List of Publications

I. Mohd. Faraz khan(First Author) and Hina Khan(September 2019).*Isolation of Bacteria from Agricultural soil and screening it for PGPR traits*.International Journal of Advance Research Ideas & Innovation in Technology.[ISSN: 2454-132X].Impact factor:4.295(Volume 5, Issue 5).

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Isolation of bacteria from agricultural soil and screening it for PGPR traits

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ABSTRACT

Rhizobacteria owning multiple plant growth-promoting activities were isolated from the rhizospheric soils of plants flourishing in a semi-arid region. Plant Growth Promoting Rhizobacterial (PGPR) strains were segregated and screened for their plant growth-promoting activities like phosphate solubilization, production of indole- acetic acid, ammonia, hydrogen cyanide (HCN). Bacteria that colonize plant roots and promote plant growth are referred to as plant growth-promoting rhizobacteria (PGPR). PGPR is highly assorted and in this review, we focus on rhizobacteria as biocontrol agents. PGPR can affect growth directly or indirectly. Direct promotion of plant growth by PGPR involves both providing plants with a compound synthesized by the bacterium or helping the uptake of certain nutrients from the environment; while mechanisms of biological control by which rhizobacteria can support plant growth indirectly, i.e., by decreasing the level of disease, include antibiosis, induction of systemic resistance, and struggle for nutrients and niches.

Keywords— *PGPR, Nutrients, Biological fertilizers, Phosphate, Solubilization*

1. INTRODUCTION

Quality and quantity of food are going to be important challenges in coming time Continuous population growth requires production of more agricultural products and to inevitably move towards increased production per unit area. This cannot be achieved without the application of either chemical or bio-based fertilizers. Since fertilizer management is considered as one of the main factors of sustainable agriculture, gradual replacement of chemical fertilizers with biological fertilizers is quite inevitable due to their advantages and cost-effectiveness. The history of plant inoculation with useful bacteria goes back to many centuries ago. For instance, by experience, farmers knew that if the soil in which legumes were planted was mixed with the soil for non-legume crops, it resulted in an increased crop yield. In late 19th century, the first license for producing a biological fertilizer known as Nitragin was issued for the production of rhizobium inoculants and after that, inoculation of legumes started to be practiced in many countries using rhizobium fertilizers [1]. The rhizosphere, the narrow zone of soil that surrounds and influences the plant roots, is home to a large number of microorganisms and is considered to be one that can have profound effects on the growth, nutrition and health of plants in agro-ecosystems [2]. The rhizosphere, microbiota can contain up to 1011 microbial cells per gram of root [3] and more than 30,000 prokaryotic species [4]. Bacteria able to colonize plant root systems and promote plant growth are referred to as plant Growth Promoting Rhizobacteria (PGPR) [5].

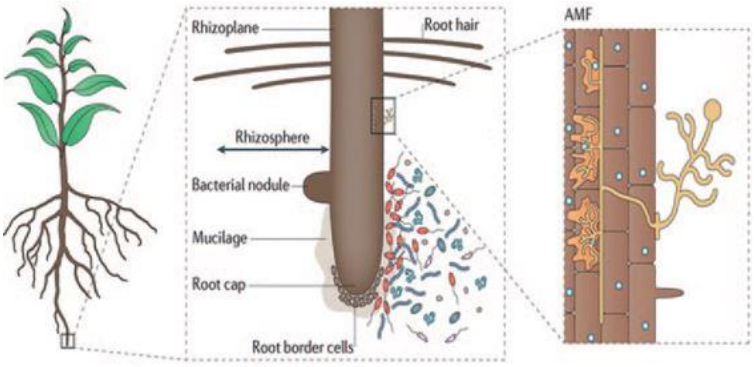

Fig. 1: Representation of a Rhizospheric Zone [6]

Table 1: PGPR and their effect on growth parameters/ yields of crop/fruit plants

PGPR	Crop parameters
Rhizobium leguminosarum	Direct growth promotion of canola and lettuce
Pseudomonas putida	Early developments of canola seedlings, growth stimulation of tomato plant
Azospirillum brasilense and *A. irakense*	Growth of wheat and maize plants
P. flurescens	Growth of pearl millet, increase in growth, leaf nutrient contents and yield of banana (*Musa*)
Azotobacter and *Azospirillum* spp.	Growth and productivity of canola
P. alcaligenes, *Bacillus polymyxa*, and *Mycobacterium phlei*	Enhances uptake of N, P and K by maize crop
Pseudomonas, *Azotobacter* and *Azospirillum* spp.	Stumulates growth and yield of chick pea (*Cicer arietinum*)
R. leguminismarum and *Pseudomonas* spp.	Improves the yield and phosphorus uptake in wheat
P. putida, *P. flurescens*, *A. brasilense* and *A. lipoferum*	Improves seed germination, seedling growth and yield of maize
P. putida, *P. fluorescens*, *P. fluorescens*, *P. putida*, *A. lipoferum*, *A. brasilense*	Improves seed germination, growth parameters of maize seedling in greenhouse and also grain yield of field grown maize

1.1 Location of study area

Unnao district represents flat topography with a general elevation of 98 m (322 ft.) covering an area of 4558 km^2 . By virtue of its geographic setting in the great (Ganga) plains, the land is highly fertile. The soil is mostly alluvial. The district is mainly drained by the river Ganga and its tributaries Kalyani, Khar, Loni and Marahai in the western part of the district and by Sai river in the eastern part of the district. All these rivers are perennial in nature. About 87% area of the net sown area (3,00,000 hectares) is irrigated both by surface water (Sharda Canal network system) and ground water through shallow and moderately deep tubewells. The share of surface water irrigation is 48% while that of ground water is 52%.

Soil found in Unnao industrial and surroundings village of Unnao district exhibit a wide variance in composition and appearance. The major part of area consists of ordinary soils known locally as Bhur or sand on the ridges, Matiar or clay in the topographic lows and Dumat or loam on the plains. Clay is dominant in the areas where "Reh" or USAR prevails. Alluvial soils of river valleys notable the "Kachhar" of the Ganga formed by repeated deposition of silt brought down by the existing river system during floods. [7]

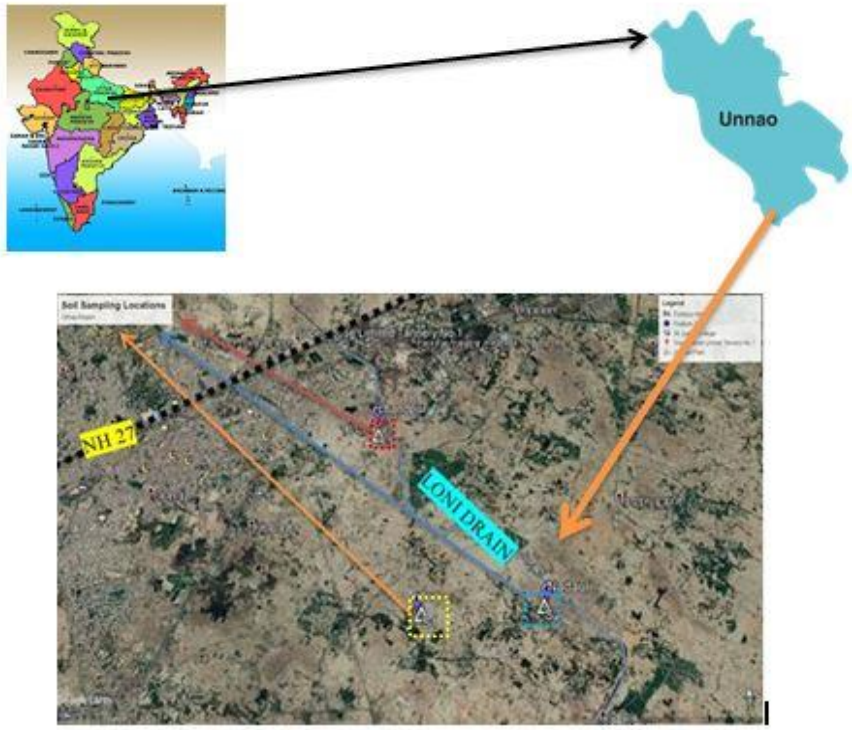

Fig. 2: Satellite Imagery of the Soil Sampling Location

2. MATERIAL AND METHOD

2.1 Sample Selection

The plant growth promoting rhizobacteria were isolated from the rhizosphere of following plants from the agricultural soils of Unnao Region:

2.1.1 Common wheat (*Triticum aestivum*): Loosely attached soil was separated from the roots. The roots were shaken tenderly to remove excess soil. After extracting the soil loosely adhering to root, the soil adhering firmly to the root of each plant was accumulated through brushing (termed rhizosphere soil sample, RSS).

2.2 Preparation of Nutrient Broth

Nutrient Broth is used for the general cultivation of less fastidious microorganisms, can be enriched with blood or other biological fluids.

Table 2: Composition of Nutrient broth Media

S no.	Ingredients	g/L
1	Peptic digest of animal tissue	5.00
2	Sodium chloride	5.00
3	Beef extract	1.50
4	Yeast extract	1.50
5	Final pH (at 25°C)	7.1±0.2

2.3 Serial Dilution for isolation of Bacteria from the soil

The Purpose of serial dilution was to determine the number of bacteria per unit volume in the original culture, determination of the culture density in cells per ml. Once the culture had been diluted it could be spread on agar plates. Agar plates allow for individual bacterial cells to be separated spatially. If done correctly, there is a low probability of having two cells very close to each other. When each of these spatially separated cells multiplies, spatially separated colonies were formed.

2.3.1 Procedure: The soil samples were serially diluted from 10^{-3} to 10^{-7} dilutions using sterile distilled water as a blank and they were inoculated on the nutrient agar medium by pour plate technique. After 24 hours of incubation at 37°C the colonies were counted.

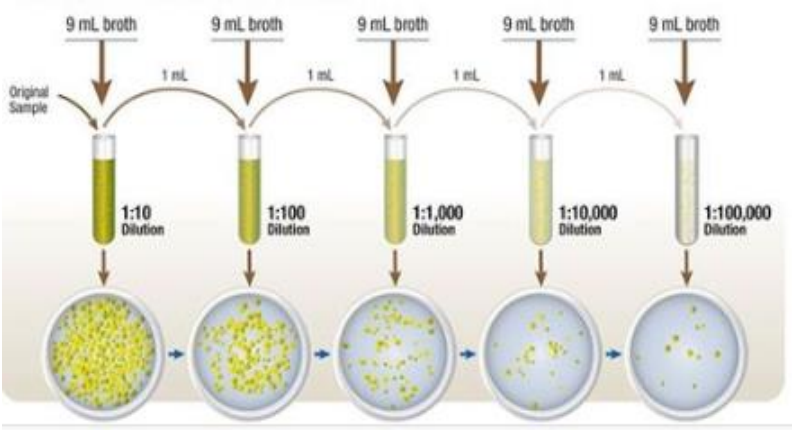

Fig. 3: Pour Plate Method

2.4 Streak Plate Method

2.4.1 Streak plate technique: is used for the isolation into pure culture of the organisms (mostly bacteria), from mixed population. The inoculum is streaked over the agar surface in such a way that it "thins out" the bacteria. Some individual bacterial cells are separated and well spaced from each other.

2.4.2 Procedure: This technique was done using an inoculating loop. 0.1 mL of the bacterial suspension was placed in the center of the plate using it.The glass rod was sterilized by first dipping it into a 70% alcohol solution and then passing it quickly through the Bunsen burner flame. The burning alcohol sterilizes the loop at a cooler temperature than holding the rod in the burner flame thus reducing the chance of burning fingers.

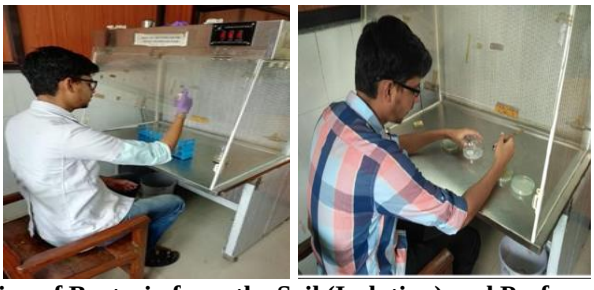

Fig. 4: Performing Serial Dilution of Bacteria from the Soil (Isolation) and Performing the Streak Plate Method in Laminar Air Flow Chamber

Screening of Phosphate Solubilizing Bacteria (PSB) .In most bacteria, mineral phosphate-dissolving capacity had been shown to be related to the production of organic acid such as Gluconic Acid (GA) [8], earlier which was reported to produce by direct oxidation of glucose. Mainly the biosynthesis of GA reported to carry out by the Glucose Dehydrogenase (GDH) enzyme and the co-factor, Pyrroloquinoline Quinone (PQQ). The p-sol ability was checked on Pikovskaya's media plate. Mainly this media contains calcium phosphate which acts as a source of phosphate. Moreover, GA producing rhizobacteria could be easily differentiated on the Pikovskaya's media plate by observing a clear halo zone due to the release of phosphorous from the media. Following steps were employed for isolation of PSB:

(a) 1 g dried Rhizospheric Soil Sample (RSS) was added to a 25 ml flask with 9.0 ml sterilized distilled water and incubated for 30 minutes (min) at 30 °C in a shaker at 250 RPM. The resulting suspension was decimally diluted (10^{-2}-10^{-6}) with sterilized distilled water.
(b) All RSS were appropriately diluted and plated on Pikovskaya agar media to get approximately 100 colonies per plate. The plates were incubated at 30 °C for 2 days. Colonies that showed halo zone was considered as PSB.
(c) Reconfirmation of PSB was done on Pikovskaya's media plates. (d) All PSB strains were streaked on nutrient agar media for colony purification.
(d) Purified strains were re-analyzed for p-sol on Pikovskaya's media plates.
(e) Glycerol stocks of all P-solubilizing strains were made in 30% glycerol and stored at -80 °C freezer.

2.5 Phosphate solubilization

Bacterial culture was spot-inoculated on the surface of the plate containing Pikovaskaya's medium and incubated in an incubator at 28 °C for 7 days. P-solubilization was determined by the development of the clearing zone around a bacterial colony [9]

2.6 Production of Ammonia

Bacterial isolate was tested for the production of ammonia in peptone water. The freshly grown culture was inoculated in 10 ml peptone water in a test tube and incubated for 48-72 h at 36±2 °C. Nessler's reagent (0.5 ml) was added to each tube. Development of brown to the yellow color indicated a positive test for ammonia production [10].

2.7 Production of Indole acetic acid

Bacterial culture was grown in LB medium amended with 100 mg L-1 tryptophan as the precursor of IAA by incubating in a shaker at 250 rpm at 28 ± 2oC for 7 days. Indole acetic acid (IAA) production was assayed colorimetrically by using Salkowski reagent (1ml of 0.5M FeCl3 in 50 ml of 35% HClO4) and the absorbance of the resultant pink color at 535nm in the colorimeter. The appearance of a pink color in test tubes indicated IAA production. The concentration of IAA was determined by comparison with a standard curve [11].

2.8 HCN Production

Briefly, the strain was streaked on the nutrient agar amended with glycine (4.4 g/L). A Whatman filter paper No.1 soaked in 2% sodium carbonate prepared in 0.5% picric acid solution was placed at the top of the plate. The plated sealed with a parafilm were incubated at 28±2oC for 4 days change of filter paper from orange to red if found, was noted down, hence confirming HCN production.[12]

Table 3: Examples of different phytohormone-producing PGPR

Phytohormones	PGPR
Indole-3-acetic acid (IAA)	*Acetobacter diazotrophicus* and *Herbaspirillum seropedicae*
Zeatin and ethylene	*Azospirillum* sp.
Gibberellic acid (GA_3)	*Azospirillum lipoferum*
Abscisic acid (ABA)	*Azospirillum brasilense*

3. RESULT AND DISCUSSION

Table 4: Plant Growth Promoting Traits of the Isolate

PGP Traits	Response
Ammonia Production	+ve
Phosphate Solubilization	+ve
IAA Production	-ve
HCN	+ve

3.1 Phosphate Solubilization

On Pikovaskaya's agar plates a clear zone was observed around the bacterial colonies. The solubilization ability of rhizosphere microorganisms is considered to be one of the most important traits associated with plant phosphate nutrition. It is generally accepted that the mechanism of mineral phosphate solubilization by PSB strains is associated with the release of low molecular weight organic acids, through which their hydroxyl and carboxyl groups chelate the cations bound to phosphate, thereby converting it into soluble forms. PSB has been introduced to the Agricultural community as phosphate Bio fertilizer.

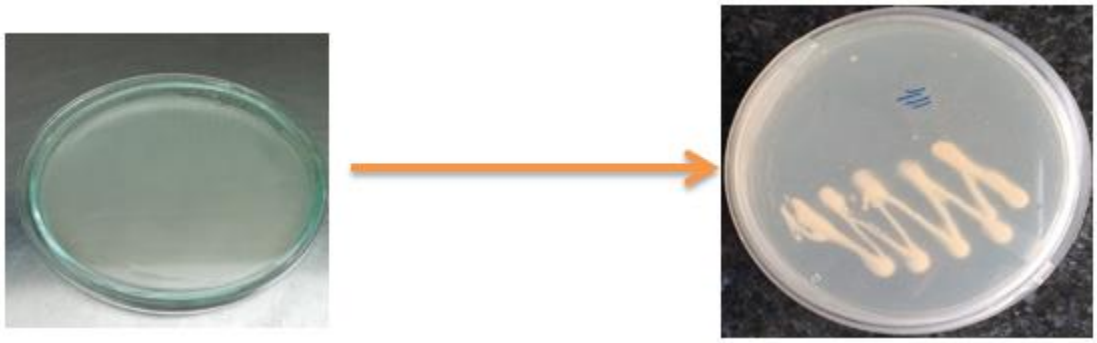

Fig. 5: Phosphate solubilization by the isolates

3.2 HCN Production

A change in color of the filter paper was noticed from light orange to reddish orange, which confirmed the production of HCN by the isolated bacterial strain. Hence the isolating strain might be PGPR. The production of the HCN by the isolates is a healthy sign that the plant will resist activities in the rhizosphere or root zone due to the pathogenic bacteria or fungal activity.

3.3 NH_3 Production

In addition, of Nessler's reagent to put-on water inoculated with bacterial strain, a considerable change in color from brown to yellow was noticed, thus confirming the production of ammonia. There are a number of sources of ammonia secretion of rhizospheric microorganisms. Ammonia and extracellular proteins are the nitrogenous secretions of nitrogen fixers in nitrogen free or deficient medium.

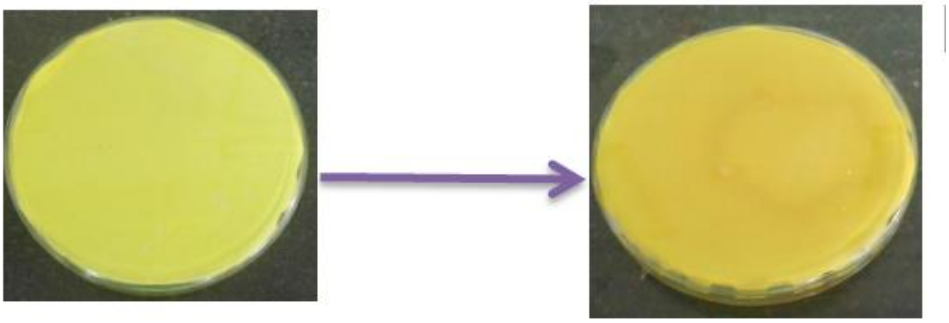

Fig. 6: HCN production by the isolates (orange color)

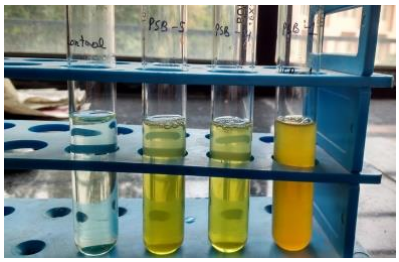

Fig. 7: Ammonia production by the isolates (yellow colour)

4. CONCLUSION AND RECOMMENDATIONS

PGPR are found in plant roots or in the adjacent soil and contribute to the plant's growth and development through multiple direct and indirect mechanisms. PGPR have been investigated in search of efficient ways to use them to improve agricultural production in a low impact ecological way. All the isolated strains in this study showed varying levels of plant growth promoting activities, and all had an overwhelmingly positive effect on the plants for all the investigated bioprocesses: IAA production, Nitrogen fixation and cellulase activity in addition to their antifungal properties. Based on these results, the isolated PGPRs in this study could constitute an efficient and more eco-friendly alternative to chemical fertilizers and fungicides in the processes of biostimulation, bio-fertilization and biological control.

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Water quality assessment of the Unnao Tannery region

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ABSTRACT

The leather industry has gained immense socio-economic importance in India. Indian leather division has contributed significant economic growth by providing job opportunities. The main reason for the development and growth of the leather industry in the country is its large animal population. India holds nearly 10% of the total global availability of raw hides and skins which are the basic raw material for the leather industry. The impact of tanning and associated activities on air, surface and groundwater and soil pollution arise from the chemicals applied, the raw materials used and the effluents, waste and off-gas releases generated in the process. The tanning industry is known to be very polluting especially through effluents high in organic and inorganic dissolved and suspended solids content followed by propensities for high oxygen demand and containing potentially toxic metal salt residues.

Keywords— *CETP, Chromium, Wastewater, Tanning, Pollution*

1. INTRODUCTION

Unnao is one of the major industrial towns adjacent to Kanpur having most of the cotton, leather, pharmaceutical, steel, and other industries. The Unnao industrial area and surrounding villages of Unnao district lie between 260 26' and 260 41' North latitudes and 800 15' and 800 33' East longitudes, falling in the survey of India Toposheet No. 63B. It is bounded on the north by Safipur block, in the east by the Bichhia block, in the south Sikandarpur Karon block, whereas the Ganga River in the west separates it from the district of Kanpur. The total area is about 220 km^2. Unnao industrial area is situated near Kanpur in the northern side of Ganga River has more than 50 industrial units mainly tannery, catering the need of the nation. The effluents discharged by the industries, after passing through a common effluent treatment plant having approx. 70% treating capacity, is finally discharged in the Ganga River. The quality of groundwater in the industrial areas is under constant threat of contamination directly or indirectly. A remarkable high concentration of chromium in some parts of groundwater of Unnao and Kanpur districts is a common feature in the region.To limit pollution of the natural environment, biological treatment, using Activated sludge process has been the common treatment process for sewage. [1]

Cr is one of the most important pollutants released from the tanning industries and the biggest problem is its disposal and recovery. Compared to the recommended permissible limit of 2 mg/L prescribed by BIS, India alone released about 2000-3000 tons of chromium into the environment annually from tanneries with chromium concentrations ranging between 40- 5000mg/L. Chromium is a potential pollutant and well known for its mutagenicity [2] and carcinogenic effects in humans, animals, and plants. Soil profile, surface water bodies such as ponds and rivers, human health, fishes and other aquatic biodiversities are at risk of serious threat due to the extensive use of chromium in tanning industries and discharge of wastewater [3].

1.1 Impact on surface water by tanneries

Surface water is not uniformly distributed over the earth's surface. According to the U.S. landmass, only about 4% is covered by rivers, lakes, and streams. The amounts of these freshwater sources depend on geographic, landscape and temporal variations and one the impact of human activities. Since surface water supplies are always in a state of transition, hydrologic models become valuable tools for estimating future water supply scenarios based on assumed sequences of hydrologic variables, such as precipitations, temperature, and evaporation and for projects physical manipulations of the surface water containment system. Thus tannery wastewater discharge pollutes surface water like drains, lake, and ponds [4].

Water bodies receiving the tannery effluent show high Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and chloride levels that are well above the stipulated concentrations prescribed by the Indian Standard Institutes [5].

Water pollution is a major problem related to the industrial growth of the country. Some of the industries like tannery release their effluents in surrounding surface water bodies contaminating the surface water. The amount and toxicity of waste released from industrial activities vary with the different industrial process. Among all the industrial wastes, those released from tanneries have the highest concentration of pollutants [6].

Fig. 1: Chrome tanning process Flowsheet

1.2 Impact on groundwater by tanneries

Groundwater acts as a reservoir by large pore space in earth materials as a conduit which can transport water over long distances and act as a mechanical filter which improves water quality by removing suspended solids and bacterial contamination. It is the source of water for wells and springs that recommended the source of rural domestic use. According to their result of anthropogenic activities, groundwater is contaminated by the constant addition of industrial, domestic and agricultural waters to it. Groundwater contamination is generally irreversible, i.e. once it is contaminated its original quality cannot be restored back. Excessive mineralization of groundwater degradeswater quality producing an objectionable taste, odor and excessive hardness [7]. The tannery industries release their effluents either on open land or surface water bodies contaminating the quality of groundwater.Groundwater is a precious natural resource. Unfortunately, it has been subjected to maximum exploitation and has been severely degraded due to tanning activities (8).

1.3 Location of the study area

Unnao district represents flat topography with a general elevation of 98 m (322 ft.) covering an area of 4558 $km^{2.}$ By virtue of its geographic setting in the great (Ganga) plains, the land is highly fertile. The soil is mostly alluvial. The district is mainly drained by the river Ganga and its tributaries Kalyani, Khar, Loni and Marahai in the western part of the district and by Sai River in the eastern part of the district. All these rivers are perennial in nature. About 87% area of the net sown area (3, 00,000 hectares) is irrigated both by surface water (Sharda Canal network system) and groundwater through shallow and moderately deep tubewells. The share of surface water irrigation is 48% while that of groundwater is 52%.

In Kanpur (& Unnao) region, Ganga River flows along NW-SE trending weak zone (a tectonic lineament) showing a prominent escarpment on the southern side and well-developed flood plain in the northern side [9]. This weak zone has also controlled the subsurface stratigraphy in the alluvium [10].

Soil found in Unnao industrial and surroundings village of Unnao district exhibit wide variance in composition and appearance. The major part of area consists of ordinary soils known locally as Bhur or sand on the ridges, Matiar or clay in the topographic lows and Dumat or loam on the plains. Clay is dominant in the areas where "Reh" or usar prevails. Alluvial soils of river valleys notable the "Kachhar" of the Ganga formed by repeated deposition of silt brought down by the existing river system during floods.

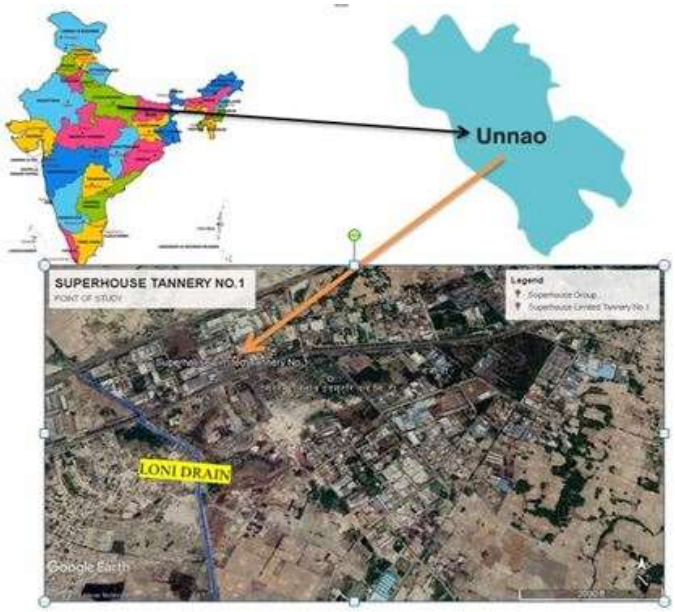

Fig. 2: Satellite imagery Location of Study Area

1.4 Industrial Area Site

1 consists of 5 units (One slaughterhouse, one meat processing unit, two fat processing units, and one metal unit). Approximately 0.75 MLD effluent is generated from all these units and is discharged in Loni Drain after treatment.

Fig. 3: CETP Unnao Outlet into Loni Drain

1.5 Industrial Area Site – 2

It consists of 21 tannery units out of which 14 are operational and 7 are non-operational since long. Out of 14 tannery units, 2 units have their own effluent treatment plant and effluent from 12 tanneries goes to the combined effluent treatment plant (CETP) after undergoing primary treatment which involves chrome recovery and suspended solids removal in general. Apart from tanneries, there is one slaughterhouse and one meat processing unit, both having their own effluent treatment plant. These units produce approximately 4.37 MLD effluent. The CETP was made operational in October 1995 at the cost of Rs. 195 lacs. The designed treatment capacity of plant is 2.15 MLD. CETP consists of bar screen, equalization tank, primary clarifier for suspended solids removal, 2-stage aerobic bioreactor and clarifiers for removal of organics, tertiary clarifier for removal of organics and suspended solids by adsorption on chemical sludge, followed by multigrade filter and activated carbon filter for final polishing. It is operated and maintained by Unnao Tanneries Pollution Control Company.

Fig. 4: Location of CETP near Superhouse Tannery Limited next to Loni Drain Satellite Imagery

Treated effluent from Site – 1 and Site – 2 are discharged in Loni drain. Approximately, 5 MLD untreated sewage from the city is also discharged in the Loni drain. Loni drain meets River Ganga in Raebareli District after traveling approximately 146 km. Water from Loni drain is utilized for irrigation by the farmers.

2. MATERIAL AND METHOD

Water and wastewater (Effluent) samples were collected in polyethylene bottles using dip/grab sampling method during pre-monsoon (May 10-12, 2017) and post-monsoon (August 3-4) season and preserved by using appropriate reagents as per standard methods. All glassware and other containers used for trace element analysis were thoroughly cleaned, soaked in 10% nitric acid for 48 h and finally rinsed with de-ionized water several times prior to use. All the testing procedures were carried out in the laboratory of Indian Institute Technology ISM Dhanbad Jharkhand. The physicochemical analysis was performed as per Standard Methods for the Examination of Water and Wastewater [11]

Table 1: Analytical Methods and Equipment Used in the Analysis

S no.	Parameter	Method	Equipment Used
1.	pH	Electrometric	pH Meter
2.	Suspended Solids	Gravemetrically	-
3.	BOD	5 days incubation at 20^{o}C followed by titration	BOD Incubator
4.	Chromium	Digestion followed by Atomic Spectrophotometer	Atomic Absorption Spectrometer

3. RESULT AND DISCUSSION

Table 2: Characteristics of Effluents of Superhouse Tannery No.1 Ltd

S no.	Parameters	Inlet (S-1)		Outlet (S-2)		Effluent Standards
		Pre-monsoon	Post-monsoon	Pre-monsoon	Post-monsoon	
1	pH	8.1	9.2	7.3	7.6	6.5-9.0
2	TSS, mg/L	2150	-	250	-	100
3	Total Chromium, mg/L	5.80	1.62	0.67	0.52	2
4	BOD, mg/L	1237	452	35	254	30

Effluent discharged by. M/S Superhouse Tannery No.1 Ltd is not in conformity with the effluent standards notified vide S.No. 57; G.S.R. 475(E) dated 5.5.1992 under Environment (Protection) Act, 1986 for discharge of effluents into inland surface water for TSS and BOD and needs appropriate statutory action by UPPCB/CPCB.

4. CONCLUSION AND RECOMMENDATIONS

The samples of wastewater/ treated water were collected from the inlet and outlet, of the sewage treatment plant and the results discussed are only pertaining those physicochemical parameters which are above the threshold limits to cause harmful effects. The concentration of chromium is reduced because it is being recycled by a chrome recovery plant that is installed within the premises of the superhouse tannery. A proper system of collection and transportation of domestic waste should be developed. A landfill site(s) should be identified and it must be scientifically designed for the disposal of domestic waste. Groundwater quality near landfill sites should be regularly monitored.

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Performance evaluation of a Sewage Treatment Plant Based on Sequential Batch Reactor Technology

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ABSTRACT

The Sequential Batch Reactor (SBR) is one of the potential options for treatment of industrial wastewater. SBR is a fill-and draw, system for aerobic and anaerobic wastewater treatment. In industrial wastewater wide variety of both, inorganic and organic, pollutants are present in the effluents which include oil, greases, metallic wastes, suspended solids, phenols, toxins, acids, dyes, colors, etc., several of which are not readily susceptible to degradation and therefore creating a dilemma during disposal. SBR is one of the latest options for the treatment of industrial wastewater. They are uniquely suited for wastewater treatment applications characterized by low or intermittent flow conditions. Operations and Maintenance (O&M) costs associated with an SBR system may be similar to a conventional activated sludge system. The overall working of the SBR is in five steps, fill, react, settle, decant, and idle. The process modification is very easy due to the amenable nature of the SBR. The cycles, hydraulic retention time (HRT), sludge retention time (SRT) can be changed and hence it provides wide scope for treatment that is too in a single reactor which is a most advantageous factor. SBRs are also used as pre or post-treatment prospects along with other treatment facilities successfully.

Keywords-*: *Activated Sludge Process,Sequencing Batch Reactor,Wastewater treatment

I. INTRODUCTION

Sewage treatment plant (STP) plays an important role in society. The chief purpose of these plants is to make the water of the sewage clean that originates from houses.The treatment of sewage water has become the need of the hour as it stops spreading the diseases and disorder caused by the sewage water. It helps the community in making the water as well as environment clean. The research area involves a 6.5 MLD capacity of sewage treatment plant based on SBR technology near Sector-6 Vrindavan Yojana, District, Lucknow, Uttar Pradesh India. This research work assessed the performance of the STP based on SBR technology in terms of physical and chemical parameters of wastewater and effectiveness of treatment.

A sequencing batch reactor (SBR) is a fill-and-draw activated sludge system. The unit processes involved in the SBR and conventional activated sludge systems are identical. Although aeration and sedimentation/clarification are

carried out in both systems, there is one important difference; in conventional plants, the processes are carried out simultaneously in separate tanks, whereas in SBR operation the processes are carried out sequentially in the same tank .[4]

Water reuse is an engaging strategy that can significantly add to water conservation in areas experiencing from water scarcity or overconsumption. This enables the use of reclaimed water for particular purposes, which depending upon the application, requires various levels of treatment. Sewage Treatment is applied to reuse treated water.

To limit pollution of the natural environment, biological treatment, using Activated sludge process has been the common treatment process for sewage.

Fig. 1-: Location of the Study Area

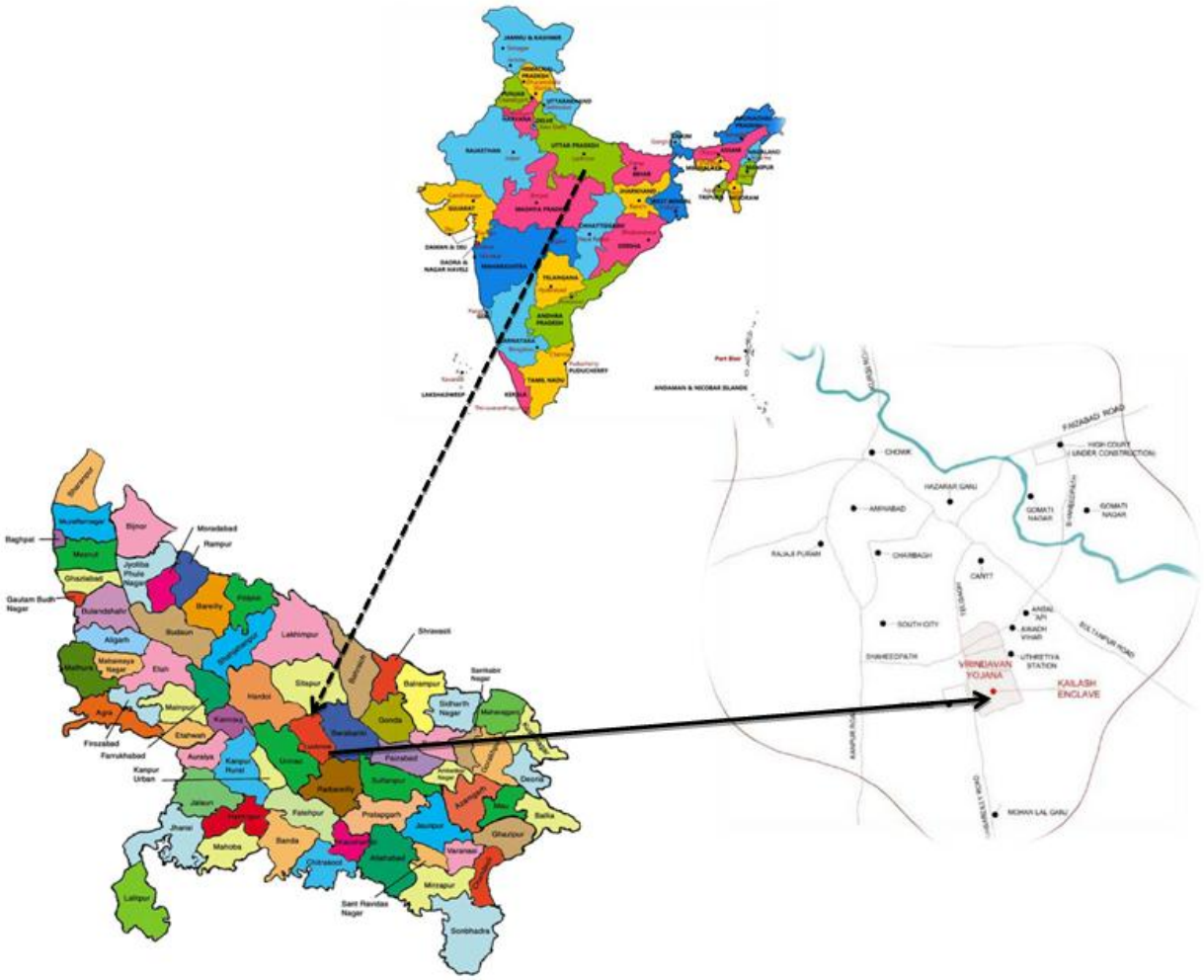

Fig. 2: Satellite Imagery of the Vrindavan Yojana STP

TABLE1:- Pictures of STP units installed at the Vrindavan Yojana

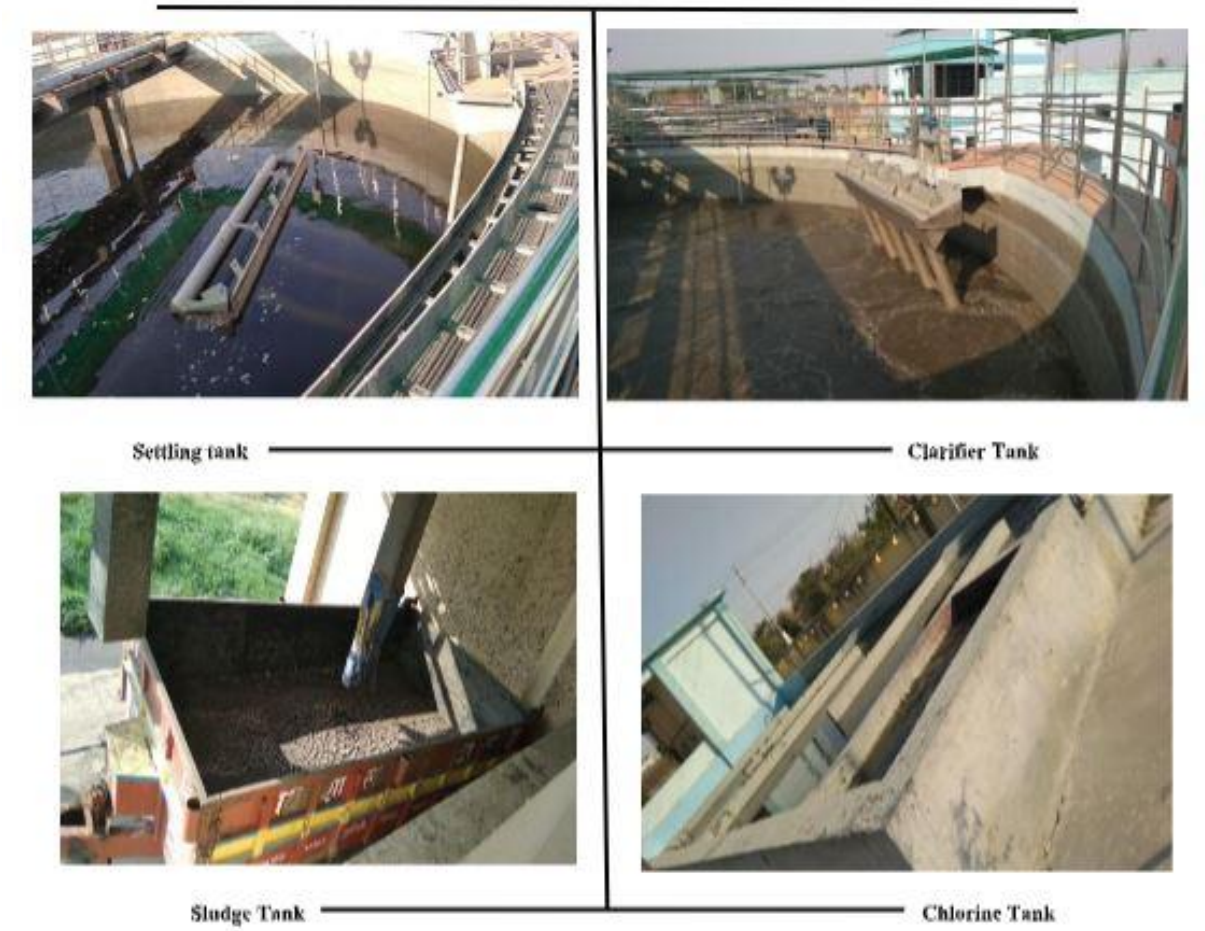

TABLE 1:- Dimensions of STP units installed at the Vrindavan Yojana

S.No.	Description	Size(mm)	Quantity
1.	Screen Chamber	800×500	1
2.	Collection Tank	2100×3650	1
3.	Aeration Tank 1 and 2	3500×3650	2
4.	Settling Tank	2800×2800	1
5.	Clarifier Water Tank	1800×2400	1
6.	Sludge holding Tank	1800×1800	1
7.	Sludge drying Beds	1100×1100	2
8.	Treated water tank	5050×4050	1

Ⅱ MATERIAL AND METHOD

The experimental method for this task includes lab analysis carried out in the Environmental Chemistry lab of Integral University Lucknow, Department of Civil Engineering, site visit to the STP, collection of sample of inlet and outlet samples, during my study have been collected from STP based on SBR technology located at sector-6, Vrindavan Yojana, Lucknow. Samples from the inlet & outlet chamber of the STP during the period from January 2019 to May 2019 were gathered. Samples were analyzed for various parameters like Turbidity, BOD, COD, TSS, and on the basis on the result, the performance of STP was assessed.

TABLE 2-: Details of some physicochemical parameters of STP with SBR Technology

S.No	Parameters	Preservation of Samples	Analysis Method	References[1]
1	Turbidity	Refrigerator below 4^0 C	Turbidity Meter	APHA,2017
2	pH	Refrigerator below 4^0 C	Potentiometry	APHA,2017
3	BOD	Refrigerator below 4^0 C	Winkler's Method	APHA,2017
4	COD	Refrigerator below 4^0 C	Dichromate Digestion	APHA,2017
5	TSS	Refrigerator below 4^0 C	Gravimetric Method	APHA,2017

There are five stages in the treatment process:

1. **Fill**
2. **React**
3. **Settle**
4. **Decant**
5. **Idle**

The inlet valve opens and the tank is being filled in, while mixing is provided by mechanical means (no air). This stage is also called the anoxic stage. Aeration of the mixed liquor is performed during the second stage by the use of fixed or floating mechanical pumps or by transferring air into fine bubble diffusers fixed to the floor of the tank. No aeration or mixing is provided in the third stage and the settling of suspended solids starts. During the fourth stage the outlet valve opens and the "clean" supernatant liquor exits the tank.[2]

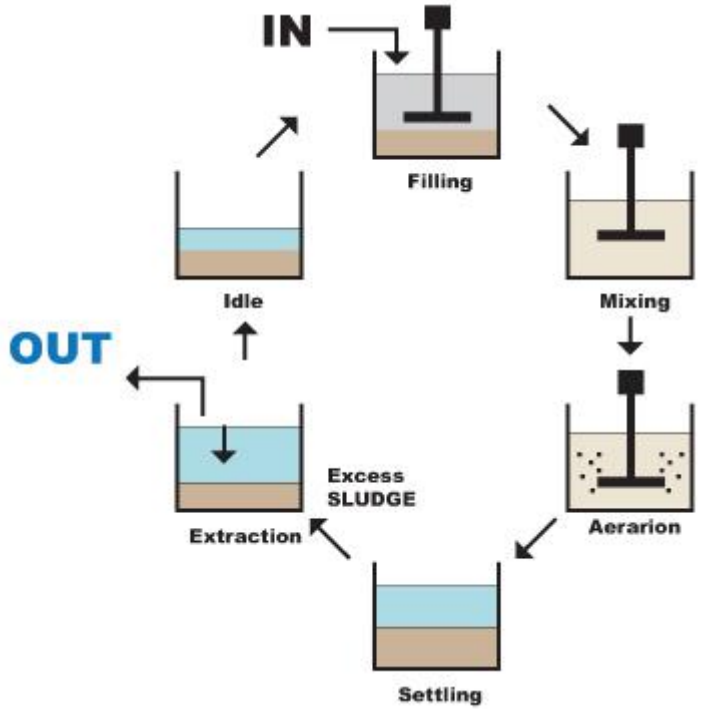

Figure 3. Process Cycle of a Sequential Batch Reactor[5]

Ⅱ.1 TECHNOLOGY DESCRIPTION

Typically the SBR system is consists of a separate tank, where all operations are carried out (Figure 1). The series of operation is divided into five discrete periods: (a) fill, (b) react (aeration), (c) settle, (d) draw, and (e) idle, which complete the whole cycle. The cycle of each SBR system undergoes one or more cycle during a day.

(a)**Fill**-: The fill process is where the reactor is filled with wastewater between a low water level & a high water level. Fill could occur under mixed, unmixed, aerated or unaerated conditions. The time of fill depends on the capacity of each reactor, the number of parallel reactors in operation, & the variations in the wastewater flow rate.[7]

(b) **React**-:The react phase begins once fill is complete. It includes mixing & aeration (dissolved oxygen (DO)>2 mg/l). In this phase, no influent flow into SBR aeration & sludge could be wasted , Aeration process serves to nitrify ammonia, oxidize organic carbon, & promote uptake of phosphorus in the sludge, while unaerated conditions support denitrification of nitrite & nitrate.

(c) **Settle**-: During the settlement period, solids and liquids are separated, which provides clarified supernatant to be discharged as effluent. In an SBR, this process is normally much more efficient than a continuous flow system because in the settle mode the reactor contents are completely quiescent. The time should be between 0.5 to 1 hour so that sludge blanket remains below the withdraw mechanisms during the draw and does not rise before a draw is completed.

(d) **Draw**-: The goal of the draw is to remove treated water from the reactor

(e) **Idle**-: Provide time for one reactor to complete its full cycle before switching to another SBR cycle [3]

III RESULTS AND DISCUSSION

Colmenarejo et al., (2006) determined the general efficiency indicator to compare overall performance of the different plants in terms of average TSS, COD, and BOD_3 removal efficiencies. The pH of waste water impacts a significance effects on rate of microbiological growth, pH (basic) after the structure of enzymes and stop growth. Favorable range of pH is 6.5 - 8.5[7].

Experimental determinations of STPs based on SBR Technology symbolize that Turbidity, BOD, COD & Total Suspended Solids (TSS), removal efficiencies were calculated to be 89.81%, 94.1%, 90.46%, 96.11%, respectively. According to Environmental protection rules 1986 [Schedule vi] published in CPCB report August 2013, treated effluent is safe against disposal on land and used in irrigation. Discharge of the final effluent from the Sewage Treatment Plant may not cause health risks or any major environmental problems.

Table 3-: Analysis of some physicochemical parameters at inlet & outlet of STP with SBR Technology

Date of Sampling	**1. pH**		**2.BOD3(mg/l)**		**3.COD(mg/l)**		**4.TSS(mg/l)**		**5.Turbidity**	
	IN	**OUT**	**IN**	**OUT**	**IN**	**OUT**	**IN**	**OUT**	**IN**	**OUT**
15/1/2018	7.24	6.69	276	7	365.18	28.14	359	11	1282	112
15/5/2018	7.94	7.26	275	25	425.12	48.27	425	20	1358	158
%Removal	**8.07**		**94.1**			**90.46**	**96.11**		**89.81**	

IV CONCLUSION

The performance of SBRs is typically comparable to conventional activated sludge systems and depends on system design and site specific criteria. Depending on their mode of operation, SBRs can achieve good BOD and nutrient removal. For SBRs, the BOD removal efficiency is generally 85 to 95 percent.[4]

The BOD, COD, and TSS in effluent are within permissible limits due to proper aeration and settling mechanisms. Discharge of the final effluent from the sewage treatment plant may not cause health risks or any major environmental problems. Guidelines of treated wastewater by different agency working for environmental protection like CPCB, WHO etc.

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Adverse impacts of Heavy Metals on Human beings and its elimination by Phytoremediation: A Current Perspective

Removal of Heavy Metals By Plant based Approach

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***Abstract*— Heavy metals are frequently cited to as those metals which hold a specific density of more than 5 g/cm3 and adversely alter the environment and living organisms (Järup, 2003). These metals are quintessential to maintain various biochemical and physiological functions in inhabiting organisms when in very low concentrations Current methods for remediation of metal contaminated soils include soil removal and washing, physical stabilization, and/or the use of chemical amendments, all of which are expensive and disruptive, with an average cost of $ 404,700 per ha (Raskin et al., 1997). USEPA (2002) recommended excavation, capping, solidification and stabilization, nitrification, soil washing/acid extraction, soil flushing, phytoremediation, etc. as current remediation technologies for heavy metal contaminated soil.**

Keywords:- Heavy metals; Phytoremediation; Rhizofiltration; PGPR.

I. INTRODUCTION

The term heavy metals have generally been used to describe those metals having an atomic number greater than iron or having a density greater than 5 g/ml. Plants require certain elements for their normal growth, which are called essential elements (micro and macro elements). But there are also some elements which are not vital for plant growth. Such elements are called non-essential elements, which include heavy metals which cause toxicity to plants. Heavy metals like Cr, Cu, Ni, Pb, and Cd are phytotoxic either at all concentrations or above levels. Toxic metals are biologically magnified through the food chain. They infect the environment by affecting the properties of soil like soil fertility, biomass, and crop yields and indirectly it affects the human health.

Table: 1 Clinical Aspects of Chronic Toxicities *(Source: Mahurpawar 2015)*

Metal	Target Organs	Primary Sources	Clinical effects
Arsenic	Pulmonary Nervous System, Skin	Industrial Dusts, Medicinal Uses Of Polluted Water	Perforation of Nasal Septum, Respiratory Cancer, Peripheral Neuropathy: Dermatomes, Skin, Cancer
Cadmium	Renal, Skeletal Pulmonary	Industrial Dust And Fumes And Polluted Water And Food	Proteinuria, Glucosuria, Osteomalacia, Aminoaciduria, Emphysema
Chromium	Pulmonary	Industrial Dust And Fumes And Polluted Food	Ulcer, Perforation of Nasal Septum, Respiratory Cancer
Manganese	Nervous System	Industrial Dust And Fumes	Central And Peripheral Neuropathies

II. REMEDIAL TECHNIQUES

A.BIOREMEDIAION

Bioremediation is a process used to treat contaminated media, including water, soil and subsurface material, by altering environmental conditions to stimulate growth of microorganisms and degrade the target pollutants. In many cases, bioremediation is less expensive and more sustainable than other remediation alternatives. Biological treatment is a similar approach used to treat wastes including wastewater, industrial waste and solid waste.

Aerobic bioremediation

Aerobic bioremediation is the most common form of oxidative bioremediation process where oxygen is provided as the electron acceptor for oxidation of petroleum, polyaromatic hydrocarbons (PAHs), phenols, and other reduced pollutants. Oxygen is generally the preferred electron acceptor because of the higher energy yield and because oxygen is required for some enzyme systems to initiate the degradation process.Numerous laboratory and field studies have shown that microorganisms can degrade a wide variety of hydrocarbons, including components of gasoline, kerosene, diesel, and jet fuel. Under ideal conditions, the biodegradation rates of the low- to moderate-weight aliphatic, alicyclic, and aromatic compounds can be very high. As the molecular weight of the compound increases, so does the resistance to biodegradation.

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Anaerobic bioremediation:

Anaerobic bioremediation can be employed to treat a broad range of oxidized contaminants including chlorinated ethenes (PCE, TCE, DCE, VC), chlorinated ethanes (TCA, DCA), chloromethanes (CT, CF), chlorinated cyclic hydrocarbons, various energetics (e.g., perchlorate, RDX, TNT), and nitrate. This process involves the addition of an electron donor to: 1) deplete background electron acceptors including oxygen, nitrate, oxidized iron and manganese and sulfate; and 2) stimulate the biological and/or chemical reduction of the oxidized pollutants. Hexavalent chromium (Cr [VI]) and uranium (U[VI]) can be reduced to less mobile and/or less toxic forms (e.g., Cr [III], U[IV]). Similarly, reduction of sulfate to sulfide (sulfidogenesis) can be used to precipitate certain metals (e.g., zinc, cadmium). The choice of substrate and the method of injection depend on the contaminant type and distribution in the aquifer, hydrogeology, and remediation objectives. Substrate can be added using conventional well installations, by direct-push technology, or by excavation and backfill such as permeable reactive barriers (PRB) or biowalls. Slow-release products composed of edible oils or solid substrates tend to stay in place for an extended treatment period. Soluble substrates or soluble fermentation products of slow-release substrates can potentially migrate via advection and diffusion, providing broader but shorter-lived treatment zones. The added organic substrates are first fermented to hydrogen (H_2) and volatile fatty acids (VFAs). The VFAs, including acetate, lactate, propionate and butyrate, provide carbon and energy for bacterial metabolism.

Natural Bioremediation:

It is also known as natural attenuation or passive bioremediation which is an environmental site management approach that relies on naturally occurring microbial processes for petroleum hydrocarbon removal from groundwater, without the engineered delivery of nutrients, electron acceptors or other stimulants. Main advantage of this method is its cost effectiveness compared to engineered conditions. Disadvantage of this technique is that it takes more time for organic biodegradation.

B.PHYTOREMEDIATION

Phytoremediation can be of many types. Phytoextraction is the name given to the process where plant roots uptake metal contaminants from the soil and translocate them to their above tissues. Rhizofiltration is similar in concept to phytoextraction but is concerned with the remediation of contaminated groundwater rather than the remediation of polluted soils. The contaminants are either adsorbed into the root surface or are absorbed by the plant roots. Phytostabilization is the use of certain plants to immobilize soil and water contaminants, which are absorbed and accumulated by the roots, absorbed into the roots or precipitated in the rhizosphere. This reduces or even prevents the mobility of the contaminants stopping their migration into the groundwater and also reduces the bioavailability of the contaminant thus preventing spread through the food chain. Phytodegradation (Phytotransformation) is the degradation or breakdown of organic contaminants by internal and external metabolic processes driven by the plant. Rhizodegradation (also called enhanced rhizosphere biodegradation, phytostimulation and plant assisted bioremediation) is the breakdown of organic contaminants in the soil by soil microbes which is enhanced by the rhizosphere's presence.

Table 2: Techniques/Strategies of Phytoremediation *(Favas et al., 2014)*

Technique	Description
Phytoextraction	Accumulation of pollutants in harvestable biomass i.e., shoots
Phytofiltration	Sequestration of pollutants from contaminated waters by plants
Phytostabilization	Limiting the mobility and bioavailability of pollutants in soil by plant roots
Rhizodegradation	Degradation of organic xenobiotics in the rhizosphere by rhizospheric microorganisms.
Phytovolatilization	Conversion of pollutants to volatile form and their subsequent release to the atmosphere
Phytodegradation	Degradation of organic xenobiotics by plant enzymes within tissues
Phytodesalination	Removal of excess salts from saline soils by halophytes

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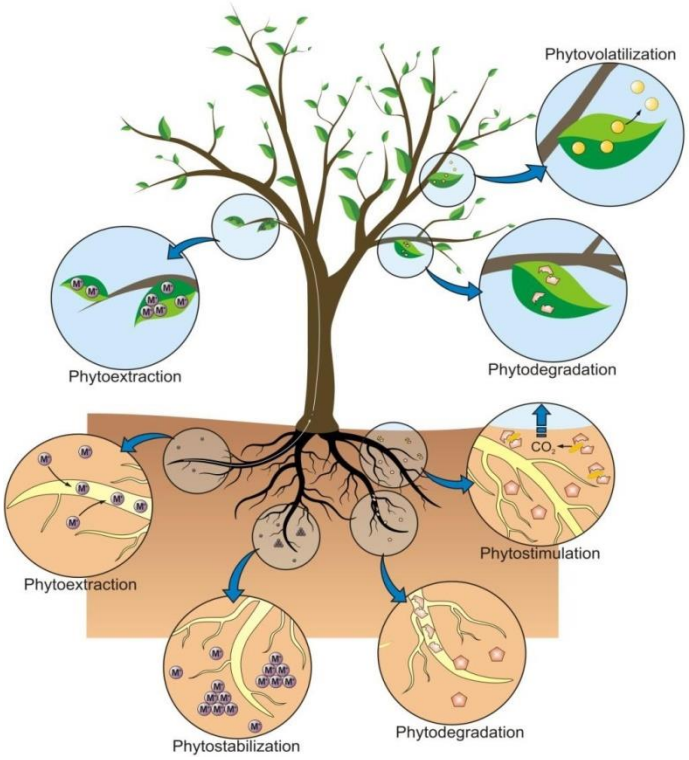
Fig. 1: Different Process of Phytoremediation

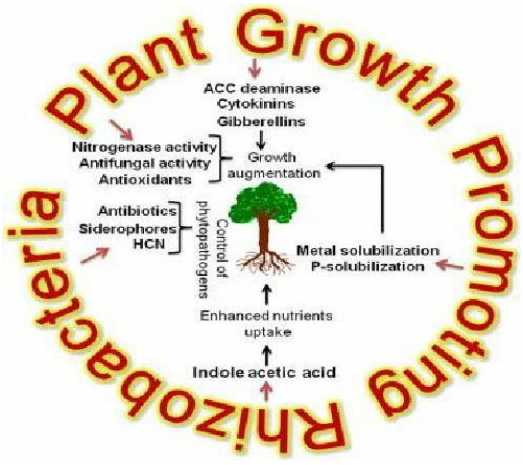
(Favas et al., 2014)



PGPR Metabolic Activities

PGPR are able to produce enzymes, which can affect plant growth under different conditions including stress. For examples, PGPR produces the enzyme 1-aminocyclopropane- 1-carboxylate (ACC)-deaminase, which is able to turn ACC, the prerequisite for the production of the stress hormone ethylene, into a-ketobutyrate and ammonium and hence alleviate the adverse effects of stress on plant growth (Glick et al., 1998). Plant growth promoting rhizobacteria can also produce plant hormones and siderophores, which can significantly affect plant growth by regulating different plant metabolisms and the availability of different soil nutrients including iron, zinc, copper, and manganese. Plant hormones can significantly affect plant growth under different conditions including stress.

Fig 2: Plant Growth Promoting Activities (*Ahemad et al., 2013*)



PGPR and Alleviation of Stress

Plant growth promoting rhizobacteria are able to alleviate the effects of different stresses on plant growth by using the following mechanisms. 1) Interacting with the other soil microbes, 2) production of plant hormones, 3) production of different enzymes such as ACC- deaminase, 4) increasing the solubility of soil nutrients, 5) controlling plant pathogens, 6) affecting heavy metals properties in the soil, and 7) their use for biofertilization. In their seven-stage experiments, Jalili et al. (2009) were able to

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isolate the strains of *P. fluorescent* and *P. putida* from saline soils to test their stress alleviating abilities. The isolated strains were then used to inoculate canola (*Brassica napus* L.) subjected to salinity stress. Under salinity, 14% of the strains were able to produce ACC- deaminase as the sole N source. The strains differ in their ability able to produce ACC- deaminase, auxin and hydrogen cyanide. Inoculation of canola seeds with *P. fluorescent* resulted in the alleviation of salinity stress on canola seed germination and seedling growth

Under stress, the process of N-fixation may be adversely affected due to the disrupting effects of stress on the production of root products including flavonoids such as genistein. Such root products can activate the bacterial genes resulting in the production of lipochitooligosaccharides and hence some morphological alteration in plant roots, including bulging and curling. It has been indicated that if *Bradyrhizobium* bacteria are preincubated with genistein, before inoculating soybean seeds, the adverse effects of stressors on the process of N-fixation can be alleviated.

Interactions with Other Soil Microbes

Plant growth promoting rhizobacteria may interact with AM fungi through binding to the fungal spore, production of some volatiles by bacteria, injection of some products into the fungal spore and degrading fungal cell wall of the bacteria. Such products can influence fungi performance and hence ecosystem efficiency by affecting the expression of fungal genes .Such interactions can be of significant importance affecting the interactions between the microbes and hence their use for biological fertilization. It is because the selection of the right microbes for the production of bioinoculants can significantly increase their efficiency.

III ADVANTAGES OF PHYTOREMEDIATION

Early research indicates that the phytoremediation technology is a promising cleanup solution for a wide assortment of pollutants and sites. (Chappell 1997). A momentous benefit of phytoremediation is that a variety of organic and inorganic compounds are amenable to the phytoremediation process. Phytoremediation can be used either as an in situ or ex situ application. In situ applications are frequently considered because reduces disturbance of the soil and surrounding environment and minimize the spread of contamination via air and waterborne wastes. An additional advantage of phytoremediation is that it is a green technology and when appropriately implemented is both environmentally friendly and aesthetically pleasing to the public

IV CONCLUSION

Today the soil pollution with heavy metals is a precarious problem for environments. Many techniques used to resolve this problem, but these techniques are a costly and less effective, so the process phytoremediation, it has green process to accumulate heavy metal with the help of plants it has safe and polluted free process. Plants have a power to clean up the environment because they need some metals for its growth so they consumed that metal easily. Phytoremediation is unaffected to people who live and work around the area while it is being cleaned up and is perceived as a more natural solution than large amounts of equipment and noisy machinery. Phytoremediation can be undertaken for less than half the cost of other technologies. Phytoremediation projects need a lesser amount of continuance (Smith 1997). Present study clearly reveals that the ornamental plants are used to clean up the heavy metals from soil by phytoremediation are a polluted frees process and cost effective. Phytoremediations related mechanism play a better role for made polluted free soil water environment. The key problem to almost to any of the methods is of course that of time and inability to handle a diverse and very large amount of contamination. As the technology is better understood and further implemented, however, it will grow in its efficiency and ability and will no doubt grow.

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