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Full Length Research Paper

Bioremediation of Uranium in contaminated water samples of Bathinda, Punjab by *Desulfovibrio* genus

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Soluble uranium salts enter human body through ground water and foodstuff. World Health Organization (2004) has set 15 µg/L as the "tolerated intake" of soluble uranium in drinking water. Uranium intake above this concentration is toxic to human body. The organ which are most affected are kidney and lungs leading to malfunctioning of kidneys and lung cancer. Soluble uranium is also known for its neuro-developmental, neuropsychological, cytotoxic, genotoxic and carcinogenic effects. So to overcome such hazardous problem in Bhatinda region (Cancer belt area- Jhajjal, Giana, Sivian, Malkana, Laliana, where cancer cases are prominent) of Punjab an effort has been done. Incubation of water samples with media specific for growth of *Desulfovibrio* genus was done for one month with soil from the same area from where water sample has been taken and with *Desulfovibrio vulgaris* subsp. *vulgaris* strain. Chemical analysis of water samples was done again to measure final uranium (VI) concentrations after incubation. In case of incubation with soil, the average reduction of uranium (VI) in the presence of Linsmaier and Skoog's (LS) media specific for the growth of *Desulfovibrio* genus is 59.08%. This concluded that the presence of one or a group of species of *Desulfovibrio* in soil is responsible for reduction of uranium (VI). In case of incubation with *D. vulgaris* subsp. *vulgaris* strain, the average reduction of uranium (VI) is 97.77%. This concludes that *D. vulgaris* subsp. *vulgaris* strain can reduce uranium at an average rate of 0.003 µg/L/h. Biochemical tests were done to find out specific species of *Desulfovibrio* present in soil responsible for uranium reduction. The results of these tests concluded that the organism present in soil responsible for reduction of uranium is *Desulfovibrio desulfuricans*.

Key words: Uranium, *Desulfovibrio*, Bathinda, cancer.

INTRODUCTION

Uranium is a toxic radioactive element that is found in traces in almost all types of rocks, soils, air and water. Uranium occurs in tetravalent (UO_2 and U^{4+}) and hexavalent (UO_3 and UO_2^{2+}) oxidation states (Banks et al., 1995). Uranium is soluble in aqueous solutions in its oxidised (U^{6+}) form and precipitates in its reduced form (U^{4+}) (Herczeg et al., 1988; Szabo and Zapecza, 1991).

The World Health Organization (2004) and UNSCEAR, (2000) have set 15 and 9 µg/L respectively, as the safe limit for uranium concentration in drinking water, whereas United States Environmental Protection Agency (2004) has recommended it as 30 µg/L. According to World Health Organization (WHO, Mulloy et al., 2001), the major hazard from uranium is lung and bone cancer. After ingestion, uranium appears in the blood (La Touche et al., 1987) and combines with red blood cells (Fisenne and Perry, 1985) to form a non-diffusible uranyl-albumin complex in equilibrium with ($UO_2HCO_3^+$) complex which has high affinity for phosphate, carboxyl, and hydroxyl groups and readily combines with proteins and nucleotides to form stable complexes (Moss, 1985). It is immediately cleared from the blood but subsequently accumulates in the kidneys, skeleton and upto some extent; in the liver (La Touche et al., 1987; Wrenn et al., 1985). In the skeleton, the uranyl ion replaces Ca in the hydrox-ylapatite complex of bone crystal (Moss, 1985).

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Figure 1. Map of Bathinda showing sample collection sites (encircled) (Singh, 2009).

It has been demonstrated *in vitro* that some microorganisms are capable of reducing hexavalent uranium [U(VI)] to tetravalent uranium [U(IV)] and precipitate a U(IV) mineral called uraninite (UO_2) (Lovley et al., 1991; Spear et al., 2000). Using this microbial approach, this present study aimed at reducing U(VI) to U(IV) present in ground water samples of Bathinda district of Punjab where the motility rate due to Cancer increasing rapidly.

The water samples were taken from hand pumps and tube wells of Bathinda area of Punjab and uranium estimation was done using fission track technique. The range of U-concentration was found out to be from 1.65±0.06 to 74.98±0.38 µg/L. This uranium concentration is above the safe limits of uranium recommended for drinking water (Kumar et al., 2003).

An investigation carried out by *The Observer* newspaper, in 2009, revealed that the cause of contamination of soil and ground water in Malwa region of Punjab is the fly ash that emanates from coal burnt at thermal power plants, which contains high levels of uranium and ash as the region has state's two biggest coal-fired power stations (Chamberlain, 2009; NDTV, 2009). In 2009, Greenpeace Research Laboratories conducted the study in 50 villages in Muktsar, Bathinda and Ludhiana districts and revealed that 20% of the samples showed nitrate levels above the safety limit of 50 mg/L, established by WHO, the study connected it with high use of synthetic nitrogen fertilizers (Garg and Balwant, 2010).

The purpose of this study reported here was to investigate the possibility that whether *D. vulgaris* subsp. *vulgaris* (Spear et al., 2000) would be able to use U(VI) as a terminal electron acceptor and immobilize it (Elias and Suflita , 2004) and to perform biochemical testings to find out specific species of *Desulfovibrio* present in soil samples, collected from corresponding villages, responsible for uranium reduction if they do so. The results indicate that *D. vulgaris* subsp. *vulgaris* can enzymatically reduce U (VI) present in water samples of villages of Bathinda, Punjab and presence of uranium reducing members of Desulfovibrio was also reported.

MATERIALS AND METHODS

Location of the study area

Bathinda District is located in the Southern part of Punjab State of India in the centre of Malwa region. It is located between 29°-33´ and 30°-36´ North latitude and 74°-38´ and 75°-46´ East longitude (Figure 1).

Sample collection

Soil and water samples were collected from the villages situated on cancer belt of Bathinda). Water samples were collected from hand pumps and tube wells in sterile alcohol rinsed capped bottles and

Table 1. Uranium (VI) concentrations before and after incubation with soil and specific strain.

Sample	Initial Uranium (VI) concentration (µg/L)	Uranium (VI) concentration after incubation with soil (µg/L)	Percentage reduction	Uranium (VI) concentration after incubation with specific strain (µg/L)	Percentage reduction
Jhajjal	54.75	5.97	89	1.47	97.31
Giana	56.133	46.84	16.5	1.38	97.54
Sivian	25.966	5.81	77.62	0.608	97.65
Malkana	14.57	3.072	78.91	0.222	98.47
Laliana	45.29	25.9	42.81	0.995	97.81
GNDTP	95.63	48.14	49.66	2.057	97.84

Table 2. Statistical analysis of data.

Water samples	Average conc. of U(VI) (µg/l)	S.D.	Coefficient of variation	Variance	Degree of freedom	t- stat	p value	Remark
Initial concentration (6)	48.72	28.24	57.96	797.56	9.219899	1.8187	0.051158	Significant
With *Desulfovibrio* (6)	22.62	20.93	92.52	438.12				
Initial concentration (6)	48.72	28.24	57.96	797.56	5.005371	4.127607	0.004553	Significant
With soil (6)	1.12	0.65	58.43	0.42				

soil samples were collected in autoclaved air tight polybags from Cancer belt area of Punjab including Jhajjal, Giana, Sivian, Malkana, Laliana and area near Guru Nanak Dev thermal plant, Bhatinda (Thakur et al., 2008. In case of soil sample collection, a shallow subsurface soil layer 10 cm below the dry soil-air interface was collected (Suzuki et al., 2003).

Estimation of U(VI) before incubation

Solid-phase extraction (SPE) technique (Madrakian and Mousavi, 2008) has been used to estimate U(VI) concentration due to its advantages of high enrichment factor, high recovery, rapid phase separation, low cost and low consumption of organic solvents.

Incubation and uranium estimation after incubation

For incubation with soil, in order to sustain growth of *Desulfovibrio* genus Lactate sulfate media specific for the growth of *Desulfovibrio* genus was used for incubation. Incubation was done for 1 month (Suzuki et al., 2003). *Desulfovibrio vulgaris* subsp. *vulgaris* was used for incubation of samples (Spear et al., 2000). Solid-phase extraction (SPE) technique (Madrakian and Mousavi, 2008) was used to estimate U (VI) concentration after incubation also.

Identification of consortium present naturally in soil

Desulfovibrio species present in soil were distinguished from each other using biochemical tests for catalase, indole, nitrate, urease, and growth on bile (Warren et al., 2005).

RESULTS AND DISCUSSION

As shown in Table 1, in case of incubation with soil, the average reduction of uranium (VI) in the presence of LS media specific for the growth of *Desulfovibrio* genus is 59.08%. This concludes that there must be one or a group of species of *Desulfovibrio* present in soil responsible for reduction of uranium (VI). In case of incubation with *D.vulgaris* subsp*. vulgaris*, the average reduction of uranium (VI) is 97.77%. This concludes that *D. vulgaris* subsp*. vulgaris* can reduce uranium (VI) at an average rate of 0.003µg/L/h.

This present study was carried out to establish the reduction rate of uranium with consortium present naturally in soil, as compared to the pure culture of *D. vulgaris* subsp. *vulgaris*. It is evident from Table 2 that the average of uranium (VI) concentration in water samples is 48.7 µg/L and that of water sample incubated with soil consortium is 1.12 µg/L while that of water sample incubated with *D. vulgaris* subsp. *vulgaris* is 22.62 µg/L. This shows highly statistically significant value of comparison of initial concentration versus water sample incubated with *D. vulgaris* subsp. *vulgaris* ($p<0.0511$) and that of initial concentration versus water sample incubated with soil consortium ($p<0.0045$) (Table 2). This can be easily concluded that the consortium present in soil is more efficient in reducing uranium (VI) as compared to pure cultures of *D. vulgaris* subsp. *vulgaris.*

Desulfovibrio species present in soil were distinguished from each other using various biochemical tests as given by (Warren et al., 2005) and the consortium naturally present in soil was identified as *Desulfovibrio desulfuricans*. Results show that the average uranium concentration in water for all the villages of Bathinda district lie above the safe limit of 1.9 µg/L suggested by ICRP (1979) and 9 µg/L suggested by UNSCEAR (2000). The average uranium concentration in the drinking waters

of only one villages (Malkana) is found to be lower than the safe limit of 15 µg/L given by WHO (1993). The waters of Jajjal, Giana, Laliana and Guru Nanak Dev Thermal Power Plant are found to have higher average uranium content in comparison to the safe limit of 30 µg/L given by USEPA (2004). Thus the presence of excessive of uranium in drinking water samples from Bathinda area can be the cause of a large number of cancer deaths in the area.

The ability to reduce U(VI) enzymatically extends to the sulfate-reducing bacteria; *D. desulfuricans* (Lovely and Philips, 1992) and *D. vulgaris* (Lovley and Philip, 1994) also reduce uranium. Enzyme system responsible for U(VI) reduction for *D. vulgaris* has been characterised in detail. Purified tetrahaem cytochrome c3 has been demonstrated to perform the function of U(VI) reductase *in vitro*, in combination with hydrogenase which is its electron donor (Lovley and Philip, 1994). *In vivo* studies were performed using a cytochrome c3 mutant of the close relative *D. desulfuricans* strain G20 and it was confirmed that cytochrome c3 plays a role in hydrogen-dependent U(VI) reduction, but suggested the existence of some additional pathways from organic electron donors to U(VI) (Payne et al., 2002). Hence, it is also evident that uranium reduction can be done by *D. desulfuricans* and hence the bacterium identified in soil is *D. desulfuricans*.

Conclusion

The results of this study reveal that the ground water of this study area is heavily contaminated with uranium (VI). This suggests that crops in such environments with high absorption coefficient for uranium (VI), may also be contaminated. This confirms earlier research by Singh (2009) that food crops in this study area are heavily contaminated with uranium and had relevance with more number of cancer cases in the region. It can also be inferred from the results that the concentration that uranium (VI) can be reduced to its precipitable uranium (IV) form using sulphur-reducing bacteria- *D. desulfuricans* and *D. vulgaris* subsp. *vulgaris.* The research also reveals that rate of U(VI) reduction by *D. desulfuricans* is more as compared to *D. vulgaris* subsp. *vulgaris*. Hence, purification of the contaminated waters by biological processes is possible, but requires extensive research to use members of *Desufovibrio* genus in order to reduce uranium (VI) to uranium (IV) state, as there are many constraints in the path like longer incubation time and pathogenicity of the strains. But such efforts can save the residents of Bathinda from serious diseases like cancer.

It is evident from this study that *D. desulfuricans* and *D. vulgaris* subsp. *vulgaris* reduce uranium from toxic soluble state to non-toxic insoluble state. Hence, there is a possibility that using this work, purification of the contaminated waters by biological processes could be worked out and the efforts could be made to provide pure water and healthy environment to the residents to save humanity from the serious diseases like cancer in the Bathinda region.

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