A COMPARATIVE ANALYSIS OF WATER COLLECTED FROM KOEL RIVER & WATER RELEASED FROM ROURKELA STEEL PLANT (RSP)

PROJECT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT OF

MASTER OF SCIENCE IN LIFE SCIENCE

By

SONITA PRADHAN

Roll No – 410LS2067

Under the guidance of

Dr. BISMITA NAYAK

DEPARTMENT OF LIFE SCIENCE

NATIONAL INSTITUTE OF TECHNOLOGY

ROURKELA -769008, ODISHA

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DECLARATION

I Sonita Pradhan, M.Sc. Life Science, Department of Life Science, N.I.T., Rourkela hereby declare that my research work incorporated in the dissertation titled **"a comparative analysis of water collected from Koel River & water released from Rourkela Steel Plant (RSP)"** is an authentic research work carried at Department of Life science, National Institute Technology, Rourkela under the direct guidance and supervision of Dr. Bismita Nayak, Asst. Professor, Department of Life science, NIT, Rourkela. The project work is original and no part of this work has been submitted for any other degree or diploma. All the given information is true to best of my knowledge.

Sonita Pradhan

Date:

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Assistant Professor

Ref. No.

Date: .....

CERTIFICATE

This is to certify that the thesis entitled "**A COMPARATIVE ANALYSIS OF WATER COLLECTED FROM KOEL RIVER & WATER RELEASED FROM ROURKELA STEEL PLANT (RSP).**" Submitted to National Institute of Technology, Rourkela for the partial fulfillment of the Master degree in Life Science is a faithful record of bonafide and original research work carried out by Sonita Pradhan under my supervisions and guidance.

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Advisor

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Last, but not the least, I would like to thank the Almighty GOD and my parents, whose dedicated and untiring efforts towards me has brought me at this stage of my life.

Sonita Pradhan

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DEDICATED TO
MY MOM &
DAD

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ABSTRACT

The expected increase in the use of coal as an energy source for the production of steel has resulted in several investigations into environmental cycling of coal unleashes pollutants. Among these is the release of various liquid effluents that are associated with coal throughout the carbonization, cleaning and combustion processes. The industries, like coal, by-product coke-plants, coal washeries and thermal power plants unleash their liquid effluents that are required urgent attention for the treatment, before they are discharged into the contemporary water streams. There's also the release of ash pond decant into the local water bodies from the coal-based industries. Such unleash pond decant tends to deposit ash all along its path thereby inflicting fugitive dust nuisance when it dries up. Additionally when such water mixes with a water body, it will increase the turbidity of the water body thereby decreasing the primary productivity. This is often harmful to the fisheries and other aquatic biota within the water body. The objective of this project work is to investigate the environmental impacts of waste water discharged from coal based industries and need to recognize the consequences. The Rourkela Steel Plant (RSP) releases the polluted water to Koel River which is the main river in Rourkela & most of the people are dependent on it for different purpose like drinking, washing, bathing etc. The present study indicated that most bacteria which were isolated from the Koel River are *Bacillus subtilis, Bacillus megaterium* & *Bacillus pumilis.*

INTRODUCTION

Water is a unique substance, because it can naturally renew and cleanse itself, by allowing pollutant to settle out or break down, or by diluting the pollutants to some extent where they' re not in harmful concentrations. However, this natural method takes time, and is tough when excessive quantities of harmful contaminants are added to the water. And humans are using more and a lot of materials that are polluting the water sources that we have a tendency to drink from. In nine of the last ten years, massive blue-green algae blooms have appeared on the northern part of Lake Winnipeg. These are caused by excess phosphorus within the water. Fertilizer use is 15 times higher these days than it was in 1945. Beach closures have become increasingly common. The list of pollutants is long and therefore the signs of water pollution surround us, however the point is this: we are dumping contaminants into the small portion of water on the planet that is fit for drinking.

Water is the most important component among the natural resources, and is crucial for the existing of all living organisms. The atmosphere, economic growth and development of Bangladesh are all highly influenced by water - its regional and seasonal availability, and therefore the quality of surface and groundwater. Spatial and seasonal availability of surface and groundwater is extremely conscious of the monsoon climate and geography of the country. Availability conjointly depends on upstream withdrawal for consumptive and non-consumptive uses. Quality includes, the surface water of the country is unprotected from untreated industrial effluents and municipal wastewater, pollution from chemical fertilizers and pesticides, and oil and lube spillage within the coastal area from the operation of ocean and river ports. Water quality also depends on effluent verities and discharge amount from completely different variety of industries; types of agrochemicals utilized include agriculture and seasonal water flow and dilution capability by the river system.

Pollution is often defined in different ways. Water pollution happens when energy and different materials are released, degrading the standard of the water for different users. Water

pollution includes all of the waste materials that can't be naturally counteracted by water. In other words, anything that's added to the water, above and beyond its capability to interrupt it down, is pollution. Pollution, in certain circumstances, is often caused by nature itself, like when water flows through soils with high acidities. However more typically than not, human actions are answerable for the pollutants that enter the water.

The water quality in this river needs to be permanently monitored, since the trace metals in acid mine drainage from active and abandoned mines probably still enter the Rimac River system in predominantly dissolved form. The objective of the project consisted in sampling and chemical analysis of river water and sediments, taken from ten sampling sites in September 2004. The analysis comprised mainly major ions, pH and heavy metals. The results showed high concentrations of Cu, Pb, Zn, As, Cd, Cr, Ni, and Hg.

In water samples high concentrations of cadmium were found at all sampling locations starting from 0.0002 mg/l at the Blanco River to 0.011 mg/l when Parac Creek at the mainstream, highlighting its threat since the water is equipped for domestic use. Alternative trace metals as Cu, Pb, Zn and As showed at the majority of sampling sites high levels of concentration and consequently, consistent with the Swedish environmental guidelines for water quality, these metals create a risk of inflicting adverse biological effects.

Samples analyzed for heavy metals in sediments demonstrated that the levels of metal concentrations in the basin exceed largely the permissible limits established by the Swedish Environmental Protection Agency. These values indicate that under the prevailing conditions and environmental regulations in Peru the basin would face major and hazardous discharges to its watercourses.

The water bodies include the Wettingen Reservoir (located on the Limmat River downstream from Zürich), the Klingnau Reservoir (on the lower Aare River), the Wohlen Reservoir (downstream from Berne), the Verbois Reservoir (downstream from Geneva) and Vidy Bay (Lake Geneva, city of Lausanne). For all sediment cores and contaminants, is observed from high contaminant values within the lower part of the cores, decreasing to lower concentrations within the higher part of the cores. However, for every site and each component, specific options are recognized. Applying the factors of the Swiss ordinance on soil protection, all sediment cores should be classified as contaminated by one or additional contaminants and at variable levels. From these data, it's concluded that: reservoirs and lakes located downstream

from major urban centres in Switzerland have accumulated important volumes of contaminated sediments within the past, representing the most important, however not the foremost intensely, contaminated sites on a national scale; the main environmental risk is remobilization of the contaminants and their come back to the food chain, significantly by infiltration into the groundwater; and though the processes of remobilization are identified, the conditions of incidence and therefore the amplitude of the processes are still poorly known. Completely different options of reservoir and lake sediment management are also mentioned and further analysis topics outlined.

All these microorganisms were found in smaller quantities within water, and in larger quantities within the bottom sediments in the Czarna Hancza River. Their number was typically beyond the amount of faecal bacteria of *Escherichia coli* cluster both in water and bottom sediments during this river at site 1 (in Stary Brod higher than Suwatki) and at sites 7-10 (in Czerwony Folwark, Mackowa Ruda, Buda Ruska, Wysoki Most, to the east of Wigry Lake). Their number was close to or less than the quantity of faecal bacteria of *Escherichia coli* cluster at the sites situated higher than and below the inflow of treated sewage from the Treatment Sewage Plant in Suwalki (2a and 2b sites), in Sobolewo (3 and 4 sites), within the region of the old river-bed of the Czarna Hancza River (5 site) and its mouth to Wigry Lake (6 site).

There were fewer at the sites of sand deposits, additional at the sites of clay-argillaceous deposits. Within the analysis period they were numerous within the second half of summer. The amount of *Pseudomonas aeruginosa*, *Aeromonas hydrophila* and *Staphylococcus* spp. ought to be taken under consideration additionally because the number of the indicators bacteria of a sanitary state (total coliforms, faecal coliforms and faecal streptococci) whereas estimating the usefulness of water within the Czarna Hancza River for recreation (Niewolak et al., 1999).

Total aerobic bacteria number in the lake was determined as $20x10^3$ cfu/ ml and fecal coliforms were determined >1100 MPN/100ml. The water samples which are from four geographically dispersed stations were screened for the *E. coli* and assessed for its resistance to twelve different antibiotics that are commonly encountered in the lake. Of the total 13 *E. coli* isolates, 0% was susceptible to all antibiotics. All of isolates were found proof against Penicillin (P) (100%). Among the twelve antibiotics tested, four patterns of antibiotic resistance were obtained and all of them were multiple antibiotic resistances with the number of antibiotics starting from 2 to 5. Three isolates had beta-lactamase detected by iodometric slide test. The results indicated that

persistent use of antibiotics against human diseases and other life forms may pollute the lake water and their impact on developing antibiotic resistant *E. coli* could also be a serious threat in both health and atmosphere (Toroglu and Toroglu, 2009).

The treatment of bacterial infections is increasingly complicated by the ability of bacteria to develop resistance to antimicrobial agents. Antimicrobial agents are usually categorized according to their principal mechanism of action. Mechanisms involve interference with cell wall synthesis (e.g., -lactams and glycopeptide agents), inhibition of protein synthesis (macrolides and tetracyclines), interference with nucleic acid synthesis (fluoroquinolones and rifampin), inhibition of a metabolic pathway (trimethoprim-sulfamethoxazole), and disruption of bacterial membrane structure (polymyxins and daptomycin). Bacteria could also be intrinsically proof against 1 class of antimicrobial agents, or could acquire resistance by de novo mutation or via the acquisition of resistance genes from alternative organisms. Acquired resistance genes could enable a bacterium to provide enzymes that destroy the antibacterial drug, to specific efflux systems that stop the drug from reaching its intracellular target, to change the drug's target site, or to produce an alternate metabolic pathway that bypasses the action of the drug. Acquisition of latest genetic material by antimicrobial-susceptible bacteria from resistant strains of bacteria could occur through conjugation, transformation, or transduction, with transposons usually facilitating the incorporation of the multiple resistance genes into the host's genome or plasmids. The using of antibacterial agents creates selective pressure for the emergence of resistant strains. From 3 case histories— one involves *Escherichia coli* resistance to third-generation cephalosporins, another specializing in the emergence of vancomycin-resistant *Staphylococcus aureus*, and finally third detailing multidrug resistance in *Pseudomonas aeruginosa*—are reviewed for example the various ways during which resistant bacteria develop.

The estimation of bacteriological quality of surface and underground waters based on classical sanitary indicators (total coliforms, faecal coliforms, faecal streptococci) may not reflect their safety for the health of bathing people and/or using water for drinking and household purposes. Numerous human diseases having bath in rivers, lakes, ponds and coastal sea waters in the area of river and sewage inflow, swimming pools are associated with the presence of opportunistic pathogens from *Pseudomonas, Aeromonas, Staphylococcus* and other microorganisms groups, being able to generate infections by contact with skin, mucous membrane, nosopharyngeal cavity, respiratory ducts, eyes, ears and urogenital passages. Wound

infections, peritonitis, meningitis, endocarditis, septicemia, corneal ulcers, nosocomial infections, urinary tract infections, gastroenteritis of people who bathe and/or use water in other ways are caused by *Aeromonas hydrophila*. Infections of skin, nosopharyngeal cavity, eyes, outer ear among bathing people could be caused by recreational waters polluted by *Staphylococcus aureus*. All the above mentioned species of bacteria survive in water longer than classical indicators of sanitary state and they are not connected with faecal contamination present in water. *Pseudomonas aeruginosa* and *Aeromonas hydrophila* occur in water and bottom sediments of river lake estuaries sea coastal waters in the zone of pollutants effluent from the land sewages soil fish food drinking water. Up till now the maximum number of these bacteria in surface water useful for recreation has not been officially stated, though the above mentioned literature suggests the necessity of including them in the system of bacteriological indicators of water quality. In Poland one cannot find published data on the number of these bacteria in surface waters. The present research shows the results on the number of *Pseudomonas aeruginosa, Aeromonas hydrophila* and *Staphylococcus* sp. in water and bottom sediments of the Czarna Hancza River in the recreational period. The river is utilized, especially in summer, for canoeing by Polish and foreign tourists for its beautiful landscape. Therefore, knowledge of sanitary-bacteriological states of water and bottom sediments of this river and potential sources of bacteriological contamination seem to be significant (Niewolak and Opieka, 1999).

Antibiotics, on the other hand are antimicrobial agents created by bacteria, fungi or of artificial in nature. Antibiotic resistance refers to the ability of microorganisms to withstand the bacteriostatic and bactericidal effects of antibiotics. It provides a survival benefit to the invading microorganisms and under such circumstances it is difficult to eliminate the infection caused by these microorganisms. The mechanisms by which microorganisms exhibit resistance to antibiotics include drug inactivation or modification, alteration of target site, alteration in the metabolic pathway, and reduced drug accumulation (Katzung, 2004). The development of antibiotic resistance in bacteria is of public concern in view of the fact that a patient could develop antibiotic resistance by contacting a resistant microorganism or the emergence of a microorganism in the patient's body when treatment with antibiotic begins (Nagulapally, 2007).

The treatment of bacterial infections is increasingly complicated by the ability of bacteria to develop resistance to antimicrobial agents. Antimicrobial agents are usually categorized according to their principal mechanism of action. Mechanisms which include interference with

cell wall synthesis (e.g. lactams and glycol-peptide agents), inhibition of protein synthesis (macrolides and tetracycline), interference with nucleic acid synthesis (fluoroquinolones and rifampin), inhibition of a metabolic pathway (trimethoprim- sulfamethoxazole), and disruption of bacterial membrane structure (polymyxins and daptomycin). Bacteria could also be intrinsically resistant to _1 class of antimicrobial agents, or may acquire resistance by de novo mutation or via the acquisition of resistance genes from alternative organisms. Acquired resistance genes may enable a bacterium to produce enzymes that destroy the antibacterial drug, to specific efflux systems that prevent the drug from reaching its intracellular target, to change the drug's target site, or to provide an alternate metabolic pathway that by passes the action of the drug. Acquisition of latest genetic material by antimicrobial-susceptible bacteria from resistant strains of bacteria may occur through conjugation, transformation, or transduction, with transposons usually facilitating the incorporation of the multiple resistance genes into the host's genome or plasmids. Antibacterial agents use creates selective pressure for the emergence of resistant strains. The 3 case histories— one involving *Escherichia coli* resistance to third-generation cephalosporins, another specializing in the emergence of vancomycin-resistant *Staphylococcus aureus*, and the last detailing multidrug resistance in *Pseudomonas aeruginosa*—are reviewed to illustrate the varied ways in which resistant bacteria develop (Tenover, 2006).

Contamination of sediments and natural aquatic recaptors with heavy metals is a major environmental problem all over the world. These inorganic micropolluants are released by effluents generated from various industries such as electroplating and metal finishing industries, metallurgy, tannery, and battery manufacturing.

Heavy-metals are widely used in chemistry and engineering. There is increasing interest in the biotechnology of extraction of rare metals from primary and secondary raw materials (Korenevskii et al., 1997). On the other hand, heavy metals are very toxic elements, which comprise the essential part of anthropogenic pollutants (Avtsyn et al., 1991). The presence of toxic heavy metal contaminants in aqueous streams, which arises from the discharge of untreated metal containing effluents into water bodies that is one of the most important environmental issues (Rehman et al., 2008). The discharge of heavy metals into the environment as a result of agricultural, industrial and military operations, and therefore the effects of this pollution on ecosystems and human health have been of concern for some years (Essa et al., 2002). These heavy metals include Cd, Cr, Co, Mn, Hg, Ni, Ag, and Zn. Metal-polluted industrial effluents

discharged into sewage treatment plants could lead to high metal concentrations in the activated sludge. Microbial populations in metal-polluted environments contain microorganisms that have adapted to the toxic concentrations of heavy metals and become "metal resistant" (Leung and Chua, 2001).

The introduction of heavy metal compounds into the environment generally induces morphological and physiological changes in the microbial communities, hence exerting a selective pressure on the micro-biota. Generally, the contaminated sites are the sources of metal resistant micro-organisms. Therefore, it is important to explore autochthonous micro-organisms from such contaminated niches for the bioremediation of heavy metals since conventional processes such as chemical precipitation; ion exchange and reverse osmosis are uneconomical and inefficient for treating effluents of dilute metal concentrations. In naturally polluted environments, the microbe's response to heavy metals toxicity depends on the concentration and the availability of metals and on the action of factors such as the type of metal, the nature of medium and microbial species. Fungi and yeast biomasses are known to tolerate heavy metals. They are a versatile group, as they can adapt and grow under various extreme conditions of pH, temperature and nutrient availability, as well as high metal concentrations. They offer the advantage of having cell wall material which shows excellent metal-binding properties. Generally, microbial biomasses have evolved various measures to respond to heavy metals stress via processes such as transport across the cell membrane, bio-sorption to cell walls, entrapment in extracellular capsules, as well as precipitation and transformation of metals. Recent studies showed that strains isolated from contaminated sites have an excellent ability of removing significant quantities of metals both from aqueous solutions and electroplating effluents (Malik, 2004). El-Morsy (2004) studied 32 fungal species isolated from polluted water in Egypt for their resistance to metals and found that *Cunninghamela echinulata* biomass could be employed as a biosorbent of metal ions in wastewater (Ezzouhri et al., 2009).

Rourkela Steel Plant (RSP) is one of the flagship steel plants of the Steel Authority of India Limited (SAIL), located in the Indian state of Orissa. It currently has the capacity to produce 1.9 MT of steel p.a. – but plans to modernize and more than double its capacity to 4.2 MT. The plans involve modifying the existing units as well as building new ones. To that purpose SAIL obtained new loans worth more than Rs. 75 billion (more than €1 billion) in the period 2008-09. Various services, including the installation of mechanical components for a new

blast furnace, project management and training are being supplied by a Dutch company, Danieli Corus BV. Atradius DSB has provided export credit insurance to Danieli Corus BV to cover just over €62.5 million through a guarantee with the State Bank of India.

RSP is an integrated plant primarily meant for producing steel through the LD (Linz-Donawitz) process or BOF (Basic Oxygen Furnace) route and has most of the raw material processing onsite. The offsite raw material sources include captive mines, fuel supply agreements with Coal India and its subsidiaries. The proposed expansion of RSP to attain a production capacity of 4.2 million tons per annum would involve the erection of several new units as well as discarding the existing or outdated machinery to combat environmental pollution using modern environmental technological upgrades.

Fig 1: Location of Rourkela Steel Plant, Rourkela.

Koel River originates from the Palamu Tiger Reserve and flows in the western portion of Palamu. The river splits into two-the North Koel River and the South Koel River. South Koel River runs across Jharkhand and Odisha states in India. The south Koel originates on the Ranchi plateau a few miles east of Ranchi. It enters Odisha and joins Sankh River at Vedveyas near Rourkela from where it is named Brahmani (Burh, 2011).

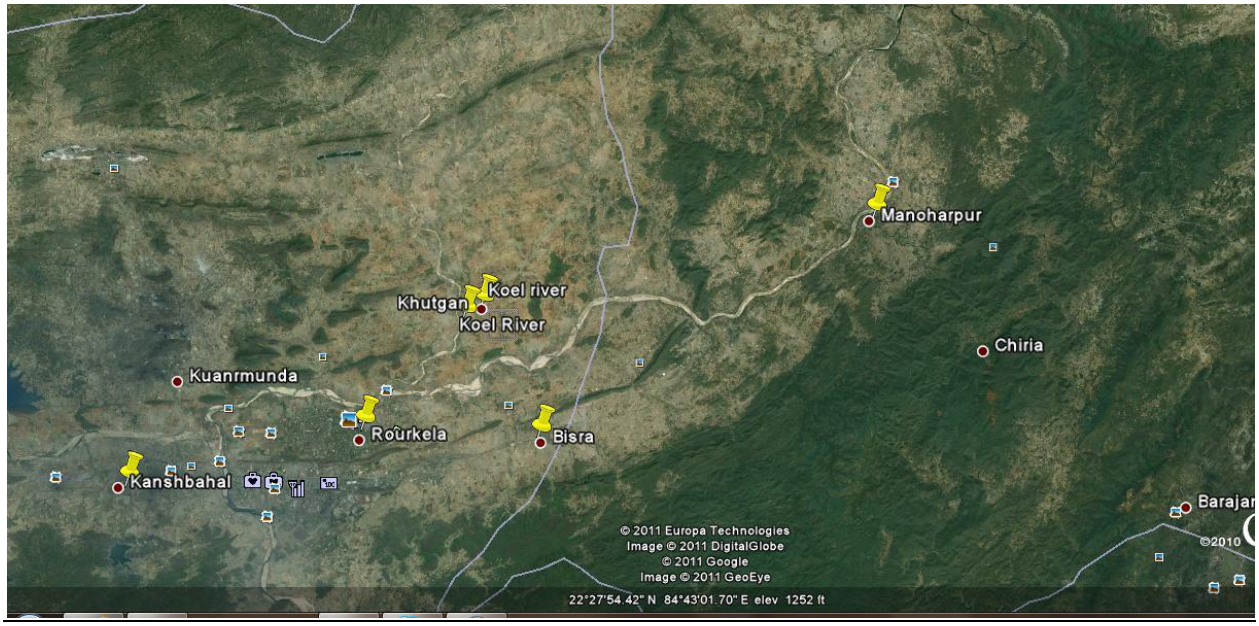


Fig 2: Site map of Koel River.

The water samples of Koel River are collected from the Leprosy colony of Tarkara Dam. The water sample collected from two different areas like polluted area & unpolluted area.

Fig 3: Sample collected from Tarkara Dam

The released polluted water of Rourkela steel plant (RSP) was collected from the main drainage system of RSP.

The comparative study is done between the two sample of released water & Koel water sample because the RSP released the polluted water which mixed with the water of Koel River. The water of Koel is used for the different uses like agriculture, drinking purpose etc. To see the pollution in the water of Koel River by RSP released water, the comparative study was done.

NEED OF THE STUDY

In RSP, iron & steel formed during combustion of coal is mixed with water and is discharged in slurry form in ash disposal ponds. If disposal ponds are not properly selected, constructed and managed and the coal ashes are not properly assessed for disposal, the risk of ground and surface water contamination due to polluting the ground water level by different metal ions. Due to this the ground water gets polluted and may become unsuitable for domestic use. There is also the release of ash pond decant into the local water bodies from the steel plant industries. Such release of ash pond decant tends to deposit ash all along its path thereby causing fugitive dust nuisance when it dries up. Also when such water mixes with a water body, it increases the turbidity of the water body thereby decreasing the primary productivity of the flora & fauna of respected water system. This is harmful to the fisheries and other aquatic biota in the water body.

REVIEW OF LITERATURE

The expected increase in the use of coal as an energy source has resulted in several investigations into environmental cycling of coal related pollutants. Among these is the release of various liquid effluents, which are associated with coal during the carbonization, cleaning and combustion processes. The coal based industries, such as by-product coke-plants, coal washeries and thermal power plants release their liquid effluents, which are needed urgent attention for the treatment, before they are discharged into the fresh water streams. There is also the release of ash pond decant into the local water bodies from the coal-based industries. Such release of ash pond decant tends to deposit ash all along its path thereby causing fugitive dust nuisance when it dries up. Also when such water mixes with a water body, it increases the turbidity of the water body thereby decreasing the primary productivity. This is harmful to the fisheries and other aquatic biota in the water body. The objective of this project work is to analyze the environmental impacts of waste water discharged from coal based industries and need to recognize that effects are both positive and negative (Patra, 2010).

The status of water quality and its propensity in the Jharia Coalfield- where about thirty major industries (mainly large sized and coal based) exit besides extensive coal mining activities. This study revealed that water is grossly polluted in the entire coal mining area. Major sources which result water quality deterioration in the region have been accounted. Inventories of water resources and its propensity have also been established (Singh, 1990).

Sediments of the river bed was characterized for heavy metals, Fe, Mn, Cd, Cr, Ni and Pb to determine total carryover of heavy metals in the river body. Heavy metal concentrations in the river sediments were higher than in the river water. The study also showed that Fe and Mn were irreversibly retained in the sediment and this effect was also observed for other metals in decreasing: Pb, Cr, Ni and Cd (Tiwary and Dhar, 1994).

The original and secondary effects on ground water quality by mining in the East Borsod Coal Basin, Hungary. They found that in all almost all Hungarian coal basins, intensive dewatering lowers the hydrostatic pressure of aquifers, reduce their water resources, unbalance water management of the area (Jambrik and Bartha, 1994).

The monitoring of water quality from six reservoirs around Mae Moh thermal power plant was conducted during January – December 2003. There was a statistical significantly differences for values of electrical conductivity, total dissolved solid, hardness, silica, arsenic and lead between natural water sources: Mae Kham and Mae Chang reservoirs and reservoirs in wastewater treatment system: Settleable solid and Oxidation pond, Bio-treatment pond, Diversion pond and South wetland pond, which receiving the effluent from the plant (Junshum et. al., 2004)).

Gendren et al (2009) found that ambient concentrations of metals in surface waters have become an important consideration when establishing water quality criteria and conducting risk assessments. Their study sought to estimate amounts of copper that may be released into fresh and estuarine waters considering ambient concentrations, toxicity thresholds, and bioavailability. Cumulative distribution functions of ambient copper concentrations were compared statistically for individual sites within 14 surface waters of North America and Europe to identify differences among mean distribution variables.

Kar et al (2008) studied the assessment of heavy water pollution in surface water. They collected a total of 96 surface water samples collected from river Ganga in West Bengal during 2004-05 was analyzed for pH, EC, Fe, Mn, Zn, Cu, Cd, Cr, Pb and Ni. They found that among the heavy metals themselves, a significant negative correlation was observed between Fe and Cr, whereas Ni exhibited a significant positive correlation with Mn and Zn.

Microbial contamination of ground water results in numerous disease outbreaks each year. Tracing their movement in ground water is therefore essential. Bacteria, viruses, yeasts & spores have been used for this purpose & to trace underground movement of water I n much the same manner as chemical tracers are used. Chemical tracers do not always reflect the movement of microorganisms in ground water. The use of certain bacteria and animal viruses is undesirable due to their pathogenic potential and difficulties in their differentiation from background, naturally occurring organisms. Bacterial viruses appear to be the microorganisms most suited as a microbial tracer because of their size, ease of assay and lack of pathogenicity. Bacteriophages have been used to trace ground water movement over distances of 1600 meters and can be used under a variety of conditions.

The term bioremediation has been introduced to describe the process of using biological agents to remove toxic waste from environment. Bioremediation is the most effective management tool to manage the polluted environment and recover contaminated soil. Bioremediation is an attractive and successful cleaning technique for polluted environment. Bioremediation has been used at a number of sites worldwide, including Europe, with varying degrees of success. Bioremediation, both in situ and ex situ have also enjoyed strong scientific growth, in part due to the increased use of natural attenuation, since most natural attenuation is due to biodegradation. Bioremediation and natural attenuation are also seen as a solution for emerging contaminant problems, e.g. endocrine disrupters, landfill stabilization, mixed waste biotreatment and biological carbon sequestration. Microbes are very helpful to remediate the contaminated environment. Number of microbes including aerobes, anaerobes and fungi are involved in bioremediation process (Kumar et al., 2011).

Intensification of agriculture and manufacturing industries has resulted in increased release of a wide range of xenobiotic compounds to the environment. Excess loading of hazardous waste has led to scarcity of clean water and disturbances of soil thus limiting crop production (Kamaludeen et al., 2003). Bioremediation uses biological agents, mainly microorganisms i.e. yeast, fungi or bacteria to clean up contaminated soil and water (Strong and Burgess, 2008). This technology relies on promoting the growth of specific microflora or microbial consortia that are indigenous to the contaminated sites that are able to perform desired activities (Agarwal, 1998). Establishment of such microbial consortia can be done in several ways e.g. by promoting growth through addition of nutrients, by adding terminal electron acceptor or by controlling moisture and temperature conditions (Hess et al., 1997; Agarwal, 1998; Smith et al., 1998). In bioremediation processes, microorganisms use the contaminants as nutrient or energy sources (Hess et al., 1997; Agarwal, 1998; Tang et al., 2007). The population explosion in the world has resulted in an increase in the area of polluted soil and water. As the number of people continues increasing day by day it also brings with it a growing pressure on our natural resources i.e. air, water and land resources. In order to outfit to the demands of the people, the rapid expansion of industries, food, health care, vehicles, etc. is necessary. But it is very difficult to maintain the quality of life with all these new developments, which are unfavorable to the environment in which we live, if proper management is not applied. In nature there are various fungi, bacteria and microorganisms that are constantly at work to break down

organic compounds but the question arises when pollution occurs, who will do this clean up job? Since the quality of life is inextricably linked to the overall quality of the environment, global attention has been focused on ways to sustain and preserve the environment. This endeavor is possible by involving biotechnology. The types of contaminants that Environmental Biotechnology investigators have expertise with include chlorinated solvents, petroleum hydrocarbons, polynuclear aromatic hydrocarbons, ketones, TNT, inorganic nitrogen (NO_3, NH_4), Tt, , Pu, Np, Cr, U and other heavy metals. Bioremediation is the term used to describe biological strategies applicable to repair of damaged environment using biological factors. In the case of oil spills, the process exploits the catabolic ability of microorganism feeding on oil. Several workers (Odu, 1978; Sloan, 1987; Ijah and Antai, 1988; Okpokwasili and Okorie, 1988; Barnhart and Meyers, 1989; Anon, 1990; Pritchard, 1991; Pritchard and Costa, 1991; Hoyle, 1992; Ijah, 2002 and Ijah, 2003) have described various application of microorganism in the bioremediation of oil pollution with encouraging results.

Bioremediation and natural attenuation area has both basic research and field application foci for the environmental biotechnology. The basic research foci are co metabolism, biotreatability, biotransformation kinetics, and modeling of biogeochemical processes. The field application foci are co-metabolic techniques, biogeochemical assessment techniques, and modeling of attenuation and environmental fate (Kumar et al., 2010). Bioremediation can be defined as any process that uses microorganisms or their enzymes to return the environment altered by contaminants to its original condition. Bioremediation may be employed in order to attack specific contaminants, such as chlorinated pesticides that are degraded by bacteria, or a more general approach may be taken, such as oil spills that are broken down using multiple techniques including the addition of fertilizer to facilitate the decomposition of crude oil by bacteria. Not all contaminants are readily treated through the use of bioremediation; heavy metals such as cadmium and lead are not readily absorbed or captured by organisms (Vidali, 2001**)**. The integration of metals such as mercury into the food chain may make things worse as organism bio-accumulate these metals. However, there are a number of advantages to bioremediation, which may be employed in areas which cannot be reached easily without excavation. The foundation of bioremediation has been the natural ability of microorganisms to degrade organic compounds. Bioremediation is not a panacea but rather a natural process alternative to such methods as incineration, catalytic destruction, the use of adsorbents, and the

physical removal and subsequent destruction of pollutants. The cost of moving and incinerating pollutants is at least ten times that of in situ biological treatment. By integrating proper utilization of natural or modified microbial capabilities with appropriate engineering designs to provide suitable growth environments, bioremediation can be successful in the field. However, a gap exists between advances in laboratory research and commercial field applications. Two major factors responsible for this gap are the lack of a sufficient knowledge base to accurately predict pollutant degradation rates and fates and sites designated as field research centers for bioremediation research and technology demonstrations. Laboratory and microcosm studies have documented the potential use of microorganisms for bioremediation. However, the physiologic potential of microbial populations to remediate environments of relevant size, heterogeneity and variability has not been adequately tested. Successful application of bioremediation techniques must address both the heterogeneous nature of many contaminated waste sites and the complexity of using living organisms. There has been progress in overcoming some of the barriers that have impeded bioremediation from being successfully applied in the field. Scientists have to put their efforts to search for organisms with better biodegradation kinetics for a variety of contaminants within broad environmental habitats. Studies examining extremophiles could result in using organisms in situ that have a high tolerance for organic solvents and alkaline soils or waters and that function at high temperatures for more efficient ex situ activity in bioreactors (Kumar et al., 2011).

Geochemical analyses of bottom sediment from rivers flowing through Orissa State, India indicated that trace element concentrations were extremely variable, and commonly higher than crustal abundance. The highest elemental concentrations were associated with the Brahmani River, followed by the Baitarani and Mahanadi Rivers. Although all three rivers drain similar geology, the Brahmani River catchment is heavily industrialized, and sediment collected downstream from industry confirms that anthropogenic activity influenced its chemical composition. A similar pattern was observed in sediments collected downstream from towns in the Mahanadi and Baitarani catchments. In both examples, the clay size fraction was shown to be the most highly reactive component of the sediments. Comparisons between metal concentrations from the upper to lower stretches of the three river systems indicated no net accumulation downstream. Apparently, trace elements discharged into the river system tend to be short-lived in the water column, rapidly settling out or becoming adsorbed into the bottom

sediment. Although for much of the year, the trace metals may remain locally incorporated as bottom sediment, during monsoonal episodes, where bed load transport can be significant, the effects of pollution may expand over regional distances (Konhauser *et al.*, 1997).

Nine rivers flowing in the Novosibirsk city were studied. Samples of water and bottom sediments were collected from upper- and downstream in each of them. Standard methods of geochemical research were used. Atomic emission spectroscopy (ICP-AES) was applied to study the liquid components (river water and pore solutions from bottom sediments). X-ray fluorescence analysis with synchrotron radiation (SR-XRF) has been used to analyze solid components (bottom sediments and suspension particles from water). Obtained results show high level of water and bottom sediments contamination in Novosibirsk Rivers. Anomalies in Fe, Mn, As, Ni, Ti, Pb concentrations are identified. Elevated concentrations of Fe and Mn in all rivers are caused by natural conditions. But wide spectrum of elements gets into the rivers from different industrial sources: metallurgical plants and coal heat plants (CHPs). Comparative mobility of the elements in the water-bottom sediment system is conditioned by their geochemical features. Orders of mobility are for major elements Ca>Sr>Mn>Fe>K>Ti and for trace elements As>Cd>Br>Ga>Zn>Pb>Cu>Th>Y>Cr>Ni>Rb>Zr. Investigation of the mineral composition of the heavy fraction in the sediment allows to identify mineral mode of occurrence of heavy metals in bottom sediments. Natural minerals from host rocks are magnetite, titaniferous magnetite, olivine, ilmenite. Minerals related to urban activites are chalcopyrite ($CuFeS_2$), bornite (Cu_5FeS_4), barite ($BaSO_4$), monacite ($CePO_4$). Obtained results allow determining the most polluted areas, which demand treatment and remediation (Bortnikova et al., 2012). The impact of industrial effluent on water quality of Omoku Creek, Rivers State of Nigeria was investigated. Physico-chemical and organic parameters of water samples of the Creek were examined to determine the quality and extent of pollution. Results indicated that TSS (Total Suspended Solid) ranged between 19.8 and 24.9mg/l; while THC (Total Hydrocarbon Concentration) was between 24.2 and 40.4. DO (Dissolved Oxygen) and BOD (Biochemical Oxygen Demand) varied between 38.2 and 41.5mg/l, and 38 and 59 mg/l respectively. The major sources of pollution were observed to be effluents from the industries and dump sites within and around the Creek catchment. These effluents were observed to impact seriously on the Creek ecosystem. In order to maintain the ecological status of the Creek, waste management practice of effluent treatment through the use of retention pond was suggested (Ewa et al., 2011).

OBJECTIVES

Koel river is an integral part of the inhabitants of the city Rourkela for their daily practices and for maintenance of their live hood. They use Koel water for many purposes like cooking, bathing and even for drinking purposes. Hence a study of the biological and biochemical parameters of the water may lead to the insight of the level of pollution in the water bodies which may affect its users. Keeping the above features in mind the present work was aimed to-

- Collection of water samples from Koel river and discharge point of Rourkela Steel Plant (RSP)
- Enumeration of the bacterial load in the water samples
- Biochemical characterization of the isolates to identify them
- To compare the microbial floral diversity in two study sites

MATERIALS & METHODS

The water samples of Koel River were collected from the Leprosy colony of Tarkara Dam. The water sample collected from two different areas like polluted area & unpolluted area. The released polluted water of Rourkela steel plant (RSP) was collected from the main drainage system of RSP.

Serial dilution

After the collection of water samples these were taken & 1 ml of this sample was added to 9 ml of sterile water to make 1:10 dilution, adding 1 ml of the 1:10 dilution of 9 ml of sterile water makes a 1:100 dilution & so on.

The number of bacteria per milliliter of fluid is reduced by 9/10 in each dilution. Subsequent dilutions are made in ratios of 1:1000, 1:10000, 1:100000, 1:1000000 or even 1:10000000 if the original culture contained an extremely large number of organism.

Spread plate method

100µl of 1:1000, 1:10000 & 1:100000 dilutions were poured into 15 ml of melted agar, which is warm enough to stay liquid but not hot enough to kill the microorganisms being mixed into it and then spread with autoclaved "L" shaped rod.

The colonies that develop are counted. A single measurement is not very reliable, so the procedure is repeated at least three times & the results are averaged. The averaged number of colonies is multiplied by the dilution factor to ascertain the total no of organism per milliliter of the original culture.

After 24 hrs of incubation, the growth of bacterial stains comes out. The single colony from the agar plates will inoculate into the nutrient broth for further utilization of pure culture by making them glycerol stocks. Then the pure cultures were done by storing them into glycerol stock. This will do by taking 250µl of glycerol & 750 µl of bacterial strains.

MORPHOLOGICAL TEST-

Gram staining-

The morphological test includes the Gram staining. In Gram staining the single colony from the agar plate was taken & placed on the slide & then heat fixed it. After that the slide was treated with crystal violet for 2 mins, Grams iodine for 1½ mins, alcohol for 2-3 secs & finally the safranin for ½ mins.

BIOCHEMICAL TESTS-

The biochemical test includes different sets of tests like as follows:

Mannitol motility test-

This experiment is generally performed to know whether the bacteria are motile or not & whether it is capable of fermenting mannitol sugar or not. Mannitol motility agar is very soft agar with smooth surface which allow the organism to move through it & shows its motility. Whenever organism ferments mannitol agar, the pH of media becomes acidic due to production of acids. The fermentation of the media from red to yellow shows positive test result.

Citrate utilisation test-

Tests for the ability of bacteria convert citrate (an intermediate of the Kreb's cycle) into oxaloacetate (another intermediate of the Kreb's cycle). In this media, citrate is the only carbon source available to the bacteria. If it cannot use citrate then it will not grow. If it can use citrate, then the bacteria will grow and the media will turn a bright blue as a result of an increase in the pH of the media.

Nitrate reduction test-

This test was performed to test whether microorganisms can able to convert nitrate to nitrite or not. 1-2 drops of sulphanilic acid and 1-2 drop of N, N-Dimethyl-1-Napthylanine Reagent. Immediate development of pinkish red colour on addition of reagent indicates positive reaction. No change in colour indicates a negative reaction.

Malonate utilisation-

Malonate test medium contains Bromothymol blue as indicator. Sodium malonate is the carbon source and ammonium sulphate is the nitrogen source .Organisms, which are able to utilise malonate, release sodium dioxide. The resulting alkaline conditions cause the indicator to change from light green to blue. Color of the medium changes from light green to blue if the test is positive. Medium remains light green in color if the test is negative.

Gas production from glucose-

Gas production from glucose was assessed by inoculating the isolated strains in MRS broth containing Durham tube in inverted condition and incubated at 37^0c for 48-72 hrs.

Carbohydrate utilization-

For carbohydrate utilisation pattern HiCarbo^{TM} Kit, (Part A, Part B and Part C) KB009 (Himedia) has been used. Bacteria produce acidic products when they ferment certain carbohydrates. The carbohydrate utilization tests are designed to detect the change in pH which would occur if fermentation of the given carbohydrate occurred. Acids lower the pH of the medium which will cause the pH indicator (phenol red) to turn yellow. If the bacteria do not ferment the carbohydrate then the media remains red.

RESULTS

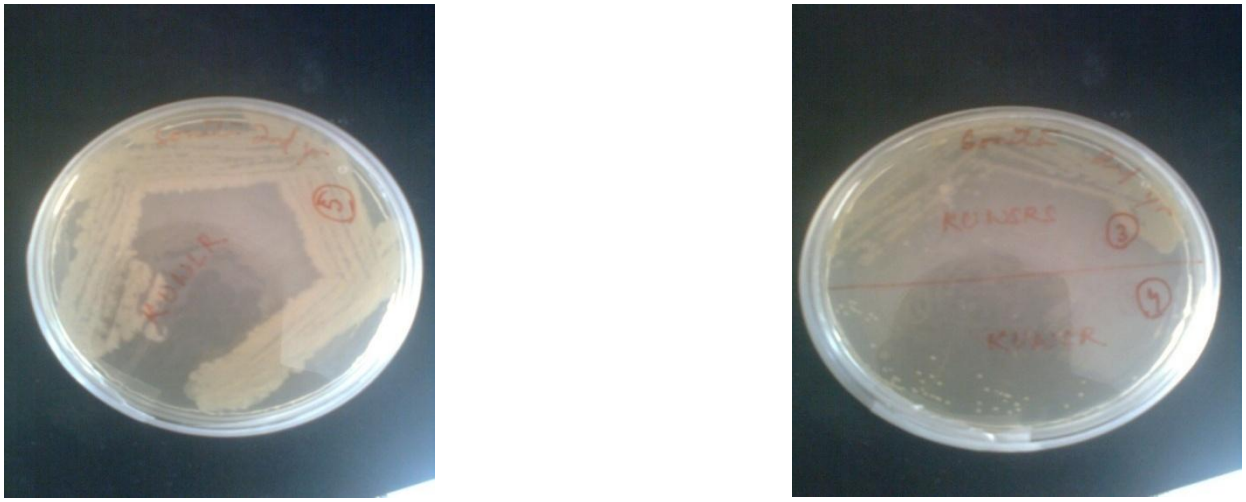
The bacterial strains are identified from the polluted as well as unpolluted area of Koel River.

Table 1. Colony Morphology of the isolates

Serial No.	Source	Dilution	Colony morphology	Code
1	Koel River polluted area	10^{-2}	Yellow, round	**KPYR**
2	Koel River polluted area	10^{-2}	White, irregular	**PKWI**
3	Koel River polluted area	10^{-2}	White, round	**KPWR**
4	Koel River unpolluted area	10^{-3}	Large, round, white	**KUWLR**
5	Koel River unpolluted area	10^{-3}	Small, round, white	**KUWSR**
6	Koel River unpolluted area	10^{-2}	Large, irregular, white	**KUWLI**
7	Koel River unpolluted area	10^{-2}	Small, round, white	**KUWSR1**
8	Koel River unpolluted area	10^{-2}	Small, round, white, shiny	**KUWSRS**

Code	Bacterial stains	Morphology
KPYR	1	Gram +ve, coccus
KPWI	2	Gram +ve, bacillus
KPWR	3	Gram +ve, bacillus
KUWLR	4	Gram –ve, coccus
KUWSR	5	Gram +ve, bacillus
KUWLI	6	Gram +ve, bacillus
KUWSR1	7	Gram +ve, bacillus
KUWSRS	8	Gram +ve, bacillus

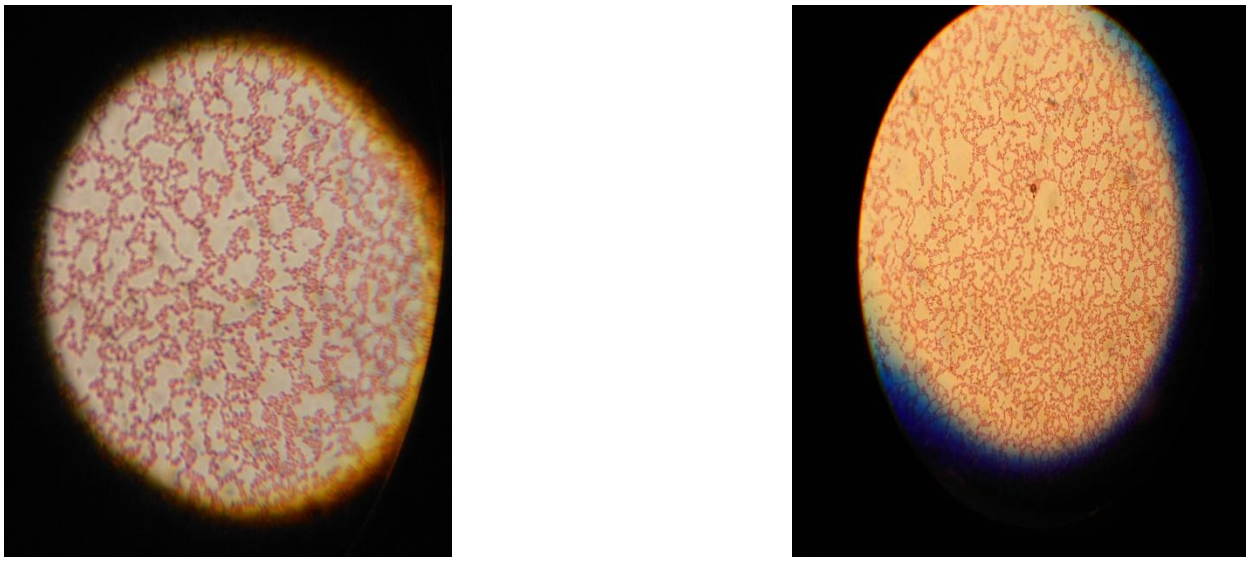
Table: 2 Morphology of identified bacteria



(A) (B)

Fig 4: strains are streaked in nutrient agar medium.

The table contains the bacterial strains which are gram +ve & Gram –ve, bacterial strains like 1,2,3,5,6,7,8 are Gram +ve & strain 4 is Gram –ve. Bacterial strains like 2, 3, 5, 6, 7, 8 are Bacillus & strains 1 & 4 are Coccus.



(A) (B)

Fig 5: (A) Gram +ve strain (B) Gram –ve strain

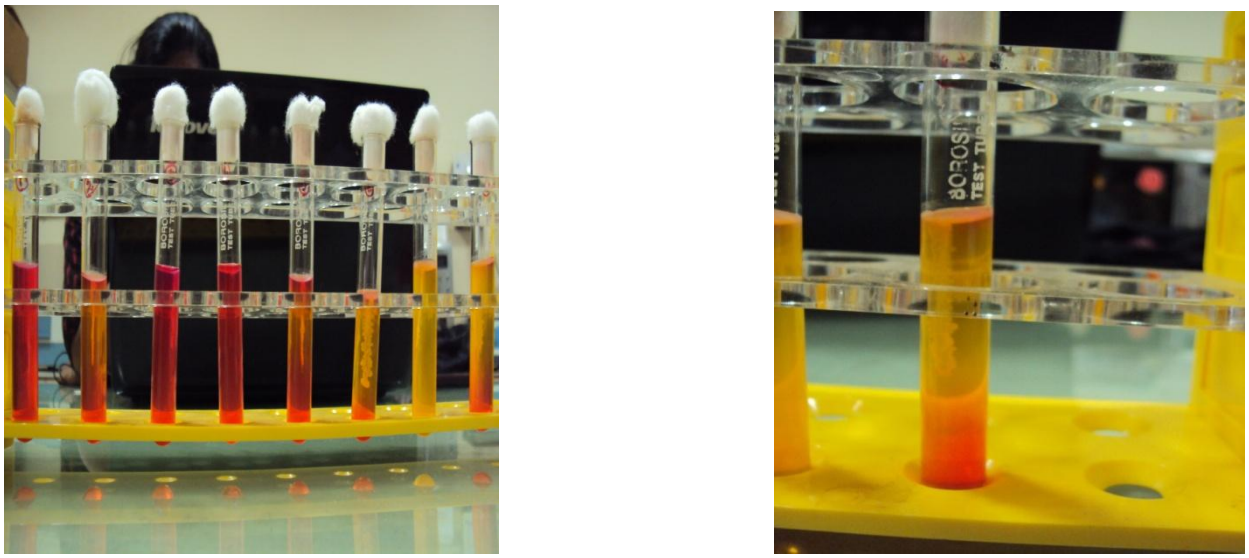
Biochemical test:

Table: 2 Different biochemical tests.

Strain no	Mannitol test	Motility test	Citrate test	Nitrate reduction test	Malonate test	Gas production from glucose
1	-ve	-ve	+ve	-ve	-ve	-ve
2	+ve	+ve	-ve	+ve	+ve	-ve
3	-ve	-ve	+ve	-ve	+ve	+ve
4	-ve	-ve	-ve	+ve	+ve	-ve
5	+ve	-ve	-ve	-ve	-ve	+ve
6	+ve	+ve	-ve	+ve	-ve	+ve
7	+ve	-ve	-ve	-ve	-ve	+ve
8	+ve	+ve	-ve	-ve	-ve	-ve

Biochemical tests shows, mannitol test includes strains 1, 3, 4 are –ve & 2, 5, 6, 7, 8 are +ve test result. Motility test shows strain 1, 3, 4, 5, 7 are –ve & 2,6,8 are +ve. Citrate test shows strains 1 & 3 are +ve & rest are –ve. Nitrate reduction test shows strains 1,3,5,7,8 are –ve & rest are +ve in nature. Malonate test shows strains 1, 5, 6, 7, 8 are –ve & rest all are +ve. The test named gas production from glucose shows 1,2,4,8 are –ve & rest are –ve.

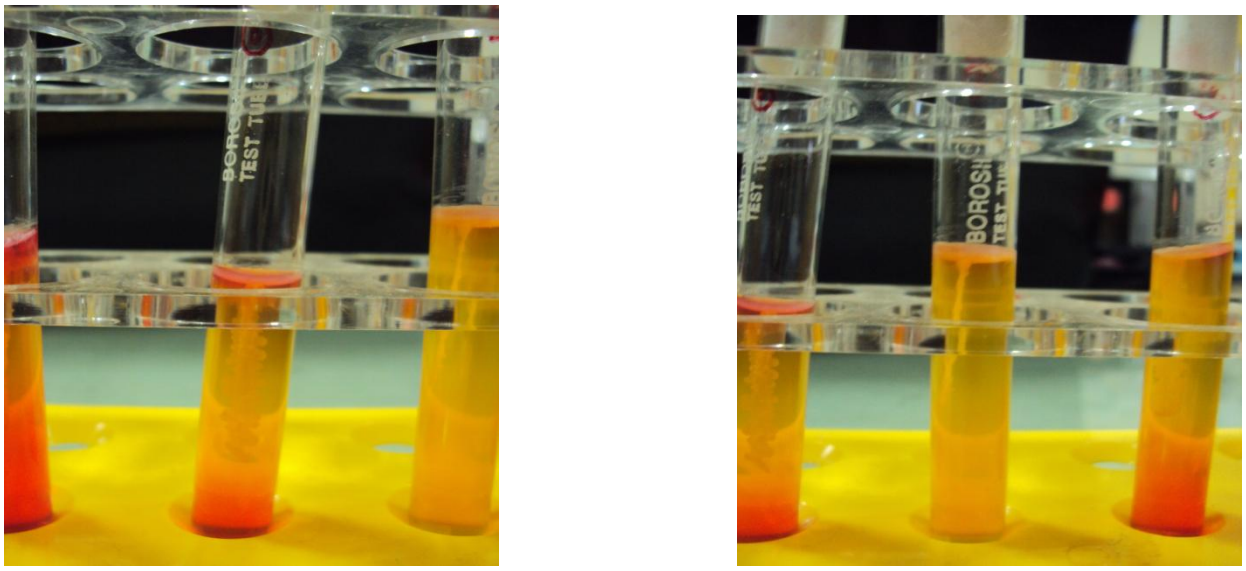
Mannitol test



(A) (B)

Fig 6: Mannitol +ve result (B)

Motility test



(A) (B)

Fig 7: Motility test (A) Motile bacteria (B) Non-motile bacteria

Citrate utilization test

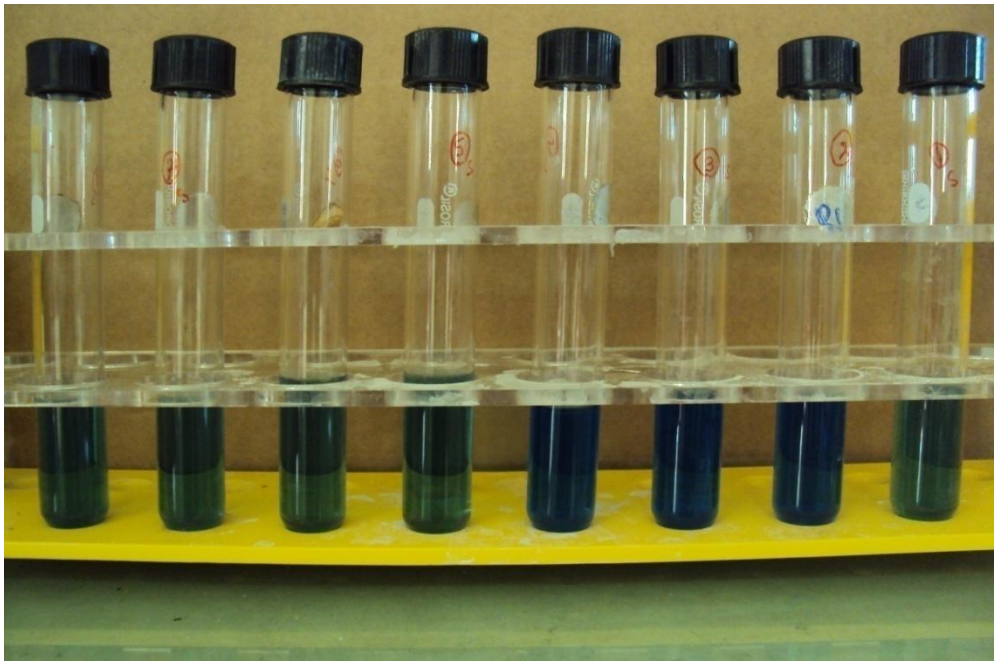
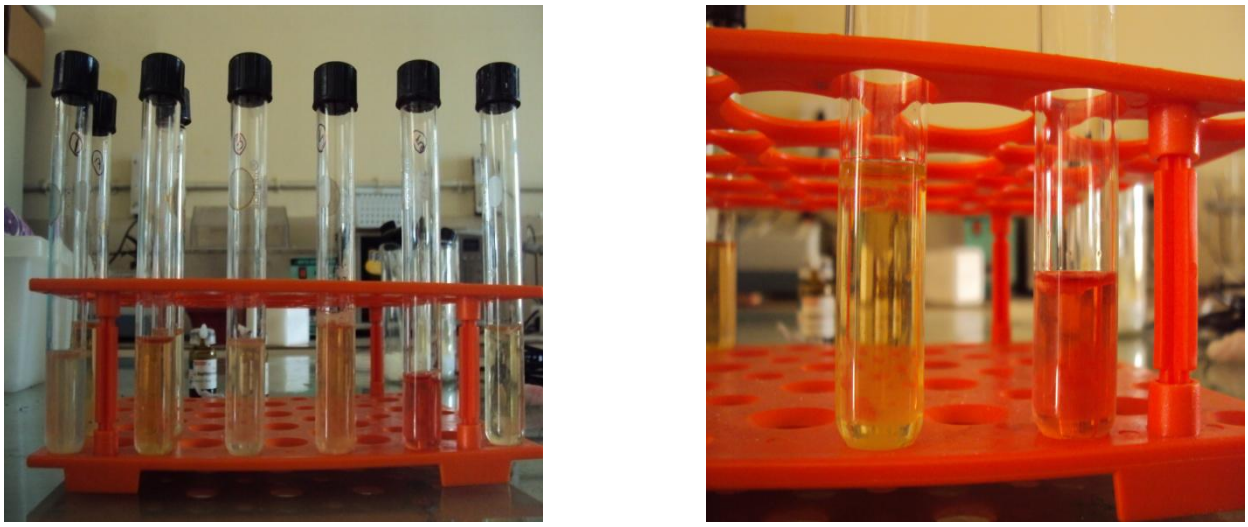


Fig 8: Citrate utilization test

Nitrate reduction test



(A) (B)

Fig 9: Nitrate reduction test

Malonate utilization test

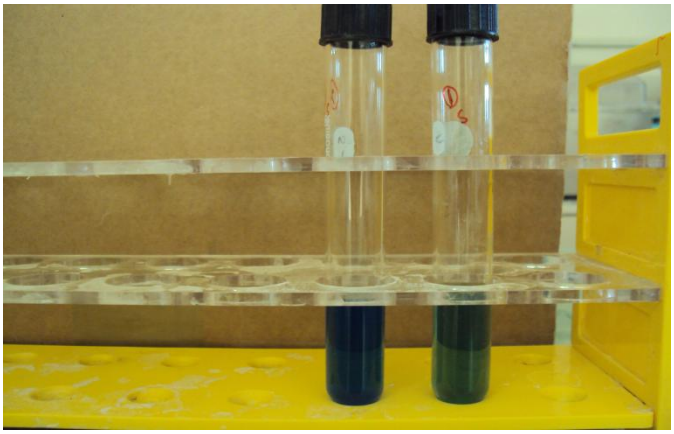


Fig 10: Malonate utilization test

Gas production from glucose

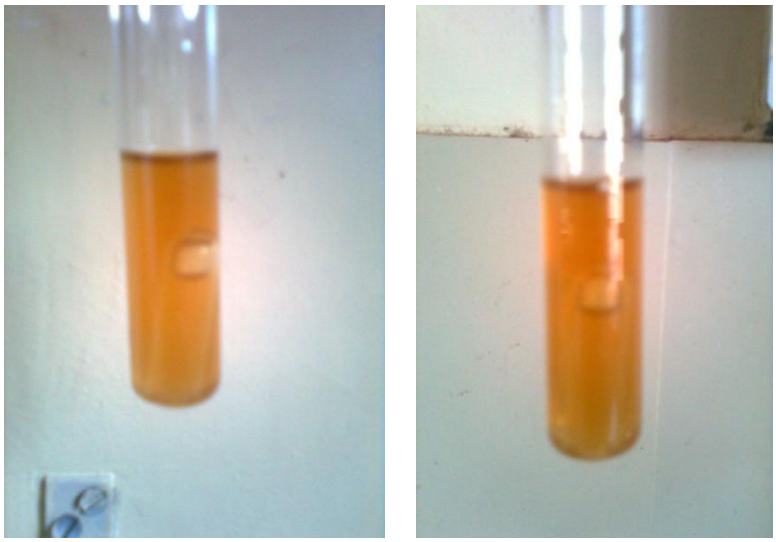


Fig 11: Gas production from glucose

Carbohydrate test:

Carbohydrates	Isolate - 2	Isolate - 5	Isolate - 7
Lactose	-ve	-ve	-ve
Xylose	+ve	-ve	-ve
Maltose	+ve	-ve	-ve
Fructose	+ve	-ve	-ve
Dextrose	+ve	-ve	-ve
Galactose	+ve	-ve	-ve
Raffinose	+ve	-ve	-ve
Trehalose	+ve	-ve	-ve
Melibiose	+ve	-ve	-ve
Sucrose	+ve	-ve	-ve
L-Hrabinose	-ve	-ve	-ve
Mannose	-ve	-ve	-ve

The table shows that strain 2 gives lactose, L-Hrabinose & mannose test –ve & all carbohydrate test +ve. Strain 5 & 7 shows all carbohydrate –ve result.

Carbohydrates	Isolate - 5	Isolate - 7
Inulin	-ve	-ve
Sodium galactose	-ve	-ve
Glycerol	-ve	-ve
Salicin	-ve	-ve
Dulcitol	-ve	-ve
Inositol	-ve	-ve
Sorbitol	-ve	-ve
Mannitol	-ve	-ve
Adonitol	-ve	-ve
Arabitol	-ve	-ve
Erythritol	-ve	-ve
α methyl D glucoside	-ve	-ve

Rhamnose	+ve	-ve
Cellobiose	+ve	+ve
α methyl- D mamoside	+ve	+ve
xylitol	+ve	+ve
ONPG	+ve	+ve
D-Arabinose	+ve	+ve
Citrate utilization	-ve	+ve
Malonate utilization	+ve	+ve
Sorbose	+ve	+ve

Table 3: Different carbohydrate tests from carbohydrate kit.

The table shows the strain 5 has +ve result in Rhamnose, Cellobiose, α methyl- D mamoside, xylitol, ONPG, D-Arabinose, Malonate utilization & Sorbose & all carbohydrate test shows –ve results. Strain 7 has +ve result in Rhamnose, Cellobiose, α methyl- D mamoside, xylitol, ONPG, D-Arabinose,Citrate utilization, Malonate utilization & Sorbose & all carbohydrate test shows –ve results.

The bacteria which are identified from the above biochemical tests are no 2 is *Bacillus subtilis*, no 5 is *Bacillus megaterium* & no 7 is *Bacillus pumilis* by the software PIBWIN (Probabilistic identification of bacteria).

Fig 12: Crabohydrate test

DISCUSSION

PIBWIN (Probabilistic identification of bacteria) programme provides probabilistic identification of unknown bacterial isolates against identification matrices of known strains. The programme has three major functions:

- The identification of an unknown isolate.
- The selection of additional tests to distinguish between possible strains if identification is not achieved.
- The storage and retrieval of results.

It also has some utility functions for assessing the usefulness of identification matrices and for converting matrices into different formats.he program makes use of Excel (2007) files to store identification matrices and archived results to achieve this, although other file formats are supported to allow backwards compatibility. The program is designed to use probabilistic identification matrices that have either published in the literature or created by the user. The matrices that are provided with PIB have been taken from the literature. These matrices have been typed in from the publication describing them and users should refer to these publications for full details of the methods used when testing isolates.

The bacteria which are identified from the above biochemical tests are *Bacillus subtilis, Bacillus megaterium* & *Bacillus pumilis* by the software PIBWIN (Probabilistic identification of bacteria).

B. subtilis can form bio-films. *Bacillus subtilis* is considered a soil organism for which endo-spore formation provides a means to ensure long-term survival in the environment. (Tam et al., 2006).

The antibacterial action of violet pigment, a mixture of violacein and deoxyviolacein, isolated from phychrotrophic bacterium RT102 strain was examined, and the operational conditions for the effective production of violet pigment were studied. The antibacterial activity of the violet pigment was confirmed for several bacteria such as *Bacillus licheniformis, Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus*, and *Pseudomonas aeruginosa*, and the high concentration of violet pigment, above about 15 mg/L, caused not only growth inhibition but also death of cells (Nakamura et al., 2003).

CONCLUSION

Due to rapid industrialization and modernization, the coal-based industries are increasing at an alarming rate. The coal based industries, such as by-product coke-plants, coal washeries and thermal power plants release their liquid effluents, which are needed urgent attention for the treatment, before they are discharged into the fresh water streams.

In this present work, water samples were collected from coal based industry namely Rourkela Steel Plant, Rourkela and *Bacillus subtilis, Bacillus megaterium, Bacillus pumilis* were identified.

So the water of the Koel River is mild polluted, however gradual monitoring of the water sample may reveal meaningful results in future. The authorities should take necessary remedial measures prior to discharge of the wastes to the river bodies as there are chances of pollution is more in this case and huge amount of inhabitants of Rourkela city are dependent on the river body for their daily purposes.

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