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Microbial Application in Heavy Metal Removal from Pharmacological Industrial Effluent

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ABSTRACT

In industrial effluents, significant amounts of metallic cations such as zinc, copper, iron, manganese, lead, nickel, and cadmium are present. Long-term irrigation with these effluents increases the amount of organic carbon and heavy metals in the soil, increasing the likelihood that they will enter the food chain and, eventually, result in significant bioaccumulation. Many techniques for the treatment and disposal of metal-containing wastes, such as ion exchange, reverse osmosis, in flotation, and evaporation, have been developed in order to reduce metal pollution issues. The main disadvantages of conventional treatments are low efficiency at low concentrations of heavy metals, expensive handling, and safe disposal of toxic sludge. In light of this, an effort has been made to investigate how tannery affects the growth of green gram.

Keywords Effluents, Heavy metals, Biological methods, Treated, Untreated, Bacteria.

INTRODUCTION

Nowadays, industrial development emphasizes the link between pollution, public health, and the environment (Mujtaba and Shahzad 2021). Industrial effluents are produced as a result of this development, and if they are not treated, they can pollute the soil, water, and sediment (Edokpayi *et al.* 2017). These effluents contain heavy metals and a variety of toxic chemicals that are harmful to human health and aquatic animals (Muedi and Meudi 2018). In particular when pharmaceutical effluents like fluoroquinone antibiotics discharged to a river, the ability of bacteria to mutate into strains that are resistant to the widely used antibiotics has been facilitated (Azam *et al.* 2020). This opens the door for infections that cannot be treated. Conventional physicochemical techniques like ion exchange, evaporation, precipitation, reverse osmosis, and electrochemical treatment are commonly used to remove heavy metals from waste streams (Mathur *et al.* 2021). However, as an alternative, cost-effective biological methods for dealing with heavy metal contamination have been considered.

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In bio-chemical processing, the proportion of biochemical to chemical oxygen demand indicates the amount of organic compounds present (Vigiak *et al.* 2019). Gram-positive rod-shaped bacteria from aerobic genera, including *Pseudomonas, Achromobacter, Alcaligens,* and *Flavobacterium,* have been observed in this treatment (Paradh and Hill 2016). The specific manganese and iron oxidizing bacteria like *Leptothrix ochracea* and *Gallionella ferruginea* removes heavy metal by oxidation process (Brooks and Field 2020). Bio surfactants are surface active substances synthesized by living cells like bacteria and yeasts (Gayathiri *et al.* 2022). This surfactants produced by *Corynebacterium* sp., *Bacillus* sp., *Pseudomonas* sp., *Micrococcus* sp., and *Mycobacterium* sp. Greater understanding of the physiology, genetics and biochemistry of bio surfactant producing strains and improved process technology can reduce the production costs (Dias and Nitschke 2023). The efficient removal of copper by terrestrial plants like *Elsholtzia splendens* and *Silene vulgaris*. There are reports on genotoxicity on plants test systems by effluents (Stainsby *et al.* 2022).

In accordance to present research findings, removing harmful heavy metals from industrial waste water is critical for preventing water pollution (Tawalbeh *et al*. 2023). Due to the inefficiency of conventional methods for removing industrial effluents, new techniques must be developed in order to meet environmental standards at reasonable costs (Sathya *et al*. 2022). As a result, the present study attempted to investigate the impact of microbial treated and untreated tannery effluent on the growth of green gram. Keeping all of these in mind, the current study seeks to:

To analyze various the physico-chemical characteristics of effluent

To isolation of metal resistant bacteria from pharmaceutical industrial effluent

To exposure of bacteria to heavy metals

To determination of heavy metal concentration in industrial effluent

To treatment of effluent with metal resistant bacteria

MATERIALS AND METHODS

A leading pharmaceutical company's industrial waste water has been collected from Chennai, India. The samples are kept at a temperature of 4°C. Furthermore, 10 ml of effluent is drawn into a conical flask with a capacity of 250 ml and 90 ml of sterile, distilled water. An electrical shaker is used to create a homogeneous suspension in the flask, and different dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}) are made by serially adding 10 ml of the water suspension to sterile distilled water. 1ml of a 10^{-5} dilution is plated in petridis containing nutrient agar medium as listed in Table 1. The pH of the medium has been adjusted to 7.

The inoculated plates are incubated for two days at 25 ± 2 °C, during which time bacteria that appeared on the medium have been accumulated, mounted on a clean slide, stained with crystal violet, grammes iodine, and safranine, are examined under a microscope. The bacteria has been identified based on colony characteristics using Gramme staining techniques. Different heavy metal salts have been added to nutrient broth during the culture of the bacteria, and the medium's metal salt concentration remained at 1 mm. Cells are inoculated in nutrient broth (100 ml/flask) and stirred in an agitator for 48 hours at a temperature of 35 ± 2°C. Centrifugation has been used to separate the cells that would be used in the bio sorption experiments.

The volumetric Winkler's method is used to estimate the dissolved oxygen contents. Water samples are taken in glasses with a narrow mouth and no air bubbles. Each sample receives 1 ml of a 40% manganese chloride solution before receiving 1 ml of an alkaline iodide solution to fix the oxygen. For the precipitate of manganese hydroxide, 1 ml of concentrated sulfuric acid has been added after 15 minutes. After acidification, 50 ml of the sample are transferred to a conical flask, and three drops of freshly made starch at 1% are added to create the blue color. After that, it is

Table 1. Composition of nutrient agar medium.

Composition	Peptone	Yeast extract	NaCl	Agar
Weight (g/l)	5	5	5	15

Table 2. Biochemical characterization of isolated bacteria.

Bacterial spp.		*Pseudomonas* spp.	*Bacillus* spp.	*Staphyococcus* spp.
	Indole	-	+	-
	MR	-	+	+
Bio-chemical characte-rizations	VP	+	-	-
	Citrate utilization	+	-	+
	Urea hydrolysis	-	+	-
	Nitrate reduction	+	+	+
	Catalase activity	+	-	+
	Oxidase test	+	-	+

titrated against solutions of sodium thiosulfate 0.025 N. When the blue color vanished, the titration turned out. Additionally, the effluent is treated with metal-resistant bacteria in Erlenmeyer flasks containing 150 ml of each sample and 15 ±1 mg of cells. The flasks are kept at 30 to 35 ± 2°C with constant agitation for 48 hours. After 48 hours, the cells and any remaining metal concentrations are removed from the medium.

RESULTS AND DISCUSSION

Three bacterial species have been isolated from the waste water, including *Bacillus* spp., *Pseudomonas* spp., *Staphylococcus* spp., and others using the serial dilution and pour plating method and nutrient agar with various heavy metal salts as dietary supplements. Table 2 provides the biochemical characteristics of the isolated bacteria. Likewise, samples are assessed for various physico-chemical parameters like pH, DO, BOD, COD and TS are listed in Table 3. The pH ranged from 6.0 to 7.9, DO ranged from 0.2 to 0.4 mg/l, BOD ranged from 250 to 480 mg/l, COD ranged from 480 to 890 mg/l, and TS ranged from 62.5 to 65.2. Similarly, the concentration of heavy metals in the effluent samples has been determined by ICPMS. The metals Cd, Cu, Pb, Hg and Co are determined in the effluent samples. The concentration of Cadmium (Cd) ranged from 0.48 to1.62 mg/l, concentration of Lead (Pb) ranged from 0.14 to 0.31mg/l, concentration of Mercury (Hg) ranged from 0.59 to 1.75 mg/l, concentration of Copper (Cu) ranged from 0.21 to 1.35 mg/l, and concentration of cobalt ranged from 0.08 to 0.91mg/l. As a result, 5 samples (S1, S2, S3, S4 and S5) are selected for this study due to high content of heavy metals as listed in Table 4.

Table 3. Physico-chemical characteristics of the effluent sample.

Sl. No.	Parameter	S1	S2	S3	S4	S5
1	pH	6.0	7.2	7.5	6.8	6.2
2	DO (mg/l)	0.2	0.3	0.2	0.4	0.3
3	BOD (mg/l)	250	360	260	480	380
4	COD (mg/l)	520	655	542	890	732
5	TS (mg/l)	63.3	64.2	63.3	65.2	62.5

Table 4. Physico-chemical characteristics of the effluent sample.

Sl. No.	Heavy metals	S1	S2	S3	S4	S5
1	Cd	0.85	-	1.54	0.09	0.48
2	Pb	0.31	0.01	0.14	-	0.22
3	Hg	1.43	0.09	1.75	-	0.59
4	Cu	0.21	-	1.35	0.06	0.42
5	Co	0.60	0.02	0.91	0.05	0.24

The reduction in heavy metal concentration in the effluent sample by *Bacillus* sp., is indicated in Table 5. The some bacterial species were reduce the metal concentration in pharmaceutical industrial effluent. The heavy metals Hg and Cu were removed by *Bacillus* sp. The average Hg reduction was 45% and Cu reduction was recorded as 62%.

The heavy metals Hg and Cu were removed by *Bacillus* sp. The average Hg reduction was 45% and Cu reduction was recorded as 62%. The reduction in heavy metal concentration in the effluent sample by *Pseudomonas* sp. is indicated in Table 6. The heavy metals Cd, Pb and Co were removed by *Pseudomonas* sp. The average Cd reduction was 56%, average Pb reduction was 34% and average Co reduction was recorded as 53%.

Table 5. Treatment of metals by *Bacillus* sp.

Sl. No.	Sample	Hg		Cu	
		Before treatment (mg/l)	After treatment (mg/l)	Before treatment (mg/l)	After treatment (mg/l)
1	S1	1.43	0.79	0.21	0.08
2	S2	1.75	0.80	1.35	0.48
3	S3	0.59	0.33	0.42	0.17
4	S4	1.15	0.69	-	-
5	S5	1.32	0.73	0.36	0.14

Table 6. Treatment of metals by *Pseudomonas* sp.,

Sl. No.	Sample	Cd		Pb		Co	
		Before treatment (mg/l)	After treatment (mg/l)	Before treatment (mg/l)	After treatment (mg/l)	Before treatment (mg/l)	After treatment (mg/l)
1	S1	0.85	0.36	0.31	0.22	0.60	0.29
2	S2	1.54	0.62	0.14	0.09	0.91	0.39
3	S3	0.48	0.22	0.22	0.5	0.29	0.11
4	S4	1.02	0.44	-	-	0.08	0.05
5	S5	1.62	0.78	0.18	0.12	0.32	0.15

Table 7. Treatment of metals by *Staphylococcus* sp.

Sl. No.	Sample	Cd		Cu	
		Before treatment (mg/l)	After treatment (mg/l)	Before treatment (mg/l)	After treatment (mg/l)
1	S1	0.85	0.50	0.23	0.14
2	S2	1.54	0.83	1.35	0.98
3	S3	0.45	0.24	0.45	0.26
4	S4	1.03	0.62	-	-
5	S5	1.65	0.85	0.35	0.25

The heavy metals Cd, Pb and Co were removed by *Pseudomonas* sp. The average Cd reduction was 56%, average Pb reduction was 34% and average Co reduction was recorded as 53%. The reduction in heavy metal concentration in the effluent sample by *Staphylococcus* sp. is indicated in Table 7. The heavy metals Cd and Cu were removed by *Staphylococcus* sp. The average Cd reduction was 44% and average Cu reduction was recorded as 34%.

CONCLUSION

The collected effluent samples used to analyze physico-chemical characteristics and isolated the metal resistant bacteria from heavy metal contaminated effluent. Initially, the physico-chemical characteristics were analyzed from the heavy metal contaminated effluent. Totally 3 species of metal resistant bacteria were recorded. Among the fungi *Pseudomonas* sp., was recorded as dominant genus with other groups. *Pseudomonas* sp., as bearing the highest potential to degrade heavy metals like Cd (56 %), Pb (34 %) and Co (53 %). *Bacillus* sp., was degrading potential through the largest removal of heavy metals like Cd (44 %), Hg (45 %) and Cu (62 %). *Staphylococcus* sp., presented the highest biodegradation efficiency as well as the removal of heavy metal like Cu (34%). As a result, this study concluded that bacteria play a very important role in the removal of heavy metals from pharmaceutical effluent.

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