Changes in soil biological activities under reduced soil pH during *Thlaspi caerulescens* phytoextraction

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Abstract

Phytoextraction of soil Cd and Zn may require reduction in soil pH in order to achieve high metal uptake. Reducing the pH of high metal soil, however, could negatively affect soil ecosystem function and health. The objectives of this study were to characterize the quantitative causal relationship between pH and soil biological activities in two Zn and Cd contaminated soils and to investigate the relationship between metals and soil biological activities under low pH. Soils were adjusted to five or six different pH levels by sulfur addition, followed by salt leaching. *Thlaspi caerulescens* was grown for 6 months, and both the rhizosphere and non-rhizosphere soil biological activities were tested after harvest. Reducing pH significantly lowered soil alkaline phosphatase activity, arylsulphatase activity, nitrification potential, and respiration. However, acid phosphatase activity was increased with decreasing pH. The relationship between soil biological activities and pH was well characterized by linear or quadratic regression models with $R^2$ values ranging from 0.57 to 0.99. In general, the three enzyme activities, nitrification potential, and the ratio of alkaline phosphatase to acid phosphatase activity were very sensitive indicators of soil pH status while soil respiration was not sensitive to pH change. The rhizosphere soil had higher biological activities than non-rhizosphere soil. The negative effects observed in the non-rhizosphere soil were alleviated by the rhizosphere influence. However, rhizosphere soil after 6 months phytoextraction showed lower nitrification potential than non-rhizosphere soil, probably due to substrate limitation in our study.

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1. Introduction

Phytoextraction uses unusual hyperaccumulator plants to accumulate high quantities of metals in plant biomass. It offers a low cost strategy to clean up contaminated soils and the plant ash may also have economic value (Baker et al., 1994; Chaney et al., 2000). *Thlaspi caerulescens* is an accumulator of Cd and Zn and is one of the most studied of all hyperaccumulators. The hyperaccumulation process of *T. caerulescens* involves rapid uptake, high rates of translocation from roots to shoots, and huge storage capacity by vacuolar compartmentalization (Chaney et al., 1997). However, the first step is uptake rate-limiting and thus critical to the effectiveness of the phytoextraction success. Plant uptake of Cd and Zn is generally limited by metal solubility. Increasing metal solubility usually results in enhanced uptake and higher shoot metal concentration (Brown et al., 1995a).

The success of phytoextraction depends on appropriate soil management practices to make metals more available to plants. Among the diverse strategies to enhance phytoextraction, pH adjustment has received the most attention, because bioavailability of Cd and Zn is largely controlled by soil pH. Theoretically, lowering pH will increase solubility of most metals. Studies conducted on other crops have shown a negative correlation between soil pH and metal transferred to plants (Narwal et al., 1983; Castilho and Chardon, 1995). Only a few studies have examined the effect of soil pH on *T. caerulescens* hyperaccumulation (Brown et al., 1994, 1995b).

Although reducing soil pH appears to be an effective strategy to enhance Cd and Zn phytoextraction, precaution is needed because low pH and elevated metal concentrations may cause negative impacts to already vulnerable soil ecological systems. How do we increase phytoextraction efficiency...
without creating a further threat to the soil quality? No ecological risk assessment work, however, has been reported to address this question.

Although pH is a master variable, the causal relationship between pH and soil biological activity is rarely studied. Studies have been conducted to observe the correlations between diverse soil properties and soil biological activities, however no conclusions about the effect of pH can be drawn, since these studies were not controlled experiments to observe the pH effect as an independent variable.

Soil is a complex ecosystem; reactions in soil are different from those in a simplified chemical solution. The complexity of the soil microenvironment, the co-existence of copious numbers of microorganisms, the extensive interaction between different physicochemical reactions make it difficult to extrapolate the results of studies in a simplified system to the soil ecosystem. For example, previous studies have found that soil aggregates may protect soil microbes from some adverse effect of acidification (Ibekwe et al., 1997).

Numerous studies have investigated liming effects on soil quality improvement (Arnold et al., 1994; Neale et al., 1997; Grego et al., 2000). Although soil pH is increased, as a result of liming, the relationship between pH and soil quality indicators is not obvious. This is because liming causes many changes in soil properties in addition to pH. Change in soil pH is also a responsive variable, therefore no effect can be discussed with pH as an explanatory variable. Lastly, studies aimed at liming combined with other soil management practices, makes it even more difficult to interpret the pH effect.

Furthermore, unlike healthy soil eco-systems, reducing soil pH in metal-rich soils may be complicated due to increased metal bioavailability. To what extent this will contribute to negative impacts on soil biological activities in addition to the low pH effect is unknown.

Soil quality is usually defined as the capacity of a soil to fulfill its unique ecosystem functions. One of the most important functions performed by soil is nutrient recycling. Carbon, nitrogen, phosphorus, and sulfur are four most fundamental elements that are essential for the development of living cells. The soil microbial-mediated transformation of these elements involves numerous biogeochemical processes. Among them, acid phosphatase and alkaline phosphatase are important in the phosphorus cycle; they may provide insight into the soil organic phosphorus mineralization potential and microbiological activity of soils. Arylsulphatase activity is important in S cycling. Nitrification is the soil microbial process in which ammonium (NH₄⁺) is transformed into nitrate (NO₃⁻). Heterotrophic CO₂ respiration is a key process regulating carbon cycling in the biosphere. These five fundamental soil biological activities were selected as indicators to investigate the effect of pH in two Cd and Zn contaminated soils in this research.

Primary objectives of this study were: (1) to obtain the quantitative causal relationship of pH and soil biological activity in soils which are acidified to increase Cd and Zn phytoextraction and to investigate and compare the sensitivity of the different soil biological activities to pH change, (2) to compare the differences in the soil biological activities of non-rhizosphere and rhizosphere soil of T. caerulescens and how they differentially respond to reduced pH, and (3) to study how metal solubility is affected by pH change and how this in turn, further affects soil biological activities.

2. Materials and methods

2.1. Site description and soil sampling

Soil samples were collected from the A horizon of two cultivated fields in the proximity of a Zn smelter that had been in operation for nearly 100 years at Palmerton, PA. Metals released to the environment were primarily Zn and Cd resulting in a metal concentration gradient according to distance and direction from the smelter. Two soils were collected. A ‘lower metal soil’ was collected about 4.5 km up wind from the smelter and characterized by relatively lower metal concentrations. The second soil was collected about 1.4 km down wind from the smelter, and contained higher metal content (Table 1). Both soils belong to the Montevallo series (loamy-skeletal, mixed, subactive, thermic, shallow Typic Dystrudepts). Soils were first passed through a 1-cm sieve to remove stones and large plant residues then passed through a 4-mm sieve. Soils were then homogenized and stored in closed containers to prevent dehydration.

2.2. Soil characterization

Total Zn and Cd concentrations were extracted by digesting with concentrated hot nitric acid and measured by flame atomic absorption spectrometry. Soil particle size distribution was determined by the hydrometer method (Gee and Bauder, 1986). Soil pH was measured in a soil water suspension (10 g soil to 20 ml deionized water) after shaking 1 h at 180 rpm on a reciprocal shaker (Eckert and Sims, 1995). Organic matter content was determined by loss on ignition (Storer, 1984). Plant available Ca²⁺, Mg²⁺, K⁺, and P were extracted with Mehlich (I) and determined on a Technicon Auto-Analyzer using a colorimeter for Mg and a flame photometer for K, Ca

Table 1
Soil properties

<table>
<thead>
<tr>
<th>Soil</th>
<th>Total Zn (mg kg⁻¹)</th>
<th>Total Cd (mg kg⁻¹)</th>
<th>Texture</th>
<th>pH</th>
<th>OM (g kg⁻¹)</th>
<th>Sand (%)</th>
<th>Silt (%)</th>
<th>Clay (%)</th>
<th>M (I)–P (mg kg⁻¹)</th>
<th>M (I)–K (mg kg⁻¹)</th>
<th>N (g kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low metal</td>
<td>450</td>
<td>5.0</td>
<td>Loam</td>
<td>7.3</td>
<td>47</td>
<td>36.5</td>
<td>38.0</td>
<td>25.5</td>
<td>68.4</td>
<td>249</td>
<td>0.75</td>
</tr>
<tr>
<td>High metal</td>
<td>1500</td>
<td>25.4</td>
<td>Loam</td>
<td>6.9</td>
<td>52</td>
<td>39.5</td>
<td>34.5</td>
<td>26.0</td>
<td>265</td>
<td>295</td>
<td>0.96</td>
</tr>
</tbody>
</table>

and P (Flannery and Markus, 1980). Total N was determined by the combustion method (Campbell, 1992).

2.3. Soil pH adjustment and salt leaching

Different amounts of elemental sulfur (S) were used to adjust soil pH to the desired levels based on a preliminary acid incubation experiment. Soil pH was monitored periodically by taking 10 g of soil and measuring the soil water suspension pH. Soil was thoroughly mixed every day to ensure equal distribution of sulfur and to aerate the soil to speed up the S oxidation process. Completion of acidification was determined when the same pH was measured for three consecutive weeks. Next, 500 ml of deionized water was used to leach salt from each pot. After all water drained and no surface water remained on the top of the soil, another 500 ml of deionized water was applied and this process was repeated a third time.

2.4. Plant growth

*T. caerulescens* used in this research was a southern French eco-type, collected from Viviez, France with high Cd hyperaccumulation potential (Reeves et al., 2001). Seeds were germinated in growth media (metro-mix) and seedlings were grown for 60 days and watered daily to maintain relatively constant moisture. Plants were grown in a controlled-environment growth chamber, which was set at 16/8 h day/night cycle at 25/22°C, light intensity exceeded 400 µmol photon m⁻² s⁻¹ and relative humidity was 65%. A general purpose N–P–K fertilizer was used as liquid spray when needed. Seedlings were then transplanted into 15 cm (diameter) by 14 cm (height) plastic pots. Each pot contained 1 kg soil and received three plants. When transplanting, seedlings were taken out of the seedling flat and the growth media attached to roots were completely removed. Pots were put into growth chambers with the same settings as for seedling growth. After transplanting, fertilizer was only supplied twice during the growth period at the rate of 0.04 g 20–20–20 general purpose fertilizer pot⁻¹. After 6 months of growth, plants were harvested.

2.5. Rhizosphere soil sampling

Rhizosphere soil is defined as that portion of soil adjacent to and influenced by plant roots (Metting, 1993). *T. caerulescens* had a very prolific root system. After 6 months of growth, all soil in the pot was filled with fine roots. Therefore, in this experiment, all the soil in the pot with plants was treated as the rhizosphere soil. At harvest, the shoot was cut using stainless steel scissors. Root and soil were manually separated.

2.6. Treatment structure and experimental design

A completely randomized block design with factorial treatment combination was used with the following factors: (1) soil type (low metal soil and high metal soil), (2) rhizosphere effect (rhizosphere soil and non-rhizosphere, i.e. with and without plant), and (3) soil pH (6.88, 6.37, 6.07, 5.28, 4.74). For the low metal soil, soil pH was adjusted to six levels; an additional pH treatment of 7.27 which was the initial soil pH was used. The initial soil pH for the high metal soil was 6.88. There were four replications for each of the treatments which were randomly placed into one of the four growth chambers.

2.7. Plant available soil metal extraction

Prior to extraction, 6–8 g of each soil was air-dried overnight, and ground to pass through a 150-µm sieve. Duplicate 2-g samples were added to 50-ml polycarbonate centrifuge tubes and extracted with 15 ml 0.1 M Sr(NO₃)₂ in a reciprocal shaker (2400 oscillations h⁻¹) for 2 h at room temperature. A successive extraction was repeated (Ahnstrom and Parker, 1999). Concentrations of Mg, Ca, Al, Mn, Cd and Zn were determined using an inductively coupled plasma spectrometer (ICP-OES). Detection limits (DL) were 0.400, 1.00, 0.060, 0.020, 0.010, and 0.020 mg L⁻¹ for Mg, Ca, Al, Mn, Cd, and Zn, respectively. Laboratory standards were routinely included for analysis.

2.8. Soil biological activities

Soil enzyme activities were measured by the colorimetric determination of *p*-nitrophenol released by referring to a calibration standard curve (Tabatabai, 1994). For each soil sample, controls without adding substrate mixture were also performed, and the *p*-nitrophenol concentration was subtracted from the sample’s value. Triplicate samples were analyzed for each soil sample.

Soil nitrification potential was measured by the shaken soil–slurry method (Hart et al., 1994). Soil (10 g) was mixed with 80 ml of nitrification substrate solution mixture and shaken at a reciprocal shaker at 300 rpm. A portion of the soil slurry was sampled at 6, 12, 18, 24, 36, and 48 h, centrifuged, and filtered using Whatman no. 40 filter paper. The NO₃⁻ in the solution was analyzed by a colorimetric method. The rate of NO₃⁻ production was then calculated by linear regression of these results.

Soil respiration was determined by closed jar incubation with NaOH traps, followed by acid titration (Zibilske, 1994).

2.9. Statistical analysis

Statistical analysis was conducted using SAS version 8.2 (SAS Institute, 2001). The assumption of normality was tested by examining the plot of residuals and calculating the Shapiro–Wilk statistic. Homogeneity of variance was tested by examining plots of predicted values versus residual values. The Spearman Test was used to test the correlations between the predicted value and absolute value of the residual. Logarithm transformation was used when needed. After checking that the data met the assumptions, the PROC MIXED procedure was used for univariate ANOVA to determine the main factor and interaction effect with block as a random factor. The pH treatment of 7.27 in the low metal soil
3. Results

3.1. Soil properties

The two soils collected had quite different levels of heavy metals (Table 1). The low metal soil had a total Zn and Cd concentration of 450 and 5.0 mg kg$^{-1}$, respectively. The high metal soil contained 1500 mg kg$^{-1}$ Zn and 25.4 mg kg$^{-1}$ of Cd. Soil pH, organic matter content, and particle size distribution were similar for the two soils. However, more elemental sulfur was needed to achieve lower pH in the low metal soil compared to the high metal soil.

3.2. pH, rhizosphere, and soil effects on biological activities

pH, rhizosphere, and soil all had significant effect on AlP, AcP, arylsulphatase, and respiration (Table 2). For nitrification, only pH effect was significant. Among the three factors, pH appeared to be the most influential factor that determined soil biological activities which was demonstrated by the highest F values of pH effect for most of the activities. For respiration, rhizosphere effect was the dominant factor while for AcP, it was soil factor.

3.3. 0.1 M Sr(NO$_3$)$_2$ extractable metal concentrations

Soluble/exchangeable forms of Cd and Zn are usually extracted with dilute salt solutions (Shuman, 1991). This form is commonly regarded as the most mobile, bioavailable form (Ahnstrom and Parker, 1999). Data showed that the concentration of this form of Cd and Zn was strongly controlled by pH (Fig. 1). Decreasing pH drastically increased the concentration of Al, Cd, Mn and Zn, while the extractable Ca and Mg were reduced at lower pH. Notably, although Al concentration increased with decreasing of pH in both soils, the extent of increase was not the same for the high and low metal soils. For the high metal soil, from the highest to the lowest pH, Al increased about 30%. However, for the low metal soil, there was an 8–11-fold increase. The final concentration in the low metal soil reached 49 and 71.8 mg kg$^{-1}$ for rhizosphere and non-rhizosphere soils, respectively. This may due to organic matter buffering capacity or the presence of different electron acceptors in the two soils. More likely, it may suggest that the two soils might have different mineralogy. This phenomenon is consistent with the higher buffering capacity of low metal soil previously observed when S was added to soil. The major differences between the high and low metal soils were Cd and Zn concentrations, where the former was much higher than the latter. However, there was not much difference in the concentrations of Ca, and Mg between these two soils. Rhizosphere soil generally had lower 0.1 M Sr(NO$_3$)$_2$ extractable metal concentrations, especially of Cd, and Zn, indicating the uptake by plant roots lowered the available metal concentrations around the roots.

3.4. pH effects on biological activities

Reducing soil pH significantly lowered AlP activity in both soils although the response was quite different. The correlation coefficient with pH was 0.77 ($P<0.0001$) (Table 3). For the high metal soil, from pH 6.88 to 6.07, activity declined slowly and from pH 6.07 to 4.74, activity decreased rapidly (Table 4). For the low metal soil, the AIP activity showed an ‘S’ curve pattern. From pH 7.27 to 6.37, both the non-rhizosphere and rhizosphere soils AIP activities were increased with decreasing pH. From pH 6.37 to 5.28, activities declined rapidly. However, from pH 5.28 to 4.74, alkaline phosphatase activity stabilized for non-rhizosphere soil and declined only slightly for rhizosphere soil. For both soils, the rhizosphere soil had higher AIP activities than non-rhizosphere soil. The relationship between pH and AIP activity was well characterized by linear or quadratic regression models (Table 5).

In contrast to AIP, acid phosphatase activities generally increased with decreasing soil pH. For this enzyme, the low
metal soil had higher activity than the high metal soil. For the high metal non-rhizosphere soil, each lower pH treatment had significantly higher activity than the previous higher pH. For the rhizosphere soil, there was no significant difference between pH treatment of 6.88 and 6.37. After that, each lower pH treatment significantly increased activity (Fig. 1). For low metal soil, from pH 6.88 to 5.28, acid phosphatase activity was increased with decreasing pH, but declined at the lowest pH for both the non-rhizosphere and rhizosphere soils. For both soils, at higher pH, rhizosphere soil had higher AcP activity than non-rhizosphere soil, and this relationship was reversed at lower pH levels.

Overall, arylsulphatase activities decreased with decreasing soil pH. For the high metal non-rhizosphere soil, each lower pH treatment had significantly lower arylsulphatase activities than its previous higher pH treatment (Table 4). This was also true for the rhizosphere soil except that there was no significant difference between pH treatment of 6.88 and 6.37. For the low

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**Table 3**

Pearson correlation coefficients between soil biological activities and metal concentrations. \( N = 88 \) Prob \( > |r| \) under \( H_0: R_{Ho} = 0 \)

<table>
<thead>
<tr>
<th>Activity</th>
<th>Al</th>
<th>Ca</th>
<th>Cd</th>
<th>Mg</th>
<th>Mn</th>
<th>Zn</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid phosphatase</td>
<td>0.42*</td>
<td>-0.83*</td>
<td>0.37*</td>
<td>-0.43*</td>
<td>0.72*</td>
<td>0.51*</td>
<td>-0.79*</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>-0.45*</td>
<td>0.76*</td>
<td>-0.51*</td>
<td>0.30**</td>
<td>-0.81*</td>
<td>-0.62*</td>
<td>0.77*</td>
</tr>
<tr>
<td>Arylsulphatase</td>
<td>-0.41*</td>
<td>0.75*</td>
<td>-0.58*</td>
<td>0.10</td>
<td>-0.86*</td>
<td>-0.70*</td>
<td>0.91*</td>
</tr>
<tr>
<td>Nitrification</td>
<td>-0.42*</td>
<td>0.53*</td>
<td>-0.37*</td>
<td>0.10</td>
<td>-0.59*</td>
<td>-0.50*</td>
<td>0.63*</td>
</tr>
<tr>
<td>Respiration</td>
<td>-0.34**</td>
<td>0.39*</td>
<td>-0.40*</td>
<td>0.21***</td>
<td>-0.30**</td>
<td>-0.23***</td>
<td>0.25***</td>
</tr>
</tbody>
</table>

* *, **, and *** indicate the significance at the 0.05, 0.01, and 0.001 probability levels, respectively.
Table 4
Means and standard errors of activities of acid phosphatase (AcP) \((n=12)\), alkaline phosphatase (AIP) \((n=12)\), arylsulphatase (Aryl) \((n=12)\), nitrification potential \((n=8)\) and soil respiration \((n=4)\) in different pH treatments

<table>
<thead>
<tr>
<th>Soil</th>
<th>pH</th>
<th>AcP PNP (mg kg(^{-1}) h(^{-1}))</th>
<th>AIP PNP (mg kg(^{-1}) h(^{-1}))</th>
<th>Aryl PNP (mg kg(^{-1}) h(^{-1}))</th>
<th>Respiration CO(_2) (µg kg(^{-1}) h(^{-1}))</th>
<th>Nitrification NO(_3) (µg kg(^{-1}) h(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>High metal soil</td>
<td>6.88</td>
<td>312 ± 10(^a)</td>
<td>447 ± 26(^a)</td>
<td>189 ± 15(^a)</td>
<td>0.76 ± 0.01(^b)</td>
<td>56.8 ± 9.2(^a)</td>
</tr>
<tr>
<td></td>
<td>6.37</td>
<td>335 ± 20(^a)</td>
<td>432 ± 12(^a)</td>
<td>149 ± 13(^b)</td>
<td>0.73 ± 0.15(^b)</td>
<td>66.4 ± 20(^a)</td>
</tr>
<tr>
<td></td>
<td>6.07</td>
<td>375 ± 20(^a)</td>
<td>421 ± 40(^a)</td>
<td>99 ± 11(^c)</td>
<td>0.61 ± 0.08(^b)</td>
<td>45.6 ± 7.8(^a)</td>
</tr>
<tr>
<td></td>
<td>5.27</td>
<td>531 ± 31(^b)</td>
<td>288 ± 24(^b)</td>
<td>57 ± 6.5(^d)</td>
<td>0.79 ± 0.16(^b)</td>
<td>10.5 ± 15(^b)</td>
</tr>
<tr>
<td></td>
<td>4.74</td>
<td>716 ± 26(^b)</td>
<td>147 ± 12(^c)</td>
<td>25 ± 3.2(^e)</td>
<td>0.48 ± 0.07(^b)</td>
<td>5.2 ± 3.5(^b)</td>
</tr>
<tr>
<td>Low metal soil</td>
<td>7.27</td>
<td>260 ± 3.5(^e)</td>
<td>444 ± 34(^a)</td>
<td>176 ± 13(^a)</td>
<td>1.07 ± 0.05(^a)</td>
<td>60.6 ± 14(^a)</td>
</tr>
<tr>
<td></td>
<td>6.88</td>
<td>374 ± 12(^d)</td>
<td>467 ± 44(^a)</td>
<td>169 ± 12(^a)</td>
<td>0.69 ± 0.05(^a)</td>
<td>48.5 ± 12(^b)</td>
</tr>
<tr>
<td></td>
<td>6.37</td>
<td>506 ± 16(^e)</td>
<td>486 ± 46(^a)</td>
<td>164 ± 14(^c)</td>
<td>0.67 ± 0.06(^a)</td>
<td>43.6 ± 8.0(^b)</td>
</tr>
<tr>
<td></td>
<td>6.07</td>
<td>664 ± 22(^e)</td>
<td>302 ± 27(^b)</td>
<td>116 ± 8.6(^d)</td>
<td>0.61 ± 0.07(^b)</td>
<td>35.9 ± 2.2(^b)</td>
</tr>
<tr>
<td></td>
<td>5.27</td>
<td>899 ± 43(^b)</td>
<td>137 ± 17(^c)</td>
<td>36 ± 3.6(^e)</td>
<td>0.61 ± 0.08(^c)</td>
<td>−3.5 ± 13(^c)</td>
</tr>
<tr>
<td></td>
<td>4.74</td>
<td>726 ± 61(^a)</td>
<td>131 ± 14(^a)</td>
<td>14 ± 2.5(^a)</td>
<td>0.43 ± 0.08(^c)</td>
<td>−14.7 ± 27(^d)</td>
</tr>
</tbody>
</table>

PNP, p-nitrophenol. Values with different superscript letters for the same soil within each column are significantly different with respect to pH treatments (FLSD t-test, \(P<0.05\)).

Table 5
Regression models of biological activities on pH in high metal soil

<table>
<thead>
<tr>
<th>Activity</th>
<th>Rhizosphere</th>
<th>Regression equation</th>
<th>(R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIP</td>
<td>Non-rhizosphere</td>
<td>(Y = -3.21 \times 10^3 + 1.10 \times 10^3 \times pH - 81.2 \times pH^2)</td>
<td>0.95*</td>
</tr>
<tr>
<td></td>
<td>Rhizosphere</td>
<td>(Y = -45.4 \times 10^3 + 1.59 \times 10^3 \times pH - 121 \times pH^2)</td>
<td>0.97*</td>
</tr>
<tr>
<td>AcP</td>
<td>Non-rhizosphere</td>
<td>(Y = 6.77 \times 10^{-1} - 1.85 \times 10^3 \times pH + 134 \times pH^2)</td>
<td>0.99*</td>
</tr>
<tr>
<td></td>
<td>Rhizosphere</td>
<td>(Y = 5.29 \times 10^3 - 1.41 \times 10^3 \times pH + 105 \times pH^2)</td>
<td>0.97*</td>
</tr>
<tr>
<td>Arylsulphatase</td>
<td>Non-rhizosphere</td>
<td>(Y = -523 + 101 \times pH)</td>
<td>0.96*</td>
</tr>
<tr>
<td></td>
<td>Rhizosphere</td>
<td>(Y = -564 + 121 \times pH)</td>
<td>0.94*</td>
</tr>
<tr>
<td>Nitrification</td>
<td>Non-rhizosphere</td>
<td>(Y = -1.66 + 0.57 \times pH - 0.045 \times pH^2)</td>
<td>0.64**</td>
</tr>
<tr>
<td></td>
<td>Rhizosphere</td>
<td>(Y = -0.20 + 0.045 \times pH)</td>
<td>0.81**</td>
</tr>
<tr>
<td>Respiration</td>
<td>Non-rhizosphere</td>
<td>(Y = -3.22 \times 10^{-4} + 1.60 \times 10^{-4} \times pH)</td>
<td>0.57**</td>
</tr>
<tr>
<td></td>
<td>Rhizosphere</td>
<td>(Y = 1.85 \times 10^{-3} + 1.91 \times 10^{-3} \times pH)</td>
<td>0.80**</td>
</tr>
</tbody>
</table>

*, **, and *** indicate the significance at the 0.05, 0.01, and 0.001 and probability levels, respectively. AIP, alkaline phosphatase; AcP, acid phosphatase.

Metal soil, the activity curve of rhizosphere soil was similar to the non-rhizosphere soil, with the former always being a little higher than the latter at each pH level.

Nitrification potential was generally decreased with descending pH. For the high metal soil, nitrification potential first increased with decreasing soil pH. The highest nitrification rate was observed at the second highest pH level. Then, it continually decreased with the decreasing of pH. For the low metal soil, the nitrification potential declined with decreasing pH with only one exception—the lowest rate for non-rhizosphere soil was at pH 5.28. Interestingly, the non-rhizosphere soil had much higher nitrification potential than the rhizosphere soil for both soils. Acidification stimulated basal respiration. For example, for the high metal rhizosphere soil, the highest respiration rate appeared at pH 5.28. Even when soil pH reached as low as 4.74, soil still had the same level of respiration as that of the highest pH. Overall, there was no statistically significant difference among the pH treatments for the rhizosphere soil. For the non-rhizosphere soil, when pH was reduced to 4.74, respiration was significantly reduced. For the low metal, non-rhizosphere and rhizosphere soils, respiration continually declined with decreasing soil pH. For both soils, the rhizosphere soil had a much higher basal respiration rate than the non-rhizosphere soil.

All of the tested biological activities had significant linear or quadratic pH regression models with \(R^2\) values above 0.80 in most cases. Table 5 listed the regression models of high metal soil, the low metal soil had similar results but the models were not presented for simplicity.

4. Discussion

4.1. Sensitivity of soil biological activities responding to pH change

There has been increasing interest in developing methods which are indicators of soil health and sustainability, reflecting changes in soil properties. The focus has switched from simple chemical approaches to more integrated biological approaches. Soil microbial-mediated processes are viewed as an integration of soil physical, chemical, and biological characteristics and therefore are excellent candidates to reflect changes in soil conditions.

Our data demonstrated that soil biological activities were sensitive to pH change. At the tested pH range—from pH 4.74...
to 6.88 or 7.27 for high or low metal soil, respectively, pH was the most important factor influencing soil biological activities. Except for AcP, lowering pH significantly reduced all activities. Fifty percent of inhibition occurred at $\Delta pH_{K1.85}$, $\Delta pH_{K1.42}$, $\Delta pH_{K1.55}$, and $\Delta pH_{K3.0}$ for AlP, arylsulphatase, nitrification potential, and respiration, respectively (Fig. 2). Opposite to other tested activities, acid phosphatase activities increased with decreasing pH; inhibition of this enzyme was calculated based on the lowest pH levels when its activity was the highest. Fifty percent of inhibition was at $\Delta pH_{2.22}$.

There are several proposed mechanisms that explain sensitivity of enzymes to pH changes. These include direct or indirect pH effects. Ionization or protonation of the acidic or basic groups in the enzyme active center is believed to account for most of the decline in enzyme activity when pH deviates from optimum. Soil pH can change the concentration of inhibitors or activators, as well as the substrate in soil. Stability of soil enzymes to pH change is also highly dependent on soil properties (Frankenberger and Johanson, 1982). Changes in enzyme activities may reflect the changes in number and relative composition of soil microbes in relation to pH change. In a study of phosphatase and arylsulphatase activities in wetland soils (Kang and Freeman, 1999), pH was found to be positively correlated with phosphatase activity. Acosta-Martínez and Tabatabai (2000) also observed a significant and positive correlation between alkaline phosphatase and arylsulphatase activity with pH—the correlation coefficient were 0.89 and 0.66—respectively, and a negative correlation of acid phosphatase and pH with a correlation coefficient $-0.69$.

Stuczyuski et al. (2003) found that changes in pH after metal salt amendments may be responsible for some of the inhibition effects in soil biological activities previously being attributed to the metal toxicity. However, in these studies, the explanatory variables were not pH, therefore strong correlations do not necessarily imply a direct pH effect.

Our data illustrated the strong pH sensitivity of nitrification suggesting these bacteria are pH-sensitive. Similarly, in a study of nitrification potential in Pb or Cu contaminated soils
(Sauvé et al., 1999), pH appeared to be one of the most influential parameters because a large part of variability in soil nitrifications was simply a function of soil pH and organic matter content. A positive correlation between pH and nitrification was also reported by Kemmitt et al. (in press).

Soil heterotrophic respiration involves numerous soil microorganisms. Under stress, such as low pH, some sensitive organisms may die, while other tolerant organisms may survive. Some acid-loving organisms may even flourish. As a sum of the various responses, it is not surprising to observe that soil respiration is not as sensitive to pH change as enzyme activities and nitrification potential. The plot of percentage of inhibition versus ΔpH had the lowest $R^2$ value, 0.40, among the five activities. Respiration also had the lowest value of correlation coefficient with pH, $r=0.25$ (P < 0.05). The effect of pH on soil respiration was investigated by several other studies. For example, in acidic aquatic ecosystems, marked inhibition of decomposition of organic matter has been observed (Traaen, 1980; McKinley and Vestal, 1982). Reduced soil respiration was also observed by Speir et al. (1999) due to metal addition and acidification. However, liming tended to increase CO2 respiration (Andersson and Nilsson, 2001).

4.2. Implications of AlP/AcP ratio

Our data on the AlP and AcP activities support other researchers’ findings that AlP activity was predominant in neutral or alkaline soils, while AcP activity was predominant in acid soils (Eivazi and Tabatabai, 1977; Dick and Tabatabai, 1984; Renella et al., in press). As pH decreased, the ratio of AlP to AcP decreased accordingly. At pH 4.74, the AlP/AcP ratio was also the lowest for all four types of soils [high metal soil non-rhizosphere, high metal soil rhizosphere, low metal soil non-rhizosphere, low metal soil rhizosphere] with a very narrow range, from 0.12 to 0.14. At the highest pH, there was an approximately nine-fold increase in the AlP/AcP ratio, with a range from 1.03 to 1.15 in the four types of soils. There were very good linear regressions for AlP/AcP ratio with pH. The $R^2$ values were 0.98, 0.94, 0.90, and 0.97 for the four soil types, respectively (Fig. 3). This indicates that it is possible to assess AlP/AcP ratio based on soil pH, and vice versa. Previously, Dick and Tabatabai (1992) proposed the idea of using AlP and AcP activities to assess the effective soil pH. This was further developed by Dick et al. (2000). They reached a conclusion that when a soil has an AlP/AcP ratio greater than 0.5, the soil pH should be approximately 6.0. Our data were consistent with their observations in that the AlP/AcP ratio is, indeed, a sensitive indicator of soil pH status. However, in our study, only one type of soil, low metal non-rhizosphere soil, reached pH 6.05 when the ratio was 0.5. For the low metal rhizosphere soil, high metal non-rhizosphere soil, high metal rhizosphere soils, the pH were 5.84, 5.49, and 5.21, respectively. At these pH levels, soil biological activities may have already been negatively affected.

4.3. Difference in soil biological activities between non-rhizosphere and rhizosphere soils of T. caerulescens

Rhizosphere soil has long been known to be different from non-rhizosphere soil. A number of rhizodeposition products (root exudates, cell lysates, mucilage, secretions, etc.) make rhizosphere soil a favorable environment for microbes to thrive. Bacteria of the rhizosphere are usually more abundant than non-rhizosphere soil (Tate, 1995). We generally observed higher biological activities in the rhizosphere soil than in the non-rhizosphere soil. Soil AlP activity, arylsulphatase, and soil respiration were consistently higher in the rhizosphere under all pH treatments. It is interesting to note that rhizosphere soil had lower nitrification potential than non-rhizosphere soil in most cases indicating a lower number of nitrifiers in the rhizosphere. This can be explained by the depletion of available $\text{NH}_4^+$ in the rhizosphere soil due to root absorption and the lack of substrate ($\text{NH}_4^+$) limited nitrifiers activity.

Plant roots can also cause considerable changes in the rhizosphere pH (Hinsinger, 1998; Jaillard et al., 2001). Depending on the forms of nitrogen used by the plant, this change could be acidification or alkalization (Smiley, 1974; Römheld, 1986; Gahoonia et al., 1992). The contribution of organic acid exudation to rhizosphere acidification varied in different studies (Dindelaker et al., 1989; Haynes, 1990; Jones and Darrah, 1994; Jones et al., 1994; Hinsinger, 1998; Jones, 1998). Our data indicate a slight pH increase (about 0.05–0.3 units, data not shown) in the rhizosphere soil after harvest. Nevertheless, although T. caerulescens was found to mobilize non-soluble forms of metals (McGrath et al., 1997; Whiting et al., 2001), it appeared that it was not achieved through acidification. This has also been observed by several other studies (Knight et al., 1997; Luo et al., 2000). The slight increase in rhizosphere pH may explain part of the reason that rhizosphere soil had higher biological activities than non-rhizosphere soil in our study.

The higher biological activities in rhizosphere soil were not only because of an abundance effect, but also due to the fact that the microbes in rhizosphere were more active. We have measured the total soil microbial biomass and calculated the ratio of biological activities versus microbial biomass. The results indicated that at most cases (except nitrification

![Fig. 3. Relationship between the alkaline phosphatase (AlP) to acid phosphatase (AcP) activity ratio ($n=12$) and pH. Closed triangles are high metal rhizosphere soil, $R^2=0.94$ (P < 0.0001). Open diamonds are high metal non-rhizosphere soil, $R^2=0.98$ (P < 0.0001). Stars are low metal rhizosphere soil, $R^2=0.96$ (P < 0.0001). Closed squares are low metal non-rhizosphere soil, $R^2=0.90$ (P < 0.0001).](attachment:image.png)
potential), rhizosphere soil indeed had higher activity ratio than non-rhizosphere soil.

4.4. Correlations between soil metal concentrations and biological activities

Numerous studies have documented the adverse effects of heavy metals on soil biological activities (Fliëfbach et al., 1994; Moreno et al., 1999). However, there is also disagreement in the current literature. Our data demonstrated the strong correlations between soil biological activities and 0.1 M Sr(NO\textsubscript{3})\textsubscript{2} extractable metal concentrations. Based on the sign of the correlation coefficients, we can partition these six metals into two groups: one group includes Al, Cd, Mn and Zn; the other group includes Ca and Mg. The correlation coefficients between the metals in the first group and AlP, arylsulphatase, nitrification potential and respiration were all negative values. While the correlation coefficients between those activities and the metals in the second group were all positive. Acid phosphatase had an opposite relationship with extractable metals from the other four activities. All correlation coefficients were significant at the 0.05 level except arylsulphatase with Mg and nitrification potential with Mg. The order of the absolute values of coefficients between these metals and soil biological activities were: Mn > Ca > Zn > Cd > Al > Mg. Magnesium was the metal least associated with microbial activities while Mn, Ca and Zn were highly associated. It appeared that the three enzyme activities were more affected by metals than nitrification potential and respiration. This was in agreement with other observations. In a study of heavy metal effect on a contaminated grassland ecosystem, significant reductions in enzyme activities were observed and the degree of reduction was closely associated with the degree of heavy metal contamination (Kuperman and Carreiro, 1997). The enzymes they tested included acid and alkaline phosphatases and several other enzymes. Arysulphatase was also found to be sensitive to heavy metals. Its activity was inhibited by a number of elements, including Cd and Zn (Al-Khafaji and Tabatabai, 1979). Effects of heavy metal stress on soil respiration are inconsistent in different studies. Metal salts added to three New Zealand soils significantly decreased soil respiration with a similar pattern: an initial sharp decline and followed by relative constant activity or even slight increase. In a study of both smelter and laboratory-contaminated soils, the soil respiration rates of the most polluted samples were 54–77% lower than control samples and were negatively correlated with the contamination level (Nordgren et al., 1988).

4.5. Conclusions

Several conclusions can be drawn from this research. Reducing pH had significant negative impacts on soil microbial activity. Soil alkaline phosphatase activity, arylsulphatase activity, nitrification potential, and respiration were significantly reduced after acidification of soil. Acid phosphatase activity responded to acidification differently from all the other tested parameters—it was increased with decreasing pH. The three enzyme activities, nitrification potential, and the ratio of AlP to AcP activity were sensitive indicators of soil pH status while soil respiration was not sensitive to pH change. The rhizosphere soil had higher biological activities than the non-rhizosphere soil. The negative effects observed in the non-rhizosphere soil were alleviated by the rhizosphere influence except for nitrification. This was due to both an abundance effect and higher physiological activities of microbes in rhizosphere. These results suggest that although reducing pH increases soluble Cd and Zn concentrations in the soil and thus possibly to enhance \textit{T. caerulescens} phytoextraction efficiency, care must be taken to avoid soil ecosystem further degradation under reduced pH. Further research is needed to determine whether the negatively affected soil biological activities can return to background levels after phytoextraction is complete. The answer to this question will largely determine the suitability of reducing soil pH as a strategy to enhance phytoextraction. Provided that soil ecosystem health can be generally restored after phytoextraction is complete and soil pH is increased to original higher levels, reducing soil pH is a recommendable measure to speed up phytoextraction process. In the other scenario, if reducing pH causes prolonged and permanent negative impacts to soil ecosystem, it should not be adopted as a good strategy.

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References


