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Bioremediation of Uranium in Contaminated Water Samples of Bathinda, Punjab by *Clostridium* Sp.

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ABSTRACT

Uranium is a naturally occurring radioactive mineral present in certain types of rocks and soils. The health hazards of uranium in drinking water in VI oxidation state of uranium are chronic. The present research work is designed for bioremediation of uranium and conversion to its less toxic states. The soil and water samples from different locations of Batinda were incubated with specific strain of *Clostridium* bacteria and the average reduction of uranium (VI) recorded is 89.68%. The anaerobic culture was identified through biochemical tests and confirmed as *Clostridium pasteurianum.* The result reveals that the contamination of uranium can be reduced by using particular soil bacteria and the probable hazards for living beings can also be reduced.

Keywords: Uranium, *Clostridium,* Bathinda, Bioremediation, Cancer.

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INTRODUCTION

Uranium has caused severe effects on reproductive system and other health issues in various animals like rodents, frogs etc. In comparison to other heavy metal such as lead or mercury, the toxicity of soluble uranium is less. Various body parts get affected due to uranium contamination in water and food materials but the organ which is most affected is the kidney. The soluble uranium salts are excreted in the urine, although some accumulation in the kidneys does occur in the case of chronic exposure. The World Health Organization has established a daily "tolerated intake" of soluble uranium salts for the general public of 0.5 µg/kg body weight (or 35 µg for a 70 kg adult) exposure at this level is not thought to lead to any significant kidney damage. Uranium was shown to have cytotoxic, genotoxic and carcinogenic effects in animal studies. It has been shown in rodents and frogs that water soluble forms of uranium are teratogenic (WHO, Mulloy et al., 2001).

In Punjab in March 2009 Uranium poisoning was noticed by South African toxicologist, Dr Carin Smit. The hair and urine samples of 149 children having birth abnormalities including physical deformities, neurological and mental disorders were taken from Faridkot city in Punjab, India, and samples, sent to Micro trace Mineral Lab, Germany. Though doctors expected heavy metal toxicity, what they were surprised to find was high levels of uranium in the samples, and in one case more than 60 times the maximum safe limit (Singh, 2010).

In another study of Malwa region of Punjab also showed similar results, study was carried out on mentally retarded children, and the results revealed 87% of children below 12 years and 82% beyond that age having uranium levels high enough to cause diseases, also uranium levels in samples of three kids from Kotkapura and Faridkot were 62, 44 and 27 times higher than normal.

For finding the reason of 100 children which are congenitally mentally and physically challenged, the samples were sent to Baba Farid Centre for Special Children, Faridkot, and results showed very high concentration of uranium in samples. It has been reported that uranium has caused reproductive effects, and other health problems in rodents, frogs and other animals (Chamberlain, 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 (UO2) (Lovley et al., 1991; Spear et al., 2000).

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.

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 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 *Clostridium* genus, Clostridial agar media specific for the growth of *Clostridium* genus was used for incubation. Incubation was done for 1 month (Suzuki et al., 2003). *Clostridium* 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:

Clostridium 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

Results reveal that the average uranium concentration in water for all the villages of Bathinda district lie above the safe limit of 1.9μg/1 given by ICRP (1979) and 9μg/1 given 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/1 given by WHO (2004). 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 30μg/1 given by USEPA (2003). Thus the presence of excess of uranium in drinking water samples from Bathinda area can be the cause of a large number of cancer deaths in the area.

The World Health Organization (2004) and UNSCEAR, (2000) have recommended 15μg/l and 9μg/l respectively as the safe limit for uranium concentration in drinking water, whereas United States Environmental Protection Agency (2003) has proposed 30μg/1 of uranium in water as the safe limit for drinking purpose. The International Commission on Radiological Protection (1979) has set the safe limit as 1.9 μg/l. These levels are proposed to represent a uranium concentration in drinking water that does not cause any significant risk to general public over the lifetime use of the water for drinking purposes.

The organism present in soil responsible for reduction of uranium is *Clostridium species*. Table 1 shows that there is reduction of uranyl nitrate in the presence of cells of *Clostridium* species there is no reduction in the uranyl nitrate from the components of media and metabolites produced during the growth of *Clostridium* species. *Clostridium* is a genus of Gram-positive rod shaped bacteria, belonging to the Firmicutes. They are obligate anaerobes capable of producing endospores. Members of which are found in soil, water, and the intestinal tracts of humans and other animals. Most species grow only in the complete absence of oxygen. Dormant cells are highly resistant to heat, desiccation, and toxic chemicals and detergents.

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).

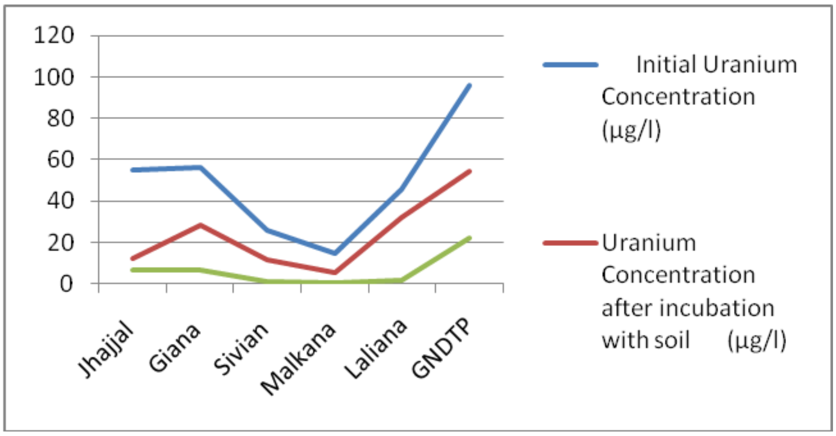

Figure 1: Graph showing the reduction of uranium (VI) after incubation with soil and specific strain

In this study, it is clear that the hydrogenase enzyme plays an important role in both uranium (VI) and iron (III) reduction by *Clostridium* species. When hydrogen gas (H_2) was provided in the headspace of the serum bottle, either uranium (VI) or iron (III) reduction occurred in the presence of whole cells without carbon source. This is in contrast to the introduction of nitrogen gas (N_2) into the headspace. In the absence of whole cells, hydrogen alone could not result in uranium (VI) or iron (III) reduction, suggesting that a hydrogenase mediated both the uranium (VI) and iron (III) reduction using hydrogen as the electron donor. When the hydrogenase activity was inhibited by adding copper (II), uranium (VI) reduction was not observed. Reduction in the concentration of uranium is well observed in the soil incubation phase with specific media for the growth of *Clostridium* species and incubation with specific strain of *Clostridium* i.e. *Clostridium pasteurianum.*

Biochemical test (citrate medium test) also identified the organism responsible for the reduction of uranium concentrations in soil incubated samples. Hence it is proved that the organism responsible for the bioremediation of uranium is *Clostridium* species particularly *Clostridium pasteurianum.* 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 *Clostridium*. It is evident from Table 1 and 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 23.5 µg/L while that of water sample incubated with *Clostridium* is 6.62 µg/L. The data is statistically analyzed with Graph Pad Software and shows highly statistically significant value of comparison of initial concentration versus water sample incubated with *Clostridium* ($p<0.0056$) and non-significant for initial concentration versus water sample incubated with soil consortium ($p<0.0967$) (Table 2).

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)	%age Reduction	Uranium Concentration after incubation with *Clostridium* (µg/l)	%age Reduction
Jhajjal	54.75	11.768	78.51%	6.59	87.96%
Giana	56.133	27.903	50.29%	7.13	87.29%
Sivian	25.966	11.091	57.28%	1.28	95.07%
Malkana	14.57	5.101	64.98%	0.70	95.19%
Laliana	45.29	31.526	30.39%	1.91	95.78%
GNDTP	95.63	54.135	43.39%	22.15	76.83%

Table 2: Statistical analysis of data

	Mean	SD	SEM	N	T	Df	standard error of difference	P value	
Initial Uranium Concentration	48.72317	28.24037	11.52908	6	1.8332	10	13.711	0.0967	Non-significant
Uranium Concentration after incubation with soil	23.58733	18.17915	7.42161	6					
Initial Uranium Concentration	48.72317	28.24037	11.52908	6	3.5102	10	11.993	0.0056	significant
Uranium Concentration after incubation with *Clostridium*	6.62667	8.08935	3.30246	6					

CONCLUSION

It is concluded from the results that uranium (VI) can be reduced to its precipitable uranium (IV) by using anaerobic bacteria *Clostridium pasteurianum* and it convert the toxic soluble state of uranium to non-toxic insoluble state. Such 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 not only in the Bathinda region but also in other affected part of world.

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