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RESEARCH ARTICLE

DIVERSITY OF BACTERIA ASSOCIATED WITH THE METAL RESISTANCE FROM THE INDUSTRIAL SOIL SAMPLES OF SALEM DISTRICT, TAMIL NADU, INDIA

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ABSTRACT

The diversity among the bacterial communities are mainly occurs in adverse environmental conditions. In this study we evaluated the bacterial diversity in a soil sample from industrial sites particularly metal based industrial factory with heavy metals. Industrial soil contains 14 different bacteria among that the dominant taxonomic groups of *Pseudomonas* and *Bacillus* were isolated. The isolated and identified bacterial diversity on genus level among the three different sites was analyzed by using PRIMER 6 (version 5.2.9). The generation of similarity matrix, Hierarchical cluster analysis (HCA), analysis of similarity (ANOSIM) and principal component analysis were carried out using SPSS package. ANOSIM was used to test for significant differences between sample groups defined by cluster tree, the percentage of cumulative dominance of bacteria with species rank calculated. The results indicated that the apparent diversity of bacterial communities from metal contaminated industrial soil was found to be sharing only 40% similarity. From that, the genera *Micrococcus* (SS2), *Bacillus* (SS3, SS12, JS43 and PS13), *Aeromonas* (JS10) and *Pseudomonas* (JS49 and PS15) appeared up to 6000 ppm of Fe(III) resistance, but in the presence of Mn (IV), 6 isolates namely *Bacillus* (SS3, JS43 and PS13) and *Pseudomonas* (JS49, PS1 and PS15) resistant to higher concentration of manganese (IV) as 6000 ppm. The present study would be a lime light for further exploration of eco-friendly bioremediation of metal contaminated soil in Salem district in turn enhances the benefits for the human welfare.

Key words: Diversity, heavy metal, similarity matrix, Hierarchical cluster analysis, cumulative dominance..

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INTRODUCTION

Metal pollution of the environment can occur through industrial processes such as mining, refining, and electroplating. In particular, heavy metals influence the microbial population by affecting their growth, morphology, biochemical activities and ultimately resulting in decreased biomass ultimately the population diversity also occurs. The selective pressure from the metal in the microbial growth environment, leads to microorganisms has evolved various mechanisms to resist the heavy metal stress due to its diversity (Kabeer*et al*.,2014).Bacteria develop heavy-metal resistance mostly for their survivals(Goering *et al.,* 1999) and long term exposure to metals leads to the selection or adaptation of a microbial community, which then thrives in polluted areas (Perez-de-Mora *et al.,* 2006).

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Generally the indigenous metal resistant bacteria have the ability to modify the physicochemical conditions of their surrounding environment either by detoxification, metal homeostasis, precipitation, redox transformations or by metabolic exploitation (Bruneel*et al*., 2006) and also ability to promote plant growth by various mechanisms like either oxidation or reduction, nitrogen fixation, solubilisation of minerals and production of siderophores (Glick *et al.*, 1999).Bacteria play an important role in maintaining soil fertility and structure. Because bacteria respond quickly and are sensitive to subtle environmental changes, they have been considered as efficient bio-indicators of soil quality. Both the structural and functional bacterial diversity are important indicators of soil health (Yu *et al*., 2014). Heavy metals include various essential elements in that Iron is an important industrial raw material and most abundant element in the earth crust (Enning and Garrelfs, 2014).Metal exposure also leads to the establishment of tolerant microbial populations, which are often represented by several Gram positives belonging to

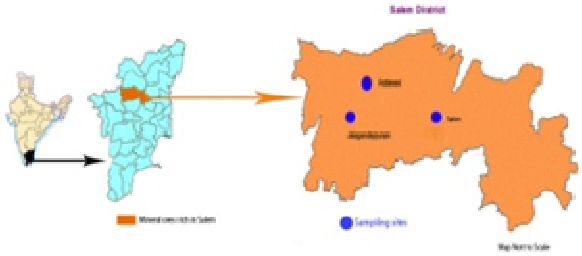
Bacillus, *Arthrobacter*, and *Corynebacterium*, as well as Gram negatives as *Pseudomonas*, *Alcaligenes* and *Ralstonia*(Elsilk*et al*., 2014). In heavy metal contaminated sites have demonstrated a high diversity of microorganisms. Lin *et al*., (2005) reported that the natural environment has two important forms of iron, i.e. ferric iron and ferrous iron can be interconvert by a redox reaction. Manganese is the second major important metal and 12th most abundant element in the earth's crust (Das et al., 2011). Metal speciation influences toxicity of a given metal because this is related to the chemical form of the element, *i.e.* speciation which depends on physical and chemical characteristics of the soil. Hence, the industrial soil containing Fe(III) and Mn(IV) is the toxic element even at low concentrations. The given iron and manganese contaminated soil containing bacterial diversity and its metal resistance also concentrated. Ranjard*et al.,* (2000) reported that the diversity of soil bacterial communities has been investigated for many years using methods based on isolating and culturing the micro-organisms. In this study also isolated bacterial isolated cultured in the various concentration of metal containing media. Heavy metal resistance is known to occur in many bacterial genera occur in metal contaminated sites, soil bacteria areusually exposed to heavy metals resulting in the establishment of heavy metal-resistance (Ellis *et al.,* 2003).The general heavymetal resistance mechanisms are an active metal efflux, synthesis of metal-binding peptides, proteins or polysaccharides such as metallothioneins, extracellular polymeric substance (EPS) and the increasing of detoxification enzymes expression (Prapagdee*et al.*, 2009). Heavy metals are able to induce increased resistance levels in soil bacteria and modify bacterial responses to environmental conditions either by inducing mutations or byaltering physiological responses leads to diversity among the population.

Biodiversity in a dynamic ecosystem provides insurance against the loss of certain species, species richness within bacterial communities engenders a corresponding range of behavioral reactions and metabolic pathways (Laplante*et al* 2011). Hill *et al*. (2003) reported that bacterial diversity from environments ranging from anaerobic sediments to aerobic soil. Particular measures can be chosen to suit the goal of the study, or a suite of measures can be applied to obtain a diversity profile. Studies on range of diversity indices have been used with bacterial communities, in particular the ubiquitous Shannon index, the evenness indices derived from it and Simpson's dominance index (Cho and Kim, 2000). The suitability and important consideration of these statistical approaches to estimating microbial diversity measures with diverse communities of bacteria reported by Hughes *et al*., (2001) and Curtis *et al.,* (2002). Microbial diversity has become an important alarm due to the importance of microorganisms in energy and also both organic and inorganic matter transformation (Torsvik, 1996). The bacterial community structure and diversity information was helpful to understand the association between environmental factors and ecosystem function. The pollution due to industrialization mainly affects the ecosystem. The adverse effect of pollution mainly leads to bacterial diversity due to environmental stress and perturbation. Laplante *et al.,* (2011) reported that the physiological status of an industrial soil ecosystem can be assessed by measuring the changes in the heterotrophic bacterial community in response to a higher level of metal oxide pollutants leads to development of metal resistant bacteria. The present study assesses the accuracy of the level for maximal metal tolerance, a valuable tool in microbial remediation, to monitor functional diversity occurring in bacterial communities. The present study aims to generate meaningful in sight son bacterial community adaptation in the presence of high metal concentration at the taxonomic level, under industrial environmental conditions.

MATERIALS AND METHODS

Sampling sites: The study area of Salem district finds very important place in the mineral map of Tamil Nadu,it lies between 11° and 12° North latitude and 77° 40' and 78° 5' East longitude. The total geographical area is 5205 sq.km and the district is comprises of seven taluks in which Mettur and Salem comprises major metal and nonmetal industries are abundant. Soil samples were collected in the depth of 1-10 cm from three different locations of industrial sites near (1) Salem Steel Plant, Salem (11º 37" N, 78º 04" E), (2) Stainless Fabrication, Jalakandapuram, Nangavalli (11º 42" N, 77 º 53" E) and (3) Jindal South West Pvt. Ltd., Potteneri, Mettur (11º 47" N, 77º 47" E) (Fig. 1). The collected soil samples were named as S1, S2 and S3 (Sample code: SS); J1, J2 and J3 (Sample code: JS); P1, P2 and P3 (Sample code: PS). Collected soil samples were passed through 2 mm sieve to remove large pieces of debris. Physical, chemical and biological parameters were analyzed from the prepared soil samples.

Fig. 1Map of sampling sites



Physicochemical parameter analysis: For pH measurement, the soil samples were processed by preparation of aqueous soil extracts (1:2.5, w/v) and were measured with a glass electrode by a pH-meter (Model: Cyberscan pH 510) at 20°C (Piotrowska-Seget*et al.,* 2005). The content of organic matter by $K_2CrO72H_2SO_4$ oxidation method, nitrogen (N) by Aalkali N-proliferation method, phosphorus (P) and potassium (K) quantified with the ASI method (Yu *et al*., 2014). For elemental analysis, to extract heavy metals from the given air-dried soil samples by acid digestion and extraction then metal concentration of each sample was analyzed by Atomic Absorption Spectrophotometry (AAS) (Begum *et al*., 2009). Soluble iron and manganese forms in the given soil were estimated by 1,10-phenanthroline method (Li *et al*., 2008) and formoldoxime method (Tebo*et al.,* 1996) respectively. The soil containing metal oxides of iron and manganese were analyzed by ammonium oxalate extraction (Arshad*et al.,* 1972; Mckeague*et al.,* 1996).

Bacterial analysis: Each of the collected triplicate soil homogenates of three different places prepared for plate count for bacterial analysis. The data of grown bacteria were subjected to one-way analysis of variance (ANOVA) to determine dominant cultivable bacterial sampling area among

the three different sites. The viable counts of bacteria were determined by counting visible colonies as colony forming unit per ml (CFU/ml). Independently growing colonies were selected based on the morphology, shape and color. The isolated bacterium was identified up to the genera by the taxonomic studies of morphological characteristics (shape, size, gram reaction and motility), cultural characteristics (nutrient agar colonies, slant culture, stab culture) and physiological characteristics (motility, oxidase, catalase reaction, utilization of glucose by oxidation and fermentation and starch hydrolysis). Identification was employed based on Bergey's Manual of Determinative Bacteriology (Holt et al., 1994), as well as molecular methods for effective metal resistant bacterial strains by Genetic identification. The sequences were submitted Gen Bank to assign accession numbers.

Bacterial diversity measures: The isolated and identified bacterial diversity on genus level among the three different sites was analyzed. The relative area data matrix generated was imported in to PRIMER 6 (Plymouth routines in multivariate Ecological research – version 5.2.9) from PRIMER-E-Ltd., Plymouth, UK. The generation of similarity matrix, Hierarchical cluster analysis (HCA), analysis of similarity (ANOSIM) and principal component analysis were carried out using SPSS package (SPSS Inc, v 17.0, Chicago, IL, USA). ANOSIM was used to test for significant differences between sample groups defined by cluster tree, the percentage of cumulative dominance of bacteria with species rank calculated (Cole *et al.,* 2013).

Screening of metal resistance: The metal tolerance pattern of each bacterial isolate was determined by the minimum inhibitory concentration (MIC) approach by using 50mM tris buffered nutrient agar (NAT) media (Manovski*et al.,* 1992). The range of concentrations used was 100 to 1000 ppm of Fe(III) and Mn(IV) in that the given isolates inoculated from initial (100 ppm) concentration of metals (Fe(III) and Mn (IV)) were consequently transferred to the next concentration (6000 ppm) and incubated at 30ºC for 48 hours. Based on the assessment of the growth, the metal tolerants were screened (Suzana*et al*., 1997). To compare metal (Fe and Mn) resistant data of industrial bacterial isolates, a joining method of the cluster analysis (CA) module (Statistica 5.5) and the clustering algorithm of Ward were applied. In this way, a dendrogram showing clustering trends among all the isolates on the site wise identification was generated.

RESULTS AND DISCUSSION

Physico-chemical parameters of collected soil: The collected soil from iron based industries in Salem district, Tamil Nadu were analyzed, in that pH of the soil samples were slightly acidic to neutral, they were ranged from 6.91 to 8.16. The light red, brown, dark black colored and rich in clay particles were noted in the study area soil. The metal concentration Fe and Mn concentration was found to be maximum as 8.4 and 6.6 ppm in the SS soil sample (Table 1). The given subsurface soils containing metal oxide treated with ammonium oxalate (pH-3) extraction gives 2.36, 1.26 and 2.71% of Fe and 0.4, 0.38 and 1.09% of Mn in site1, 2 and 3 respectively. The soil containing total iron and manganese determined by ammonium oxalate extraction followed by acid digestion respectively showed 8134.76 and 1084.79 mg/kg as higher amount in Salem site (SS) soil, 5879.4 and 637.41 mg/kg in JS soil and in PS soil estimated as 7720.5 and 436.04 mg/kg respectively. Salem district finds very important place in the mineral map of Tamilnadu, because of bauxite, dunite, magnetite, quartz, limestone, soapstone and granite are important minerals available in this district. Srinivasamoorthy *et al.* (2011) reported that iron was found to be higher in locations like Pappambatti and Nangavalli which recorded low resistivity values and higher total dissolved solids (TDS).The presence of iron oxide in soil properties leads to reddish brown to dark brown mottles arising from the enrichment of different Fe oxides (Baudouin-Cornu*et al.,* 2009).

Bacterial isolation and identification from collected soil samples: Sampling environments that contain prominent concentrations of mineral mixture are a potential source of bacteria, because large numbers of bacterial strains were isolated from lower dilutions of soil from three different metal based industrial sites of Salem district. Heterotrophic bacterial counts range from 10^4 and 10^5 dilutions, in that SS (site 2), JS (site 2) and PS (site 1) soil has the higher bacterial colonies. They were 37, 39 and 36 x 10^5 CFU/g respectively. The ANOVA gives the F ratio of SS sample value was 103.40, PS soil samples has 11.19 and JS sample value has 22.48, the P values of PS, JS and SS soil sample ranges like 0.0094, 0.0016 and 0 respectively (Fig. 2).Initially, 90 bacterial strains were purified and characterizations and variability among all the isolated strains was observed with respect to all the studied parameters. Kakimoto *et al.* (1974) reported a simplified schematic representation of heterotrophic bacterial identification based on the staining reaction, motility, catalase, oxidase and glucose fermentation result are regard as to identification. In the given results, bacteria belonging to the dominant taxonomic groups of *Pseudomonas* and *Bacillus* were isolated from the culture media. The majority of isolates, total 17% of *Pseudomonas*, next to this16.33% representatives of *Bacillus*, *Acetobacter* 14.33%, *Lactobacillus* 10.33%, *Micrococcus* 8.66%, *Corynebacterium* 6.6%, *Alcaligenes* and facultative aerobic *Clostridium* both 6.33%, *Acinetobacter* 4.33%, *Azotobacter* 3.33%, *Acidophilum* 2.33%, *Entero-bacteriaceae* and *Planococcus* both 1.66% finally 0.66% *Dexia* identified among the 90 isolates from three different sites (Fig.3). Geo microbiology is an important branch of earth sciences since the end of the twentieth century itself.

In this field major studies on the microbial processes in geologic environment and all kinds of geochemical records generated in these processes, i.e., interaction of microorganisms with minerals, microbial ecology at the extreme environments and molecular geomicrobiology (Xuezheng *et al.,* 2008).In the given 3 industrial sites, *Bacillus*>*Pseudomonas*> *Acetobacter*> *Micrococcus*> *Corynebacterium* then others occur in site 2 samples but in site 2 and 3 *Pseudomonas* isolates dominant it could be revealed that the conditions of bacterial isolation and the nature and physiological characteristics of each bacterial isolate mainly depends on the physiology of the sampling site (Hassan *et al.,* 2008). The selected both Fe(III) and Mn(IV) resistant isolates taxonomic groups conformed by molecular identification sequencing of amplified 16S rDNAssequences of SS3 and SS12 isolates were submitted to confirmed NCBI database search using Blastn to confirm the species of the bacterium respectively. The result revealed that the isolated strain SS3 is *Bacillus methylotrophicus*of Gram positive bacteria had the highest similarity (96%) and accession number obtained is KM001603. *Bacillus cereus* (SS12) had 85% similarity and the

Table. 1. Physiochemical parameters of collected soil

S. No.	Parameter	SS soil (Salem)	JS soil (Jalakandapuram)	PS soil (Pottaneri)
1	pH	6.91	7.6	8.16
2	Ec(dSm^{-1})	0.6	0.3	0.1
3	Color	Reddish brown	Light red	Brownish red
4	Texture	Clay	Loamy	Clay
5	Fe (ppm)	8.4	4.6	5.4
6	Mn (ppm)	6.6	2.4	1.2
7	Oxalate extractable Fe (%)	2.36	1.26	2.71
8	Oxalate extractable Mn (%)	1.4	0.38	1.09
9	Total Fe (mg/kg)	8134.76	5879.4	7720.5
10	Total Mn (mg/kg)	1084.79	637.41	436.04

Fig 2. Bacterial isolation from Soil

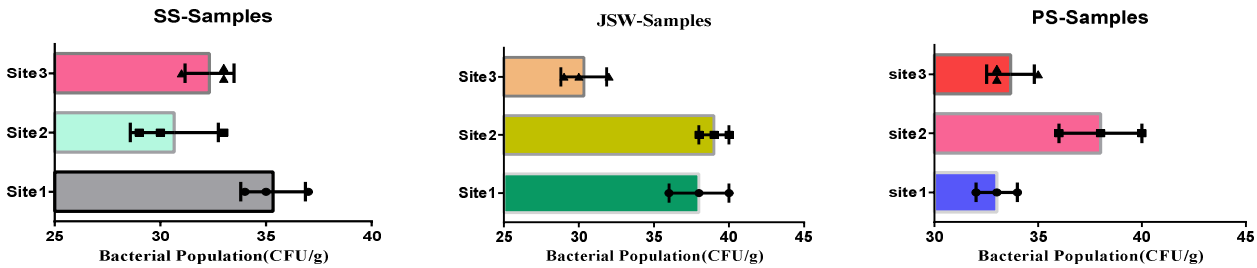
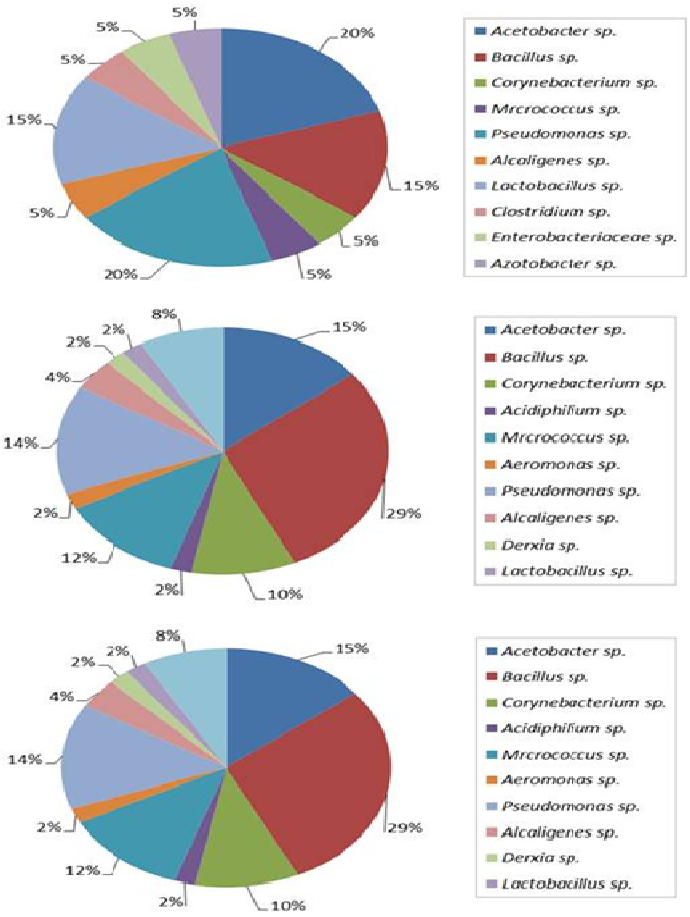


Fig 3. Percentage of Bacterial genera: a.SS (Site 1), b. JS (site 2), c. PS (site 3)



accession number is KM001604 (Fig.4 and 5).The genome were deposited in Gen Bank nucleotide sequence database and obtained accession numbers of KM001603 and KM001604 respectively.

Fig. 4. Sequencing matching of *Bacillus , methylotrophicus* KM001603 (SS3)

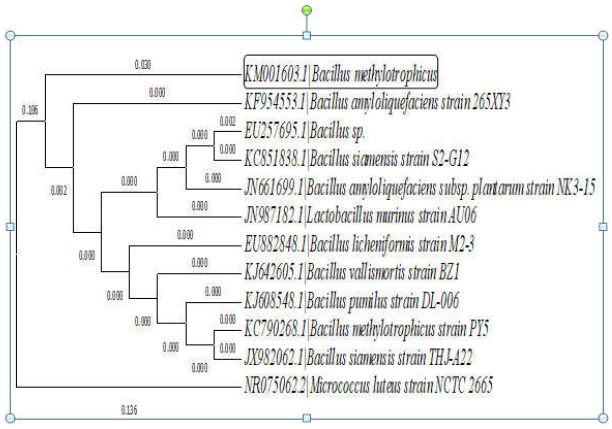
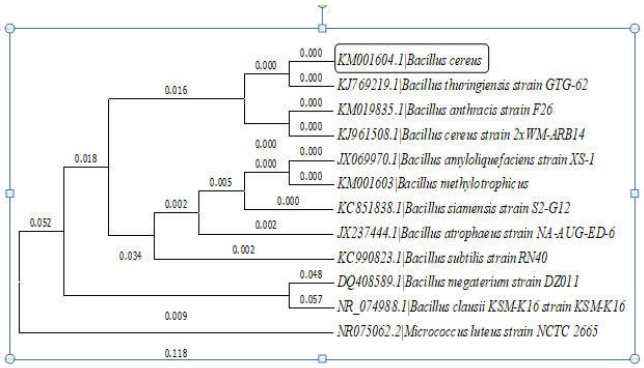


Fig 5.Sequencing matching of *Bacillus cereus* KM001603 (SS12)



Fredrickson and Gorby (1996) described about the phylogenetic diversity of dissimilatory iron reducing bacteria, in that they stated that a number of studies have focused on the isolation and taxonomic characterization of DIRB. In the given study, the isolated dominant bacterial community also having ecological impact because which has Fe and Mn resistance and also reducing capability.

Fe(III) and Mn(IV) tolerant bacteria: The isolates were examined tolerant limits for Fe(III) and Mn(IV) and showed resistance up to 6000 ppm concentrations. Among the 90 isolates obtained from the three different sites the 14 bacterial isolates able to grow in a NAT containing Fe(III) at 1000 ppm. In that, the three different strains of *Bacillus* and *Micrococcous* from SS soil, 5 strains of *Bacillus*, *Pseudomonas*, *Aeromonas* and *Corynebacterium* from JS soil in addition to this *Bacillus* and *Pseudomonas* from PS soil having higher resistance to Ferric iron. But 8 isolates from three sites in that 4 strains of *Bacillus*, 2 strains of *Pseudomonas, Aeromonas*and *Micrococcou s*having tolerance up to 6000 ppm Fe(III) (Fig. 6).In the Mn resistant pattern, the growth of *Bacillus* (SS1) and *Pseudomonas* (PS1) in the manganese (6000 ppm) containing media were negligible. But, the 6 isolates namely, *Bacillus* (SS3, JS43 and PS13) and *Pseudomonas* (JS49, PS1 and PS15) have higher tolerance up to 6000 ppm. Cluster analysis of Fe(III) and Mn(IV) resistant patterns of all the 90 isolates from 3 different sites were showed two major clusters according to the concentration of Fe(III) and Mn(IV) added to the medium e.g. a composite up to 500 ppm and above 500 ppm of metal and also an additional grouping of the strains according to genus level was found in the form of sub-clusters among the two major clusters (Fig. 7).In metal resistance, comparable observations were made by earlier researchers (Rajbanshi, 2008; Ceylan*et al*., 2012; Gupta *et al.,* 2012).In the given study, *Bacillus* populations precisely modified to high concentrations of heavy metals will increase the capability of bioremediation of Fe and Mn metal contaminated soils, because often the inhibitory effect of higher concentration of metal. These adaptations mainly recognized due to a variety of chromosomal, transposon and plasmid mediated resistance systems in bacteria (Bruins *et al*., 2000).

Fig 6. Dendrogram representing similarities of tolerance of Fe(III) up to 6000 ppm containing bacterial strains isolated from three different (SS,JS,PS) iron based industrial soils. Abbreviations: SS= Salem soil, JS= Jalakandapuram soil, PS= Potteneri soil isolate with ID number, B= *Bacillus,* P = *Pseudomonas,* M= *Micrococcus*, AE= *Aeromonas*, C= *Corynebacterium*

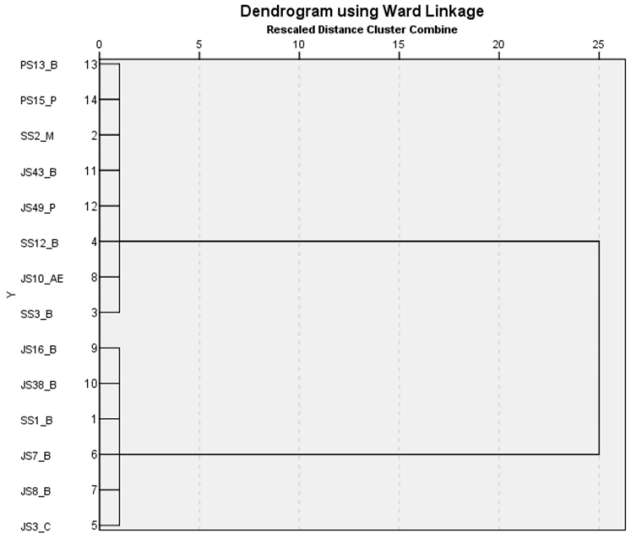
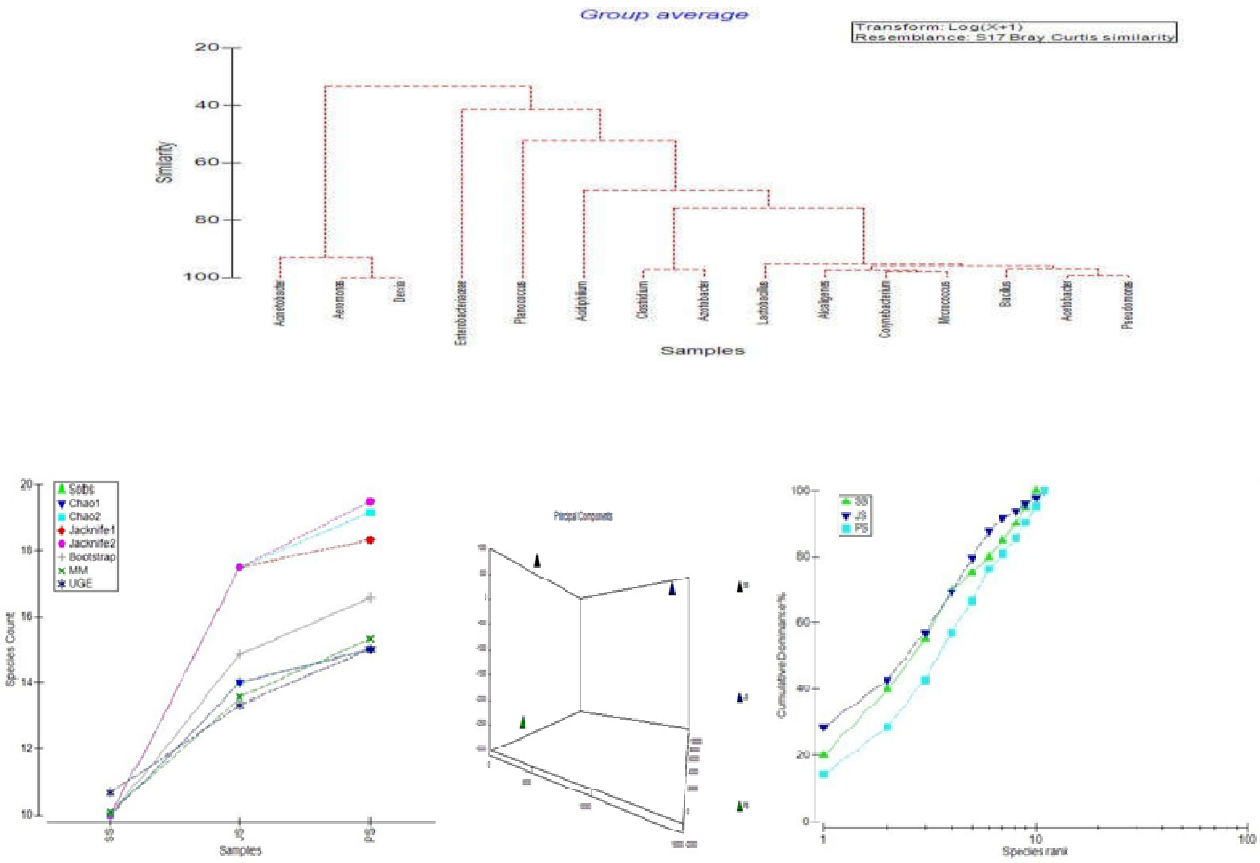


Fig 7. Dendrogram representing similarities of tolerance of Mn(IV) up to 6000 ppm containing bacterial strains isolated from three different (SS,JS,PS) iron based industrial soils. Abbreviations: SS= Salem soil, JS= Jalakandapuram soil, PS= Potteneri soil isolate with ID number, B= *Bacillus,* P = *Pseudomonas,* M= *Micrococcus*, C= *Corynebacterium,* AE= *Aeromonas*

Diversityamong the isolates: The bacterial communities growing in the metal contaminated soil sites are dominated by fewer, possibly specialized genera present in very high abundance, accompanied by a suite of rarer species. The abundance is distributed unevenly among the species present. The bacterial communities from metal contaminated industrial soil were found to be sharing only 40% similarity. The results of the multivariate cluster analysis of the present study have been summarized. A single link bray curtis cluster analysis dendrogram was constructed among the genus level identified isolates from three different sites (Fig. 3.8). The distinctly repeated genus *Pseudomonas* and *Acetobacter*additionally *Clostridium* and *Lactobacillus* dominantly isolated in all the three sites. Different characteristics of *Bacillus* in the same site also identified. The relative abundance of the 15 bacterial genera associated with soil bacterial communities. In that the major bacterial flora distribution showed as *Bacillus* and *Acetobacter*was the most dominant bacteria strains in the given soil communities, with the other two dominant phyla

Fig 8. The similarity of samples based on their community composition, considered at the genus level for identified bacterial isolates. A & B. Cluster tree calculated using Bray–Curtis similarity with jackknife values, C. Principal Component analysis, D. Cumulative dominance



Corynebacterium and *Pseudomonas*.Shange*et al*., (2012) reported that assessing the diversity and composition of bacterial communities across a wetland, transition and upland gradient in Macon County Alabama, where the most dominant soil bacterial communities may eventually be an important indicator of ecological impact in wetland ecosystems. In the given study, the isolated dominant bacterial community also having ecological impact because which has Fe and Mn resistance and also reducing capability. A wide metabolic diversity was established, including a higher resistance against metal oxide. The reported results will contribute to our understanding of microbial activity and strategies for cell survival and growth in metal contaminated ecosystems. This might also contribute to unravel prerequisites for bioremediation habitats in metalized soil.

REFERENCES

Arshad, M.A., St. Arnaud, R.J. and Huang, P.M., 1972. Dissolution of trioctahedral layer silicates by ammonium oxalate, sodium dithionite-citrate-bicarbonate, and potassium pyrophosphate.*Can. J. Soil Sci.,* 52: 19-26.

Baudouin-Cornu, P., Lagniel, G., Chedin, S. and Labarre, J. 2009.Development of a new method for absolute protein quantification on 2-D gels. *Proteomics,* 9: 4606–4615.

Begum, M., Harikrishna, R., Khan, I. and Veena, K., 2009.Analysis of heavy metals concentration in soil and lichens from various localities of Hosur road, Bangalore, India .*E-Journal of Chemistry*, 6(1): 13-22.

Bruneel, O., Duran, R., Casiot, C., Elbaz-Poulichet, F. and Personne, J.-C. 2006. Diversity of Microorganisms in Fe-As-Rich Acid Mine Drainage Water of Carnoulès, France. *Applied and Environmental Microbiology.,*72: 551-556.

Ceylan, O. and Ugur, A. 2012. Bio-Monitoring of Heavy Metal Resistance in *Pseudomonas* and *Pseudomonas* related genus. *J. Biol. Environ. Sci.*, 6(18): 233-242.

Cho, J. C. and Kim, S. J. 2000. Increase in bacterial community diversity in subsurface aquifers receiving livestock wastewater input. *Appl. Environ. Microbiol*., 66: 956-965.

Cole, JK., Peacock, JP., Dodsworth, JA., Williams, AJ., Thompson, DB. and Dong, H. 2013. Sediment microbial communities in great boiling spring are controlled by temperature and distinct from water communities. *ISMEJ.,* 7: 718-729.

Curtis, T.P., Sloan, W.T. and Scannell, J.W. 2002. Estimating prokaryotic diversity and its limits. *Proc. Natl. Acad. Sci.,* USA 99: 10494-10499.

Das, A.P., Sukla, L.B., Pradhan, N. and Nayak, S. 2013. Manganese biomining: A review. *Bioresource Technology*, 102: 7381–7387.

Ellis, R.J., Morgan, P., Weightman, A.J. and Fry, J.C. 2003. Cultivation dependent approaches for determining bacterial diversity in heavy metal-contaminated soil. *Appl. Environ. Microbiol*., 69; 3223-3230.

Elsilk, SE., Shanshoury, AERR El. and Ateya, PS. 2014. Accumulation of some heavy metals by metal resistant avirulent Bacillus anthracis PS2010 isolated from Egypt.

African Journal of Microbiology Research, 8 (12); 1266-1276.

Enning, D. and Garrelfs, J. 2014. Corrosion of Iron by Sulfate-Reducing Bacteria: New Views of an Old Problem. *Appl. Environ. Microbiol.,* 80(4): 1226–1236.

Fredrickson, J.K., Zachara, J.M., Kennedy, D.W., Dong, H., Onstott, T.C., Hinman, N.W. and Li, S.M., 1998. Biogenic iron mineralization accompanying the dissimilatory reduction of hydrous ferric oxide by a groundwater bacterium. *Geochim. Cosmochim. Acta*., 62(19/20): 3239 −3257.

Glick, BR., Patten, CL., Holguin, G. and Penrose, GM. 1999. Biochemical and genetic mechanisms used by plant growth promoting bacteria. *Imperial College Press*, London.

Goering, PL., Aposhian, HV., Mass, MJ., Cebrián, M., Beck, BD. and Waalkes, MP., 1999. The enigma of arsenic carcinogenesis: role of metabolism. *Toxicol. Sci.,* 49(1): 5-14.

Gupta, N. and Vijay Kumar, 2012. Identification and isolation of heavy metal (copper) resistant bacteria. *Archives of Applied Science Research*, 4 (1): 577-583.

Hassan, S.H.A., Abskharon, R.N.N., Gad El-Rab, S.M.F. and Shoreit, A.A.M. 2008. Isolation, characterization of heavy metal resistant strain of *Pseudomonas aeruginosa*isolated from polluted sites in Assiut city, Egypt. *J. Basic Microbiol*., 48:168-176.

Hill, G.T., Mitkowski, N.A., Aldrich-Wolfe, L., Emele, L.R., Jurkonie, D.D., Ficke, A., Maldonado-Ramirez, S., Lynch, S.T. and Nelson, E.B., 2003. Methods for assessing the composition and diversity of soil microbial communities. *Applied Soil Ecology,* 15: 25–36.

Holt, J.G., Kreing, N.R., Sneath, P.H.A., Staley, J.T. and Williams, S.T. 1994. Bergey's manual of determinative bacteriology. Lippincott Willams and Willkins, Baltimore. USA. ISBN-13:978.0683006032. 9: 787.

Hughes, J.B., Hellmann, J.J., Ricketts, T.H. and Bohannan, B.J.M. 2001. Counting the uncountable: statistical approaches to estimating microbial diversity. Appl. Environ. *Microbiol*. 67: 4399-4406.

Kabeer, R., Varghese, R., Kannan ,V.M., John Richard Thomas. andPoulose, SV. 2014. Rhizosphere bacterial diversity &heavy metal accumulation in Nymphaeapubescens in aid of phytoremediation potential. *J. BioSci. Biotech.,* 3(1): 89-95.

Kakimoto, D., Okita, K. and Inai, M. 1974. Mineral requirements of marine bacteria. In: Effect of ocean environment on microbial activities. R. R. Colwell and R. Y. Morita (eds.): 16-37, University Park Press, USA.

Laplante, K. and Derome, N. 2011. Parallel changes in the taxonomical structure of bacterial communities exposed to a similar environmental disturbance. *Ecology and evolution,* 37: 481-501.

Li, X.M., Zhou, S.G., Li, F.B., Wu, C.Y., Zhuang, L., Xu, W. and Liu, L. 2008. Fe (III) oxide reduction and carbon tetrachloride dechlorination by a newly isolated *Klebsiella pneumonia* strain L17. *Journal of Applied Microbiology*, 106: 130–139.

Lin, B., Braster, M., Van Breukelen, B.M., Van Verseveld, H.W., Westerhoff, H.V. and Roling, W.F.M. 2005.*Geobacteraceae*community composition relates to hydrochemistry and biodegradation in an iron-reducing aquifer polluted by a neighboring landfill. *Appl Environ Microbiol.,*71: 5983–5991.

Manovski, S., Roddick , F.A. and Britz M.L. 1992. Isolation of lead tolerant microbes from a contaminated site in Melbourne, Australia. In: Soil Decontamination Using Biological Processes. EFB Task Group on Soil Decontamination Using Biological Processes DECHEMA, Frankfurt. pp 689–695.

McKeague, J.A. and Day, J.H. 1996. Dithionite and oxalate extractable Fe and Al As aids in differentiating various classes of soils. *Cn. Soil. Sci*., 46: 13-24.

Perez-de-Mora, A., Burgos, P., Madejón, E., Cabrera, F., Jaeckel, P. and Schloter, M. 2006. Microbial community structure and function in a soil contaminated by heavy metals: effects of plant growth and different amendments. *Soil Biology and Biochemistry,* 38(2): 327-341.

Piotrowska-Seget, Z., Cycon, M. and Kozdroj, J. 2005. Metal-tolerant bacteria occurring in heavily polluted soil and mine spoil. *J. Appl. Soil Ecology,* 28: 237–246.

Prapagdee, B. and Watcharamusik, A. 2009. Adaptive and cross-protective responses against cadmium and zinctoxicity incadmium-resistant bacterium isolated from a zinc mine. *Brazilian Journal of Microbiology*, 40: 838-845.

R., Kapil, S. and Oehme, F.W. 2000.Microbial resistance to metals in the environment. *Ecotoxicol. Environ. Saf*., 45: 198-207.

Rajbanshi, A. 2008. Study on heavy metal resistant bacteria in Guheswori sewage treatment plant. *Our Nature,* 6: 52-57.

Ranjard, L., Brothier, E. and Nazaret, S. 2000. Sequencing bands of ribosomal intergenic spacer analysis fingerprints for characterization and micro scale distribution of soil bacterium populations responding to mercury spiking. *Appl. Environ. Microbiol*., 66: 5334–5339.

Shange, R.S., Ankumah, R.O., Ibekwe, A.M., Zabawa, R. and Dowd, S.E. 2012. Distinct soil bacterial communities revealed under a diversely managed agro ecosystem. *PLOS One,* 7: 403-38.

Srinivasamoorthy, K., Vijayaraghavan. K., Vasanthavigar, M., Rajivgandhi, R., Sarma, V.S. 2011. Integrated techniques to identify groundwater vulnerability to pollution in a highly industrialized terrain, Tamilnadu, India. *Environ Monit Assess*, 182: 47–60.

Suzana, T., Britz, L.M. and Bhave, M. 1997. Detection of heavy metal ion resistance genes in gram-positive and gram-negative bacteria isolated from a lead-contaminated site. *Biodegradation*, 8: 113–124.

Tebo, B.M., Bargar, J.R., Clement, B.G., Dick, G.J., Murray, K.J. Parker, D., 2004. Biogenic manganese oxides: Properties and mechanisms of formation. *Annu. Rev. Earth Planet. Sci*., 32: 287–328.

Torsvik, V., Sorheim, R. and Goksoyr, J. 1996. Total bacterial diversity in soil and sediment communities - a review. *J. Ind. Microbiol.,* 17: 170 -178.

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.

Yu, X., Li, Y., Zhang, C., Liu, H., Liu, J. and Chen, Q., 2014.Culturable Heavy Metal-Resistant and Plant Growth Promoting Bacteria in V-Ti Magnetite Mine Tailing Soil from Panzhihua, China. *PLoS ONE*, 9(9): e106618.