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Metal Detoxification in Hypersaline Environments

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Abstract

Traditional salt farming has been practised in Goa, India for the past 1,500 years. The presence of mineral impurities in these salts is known to enhance the flavor, color and texture of foods. Organisms ranging from bacteria, fungi, algae, to archaea, are known to colonise salterns and influence the quality of salt produced. What is not known, however, is that these microorganisms also play an important role in detoxification of metal impurities in such hypersaline environments.

Goa's Mandovi estuary faces the threat of anthropogenic pollution, consequently the salterns 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. The chronic exposure of humans to heavy metals has been linked to various diseases, including neurodegenerative conditions, dysfunction of vital organs like liver and kidney, and even cancer. However, this was not the case in the Ribandar salterns. Heterotrophic bacteria were found to play a pivotal role in the cycling of metals. In the salterns due to continuous exposure of these bacteria to heavy metals, there was an emergence of metal tolerant strains. It was seen that these tolerant bacteria employed various mechanisms for detoxification of metals. Multiple mechanisms employed for metal tolerance were - secretion of EPS, biosorption, bio precipitation, regulation of protein expression, presence of mnxG, metallothionein and groEL genes. The metals were therefore removed from the overlying water of the salterns and were found to accumulate in the sediment, thereby keeping the salt prepared from the overlying water free from metal contamination and safe for human consumption. Therefore, protecting the environment is dependent on our understanding of the detoxification mechanisms employed by organisms for remediating metal-polluted environments.

Key words: Metal, pollution, bacteria, remediation

Introduction:

Goa lies between 14°542 to 15°482 North and 73°412 to 74°262 East, with a coastline of about 110 km and an area of 3,702 sq. km. The coastline of Goa comprises of estuaries, river outlets, mangroves, saltpans and creeks. Being blessed with a vast coastline and a tropical climate with high intensity sunlight and strong winds, the traditional salterns of Goa are ideal for salt extraction, as being adjacent to the coast they get inundated by tidal waters from riverine and marine sources. Sea salt, bay salt or solar salt obtained from the evaporation of sea water has 85.62% sodium chloride and 14.38% other trace minerals: sulphate, magnesium, calcium, potassium, bicarbonate, bromide, borate, strontium, and fluoride. Unrefined sea salt is usually not processed, or undergoes minimal processing, and therefore retains trace levels of minerals like magnesium, potassium, calcium and other nutrients, as well as some microorganisms (http://sodiumbreakup.heart.org/). For centuries, salt produced in the four talukas of Pernem, Bardez, Tiswadi and Salcete had been adequate for the needs of the local populace, whether for consumption or commercial use. However, this salt production received a set back after liberation due to breaching of bunds, land reclamation and water pollution caused due to industries and tourism (Prabhudesai, 1997). 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. The quality of water and sediment affects all the living organisms in this diverse and complex region. Therefore these salterns are a good example of a site where human pressures and ecological values collide with each other.

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. Since the salt from these salterns is consumed as well as used for various commercial purposes by the local Goan population, a study of the distribution of trace metals in the sediments and waters of the saltern becomes pertinent, in order to assess the probable influence of mining on the salt produced.

It is well known that solar salterns act as a niche for extremophilic organisms which thrive over a range of salinities, temperatures, pH, nutrient concentrations, oxygen availability, water activity and solar radiation (Sequiera, 1992). What needs to be determined is if these extremophilic heterotrophic bacteria play any role in regulating the concentrations of metals in these salterns. This paper attempts to determine if this hypothesis really holds true and if so to study the mechanisms employed.

Materials and Methods:

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. The climate on an average is generally warm and humid, fluctuating from a minimum of 20 °C in the month of December to 42 °C in May, hence highly conducive to salt-making. 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.

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). Briefly, a known quantity (0.2 g) of sediment was digested in a Teflon vessel with a solution (10 ml) of concentrated HF (48 % GR; Merck), HNO3 (69 % GR; Merck) and HClO4 (35 % GR; Merck) in the ratio 7:3:1. The mixture was digested on a hot plate in a fume hood chamber at 70 °C for 4–6 h. The procedure was repeated with 5 ml of acid mixture. A further 2 ml of concentrated HCl (35 % GR; Merck) was added followed by 10 ml of HNO3 (69 % GR; Merck). The residue was warmed and transferred to a clean, dry standard flask to make a final volume of 50 ml with double distilled water. The concentration of the metals was analysed on an atomic absorption spectrophotometer (AAS; GBC 932AA model) at wavelengths, ë: 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).

Heterotrophic metal tolerant bacterial counts:

The sediment and water samples from the Ribandar salterns were serially diluted in sterile saltern water and plated on modified salt agar (25% nutrient agar) amended with different metals and incubated at 38°C for 15 days (Rodriguez-Valera, 1981). The heterotrophic metal tolerant bacterial counts were enumerated as CFU/g and in water as CFU/ml respectively.

Studies on mechanisms of metal tolerance:

Cellular analysis was performed by light microscopy and SEM-EDS. Regulation of protein expression was studied using SDS-PAGE. Genetic basis of heavy metal tolerance was analyzed by screening for known genes related to heavy metal tolerance within the genome of isolated strains. The genes screened included mnx for Manganese tolerance (Dick et al. 2006) and ncc (Abdelatey et al. 2011), nik and cnr operons (van Vliet et al. 2002) for nickel and Cobalt tolerance, as also metallothionein genes (Naz et al. 2005).

Results and discussion:

Metal concentrations:

Coastal areas are sites of discharge and accumulation of a range of environmental contaminants. Anthropogenic activities like urbanization and industrialization, including mining, agriculture, and waste disposal are the main contributors of metal pollution in estuaries and rivers (Tabak et al. 2005; Ross, 1994). 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, 2010). 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).The manifold increase in metal concentrations in the sediment could also be due to the absorptive nature of clayey soil. According to Martincic et al. (1990) and Biksham et al. (1991), this phenomenon is common as it is generally recorded that metals are associated with smaller grain size particles. 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 Reference Tables (SQuiRTs) (Buchman, 1999).

According to NOAA SQuiRT (Table 1), Fe was below the AET during the monsoon and post monsoon season, but above AET during pre monsoon. 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).

Table1: Screening quick reference table (SQuiRT) for metals in marine sediments (Buchman, 1999).

Elements	Background	Threshold effect level (TEL)	Effect range low (ERL)	Probable effect level (PEL)	Effect range medium (ERM)	Apparent effect threshold (AET)
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

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 (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. Heavy metal toxicity causes toxicity of blood, causing 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.

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. The count of Fe, Mn, Ni, Co, Pb, Cd and Zn-tolerant bacteria is given in Table 3.

Table 3: Count of heterotrophic metal tolerant bacteria in Ribandar saltern sediment

	Depth	Nov	Jan	Feb	Mar	Apr	May	Aug
Fe	0-5cms	5.00E+06	5.50E+05	3.00E+05	1.56E+07	1.35E+06	3.50E+05	5.09E+07
	5-10cms	3.00E+06	0.00E+00	1.40E+06	7.00E+05	3.65E+06	3.00E+05	6.00E+05
Mn	0-5cms	1.50E+06	1.15E+06	0.00E+00	1.33E+07	1.55E+06	3.00E+05	7.00E+05
	5-10cms	5.00E+05	0.00E+00	2.50E+05	1.00E+05	2.60E+06	2.00E+05	5.00E+04
Ni	0-5cms	4.60E+07	4.00E+05	0.00E+00	1.20E+06	2.55E+06	3.00E+05	1.30E+06
	5-10cms	1.30E+07	0.00E+00	4.00E+05	6.60E+06	2.60E+06	4.00E+08	0.00E+00
Co	0-5cms	4.00E+06	4.50E+05	4.00E+05	1.00E+06	1.90E+06	2.50E+05	6.50E+04
	5-10cms	7.50E+06	5.00E+04	1.25E+06	2.50E+05	2.90E+06	3.00E+05	0.00E+00
Pb	0-5cms	6.50E+06	5.00E+05	0.00E+00	7.00E+05	5.00E+04	2.00E+05	1.85E+06
	5-10cms	3.50E+06	5.00E+04	1.00E+06	2.75E+07	0.00E+00	2.00E+05	2.00E+05
Zn	0-5cms	0.00E+00	0.00E+00	0.00E+00	1.00E+05	0.00E+00	0.00E+00	1.00E+05
	5-10cms	0.00E+00	0.00E+00	0.00E+00	9.00E+05	0.00E+00	0.00E+00	0.00E+00
Cd	0-5cms	0.00E+00	0.00E+00	0.00E+00	0.00E+00	5.00E+04	0.00E+00	0.00E+00
	5-10cms	0.00E+00	0.00E+00	0.00E+00	0.00E+00	2.50E+05	0.00E+00	0.00E+00
Hg	0-5cms	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
	5-10cms	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00

Highest counts of Fe tolerant bacteria (10^{7} CFU g$^{”1}$ sediment) were seen at 0–5 cm depth during non salt-making season while at 5-10 cm depth the counts were less by one order. In the salt making season the retrievable count of Fe tolerant bacteria was one order lower than that during the non salt-making season. At 0–5 and 5–10 cm, irrespective of the season, the counts of Mn-tolerant bacteria were mostly in the order of 10^{7} and 10^{5} CFU g$^{”1}$ sediment. The counts of Co-tolerant bacteria during both non salt-making season and salt-making season were in the order of 10^{4-6} CFU g$^{”1}$ sediment. At 0-5 cm depth interval, the counts of Ni-tolerant bacteria in non salt-making season were lower by an order (~10^{7} CFU g$^{”1}$ sediment) compared to the salt-making season. However, the counts were higher by three orders in the depth interval of 5–10 cm in the non- salt making season. In general, the counts of Zn- and Cd tolerant bacteria ranged from $10^{4"5}$ CFU g$^{”1}$ sediment. During non salt-making season, the counts of Pb-tolerant bacteria were in the order of 10^{6} CFU g$^{”1}$ sediment; however, in the salt-making season, it ranged from $10^{4“7}$ CFU g$^{”1}$ sediment, with higher counts observed at 5–10 cm. 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, 2006; Sehgal and Gibbons, 1960; Nieto et al. 1987; Popescu and Dumitru 2009. At low concentrations, certain metal ions like Mn(II), Fe(II), Co(II), Ni(II), and Zn(II) were found to enhance growth of metal tolerant bacteria, though interestingly, at high concentrations, metals must be quickly and efficiently eliminated. Thus in order to avoid toxicity, bacteria activate metal resistance mechanisms to overcome metal stress (Kaur, 2006; Sehgal and Gibbons 1960). These microbiological processes can regulate the solubility of metals thereby governing bioavailability and potential toxicity. 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 not only as a fundamental process but also as a potential bio remedial technology.

Conclusion:

Heavy metals entering into the Ribandar saltern are most likely scavenged by heterotrophic metal tolerant bacteria employing various resistance mechanisms, and by suspended particles leading to their removal from the surface waters and their accumulation in sediments. These sediments, however, could become a reservoir of metals and a source to the overlying water column long after their input to the ecosystem has ceased, potentially leading to adverse ecologic effects. Since the extent of the risks is difficult to assess accurately because of the complexity of biologic and chemical interactions that alter the bioavailability of metals, a continuous monitoring is recommended so as to ascertain that the heavy metals incorporated in the salt crystals are not at a level that could cause potential toxicity to human beings.

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