
Bioremediation of Heavy Metals from Saline Water Using Hypersaline Dissimilatory Sulfate-Reducing Bacteria

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Savita Kerkar and Kirti Ranjan Das

Abstract

Salt pans are man-made ecosystems which are fed by the tidal influx of seawater through the estuaries. Most heavy metal contaminants from industries and anthropogenic processes dissolve in water and thus gain entry into the sea. Heavy metals are high-density metallic chemicals that are potentially toxic at low concentrations and present a danger to human and environmental health. The removal of these metals by general physical separation techniques is a crucial issue and chemical treatment is not always environmentally friendly. Biological methods provide an alternative to heavy metal remediation. In the present study, hypersaline dissimilatory sulfate-reducing bacteria (SRB) were found to remediate barium, calcium, cadmium, cobalt, copper, iron, magnesium, molybdenum, zinc, mercury, nickel, and lead metals from saline waters. SRB produce H_2S by utilizing sulfate as electron acceptor, which helps in oxidizing organic matter, and reactive H_2S precipitates dissolved heavy metals as their metal sulfides and thus play an important role in detoxifying saline waters. Among the 11 heavy metals found in the adjoining estuarine seawater, 9 metals were detected in the salt pan water of Ribandar, Goa. Fe, Mn, and Pb were observed in dissolved and particulate form, whereas Hg and Sb were absent. In the salt manufacturing process, the brine starts crystallizing the salt and metal concentrations increase by 10^3 fold in brine and 10^4 in salt crystals. SRB precipitate almost 50% concentrations of the dissolved metals (from the overlying salt pan water) as their metal sulfides, which gradually get deposited in the underlying salt pan sediments. Hypersaline SRB show optimal sulfate-reducing activity from 80 to 115 psu and are thus potential bioremediators in salt pan ecosystems and in turn have an application in detoxifying industrial effluents containing

S. Kerkar (✉) • K. Ranjan Das
Department of Biotechnology, Goa University,
Goa 403206, India
e-mail: savitakerkar@gmail.com

heavy metals. This study assesses the role of hypersaline SRB strains isolated from salt pans in remediating heavy metal containing saline waters.

2.1 Heavy Metal Influx in Saline Waters

Coastal areas are sites of discharge and accumulation of a range of environmental contaminants due to urbanization and industrialization, which include mining, agriculture, and waste disposal, being the main contributors of metal pollution in estuaries and rivers (Tabak et al. 2005; Ross 1994) which elevate metal concentrations in estuaries (Kumar et al. 2010) and salt crystallizer ponds (Pereira et al. 2013).

Metals, at high concentrations, influence the biochemical activity, growth, and morphology of microbes (Pereira et al. 2012) even at low (5–10 ppm) concentrations. Metals form complexes and combine with inorganic or organic metals and get accumulated in the sediments. Microorganisms use various strategies, like complex formation and extracellular precipitation, reducing metal transport across cell membranes or impermeability (Atlas and Bartha 1997). In some studies, microorganisms have been utilized to remove metal contamination from wastewaters, to separate metals from sediments and soil or to foster metal solubilization for extraction (Lovley and Coates 1997).

The Mandovi estuary of Goa faces a threat of anthropogenic pollution. Consequently the salt pans fed by the estuary would get affected. In the salt pans, metals get concentrated along with the formation of brine. Organisms like Bacteria, Archaea, and Eukarya are known to inhabit and influence the salt pan water and thus the quality of the salt produced. Solar salt obtained from the evaporation of seawater has 86% sodium chloride and 14% other trace minerals, viz., SO_4 , Mg, Ca, K, HCO_3 , Br, Sr, and F (Kerkar and Fernandes 2013).

The Ribandar solar salt pans are fed by the Mandovi estuary and in turn are vulnerable to metal effluent influx from ferromanganese ore mining activity, barge traffic, and sewage dis-

posal, affecting the water and sediment quality in the salt pan and its inhabiting organisms. Solar salt pan is a niche for extremophiles thriving on temperature variation, oxygen availability, solar radiations, pH, nutrient concentration, salinity variation, and water activity. Besides other functions, these extremophilic microbes play a key role in modulating the metal concentrations in the salt produced.

White et al. (1998) have reported comfortable leaching of metal contaminants, viz., Zn, Ni, Mn, Cr, Co, and Cd, from artificially contaminated soil. In wastewater treatment, physiochemical methods, viz, chemical precipitation, carbon absorption, ion exchange, and electrochemistry, are generally used, but still have some disadvantages. When higher concentrations of heavy metals (1–100 mg/l) are present, proportionately the treatment and material cost increases. In some treatment processes, poor selectivity was observed for competitive metal absorption. Biological treatment has an advantage over traditional chemical treatment due to low operational cost, steady effect, and a smooth recovery of the desired metals (Wang et al. 2001; Rehman and Shakoori 2001).

Biosorption and bioaccumulation of metals by microorganisms are probably one of nature's safeguards for reducing metal ion toxicity in the surrounding microbial niche. Potential applications of these phenomena, however, are governed by certain criteria or characteristics of the biosorbent. These include metal affinity, rate of metal uptake, selectivity, temperature tolerance, versatility, and robustness (Eccles 1995).

Bacterial resistance to metals may be due to properties like metal precipitation, metal detoxification, absorption, or accumulation. Bioabsorption involves the cell surface with a complex formation between functional groups like phosphoryl, carbonyl, and hydroxyl present on the cell surface with metal ions. Bioaccumulation involves transport systems and

depends on active metabolism of the cell (Mohan and Pittman 2007).

In the Ribandar salt pans of Goa, due to continuous exposure of heavy metals, there is an emergence of metal-tolerant bacterial strains. It was seen that these tolerant bacteria employed specific and multiple mechanisms for detoxification of metals. These metals were thus removed from the overlying water of the salt pans and were found to accumulate in the sediment (Pereira and Kerkar 2014).

The average ranges of metal concentrations recorded in the Ribandar salt pan water, salt crystals, and sediment were listed below in Table 2.1 which shows the concentration ranges of toxic heavy metals such as cadmium, zinc, and lead were well within the permissible limits of 0.001–0.05 ppm, 0.005–5 ppm, and 2–20 ppm in water and 0.03–0.3 ppm, 50–300 ppm, and 2–20 ppm in the sediment, respectively (RSMENR 2002).

Our previous assessment of the concentration of metals in the Ribandar salt pan sediment for all seasons revealed that the metal concentration increases by 52% during salt harvesting season. Attri and Kerkar (2011) reported the metal con-

centrations in the Mandovi estuary are $18.3 \pm 1.9\%$ Fe (Attri et al. 2011), $0.19 \pm 0.002\%$ Mn, 36.2 ± 4.2 ppm Co, and 102.3 ± 9.8 ppm Zn.

Attempts were made to precipitate high levels of Co using hypersaline SRB which revealed 90% of Co was precipitated (where 3% was by the SRB cells and 87% by SRA) and hence established the role of SRB in bioremediating Co. It was observed that SRB cells could bio-adsorb cobalt minimally; however the actively growing SRB cells actually mediate the bioremediation of Co by utilizing the sulfide metabolized to precipitate Co as cobalt sulfide in the medium.

2.2 Sulfate-Reducing Bacteria

SRB are members of delta subdivision of Proteobacteria. They are strict anaerobes and their permanent habitats being estuarine, marine and salt marsh sediments, saline and hypersaline ponds, and lakes. Due to the high and almost inexhaustible supply of sulfate, SRB are able to produce sulfide in high concentrations which

Table 2.1 Metal concentration in salt pan water, salt crystals, and salt pan sediment

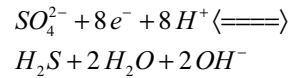
Metals	Dissolved form in salt pan water (in ppm)	Particulate form in salt pan water $\times 10^3$ (in ppm)	Metal concentration in salt crystals $\times 10^4$ (in ppm)	Metal concentration in sediment $\times 10^4$ (in ppm)
Li	–	–	–	0.0044
Mg	1.109	4	1.400	1.3190
Al	0.062	0.359	4.400	3.5245
Ti	0.010	1.0	0.620	0.51
Sc	–	0.001	–	–
V	0.047	0.121	0.013	–
Cr	0.021	0.009	0.018	0.0089
Mn	0.255	0.882	0.064	0.0258
Fe	0.173	2.749	5.900	3.037
Co	–	0.024	0.003	0.002
Ni	0.005	0.041	0.007	0.005
Cu	0.058	0.021	0.005	0.003
Zn	0.136	0.030	0.067	0.004
Sr	0.046	0.120	0.013	0.027
Cd	0.002	–	–	–
Ba	–	–	0.095	0.005
Hg	–	–	–	–
Pb	0.116	0.294	0.002	6.750

precipitate most of the metals present in ionic form into their corresponding metal sulfides. The role of sulfate-reducing bacteria in coastal marine sediments amounts to almost 50% of the organic material degradation (Jorgensen 1982), and also their involvement in anaerobic turnover of certain metals makes them important as metal contamination detoxifiers.

SRB comprise of anaerobic bacteria that use sulfate as their terminal electron acceptor forming a mixed group which is morphologically and nutritionally diverse. SRB oxidize a range of compounds including fatty acids, organic acids, alcohols, and H_2 as an electron donor and carbon sources. A symbolic expression of SRB metabolism characterized by production of a strong reducing agent, hydrogen sulfide, is able to inhibit growth of other microorganisms present in its environment (Gibson and Suflita 1990). The SRB perform dissimilatory and assimilatory sulfate reduction, with the dissimilatory process far exceeding the assimilatory reduction.

The genera of SRB are generally defined in terms of their morphology rather than physiology. The majority of SRB are reported to stain gram-negative with *Desulfovibrio* being the most encountered genus and *Desulfotomaculum* being the sole gram-positive genus. However gram-staining behavior of SRB is diagnostically unreliable (Boopathy et al. 1998a; Zehnder 1988). Although morphologically diverse, SRB are considered to be physiologically unified. Currently 18 dissimilatory SRB genera are placed into two physiological subgroups. The first group contains *Desulfonema*, *Desulfovibrio*, *Desulfobulbus*, and *Desulfotomaculum* genera as they can utilize ethanol, lactate, and pyruvate or certain fatty acids as carbon and energy sources. The second group contains genera that are specialized in oxidation of acetate and fatty acids such as *Desulfobacter*, *Desulfococcus*, and *Desulfosarcina* (Madigan et al. 1997).

The reduction of sulfate to hydrogen sulfide is an eight-electron reduction reaction:



The reaction proceeds through a number of intermediate stages. The stable sulfate ion is first activated by the enzyme adenosine triphosphate (ATP) sulfurylase to give adenosine phosphosulfate (APS). In dissimilatory sulfate reduction, the sulfate in adenosine phosphosulfate is then reduced to sulfite releasing adenosine monophosphate (AMP). In assimilatory reduction another phosphate molecule adds to APS to form phosphoadenosine phosphosulfate (PAPS), after which the sulfate is reduced. Sulfite is the first product of sulfate reduction in both cases (Madigan et al. 1997). SRB are associated with the systems that are characterized from fouling problems and a pungent smell of H_2S . *Desulfotomaculum nigrificans* and *Desulfovibrio desulfuricans* are the two widely distributed, most common SRB species found in the anaerobic environment. Though SRB favor anaerobic conditions, they still can grow in oxygenated environments, even in slimy deposits where aerobic conditions persist. They also establish themselves in the well water beneath the aerobes like IRB (iron-reducing bacteria) which form biofilms on the surface and use up oxygen, while SRB thrive in the anaerobic condition below the IRB biofilm. The presence of SRB is detected by visualizing yellowish or reddish nodules on metal surfaces and exhibits a black color due to the production of iron sulfide when nodules are broken open. A bright metallic pit on the metal forms on the removal of nodules and releases H_2S when hydrochloric acid is added to it which is characterized by a rotten egg smell.

SRB reduce sulfate via dissimilatory pathway to obtain its energy. It can grow by utilizing miniscule amounts of grease and oil as a nutrient source. Low flow or stagnant water favors its chances of growing. SRB are also considered as biocorrosion agents, as the produced hydrogenase enzyme enables them to use elemental

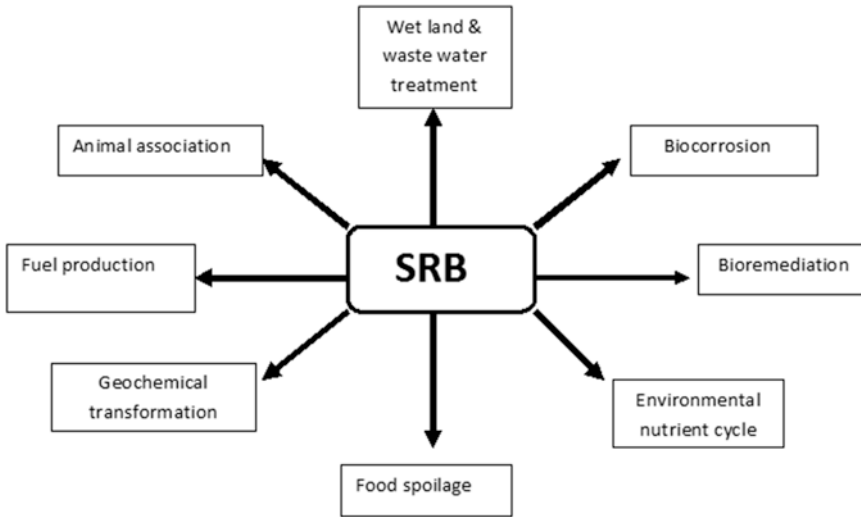


Fig. 2.1 Role of SRB

hydrogen to reduce sulfate and generate H_2S which triggers biocorrosion. Hence iron corrosion through such biological processes occurs very rapidly as compared to normal iron rusting (Fig. 2.1).

2.3 Bioremediation of Heavy Metals

Bioremediation is an ancient technology, dates back to 6000 BC, as evident from compost pile and kitchen middens (NABIR primer 2003), and demonstrates an ancient bioremediation practice by human beings. Bioremediation techniques utilize microbes to remove or convert toxic contaminants present in the environments like water, sediments, soil, and air to a less toxic form. The sewage treatment plant in Sussex, UK, in 1891 (considered to be the first biological treatment system), demonstrates bioremediation process application that started more than 100 years ago (NABIR Primer 2003). The wastewater treatment, using microorganisms to remove heavy metals, is one of the most investigated research areas in the current scenario (Leusch et al. 1995; Kaewsarn 2002; Wu et al. 1996; Xu et al. 2005; Adeniji 2004). There are various strategies being adopted by microorganisms in order to counter-

act the presence of heavy metals in its surrounding to facilitate bioremediation:

- (a) *Bio-mineralization*: Bio-mineralization is a process of precipitation of insoluble metal with the interaction of metabolic products of microbes. Such processes result in mineral formation and geochemical depositions. Bio-mineralization adds value to metal microbe interaction research (Ehrlich 1999; Banfield et al. 2000; White and Gadd 2000).
- (b) *Bio-accumulation*: Bio-accumulation is a metal uptake process which requires external energy to enter into the cells and get accumulated. Some physiologically essential metal ions, toxic metals, and radionuclides have been reported to enter into the cell using the energy transport system. For example, K^+ ion uptake is linked with H^+ ion bound to the plasma membrane and ATPase through membrane potential. Such processes get affected by the factors that inhibit energy metabolism of cells. As explained by White and Gadd (1987), absence of substrates, anaerobiosis, low incubation temperature, and respiratory inhibitors like cyanide could affect this metabolism.
- (c) *Bio-sorption*: It is a widely used approach to bioremediate metals and radionuclides, involving passive sequestration of metals by

their interaction with living or dead biological entities. It is effectively used in wastewater treatment (Schiewer and Volesky 2000; Jang et al. 2001).

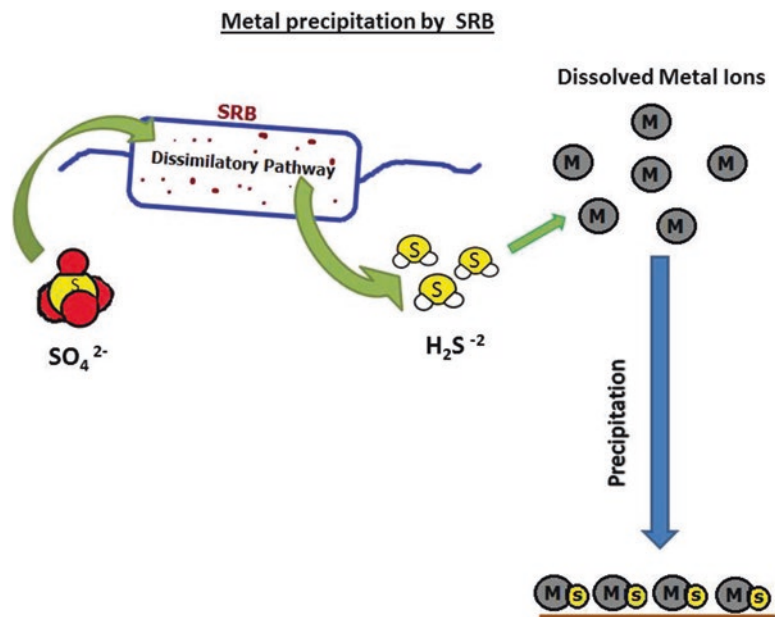
- (d) *Biotransformation*: Microbes can transform toxic metals to less toxic forms by catalyzing them to more volatile or less soluble form. For example, metal precipitation achieved by the microbial reduction of Cr(VI) to Cr(III), Se(VI) to Se(0), V(V) to V(III), Au(III) to Au(0), Pd(II) to Pd(0), and U(VI) to U(IV) has been reviewed by Lloyd (2003).
- (e) *Microbially enhanced chemisorption of heavy metals (MECHM)*: This is a class of microbial cell-mediated reactions, which involve the precipitation of metal biomineral called “priming deposit” acting as a nucleation center on which subsequently targeted heavy metals get deposited and promote a targeted metal precipitation reaction (Macaskie et al. 1996). Generally priming deposit is initiated by sulfide or phosphate biomineralization route. SRB adapt such a strategy when Fe is present on a precipitant metal to H₂S produced and then FeS acts as primary deposit and as an adsorbent for target metals (Ellwood et al. 1992; Watson and Ellwood 1994, 1988).

When metal biosorption strategies are not feasible, then a consortium of metal-resistant cells can ensure better remediation, combining biosorption, bioprecipitation, and bioaccumulation. This approach can be used simultaneously in the removal of toxic metals and organic and inorganic pollutants from water. But these living cells have some constraints like sensitivity to extreme pH, high metal concentration, and metabolic energy production requirements limiting their use in bioremediation. Therefore efforts are being made to meet such challenges by metal-resistant microbial strains.

2.4 SRB as Bioremediators of Heavy Metals

The use of microorganisms in metal polluted water remediation was successfully used for the reduction and precipitation of soluble metal sulfates as insoluble sulfides in liquid wastes by using SRB, as they use sulfate as their electron acceptor to produce H₂S, binding with metals to give their metal sulfide. Nowadays this procedure is effectively used for surface water treatment, underground water cleaning up, and even in commercial treatment plants (Fig. 2.2).

Fig. 2.2 Mechanism of metal precipitation by SRB



Microbiologically produced H_2S is reported to be an effective way to immobilize metals like iron, cadmium, nickel, lead, copper, and zinc in bioprecipitating metals as their insoluble sulfides, e.g., FeS , CdS , NiS , CoS , ZnS , and CuS (White et al. 1998; White and Gadd 2000; Labrenz et al. 2000; Wang et al. 2001; Drzyzga et al. 2002; Valls and Lorenzo 2002; Utgikar et al. 2002; White et al. 2003; Krumholz et al. 2003). Precipitating toxic concentrations of metals as metal sulfides adds value to anoxic wetland and sediment bioremediation (White et al. 1998; Kaksonen et al. 2003; Labrenz and Banfield 2004). Jalali and Baldwin (2000) were able to grow SRB in a solution up to 150 mg L^{-1} of copper and remove copper to levels below 0.1 mg/L . Mining and mineral processing of polymetallic ore in Vromos bay area near the Black Sea coast of southeastern Bulgaria have resulted in the contamination of the surrounding agricultural land with Th, Ra, and U radioactive elements. Other toxic heavy metals (e.g. Co, Cd, and Pb) are also present. Laboratory experiments demonstrated efficient treatment of the soils by using in situ treatment method, where acidified water was used to solubilize the metals, and the SRB in turn immobilized the metals. Real field application of this process gave promising results (Groudev et al. 2001). The sulfate-reducing bacterium, *Desulfovibrio desulfuricans*, was found to be capable in coupling the oxidation of an organic compound with an enzymatic reduction of uranium (VI) to uranium (IV), which precipitated out of solution from groundwater contaminated with uranium (VI) as uraninite (Abdelouas et al. 1999, 2000). SRB are a physiologically important group of microbes that are used extensively in metal bioremediation of water and soil with their produced sulfides to remediate toxic metal contaminants in soil.

Many sulfate-reducing microorganisms can also reduce ferric ions and may preferentially reduce $Fe(III)$ at low electron donor concentrations, typically in the sediments (Coleman et al. 1993; Lovley et al. 1993). Analysis of microbial lipids suggested that sulfate-reducing bacteria belonging to genus *Desulfovibrio* were enriched in zones of ferric reduction in salt pan sedi-

ments, suggesting that they might be involved in ferric ion reduction in these environments (Coleman et al. 1993). The biosulfide process described by Rowley et al. (1994) involved the separation of chemical precipitation of sulfide metals from the biological conversion of sulfate to sulfide. The advantage of this process is that the SRB biomass is not exposed to the fluctuating conditions of the wastewater effluent, which means bacterial sensitivity to toxic compounds is eliminated.

2.5 Hypersaline SRB and Metal Bioremediation

Hypersaline environments are those which contain high concentration of salt. These include mainly inland lakes (the Dead Sea, Great Salt Lake, etc.), soda brines, deep sea brines, polynias, and marine salt pans. SRB are omnipresent and are hyperactive in ecosystems with high sulfate concentrations and are considered to be one of the oldest forms of bacterial life on earth. Hypersaline environments support the growth of salt-loving organisms and these halophilic organisms can tolerate such environments which limit the growth of other organisms. Halophilic organisms produce a variety of stable and unique biomolecules during their growth that may be useful in various biotechnological applications (Kerkar 2004). Literature reveals that halophiles possess excellent metal-scavenging capability. Metal-tolerant strains of bacteria from hypersaline niches were found to tolerate various metals at higher concentrations and can be considered as a potential candidate for metal removal from wastes. In hypersaline environments like solar lakes and salt pans, SRB communities carry out extremely active sulfate reduction with a temporal, spatial, and functional separation of activities among the species present. SRB maintain an osmotically isotonic cytoplasm to cope up with the outside medium osmolarity, as their survival strategy in order to thrive in hypersaline environments (Kerkar 2004). We have detected *Desulfobacteraceae* at salt concentrations of 475 g/l which indicates

the existence of a unique oxidizing SRB with an inorganic osmoadaptation strategy or the presence of spatial microniches of lower salt concentrations in the salt pan. Harithsa et al. (2002) assessed the tolerance of HgCl_2 and $\text{Pb}(\text{NO}_3)_2$ salts using three mesohaline SRB (HSR1, HSR4, HSR14) at 95 psu with concentrations ranging from 50,100 to 500 $\mu\text{g/ml}$. Growth and SRA were the assessed parameters. Growth of HSR1 strain was 80 % at 100–200 $\mu\text{g/ml}$ of mercury, while sulfate-reducing activity decreased to 60 % as compared to the control. HSR14 could grow normally at 200 $\mu\text{g/ml}$ of HgCl_2 , but SRA was inhibited by 60 %. In the presence of 500 $\mu\text{g/ml}$ of $\text{Pb}(\text{NO}_3)_2$, HSR4 growth was stimulated by 160 % and SRA by 170 % as compared to the control. There is a possibility that some hypersaline SRB strains can tolerate heavy metals more efficiently than their mesohaline counterparts and thus can be a better candidate for metal bioremediation (Kerkar and Lokabharathi 2007, 2011).

2.6 Measurement of SRA and Its Variation with Salinity

In hypersaline anoxic sediments (Oren 1999; Skyring 1987; Ollivier et al 1994) and hypersaline microbial mats (Canfield and Des Marais 1993; Caumette et al 1994), sulfate reduction governed by bacteria is of great ecological and biochemical importance. Changes in biological lability and the amount of organic matter that undergoes decomposition show a large variation in the sulfate reduction rate in marine sediments (Goldhaber and Kaplan 1974). In the marine coastal ecosystem, SRA contributes almost 50 % of organic carbon turnover in the sediments, while the total sediment respiration rate is estimated to be 2.5–5.5 $\text{g cm}^{-2} \text{day}^{-1}$. Therefore SRB play an important role in hypersaline ecosystems like solar salt pans of Goa.

To ascertain the sulfate reduction rate in this complex system, multiple methods were carried out to determine an integrated and comparative estimate of SRA. Four different methods were followed:

1. Spectroscopic method for fatty acid-amended sediment
2. Radio isotope ^{35}S method
3. Monthly measurement of increase in natural sulfide content
4. Monthly assessment of decrease in natural sulfate content

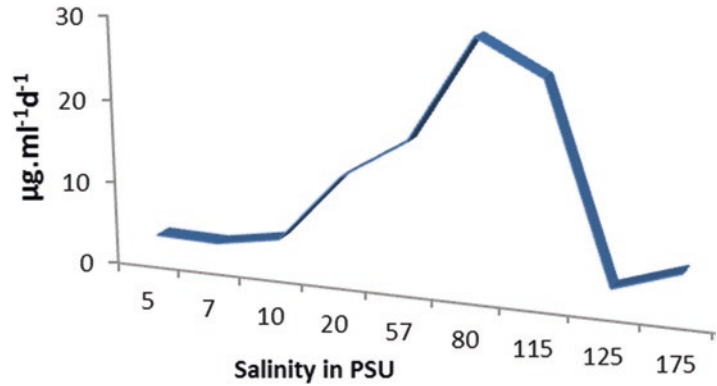
All the estimations were carried out during the peak salt manufacturing seasons from 0 to 2, 2 to 5, and 5 to 10 cm depth of sediment cores and the values were expressed as $\text{ng g}^{-1} \text{h}^{-1}$.

From fatty acid-amended sediment method, SRA at three depths were 2929, 1379, and 1342 $\text{ng g}^{-1} \text{h}^{-1}$, while ^{35}S method at 85 psu was found to be 3,713,721 and 200 $\text{ng g}^{-1} \text{h}^{-1}$ but 330 psu. SRA varied from 108, 25, and 13 $\text{ng g}^{-1} \text{h}^{-1}$, respectively, along the depth. SRA based on increase in natural sulfide was found to be 65, 9, and 33 $\text{ng g}^{-1} \text{h}^{-1}$, while natural decrease in sulfate was measured to be 6.2, 0.1, and 3.5 $\text{ng g}^{-1} \text{h}^{-1}$, respectively, at the three depths. Results showed a trend of depth-wise decrease in SRA measured by all four methods and higher values were obtained for surficial sediment at 0–2 cm depth (Fig. 2.3).

SRA measurements by fatty acid amended and ^{35}S methods were comparable, while values at 330 psu were very low. Thus 330 psu sediment was amended with 10 mM of fatty acid cocktail which helped in measuring SRA by ^{35}S within 24 h. To overcome auto-oxidation of sulfide and other artifacts due to long incubation time, short-period incubation is generally recommended. SRA measured by fatty acid-amended technique (3190 $\text{nM cm}^{-3} \text{day}^{-1}$) are comparable to the tracer technique (2050 $\text{nM cm}^{-3} \text{day}^{-1}$) which was 1.3 times higher in SRA measured by ^{35}S method (85 psu) at surficial sediments, which were four orders higher from the values given by Li et al. (1999) of 0.5 $\text{nM ml}^{-1} \text{day}^{-1}$.

The rise in sulfide concentration revealed an SRA of 65 $\text{ng g}^{-1} \text{h}^{-1}$, which was two orders lower than the values of above two methods, probably because of the generated sulfides that rapidly get oxidized in the system chemically or biologically in their natural conditions. Moreover H_2S easily escapes out of the system. SRA based on decrease

Fig. 2.3 Effect of salinity on sulfate reduction activity



in sulfate concentration level yielded a very low value of $6.2 \text{ ng g}^{-1} \text{ h}^{-1}$, which is one order lower than the sulfide increase value. Natural sulfide and sulfate concentration analyses showed a 100-fold difference between the sulfide formed and the increase in sulfate concentration. Such a variation in a chemically stable compound could be explained through biological oxidation in the natural system and is much faster than biological reduction. Salinity plays a key role in the process as 85 psu salinity yielded higher SRA than 330 psu. Thus we measured SRA on different salinity gradients to obtain an optimal value for SRA. It was found that salinity enhances SRA as presented in Fig. 2.4. Increase in SRA was observed from 20 to 115 psu. Maximum activity was observed at 80 psu; however the activity drastically decreased at 125 psu. Highest values of 29.96 and $25.87 \text{ } \mu\text{g ml}^{-1}\text{day}^{-1}$ were obtained for 80 and 115 psu suggesting it to be the optimal salinity to foster higher SRA. We observed the sulfate reduction rate is somewhat related to the optimal salinity range of 80–115 psu as the values are significantly higher (Fig. 2.3).

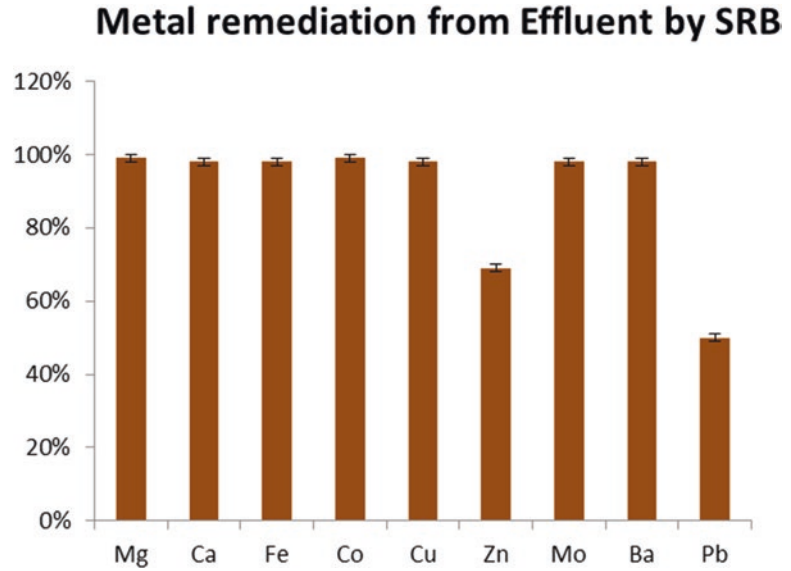
From the obtained data, it could be a promising alternative to use a consortium of hypersaline SRB from Ribandar salt pan in heavy metal remediation as they resist and precipitate more heavy metals than its mesohaline counterpart,

thus enabling them as a better candidate for saline wastewater treatment.

2.7 Effect of Metals on SRA

To access the impact of certain metals influencing activity of hypersaline SRB, lead (Pb) at concentrations of 0, 100–500 psu, was used at incubation periods of 7 and 14 days with different carbon sources (individually) like formate, acetate, lactate, butyrate, etc. Formate was found to stimulate the growth and respiration after 14 days. Similarly when selected strains were grown with mercury (Hg) at 0–500 ppm. it was found that the activity was inhibited. However formate and lactate stimulated the activity up to 100 ppm of Hg and SRA was observed up to 400 ppm of Hg. These experiments indicated that different metals influence the SRA, whereas the presence of low concentrations of specific carbon sources with the respective metal stimulates the SRA. Therefore addition or the presence of certain carbon sources in an environment contaminated with metals stimulates the SRA and thus could be used to enhance the rate of metal bioremediation, i.e., in the formation of metal sulfides, and to increase the level of tolerance to higher concentrations of the metal. The higher the SRA, the higher the production of

Fig. 2.4 Metal remediation from industrial effluent



H₂S which ultimately acts upon the heavy metals and precipitates them as metal sulfide, thus increasing bioremediation efficiency.

2.8 Bioremediation of Industrial Effluent with SRB

Organisms in hypersaline ecosystems can tolerate high metal concentrations in their surrounding and adapt various strategies for survival in such extreme conditions. Thus SRB from such environments are considered to be ideal in mitigating problems with environments having high metal contaminations. This is due to their adaptability to extreme saline condition and metal precipitation capabilities. Researchers worldwide have surveyed the heavy metal contents of the sediments from rivers, salt pans, bays, lagoons, and harbors and mostly detected sulfur in the deposits of heavy metals and attributed it to the role of SRB. H₂S gets discharged into the environment as the final product of sulfate respiration via SRB which are responsible for precipitation of metal ions as less soluble metal sulfides. The H₂S production is generally considered to be the main reaction that governs the metal sulfide precipitation (Amacher et al. 1993).

Modified Hatchikians media (1972) of 100 psu were prepared by dissolving NaCl in seawater to isolate hypersaline SRB from the Ribandar salt pans. SRB tolerating higher concentration of Pb, Hg, Ni, and Co were used in bioremediation resulting in almost 100% heavy metal precipitation from industrial effluents with a salinity ranging from 8 to 90 psu and containing various metals.

Bioprecipitation of nine metals (viz., Mg, Ca, Fe, Co, Cu, Zn, Mo, Ba, Pb) using mix consortia (three strains) of potential SRB out of 15 different metals in varying concentration (listed in Table 2.2) from an industrial effluent was achieved over an incubation period of 17 days. Experimental results show that the consortium was capable of precipitating out nine metals at 50–99% concentrations.

Tolerance of SRB to environment stress factors like salinity and temperature in the salt pans and their capability of reducing toxic metals at neutral to alkaline pH has an added advantage over traditional metal remediation techniques requiring acidic condition for metal removal. This process of using hypersaline SRB for bioremediation of metals is clearly a more attractive option as the anaerobic waste treatment systems are advantageous as they do not require oxygen and mixing as the aerobic counterparts.

Table 2.2 Different metal concentrations in industrial effluent

Metals in effluent	Mg	Al	Ca	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Ba	Pb
Concentration in mg/L)	3.41	2.54	12.53	0.20	22.17	0.13	7.98	33.35	0.06	0.75	11.30	76.20	3.39	0.04	0.06

2.9 Conclusion

Hypersaline SRB develop tolerance and their metabolic activity gets stimulated in the presence of metals in its surrounding environment by virtue of its survival strategy in this natural habitat bearing higher metal concentrations. Since SRA is the main functional process in SRB which controls the bioremediation of heavy metals from saline waters, stimulating the sulfate-reducing activity would enhance the formation of high levels of sulfides which in turn would lead to the rapid precipitation of metal sulfides and thus remediate the heavy metals from the surrounding waters. Saline waters have limited types and low concentrations of carbon sources. Hypersaline sulfate-reducing bacteria have a tremendous potential and application in bioremediating heavy metals from saline waters, and their activity can be enhanced on supplementing with low concentrations of a specific carbon source according to the strain's requirement. Depending upon the type of metal contamination, individual hypersaline strains with specific metal tolerance levels or mixed consortia with multiple metal tolerance and high SRA could be used to bioremediate metal-contaminated saline waters.

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