Pyrosequencing Analysis of Bacterial and Fungal Diversity in Reclaimed Lands in a Mining Region

Ramya Narendrula[1] and Kabwe K. Nkongolo[1,2]

[1]Biomolecular Science Program, [2]Department of Biology, Laurentian University, Sudbury, Ontario, P3E 2C6.

ABSTRACT

Metals in the environment are known to alter soil ecosystem diversity, structure, and function. The objective of this study was to assess the effects of long-term soil metal contamination on microbial community abundance and diversity. Phospholipid fatty acid (PLFA) and pyrosequencing analyses were used to determine microbial abundance and diversity. No significant differences between metal contaminated stable upland (with the highest level of metal contamination) and reference sites were observed for total microbial biomass, bacteria, fungi, arbuscular fungi, eukaryote and actinomycetes abundance. *Acidobacteria* and *Proteobacteria* were the dominant bacterial taxonomic groups in all sites. For fungi, *Ascomycota* were more predominant in metal contaminated disturbed soils (45%) while *Basidiomycota* represent 69% of all fungi in reference areas. Although 80% of bacteria and fungi were similar in the three sites, no significant difference was observed between metal contaminated stable upland and reference samples for microbial diversity. Shannon's diversity index was 2.73 for metal contaminated disturbed, 3.68 for metal contaminated stable upland and 3.70 for references soils, respectively. Overall, both PLFA and pyrosequencing indicate that the soil microbial community had adapted to the elevated metal concentrations in the targeted region polluted with metals for a century as evidenced by microbial biomass, abundance and diversity.

OBJECTIVES

The main objectives of the present study were to determine the effects of long term soil metal contamination on microbial diversity and abundance using PLFA and pyrosequencing analyses.

MATERIALS AND METHODS

Sampling and Metal Analysis

- Nine sites were selected from the Northern Ontario and grouped based on distance from smelters. Total and bioavailable metals were determined based on established protocols.

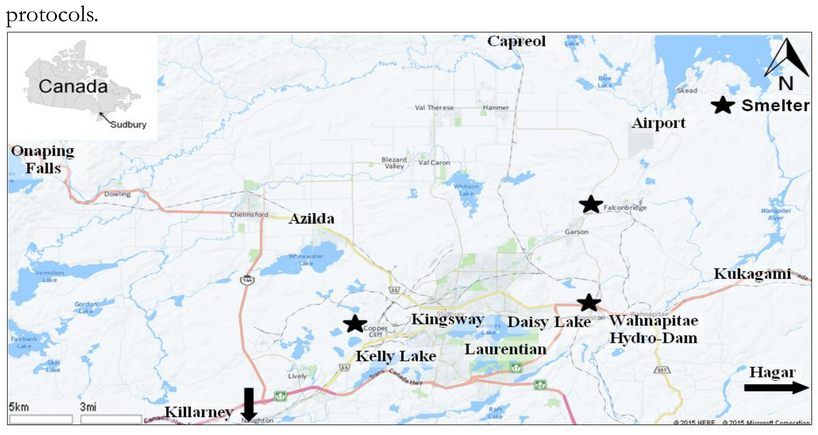


Figure 1: Geographical locations of the sampling area from the GSR in Northern Ontario.

Phospholipid Fatty Acid (PLFA) Analysis

- PLFA analysis was performed as described by Buyer and Sasser.
- Mole percentage of each PLFA was used to indicate the relative abundance of bacteria.
- Total PLFA extracted from soil was used as an index of living microbial biomass.

PCR Amplification and Pyrosequencing

- DNA was extracted from 10 g of soil per sample according to the manufacturer's instructions.
- Bacterial and fungal microbiota were assessed using high throughout sequencing of 16S and internal transcribed spacer (ITS) genes.
- Tag-encoded FLX-titanium 16S rDNA gene amplicon pyrosequencing (bTEFAP) was performed using 16S universal eubacterial primers 530F (5' GTG CCA GCM GCN GCG G) and 1100R (5' GGG TTN CGN TCG TTR).
- Fungal tag-encoded FLX amplicon pyrosequence (fTEFAP) was performed using ITS specific primers ITSF (5' TCC GTA GGT GAA CCT GCG G) and ITSR (5' TCC TCC GCT TAT TGA TAT GC).

Statistical Analysis

- Microbial biomass (total PLFA), abundance, and composition (bacterial, actinomycetes and fungal PLFAs) were analyzed by ANOVA using SPSS version 20.
- The OTUs were used to calculate community diversity (Shannon and Simpson diversity indices), evenness (Shannon equitability index) and richness (abundance based coverage estimator, Chao1).

RESULTS

Table 1: Microbial organisms identified using phospholipid fatty acid (PLFA) analysis in soil samples from the Greater Sudbury Region. Data in ng/g.

Sites	Metal contaminated disturbed sites (0-5 km)	Metal contaminated stable upland sites (5-15 km)	Reference sites (> 15 km)
Total Microbial Biomass	136.40a ±49.28	366.03b ±46.90	464.92b ±58.09
AM Fungi	5.20a ±1.67	11.47b ±1.55	18.18c ±2.21
Fungi	14.27a ± 6.08	50.03ab ±9.04	62.50b ±12.89
Gram Negative	53.51a ±23.52	177.37b ±22.01	213.78b ±23.88
Gram Positive	38.06a ±12.50	94.39b ±8.02	102.14b ±11.28
Eukaryote	4.29a ±1.48	13.41b ±2.40	22.12b ±5.60
Anaerobe	2.06a ±0.53	4.93b ±1.02	5.70b ±0.92
Actinomycetes	19.01a ±4.30	38.93b ±2.21	40.51b ±4.01

Means in rows with a common subscript are not significantly different based on Tukey multiple comparison test (P ≥ 0.05).

Table 2: The five main phylogenetic groups of bacteria and their relative abundance identified in soil samples from the Greater Sudbury Region (GSR).

Phylum	Metal contaminated disturbed sites (0-5 km)	Metal contaminated stable upland sites (5-15 km)	Reference sites (>15 km)
Actinobacteria	4.27%	6.77%	4.71%
Acidobacteria	42.53%	45.67%	47.24%
Chloroflexi	7.80%	1.86%	0.08%
Firmicutes	4.45%	1.20%	1.82%
Proteobacteria	37.87%	42.35%	43.90%

Table 3: Phylogenetic groups of fungi and their relative abundance identified in soil samples from the Greater Sudbury Region (GSR).

Phylum	Metal contaminated disturbed sites (0-5 km)	Metal contaminated stable upland sites (5-15 km)	Reference sites (>15 km)
Ascomycota	45.26%	31.46%	24.60%
Basidiomycota	49.86%	65.41%	69.05%
Glomeromycota	0.00%	0.00%	4.55%
Zygomycota	4.88%	3.13%	1.80%

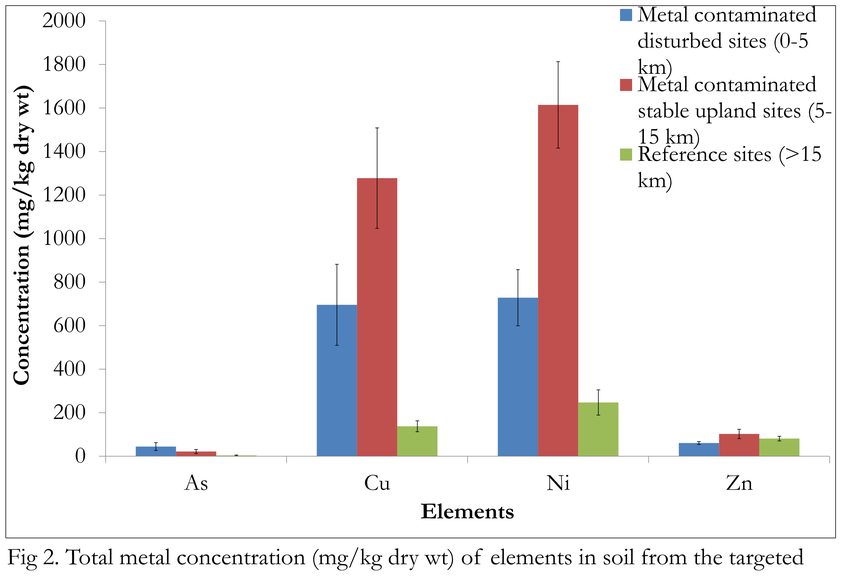
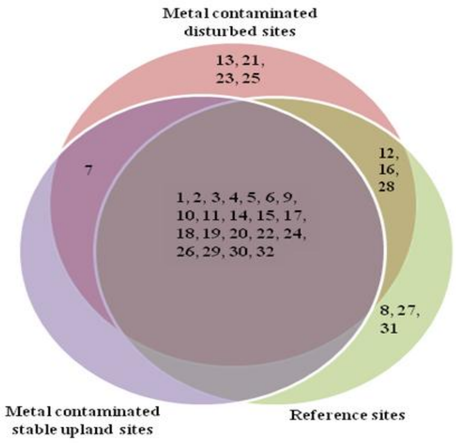


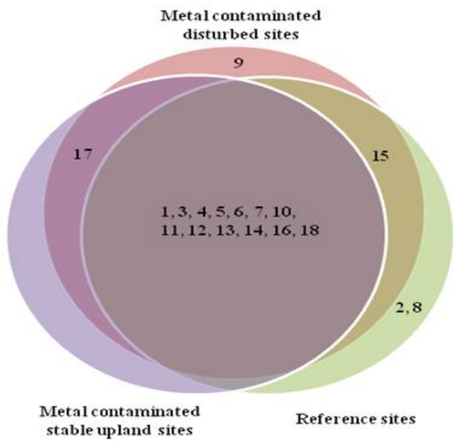
Fig 2. Total metal concentration (mg/kg dry wt) of elements in soil from the targeted sites.

RESULTS



1. *Acidimicrobiia*
2. *Acidobacteria*
3. *Acidobacteria*
4. *Actinobacteria*
5. *Actinobacteria*
6. *Alphaproteobacteria*
7. *Anaerolineae*
8. *Armatimonadia*
9. *Bacilli*
10. *Betaproteobacteria*
11. *Bryopsida*
12. *Caldilineae*
13. *Chloroflexi*
14. Class unspecified
15. *Clostridia*
16. *Cytophagia*
17. *Deltaproteobacteria*
18. *Flavobacteria*
19. *Gammaproteobacteria*
20. *Gemmatimonadetes*
21. *Jungermanniopsida*
22. *Ktedonobacteria*
23. *Lentisphaeria*
24. *Opitutae*
25. *Oscillatoriophycideae*
26. *Planctomycetacia*
27. *Spam* (*Candidate division*)
28. *Spartobacteria*
29. *Sphingobacteria*
30. *Thermoleophilia*
31. *TM6*
32. *TM7*

Fig 3. Venn diagram showing distribution of bacterial classes identified in metal contaminated disturbed, metal contaminated stable upland and reference soil samples.



1. *Agaricomycetes*
2. *Archaeosporomycetes*
3. *Ascomycota*
4. *Basidiomycota*
5. *Dothideomycetes*
6. *Eurotiomycetes*
7. *Fungi*
8. *Geoglossomycetes*
9. *Lecanoromycetes*
10. *Leotiomycetes*
11. *Mortierellomycotina*
12. *Mucoromycotina*
13. *Pezizomycetes*
14. *Pezizomycotina*
15. *Saccharomycetes*
16. *Sordariomycetes*
17. *Taphrinomycotina*
18. *Tremellomycetes*

Fig 4. Venn diagram showing distribution of fungal classes identified in metal contaminated disturbed, metal contaminated stable upland and reference soil samples.

CONCLUSIONS

- Our results demonstrated that long-term exposure to metals (mostly nickel and copper) for almost 100 years does not reduce microbial biomass, abundance, and diversity. Other factors mostly low CEC and organic matter are directly associated to ecosystem complexity, and reduction of microbial biomass and diversity.
- Site-specific bacteria and fungi were identified.

AKNOWLEDMENT

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REFERENCES

Buyer J, Sasser M (2012) High throughout phospholipid fatty acid analysis of soils. Appl Soil Ecol 61:127–130.