Ecotoxicology and Environmental Safety 140 (2017) 241–248

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Changes in enzymatic activities in metal contaminated and reclaimed lands in Northern Ontario (Canada)

Ramya Narendrula-Kotha[a], Kabwe K. Nkongolo[a,b,*]

[a] Biomolecular Sciences Program, Laurentian University, Sudbury, Ontario, Canada P3E 2C6
[b] Department of Biology, Laurentian University, Sudbury, Ontario, Canada P3E 2C6

ARTICLE INFO

Keywords:
Soil respiration
Soil enzyme activity
pH
Soil organic matter
Soil quality
Liming
Metal contamination

ABSTRACT

Metal and sulfur dioxide (SO_2) contaminations in Northern Ontario (Canada), especially in the Greater Sudbury Region (GSR) caused by mining activities have resulted in severe environmental degradations. A long term restoration program has led to significant landscape changes and healthy ecosystems. The objective of this study was to assess variation in enzymatic activities and soil respiration in metal contaminated and reclaimed ecosystems. Soil analysis revealed that respiration rates were higher in metal contaminated limed soils (65 ppm) compared to adjacent unlimed areas (35 ppm). The respiration rates in metal contaminated sites (55 ppm) were significantly lower compared to reference (metal-uncontaminated) areas (90 ppm). β-glucosidase (BG), cellobiohydrolase (CBH), β-N-acetylglucosaminidase (NAGase), aryl sulfatase (AS), acid phosphatase (AP), alkaline phosphatase (AlP), glycine aminopeptidase (GAP), and leucine aminopeptidase (LAP) activities were significantly higher in limed compared to unlimed sites. Metal contamination significantly reduced the activities of these enzymes with the exception of LAP. An opposite trend was observed for peroxidase (PER) activity that was lower in limed compared to corresponding unlimed areas. Likewise, PER activity values were significantly lower in metal contaminated than in uncontaminated reference sites.

1. Introduction

Mining activities for over a century have led to a decrease in soil organic matter (SOM) content and hence to a decline in soil quality in the Greater Sudbury Region (GSR) (Narendrula and Nkongolo, 2015; Nkongolo et al., 2016, 2013). This was a direct effect of metalliferous ores exploitations (since 1800) that released enormous amounts of sulfur dioxide (SO_2) and various metals into the atmosphere resulting in severe contamination and acidification of soils and water in the GSR (Narendrula and Nkongolo, 2015; Nkongolo et al., 2016, 2013). In the last 40 years, legislated controls and industrial technology development reduced SO_2 emissions by 90% which resulted in improved air quality and natural recovery of damaged ecosystems (Narendrula and Nkongolo, 2015; Nkongolo et al., 2013). In addition, a regreening program that consisted in soil liming and tree planting in the GSR led to an increase of SOM and microbial biomass (Gunn et al., 2007; Goupil et al., 2015; Narendrula and Nkongolo, 2015; Theriault et al., 2013).

Recent chemical analysis revealed that soil pH and cation exchange capacity (CEC) are still lower in metal contaminated unlimed soils compared to adjacent limed areas (Goupil et al., 2015; Goupil and Nkongolo, 2014; Nkongolo et al., 2016, 2013). Also, low pH values were observed in these unlimed soils (pH = 3.90) compared to reference (metal uncontaminated) sites (pH = 4.80) (Narendrula and Nkongolo, 2015; Nkongolo et al., 2013). Other studies on plant population diversity showed a similar trend where these unlimed sites had lower tree species richness compared to limed and reference sites (Narendrula and Nkongolo, 2015; Nkongolo et al., 2016, 2013). Moreover, phospholipid fatty acid analysis (PLFA) revealed that total microbial biomass, fungal, and bacterial abundance were significantly lower in contaminated unlimed sites compared to limed and reference areas within the GSR (Goupil et al., 2015; Narendrula and Nkongolo, 2015; Nkongolo et al., 2016). These studies did contribute to our understanding of the dynamics of soil chemistry and ecosystems in the GSR. But biochemical indicators are more informative as they provide information on microbial activities and functions (Acosta-Martinez and Tabatabai, 2000; Creamer et al., 2009; Zhou et al., 2013). Soil respiration and enzyme activities involved in organic matter turnover, nutrient cycling, and plant nutrition have been used to study soil fertility and health (Acosta-Martinez and Tabatabai, 2000; Creamer et al., 2009; Zhou et al., 2013).

Enzyme activities in soils have been useful tools to our understanding of the biochemistry of decomposition and nutrient cycling

* Corresponding author at: Laurentian University, Department of Biology, 935 Ramsey Lake Road, Sudbury, ON, Canada P3E 2C6.
E-mail address: knkongolo@laurentian.ca (K.K. Nkongolo).

http://dx.doi.org/10.1016/j.ecoenv.2017.02.040
Received 22 October 2016; Received in revised form 22 February 2017; Accepted 24 February 2017
Available online 08 March 2017
0147-6513/

(carbon: C, nitrogen: N, phosphorous: P and sulfur: S) (Acosta-Martinez and Tabatabai, 2000; Creamer et al., 2009; Sinsabaugh et al., 2008). In addition, enzyme activities have been associated with microbial ecology, biogeochemical, and soil processes and health (Creamer et al., 2009; Hagmann et al., 2015; Sinsabaugh et al., 2008). The most widely used enzymes are those involved in the degradation of cellulose and lignin (abundant components of plant litter), as well as enzymes that hydrolyze proteins, chitin, and peptidoglycan (reservoirs of organic N) (Acosta-Martinez and Tabatabai, 2000). In addition, phosphatases which play a role in mineralizing P from nucleic acids, phospholipids, and other ester phosphates have been studied under different agricultural conditions (Acosta-Martinez and Tabatabai, 2000; Hagmann et al., 2015).

The main objective of this study was to assess the effect of liming and metal contamination on soil enzymatic activities and respiration.

2. Experimental procedures

2.1. Study sites

All the study sites were located at Laurentian University research areas and Crown (public) lands that are not within a park or conservation reserve in the GSR (Table 1). Reclaimed mining sites within the GSR were selected for this study. Liming was performed > 30 years ago and consisted in manual and aerial applications of crushed dolomitic limestone (10 t/ha). Soil sampling was performed at four distant locations in reclaimed sites (metal contaminated limed) and their respective adjacent un-reclaimed areas (metal contaminated unlimed). These sites included Daisy Lake 2 (site 1), Wahnapitae Hydro-Dam (site 2), Kelly Lake (site 3), and Kingsway (site 4) (Table 1) (Theriault et al., 2013).

To determine the effects of metals on enzymatic activities, three sites close to smelters with high levels of metal contaminations (unlimed sites) were selected. They include Laurentian, Kelly Lake and Kingsway. Three metal uncontaminated sites located > 50 km from smelters (Onaping Falls, Capreol and Killarney) were used as references (Table 1). These sites have been described in Nkongolo et al. (2016).

Weather conditions such as temperature and rain falls, were presumed similar at all the sites, based on literature (HistoricalWeather, 2015; Time and Date, 1995). Data on metal concentration, pH, organic matter contents, cation exchange capacity (CEC), and microbial abundance for these sites have been previously reported (Narendrula-Kotha and Nkongolo, 2017a, 2017b; Narendrula and Nkongolo, 2015; Nkongolo et al., 2013).

2.2. Soil sampling

At every site of approximately 5 km^2 in size, three soil samples (each consisting of 15 sub-samples) were collected from organic layer (0–5 cm in depth). Plant materials, stones and residues were removed and sieved using a 2 mm mesh. Soil samples were divided into two equal parts for further analysis.

2.3. Soil respiration

For soil respiration, all the samples were completely dried, labeled and stored prior to analysis. Soil respiration analysis was performed using the procedure described by Narendrula and Nkongolo (Narendrula and Nkongolo, 2015). Each soil sample was analyzed in triplicates.

2.4. Enzyme activities

Soil samples from all the 12 sites were stored at 4 °C and enzyme activities were analyzed within six days. Potential activities of nine enzymes involved in catalyzing the cleavage of a range of organic matter compounds and their importance in nutrient cycling in soils were investigated (Table 2). All enzymes were assayed at their optimal pH values. β-glucosidase (BG), cellobiohydrolase (CBH), β-N-acetylglucosaminidase (NAGase), aryl sulfatase (AS), acid phosphatase (AP), alkaline phosphatase (AlP), glycine aminopeptidase (GAP), leucine aminopeptidase (LAP), and peroxidase (PER) activities were analyzed in details.

Assays were conducted using *p*-nitrophenol (*p*NP) linked substrates and measured in a 96-well plate reader (Fluostar optima). Glycine aminopeptidase and leucine aminopeptidase activities were assayed using *p*-nitroanilide whereas peroxidase was assessed using DOPA (L-3,4-dihydroxyphenylalanine) (Table 2). Substrate concentrations of 5 mM were used for all enzymes, except for cellobiohydrolase (CBH) and β-N-acetylglucosaminidase (NAGase) due to their solubility (2 mM) and cost. Original protocols for all assays are available on the Environment RCN webpage (http://enzymes.nrel.colostate.edu).

For these assays, 4g of soil were mixed with 40 mls of 50 mM NaOAc (sodium acetate) buffer (pH 5.0) and vortexed at high speed for 3 min. Aliquots (200 µl) of slurry were transferred to polypropylene tubes to which 200 µl of substrates were added. For the peroxidase activity, 10 µl of 0.3% H_2O_2 (hydrogen peroxide) were added. The tubes were then capped and placed on a rotary shaker for 2 h at 25 °C. Following incubation, tubes were centrifuged at 3200 × *g* for 4 min and aliquots (100 µl) of supernatant were taken from each tube and transferred into microplate. For *p*NP substrates, 5 µl of 1.0 M NaOH (sodium hydroxide) were added to the wells to stop the reaction. Microplates were read at 405 nm for *p*NP and *p*-nitroanilide and at 450 nm for peroxidase. Substrate and sample controls were used. All assays were done in triplicates, and repeated twice. The absorbance was corrected by subtracting the combined absorption results for the sample and substrate controls. Enzyme activity was expressed as nmol h^{-1} g $soil^{-1}$.

2.5. Statistical analysis

Respiration and enzyme activity data were tested for normality using the Shapiro–Wilk test ($p \leq 0.05$). Both sets of data showed normal distribution, hence statistical analyses were conducted using SPSS version 20 for windows (IBM, NY, USA). Independent sample *t*-tests were used to determine significant differences for soil respiration and

Table 1
Study locations and their coordinates.

Sites	Description	GPS coordinates
Daisy Lake 2	Metal contaminated limed and unlimed sites	46′27′50′N/80′53′1′W
Wahnapitae Hydro-Dam	Metal contaminated limed and unlimed sites	46′28′31′N/80′49′14′W
Kelly Lake	Metal contaminated limed and unlimed sites	46′26′42′N/81′3′18′W
Kingsway	Metal contaminated limed and unlimed sites	46′29′54′N/80′58′14′W
Laurentian	Metal contaminated unlimed site	46′28′5′N/80′58′35′W
Onaping Falls	Reference site	46′35′32′N/81′23′3′W
Capreol	Reference site	46′45′28′N/80′55′21′W
Killarney	Reference site	46′13′12′N/80′47′43′W