Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2021; 9(1): 1045-1050

Received: 18-11-2020
Accepted: 23-12-2020

Rajveer Kaur
Research Scholar, College of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Science University, Ludhiana, Punjab, India

Navdeep Sidhu
Assistant Professor, Department of Biotechnology, Sri Guru Gobind Singh College, sec 26, Chandigarh, India

Gurjot Kaur Mavi
Assistant Professor, Department of Veterinary Extension, College of Veterinary Sciences, Guru Angad Dev Veterinary and Animal Science University, Ludhiana, Punjab, India

Corresponding Author:
Rajveer Kaur
Research Scholar, College of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Science University, Ludhiana, Punjab, India

Estimation and removal of heavy metal from industrial sludge by bacterial strain

Rajveer Kaur, Navdeep Sidhu and Gurjot Kaur Mavi

Abstract
Sludge refers to the residual, semi-solid material left from industrial wastewater or sewage treatment processes. The present investigation for the bioremediation of sludge the samples were collected from different places near Jalandhar city of the Punjab (India). Experimental studies revealed a broad range in metal extraction efficiencies of the different extraction technologies. In order to analyse the sludge, the physiochemical parameters like pH, Moisture and heavy metal are determined. The bacterial strain Pseudomonas aeruginosa was screened for the removal of heavy metal like LEAD and COPPER from the industrial sludge. The effect on pH and moisture was determined. Maximum lead removal was noted to be 2.09 by Pseudomonas aeruginosa species from the sludge sample and copper removal was noted to be 2.65 by *P. aeruginosa* species. The present study depicts that the bacterial species remove heavy metal from sludge and can be used for the industrial waste management and other environmental maintenance.

Keywords: heavy metal, *Pseudomonas aeruginosa*, bioremediation, industrial sludge

Introduction
Sludge refers to the residual, semi-solid material left from industrial wastewater or sewage treatment processes. It can also refer to the settled suspension obtained from conventional drinking water treatment and numerous other industrial processes. Sludge can be used for agriculture, land restoration, soil improvement and for construction materials such as cement and bricks but after proper treatment. Suspended solids are the most visible of all impurities in waste water and may be either Organic or inorganic in nature. The active sludge is generated from synthesis of influent organic material. The micro-organisms in the activated sludge system are composed of a very large number of species of bacteria, fungi and protozoa. Depending on the operational conditions, more complex organisms like ciliates and rotifers may also be present. The composition of the active sludge may differ considerably from one system to the other, depending on the nature of the influent waste water and the operational conditions and the inactivated sludge. The inert sludge and endogenous residue fraction is generated from the accumulation of particulate non-biodegradable organic material present in the influent. This material is flocculated and becomes part of the solid phase, forming the inert fraction. The endogenous residue has its origin in the decay of living bacteria cells, a process occurring continuously in the activated sludge system. During the decay process of the active sludge, part of the microbial mass is oxidized in a process called endogenous respiration. Bioremediation has become an important method for the restoration of oil-polluted environments by the use of indigenous or selected microbial flora. Several factors such as aeration, use of inorganic nutrients or fertilizers and the type of microbial species play a major role in the remediation of oil-contaminated sites [1]. Bioremediation is the process of utilizing living organisms, microorganisms to degrade pollutants and contaminants from the environment and transform them into less toxic form. Bioremediation is based on the ability of a microorganism to degrade the hydrocarbons into components that can be taken up by other micro-organisms as a nutrient source or can be safely returned to the environment [2]. An effective bioremediation requires enzymatic attack by microorganisms to convert pollutants into harmless products. Environmental parameters should be optimum to help the microorganisms to grow and degrade the pollutants at a rapid rate [3]. The indigenous microorganisms normally carry out bioremediation and their activity can be enhanced by a more suitable supply of nutrients and/or by enhancing their population. Therefore, this process exploits such microorganisms and their enzymatic activities to effectively remove contaminants from contaminated sites.

This process is a cost effective means of cleanup of hydrocarbon spills from contaminated sites as it involves simple procedures only and it is an environmentally friendly technology which optimizes microbial degradation activity via control of the pH, nutrient balance, aeration and mixing. Also, bioremediation is a versatile alternative to physicochemical treatments and produces non-toxic end products such as CO2, water and methane from petroleum hydrocarbons. Bioremediation has been proven to be an effective, environmentally friendly and less expensive treatment option for remediation of aquifers contaminated with hydrocarbons [4, 5, 6].

In situ bioremediation: The most effective means of implementing in situ bioremediation depends on the hydrology of the subsurface area, the extent of the contaminated area and the nature of the contamination. In general, this method is effective only when the subsurface soils are highly permeable, the soil horizon to be treated falls within a depth of 8-10 m and shallow groundwater is present at 10 m or less below ground surface.

On site (ex site) bioremediation Here the contaminated soil is excavated and placed into a lined treatment cell. Thus, it is possible to sample the site in a more thorough and, therefore, representative manner. On site treatment involves land treatment or land farming.

Material and Methods

The study was carried out at Biotechnology Laboratory, Department of Biotechnology, CT Institution of Pharmaceutical Sciences Jalandhar.

Sample Collection

Sludge samples were collected from different industrial drains like Maqsudan, Kala Singhia, Chaheru, Basti Bawa and Hamira near Jalandhar City.

Different parameters of sludge

1. Moisture test
2. pH test
3. Lead test
4. Copper test
5. Phosphorus test
6. Solid test

Analysis of sludge sample

The physical chemical parameters (pH, color, moisture, phosphorus and heavy metal like lead and copper) were determined. Ph is determined by electronic digital ph meter. Phosphorus was determined by the colorimeter. lead was determined by colorimeter and Copper was determined by colorimeter for metal analysis the effluent sample were digested with HNO3.

Estimation of lead: In the present study we have used Dithizone method for Lead estimation. An acidified sludge sample containing microgram quantities of lead is mixed with ammonium citrate-cyanide reducing solution and extracted with dithizone in chloroform (CHCl3) to form a cherry-red lead dithizonate. The color of the mixed color solution is measured photo metrically. Sample volume taken for analysis may be 2L when digestion is used.

In a weekly ammonium cyanide solution (pH 8.5 to 9.5) dithizone forms colored complexes with bismuth, stannous tin and monovalent thallium. In strongly ammonia citrate cyanide solution (pH 10 to 11.5) the dithizonates of these ions are unstable and are extracted only partially. This method uses a high pH, mixed color and single dithizone Extraction. Interference from stannous tin and mono-valent thallium is reduced further when these ions are oxidized during preliminary digestion. A modification of the method allows detection and eliminate of bismuth interference. Excessive quantities of bismuth, thallium and tin may be removed. Dithizone in CHCl3 absorbs at 510nm; control its interference by using nearly equal concentrations of excess dithizone in samples, standards and bland. The method is without interference for the determination of 0 to 30µg Pb in the presence of Tl+, 100 µg Sn2+, 200µg ln3+ and 1000µg each of Ba2+, Cd2+. Cp2+, Cu2+, Mg2+, Mn2+, Hg2+, Sr2+, Zn2+, Al3+, Sb3+, As3+, Cr3+, Fe3+, V3+, PO4.

Copper estimation

Neocuproine Method

Sampling and storage:-Copper ion tends to be absorbed on the surface of sample containers. Therefore, analyses samples as soon as possible after collection. If storage is necessary, use 0.5 ml 1+1 HCl/100 ml samples, or acidify to pH 2 with HNO3, to prevent this adsorption

- A 25mL volumetric flask, taking care not to transfer any of the aqueous layer. Repeat extraction of the water layer with an additional 10mL CHCl3 and combine extracts. Dilute combined extracts to 25mL with CH3OH, stopper, and mix thoroughly. Preparation of calibration curve.Added20gm of sludge into a 125mL separatory funnel for use as a reagent blank. Prepare standards by pipetting 1 to 10mL (20.0 to 200µg Cu) standard copper solution into a series of 125mL separatory funnels, and dilute to 50mL with water. Add 1mL of conc. H2SO4 and use the extraction procedure. Construct a calibration curve by plotting absorbance versus micrograms of copper. To prepare a calibration curve for smaller amounts of copper, dilute 10mL standard copper solution through the previously described procedure, but use 5cm cells to measure absorbance.
- Treatment of sample: Transfer 100mL sample to a 250mL beaker, add 1mL conc. H2SO4 and 5mL conc. HNO3. Add a few boiling chips and cautiously evaporate to dense white SO3 fumes on a hot plate. If solution remains colored, cool, add another 5mL conc. HNO3, and again evaporate to dense white fumes. Repeat, if necessary, until solution becomes colourless. Cool, add about 80mL water, and bring to a boil. Cool and filter into a 100mL volumetric flask. Make up to 100mL with water using mostly beaker and filter washings. Pipette 50mL or other suitable portion containing 4 to 200µg Cu, from the solution obtained from
- Preliminary treatment:-, into a 125mL separatory funnel. Dilute, if necessary, to 50mL with water. Add 5mL NH3OH.HCl solution and 10mL sodium citrate solution, and mix thoroughly.
- Adjusted pH to approximately 4 by adding 1mL increments of NH4OH until pH test paper indicates a value between 4 and 6.
- Added 10mL neocuproine reagent and 10mL CHCl3. Stopper and shake vigorously for 30s or more to extract the copper neocuproine complex into the CHCl3 layer into

- Transferred an appropriate portion of extract to a suitable absorption cell (1cm for 40 to 200 µg Cu, 5cm for lesser amounts) and measure absorbance at 457nm or with a 450 to 460nm filter.
- Use a sample blank prepared by carrying 50mL water through the complete digestion and analytical procedure.

Phosphorus test

Determining phosphate-phosphorus is the Ascorbic Acid Procedure. The procedure is suitable for concentrations of 0.01 to 6 mg/L PO4.Ammonium molybdate and sulfuric acid solution react in acidic solution which can be reduced by ascorbic acid to form an intense blue color.

For the bioremediation of lead sludge sample were collected from the dumping site and nearby area of textile industries.

Micro organism used for bioremediation

Pseudomonas aeruginosa (ATCC NO.2453)

KINGDOM BACTERIA
PHYLLUM PROTEOBACTERIUM
CLASS GAMMA PROTEOBACTER
ORDER *Pseudomonadace*
FAMILY *Pseudomonadaces*
GENUS *Pseudomonas*
SPCIES *P.aeruginosa*
(STRAIN (ATCC 2453) Rifampicin bacterial strain)

Revival of lyophlized culture

From pre-scored ampules

Disinfect the sample by wiping with 70% alcohol. Wrap the scored area (arrow at the narrow neck below the gold colored band) with the ethanol dampened tissue to protect your fingers. The tissue should not so wet that alcohol enters the ampoule. Bend and break the ampoule at the narrow, pre scored area. The alcohol damped tissue provides good cushioning and protection against cuts for this step. Aseptically added 0.2-0.5 ml of sterile water. Using a sterile pipette gently aspired the contents several times to mix the suspension thoroughly. Let the suspension to rest for 15-30 minutes. Inoculate the suspension onto an appropriate medium and incubate.

Results and Discussion

The sample was taken from various drains surrounding Jalandhar and as well as from the dumping site of industries. Then they were analyzed for physico-chemical properties such as pH, moisture content, phosphorus and heavy metal like lead and copper). The pH of the sludge was varied according to their origin ranging between 5.6-8.9. The ph was determined by using calibrated pH meter. The higher value of moisture and solid were observed in sample collected from Hamira and Kala Sanghian drains. The bacterial cultures exhibited removal even at higher levels of lead and the bacterial growth decreased with increase in the metal concentration. Similarly, sludge samples were analyzed for heavy metals. Nine different bacterial species were screened on the basis of morphological characteristics which grew in 10-50 mg/l of lead concentration. After screening, *Pseudomonas aeruginosa was* found capable to remove lead and used for further study. It showed consistent growth, both in nutrient broth and nutrient. The data was observed for the uptake of metal ions vs contact time for different conc. The metal removal efficiency increased with increase in time. However, a remarkably increased in percent lead removal was estimated 75.0 ± 2.27% by *Pseudomonas species.* Different concentration of broth were used like 10mg/l, and 69.70 ± 0.80% removal by *Pseudomonas aeruginosa* at 40mg/l and 90.88 ± 0.87 % by *Pseudomonas aeruginosa. Pseudomonas aeruginosa* removed considerable amount of lead and showed significant efficiency for bioremediation. From the above results it is observed that *Pseudomonas aeruginosa* can be used for the removal of lead from waste generated by industries. Further study can be carried out different concentrations and the strain can be selected for further removal of lead from effluent and sludge. pH of active sludge effluent was 8.0 and atmospheric temperature was 25°C, while ambient temperature was 20°C. Several mesophilic gram-negative and copper resistant bacteria were also isolated. The enrichment media showed better growth in comparison to direct culture method for the isolation of copper resistant bacteria and less time was taken by the organisms. Also, the isolates in primary enrichment method could grow on 6 mM concentration of Coppercontaining medium. Majority of the bacterial isolates were belonging to gram-negative non-fermentive *Pseudomonas* (4 isolates). One gram-negative coccus was also capable to grow on 2mM concentrations of Copper; but on subsequent inoculation, the strain lost its ability to grow on more than 2ml copper. The data obtained in this study clearly shows that with use of cadmium resistant mutated biomass, bioaccumulation of Copper solution considerably increased. *P. aeruginosa*one of the isolate was able to efficiently remove 94.7% in 30 mg/L of coppersolution within 60 min. cadmium toxicity. Bio-chemical tests were performed to characterize microorganisms on basis of morphological and biochemical properties. The tests performed in the present study were Gram staining, Citrate utilization test, H_2S production, Nitrate reduction test, Indole test, Methyl red test.

Oleszkiewicz *et al.* (1993) [7] described the moisture content by dry method of sludge in which sludge drying is really a necessity, through discussing the results of sludge drying, the process of sludge drying. Yoshizaki *et al.* (2000) [8] worked on Principle and Process of Heavy Metal Removal from Sewage Sludge. The sufficient removal of heavy metals from sewage sludge remains to be achieved. Yang *et al.* (2005) [9] discussed about the potential adsorbent for phosphorus removal Alum sludge refers to the byproduct from the processing of drinking water in Water Treatment Works. Zheng *et al*. (2009) [10] described the Sludge Phosphorus Tests in Phosphorus is an essential element for plant growth and development, as it plays key roles in plant metabolism, structure and energy transformation.Nezhad Kermani *et al*. (2010) [11] describe that Lead bioremediation by metal-resistant mutated bacteria isolated from active sludge of industrial effluent in which Bioremediation of metal pollutants from industrial wastewater using metal resistant bacteria is a very important aspect of environmental biotechnology. Krishnaveni1 *et al.* (2013) [12] explained that bioremediation of steel industrial effluents using sludge microorganisms in which Bioremediation is treatment processes that uses naturally occurring microorganisms as well as plants to breakdown,or degrade hazardous substances into less toxic or nontoxic substances. Ghazali *et al.* (2004) [13] investigated the bioremediation of hydrocarbon in contaminated soils by mixed cultures of hydrocarbon-degrading bacteria. The strains were selected based on the criteria that they were able to display good growth in crude oil, individual hydrocarbon compounds or both. Their ability

to degrade hydrocarbon contamination in the environment was investigated using soil samples that were contaminated with diesel, crude oil 52 or engine oil. Vezzulli *et al.* 2004 [14] evaluated the potential of bioremediation for mobilization of carbon in organic-rich sediments. Both bioaugmentation (bio-fixed microorganisms) and bio-stimulation (oxygen release compounds ORC) protocols had been tested and the response of the bacterial community has been described to assess the baseline for bioremediation potential. Mrayyan & Battikhi (2005) [15] described bioremediation as cost-effective, environmentally friendly treatment for oily contaminated sites by the use of microorganisms. In their study, laboratory experiments were conducted to establish the performance of bacterial isolates in degradation of organic compounds contained in oily sludge from the Jordanian Oil Refinery plant. As a result of the laboratory screening, three natural bacterial consortia capable of degrading total organic carbons (TOC) were prepared from isolates enriched from the oil sludge. Shuchi *et al.* (2006) [16] tested the ability of three bacterial strains, Bacillus sp. SV9, Acinetobacter s., SV4 and Pseudomonas sp. SV17 from contaminated soil in Ankleshwar, India to degrade the complex mixture of petroleum hydrocarbons (such as alkanes, aromatics, resins and asphaltenes), sediments, heavy metals and water known as oily sludge. Margesin *et al.* (2005) [17] evaluated soil biological activities as a monitoring instrument for the decontamination process of a mineral oil contaminated soil was made using measurements of microbial counts, soil respiration, soil biomass and several enzyme activities.

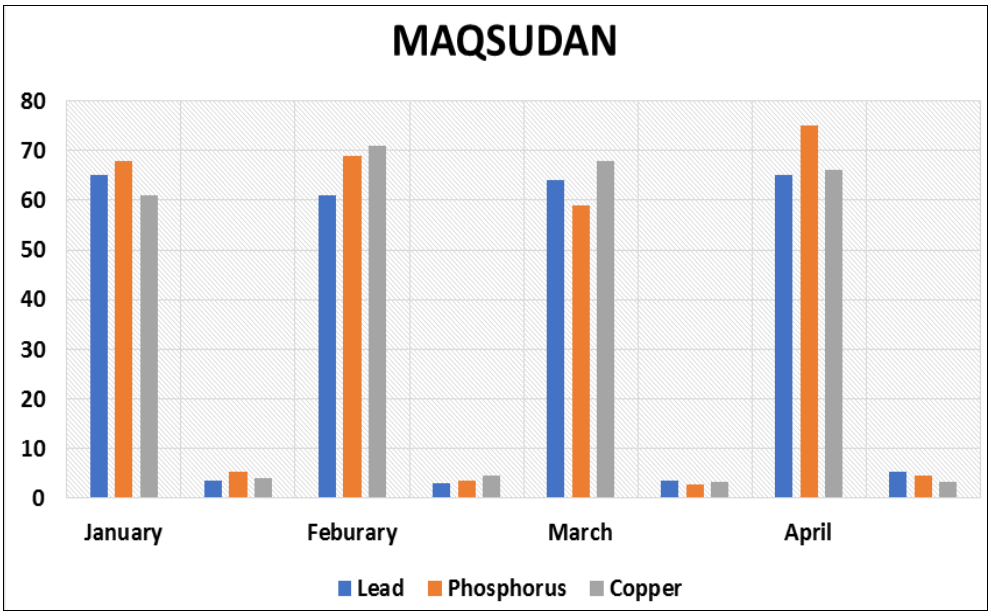

Fig 1: Removal of heavy metal before and after bioremediation

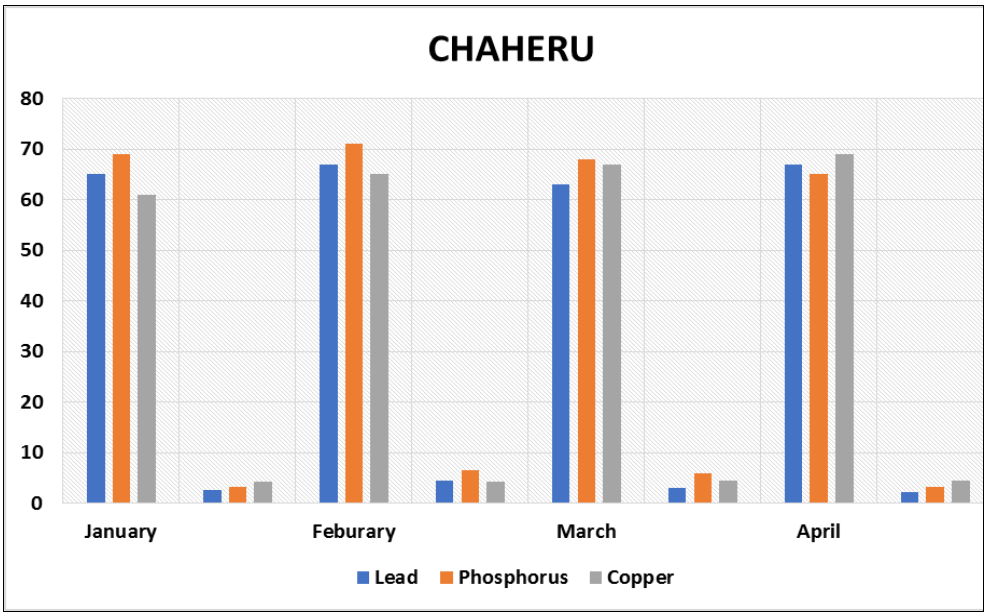

Fig 2: Removal of heavy metal before and after bioremediation

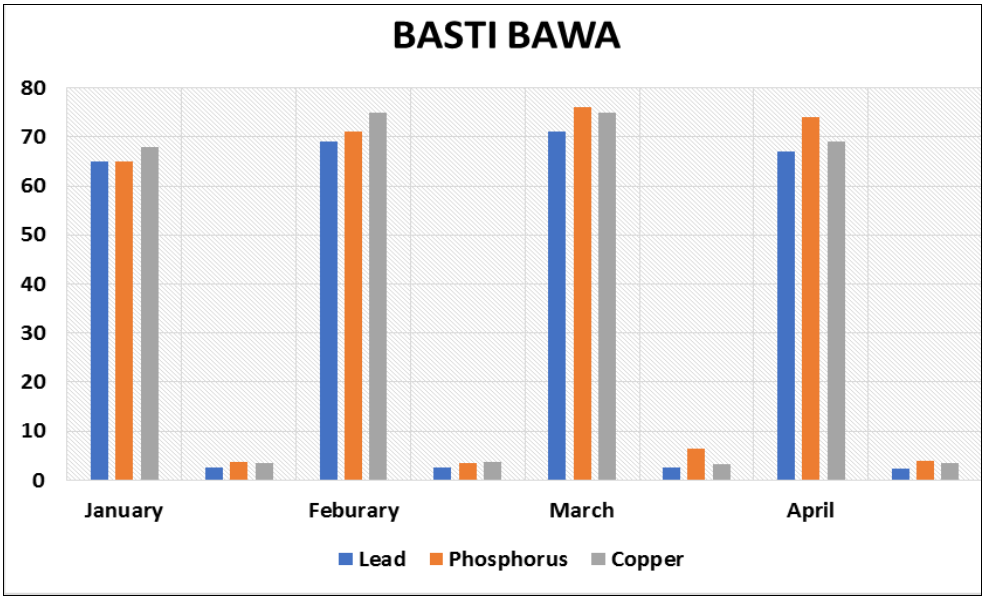

Fig 3: Removal of heavy metal before and after bioremediation

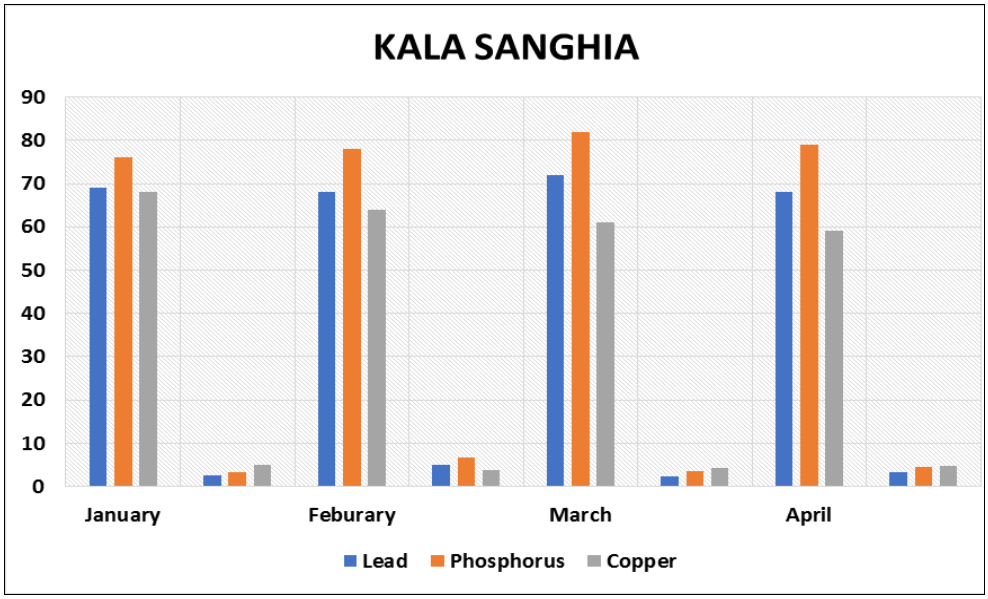

Fig 4: Removal of heavy metal before and after bioremediation

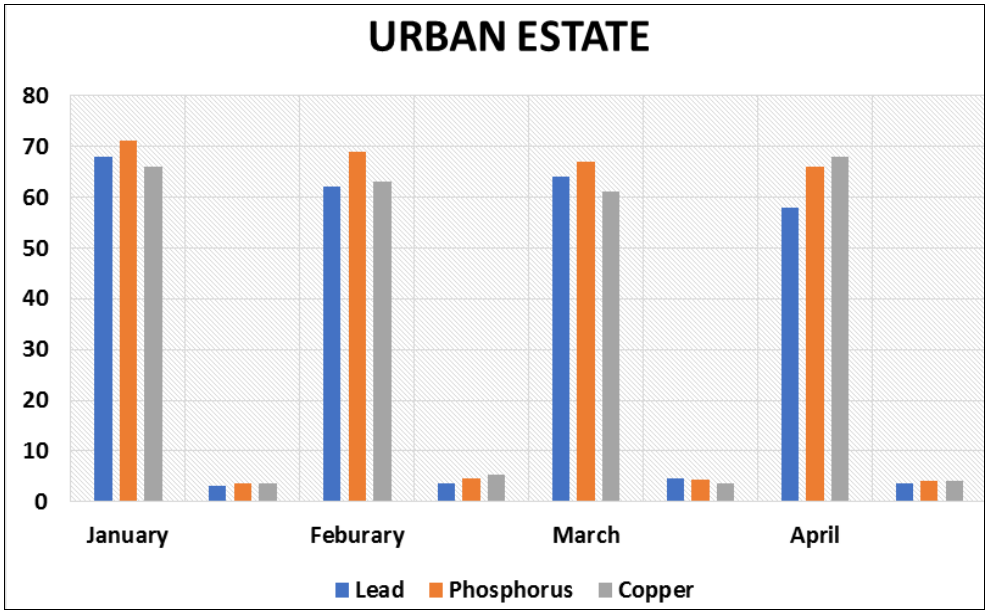

Fig 5: Removal of heavy metal before and after bioremediation

Conclusion

It can be concluded from the present study "Bioremediation of sludge using *Pseudomonas aeruginosa* strain that *Pseudomonas aeruginosa* has great potential to remove the heavy metals like lead and copper from the sludge sample. The strain of *Pseudomonas aeruginosa* can be successfully used for the removal of lead, copper, cadmium and chromium. These bacteria were found very effectively in bioremediation of heavy metal because metals are directly and indirectly involved in the all aspect of microbial growth metabolism. Bioremediation of heavy metal by bacterial cell has been recognized as potential alternative to existing technologies for the removal of heavy metal from the industrial waste. This is an attempt to explore a new innovative, cost effective and environment friendly technology for the bioremediation of sludge containing heavy metals as contaminants by using microorganisms.

Acknowledgement

I would like to acknowledge the Head of the Department, Dr. Navdeep Kaur Sidhu for providing facilities to conduct the research work.

References

1. Vasudevan N, Rajaram P. Bioremediation of oil sludge-contaminated soil. Environ Int 2001;26(5-6):409-411.
2. Kaur R, Mavi GK, Raghav S. Bioremediation of Sludge using *Pseudomonas aeruginosa*. Int. J Curr. Microbiol. App. Sci 2019;8(04):69-79.
3. Cueva Dl, Rodríguez SC, Cruz CH, Contreras NOS, Miranda JL. Changes in Bacterial Populations During Bioremediation of Soil Contaminated with Petroleum Hydrocarbons. Water, Air, & Soil Pollution 2016;227(3):1-12.
4. Chen JM, Hao OJ. Microbial chromium (VI) reduction. Crit. Rev. Environ. Sci. Technol 1998;28(3):219-251.
5. Jardine PM, Taylor DL. Kinetics and mechanisms of Co (II) EDTA oxidation by pyrolusite. Geochim. Cosmochim. Acta 1995;59:4193-4203.
6. Ganguli A, Tripathi AK. Bioremediation of toxic chromium from electroplating effluent by chromate-reducing *Pseudomonas aeruginosa* A2Chr in two bioreactors. Appl. Microbiol. Biotechnol 2002;58:416-420.
7. Oleszkiewicz JA, Elektorowiz M. Groundwater contamination with trichloroethylene: the problem and some solutions. Journal of Soil Contamination 1993;2(3):205-228.
8. Yoshizaki S, Tomida T. Principle and Process of Heavy Metal Removal from Sewage Sludge Environ. Sci. Technol 2000;34(8):1572–1575. DOI: 10.1021/es990979s.
9. Zhang FS, Nriagu JO, Itoh H. Mercury removal from water using activated carbons derived from organic sewage sludge. Water Res 2005;39:389-395.
10. Zheng X, Kong H, Wu D, Wang C, Li Y, Ye H. Phosphate removal from source separated urine by electrocoagulation using iron plate electrodes. Water Sci Technol 2009;60:2929-2937.
11. Kermani JN, Ghasemi F, Khosravan A, Farahmand A, Shakibaie MR. Cadmium bioremediation by metal-resistant mutated bacteria isolated from active sludge of industrial effluent. Iran. J Environ. Health. Sci. Eng. 2010;7(4):279-286.
12. Krishnaveni R, Devi YP, Rao SR. Bioremediation of steel industrial effluents using soil microorganisms. International Journal of Advanced Biotechnology and Research 2013;4(1):914-919.
13. Ghazali FM, Rahman NZA, Salleh AB, Basri M. Biodegradation ofhydrocarbons in soil by microbial consortium International Biodeterioration & Biodegradation 2004;54:61-67.
14. Vezzulli L, Pruzzo C, Fabiano M. Response of the bacterial community to in situ bioremediation of organic-rich sediments. Marine Pollution Bulletin 2004;49(9-10):740-751.
15. Mrayyan B, Battikhi MN. Biodegradation of total organic carbons (TOC) in Jordanian petroleum sludge. J Hazard Mater 2005;120(1-3):127-134.
16. Verma S, Bhargava R, Pruthi V. Oily Sludge Degradation by Bacteria from Ankleshwar. International Biodeterioration & Biodegradation 2006;57(4):207-213. DOI: 10.1016/j.ibiod.2006.02.004
17. Margesin R, Schinner F. Manual for Soil Analysis—Monitoring and Assessing Soil Bioremediation. Soil Biology 2005;5:359.