fresh weight, dry weight and chlorophyll content of maize and green gram showed an increase when treated effluent by aerobic bacterial consortium composed of Alcaligenes, Bacillus and Corynebacterium was used, whereas, a decrease of growth was noticed in untreated effluent tested seedlings (Ayasamy et al., 2008). Lakshmi and Sundaramoorthy, (2010) has explained that the microbes (PGPR) mixed polluted soil showed good results in morphological, biochemical and yield parameters of blackgram than the untreated soil.

Albino Wins and Murugan, (2010) has stated that the textile mill effluent can be safely used for irrigation purposes with proper treatment and dilution at 25 percent for growing blackgram Vigna mungo. Similarly germination of kidney bean (Phaseolus aureus) and lady’s finger (Abelmoschus esculentus) seeds where affected adversely when raw textile effluent was used, were as the bioremediated effluent increased the seed germination, total sugars, starch, reducing sugars and chlorophyll content of the plant than control (Priya Kaushik et al., 2004).

Bacteria viz., B. cereus and B. thuringensis played an important role in degrading Cd, Ni and Zn in soil and increasing soil fertility by removal of heavy metals (Cd, Cu, Ni, Pb and Zn) for Millet and Green gram plants (Ajaz Haja Mohideen et al., 2010). The dry weight of root and shoot of
*Phaseolus radiatus* exhibited progressive increase from the control up to 9.25 percent concentration when irrigated with *Aspergillus terreus* treated dairy effluent (Ramana et al., 2002).

### 2.9 INFLUENCE OF PAPER INDUSTRIAL EFFLUENTS ON CROP PERFORMANCE

Ben *et al.* (1965) studied the effect of pulp mill effluents on different crop rotations and found that corn in rotation with oats, cowpea and vetch was greatly benefited by use of waste water from paper mill as a supplemental irrigation. Irrigation with waste water yielded 92 bushels per acre and the one with well water yielded 94 bushels per acre during eight year period. Further they found that irrigation with waste water from paper mill could be carried out on rice without any harmful effects. The rice irrigated with waste water yielded 63 bushels per acre, while that irrigated with good water from wells produced 62 bushels per acre.

Malleshappa, (1979) observed a slight reduction in the dry matter production in soybean due to irrigation with paper mill effluent as such or diluted (50 percent), when compared to that obtained from irrigation with river water or and paper mill effluent, alternatively. Further, he reported that the important yield contributing characters of soybean such as seed weight per plant, number of seeds per plant, and hundred seed weight did not differ significantly due to irrigation with paper mill effluents as compared to those observed due to irrigation with river water.
Rajanan and Oblisami, (1979) studied the effect of paper factory effluents on germination and seedling growth in rice, black gram and tomato crops. The effluents samples from paper factories were alkaline and contained considerable amount of nitrate, nitrogen (313 to 368 mg/l). They observed that the undiluted paper factory effluent had detrimental effect on the germination of all the three crop seeds. However, diluted effluents showed increasing germination percentage with increasing dilution level. The vigour index of all the seedlings of all the three crop seeds was significantly poor in undiluted effluent compared to water control and it increased with the decrease in the concentration of the effluents. The presence of excess amounts of soluble salts like Ca and Mg in irrigation water caused injuries to the plant system. The reduction in growth of seedling was attributed to the greater amounts of Ca, Mg and solid materials in the effluents. In certain cases, diluted effluents enhanced the growth of plant and this was reasoned out to the decrease in the concentration of various chemicals in the effluents and presence of root promoting phenolic compounds which might have played a role in influencing the beneficial effect on the plant growth.

Somashekar et al. (1984) studied the effect of paper industry effluent on jowar, bajra and paddy crops. The effluents were diluted up to 50 per cent and 25 per cent concentration. The germination percentage of control seeds except paddy was above 90 per cent. Effluents of paper factory almost
completely inhibited germination of paddy seeds and even at 25 per cent concentration, the germination percentage was significantly less. The inhibition of germination of jowar, bajra, and paddy seeds in treatment with 100 per cent of effluents were 31, 38 and 82 per cent, respectively. The values come down 27, 26 and 75 per cent in case of 25 per cent effluents. Hence it is clear that the per cent inhibition is directly proportional to the concentration of the effluents and germination being highest at 25 per cent concentration. The vigour index of all the three crop plants was significantly poor in undiluted effluents and it increased with a decrease in their concentration.

Dhevagi et al. (2000) studied the effect of paper mill effluent on spermosphere microflora of maize, sunflower, greengram, blackgram, soybean and groundnut with different dilution levels. Among these crops studied, groundnut recorded the maximum microbial population (104.1 x 10^6 g of soil), followed by soybean (50.7 x 10^6 g of soil). In case of blackgram, greengram, and soybean up to 100 per cent effluent concentration, the total microbial population was higher than the control. As the effluent concentration increases in case of maize, blackgram, greengram, soybean and sunflower, the population load as well as the germination percentage of the seeds also increased till 50 to 75 per cent effluent concentration. The reason for more number of microbes at lower concentrations was pointed to the reduced level of toxic metabolites due to dilution factor.
Dhevagi and Oblisami, (2000) noticed that paper factory raw effluent (as such) affected the germination percentage to the level of 19, 5.4, 13 and 9.9 per cent for the maize, groundnut, soybean and blackgram respectively. It is because the effluent analysis showed the presence of lower concentration of major organic nutrients and higher EC which has negative correlation with seed germination.

Agricultural practices and the discharge of effluents from the treatment plants are the major contributors to water and other environmental pollution problems (Sharif and George, 2000). Most of the Indian rivers are already polluted by the industrial effluents. Due to scarcity of good water for irrigation, the farmers have started to irrigate their agricultural crops by using the industrial waste water (Rathore et al., 2000). Sharma et al. (2004) reported that the soil samples collected from the agricultural land irrigated with effluents had high level of several toxic metals as Cu, Cd, Cr, Zn, Ni, Fe, Mn and Pb. Similarly, soil analysis showed higher values of Zn, Fe, Mn, Cd, Ni and Pb and plant samples had greater concentration of many heavy metals than recommended values in areas where industrial effluent was used as irrigation for vegetable cultivation (Saleem Saif et al., 2005).

Heavy metal contamination in effluents has been on the rise in proportion to the peace of worldwide industrialization, leading to significant health problems and toxic effects on plant and microbial biodiversity (George Garrity and Terry Marsh, 2007). The untreated petroleum refinery
effluents contained high concentration of COD (112mg/L); BOD (30.8mg/L); TDS (157.4mg/L); Fe (5.065mg/L); oil/ grease (4.04mg/L) and low DO (1.78mg/L) (Atuanya, et al., 2009). Likewise, Globally, pulp and paper industry is considered as one of the most polluting industry contributing 100 million kg of toxic pollutants that are being released every year in the environment (Sumathi and Hung, 2006).

In India, the paper industry has been one of the major sources of aquatic pollution (Pathan et al., 2009). The pulp and paper Industry is one of the oldest industries in this country and there has been tremendous expansion of these industries during the last 25 years. Effluents from pulp and paper mills are highly toxic and are a major source of aquatic pollution. Many chemicals have been identified in effluents which are produced at different stages of papermaking. Their toxic nature is derived from the presence of several naturally occurring and xenobiotic compounds which are formed and released during various stages of papermaking. The pulp and paper mills rank high in terms of water use during paper production on the other hand they contributing to pollution loads in rivers through effluent discharge (Jayabalakrishnan, 2007). Waste and wastewaters are generated from both of pulp and bleaching processes. The major problems of the wastewaters are high organic content, dark brown coloration, adsorbable organic halide and toxic pollutants. Toxic dyes, bleaching agents, salts, acids and alkalis are present in effluents discharged from pulp and paper
industries. Heavy metals such as cadmium, copper, zinc, chromium are present in pulp and paper mill effluent that ultimately released into aquatic environment (Zahrim et al., 2007). Dichloroguicicol, trichloroguicicol, tetrachloroguicicol and chlorinated phenols are major contaminants found in the effluent released from pulp and paper mill which are toxic to fish fauna (Mellanen et al., 1996). Various acute and chronic effects of pulp and paper mill effluent in fish fauna have been noticed by several workers.

In the past two decades, there has been a notable increase in the use of wastewater for crop irrigation, especially in arid and seasonally arid areas of both industrialized and developing countries (Baghel, 2008; Biswas et al., 2009; Chidankumar et al., 2009). It may be due to the increasing scarcity of alternative water for irrigation and high cost of chemical fertilizers (Ramana et al., 2002; Sukanya and Meli, 2004; Rath et al., 2011). In agriculture, irrigation water quality is believed to have effects on the soils and crops (Bharagava et al., 2008; Biswas et al., 2009; Tharakeshwari and Jagannath, 2011a; Mohamed and Ebead, 2013). The use of saline water may result in the reduction of crop yield, while the sodic water may deteriorate the physical properties of the soil with consequent reduction in the yield (Chandra et al., 2009; Chidankumar et al., 2009; Shenbagavalli et al., 2011). Thus considerable attention is presently focused towards the aspects of wastewater application, including the possible causes of potentially harmful agents in the environment (Chonker et al., 2000; Hati et al., 2007; Kalaiselvi
et al., 2009). Although, water effects on soils, crops and water management are of more concern to people when the irrigant is wastewater, which may contain agents capable of inducing adverse effects on the soil media and the agricultural products (Kaushik et al., 2005; Nath et al., 2007; Kannan and Upreti, 2008). The disposal of wastewater is a major problem faced by industries, due to its generation of high volume of effluent and with limited space for land based treatment and disposal (Pandey et al., 2008; Chidankumar et al., 2009; Shenbagavalli et al., 2011). On the other hand, wastewater is also a resource that can be applied for productive uses, since wastewater contains nutrients that can be used for the cultivation of agricultural crops (Hati et al., 2007; Chandra et al., 2009; Rath et al., 2011). Irrigation with effluents is known to contribute significantly to the heavy metals content of soil as well as crop plants (Kumar and Chopra, 2012). Excessive accumulation of heavy metals in agricultural soils through wastewater irrigation may not only result in soil contamination, but also affect food quality and safety (Hati et al., 2007; Bharagava et al., 2008; Chopra et al., 2009). Vegetables take in heavy metals and accumulate them in their edible and inedible parts, in quantities high enough to cause clinical problems both to animals and human beings that consume them (Muchuweti et al., 2006; Bharagava et al., 2008). India has nearly 330 distilleries, producing about 3,500 million liter alcohol (Kaul et al., 1995; Hati et al., 2007). They generate a huge amount of wastewater (spent wash) with high
chemical oxygen demand (COD) and biochemical oxygen demand (BOD). The utilization of industrial waste as soil amendment and irrigation of agricultural crops has generated interest in recent time. In agriculture, irrigation water quality is believed to have effects on the soils and crops. (Ramana et al., 2002; Kaushik et al., 2005; Muchuweti et al., 2006; Hati et al., 2007; Bharagava et al., 2008; Kannan and Upreti, 2008; Kumar, 2010; Mohamed and Ebead, 2013).