

[10.1071/SR22135](https://doi.org/10.1071/SR22135)

Soil Research

Supplementary Material

Bacterial succession in an abandoned circum-neutral opencast coal mine in India

Sohini Banerjee^{A,B,C}, Arijit Misra^A, Abhijit Sar^A, Srikanta Pal^A, Shibani Chaudhury^B, and Bomba Dam^{A,}*

^AMicrobiology Laboratory, Department of Botany (DST-FIST & UGC-DRS Funded), Institute of Science, Visva-Bharati (A Central University), Santiniketan, West Bengal 731235, India.

^BDepartment of Environmental Studies, Institute of Science, Visva-Bharati (A Central University), Santiniketan, West Bengal 731235, India.

^CPresent Address: Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX 77030, USA.

*Correspondence to: Bomba Dam Microbiology Laboratory, Department of Botany (DST-FIST & UGC-DRS Funded), Institute of Science, Visva-Bharati (A Central University), Santiniketan, West Bengal 731235, India Email: bomba.dam@visva-bharati.ac.in

Table S1. Notable works pertaining to physicochemical &/or microbial studies in mines of Europe, America, Africa and Asia. Studies on Indian coalmines are tabulated at the end.

Location	Mine	Soil pH	Sample used		Physico-chemical study	Microbial study type		Reference	
			Temporal	Spatial		Culture dependent	Culture independent		
EUROPE	Iberian Belt, Spain	Pyrite	Acidic	No	No	Yes	No	Clone library sequencing	(González-Toril et al., 2011)
	Iberian Belt, Spain	Cu	Acidic	No	Yes	Yes	No	No	(Nieto et al., 2013)
	Carnoules, France	Pb, Zn	Acidic	3 Years	Yes	Yes	No	TRFLP [#] , Amplicon sequencing	(Volant et al., 2014)
	Botswana, Germany, & Sweden	Pyrite	Acidic	3 Years	Yes	Yes	No	Clone library sequencing, qPCR [#]	(Korehi et al., 2014)
	Sokolov, Czech Republic	Coal	Neutral	6, 12, 21 & 45 Years	No	Yes	No	PLFA [#]	(Urbanov' et al., 2011)
	Sokolov, Czech Republic	Coal	Alkaline	1 - 44 Years	No	Yes	Yes	Total Count	RFLP
AMERICA	California, US	Coal	Acidic	11 Month	No	Yes	No	FISH [#]	(Edwards et al., 1999)
	Arizona, US	Pb, Zn	Acidic	No	Yes	Yes	No	Clone library sequencing	(Mendez et al., 2008)
	Pennsylvania, US	Coal	Acidic	No	Yes	Yes	No	Amplicon sequencing, qPCR	(Lee et al., 2017)
	Wisconsin, US	Pb, Zn	Neutral	No	No	Yes	SRB	FISH	(Labrenz and Banfield, 2004)
	Greens Creek, Alaska	Ag	Alkaline	No	Yes	Yes	SRB, SOB, MPN, IOB	No	(Lindsay et al., 2009)
	Sossego, Brazil	Cu	Neutral	No	Yes	Yes	No	Amplicon sequencing	(Pereira et al., 2014)
AFRICA	Mpumalanga Province, South Africa	Coal	Acidic	No	Yes	Yes	No	No	(Fosso-Kankeu et al., 2017)
	Jwaneng, Botswana	Diamond	Acidic	No	Yes	Yes	No	No	(Murty and Karunakara, 2008)
	South Africa	Coal	Acidic	1 - 8 years	No	Yes	Total Count	PLFA	(Claassens et al., 2008)
ASIA	Kerman–Bahabad, Iran	Coal	Acidic	No	Yes	Yes	No	No	(Shahabpour et al., 2005)
	Kohistan, Pakistan	Pb, Zn	Acidic	No	Yes	Yes	No	No	(Muhammad et al., 2011)

	Zhuji City, China	Coal	Acidic	No	Yes	Yes	Total Count	No	(Liao and Xie, 2007)
	Fankou, China	Pb, Zn	Acidic	No	Yes	Yes	No	T-RFLP	(Huang et al., 2011)
	Tongchang, Yinshan, & Yongping, China	Cu	Acidic	No	Yes	Yes	No	Microarray	(Xie et al., 2011)
	Southeast, China	Cu, Pb, Zn, Pyrite	Acidic	No	Yes	Yes	No	No	(Kuang et al., 2013)
	Southeast China	Cu, Pb, Zn, Pyrite	Acidic	No	Yes	Yes	No	Meta-genomics, transcriptomics	(Hua et al., 2015)
	Guangdong Province, China	Poly-metals	Acidic	No	Yes	No	No	Meta-genomics, transcriptomics	(Chen et al., 2015)
	Antaiba, China	Coal	Neutral	2 - 30 Years	No	Yes	No	PLFA, Microarray, Amplicon sequencing	(Li et al., 2016)
	Shuimuchong, China	Cu	Neutral	No	Yes	Yes	No	No	(Liu et al., 2014)
	Hubei, China	Coal	Alkaline	No	No	Yes	No	Clone library sequencing, RT-PCR	(Wei et al., 2014)
INDIA	Madhya Pradesh	Coal	Acidic	No	No	Yes	No	No	(Jamal et al., 1991)
	Jharkhand	Coal	Acidic	No	No	Yes	Total count	No	(Ghose, 2004)
	Assam	Coal	Acidic	No	Yes	Yes	No	No	(Saikia et al., 2014)
	Orissa	Coal	Neutral	2 - 10 Years	No	Yes	No	RAPD [#]	(Maharana and Patel, 2015)
	Jharkhand	Coal	Neutral	No	Yes	Yes	No	No	(Sinha and SINha, 1987)
	Jharkhand	Coal	Neutral	No	Yes	Yes	No	No	(Masto et al., 2011)
	Jharkhand	Coal	Neutral	No	Yes	Yes	No	No	(Kumar and Singh, 2016)
	Jharkhand	Coal	Neutral	No	Yes	Yes	No	No	(Pandey et al., 2016)
	West Bengal	Coal	Alkaline	No	Yes	Yes	No	No	(Sadhu et al., 2012)

[#] TRFLP: Terminal restriction fragment length polymorphism, qPCR: Quantitative polymerase chain reaction, PLFA: Phospholipid fatty acid analysis, FISH: Fluorescence in situ hybridization

Table S2. Relative abundance of phyla in Tasra colliery soil samples.

SI No	Phylum	T.13	T.14	T.15	T.16
1	Acidobacteria	0.0925315	0.0268506	0.0700743	0.0232951
2	Actinobacteria	0.2081653	0.4112589	0.2010495	0.4098296
3	AD3	0.0003803	0.0004322	0.0001558	0.0000000
4	Armatimonadetes	0.0000272	0.0000000	0.0000000	0.0000000
5	Bacteroidetes	0.0308574	0.0079857	0.0323168	0.0370104
6	BRC1	0.0000000	0.0000251	0.0000107	0.0000000
7	Chlamydiae	0.0006383	0.0000063	0.0000000	0.0000000
8	Chlorobi	0.0432438	0.0096642	0.0000000	0.0007852
9	Chloroflexi	0.0809192	0.0487157	0.1436720	0.0708426
10	Cyanobacteria	0.0016570	0.0026619	0.0101188	0.0030063
11	Elusimicrobia	0.0001087	0.0000000	0.0000000	0.0000000
12	FBP	0.0003260	0.0000626	0.0002202	0.0000000
13	Fibrobacteres	0.0000000	0.0001816	0.0003813	0.0000000
14	Firmicutes	0.0178055	0.0334960	0.0779911	0.0087198
15	Fusobacteria	0.0000000	0.0000313	0.0000000	0.0000000
16	Gemmatimonadetes	0.0757989	0.0237628	0.0269351	0.0700873
17	GN02	0.0012088	0.0002881	0.0000161	0.0001047
18	GOUTA4	0.0000272	0.0000000	0.0000000	0.0000000
19	MVP-21	0.0000000	0.0000000	0.0000000	0.0002244
20	Nitrospirae	0.0114085	0.0028373	0.0031151	0.0057359
21	OD1	0.0058265	0.0008706	0.0001128	0.0000075
22	OP11	0.0001494	0.0001942	0.0000107	0.0000374
23	OP3	0.0001901	0.0000752	0.0000000	0.0000000
24	OP8	0.0001766	0.0000063	0.0000000	0.0000000
25	Planctomycetes	0.0000679	0.0000063	0.0012514	0.0000000
26	Proteobacteria	0.3120645	0.2865759	0.2975487	0.2636948
27	Spirochaetes	0.0269595	0.0015971	0.0000000	0.0000000
28	TM6	0.0000272	0.0001816	0.0000269	0.0000075
29	TM7	0.0077551	0.0038018	0.0611694	0.0031933
30	Verrucomicrobia	0.0008013	0.0000125	0.0000752	0.0002543
31	WPS-2	0.0074291	0.0005637	0.0012353	0.0000150
32	WS2	0.0000000	0.0000063	0.0000000	0.0003814
33	WS3	0.0000815	0.0003382	0.0000000	0.0000000
34	WWE1	0.0000815	0.0000000	0.0000000	0.0000000

Figure S1. Tasra colliery as observed during the yearly soil sampling. a, b, c and d represent the view of the site during sampling in the four consecutive years, (2013-2016) and named as T.13-T.16.



Figure S2. Box-whiskers plot representing total culturable and Cr(VI) tolerating bacterial load enumerated in Luria-Bertanni agar plates and LB supplemented with 100 mg L⁻¹ K₂Cr₂O₇ respectively, and measured as colony forming units (CFU) per gm of soil (n= 5).

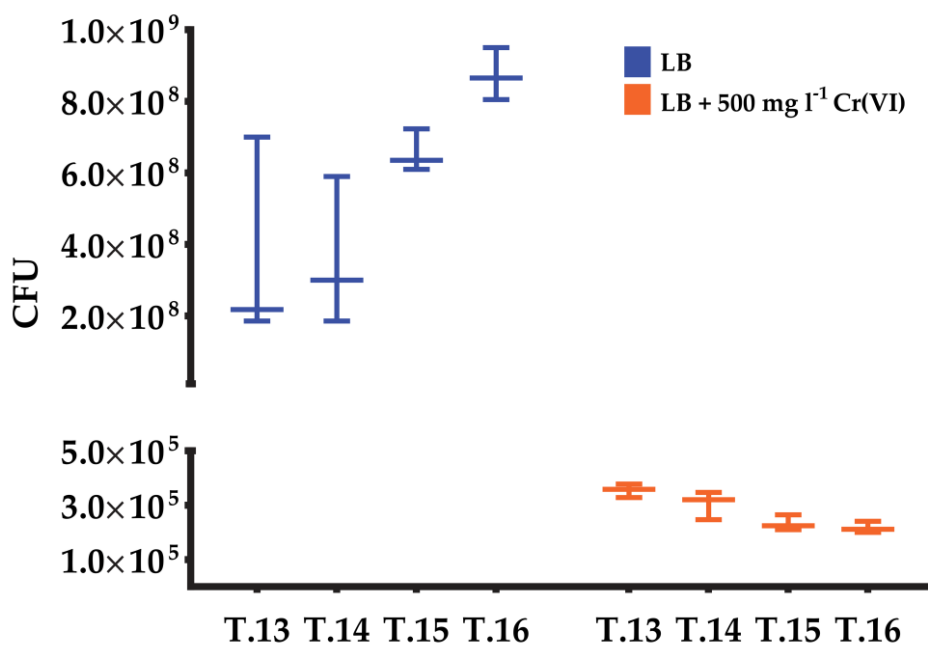


Figure S3. Bacterial phyla distribution observed for each sampling time point in Tasra colliery. For clearer resolution, only topmost abundant (0.1%) phyla are represented. Proteobacterial members are represented either as respective class (represented by pattern-filling), and those which can't be assigned to a specific class are clustered together and labeled as Proteobacteria.

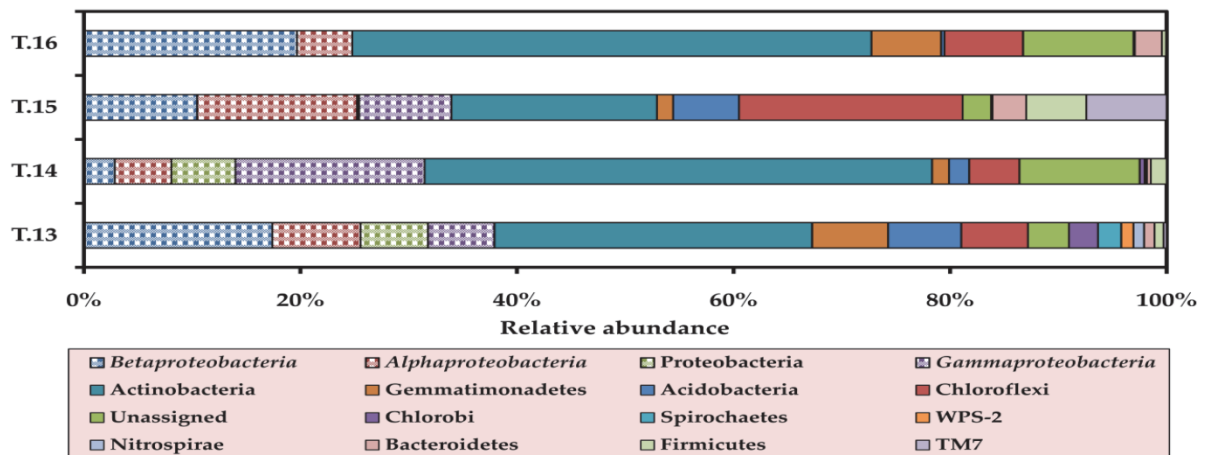
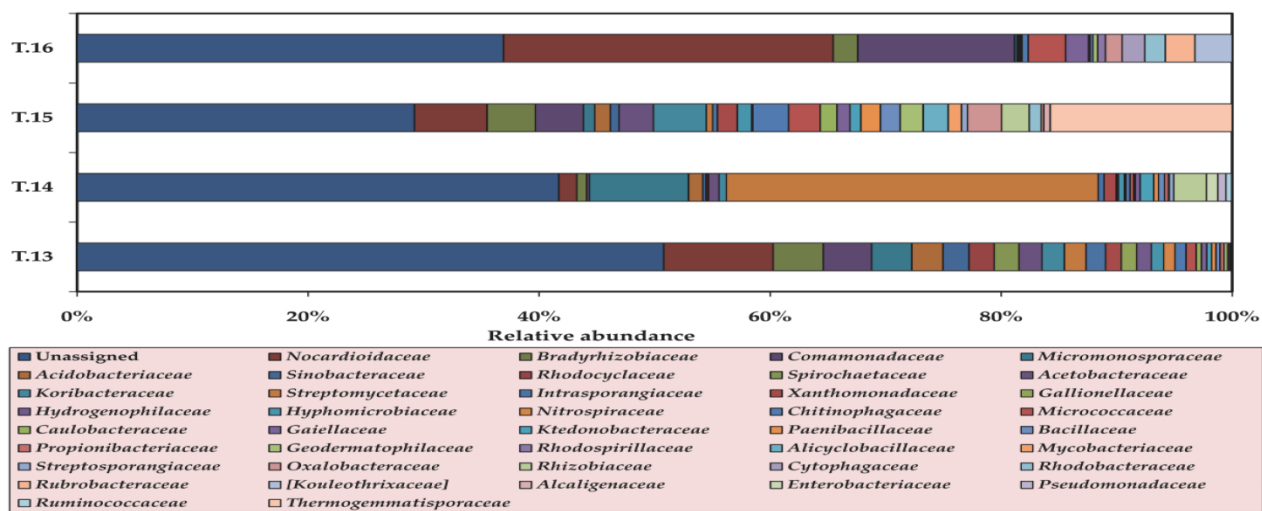


Figure S4. Relative abundance of families with OTUs with > 0.1% occurrence in Tasra Colliery.



References:

- Chen, L.X., Hu, M., Huang, L.N., Hua, Z.S., Kuang, J.L., Li, S.J., Shu, W.S., 2015. Comparative metagenomic and metatranscriptomic analyses of microbial communities in acid mine drainage. *The ISME Journal* 9, 1579.
- Chrončáková, A., Krisťufek, V.c., Tichý, M., Elhottová, D., 2009. Biodiversity of streptomycetes isolated from a succession sequence at a post-mining site and their evidence in Miocene lacustrine sediment. *Microbiological Research* 165, 594-608.
- Claassens, S., Van Rensburg, P.J., Maboeta, M., Van Rensburg, L., 2008. Soil microbial community function and structure in a post-mining chronosequence. *Water, Air, and Soil Pollution* 194, 315-329.
- Edwards, K.J., Gihring, T.M., Banfield, J.F., 1999. Seasonal variations in microbial populations and environmental conditions in an extreme acid mine drainage environment. *Applied and Environmental Microbiology* 65, 3627-3632.
- Fosso-Kankeu, E., Manyatshe, A., Waanders, F., 2017. Mobility potential of metals in acid mine drainage occurring in the Highveld area of Mpumalanga Province in South Africa: Implication of sediments and efflorescent crusts. *International Biodeterioration & Biodegradation* 119, 661-670.
- Ghose, M.K., 2004. Effect of opencast mining on soil fertility. *Journal of Scientific and Industrial Research*.
- González-Toril, E., Aguilera, A., Souza-Egipsy, V., Pamo, E.L., España, J.S., Amils, R., 2011. Geomicrobiology of an acid mine effluent, La Zarza-Perrunal (Iberian Pyritic Belt, Spain). *Applied and Environmental Microbiology*.
- Hua, Z.-S., Han, Y.-J., Chen, L.-X., Liu, J., Hu, M., Li, S.-J., Kuang, J.-L., Chain, P.S.G., Huang, L.-N., Shu, W.-S., 2015. Ecological roles of dominant and rare prokaryotes in acid mine drainage revealed by metagenomics and metatranscriptomics. *The ISME Journal* 9, 1280.
- Huang, L.-N., Zhou, W.-H., Hallberg, K.B., Wan, C.-Y., Li, J., Shu, W.-S., 2011. Spatial and temporal analysis of the microbial community in the tailings of a Pb/Zn mine generating acid drainage. *Applied and Environmental Microbiology*, AEM. 02458-02410.
- Jamal, A., Dhar, B.B., Ratan, S., 1991. Acid mine drainage control in an opencast coal mine. *Mine Water and the Environment* 10, 1-16.
- Korehi, H., Blöthe, M., Schippers, A., 2014. Microbial diversity at the moderate acidic stage in three different sulfidic mine tailings dumps generating acid mine drainage. *Research in Microbiology* 165, 713-718.
- Kuang, J.-L., Huang, L.-N., Chen, L.-X., Hua, Z.-S., Li, S.-J., Hu, M., Li, J.-T., Shu, W.-S., 2013. Contemporary environmental variation determines microbial diversity patterns in acid mine drainage. *The ISME Journal* 7, 1038.
- Kumar, A., Singh, P.K., 2016. Qualitative assessment of mine water of the western Jharia coalfield area, Jharkhand, India. *Current World Environment* 11, 301.
- Labrenz, M., Banfield, J.F., 2004. Sulfate-reducing bacteria-dominated biofilms that precipitate ZnS in a subsurface circumneutral-pH mine drainage system. *Microbial Ecology* 47, 205-217.

Lee, S.-H., Sorensen, J.W., Grady, K.L., Tobin, T.C., Shade, A., 2017. Divergent extremes but convergent recovery of bacterial and archaeal soil communities to an ongoing subterranean coal mine fire. *The ISME Journal* 11, 1447.

Li, J., Liu, F., Chen, J., 2016. The effects of various land reclamation scenarios on the succession of soil bacteria, Archaea, and Fungi over the short and long term. *Frontiers in Ecology and Evolution* 4, 32.

Liao, M., Xie, X.M., 2007. Effect of heavy metals on substrate utilization pattern, biomass, and activity of microbial communities in a reclaimed mining wasteland of red soil area. *Ecotoxicology and environmental safety* 66, 217-223.

Lindsay, M.B.J., Condon, P.D., Jambor, J.L., Lear, K.G., Blowes, D.W., Ptacek, C.J., 2009. Mineralogical, geochemical, and microbial investigation of a sulfide-rich tailings deposit characterized by neutral drainage. *Applied Geochemistry* 24, 2212-2221.

Liu, J., Hua, Z.-S., Chen, L.-X., Kuang, J.-L., Li, S.-J., Shu, W.-S., Huang, L.-N., 2014. Correlating microbial diversity patterns with geochemistry in an extreme and heterogeneous mine tailings environment. *Applied and Environmental Microbiology*, AEM. 00294-00214.

Maharana, J.K., Patel, A.K., 2015. Assessment of microbial diversity associated with chronosequence coal mine overburden spoil using random amplified polymorphic DNA markers. *International Journal Recent Scientific Research* 6, 4291-4301.

Masto, R.E., Ram, L.C., George, J., Selvi, V.A., Sinha, A.K., Verma, S.K., Rout, T.K., Priyadarshini, Prabal, P., 2011. Impacts of opencast coal mine and mine fire on the trace elements' content of the surrounding soil vis-a-vis human health risk. *Toxicological & Environmental Chemistry* 93, 223-237.

Mendez, M.O., Neilson, J.W., Maier, R.M., 2008. Characterization of a Bacterial Community in an Abandoned Semiarid Lead-Zinc Mine Tailing Site. *Applied and Environmental Microbiology* 74, 3899-3907.

Muhammad, S., Shah, M.T., Khan, S., 2011. Heavy metal concentrations in soil and wild plants growing around Pb-Zn sulfide terrain in the Kohistan region, northern Pakistan. *Microchemical Journal* 99, 67-75.

Murty, V., Karunakara, N., 2008. Natural radioactivity in the soil samples of Botswana. *Radiation Measurements* 43, 1541-1545.

Nieto, J.M., Sarmiento, A.M., Canovas, C.R., Olias, M., Ayora, C., 2013. Acid mine drainage in the Iberian Pyrite Belt: 1. Hydrochemical characteristics and pollutant load of the Tinto and Odiel rivers. *Environmental Science and Pollution Research* 20, 7509-7519.

Pandey, B., Agrawal, M., Singh, S., 2016. Ecological risk assessment of soil contamination by trace elements around coal mining area. *Journal of Soils and Sediments* 16, 159-168.

Pereira, L.B., Vicentini, R., Ottoboni, L.M.M., 2014. Changes in the bacterial community of soil from a neutral mine drainage channel. *PLoS One* 9, e96605.

Sadhu, K., Adhikari, K., Gangopadhyay, A., 2012. Effect of mine spoil on native soil of Lower Gondwana coal fields: Raniganj coal mines areas, India. *International Journal of Environmental Sciences* 2, 1675-1687.

Saikia, B.K., Ward, C.R., Oliveira, M.L.S., Hower, J.C., Baruah, B.P., Braga, M., Silva, L.F., 2014. Geochemistry and nano-mineralogy of two medium-sulfur northeast Indian coals. *International Journal of Coal Geology* 121, 26-34.

Shahabpour, J., Doorandish, M., Abbasnejad, A., 2005. Mine-drainage water from coal mines of Kerman region, Iran. *Environmental Geology* 47, 915-925.

Sinha, M.P., SINha, K., 1987. Characterisation of coal mine effluents from Jharia coalfields, Bihar, India.

Urbanov', M., Kopecky', J., Val'as'kov', V., S'agov'a-Marec'kov', M.e., Elhottov', D., Kyselkov', M., Moe'`nne-Loccoz, Y., Baldrian, P., 2011. Development of bacterial community during spontaneous succession on spoil heaps after brown coal mining. *FEMS Microbiology and Ecology* 78, 59-69.

Volant, A.e., Bruneel, O., Desoeuvre, A., Hery, M., Casiot, C., Bru, N., Delpoux, S., Fahy, A., Javerliat, F., Bouchez, O., 2014. Diversity and spatiotemporal dynamics of bacterial communities: physicochemical and other drivers along an acid mine drainage. *FEMS Microbiology and Ecology* 90, 247-263.

Wei, M., Yu, Z., Jiang, Z., Zhang, H., 2014. Microbial diversity and biogenic methane potential of a thermogenic-gas coal mine. *International Journal of Coal Geology* 134, 96-107.

Xie, J., He, Z., Liu, X., Liu, X., Van Nostrand, J.D., Deng, Y., Wu, L., Zhou, J., Qiu, G., 2011. GeoChip-based analysis of the functional gene diversity and metabolic potential of microbial communities in acid mine drainage. *Applied and Environmental Microbiology* 77, 991-999.