Studies on the abundance and diversity of manganese and copper tolerant bacteria in ridge ecosystem and their role in metal uptake

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By

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Under the Guidance of

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Declaration

I declare that the thesis entitled **"Studies on the abundance and diversity of manganese and copper tolerant bacteria in Ridge ecosystem and their role in metal uptake"** is my own work conducted under the supervision of **Dr. Shyama S.K.**, Professor and Head, Department of Zoology, Goa University, Goa, under the University Ordinance OB 9A.

I further declare that to the best of my knowledge, the thesis does not contain any part of any work which has been submitted for the award for any degree either in this University or in any other University / Deemed University without proper citation.

Babu Shashikant Mourya

Certificate

This is to certify that the thesis entitled "**Studies on the abundance and diversity of manganese and copper tolerant bacteria in Ridge ecosystem and their role in metal uptake"** submitted by **Mr. Babu Shashikant Mourya** for the award of the **degree of Doctor of Philosophy in Zoology** is based on his original studies carried out by him under my supervision. The thesis or any part thereof has not been previously submitted for any degree or diploma in any University or Institution.

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CONTENTS

INTRODUCTION

1.1 Carlsberg Ridges as an ecological niche

Seafloor ridges are elevated underwater chain of mountains formed by geological processes associated with floor spreading and plate tectonics. Following are the important major level ridges of our earth.

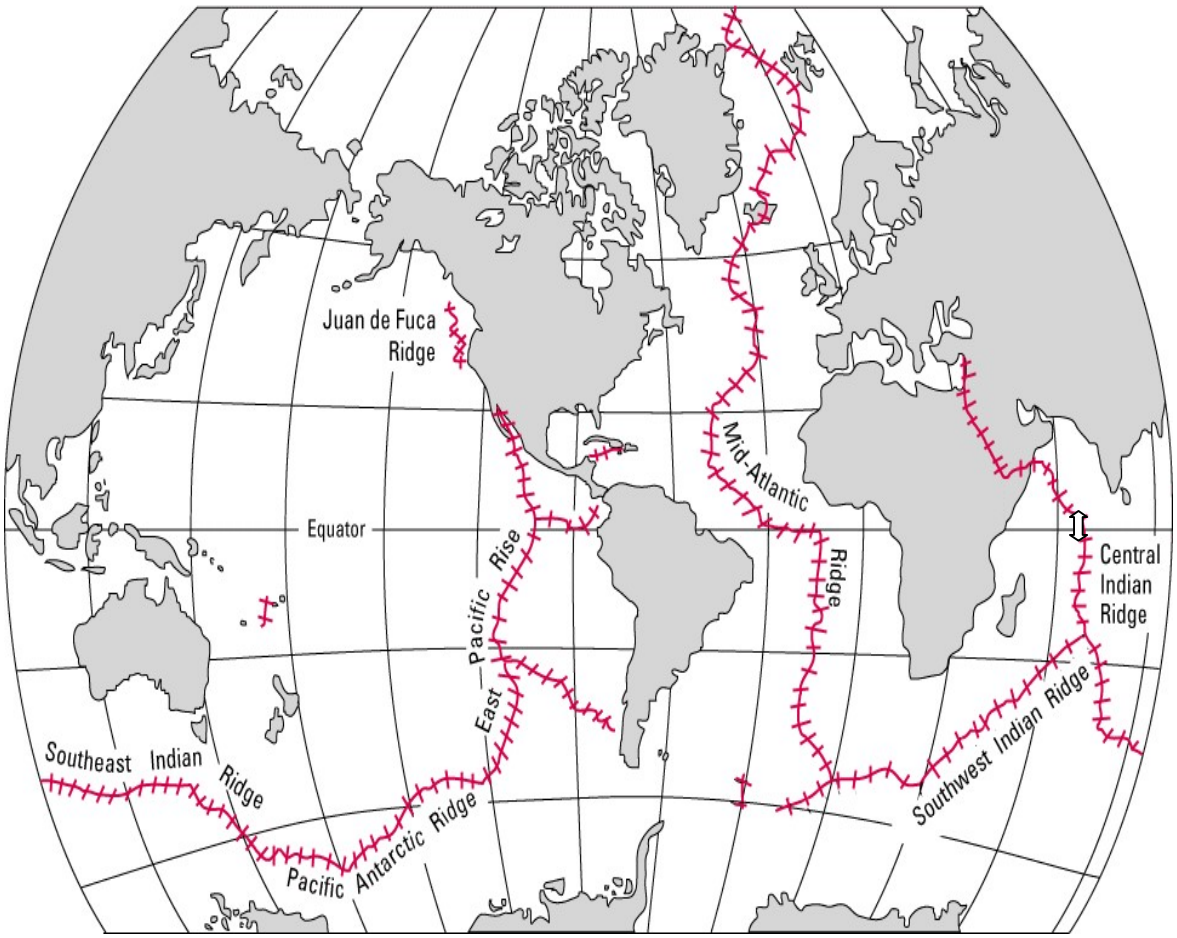


Fig. 1.1: Distribution of the Ridges in the world

Source - https://www.thoughtco.com/map-of-the-mid-ocean-ridges-1441097

Arrow indicates Carlsberg ridge

- The Southwest Indian Ridge
- Mid Atlantic ridge
- Juan de Fuca ridge
- East Pacific ridge
- Pacific Antarctic ridge
- South East Indian ridge

The northern section of the Central Indian Ridge (CIR) between the African Plate and the Indo-Australian Plate, traversing the western regions of the Indian Ocean is known as the Carlsberg Ridge. This ridge is characteristically seismic because of its divergent tectonic plate. Ridges are formed due to the release of hydrothermal fluids associated with venting as these locations act as a source of elevated Mn (II), Cu II and Fe (II) concentrations. These elements exert an influence on the geochemical characteristics of the water column and thereby support unique forms of microbial life that catalyze the oxidation/reduction of metal cations leading to the precipitation or dissolution of minerals. Carlsberg Ridge (CR) in the Arabian Sea is a segment of the Central-Indian Ridge near Rodrigues Island which is known as a unique and extreme ecosystem due to low temperature, high pressure, very low organic matter and high availability of inorganic ions. All these environmental characters lead to extreme kind of ecosystem and presence of microbes like bacteria as interlink between inorganic ions and fauna present in ridges near hydrothermal vent by chemosynthetic activities resulting in fixing of organic carbon from inorganic ions (Das *et al*., 2012).

Hansell and Carlson (1998) reported 42.8 µM of dissolved organic carbon (DOC) at water depths of 1000 to 4300 m which support microbial oxidation as a dominant process. DOC concentration varies from 70-80 µM during the northeast

monsoon and 75-90 μM during the spring inter-monsoon period (Hansell and Peltzer, 1998) which may due to microbial mobilization at the same environment. Ecologically and chemically, Deep Sea Hydrothermal Vents (DSHVs) play an important role in several global chemical budgets by introducing as unique and important ecosystems (Seyfried and Mottl, 1995; Van Dover, 2000). Formation of DSHV is a common phenomenon in ridges due to constructive margins of oceanic plates on the sea surfaces, where the new oceanic lithosphere is continually generated due to rifting and is characterized by high heat flow and marked seismicity (Iyer *et al*., 2003). DSHVs are the source for hydrothermal fluids to emerge from the seafloor. Direct mixing of these hydrothermal floods with the surrounding seawater creates strong chemical and temperature gradients. Due to this chemical and temperature gradients, fluids become enriched in reduced metal ions which can be used by chemolithotrophic bacteria as a source of energy (Van Dover, 2000). These chemolithotrophs work as base of the food chain in ridge ecosystem which also supports chemoorganotrophic microorganisms and diverse macro-fauna. Bacteria also play a potential role in the mobilization/weathering of minerals and oxides in many ways (Ehrlich, 1996). Direct Mobilization of primary minerals can be induced by chemolithotrophic microbes and at the same time dissolution may also happen from the precipitation of secondary minerals through enzyme-catalyzed oxidation or reduction (Lovley and Phillips, 1988; Francis and Dodge, 1990, 1991; Zachara *et al.,* 1998). Production of ligands by chemolithotrophic or chemoorganotrophic microbes is an indirect way to solubilize minerals (e.g. organic acids, metabolites, siderophores and polysaccharides) which form complexes with mineral-forming ions, which further induce ligand-promoted mineral dissolution (Francis and Dodge, 1990, 1991; Barker and Banfield, 1996; Kalinowski *et al*., 2000; Liermann *et al*., 2000; Welch and Banfield, 2002). Relatively few reports are available regarding Mn mobilization by

bacteria in basalt rocks, particularly in marine environment or near DSHVs. Sujith *et al*. (2014) have investigated Mn mobilization in ridge basalt rock by microbial community in the laboratory conditions. Whereas, Thorseth *et al*. (1991) have demonstrated the microbial alteration of basaltic glass in the marine environment and in the laboratory (Thorseth *et al*., 1991, 1992, 1995a, 1995b; Fisk *et al*., 1998). At the same time Fortin *et al*. (1998) reported that bacteria near DSHVs were coated with secondary Fe and Mn oxides and iron silicates, although it was not clear if the bacteria played a direct (enzymatic) or passive role in the formation of the precipitates (Juniper and Tebo, 1995).

Hence, we hypothesize that along with certain environmental and chemical process for mineral deposition, immobilizations by microorganisms associated with these basalts in ridges also have a big contribution. The present study reveals that the bacteria are capable to mobilize Mn from basaltic minerals and oxides in their pure form of metal which strongly supports biomining in ridges. So, R6 was used an identified culturable Mn (II)-oxidizing bacteria to check an interactive role in the mobilization of Mn in the ridge ecosystem.

1.2 Metal-rich mineral deposits and their formation

Ridges are marine environments where the migration of Fe and Mn from less oxidizing to more oxidizing conditions contributes to the formation of Fe, Mn and Fe-Mn mineral deposits (Glasby, 2006). These deposits occur in almost all geomorphologic and tectonic environments of the ocean basins in the form of nodules, crusts, cement, mounds and sediment-hosted strata-bound layers. They are formed by one or more of four processes namely (1) hydrogenetic precipitation from cold ambient seawater (2) precipitation from hydrothermal fluids (3) precipitation from sediment pore waters by

diagenetic reactions and (4) replacement of rocks and sediments (Hein *et al*.,1997). The three major prerequisites that underpin the formation of Fe-Mn crusts are sediment free hard and exposed surfaces, availability of large pool of dissolved Mn and Fe and oxygenated ambient seawater (Banakar *et al*., 1997). Depending on the type of reactions or the combination of the said processes, they vary in their mineralogy, Fe/Mn ratio, trace metal contents, rare-earth element patterns and growth rates. The hydrogenetic crusts are normally formed by a continuous process on tectonically stable and geologically old seamounts and exhibit Fe/Mn ratio near unity (1±0.3). Hydrothermal deposits are episodic, have distinctly different composition from the hydrogenetic type of crusts i.e. vary highly in their Fe/Mn ratio from 24000 to 0.001 (Hein *et al*., 1997). On the other hand, the precipitation of metal ions from sediment pore waters by diagenetic reactions lead to the formation of Fe-Mn concretions/nodules (Reyss *et al.*, 1982). They differ from hydrogenetic nodules and crusts in their Mn/Fe ratio.

1.3 Carlsberg ridge: a potential site for hydrogenetic Fe-Mn crust

The Carlsberg ridge (CR) in the Northern Central Indian Ocean is one of the low productive environments characterized by seasonal thermohaline alteration, voluminous precipitation, and freshwater inflow (Sardessai *et al*., 2010). It extends from the near Rodrigues Island to the Gulf of Aden, trending Northwest to Southeast and separates the Arabian Sea in the Northeast from the Somali Basin in the Southwest (Fournier *et al*., 2008). The mean elevation of the Carlsberg Ridge crest is 2100 m and the water depth is 1800 to 3600 m (Mudholkar *et al*., 2000). A ridge rock sample bearing oxide coating was collected at 3°39.718N (lat.) and 63°49.922 E (long.) (Figs. 4.1 A and B). The CR is a potential site for the formation of Cu enriched Fe-Mn crusts. Approximate resource evaluation states 0.9 million tonnes of Cu metal for 180 million

tonnes of Fe-Mn crust assuming an average crust thickness of 2 cm and Cu content of 0.34% covering the whole 5000 km^2 area. The significance of the CR is not only because of its tectonic history but also due to the presence of potentially economically important Cu-enriched Fe-Mn crusts (Parthiban and Banakar, 1999). Mn and Fe which constitute the major (oxy)-hydroxide mineral phases of the Fe-Mn crust. The substrate of the crust is composed of fresh-water phreatic calcite cement, terebratulina casts, rounded and ferruginised basalt clasts and weathered coralline algal fragments (Banakar *et al*., 1997).

The Fe-Mn deposition on the CR is formed by hydrogenetic process and contains a promising source of Cu (0.5-1%), Ni (~0.26%) and other rare earth elements. The mineral phases are composed of poorly crystalline vernadite (MnO_2) (Parthiban and Banakar, 1999) resembling the amorphous mineral oxide phases of Mn produced by bacteria. Here the bacterial production accounts for the overall production of the region whereas primary production by phytoplankton is very low. The region exhibits sub-surface maxima for chlorophyll 'a' over seasons (Fernandes *et al*., 2008). The summit of the CR protrudes above the Calcite compensation depth (CCD) at 1550m (Rudenko, 1994) and lies at a shallower depth beneath the intermediate oxygen minimum zone (OMZ) (Banakar *et al*., 1997). This is the most suitable condition for the colloidal precipitation of Fe-Mn hydroxides and an ideal environment that suits the biological processes.

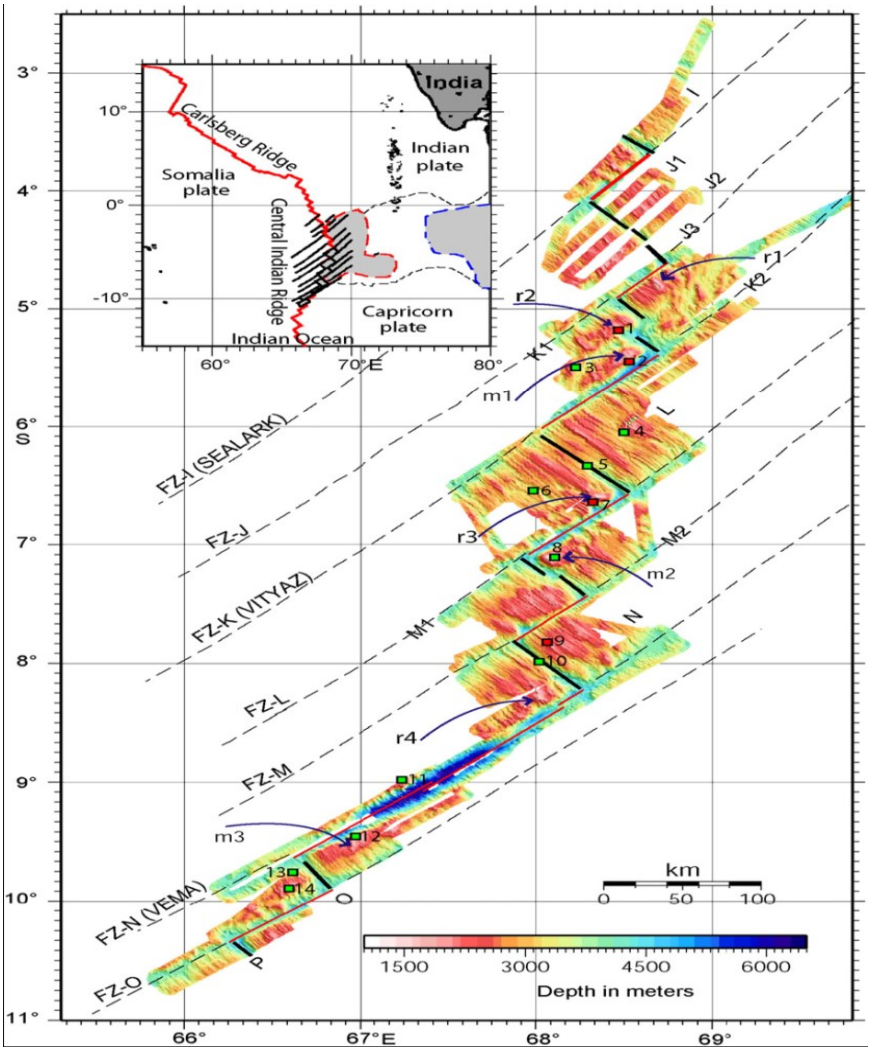


Fig. 1.2: Depth profile map of Carlsberg ridge

1.4 Interdependence of manganese and copper in marine microorganisms

Manganese is an essential micronutrient and its role in bacterial nutrition has been well established (Cowen, 1989). Most of the studies with Mn have been focused on its oxidation by bacterial isolates (Fernandes *et al*., 2005; Tebo *et al*., 2010; Sujith and Loka, 2011) and a few by non bacterial microbial communities (Sujith *et al*., 2011).

Studies on the association of Mn and bacteria are mostly dealt with metal uptake (biosorption/ bioprecipitation/ bioaccumulation), toxicity, transport mechanisms and mobilization (Fernandes *et al*., 2005, Sujith *et al*., 2011).

Reports are available on the Co and Ni immobilizing potential of Mn oxidizing bacterial isolates (Antony *et al*., 2011, Sujith *et al*., 2011; Krishnan *et al*., 2007) as well as the importance of plasmids and enzymes in metal tolerance (De Souza *et al*., 2006). The involvement of multicopper oxidases (MCOs) in Mn oxidation has been reported in various species of bacteria dominating in the water column of ridge ecosystem (Brouwers *et al*., 2000; Sujith and Loka, 2011). However, no reports are available on the participation of MCOs in Mn oxidation in fauna associated bacteria of ridge ecosystem. Hence, in order to know the mechanism of oxidation of Mn in fauna associated bacteria of ridge ecosystem, it is attempted to know the role of MCOs in the present study.

1.5 Interactions between microorganisms and metals

Metals do exist in almost all ecosystems and occur either in elemental and/or ore forms in nature (Tripathi and Srivastava, 2007). The microorganisms adapted to such metal rich deep-sea environments are expected to show metal immobilization/mobilization activity because of the restricted organic food supplies (Runa *et al*., 2011) in the open-ocean. These ecosystems exhibit surprising host diversity and show multiple abilities in organisms to tolerate metals. In order to adapt, survive and reproduce in such metal predominant, light deficient, special ecosystems, organisms need to make use of these metallic elements and procure energy for their metabolism/existence. Different mechanisms for the uptake and release of metal ions from their surrounding environment could, therefore, operate in these organisms. The

biogenic oxides of Mn for example produced by one organism with the potential to degrade humic substances to simpler compounds feed the growth of several other organisms with the required substrates (Sunda and Kieber, 1994). In the absence of carbon or energy, the storage of the metal serves as a respiratory substrate and energy for growth through the oxidation of reduced Mn (Tebo *et al*., 1997). Besides, bacterial cells couple the oxidation of organic matter to the dissimilatory reduction of metal oxides (Lovley and Phillips, 1988; Nelson *et al*., 1988). The dissolution of the oxides could occur by any one of the two processes wherein direct contact between the cell surface and the oxide leads to solubilization or indirectly through microbially mediated changes in the reducing conditions (Baglin *et al*., 1992; Sand *et al*., 2001). In some cases, the metal may be reduced to fulfill a nutritional need for soluble ions (de Vrind *et al*., 1986) or to scavenge excess reducing power as in the case of nitrate and iron reduction (Robertson *et al*., 1988; Lovley, 1991). The microorganisms that are associated with the metal-rich deep sea habitats belong to divergent phylogenetic lineages such as *Firmicutes, Proteobacteria*, *Actinobacteria,* and *Bacteroidetes* (Mourya *et al.,* 2015). This broad phylogenetic diversity mirrors their physiological diversity (Tebo *et al*., 2005). The elements like Mn, Cu, and Fe are essential micronutrients required for most, if not all, living organisms as a constituent of many metalloenzymes and proteins involved in electron transport, redox, and other important reactions (Ford *et al*., 1995). The primitive size and larger surface area to volume ratio permit microbial cells to interact with metal ions at different rates (Ledin, 2000). It is interesting that only prokaryotes have the ability to oxidize metals and conserve energy in those reactions.

Inevitably, certain microbes (eubacteria and archaea) use some metals or metalloids as electron donors or acceptors in their energy metabolism (Ehrlich, 2002).

Certain organisms have the ability to bind metal ions at the cell surface or to transport them into the cell for various intracellular functions (Ehrlich, 1997). The bacterial adaptation to heavy metal rich environments show activities involving biosorption, bioprecipitation, extracellular sequestration, transport mechanisms and/or chelation (Haferburg and Kothe, 2007). Although the requirements of metal ions for growth are generally small, the heavy metal uptake systems may often operate at higher concentrations and may also pose toxicity of metal ions to microorganisms at the individual and community level.

The different strategies that bacteria engage in order to tolerate or adapt to excess metal concentrations includes a) the production of stable precipitates or chelates with essential metabolites, b) catalyzing the decomposition of essential metabolites and thereby making the metabolites unavailable to the cells, c) acting as antimetabolites, d) replacing structurally or electrochemically important elements and thereby interfering with enzymatic or cellular function (Ehrlich, 1978). The metal uptake by bacteria either involves a) the nonspecific binding of the metal cations to cell surfaces, slime layers, extracellular matrices, or b) metabolism-dependent intracellular uptake (Gadd, 1990). Often the intracellular heavy metal uptake may precipitate or compartmentalize to a more innocuous form such as phosphide, sulfide, carbide or hydroxide deposits (Ehrlich, 1997). The interactions, however, when deadly, exerts morphological changes, altered cell metabolism, bacteriostasis or lethality in bacteria (Ehrlich, 1978). Interactions between bacteria and metal ions would also lead to the formation of biogenic minerals. The interactions of bacteria with metals may have several implications on the environment, because of the fact that metals play an essential role in the biogeochemical cycling of carbon, nitrogen, phosphorous and several associated elements in the

seamount ecosystem. Besides, they could also serve as suitable candidates for cleaning up or remediating metal-contaminated environments.

Substantial studies have reported the bacterial diversity of hydrothermally active seamounts of Pacific and Atlantic Oceans (Emerson and Moyer, 2010; Clark *et al*., 2010) but very little evidence exists on the bacterial ecology of hydrogenetic seamount Fe-Mn crusts and the associated sediments (Kato and Yamagishi, 2011). Studies related to hydrothermal systems report the dominance of α and β Proteobacteria (Emerson and Moyer, 2010) and a study from the Takuyo-Daigo Seamount, northwest Pacific report α- and β-Proteobacteria in the hydrogenetic crusts (Nitahara *et al*., 2011). The bacterial diversity of the mid-ocean ridge systems are composed of known, nonphotosynthetic lineages of organisms like the Firmicutes, Verrucomicrobia, Thermales and the Cytophaga– Flexibacter– Bacteroides groups (Takai *et al*., 2006). On the other hand, Mn and S oxidizing bacterial communities belonging to class λ-Proteobacteria, α-Proteobacteria, β-Proteobacteria, Actinobacteria, and Bacilli dominate the deep-sea low-temperature influenced oceanic crust basalts and sediments along the ridge axis (Rathsack *et al*., 2009). The hydrogenetic Fe-Mn crusts are potential ore-deposits because of their widespread occurrence and high concentrations of valuable trace and rare earth elements present in this. Yet, investigations of hydrogenetic crusts are far less numerous than those of hydrothermal crusts and equivalent insights have not been made. Several studies addressed the capacity of bacteria in forming minerals by induced and controlled mechanisms. However their direct role in the process has only been accepted for manganese nodules where bacteria with surface layers arranged on biofilm like structures initiate the deposition of Mn. It has been found that the enrichment of trace and rare earth elements in diagenetic deposits could occur by abiogenic or biogenic

routes that progress and proceeds in close association with organic molecules and matrices (Lowenstam and Weiner, 1989). Studies have also identified manganese-mineralized and silicified textures resembling fossil biofilms and microbial sites in Mn oxide and barite deposits (Ivarsson *et al.,* 2010; Kilias, 2011). Such interactions between microorganisms and metals were found to yield oxides and hydroxides of low crystallinity (Hein *et al*., 2000). However, the origin of cobalt enriched Fe-Mn crusts is thought to be of pure abiogenic reaction even though coccolithophores are reported to act as bio-seeds for initial Mn deposition in crusts (Wang and Muller, 2010). In spite of the recognized importance of microbial participation in mineral formation (Ferris *et al*., 1987), studies on cobalt-enriched Fe- Mn crusts are scanty.

As in diagenetic deposits where there is bacterial involvement, it is hypothesized that the hydrogenetic crust formation may also be triggered or accelerated by bacterial activity. Information on the different ecological types of bacteria prevailing around crust deposits would throw light on the predominant groups participating in these processes. It was, therefore, one of the primary objectives of the present study to understand the role of bacteria in the mobilization/immobilization of the Fe-Mn crust containing high concentrations of Cu and Mn. The study also compares and contrasts the culturability and in vitro metal immobilizing/mobilizing activity of bacteria under simulated deep-sea temperature and/or pressure conditions in microcosm and/or with bacterial isolates for samples from the Carlsberg Ridge (CR). Understanding of the microbe-metal interactions provides insight into the potential influence of microorganisms to alter oxidation states of heavy metals, and to influence their behavior in the environment (Nedelkova *et al*., 2007). The findings of the present study would aid

in exploration studies for removal and/or recovery of economically important metals like Cu and Mn from valuable ores by bioleaching/precipitation.

1.6 Fauna in Carlsberg Ridges

Carlsberg ridges are elevated underwater chain of mountains formed by geological processes associated with floor spreading and plate tectonics. Foraminifera are eukaryotic unicellular amoeboid protists and are classified in the Kingdom Protista, Phylum Granuloreticulosa, Class Foraminifera (Sen and Gupta, 1999). There are thought to be approximately 10,000 extinct foraminiferal species, the majority of these being benthic, with only 50 planktonic species known (Vickerman, 1992). The size range of adults is relatively large varying from approximately 50 μm to greater than 12 cm in some tropical forms, spanning four orders of magnitude (Giere, 1993). Foraminifera are usually encased by the protective shell or test that is composed of organic, agglutinated or calcareous materials. These animals are importantly classified on the basis of the test which may be one or more chambers, chamber arrangement and aperture style. Due to their general abundance, high preservation potential in marine sediments and long geological records (benthic: Cambrian to Recent, planktonic: Jurassic to Recent) foraminifera have historically been the focus of palaeoecological studies. Although their significance in terms of numerical abundance and biomass has been demonstrated in the deep-sea (Gooday *et al*., 1992) and for some intertidal areas (Ellison, 1984; Chandler, 1989; Murray and Alve, 2000; Moodley *et al*., 2000) foraminifera remain a frequently overlooked group in ecological studies of coastal regions. They also have been shown to

be important food items for a large variety of invertebrates (Lipps and Valentine, 1970; Capriulo, 1990; Murray 1991; Berry 1994) (Fig. 1.3).

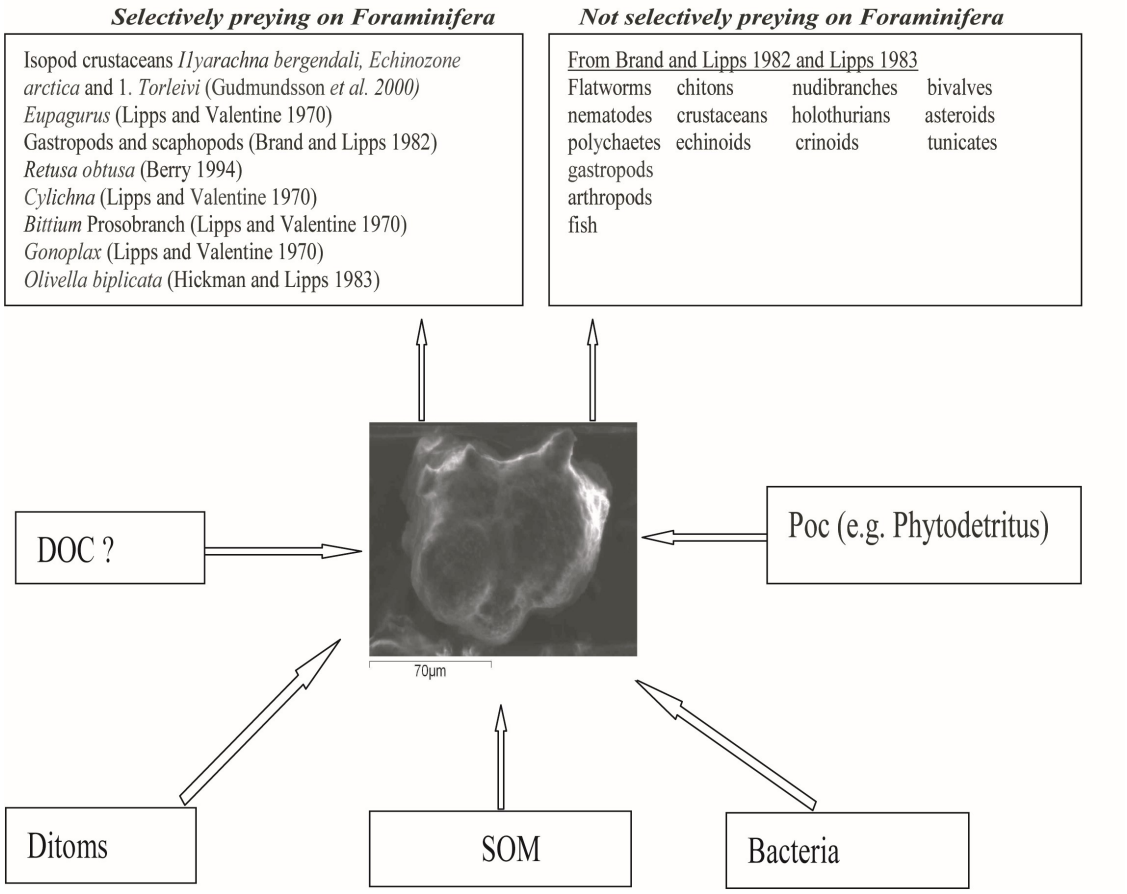


Fig. 1.3: Schematic diagram of the major food sources and predators of foraminifera. DOC = Dissolved organic carbon, POC= Particulate organic carbon, SOM = Sediment organic matter

More recently, the multi-nucleated deep-sea Xenophyophores, previously placed in a separate class, have again been suggested as belonging to the Phylum *Granuloreticulosa* and these may measure more than 25 µm in diameter (Gooday, 1999). However, the vast majority of adult foraminifera in temperate intertidal mudflats fall

within the size classification for meiofauna (63 to 500 μm) (Giere, 1993).These fauna are distributed in full marine environments including extreme ecosystems like ridges and underwater mountains which basically contains the majority of metal ions in oxides and mineral forms.

Various benthic foraminifera are often used for different kinds of biological, environmental and pollution monitoring studies. Microfossils, especially foraminifera, became the prime source to understand several environmental issues (Nigam, 2005; Muruganantham and Mohan, 2015). Guimerans and Currado (1999) reported the distribution of Foraminifera in relation to water depth, sediment texture, and sedimentation rate. Further temperature is the main factor to determine the different faunal zones at 100 m depth for benthic foraminifera (Bandy, 1954). During the present study different foraminiferal groups were collected from Carlsberg Ridge and identified for the first time.

1.7 Faunal Symbiosis in Carlsberg Ridges

Some foraminifera are known to have a symbiotic relationship with algae, bacteria, and dinoflagellates. Foraminiferal families which are considered to have a truly symbiotic relationship tend to be the large tropical species (e.g. *Amphistegina* sp. Symbiont Class *Bacillariophyceae)* and planktonic foraminifera (e.g. *Orbulina universa* symbiont, the dinoflagellate *Gymnodinium beb*) (Lee and Anderson, 1991; Hallock, 1999). Few species reported the deep sea like (*Slainjorlhia jusiformis, Epislominella exigua* and *Alabaminella weddellensis*) are described as being small, thin walled and displaying opportunistic characteristics (Gooday, 1993; Alve, 1994). However, Hughes *et al*. (2000) overlooked foraminifera in meiofaunal studies despite the fact that they play

a significant role in the rapid transfer of autotrophic carbon to higher trophic levels within benthic marine food webs. Ridges are having extreme environment characterized by no sunlight, very low organic matter, low temperature, high atmospheric pressure and highly metal rich surroundings. In such an environment, foraminifera exists symbiosis relationship with bacteria and contribute a significant role in the maintainance of the higher food chain. Thus, in deep sea extreme environments, benthic foraminifera contribute a significant role in the food chain. In this study, we report benthic foraminifera from the Carlsberg ridges which can directly or indirectly contribute to the information of the previous environment existed in these mountain ranges. In these types of extreme ecosystems, with lack of light and organic matter in the environment and highly rich in metals, foraminiferans play important role at the higher trophic levels as primary consumers.

Study was undertaken with the following objectives:

- To assess the abundance and diversity of Mn and Cu tolerant bacteria in water, rock and fauna of the ridge ecosystems.
- To assess the faunal diversity of Carlsberg ridge ecosystem.
- To estimate the activity of metal tolerant bacterial population in terms of rates of mobilizing and immobilizing metal ions under ambient conditions and it also includes the study on Foraminifera associated bacteria.

REVIEW OF LITERATURE

2.1 Manganese (Mn)

2.1.1 Mn and its importance

Manganese contributes to about 0.10% of total the mass of Earth (Nealson, 1983) and basically found as $MnAl_2O_4$ (Zajic, 1969) which is the second abundant trace metal after iron and fifth abundant transition metal in the Earth's crust (Tebo *et al*., 1997, 2007). Manganese is reported as a vital trace metal, essential nutrient for the growth and survival of organisms and at same time plays a key role in oxygenic photosynthesis in cyanobacteria (Yocum and Pecoraro, 1999; Ogawa *et al*., 2002). Mn has also been reported to protect living organisms from metal toxicity, UV light, predation, scavenging of trace metals as a micronutrient and the breakdown of organic matter into metabolizable in bacteria (Christianson, 1997; Spiro *et al*., 2010). The dissolved Mn (Mn^{2+}) concentration ranges from 0.2–3 nmol in the ocean, 30–100 pmol in river and 1–10 pmol in groundwater kg^{-1} of water (Bowen, 1979; Nealson, 1983; Glasby, 2006) while 8 mg kg^{-1} in fresh water. Mn because of its higher redox potential its reduced faster than iron or other metals and is tough to get oxidize. Soluble Mn (Mn^{2+}) is found at a higher level in the oxygen gradient than iron (Kirchner and Grabowski, 1972). Mn controls nutrient availability in water significantly by complexing with iron (Kirchner and Grabowski, 1972). Further, Mn plays an important role in bacterial metabolism (anabolism and catabolism) for functions in anaerobiosis and aerobiosis (Crowley *et al*., 2000) and in carbohydrate metabolism. Mn works as four metalloenzymes manganese superoxide dismutase (MnSOD), manganese-catalase, arginase, and O-phosphatases (Christianson, 1997; Shi, 2004). Mn^{2+} presence containing O-phosphatases controls

metabolic activities like spore formation, stress-response, cell density in stationary phase, carbon and nitrogen integration, vegetative growth, development of fruiting bodies, and cell segregation (Shi, 2004). Mn enrichment can happen by different modes. Mostly it may reach ocean from the atmospheric input, scavenging at mid-depth, fluxes from reducing shelf and direct flow of magma from hydrothermal vents (Saager *et al*., 1989). Mn^{2+} also contributes to the several bacterial products, including secreted antibiotics (Archibald, 1986) and also plays role in bacterial cell wall's stability with signal transduction (Doyle, 1989; Jakubovics and Jenkinson, 2001). Mn^{2+} is required in enzyme activity like glycolysis for the activity of 3-phosphoglycerate mutase in endospore-forming gram positive bacteria and also plays a key role in multi copper oxidase enzymes (Chander *et al*., 1998).

2.1.2 Biogeochemistry of Manganese

The Mn geochemistry shows a composite pattern in oxidation and reduction chemical reactions (Kirchner and Grabowski, 1972) which is environment depended on shows a gradation in oxygen profile (Roitz *et al*., 2002). Mn exists in the soluble Mn^{2+} form in a low oxygen environment and as insoluble oxyhydroxides in well-oxygenated surroundings (Calvert and Pedersen, 1996). The Mn^{2+} concentration depends on environmental type with indigenous redox changes and microbial communities. The immobilization of Mn by bacteria reduces the soluble Mn and increases the particulate/oxidation states (Mn^{3+} and Mn^{4+}) of Mn (Ehrlich, 1976 and 1978). At the same time a particular microorganism group induced mobilization/reduction of Mn^{3+} and Mn^{4+} in the same environment which reduces Mn oxides/Minerals that increases the soluble form of metal (Sunda and Huntsman, 1990). Mn having sorptive property in the natural environment is a strong oxidant which controls the distributions and

bioavailability of many toxic and necessary trace elements (Tebo *et al*., 2004). Bacteria and fungi play an important role in oxidation and reduction of Mn, in presence of Mn^{2+} as oxidizers and in presence of Mn oxide-containing minerals as reducers. Bacteria perform Mn oxidation/reduction for conservation energy for carbon oxidation as well as growth (Nealson and Myers, 1992; Tebo *et al*., 2005). In natural environment, bacteria induced Mn^{2+} oxidation at pH range of 5.5–8.0, Eh value above 200 mV and with oxygen concentration of 3–5 mg L^{-1} (Schweisfurth *et al*., 1978).

Ehrlich (1976, 1978) reported bacterial energy extraction from Mn^{2+} for mixotrophic and autotrophic growth where bacteria could yield ΔFr of +2.79 and DF0r of -16.31 kcal.

$$Mn^{2+} + 1/2O^{2+}H_2O \rightarrow MnO^{2+}2H^{+}$$

and yield DG -18.5 kcal in the reverse reaction

$$MnO_2 + 4H^{+} + 2^{e-} \rightarrow 2Mn^{+} + 2H_2O$$

when allowance for physiological pH of 7.0 is made (Ehrlich, 1987). The key steps in the reduction of Mn^{4+} to Mn^{2+} involve the reactions of strongly bound Mn^{3+} $\{\{Mn^{3+}\}\}$ and weakly bound Mn^{2+} $\{Mn^{2+}\}$ on the surface of MnO_2 (Ehrlich, 2002b). The following steps explain the process-

$$\{Mn^{2+}\} + MnO^{2+}2H_2O \rightarrow 2\{\{Mn^{3+}\}\} + 4OH^{-}$$

$$2\{\{Mn^{3+}\}\} + 2e^{-} \rightarrow \{Mn^{2+}\} + Mn^{2+}$$

The reduction of Mn oxide is predicted based on standard redox potential of +1.29 mV (Rusin and Ehrlich, 1995) based on the equation

$$1/2MnO + 2H^{+} + e^{-} \rightarrow 1/2Mn^{+} + H_2O$$

In ridges where Mn and iron oxides coexist, bacteria preferentially used manganese minerals. It may be due to Mn over Fe by bacteria due to lower midpoint potential for the Fe (III)/Fe(II) couple relative to the Mn (IV)/Mn (II) couple (Ehrlich, 1987).

2.1.3 Manganese oxidation/reduction by Marine Bacteria

The oxidation/reduction of Mn by marine bacteria is at adaptable process reported by Dick *et al.* (2008). The bacterial isolate *Aurantimonas* sp., from α-proteobacterium carrying genes responsible for organo-heterotrophy, methylotrophy, oxidation of sulfur and carbon monoxide and the complete Calvin cycle for carbon fixation. Mn^{2+} adsorption occurring by bacteria in the presence of peptone in Mn nodules and bacteria shows vital role in nodules formation as reported by Ehrlich (1963). In hydrothermal area bacterial oxidation of Mn^{2+} could happen through an inducible enzyme system. (Ehrlich, 1983). Further, psychrotrophic heterotrophic bacteria showed the ability of mobilizing and immobilizing Mn from ridges (Sujith *et al*., 2013). Rosson and Nealson (1982) reported that bound *Bacillus* spores strain SG-1 could oxidize Mn^{2+} but not in free condition. They hypothesized that complex formation occurred with exosporium or a spore coat protein due to the presence of Mn^{2+} while Kepkay and Nealson (1982) reported that in isolate SG-1, spores except vegetative cells are responsible for Mn^{2+} oxidation. Observations in laboratory on microbially mediated Mn^{2+} oxidation in bacterial isolates belonging to *Halomonas* sp., *Bacillus* sp., *Imtechella* sp. and *Actinobacteria* sp from Carlsberg Ridge (Fernandes *et al*., 2005, Sujith *et al*., 2013) showed that Mn is precipitated extracellularly.

2.1.4 Biotechnological applications of Manganese

Manganese shows low toxic effects on human compared to other metals until it crosses its lethal quantity at domestic level which is under the EPA permissible levels (0.05 mg L^{-1}) (Sujith *et al*., 2011). Hence, Mn oxidizers and their products introduced a solution for such problems (Czekalla *et al*. 1985). Mn oxides show effective role in anaerobic respiration by electron acceptor (Nealson *et al*., 1989). Mn oxides being potent chelators for many trace metals are further used for the removal of radium from water supplies (Moore and Reid, 1973). In 1986, Ghiorse introduced the metal recovery methods by Mn-precipitating microorganisms (Vodyanitskii *et al*., 2009). The Mn-oxidizing bacteria may be useful for effluents treatment to reduce unwanted by-product formation. The mechanisms like adsorption, accumulation, precipitation, and oxidation can be easily performed by Mn oxidizers in the active and passive process in wastewater treatment however; the slow rate of Mn oxidation is the drawback of using these bacteria for the treatment of effluent biotechnologically (Stuetz *et al*., 1996).

2.2 Copper (Cu)

2.2.1 Cu and their Biogeochemistry

Copper contributes about 0.00681% of total mass of Earth and basically found as $CuFeS_2$ and CuS_2 (Zajic, 1969) and 0.34% in ridges. Cu exists in diverse forms, associated with iron and manganese hydroxides (e.g., tenorite, and cuprite), sulfides (e.g., chalcopyrite, bornite, chalcocite, and covellite), and carbonates (e.g., chrysocolla, dioptase, and malachite). Copper exists in the environment (geosphere, atmosphere, and biosphere) as soluble (ionic copper) or insoluble (copper minerals and copper sorbed to

particulate organic and inorganic matter) forms. Copper biogeochemistry is widely explained by Ernest Chi Fru (2009). In rare cases, only copper is found in its pure form in nature. It plays a key role in biological oxidation/reduction in both aquatic and terrestrial ecosystems (Bull *et al*., 2000; Dedysh *et al*., 2004). Copper is a fundamental micronutrient for all domains of life. Copper is also known as an essential trace element for life because it contributes as a prosthetic group with oxidizing enzymes during oxidation and reduction processes. Presently thirty copper-containing enzymes are known which have key role in different metabolic/biogeochemical pathways (Mokhele *et al*., 1987; Flemming and Trevors, 1989; Bertini *et al*., 2007). The methanotrophic bacteria require copper for their metabolism as expression of methane monooxygenase gene (MMO) is strongly regulated by copper bioavailability (Knapp *et al*., 2007).

2.2.2 Copper oxidation/reduction by Marine Bacteria

The reductive changes occurs in heavy metals during the chemically mediated reactions such as Cu(II) reduction to Cu(I) by Fe^{2+} or H_2S and reduction of Cu(II) to elemental forms by Fe(II)- bearing green-rust (Borch *et al*., 2010). Acidophilic bacteria grows optimally at pH 3.0, and at the same time these bacteria showed greater susceptibility for chemolithotrophy compared to other bacterial groups and archaea that have higher pH growth optima (Johnson and Aguilera, 2015; Dopson, 2016). This tendency of bacteria is due to the richness in reduced sulfur, iron, and sulfide minerals, but relatively small concentrations of dissolved organic carbon (Rawlings *et al*., 1999). In addition, the extreme acidity means that the solubility and bioavailability of cationic metals are much greater than in circumneutral pH environments. The most well studied chemo-lithotrophic life-styles (amongst acidophiles) are those based on the oxidation of reduced iron (Fe_2C) by, for example, *Leptospirillum sp.* and also of elemental sulfur and

reduced inorganic sulfur compounds (RISCs) such as tetrathionate by *Acidithiobacillus sp.* and others (Dopson and Johnson, 2012). Nielsen and Beck (1972) reported acidophilic bacteria which catalyze redox transformations of copper other than iron as ferrooxidants could develop on chalcocite (Cu_2S) and produced Cu_2C. Lewis and Miller (1977) reported that CuC could be oxidized by ferrooxidans but could not behave as a sole energy source. Sugio *et al*. (1990) observed that *Acidithiobacillus sp*. united the copper (II) reduction to the oxidation of sulfur, and explained direct (enzymatic) reaction mechanism with a pH optimum (5.0), which is not suitable for growth of acidophiles. This thesis reports the indirect redox transformations of metal copper mediated by different species of bacteria like *Acidithiobacillus, Leptospirillum,* and *Acidiphilium.*

2.2.3 Biotechnological applications of Copper

Copper compounds have been used extensively in agriculture to control plant diseases. Their relatively high toxicity to plant pathogens, low cost, and low toxicity to mammals have made them economically important. Copper compounds are the most common bactericides for control of plant bacterial diseases, especially since antibiotics are not registered for use on most edible crops. The effectiveness of copper sprays for control of certain plant bacterial diseases is reduced by the appearance of copper-resistant bacterial strains. With the help of biomining of copper from nature can provide pure form of metal without any chemical pollution by using these bacterial isolates.

2.3 Fauna

2.3.1 Fauna in Ridges

Ridges being underwater elevated mountains chains formed due to plate tectonic activities lead to the creation of hydrothermal vents. These vents are 1 m. craters are

continuously extrude magma. These types of extreme environments lead to the exclusive type of ecosystem. With depths ranging from 3300 to 4600 m (Sujith *et al*., 2014) vertically from the surface of sea water, with no sunlight, low amount of organic carbon and high pressure with metal rich surroundings. Because of this type of environment higher organisms depend on bacteria and in fauna bacteria are associated with higher organisms like benthic foraminifera, tube worm, seeps and other faunal specimens. Hydrothermal vents and their fauna typically exist at depths of 3000 m below the end of the photic zone of 450 m (Dover *et al*., 2002). Lonsdale discovered first hydrothermal vent in 1977 (Lonsdale, 1977) and their communities in deep sea before that scientist assumed that deep sea is a desert of sparsely populated scavengers those relied on the low (less than 1%) of detritus that rained down from the photic zone. Later on 1985, Jannasch & Mottl (1985) reported that the ridges and deep sea are also having productive ecosystems even through these depths are situated away from sunlight and having high pressure.

2.3.2 Benthic foraminifera in ridges and their association with bacteria

In ridges have unique kind of extreme ecosystem where conditions are oligotrophic, aphotic and highly mineral rich surroundings, bacteria and other microorganism's plays linker role between minerals and living organisms. This type of symbiotic association of bacteria with benthic foraminifera and other living organisms leads to unique kind of ecosystem. The common benthic foraminiferans exists only in association with sediments (either as epifaunal or infaunal populations) but they are also found on other surfaces such as a shell, rock (epilithic), and macroalgae or seagrass (epiphytic). Foraminifera are related to prevail coastal habitats (Boltovskoy, 1966) as well as in deep sea environment (Corliss, 1985) reported their presence. Bacteria exhibit

connecting link between inorganic metal ions and organic matter. Gooday (1993) reported that the benthic food chain often depends on primary activity and the flow of organic matter that reaches the sediment. Specific foraminifera species depend on bacteria or on bacterial activity to survive in anoxic conditions or in other hostile conditions (Duijnstee, 2001). However, the specific role that bacteria play in the control of foraminiferal occurrence is still not conclusive. Lee *et al*. (1991) suggest that many coastal foraminiferas feed on algal blooms, indicating that they benefit from a food source when it is abundant, but feed and reproduce more slowly when food is scarce. This could be the situation in the estuary, i.e., the foraminifera species might be benefiting from the presence of bacteria and could be feeding on them. Heeger (1990) showed that some species change their food sources based up to their availability. Polychaete worms and shrimp showed epi-symbiotic relationships with bacteria, hosting them on their exterior. Sibuet and Olu (1998) reported the relatedness between the biota of vents, seeps, and other chemosynthetic environments. Other fauna such as *Riftia pachyptila* (Giant Tube Worm) also existed in deep sea vent region which used to grow up to 2 meters long in dense colonies and lives within a chitinous tube attached to the seafloor. In deep sea fauna, some invertebrate's head containing hemoglobin which can bind both oxygen and hydrogen sulfide, transporting this normally toxic chemical to the trophosome (Howe, 2009). This special organ contains endosymbiont bacteria which are responsible for quantities of carbohydrates in exchange for other nutrients and a stable habitat (Childress and Fisher, 1992; Van Dover, 2000). *Rimicaris exoculata* is a species of shrimp that is often found on or near the chimneys at hydrothermal vents. Unlike most other species of shrimp in the deep ocean, *R. exoculata* has no eye-stalks and instead has a pair of fused eyes that see in the infrared spectrum, allowing them to locate the superheated plumes of hydrothermal vent fluids (Van Dover, 2000).

2.3.3 Distinction between live and dead foraminiferans

Walton (1952) introduced a method for staining foraminiferal protoplasm with protein stain rose Bengal solution that brought revolution in isolation of living foraminifera (Walton, 1952). Although rose Bengal is a non-vital stain, it does stain foraminiferal protoplasm red, indicating a living or recently dead individual (Murray, 1991, Bernhard, 2000, Murray and Bowser, 2000). This was followed by the use of Sudan black B (Walker *et al*., 1974) and both techniques are used today. More recently, some authors have suggested the use of a luciferase-based adenosine-5'-triphosphate (ATP) assay to give a more accurate estimate of foraminiferal standing stock (Holm-Hansen and Booth, 1966, DeLaca 1986, Bernhard, 1988 and Bernhard, 2000). The major drawback with this method is the high cost of reagents and specialist equipment (Photon-counter) required. All of the above methods of determining "live" foraminifera are mortal, killing the foraminifera. Bernhard *et al*. (1995) investigated a vital procedure to distinguish live foraminifera from dead using epifluorescence microscopy and spectrofluorometry, with best results. Again, the intricacy and costs of using these methods is a major limiting factor.

2.3.4 Importance and applications for foraminifera

In deep-sea environments (food-limited), some phytodetritus species of foraminiferans like *Alabaminella weddellensis*, *Epistominella exigua* are known to respond rapidly (within 4 weeks) to seasonal pulses of phytodetritus (Gooday and Turley, 1990; Gooday, 1993; Gooday and Rathburn, 1999) their life history

(reproduction and growth) closely reflect the primary productivity of the surface ocean (Gooday and Hughes, 2002). These species respond to phytodetritus events by rapid reproduction, resulting in an increased abundance and colonization of the phytodetritus deposits. In general, foraminifera have been described as obtaining nutrients by herbivory, carnivory, omnivory, cannibalism, parasitism, scavenging, mixotrophy (an ability to feed and photosynthesize) and from dissolved organic matter (Capriulo *et al.*, 1991).

MATERIALS AND METHODS

3.1 Study area

The Carlsberg Ridge situated in the Arabian Sea is a segment of the Central-Indian Ridge. It extends from near Rodrigues Island to the Gulf of Aden, trending Northwest to Southeast and separates the Arabian Sea in the Northeast from the Somali Basin in the Southwest (Fournier *et al*., 2008; Sujith *et al*., 2014). The elevation of the Carlsberg Ridge crest ranges from 1800 to 3000 m and the water depth is 1800–3600 m (Mudholkar *et al*., 2000). Rock samples bearing oxide coating was collected at 3°39.718N (lat.) and 63°49.922E (long.) (Fig. 3.1) during the Akademic Boris Petrov cruise 36 (2009), using a chain-bag dredge at a water depth of 3390 m.

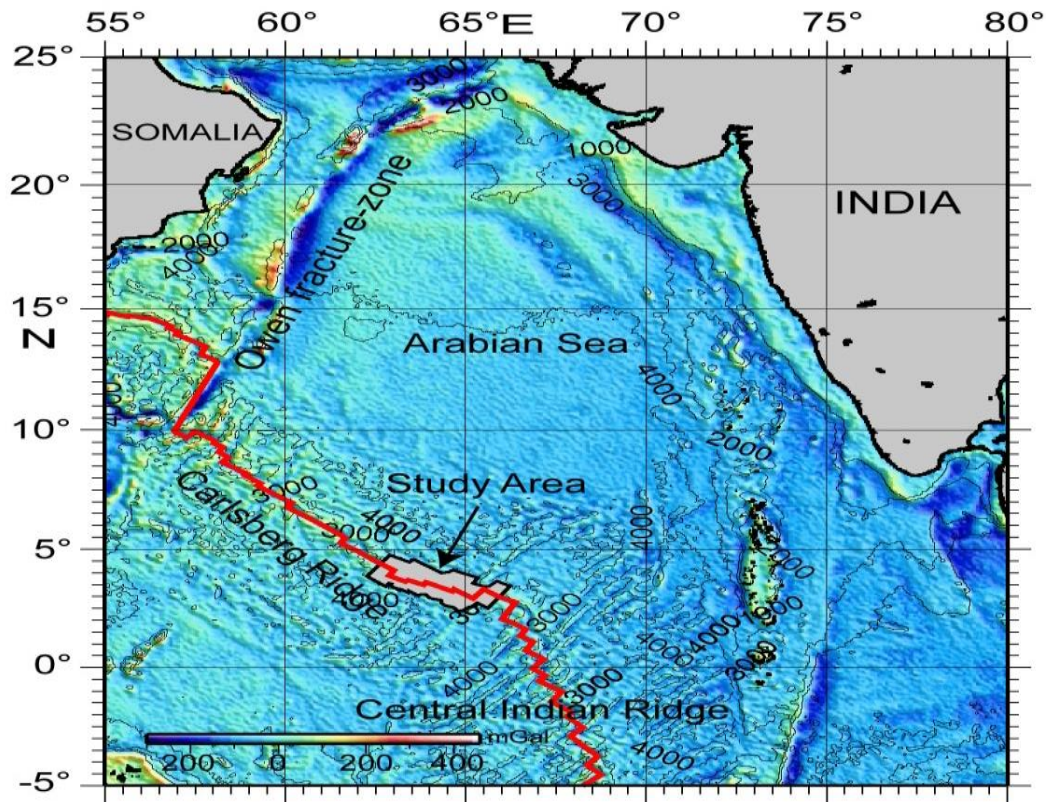


Fig. 3.1: Study area showing the location of the sampling site in Carlsberg Ridge

3.1.2 Sample collection: Sampling area and frequency

Rock and water samples were collected on board R.V. ABP 37 and O. R.V. Sagar Nidhi- 48 as a part of "Tectonics controls and hydrothermal processes along the slow spread mid-ocean ridges and Andaman trench-backarc system, Indian Ocean" program O. R.V. Sagar Nidhi 48 was undertaken during the austral winter of November-December.

3.1.3 Location of the stations:

Sediment and water samples were collected from 12 different locations in study area as detailed below in Table 3.1.

Table 3.1: Showing sampling stations and sample types collected at various depths

Sr.No	Station	Sample type	Lat.	Long.	Depth (m)
1	1	Rock+water	5ð14' 1617E	61ð57' 989N	3795
2	2	Rock+water	5ð14' 628E	61ð57' 995N	3694
3	3	Rock+water	5ð14'608E	61ð58' 014N	3590
4	4	Water	4ð35' 07N	62ð24' 30E	3488
5	5	Water	4ð35' 064N	62ð24' 200E	3385
6	6	Water	3ð45'233N	63ð37' 255E	3335
7	7	Water	3ð45'251N	63ð37' 250 E	3314
8	8	Water	3ð45' 233N	63ð35' 508E	3282
9	9	Water	3ð42' 989N	63ð35' 483 E	3262
10	10	Water	3ð47' 000N	63ð38'50E	3240
11	11	Water	3ð44' 878N	63ð37'50E	3182
12	12	Water	3ð40' 170N	63ð36'40E	2978

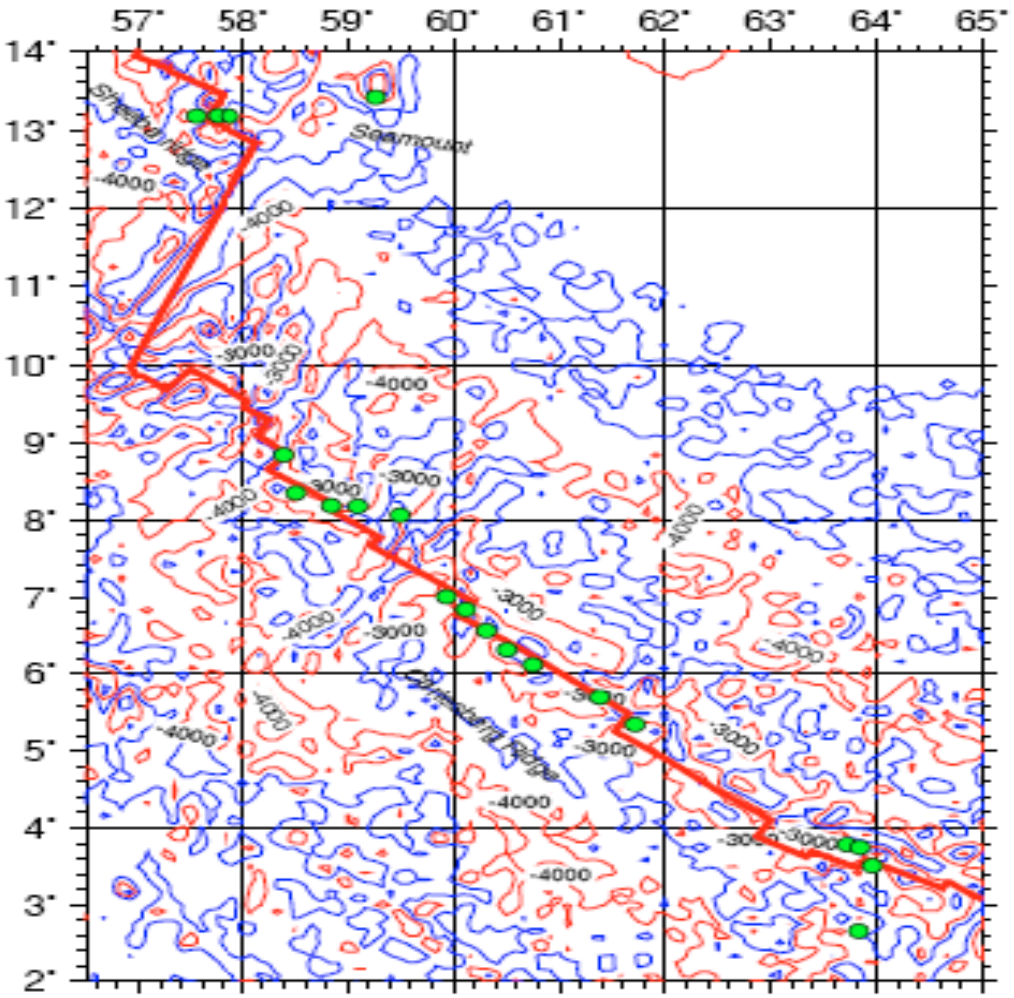


Fig. 3.2: Sampling area and topographic features with locations

(Courtesy: GAP-2157, NIO, Goa)

3.2 Water Sample collection and preservation

The samplings were carried out at 3°39.718 N (Lat.) and 63°49.922 E (Long.) in the north western region of the Indian Ocean. Near-bottom water samples were retrieved with Niskin samplers mounted onto a CTD rosette from the Carlsberg Ridge waters during the cruise SN-48 onboard ORV Sagar Nidhi (November-December). A total of 120 samples along the ridge axis and flanks were collected in 100 ml sterile polypropylene bottles at 12 different stations.

3.3 Rock/Sediment Sample collection and preservation

The region has a water depth of 1800 to 3600 meters. The sampling was carried out at 3°39.718 N (Lat.) and 63°49.922 E (Long.) in the north western region of the Indian Ocean. A total of 3 rock samples were collected onboard ORV Sagar Nidhi (November, 2010) using a chain-bag dredge from a water depth of 3390 m and were preserved in triplicate one set with formaldehyde and other two set without any preservatives in new sterile polypropylene bottles at 4±2°C until further investigations.

3.4 Physical Environmental parameters

3.4.1 Temperature

The temperature of sediment/rock and water samples from the Carlsberg ridge were measured onboard as soon as the samples were retrieved using a thermometer. In situ temperature from the sea-bed was not available.

3.4.2 Depth and pressure

The depth from which the samples were collected from the cruise reports (Courtesy: GAP-2157, CSIR-NIO Goa, India). Data was collected on-board from hydro sweep/para sound measurements. The depth of most of the samples varied from 2600 m to 3600 m in the deep sea. Difference between given and touch- position was usually within 10-100 m, thus providing reliability in sampling repeats between different stations and seasons. In situ pressure measurements were unavailable. The pressure was calculated as 1-atmosphere increase per 10 m in depth.

3.4.3 pH and Eh

The pH and Eh were measured using a Lab India Pvt. Ltd. Controlled pH analyzer probe directly from sediment as soon as the samples were retrieved. The pH meter was calibrated using standard buffers of pH 4, 7 and 9.2 (Hi-media) respectively. Relative Eh were also measured. Eh was calibrated by using standard reference solutions (Appendix VI). Reference solution A was 192 mV and reference solution B was 258 mV. Approximately 60-66 mV difference is acceptable between the two solutions where mV of solution B is greater than solution A.

3.4.4 Dissolved Oxygen (DO)

The DO in sediment pore water and water was measured using Winkler's titrimetric (Carpenter, 1965) method followed by spectrophotometric method (Pai *et al*., 1993). A measured amount of fresh wet sediment was introduced immediately after retrieval into deoxygenated cold seawater using a cut syringe. The blank value was measured and corrected. Sediment was taken not to introduce bubbles. Winkler's A and B solutions (Appendix VI) were added and the closed bottles kept in dark for 1 hour (Carpenter, 1965). Acid digestion gave a yellow colour whose intensity was directly proportional to the intensity of dissolved oxygen (Appendix VI). The spectrophotometric measurement was done at 450 nm and oxygen was expressed as $\mu M\ L^{-1}$ of water.

3.4.5 Salinity

Salinity was measured in waters extracted from the sediment. The measurement was done by using a hand-held refractometer (ATGO 2442-W01 S/MILL-E) calibrated to zero with distilled water. About a gram of fresh wet sediment was centrifuged at 5000 rpm for 10 min at 4˚C.

3.4.6 Total organic carbon

Organic carbon was determined by the wet oxidation method with a precision of 0.01% (Walkley-Black, 1934; Loring and Rantala, 1992) (Appendix V).

3.5 Microbiological parameters of Sediment samples

The sediment sample was thawed and a subsample of ~5 g wet sediment was transferred to 45 ml of filter sterilized half strength seawater (10^{-1} dilution). An aliquot of this suspension was used to enumerate total bacterial abundance, total direct viability, and retrievability. The initial weight of the sediment used in the dilution was determined by drying the filtered sediment at 60 °C.

3.5.1 Bacterial counts of Sediment samples

Bacterial counts include Total bacterial counts and Retrieval counts of Heterotrophs and Mn & Cu Oxidizers.

3.5.1.1 Total counts of bacteria of Sediment samples

Total counts of bacteria were estimated according to Hobbie *et al*. (1977). About 1 g of sediment was diluted with 9 ml of sterile seawater and 3 ml of their slurry was fixed with buffered formalin at a final concentration of 2% and stored at 4°C until further analysis. At the onshore laboratory, the aliquot was sonicated at 15 hertz for 15 seconds. The supernatant (1 ml) was stained with 0.75 ml of 0.01% acridine orange (3 mins, in dark) and filtered through 0.22 m black polycarbonate filter paper (Millipore, USA). This procedure minimized masking by sedimentary particles. About 10-15 microscopic fields per sample were counted on Nikon 80i epifluorescence microscope. The counts were normalized to cells per gram dry sediment.

3.5.1.2 Culturable Heterotrophic Bacteria of Sediment samples

The 10^{-1} dilution was used with autoclaved seawater for Retrievable counts (RC). Colony forming units (CFU) on varying concentrations of Nutrient Agar (NA) were assessed using 1% Nutrient Marine broth in 1.5% agar. Previous studies have shown that dilute Nutrient Marine Agar (1% Nutrient Broth, Himedia, Mumbai, amended with 1.5% agar) yield maximum CFU in ridge rocks/sediments (Nair *et al*., 2000; Raghukumar *et al*., 2001; Loka and Nair, 2005). The plates were incubated at 2-5°C. Heterotrophic colonies appeared within 4-10 days. The retrievable heterotrophic population was assessed on NA plates, normalized per gram dry sediment and expressed as CFU / gram dry sediment.

3.5.1.3 Cu and Mn oxidizers of Sediment samples

Cu-oxidizers were cultivated on 0.01% NA medium containing 100 uM $CuSO_4$ in Sea water medium (Himedia, Mumbai, India). In order to maintain a mild acidic to near neutral condition (Rodina, 1972), the salinity was adjusted to 35 ppt while the pH was adjusted to 6.8± 0.2. Mn-oxidizers were cultivated on modified Beijerinck's medium using 100µM $MnSO_4$ (Rodina, 1972; Havert, 1992). In order to maintain a near neutral condition, the salinity was adjusted to 35 ppt and pH was adjusted to 7.8+ 0.2 (See Appendix V for media compositions).

3.6 Microbial Diversity of Sediment samples

It includes the studies on the diversity of culturable bacterial isolates from sediment samples and their phylogeny.

3.6.1 Identification using biochemical method

Well isolated colonies were randomly picked up from plates. Nearly 120 colonies were isolated representing the different concentration of media. These isolates represented different morphotypes from all the cores. The isolates were subjected to various biochemical and physiological tests (Gerhardt, 1981) (Appendix IV). Identification was carried out up to generic levels using phenotypic traits (Oliver, 1982; Bergey's Manual of Determinative Bacteriology, 1984).

3.6.2 Phylogenetic diversity of bacterial isolates using 16S rRNA of Sediment samples

For genotypic analysis, DNA was extracted from the isolate as per the method described by Maniatis *et al*. (1982). The primers used for 16S ribotyping analysis were as per MicroSeq 500 kit (Applied Biosystems, USA). Amplification of DNA was carried out on a thermocycler (GeneAmp PCR, Applied Biosystems, USA). The mixture was incubated through initial denaturation at 94 °C for 2 min, followed by 30 cycles of 94 °C for 30s, 54 °C for 1 min and 72° C for 1 min. A final extension period consisted of 1 min at 72 °C. The entire 500bp long sequence generated was used for further analysis. The amplified DNA fragment was purified using the PCR purification kit (Vivantis, Malaysia). The purified PCR product was sequenced using the BDT v3.1 cycle sequencing PCR kit (Applied Biosystems, USA) on a 3130 Genetic Analyzer (Applied Biosystems Inc, USA) at the genomic Technologies sequencing facility. Their phylogenetic affiliation was carried out by establishing the closest genetic neighbors using the RDP-II program (release number 10.0) and available nucleotide database.

3.6.3 Metabolic diversity of bacterial isolates of Sediment samples

Cultures were examined for gram-stain reaction and tested for motility, catalase, oxidase and DNase activity according to Gerhardt *et al*. (1981). The Marine oxidation fermentation (MOF) medium test was done with 1% glucose as carbon source. Results of the MOF test were interpreted based on the color change of the pH indicator bromothymol blue after incubation at 28 ± 2 °C for 72 h (Hugh and Leifson, 1953). The screening of bacterial isolates for amylase and lipase enzymes was done on plates of nutrient agar supplemented with 1% starch and 1% tributyrin respectively (Bairagi *et al*., 2002). The plates were flooded with Lugol's iodine (1%) after growth for detecting amylase activity. The hydrolysis of starch and tributyrin was observed as clear halos surrounding the colony. Similarly, screening for cellulase producers was carried out on carboxymethylcellulose (CMC) agar (0.2% $NaNO_3$, 0.1% K_2HPO4, 0.05% $MgSO_4$, 0.05% KCl, 0.02% peptone and 1.7% agar) with 1% CMC sodium salt as substrate. Enzyme activity was detected by the use of Gram's iodine reagent (Kasana *et al.,* 2008). For the extracellular caseinase production, bacterial isolates were spot inoculated on soybean-casein digest agar medium (Hi-media, Mumbai, India). Regions of enzyme activity were detected as clear halos after the addition of 0.1% amido black in methanol acetic acid water mixture in the ratio 30:10:60 (Vermelho *et al*., 1996).

3.6.4 Extracellular enzymes of Sediment samples

The isolates ability to elaborate amylase, DNase, lipase, phosphatase, and protease were checked in nutrient medium amended with starch (Amy), DNA (Dna),

tween 80 (Lip), p-nitrophenyl phosphate (Phos) and casein (Protein) as substrate (Appendix I).

3. 7 Water samples

Water samples were collected at different water from depths using Niskin bottle sampler fixed on CTD rosette.

3.7.1. Microbiological parameters –This include bacterial counts along with the following parameters:

3.7.1.1 Total counts of bacteria of Water samples

Total counts of bacteria were estimated according to Hobbie *et al*. (1977). About 1 g of sediment was diluted with 9 ml of sterile seawater. Three ml of this slurry was fixed with buffered formalin at a final concentration of 2% and stored at 4°C until further analysis. At the onshore laboratory, the aliquot was sonicated at 15 hertz for 15 seconds. The supernatant (1 ml) was stained with 0.75 ml of 0.01% acridine orange (3 mins, in dark) and filtered through 0.22 m black polycarbonate filter paper (Millipore, USA). This procedure minimized masking by sedimentary particles. About 10-15 microscopic fields per sample were counted on Nikon 80i epifluorescence microscope. The counts were normalized to cells per gram dry sediment.

3.7.1.2 Culturable Heterotrophic Bacteria of Water samples

The 10^{-1} dilution was used with autoclaved seawater for RC. Colony forming units on varying concentrations of Nutrient Agar (NA) were assessed using 1% Nutrient Marine broth in 1.5% agar. Previous studies have shown that dilute Nutrient Marine

Agar (1% Nutrient Broth, Himedia, Mumbai, amended with 1.5% agar) yield maximum CFU in ridge rocks/sediments (Nair *et al*., 2000; Raghukumar *et al*., 2001 ;Loka and Nair, 2005). The plates were incubated at 2-5°C. Heterotrophic colonies appeared within 4-10 days. The retrievable heterotrophic population was assessed on NA plates, normalized per ml of seawater and expressed as CFU per gram dry sediment.

3.7.1.3 Cu and Mn oxidizers of Water samples

Cu-oxidizers were cultivated on 0.01% NA medium containing 100 µM $CuSO_4$ in Sea water medium (Himedia, Mumbai, India). In order to maintain a mild acidic to near neutral condition (Rodina, 1972), the salinity was adjusted to 35 ppt while the pH was adjusted to 6.8± 0.2. Mn-oxidizers were cultivated on modified Beijerinck's medium using with 100µM $MnSO_4$ (Rodina, 1972; Havert, 1992). In order to maintain a near neutral condition, the salinity was adjusted to 35 ppt and pH was adjusted to 7.8+ 0.2 (See Appendix V for media compositions).

3.7.2 Microbial Diversity of water samples

This includes Diversity of bacterial isolates and their Phylogenetic analysis.

3.7.2.1 Identification using biochemical method

Well, isolated colonies were randomly isolated from plates. Nearly 160 colonies were isolated representing the different concentration of media. These isolates represented different morphotypes from all the cores. The isolates were subjected to various biochemical and physiological tests (Gerhardt, 1981) (Appendix I). Identification was carried out up to generic levels using phenotypic traits (Oliver, 1982; Bergey's Manual of Determinative Bacteriology, 1984).

3.7.2.2 Phylogenetic diversity of bacterial isolates using 16S rRNA of Water samples

For genotypic analysis, DNA was extracted from the isolate by the method described by Maniatis *et al*. (1982). The primers used for 16S ribotyping analysis were as per MicroSeq 500 kit (Applied Biosystems, USA). Amplification of DNA was carried out on a thermocycler (GeneAmp PCR, Applied Biosystems, USA). The mixture was incubated through initial denaturation at 94 °C for 2 min, followed by 30 cycles of 94 °C for 30s, 54 °C for 1 min and 72 °C for 1 min. A final extension period consisted of 1 min at 72 °C. The entire 500bp long sequence generated was used for further analysis. The amplified DNA fragment was purified using the PCR purification kit (Vivantis, Malaysia). The purified PCR product was sequenced using the BDT v3.1 cycle sequencing PCR kit (Applied Biosystems, USA) on a 3130 Genetic Analyser (Applied Biosystems Inc, USA) at the genomic Technologies sequencing facility. A phylogenetic affiliation by establishing the closest genetic neighbors was carried out using the RDP-II program (release number 10.0) and available nucleotide databases.

3.7.2.3 Metabolic diversity of bacterial isolates of Water samples

Cultures were examined for gram-stain reaction, and tested for motility, catalase, oxidase and DNase activity according to Gerhardt *et al*. (1981). The MOF (Marine oxidation fermentation medium) test was done with 1% glucose as carbon source. Results from the MOF test were interpreted based on the color change o the pH indicator bromothymol blue after incubation at 28 ± 2 °C for 72 h (Hugh and Leifson, 1953). The screening of bacterial isolates for amylase and lipase enzymes was done on plates of

nutrient agar supplemented with 1% starch and 1% tributyrin respectively (Bairagi *et al*., 2002). The plates were flooded with Lugol's iodine (1%) after growth for detecting amylase activity. The hydrolysis of starch and tributyrin was observed as clear halos surrounding the colony. Similarly, screening for cellulase producers was on carboxymethyl cellulose (CMC) agar (0.2% $NaNO_3$, 0.1% K_2HPO_4, 0.05% $MgSO_4$, 0.05% KCl, 0.02% peptone and 1.7% agar) with 1% CMC sodium salt as substrate. Enzyme activity was detected by the use of Gram's iodine reagent (Kasana *et al*., 2008). For the extracellular caseinase production, bacterial isolates were spot inoculated on soybean-casein digest agar medium (Himedia, Mumbai, India). Regions of enzyme activity were detected as clear halos after the addition of 0.1% amido black in methanol–acetic acid–water mixture in the ratio 30:10:60 (Vermelho *et al*., 1996)

3.7.2.4 Extracellular enzymes of Water samples

The isolates ability to elaborate amylase, DNase, lipase, phosphatase, and protease were checked in nutrient medium amended with starch (Amy), DNA (Dna), tween 80 (Lip), p-nitrophenyl phosphate (Phos) and casein (Protein) as substrate (Appendix I)

3.8 Quantification of Mn and Cu in Water samples using voltammetry

3.8.1 Mn

The quantification of Mn was performed by adsorptive stripping voltammetry in the interface with 797 VA computrace (Metrohm, Switzerland) in the differential pulse mode according to Colombini and Fuoco (1983) and as modified by Sujith *et al*. (2011). To deduce the change in metal concentration 1ml supernatant from each tube was diluted with 9ml of Milli-Q water (18.2Ω resistance) and then acidified to pH 2 with 1N HCl.

The acidified samples were UV digested in a quartz cuvette at 90°C for one hour. The UV treated samples were analyzed immediately after cooling or was stored in dark at 4°C for later analysis. The solid phase concentration of Mn in ridge rock was determined from 50 mg of powdered rock by closed vessel digestion method (Roy *et al*., 2007). The instrument parameters and sample processing for Mn analysis were same as above but without UV digestion. The analysis was quantified with two standard additions of Mn^{2+} ($MnCl_2 \bullet 4H_2O$, 40 $\mu g L^{-1}$). The gram dry weight concentration of Mn mobilized was normalized after correcting for the respective blanks.

3.8.2 Cu

The quantification of Cu was performed by adsorptive stripping voltammetry in the interface with 797VA computrace (Metrohm, Switzerland) in the differential pulse mode according to Ostapczuk (1984) and as modified by Sujith *et al*. (2014). To deduce the change in metal concentration 1ml supernatant from each tube was diluted with 9 ml of Milli-Q water (18.2Ω resistance) and then acidified to pH 2 with 1N HCl. The acidified samples were UV digested in a quartz cuvette at 90°C for one hour. The UV treated samples were analyzed immediately after cooling or was stored in dark at 4°C for later analysis. The solid phase concentration of Cu in ridge rock was determined from 50 mg of powdered rock by closed vessel digestion method (Roy *et al*., 2007). The instrument parameters and sample processing for Cu analysis were same as above but without UV digestion. The analysis was quantified with two standard additions of Cu^{2+} ($CuCl_2 \bullet 4H_2O$, 40 $\mu g\ L^{-1}$). The gram dry weight concentration of Cu mobilized was normalized after correcting for the respective blanks.

3.9 Spectroscopy of sediments and water samples:

3.9.1 Scan Electron microscopy conjugate with Electron dispersion spectra (SEM/EDS)

Suspensions of Mn and Cu oxidizing colonies cultured on 1 mM Mn and Cu amended dilNA medium were prepared in 0.9% sterile saline. Clean glass pieces were immersed in the suspensions and left overnight for the cells to form a biofilm on the glass surface. The glass pieces were retrieved and the biofilm was then subjected to dehydration by running it through a series of increasing acetone concentrations from 10, 30, 50, 70, 90 and 100%. The samples were then air dried, mounted on a stub and sputter coated with Au/Pd. The specimens were then visualized with a JEOL JSM-5800 Scanning electron microscope (SEM). The presence of Mn-oxide deposits in the culture was corroborated using an energy dispersive X-ray spectrometer (EDS) in conjunction with the SEM.

3.9.2 X-ray diffraction (XRD)

The mineralogy of natural Mn-oxide was characterized by using X-ray diffraction analysis (XRD) as described by Villalobos *et al*., (2003). For XRD analysis, oxide coatings were removed from a single large rock fragment using scalpel and then finely powdered using an agate mortar and pestle. The XRD patterns of the Mn-oxides were recorded in a Rigaku X-ray powder diffractometer using a monochromatic Cu Ka1 radiation (operating at 40 kV and 20 mA) and a scintillation detector. All samples were

run in scan mode over a 2h range of 0-80 with a scanning rate of 1.2 min 1. The interpretation of the peaks was done according to Burns and Burns (1977).

3.9.3 Light Microscopy

The bacterial samples bio mass were observed light Microscope (Olympus CKX41 manufactured in Japan) (Muruganantham and Mohan, 2015).

3.9.4 Determination of cell morphology and intracellular metal accumulation studies

To detect changes in the cell morphology brought about by increasing concentration of iron, images of cells from iron amended and unamended seawater were captured and analyzed by scanning electron microscopy (SEM) according to **section 3.4.3.3.** An energy-dispersive X-ray spectrometer (EDS) was carried out in conjunction with the SEM on selected cells and on metal precipitates at an accelerating voltage of 15 keV. Additionally, powder X-ray diffraction (XRD) scans were performed to distinguish the mineral type contained in the sample. The bacterial cells were harvested at the end of the experimental period, by centrifuging the medium at 10000 x g for 10 min. The cell pellet was washed thrice with distilled water followed by acetone and then air-dried and crushed (Deplanche and Macaskie, 2008). The crushed dried samples were scanned using a Rigaku X-Ray powder diffractometer using monochromatic Cu Kα1 radiation (λ = 1.54050 Å). The diffraction spectrum was recorded from 10º to 100º (2θ) with a step size of 0.02º (2θ). The d values were determined with the aid of charts prepared by Brown (1980).

3.10 Microcosm experiments

3.10.1 Microcosm experiments to determine the Mobilization rate of Mn in natural ridge rock with the indigenous microbial community.

Ridge rock samples collected from Carlsberg ridge from station 1 and divided into four parts and each set was run in triplicates. One part was used to detect the release of Mn from rock and three parts were used as controls. Controls were in the form of blank, heat and azide inactivated rock. The experimental set up is described below in table 3.2.

Table 3.2 Experimental set up for microcosm studies with whole rock to determine the ability of the bacterial community to remove Mn in soluble form from the rock

Experimental set up	Set I	Set II	Set III	Set IV
Ridge rock with native bacteria	√	√	√	√
Blank (sterile natural high seawater)	20 mL	20 mL	20 mL	20 mL
Heat-killed (Autoclave- 121 °C,15 min,	--	√	--	√
Sodium azide (15 mM final conc.)	--	--	√	√

Approximately 1 g of rock was weighed and added to 20 mL of Sea water medium I. Individual sets were maintained and sacrificed for each monitoring day. The experiment was run for 150 d. The bottles were incubated in the dark at RT to avoid photo-oxidation. Aliquots of the inoculated media as well as the control medium was taken from each tube and amount of soluble Mn (II) in the media was determined by voltammetry.

3.10.2 Microcosm experiments to determine the Mobilization rate of Mn in natural ridge rock with potential bacterial isolate

Ridge rock samples were collected from Carlsberg ridge from station 1 and divided into four parts and each set was run in triplicates. One part was used to detect the release of Mn from rock and three parts were used as controls. Controls were in the form of a blank, heat and azide inactivated rock. The experimental set up is described in table 3.3.

Table 3.3 Experimental setup for microcosm studies with whole rock to determine the ability of native bacteria to remove Mn in soluble form from the rock

Experimental set up	Set I	Set II	Set III	Set IV
Ridge rock with bacteria	√	√	√	√
Blank (sterile natural high seawater)	20 mL	20 mL	20 mL	20 mL
Heat-killed (Autoclave- 121 °C,15 min,	--	√	--	√
Sodium azide (15 mM)	--	--	√	√

Approximately 1 g of rock was weighed and added to 20 mL of Sea water medium I. Individual sets were maintained and sacrificed for each monitoring day. The experiment was run for 150 d. The bottles were incubated in the dark at RT to avoid photo-oxidation. Aliquots of the inoculated media, as well as the control medium, was taken from each tube and amount of soluble Mn (II) in the media was determined by voltammetry.

3.10.3 Microcosm experiments to determine the Immobilization rate of Mn in natural ridge rock with the indigenous bacterial community

Ridge rock samples were collected from Carlsberg ridge from station 1 and divided into four parts and each set was run in triplicates. One part was used to detect the release of Mn from rock and three parts were used as controls. Controls were in the form of blank, heat and azide inactivated rock. Medium was amended with 1000 mg $MnCl_2$. The experimental set up is described in table 3.4

Table 3.4 Experimental setup for microcosm studies with whole rock to determine the ability of native bacteria to remove Mn in soluble form from the rock

Experimental set up	Set I	Set II	Set III	Set IV
Ridge rock with native bacteria	√	√	√	√
Blank (sterile natural high seawater)	20 mL	20 mL	20 mL	20 mL
Heat-killed (Autoclave- 121 °C,15 min,	--	√	--	√
Sodium azide (15 mM)	--	--	√	√

Approximately 1 g of rock was weighed and added to 20 mL of Sea water medium I. Individual sets were maintained and sacrificed for each monitoring day. The experiment was run for 150 d. The bottles were incubated in the dark at RT to avoid photo-oxidation. Aliquots of the inoculated media as well as the control medium was taken from each tube and amount of soluble Mn (II) in the media was determined by voltammetry.

3.10.4 Microcosm experiments to determine the Immobilization rate of Mn in natural ridge rock with potential Bacteria

Ridge rock samples were collected from Carlsberg ridge from station 1 and divided into four parts and each set was run in triplicates. One part was used to detect the release of Mn from rock and three parts were used as controls. Controls were in the form of a blank, heat and azide inactivated rock. Medium was amended with 1000 mg $MnCl_2$. The experimental set up is described in table 3.5.

Table 3.5 Experimental setup for microcosm studies with whole rock to determine the ability of the bacterial community to remove Mn in soluble form from the rock

Experimental set up	Set I	Set II	Set III	Set IV
Ridge rock with bacteria	√	√	√	√
Blank (sterile natural high seawater)	20 mL	20 mL	20 mL	20 mL
Heat-killed (Autoclave- 121 °C,15 min,	--	√	--	√
Sodium azide (15 mM)	--	--	√	√

Approximately 1 g of rock was weighed and added to 20 mL of Sea water medium I. Individual sets were maintained and sacrificed for each monitoring day. The experiment was run for 150 d. The bottles were incubated in the dark at RT to avoid photo-oxidation. Aliquots of the inoculated media, as well as the control medium, was taken from each tube and amount of soluble Mn (II) in the media was determined by voltammetry.

3.10.5 Microcosm experiments to determine the mobilization rate of Cu in natural ridge rock with the indigenous microbial community

Ridge rock samples were collected from Carlsberg ridge from station 1 and divided into four parts and each set was run in triplicates. One part was used to detect the release of Cu from rock and three parts were used as controls. Controls were in the form of a blank, heat and azide inactivated rock.. The experimental set up is described in table 3.6

Table 3.6 Experimental setup for microcosm studies with whole rock to determine the ability of the bacterial community to remove Cu in soluble form from the rock

Experimental set up	Set I	Set II	Set III	Set IV
Ridge rock with community	√	√	√	√
Blank (sterile natural high seawater)	20 mL	20 mL	20 mL	20 mL
Heat-killed (Autoclave- 121 °C,15 min,	--	√	--	√
Sodium azide (15 mM)	--	--	√	√

Approximately 1 g of rock was weighed and added to 20 mL of Sea water medium I. Individual sets were maintained and sacrificed for each monitoring day. The experiment was run for 150 d. The bottles were incubated in the dark at RT to avoid photo-oxidation.

Aliquots of the inoculated media as well as the control medium was taken from each tube and amount of soluble Cu (II) in the media was determined by voltammetry.

3.10.6 Microcosm experiments to determine the Mobilization rate of Cu in natural ridge rock with potential bacterial isolate

Ridge rock samples were collected from Carlsberg ridge from station 1 and divided into four parts and each set was run in triplicates. One part was used to detect the release of Cu from rock and three parts were used as controls. Controls were in the form of blank, heat and azide inactivated rock. The experimental set up is described in table 3.7

Table 3.7 Experimental set up for microcosm studies with whole rock to determine the ability of native bacteria to remove Cu in soluble form from the rock

Experimental set up	Set I	Set II	Set III	Set IV
Ridge rock with bacteria	√	√	√	√
Blank (sterile natural high seawater)	20 mL	20 mL	20 mL	20 mL
Heat-killed (Autoclave- 121 °C,15 min,	--	√	--	√
Sodium azide (15 mM)	--	--	√	√

Approximately 1 g of rock was weighed and added to 20 mL of Sea water medium I. Individual sets were maintained and sacrificed for each monitoring day. The experiment was run for 150 d. The bottles were incubated in the dark at RT to avoid photo-oxidation.

Aliquots of the inoculated media as well as the control medium was taken from each tube and amount of soluble Cu (II) in the media was determined by voltammetry.

3.10.7 Microcosm experiments to determine the Immobilization rate of Cu in natural ridge rock with potential bacterial isolate

Ridge rock samples were collected from Carlsberg ridge from station 1 and divided into four parts and each set was run in triplicates. One part was used to detect the release of Cu from rock and three parts were used as controls. Controls were in the form of a blank, heat and azide inactivated rock. Medium was amended with 100 mg $CuCl_2$. The experimental set up is described in table 3.8

Table 3.8 Experimental setup for microcosm studies with whole rock to determine the ability of native bacteria to remove Cu in soluble form from the rock

Experimental set up	Set I	Set II	Set III	Set IV
Ridge rock with bacteria	√	√	√	√
Blank (sterile natural high seawater)	20 mL	20 mL	20 mL	20 mL
Heat-killed (Autoclave- 121 °C,15 min,	--	√	--	√
Sodium azide (15 mM)	--	--	√	√

Approximately 1 g of rock was weighed and added to 20 mL of sea water medium I. Individual sets were maintained and sacrificed for each monitoring day. The experiment was run for 150 d. The bottles were incubated in the dark at RT to avoid photo-oxidation.

Aliquots of the inoculated media, as well as the control medium, was taken from each tube and amount of soluble Cu (II) in the media was determined by voltammetry.

3.11 Benthic Foraminifera Samples Analysis

3.11.1 Sample preservation and analysis

Apart of sediment was kept in plastic covers preserved with 10 % formaldehyde and this sediment ample were stained with a solution of Rose Bengal (1g/ 1-litre ethanol) following the method of Boltovskoy and Wright (1976). After 14 days of preservation, 100 ml (approx.) of sediments were sieved through 500 µm and 63 µm standard sieves. The samples retained in the 63 µm sieve were taken for the faunal analysis under a Binocular Stereoscopic Microscope (Olympus CKX41 manufactured in Japan) (Muruganantham and Mohan, 2015).

3.11.2 Light Microscopy

The samples retained in the 63 µm sieve were taken for the faunal analysis under a Binocular Stereoscopic Microscope (Olympus CKX41 manufactured in Japan) (Muruganantham and Mohan, 2015).

3.11.3 Scanning electron microscope

Sieved and isolated foraminifera samples from basalt placed in sterile sea water wet glass plates randomly and were subsequently kept in laminar air flow in white light for drying. Dried samples were placed on conductive adhesive tape prior to gold coating just before analysis using JEOL JSM-5800 scanning electron microscope (SEM). The

Foraminiferal morphology was visualized under SEM at different magnifications. The details of the procedures was followed according to Sujith *et al*. (2014) and Mourya *et al*. (2015).

3.11.4 Bacteria isolation from foraminiferal samples

Collected sediment samples were preserved in polythene bag at 4°C in sterile condition till analysis. In laboratory 1 gram of sediment was soaked in 0.22 µm filter paper filtered autoclaved seawater for next 24 hours in sterile conditions. The sample was sieved with 63 µm sieve and keep in laminar flow in white light for drying. The dry sample was weighed and put in 9.9 ml of autoclaved seawater prior to sonication at 15 Htz for five seconds thrice on the interval of 10 seconds. The method given in **section 3.5.3.3** was followed for general heterotrophs, Mn and Cu oxidizers.

RESULTS

4.1. Rock samples morphological characteristics

Visual, Microscopic and Physical characterization of the natural basalts mostly showed black-brownish colored coatings with rough surface, numerous pits, fissures and fractures. Brown and black coating of varying thicken were observed on surface of rock (Fig 4.1A and 4.1B). The thickness of the coatings varied from 1.0 - 3.6 mm with greater thickness on irregular surfaces and vice versa on regular surfaces.

4. 2 Physical Environmental parameters

4.2.1 Temperature and Salinity

Temperature of rock/sediment samples ranged from 6° to10°C on board while those water samples varied temperature between 1.5 to 8°C (Fig 4.2 A-L). In situ temperature of the Carlsberg ridge ranged between 1 to 2°C. Salinity concentrations of water columns are presented in Fig. 4.2 A-L. It varied from 33 to 35 psu at 10°C.

4.2.2 Depth and pressure

Water samples were procured from depth ranging from 3000 to 4000 m at particular known intervals. In situ pressure varied 300 to 400 atmospheres.

4.2.3 pH

The pH data are presented in Fig. 4.2 A-L for water samples. The pH of water samples ranged from 6 to 8.

4.2.4 Eh

The Eh data are presented in Fig. 4.3 A-L for water samples. Eh varied from +150 mV to -370 mV.

4.2.5 Dissolved Oxygen (DO)

The oxygen concentrations in water columns are presented in Fig. 4.2 A-L and it varied from 3 to 5ml L^{-1}.

4.2.6 Total organic carbon (TOC)

TOC in water columns are presented in Fig. 4.4 A-L where it varied from 0.07-0.12%. Total inorganic carbon (TIC) was below detection limit.

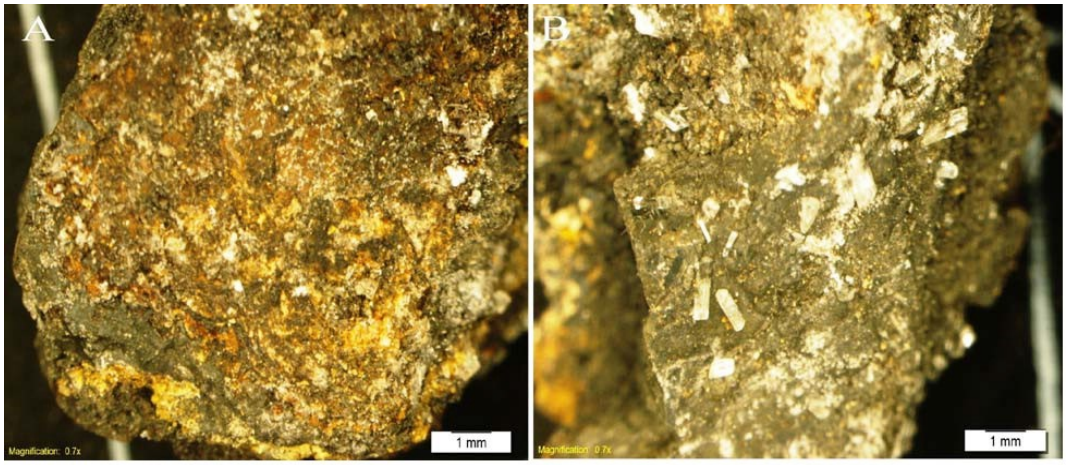
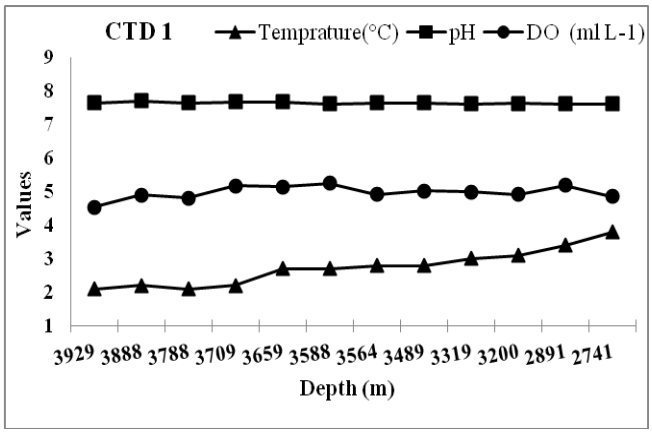
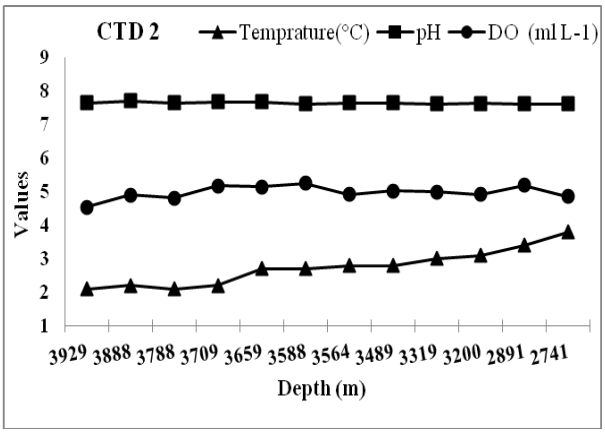


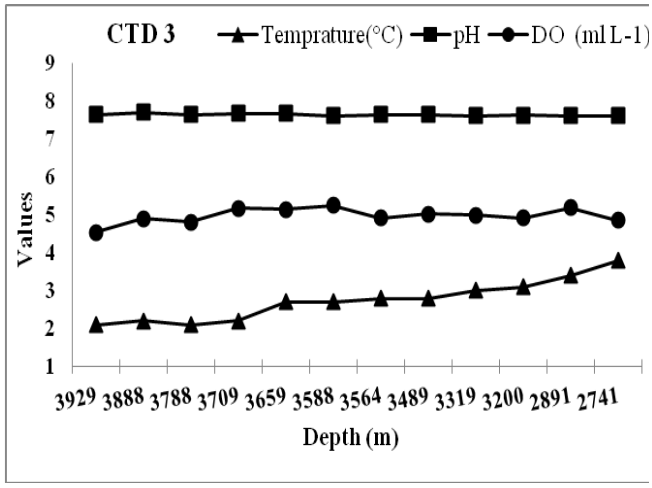
Fig. 4.1 A and B: Basalt rock sample bearing Mn-Fe oxides and mineral deposits used in present laboratory experiment. A) Before incubation B) After incubation

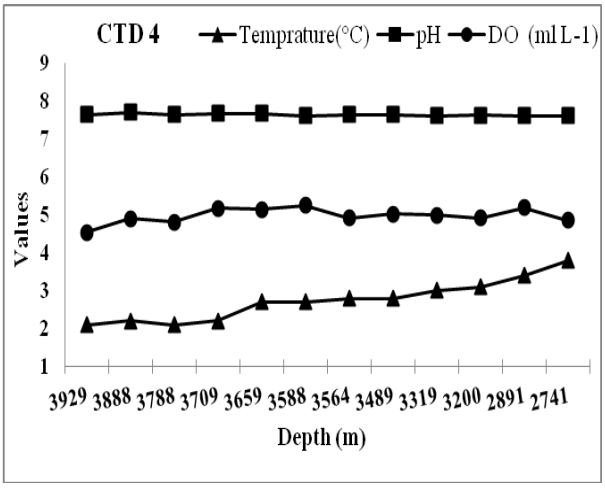
Table 4.1: Rock/sediment samples physical data presentation

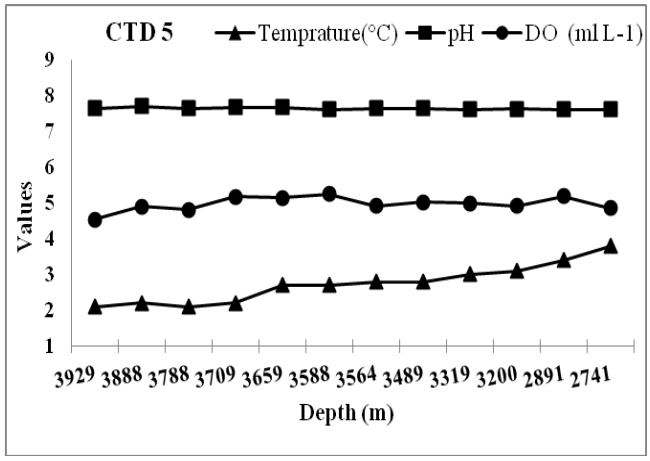
Sample	pH (Avg.)	Eh (Avg.)	TOC (%)	Temperature (°C)
Ridge 1	7.82	-125	0.12	7
G1	7.88	-133	0.10	8
G14	7.60	-128	0.14	7

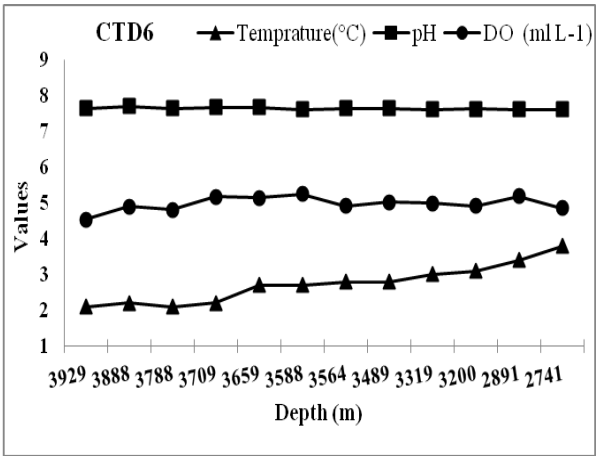

CTD 1
Temprature(°C)
pH
DO (ml L-1)
Values
9
8
7
6
5
4
3
2
1
3929 3888 3788 3709 3659 3588 3564 3489 3319 3200 2891 2741
Depth (m)


CTD 2
Temprature(°C)
pH
DO (ml L-1)
Values
9
8
7
6
5
4
3
2
1
3929 3888 3788 3709 3659 3588 3564 3489 3319 3200 2891 2741
Depth (m)


CTD 3
Temprature(°C)
pH
DO (ml L-1)
Values
9
8
7
6
5
4
3
2
1
3929 3888 3788 3709 3659 3588 3564 3489 3319 3200 2891 2741
Depth (m)


CTD 4
Temprature(°C)
pH
DO (ml L-1)
Values
9
8
7
6
5
4
3
2
1
3929 3888 3788 3709 3659 3588 3564 3489 3319 3200 2891 2741
Depth (m)


CTD 5
Temprature(°C)
pH
DO (ml L-1)
Values
9
8
7
6
5
4
3
2
1
3929 3888 3788 3709 3659 3588 3564 3489 3319 3200 2891 2741
Depth (m)


CTD6
Temprature(°C)
pH
DO (ml L-1)
Values
9
8
7
6
5
4
3
2
1
3929 3888 3788 3709 3659 3588 3564 3489 3319 3200 2891 2741
Depth (m)

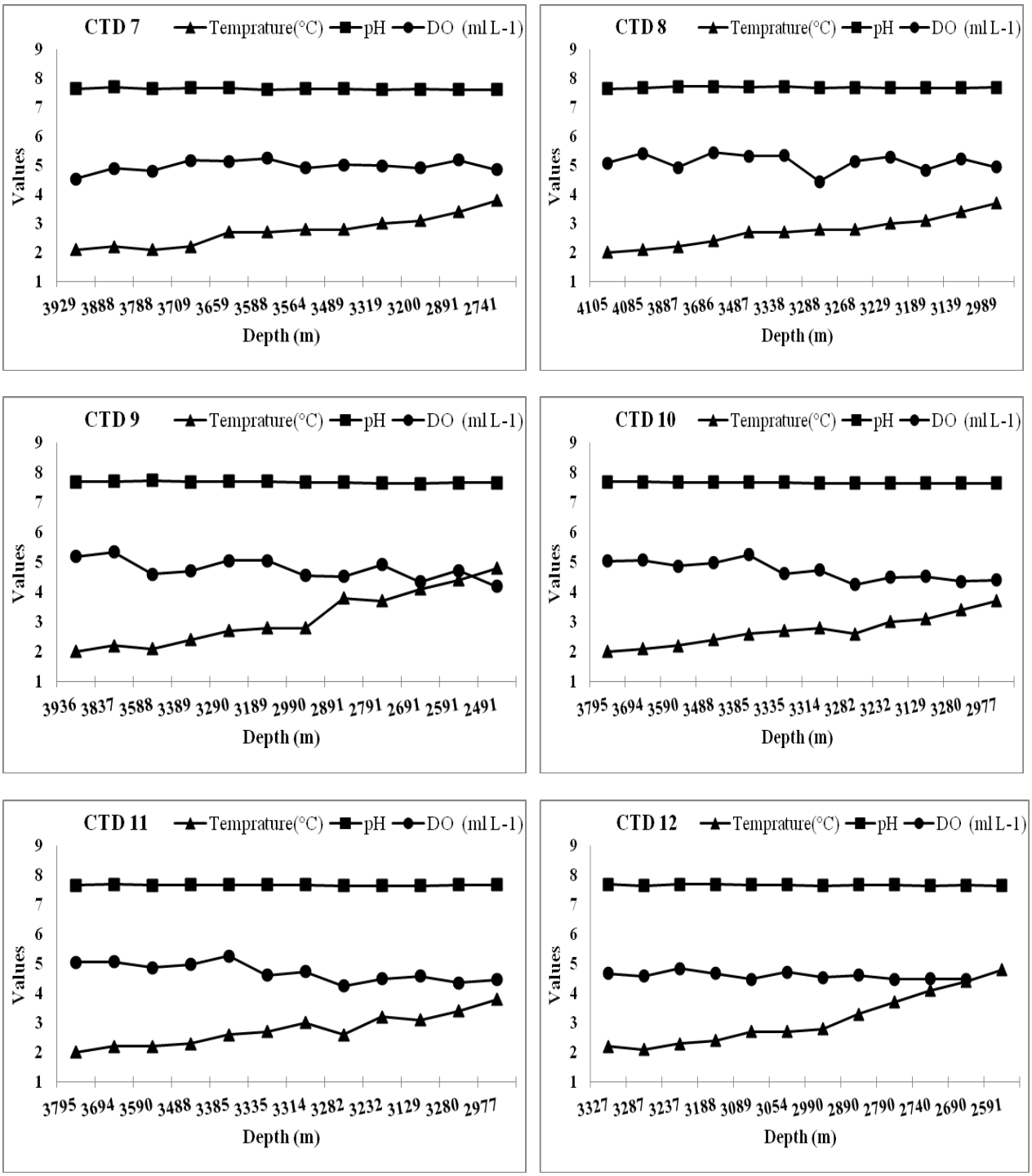
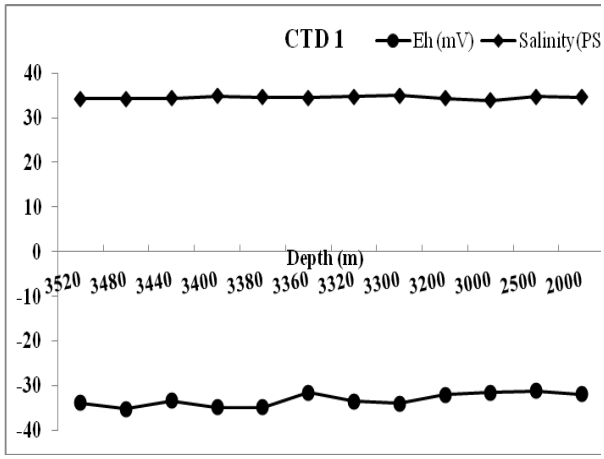
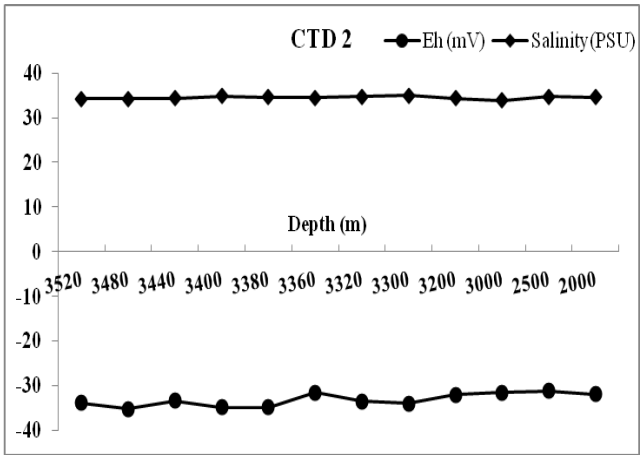
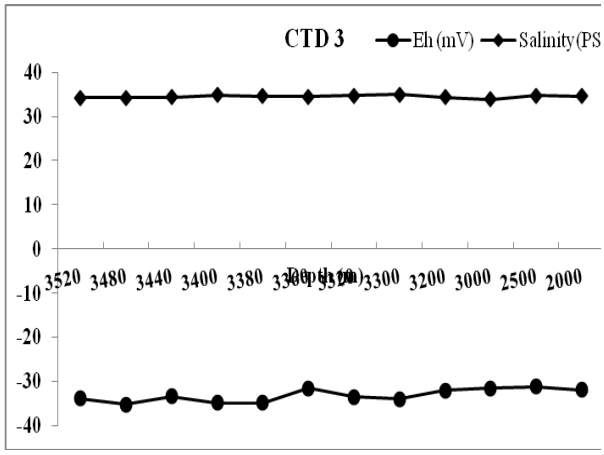
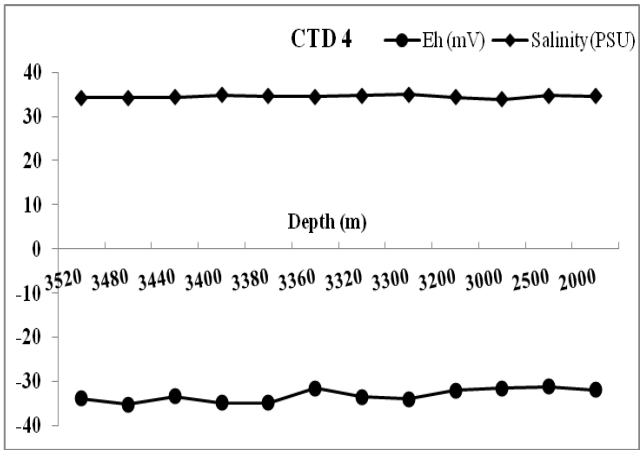


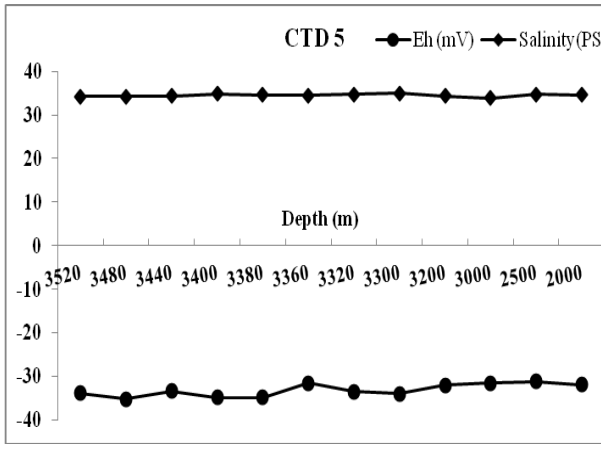
Fig.4.2 A-L: Temperature, pH and Dissolve oxygen in ridges water column in 12 different stations

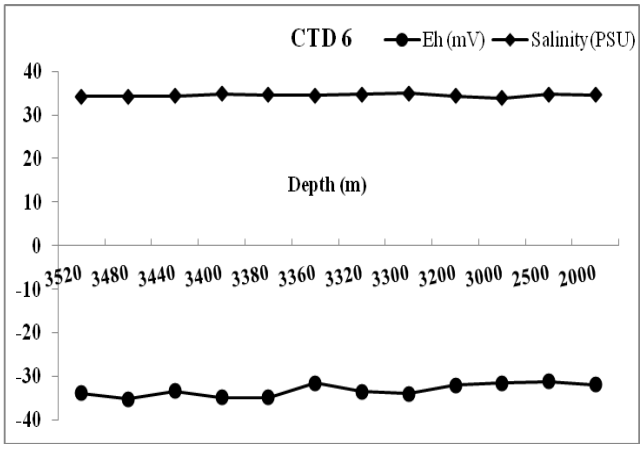

CTD 1
Eh (mV)
Salinity (PS
40
30
20
10
0
-10
-20
-30
-40
Depth (m)
3520 3480 3440 3400 3380 3360 3320 3300 3200 3000 2500 2000


CTD 2
Eh (mV)
Salinity (PSU)
40
30
20
10
0
-10
-20
-30
-40
Depth (m)
3520 3480 3440 3400 3380 3360 3320 3300 3200 3000 2500 2000


CTD 3
Eh (mV)
Salinity (PS
40
30
20
10
0
-10
-20
-30
-40
3520 3480 3440 3400 3380 3300 3200 3000 2500 2000


CTD 4
Eh (mV)
Salinity (PSU)
40
30
20
10
0
-10
-20
-30
-40
Depth (m)
3520 3480 3440 3400 3380 3360 3320 3300 3200 3000 2500 2000


CTD 5
Eh (mV)
Salinity (PS
40
30
20
10
0
-10
-20
-30
-40
Depth (m)
3520 3480 3440 3400 3380 3360 3320 3300 3200 3000 2500 2000


CTD 6
Eh (mV)
Salinity (PSU)
40
30
20
10
0
-10
-20
-30
-40
Depth (m)
3520 3480 3440 3400 3380 3360 3320 3300 3200 3000 2500 2000

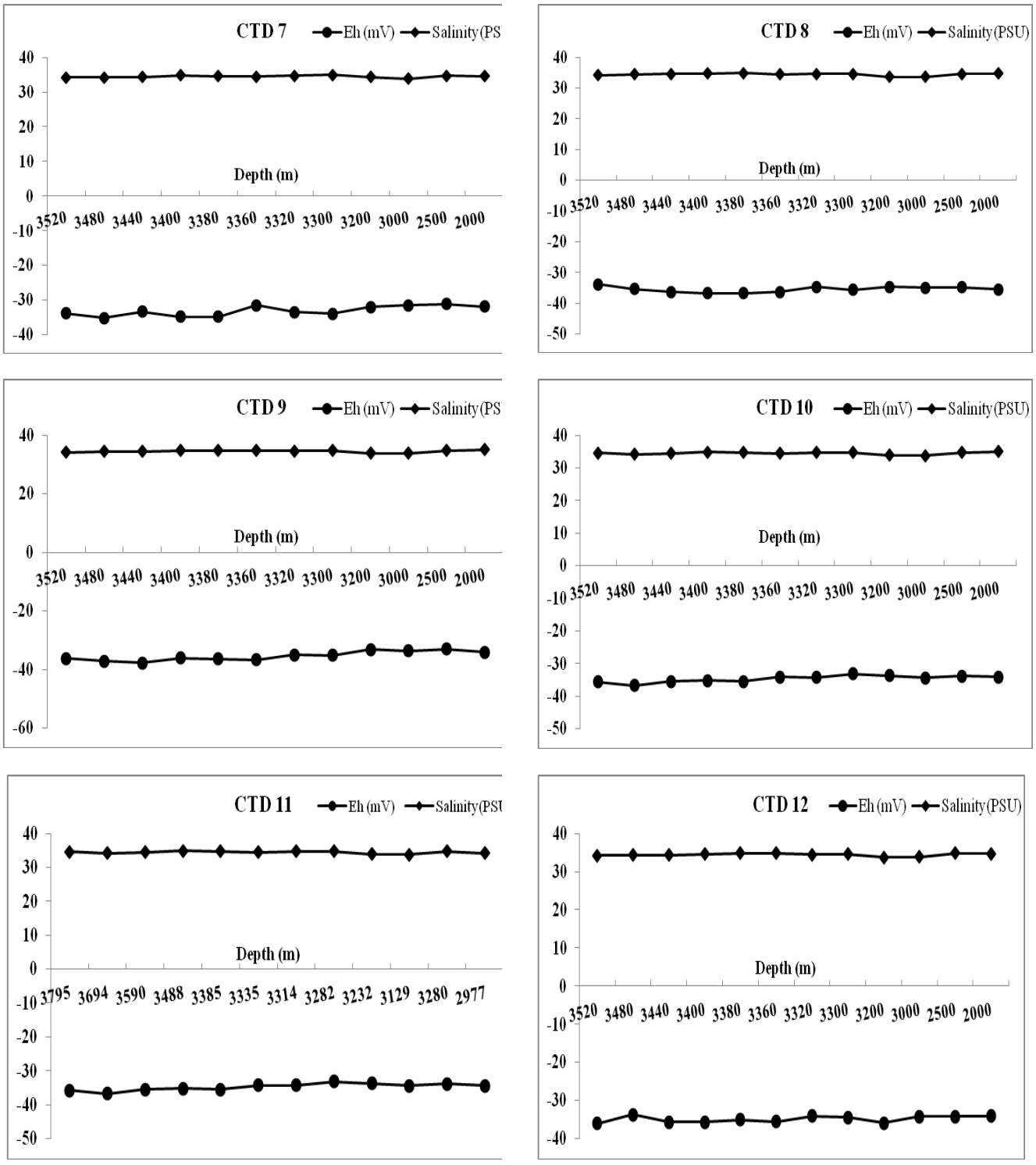
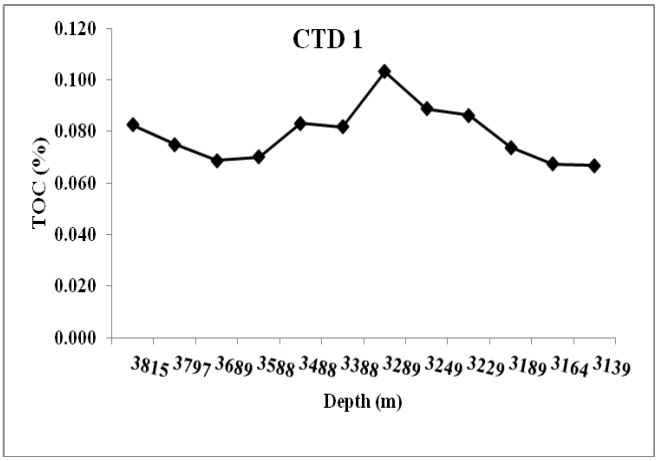
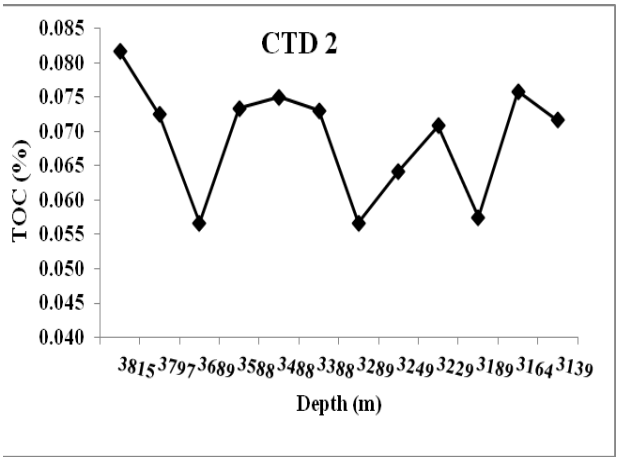
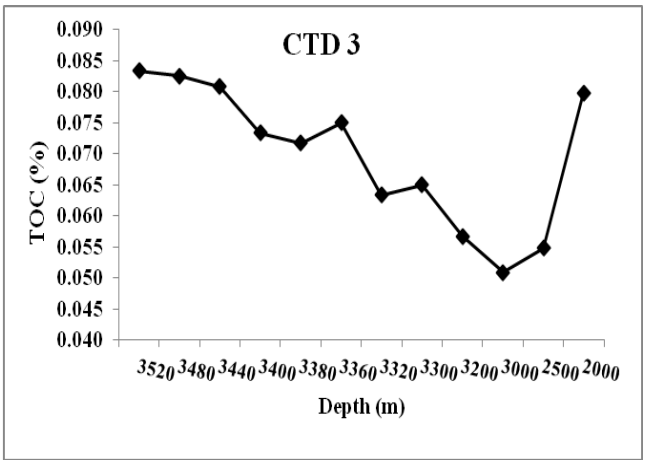
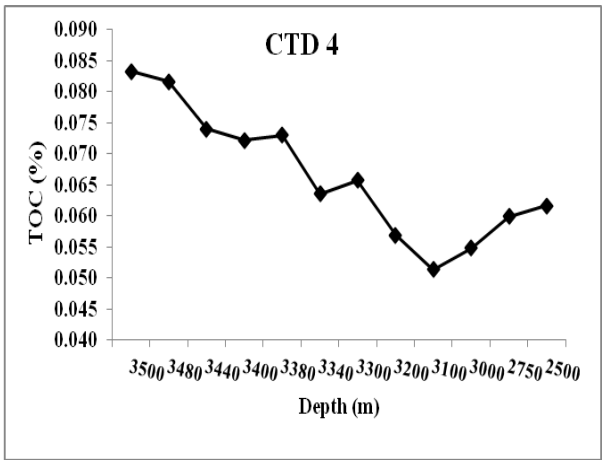


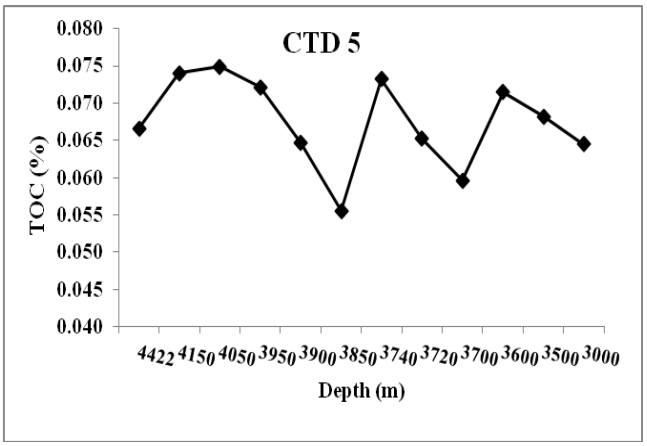
Fig. 4.3 A-L: Salinity and Eh (Redox potential) in ridges water column in 12 different stations

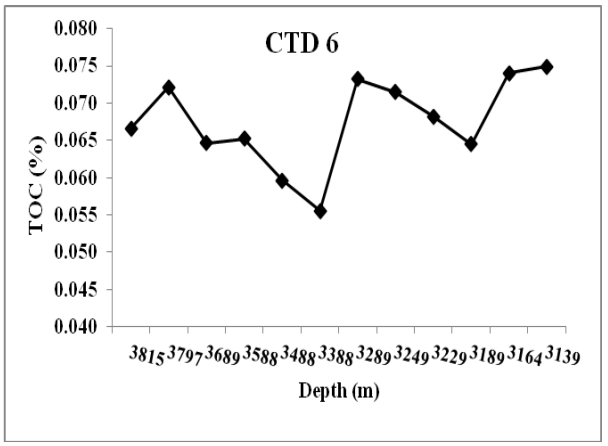

CTD 1
TOC (%)
0.120
0.100
0.080
0.060
0.040
0.020
0.000
3815 3797 3689 3588 3488 3388 3289 3249 3229 3189 3164 3139
Depth (m)


CTD 2
TOC (%)
0.085
0.080
0.075
0.070
0.065
0.060
0.055
0.050
0.045
0.040
3815 3797 3689 3588 3488 3388 3289 3249 3229 3189 3164 3139
Depth (m)


CTD 3
TOC (%)
0.090
0.085
0.080
0.075
0.070
0.065
0.060
0.055
0.050
0.045
0.040
3520 3480 3440 3400 3380 3360 3320 3300 3200 3000 2500 2000
Depth (m)


CTD 4
TOC (%)
0.090
0.085
0.080
0.075
0.070
0.065
0.060
0.055
0.050
0.045
0.040
3500 3480 3440 3400 3380 3340 3300 3200 3100 3000 2750 2500
Depth (m)


CTD 5
TOC (%)
0.080
0.075
0.070
0.065
0.060
0.055
0.050
0.045
0.040
4422 4150 4050 3950 3900 3850 3740 3720 3700 3600 3500 3000
Depth (m)


CTD 6
TOC (%)
0.080
0.075
0.070
0.065
0.060
0.055
0.050
0.045
0.040
3815 3797 3689 3588 3488 3388 3289 3249 3229 3189 3164 3139
Depth (m)

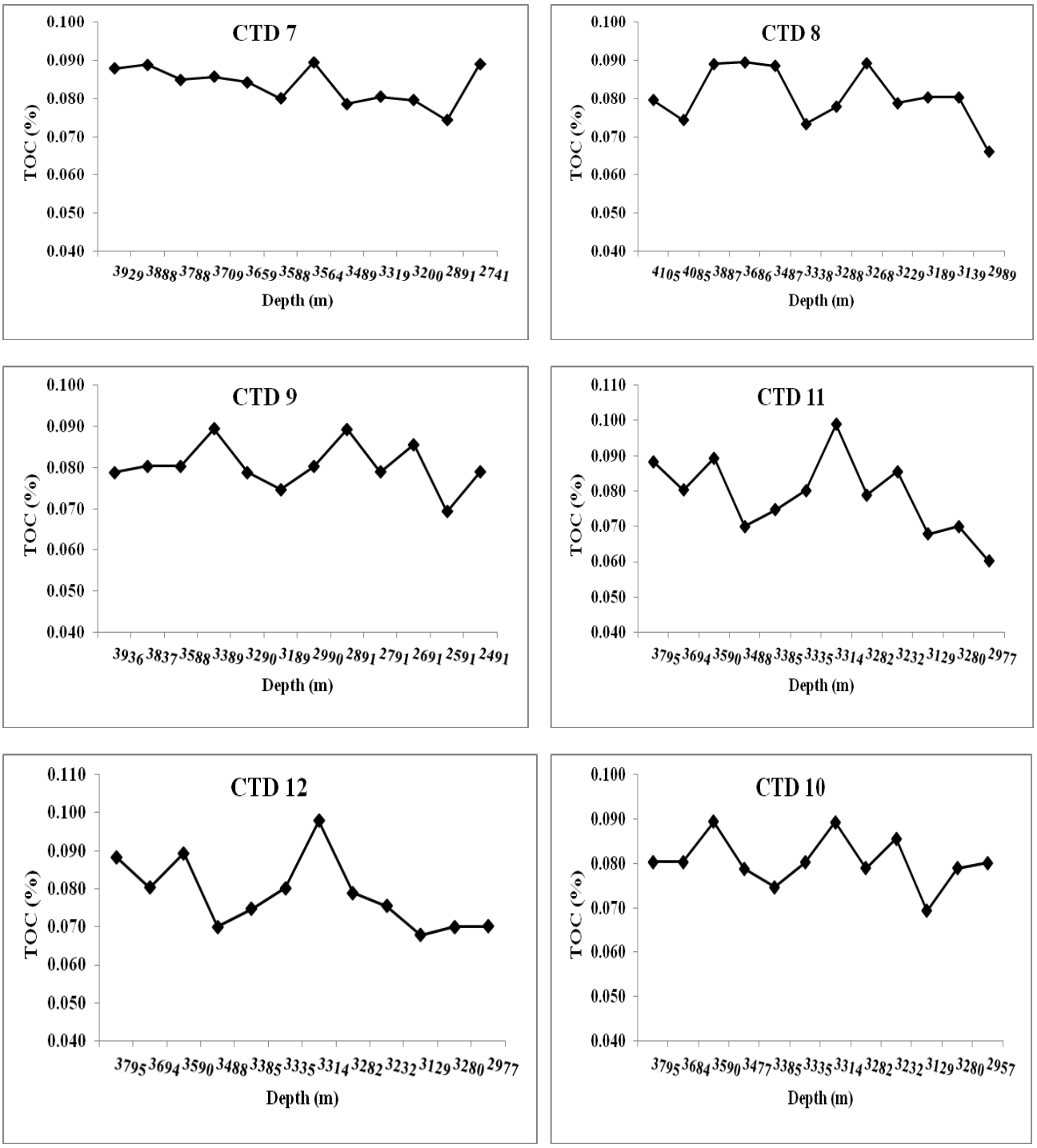


Fig.4.4 A-L: Total Organic Carbon in ridges water column in 12 different stations

4.3 Total bacterial counts (TBC) and Retrieval counts (RC) in sediment

The total counts in rock and sediments varied from 2.04E+05 to 2.67E+05. The results for Total counts are presented in the table 4.2 for rock/sediment samples. Retrieval counts (RC) for Heterotrophic and metal resistant bacteria (Cu & Mn) from Fe-Mn crust samples were observed from 9.69E+03 to 3.78E+03, 7.00E+02 to 1.30E+03 and 2.76E+03 to 1.22E+03 respectively presented in Table 4.3.

4.4 Total bacterial counts and Retrievable counts (RC) in water samples

The total counts in water samples varied from 1.88E+06 to 9.82E+06. The results for Total counts are presented in the Table 4.4 and Fig 1 while individual results are presented in Table 4.4 and Fig. 2 for water samples.

4.5 Retrievable counts (RC)

Retrieval counts (RC) for metal resistant bacteria (Mn & Cu) from water samples were observed from 1.00E+02 to 2.58E+04 and 1.00E+02 to 1.37E+04 respectively presented in Table 4.6 and 4.7. The average results for R counts are presented in the table 4.8 and Fig. 4.8 for Mn while for Cu in Table 4.9 and Fig. 4.9.

Comparative Mn, Cu and TBC of bacterial counts were presented in Fig 4.10

Table 4.2: Total Bacterial Counts (Rock)

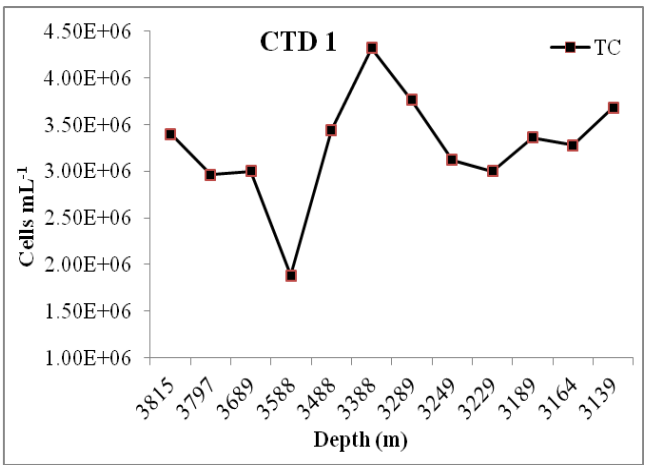
Sample	TC cell g-1	Avg. cell g-1	Std. deviation
Ridge 1	2.44E+05- 2.67E+05	2.57E+05	1.18E+04
G1	2.36E+05- 2.44E+05	2.36E+05	8.00E+03
G14	2.04E+05- 2.40E+05	2.20E+05	1.83E+04

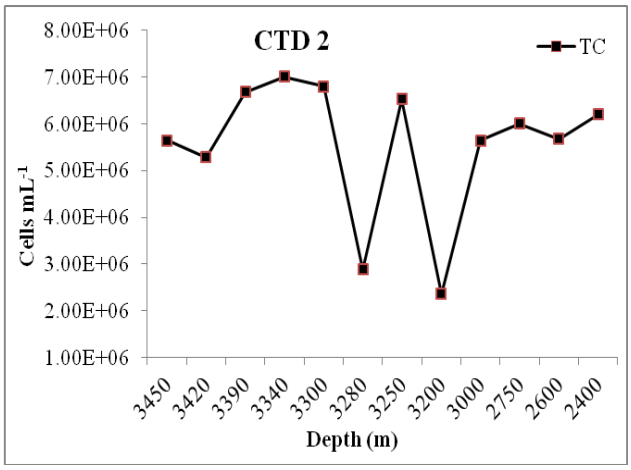
Table 4.3: Retrievable counts (RC) (Rock)

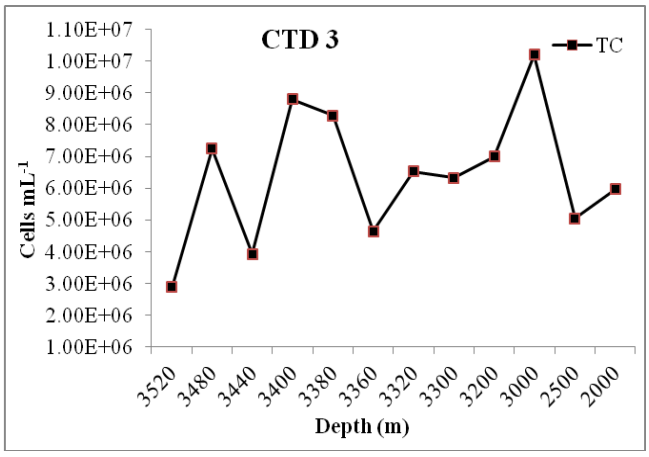
Sample	RC cell g-1	Avg. cell g-1	Std. deviation
Mn oxidiser	2.76E+03 to 1.22E+03	1.94E+03	7.70E+02
Cu oxidisers	7.00E+02 to 1.30E+03	1.00E+03	4.24E+2
Hetrotrophs	9.69E+03 to 3.78E+03	6.09E+03	3.16E+03

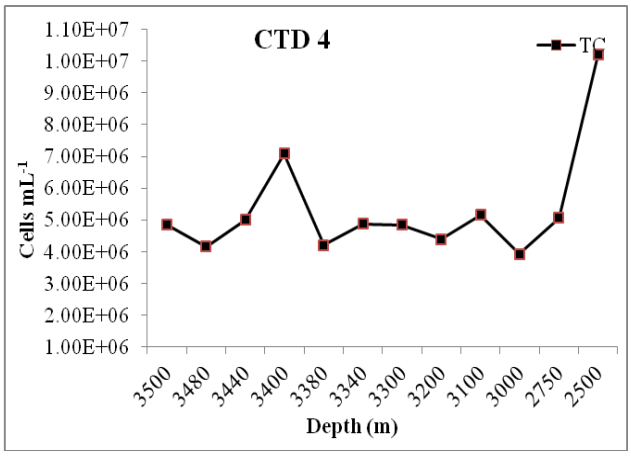
Table 4.4: Total bacterial Counts (Water)

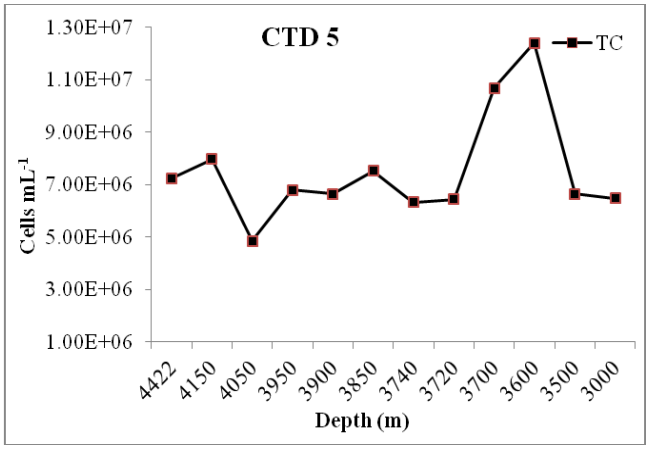
Sample	TC cell g^{-1}	Avg. cell g^{-1}	Std. deviatio
Station 1	1.88E+06 to 3.76E+06	3.27E+06	5.85E+05
Station 2	2.36E+06 to 7.00E+06	5.56E+06	1.47E+06
Station 3	2.88E+06 to 7.02E+06	6.40E+06	2.10E+06
Station 4	3.92E+06 to 7.08E+06	4.86E+06	8.41E+05
Station 5	2.56E+06 to 5.28E+06	4.16E+06	9.30E+05
Station 6	2.20E+06 to 5.56E+06	4.13E+06	7.41E+05
Station 7	3.64E+06 to 5.44E+06	4.42E+06	7.42E+05
Station 8	5.04E+06 to 8.06E+06	6.60E+06	1.71E+06
Station 9	5.20E+06 to 8.86E+06	8.15E+06	4.09E+06
Station 10	2.80E+06 to 8.80E+06	6.20E+06	2.04E+06
Station 11	4.84E+06 to 8.24E+06	7.50E+06	2.07E+06
Station 12	6.64E+06 to 9.82E+06	9.32E+06	3.83E+06

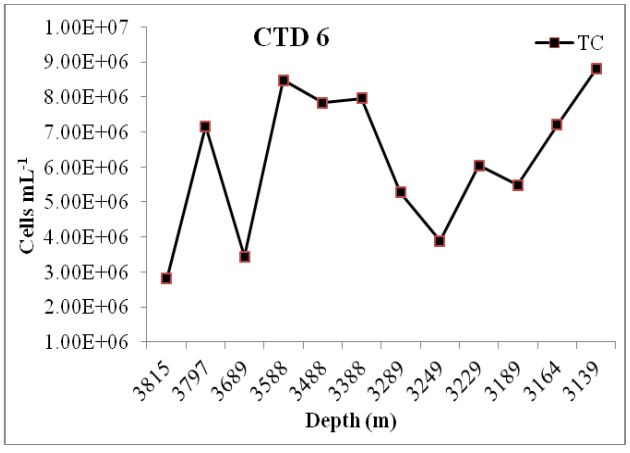

CTD 1
TC
Cells mL-1
4.50E+06
4.00E+06
3.50E+06
3.00E+06
2.50E+06
2.00E+06
1.50E+06
1.00E+06
3815 3797 3689 3588 3488 3388 3289 3249 3229 3189 3164 3139
Depth (m)


CTD 2
TC
Cells mL-1
8.00E+06
7.00E+06
6.00E+06
5.00E+06
4.00E+06
3.00E+06
2.00E+06
1.00E+06
3450 3420 3390 3340 3300 3280 3250 3200 3000 2750 2600 2400
Depth (m)


CTD 3
TC
Cells mL-1
1.10E+07
1.00E+07
9.00E+06
8.00E+06
7.00E+06
6.00E+06
5.00E+06
4.00E+06
3.00E+06
2.00E+06
1.00E+06
3520 3480 3440 3400 3380 3360 3320 3300 3200 3000 2500 2000
Depth (m)


CTD 4
TC
Cells mL-1
1.10E+07
1.00E+07
9.00E+06
8.00E+06
7.00E+06
6.00E+06
5.00E+06
4.00E+06
3.00E+06
2.00E+06
1.00E+06
3500 3480 3440 3400 3380 3340 3300 3200 3100 3000 2750 2500
Depth (m)


CTD 5
TC
Cells mL-1
1.30E+07
1.10E+07
9.00E+06
7.00E+06
5.00E+06
3.00E+06
1.00E+06
4422 4150 4050 3950 3900 3850 3740 3720 3700 3600 3500 3000
Depth (m)


CTD 6
TC
Cells mL-1
1.00E+07
9.00E+06
8.00E+06
7.00E+06
6.00E+06
5.00E+06
4.00E+06
3.00E+06
2.00E+06
1.00E+06
3815 3797 3689 3588 3488 3388 3289 3249 3229 3189 3164 3139
Depth (m)

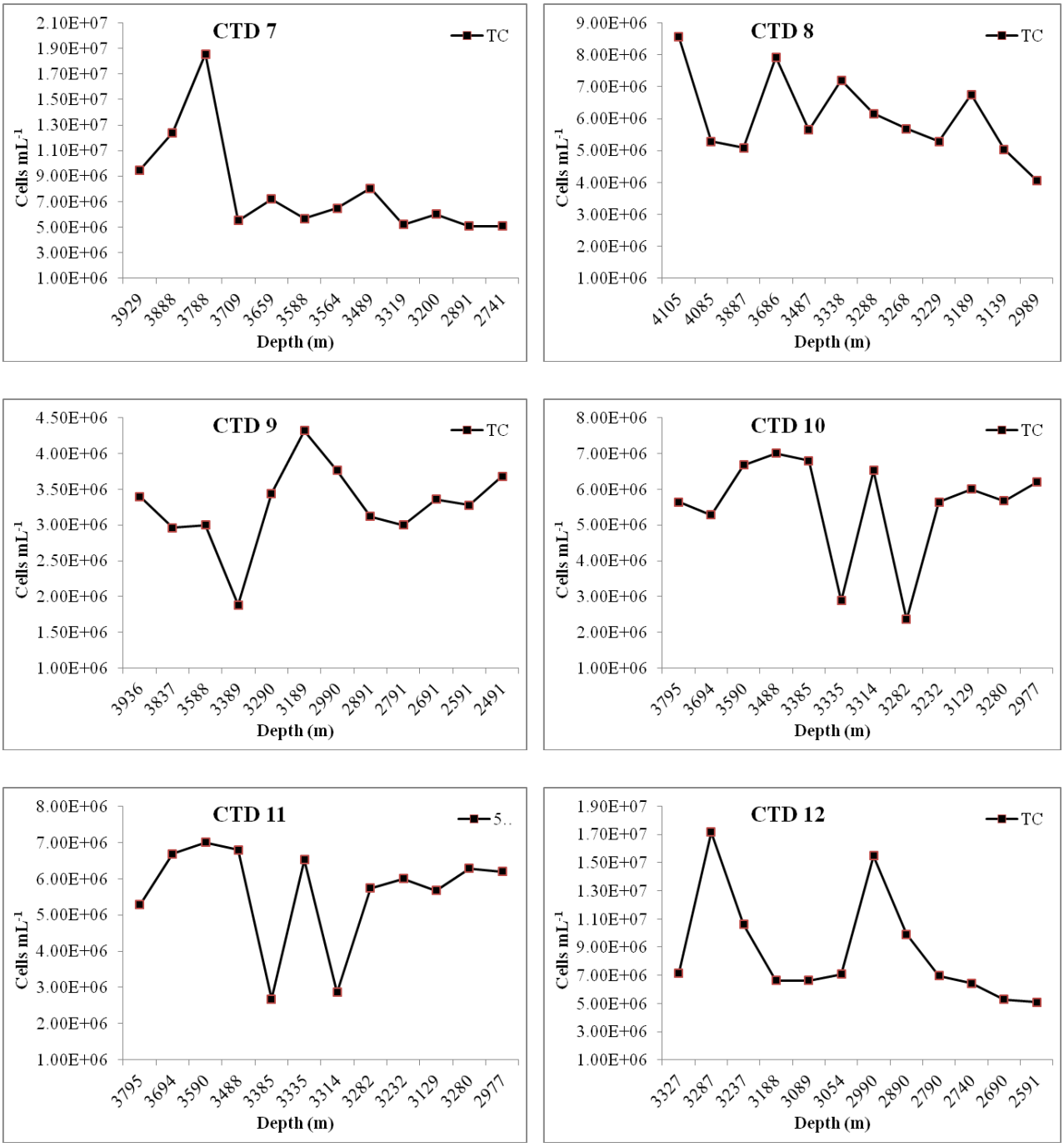


Fig. 4.5: Total bacterial counts in ridges water column in 12 different stations

Table 4.5 Total bacterial Counts (Water)

Sample	TC cell ml^{-1}	Avg. cell g-1	Std. deviation
Stations	1.88E+06 to 9.82E+06	5.60E+06	3.88E+05

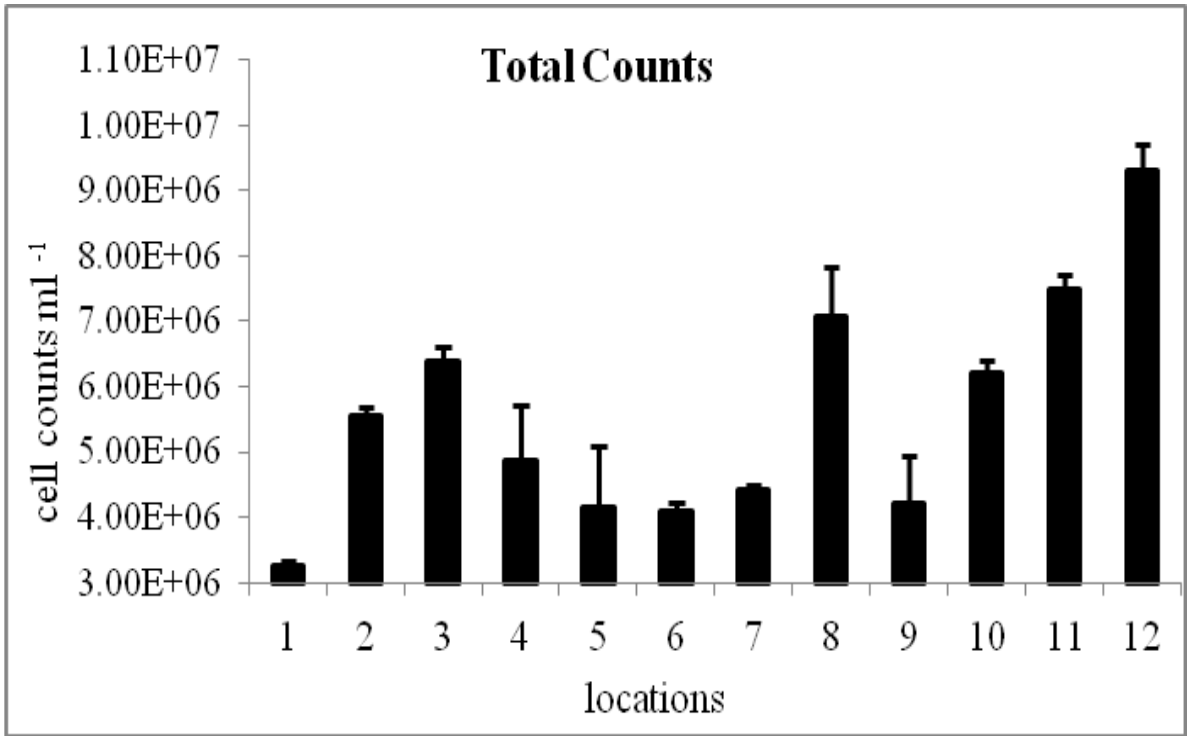


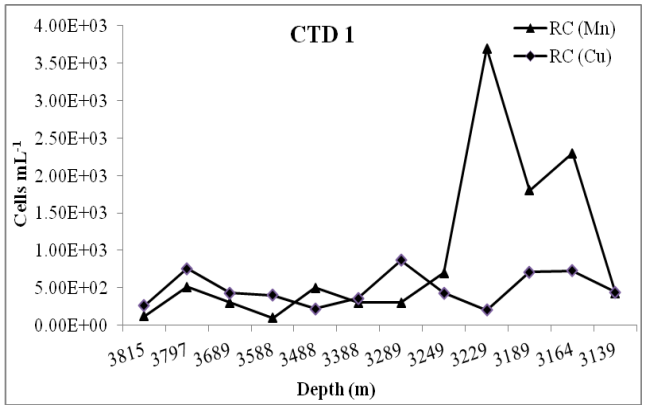
Fig. 4.6: Total bacterial counts in ridges water column in 12 different stations

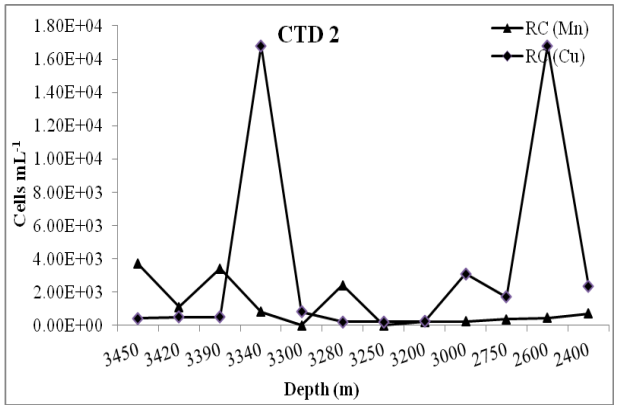
Table 4.6: Retrievable counts (RC) Mn tolerant (Water)

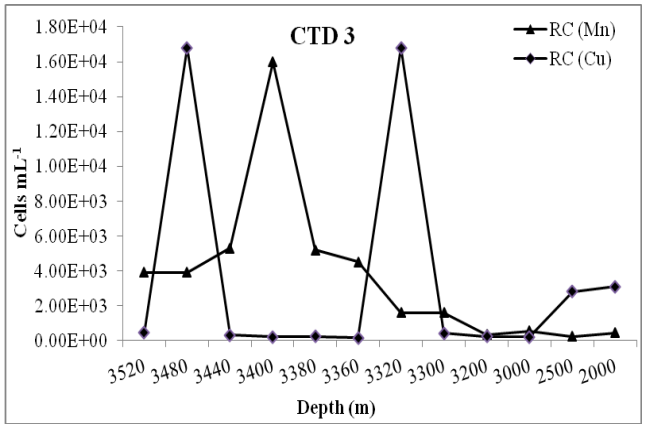
Sample	RC cell mL-1	Avg. cell mL-1	Std. deviation
Station 1	4.00E+02 to 4.50E+03	1.82E+03	1.39E+03
Station 2	4.00E+02 to 1.07E+04	2.75E+03	3.00E+03
Station 3	3.00E+02 to 2.10E+03	1.03E+03	5.85E+02
Station 4	3.00E+03 to 4.40E+03	1.02E+04	4.38E+03
Station 5	3.00E+02 to 3.40E+03	5.91E+03	5.36E+03
Station 6	4.00E+02 to 4.60E+03	3.55E+03	3.45E+03
Station 7	2.00E+02 to 3.30E+03	3.62E+03	7.60E+03
Station 8	2.00E+02 to 2.58E+04	9.25E+03	1.05E+03
Station 9	1.00E+02 to 3.60E+03	9.96E+02	1.10E+03
Station 10	3.00E+02 to 3.40E+03	1.74E+03	9.90E+02
Station 11	1.00E+02 to 2.80E+03	6.04E+03	1.94E+03
Station 12	2.00E+02 to 1.37E+04	3.23E+03	3.77E+03

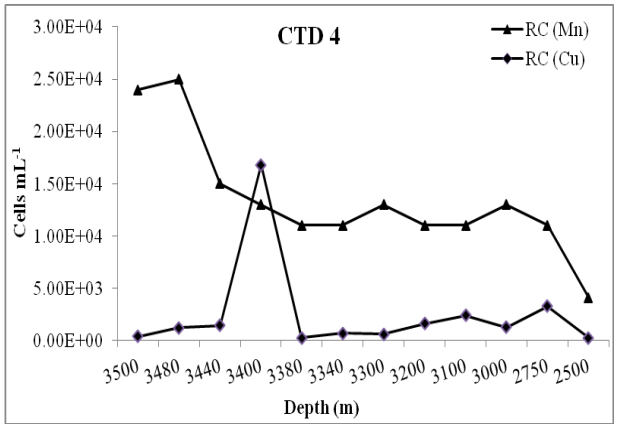
Table 4.7: Retrievable counts (RC) Cu tolerant (Water)

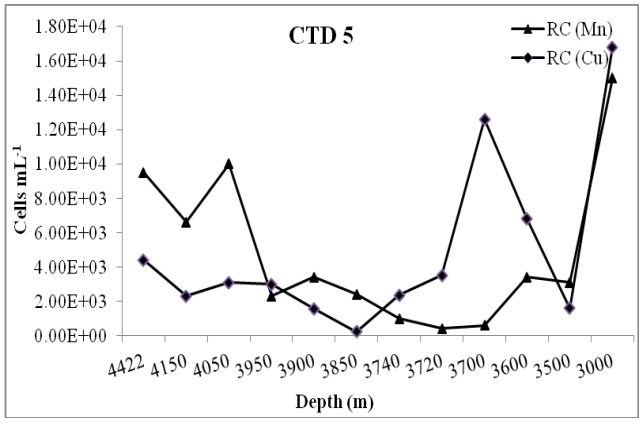
Sample	RC cell mL-1	Avg. cell mL-1	Std. deviation
Station 1	1.00E+02 to 3.20E+03	1.33E+03	1.25E+03
Station 2	2.00E+02 to 1.60E+03	8.17E+02	5.08E+02
Station 5	2.00E+02 to 2.36E+03	7.64E+02	7.61E+02
Station 11	6.00E+02 to 4.40E+03	1.58E+03	1.89E+03
Station 12	2.36E+03 to 1.26E+04	4.57E+03	3.63E+03
Station 13	4.00E+02 to 1.33E+04	3.84E+03	4.15E+03
Station 14	2.00E+02 to 2.80E+03	9.60E+02	1.17E+03
Station 17	2.00E+02 to 3.00E+03	5.40E+02	2.89E+02
Station 18	6.70E+02 to 8.00E+02	1.42E+03	1.39E+03
Station 19	3.70E+02 to 3.35E+03	1.40E+03	9.82E+02
Station 21	1.50E+02 to 3.27E+03	6.42E+02	9.58E+02
Station 23	7.00E+02 to 4.10E+03	3.48E+03	3.85E+03

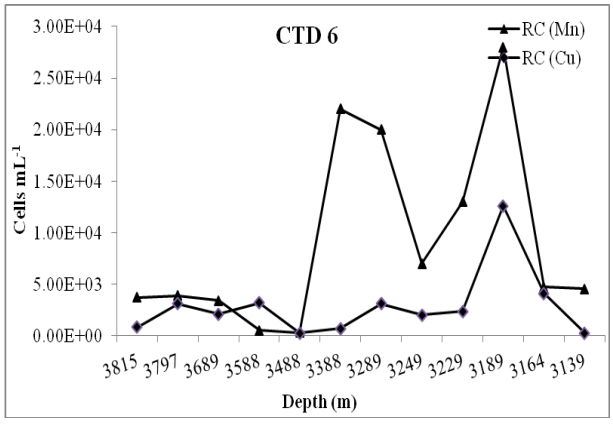

CTD 1
RC (Mn)
RC (Cu)
Cells mL-1
4.00E+03
3.50E+03
3.00E+03
2.50E+03
2.00E+03
1.50E+03
1.00E+03
5.00E+02
0.00E+00
3815 3797 3689 3588 3488 3388 3289 3249 3229 3189 3164 3139
Depth (m)


CTD 2
RC (Mn)
RC (Cu)
Cells mL-1
1.80E+04
1.60E+04
1.40E+04
1.20E+04
1.00E+04
8.00E+03
6.00E+03
4.00E+03
2.00E+03
0.00E+00
3450 3420 3390 3340 3300 3280 3250 3200 3000 2750 2600 2400
Depth (m)


CTD 3
RC (Mn)
RC (Cu)
Cells mL-1
1.80E+04
1.60E+04
1.40E+04
1.20E+04
1.00E+04
8.00E+03
6.00E+03
4.00E+03
2.00E+03
0.00E+00
3520 3480 3440 3400 3380 3360 3320 3300 3200 3000 2500 2000
Depth (m)


CTD 4
RC (Mn)
RC (Cu)
Cells mL-1
3.00E+04
2.50E+04
2.00E+04
1.50E+04
1.00E+04
5.00E+03
0.00E+00
3500 3480 3440 3400 3380 3340 3300 3200 3100 3000 2750 2500
Depth (m)


CTD 5
RC (Mn)
RC (Cu)
Cells mL-1
1.80E+04
1.60E+04
1.40E+04
1.20E+04
1.00E+04
8.00E+03
6.00E+03
4.00E+03
2.00E+03
0.00E+00
4422 4150 4050 3950 3900 3850 3740 3720 3700 3600 3500 3000
Depth (m)


CTD 6
RC (Mn)
RC (Cu)
Cells mL-1
3.00E+04
2.50E+04
2.00E+04
1.50E+04
1.00E+04
5.00E+03
0.00E+00
3815 3797 3689 3588 3488 3388 3289 3249 3229 3189 3164 3139
Depth (m)

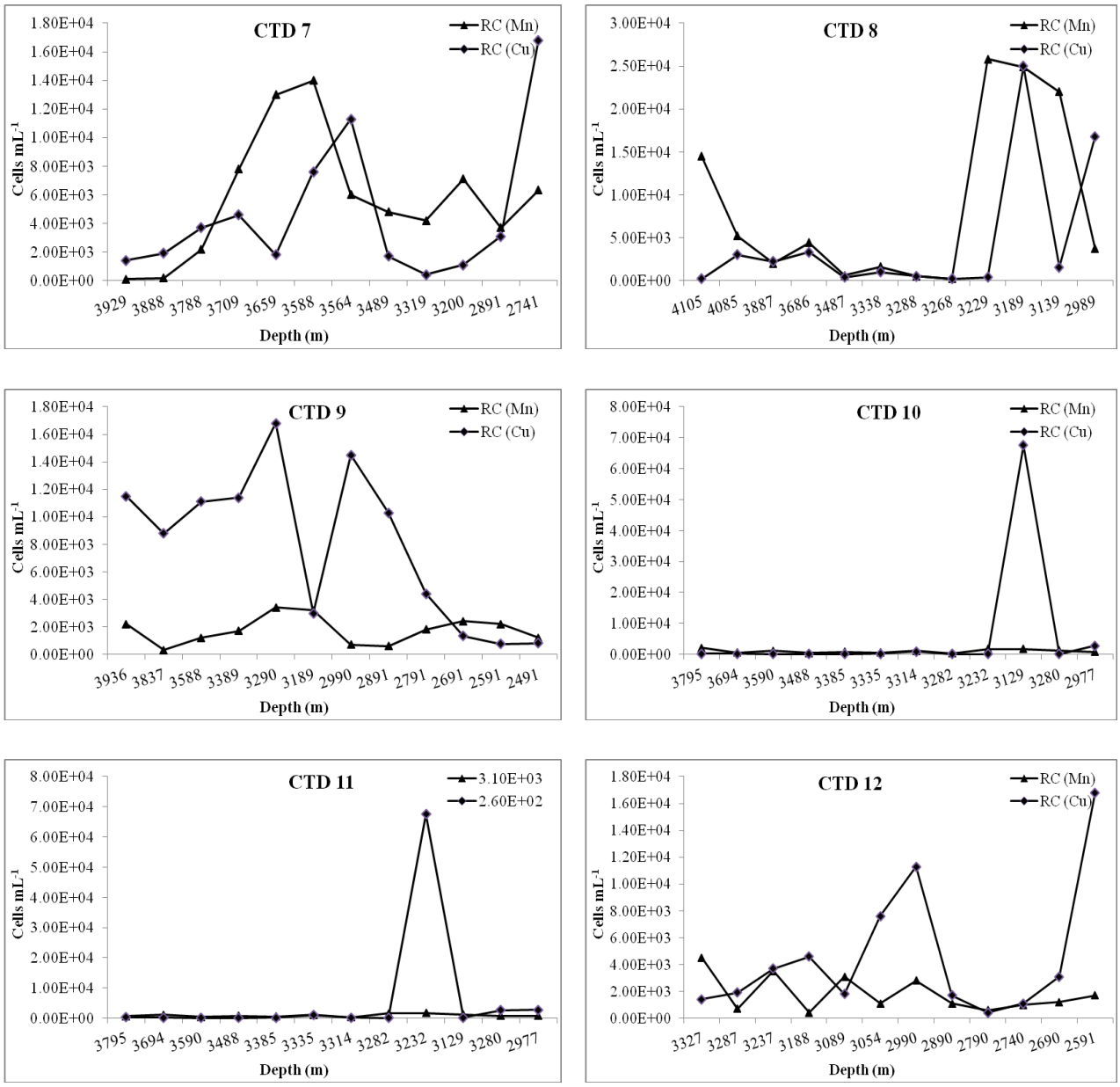


Fig. 4.7: Mn and Cu Retrievable bacterial counts in ridges water column in 12 different stations

Table 4.8 Retrievable counts (RC) (Mn Oxidisers)

Sample	RC cell ml^{-1}	Avg. cell g^{-1}	Std. deviation
Stations	1.00E+02 to 1.37E+04	6.28E+03	1.05E+03

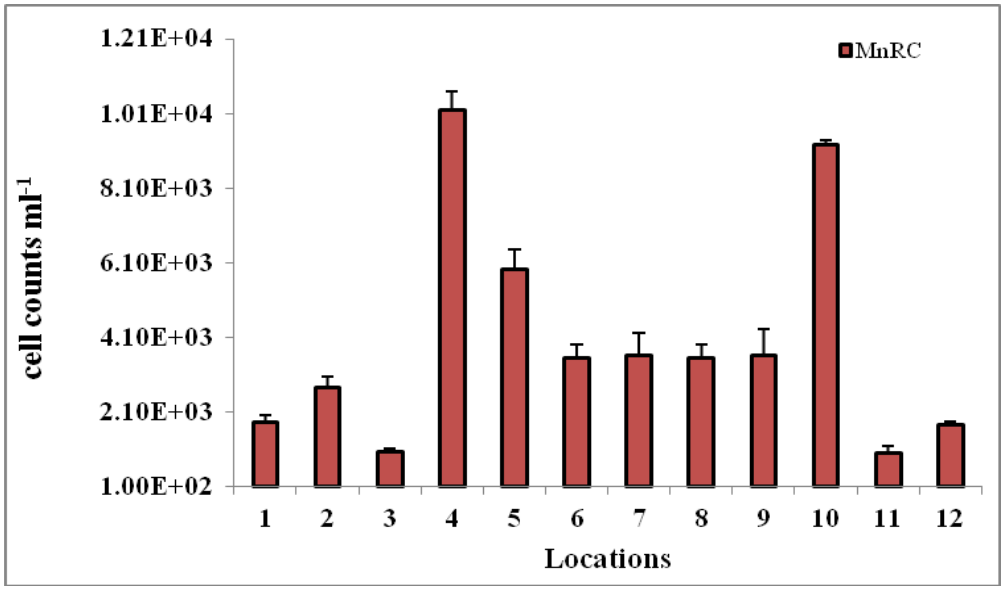


Fig. 4.8: Total retrieval bacterial counts for Mn oxidisers in ridges water column in 12 different stations

Table 4.9: Retrievable counts (RC) (Cu Oxidisers)

Sample	RC cell ml^{-1}	Avg. cell g^{-1}	Std. deviation
Stations	1.00E+02 to 1.33E+04	6.13E+03	1.67E+03

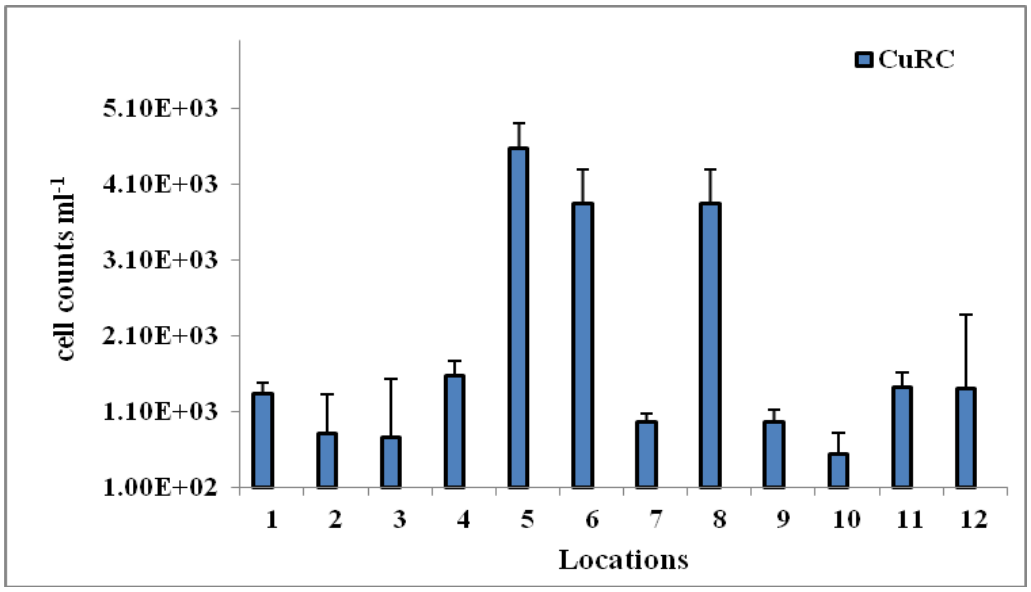


Fig. 4.9: Total retrieval bacterial counts for Cu oxidisers in ridges water column in 12 different stations

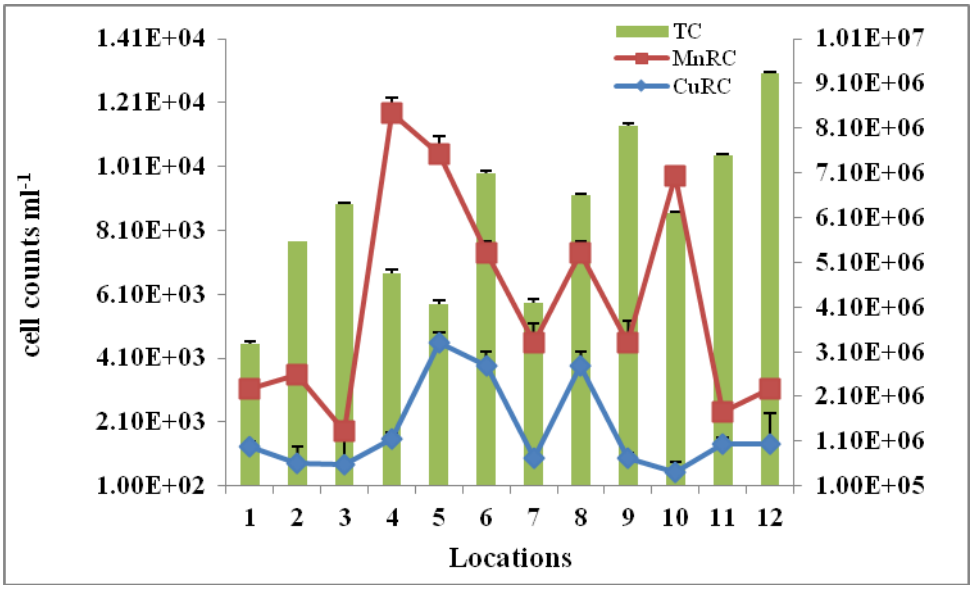


Fig. 4.10: Comparetive presentation of Mn and Cu oxidisers with total bacterial counts in ridges water Columns

4.6 Biochemical analysis of Bacterial Isolates

A total 180 bacterial isolates including Mn and Cu oxidisers isolated from rocks, water and benthic fauna and heterotrophic bacterial isolates were chosen for further study. Biochemical analysis and enzymatic activities were carried out for all the bacterial isolates.

4.6.1 Phylogenetic analysis of Bacterial Isolates

Forty nine bacterial isolates (Table 4.11) were identified with 16S rRNA technique out of 180 isolates. Genomic DNA was extracted from all bacterial isolates followed by PCR amplification. The amplified products were sequenced using ABI 3130 XL analyzer sequencer in CSIR- National Institute of Oceanography Goa, India. Phylogenetic relationship among samples was carried out in the form of neighbor joining tree using MEGA 4 (Sujith *et al.*, 2014). In the present Study, *Proteobacteria* (50%) were the dominant general followed by *Firmicutes* (41%) and *Actinobacteria* (9%) (Fig. 4.12).

4.6.1.1 Bacteria isolated from Rock and water

Phylogenetic analysis of ridge isolates (29) from rock and sediment indicated that λ *Proteobacteria* is dominant class *(48%)* in the ridge followed by *Bacilli* (38%), *Actinobacteria (9%) and Bacteroidetes* (5%) Fig 11.C and D. R4 [*Bacillus oceanisediminis* H2 (T), (GQ292772)], R6 [*Imtechella sp* (FR774044)] and NA1 [*Pseudomonas parafulva* AJ 2129(T), (AB060132)] at 97.52, 90.24 and 92.50% similarity represented by novel isolates in ridge rock and water samples.

4.6.1.2 Bacteria isolated from Fauna

Out of 49 bacterial isolates 8 bacterial isolates isolated from different benthic foraminifera morphotypes. In which *Proteobacteria* was most dominant at 53% and included *Salinicola*, *Halomonas*, *Nitratireductor, Bordetella*, *Pseudomonas.* The next dominant group *Firmicutes at* 40% included *Bacillus* and *Oceanobacillus. Actinobacteria* formed only 7% and included the single genus *Brachybacterium.* RF1 bacterial isolate from ridge had shown 95.2% similarity with *Bacillus vietnamensis* 15-1(T) (AB099708).

4.6.2 Fatty acid analysis of Bacterial Isolates

Six bacterial isolates were identified using Sherlock software by Agilent 8800 GC-MS on the basis of Fatty acid profiling in which, Bacterial isolates NiB25/3 and 30M did not find any match with Sherlok RSTBA6 software while remains presents *Firmicutes* introduced 4 *bacillus* sp. (Table 4.10).

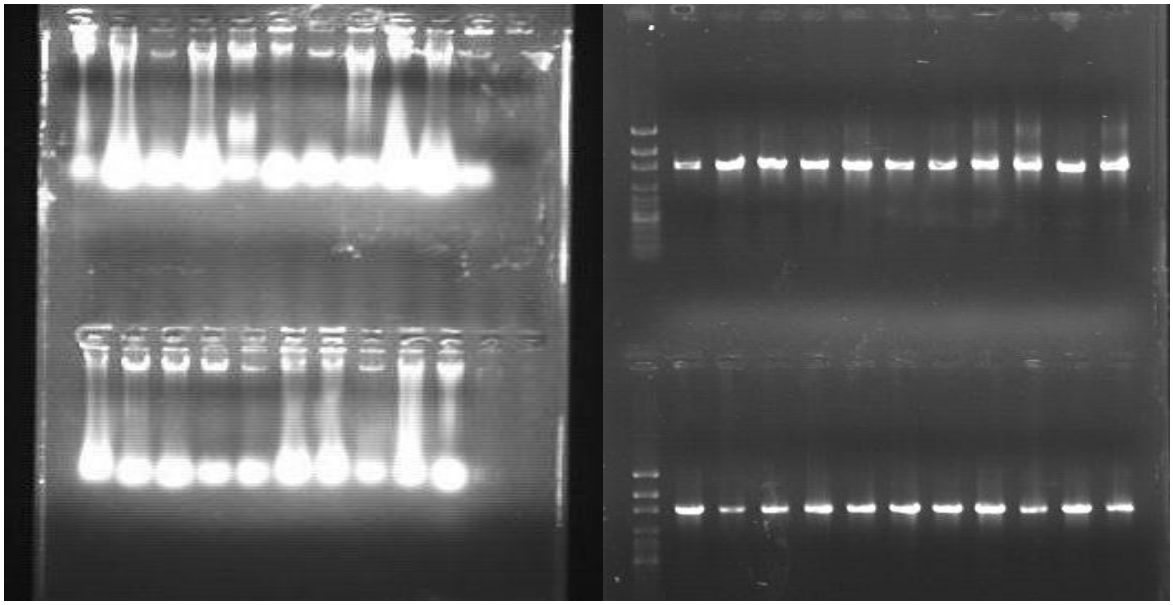


Fig 4.11 A: Bacterial Isolates gel presentation from ridge samples

Table 4.10: Fatty acid analysis of Bacterial Isolates

Sr. No.	Isolates	Similarity index	Identified name
1	NiB25/3	0.00	No match
2	1B1O	0.41	*Ewingella americana*
3	MF214	0.212	*Microbactrium flavescens*
4	R6	4.19	*Grimontia holliisac*
5	30M	0.00	No match Found
6	1B2F	0.570	*Ewingella americana*

Table 4.11: Bacterial Isolates identified from ridge samples (Rock and Water)

Sr. No	Isolates	Ez Taxon	Similarity	Source/Function
1	CoA (708) (in Process)	*Thalassospira xianhensis* P-4(T), (EU017546)	99.01%	Water /Copper immobilization
2	NAI (610) (in Process)	*Lysinibacillus fusiformis* NBRC 15717(T), (AB271743)	99.50%	Water /Copper immobilization
3	NiB (1470) (in Process)	*Staphylococcus warneri* ATCC 27836(T) (L37603)	100.0%	Water /Copper immobilization
4	R1 (999) HE681807	*Bacillus oceanisediminis* H2(T), (GQ292772)	99.29%	Rock/Mn immobilization
5	R2 (1398) HE681808	*Exiguobacterium indicum* HHS31(T (AJ846291)	99.42%	Rock/Mn immobilization
7	R3 (1247) HE681809	Bacillus aryabhattai B8W22(T), (EF114313)	99.27%	Rock/Mn immobilization
8	R4 (1375)* HE681810	*Bacillus oceanisediminis* H2(T), (GQ292772)	97.52%	Rock/Mn immobilization
9	R5 (1039) (in Process)	*Idiomarina abyssalis* KMM 227(T), (AF052740)	99.71%	Rock/Mn immobilization
10	R6 (1321) (in Process)*	*Imtechella halotolerans* K1(T), (FR774044)	92.50%	Rock/Mn immobilization
11	R7 (1049) (in Process)	*Bacillus flexus* IFO 15715(T), (AB021185)	100.0%	Rock/Mn immobilization
12	R8 (1036) (in Process)	*Idiomarina abyssalis* KMM 227(T), (AF052740)	99.61%	Rock/Mn immobilization
13	R9 (967) (in Process)	*Thalassospira permensis* SMB34(T) (FJ860275)	99.48%	Rock/Mn immobilization
14	R10 (1034) (in Process)	*Serratia nematodiphila* DZ0503SBS1(T), (EU036987)	99.32%	Rock/Mn immobilization

15	NA1 (1374)* HE681811	*Pseudomonas parafulva* AJ 2129(T) (AB060132)	90.24%	Rock/Mn immobilization
16	NA2 (1356) HE681812	*Bacillus flexus IFO* 15715(T), (AB02118)	100.0%	Rock/Mn immobilization
17	NA3 (1272) HE681813	*Staphylococcus sciuri* subsp. sciuri DSM 20345(T), (AJ421446)	100.0%	Rock/Mn immobilization
18	NA5 (1360) HE681814	*Brevibacterium epidermidis* NCDO 2286(T), (X76565)	99.63%	Rock/Mn immobilization
19	NA6 (1038) (in Process)	*Idiomarina abyssalis* KMM 227(T), (AF052740)	99.61%	Rock/Mn immobilization
20	NA7 (1004) (in Process)	*Idiomarina abyssalis* KMM 227(T), (AF052740)	99.10%	Rock/Mn immobilization
21	NA9 (1308) (in Process)	*Marinobacter algicola* DG893(T), (ABCP01000031)	99.08%	Rock/Mn immobilization
22	NA10 (1035) (in Process)	*Marinobacter algicola* DG893(T), (ABCP01000031)	99.03%	Rock/Mn immobilization
23	NA11 (1270) (in Process)	*Brevibacterium casei* NCDO 2048(T), (X76564)	99.68%	Rock/Mn immobilization
24	NA12 (1057) (in Process)	*Serratia nematodiphila* DZ0503SBS1(T), (EU036987)	98.67%	Heterotroph
25	CuR1 (1267) (in Process)	*Bacillus oceanisediminis* H2(T)(GQ292772)	99.28%	Rock/Copper immobilization
26	CuR2 (991) (in Process	*Halomonas rifensis* DSM 4740(T)(L42615)	99.49%	Water/Copper immobilization
27	CuR3(963) (in Process)	*Imtechella halotolerans* K1(T), (FR774044)	98.10%	Rock/Copper immobilization
28	CuR4(1063) (in Process)	*Bacillus aryabhattai* B8W22(T)	93.03%	Rock/Copper immobilization
29	CuR6 (712) (in Process)	*Thalassospira xianhesis* EU017546 (T)	99.01%	Rock/Copper immobilization

Table 4.12: Bacterial Isolates identified from ridge samples (Foraminifera)

Sr.No	S. Name	Closest match	Similarity	Source
1	RF1 (1032)	*Bacillus vietnamensis* 15-1(T)(AB099708)	95.800%	Benthic Foraminifera
2	RF6(1058)	*Oceanobacillus picturae* LMG 19492(T)(AJ315060)	99.905%	Benthic Foraminifera
3	RF8(988)	*Halomonas cupida* DSM 4740(T)(L42615)	99.493%	Benthic Foraminifera
4	RF7(993)	*Brachybacterium phenoliresistens phenol-*A(T)(DQ822566)	99.697%	Benthic Foraminifera
5	RF3(998)	*Bacillus oceanisediminis* H2(T)(GQ292772)	99.298%	Benthic Foraminifera
6	RF2 (1028)	*Salinicola salarius* M27(T)(AM229316)	98.443%	Benthic Foraminifera
7	RF5(992)	*Halomonas cupida* DSM 4740(T)(L42615)	99.4959%	Benthic Foraminifera
8	RF4(1040)	*Bacillus vietnamensis 15-1(T)(AB099708)*	99.134%	Benthic Foraminifera

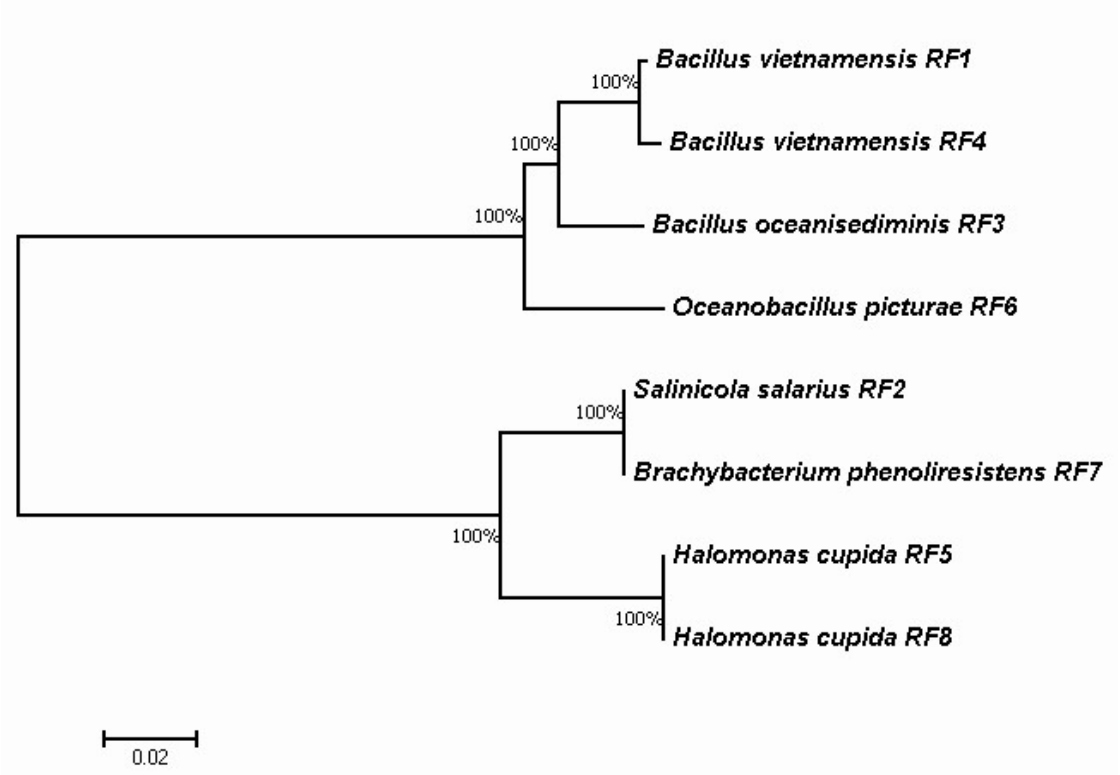


Fig 4.11 B: Phylogenetic tree showing the relationship between ridge Fauna isolates and closely related species. Bootstrap values as a percentage of 1000 replications are shown at the branch

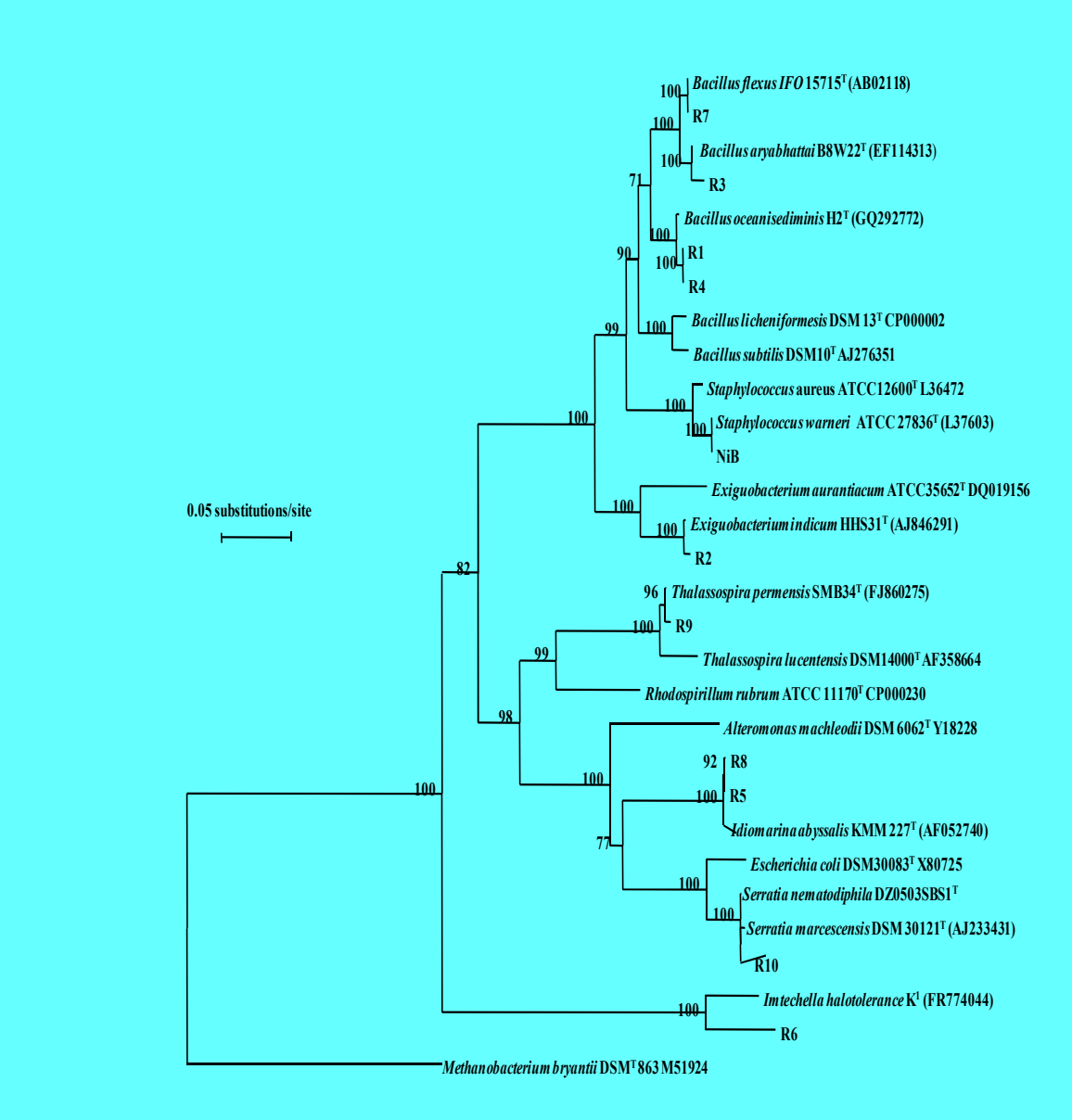


Fig 4.11 C: Phylogenetic tree based upon neighbor-joining method showing the relationship between ridge isolates and closely related species. Bootstrap values as a percentage of 1000 replications are shown at the branch. The sequence from *Methobacterium bryantii* DSM 863^T was used as out group. Bar represents 0.05 substitutions per site

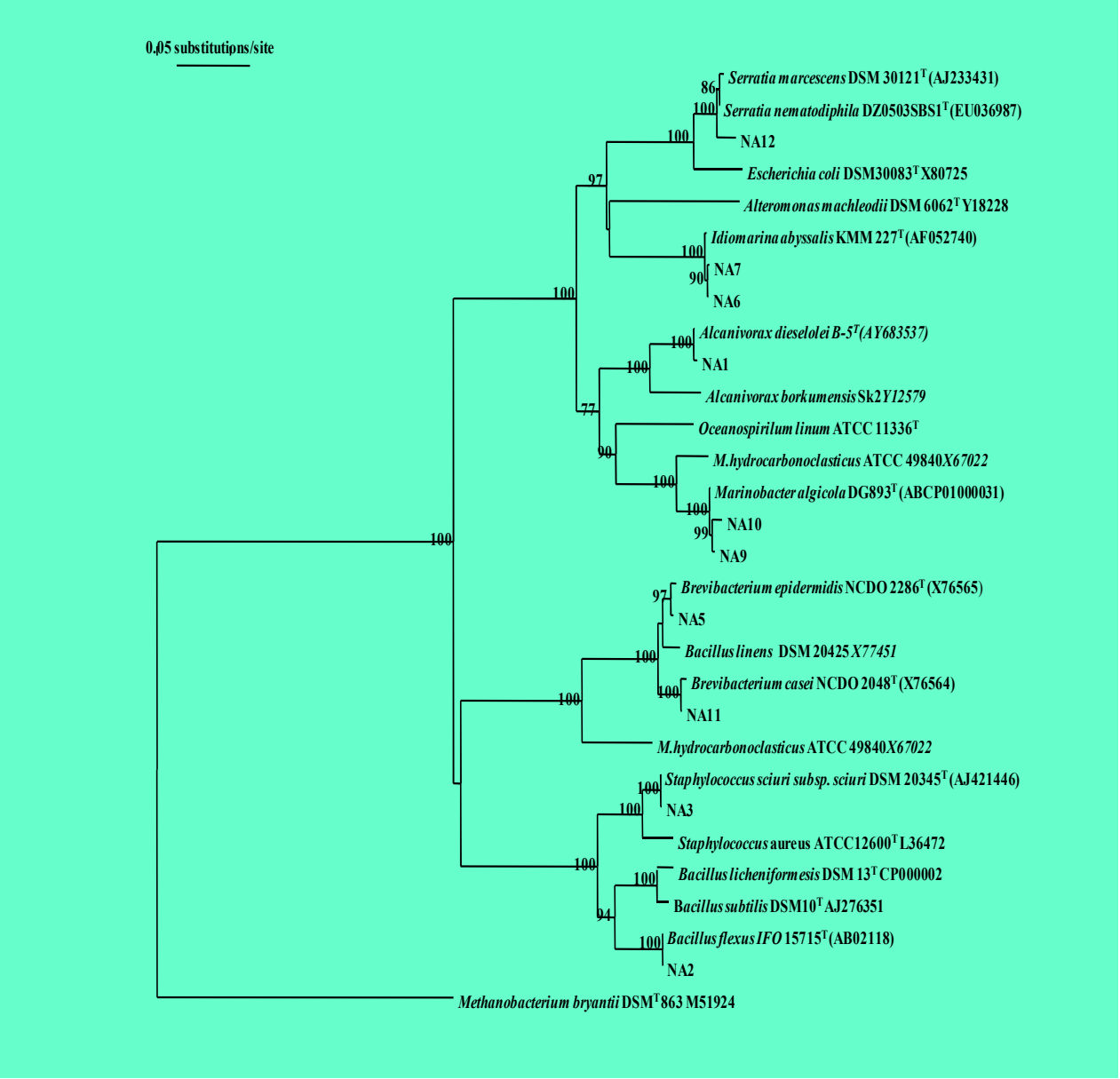


Fig 4.11 D: Phylogenetic tree based upon neighbor-joining method showing the relationship between heterotrophic ridge isolates and closely related species. Bootstrap values as a percentage of 1000 replications are shown at the branch. The sequence from *Methobacterium bryantii* DSM 863^T was used as out group. Bar represents 0.05 substitutions per site

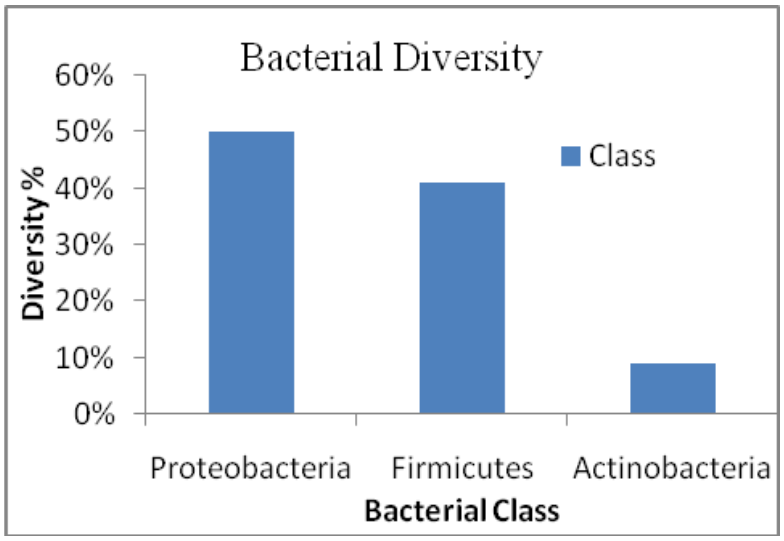


Fig 4.12: Total Bacterial Diversity in ridge samples

4.7 Cultivability of Mn-oxidizers, reducers and heterotrophs

All CFU formed on Mn-oxidizing media were brown and their average number was 5.47 x103 CFU g-1. However, none of the 379 CFU (3.18x103 CFU g-1) in Mn-reducing medium showed halos (Fig. 4.11). The average yield of heterotrophic bacteria was 7.92 x103 CFU g-1. The isolates from Mn-reducing plates were unable to reduce Mn-oxide but those from Mn-oxidizing plates were able to reduce Mn oxide. Mn oxidizing bacterial isolates R1, R2, R3, R4, R5, R6, R7, R8,R9, R10, NIB10, CoA 25 and NiC 25 constituted 2%, 2%, 7%, 10%, 12%, 8%, 6%, 8%, 10%, 11% and 6% respectively of the total CFU in Mn^{2+} amended media. Whereas, isolates NA1, NA2, NA3, NA4, NA5, NA6, NA7, NA8, NA9 NA10, NA11 and NA12 representing the heterotrophs

constituted 10%, 8%, 10%, 12%, 16%, 6%, 7%, 11%, 12%, 14% and 25% respectively of the total CFU in non-metal amended media. Bacterial colonies on 1% nutrient agar medium for heterotrophs were larger (3–5 mm) compared to colonies on 0.01% nutrient agar medium amended with Mn^{2+} and the other medium for reducers containing Mn-oxides (1–3 mm). The colonies were mostly circular, convex and opaque with smooth edges on the different types of media used (Appendix IV). Bacterial colonies developing on Mn (II) supplemented medium were however brown, suggesting that they promoted Mn-oxidation.

4.8 Culturability of Cu-oxidizers and reducers

All CFU formed on Cu-oxidizing media were brown pinkish and their average number was 1.00E+02 to 1.33E+04 CFU g^{-1}. However, none of the 67 CFU ($6.7x10^1$ CFU g^{-1}) in Cu-reducing medium showed halos Fig 4.12. The average yield of heterotrophic bacteria was 7.92 $x10^3$ CFU g^{-1}. The isolates from Cu-reducing plates were unable to reduce Cu-oxide but those from Cu-oxidizing plates were able to reduce Cu oxide. Cu oxidizing bacterial isolates CuR1, CuR2, CuR3 and CuR4 constituted 22%, 16%, 34% and 28% respectively of the total CFU in Cu^{2+} amended media. The colonies were mostly circular, convex and opaque with smooth edges on the different types of media used (for details please refer Appendix IV). Bacterial colonies developing on Cu (II) supplemented medium were however brown, suggesting that they promoted Cu-oxidation.

4.9 Microbiological experiments

4.9.1 Mobilization of manganese with microbial community

4.9.1.1 Mobilization

The results of mobilization rate of Mn is represented in Fig. 4.13. the mobilization rate was found to be maximum at the end of 150 d incubation. In the experiment, the rate of Mn mobilization in 'G+' was 1.76 mg g^{-1} d^{-1} and 0.17 mg g^{-1} d^{-1} in 'G' media The experimental rates in 'G+' were 13 times and in 'G-' 4 times more than the respective azide poisoned controls. Compared to heat killed controls, the respective experimental rates in 'G+' were 16 times and in 'G-, 24 times more.

4.9.1.2 Cell counts

The results of TC are presented in Fig. 4.14. An increase by more than an order of magnitude in the presence of added glucose and less than an order of magnitude in the absence of added glucose was observed. It ranged from 1.63 -10^6 to 6.71 10^7 cells g^{-1} in 'G+' and 3.52-10^7 cells g^{-1} to 1.41-10^7 cells g^{-1} in 'G-' incubations. The maximum cell counts in the presence of added glucose occurred on the 120 d of incubation (6.71 -10^7 cells g^{-1}) and in the absence of added glucose (1.41-10^7 cells g^{-1}) on the 30 d. In the azide poisoned control the counts ranged from 4.59-10^6 to 2.01-10^7 cells g^{-1} in the 'G+' and 4.27-10^6 cells g^{-1} to 2.61-10^7 cells g^{-1} in the 'G-'.

4.9.1.3 pH &Eh

The results of pH and Eh are given in Fig 4.15 and 4.16. Significant difference in pH and Eh was not apparent between the experiments and the corresponding controls. The pH decreased from 8.1 to 7.2 in the 'G+' and from 7.9 to 7.2 in the 'G-'. The Eh shifted from positive to negative redox potentials toward the end of incubation (150 d). It varied from 117 to -116 mV in the 'G+' and 137 to -118 mV in the 'G-' incubations.

4.9.1.4 SEM and EDS analysis

The results of SEM and EDS are presented in Fig. 4.17. The cells in medium with added glucose varied in morphology after incubation. The size of the cells increased from 0.5 ± 0.1 lm to 5.0 ± 1 lm. The shape of the cells varied from circular to oval on the initial day to long slender rods at the end of 150 d (Fig. 4.17 C and D). The cells attached to the rock surfaces produced EPS in 'G+' medium (Fig. 4.17 E and F). In contrast, in 'G-' medium, there was no EPS production, variation in cell size or change in morphology (4.20). Compositional analyses of basalt fragments after incubation showed the loss of Mn and K and gain of Na and Mg in the experimental tubes (Table 4.14). On the other hand, the basalt fragments from the controls appeared unchanged and seemed similar in texture and composition to the natural samples (Fig. 4.18). These observations strongly suggest the bacterial community's participation in the mobilization of Mn.

4.9.1.5 Experiment on the Mn (IV)-mobilizing activity of Mn(II)-oxidizing bacterial isolates

Demonstration of Mn(IV)-mobilizing activity with selected isolates of Mn(II)-oxidizing bacteria presented in Fig. 4.19 which shows isolate R2 to be positive for Mn-oxide mobilization/reduction. Mobilization was evident from the disappearance of the Mn-oxide.

4.9.1.6 Characterization and identification of bacterial isolates

Characterization and identification of bacterial isolates results are given in Table 4.13 and Fig 4.19. Of the 8 representative bacterial isolates identified, all were Gram-positive except isolate NA1. Tests for extracellular enzyme activity showed isolate R1 to be positive for 6/7 different enzymes that were screened. Positive enzyme test included those for catalase, oxidase, amylase, caseinase, lipase and cellulase. Isolates R2, R3, R4 and NA1 showed activity for 5 different enzymes. Tests for oxidative fermentative reaction showed isolate R1 as fermentative and the rest oxidative in the metabolism of glucose. Based on phylogenetic analysis, each of the above bacterial isolates could be assigned to one of three bacterial phyla: Firmicutes, Actinobacteria and Proteobacteria. Half of the isolates were identified as *Bacillus* sp., with the rest, one each belonged to the genus *Exiguobacterium, Staphylococcus, Brevibacterium* or *Alcanivorax sp.* A neighbor-joining phylogenetic tree obtained with Jukes-Cantor one-parameter model (Fig. 4.19) showed that strain NA1 forms a separate cluster with *Alcanivorax dieselolei* B-5T with

maximum sequence similarity of 99.7%. On the other hand, strains NA2 and NA3 showed 100% sequence similarity with *Bacillus flexus* IFO 15715T and *Staphylococcus sciuri* sub sp. *sciuri* DSM 20345T respectively. Isolate NA5 fell in 99.6% sequence similarity with *Brevibacterium epidermidis* NCDO 2286T. Isolates R1 and R4 resembled to *Bacillus firmus* NCIMB 9366T with sequence similarity of 93.6%. Isolates R2 and R3 formed clusters with *Exiguobacterium indicum* HHS31T and *Bacillus aryabhattai* B8W22T with maximum sequence similarity of 99.4% and 99.2% respectively.

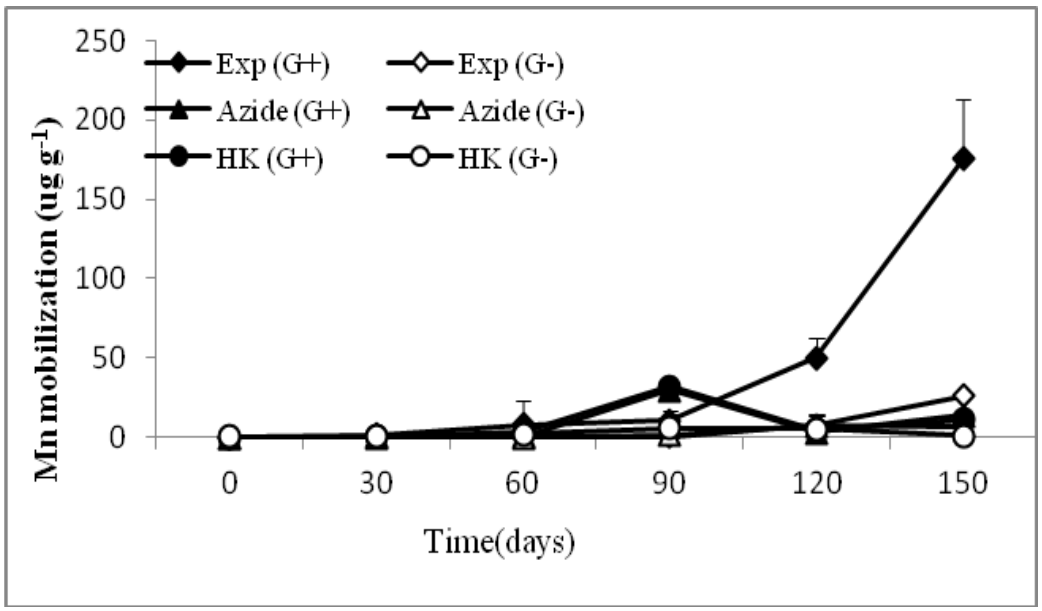


Fig. 4.13: Mobilization rate of Mn by Bacterial community. (E: experiment, Az: azide poisoned control, Hk: heat killed control, G+: with added glucose and G-: without added glucose) The values in the figure are mean values ± SD, n = 3

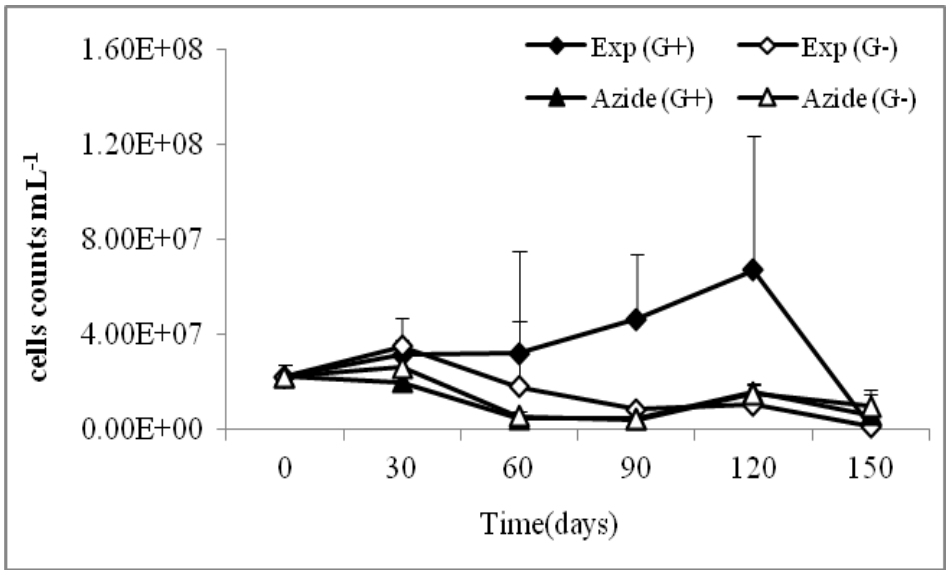


Fig. 4.14: Variation in cell numbers in relation to the mobilization of Mn (E: experiment, Az: azide poisoned control, Hk: heat killed control, G+: with added glucose and G-: without added glucose)

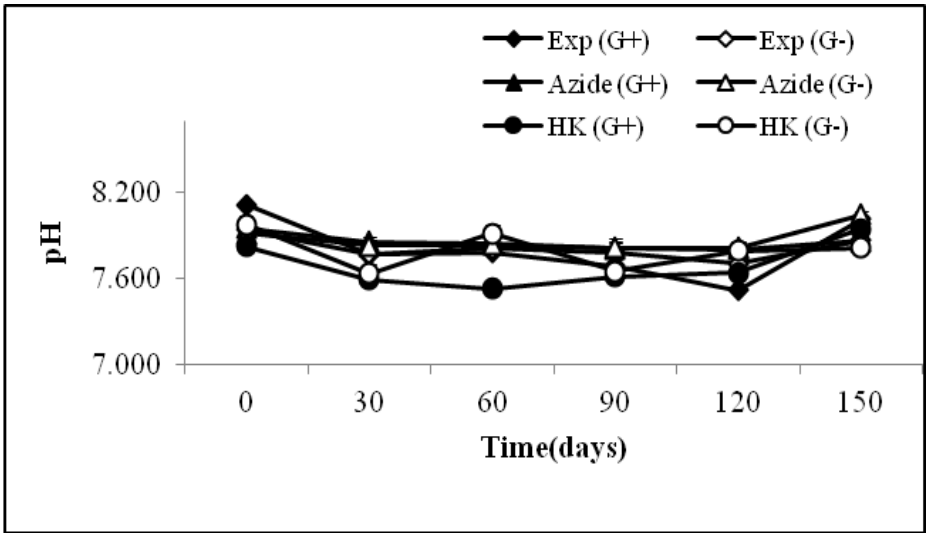


Fig 4.15: Variation in pH in relation to the mobilization of Mn (E: experiment and Az: azide poisoned control, G+: with added glucose and G-: without added glucose)

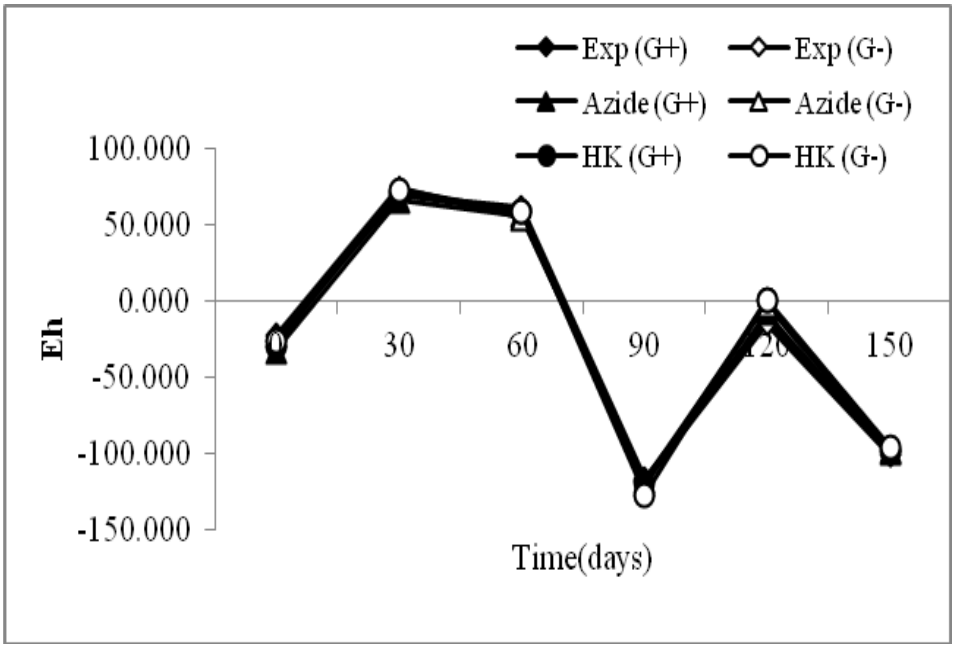


Fig 4.16: Variation in Eh in relation to the mobilization of Mn (E: experiment and Az: azide poison control :G+ with glucose and G-: without glucose

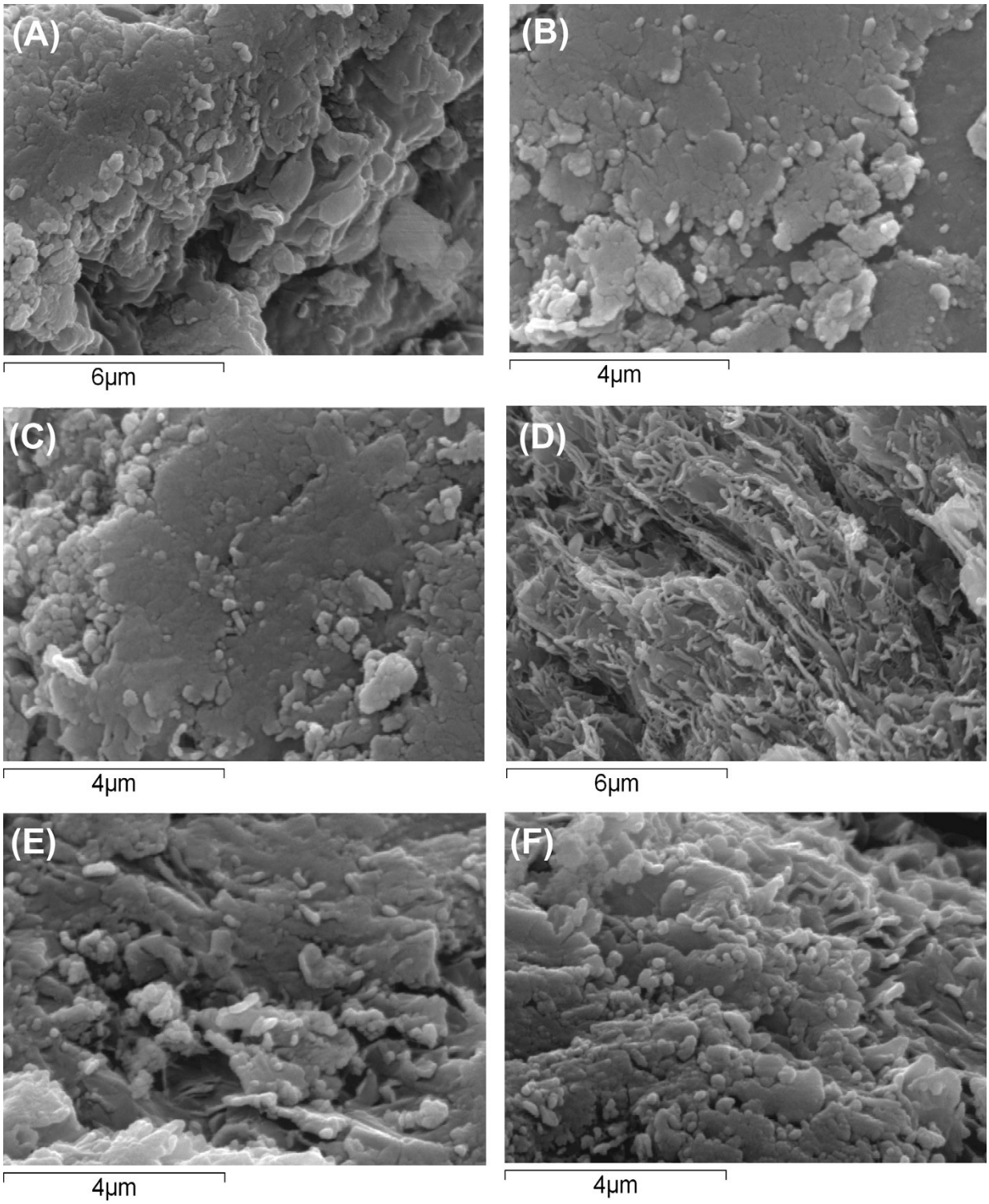


Fig 4.17: SEM images of bacterial cells associated with basalt. Image of before incubation (A) control and (B) experiment. After incubation (C) control (G-) (D) experiment (G-), (E) control (G+), (F) experiment (G+). (Legend G+: with added glucose and G-: without added glucose)

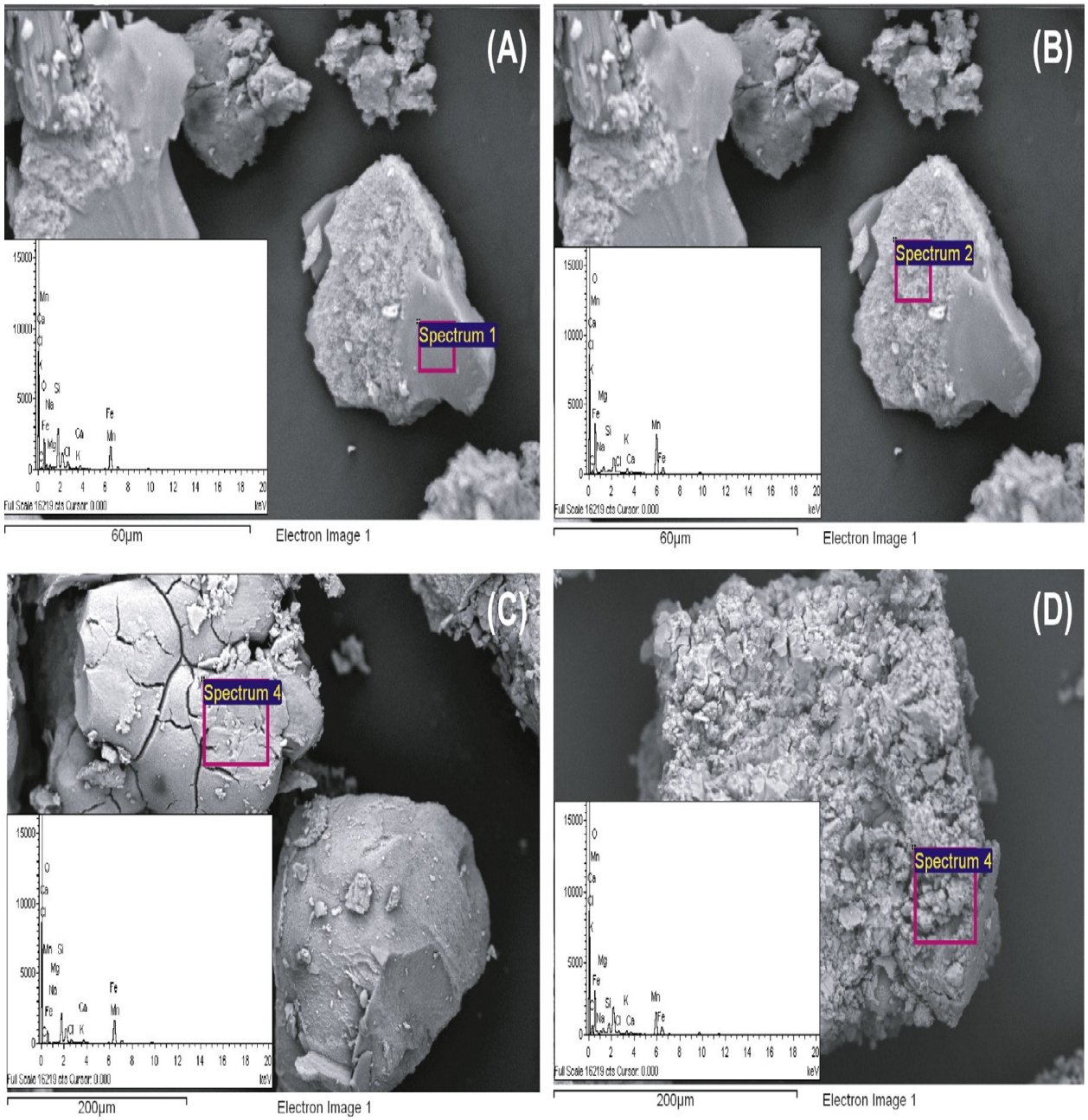


Fig 4.18: SEM images showing the surface texture and EDS spectra the chemical composition of basalt fragments before (A and B) and after incubation (C and D) for 150 d. The rough surface show higher concentration of Mn relative to smooth surface and tend to decrease with time on rough surface

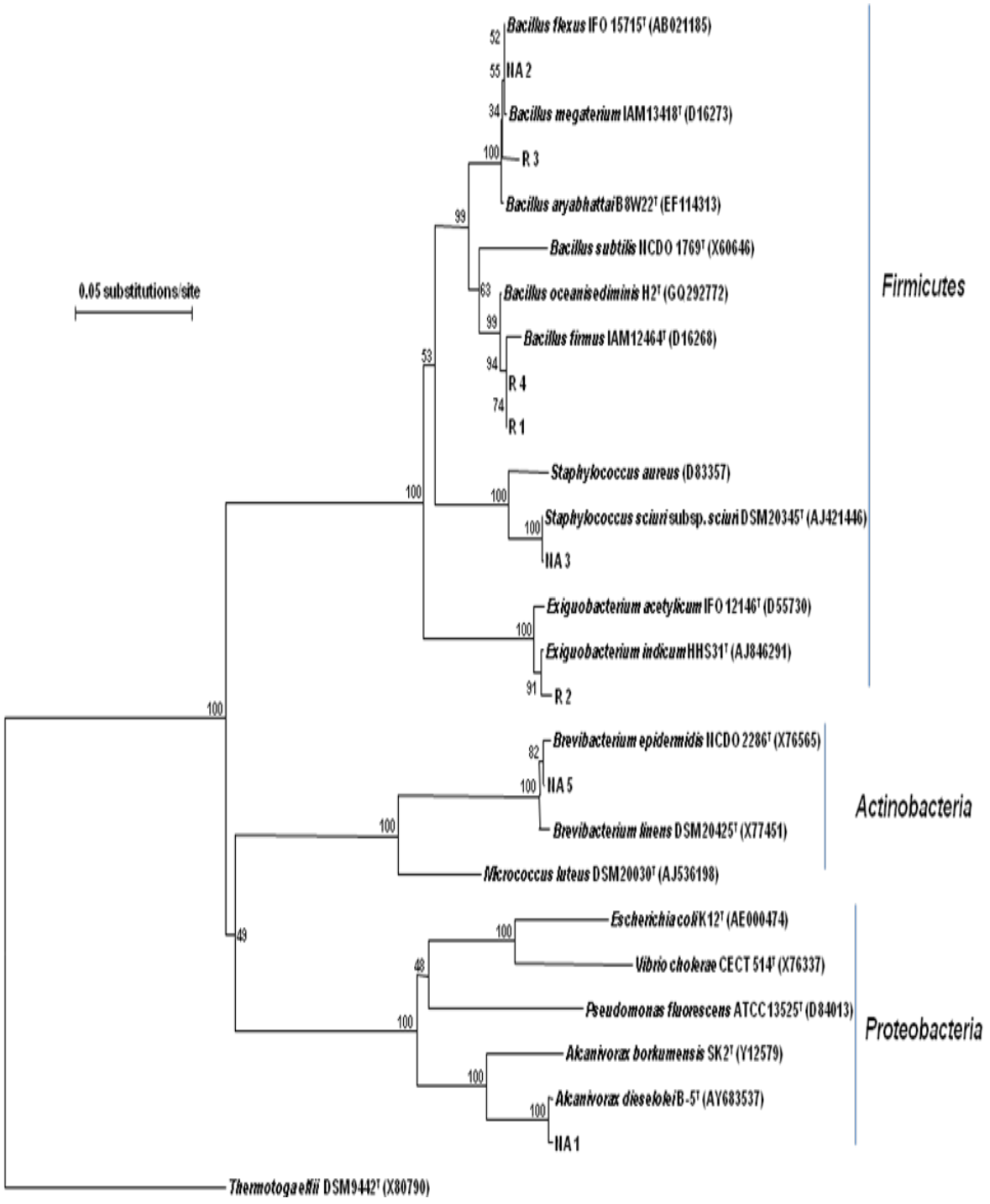


Fig 4.19A: Phylogenetic tree of bacterial isolates. The bootstrap values were calculated as percentage of 1000 replicates. The bar indicates 0.05 substitutions per site. The sequence from *Thermotoga elfii* was taken as out-group

Table 4.13: Phenotypic characteristics of culturable bacteria associated with the ridge rock and their phylogenetic identity by 16S rRNA analysis

Isolates	Colony colour	Gram ±	KOH	Motility	OF Test	Catalase	Oxidase	Amylase	Dnase	Protease	Lipase	Cellulase	16S Identity
R1	Yellow	+	NT	Motile	F	+	+	+	−	+	+	+	*Bacillus* sp.
R2	Brown	+	NT	Motile	O	+	+	+	−	+	+	−	*Exiguobacterium* Sp.
R3	Brown	+	NT	Motile	O	+	+	−	−	+	+	+	*Bacillus* sp.
R4	Brown	+	NT	Motile	O	+	−	+	−	+	+	+	*Bacillus* sp.
NA1	Cream	−	T	Non-motile	O	+	−	+	−	+	+	+	*Alcanivorax* sp.
NA2	Cream	+	NT	Non-motile	O	+	+	−	−	−	−	−	*Bacillus* sp.
NA3	Cream	+	NT	Motile	O	+	+	−	−	−	−	−	*Staphylococcus* Sp.
NA5	White	+	NT	Motile	O	+	+	−	−	−	−	−	*Brevibacterium* Sp.

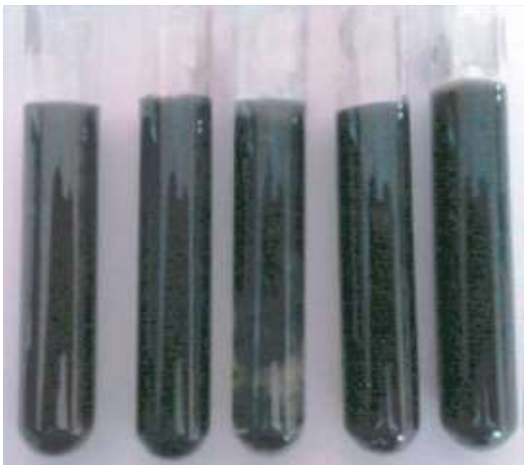


Fig 4.19 Reduction of synthetic Mn-oxide by Mn(II)-oxidizing bacterial isolates (C: control, R1, R3 and R4: negative for Mn reduction, R2: positive for Mn reduction).

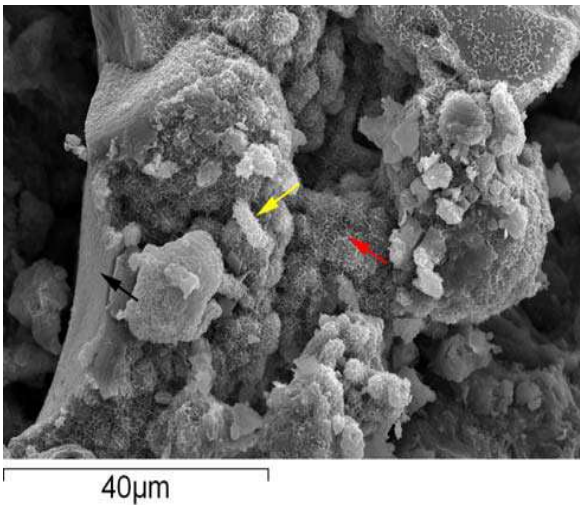


Fig 4.20 Exopolysaccharides associated with the bacterial cells on basalt surfaces. Arrow in yellow indicates the bacterial cell, red the exopolysaccharide and black the basalt.

Table 4.14: EDS point analysis of Mn oxide coatings on basalt

Element (Wt %) Random EDS points				
	Before incubation		After incubation	
	1	2	4	5
Na	5.86	4.52	7.45	6.23
Mg	2.44	4.48	2.75	3.54
Si	2.44	0.45	5.6	5.02
Cl	8.46	8.21	9.02	9.66
K	1.40	0.99	0.84	0.69
Ca	0.81	1.53	1.17	0.61
Mn	32.62	40.87	23.0	16.13
Fe	1.82	4.03	4.81	4.97
O	46.14	25.74	45.94	55.16
Total	100	100	100	100

4.9.2 Microcosm Investigation of Mn mobilization in basalt rock by Potential Bacteria R6 from Carlsberg ridge eco system

4.9.2.1 Microscopic and X-ray characterization

The results of texture and morphology changes in natural samples presented in Fig 4.21 A and B. Natural basalts mostly showed black coloured coatings with rough surface, numerous pits, fissures and fractures as physical characters. EDS spot analyses indicated heterogeneity in chemical composition with rough surfaces particularly high in Fe-Mn oxides.

The thickness of the coatings vary from 1-3.6 mm with greater thickness on irregular surfaces and vice versa on regular surfaces. The solid phase Mn concentration in the rock bearing Mn-oxide varied from 56.16 to 288.69 mg g^{-1}. Mineralogical investigations showed todorokite [(Na, Ca, Mn)$_2$ Mn_5O_{12}. $3H_2O$] as the major and birnesite ($Na_4Mn_{14}O_{27}.9H_2O$) as the minor Mn mineral. Halite (NaCl) was due to the background of seawater (Fig. 4.28 A and B).

4.9.2.2 Mobilization of Manganese

The results of mobilization rate of Mn is represented in Fig. 4.23. The mobilization rate of Mn was maximum at the end of 120 d incubation in 'G+' 27985.91 μg g^{-1} d^{-1} and 4797.37 μg g^{-1} d^{-1} in 'G-' media. The experimental rates in 'G+' were 43 times and in 'G-', 22 times more than the respective azide poisoned controls. Compared to heat killed controls, the respective experimental rates in 'G+' were 478 times and in 'G-', 27 times more.

4.9.2.3 Total counts

Total Counts bacterial growth during experiment are presented in Fig 4.24. Bacteria increased by more than an order of magnitude in the presence of added glucose and less than an order of magnitude in the absence of added glucose. It ranged from 2.13 × 10^6 to1.99 × 10^8 cells g^{-1} in 'G+' and 2.27 × 10^6 cells g^{-1} to 9.37 × 10^7 cells g^{-1} in 'G-' incubations. The maximum cell counts in the presence of added glucose occurred on the 60 d of incubation (1.99 × 10^8 cells g^{-1}) and in the absence of added glucose (9.37 × 10^7 cells g^{-1}) on the 60 d. In the azide poisoned control the counts ranged from 2.05 × 10^6 to 4.37 × 10^7 cells g^{-1} in the 'G+' and 2.92× 10^6 cells g^{-1} to 2.54 × 10^7 cells g^{-1} in the 'G-'.

4.9.2.4 pH and Eh Variation

The pH and Eh Variation are presented in Fig 4.27 and Fig 4.22. The difference in pH and Eh was not perceptible between the experiments and corresponding controls. The pH decreased from 7.85 to 6.21 in the 'G+' and from 8.24 to 8.01 in the 'G-'. The Eh shifted from negative to positive redox potentials at the end of incubation (60 d). It varied from -69.96 to 126.2 mV in the 'G+' and -68.63 to 106.88 mV in the 'G' incubations.

4.9.2.5 SEM and EDS analysis

Bacteria R6 morphology varied comparing to that of 0 day and 120th day in G+ media are presented in Fig. 4.27. The size of the cells increased from 0.55 ± 0.10 µm to 5.0 ± 1 µm. The shape of the cells varied from circular to oval on the initial day to long slender rods at the end of 120 d and produced EPS around cells which directly indicates

higher mobilization of metal in media (Fig. 4.27A and B). The cells in 'G+' medium directly attached/sitting to basalt surfaces on 0 day but on 120th day they produced EPS on rock surfaces (Fig. 4.27 C and D). In contrast, in 'G-' medium, there was no EPS production, variation in cell size or change in morphology when comparing 0 day with 120th day incubation. Compositional analyses of basalt fragments after incubation showed the loss of Mn and Cu and gain of Na and Mg in the experimental flasks (Table 4.15). At the same time, the basalt rock fragments from the controls appeared unchanged and seemed similar in texture and composition to the natural samples (Fig. 4.21). These observations strongly suggest the bacterial participation in the mobilization of Mn.

4.9.2.6 Characterization and identification of bacterial isolate

Bacterial isolated R6 characterization and identification presented in Fig. 4.29 and Table 4.16 where its appeared Gram positive rod shaped bacteria. They showed positive results for some of the extracellular enzymes such as catalase, oxidase, amylase, caseinase, lipase and cellulose. The strain also showed oxidative fermentation and motility. It was later identified as *Imtechella halotolerans* () with 92.6% similarity and the sequence uploaded (accession number: LK934699) to EMBL database.

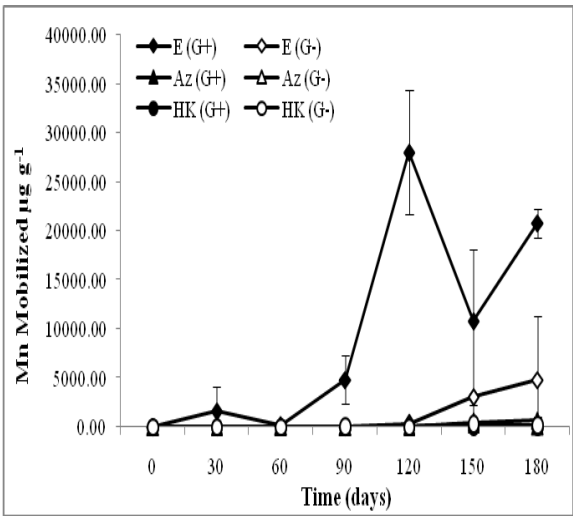


Fig. 4.23: Mobilization rate of Mn by Bacterial potential isolate R6. Numbers 1-2 in the secondary X-axis indicates heat killed controls, 3-4 azide poisoned controls and 5-6 experimental samples without and with added glucose respectively. The values in the figure are mean values ± SD, n = 3

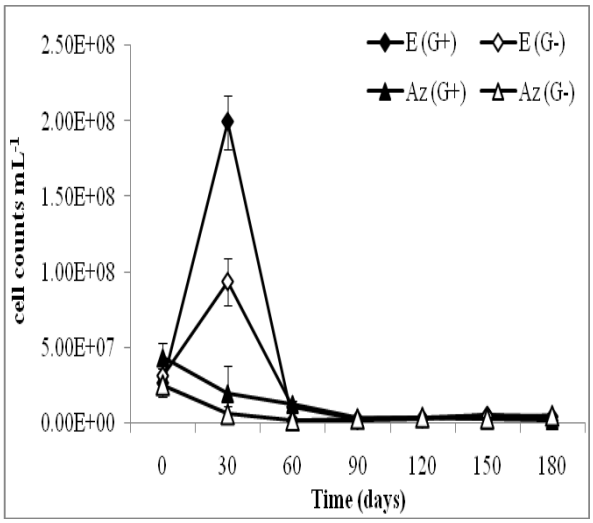


Fig. 4.24: Variation in cell numbers in relation to the mobilization of Mn (E: experiment, Az: azide poisoned control, Hk: heat killed control, G+: with added glucose and G-: without added glucose)

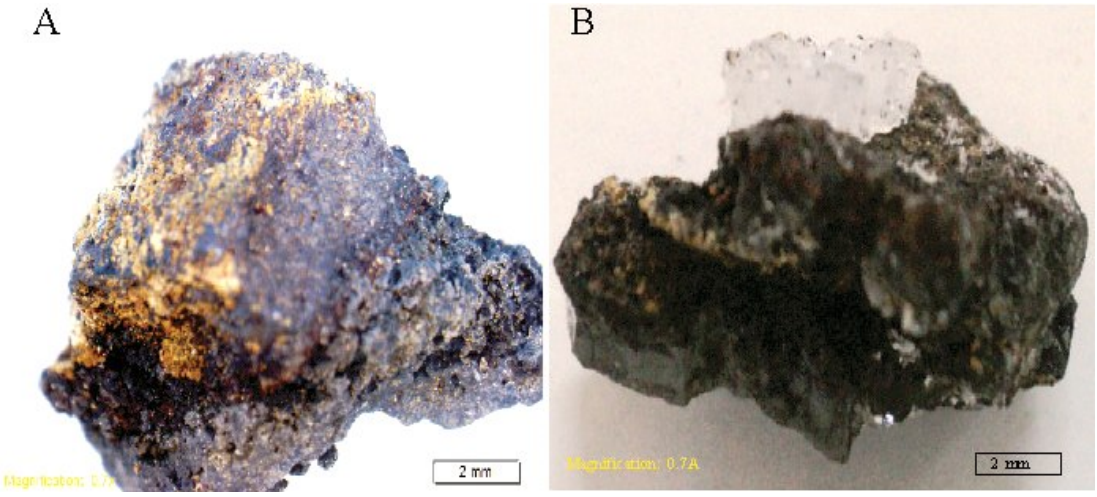


Fig. 4.21: Basalt rock sample A) Before incubation and B) After incubation

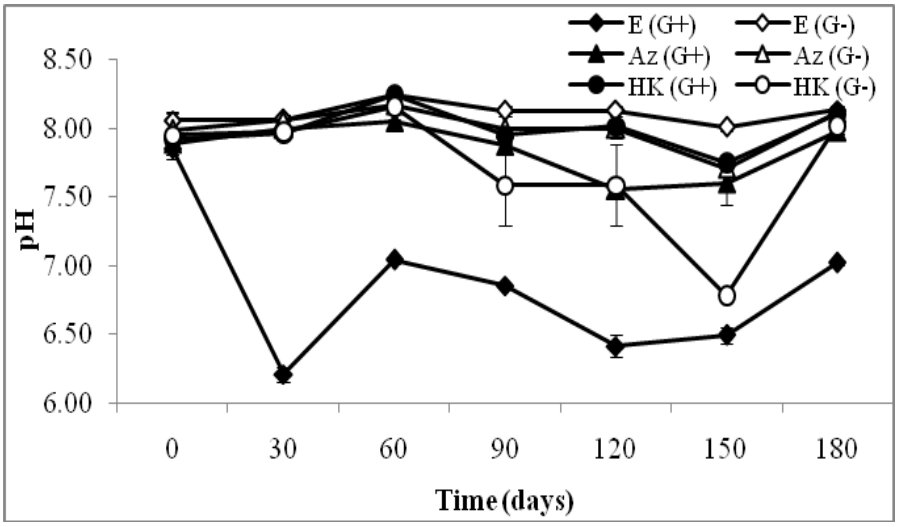


Fig 4.25: Variation in pH in relation to the mobilization of Mn (E: experiment and Az: azide poisoned control, G+: with added glucose and G-: without added glucose)

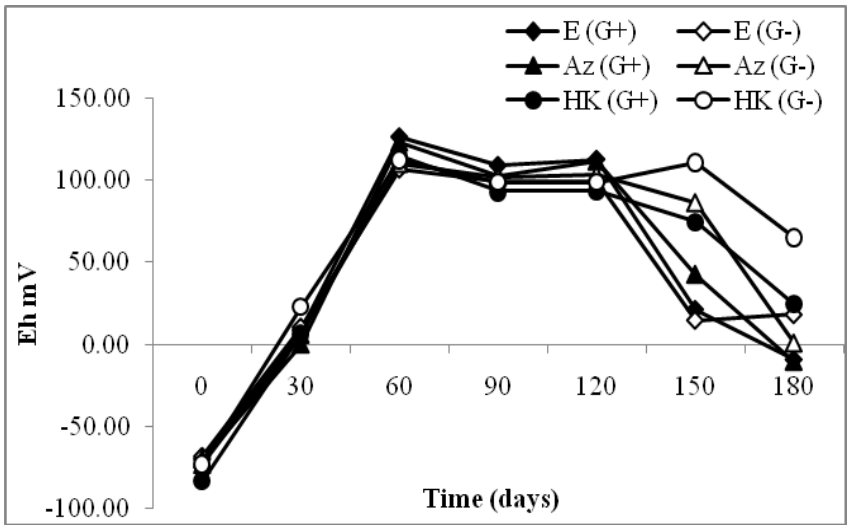


Fig 4.26: Variation in Eh in relation to the mobilization of Mn (E: experiment and Az: azide poisoned control, G+: with added glucose and G-: without added glucose)

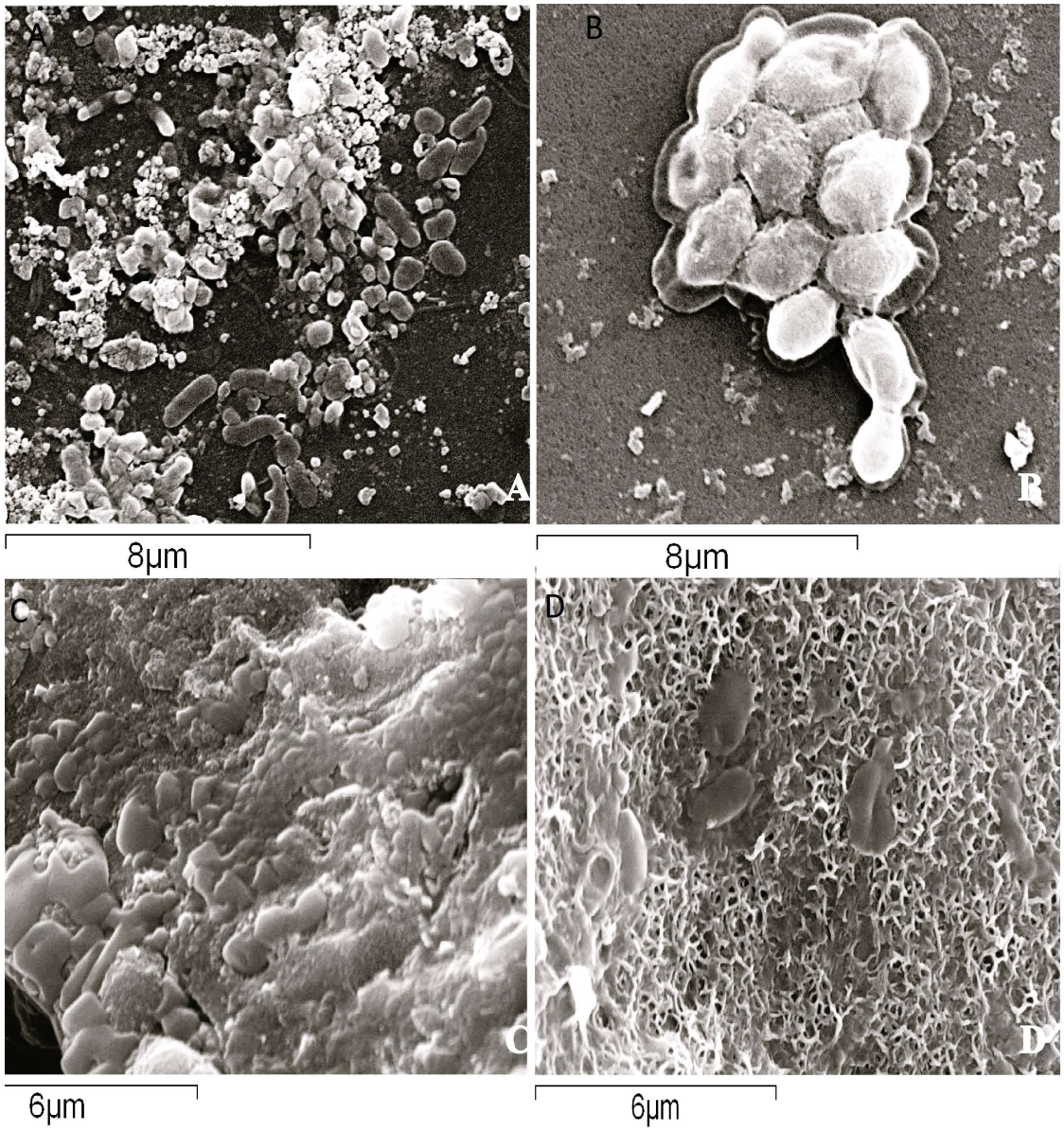


Fig. 4.27: SEM images of R6 bacterial cells in glucose amended medium and associated with basalt. Bacterial cells Image before incubation A) control and B) experiment. After Incubation C) control D) experiment

Table 4.15: EDS point analysis of Mn oxide coatings on basalt

Element (Wt %) Random EDS points

	Before incubation		After incubation	
Element	**1**	**2**	**3**	**4**
Na	1.41	2.25	3.13	3.74
Mg	1.31	2.35	2.19	4.93
Al	0.35	0.85	1.75	2.57
Si	3.81	3.33	11.69	8.14
K	0.61	0.95	1.33	0.42
Ca	0.98	1.01	1.18	2.06
Mn	17.78	28.83	14.96	16.44
Fe	12.41	4.42	14.13	10.82
Cu	0.87	1.78	0.77	0.44
Zn	0.64	1.30	0.70	0.39
Oxygen	62.55	52.93	48.44	50.06
Total	100	100	100	100

Table 4.16: Phenotypic characteristics of culturable bacteria R6 associated with basalt

Isolates	Gram ±	KOH	Motility	OF Test	Catalase	Oxidase	Amylase	Dnase	Protease	Lipase	Cellulase
R6	+	NT	Motile	F	+	+	+	-	+	+	+

Isolate numbers R6 represent Mn oxidizing bacteria. OF = oxidation fermentation, O= oxidative, F= fermentative. NT= no thread formation and T = thread formation in KOH test

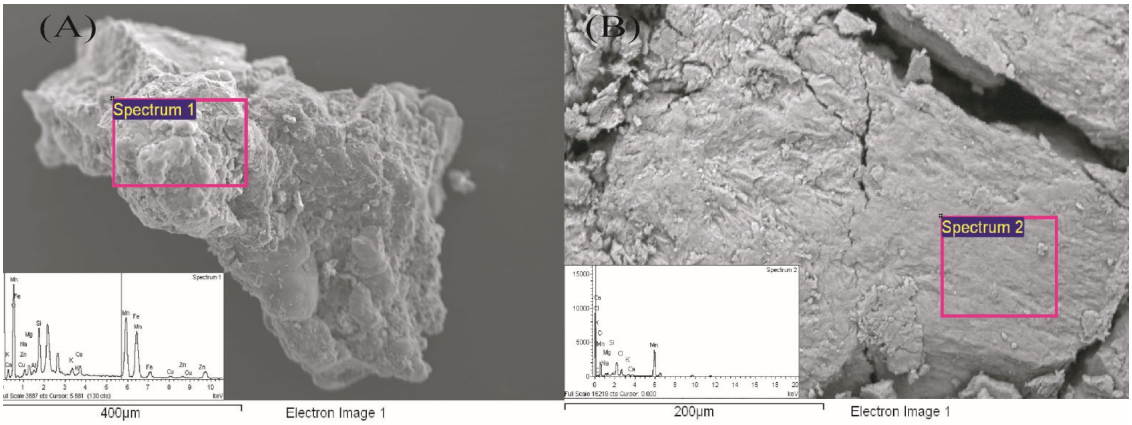


Fig. 4.22: SEM images show the surface texture and EDS spectra the chemical composition of basalt fragments before (A) and after incubation (B) for 150 d. The rough surface show higher concentration of Mn relative to smooth surface and tend to decrease with time on rough surface

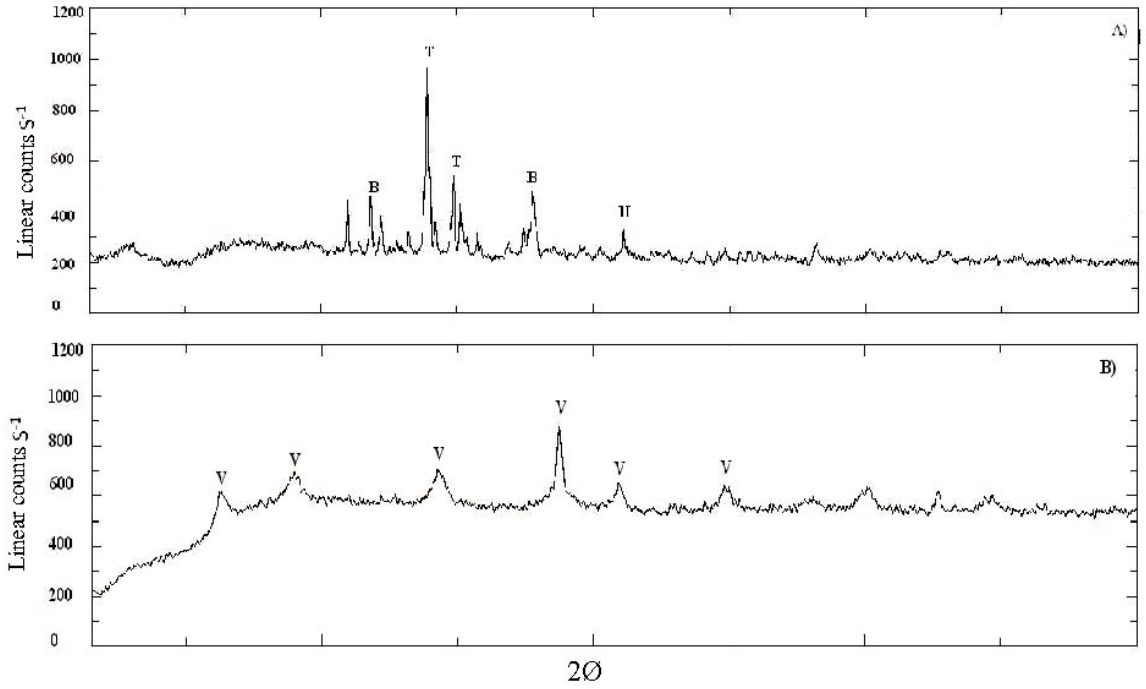


Fig. 4.28: X-ray diffraction analysis results. A) Mn-oxide coatings removed from basalt surface and B) chemically synthesized Mn oxide (Peak labels B: birnessite, H: halite, T: todorokite and V: Vernadite)

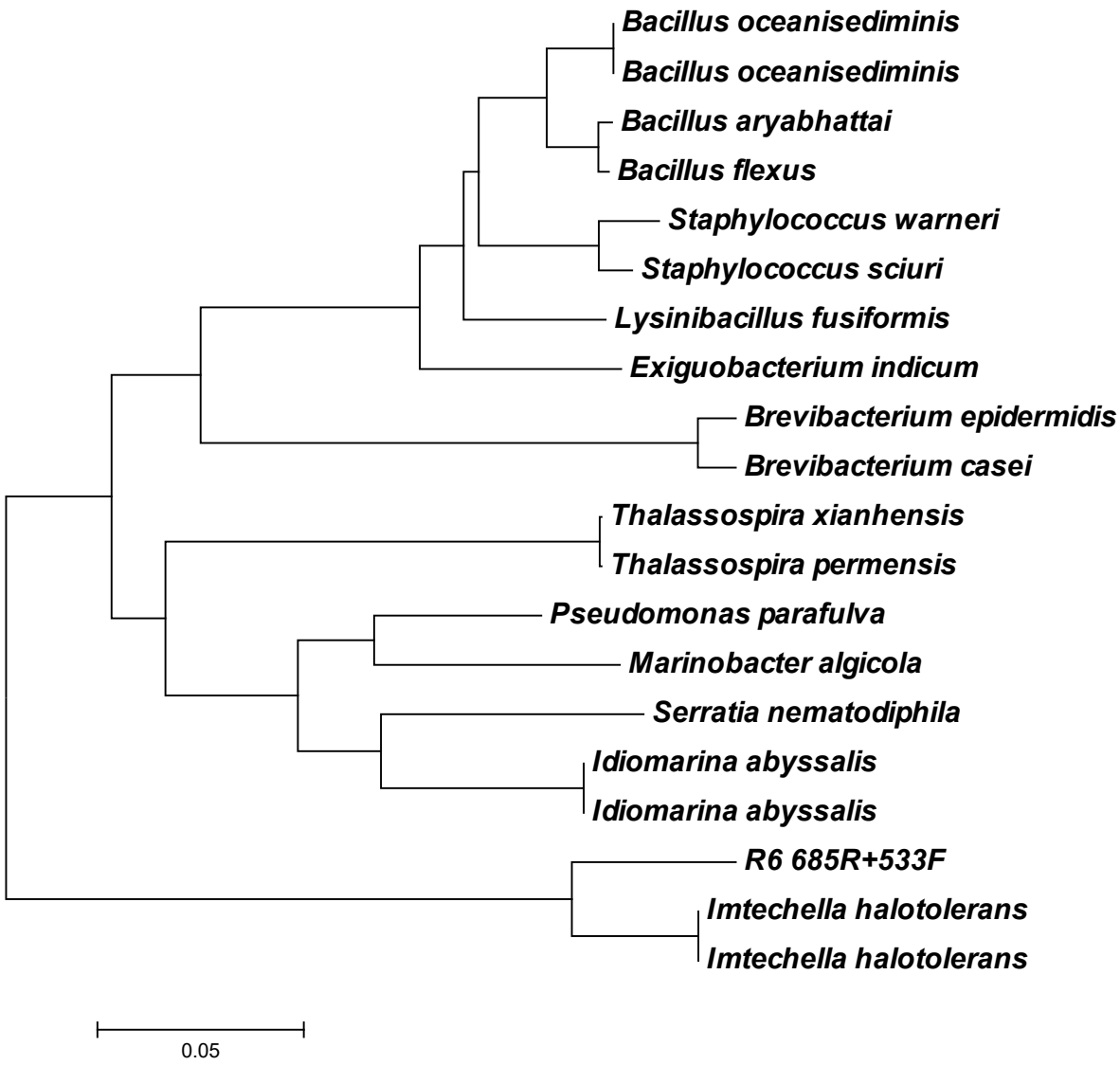


Fig. 4.29: Phylogenetic tree of bacterial isolate R6 and their closely related species

4.9.3 Immobilization of manganese with microbial community

4.9.3.1 Immobilization

The Immobilization rate of Mn is presented in Fig. 4.30. The observations shows maximum at the 0 day of incubation. In the experiment, the rate of Mn immobilization in 'G+' was 10007.63 µg g^{-1} d^{-1} and 306.61 ug g^{-1} d^{-1} in 'G-' media. The experimental rates in 'G+' were 2 times and in 'G-' negative than the respective azide poisoned controls. Compared to heat killed controls, the respective experimental rates in 'G+' were 1.5 times. The net biotic immobilization of Mn was maximum on 20th day of incubation. In G+ biotic, the rate of immobilization was 10.296µg $g^{-1}d^{-1}$ which was more than Az abiotic control and 2.663µg $g^{-1}d^{-1}$ more than HK abiotic control. In G- biotic, the values were 3.194µg g^{-1} d^{-1} more than Az abiotic control.

4.9.3.2 Cell counts

Total Counts bacterial growth during experiment are presented in Fig. 4.31. TC increased by more than an order of magnitude in the presence of added glucose and less than an order of magnitude in the absence of added glucose. It ranged from 1.63 -10^6 to 6.71 10^7 cells g^{-1} in 'G+' and 3.52-10^7 cells g^{-1} to 1.41-10^7 cells g^{-1} in 'G-' incubations. The maximum cell counts in the presence of added glucose occurred on the 120 d of incubation (6.71 -10^7 cells g^{-1}) and in the absence of added glucose (1.41-10^7 cells g^{-1}) on the 30 d. In the azide poisoned control the counts ranged from 4.59-10^6 to 2.01-10^7 cells g^{-1} in the 'G+' and 4.27-10^6 cells g^{-1} to 2.61-10^7 cells g^{-1} in the 'G-'.

4.9.3.4 pH &Eh

The pH and Eh Variation are presented in Fig. 4.32 and Fig. 4.33. The pH and Eh was not apparent between the experiments and the corresponding controls. The pH decreased from 8.1 to 7.2 in the 'G+' and from 7.9 to 7.2 in the 'G-'. The Eh shifted from positive to negative redox potentials toward the end of incubation (25 d). It varied from 117 to -116 mV in the 'G+' and 137 to -118 mV in the 'G-' incubations.

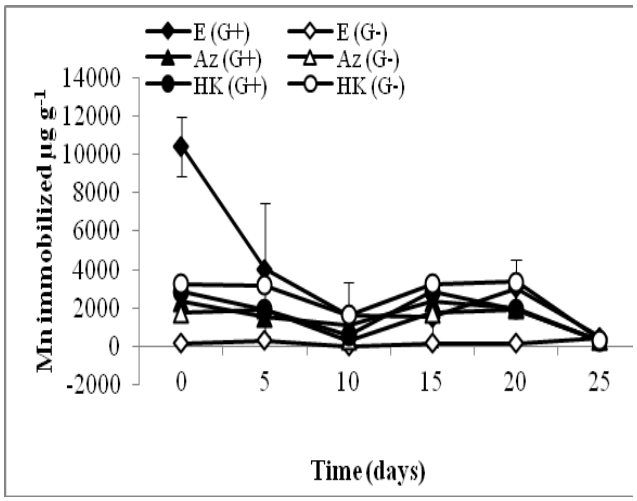


Fig. 4.30: Imobilization rate of Mn by Bacterial community. controls and experimental samples without and with added glucose. The values in the figure are mean values ± SD, n = 3

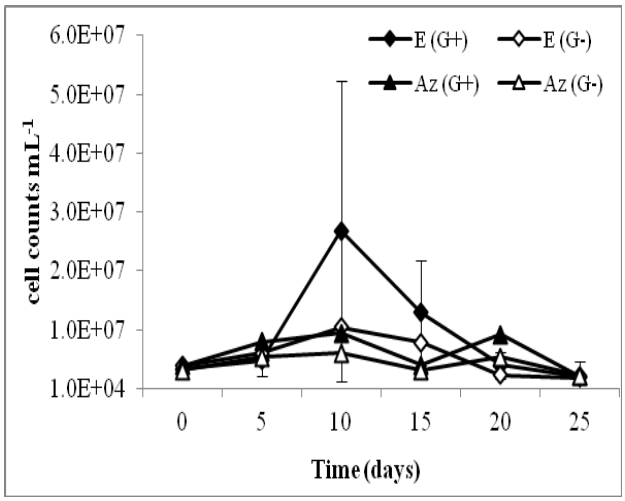


Fig. 4.31: Variation in cell numbers in relation to the immobilization of Mn (E: experiment, Az: azide poisoned control, Hk: heat killed control, G+: with added glucose and G-: without added glucose)

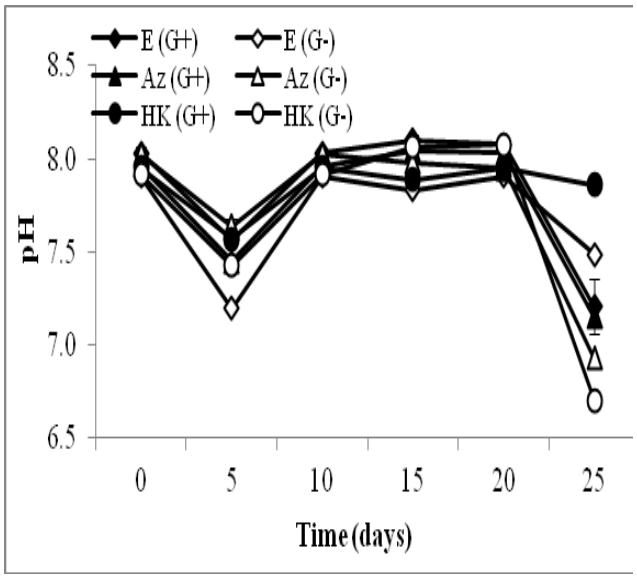


Fig 4.32: Variation in pH in relation to the immobilization of Mn (E: experiment and Az: azide poisoned control, G+: with added glucose and G-: without added glucose)

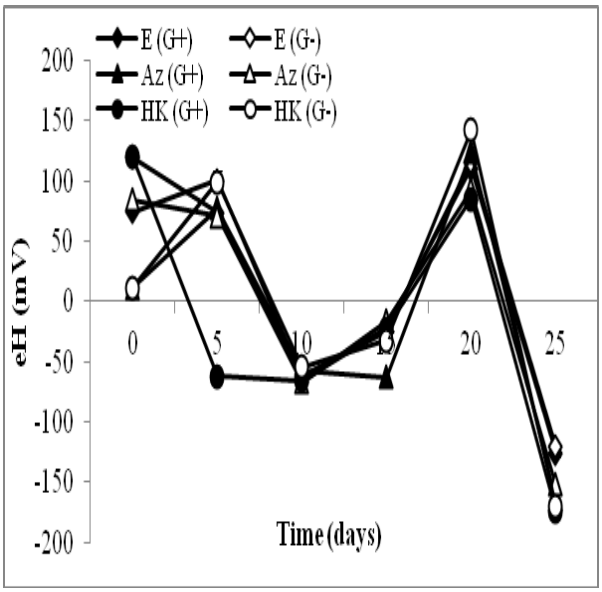


Fig 4.33: Variation in Eh (mV) in relation to the immobilization of Mn (E: experiment and Az: azide poisoned control, G+: with added glucose and G-: without added glucose)

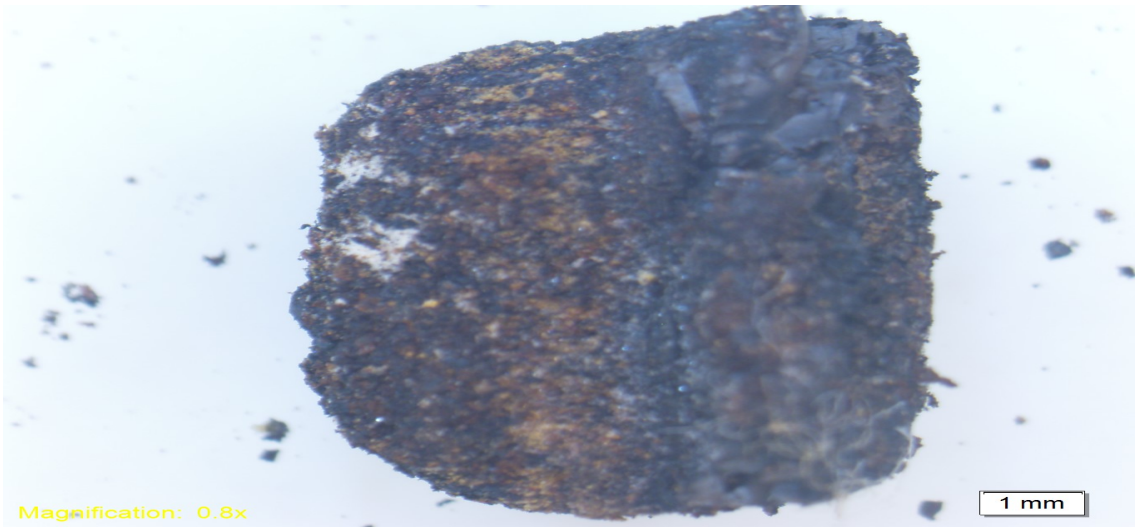


Fig. 4.34: Ridge rock surface collected from sampling site and used for present study work

4.9.4 Immobilization of Mn with potential isolate

4.9.4.1 *Microscopic and X-ray characterization*

Ridge rock samples mostly showed brownish black coloured coatings with rough surface, numerous pits, fissures and fractures as physical characters as given in section 4.9.1. EDS spot analyses indicated heterogeneity in chemical composition with rough surfaces particularly high in Fe-Mn oxides (Fig. 4.34 A and B). The thickness of the coatings vary from 1-3.6 mm with greater thickness on irregular surfaces and vice versa on regular surfaces. The solid phase Mn concentration in the rock bearing Mn-oxide varied from 56.16 to 288.69 mg g^{1}.

4.9.4.2 Immobilization

The Immobilization rate of Mn are presented in Fig. 4.35. The immobilization rate of Mn was maximum at the end of 150 d incubation in 'G+' 164.92 µg g^{-1} d^{-1} and 212.92 µg g^{-1} d^{-1} in 'G-' media. (Fig 4.35). The experimental rates in 'G+' were 2 times and in 'G-', 3 times more than the respective azide poisoned controls. Compared to heat killed controls, the respective experimental rates in 'G+' were 2 times and in 'G-', 3 times more.

4.9.4.3 Cell counts

Total Counts bacterial growth during experiment are presented in Fig. 4.36. TC increased by more than an order of magnitude in the presence of added glucose and less than an order of magnitude in the absence of added glucose. It ranged from 1.63×10^4 to 6.96×10^5 cells g^{-1} in 'G+' and 1.41×10^4 cells g^{-1} to 6.96×10^5 cells g^{-1} in 'G-'

incubations. The maximum cell counts in the presence of added glucose occurred on the 0 d of incubation (6.96×10^5 cells g^{-1}) and in the absence of added glucose (6.96×10^5 cells g^{-1}) on the 0 d. In the azide poisoned control the counts ranged from 6.91×10^4 to 6.96×10^5 cells g^{-1} in the 'G+' and 4.77×10^4 cells g^{-1} to 6.96×10^5 cells g^{-1} in the 'G-'.

4.9.4.4 pH &Eh

The pH and Eh Variation are presented in Fig. 4.37 and Fig. 4.38. Difference in pH and Eh was not perceptible between the experiments and the corresponding controls. The pH decreased from 7.62 to 5.56 in the 'G+' and from 7.91 to 5.14 in the 'G-'. The Eh shifted from negative to positive redox potentials at the end of incubation (60 d). It varied from -123.00 to 131.37 mV in the 'G+' and -120.18 to 106.88 mV in the 'G-' incubations.

4.9.4.5 SEM and EDS analysis

Bacteria CR 35 morphology varied comparing to that of 0 day and 120th day in G+ media are presented in Fig. 4.39. Comparing bacteria CR-35 on 0 day and 120th day in G+ media, where on 120th day the cells in medium with added glucose varied in morphology (Fig 4.39 A, B). The size of the cells increased from 0.25 ± 0.10 μm to 0.6 ± 1 μm. The shape of the cells varied from circular to oval on the initial day to long short rods at the end of 60 d and produces EPS around cells which directly indicates higher mobilization of metal in media (Fig. 4.39 C and D). The cells in 'G+' medium directly attached/sitting to basalt surfaces on 0 day but on 60th day they produced EPS on rock surfaces (Fig. 4.39 C and D). In contrast, in 'G-' medium, there was no EPS production,

variation in cell size or change in morphology when compared 0 day with 60th day incubation.

4.9.4.6 Characterization and identification of bacterial isolate

Bacterial isolate CR35 was found to be Gram negative short rod shaped. They showed positive results for some of the extracellular enzymes such as catalase, oxidase, amylase, caseinase, lipase and cellulose. The strain also showed oxidative fermentation and motility.

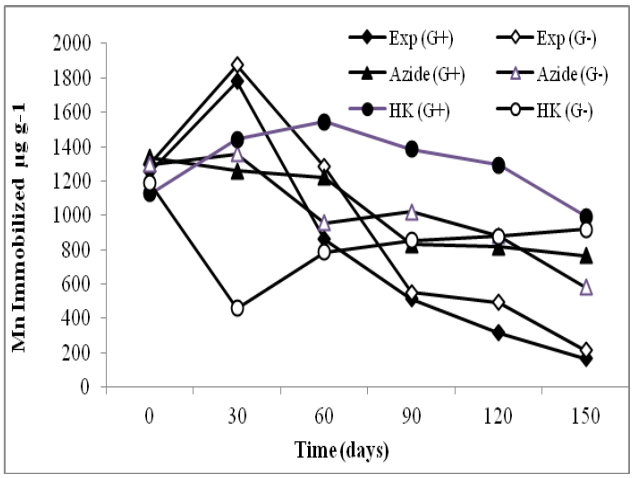


Fig. 4.35: Immobilization rate of Mn by Bacterial potential isolate CR-35. Numbers 1-2 in the secondary X-axis indicates heat killed controls, 3-4 azide poisoned controls and 5-6 experimental samples without and with added glucose respectively. The values in the figure are mean values ± SD,n = 3

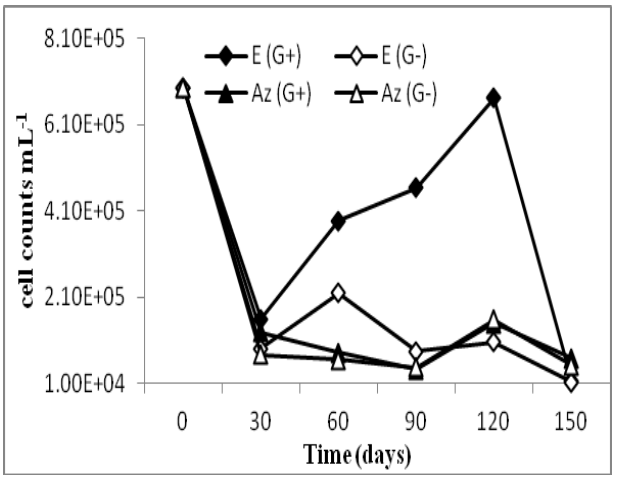


Fig. 4.36: Variation in cell numbers in relation to the immobilization of Mn (E: experiment, Az: azide poisoned control, Hk: heat killed control, G+: with added glucose and G-: without added glucose)

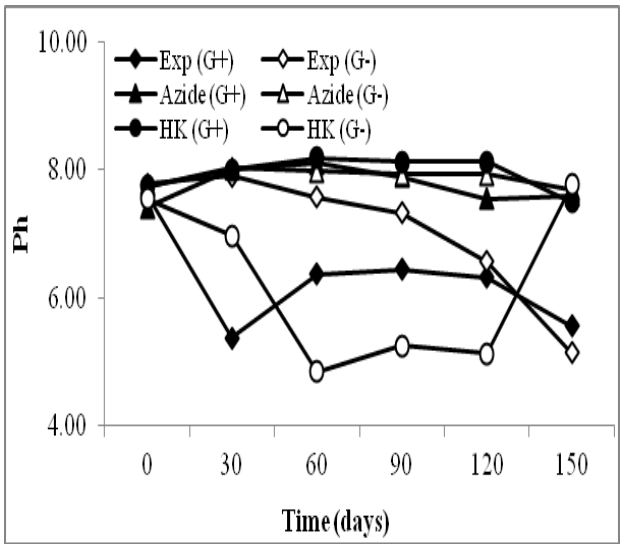


Fig. 4.37: Variation in pH in relation to the immobilization of Mn (E: experiment and Az: azide poisoned control, G+: with added glucose and G-: without added glucose)

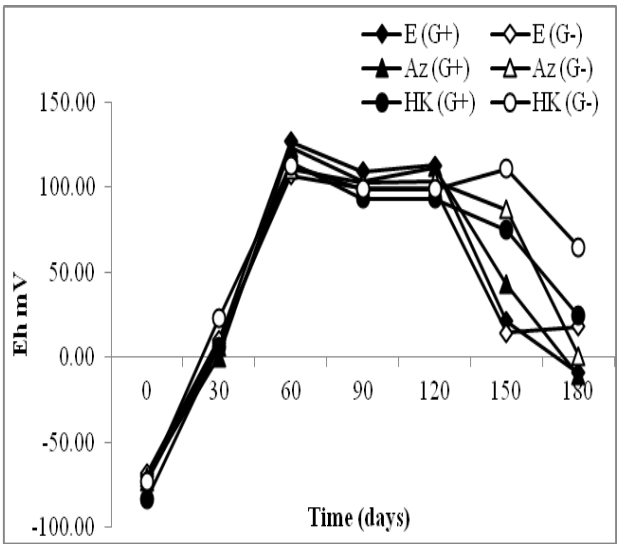


Fig. 4.38: Variation in Eh (mV) in relation to the immobilization of Mn (E: experiment and Az: azide poisoned control, G+: with added glucose and G-: without added glucose)

Table 4.17: Phenotypic characteristics of culturable bacteria CR-35 associated with basalt

Isolates	Gram ±	KOH	Motility	OF Test	Catalase	Oxidase	Amylase	Dnase	Protease	Lipase	Cellulase
CR-35	-	T	Motile	F	+	+	+	-	+	-	+

Isolate numbers CR-35 represent Mn oxidizing bacteria. OF = oxidation fermentation, O= oxidative, F= fermentative. NT= no thread formation and T = thread formation in KOH test.

4.9.5 Copper mobilization by basalt associated Cu (II) - oxidizing bacteria from the Carlsberg Ridge ecosystem

4.9.5.1 Microscopic and solid phase metal analysis

Morphologically, these rocks contains Mn and Fe oxides and their minerals those appeared as black and brownish colored coatings with rough surface containing abundant of pits, fissures and fractures. Whereas, EDS presented chemical heterogeneity with specifically in Fe-Mn oxides on its rough surfaces (Fig. 4.34). The Cu concentration present in the ridge rock basalt varied from 3.36 to 11.69 mg g^{-1} in solid phase.

4.9.5.2 Culturability of Cu as oxidizers, reducers and heterotrophs

CFU appeared on Cu-oxidizing media were brown in colour with average number calculated was 2 $\times 10^2$ CFU g^{-1}. Whereas, CFU (3.2$\times$ 10^2 CFU g^{-1}) in Cu-reducing medium showed no halos. Heterotrophic bacteria showed an average yield of 7.92 $\times$ 10^3 CFU g^{-1}. Cu-oxidizing isolates were observed to reduce Cu oxide in contrast to isolates from Cu-reducing plates. Cu-oxidizing bacterial isolates CuR1, CuR2, CuR3 and CuR4 constituted 20, 14, 23 and 43% respectively of the total CFU in Cu^{2+} amended media.

Whereas, isolates NA6, NA7, NA9 and NA10 representing the heterotrophs constituted 33, 28, 17 and 22% respectively of the total CFU in non-metal amended media. Bacterial colonies grown in 1% nutrient agar medium for heterotrophs were larger (3-5 mm) compared to colonies grown in 0.01% nutrient agar medium amended with Cu^{2+} and the other medium for reducers containing Cu-oxides (1-3 mm). The colonies were mostly circular, convex and opaque with smooth edges on the different types of media used. Bacterial colonies appeared on Cu (II) supplemented medium were however brown, suggesting that they promoted Cu-oxidation.

4.9.5.3 Copper mobilization

The results of mobilization rate of Cu is represented in Fig. 4.43. Maximum mobilization rate of Cu was observed at the end of 150 d incubation. During experiment, the Cu mobilization rate in 'G+' was 980.2 μg g^{-1} d^{-1} and 430.1 μg g^{-1} d^{-1} in 'G-' media. The rate of experimental in 'G+' was observed 6 times more as comparison with 'G-' and 2 times more than the controls (azide poisoned). On Comparison with controls (heat killed), the respective rate of experimental rates is 9 times and 4 times more in 'G+' and 'G-', 4, respectively.

4.9.5.4 Study on Bacterial growth

The results of TC are presented in Fig. 4.44. Bacterial total count observed to be increased in the presence of as its order of magnitude with added glucose as compared to the order of magnitude in the absence of added glucose. It showed variation from 1.64 × 10^6 to 3.88 × 10^7 cells g^{-1} in 'G+' and 1.40 × 10^6 cells g^{-1} to 1.88 × 10^7 cells g^{-1} in 'G-'

incubations. The cell counts was maximum in the presence of added glucose observed was 3.88×10^7 cells g-1 on the 90 d of incubation and 1.88×10^7 cells g^{-1} in the absence of added glucose on the 0 d. Whereas control (azide poisoned) the counts varied from 1.44×10^6 to 2.12×10^7 cells g^{-1} in the 'G+' and 4.60×10^6 cells g^{-1} to 2.32×10^7 cells g^{-1} in the 'G-'.

4.9.5.5 pH and Eh alterations

The results of pH and Eh are given in Fig. 4.45 and 4.46. pH and Eh values does not showed any apparent difference between the experiments and the corresponding controls. The pH value was observed to be decreased from 8.13 to 7.12 and 7.92 to 7.60 in the 'G+' and 'G', respectively. The Eh observed to be shifted from positive to negative redox potentials at 150 d (the final stage of incubation) and showed variation from 69 to -115 mV and 68 to -120 mV in the 'G+' and 'G-', respectively.

4.9.5.6 SEM and EDS analysis

SEM investigation showed minerals and oxides textural and chemical composition on natural basalt samples and compared with the control experimentally incubated basalt trashes revealed abundant of microbial cells attached to the surface (Fig. 4.47 A and B). The microbial cells in presence and absence of added glucose showed variation in size and morphology of cells after incubation. The cells size was observed to be increased from 0.5 ± 0.1 to 3.0 ± 1 μm in absence of added glucose while in presence of added glucose the cell size varied from 0.5 ± 0.1 to 6.0 ± 1 μm. The cell shape showed changes from the initial to final day of incubation were circular to oval and cylindrical. On basalt,

bacteria produced more EPS in glucose added medium than medium without added glucose (Fig. 4.47 C, D, E and F). EDX analyses performed for basalt sample before and after incubation in medium showed peaks for Copper, Manganese, Iron, Calcium, Sodium, Potassium and Oxygen observed approximately similar element (wt%) but in case of Cu Element (Wt%) reduced significantly. This result confirms Cu leaching in medium by bacterial activities as shown in Fig. 4.48 and Table 4.19.

4.9.5.7 Cu Sequestration: Partial experiment

Of 8 bacterial isolates 4 were chosen CuR1, CuR2, CuR3 and CuR4 for metal sequestration and tolerance experiment. The Cu sequestration by Cu oxidizing bacteria CuR1 was observed under SEM to investigate morphological changes and Cu accumulation inside cells in Cu exposed and unexposed condition. Cells grown in seawater without any nutrient or Cu amendment did not show any morphological variation after incubation for 7 days (Fig. 4.49 A and B). The average cell size (l×w) of CuR1 was from 1.42 ±0.2 to 1.10±0.10 μm. Bacterial cells were grown in 100 μM Cu showed healthy dividing cells (Fig. 4.49C), but average cell size increases to 2.02±0.38 to 3.58±0.10 μm. At 1000 μM Cu concentration, increase in cell size without morphological changes and subsequent rupture was observed (Fig. 4.49D). At the same time at 10,000 μM Cu Cells increases in size, changes their morphology and ruptured due to very high metal sequestration and cells size of CuR1 showed variation range from 1.37±0.42 to

3.02±0.24 μm in presence and absence of glucose (Fig. 4.49 E and F). EDS analyses performed for cells grown without Cu in medium showed peaks for calcium, Sodium, potassium and oxygen. Cu (1000 μM) exposed bacterial cells showed peaks for Cu with decrease in intensity of the peak for Oxygen and loss of K peak that indicates cation efflux in CuR1. It confirms that bacterial cells have sequestered the metal Table 4.19.

4.9.5.8 Cu (III) mobilizing activity of Cu (II) oxidizing bacterial isolates an experimental approach

Experiments were carried out to understand the Cu (III)-mobilizing activity of selected Cu (II)-oxidizing isolates. Bacterial isolates CuR2 and CuR3 are found positive for Cu-oxide mobilization/reduction. During the experiment, halo appeared and oxide disappearance surrounding to bacterial colony CuR2 and CuR3 which proves the reduction of Cu oxide by bacterial cell. At the same time CuR1 and CuR4 bacterial colonies grew well but no halo zone formation occurred which proves their oxidative nature.

4.9.5.9 Characterization and identification of bacterial isolates

Eight representative bacterial isolate, CuR1, CuR3, NA9 and NA10 which showed Gram-positive character and others observed as gram negative bacterial isolates were identified by 16S rRNA sequencing method. CuR2, NA9 and NA10 observed as oxidative and remaining showed fermentative reaction showed isolate CuR1 as fermentative nature in the metabolism of glucose (Table 4.18). In screening of

extracellular enzyme activity all isolates found positive for oxidase and amylase, except CuR2 other were observed to be positive for DNase (Table 4.18).

Phylogenetic analysis reports that above bacterial isolates could be assigned to one of three bacterial phyla: *Firmicutes*, *Actinobacteria* and *Proteobacteria*. CuR1 isolate was identified as *Bacillus* sp., while one each belonged to the genus *Idiomarina, marinabactor, Halomonas, Sinomicrobium and Erwinia.* Jukes-Cantor one-parameter model was used for construction of neighbor-joining phylogenetic tree (Fig. 4.50) and it is reported that strain NA6 and NA7 are similar bacterial isolate *Idiomarina abyssalis* with slight difference of similarity of 99.61 and 99.10% respectively. On the other hand, strains NA9 and NA10 were found as *Marinabactor algicola* with similarity of 99.08 and 99.03%. CuR1 showed 100 % similarity with *Bacillus aryabhattai* and CuR2 belongs to *Halomonas rifiensis* with similarity of 99.61%. Isolates CuR3 and CuR4 formed clusters with *Sinomicrobium oceani* and *Erwinia rhapontici* with similarity of 99.48 and 99.32% respectively. (Table 4.20).

Table 4.18: Phenotypic characterization of identified bacteria isolated from basalt

Isolates	Gram	KOH	Motility	OF Test	Catalase	Oxidase	Amylase	Dnase	Protease	Lipase	Cellulase
CuR 1	+	NT	+	F	-	+	+	+	-	+	+
CuR 2	-	T	+	O	+	+	+	-	-	-	+
CuR 3	+	NT	-	F	-	+	+	+	+	+	+
CuR 4	-	T	+	F	+	+	+	+	-	-	+
NA 6	-	NT	-	F	-	+	+	+	+	+	+

NA 7	-	NT	-	F	-	+	+	+	+	+	+
NA 9	+	NT	+	O	-	+	+	+	+	+	-
NA10	+	NT	+	O	-	+	+	+	+	+	-

Cu oxidizing bacteria represents by isolates. OF = oxidation fermentation, O= oxidative, F= fermentative. NT= no thread formation and T = thread formation in KOH test

Table 4.19: EDS point analysis in bacterial cells amended with Cu. Element (Wt %) Random EDS points

	Before incubation		**After incubation**	
Element	**1**	**2**	**3**	**4**
Na	2.51	2.21	2.13	3.64
K	0.64	0.88	1.33	0.33
Ca	0.88	1.02	0.14	2.00
Cu	06.08	08.69	38.96	44.43
Oxygen	90.00	87.20	57.44	50.06
Total	100	100	100	100

Table 4.20: 16S rRNA identified culturable bacteria with their accession numbers given by European molecular biology laboratory (EMBL) associated with basalt

Isolate	**Closest match**	**Similarity**	**Accession Number**
CuR 1	*Bacillus aryabhattai* B8W22(T), (EF114313)	100.0%	LK934690

CuR 2	*Halomonas rifensis* HK31(T)(HM026177)	99.61%	LK934691
CuR 3	*Sinomicrobium oceani* SCSIO 03483(T)(JQ352762)	99.48%	LK934692
CuR 4	*Erwinia rhapontici* ATCC 29283(T)(U80206)	99.32%	LK934693
NA 6	*Idiomarina abyssalis* 227(T), KMM (AF052740)	99.61%	LK934704
NA 7	*Idiomarina abyssalis* 227(T), KMM (AF052740)	99.10%	LK934705
NA 9	*Marinobacter algicola* DG893(T), (ABCP01000031)	99.08%	LK934706
NA10	*Marinobacter algicola* DG893(T), (ABCP01000031)	99.03%	LK934707

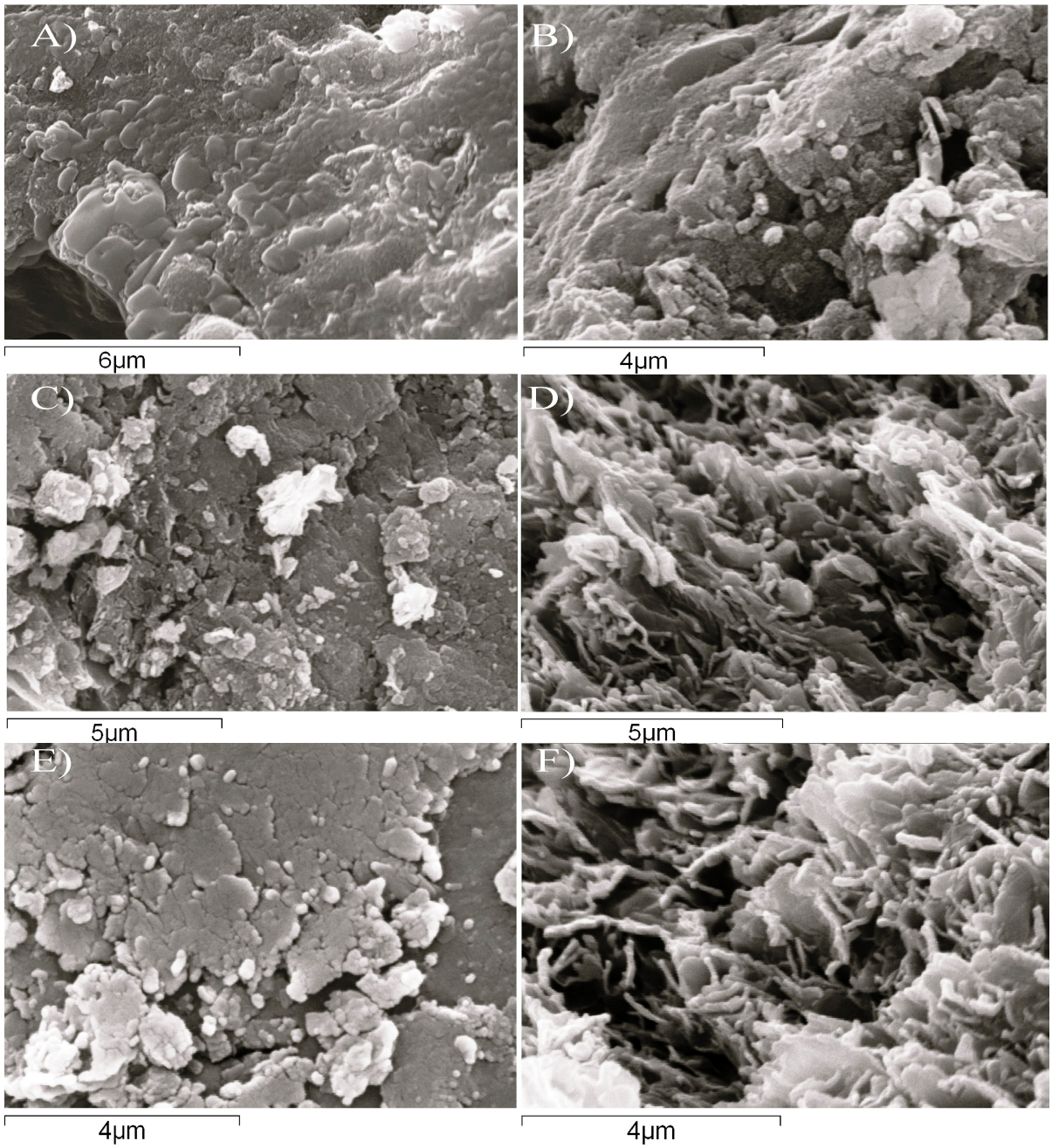


Fig 4.43: SEM images of indigenous Cu oxidiser bacterial community present in basalt. Image 0 day A) control and B) experiment. SEM end day C) control (G-) D) experiment (G-), E) control (G+), F) experiment (G+). (Legend G+: with glucose and G- : without glucose)

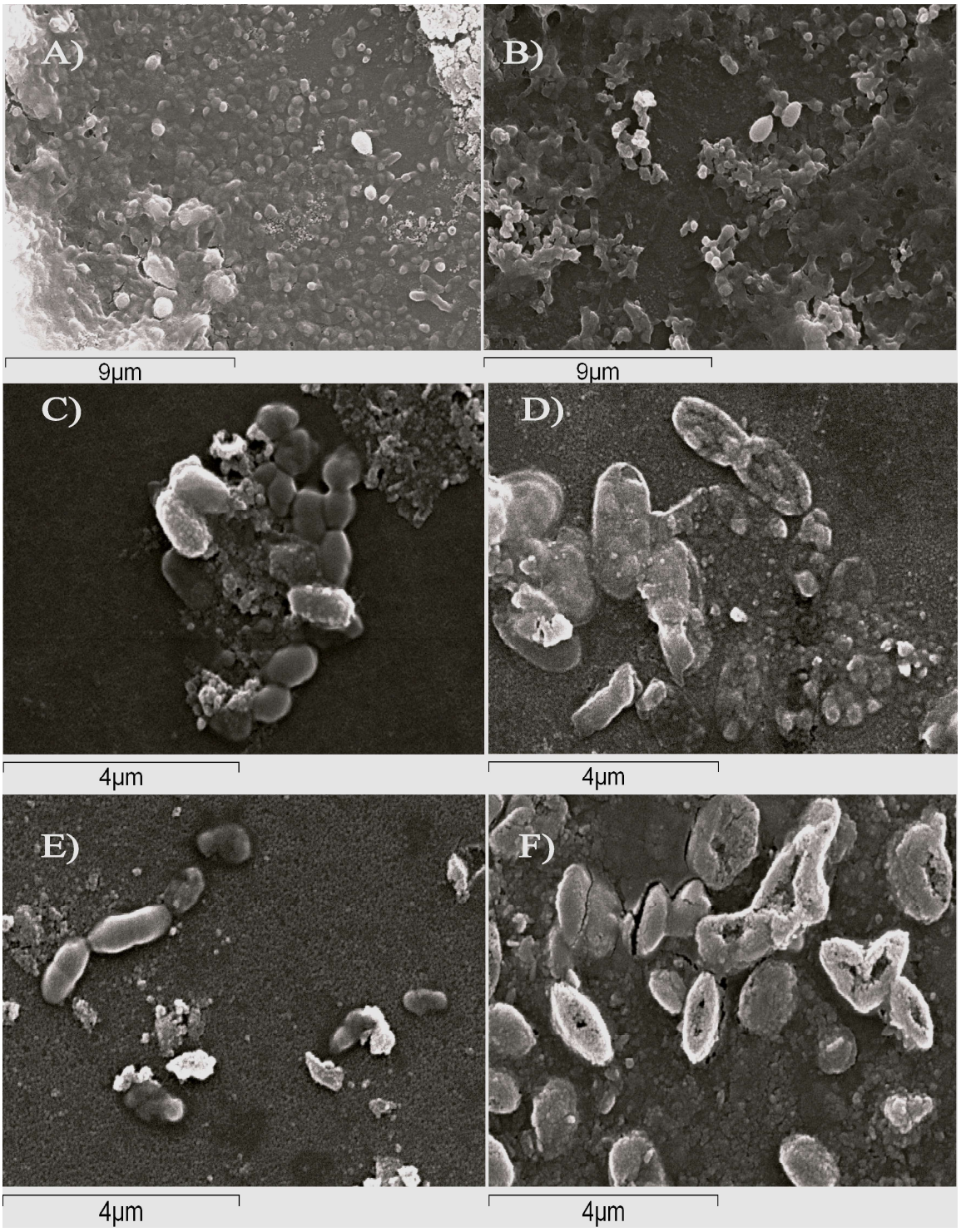


Fig 4.45: SEM images shows morphological alteration in CuR1 bacterial cell grown in seawater medium. A) with no Cu and glucose B) 0.01% glucose C) 1000 μM $CuCl_2$ and 0.01% glucose D) 1000 μM added $CuCl_2$ E) 10000 μM $CuCl_2$ and 0.01% glucose F) 10000 μM added $CuCl_2$

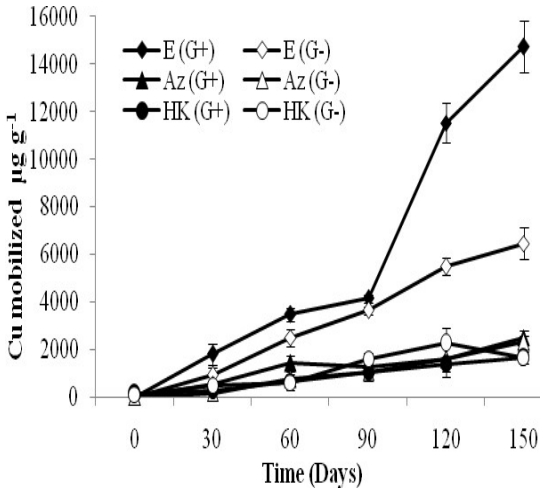


Fig 4.39: Biotic mobilization rate of Cu from ridge rock. (E: experiment, Az: azide poisoned control, Hk: heat killed control, G+: with glucose and G-: without glucose)

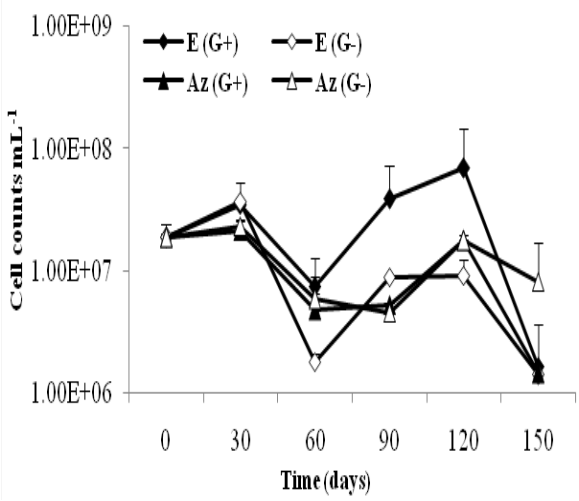


Fig 4.40: Growth rate of microbial biomass with respect to time. (E: experiment, Az: azide poisoned control, Hk: heat killed control, G+: with glucose and G-: without glucose)

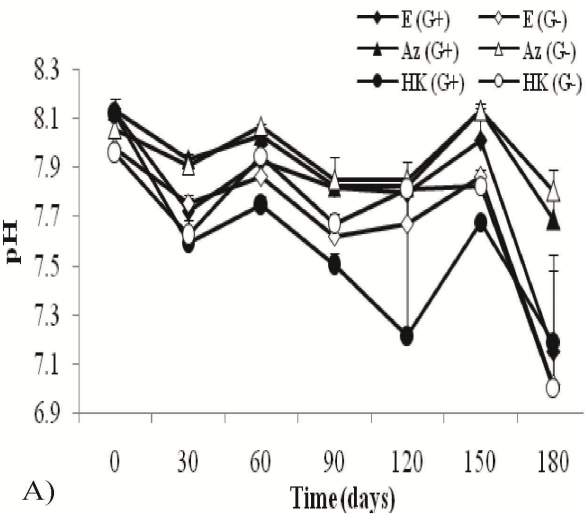


Fig 4.41: Variation in pH (E: experiment, Az: azide poisoned control, Hk: heat killed control, G+: with glucose and G-: without glucose)

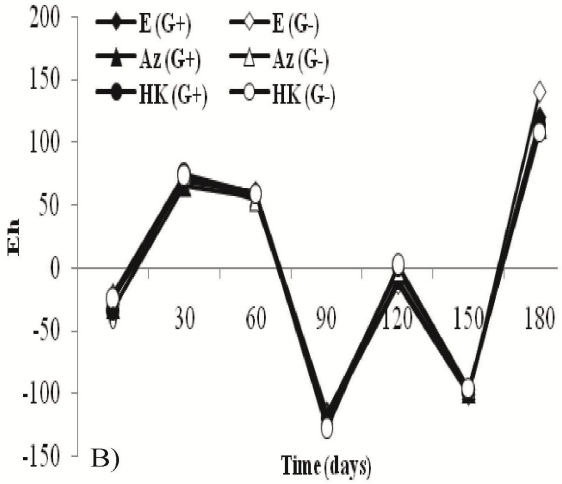


Fig 4.42: Variation in Eh(E: experiment, Az:azide poisoned control, Hk: heat killed control, G+: with glucose and G-: without glucose)

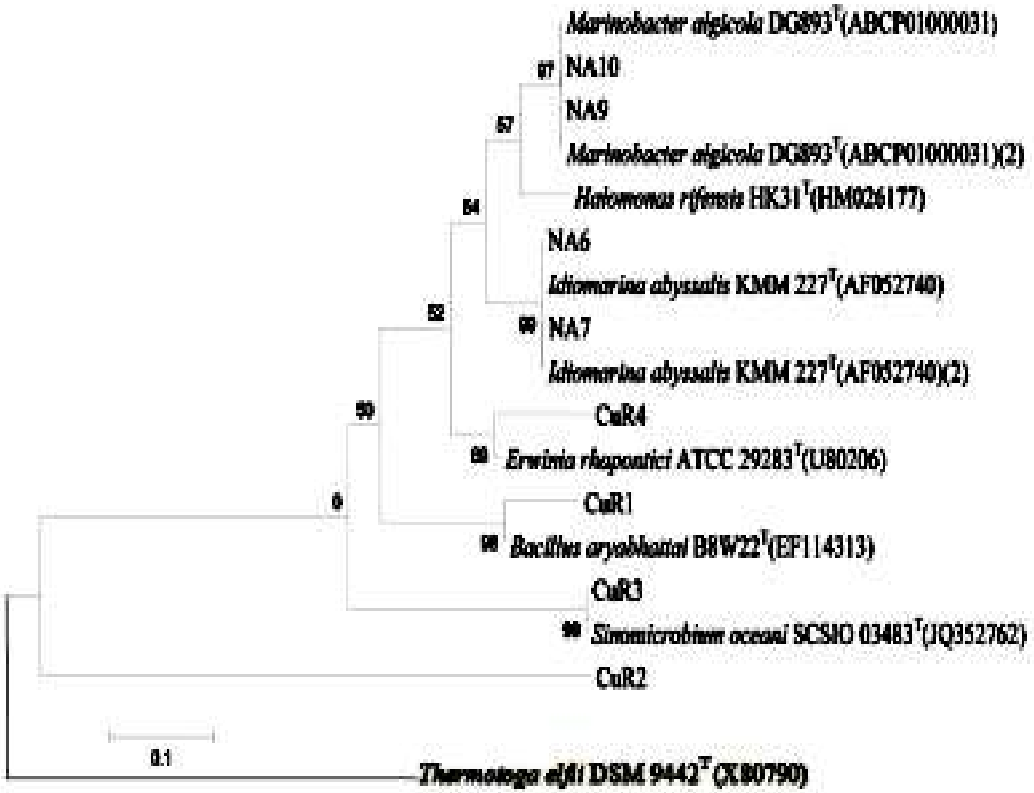
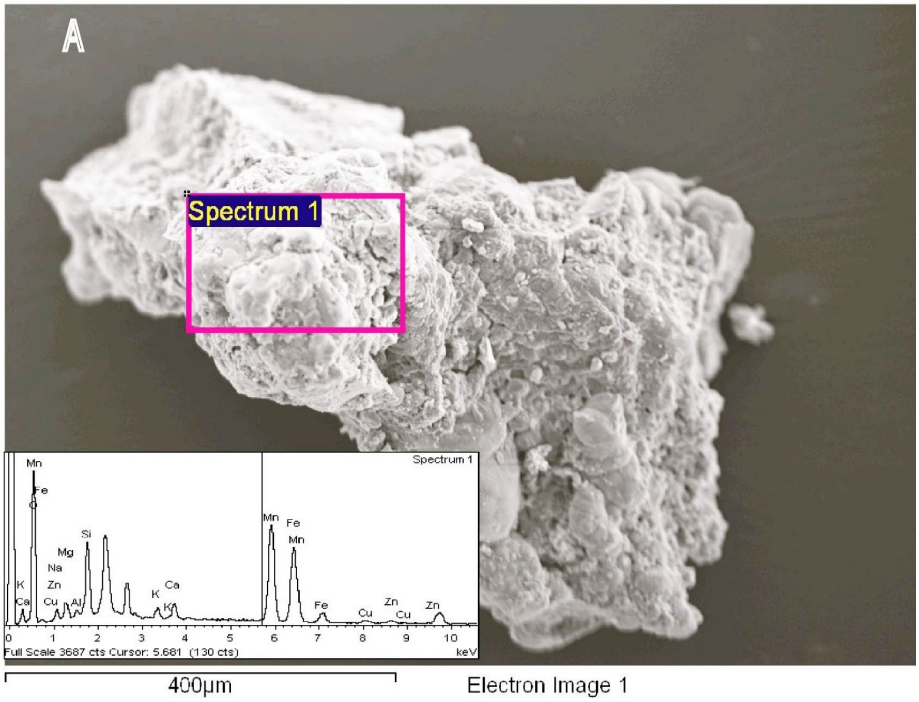


Fig.4.47: Phylogenetic tree of Cu oxidizing bacterial isolates and their closely related species



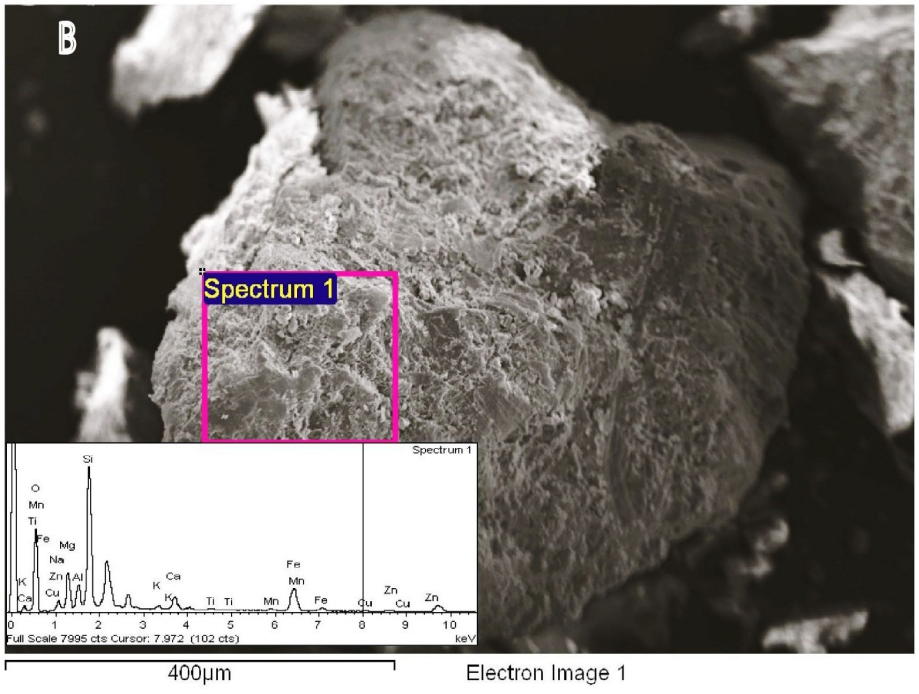


Fig 4.44: SEM images of basalt surface and EDS spectra

(A) Before incubation (B) After incubation

4.9.6.1 Immobilization of Cu with potential isolate

4.9.6.1 Immobilization

The results of immobilization rate of Cu is represented in Fig. 4.51. The immobilization rate of Cu was maximum at the end of 150 d incubation in 'G+' 464.33 μg g^{-1} and 660.02 μg g^{-1} in 'G-' media. The experimental rates in 'G+' were 3 times and in 'G-', 3 times more than the respective azide poisoned controls. Compared to heat killed controls, the respective experimental rates in 'G+' and in 'G-' were 3 times more.

4.9.6.2 Cell counts

The results of TC are presented in Fig. 4.52. TC increased by more than an order of magnitude in the presence of added glucose and less than an order of magnitude in the absence of added glucose. It ranged from 2.09×10^6 to 6.75×10^7 cells g^{-1} in 'G+' and 7.33×10^5 cells g^{-1} to 1.66×10^7 cells g^{-1} in 'G-' incubations. The maximum cell counts in the presence of added glucose occurred on the 0 d of incubation (2.12×10^7 cells g-1) and in the absence of added glucose (2.95×10^7 cells g^{-1}) on the 0 d. In the azide poisoned control the counts ranged from 1.88×10^6 to 2.12×10^7 cells g^{-1} in the 'G+' and 2.61×10^6 cells g^{-1} to 2.95×10^7 cells g^{-1} in the 'G-'.

4.9.6.3 pH &Eh

The results of pH and Eh are given in Fig. 4.53 and 4.54. Difference in pH and Eh was not perceivable between the experiments and corresponding controls. The pH decreased from 7.16 to 6.42 in the 'G+' and from 7.24 to 7.01 in the 'G-' . The Eh shifted

from negative to positive redox potentials at the end of incubation (60 d). It varied from -19.00 to 117.37 mV in the 'G+' and -18.10 to 107.88 mV in the 'G-' incubations.

4.9.6.4 Characterization and identification of bacterial isolate

Bacterial isolate CR35 was Gram negative short rod shaped. They showed positive results for some of the extracellular enzymes such as catalase, oxidase, amylase, caseinase, lipase and cellulose. The strain also showed oxidative fermentation and motility.

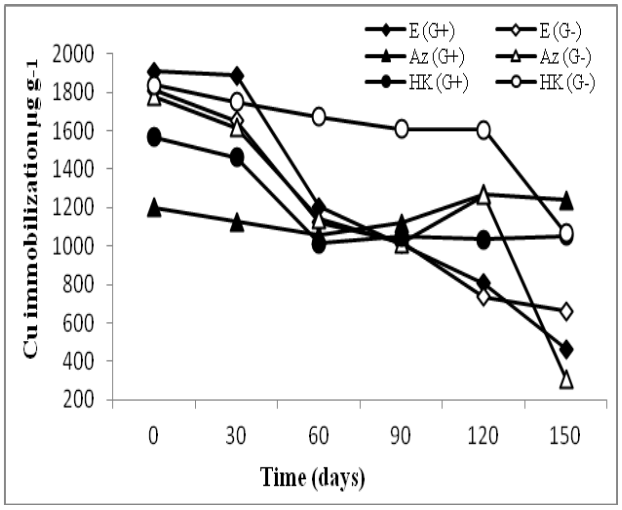


Fig 4.48: Immobilization rate of Cu from basalt by Bacterial potential isolate CR-35. The values in the figure are mean values ± SD, n = 3

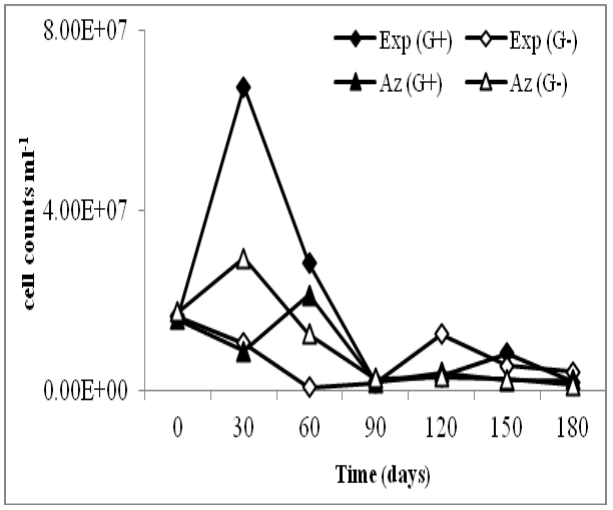


Fig 4.49: Variation in cell numbers in relation to the immobilization of Cu (E: experiment, Az: azide poisoned control, Hk: heat killed control, G+: with added glucose and G-: without added glucose)

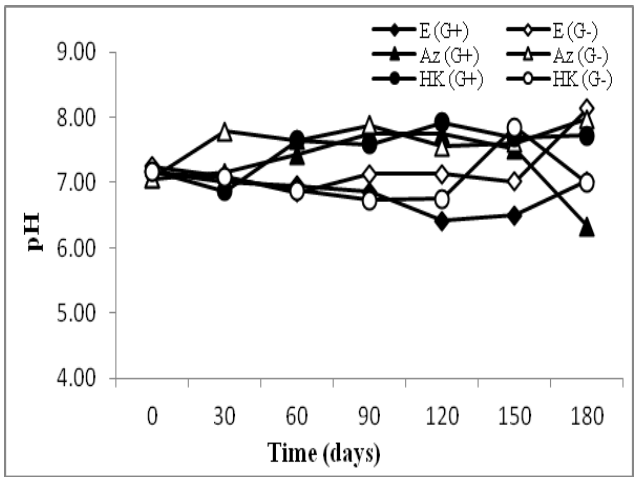


Fig 4.50: Variation in pH in relation to the immobilization of Cu (E: experiment and Az: azide poisoned control, G+: with added glucose and G-: without added glucose)

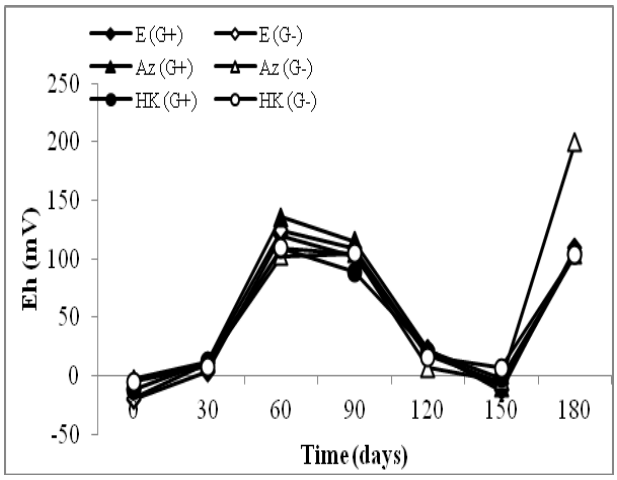


Fig 4.51: Variation in Eh in relation to the immobilization of Cu (E: experiment and Az: azide poisoned control, G+: with added glucose and G-: without added glucose)

4.9.7 Immobilization of Copper with microbial community

4.9.7.1 Immobilization

The results of mobilization rate of Cu is represented in Fig. 4.55. The immobilization of Cu was reported maximum on 60d of incubation at 9.92 µg g^{-1} d^{-1} in the presence of glucose. The immobilization of Cu was maximum on 120d of incubation at 6.58 µg g^{-1} d^{-1} in the absence of glucose.

4.9.7.2 Cell counts

The results of TC are presented in Fig. 4.56. TC increased by more than an order of magnitude in the presence of added glucose and less than an order of magnitude in the absence of added glucose (Fig. 5.56). It ranged from $3.76x10^5$ to $2.47x\ 10^7$ cells g^{-1} in

'G+' and $5.36x10^5$ cells g^{-1} to $3.36x10^7$ cells g^{-1} in 'G-' incubations. The maximum cell counts in the presence of added glucose occurred on the 0 d of incubation ($2.47x10^6$ cells g^{-1}) and in the absence of added glucose ($3.36x10^6$ cells g^{-1}) on the 120 d. In the azide poisoned control the counts ranged from $3.25x10^5$ to 2.4710^6 cells g^{-1} in the 'G+' and $4.21x10^5$ cells g^{-1} to $2.47x10^6$ cells g^{-1} in the 'G-'.

4.9.7.3 pH &Eh

The results of pH and Eh are given in Fig 4.57 and 4.58. Difference in pH and Eh was not apparent between the experiments and the corresponding controls. The pH decreased from 6.81 to 6.17 in the 'G+' and from 6.89 to 5.79 in the 'G-' (Fig 4.57). The Eh shifted from positive to negative redox potentials toward the end of incubation (30 d) in G+ while in G- it shifted from positive to negative end of experiment. It varied from 133 to -167 mV in the 'G+' and 162 to -164 mV in the 'G-' incubations (Fig. 4.58).

4.9.7.4 SEM

SEM examination of microbial cells adhering to the rock surfaces showed increase in their cell size 0.3 to 1µm and change in morphology (Fig. 4.59). Exopolysaccharide-like material surrounding the bacterial cells suggest that Cu sequestration could exist outside the cell.

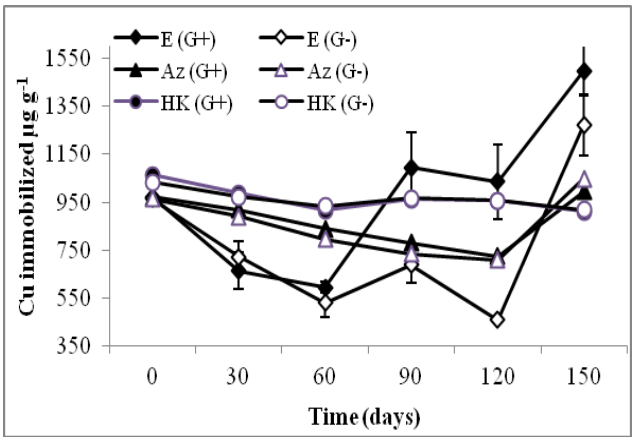


Fig. 4.52: Immobilization rate of Cu from basalt by Bacterial community. The values in the figure are mean values ± SD, n = 3

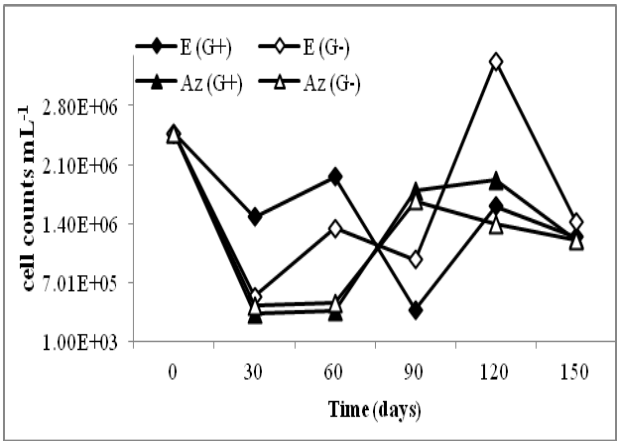


Fig. 4.53: Variation in cell numbers in relation to the immobilization of Cu (E: experiment, Az: azide poisoned control, Hk: heat killed control, G+: with added glucose and G-: without added glucose)

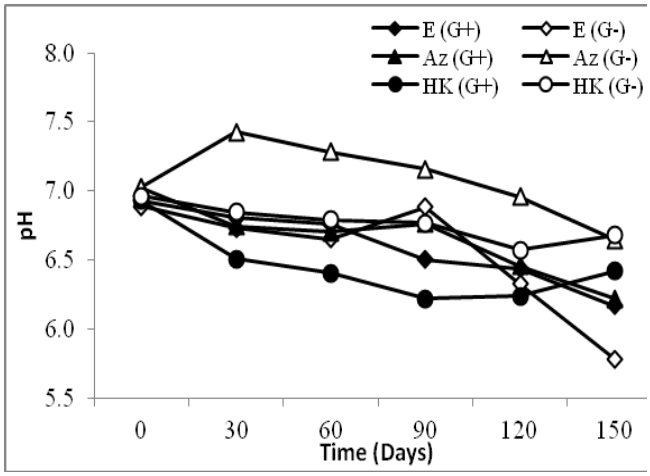


Fig. 4.54: Variation in pH in relation to the immobilization of Cu (E: experiment and Az: azide poisoned control, G+: with added glucose and G-: without added glucose)

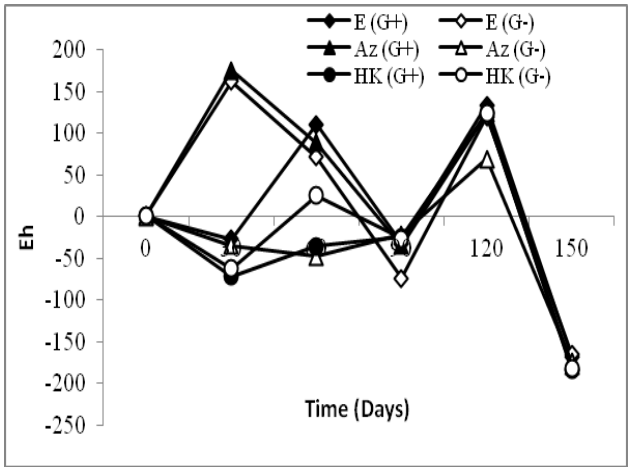


Fig. 4.55: Variation in Eh in relation to the immobilization of Cu (E: experiment and Az: azide poisoned control, G+: with added glucose and G-: without added glucose)

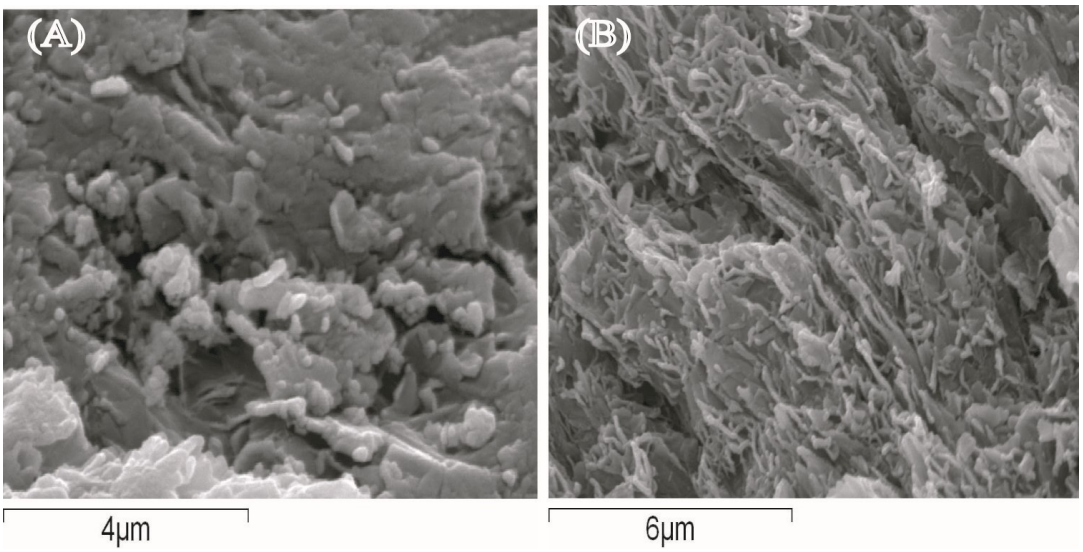


Fig. 4.56: SEM images shows morphological alteration in bacterial cell grown in seawater medium. A) Before incubation B) After incubation

4.10 Novel benthic foraminiferal diversity and their identification from ridge ecosystem

Benthic Foraminifera data are presented in Fig. 4.58. During this study 5 foraminifera species namely *Spirillina guttata, Lagena hexagona, Trochammina inflate, Globigerina bulloides, Globocassidulina cabalisuturata* and three additional unidentified species. *Spirillina guttata* (n=323) was found to be the dominant foraminifera among the three sampling ridges followed by *Globigerina bulloides* (n=264). Further, *Lagena hexagona* (n=48) was found to be the least occurring species. Out of the three Unidentified species, unidentified species 1 is observed to be occurring among all the 3 ridges, unidentified species 2 was found in R-2 and R-3 and unidentified species 3 was observed only at R-3 sampling station.

Graphic distribution of forminifera among the sampling stations are given in Fig. 4.58 A, B and C and the overall foraminifera recorded from these three sampling areas R1, R2 and R3 are presented in Fig. 4.58 D.

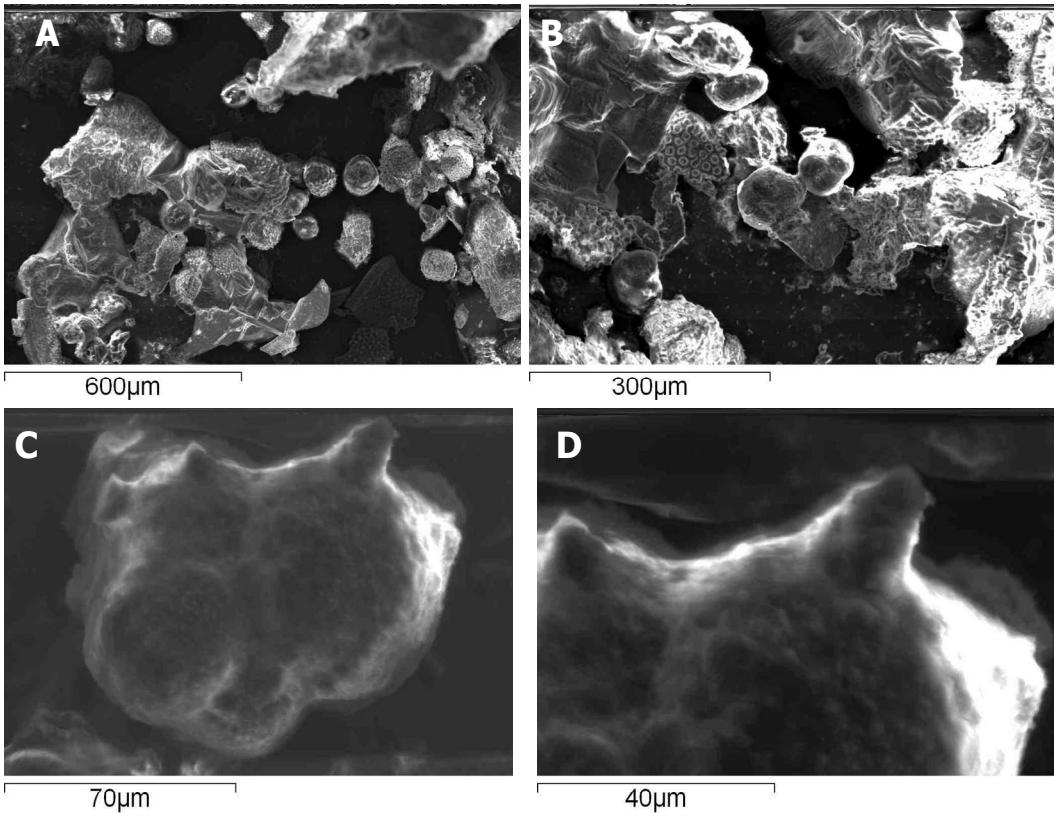


Fig.4.57: SEM images of the Ridge sediments containing foraminiferans at different magnifications

4.10.1 Systematics of Foraminifera collected and identified

Descriptions of the 5 species identified are as follows:

1. *Spirillina guttata:*

Phylum – Foraminifera
Class – Tubothalamea
Order – Spirillinida
Family – Spirillinidae

Descriptions -

Test elongate, calcareous, porcellaneous, imperforate. Test surface smooth and very delicate, the earlier chambers are clearly visible. The latest chamber envelopes the older chambers; Earliest chambers are triloculine and the final chamber biloculine. The aperture consists in the terminal end of the final chamber and is arranged at each opposite end of the test (Fig. 4.59 A).

2. *Trochammina inflate* :

Class – Globothalamea

Order - Lituolida

Family – Trochamminidae

Description

Porcelaneous shell walls are composed of microscopic rod-shaped crystals of $CaCO_3$. These have a milky, translucent to opaque look and generally lack pores beyond the initial chambers. In some porcelaneous species, small depressions in the surface ornamentation give the appearance of pores. (Fig. 4.59 B).

3. *Lagena hexagona:*

Class – Forminiferaincertaesedia

Order – Lagenida

Family- Lagenidae

Description

The elongated test consists of Hexagonal structurs with circular cavity in centre of these hexagonal structures. The shell is made of calcareous hyaline materials (Fig.59 C).

4. *Globigerina bulloides:*

Order – Rotaliida

Family- Globigerinidae

Description

The test is calcareous, porcellaneous, ovate to sub-circular, showing eight ovule with throne like structure. Test may show numerous longitudinal structure on the surface. (Fig. 59 D).

5. *Globocassidulina cabalisuturata:*

Family – Cassidulinidae

Description

Porcelaneous forms have a wall composed of thin inner and outer veneers enclosing a thick middle layer of crystal laths; they are imperforate and made from high magnesium calcite. The hyaline foraminifera add a new lamella to the entire test each time a new chamber is formed; various types of lamellar wall structure have been recognized (Fig. 4.59E).

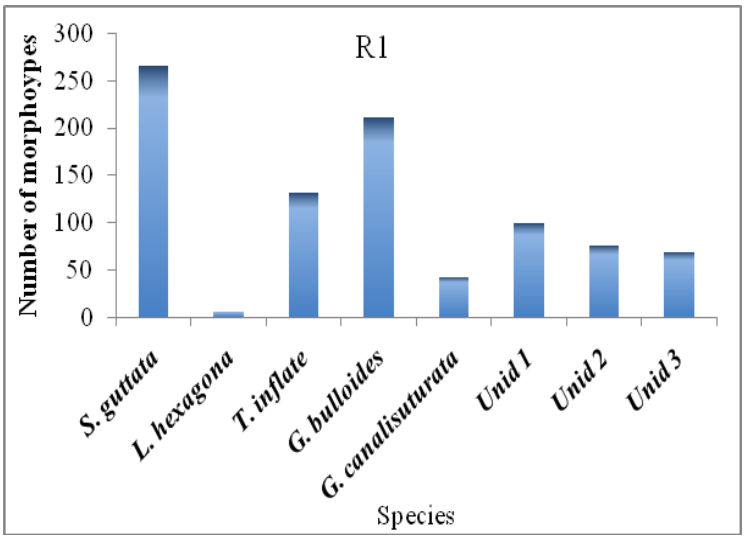


Fig. 4.58 A: The distribution and abundance of fominifera in R1 sampling station at Carlsberg ridge

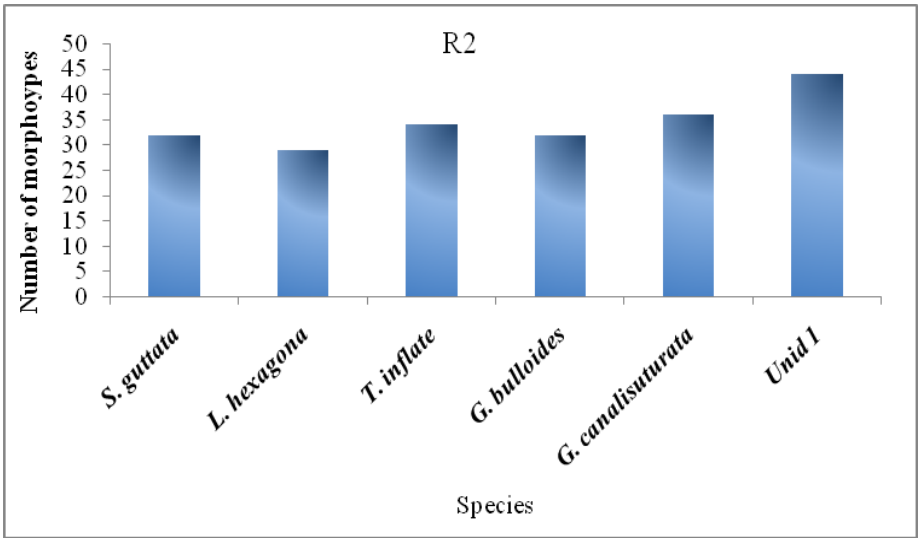


Fig. 4.58 B: The distribution and abundance of fominifera in R2 sampling station at Carlsberg ridge

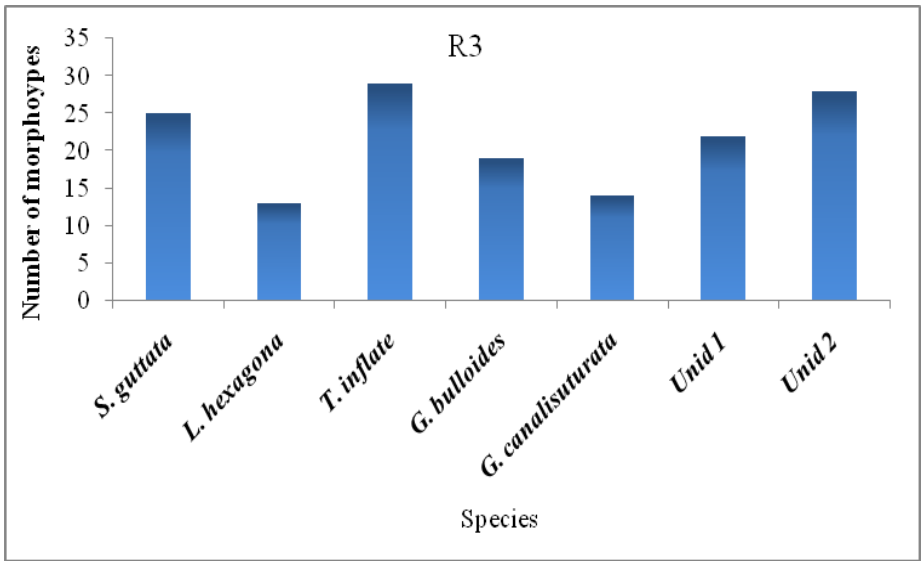


Fig.4.58 C: The distribution and abundance of fominifera in R3 sampling station at Carlsberg ridge

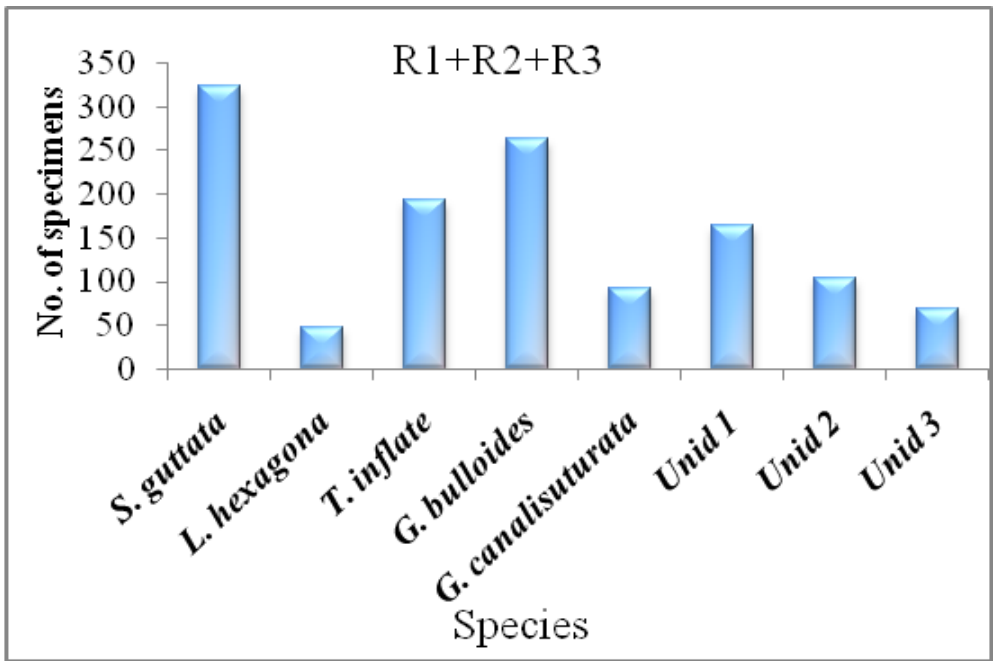
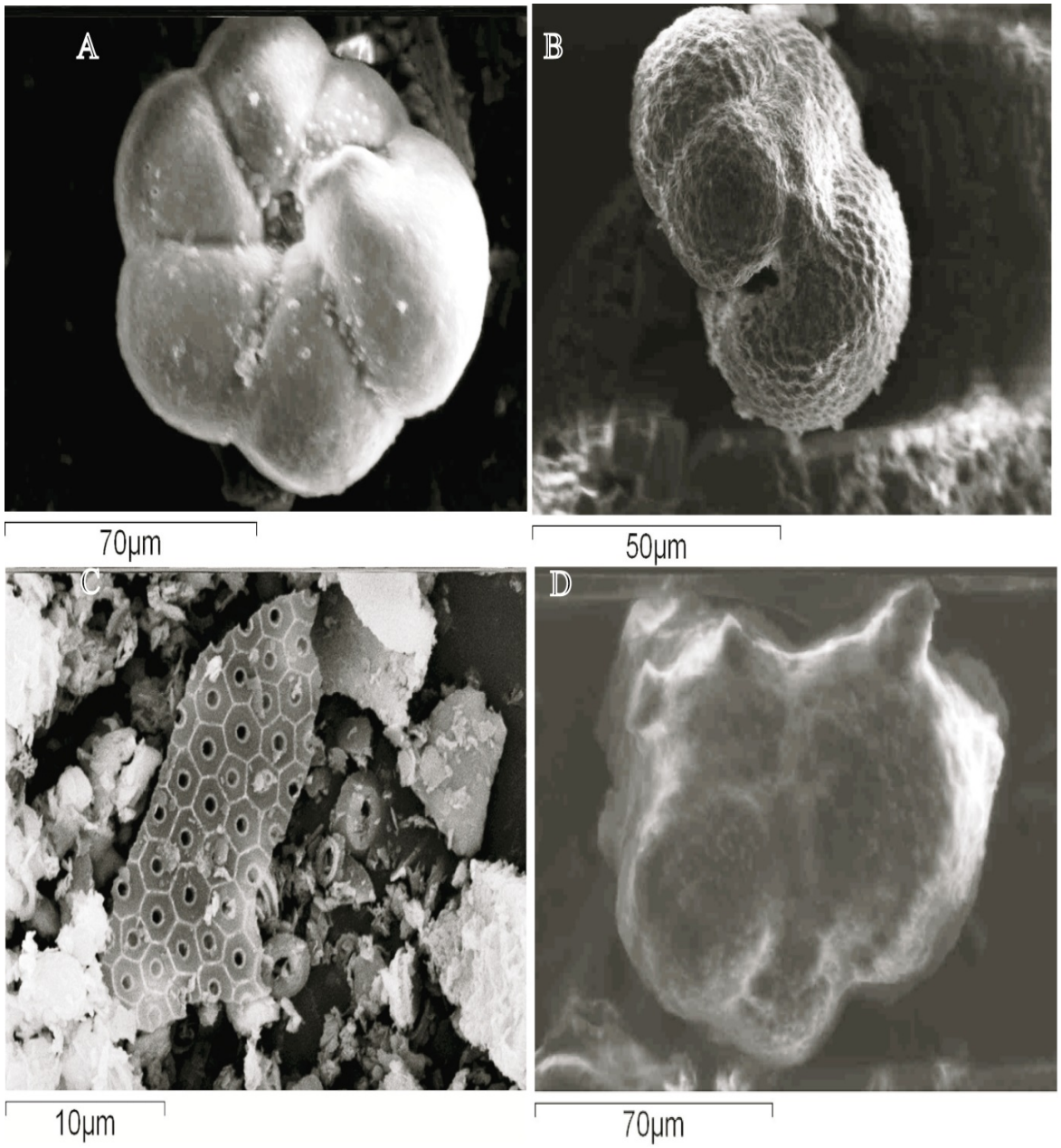


Fig. 4.58 D: The distribution and abundance of fominifera in Carlsberg ridge


A
B
70µm
50µm
C
D
10µm
70µm

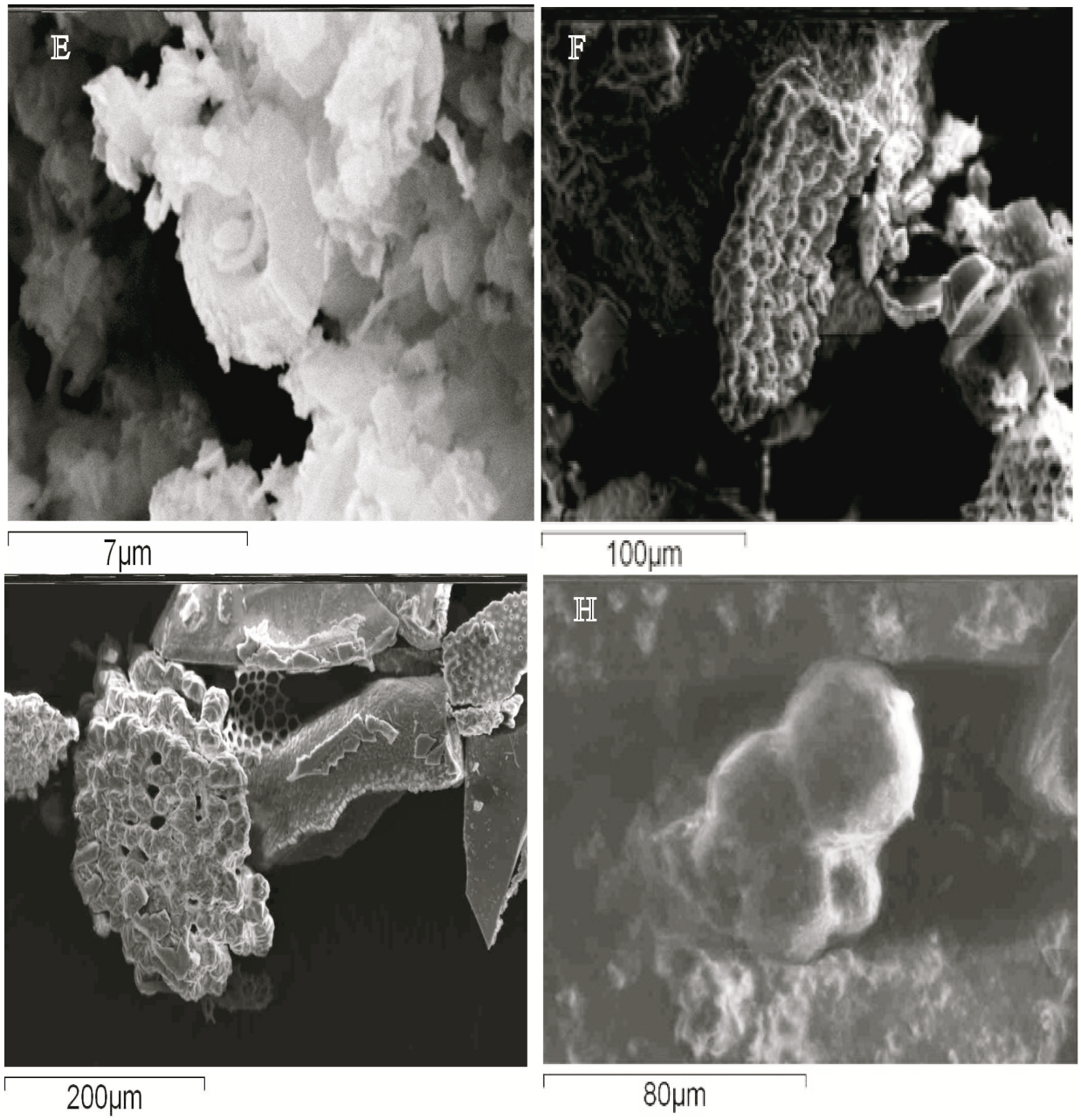


Fig. 4.59: SEM images Foraminifera A) *S. guttata* B) *T. inflate* C) *L. hexagona and* D) *G. bulloides* E) *G. cabalisuturata* F) unidentified species 1 G) unidentified species 2 & H) unidentified species 3

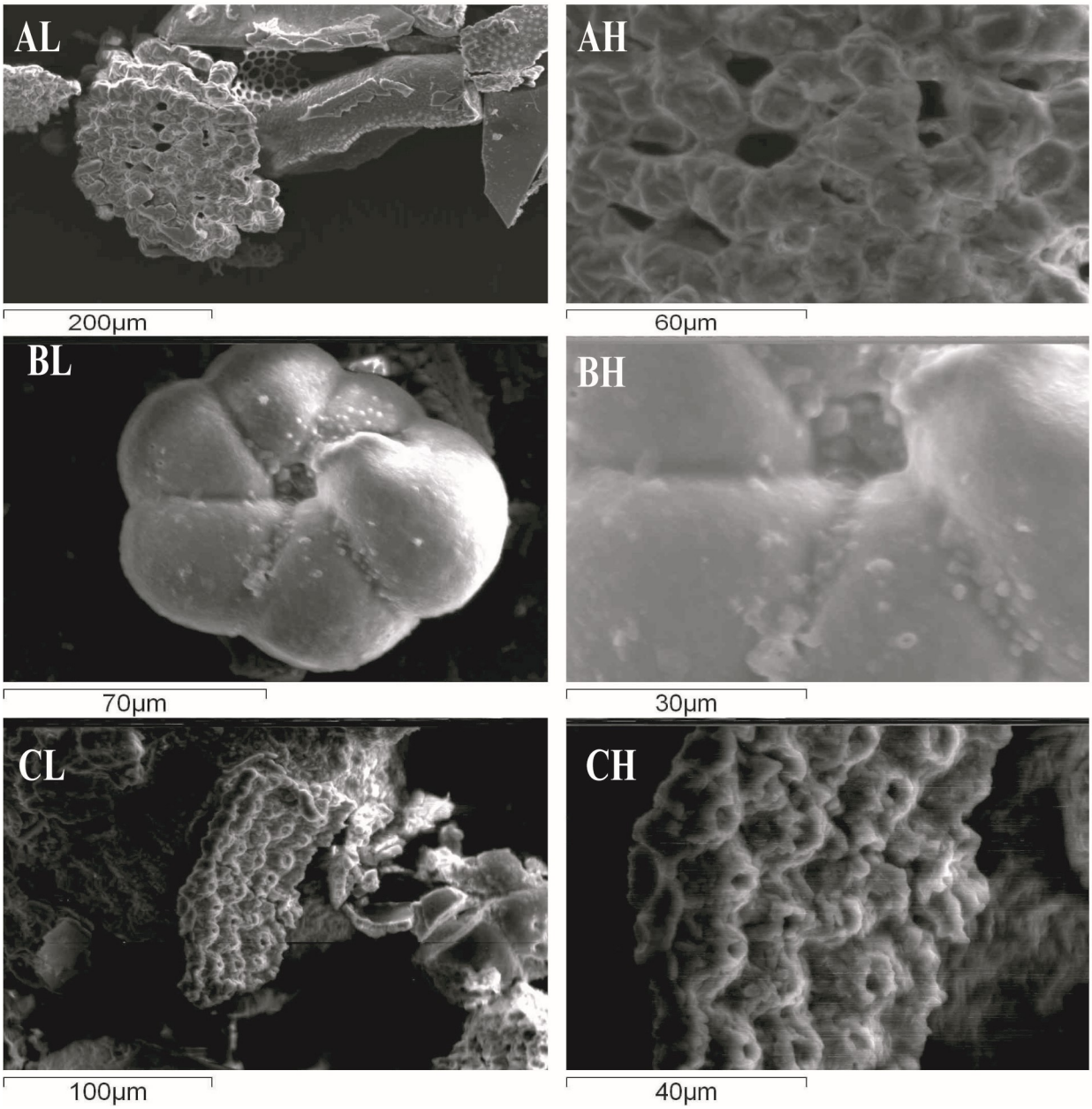


Fig. 4.60: SEM images of 3 foraminiferans at lower (L) and higher (H) resolution. AL and AH (A: Unidentified specimen); BL and BH (B : *S. guttata); CL and CH (C:* Unidentified specimen)

4.10.2 Foraminifera associated bacteria

Scanning electron microscopy (SEM) and electron dispersion spectra (EDS) study was carried out to understand the association between foraminifera and bacteria. Five foraminifera taxa (*Spirillina guttata, Lagena hexagona, Trochammina inflate, Globigerina bulloides, Globocassidulina cabalisuturata*) were scanned and eight different bacteria were found associated with them.

Bacillus vietnamensis, Oceanobacillus picturae, Halomonas cupida, Brachybacterium phenoliresistens phenol-A, *Bacillus oceanisediminis, Salinicola salaries, Halomonas cupida* and *Bacillus vietnamensis* species of bacteria were identified as in symbiotic associations with foraminifera (table 4.12). SEM images showed the surface details of foraminifera with the presence/occurrence of bacteria.The rod shaped bacteria was found scattered on the surface of foraminifera in spectrum 1 (Fig. 4.61 A), similarly round shaped bacteria was observed densely arranged at the apical end or opening of *S. guttata* in spectrum 2 (Fig. 4.61B and C). Fig. 4.61D shows spectra on foraminifera shell.

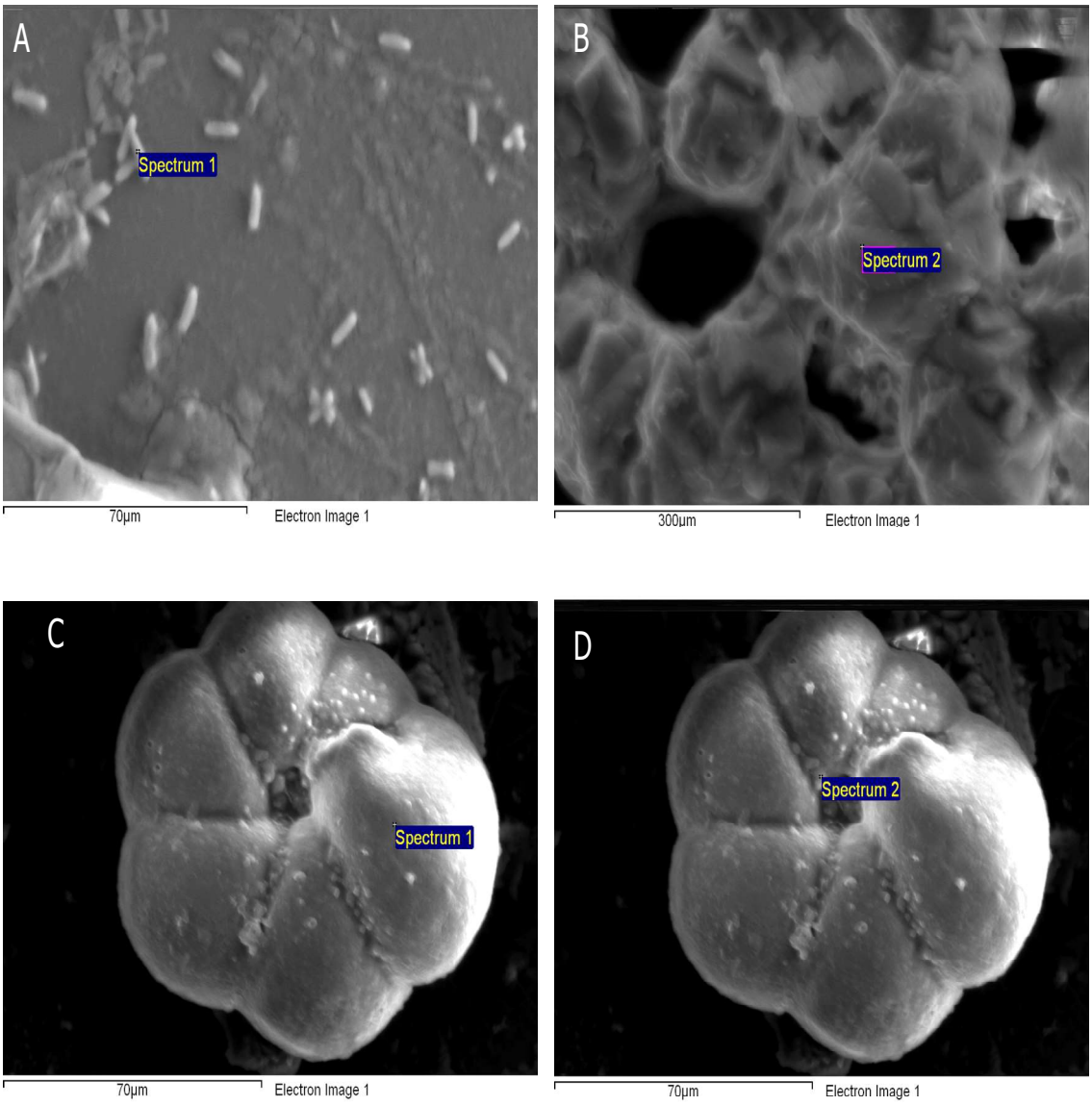


Fig. 4.61: EDS data to compare chemical composition of A) bacteria, B) Bacteria associated on fauna C) Bacteria associated on fauna and D) Fauna shell

The compositional comparison by EDS on pure R6 bacterial culture form and bacterial associated with foraminifera (Figs. 4.62 A, B, C), which does not show any specific difference in their composition and revealing the same. The EDS analysis of foraminifera cells was represented in Fig. 4.62 D showed slight variation in their composition which may be due to the cell masses of foraminifera only.

A

Element	Weight %	Atomic %	Compd %	Formula
Na K	5.17	5.15	6.97	Na2O
Si K	4.31	3.51	9.22	SiO2
S K	18.78	13.41	46.90	SO3
Cl K	3.45	2.23	0.00	
K K	2.80	1.64	3.38	K2O
Ca K	17.90	10.22	25.04	CaO
Ti K	0.34	0.16	0.56	TiO2
Zn K	1.67	0.59	2.08	ZnO
As L	1.24	0.38	1.64	As2O3
Rh L	0.61	0.14	0.76	Rh2O3
O	43.72	62.57		
Totals	100.00			

B

Element	Weight %	Atomic %	Compd %	Formula
Si K	1.04	1.06	2.23	SiO2
Cl K	0.66	0.53	0.00	
Ca K	65.13	46.36	91.13	CaO
Cu K	1.45	0.65	1.82	CuO
Zn K	1.33	0.58	1.66	ZnO
Rh L	0.84	0.23	1.03	Rh2O3
Yb L	1.29	0.21	1.47	Yb2O3
O	28.25	50.37		
Totals	100.00			

C

Element	Weight %	Atomic %	Compd %	Formula
Na K	3.17	3.93	4.28	Na2O
Al K	2.71	2.86	5.13	Al2O3
Si K	11.05	11.20	23.63	SiO2
Cl K	5.47	4.40	0.00	
Ca K	26.39	18.75	36.93	CaO
Fe K	1.22	0.62	1.57	FeO
Zn K	1.22	0.53	1.51	ZnO
Rh L	0.69	0.19	0.85	Rh2O3
In L	8.73	2.17	10.56	In2O3
Au M	8.98	1.30	10.07	Au2O3
O	30.36	54.05		
Totals	100.00			

D

Element	Weight %	Atomic %	Compd %	Formula
Na K	1.10	1.38	1.48	Na2O
Cl K	3.43	2.79	0.00	
K K	1.49	1.10	1.80	K2O
Ca K	61.11	43.99	85.50	CaO
Ni K	0.27	0.13	0.34	NiO
Zn K	1.63	0.72	2.03	ZnO
Zr L	4.01	1.27	5.42	ZrO2
O	26.96	48.62		
Totals	100.00			

Fig. 4.62: EDS data to compare chemical composition of A) bacteria, B) and C) Bacteria associated on fauna and D) Faunal shell.

DISCUSSION

In the present work, the abundance and diversity of Mn and Cu tolerant bacteria in Carlsberg ridge ecosystem was studied along with their role in metal uptake in the Carlsberg ridge. The findings of the present work are discussed under the following headings:

5.1 Environmental setting of the Ridges

5.1.1 Characteristics of the deep-sea

The deep-sea benthic ecosystems, can be classified into three kinds based on their composition and bacterial response. They are 1) detrital, which is dependent on the organic rain from above (including the nodule provinces of the deep-ocean basins), 2) non-detrital and chemosynthetic, as in the hydrothermal vent areas and 3) both detrital and non-detrital as in passive margins and cold seeps (Sibuet, 2005). Most of these often inaccessible realms are still vastly unknown in terms of baseline knowledge (Morgan, 2005). Jannasch *et al*. (1971) and Jannasch and Wirsen (1973) reported 50 times lower metabolic activities of free-living bacteria in deep-sea sediments as compared to the microorganisms in shallow waters or on sediments at shallow depths. Environmental factors contributing to this slow rate of bacterial metabolism may be due to low temperature (<5°C), and elevated pressure (500 bars), with a greater proportion of metabolic activity devoted to cellular maintenance (Schwartz and Colwell, 1975). On the contrary, there are reports that the deep-sea forms are as active as their counterparts in the shallow regions (Lochte *et al.*, 1999). The environments that are predominantly detrital are influenced by seasonal variations and chemosynthetic activity (Jon Copley,

2005). The deep-sea environments are no longer monotonously homogenous but show large-scale spatiotemporal variations. The variations in the benthic community also contribute to topographic and sediment pattern differences.

5.1.2 Depth, temperature, and pressure

The Ridges are one of the deepest parts of the Indian Ocean where rocks/sediments are recovered from below 3500 m (Fig. 3.1 and Table 3.1). Warren *et al*. (1982) reported in situ temperature in the vicinity of the deep sea. However, due to high volcanic activity through Deep Sea Hydrothermal Vents (DSHV) there are possibilities of higher temperature than the observed in unit temperature of 2°C whereas the pressure measured around 500 bars at these depths. It is also evident that due to the hydrothermal flow, fluids pass through the sediment column and reveal that the flow interacts with the hydrostatic pressure, contributing to changes in the phase chemistry. As a result of this, the microbes may be residing at a lower pressure and higher temperature in these rocks/sediment. This could explain a large number of mesophiles culturable from these sediments.

5.1.3 Elemental Carbon

In general total organic carbon increased from the northern sampling site to southern sites. Across the Carlsberg ridge our values are in the consistent range as reported earlier by Gupta and Jauhari (1994). However, the present study, station 04 in the central part of the ridges showed high TOC irrespective of being relatively far from the effects of terrigenous influx. They are also high in C/N ratios (Das *et al*., 2014). The factors governing the variation in organic carbon are surface water productivity, bottom water oxygen content, degradation, sedimentation rate and bioturbation (Feeney

et al., 1998). TOC is the key factors governing the microbial distribution and which in turn is also affected by the microbial activity.

5.2 Abundance of bacteria in ridges

The deep sub-seafloor biosphere supports a diverse population of prokaryotes belonging to the Bacteria and Archaea (Parkes *et al.,* 1994, 2000, 2005). Most of the taxonomic groups identified by molecular methods contain mainly uncultured phylotypes. Despite this, we could isolates several culturable strains have been isolated from this habitat, but they probably do not represent the majority of the population. This suggest that some of the activities measured, such as sulfate reduction and methanogenesis, reflected in geochemical profiles, are carried out by a small subset of the community detected by molecular methods (Fry *et al*., 2008). It is further possible that heterotrophy may not be totally ruled out as a mode of metabolism in subsurface sediments and heterotrophic microorganisms could dominate the uncultured prokaryotic population. Although heterotrophy is limited by the increasing recalcitrance of organic matter with depth, thermal activation of buried organic matter improves heterotrophy by providing additional substrates at depth (Fry *et al*., 2008). In addition, a high proportion of the cells detected by AODC were also shown to be viable (Inagaki *et al*., 2006).

5.2.1 Total counts and frequency of dividing cells

During the present study, the total counts of bacteria varied from 10^6 to 10^7 cells in dry rock. Although geographically distinct and geologically contrasting bacteria from some stations viz. 2 and 8 are clustered together for total count and frequency of dividing cells. Low TC was observed in the water samples from station 1 to 12 while in rock samples highest TC was found from station 12 (Fig. 4.6 and Table 4.2). Maximum TOC

was observed in station 11 and 12 (Fig. 4.4) region may be because it is under the influence of grazing macro and meiobenthos. In general total counts are consistent with the values reported earlier (D'Hondt *et al*., 2002, 2004).

5.2.2 Culturable counts and endeavour to improve culturability

Although it was argued that the culturable fraction could exert greater influence on the environment, such inferences remained controversial for two decades, as only 0.001 to 0.01% of the total bacterial count is amenable to cultivation techniques (Van Es and Meyer Reil, 1982). According to D'Hondt *et al*. (2002, 2004), the culturable bacteria are now known to be consistent over large areas on the ocean floor.

5.2.4 Aerobic culturability

In the abyssal depths of ridges, chemolithotrophy is expected to flourish out of compulsion. However, the organisms growing at the expense of organic energy as a source are known as facultative autotrophs. These prokaryotes grow better when small amounts of organic compounds are present in the otherwise inorganic medium and are known as mixotrophs. The heterotrophic bacteria obtain the carbon from organic nutrients. Here, the carbon source in most cases is also the energy source. Some bacteria like the Pseudomonads are versatile and are known to utilize more than hundred different carbon compounds as the sole source of carbon and energy. In contrast, few versatile bacteria are more fastidious ones that grow on a relatively smaller number of organic compounds like the obligate methylotrophs that grow on methane, methanol, and dimethyl ethers etc. (Gottschalk *et al*., 1991). To the extent that carbon and energy limitations are the rules of natural habitats, mixotrophic organisms may have a selective advantage over the obligate heterotrophs or obligate autotrophs (Leefeldt and Martin, 1980).

5.2.5 Heterotrophs

Heterotrophs were enumerated on 1% strength of Nutrient Agar (NA). The bacteria in ridges are known to grow best at this concentration (Nair *et al*., 2000; Loka and Nair, 2005). During the present study, heterotrophs were observed as ubiquitous in the ridges and often found in higher numbers in the organically depleted southern region (Table 4.3). These heterotrophs might have higher adaptability, versatility, and resilience as they are associated with relatively oligotrophic sediments (Harder and Dijkhuizen, 1982; Goltekar *et al.,* 2006). Similar unexplained high heterotrophic populations have been reported in some earlier findings in vent sites with low organic carbon (Karl, 1995).

5.2.6 Cu and Mn oxidizers

The ubiquity of Cu and Mn oxidizers is expected on the higher side due to the presence of hydrothermal fluid containing metals in the ridge region. The homogenous distribution of both Cu and Mn oxidizers indicate that the reduced ions are available at more or fewer uniform concentrations in water columns. We observed during the present study, that Mn oxidizers are marginally more in abundance than Cu-oxidizers (Fig. 4.7 and Table 4.6 and 4.7). Copper and manganese oxidizers are widespread in both coastal and oceanic environments. Deep-sea bacteria participate in the redox coupling of Cu and Mn along with oxygen, sulfur, nitrogen, and carbon (Van Cappellen and Wang, 1996). The net rate of organic carbon oxidation could be broken down into the contributions from aerobic respiration, denitrification, dissimilatory Mn (IV) reduction, dissimilatory Cu (II) reduction, sulfate reduction and methanogenesis. Edwards *et al*. (2003) revealed physiological characterization of novel, psychrophilic, Mn-oxidizing bacterial (MnOB) isolates from low-temperature weathering habitats in the vicinity of the Juan de Fuca deep-sea hydrothermal. The MnOB were cultured from the surfaces of weathered rock

and metalliferous sediments. These oxidizers are capable of immobilizing metal ions in the natural environment which was performed in microcosm environment in lab conditions. In mobilization and immobilization experiments with the microbial community, mobilization rate observed higher than immobilization which proves the presence of Mn and Cu oxidizers/reducers in the deep sea environment. Present results also reported bacteria with obligate chemolithoautotrophic and microaerobic character under optimum conditions which are supported with the similar results of Edwards *et al*. (2003). Similarly, the geochemical analysis of ridge rock samples after mobilization/immobilization experiments showed a reduction by bacteria in present minerals and perform mineralization until the end of incubation (Fig. 4.21). This indicates that microbial Mn-oxidizing bacteria play a critical factor in the kinetics of mineral dissolution at the seafloor by accelerated dissolution of minerals approximately 6-8 times over abiotic rates. Minerals released by the bacterial action causes Mn or Cu reduction and thus getting oxidized further by Mn or Cu-oxidizing bacteria (Edwards *et al*., 2004).

Likewise, microbial Cu-oxidation is also widespread in the deep-sea. Mn (II)-oxidizing microbes play an integral role in the biogeochemical cycling of manganese, iron, nitrogen, carbon, sulfur and several nutrients and trace metals. Linking Mn (II) oxidation to cellular function, although still unknown, continues to drive efforts to characterize manganese biomineralization i.e. formation and deposition of oxides.

During the present study, complexed - Mn (III) revealed transient intermediate state from Mn (II) oxidation to Mn (IV), suggesting that the reaction might involve a unique multicopper oxidase system capable of a two-electron oxidation of the substrate. During biogenic and abiotic synthesis experiments, the application of synchrotron-based

X-ray scattering and spectroscopic techniques have been significantly important in order to understand the oxidation state and relatively amorphous structure (i.e. MnO_7). This also provides a new blueprint for knowing the structure of biogenic Mn-oxides (Tebo *et al*., 2005). Mn (II)-oxidizing bacteria was also identified as a divergent phylogenetic lineage group in the bacterial domain, such as *Firmicutes, Proteobacteria,* and *Actinobacteria* (Tebo *et al*., 2005).

5.3 Diversity of Bacteria

5.3.1 Biochemical identification

Although culturable bacteria form a very small fraction of 0.1% of the total diversity, they are often functionally important groups that dominate biogeochemical processes. A few examples of such ecological functions are given in Table 4.13 and 4.18. The heterotrophs like *Pseudomonas alcaligenes* group break larger particles of organic matter to smaller ones (Loka and Nair, 2005). *Pseudoalteromonas sp.* is a psychrotolerant bacterium isolated from deep-sea sediment which is known to produce an exopolysaccharide (EPS) Fig. 4.20. This EPS could enhance the stability of the cold-adapted protease secreted by the same bacteria through preventing their autolysis. The EPS could bind many metal ions, including Fe^{2+}, Zn^{2+}, Cu^{2+}, and Co^{2+}. It is also a very good flocculating agent and could be colloidal and suspended particles. The EPS secreted might help these bacteria enrich the protein aqueous particles reported by Qin *et al. (*2007). These bacteria may have various roles in the growth of ridge in the deep sea.

5.3.2 Hydrolytic enzyme activity

The bacterial from Carlsberg ridge isolates from sediments, water and foraminifera hydrolyzed DNase, protease, amylase, and lipase (Table 4.13 and 4.18). Bacteria are known to digest various organic compounds with specific extracellular

hydrolases (Chrost, 1991). Only monomers and small oligomers can be incorporated into bacterial cells and participate in their physiological pathways. Therefore its well defined that extracellular enzymatic hydrolysis is the key process involved in the degradation of organic material (Billen, 1982; Meyer-Reil, 1991). Since the production of most of the extracellular hydrolases is substrate inducible (Priest, 1984) the potential hydrolytic activity of specific enzymes may reflect the availability of their respective organic substrates.

5.3.3 I6S rDNA phylogenetic affinities of Ridges cultures

In this study bacterial isolates R1, R3, R4 and NA2 (Fig 4.19A) *Firmicutes* belonging to the genus *Bacillus*. Other related species like *Alcanivorax sp*. are psychrotolerant bacteria isolated from deep-sea sediment. The structural characterization and ecological roles of the exopolysaccharide (EPS) secreted by these strains could serve as enhancers for the survival of deep-sea bacteria in a fluctuating environment (Qin *et al*., 2007).

Culture CuR1 (Fig 4.47) isolate from Carlsberg ridge is other Firmicute showing affinity to *Bacillus* sp. Manganese (II) oxidation mediated by heterotrophic *Bacillus* species in hydrothermal plumes was reported from the Guaymas Basin hydrothermal field (Dick *et al.,* 2006). Spores of *Bacillus*-like organisms can rest dormant for long time periods and they are resistant to damage through desiccation and radiation. *Bacillus*-like organisms have been isolated from deep subsurface sediments and from inclusions inside materials like amber, salt crystals or glacial ice, where the spores must have been included since the time of deposition. The age of the inclusions in the salt crystals and amber was estimated in the range of several million years, the age of those in glacial ice in the range of 5 to 750,000 years. *Bacillus*-like strains that are physiologically capable

of growing under these conditions in the brine, i.e. extremely high salinity combined with anoxia and a sulfide concentration of several millimolar per liter (Sass *et al*., 2008). Culture R6 (Fig. 4.29) showed affinity to *Imtechella* which have a similar ecological role in biofilm formation with that of *Pseudoalteromonas.* Culture R10 (Fig. 4.11C) showed affinity to *Alcanivorax sp. the* same *sp.* belongs to y-proteobacteria. *Alcanivorax, Marinobacter, Thalassolituus, Cycloclasticus, Oleispira* and few others belong to the obligate hydrocarbon clastic bacteria (OHCB). Hydrocarbons and their derivatives, not only include solid, liquid and gaseous fossil carbon deposits, but also compounds of biological origin, such as lipids and fatty acids from plants, animals, and microbes. The products of their conversion in anoxic zones are ubiquitous in the biosphere, though highly heterogeneous in type and concentration, and in time and space. Given the high carbon content available for biomass production, and the high energy content of such highly reduced compounds, it is hardly surprising that many microbes have evolved or acquired the ability to utilize hydrocarbons as sources of carbon and energy. More than 250 Alcanivorax-affiliated bacteria have been isolated or detected as 16S rRNA gene sequences in all types of marine environment: surface water, shallow and deep-sea water bodies, sediments, hydrothermal vents and mud volcanoes, ridge flank crustal fluids and gray whale carcass. Interestingly, although *Alcanivorax* related 16S rRNA gene sequences have been retrieved from microbial communities inhabiting cold polar areas, the organism itself has so far only been isolated from more temperate latitudes. Despite these qualifications about experimental approaches, it is clear that oil hydrocarbon degradation in marine systems is carried out by microorganisms belonging to a relatively small group of genera, and that there are certain important differences in the compositions of oil-degrading communities at high and low latitudes that need to be

considered when developing potential mitigation strategies to combat oil pollution in marine systems (Yakimov *et al*., 2007).

5.4 Mn mobilization/immobilization in terms of bacterial population

5.4.1 Mn Activity with microbial community

In the present study, we demonstrated the Mn activity (Mobilization /Immobilization) in microcosm environment in terms of bacterial population in ridges. This activity was performed in laboratory conditions with the indigenous microbial community and potential bacterial isolate for Mn and Cu.

The deep-sea hydrothermal plumes at CR exert an influence on the geochemical characteristics of the water column and support unique forms of microbial life (Quadfasel *et al*., 1997; Ray *et al*., 2012). They thereby offer favorable conditions for the growth and activity of metal immobilizing/mobilizing microorganisms. The concentration of Mn in basalt used in the present study varied between 5.9% and 28.76%. These values are in agreement with the range of those reported (25.5–65.2%) from the Galapagos spreading axis (Moore and Vogt, 1976). The lower enrichment of Mn in the CR samples compared to that found in the Galapagos could be attributed to the greater distance of the sampling point from the potential volcanic sources. Also, we observed a large number of Mn-oxidizing bacteria associated with the Mn-oxide coatings on basalt surfaces. The culturability of Mn-oxidizing bacteria from these samples equaled that of general heterotrophic bacteria. Their colony characteristics except for the color were similar on medium amended and unamended with Mn. These results are on par with the reports of Ehrlich *et al*. (1972) and Schutt and Ottow (1978) on the culturability of Mn-oxidizing bacteria from manganese nodules.

The mobilization of Mn was evident despite the non-detectable number of reducers on Mn-oxide plates. Of the four representatives Mn (II)-oxidizing bacterial isolates, one was positive for Mn-mobilization. These observations suggest that mobilization of Mn from the basalt surfaces could be mediated by bacteria that could either oxidize Mn (II) or reduce Mn-oxide, depending on the ambient conditions. The current results agree with the report of Bromfield and David (1976) on the mobilization of Mn from Mn-oxide by *Arthrobacter sp*. that had the ability to either mobilize or immobilize Mn, depending on conditions. Furthermore, Thamdrup (2000) reported different strains of Mn and Fe oxidizing *Bacillus* sp. that were able to reduce oxidized forms of Fe and Mn under anaerobic conditions. Similarly, de Vrind *et al*. (1986) reported Mn-oxide reduction by the vegetative cells of Mn-oxidizing *Bacillus* SG-1. The different bacterial genera so far known to reduce Mn-oxides include *Bacillus, Deferribacter, Thermoanaerobacter, Fervidobacterium, Desulfovibrio, Shewanella, Achromobacter, Enterobacter and Salmonella sp.* (Ehrlich, 1980; Pak *et al*., 2002; Das *et al*., 2011).

The bacterial communities living in the basaltic environment may, therefore, shift to high-affinity transport systems (Geesey and Morita, 1979) as they contain no apparent energy reserves (lipids and poly-b hydroxybutyrate) required for the maintenance of life (Morita, 1988). The nutritional requirements of such microbes are met by the production of suitable exoenzymes or depend upon syntrophism for their energy needs. In our study, the bacterial isolates expressed multiple exoenzymes with different growth substrates and also oxidized Mn. It is suggested that bacteria associated with the basalt surfaces are highly efficient in adapting to the nutrient poor environment and in meeting the metabolic requirements. Our results agree with that of Lorenz *et al*. (2006) and De Souza *et al*. (2006) on the tolerance of bacteria to heavy metals and their ability to express

multiple enzymes. Under low nutrient availability, bacteria lose the ability to multiply but energy-yielding mechanism tends to remain intact (Novitsky and Morita, 1976). Such an adaptation for nutrient concentrations was observed in the current study under experimental conditions. The onset of growth was faster in 'G-' and slower in 'G+' media. The faster growth in 'G-' could be because the laboratory conditions simulated the natural conditions more in terms of temperature and ambient organic carbon concentration (glucose). In contrast, the delay in the onset of growth with 'G+' could be the adaptation period required by those organisms in a non-energized state. The higher mobilization of Mn in the presence of glucose could have been because the active cells were chemotactically more active than the non-energized cells (Torrella and Morita, 1981).

The oxidation of glucose by cultured bacteria from basalts further confirms that microbial cells indigenous to the rock surface couple the oxidation of glucose to the reduction of Mn-oxide. Our results are in agreement with that of Roh *et al*. (2002) in which the utilization of glucose by heterotrophic bacteria as an electron donor for the reduction of Mn oxide was observed. Our results are also on par with Madgwick (1987) and Baglin *et al.* (1992) regarding the solubilization of Mn from ores. Although the pH of seawater is typically in the range of 7.5–8.4, a decrease in pH may occur during bacterial growth under experimental conditions. The drop in pH is strongly influenced by the growth of bacteria (Li *et al*., 2006) and facilitates the release of metal cations by promoting an increase in the solubility of metal oxides (Burkhardt *et al*., 2011). Following incubation, the pH of the media in the present study decreased from 8.1 to 7.2 in the 'G+' and from 7.9 to 7.2 in the 'G-'. Our results agree with Russin and Ehrlich (1995) on Mn-oxide reduction by bacteria in the pH range of 6–8. This reduction could be significant because the bacterial groups associated with basalt participate in the

cycling of Mn and vary in their tolerance to different pH levels and metal concentrations. The redox cycling of Mn could also be bacterially mediated under optimal Eh conditions (Webb *et al*., 2005) because of the general relationship between pH and Eh (Pareuil *et al*., 2008) wherein the growth and metabolic activity of bacteria either increases or decreases the pH of the surrounding. The increase in H+ activity (drop in pH) leads to Eh decrease and consequently to the increased mobilization of metallic elements and vice versa with decrease in H+ and an increase in Eh (DeLaune and Reddy, 2005; Pareuil *et al*., 2008). In our study, the Eh varied between +117 to -116 mV in media with added glucose and +137 to -118 mV in media without added glucose. The Eh values indirectly show that some portions of Mn and/or Fe compounds are present in their reduced state and a large portion in their oxidized or bound state. The results most likely suggest that bacteria present in the optimal pH and Eh range are responsible for the Mn-oxide reduction. Bacteria exhibit morphological variation in response to heavy metal stress, nutrient deprivation and excess nutrient availability (Shehata and Marr, 1971; Amy and Morita, 1983; Antony *et al*., 2011). Low metal concentrations i.e., <10 μM stimulate bacterial growth (Sujith *et al*., 2010) but exert a toxic effect on them at higher concentrations (Antony *et al*., 2011). Some bacteria adapt to high metal concentrations just by merely decreasing their cell surface area, as, for instance, by increasing their cell size and by forming cell aggregates (Sujith *et al*., 2010; Antony *et al*., 2011). In the present study, the cells that were tiny and coccoid in shape on the initial day of incubation became long slender rods in medium with added glucose at the end of incubation. However, there was no variation in size or change in morphology of cells in medium without added glucose. These observations are in line with that of Shehata and Marr (1971) with respect to an increase in bacterial size and numbers during growth in the presence of 0.1% glucose. Further, the presence of EPS associated with the bacterial

cells on medium amended with glucose explains the ability of bacteria to withstand heavy metal stress and promote Mn dissolution. The EPS or slime layers have been shown earlier to help bacteria in attaching to the surfaces, alleviating the toxic effect of metals and in the dissolution of minerals (Geesey and Jang, 1989; Vandevivere and Kirchman, 1993). Following incubation, EDS analysis of the basalt surfaces showed the loss and gain of elements in addition to colonization by abundant rod-shaped bacteria in the experiment relative to the controls. It thus indicates the direct participation of bacteria in the mobilization of Mn from the basalt surfaces. The mineralogy of the Mn-oxide coatings on basalt showed characteristic peaks for Mn minerals todorokite and birnessite apart from background peak for halite. The present results are in agreement with the reported mineralogy of Mn-oxides from the mid-ocean ridge systems (Rao and Pattan, 1989). The identification of culturable bacteria by 16S rDNA method showed taxonomic affinities to *Bacillus, Exiguobacterium, Staphylococcus, Brevibacterium and Alcanivorax sp.* Among them, *Bacillus* represents the predominant genus, which signifies its importance in the redox cycling of Mn in the CR ecosystem. Our results are also in agreement with the reports of Rusin *et al*. (1991), Ehrlich (1993) and Boone *et al*., (1995) on the capacity of *Bacillus* to reduce Mn and Fe under aerobic and anaerobic conditions. The induction of MnO_2-reductase system in *Bacillus* by Mn^{2+} (Trimble and Ehrlich, 1970; Chandramohan *et al*., 1987) further explains its importance in controlling the redox speciation of Mn. The lack of detection of other known Mn reducing bacterial groups may not indicate their absence in our samples but may have been due to the lesser abundance or lack of some required nutrients in the culture medium employed. However, *Exiguobacterium sp*. a member of the Firmicutes was found associated with the Mn-oxide surfaces of basalt. Apart from Mn (II)-oxidation, this strain exhibited the property of Mn-oxide reduction under laboratory conditions in the present study. Their occurrence

has also been reported from the oligotrophic seas to the deep-sea hydrothermal vents (Vishnivetskaya *et al*., 2009). They could be candidate organisms for catalyzing the oxidation/ reduction of Mn in the ridge ecosystem because of their potential for tolerating and reducing heavy metals in solution (Okeke, 2008). Members of the genus *Staphylococcus* sp. show a diverse spectrum of metabolic activities, which is indicative of the ability to adapt to various environmental niches. They are efficient members in the mobilization of Mn from low grade ores (Das *et al*., 2012). They also contain the enzymes manganese superoxide dismutase and urease with Mn and Ni as the cofactors respectively (Horsburgh *et al*., 2002). On the other hand, *Brevibacterium* has a specific requirement of Mn for the activity of the enzyme ribonucleotide reductase (Willing *et al*., 1988). It is efficient in energy dependent Mn uptake (Schmid and Auling, 1987). The metabolic flexibility and the versatile nature of these bacterial isolates therefore could contribute to the biogeochemical cycling of redox sensitive elements like Mn/Fe and thereby the fate of several other elements in the ridge ecosystem. The occurrence of *Alcanivorax sp.* a marine petroleum oil-degrading bacterium known for alkane metabolism (Sabirova *et al*., 2006) in the CR environment may be due to their role in Mn cycling as was reported in Arctic sediments (Xuezheng *et al*., 2008). The results of the present study suggest that the Mn (II)-oxidizing bacteria associated with basalt from the CR actively participate in promoting Mn-accretion at ambient concentrations of organic carbon and Mn-mobilization under conditions of organic carbon enrichment. This could be bio-geochemically significant because the redox speciation of Mn controls the fate of several other associated elements in the ridge ecosystem by consuming or releasing electrons or protons. Experiments conducted under microaerophilic and anaerobic conditions would throw more light on particle associated bacterial contribution from the water column above to the redox changes in Mn chemistry in ridge ecosystems.

5.4.2 Mn Activity of potential isolate

The deep-sea hydrothermal plumes at CR wield an influence on the water column characteristics and support unique kind of microbial life (Quadfasel *et al*., 1997; Ray *et al*., 2012). These geochemical characteristics of water column and deposition of the plume on sea surfaces offer encouraging conditions for the growth and activity of metal immobilizing/mobilizing microorganisms. In the present study Mn concentration in basalt was reported around 6.2 to 26.96%, which is similar to the previous reports (25.5 to 65.2%) from the Galapagos spreading axis (Moore and Vogt, 1976). In present samples, Mn concentration was observed to be lower than Galapagos due to more distance of the sampling point from the potential volcanic sources. Fernandes *et al*. (2005) and Sujith *et al*. (2014) observed a large number of Mn oxidizing bacteria allied with the Mn-oxide coatings on basalt rock surfaces. The culturability of Mn oxidizing and general heterotrophic bacteria from these samples showed equal kind of physical characters except for the color on medium amended and unamended with Mn. R6 isolated from the same area on Mn amended media plate. The same Mn oxidizing bacteria R6 was used in the present study for Mn mobilization in laboratory condition to solve the purpose of Mn biomining from deep sea ridge basalts. The mobilization of Mn was shown on Mn-oxide plate when R6 reduced Mn oxide and developed a zone around the bacterial colony. So, these observations suggest that mobilization of Mn from the basalt surfaces could be mediated by bacteria that could either oxidize Mn (II) or reduce Mn-oxide, depending on the ambient conditions. The present results follow the report of Bromfield and David (1976) on Mn mobilization from Mn-oxide by *Arthrobacter sp*. that had the ability to either mobilize or immobilize Mn, depending on conditions. Furthermore, Thamdrup (2000) reported different strains of Mn and Fe oxidizing *Bacillus* sp. and Mn-oxidizing *Bacillus SG1* which were able to reduce or oxidized metal

under anaerobic conditions. Mn-oxides reduction is reported by different bacterial genera includes Bacillus, *Deferribacter, Thermoanaerobacter, Fervidobacterium, Desulfovibrio, Shewanella, Achromobacter, Enterobacter Salmonella and Halotolerance sp* (Ehrlich, 1980; Pak *et al*., 2002; Das *et al*., 2011; Sujith *et al*., 2014).The bacterial communities present in the basaltic environment may transfer to high-affinity transport systems (Geesey and Morita, 1979) as they do not contain any energy reserves (lipids and poly-β hydroxyl butyrate) essential for the life maintenance (Morita, 1988). So, nutritional requirements of such microbes depend on exoenzymes production or depend upon syntrophism for their energy needs.

In above study bacterial isolate, R6 expressed multiple exoenzymes with different growth substrates and also oxidized Mn. It directly indicates R6 having high efficiency to adapt nutrient poor environment and meeting the metabolic requirements. Our results favoring the work of Lorenz *et al*. (2006) and De Souza *et al.* (2006) towards the bacterial tolerance to the heavy metals and their ability to express multiple enzymes. Novitsky and Morita (1978) suggested that in low nutrient availability, bacteria may lose the ability of multiplication but energy-yielding mechanism tends to remain intact. Our results followed the same pattern as G- shows higher onset growth than G+ in media may be due to the laboratory conditions mimicked the natural conditions to some extent more in terms of temperature and ambient organic carbon concentration (glucose). In contrast, organisms in 'G+' media may required adaptation period in a non-energized state. While in presence of glucose R6 became chemotactically more active than the non-energized cells present in media without glucose this could be directly responsible for higher mobilization of Mn (Torrella and Morita, 1981). Glucose oxidation by R6 in presence of basalt leads to the reduction of Mn-oxide which directly supports Roh *et al*., (2002) theory of glucose utilization by heterotrophic bacteria as an electron donor for the

reduction of Mn oxide proposed by Madgwick (1987) and Baglin *et al*. (1992) with reference to the solubilisation of Mn from ores. Although the pH of seawater ranged from 7.5 to 8.4 and pH decrease may occur during bacterial growth under experimental conditions. The pH drop is strongly influenced by bacterial growth (Li *et al*., 2006) and influence the release of metal cations by promoting an increase in the solubility of metal oxides (Burkhardt *et al*., 2011). In the present study, the pH decreased from 8.1 to 7.4 in the 'G+' and from 7.9 to 7.4 in the 'G-' in media. Our results support Rusin and Ehrlich (1995) on Mn-oxide reduction by bacteria in the pH range of 6 to 8. Here R6 shows significant reduction associated with basalt and participate in the cycling of Mn vary in their tolerance to different pH levels and metal concentrations. Bacteria also affects or mediates redox cycling of Mn under optimal Eh conditions (Webb *et al*., 2005) because of the general relationship between pH and Eh (Pareuil *et al*., 2008). In bacterial growth and metabolic activity either pH of surroundings either increases or decreases. Mobilization of metallic elements directly influenced by the increase in H+ activity (drop in pH) which leads to Eh decreases (DeLaune and Reddy, 2005; Pareuil *et al*., 2008).

In our study, the Eh varied between +117 to - 116 mV in media with added glucose and +137 to -118 mV in media without added glucose. Our results support the Mn-oxide reduction by bacteria in the optimal pH and Eh range. The Eh values indirectly indicate a lower amount of reduced Mn and/or Fe compounds and higher amount of oxidized or bounds state compounds in basalt. Heavy metal stress, nutrient deprivation, and excess nutrient availability directly influence morphological variation in bacteria (Shehata and Marr, 1971; Amy and Morita, 1983; Antony *et al*., 2011). Sujith *et al*. (2010) and Antony *et al.* (2011) reported that low metal concentrations i.e., <10 µM stimulate bacterial growth but higher concentrations may lead to toxicity. R6 shows the similar results, reported by Sujith *et al.* (2010) and Antony *et al.* (2011) that some

bacteria decrease their cell surface area to adapt to higher metal concentrations for instance whereas some increase their cell size by forming cell aggregates. In this study, R6 bacteria were very tiny coccoids on the initial day of incubation which subsequently became long slender rods in medium with added glucose at the end of the incubation. There was no change in cell size and shape in medium without added glucose. These observations are supported by those of Shehata and Marr (1971) in which they observed an increase in bacterial size and numbers during growth in the presence of 0.1% glucose. EPS secretion and association with the bacterial cells in medium indicates metal stress tolerance and promote Mn dissolution. Similar results were shown by R6 in SEM analysis. R6 bacteria withstand heavy metal stress in the presence of medium amended with glucose. The secretion of EPS or slime layers helps bacteria in attaching to the surfaces, dissolution of minerals and in the alleviating the toxic effect of metals (Geesey and Jang, 1989; Vandevivere and Kirchman, 1993). Following incubation, EDS analysis of the basalt surfaces explains the elemental loss and gain in addition to colonization by R6 bacteria in the experiment relative to the controls. It showed the direct participation of R6 bacteria in the mobilization of Mn from the basalt surfaces. The mineralogy of the Mn-oxide coatings on basalt showed characteristic peaks for Mn minerals todorokite and birnessite apart from background peak for halite. The present results support the reported mineralogy of Mn-oxides from the mid-ocean ridge systems (Rao and Pattan, 1989 and Gitanjali, 2015).

The identification of culturable bacteria R6 showed taxonomic affinities to *Imtechela halotolerance*. The present study suggests that the Mn (II)-oxidizing bacteria R6 from the CR actively participate in promoting Mn-accretion at ambient concentrations of organic carbon. It also describes Mn-mobilization under conditions of organic carbon enrichment. This could be bio geochemically significant because of the

redox speciation of Mn controls and the fate of several other associated elements in the ridge ecosystem by consuming or releasing electrons or protons. Experiments conducted under microaerophilic and anaerobic conditions would throw more light on particle associated bacterial contribution from the water column above the redox changes in Mn chemistry in ridge ecosystems.

5.5 Cu mobilization/immobilization in terms of bacterial population

5.5.1 Cu Activity with microbial community

The hydrothermal magma at CR (Ray *et al*., 2012) creates influence on the geochemical characterization of the water column and microbial diversity. They are a favorable site for the growth and activity like metal immobilizing/mobilizing microorganisms. During the present study, the concentration of Cu in basalt varied between 0.20 to 0.34%. Basalt's copper concentration was reported around 116 ppm (Prinz, 1967), 100 ppm (Vinogradov, 1962) 88 ppm (Wedepohl, 1962) and 87 ppm (Turekian and Wedepohl, 1961). During the present study, it was reported 57.12 to 288.69 mg g^{-1}. This study also highlighted the presence of Cu oxidizing bacteria those associated with basalt surfaces in ridges. The heterotrophic bacterial colony and bacterial colony appeared to be similar in both Cu amended and unamended medium except showing variation in color. Similarly, Ehrlich *et al*. (1972) observed in Mn-oxidizing bacteria from manganese nodules.

CuR1 isolates were positive for Cu-mobilization and used for Cu sequestration study in a partial experiment where isolate morphology changes and their size increases from 0.50 to 5±1 µm as observed under SEM. In the presence of glucose, increased cell size from 0.50 to 6.0 ±1 µm was observed whereas some cells showed ruptured cell wall. However, cell size in absence of glucose increased from 0.50 to 4±1 µm without any

rupturation. EPS production was also observed in both media with and without glucose. Similar findings were reported by Sujith *et al*. (2011) and Antony *et al*. (2011) showing CR-35 and CR-48 isolates for Ni and Co accumulation. These observations strongly reveal that Cu mobilization from the basalt surfaces was influenced by bacteria that can be either oxidize Cu(I) or reduce Cu-oxide, depending on surrounding environment. In Cu mobilization experiment, bacteria showed morphological changes in response to metal stress, nutrient deficiency and overload nutrient accessibility (Amy and Morita, 1983; Antony *et al*., 2011). Metal concentrations up to 10 µM encourage bacteria growth (Sujith *et al*., 2010) but higher concentrations showed a toxic effect on bacterial isolates (Antony *et al*., 2011). Few bacteria have a tendency to forming cell aggregates by growing their cell size and decrease their cell surfaces in order to protect them from higher metal toxicity (Sujith *et al*., 2010; Antony *et al*., 2011). In this study, bacteria were the small and coccoid shape on the initial day of incubation and at the end of incubation bacteria became long slender and rod-shaped in presence of glucose.

Bacteria increase in size and numbers during experimental incubation in the glucose added medium. No notable changes occurred in bacterial cells' morphology and size without added glucose during the experiment. SEM showed, in added glucose medium bacterial cells produce EPS which reveals that the bacterial resistance to metal stress and support metal dissolution. The EPS or slime layers provided a base for bacterial attachment to surfaces, alleviating the toxic effect of metal and dissolution of minerals (Vandevivere and Kirchman, 1993). Seawater pH is typically reported in the range of 7.5 to 8.4, notable pH decrease occurs throughout bacterial growth under experimental environment. The pH drop is positively inclined by bacterial growth (Li *et al*., 2006) and facilitates the metal cations discharge by promoting enhancement of the mobilization of metal oxides (Burkhardt *et al*., 2011).

Following incubation, the pH was observed in the range of 8.1 to 7.2 with added glucose and from 7.9 to 7.2 without added glucose in the medium. Our results are supported by that of Rawlings (2005) and Rusin and Ehrlich (1995) in which they observed the bacterial reduction of Cu ore and Mn-oxide in the pH range of 6 to 8. The basalt associated bacterial groups play role in Cu dissolution by redox cycling of Cu on different pH and Eh level and metal concentration. So, pH and Eh relationship (Pareuil *et al.,* 2008) is a responsible factor which directly increases or decreases due to bacterial growth and their metabolic activity. Thus, increase in H+ activity (drops in pH) is reported inversely proportional to Eh (DeLaune and Reddy, 2005; Pareuil *et al*., 2008). In the present analysis, with added glucose, Eh varied between +117 to -116 mV and without glucose from +137 to -118 mV which followed reports given by Gadd (2000) on mobilization of Cu from Ferromanganese oxide by *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* that showed the ability to either reduction or oxidation of Cu, depending on the environment.

This study shows significant reduction because the bacterial groups associated with basalt plays a vital role in the Cu cycling and differ in their metal tolerance to diverse pH levels and metal concentrations. Our results are in agreement with the observations of Mercier (1999) in which Mn and Fe oxidizing *Bacillus sp.* reduced oxidized forms of Fe, Mn, and Cu. Bacterial genera like *Bacillus, Shewanella, Fervidobacterium, Thermoanaerobacter, Desulfovibrio, Enterobacter, and Salmonella sp.* are reported for basalt and ferromanganese reduction (Pak *et al*., 2002; Das *et al*., 2011). Morita (1988) reported that energy forms (lipids and poly-β hydroxyl butyrate) required for the metabolic activities are absent in basalt bacterial community which leads to transformation in high-affinity transport systems. So, such bacteria fulfill their nutritional need by the production of required exoenzymes. In this study, bacteria

supports above results by producing various exoenzymes with substrates and also immobilized Cu. Our results are directly supported by that of Sujith *et al.* (2014), Lorenz *et al*. (2006) and De Souza *et al*. (2006) regarding bacterial heavy metals tolerance and their capability to express various enzymes. In low nutrient, environment bacteria lose their multiply capacity but energy-yielding system tends to remain integral (Novitsky and Morita, 1978). In this study, bacteria expressed hydrolytic enzyme characters which suggests that bacteria in basalt rocks are highly capable to adapt the low nutrient environment to meet their metabolic activities (Gonsalves *et al.,* 2005). Same results were observed in microcosm investigation in the presence (G+) and absence (G-) of nutrient (0.1% glucose). During incubation, bacteria show faster growth rate in 'G-' and slower in 'G+' media which may be because of laboratory condition which mimic the natural surroundings in terms of organic carbon concentration (glucose) and temperature. The mobilization of Cu was higher in the added glucose media which could be because the active bacteria were chemotactically more energetic than the non-energized cells (Torrella and Morita, 1981). Our results followed the observations of Roh *et al*. (2002) where glucose utilization by heterotrophic bacteria as an electron donor for the metal oxide reduction and Bausciker (1999) and Baglin *et al*. (1992) for the metal dissolution from ores. The identification of culturable bacteria by 16S rDNA method showed taxonomic affinities to *Bacillus aryabhattai, Erwinia rhapontici, Sinomicrobium oceani, Halomonas rifensis, Idiomarina abyssalis,* and *Marinobacter algicola.* Among them, *Bacillus* sp. signifies its important role in the redox cycling of Cu in the ridge ecosystem. Present results followed the reports of Ehrlich (1997) for the capacity of Fe-Mn oxide and Cu reduction by *Bacillus* under aerobic and anaerobic conditions. *Exiguobacterium* sp was found on the basalt surfaces in rock sample also reported from the oligotrophic marine environment from hydrothermal vents (Sujith *et al*., 2014).

These bacteria could be catalyzing representatives of Cu oxidation/reduction in the ridge ecosystem because of heavy metal tolerance potential (Sujith *et al*., 2014). *Staphylococcus* sp. shows a diverse continuum of metabolic routes, which reduces the ability to adapt diverse environmental groups. *Staphylococcus* sp. has efficient potential to mobilize Cu from low-grade ores (Singh *et al*., 2011). *Brevibacterium* sp. and *Pseudomonas aeruginosa* AT18 shows the precise requirement of Cu for the action of the enzyme oxidoreductive (Wang *et al*., 1997). Sujith *et al*. (2014) reported similar results which showed isolated bacterial strains resourceful nature and metabolic flexibility that could contribute in the biogeochemical redox cycling of sensitive metals like Cu/Fe and thereby the chance of other elements in the same environment. A series of heterotrophic microorganisms (bacteria, fungi) is also part of microbial bioleaching communities. This group of organisms uses extracellular metabolites and cell lysates from autotrophs as carbon source resulting in the removal of an inhibitory excess of carbon and stimulating, therefore, growth and iron oxidation of *Thiobacillus* (Butler and Kempton, 1987; Fournier *et al*., 1998). In addition, several heterotrophs can also contribute to metal solubilization by the excretion of organic acids such as citrate, gluconate, oxalate, or succinate.

The present study suggests Cu (II)-oxidizing bacteria in ridge rocks or basalts from the CR actively participate in Cu mobilization and accretion at ambient concentrations of nutrient and organic carbon. This could be bio geochemically significant because the rate of mobilization was observed higher side without any chemical pollution to the environment. Microaerophilic and anaerobic experiment setup would more successful to get higher mobilization rate of pure metal.

5.5.2 Cu Activity with potential isolate

Cu mobilization/immobilization by potential bacterial isolate was performed in laboratory microcosm environment to produce a picture of natural ridge ecosystem. Basically, bacteria perform mobilization/immobilization by two mechanisms reported by (Ewart and Hughes, 1991; Silverman and Ehrlich, 1964): (1) Microorganisms can oxidize metal sulfides by a direct mechanism by obtaining electrons directly from the reduced minerals. Cells have to be attached to the mineral surface and a close contact is needed. The adsorption of cells to suspended mineral particles takes place within minutes or hours. This has been demonstrated using either radioactively labeled *Thiobacillus ferrooxidans* cells grown on $NaH_{14}CO_3$ or the oxidative capacity of bacteria attached to the mineral surface (Escobar *et al*., 1996). Cells adhere selectively to mineral surfaces occupying preferential irregularities of the surface structure (Edwards *et al*., 1999; Ewart and Hughes, 1991). In addition, a chemotactic behavior to copper, iron, or nickel ions has been demonstrated for *Leptospirillum ferrooxidans* (Acuna *et al*., 1992) and *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* (Acuna *et al*., 1992). Same results were produced by potential bacterial isolate CuR1 when we treated them with different concentration of Cu metal.

SEM & EDS was performed to investigate morphological changes and Cu accumulation inside cells in Cu exposed and unexposed condition. Cells grown in seawater without any nutrient or Cu amendment did not show any morphological variation after incubation of 7 days (Fig 4.45 A, B). The average cell size (l×w) of CuR1 was from 1.42 ±0.2×1.10±0.10 µm. Bacterial cells were grown in 100 µM Cu showed healthy dividing cells (Fig 4.45 C), but average cell size increases to 2.02±0.38 to 3.58±0.10 µm. At 1000 µM Cu concentration, increase in cell size without morphological changes and subsequent rupture was observed (Fig 4.45 D). At the same time at 10,000 µM Cu, cells increase in size, change their morphology and ruptured due

to very high metal sequestration and cells size of CuR1 showed variation range from 1.37±0.42 to 3.02±0.24 μm in presence and absence of glucose (Fig 4.45 E, F). EDX analyses performed for cells grown without Cu in medium showed peaks for calcium, sodium, potassium, and oxygen. Cu (1000 μM) exposed bacterial cells showed peaks for Cu with a decrease in intensity of the peak for Oxygen and loss of K peak that indicates cation efflux in CuR1. This confirms that bacterial cells have sequestered the metal. The present study also supports the observation and theory proposed by (Ewart and Hughes, 1991) for iron mobilization/immobilization from ore, according to the above theory - The oxidation of reduced metals through the "indirect" mechanism is mediated by ferric iron originating from the microbial oxidation of ferrous iron compounds present in the minerals. Ferric iron is an oxidizing agent and can oxidize, e.g., metal sulfides and is (chemically) reduced to ferrous iron which, in turn, can be microbially oxidized again, In this case, iron has a role as an electron carrier. It was proposed that no direct physical contact is needed for the oxidation of metal with bacteria.

Mobilization/immobilization experiment performed with a potential isolate that produced significant results. Bacterial isolates produces EPS during metal mobilization/immobilization experiments this results directly supported by Escobar *et al*. (1997), Gehrke *et al*. (1998) reports where the formation of extracellular polymeric substances plays an important role in the attachment of *Thiobacillus* to mineral surfaces such as, e.g., sulfur, pyrite, or covellite. Extraction or loss of these exopolymers prevents cell attachment resulting in decreased metal leaching efficiencies. It was concluded that a direct contact between bacterial cells and solid surfaces is needed and represents an important prerequisite for an effective metal mobilization (Ostrowski and Sklodowska, 1993).

5.6 Foraminifera in ridges

In the present study, an attempt was made to identify the foraminiferal diversity in Carlsberg ridge sediments. Muruganantham & Mohan (2015) reported for the first time the living benthic foraminifera *Nevillina coronata* (Millet, 1898), *Sigmoihauerinain volute* (Cushman, 1946) and *Loxostomina limbata* (Brady, 1881) from the inner shelf regions of Andaman Islands, India. Kroon *et al*. (1991) revealed the abundance of *Globigerina bulloides* in the upwelling environment while in this study, the same foraminifera is found in deep sea ridges which indicate the wide distribution of this species.

Clague *et al*. (1984) found that agglutinated foraminiferal species dominated most of the dredge samples from the northern part of Gorda Ridge (Escanaba Trough). Jonasson *et al*. (2005) described benthic foraminiferal distribution in Juan de Fuca Ridge of northeast Pacific Ocean, and described species of the family Hormosinidae, subfamily Reophacinae, as a dominate in the hydrothermal habitat because of their success at colonization. Thomas *et al*. (2000) described that the distribution of benthic foraminiferal fauna in Long Island Sound (LIS) are less diverse and are dominated by *Elphidium excavatum clavatum* species of the genus *Elphidium* and also revealed their distribution is positively correlated with depth. Guimerans and Currado (1999) reported the distribution of foraminifera in relation to water depth, sediment texture, and sedimentation rate, whereas Bandy (1954) stated temperature as the main factor to determine the different faunal zones at 100 m depth for benthic foraminifera. In this study, we report benthic foraminifera from Carlsberg ridges which can directly or indirectly contribute to the information on the previous environment of these mountain ranges. In this type of extreme ecosystem, Foraminifera plays an important role in higher

trophic levels in the absence of light and organic matter in environments that are highly rich in metals due to volcanic activities. Deep sea foraminifera, by virtue of their often large standing stocks and high resistance to the extreme environment, are important key factors in the food chain of many invertebrates. This study supports the hypothesis that there is a close trophic link between microphytobenthos and foraminifera in the transfer of organic carbon towards higher trophic levels in ridge sediments. Therefore foraminifera play a key role in the rapid transfer of autotrophic carbon to higher trophic levels and can get recycled after death. A better understanding of the global carbon cycle will be required to have an improved quantitative knowledge of carbon transfer within such extreme environments.

SUMMARY

6 Summary and Future scope

The present work, submitted under the title **"Studies on the abundance and diversity of manganese and copper tolerant bacteria in ridge ecosystem and their role in metal uptake"**, incorporates the results of studies carried out to understand the diversity of Foraminiferans and metal tolerant bacteria of the Carlsberg Ridges. It would also endeavour to improve the cultivability of bacteria and delineate their role under in vitro conditions.

The Study area is the Carlsberg Ridge situated in the Arabian Sea, a segment of the Central-Indian Ridge. Sediment and water samples were collected from 12 different locations in study area on board R.V. ABP 37 and O. R.V. Sagar Nidhi-48 as a part of "Tectonics controls and hydrothermal processes along the slow spread mid-ocean ridges and Andaman trench-backarc system, Indian Ocean" program O. R.V. Sagar Nidhi 48 was undertaken during the austral winter of November-December.

Several environmental parameters of the study sites such as temperature, depth, pressure, pH and Eh, dissolved Oxygen, salinity and Total organic carbon were recorded. Microbiological parameters of sediment and water samples such as bacterial counts, total counts, Culturable Heterotrophic Bacteria, Cu and Mn oxidizers, Retrieval counts of Heterotrophs and their phylogeny were analysed. Bacterial identifications were carried out using various biochemical and physiological tests. Phylogenetic diversity of bacterial isolates was carried out using 16S rRNA. Metabolic diversity of bacterial isolates were examined for

gram-stain reaction and tested for motility, catalase, oxidase and DNase activity. The isolates ability to elaborate amylase, DNase, lipase, phosphatase, and protease were checked in nutrient medium amended with starch (Amy), DNA (Dna), tween 80 (Lip), p-nitrophenyl phosphate (Phos) and casein (Protein) as substrate.

Quantification of Mn and Cu in Water samples using voltammetry. Spectroscopy of sediments and water samples was carried out. Scan Electron microscopy conjugate with Electron dispersion spectra (SEM/EDS), X-ray diffraction (XRD) analysis were undertaken.

The bacterial samples bio mass were observed using light Microscope (Olympus CKX41 manufactured in Japan) (Muruganantham & Mohan 2015). Cell morphology and intracellular metal accumulations were also studied.

Microcosm experiments were carried out to determine the Mobilization/Immobilization rate of Mn/Cu in natural ridge rock by the indigenous microbial community, potential bacterial isolates and indigenous bacterial communities.

Benthic Foraminiferans collected from these sites were preserved and analysed. They were observed under a Binocular Stereoscopic Microscope as well as under Scanning electron microscope and identified. Bacteria associated with these foraminiferal samples were also analyzed.

6.1 Following points presented here emphasize the major findings of each of the objectives studied:

I. First objective is to assess the abundance and diversity of Mn and Cu tolerant bacteria in water, rock and fauna of the ridge ecosystems.

This includes the results on the physical parameters. Temperature of rock/sediment samples observed on board varied from 6 to10°C while water samples varied between 1.5 to 8°C. The pH and Eh of water samples were observed to be in the range of 5 to 8 and +150 to -370 mV respectively in rock samples. The oxygen concentrations in water columns in ridges varied from 3 to 5mg L^{-1}.

1. Under the microbiological parameters, culture-dependent bacterial abundance and diversity from the hydrothermal vent basalt and water columns were addressed. Total counts (TC) of rock samples varied from 2.04E+05 to 3.30E+05 in water samples and 1.12E+06 to 3.48E+06 mL^{-1} in rock samples.

2. With regard to Retrieval counts (RC) on rock samples, heterotrophic, Mn oxidizers and Cu oxidizers ranged from 2.76E+03 to 1.22E+03, 1.53E+03 to 1.88+03 and 9.69E+03 to 3.78E+03 respectively.

3. RC observed in water samples for Heterotrophic, Mn oxidizers and Cu oxidizers ranged from 2.30E+03 to 1.46E+04, 1.45E+04 to 3.00E+03 and 8.00E+02 to 2.00E+02 $gram^{-1}$ respectively.

II. Second objective is to assess the faunal diversity of Carlsberg ridge ecosystem and the associated bacteria.

1. Analysis of Culture-dependent bacterial diversity was analyzed by 16S rRNA gene technique, resulted in 76 distinct phylotypes exclusively from the vent. *Proteobacteria* (α, β, and γ) was observed as a dominant phylum followed by *Firmicutes,* and *Actinobacteria.*

2. In this study, more than 5 novel bacterial taxa which could be potential bacteria for bioremediation have been reported. Bacteria isolated and identified from benthic foraminifera morphotypes have been reported.

3. Benthic foraminifera were identified based on their morphotypes. 8 morphotypes were isolated, out of which 5 were identified as *Spirillina guttata, Lagena hexagona, Trochammina inflate, Globigerina bulloides, Globocassidulina cabalisuturata* while the remaining three unidentified species were also documented.

4. *Spirillina guttata* (n=323) was the dominant foraminifera among three sampling ridges followed by *Globigerina bulloides* (n=264) whereas *Lagena hexagona* (n=48) was the least occurring species.

5. The Bacterial isolates procured from sediment and water samples are as follows: R1: *Bacillus oceanisediminis* H2 (T), (GQ292772) was isolated from ridge rock. Cells are Gram-negative, motile and small rod-shaped.

   R2: *Exiguobacterium indicium* HHS31 (T), (AJ846291) was isolated from ridge rock. Cells are Gram-negative, motile and small rod-shaped.

   R3: Bacillus aryabhattai B8W22 (T), (EF114313) was identified as ridge rock isolate. Cells are Gram-negative, motile and small rod-shaped.

R6: *Imtechella halotolerace* sp. nov.: The strain, K1 (T), (FR774044), was isolated from ridge rock. Cells are Gram-negative, motile and small rod-shaped.

NA1: *Pseudomonas parafulva sp*.: The strain, AJ 2129(T), (AB060132), was isolated from ridge rock surface. Cells are Gram-positive, rod-shaped, motile.

NA12: *Serratia nematodiphila* sp. nov.: The strain is DZ0503SBS1 (T), (EU036987). Cells are Gram-negative, rod-shaped, motile.

6. Bacterial isolates from benthic foraminifera included:

RF1: *Bacillus vietnamensis* sp.: The strain, 15-1(T) (AB099708) was isolated from surface seawater at a hydrothermal vent in CR. Cells are aerobic, Gram-positive, spore-forming and small rod-shaped.

RF2: *Salinicola salarius* sp.: The strain, M27 (T) (AM229316), was isolated from shallow water hydrothermal vent water from CR. Cells are Gram-negative, curved-rod shaped, motile and non-spore forming.

RF3: *Bacillus oceanisediminis* sp.: H2 (T) (GQ292772). The strain was isolated from the body of benthic foraminiferons from CR sample.

RF6: *Bacillus oceanobacillus* LMG 19492(T) (AJ315060). The strain was isolated from the body of benthic foraminiferans from CR sample.

III. Third and final objective is to estimate the activity of metal tolerant bacterial population in terms of rates of mobilizing and immobilizing

metal ions under ambient conditions and it also includes the study on Foraminifera associated bacteria.

1. Mn mobilization and immobilization activity by bacterial community and isolate showed the mobilization rate of Mn from the ridge was maximum in the presence of glucose (0.1%) and occurred on 150th day of incubation at 4±1°C. Mn was mobilized at the rate of 1.71µg g^{-1} d^{-1} with glucose and 0.17 µg g^{-1} d^{-1} without glucose.

2. In the presence of isolate CR 35, the mobilization rate of Mn from the ridge was maximum in the presence of glucose (0.1%) and occurred on 120th day of incubation at 4±1°C. Mn was mobilized at the rate of 900µg g^{-1} d^{-1} with glucose and 27µg g^{-1} d^{-1} without glucose.

3. The maximum net biotic immobilization of Mn with isolate CR 35 was 527 and 621. µg ^{1} with and without glucose (0.1%) respectively maximum after 25 days of incubation.

4. Cu mobilization and immobilization activity by bacterial community and isolate showed the amount of Cu mobilized from the ridge (rock) sample was 1474. µg g^{-1} with glucose and 650 µg g^{-1} without glucose after 150 days of incubation using indigenous microbial communities at 4±1°C.

5. Mobilization rate of Cu from ridge rock by bacterial isolate was maximum in the presence of glucose (0.1%) which occurred on 150th day of incubation at 4±1°C. The rate of Cu mobilization was 2546.33 µg g^{-1} with glucose and 105.67 µg g^{-1} without glucose.

6. The net biotic immobilization of Cu with microbial community was maximum after 150 days of incubation. In G+ biotic, the rate of immobilization was 377.33μg g^{-1} and 504.81 μg g^{-1} without glucose.

7. Whereas the net biotic immobilization of Cu was maximum with bacterial isolate on 150 days of incubation. In G+ biotic, the rate of immobilization was 6.68 and 9.26 μg $g^{-1}d^{-1}$ with and without glucose.

8. Scanning electron microscopy (SEM) and electron dispersion spectra (EDS) study was also carried out to understand the association between foraminifera and bacteria. Five foraminifera taxa (*Spirillina guttata, Lagena hexagona, Trochammina inflate, Globigerina bulloides, Globocassidulina cabalisuturata*) were scanned and eight different bacteria were found associated with them. *Bacillus vietnamensis, Oceanobacillus picturae, Halomonas cupida, Brachybacterium phenoliresistens phenol*-A, *Bacillus oceanisediminis, Salinicola salaries, Halomonas cupida* and *Bacillus vietnamensis* species of bacteria were identified as in symbiotic associations with foraminifera

9. In conclusion, it can be said that hydrothermal activity and associated rock alteration processes may be more relevant than organic matter delivery in Ridges. Microbes could be involved in diagenetic and hydrothermal alterations on varying timescales.

10. The biogeochemical analyses of ridges rock/sediments suggests that the minor components like the rod morphotypes amongst bacteria and sand

component in rock/sediment texture of ridges, wield a large influence on the variability of other parameters.

11. In ridges rock/sediments, inorganic phosphate is possibly made available to biota, by variable rates of mobilization/immobilization activity. Oxidative and reductive processes operate in close tandem one feeding the other during diagenesis.

12. Present work could be biogeochemically significant because rate of mobilization was observed on the higher side without any chemical pollution to environment. Experiments conducted under microaerophilic and anaerobic environment would be more successful to get higher mobilization rate for pure form of metal.

13. The extent of bioreduction/mobilization of Mn natural oxide by R6 bacteria *Halotolerance* sp. was dependant on the pH, initial Mn natural oxide concentration and metabolic activity of the organism. Mn is found to play an important role in bacteria metabolic activity development of crystal particle on the bacteria associated rock samples. Hence, R6 may be recommended for the use in bio mining without inducing any chemical pollution of the environment.

6.2 Future scope

- Present study revealed for the First time that bacteria are associated benthic foraminifera in the deep sea ridge ecosystem and these bacteria can/may be used for future study for bioremediation or biotechnological aspects.

- The knowledge on bacterial diversity and activity acquired in this study will be useful for the better understanding and management of deep-sea ecosystems for marine mining of metallic resources in association with microbial community.

- The present study also suggests the plausible role of the Aerobic Anoxygenic bacteria in organically depleted and hydrothermally altered site in promoting chemosynthesis. As a part of future research, more robust numerical simulations explaining thermodynamics of the biogeochemical reactions in ridges would be possible with the availability of data on heat flux and fluid flow. Kinetic models could also add to the better understanding of the process occurring in DSHV ecosystem. It would also trigger interesting research on the deep-biosphere in ridges

REFERENCES

- Acuna J, Rojas J, Amaro AM, Toledo H, Jerez CA. Chemotaxis of Leptospirillum ferrooxidans and other acidophilic chemolithotrophs: comparison with the Escherichia coli chemosensory system. Fems Microbiol Lett. 1992;75:37–42.
- Alexander, P.O., Thomas, H. 2011. Copper in Deccan Basalts (India): review of the abundance and patterns of distribution.
- Altimira, F., Yáñez, C., Bravo, G., González, M., Rojas, L.A., Seeger, M. 2012. Characterization of copper-resistant bacteria and bacterial communities from copper-polluted agricultural soils of central Chile. BMC microbiology, 12(1), 193.
- Amy, P.S., Morita, R.Y. 1983. Starvation-survival patterns of sixteen freshly isolated open-ocean bacteria. Applied and Environmental Microbiology, 45(3), 1109-1115.
- Anderson, C.R., Cook, G.M. 2004. Isolation and characterization of arsenate-reducing bacteria from arsenic-contaminated sites in New Zealand. Current microbiology, 48(5), 341-347.
- Antony, R., Sujith, P., Fernandes, S.O., Verma, P., Khedekar, V., Bharathi, P.L. 2011. Cobalt immobilization by manganese oxidizing bacteria from the Indian Ridge System. Current microbiology, 62(3), 840-849.
- Archibald F 1986. Manganese: its acquisition by and function in the lactic acid bacteria. Crit Rev Microbiol 13:63–109
- Baglin, E., Noble, E., Lamsphire, D., Eisele, J. 1992. Solubilization of manganese from ores by heterotrophic micro-organisms. Hydrometallurgy, 29(1-3), 131-144.
- Bairagi, A., Ghosh, K.S., Sen, S.K., Ray, A.K. 2002. Enzyme producing bacterial flora isolated from fish digestive tracts. Aquaculture International, 10(2), 109-121.
- Bandy O.L., 1954. Distribution of some shallow-water foraminifera in the Gulf of Mexico. Geological Survey Professional Paper, 254-F, 132 pp.
- Benzerara, K., Menguy, N., Banerjee, N., Tyliszczak, T., Brown, G., Guyot, F. 2007. Alteration of submarine basaltic glass from the Ontong Java Plateau: a STXM and TEM study. Earth and Planetary Science Letters, 260(1), 187-200.
- Bernhard, J. M., Distinguishing live from dead foraminifera: Methods review and proper applications, *Micropaleontology*, **46**, suppl. 1, 38–46, 2000

- Bernhard, J. M., Microaerophilic and facultative anaerobic benthic foraminifera: A review of experimental and ultrastructural evidence, *Rev. Paléobiol.*, **15**, 261–275, 1996
- Bernhard, J.-M., 1988, Postmortem vital staining in benthic foraminifera: Duration and importance in population and distributional studies: Journal of Foraminiferal Research, v. 18, p. 143–146.
- Berry, A. 1994. Foraminiferan prey in the annual life-cycle of the predatory opisthobranch gastropod Retusa obtusa (Montagu). Estuarine, Coastal and Shelf Science, 38(6), 603-612.
- Bharathi, P.L. 1989. The occurrence of denitrifying colourless sulphur-oxidising bacteria in marine waters and sediments as shown by the agar shake technique. FEMS Microbiology Letters, 62(6), 335-342.
- Billen, G. 1982. Modelling the processes of organic matter degradation and nutrients recycling in sedimentary systems. Sediment microbiology, 15-52.
- Boetius, A., Scheibej, S., Tselepides, A., Thiel, H. 1996. Microbial biomass and activities in deep-sea sediments of the Eastern Mediterranean: trenches are benthic hotspots. Deep Sea Research Part I: Oceanographic Research Papers, 43(9), 1439-1460.
- Boltovskoy, E. 1978. Late Cenozoic benthonic foraminifera of the Ninetyeast Ridge (Indian Ocean). Marine Geology, 26(1-2), 139-175.
- Boone, D.R., Liu, Y., Zhao, Z.-J., Balkwill, D.L., Drake, G.R., Stevens, T.O., Aldrich, H.C. 1995. Bacillus infernus sp. nov., an Fe (III)-and Mn (IV)-reducing anaerobe from the deep terrestrial subsurface. International Journal of Systematic and Evolutionary Microbiology, 45(3), 441-448.
- Bosecker, K. 1997. Bioleaching: metal solubilization by microorganisms. FEMS Microbiology reviews, 20(3-4), 591-604.
- Bowen HJM 1979. Environmental chemistry of the elements. Academic, London
- Brady, H. 1879. Notes on some of the reticularian Rhizopoda of the Challenger Expedition II: Additions to the knowledge of porcellaneous and hyaline types. Quart. Jour. Micro. Sci, London, ns, 19, 286.

- Brandl, H., Faramarzi, M.A. 2006. Microbe-metal-interactions for the biotechnological treatment of metal-containing solid waste. China Particuology, 4(2), 93-97.

- Bratina, B.J., Stevenson, B.S., Green, W.J., Schmidt, T.M. 1998. Manganese reduction by microbes from oxic regions of the Lake Vanda (Antarctica) water column. Applied and environmental microbiology, 64(10), 3791-3797.

- Bromfield, S., David, D. 1976. Sorption and oxidation of manganous ions and reduction of manganese oxide by cell suspensions of a manganese oxidizing bacterium. Soil Biology and Biochemistry, 8(1), 37-43.

- Burkhardt, E.-M., Bischoff, S., Akob, D.M., Büchel, G., Küsel, K. 2011. Heavy metal tolerance of Fe (III)-reducing microbial communities in contaminated creek bank soils. Applied and environmental microbiology, 77(9), 3132-3136.

- Banakar V K, Pattan J N and Mudholkar A V 1997 Palae oceanographic conditions during the formation of ferromanganese crusts on the Afanasiy—Nikitin seamount, North central Indian Ocean: Geochemical evidences; Mar. Geol. 136 299-315.

- Burns, R. G., Burns, V. M., and Stockman, H.(1983) A review of the odorokite-buserite problem: Implications to the mineralogy of marine manganese nodules: Amer. Mineral 68, 972-980.

- Calvert, S., Cronan, D. 1978. Geochemistry of oceanic ferromanganese deposits. Philosophical Transactions of the Royal Society of London A: Mathematical, Physical and Engineering Sciences, 290(1366), 43-73.

- Calvert, S., Piper, D. 1984. Geochemistry of ferromanganese nodules from DOMES Site A, Northern Equatorial Pacific: Multiple diagenetic metal sources in the deep sea. Geochimica et Cosmochimica Acta, 48(10), 1913-1928.

- Calvert SE, Pedersen TF (1996) Sedimentary geochemistry of manganese: implications for the environment of formation of manganiferous black shales. Econ Geol 91:36–47

- Capriulo, G.M. 1990. Feeding-related ecology of marine protozoa. Ecology of marine protozoa, 186-259.

- Capriulo, G. M., Sherr, E. D., and Sherr, B. F. (1991). Trophic behaviour an related community feeding activities of heterotrophic marine protists. In *"Protozoa and their role in marine processes."* (P. C. Reid, Ed.), pp. 219- 265. Springer-Verlag, Berlin.

- Chander M, Setlow B, Setlow P (1998) The enzymatic activity of phosphoglycerate mutase from gram-positive endospore-forming bacteria requires Mn2+ and is pH sensitive. Can J Microbiol 44:759–767

- Chen, G., Chen, X., Yang, Y., Hay, A.G., Yu, X., Chen, Y. 2011. Sorption and distribution of copper in unsaturated Pseudomonas putida CZ1 biofilms as determined by X-ray fluorescence microscopy. Applied and environmental microbiology, 77(14), 4719-4727.

- Chesworth, W. 2008. Encyclopedia of soil science.

- Childress JJ, Fisher CR. (1992). The biology of hydrothermal vent animals – physiology, biochemistry, and autotrophic symbioses. *Oceanogr Mar Biol* **30**: 337–441.

- Christians, S., Jose, J., Schäfer, U., Kaltwasser, H. 1991. Purification and subunit determination of the nickel-dependent Staphylococcus xylosus urease. FEMS microbiology letters, 80(2-3), 271-275.

- Christianson DW 1997. Structural chemistry and biology of manganese metalloenzymes. Prog Biophys Mol Biol 67:217–252

- Chrost, R.J. 1991. Environmental control of the synthesis and activity of aquatic microbial ectoenzymes. in: Microbial enzymes in aquatic environments, Springer, pp. 29-59.

- Clague, D., Friesen, W., Quinterno, P., Morgensson, L., Holmes, M.L., Morton, J., Bouse, R.M., Davis, A. 1984. Preliminary geological, geophysical, and biological data from the Gorda Ridge. US Geological Survey. 2331-1258.

- Colombini, M., Fuoco, R. 1983. Determination of manganese at ngml levels in natural waters by differential pulse polarography. Talanta, 30(12), 901-905.

- Corliss, B. H., Morphology and microhabitat preferences of benthic foraminifera from the northwest Atlantic Ocean, *Mar. Micropaleontol.*, **17**, 195–236, 1991

- CORLISS, B. H., 1985, Microhabitats of benthic foraminifera within deep-sea sediments: Nature, v. 314, p. 435–438.
- Crowley JD, Traynor DA, Weatherburn DC 2000. Enzymes and proteins containing manganese: an overview. Met Ions Biol Syst 37:209–278.
- Cushman J.A., 1942. The foraminifera of the Tropical Pacific Collections of the "Albatross," 1899-1900, Part-3. Hetrerohelicidae and Buliminidae. Smithsonian Institution United States National Museum, 161: 1–98.
- Czekalla C, Mevius W, Hanert H (1985) Quantitative removal of iron and manganese by microorganisms in rapid sand filters. Wat Suppl 3:111–123
- D'Hondt, S. Rutherford, A.J. SpivackMetabolic activity of subsurface life in deep-sea sediments Science, 295 (2002), pp. 2067-2070
- D'Hondt et al., 2004 S. D'Hondt, B.B. Jorgensen, D.J. Miller, *et al.*Distributions of microbial activities in deep subseafloor sediments Science, 306 (2004), pp. 2216-2221
- Das, A., Fernandes, C.E., Naik, S.S., Nagender Nath, B., Suresh, I., Mascarenhas-Pereira, M., Gupta, S., Khadge, N., Prakash Babu, C., Borole, D. 2011. Bacterial response to contrasting sediment geochemistry in the Central Indian Basin. Sedimentology, 58(3), 756-784.
- Das, A., Sujith, P., Mourya, B.S., Biche, S.U., LokaBharathi, P. 2011. Chemosynthetic activity prevails in deep-sea sediments of the Central Indian Basin. Extremophiles, 15(2), 177-189.
- Das, A., Sukla, L., Pradhan, N., Nayak, S. 2011. Manganese biomining: a review. Bioresource technology, 102(16), 7381-7387.
- Das, A.P., Sukla, L.B., Pradhan, N. 2012. Microbial recovery of manganese using Staphylococcus epidermidis. International Journal of Nonferrous Metallurgy, 1(02), 9.
- Davidson, D.E., Hill, B.C. 2009. Stability of oxidized, reduced and copper bound forms of Bacillus subtilis Sco. Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics, 1794(2), 275-281.

- Dedysh SN, Berestovskaya YY, Vasylieva LV, Belova SE, Khmelenina VN, Suzina NE, Trotsenko YA, Liesack W, Zavarzin GA. 2004. Methylocella tundrae sp. nov., a novel methanotrophic bacterium from acidic tundra peatlands. Int J Syst Evol Microbiol. 54(Pt 1):151-6.

- DeLaca, T. E. (1986). Detenniniation of benthic rhizopod biomass using ATP analyses. *Journal of Foraminiferal Research* 16,285-292.

- De Souza, M.-J., Nair, S., Bharathi, P.L., Chandramohan, D. 2006. Metal and antibiotic-resistance in psychrotrophic bacteria from Antarctic Marine waters. Ecotoxicology, 15(4), 379-384.

- de Vrind, J., Boogerd, F.C., de Vrind-de Jong, E.W. 1986. Manganese reduction by a marine Bacillus species. Journal of bacteriology, 167(1), 30-34.

- Di Simine, D., Finoli, C., Vecchio, A., Andreoni, V. 1998. Metal ion accumulation by immobilised cells of Brevibacterium sp. Journal of Industrial Microbiology and Biotechnology, 20(2), 116-120.

- Dick, G.J., Lee, Y.E., Tebo, B.M. 2006. Manganese (II)-oxidizing Bacillus spores in Guaymas Basin hydrothermal sediments and plumes. Applied and Environmental Microbiology, 72(5), 3184-3190.

- Dopson, M. (2016). "Physiological and phylogenetic diversity of acidophilic bacteria," in Acidophiles: Life in Extremely Acidic Environments, eds R. Quatrini and D. B. Johnson (Haverhill: Caistor Academic Press), 79–92.

- Dopson, M., and Johnson, D. B. (2012). Biodiversity, metabolism and applications of acidophilic sulfur- metabolizing micro-organisms. Environ. Microbiol. 14, 2620–2631. doi: 10.1111/j.1462-2920.2012.02749

- Doyle RJ 1989. How cell walls of gram-positive bacteria interact with metal ions. In: Beveridge TJ, Doyle RJ (eds) Metal Ions and Bacteria. Wiley, New York, pp 275–293

- Duijnstee, I., De lugt, I., Vonk Noordegraaf, H., and Van Der Zwaan, G. J., 2001. Dynamics of benthic foraminifera from northern Adriatic Sea:

- Edwards, K.J., Bach, W., McCollom, T.M., Rogers, D.R. 2004. Neutrophilic iron-oxidizing bacteria in the ocean: their habitats, diversity, and roles in mineral

deposition, rock alteration, and biomass production in the deep-sea. Geomicrobiology Journal, 21(6), 393-404.

- Edwards, K.J., Rogers, D.R., Wirsen, C.O., McCollom, T.M. 2003. Isolation and characterization of novel psychrophilic, neutrophilic, Fe-oxidizing, chemolithoautotrophic α-and γ-Proteobacteria from the deep sea. Applied and Environmental Microbiology, 69(5), 2906-2913.
- Ehrlich, H. 1971. Bacteriology of Manganese Nodules V. Effect of Hydrostatic Pressure on Bacterial Oxidation of Mn II and Reduction of MnO2. Applied microbiology, 21(2), 306-310.
- Ehrlich, H. 1988. Bioleaching of manganese by marine bacteria. Proceedings 8th International Biotechnology Symposium, Paris, France. pp. 1094-1105.
- Ehrlich HL 1987. Manganese oxide reduction as a form of anaerobic respiration. Geomicrobiol J 5:423–431
- Ehrlich, H. 1997. Microbes and metals. Applied Microbiology and Biotechnology, 48(6), 687-692.
- Ehrlich, H., Ghiorse, W., Johnson, G. 1972. Distribution of microbes in manganese nodules from the Atlantic and Pacific Oceans. Dev. Ind. Microbiol, 13, 57-65.
- Ehrlich HL 1976. Manganese as an energy source for bacteria. In: Nriagu JO (ed) Environmental biogeochemistry. Ann Arbor Science, Michigan, pp 633–644
- Ehrlich HL 1978. Inorganic energy sources for chemolithotrophic and mixotrophic bacteria. Geomicrobiol J 1:65–83
- Ehrlich, H.L. 1980. Bacterial leaching of manganese ores. in: Biogeochemistry of ancient and modern environments, Springer, pp. 609-614.
- Ehrlich, H.L. 1993. Electron transfer from acetate to the surface of MnO 2 particles by a marine bacterium. Journal of Industrial Microbiology & Biotechnology, 12(2), 121-128.
- Ehrlich HL 2002. How microbes mobilize metals in ores: a review of current understandings and proposals for future research. Miner Metall Proc 19:220–224

- Ehrlich, H.L. 2012. Reminiscences from a career in geomicrobiology. Annual Review of Earth and Planetary Sciences, 40, 1-21.

- Ewart, D.K., Hughes, M.N., 1991. The extraction of metals from ores using bacteria. Adv. Inorg. Chem. 36, 103–135.

- Chi Fru, Ernest 2009. Iron oxides influence bacterial community structure and the spatial distribution of the aerobic methanotrophs and sulphate reducers in granitic aquifers. *Geomicrobiology Journal* 26 (6) , pp. 415-429.

- Fabiano, M., Pusceddu, A., Dell'Anno, A., Armeni, M., Vanucci, S., Lampitt, R., Wolff, G., Danovaro, R. 2001. Fluxes of phytopigments and labile organic matter to the deep ocean in the NE Atlantic Ocean. Progress in oceanography, 50(1), 89-104.

- Fernandes, S.O., Krishnan, K., Khedekar, V., Bharathi, P.L. 2005. Manganese oxidation by bacterial isolates from the Indian Ridge System. Biometals, 18(5), 483.

- Flemming, C.A. & Trevors, J.T. Water Air Soil Pollut (1989) 44: 143. https://doi.org/10.1007/BF00228784

- Fournier, M., Petit, C., Chamot-Rooke, N., Fabbri, O., Huchon, P., Maillot, B., Lepvrier, C. 2008. Do ridge–ridge–fault triple junctions exist on Earth? Evidence from the Aden–Owen–Carlsberg junction in the NW Indian Ocean. Basin Research, 20(4), 575-590.

- Fry, J.C., Parkes, R.J., Cragg, B.A., Weightman, A.J., Webster, G. 2008. Prokaryotic biodiversity and activity in the deep subseafloor biosphere. FEMS Microbiology Ecology, 66(2), 181-196.

- Gadd, G. 1992. Heavy metal pollutants: environmental and biotechnological aspects. Encyclopedia of microbiology, 2, 351-360.

- Gadd, G.M. 2007. Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. Mycological research, 111(1), 3-49.

- Geesey, G., Jang, L. 1989. Interactions between metal ions and capsular polymers. Metal ions and bacteria, 325-357.

- Geesey, G., Jang, L., Jolley, J., Hankins, M., Iwaoka, T., Griffiths, P. 1988. Binding of metal ions by extracellular polymers of biofilm bacteria. Water Science and Technology, 20(11-12), 161-165.
- Gier, O. 1993. Meiobenthology: the microscopic fauna in aquatic sediment. Spr. Fer. Ber, 1-328.
- Giere, O. 2013. Meiobenthology: the microscopic fauna in aquatic sediments. Springer Science & Business Media.
- Glasby, G.P. 1977. Marine manganese deposits. Elsevier.
- Goltekar, R.C., Krishnan, K., De Souza, M.-J.B., Paropkari, A., Bharathi, P.L. 2006. Effect of carbon source concentration and culture duration on retrievability of bacteria from certain estuarine, coastal and offshore areas around peninsular India. Current Science, 103-106.
- Gonsalves, M.-J., Paropkari, A., Fernandes, C., Bharathi, P.L., Krishnakumari, L., Fernando, V., Nampoothiri, G. 2011. Predominance of anaerobic bacterial community over aerobic community contribute to intensify 'oxygen minimum zone'in the eastern Arabian Sea. Continental Shelf Research, 31(11), 1224-1235.
- Gooday, A.J. 1999. Biodiversity of foraminifera and other protists in the deep sea: scales and patterns. Belgian Journal of Zoology(1).
- Gooday, A. J., and A. E. Rathburn, Temporal variability in living deep-sea benthic foraminifera: A review, *Earth Sci. Rev.*, **46**, 187–212, 1999.
- Gooday, A.J. 1993. Deep-sea benthic foraminiferal species which exploit phytodetritus: characteristic features and controls on distribution. Marine Micropaleontology, 22(3), 187-205.
- Gooday, A.J. & Turley, C.M. (1990) Responses by benthic organisms to inputs of organic material to the ocean floor: a review. Philosophical Transactions of the Royal Society of London, A 331, 119-138.
- Gooday, A.J., Hughes, J.A., 2002. Foraminifera associated with phytodetritus deposits at a bathyal site in the northern Rockall Trough (NE Atlantic): seasonal contrasts and a comparison of stained and dead assemblages. Marine Micropaleontology, 46: 83-110

- Graetz, D.A., Keeney, D.R., Aspiras, R.B. 1973. Eh Status Of Lake Sediment-Water Systems In Relation To Nitrogen Transformations. Limnology and oceanography, 18(6), 908-917.
- Guimerans, P.V., Currado, J.C. 1999. Distribution of Planorbulinacea (benthic foraminifera) assemblages in surface sediments on the northern margin of the Gulf of Cadiz. BOLETIN-INSTITUTO ESPANOL DE OCEANOGRAFIA, 15(1/4), 181-190.
- Gupta, S.M., Jauhari, P. 1994. Radiolarian abundance and geochemistry of the surface-sediments from the Central Indian Basin: inferences to Antarctic bottom water current. Current Science, 659-663.
- Hansell, D.A., Peltzer, E.T. 1998. Spatial and temporal variations of total organic carbon in the Arabian Sea. Deep Sea Research Part II: Topical Studies in Oceanography, 45(10), 2171-2193.
- Harder, W., Dijkhuizen, L., Postgate, J. 1982. Strategies of mixed substrate utilization in microorganisms. Philosophical Transactions of the Royal Society of London B: Biological Sciences, 297(1088), 459-480.
- Heeger, T., 1990. Electronenmikroskopische Untersuchungen zur Ern¨ahrungsbiologie benthischer Foraminiferen. Ber. Sonderforschungsbereich 313(31): 1−139
- Holm-Hansen, C. R. BOOTII. 1966. The measurement of adenosine triphosphate in the ocean and its ecological significance. Limnol. Occanog., 11: 510-519.
- Horsburgh, M.J., Wharton, S.J., Cox, A.G., Ingham, E., Peacock, S., Foster, S.J. 2002. MntR modulates expression of the PerR regulon and superoxide resistance in Staphylococcus aureus through control of manganese uptake. Molecular microbiology, 44(5), 1269-1286.
- Hugh, R., Leifson, E. 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. Journal of bacteriology, 66(1), 24.
- Ibrahim, A.S., El-Tayeb, M.A., Elbadawi, Y.B., Al-Salamah, A.A. 2011. Isolation and characterization of novel potent Cr (VI) reducing alkaliphilic Amphibacillus sp. KSUCr3 from hypersaline soda lakes. Electronic Journal of Biotechnology, 14(4), 4-4.

- Inagaki, F., Nunoura, T., Nakagawa, S., Teske, A., Lever, M., Lauer, A., Suzuki, M., Takai, K., Delwiche, M., Colwell, F. 2006. In search of the deep biosphere: biogeographical distribution and diversity of microbes in deep marine sediments associated with methane hydrates on the Pacific Ocean Margin. Proc Natl Acad Sci USA, 103, 2815-2820.
- Ishibashi, J.-i., Okino, K., Sunamura, M. 2015. Subseafloor Biosphere Linked to Hydrothermal Systems. Springer Japan: Imprint: Springer.
- Iyer, S.D., Mukhopadhyay, R., Drolia, R.K., Ray, D. 2003. Mid-ocean ridges, InRidge and the future. Current Science, 272-276.
- Jahnke, R.A., Jahnke, D.B. 2004. Calcium carbonate dissolution in deep sea sediments: reconciling microelectrode, pore water and benthic flux chamber results. Geochimica et Cosmochimica Acta, 68(1), 47-59.
- Jahnke, R.A., Reimers, C.E., Craven, D.B. 1990. Intensification of recycling of organic matter at the sea floor near ocean margins. Nature, 348(6296), 50.
- Jakubovics NS, Jenkinson HF 2001. Out of the iron age: new insights into the critical role of manganese homeostasis in bacteria. Microbiology 147:1709–1718
- Jannasch, H.W. 1979. Microbial turnover of organic matter in the deep sea. BioScience, 29(4), 228-232.
- Jannasch, H.W., Eimhjellen, K., Farmanfarmalan, A. 1971. Microbial degradation of organic matter in the deep sea. Science, 171(3972), 672-675.
- Jannasch, H.W., Wirsen, C.O. 1973. Deep-sea microorganisms: in situ response to nutrient enrichment. Science, 180(4086), 641-643.
- Jannasch, H.W., Mottl, M.J.1985. Geomicrobiology of deep-sea hydrothermal vents. *Science* **229**:717.
- Joe Copley, (2005). Hidden depths (book reviews: Fathoming the Ocean:the discovery and exploration of the deep sea (H.M. Rozwadowski, Belknap 2005); The Remarkable Life of William Beebe: explorer and naturalist (C.G. Gould, Shearwater 2004); Descent: The Heroic Discovery of the Abyss, (B. Matsen, Pantheon 2005). Nature, 435(7040), 279-280. DOI: 10.1038/435279a

- Johnson, D. B., and Aguilera, A. (2015). "The microbiology of extremely acidic environments," in Manual of environmental microbiology, 4th Edn, eds M. V. Yates, H. Cindy, C. H. Nakatsu, R. V. Miller, and S. D. Pillai (New York, NY: ASM Press), doi:10.1128/9781555818821.ch4.3.1

- Johnson, K.S., Berelson, W.M., Coale, K.H., Coley, T.L., Elrod, V.A., Fairey, W.R., Iams, H.D., Kilgore, T.E., Nowicki, J.L. 1992. Manganese flux from continental margin sediments in a transect through the oxygen minimum. Science, 257(5074), 1242-1245.

- Johnston, C.G., Kipphut, G.W. 1988. Microbially mediated Mn (II) oxidation in an oligotrophic arctic lake. Applied and environmental microbiology, 54(6), 1440-1445.

- Jonasson, K.E., Schro, C., Patterson, R. 1995. Benthic foraminiferal distribution at Middle Valley, Juan de Fuca Ridge, a northeast Pacific hydrothermal venting site. Marine Micropaleontology, 25(2-3), 151-167.

- Jost, G., Pollehne, F. 2011. The energetic balance of microbial exploitation of pelagic redox gradients. in: Chemical Structure of Pelagic Redox Interfaces, Springer, pp. 47-65.

- Joux, F., Lebaron, P. 1997. Ecological implications of an improved direct viable count method for aquatic bacteria. Applied and Environmental Microbiology, 63(9), 3643-3647.

- Jukes, T.H., Cantor, C.R., Munro, H. 1969. Evolution of protein molecules. Mammalian protein metabolism, 3(21), 132.

- Karl, D.M. 1995. The microbiology of deep-sea hydrothermal vents. CRC.

- Kasana, R.C., Salwan, R., Dhar, H., Dutt, S., Gulati, A. 2008. A rapid and easy method for the detection of microbial cellulases on agar plates using Gram's iodine. Current microbiology, 57(5), 503-507.

- Kim, W., Lee, S. 2001. Data standards utilized in the environmental studies of the Korea Ocean Research and Development Institute (KORDI). Standardization of environmental data and information: development of guidelines, proceedings of the International Seabed Authority's workshop held in Kingston, Jamaica. pp. 234-263.

- Kirchner WB, Grabowski S 1972. Manganese in lacustrine ecosystems: a review. Am Water Resour Assoc 8:1259–1264

- Kogure, K., Simidu, U., Taga, N. 1984. An improved direct viable count method for aquatic bacteria. Archiv für Hydrobiologie, 102(1), 117-122.
- Kogure, K., Simidu, U., Taga, N. 1979. A tentative direct microscopic method for counting living marine bacteria. Canadian Journal of Microbiology, 25(3), 415-420.
- Kondo, I., Ishikawa, T., Nakahara, H. 1974. Mercury and cadmium resistances mediated by the penicillinase plasmid in Staphylococcus aureus. Journal of Bacteriology, 117(1), 1-7.
- Kostka, J., Nealson, K.H. 1998. Isolation, cultivation and characterization of iron-and manganese-reducing bacteria. Techniques in microbial ecology, 58-78.
- Kostka, J.E., Luther, G.W., Nealson, K.H. 1995. Chemical and biological reduction of Mn (III)-pyrophosphate complexes: potential importance of dissolved Mn (III) as an environmental oxidant. Geochimica et Cosmochimica Acta, 59(5), 885-894.
- Krishnamurthi, S., Bhattacharya, A., Mayilraj, S., Saha, P., Schumann, P., Chakrabarti, T. 2009. Description of Paenisporosarcina quisquiliarum gen. nov., sp. nov., and reclassification of Sporosarcina macmurdoensis Reddy et al. 2003 as Paenisporosarcina macmurdoensis comb. nov. International journal of systematic and evolutionary microbiology, 59(6), 1364-1370.
- Kröncke, I., Bergfeld, C. 2003. North Sea benthos: a review. Marine Biodiversity, 33(1), 205-268.
- Kroon, D., Steens, T., Troelstra, S.R. 1991. 13. Onset Of Monsoonal Related Upwelling In The Western Arabian Sea As Revealed By Planktonic Foraminifers1.
- Krumbein, W.E., Jens, K. 1981. Biogenic rock varnishes of the Negev Desert (Israel) an ecological study of iron and manganese transformation by cyanobacteria and fungi. Oecologia, 50(1), 25-38.
- Kumar, A., Singh, V., Kumar, R. 2006. Characterization of an alkaliphile, Exiguobacterium sp. and it's application in bioremediation. Book of Abstracts. pp. 115.
- Lee, J.J., Anderson, O.R., Karim, B. and Beti, 1.. 1991. Additionalinsight into the structure and biology of Abyssofherma *pncificn* (Bronnimann, Van Dover and Whittaker) from the East Pacific Rise.Micropaleontology, 37(3): 303-312.

- Leefeldt, R., Matin, A. 1980. Growth and physiology of Thiobacillus novellus under nutrient-limited mixotrophic conditions. Journal of bacteriology, 142(2), 645-650.
- Levin, L.A., Gage, J.D., Martin, C., Lamont, P.A. 2000. Macrobenthic community structure within and beneath the oxygen minimum zone, NW Arabian Sea. Deep Sea Research Part II: Topical Studies in Oceanography, 47(1), 189-226.
- Lewis, A. J., and Miller, J. D. A. (1977). Stannous and cuprous ion oxidation by Thiobacillus ferrooxidans. Can. J. Microbiol. 23, 319–324. doi: 10.1139/m77-047
- Li, Y., Low, G.K.-C., Scott, J.A., Amal, R. 2007. Microbial reduction of hexavalent chromium by landfill leachate. Journal of hazardous materials, 142(1), 153-159.
- LIKENS, E. 1972. Eutrophication and aquatic ecosystems.
- Lin, Q., Brookes, P. 1996. Comparison of methods to measure microbial biomass in unamended, ryegrass-amended and fumigated soils. Soil Biology and Biochemistry, 28(7), 933-939.
- LIPPS, J.H., VALENTINE, J.W. 1970. The role of foraminifera in the trophic structure of marine communities. Lethaia, 3(3), 279-286.
- Lochte, K. 1992. Bacterial standing stock and consumption of organic carbon in the benthic boundary layer of the abyssal North Atlantic. in: Deep-sea food chains and the global carbon cycle, Springer, pp. 1-10.
- Loeblich Jr, A.R., Tappan, H. 2015. Foraminiferal genera and their classification. Springer.
- Loka Bharathi, P., Nair, S. 2005. Rise of the dormant: simulated disturbance improves culturable abundance, diversity, and functions of deep-sea bacteria of Central Indian Ocean Basin. Marine georesources & geotechnology, 23(4), 419-428.
- Lopez, L., Pozo, C., Rodelas, B., Calvo, C., Juarez, B., Martinez-Toledo, M., Gonzalez-Lopez, J. 2005. Identification of bacteria isolated from an oligotrophic lake with pesticide removal capacities. Ecotoxicology, 14(3), 299-312.
- Lorenz, N., Hintemann, T., Kramarewa, T., Katayama, A., Yasuta, T., Marschner, P., Kandeler, E. 2006. Response of microbial activity and microbial community composition in soils to long-term arsenic and cadmium exposure. Soil Biology and Biochemistry, 38(6), 1430-1437.

- Madgwick, J. 1987. Microbial processing of manganese. Aust. J. Biotechnol, 1(2), 41-43.
- Meyer-Reil, L.-A. 1991. Ecological aspects of enzymatic activity in marine sediments. in: Microbial enzymes in aquatic environments, Springer, pp. 84-95.
- Millett, F.W. 1898. Report on the Recent Foraminifera of the Malay Archipelago collected by Mr. A. Durrand, FRMS. Journal of Microscopy, 18(3), 258-269.
- Millett, F.W. 1899. Report on the Recent Foraminifera of the Malay Archipelago collected by Mr. A. Durrand, FRMS—Part IV. Journal of Microscopy, 19(3), 249-255.
- Moodley, L., Boschker, H., Middelburg, J., Pel, R., Herman, P., De Deckere, E., Heip, C. 2000. Ecological significance of benthic foraminifera: 13C labelling experiments. Marine Ecology Progress Series, 202, 289-295.
- Moore WS, Reid DF (1973) Extraction of radium from natural waters using manganeseimpregnated acrylic fibers. J Geophys Res 78:8880–8886
- Moore, W.S., Vogt, P.R. 1976. Hydrothermal manganese crusts from two sites near the Galapagos spreading axis. Earth and Planetary Science Letters, 29(2), 349-356.
- Morita, R.Y. 1988. Bioavailability of energy and its relationship to growth and starvation survival in nature. Canadian Journal of Microbiology, 34(4), 436-441.
- Mudholkar A.V., Kodagali V.N., Kamesh Raju K.A., Valsangkar A.B., Ranade, G.H. and Ambre N.V., 2002. Geological and geophysical observations along a segment of slow-spreading Carlsberg Ridge, Curr. Sci. v.82, pp: 2982-989.
- Murray, J.W., Bowser, S.S., 2000. Mortality, protoplasm decay rate, and reliability of staining techniques to recognize 'living' foraminifera: a review. Journal of Foraminiferal Research 30, 66 – 70
- Murray, J.W. 1991. Ecology and distribution of benthic foraminifera. Biology of Foraminifera. Academic Press, London, 221-253.
- Murray, J.W., Alve, E. 1999. Taphonomic experiments on marginal marine foraminiferal assemblages: how much ecological information is preserved? Palaeogeography, Palaeoclimatology, Palaeoecology, 149(1), 183-197.

- Murray, R., Costilow, R.N., Nester, E., Wood, W., Krieg, N., Phillips, G. 1981. Manual of methods for general bacteriology. American society for microbiology, Washington, DC, 31.
- Muruganantham, M., Mohan, P. First report of three benthic foraminifera from the waters of Andaman Islands, India.
- Myers, C.R., Nealson, K.H. 1988. Bacterial manganese reduction and growth with manganese oxide as the sole electron acceptor. Science, 240(4857), 1319.
- Nair, S., Mohandass, C., Loka Bharathi, P., Sheelu, G., Chandralata, R. 2000. Microscale response of sediment variables to benthic disturbance in the Central Indian Ocean Basin. Marine georesources & geotechnology, 18(3), 273-283.
- Nath, B.N., Mudholkar, A. 1989. Early diagenetic processes affecting nutrients in the pore waters of Central Indian Ocean cores. Marine Geology, 86(1), 57-66.
- Nealson, K.H., Tebo, B. 1980. Structural features of manganese precipitating bacteria. Limits of Life. Dl. Reidel, Holland, 173-182.
- Nealson, K.H., 1983. The microbial manganese cycle. In: Krumbein WE (ed) Microbial geochemistry. Blackwell Scientific Publications, Oxford, pp 191–221
- Nealson KH, Rosson RA, Myers CR (1989) Mechanisms of oxidation and reduction of manganese. In: Beveridge T, Doyle R (eds) Metal ions and bacteria. Wiley, New York, pp 383–411
- Nielsen, A. M., and Beck, J. V. (1972). Chalcocite oxidation and coupled carbon dioxide fixation by Thiobacillus ferrooxidans. Science 175, 1124–1126. doi: 10.1126/science.175.4026.1124
- Nigam, R., Panchang, R., Banerjee, P. 2005. Foraminifera in surface sediments of Mandovi River estuary: indicators for mining pollution and high sea stand in Goa, India. Journal of Coastal Research, 853-859.
- Novitsky, J.A., Morita, R.Y. 1976. Morphological characterization of small cells resulting from nutrient starvation of a psychrophilic marine vibrio. Applied and Environmental Microbiology, 32(4), 617-622.

- Ogawa T, Bao DH, Katoh H, Shibata M, Pakrasi HB, Bhattacharyya-Pakrasi M 2002). A twocomponent signal transduction pathway regulates manganese homeostasis in Synechocystis
- 6803, a photosynthetic organism. J Biol Chem 277:28981–28986
- Okeke, B., Laymon, J., Oji, C., Crenshaw, S. 2007. Rapid bioreduction of hexavalent chromium in water by Exiguobacterium sp. GS1. ASM general 107th meeting, ASM Press, Toronto, ON, Canada, pp Q-199.
- Ostapczuk, P., Goedde, M., Stoeppler, M., Nürnberg, H. 1984. Kontroll-und Routinebestimmung von Zn, Cd, Pb, Cu, Ni und Co mit differentieller Pulsvoltammetrie in Materialien der Deutschen Umweltprobenbank. Fresenius' Zeitschrift für analytische Chemie, 317(3-4), 252-256.
- Ostrowski, M., Skłodowska, A. 1993. Bacterial and chemical leaching pattern on copper ores of sandstone and limestone type. World Journal of Microbiology and Biotechnology, 9(3), 328-331.
- Pak, K.-R., Lim, O.-Y., Lee, H.-K., Choi, S.-C. 2002. Aerobic reduction of manganese oxide by Salmonella sp. strain MR4. Biotechnology letters, 24(14), 1181-1184.
- Pareuil, P., Pénilla, S., Ozkan, N., Bordas, F., Bollinger, J.-C. 2008. Influence of reducing conditions on metallic elements released from various contaminated soil samples. Environmental science & technology, 42(20), 7615-7621.
- Parkes, R.J., Cragg, B.A., Bale, S., Getlifff, J., Goodman, K., Rochelle, P.A., Fry, J.C., Weightman, A.J., Harvey, S. 1994. Deep bacterial biosphere in Pacific Ocean sediments. Nature, 371(6496), 410-413.
- Parkes, R.J., Cragg, B.A., Bale, S., Getlifff, J., Goodman, K., Rochelle, P.A., Fry, J.C., Weightman, A.J., Harvey, S. 1994. Deep bacterial biosphere in Pacific Ocean sediments. Nature, 371(6496), 410-413.
- Parkes, R.J., Cragg, B.A., Wellsbury, P. 2000. Recent studies on bacterial populations and processes in subseafloor sediments: a review. Hydrogeology Journal, 8(1), 11-28.
- Parkes, R.J., Webster, G., Cragg, B.A., Weightman, A.J. 2005. Deep sub-seafloor prokaryotes stimulated at interfaces over geological time. Nature, 436(7049), 390.

- Parkes, R.J., Webster, G., Cragg, B.A., Weightman, A.J. 2005. Deep sub-seafloor prokaryotes stimulated at interfaces over geological time. Nature, 436(7049), 390.

- Parkes, R.J., Webster, G., Cragg, B.A., Weightman, A.J. 2005. Deep sub-seafloor prokaryotes stimulated at interfaces over geological time. Nature, 436(7049), 390.

- Parkes, R.J., Wellsbury, P., Mather, I.D., Cobb, S.J., Cragg, B.A., Hornibrook, E.R., Horsfield, B. 2007. Temperature activation of organic matter and minerals during burial has the potential to sustain the deep biosphere over geological timescales. Organic Geochemistry, 38(6), 845-852.
- Pattan, J., Jauhari, P. 2001. Major, trace, and rare earth elements in the sediments of the Central Indian Ocean Basin: Their source and distribution. Marine georesources & geotechnology, 19(2), 85-106.
- Priest, F.G. 1989. Products and applications. in: Bacillus, Springer, pp. 293-320.
- Prinz, M. 1967. Geochemistry of basaltic rocks: trace elements. Basalts, 1, 271-323.
- Qin, G., Zhu, L., Chen, X., Wang, P.G., Zhang, Y. 2007. Structural characterization and ecological roles of a novel exopolysaccharide from the deep-sea psychrotolerant bacterium Pseudoalteromonas sp. SM9913. Microbiology, 153(5), 1566-1572.
- Quadfasel, D., Fischer, J., Schott, F., Stramma, L. 1997. Deep water exchange through the Owen Fracture Zone in the Arabian Sea. Geophysical Research Letters, 24(22), 2805-2808.
- Ram, A.P., Bharathi, P.L., Nair, S., Chandramohan, D. 2001. A deep-sea bacterium with unique nitrifying property. Current Science, 1222-1224.
- Rao, P., Pattan, J. 1989. Ferromanganese oxides from Mid-Indian Ridge, seamounts and abyssal plains from the Indian Ocean.
- Rathsack, K., Stackebrandt, E., Reitner, J., Schumann, G. 2009. Microorganisms isolated from deep sea low-temperature influenced oceanic crust basalts and sediment samples collected along the Mid-Atlantic Ridge. Geomicrobiology Journal, 26(4), 264-274.
- Rattanachot, E., Prathep, A. 2015. Species-specific effects of seagrass on belowground biomass, redox potential and Pillucina vietnamica (Lucinidae). Journal of the Marine Biological Association of the United Kingdom, 95(8), 1693-1704.

- Rawlings, D. E., Tributsch, H., and Hansford, G. S. (1999). Reasons why 'Leptospirillum'-like species rather than Thiobacillus ferrooxidans are the dominant iron-oxidizing bacteria in many commercial processes for the biooxidation of pyrite and related ores. Microbiology 145, 5–13. doi: 10.1099/ 13500872-145-1-5

- Ray, D., Kamesh Raju, K., Baker, E.T., Srinivas Rao, A., Mudholkar, A.V., Lupton, J.E., Surya Prakash, L., Gawas, R.B., Vijaya Kumar, T. 2012. Hydrothermal plumes over the Carlsberg Ridge, Indian Ocean. Geochemistry, Geophysics, Geosystems, 13(1).

- Reyss, J. L., V. Marchig, and T. L. Ku (1982), Rapid growth of a deep-sea manganese nodule, Nature, 295, 401–403

- Rodriguez, F., Oliver, J., Marin, A., Medina, J.R. 1990. The general stochastic model of nucleotide substitution. Journal of theoretical biology, 142(4), 485-501.

- Roh, Y., Liu, S.V., Li, G., Huang, H., Phelps, T.J., Zhou, J. 2002. Isolation and characterization of metal-reducing Thermoanaerobacter strains from deep subsurface environments of the Piceance Basin, Colorado. Applied and Environmental Microbiology, 68(12), 6013-6020.

- Roitz JS, Flegal AR, Bruland KW (2002) The biogeochemical cycling of manganese in San Francisco Bay: temporal and spatial variations in surface water concentrations. Estuar Coast Shelf Sci 54:227–239

- Rowe, G.T., Pariente, V. 2012. Deep-sea food chains and the global carbon cycle. Springer Science & Business Media.

- Roy, P., Balaram, V., Kumar, A., Satyanarayanan, M., Gnaneshwar Rao, T. 2007. New REE and Trace Element Data on Two Kimberlitic Reference Materials by ICP-MS. Geostandards and Geoanalytical Research, 31(3), 261-273.

- Rodina, A.G. (1972) Methods of culturing iron bacteria In: Methods in aquatic microbiolog.y Eds. Colwell, R.R.and Zambruski, M.S. Univ. Park Press, Baltimore, USA, pp 358-367.

- Rusin, P., Ehrlich, H. 1995. Developments in mcirobial leaching—mechanisms of manganese solubilization. Microbial and Eznymatic Bioproducts, 1-26.

- Rusin, P.A., Quintana, L., Sinclair, N.A., Arnold, R.G., Oden, K.L. 1991. Physiology and kinetics of manganese-reducing bacillus polymyxa strain D1 isolated from manganiferous silver ore. Geomicrobiology Journal, 9(1), 13-25.
- Sabirova, J.S., Ferrer, M., Regenhardt, D., Timmis, K.N., Golyshin, P.N. 2006. Proteomic insights into metabolic adaptations in Alcanivorax borkumensis induced by alkane utilization. Journal of bacteriology, 188(11), 3763-3773.
- Saager PM, De Baar HJW, Burkill PH 1989. Manganese and iron in Indian Ocean waters. Geochim Cosmochim Acta 53:2259–2267
- Saitou, N., Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular biology and evolution, 4(4), 406-425.
- Santoro, A.L., Enrich-Prast, A. 2011. Regulação microbiológica da disponibilidade de nitrogênio em ecossistemas aquáticos continentais. Oecologia Australis, 15(2), 213-235.
- Sass, A.M., McKew, B.A., Sass, H., Fichtel, J., Timmis, K.N., McGenity, T.J. 2008. Diversity of Bacillus-like organisms isolated from deep-sea hypersaline anoxic sediments. Saline systems, 4(1), 8.
- Schmid, J., Auling, G. 1987. Manganese transport in Brevibacterium ammoniagenes ATCC 6872. Journal of bacteriology, 169(7), 3385-3387.
- Schut, C. 1978. Distribution and identification of manganese-precipitating bacteria from noncontaminated ferromanganese nodules. Environmental biogeochemistry and geomicrobiology, 3, 869-878.
- Scott, M.R., Scott, R.B., Rona, P.A., Butler, L.W., Nalwalk, A.J. 1974. Rapidly accumulating manganese deposit from the Median Valley of the Mid-Atlantic Ridge. Geophysical Research Letters, 1(8), 355-358.
- Schwarz J.R., Walder J.D., Colwell R.R. Deep-sea bacteria: growth and utilization of hydrocarbons at ambient and in situ pressure, Applied Microbiology 28 (1974) 982 986. [126]
- Shehata, T.E., Marr, A.G. 1971. Effect of nutrient concentration on the growth of Escherichia coli. Journal of bacteriology, 107(1), 210-216.
- Shi L 2004. Manganese-dependent protein o-phosphatases in prokaryotes and their biological functions. Front Biosci 9:1382–1397

- Sibuet M, Olu K (1998) Biogeography, biodiversity and fluid dependence of deep-sea cold seep communities at active and passive margins. Deep-Sea Res 45:517–567
- Silver, S., Budd, K., Leahy, K., Shaw, W., Hammond, D., Novick, R., Willsky, G., Malamy, M., Rosenberg, H. 1981. Inducible plasmid-determined resistance to arsenate, arsenite, and antimony (III) in escherichia coli and Staphylococcus aureus. Journal of Bacteriology, 146(3), 983-996.
- Silverman, M. P., and Ehrlich, H. L. (1964). Microbial Formation and Degradation of Minerals. Adv. Appl. Microbiol. 6, 153–206.
- Singh, S., Sukla, L.B., Mishra, B.K. 2011. Extraction of Copper from Malanjkhand Low-Grade Ore by Bacillus stearothermophilus. Indian journal of microbiology, 51(4), 477-481.
- Sklodowska, A., Matlakowska, R. 1997. Influence of exopolymers produced by bacterial cells on hydrophobicity of substrate surface. Biotechnology techniques, 11(11), 837-840.
- Spiro TG, Bargar JR, Sposito G, Tebo BM 2010. Bacteriogenic manganese oxides. Acc Chem Res 43:2–9
- Stuetz RM, Greene AC, Madgwick JC (1996) The potential use of manganese oxidation in treating metal effluents. Miner Eng 9:1253–1261
- Sugio, T., Tsujita, Y., Inagaki, K., and Tano, T. (1990). Reduction of cupric ions with elemental sulphur by Thiobacillus ferrooxidans. Appl. Environ. Microbiol. 56, 693–696.
- Sujith, P., Das, A., Mourya, B.S., Loka Bharathi, P. 2011. Immobilisation of manganese, cobalt and nickel by deep-sea-sediment microbial communities. Chemistry and Ecology, 27(3), 189-206.
- Sujith, P., Khedekar, V., Girish, A., Bharathi, P.L. 2010. Immobilization of nickel by bacterial isolates from the Indian ridge system and the chemical nature of the accumulated metal. Geomicrobiology Journal, 27(5), 424-434.

- Sujith, P., Mourya, B., Krishnamurthi, S., Meena, R., Bharathi, P.L. 2014. Mobilization of manganese by basalt associated Mn (II)-oxidizing bacteria from the Indian Ridge System. Chemosphere, 95, 486-495.

- Sujith, P., Mourya, B., Krishnamurthi, S., Meena, R., Bharathi, P.L. 2014. Mobilization of manganese by basalt associated Mn (II)-oxidizing bacteria from the Indian Ridge System. Chemosphere, 95, 486-495.

- Sunda, W.G., Huntsman, S.A. 1988. Effect of sunlight on redox cycles of manganese in the southwestern Sargasso Sea. Deep Sea Research Part A. Oceanographic Research Papers, 35(8), 1297-1317.

- Sunda WG, Huntsman SA (1990) Diel cycles in microbial manganese oxidation and manganese redox speciation in coastal waters of the Bahama Islands. Limnol Oceanogr 35:325–338

- Takai, K., Nakagawa, S., Reysenbach, A.L., Hoek, J. 2006. Microbial Ecology of Mid-Ocean Ridges and Back-Arc Basins. Back-Arc Spreading Systems: geological, biological, chemical, and physical interactions, 185-213.

- Tebo, B.M., Ghiorse, W.C, van Waasbergen LG, Siering PL, Caspi R 1997. Bacterially mediated mineral formation: insights into manganese(II) oxidation from molecular genetic and biochemical studies. In: Banfield JF, Nealson KH (eds) Geomicrobiology: interactions between microbes and minerals. Mineral Soc Am, Washington, DC, pp 225–266

- Tebo BM, Bargar JR, Clement BG, Dick GJ, Murray KJ, Parker D, Verity R, Webb SM 2004. Biogenic manganese oxides: properties and mechanisms of formation. Annu Rev Earth Planet Sci 32:287–328

- Tebo BM, Clement BG, Dick GJ 2007. Biotransformations of manganese. In: Hurst CJ, Crawford RL, Garland JL, Lipson DA, Mills AL, Stetzenbach LD (eds) Manual of environmental microbiology. ASM Press, Washington, DC, pp 1223–1238

- Tebo, B.M., Johnson, H.A., McCarthy, J.K., Templeton, A.S. 2005. Geomicrobiology of manganese (II) oxidation. TRENDS in Microbiology, 13(9), 421-428.

- Thamdrup, B. 2000. Bacterial manganese and iron reduction in aquatic sediments. in: Advances in microbial ecology, Springer, pp. 41-84.

- Thomas, E., Booth, L., Maslin, M., Shackleton, N. 1995. Northeastern Atlantic benthic foraminifera during the last 45,000 years: changes in productivity seen from the bottom up. Paleoceanography, 10(3), 545-562.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic acids research, 25(24), 4876-4882.
- Trimble, R., Ehrlich, H. 1970. Bacteriology of manganese nodules IV. Induction of an MnO2-reductase system in a marine Bacillus. Applied microbiology, 19(6), 966-972.
- Trudinger, P.A., Walter, M.R., Ralph, B. 2013. Biogeochemistry of Ancient and Modern Environments: Proceedings of the Fourth International Symposium on Environmental Biogeochemistry (ISEB) and, Conference on Biogeochemistry in Relation to the Mining Industry and Environmental Pollution (Leaching Conference), held in Canberra, Australia, 26 August–4 September 1979. Springer Science & Business Media.
- Turekian, K.K., Wedepohl, K.H. 1961. Distribution of the elements in some major units of the earth's crust. Geological Society of America Bulletin, 72(2), 175-192.
- Van de Peer, Y., De Wachter, R. 1997. Construction of evolutionary distance trees with TREECON for Windows: accounting for variation in nucleotide substitution rate among sites. Bioinformatics, 13(3), 227-230.
- Vandevivere, P., Kirchman, D.L. 1993. Attachment stimulates exopolysaccharide synthesis by a bacterium. Applied and Environmental Microbiology, 59(10), 3280-3286.
- Van Dover CL. 2000. *The Ecology of Deep-sea Hydrothermal Vents*. Princeton University Press: New Jersey.
- Vermelho, A.B., Meirelles, M.N.L., Lopes, A., Petinate, S.D.G., Chaia, A.A., Branquinha, M.H. 1996. Detection of extracellular proteases from microorganisms on agar plates. Memorias do Instituto Oswaldo Cruz, 91(6), 755-760.
- Vickerman, K. 1992. The diversity and ecological significance of Protozoa. Biodiversity and Conservation, 1(4), 334-341.

- Villalobos, M., Toner, B., Bargar, J., Sposito, G. 2003. Characterization of the manganese oxide produced by Pseudomonas putida strain MnB1. Geochimica et Cosmochimica Acta, 67(14), 2649-2662.
- Vinogradov, A. 1962. Average contents of chemical elements in the principal types of igneous rocks of the earth's crust. Geochemistry, 7(1962), 641-664.
- Vishnivetskaya, T.A., Kathariou, S., Tiedje, J.M. 2009. The Exiguobacterium genus: biodiversity and biogeography. Extremophiles, 13(3), 541-555.
- Von Damm, K., Edmond, J., Grant, B., Measures, C., Walden, B., Weiss, R. 1985. Chemistry of submarine hydrothermal solutions at 21 N, East Pacific Rise. Geochimica et Cosmochimica Acta, 49(11), 2197-2220.
- Vodyanitskii YN (2009) Mineralogy and geochemistry of manganese: a review of publications. Eurasian Soil Sci 42:1170–1178
- Walker, D. A., Linton, A. E., and Schafer, C. T. (1974). Sudan black B: A superior stain to rose Bengal for distinguishing living from non-living foraminifera *Journal of Foraminiferal Research* 4, 205-215.
- Walkley, A. and I. A. Black. 1934. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. 37: 29-37.
- Walton, W. R., 1952, Techniques for recognition of living Foraminifera: Contributions to the Cushman Foundation for Foraminiferal Research, v. 3, p. 56–60.
- Wang, G., Huang, L., Zhang, Y. 2008. Cathodic reduction of hexavalent chromium [Cr (VI)] coupled with electricity generation in microbial fuel cells. Biotechnology letters, 30(11), 1959.
- Wedepohl, K. 1962. Beiträge zur Geochemie des Kupfers. Geologische Rundschau, 52(1), 492-504.
- Williams, R.A. 2009. Comparing reef bioindicators on benthic environments off southeast Florida.
- Xiong, A., Jayaswal, R.K. 1998. Molecular Characterization of a Chromosomal Determinant Conferring Resistance to Zinc and Cobalt Ions inStaphylococcus aureus. Journal of bacteriology, 180(16), 4024-4029.

- Xuezheng, L., Aiguo, G., Haowen, C. 2008. Isolation and phylogenetic analysis of cultivable manganese bacteria in sediments from the Arctic Ocean. Acta Ecologica Sinica, 28(12), 6364-6370.
- Yakimov, M.M., Timmis, K.N., Golyshin, P.N. 2007. Obligate oil-degrading marine bacteria. Current opinion in biotechnology, 18(3), 257-266.
- Yocum CF, Pecoraro V 1999. Recent advances in the understanding of the biological chemistry of manganese. Curr Opin Chem Biol 3:182–187
- Zajic, J.E., 1969. Microbial biogeochemistry. Academic, New York

Appendix I

LIST OF FIGURES

Bacteria associated on fauna) and D) Fauna shell.

Fig. 4.62. EDS data to compare chemical composition of A) bacteria, B and C) Bacteria associated on fauna) and D) Fauna shell.

Appendix II

LIST OF TABLES

Appendix III

List of abbreviations

CIOB	Central Indian Ocean Basin
IONF	Indian Ocean Nodule Formation
CCD	Calcite compensation depth
TIC	Total inorganic carbon
TOC	Total organic carbon
TOM	Total organic matter
C/N	Carbon/ Nitrogen
LOC	Labile organic carbon
LOM	Labile organic matter
TC	Total counts
FDC/NVC	Frequency of dividing cells/ Naturally viable cells
DVC-a	Direct viable counts -aerobic
DVC-an	Direct viable counts -anaerobic
ATP	Adenosine triphosphate
PRIMER	Plymouth routines in multivariate ecological research
RTJ-IO	Rodrigues Triple Junction-Indian Ocean
BC	Box-core
TVBC	Temporal variability box-core
SVBC	Seasonal variability box-core
IVBC	Intermediate variability box-core
EVDC	Environmental variability data collection
ZMA	ZoBell Marine Agar
NI	Nitrifiers I
NII	Nitrifiers II
Ndl	Non-detectable leevels
TDLO	Thiobacillus Denitrificans like organisms
NRB	Nitrate reducing bacteria
RuBisCO	Ribulose bis-phosphate carboxylase/ oxigenase

Appendix IV

List of media used for culturable bacteria

The Aerobes

1. ZoBell Marine Broth and Agar (Himedia) as per manufacturer's instructions.
The broth concentrations 1, 25, 50 and 100% were amended with 1.5% agar agar.

2. 1. Nutrient Marine Broth and Agar (Himedia) as per manufacturer's instructions.
The broth concentrations 1, 25, 50 and 100% were amended with 1.5% agar agar.

3. Ammonium oxidizers (NI)

KH2PO4	0.25g
MgSO4 7H20	1.5g
FeSO4 7H20	0.1g
MnSO4	0.1g
Na2Mo04	0.05g
Pure agar	15g
Aged sea water	1 litre
pH 7.5-8	
$(NH_4)2SO4$	1.32g/50m1 of distilled water

10m1 1 1 of (NH4)2SO4 solution to be autoclaved separately and added just before pouring.

4. Mn-oxidizers

Two parts A and B of the medium were prepared separately.

Part A

NaHCO3	0.1g,
(NH4)2SO4	0.1 g,
K2HPO4	0.5g,
MgSO4	0.5g, was dissolved in 900 ml of seawater.

Part B

MnC12	12.5g was dissolved in 100m1 of distilled water and mixed to Part A.

Final salinity 35ppt

Final pH was adjusted to 7.8+ 0.2 in order to maintain a near neutral condition.

5. Cu-oxidizers

Two parts A and B of the medium were prepared separately.

Part A

NaHCO3 0.1g,

(NH4)2SO4 0.1 g,

K_2HPO4 0.5g,

MgSO4 0.5g, was dissolved in 900 ml of seawater.

Part B

$CuC1_2$ 12.5g was dissolved in 100m1 of distilled water and mixed to Part A.

Final salinity 35ppt

Final pH was adjusted to 7.8+ 0.2 in order to maintain a near neutral condition.

The Anaerobes

Sodium bicarbonate solution was filter sterilized and added to medium just before pouring.

2. Nitrate Reducers

Medium composition per litre of aged sea water:

KNO3 -0.101g

Nutrient agar 3.5 g 1 -1

The original 14 g of nutrient agar was modified to 25% strength of nutrient broth amended with pure agar to give a final agar concentration of 0.8%. pH 7.5-8.

Appendix V

List of important chemicals and buffers

1. Eh reference solutions

Reference solution A: (192mV)

Potassium ferrocyanide (K4Fe (CN)	6 0.1M
Potassium ferricyanide (K3Fe (CN)	6 0.05M

Reference solution B: (258mV)

Potassium ferrocyanide (K4Fe (CN)	6 0.01M
Potassium ferricyanide (K3Fe (CN)	6 0.05M
Potassium fluoride KF2. H2O	0.36M

2. Dissolved oxygen fixative and other reagents

i. Winkler A- Dissolve manganese (II) chloride (MnC1 5H20) (40g) in dissilled water (100m1).

ii. Winkler B- Dissolve separately potassium iodide (KI) (10g) and sodium hydroxide (NaOH) (36g) in minimum amount of distilled water. Mix two solutions in a 100 ml volumetric flask and make up to the volume with distilled water.

iii. Hydrochloric acid (50%)- Carefully add the concentrated HC1 (50m1) to distilled water (50m1).

iv. Sodium thiosulphate solution (approximately 0.01N): Dissolve Na2S203 .5H20 (2.49g) in distilled water and make up to 1 litre with distilled water.

v. Starch indicator solution: Dispense 1g of starch powder in 100m1 of distilled water and quickly heat the suspension to boiling. This solution should not be kept longer than a week.

vi. Standard iodate solution (0.01): Weigh accurately KI03 A.R. (0.35g) and dissolve in 1 litre of distilled water.

3. Total Mn

i.Citrate solution – A 1M citric acid solution, adjusted to pH 9 with concentrated sodium hydroxide solution

ii.Ascorbate solution- A 1M ascorbic solution adjusted to pH 9 with concentrated sodium hydroxide solution.

iii.Ammonium buffer solution- A 6M ammonia / 3M ammonium chloride buffer solution (pH 9.8).

iv.Borate buffer solution- A 0.5M borax/0.34M sodium hydroxide buffer (pH 9.5)

v.Synthetic sea-water – NaCl (30g), MgSO4 7H2O (3.5g) and CaCl2 6H2O (2.2g) were dissolved in 1 litre of pure water. The concentration of nitrate ions was 1ppm, except where otherwise specified.

vi.Nitrate solution 0.01M.

3. Total Cu

i.Citrate solution – A 1M citric acid solution, adjusted to pH 9 with concentrated sodium hydroxide solution

ii.Ascorbate solution- A 1M ascorbic solution adjusted to pH 9 with concentrated sodium hydroxide solution.

iii.Ammonium buffer solution- A 6M ammonia / 3M ammonium chloride buffer solution (pH 9.8).

iv.Borate buffer solution- A 0.5M borax/0.34M sodium hydroxide buffer (pH 9.5)

v.Synthetic sea-water – NaCl (30g), MgSO4 7H2O (3.5g) and CaCl2 6H2O (2.2g) were dissolved in 1 litre of pure water. The concentration of nitrate ions was 1ppm, except where otherwise specified.

vi.Nitrate solution 0.01M.

5. Bacterial counts

Acridine orange solution 0.01%

Acridine orange	0.1 g
Formaldehyde (5%)	100 ml

Filter through 0.22 m polycarbonate paper

Store in amber colored bottle at 4(±2) °C

Yeast extract solution 0.001%

DW	30 ml
Yeast extract	0.3 g

The yeast extract is dissolved in DW.

The solution is autoclaved, filter sterilized and stored in vials at 4(±2) °C.

Antibiotic cocktail of nalidixic, piromedic and pipemedic acid 0.0016%

DW	30 ml
Nalixidic acid	0.024 g
Piromedic acid	0.012 g
Pipemedic acid	0.012g
Saturated NaOH solution	150 L

The antibiotics are dissolved in saturated NaOH solution and DW

The solution is filter sterilized and stored in vials at 4(±2) °C

Na_2S $9H_2O$ 0.125%

DW	100 ml
$Na_2S.9H_2O$	5 g

The compound is mixed in autoclaved DW, filter sterilized and used immediately.

Buffered Formaldehyde 2%

Formaldehyde (38%)

Hexamine

Saturate formalin with hexamine.

Filter sterilize and store at room temperature

Additions for incubation and fixing for direct total counts

	TCL	**DVCa**	**DVCan**
	µl	**µl**	**µl**
Sample	5000	5000	5000
Yeast extract	-	50	50
Antibiotic cocktail	-	50	50
Sulfide solution	-	-	20
Buffered formalin (0 hr)	250	-	-
Buffered formalin (after 7hrs)	-	250	250

Biochemical characterization

Cultural Characteristics:

The following characteristics were noted:

Size, Shape, Color, Margin, Elevation, Opacity

Morphological and biochemical tests

Gram staining

a) Crystal violet

Crystal violet	2 g
Ethyl alcohol (95%)	20 ml

b) Gram's iodine

Iodine	1 g
Potassium iodide	2 g
Distilled water	300 ml

c) Ethyl alcohol 70%

d) Safranine

Smear of the isolates was prepared on slides, air-dried and heat fixed.

The slides were treated with crystal violet (1min) followed by Gram's iodine (1min).

Slides were then washed with decolorizing solution (ethyl alcohol) till the blue color disappears and counter stained with safranine for 30s.

Slides were washed with water, dried and observed under oil- immersion

Gram positive bacteria are stained purple while gram negative bacteria are stained pink

Motility

Motility was observed using hanging drop method.

Oxidase test

The enzyme oxidase is a part of the electron transfer system used by some organisms that use molecular oxygen as a terminal electron acceptor. Oxidase interacts with the membrane bound cytochromes and delivers cytochromes to oxygen. As a result H2O2 or H2O is generated. Strict anaerobes do not use oxygen and hence do not possess the oxidase enzyme. Most gram positive bacteria are oxidase negative as well as the members of the family Enterobacteriaceae. Oxidase positive bacteria possess cytochrome oxidase or indophenol oxidase (an iron containing haemoprotein). These both catalyze the transport of electrons from donor compounds (NADH) to electron acceptors (usually oxygen). The test reagent, N, N, N', N'-tetra-methyl-p-phenylenediamine dihydrochloride acts as an artificial electron acceptor for the enzyme oxidase. The oxidised reagent forms the coloured compound indophenol blue. The cytochrome system is usually only present in aerobic organisms which are capable of utilizing oxygen as the final hydrogen receptor. The end product of this metabolism is either water or hydrogen peroxide (broken down by catalase).

Oxidase reagent

DW 100 ml

N, N, N', N'-tetra-methyl-p-phenylenediamine dihydrochloride 1 g

A drop of oxidase reagent was placed on Whatman filter paper no.1

Isolates were picked using sterile toothpicks and smeared on treated filter paper to check for the presence of cytochrome oxidase in the isolates.

The observations were inferred from the following table

Observation	**Report**
1. Deep violet color developed immediately after smearing	Oxidase positive
2. Deep violet color developed after 30 sec	delayed positiveness
3. No color change.	Oxidase negative

Catalase test

The catalase test is used to detect the presence of catalase enzymes by the decomposition of hydrogen peroxide to release oxygen and water. Hydrogen peroxide is formed by some bacteria as an oxidative end product of the aerobic breakdown of sugars and if allowed to accumulate, is highly toxic. Catalase either decomposes hydrogen peroxide or oxidizes secondary substrates.

Hydrogen peroxide (3%)

Scrape the growth from a slant or plate with a non- metallic instrument.

Suspend it in 3 % hydrogen peroxide on a slide.

Examine for effervescence, presence of effervescence denotes catalase positive and absence denotes negative reaction.

Hugh and Leifson's medium (OF test)

Oxidative organisms can only metabolize glucose or other carbohydrates under aerobic conditions ie oxygen is the ultimate hydrogen acceptor. Other organisms ferment glucose and the hydrogen acceptor is then another substance eg sulphur. This fermentative process is independent of oxygen and cultures of organisms may be aerobic or anaerobic. The end product of metabolizing a carbohydrate is acid. Oxidizing organisms produce an acid reaction towards the top of the tube. Fermenting organisms produce an acid reaction throughout the medium. Organisms that cannot break down the carbohydrate aerobically or anaerobically, produce an alkaline reaction in the tube. Hugh and Leifson's medium can also be used for recording gas production and motility.

OF medium

1	Dextrose	1 g
2	Peptone	0.2 g
3	KH2PO4	0.03 g
4	Agar	1.5 g
5	Bromothymol blue	0.002 g
6	50% SW	100 ml

* Dextrose was filter sterilized and added to the medium later

The tubes containing the OF medium was stab inoculated with the cultures and incubated at room temperature for 48 hrs.

The observations were inferred from the following table

Observation	Report
1. Bottom to top yellow/ Bottom yellow	Fermentative (with or without gas)
2. Yellow only on top	Oxidative
3. Blue color	Alkaline

4. Growth, no color Growth only

5. No Growth Inert

Metabolic diversity of bacterial isolates

Screening for Amylase

Amylolytic medium

Nutrient Agar 28 g

Starch 2 g

50% SW 1000 ml

pH 7.5-7.8

Culture was spot inoculated on the medium and incubated for 24 –48 hrs at room temperature. On addition of iodine to the plate, the whole plate turns dark blue except for yellow/colourless halos around the colonies indicating amylase production.

Screening for Protease

a) Proteolytic medium

Nutrient Agar 28 g

Casein 2 g

50% Sea water 1000 ml

pH 7.5-7.8

b) HgCl2 solution

HgCl2 15 g

HCl 20 ml

DW 100 ml

Culture was spot inoculated on the proteolytic medium and incubated for 24 –48 hrs at room temperature.

After the colonies have grown, overlay the plate with HgCl2 solution

Observe for the clearance zone around the colony.

Screening for Lipase

Lipolytic medium

Peptone	10 g
NaCl	5 g
CaCl2	0.1 g
*Tween	10 ml
Agar	15 g
50% SW	1000 ml
pH	7.0-7.4

* Tween was autoclaved separately and added to the medium just before pouring

Culture was spot inoculated on the medium and incubated for 24 –48 hrs at room temperature. Observe for precipitate around the colony.

Screening for Phosphatase

Phosphatase medium

Nutrient Agar	28.0 g
50% SW	120 ml

After autoclaving and just before pouring the substrate, filter sterilized p-nitrophenyl phosphatase (sigma) was added to the medium so as to obtain a final concentration of 0.02%.Culture was spot inoculated on the medium and incubated for 24 –48 hrs at room temperature. Presence of a greenish yellow color around the colony is indicative of phosphatase production.

Screening for DNase

DNase test medium

DNase test agar	5.04 g
Toluidine blue	0.012 g

50% SW 120 ml

pH 7.5-7.8

Culture was spot inoculated on the medium and incubated for 24 –48 hrs at room temperature. Presence of a clearance zone indicated DNase production

Appendix VII
List of publications

I. Full length papers - (10)

From Thesis:

Mourya B.S. and Shyama S.K., (2017) Copper Mobilization by basalt associated Cu (II)-oxidizing bacteria from the Indian Ridge System. (Accepted in F1000 Research for publication).

Mourya B.S, and Shyama S.K., (2017). Novel benthic foraminifera diversity and their identification from carlsberg ridge eco system. (Accepted for publication in journal of Bio life).

Mourya B.S, Shyama S.K., Sujith P.P, Krishanmurthi S., Meena R.M. and Loka Bharathi (2015). Microcosm Investigation of Mn mobilization in basalt rock by Potential Bacteria R6 from carlsberg ridge eco system. Journal of Biolife, 3(2):415-427.

Sujith P.P, **Mourya B.S**, Krishanmurthi S., Meena R.M. and Loka Bharathi P.A*. (2014) Mobilization of manganese by basalt associated Mn(II)-oxidizing bacteria from the Indian Ridge System. Chemosphere. 95:486-495.

Others publications:

Das A, Sujith P.P, **Mourya B.S,** Bichhe S.U and Loka Bharathi P.A. (2011) Chemosynthetic activity prevails in deep-sea sediments of Central Indian Basin. Extremophiles, 15: 177-189.

Das A, Sujith P.P, **Mourya B.S**, Bichhe S.U and Loka Bharathi P.A. (2011). Bacterial response to contrasting sediment geochemistry in the Central Indian Basin. Sedimentology, 58: 756-784.

Sujith P.P, Anindita Das, **Mourya B.S** and Loka Bharathi P.A*. (2011). Immobilization of manganese, cobalt and nickel by deep-sea sediment microbial communities.Chemistry and Ecology. 27 (3):189–206.

Fernandes E.G., Dhillan V., **Mourya B.S.**, Shagufta S., Das A. and. Loka Bharathi P.A. (2011) Iron Released from ilmenite mineral sustains a phytoplankton community in microcosoms. Botanica Marina. DOI 10.1515/BOT.2011.051.

Das A, C.E.G. Fernandes, S.S. Naik, , **B.S. Mourya** P.P. Sujith, R. Sharma, and P.A. Loka Bharathi (2016). Microbial and biochemical alteration due to storage of deep sea under ambient tropical conditions. Indian journal of geo marine Sciences. 45, 733–741.

Das A, B.N. Nath, I. Suresh, S.M. Gupta, N.H. Khadge, C. Prakash Babu, **B.S. Mourya**, S.U. Biche, R. Sharma, and P.A. Loka Bharathi, Astrobiological implications of dim light phototrophy in marine clays (Accepted in LIFE SCIENCE AND SPACE RESEARCH for publication**).**

II. Symposium Abstracts- (10)

From Thesis:

- **Mourya B.S**, Sujith P.P, Krishnamurthi S, Meena R.M, Shyama S.K and Loka Bharathi P.A*. (2014). COPPER IMMOBILIZATION BY BACTERIA IN CARLSBERG RIDGE ROCK: ACTIVITY, CULTURABILITY AND IDENTITY. International Conference on Environmental Biology and Ecological Modelling (ICEBEM – 2014) February 24 to 26, 2014. Department of Zoology, Centre for Advanced Studies, Visva-Bharati (A Central University) Santiniketan - 731235, WB, India.

- **Mourya B.S,** Sujith P.P, Krishnamurthi S, Meena R.M and Loka Bharathi P.A*. (2012). MANGANESE IMMOBILIZING BACTERIA IN CARLSBERG RIDGE ROCK: DOMINANT GENERA AND THEIR ACTIVITY. Ridges and Hotspots around the Mascarene Islands: present activity, past evolution, 03-07 September, 2012, Bel Ombre, Mauritius.

- Sujith P.P, **Mourya B.S**, Krishnamurthi S, Meena R.M and Loka Bharathi P.A*. (2013).Mobilization of manganese from ridge rock by indigenous microbial communities. Ridges and Hotspots around the Mascarene Islands: present activity, past evolution, 03-07 September, 2012, Bel Ombre, Mauritius.

Others:

- **Mourya B.S.** and Guchhit Purbasha (2006) "Biofuel Technology: A Growing Source of Energy (Jatropha Curcus) Research" 47th Annual Conference of Association of microbiologist of India December-2006.

III Technical Reports and presentations - (3)

- I submitted my first year CSIR- SRF progress report in April 2013 entiled "**Ecological studies on the metal tolerant marine bacteria: A comparison between Mn and Cu tolerant bacteria from coastal and oceanic systems"** to HRDG-CSIR New Delhi, India.

- I submitted my two years CSIR- SRF progress report from April 2012 to April 2014 entiled "**Ecological studies on the metal tolerant marine bacteria: A comparison between Mn and Cu tolerant bacteria from coastal and oceanic systems"** to HRDG-CSIR New Delhi, India.