Bacillus aryabhattai: A Multi Metal Resistant Sewage Water Bacteria and Bioremediatory Tool for Sewage Water Pollutants

Sapna Kalra, Anita Bhatnagar*, Nisha Thakral
Department of Zoology, Kurukshetra University, Kurukshetra, Haryana (India) -136119

*Corresponding Author: anitabhatnagar@gmail.com

ARTICLE INFO

ABSTRACT

Sewage water contamination is a significant health risk factor that affects plant development, aquatic environment, and human life quality. Among different mitigation strategies, bioremediation is thought to be the most sustainable and cost-effective. Thus, the current study aimed at finding new bacteria with a high potential for reducing sewage water contaminants, as well as heavy metal sorption capabilities. Only one bacterium (Strain DDN) was selected from a large number of isolated bacteria due to its high proteolysis and antibacterial activity. This bacterium was identified using biochemical and 16S rDNA-based molecular analysis. The phylogenetic tree revealed the strongest resemblance to *Bacillus aryabhattai* strain QH 16-25 (here called as *Bacillus aryabhattai* DDN). Furthermore, the biodegradation capability of bacteria was assessed, and it was discovered that there was a significant reduction in certain pollution indicating parameters including BOD (65.81%), COD (58.02%), alkalinity (53.65%), hardness (56.91%), calcium (71.56%) ammonia (43.34%) and orthophosphate (40.37%) content within 14 days. Moreover, the concentration of heavy metals, such as Hg, Cd, Cr, Fe and Mn decreased up to 61.69, 60.77, 88.67, 24.69, and 81.95%, respectively, when compared to the initial concentration. Thus, this novel bacterium proved to be a multi metal tolerant and a potent bioremediatory tool for environment safeguard.

INTRODUCTION

Water plays a keystone role in the world economy and retains the living standards of all biota. It covers 71% of earth surface, of which 96.5% covers the saline water, while 1.7% is only available as fresh water which is also on the urge of depletion by the addition of toxic chemicals, agricultural runoff, heavy metals and many more effluents from varied industries **(Chandnani *et al.,* 2022)**. Among all these, dumping of untreated sewage water into waterways due to insufficient sewage treatment plants is one of the most hazardous activities since it diminishes the water quality of fresh water available on Earth. Untreated sewage and industrial effluents discharged into surface water bodies causing significant contamination, which in turn pose threat to water bodies **(Kulshrestha *et al.*, 1989; Wang *et al.,* 2021)**. Water pollution can be examined by changes in physical, chemical, and biological parameters, including color, organic/ inorganic contents, and microbial stress. Harmful compounds present in sewage water have properties of carcinogenicity, immunotoxicity and

mutagenicity **(Kuppusamy et al., 2020)**. Toxic heavy metals found in sewage are absorbed and accumulate in plant components as free metals. These metals go up the food chain and eventually reach humans **(Ghosh et al., 2018)**.

Traditional technologies are extremely costly for this purpose. There is a necessity for novel treatment technologies for the detoxification from wastewater. Therefore, finding the better adaptive and effective strategies are fundamental to clean the sewage water. Sewage treatment plants give three basic types of reclamation techniques that include physical (1˚), biological (2˚) and chemical (3˚) treatment. Biological and chemical treatment offers more effective treatment to the wastewater, however chemical treatments have some limitations. The chemicals used during treatment may remain intact, or sometimes intermediates of the reaction, becoming more dangerous and eventually reach humans, potentially causing drug resistance. This approach is not cost-effective. On the other hand, secondary treatment offers no ill effects of treatment as it employs the naturally occurring microorganisms to treat the sewage water. Furthermore, biological method is cost- effective **(Younas et al., 2021)**.

In recent years, microbial approaches for the wastewater degradation process to optimize different parameters under laboratory conditions have drawn the significant attention of many researchers around the world. This bioremediation technology involves biodegradation which completely breaks down organic materials into CO_2 and water. Microorganisms have been shown to biodegrade both industrial and home waste effluents. Adding microbes as bioenhancers to sewage treatment improves the efficiency for the BOD, COD, detergent, oil, and grease removal. Microbes, mainly bacteria, produce enzymes such as protease which is capable of breaking down complicated chemical molecules.

Chettri et al. (2021) reported the augmentation of potential hydrocarbon biodegraders on petroleum hydrocarbons sullied sites as propitious strategy to extend organic degradation of petroleum hydrocarbons. In addition to bioaugmentation, biostimulation with organic and inorganic substrates has also been reported for enhancing the biodegradation of petroleum hydrocarbons and/or other pollutants **(Zhang et al., 2017)**. In the past decades, several studies have been done on the bioremediation of sewage wastewater using strains, viz. *Arthobacter, Rhodobacter, Bacillus, Burkholderia, Pseudomonas* etc. that have the ability to utilize contaminants as a carbon and nitrogen source **(Deepanraj et al., 2016)**. Similarly, *Achromobacter denitrifacians* was isolated from industrial wastewater that has the potential to degrade para nitro-phenol **(S.M. et al., 2021)**. Textile effluent adapted *Aeromonas hydrophila* SK16 bacteria has a potential to decolorizing and mineralizing dyes, utilizing them as energy source **(Srinivasan & Sadasivam, 2021)**. These multifunctional bacteria such as *Pseudomonas*, *Flavobacterium*, *Methylotenera* and *Bacillus* are well known potential PAHs (Poly Aromatic Hydrocarbons) biodegraders which can use PAHs as their carbon and energy source **(Ma et al., 2021)**.

Lima e Silva et al. (2012) isolated bacterial strains from sewage water such as *Bacillus* sp. and *P. aeruginosa* to combat the toxic chemical impact of pollutants that were also resistant to Ag and Hg. **Ghosh et al. (2018)** suggested that such mechanisms might help to detoxify and remove heavy metals from contaminated environments. The probable mechanism of biodegradation includes four major processes. The first step is for bacteria to produce biosurfactants, which emulsify harmful substances. These emulsified contaminants are absorbed on the cell surface of microorganisms in the second stage. Later, this ingested

compound is internalized and eventually degraded by different enzymes. Bacteria-derived ligninolytic laccase enzymes catalyze the mono-electronic oxidation of substrates and degrade a wide spectrum of pollutants from factories **(Rahman *et al.,* 2013)**. Till date, various bacterial strains have been isolated showing varying capabilities for the dissolution of varied pollutants or heavy metals; thus, it is necessary to identify a single bacterial strain capable of reducing the majority of pollutants as well as heavy metals. Therefore, the current work focused on the isolation, screening, and characterization of protease producing, metal tolerant, and antimicrobial strains of bacteria for the evaluation of their potential for degradation of sewage water impurities for environmental safety.

MATERIALS AND METHODS

1. Collection of water samples and its physiochemical analysis

Sewage water samples were collected in pre-sterilized plastic bottles from DD colony sewage treatment plant (29.976766° N, 76.86490° E) located in Kurukshetra City, India, for physiochemical examination. Water quality parameters such as pH, BOD (biochemical oxygen demand), COD (chemical oxygen demand), DO (dissolved oxygen), alkalinity, hardness, calcium, magnesium, chloride concentration, TSS (total suspended solid), TDS (total dissolved solid), ammonia, nitrate, and orthophosphate were determined before and after bacterial treatment using APHA-standard procedures **(APHA, 2017)**. Heavy metals content was determined before and after the bacterial treatment by ICP-OES (Inductively Coupled Plasma-Optical Emission Spectroscopy) (SHIMADZU ICP-OES 9000).

2. Isolation and selection of bacterial strain

The procedure was centered on selecting the foremost potent bacterial strain competent of producing a protease enzyme, together with antibacterial activity against *E. coli* (procured earlier from Institute of Microbial Technology (IMTECH), Chandigarh. Isolation of bacteria was performed using spread plate and streak plate technique after serially diluting the sewage water sample. Biodegrading bacteria were selected on basis of protease production (on skim milk agar plate) and antimicrobial activity (against *E.coli*) possessing bacteria via a well diffusion assay **(Perez, 1990)**.

3. Identification of bacteria

The bacterial isolation samples were processed in the laboratory and characterized using cultural characterization, biochemical identification, and microscopic observations. The cultures were differentiated into Gram- positive and Gram- negative based on the stains (crystal violet and safranin) retained by the bacterial cells and their structural characterization under the microscope. The Schaeffer-Fulton`s method was used to observe endospore formation. The procedure employs malachite green as the primary stain and safranin to serve as counterstain. Various other biochemical tests, such as starch hydrolysis, glucose hydrolysis, urease test, catalase test, and motility testing were performed according to Bergey's manual of systematic **(Bacteriology, 1994)**.

4. 16S rDNA molecular characterization

For DNA extraction, the bacterial sample was scooped up and put in a mortar, then homogenized with 1ml of extraction buffer and transferred to a microfuge tube. The tubes were filled with an equivalent volume of phenol with chloroform and isoamyl alcohol (25:24:1) and gently shaken to blend well. The tubes were centrifuged at room temperatures at 14,000rpm for 15 minutes. The top aqueous phase was transferred to a separate tube and an equal amount of chloroform: isoamyl alcohol (24:1) was then added and stirred. The top aqueous phase, produced by centrifuging at room temperature for 10 minutes at 14,000rpm, was transferred to a fresh tube. The DNA was precipitated from the solution by adding 0.1 volume of 3M sodium acetate pH 7.0 and 0.7 volume of isopropanol. After 15min of incubation at room temperature, the tubes were centrifuged at 4ºC for 15min at 14,000rpm. The DNA pellet was washed twice with 70% ethanol, followed by 100% ethanol and then air dried. The DNA was dissolved in TE (Tris-Cl 10mM pH 8.0, EDTA 1mM). To remove RNA 5μl of DNAse free RNAse A (10mg/ ml) was added to the DNA. Further PCR amplification of 16S Gene in which 136ng of extracted DNA was used for amplification along with 10pM of each primer. Composition of TAQ Master MIX: 1) High-Fidelity DNA Polymerase 2) 0.5mM dNTPs 3) 3.2mM $MgCl_2$ 4) PCR Enzyme Buffer.

The 800bp test amplicon was purified by column purification, and bidirectional cycle sequencing was performed using forward and reverse primers on an ABI 3130 genetic analyzer employing the BDT Cycle sequencing kit.

16s Forward GGATGAGCCCGCGGCCTA

16s Reverse CGGTGTGTACAAGGCCCGG

Amplified product was then checked for its size and purity using agarose gel electrophoresis employing tris acetate EDTA buffer (pH 8.3). Sequences were aligned utilizing the system software aligner in phylogenetic tree builder, and BLAST analysis was used to compare the sequences to those accessible in the NCBI GenBank database. The first 10 sequences in the database with the highest similarity were chosen based on their maximum identity score and phylogenetic tree results.

5. Estimation of bioremediation potential of bacteria

To measure biodegradation capacity, the selected bacterial strain inoculum was generated by inoculating nutrient broth with bacterial colonies, which were then incubated in an incubator for 24 hours to allow the mother culture to grow. After centrifugation at 7000rpm for 15 minutes at 4˚C, the supernatant was discarded. The pellets were then suspended in 1% normal saline to achieve the appropriate optical density at 600nm. The concentration of the bacterial inoculum was selected on the basis of preliminary studies using the optical density of the bacterial solution at 600nm. OD_{600} of 0.5nm was used having bacterial count 4x 10^8CFU/ ml for removing pollutants from sewage water. Removal efficiencies of all parameters were calculated after the interval of 7 days according to the following equation **(El Bestawy *et al.,* 2014)**:

Removal Efficiency = C_i-C_f/C_f × 100

Where, C_i =Initial concentration before treatment,

C_f= Final concentration after treatment.

6. Statistical analysis

All experimental data was examined using analysis of variance (ANOVA), followed by the multiple range test (Duncan), with a level of significance of 5% for actual data. Statistical software SPSS 16.0 was used.

RESULTS

1. Isolation, screening, and characterization of bacteria

A total of 18 bacterial cultures (DDA-DDS) were isolated from sewage water sample and then underwent screening. Selected bacteria (DDN) showed both the protease activity by forming zone of proteolysis around bacterial growth (Fig. 1a), as well as the antimicrobial activity by forming zone of inhibition around it, as shown in Fig. (1b). Later, the morphological characterizations and biochemical characterization were performed, and data are shown in Tables (1, 2).
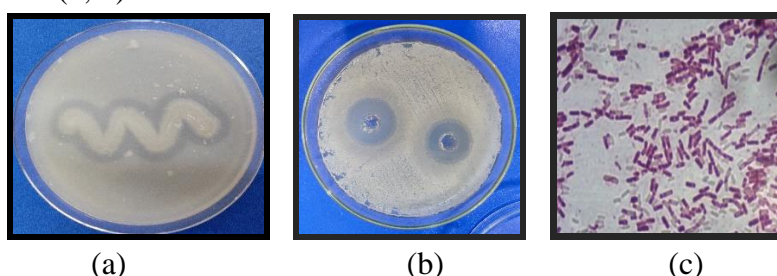

(a)   (b)   (c)

Fig. 1. (a): Zone of proteolysis formed by bacterial strain DDN;
(b): Zone of inhibition formed by bacterial strain DDN;
(c): Gram staining of bacterial strain DDN

Table 1. Morphological characteristics of the bacterial isolates

Sr. no	Morphological characteristic	Bacterial strain DDN
1	Color	White
2	Size (in mm)	4mm
3	Nature of colony	Rounded
4	Shape	Rod shaped
5	Gram nature	Gram positive
6	Motile/non motile	Motile

Table 2. Biochemical characterization of the bacterial isolates

Sr no.	Biochemical characterization	Bacterial strain DDN
1	Peroxidase	+
2	Starch hydrolysis test	+
3	Methyl red test	-
4	Vogues parker test	+
5	Lactose fermentation	-
6	Glucose fermentation	+
7	Maltose fermentation	+

Comparative BLAST analysis of the sequence obtained with the available database revealed that the isolated bacterial strain DDN showed closest similarity (99.57) with *Bacillus aryabhattai* strain QH 16-25(Accession no: MT539739.1), as shown in Fig. (2).
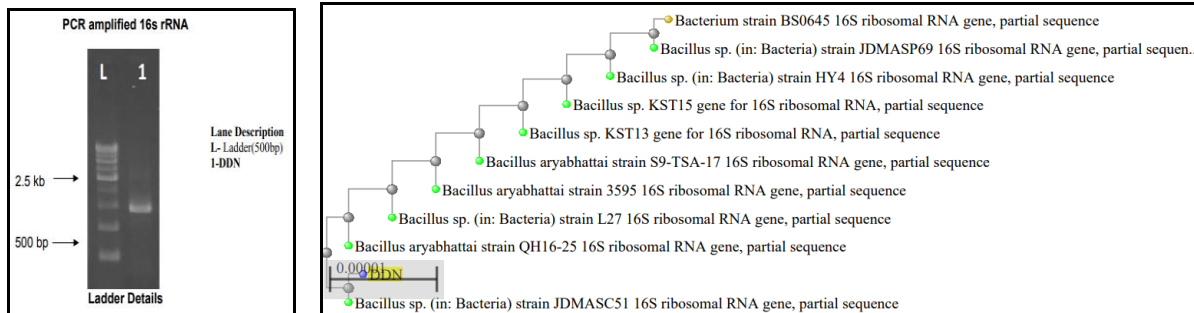

Fig. 2. Dendrogram comprising the phylogenetic tree of bacterial strain DDN showing relationship with other closely related strains acquired from NCBI GenBank database with their accession number along with the agarose gel electrophoresis image depicting lane L- Ladder (500bp) and 1-Sample DNA

2. Biodegrading potential of bacteria

Sewage water sample were examined on the 7th, 14th, and 21st days following bacterial inoculation, and compared to the initial findings to assess the ability of the bacterial strain to reduce pollutant load by calculating % removal efficiency. All the data were provided as means ± standard errors (Table 3). The pH of the samples ranged from 8 to 8.7 on different days. *Bacillus aryabhattai* strain DDN decreased the BOD concentration and COD content of untreated sewage water by up to 65 and 58%, respectively, after 21 days (Fig. 3). Chloride content decreased by up to 90%. When the days of incubation were increased, the rate of degradation was enhanced; nevertheless, it eventually slowed down due to the limited amount of organic matter left in it. Similarly, ammonia and orthophosphate decreased by up to 43 and 40%, respectively (Fig. 4).

Table 3. Mean ±SEM value of physiochemical parameter of sewage water before and after bacterial treatment along with the percent reduction in values after treatment

Physio- chemical parameter	Initial concentration (pre treated sewage water)	Control	Final concentration (after DDN bacterial treatment)	Percent reduction
BOD (mgL^{-1})	130.67±1.33^A	121.33±1.33^A	64±2.3^B	65.81
COD (mgL^{-1})	182.67±7.05^A	164.67±2.66^B	86±2^C	58.02
ALKALINITY (mgL^{-1})	447.33±4.67^A	388.67±5.69^B	214.67±5.3^C	53.65
CHLORIDE (mgL^{-1})	140.97±2.91^A	101.4±4.33^B	16.65±0.87^C	88.32
HARDNESS (mgL^{-1})	255.33±2.4^A	232.67±1.76^B	125.33±2.9^C	56.91
CALCIUM (mgL^{-1})	72.33±1.93^A	61.63±0.31^B	19.7±0.6^C	71.56
MAGNESIUM (mgL^{-1})	36.30±0.93^A	30.78±0.24^A	13.3±0.4^B	70.68
TDS (mgL^{-1})	580±20^A	540±5.77^B	216.67±3.3^C	62.06
TSS (mgL^{-1})	2880±36.05^A	2650±40.41^B	1190±40.4^C	56.13
AMMONIA (mgL^{-1})	3.8667±0.094^A	3.62±0.034^A	2.2±0.05^B	43.34
NITRATE (mgL^{-1})	2.60±0.042^A	2.48±0.02^B	2.27±0.07^C	13.52
ORTHOPHOSPHATE (mgL^{-1})	2.89±0.028^A	2.77±0.055^B	1.76±0.04^C	40.37

All values are mean ± S.E. of mean. Means with different letters in the same column are significantly (*P*< 0.05) different (Duncan's multiple range test).

As indicated in Table (4), the *B. aryabhattai* DDN strain has a significant reduction in heavy metal concentration, particularly in Cd, Cr, Hg, Mn, Ni, and Zn. Pb and Zn concentrations in sewage water samples that were not significantly lowered following bacterial treatment.
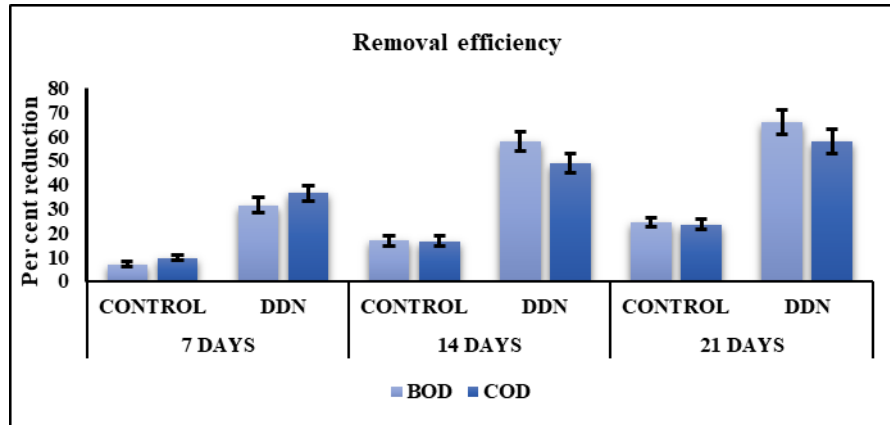

Fig. 3. Removal efficiency in terms of the percent reduction of BOD and COD in control and bacterial strain DDN treated sewage water sample
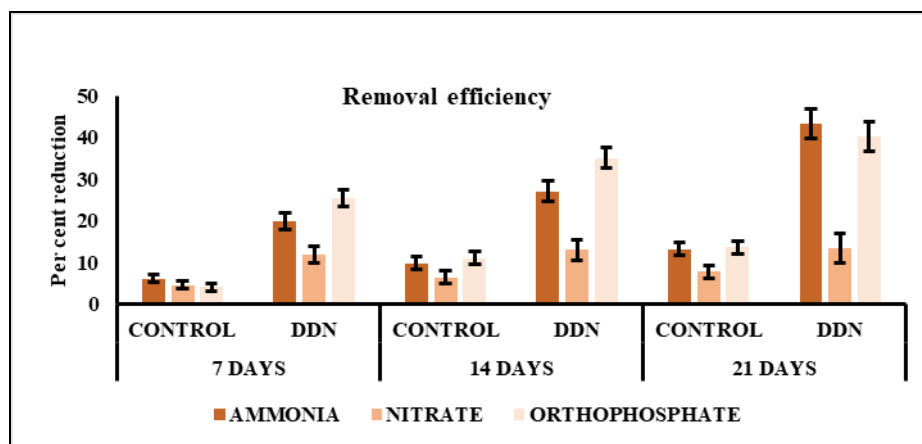

Fig. 4. Removal efficiency in terms of the percent reduction of ammonia, orthophosphate and nitrate contents in control and DDN treated sewage water sample

DISCUSSION

A significant amount of sewage water production and its improper treatment and release into aquatic bodies cause physiochemical parameters to change over the acceptable limit. The high concentration of toxins in sewage water has resulted in considerable contamination of the aquatic environment, and techniques are being developed to eliminate these compounds in an economical and environmentally friendly manner. Bacteria are key agent for environmental pollution remediation, with *Bacillus* sp. being regarded as one potential bioremediatory agent among them.

Table 4. Mean ± SEM of concentration of heavy metals in sewage water analyzed using ICP-OES before and after treatment, along with the percent removal efficiency

Heavy metal (ppb)	Initial concentration (Pre treated sewage water)	Control	Final concentration (After DDN bacterial treatment)	Percent reduction
Cd	0.039±0.0008[A]	0.02±0.001[B]	0.015±0.0017[C]	60.77
Cr	0.175± 0.002[A]	0.12± 0.01[B]	0.014±0.002[C]	88.67
Fe	0.817±0.002[A]	0.72±0.04[AB]	0.62±0.04[B]	24.69
Hg	0.201±0.002[A]	0.12±0.01[B]	0.08±0003[C]	61.69
Mn	1.64±0.011[A]	1.26±0.15[B]	0.3±0.03[C]	81.95
Ni	0.422±0.0015[A]	0.04±0.001[B]	0.04±0.003[B]	91.07
Pb	0.036±0.001[A]	0.03±0.003[A]	0.03±0.004[A]	22.22
Zn	0.395±0.001[A]	0.207±0.03[B]	0.21±0.04[B]	46.76

All values are mean ± S.E. of mean. Means with different letters in the same column are significantly ($P< 0.05$) different. (Duncan's multiple range test).

Thus, the current study illustrates the potential of using selected bioremediating bacteria to enhance wastewater quality by lowering BOD, COD, and heavy metal concentration. The mechanism underline biological treatment is the decomposition of finely dispersed matter, colloidal and dissolved substances by aerobic microorganisms. The current study reported that *Bacillus aryabhattai*, which is a native sewage water bacterium and is common to most of the wastewater, possesses biodegrading capability toward sewage water pollutants, along with heavy metal resistance. Earlier, *Bacillus aryabhattai* has been isolated from paper mill effluents, dye industry effluents and several other wastewaters and studied for resistance toward a single heavy metal, but in our study, it was isolated from sewage water and studied for pollution reduction potential and resistance toward various heavy metals. *B. aryabhattai* was selected because of its enzyme producing activities (protease, amylase, peroxidase) and antibacterial activity against coliform bacteria. After 21 days of bacterial treatment, a significant drop in BOD and COD content was observed in the sewage water sample. This might be related to the production of enzymes that help in the biodegradation of organic molecules. These results are reinforced in the study of **Paz and coworkers (2019)**, who isolated *B. aryabhattai* BA03 from paper and pulp mill effluent that has the potential to generate enzyme laccase and lignin peroxidase, which may be used as tool for cleaning effluents with a high content of phenolic compounds. *B. aryabhattai* also produces enzymes, such as superoxide dismutase and catalase, making it extremely resistant to an oxidative stress generated by O_2^- radicals **(Park *et al*., 2017)**. In the current study, it was noticed that, *B. aryabhattai* strain DDN decreased TDS levels in sewage water by up to 62%. This might be owing to the presence of enzymes like tyrosinase and chitinase generated by *B. aryabhattai*, as described by **Wang *et al*. (2021)**. Tyrosinase rTYR from *B. aryabhattai* demonstrated strong catalytic activity throughout a wide temperature and pH range, as well as great resistance to salt and chloride. Furthermore, rTYR displayed significant benefits in efficiently decolorizing a variety of dyes.

In accordance with **Krishnaswamy *et al*. (2009)**, *Bacillus* sp. is considered to be a phosphate solubilizing species that uses phosphate-containing chemicals as a source of carbon in synthetic phosphate-containing mediums. **Sunar *et al*. (2015)** isolated *Bacillus altitudinis* from soil, which is an influential phosphate biodegrading bacteria. Similarly, in

our treatments, phosphate and ammonia contents of the sewage water after bacterial inoculation reduced by up to 40 and 43%, respectively. This finding concur with the results of **Sonune and Garode (2015)**. They isolated *Bacillus* sp. from municipal wastewater and observed a reduction in BOD, COD, and phosphate content after treatment.

In addition, it has been observed that the strain *Bacillus aryabhattai* improves zinc absorption, resulting in enhanced maize development (**Mumtaz *et al.,* 2017**). Similarly, in our experiment, *B. aryabhattai* lowered the zinc concentration by a percentage up to 46. **Singh *et al.* (2016)** found that, *Bacillus aryabhattai* (NBRI014) has arsenic detoxification activities through intracellular As buildup and volatilization. The bacteria contain an As operon, with 7 differently expressed proteins that are up-regulated in bacterial cells after As exposure, which may play a role in lowering As toxicity within the bacterial cells. Similarly, the reduction in Cd concentration in our findings is verified by the outcome of **Liu *et al.* (2019)** who indicated that, the *B. aryabhattai* strain is resistant to Cd, therefore it aids in cadmium bioremediation. *B. aryabhattai* has also been studied for additional uses, including calcite dissolution (**Tamilselvi *et al.,* 2016**), glyphosate (herbicide) degradation (**Elarabi *et al.,* 2020**), and enzymatic degradation of synthetic pyrethroids (**Farooq *et al.,* 2022**). Thus, *B. aryabhattai* represents a multi-metal tolerant bacterium that decreases the BOD and COD contents of sewage water, making it an emerging bioremediation agent for pollutants found in sewage water.

CONCLUSION

Sewage water comprises an extensive variety of microorganisms that have an inherent ability to remove pollutants from the sewage water over time. Thus, in the current investigation, a novel bacterial strain, *B. aryabhattai* DDN*,* was isolated from sewage water samples and examined for the production of several enzymes that allow the bacteria to dissolve the majority of contaminants and lower BOD, COD, and other parameters. Additionally, this bacterium demonstrated heavy metals' tolerance and the capacity to lower their concentration in sewage water by disinfecting it. Further studies require more evaluation to determine its optimal operating conditions.

REFERENCES

APHA (American Public Health Association) (2017). Standard methods for the examination of water and waste water. 21th edn. American Public Health Association, New York.

Bergey, D.H. (1994). Bergey's manual of determinative bacteriology. Lippincott Williams & Wilkins.

Chandnani, G.; Gandhi, P.; Kanpariya, D.; Parikh, D. and Shah, M. (2022). A comprehensive analysis of contaminated groundwater: Special emphasis on nature-ecosystem and socio-economic impacts. Groundwater for Sustainable Development., 19: 100813. https://doi.org/10.1016/j.gsd.2022.100813

Chettri, B.; Singha, N.A. and Singh, A.K. (2021). Efficiency and kinetics of Assam crude oil degradation by *Pseudomonas aeruginosa* and *Bacillus sp*. Archives of Microbiology, 203: 5793-5803.

Deepanraj, B.; Lawrence, P.; Sivashankar, R. and Sivasubramanian, V. (2016). Analysis of pre-heated crude palm oil, palm oil methyl ester and its blends as fuel in a diesel engine. International Journal of Ambient Energy, 37(5): 495-500. https://doi.org/10.1080/01430750.2015.1004106

El Bestawy E.; Ahmed A.H.; Amer, R. and Kashmeri, R.A. (2014). Decontamination of domestic wastewater using suspended individual and mixed bacteria in batch system. Journal of Bioremediation & Biodegredation, 5(5): 1. http://dx.doi.org/10.4172/2155-6199.1000231

Elarabi, N.I.; Abdelhadi, A.A.; Ahmed, R.H.; Saleh, I.; Arif, I.A.; Osman, G. and Ahmed, D.S. (2020). *Bacillus aryabhattai* FACU: A promising bacterial strain capable of manipulate the glyphosate herbicide residues. Saudi journal of biological sciences, 27(9): 2207-2214. https://doi.org/10.1016/j.sjbs.2020.06.050

Farooq, H.; Khalid, M. and Hashmi; I. (2022). Bioremediation of Synthetic Pyrethroid by Hydrolases of *Bacillus aryabhattai* and *Bacillus circulans* Derived from Indigenous Soil. Journal of Environmental Treatment Techniques, 10(3): 187-194. https://doi.org/10.47277/JETT/10(3)194

Ghosh, P.K.; Maiti, T.K.; Pramanik, K.; Ghosh, S.K.; Mitra, S. and De, T.K. (2018). The role of arsenic resistant *Bacillus aryabhattai* MCC3374 in promotion of rice seedlings growth and alleviation of arsenic phytotoxicity. Chemosphere, 211: 407-419. https://doi.org/10.1016/j.chemosphere.2018.07.148

Krishnaswamy, U.; Muthusamy, M. and Perumalsamy, L. (2009). Studies on the efficiency of the removal of phosphate using bacterial consortium for the biotreatment of phosphate wastewater. Eur. J. Appl. Sci, 1(1): 6-15.

Kulshrestha, S.K., Adholia, U.N., Bhatnagar, Anita and Khan, A.A. (1989). Studies on pollution in river Kshipra: Zooplankton in relation to water quality. International Journal of Ecology and Environmental science,15: 27-36.

Kuppusamy, S.; Maddela, N.R.; Megharaj, M.; Venkateswarlu, K.; Kuppusamy, S.; Maddela, N.R.; Megharaj, M. and Venkateswarlu, K. (2020). Ecological impacts of total petroleum hydrocarbons. Total Petroleum Hydrocarbons: Environmental Fate, Toxicity, and Remediation, 10: 95-138.

Lima e Silva, A.A.D.; Carvalho, M.A.; de Souza, S.A.; Dias, P.M.T.; Silva Filho, R.G.D.; Saramago, C.S.; Bento, C.A. and Hofer, E. (2012). Heavy metal tolerance (Cr, Ag and Hg) in bacteria isolated from sewage. Brazilian Journal of Microbiology, 43: 1620-1631. https://doi.org/10.1590/S1517-83822012000400047

Liu, J.S.; Xie, X.C.; Luo, Y.L.; Deng, B.W.; Bai, Q.Y.; Yan, M.C. and Bai, X. (2019). Isolation, identification, and biological characteristics of a cadmium-resistant endophytic bacteria *Bacillus aryabhattai*. Biotechnology Bulletin, 35(2): 64. https://doi.org/10.13560/j.cnki.biotech.bull.1985.2018-0803

Ma, X.; Li, X.; Liu, J.; Cheng, Y.; Zou, J.; Zhai, F.; Sun, Z. and Han, L. (2021). Soil microbial community succession and interactions during combined plant/white-rot

fungus remediation of polycyclic aromatic hydrocarbons. Science of The Total Environment, 752: 142224. https://doi.org/10.1016/j.scitotenv.2020.142224

Mumtaz, M.Z.; Ahmad, M.; Jamil, M. and Hussain, T. (2017). Zinc solubilizing *Bacillus spp.* potential candidates for biofortification in maize. Microbiological research, 202: 51-60. https://doi.org/10.1016/j.micres.2017.06.001

Park, Y.G.; Mun, B.G.; Kang, S.M.; Hussain, A.; Shahzad, R.; Seo, C.W.; Kim, A.Y.; Lee, S.U.; Oh, K.Y.; Lee, D.Y. and Lee, I.J. (2017). *Bacillus aryabhattai* SRB02 tolerates oxidative and nitrosative stress and promotes the growth of soybean by modulating the production of phytohormones. PLoS One, 12(3): 0173203. https://doi.org/10.1371/journal.pone.0173203

Paz, A.; Costa-Trigo, I.; Tugores, F.; Míguez, M.; de la Montaña, J. and Domínguez, J.M. (2019). Biotransformation of phenolic compounds by *Bacillus aryabhattai.* Bioprocess and biosystems engineering, 42: 1671-1679.

Perez, C. (1990). Antibiotic assay by agar-well diffusion method. Acta Biol Med Exp, 15: 113-115.

Rahman, N.H.A.; Abd Aziz, S. and Hassan, M.A. (2013). Production of ligninolytic enzymes by newly isolated bacteria from palm oil plantation soils. BioResources, 8(4): 6136-6150.

Raja, C.E.; Selvam, G.S. and Omine, K.I.Y.O.S.H.I. (2009). Isolation, identification and characterization of heavy metal resistant bacteria from sewage. In Int Joint Symp on Geodisaster Prevention and Geoenvironment in Asia (pp. 205-211).

S. S, S.M.; Vijayan, D.S.; Anand, M.; Ajona, M. and Jarin, T. (2021). Biodegradation of P-nitro phenol using a novel bacterium *Achromobacter denitrifacians* isolated from industrial effluent water. Water Science and Technology, 84(10-11): 3334-3345.

Singh, N.; Gupta, S.; Marwa, N.; Pandey, V.; Verma, P.C.; Rathaur, S. and Singh, N. (2016). Arsenic mediated modifications in *Bacillus aryabhattai* and their biotechnological applications for arsenic bioremediation. Chemosphere, 164: 524-534. https://doi.org/10.1016/j.chemosphere.2016.08.119

Sonune, N.A. and Garode, A.M. (2015). Bioremediation potential of bacterial isolates for municipal wastewater treatment. Current world environment. 10(2). http://dx.doi.org/10.12944/CWE.10.2.27

Srinivasan, S. and Sadasivam, S.K. (2021). Biodegradation of textile azo dyes by textile effluent non-adapted and adapted *Aeromonas hydrophila.* Environmental Research, 194:110643.

Sunar, K.; Dey, P.; Chakraborty, U. and Chakraborty, B. (2015). Biocontrol efficacy and plant growth promoting activity of *Bacillus altitudinis* isolated from Darjeeling hills, India. Journal of basic microbiology, 55(1): 91-104. https://doi.org/10.1002/jobm.201300227

Tamilselvi, S.M.; Thiyagarajan, C. and Uthandi, S. (2016). Calcite dissolution by *Brevibacterium sp.* SOTI06: a futuristic approach for the reclamation of calcareous sodic soils. Frontiers in Plant Science, 7: 1828.

Wang, F.; Xu, Z.; Wang, C.; Guo, Z.; Yuan, Z.; Kang, H.; Li, J.; Lu, F. and Liu, Y. (2021). Biochemical characterization of a tyrosinase from *Bacillus aryabhattai* and its

application. International Journal of Biological Macromolecules, 176: 37-46. https://doi.org/10.1016/j.ijbiomac.2021.02.042

Younas, F.; Mustafa, A.; Farooqi, Z.U.R.; Wang, X.; Younas, S.; Mohy-Ud-Din, W.; Ashir Hameed, M.; Mohsin Abrar, M.; Maitlo, A.A.; Noreen, S. and Hussain, M.M. (2021). Current and emerging adsorbent technologies for wastewater treatment: trends, limitations, and environmental implications. Water, 13(2): 215. https://doi.org/10.3390/w13020215

Zhang, Q.Q.; Yang, G.F.; Zhang, L.; Zhang, Z.Z.; Tian, G.M. and Jin, R.C. (2017). Bioaugmentation as a useful strategy for performance enhancement in biological wastewater treatment undergoing different stresses: Application and mechanisms. Critical Reviews in Environmental Science and Technology, 47(19):1877-1899. https://doi.org/10.1080/10643389.2017.1400851