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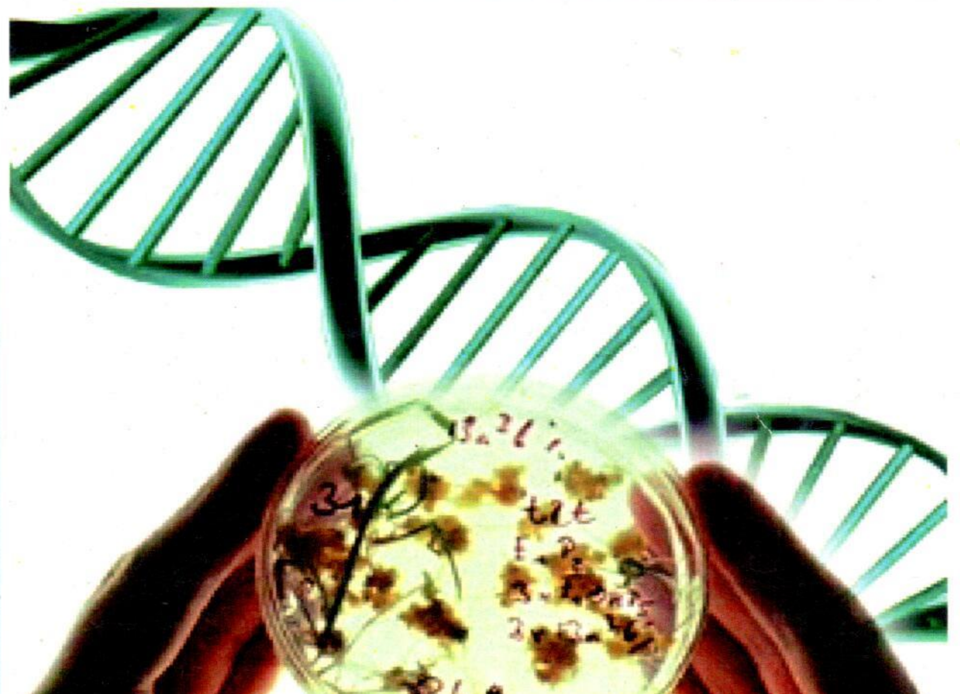

Organized by

DEPARTMENT OF BIOTECHNOLOGY

ST. XAVIER'S COLLEGE, MAPUSA-GOA

09-10 MARCH, 2015

PROCEEDINGS
OF
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ISBN: 978-93-84298-28-9

Published by

Department of Biotechnology, St. Xavier's College, Mapusa –Goa

Note: Views expressed by the authors are their own and do not necessarily reflect those of the Institution or of the Editors

Printed at JESEL WORLD, Cuncolim - Goa. Mobile: 9822687323 www.jeselworld.in

ISBN: 978-93-84298-28-9

Biotechnology for the treatment of metal pollution

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Abstract: Mining by its nature consumes, diverts and can seriously pollute water resources. In Goa pollution due to mining and other industries is of serious environmental concern. Goa's Mandovi estuary faces the threat of anthropogenic pollution, consequently the salterns adjacent to and fed by the estuary would obviously also get affected. In the salterns metals get concentrated along with the brine. Therefore, theoretically speaking, the salt from these salterns should also contain high concentrations of metals. Since the salt is used for local consumption, there could be chronic exposure of humans to heavy metals. This has been linked to various diseases, including neurodegenerative conditions, dysfunction of vital organs like liver and kidney, and even cancer. However, the metal content in the Ribandar salterns was found to be mitigated. Metal tolerant heterotrophic bacteria were found to play a pivotal role in the cycling of metals. Therefore the practical significance of the applicability of these bacteria in the removal of heavy metals from salterns has been discussed in this paper.

Key words: Pollution, Metal, Heterotrophic bacteria, Salterns.

1. Introduction

The dispute over humanity's impact on the environment has come of age. Our destructive activities can no longer be denied, but we also depend crucially on the continuation of our economic activities. Hence, a compromise has to be worked out: sustainable development. Mining related activities in Goa, especially, its influence on the aquatic environment of the Mandovi estuary, have been in focus during the past decade because of the large amount of effluents released into it. Regardless of the source or original intended use, substantial amounts of chemicals end up in the Mandovi estuary due to anthropogenic activities. The mining activities in this region have had a considerable influence on the biological and geochemical conditions of the estuarine waters and consequently on the adjacent salterns. The Ribandar solar salterns along the Mandovi estuary in Goa are exposed to an influx of metal effluents from the ferromanganese ore mining activities, barge traffic and sewage disposal activities, since they are fed by this estuary. It is surmised that as a consequence of metal pollution of the waters from the estuary feeding the salterns, the metals probably would concentrate with the brine during evaporation. The salt from these salterns is consumed as well as used for various commercial purposes by the local Goan population. Some of these metals are

 ISBN: 978-93-84298-28-9

useful to us at low concentrations but are highly toxic at higher concentrations (Ge *et al.* 2009). Metals cause detrimental effects if they are taken up and can reach a target site where they can do harm (Escher and Hermens 2004, Schwarzenbach *et al.* 2006). Heavy-metal accumulation throughout the food chain leads to serious ecological and health problems. Therefore, the food and water we consume are often associated with numerous diseases.

Metal remediation through common physico-chemical techniques is expensive and unsuitable in case of voluminous effluents containing complexing organic matter and metal contamination. In such cases, a number of bio materials such as molds, yeasts, bacteria, and seaweeds find use in the removal of metals from the water (Vieira and Volesky 2000). Biotechnological approaches employing biomaterials have received great deal of attention in the recent years. Studies show that bacteria isolated from contaminated sites show better metal resistance and possess excellent capability of metal scavenging. Some bacterial strains possess high tolerance to multiple metals and may be potential candidates for bioremediation. In terms of biotechnology, the most promising targets in the genetic engineering of metal tolerant bacteria include mechanisms to immobilize, mobilize or transform heavy metals. Microorganisms may be engineered to enhance mobilization of metals through autotrophic and heterotrophic leaching, or chelation by microbial metabolites and siderophores, or methylation which can result in volatilization, thereby providing a route for removal from solid matrices such as soils, sediments, dumps and industrial wastes.

Conversely, enhanced immobilization is also particularly applicable to removing metals from mobile aqueous phases. This can result from sorption to cell components or exopolymers, transport into cells and intracellular sequestration or precipitation as insoluble organic and inorganic compounds, like oxalates, sulphides or phosphates (Gadd and White 1993).

This study focuses on the feasibility of using bacterial cells for mitigating metal pollution and the efforts directed towards process development to make this option technically and economically viable for the comprehensive treatment of metal-rich effluents.

2. Methodology

2.1. Study area and sampling site

The study site was the Ribandar saltern (15° 30.166 N and 73° 51.245 E) Goa, India, situated along the Mandovi estuary. Sediment cores (0-10 cm) from the Ribandar saltern were collected during the pre-monsoon season (January–May), the monsoon season (August) and post monsoon season (November) in triplicates using 1.5-inch diameter graduated PVC hand-held corers. The corers were sealed at both ends with sterile core caps to prevent direct contact with air and transported to the laboratory in an icebox for further physico-chemical analysis.

2.2. Metal concentrations

Sub-samples for metal analysis were dried at 60(±2)°C for 48 h and disaggregated in an agate mortar before chemical treatment for the measurement of Fe, Mn, Ni, Co, Pb, Zn, Cd and Hg following sediment digestion methods as described by Balaram *et al.* (1995). The concentration of the metals was analysed on an atomic

ISBN: 978-93-84298-28-9

absorption spectrophotometer (AAS; GBC 932AA model) at wavelengths, λ: Fe = 372.0 nm; Mn = 279.5; Ni = 232.0 nm; Co = 240.7 nm; Pb = 217.0 nm; Zn = 213.9 nm; Cd=228.8 nm and Hg=253.7 nm using air acetylene flame. Blank corrections were applied wherever necessary and the accuracy was tested using standard reference material MAG-1 (United Geological Survey).

2.3. Metal –Microbe interactions

Counts of metal tolerant microbes in response to various concentrations of metals were as per the procedure of Pereira *et al.* (2013) and the study of metal microbe interactions was done as described by Pereira (2013).

3. Results and Discussion

3.1. Metal concentrations

Coastal areas are sites of discharge and accumulation of a range of environmental contaminants. Econiches like estuaries (Kumar *et al.* 2010) and solar crystallizer ponds (Pereira *et al.* 2013) therefore may contain high concentrations of metals, since they serve as ecological sinks for metals and as effective traps for river borne metals (Chapman and Wang 2001). In the present study, the average metal concentrations recorded in the Ribandar saltern sediment were 17.2±2.8 to 26.3±6.7 % Fe, 0.60±0.2 to 0.9±0.2 % Mn, 27.6±7.3 to 51±8.3 ppm Ni, 28.4±8.9 to 35.2± 10.6 ppm Co, 44.0±21.6 to 62.8±23.6 ppm Zn, 0.06± 0.01 ppm Cd, 1.7±1.0 to 2.6±0.7 ppm Pb and below detection limit Hg, whereas the average values recorded in the overlying saltern water were 4.6±3.2 ppm Fe, 0.5±0.1 ppm Mn, 0.5±0.5 ppm Ni, 1.1±1.0 ppm Co, 1.2±0.84 ppm Zn, 0.01±0.01 ppm Cd and 0.26±0.08 ppm Pb. The concentrations of toxic metals such as Cd, Zn and Pb were well within the permissible limits of 0.03-0.3ppm, 50-300ppm and 2-20ppm respectively in the sediment and 0.001-0.05ppm, 0.005-5ppm and 2-20ppm in water respectively (RSMENR 2002).

The higher concentrations of metals measured in sediment than in water indicate that lower pH (6.5 to 7.5) encountered in the saltern favoured metal accumulation and is in agreement with the report that sediments are the major depository of metals holding more than 99% of total amount of a metal present in the aquatic system (Campbell 1995). An assessment of the concentration of metals in the Ribandar saltern sediment for all seasons revealed that the metal concentrations were higher in the salt-making season by 52 % for Fe, 42 % for Mn, 85 % for Ni, 23 % for Co, 42 % for Zn and 47 % for Pb, compared to the non salt-making season. The Ribandar saltern with salinity varying between 5 to 300 is therefore a classic example of several solubilised elements getting magnified with increasing gradients of salinity. Interestingly, the metal concentrations obtained in the Mandovi estuary which feeds the Ribandar saltern were lesser than the metal concentrations recorded in present study in the Ribandar saltern sediment during the salt-making season except in the case of Zn. According to Attri and Kerkar (2011), the concentration of metals in the Mandovi estuary were 18.3±1.9 % Fe, 0.19±0.002 % Mn 36.2±4.2 ppm Co and 102.3±9.8 ppm Zn.

In order to estimate the possible environmental consequences of metal pollution, our results were compared with Sediments Quality Values (SQV) using National Oceanic and Atmospheric Administration (NOAA) Screening Quick

 ISBN: 978-93-84298-28-9

Reference Tables (SQuiRTs) (Buchman 1999).

According to NOAA SQuiRT (Table 1), Fe was below the AET (Apparent effect threshold) during the monsoon and postmonsoon season, but above AET during premonsoon. Mn and Co concentration were above the AET for all seasons, while Ni, Zn , Cd and Pb were below AET for all seasons. Hg was below detectable levels. High Mn, Co and Fe indicate their possible toxicity which may impart an adverse effect on the biota (Buchman 1999).

However, the metal concentrations from salt obtained from the same saltern were upto 2.58 ppm Fe, 0.24 ppm Mn, 0.065 ppm Ni, 0.012 ppm Cu and 0.032 ppm Zn (Kerkar and Fernandes 2013). Surprisingly these concentrations were found to be well within the safe levels for human consumption (Table 2).

Table 1 Screening quick reference table (SQuiRT) for metals in marine sediments (Buchman 1999)

Element	Background	Threshold effect level	Effect range low	Probable effect level	Effect range medium	Apparent effect threshold
Fe		-	-	-	-	22 (Neanthes)
Mn		-	-	-	-	0.026 (Neanthes)
Ni		15.9	20.9	42.8	51.6	110(Echinoderm larvae)
Co		-	-	-	-	10 (Neanthes)
Pb	4-17	30.2	46.7	112	218	400 (Bivalve)
Zn	7-38	124	150	271	410	410 (Infaunal community)
Cd		0.68	1.2	4.2	9.6	3.0 (Neanthes)

Table 2 Health based guideline values of heavy metals (http://www.who.int/water_sanitation_health)

Heavy metals	Ppm
Hg	0.001
Cu	0.05-2
Zn	5
Ni	0.02-0.1
Pb	0.1
Cd	0.01
Fe	1-3
Mn	5

ISBN: 978-93-84298-28-9

Though some of the metals like Cu, Fe, Mn, Ni and Zn are essential as micronutrients for life processes, they are proved detrimental beyond a certain limit (Marschner 1995, Bruins *et al.* 2000), which is low for some elements like Cd (0.01 mg/L), Pb (0.10 mg/L) and Cu (0.050 mg/L). Some of the serious toxic effects of metals like mercury, lead, copper, cadmium, arsenic, chromium, nickel and manganese are mental retardation in children, dementia in adults, CNS disorders, renal diseases, hepatic diseases, insomnia, personality changes, emotional instability, depression, panic attacks, memory loss, headaches, vision disturbances, excessive salivation, excess sweating, lack of co-ordination. Death due to encephalopathy or cardiovascular diseases may occur. Increased heavy metal concentration causes toxicity of blood, leading to extraction of calcium from bones to buffer the acidity. This calcium accumulates in soft tissue of arteries causing hardening of arteries (Hu 2002). In the Ribandar saltern microbial processes could be important and even dominating factors in the mitigation and fate of specific metals.

3.2. Role of heterotrophic metal tolerant bacteria in mitigation of metals

Organisms inhabiting metal polluted environments develop resistance mechanisms that enable efficient detoxification and transformation of toxic forms to nontoxic forms. Heterotrophic metal-tolerant bacteria were abundant in the Ribandar saltern both during the salt-making as well as the non salt-making season (Pereira *et al.* 2013). The bacteria were found to exhibit a high degree of variation in the concentration of metals that they can tolerate. Similar observations were also made by Kaur *et al.* (2006) and Popescu and Dumitru (2009). We have attempted to understand this aspect by identifying the genes responsible for tolerance to metals. It was seen that the bacteria employed multiple mechanisms for metal tolerance such as secretion of EPS, biosorption, bioprecipitation, regulation of protein expression, presence of mnxG, metallothionein and groEL genes (Pereira 2013). In addition to these biological factors, an interplay of a large number of physico-chemical factors that enhances the settling and mitigation of locally introduced pollutants was also evident, thereby enabling the deposition of pollutants in the sediment rather than being available in the overlying waters. This proves beneficial especially in the saltern, since the metals are removed from the water and accumulated in the sediment and the salt obtained from this overlying water therefore remains safe for consumption. Interactions between bacteria and heavy metal ions are therefore of great interest for mitigating metal pollution, not only as a fundamental process but also as a potential bio remedial technology, making this option technically and economically viable for the comprehensive treatment of metal-rich effluents.

4. Conclusion

Heavy metals entering into the Ribandar saltern are most likely scavenged by heterotrophic metal tolerant bacteria employing various resistance mechanisms. It is feasible to use bacterial cells as an economically viable option for the comprehensive treatment of metal-rich effluents.

Acknowledgement

We thank H.O.D. Department of Biotechnology, Goa University and Shri Rasik Ravindra, ex-Director, National Centre for Antarctic and Ocean Research, Goa, for providing the lab facilities and also Dr. K.P.Krishnan for his constant support.

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