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Studies on Chromium Resistance Bacteria Isolated from Damanganga River Water

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ABSTRACT

The very hazardous metal chromium (VI) makes up a significant portion of industrial waste. In the environment, hexavalent chromium (Cr (VI)) is highly mobile and water-soluble. Because of its continuous release into the water and the problems it causes to the environment and human health, it is a matter of serious concern in developing nations. Chromium-resistant bacteria were isolated from 15 different regions of Silvassa (UT of DNHDD), Bhilad, and Vapi (Gujarat) along the Daman Ganga River. A total of 28 chromium-resistant isolates were selected by using nutrient agar plates containing 50 mg/l of ($K_2Cr_2O_7$) and primarily differentiated based on morphological characteristics. Among the 28 isolates, 22 (CrR 1, CrR 3, CrR 4a, CrR 4b, CrR 5, CrR 7, CrR 8, CrR 9, CrR 10, CrR 11, CrR 12, CrR 13, CrR 14a, CrR 14b, CrR 15a, CrR 15b, CrR 17, CrR 20, CrR 21, CrR 23, CrR 24, CrR 25) can grow well up to 1,000 mg/l of Cr (as $K_2Cr_2O_7$) in nutrient broth. Native bacterial strains from sites contaminated with industrial wastewater make use of their natural capacity to change toxic heavy metals into less harmful forms and can be a useful instrument for tracking environmental heavy metal contamination.

Keywords- Industrial waste, Chromium, Damanganga

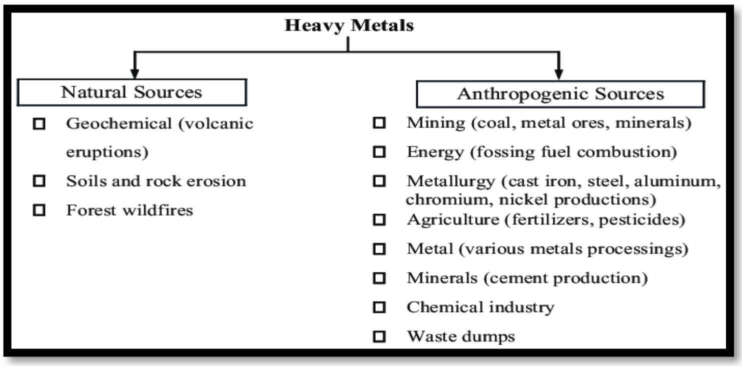
INTRODUCTION

Metallic elements with a density higher than that of water are referred to as heavy metals. Environmental pollution by these metals has been a growing global public health and ecological problem in recent years. NIHMS (Tchounwou et al., 2012)

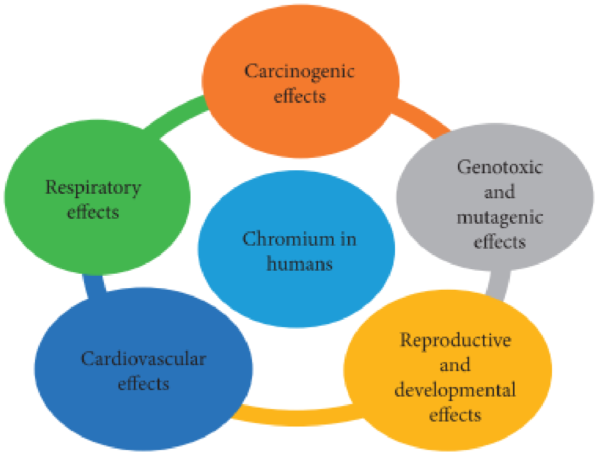
Industrial Heavy Metals as Major Water Pollutant in Damanganga River.

Heavy metals are one of the primary sources of water contamination. In general, industrial activity is responsible for introducing a greater amount of toxic heavy metal waste into water bodies directly or indirectly (McNeill et al., 2012). As per the report of Nigrude et al, Vapi GIDC was the second most polluted industrial area in India, with a CEPI index of 88.29, and Ankleshwar was the first, with a CEPI index of 88.5. (Nirgude et al., n.d.) Industrial effluents from DNH, Vapi (Gujarat), and Daman, as well as domestic sewage from towns, cities, and villages along the river's edge, are the main sources of wastewater discharges into the Damanganga River through several drains.

Sources of chromium



***Disease associated with Chromium*:**



Source - (Deb & Roy, 2021)(Mishra & Bharagava, 2016)

To reduce the toxicity of chromium in the environment

Many conventional methods have been adopted for removing metals from industrial effluents i.e. chemical and physical (Ahluwalia & Goyal, 2007). These technologies are efficient and cost-effective. (Chen et al., 2014) (Nourbakhsh et al., 1994) Therefore, replacing costly technologies with eco-friendly methods for efficiently removing low concentrations of toxic heavy metals is much needed**.**

Microbial resistance toward chromium

Chromium Bioremediation is widely acknowledged as the best and most cost-effective technology. A large number of microorganisms can be found naturally in water that has received industrial effluents. These microorganisms have adopted and developed a variety of defense mechanisms to protect themselves from heavy metal toxicity. Some chromium-resistant bacteria were found to have the ability to reduce chromium using reductase enzymes. (Kanmani et al., 2012) Chromate reductases found in chromium-resistant bacteria have been shown to reduce hexavalent chromium Cr (VI) to trivalent chromium Cr (III) (Marsh & McInerney, 2001)(Wani et al., 2019)

MATERIALS AND METHODS

Study area: -

The river originates in the Sahyadri Hills of Maharashtra and covers the hilly regions of Gujarat, and the Union Territories (UT) of Dadra and Nagar Haveli. The river basin is located in the Western Ghats between latitudes 19° 51' to 20° 28'N and longitudes 72° 50' to 73° 38'E. The Damanganga River is home to about 5105 large, medium, and small-scale industries in Dadra & Nagar Haveli, Vapi (Gujarat), and Daman industrial clusters.

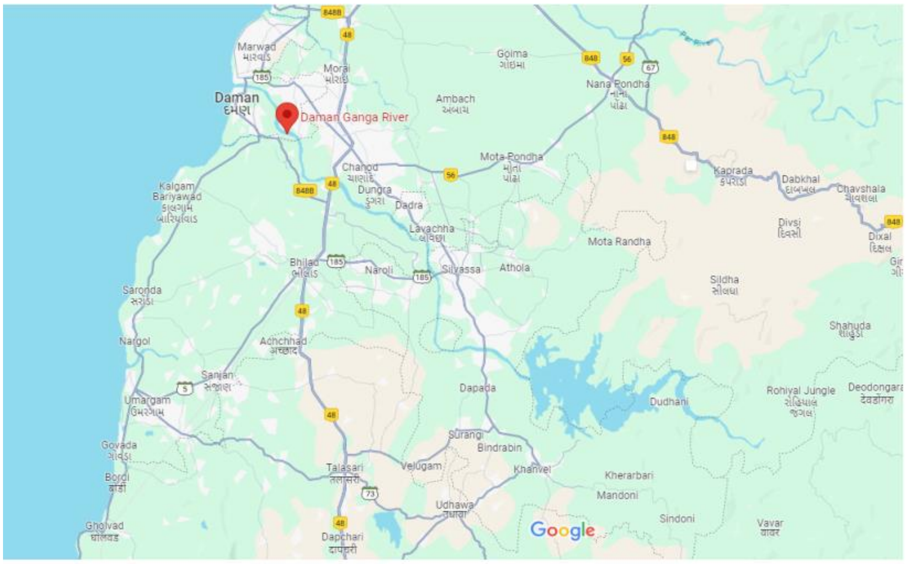


Fig -1 Damanganga River

Sample collection

The Damanganga river water samples were collected from 13 different locations (D01-D13) (Table. 1) of Dadra Nagar Haveli (D01-D10), Bhilad (D11-D12), and Vapi (D13) as per standard sampling methods (IS: 2498, 1966 – Part - 1) and immediately brought in laboratory.

Table -1. Sampling location details

Sr. no	District/ Town	Location name	Location no.	Location detail
1	Dadra Nagar Haveli	A. Masat Industrial Estate	D-01	i. Industrial drain at khadipada village
			D-02	ii. Damanganga River water near khadipada drain
			D-03	iii. Industrial drain at Ozarpada
			D-04	iv .Damanganga River water at Ambapada village
		B. Rakholi Industrial Estate	D-05	i. Industrial drain under karad bridge
			D-06	ii .Industrial drain near Damanganga river
			D-07	iii. Damanganga river water sample near rakholi bridge
		C. Piparia Industrial Estate	D-08	i. Industrial drain under bridge near Ganesh Canteen
			D-09	ii .Industrial drain near Damanganga river at Piparia
			D-10	iii. Damanganga river water sample near Piparia industrial estate
2	Bhilad	Gujarat Heavy Chemical Limited (Old)	D-11	i. Industrial drain near old GHCL flowing to Darotha River
			D-12	ii. Darotha River water sample (tributary to Damanganga River)
3	VAPI	GIDC	D-13	Damanganga river water sample at CETP outlet

Isolation and Characterization of chromium-resistant bacteria

The sample was serially diluted and plated on a sterile Nutrient agar medium containing 50 mg/l of chromium. The plates were incubated for 24-48 hours at 30°C. After incubation, the Nutrient agar plates were observed for Bacterial Colonies showing different morphological features.

Screening of chromium-resistant bacteria

Each bacterial isolate was spread out individually on nutrient agar plates with 100 mg/l of chromium. The plates were incubated at 30° C for 24 hours and observed for the development of bacterial colonies.

Tolerance of isolates

Resistance of isolates to chromium was determined by inoculating the isolates in nutrient broth tubes containing increasing concentrations of chromium (100-1000mg/l). Isolates were inoculated in a Nutrient broth tube containing increasing concentrations of chromium and incubated for 24 hours at 30°C. After 24 hours the nutrient broth tubes were observed for turbidity against control.

RESULT AND DISCUSSION

Isolation of chromium-resistant bacteria

Based on variations in morphological features shown in Table 2, A total of 28 bacteria were isolated from 13 distinct locations of which six isolates came from the GIDC Vapi region, twelve from the Piparia Industrial Estate region, seven from the Masat Industrial Estate region, and two from Rakholi and one from Bhilad region samples. (Table 3) They were arbitrarily named as CrR1, CrR2, CrR3….CrR25). The river effluent primarily consisted of chromium and protein which makes it an ideal medium for many bacterial species to grow, in order to give a considerably high CFU/ml (Kalsoom et al., 2023)

Table -2 . Cultural characteristics of chromium resistant bacterial isolates

Sr.no	Isolate	Size	shape	elevation	opacity	Color/ Pigmentation
1	Cr R-1	2mm	round	flat	opaque	Red
2	CrR-2	2 mm	Round	flat	Translucent	Golden white
3	Cr R-3	3mm	round	convex	opaque	Yellow
4	CrR-4a	1mm	round	convex	opaque	Golden
5	CrR-4b	2mm	round	convex	Translucent	Golden
6	CrR-5	1mm	round	raised	translucent	White
7	CrR-6	1.5mm	round	flat	translucent	Transparent
8	CrR-7	0.5mm	round	flat	opaque	White
9	CrR-8	2mm	round	raised	opaque	White
10	CrR-9	1mm	round	pulvinate	opaque	Bluish
11	CrR-10	4mm	round	flat	translucent	creamish-white
12	CrR-11	3mm	round	flat	opaque	White
13	CrR-12	2mm	round	flat	translucent	White
14	CrR-13	1mm	round	raised	opaque	White
15	CrR-14-a	2mm	round	raised	translucent	golden white
16	CrR-14 -b	1mm	round	raised	opaque	White
17	CrR-15a	6mm	irregular	flat	opaque	White
18	CrR-15b	3mm	irregular	flat	Translucent	White
19	CrR-16	2mm	round	raised	Translucent	Off white
20	CrR-17	2mm	round	convex	opaque	creamish white
21	CrR -18	0.5mm	round	flat	opaque	Off white
22	CrR-19	9mm	Irregular	flat	opaque	creamish white
23	CrR-20	7mm	round	convex	opaque	creamish white
24	CrR-21	2mm	round	flat	translucent	off-white
25	CrR-22	1mm	round	flat	opaque	Off white
26	CrR-23	3mm	round	flat	opaque	Orange

27	CrR-24	9mm	irregular	pulvinate	opaque	creamish white
28	CrR-25	8mm	irregular	flat	translucent	creamish white

Table -3 . Location details of the chromium resistant isolates

Sr. no	Isolate.	Location
1	Cr R-1*	D-08
2	CrR-2	D-08
3	Cr R-3*	D-13
4	CrR-4a*	D-13
5	CrR-4b*	D-13
6	CrR-5*	D-13
7	CrR-6	D-01
8	CrR-7*	D-03
9	CrR-8*	D-03
10	CrR-9*	D-02
11	CrR-10*	D-02
12	CrR-11*	D-04
13	CrR-12*	D-04
14	CrR-13*	D-08
15	CrR-14-a*	D-08
16	CrR-14 –b*	D-08
17	CrR-15a*	D-08
18	CrR-15b*	D-08
19	CrR-16	D-09
20	CrR-17*	D-09
21	CrR -18	D-09
22	CrR-19	D-10
23	CrR-20*	D-10
24	CrR-21*	D-05
25	CrR-22	D-05
26	CrR-23*	D-12
27	CrR-24*	D-13
28	CrR-25*	D-13

Screening of chromium-resistant bacteria

To screen for chromium resistance, all 28 isolates have been grown on media supplemented with 100 mg/l of chromium. It was found that Among 28 isolates, 22 (*marked in Table 3) were resistant to chromium at a concentration of 100 mg/l. In a related investigation carried out in 2015 from industrial waste collected from Korangi and Lyari, Karachi by Kalsoom et al, Seven of the 53 isolated bacterial strains were identified based on morphological and biochemical characteristics. The designations S11, S13, S17, S18, S30, S35, and S48 were given to these strains, and all are found resistant to chromium at a concentration of 100ppm. Using the 16S rRNA sequence, the bacterial strains S35 and S48 were identified. which revealed 99% similarity to *Bacillus paranthracis* and *Bacillus paramycoides*. .(Kalsoom et al., 2023). Similar studies were carried out by Jishnu Sarathi Deb and Dr. Atanu Roy,in which eleven distinct single colonies were isolated from the plate containing 15 mM potassium chromate from a loam soil sample of river Ganga (Deb & Roy, 2021). Numerous studies have documented metal resistance in bacteria taken from soil and wastewater that has been contaminated with various heavy metals. (Sanjay et al., 2020),(Mustapha & Halimoon, 2015)

Tolerance of isolates

The resistance of isolates was determined by using varying concentrations of chromium. between 100 and 1,000 mg/l. 19 isolates showed highest level of tolerance, growing well in presence of 1,000 mg/l chromium (Table 4) whereas other isolate CrR1 did not grow well above 600 mg/l Cr and isolates CrR 4a, 4b , showed tolerance up to 900 mg/l Cr. In a study Cr(VI) resistant isolates were selected from sum of 40 separates in which the microorganisms have been identified as Atlantibacter sp., Pseudomonas sp., Exigobacterium sp., Brevibacillus agri, staphylococcus sp. and Includes three Bacillus sp. Strains. The staphylococcus sp. and Brevibacillus agri. exhibited the highest chromium tolerance to 2500mg/l while others sp. showed resistance up to 1500mg/ Camargo et al. also isolated some chromium resistant bacteria that can tolerate Cr(VI) at concentrations of 1,500–2,500 mg/l. (Camargo et al., 2003)

Table -4 Determination of Tolerance to Chromium

Sr no	Isolate	Highest chromium tolerance mg/l
1	CrR-1	600
2	CrR-3	1000
3	CrR-4a	900
4	CrR-4b	900
5	CrR-5	1000
6	CrR-7	1000
7	CrR-8	1000
8	CrR-9	1000
9	CrR-10	1000
10	CrR-11	1000
11	CrR-12	1000
12	CrR-13	1000

13	CrR-14a	1000
14	CrR-14b	1000
15	CrR-15a	1000
16	CrR -15b	1000
17	CrR-17	1000
18	CrR -20	1000
19	CrR -21	1000
20	CrR-23	1000
21	CrR-24	1000
22	CrR-25	1000

CONCLUSION

Heavy metal discharge from the industrial effluent has a detrimental effect on the environment. Traditional methods of treating heavy metals in aqueous solutions are expensive and produce large amounts of hazardous chemical sludge. Native bacterial species in contaminated samples and effluents utilize their natural capacity to break down contaminants like chromium, which is also economically viable when compared to conventional methods. These isolates that exhibit a high chromium tolerance may be able to significantly lower the hazardous hexavalent chromium levels in contaminated areas as well as they can also be practiced in the metal recovery techniques.

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