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Levels of Metals and Microbial Biomass in Cobalt Coleman Mine Tailings (Canada) Three Decades after Land Reclamation

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Abstract: Investigations of restored metal contaminated tailings in Canada with regard to their long-term ability to sustain plant and associated microbiological populations are limited. The specific objectives of the present study were to assess (1) The current levels of total and bioavailable metals and (2) Microbial biomass in Cobalt Coleman mine tailings reclaimed in 1990. The level of microbial biomass was determined using Phospholipid Fatty Acid analysis (PLFA). Metal analysis revealed that the concentration of total Arsenic (As) was >100 and 20 × higher in the Nipissing tailing compared to the non-tailing site and the Cart Lake tailing. The same trend was observed to a lesser degree for total Cobalt (Co) and Copper (Cu) concentrations. Overall, the total metal levels remain high >30 years after phytoremediation, but the bioavailable metal amount was low. This suggests that soil metal impacts on biota are minimal. The reclaimed tailing exhibited significantly lower levels ($p \leq 0.05$) of organic matter compared to non-tailing reference areas. More importantly, the analyses revealed significantly high ($p \leq 0.05$) total microbial biomass in non-tailing soil samples (with higher organic matter content) compared to tailing soils. A strong positive correlation ($r = 0.87$) was observed between organic matter and total microbial biomass. In contrast to other studies, the pH of the two tailing sites was neutral (7.1 and 7.5) and negatively correlated ($r = -95$) with bacterial and fungal abundance. Bacteria dominated the microbial communities in all the sites including the non-tailing area, indicating that the targeted region is still under severe environmental stress. Overall, the metal levels in the targeted tailings remain high and the phytoremediation did not improve significantly the soil quality (organic matter, microbial biomass) over the last three decades.

Keywords: Tailings, Cobalt Coleman Mines, Northern Ontario, Metal Contamination, Microbial Biomass

Introduction

Mine tailings contain relatively high concentrations of metals, metalloids, sulfates, and other salts and minerals. Their levels of organic matter and available nutrients are low (Lottermoser and Lottermoser, 2010). Successful phytostabilisation of taillings landscapes has been difficult to achieve (Mendez and Maier, 2008). Microorganisms rapidly respond to variations in environmental conditions during tailing restoration processes (Mendez and Maier, 2008; Li *et al*., 2018). Feketeová *et al*. (2021) showed a negative correlation between microbial biomass, carbon content, and concentrations of several metals (cobalt, copper, nickel, and zinc) in the soil ecosystem at a reclaimed tailing pond in Slovakia. Nkongolo *et al*. (2022a) analyzed the levels of microbial relative abundance and diversity in a sup hide tailing using amplicon Illumina sequencing. They identified a high number of bacterial (775) and fungal (905) species. They reported no significant differences for # of OTUs, Chao 1, Simpson index, Shannon index, and species richness among the bacterial communities from reclaimed tailing areas in the City of Greater Sudbury (CGS) (Ontario). On the other hand, Nkongolo *et al*. (2022b) highlighted a higher # of OTUs, Chao 1, Shannon index, and species richness in microbial populations from newly reclaimed tailing sites compared to reference areas.

Cobalt Coleman City is located 200 km northeast of the CGS. It was developed in 1903 along the shores of long lake, now cobalt lake. Previous investigations in the Cobalt areas have shown the impact of the historical mining and milling operations on the environment (OME, 2011; Boyle and Dass, 1971; Rainville *et al*., 2017). These investigations revealed elevated levels of metals, metalloids, and mercury at or adjacent to mine and mill sites in the Cobalt Coleman mining areas (OME, 2011).

No detailed studies have been conducted on Cobalt Coleman tailings with regard to soil quality, metal content, and their long-term ability to sustain both plant and associated microbiological populations. This study aims at determining the current levels of total and bioavailable metals and microbial biomass and abundance at remediated tailing areas. We hypothesize that soil pH and organic matter will be lower while metal content will be higher in tailing areas compared to surrounding reference sites. The abundance of microbial groups was analyzed for the first time in relation to soil chemistry.

Materials and Methods

Site Characterization and Sampling

Two main mining tailing areas were targeted at Cobalt based on the accessibility and reclamation status (Fig. 1). Cart Lake is a small lake southeast of Cobalt (Cobalt 1). It has been used for tailings (N47° 38.353', W79° 68.457') disposal since 1910 for several years. The second targeted tailing was the Nipissing (Cobalt 2) high-grade mill (N47° 23.466’, W79° and 40.797’) that was built in 1911. These two tailings were revegetated in the late 1990 s. The soil was largely covered with grass and herbaceous vegetation during the time of sampling. A non-tailing area located within the vicinity of the two tailings was used as a reference site. Fresh soil samples were collected and processed as described in (Nkongolo *et al*., 2022a).

Analysis of Soil pH, Organic Matter, and Metal Content

Soil pH, Organic Matter (OM), and metal analyses were performed at Tesmark Inc. (Sudbury). The organic matter content was determined using the Loss on Ignition (LoI) analysis. Metal analysis was performed as described in Nkongolo *et al*. (2013; 2016).

Phospholipid Fatty Acid (PLFA) Analysis

Soil samples were analyzed following the protocol described by Buyer and Sasser (2012); Narendrula-Kotha and Nkongolo (2017a-b). A summary of this method is also reported in Nkongolo *et al*., (2022b).

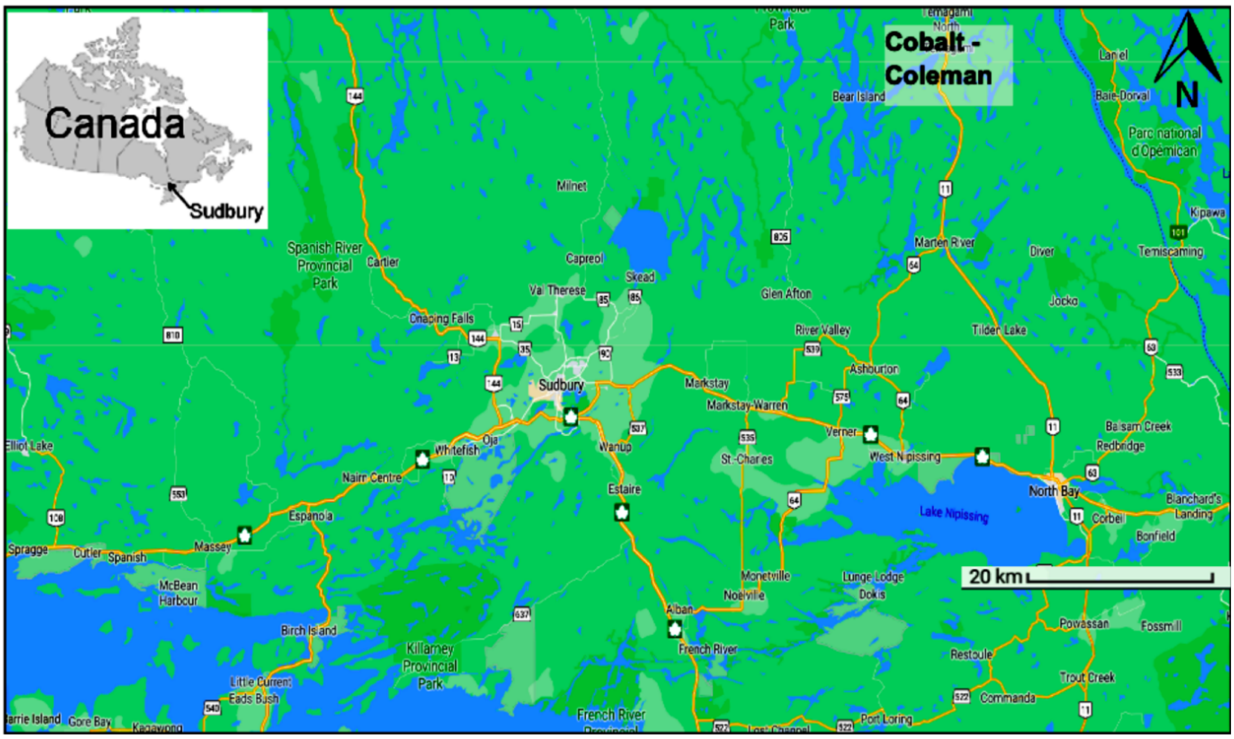

Fig. 1: Northern Ontario map showing the Cobalt tailing location. The coordinates of the tailings are N47° 38.353’, W79° 68.457’ and N47° 23.466’, W79° 40.797’, for Cart Lake and Nipissing tailings, respectively. The reference site was located between the two tailings

Statistical Analyses

Metal and microbial data were analyzed using SPSS statistics version 20 for Windows. The normal distribution of the data was verified using the Shapiro-Wilk test. Data sets that did not meet requirements were transformed using inverse transformation to achieve normality. This was followed by the Analysis of Variance (ANOVA) and Tukey's HSD multiple comparison tests to determine significant differences ($p \leq 0.05$) among means.

Results

Soil Organic Matters, Ph, and Metal Contents

Soil pH, organic matter, and total and bioavailable metal data are described in Table 1-2. Surprisingly, the pH at tailing areas covered with mixed grasses was neutral (7.1 and 7.5) and higher compared to the non-tailing reference site. But, the levels of organic matter were higher in samples from this reference site compared to the tailings. The Nipissing site (Cobalt 2) contained a significantly higher amount of total metals compared to the Cart Lake (Cobalt 1) and the non-tailing reference site (Table 1). The concentration of total Arsenic (As) was >100 × higher compared to the reference and 20 × more than the cobalt 1 (Nipissing) tailing. Total Cobalt (Co) content was 67 and 12 × higher in Cobalt 2 (Cart Lake) compared to the reference site and the Cobalt 1 (Nipissing) tailing, respectively. Total Nickel (Ni) amount was 37 × higher in Cobalt 2 (Cart Lake) compared to the reference and 14 × compared to Cobalt 1 (Nipissing). The same trend was observed for total Copper (Cu) concentration which was 12 and 7.5 × higher in Cobalt 2 compared to the reference and Cobalt 1, respectively. The amount of total zinc (Zn) was also higher (8.4 ×) in cobalt 2 compared to the reference and 3.5 × compared to Cobalt 1.

The levels of other bioavailable elements are described in Table 2. The bioavailable levels of most of the elements including Zin (Zn), lead (Pb), Sodium (Na), Potassium (K), iron (Fe), Chrome (Cr), Cobalt (Co), Barium (Ba) and Arsenic (As), were below 1 mg/kg. The bioavailable amount of phosphorus, manganese, Nickel (Ni), and Copper (Cu) were less than 30 mg/kg. The bioavailable Titanium (Ti), magnesium and Calcium (Ca) levels were significantly higher than other elements. Bioavailable Mg ranged from 38 to 138 mg/kg while Ca ranged from 128 to 447 mg/kg (Table 2).

Phospholipid Fatty Acid Analysis

Results of the PLFA analysis are described in Tables 3-4 and in Fig. 2. The level of total microbial biomass was significantly higher ($P \leq 0.05$) in reference soil samples compared to Cobalt 1 (Cart Lake) and Cobalt 2 (Nipissing) tailings. The same trend was observed for the abundance of bacteria, AM fungi, other fungi, and other eukaryotes. Overall, there were up to 9 × more bacteria than fungi in the soil samples analyzed. In fact, bacteria represented 70% of the total microbial biomass at both tailings (Cobalt 1 and Cobalt 2) and >75% at the reference site.

Palmitic acid (18:1), palmitoleic acid (16:1), and palmitic acid (16:0) were the most prevalent of all the fatty acids identified in all the soil samples as they accounted for 45% of Fatty Acids (FA) for all the samples. FA 18:1 (Oleic acid) was the most prevalent (19%) followed by FA 16:0 (palmitic acid) with 15% and FA 16:01 (palmitoleic acid) with 11.5%. The amount of these FA was much lower at the two tailings compared to the reference site from Cobalt (Table 4).

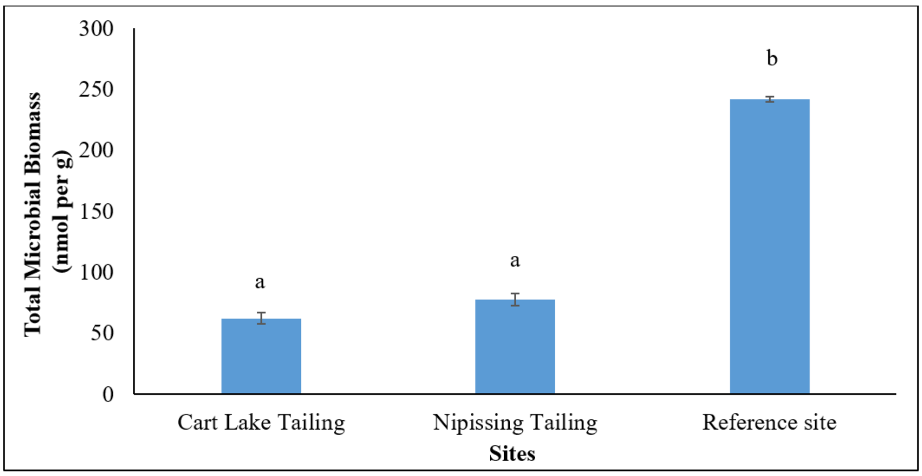

Fig. 2: Total microbial biomass in soils from Cart Lake and Nipissing tailings, as well as reference sites in Cobalt Coleman (Northern Ontario, Canada) based on Phospholipid Fatty Acid (PLFA) analysis. Means with different letters are significantly different ($p \leq 0.05$)

Table 1: Total metal concentration† (mg/kg dry wt) in soil samples from Cobalt Coleman tailings in Northern Ontario

Sites	As	Ba	Ca	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	P	Pb	Ti	Zn
Cobalt tailings																
Cobalt 1 -Cart Lake	174^{a}	9.1^{a}	6790^{a}	155^{a}	122^{b}	61.5^{a}	43800^{b}	867^{a}	20500^{b}	734^{a}	286^{a}	316^{a}	467^{a}	148^{b}	1100^{b}	17.9^{b}
Cobalt 2-Nipissing	3740^{b}	66.4^{c}	20100^{b}	1950^{b}	121^{b}	465^{b}	45500^{b}	2510^{b}	21400^{b}	1010^{b}	620^{b}	4340^{b}	533^{a}	238^{c}	788^{ab}	62.9^{b}
Cobalt 3 (reference)	35^{a}	42.1^{b}	4730^{a}	29.2^{a}	44.9^{a}	38.3^{a}	19000^{a}	1480^{a}	5710^{a}	516^{a}	216^{a}	118^{a}	588^{a}	24.7^{a}	521^{a}	7.5^{a}

†Means in columns with a common subscript are not significantly different based on Tukey multiple comparison test ($p \geq 0.05$)

Table 2: Organic matters, pH, and bioavailable metal concentrations† (mg/kg dry wt) in soil samples from Cobalt-Coleman tailings in Northern Ontario (Canada)

	Bioavailable metals (mg/kg)±								
Sites	pH	OM (%)*,	Ca	Cu	Mg	Ni	Ti	Mn	P
Cobalt tailings									
Cobalt 1-Cart Lake	7.1^{b}	4.5^{a}	183.3^{a}	0.9^{a}	138.3^{b}	1.30^{a}	89.4^{b}	7.3^{a}	23.4^{a}
Cobalt 2-Nipissing	7.5^{b}	1.9^{a}	443^{b}	7.1^{b}	143.4^{b}	17.8^{b}	64.1^{ab}	12.5^{a}	26.7^{a}
Cobalt 3 (reference)	6.3^{a}	8.0^{b}	128^{a}	0.5^{a}	38.3^{a}	0.48^{a}	42.4^{a}	5.6^{a}	29.4^{a}

†The bioavailable amounts of As, Ba, Co, Cr, Fe, K, Na, Pb and Zn were < 1 mg/kg. *OM represents organic matter. Means in columns with a common superscript are not significantly different based on Tukey multiple comparison test ($p \geq 0.05$)

Table 3: Microbial organisms identified using Phospholipid Fatty Acid (PLFA) analysis in soil samples from Cobalt Coleman tailing (Northern Ontario). Data in nMole/g*

Sites	Total	AM fungi	Fungi	Gram-negative	Gram-positive	Eukaryote	Anaerobe	Actinomycetes
Cobalt 1	62.48^{a} ±3.88	5.19^{a}±0.33	3.49^{a}±0.33	30.12^{a}±1.85	15.02^{a}±0.90	1.65^{a}±0.14	0.60^{a}±0.04	6.40^{a}±0.31
Cobalt 2	77.86^{ab}±4.46	6.03^{a}±0.24	3.88^{a}±0.23	37.85^{a}±1.58	17.80^{a}±0.62	1.58^{a}±0.06	0.93^{a}±0.11	8.11^{a}±0.27
Cobalt 3	242.04^{b}±2.05	12.79^{b}±0.14	7.40^{a}±0.23	128.15^{b}±0.40	56.29^{b}±0.77	3.64^{a}±0.24	1.74^{a}±0.12	32.03^{b}±0.34
Non-tailing reference								

*Means in columns with a common superscript are not significantly different based on Tukey multiple comparison test ($p \geq 0.05$)

Table 4: Phospholipid Fatty Acid (PLFA) composition of the total microbial profile analyzed in soil samples from Cobalt Coleman, Data in nMol/g

Sites	Total	16:00	16:01	17:0	17:1	18:0	18:1	19:0	Others
Cobalt tailings									
Cobalt 1-Court Lake	56.5^{a}	13.9^{a}	10.8^{a}	3.9^{a}	1.2^{a}	3.5^{a}	13.0^{a}	2.4^{a}	7.80
Cobalt 2-Nipising	71.3^{a}	15.5^{a}	14.0^{a}	5.0^{a}	1.9^{a}	4.7^{a}	17.0^{a}	2.4^{a}	10.80
Cobalt 3 (non-tailing reference)	359.7^{b}	44.6^{b}	32.4^{b}	16.1^{b}	4.4^{b}	11.6^{b}	62.6^{b}	16.9^{b}	171.10

Means in columns with a common superscript are not significantly different based on Tukey multiple comparison test ($p \geq 0.05$)

Discussion

This study revealed that the level of total metals in reclaimed tailings in Cobalt Coleman is still very high >30 years after phytoremediation. Detailed analysis showed that metal contamination levels including arsenic, cobalt, nickel, and copper have no effects on microbial biomass. In fact, the Nipissing tailing in Cobalt contained 20 As, 13 Co, 8 Cu, and 14 × Ni compared to the Court Lake tailing but both tailings have a similar level of microbial biomass. On the other hand, the reference site had a higher level of microbial biomass compared to the two tailings. This non-tailing reference site had less metal content, but higher above ground vegetation and elevated level of soil organic matter. The low level of bioavailable metals in soil suggests that soil metal impacts on biota are minimal (Narendrula and Nkongolo 2013; Narendrula-Kotha and Nkongolo, 2017a-b; Ghazouani *et al*. 2020). Kwon *et al*. (2015) showed that over 90% of total metals were not bioavailable. Hence, they were below the levels known to cause any effect on bacteria and fungi for example. This indicates that metals may have not been easily leachable from the tailing samples (Kwon *et al*. 2015).

Different techniques can be used to determine the number of bioavailable metals. In the present investigation, bioavailability is described as the level of a metal or a nutrient absorbable by a living organism that trigger physiological and/or toxicological responses US Environmental Protection Agency (EPA). Ettler *et al*. (2005; 2011), reported high percentages of exchangeable (bioavailable) metals using single and sequential extractions. Metal bioavailability can also be measured by the number of metals that is present in human urine. Morrison and Gulson (2007); Ettler *et al*. (2012) described the levels of bioavailable elements as the portion of total metals entering the blood steam. These techniques underline human risk assessment in mining regions (Banza *et al*., 2009; Ettler *et al*., 2012). This study emphasizes rather on risk assessment at the environmental levels with an analysis of total metals in soil and the portion that is potentially accessible for plant uptake from soil solutions.

Several studies have documented a strong positive correlation between Microbial Biomass Carbon (MBC) and Soil Organic Carbon (SOC) (Narendrula-Kotha and Nkongolo 2017a-b; Dimitriu and Grayston, 2010; Li *et al*., 2016; McKergow *et al*., 2021; Nkongolo *et al*., 2022b). Data from this study are consistent with previous findings since higher microbial biomass in sites was associated with high organic matter. Surprisingly, the correlation between microbial biomass and soil acidity (pH) was negative and significant (-0.90). This is due to neutral pH values in tailing sites. In other studies, the levels of microbial biomass have been correlated to physical/chemical conditions (Londry and Sherriff, 2005). Changes in vegetation cover can also affect soil microbial biomasses. In the present study, the overall microbial populations were dominated by bacteria, suggesting that the entire targeted region is still under severe environmental stress. This is consistent with the low ratios between fungal and bacterial biomass. These data corroborate with Mhete *et al*. (2020) finding indicating that high degrees of disturbances and neutral to mildly acidic pH are associated with a preponderance of bacteria. $Gram^{+}$ bacteria resist more stress and harsh conditions than a $Gram^{-}$ bacteria. This is because of their acid resistance systems that can help them to overcome the challenge posed by acidic environments (Cotter and Hill, 2003; De Vries and Shade, 2013).

A recent analysis of sulfide tailing by amplicon sequencing, a powerful approach to detecting microbial composition at a species level revealed the difficulty of linking specific species to ecological conditions (Nkongolo *et al*., 2022a). However, it is established that spatial variations in community diversity and structure are mainly driven by soil physicochemical parameters (Ren *et al*., 2018; Xue *et al*., 2018; Nkongolo *et al*., 2022a). An increase in soil organic matter is usually associated with high microbial diversity. It is therefore expected that bacterial and fungal relative abundance, alpha, and beta diversity indices will be higher in soil samples from the reference site compared to the tailing sites in this study. This is inferred to a certain degree from the results of the PLFA (a cost-effective technique) of different microbial groups (bacteria, fungi, etc.,) used in the present study to compare the targeted tailing and reference site.

Conclusion

Soil chemistry and microbial communities in three targeted sites at Cobalt Coleman City were characterized. This study provides novel information on soil quality in tailings after reclamation. The total metal content at tailing sites remains elevated >30 years after phytoremediation. The levels of total AS, Co, Cu, and Ni were extremely high at Nipissing tailing compared to the Cart Lake tailing and the non-tailing area. Only <15% of total metals were phytoavailable. The level of total microbial biomass was significantly higher in reference non-tailing areas (with higher organic matter content) compared to the reclaimed tailing sites. There was a strong positive correlation between organic matter and microbial biomass. In contrast to other studies, the pH at the targeted tailing sites was neutral (7.1 and 7.5) and negatively correlated with the level of microbial biomass. Gram-negative bacteria Palmitic acid (18.1), palmitoleic acid (16.1), and palmitic acid (16:0) were the most prevalent of all the fatty acids identified in all the soil samples. Surprisingly, the phytoremediation of the targeted tailings did not improve significantly soil quality (organic matter, microbial biomass) over the last 30 years. Further studies can characterize microbial diversity and relative abundance using next generation sequencing analysis.

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Author's Contributions

Kabwe Nkongolo: Designed the experiments, collected the samples, analyzed the data, and wrote the manuscript.

Graeme Spiers: Collected and analyzed data, revised the manuscript.

Peter Beckett: Collected the samples, and revised the manuscript.

Ramya Narendrula-Kotha: Analyzed the data and revised the manuscript.

Ethics

Competing Interests: The authors declare that they have no competing interests.

Data Availability

All data generated or analyzed during this study are included in this published article.

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