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A Preliminary Study on Bioremediation of Zinc, Copper and Lead using Rumen Fluid

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A B S T R A C T

Rumen fluid a major slaughter house waste is known to have numerous microorganisms which include bacteria, fungi, protozoa, Archaea and viruses. These Microbes in the rumen fluid not only have the ability to ferment a variety of carbohydrates but also degrades and ferment a significant portion of dietary protein. In the present study the rumen fluid is evaluated for its ability to reduce heavy metals- Zinc, Copper and Lead. Rumen fluid collected from slaughter house was inoculated in Nutrient broth added with heavy metals- Zinc, Copper and Lead. The medium was collected for every 24hrs and was centrifuged and the supernatant was analyzed for the heavy metal reduction. The Bioremediation of heavy metals- Zinc, Copper and Lead was evaluated for 96hours and was found to be reduced in the medium at 96th hr by 40%, 33% and 15% respectively.

Introduction

Heavy metals are essential trace elements, but most of them can be toxic to all forms of life at high concentrations due to formation of complex compounds within the cell. Unlike other organic pollutants, heavy metals once introduced into the environment cannot be biodegraded causing pollution of air, water, and soils. Heavy metals enter plant, animal and human tissues via air inhalation, food chain and manual handling. These Heavy metals once entered can bind to vital cellular components, such as structural proteins, enzymes, and nucleic acids, and interfere with their functioning. The long-term exposure to heavy metals can have carcinogenic, central and peripheral nervous system and circulatory effects in humans. Thus, the bioavailability, mobility, and toxicity of metals in the environment should be reduced. Commonly used methods of remediating heavy metal-contaminated environments are *in situ* vitrification and soil incineration and land filling, soil washing, soil flushing and solidification and stabilization by electrokinetic systems. These physicochemical methods of remediation are expensive and environmentally destructive. Thus bioremediation- an environmentally friendly approach is used as an alternative to physicochemical methods of remediation

(Madhu Choudary *et al.,* 2017). Bioremediation is the process of utilizing microbes such as bacteria, yeast, fungi, algae and higher plants as main tools in treating oil spills and heavy metals existing in the environment (Gupta et al, 2016). Due to natural and industrial processes heavy metals are found increasingly in microbial habitats. Thus, microorganisms have evolved several mechanisms to tolerate the heavy metal presence by adsorption, complexation, or chemical reduction of metal ions or to use them as terminal electron acceptors during anaerobic respiration (Mohammed *et al.,* 2011). The rumen fluid comprises of obligately anaerobic bacteria, fungi and protozoa (Forsberg and Cheng, 1992). *Bacillus* sp. commonly present in rumen fluid has been known in uptake of Copper and Zinc by 15% and 14% dry weight (Rajendran *et al.,* 2003). Rumen consortia have been used for chromium reduction by biosorption (Meignanalaksmi *et al.,* 2013). In the present study microbial consortia in the rumen fluid is evaluated for its ability to bioremediate the three commonly polluting heavy metals – Zinc, Lead and Copper.

Materials and Methods

Collection of Rumen fluid- Slaughter house waste

Rumen fluid was collected from Perambur slaughter house.

Analysis of heavy metal reduction

Rumen fluid of about 1ml was inoculated to 100ml of nutrient broth added with heavy metals Zinc, Copper and lead (2ppm, 6.5ppm and 210ppm) in separate flasks (Sujitha and Jayanthi, 2014). The medium was incubated in shaking condition at 37°C for 4 days. All the experiments were carried out in triplicates. The medium was collected at regular intervals- 0^{th}, 24^{th}, 48^{th}, 72^{nd} and 96^{th} hours respectively. The collected medium was centrifuged at 6000rpm for 10min and the supernatant was collected. The supernatant was estimated for the heavy metals- Zinc (Tazul Islam and Jammaludin Ahmed, 2013), Copper (Vadiraj and Belagali, 2014) and lead (Humaria Khan *et al.,* 2006).

Results and Discussion

The heavy metals inoculated with rumen fluid were evaluated for its ability to bioremediate heavy metals and the heavy metal reduction studies are given in table 1. The percent reduction of heavy metals- Zinc, Copper and Lead was calculated and are given in table 2. The graphical representation of heavy metal reduction is shown in figure 1 and 2.

In the present study heavy metals Zinc, Copper and Lead was found to be reduced by 40%, 33% and 15% when rumen fluid was used. Kang *et al.,* 2016 showed remediation of Pb by 98.3%, Cd by 85.4% and Cu by 5.6% using cocultures of *Viridibacillus arenosi* B-21, *Sporosarcina* soli B-22, *Enterobacter cloacae* KJ-46, and *E. cloacae* KJ-47, which are isolated from an abandoned mine site. *Saccharomyces cerevisiae* was used by Dilna Damodaran *et al.,* (2011) and reported the reduction of lead by 67-82% and Cadmium by 73-79 % in 30 days.

Maximum heavy metal adsorption by the immobilized bacterial cell *Bacillus Subtilis* (78.4 mg/L for Zn^{2+}, 76.8 mg/L for Cu^{2+} and 74.2 mg/L for Pb^{2+}) has been shown by Sujitha and Jayanthi (2014). Immobilized *Serratia marcescens* showed maximum absorption of Zinc (74.8 mg/L), Copper (72.3 mg/L) and Lead (71.4 mg/L) (Sujitha and Jayanthi, 2014). *Pseudomonas fluorescens* was immobilized by Sujitha and Jayanthi (2014) and reported maximum absorption (70.3 mg/L for Zn^{2+}, 69.8 mg/L for Cu^{2+} and 68.4 mg/L for Pb^{2+}).

Table.1 Zinc, copper and lead reduction by rumen fluid for every 24hrs

Time in hours	Zinc (ppm)	Copper (ppm)	Lead (ppm)
Mean ±S.D.			
0 (Initial value)	2.00±0.01	6.5± 0.01	210± 1.00
24	1.84± 0.01	6.36± 0.01	208± 1.00
48	1.2± 0.1	4.36± 0.01	178± 1.00
72	1.2± 0.1	4.36± 0.01	178± 1.00
96	1.2± 0.1	4.36± 0.01	178± 1.00

Table.2 Percent reduction of heavy metals by rumen fluid

Heavy metal	Percent reduction at the end of 96th hour
Zinc	40%
Lead	15%
Copper	33%

Fig.1 Reduction of zinc and copper by rumen fluid at every 24hrs

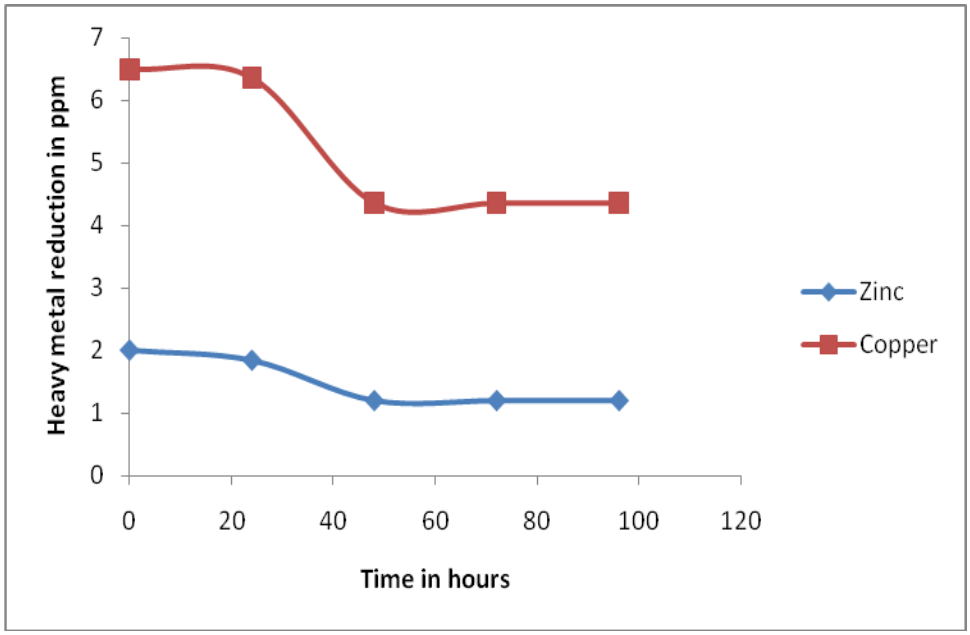
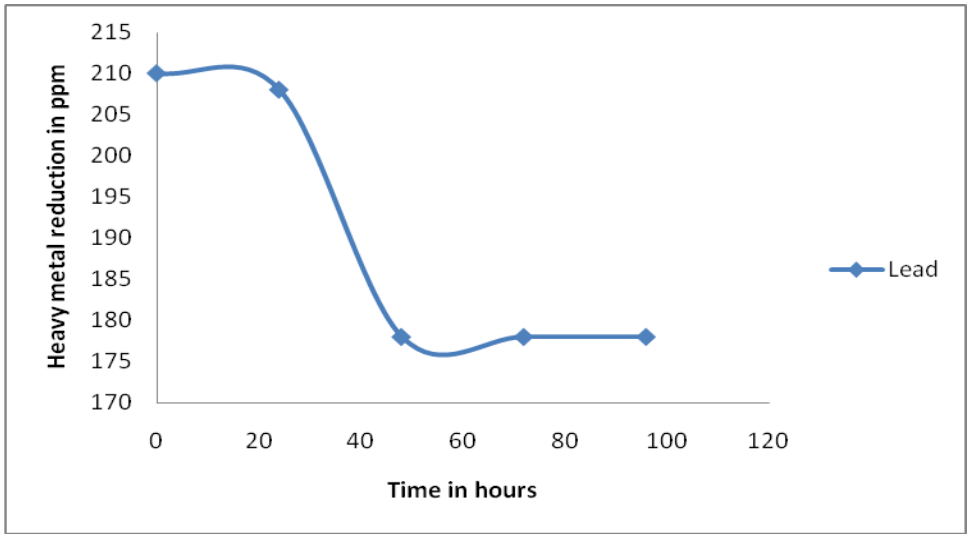


Fig.2 Reduction of lead by rumen fluid at every 24hrs



Krishna et al, 2013 used *Bacillus* sp to reduce Zinc to the maximum of about 40%. Reduction of copper levels in soil added with bacteria when compared to raw soil sample was shown by Bini Samal and Parul Bhatt Kotiyal (2013). Arun Karnwal and Vaishali Bhardwaj (2014) isolated bacterial strain vb4 from metal contaminated areas in and around Baddi and was found to be the potent producer of biosurfactant as well as found to efficiently remove zinc and chromium.

Sayed *et al.,* (2011) showed maximum uptake of Zinc by using *Streptomyces aureofacienes* (734.8 Zinc µg/g of biomass). Arpita Ghosha and Papita Das Saha (2012) isolated Copper resistant bacteria *Stenotrophomonas* sp. PD2 from soil of Dhapa and had shown maximum copper resistance upto 200 mg/l of Cu(II). Biosorption of Chromium by rumen fluid microbes was reported by Meignanalakshmi *et al.,* (2013).

In the present study rumen fluid containing millions of microbes was used for Bioremediation of copper, zinc and lead when compared to other studies where individual microbes have been used. As rumen fluid contains diverse of microbes it could able to reduce all the three heavy metals.

Hence, concluded in the present study rumen fluid a major slaughter house waste has been proved to bioremediate heavy metals- Zinc, Copper and Lead and can be used in future as a method of low cost and ecofriendly remediation technique.

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