Microbial cellulose decomposition in soils from a rifle range contaminated with heavy metals

I. Chew, J.P. Obbard *, R.R. Stanforth

Department of Chemical and Environmental Engineering, National University of Singapore, 10 Kent Ridge Crescent, 119260, Singapore

Received 24 May 1999; accepted 21 February 2000

“Capsule”: Microorganisms were able to adapt to high concentrations of metals, where the bioavailability of the metals was reduced.

Abstract

The objective of this study was to assess the effects of heavy metals on microbial decomposition of cellulose in heavy metal-contaminated soils using a cotton strip assay. The assay is a measure of the potential of soil microorganisms to decompose the plant polymer, cellulose. Cellulolytic activity in soil was assessed by determining the reduction in tensile strength of the buried cotton strips over a 25- and 45-day period. Soils were obtained from a rifle range that contain high levels of lead, copper and zinc. The site has been used for approximately 50 years, resulting in metal levels of up to 30,000 mg/kg of lead, 4000 mg/kg of copper and 600 mg/kg of zinc in the most contaminated soils. All the metal-contaminated soils had lower degradation rates than the uncontaminated soils tested. Among the contaminated soils, however, the heavy metal concentration was not the major factor in determining the loss in tensile strength of the cotton strips, where cellulose decomposition was governed by other soil physicochemical properties. Soil with a higher cation exchange capacity, readily oxidisable material and volatile solids content had the greatest loss in tensile strength of cotton strips. Microbial adaptation to the presence of high concentrations of soil heavy metals and reduced bioavailability of metals is the likely explanation for this phenomenon. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Cellulose; Heavy metal-contaminated soils; Tensile strength; Cotton strip; Decomposition

1. Introduction

The soil microbial ecosystem plays an integral role in maintaining soil fertility through the biogeochemical cycling of essential plant nutrients and the mineralisation of organic matter (Domsch, 1984; Vaughan and Ord, 1985; Paul and Clark, 1996; Sylvia et al., 1998). Impacts on these microbial processes from environmental contaminants, including heavy metals, are therefore of concern. Many metals are essential for microbial metabolism [e.g. zinc (Zn), copper (Cu)], but others have no metabolic function [e.g. lead (Pb), chromium (Cr), cadmium (Cd)]. All metals do, however, become toxic above threshold concentrations, although the effects are often dependent on the specificity of microorganisms in mediating a particular process. For example, nitrification is often more sensitive than ammonification due to the restricted number of microbial species that conduct the former process (Coppola et al., 1988; Naidu and Reddy, 1988).

Decomposition of organic carbonaceous materials in soils is undertaken by groups of soil organisms acting in concert (Sagar, 1988; Sylvia et al., 1998). Carbonaceous materials subjected to microbial decomposition include cellulose, hemicelluloses and lignin. Cellulose can account for between 30 and 60% of plant material (dry wt.), and its decomposition is of major importance to the biogeochemical cycling of carbon (C) and essential plant nutrients (Paul and Clark, 1996). In structure, cellulose is a carbohydrate composed of glucose units bound together in a long, linear chain by β-linkages at C atoms 1 and 4 of the sugar molecule. Cellulolytic enzymes for degrading the polymer are produced by a host of microorganisms (bacteria, actinomycetes and microfungi). The enzyme system, collectively known as cellulases, breaks down insoluble cellulose molecules into simple water-soluble mono- or disaccharides that can be
transported into the cell. Once inside the cell, these simple sugars are oxidised to provide energy and to biosynthesise microbial biomass. The sensitivity of microbial decomposition to the presence of heavy metals is often substrate dependent, e.g. straw, starch and cellulose decomposition are sensitive (Doelman and Haanstra, 1979a), but manure and sewage sludge decomposition are not (Cornfield, 1977). Reductions in leaf litter decomposition have been found in forest soils contaminated with heavy metals (Strojan, 1978; Coughtry et al., 1979; Freedman and Hutchinson, 1980). The C cycle, via the process of microbial decomposition, interacts strongly with other elemental cycles, including the nitrogen (N) and phosphorous (P) cycles, to release inorganic nutrients for plant uptake. Hence, this interaction of major plant nutrient cycles is an important area to evaluate potential ecotoxicological effects induced by heavy metals (Hendricks, 1996).

Microbial adaption is an important mechanism in response to the presence of soil contaminants (Doelman, 1986; Silver et al., 1989), and may result in the compensation of an adverse effect by the increased activity of the remaining microflora (Duxbury and Bicknell, 1983). Soil microorganisms may also resist toxicity by transforming metals into less toxic forms, immobilising metals at the cell surface or in intracellular polymers, by precipitation or biomethylation (Babich and Stotzky, 1985; Doelman, 1986).

The aim of this investigation was to assess the effect of heavy metals on the decomposition of cellulose in soils contaminated with heavy metals, particularly Pb and Cu, in a military rifle range soil using the cotton-strip assay. The assay was originally developed for use in the International Biological Programme (Latter and Walton, 1988) to assess decomposition rates in a variety of soil systems. Decomposition is measured as the loss over time of tensile strength in soil-buried cotton fabric consisting of 96% pure cellulose (Barr, 1988; Gillespie et al., 1988). Degradation of buried cellulose may be divided into three stages (Smith and Maw, 1988), i.e.: (1) initial colonisation by microorganisms; (2) initial degradation by enzymes released by the pioneer colonisers, resulting in tensile strength loss; and (3) proliferation of the pioneer community and other secondary organisms, resulting in maximum enzyme activity and complete degradation of the material.

The assay has been previously used to assess the effect of heavy metals and other factors in soils amended with contaminated sewage sludges (Obbard and Jones, 1993) and is well suited to determining comparative rates of decomposition. The assay has several points of merit for our studies, i.e. the assay is simple to perform and a large number of replicates can be used; cotton is a natural substrate of high degree of uniformity and it is also highly suitable for tensile strength testing and; the assay integrates decomposition activity by microorganisms over time.

## 2. Materials and methods

### 2.1. The experimental site

The rifle range, located in Singapore, is approximately 5 ha in size and has been in service for over 50 years. Soils consist of decomposed granites with iron (Fe) and aluminium (Al) oxides on the surface, and contain elevated levels of heavy metals, particularly Pb and Cu. Weathered soil from a contaminated earth-mound ‘backstop’ (used behind the targets), is currently being eroded by rainfall runoff, and the eroded material is deposited as a delta in an adjacent marsh area. The heavy metal contamination is derived from cartridge bullets fired into the backstop. Although organic contamination of soils from the propellants is a possibility at such sites, such contamination at this site was not considered as firstly, bullets are fired from a distance of 500 m and, secondly, the soils are strongly leached by tropical rainfall and also strongly oxidised. Organic contaminants associated with explosives in fired cartridges are therefore unlikely to be present at significant concentrations.

### 2.2. Field sampling and preparation

Sample locations were selected on the evaluation of data obtained from previous soil sampling at the site (unpublished data) in order to provide a gradient of metal concentrations for experimental purposes. Contaminated soil samples (0–15 cm depth) were collected from three separate site locations, i.e. within the delta area (total of three samples about 50 m apart, referred to as D1–D3); from an area of dumped contaminated soil adjacent to the rifle range (A1); and from the backstop itself (B1). The relative sampling locations are shown schematically in Fig. 1.

Soil from the surface to 15 cm depth were collected and transported back to the laboratory for analysis. Soil samples for chemical analysis were air-dried and sieved to <2 mm. Soils used for the cotton-strip assay were kept moist, and plant debris, stones and soil fauna removed. Triplicate soil samples were then placed in the clean plastic trays used for the cotton strip experiment (see below). The moisture contents of the soils were adjusted to 40% of the maximum water-holding capacity. An uncontaminated background soil from the immediate area and a commercially available nursery soil were used as control samples.

### 2.3. Soil analysis

The soil properties analysed included pH, volatile solids (loss-on-ignition), particle size distribution, readily oxidisable carbon (ROC), and cation exchange capacity (CEC). British Standards (BS), United States Environmental Protection Agency (USEPA), or American Society of
Testing and Materials (ASTM) methods were used for soil analysis, and all analyses were conducted in triplicate. ROC was measured using a modified Walkley and Black method (Soil Science Department, Reading University, 1989) to determine the levels of readily available substrate for microbial growth. Total heavy metal concentrations were determined by hot nitric acid digestion on 2 g of soil sample (duplicate samples), followed by inductively coupled plasma emission spectrophotometric analysis for Pb, Cu, Zn, nickel (Ni), Cr and Cd. The non-digestable material (presumably mostly silicate) was determined by measuring the weight of the solids left after the acid digestion for the metals.

2.4. Procedure for cotton-strip assay

Shirley Soil Burial Test Fabric (Shirley Institute, Manchester, UK) was used in this experiment. This is an unbleached 100% cotton fabric (96% pure cellulose) and is the standard fabric for assessing cellulytic activity in soils (Latter and Walton, 1988). Three replicate soil trays, each containing about 12 kg of soil (wet wt. equivalent), were used per soil sample. Cotton strips were prepared (3.5×12 cm) and sterilised by autoclaving (121°C for 20 min). A layer of soil was placed in the trays (5–6 kg wet wt.), and the cotton strips were placed uniformly on the soil surface and covered with the remainder of the soil. The duration of the experimental period was based on experiments conducted by Obbard and Jones (1993), where approximately 80% of the original tensile strength was lost in 25 days. Five cotton strip harvests were taken over the 25-day period (i.e. 5, 10, 15, 20 and 25 days) from each tray to make a total of nine cotton strip replicates from each type of soil per harvest. Trays were placed in a room at ambient temperature and humidity and watered to the initial weight every 3 days using sterilised, deionised water.

At each harvest, cotton strips were retrieved from the soil trays with minimal disturbance to the other strips. The harvested strips were washed with deionised water, soaked in 70% ethanol for at least 4 h, air-dried and stored in sealed polythene bags until ready for tensile testing. Prior to testing, the strips were frayed down by 0.5 cm on each side to remove ‘edge’ effects. The strips were then dried at 50°C for 14 h and conditioned at constant humidity by placing the strips in a humidified incubator at 20°C for 24 h. Tensile strength loss was then measured in the warp direction on a pre-calibrated spring beam 600N Type W Monsanto Tensometer.

Two rounds of testing were undertaken. Soils were replicated in both rounds, with a mean of <9% difference in cotton strip tensile strength loss between the soils on each sampling occasion, thereby indicating good experimental reproducibility. The first round was conducted for 25 days, and the second for 45 days. For the second round, the 25-day results were used for comparison with the first round.
3. Results and discussion

3.1. Soil physicochemical data

Physicochemical data for the soils are given in Table 1. All soils comprised predominantly sand (66.3–96.0%), with lesser amounts of silt (3.3–26.9%) and clay (0.7–6.8%). Delta soils (D1–D3) had pH values in the range of 4.4–6.2, and the backstop soil was pH 5.3. CEC, volatile solids (loss-on-ignition) and ROC levels in the delta soils ranged from 1.6 to 14.4 m.e./100 g soil, 3.9–5.3% and 0.5–4.4 mg/g soil, respectively. Respective values for the backstop soil were 10.5 m.e./100 g soil, 5.7% and 6.4 mg/g soil.

Heavy metal concentrations are compared to known ranges and typical mean values concentrations for soils world-wide in Table 1. Highest Pb concentrations were found in the backstop soil, i.e. 29,800 mg/kg, although the highest Cu and Zn concentrations were found in the adjacent sample, A1, at 4060 and 587 mg/kg, respectively. Overall, the site background and nursery soils had significantly less heavy metal concentration than the delta and backstop soils, and were generally more acidic with higher volatile solid, ROC and CEC levels. When compared to the typical range and mean of heavy metal concentrations found in soils world-wide (Lindsay, 1979, Table 1), it is clear that Pb and Cu are present at high concentrations in all soils at the rifle range site, with slight elevations for Cd and Zn over typical background levels.

3.2. Cellulose decomposition

Cotton tensile strength loss (CTSL) was calculated as the original tensile strength of the cotton strips (controls) minus the tensile strength remaining at each harvest. CTSL results for all the soils are given in Table 2.

The nursery soil had a very rapid decrease in cotton tensile strength, with an 80% loss after 10 days. The sample was discontinued after 10 days. The site background soil had a slightly slower loss of tensile strength, with 76% of the strength lost after 25 days. All of the metal-contaminated samples showed considerably slower losses in tensile strength than did the uncontaminated soils. Fig. 2 shows a comparison of the tensile strength loss with time (as a per cent of the initial strength) for the nursery, uncontaminated and averaged contaminated soils. The reduced rate of tensile strength loss in the contaminated soils suggests that the metal content of the soils is having a major impact on the microbial decomposition of cellulose.

Over the 25-day experimental period, the final loss in cotton tensile strength for the contaminated soils ranged from 18 to 41% of the original tensile strength, with the adjacent soil sample, A1, giving maximum loss and the D2 sample giving minimum loss (Fig. 3). Referring to Table 2 and Fig. 3, from the 2nd to the 10th day the differences in the CTSL (%) of the different soils remained low (about 5%). This is probably due to the time lag taken for microorganisms, particularly microfungi, to colonise the substrate and commence cellulose degradation. It was only after the 15th day that there was significant variation in the CTSL (%) between the different soils. The CTSL of delta sample, D3, increased more rapidly than other soil samples, but slowed down at the end of the experiment. The CTSL of the delta sample D1 increased rather rapidly after a lag of 10 days. Although the delta sample D1 was the least contaminated, it did not have the highest CTSL.

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>A1</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>Site background</th>
<th>Nursery soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.2</td>
<td>4.4</td>
</tr>
<tr>
<td>PSD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.9</td>
<td>6.2</td>
</tr>
<tr>
<td>Sand %</td>
<td>84.8</td>
<td>77.3</td>
<td>84.6</td>
<td>66.3</td>
<td>90.3</td>
<td>96.0</td>
</tr>
<tr>
<td>Silt %</td>
<td>12.3</td>
<td>18.2</td>
<td>13.9</td>
<td>26.8</td>
<td>8.0</td>
<td>5.3</td>
</tr>
<tr>
<td>Clay %</td>
<td>2.9</td>
<td>4.5</td>
<td>1.5</td>
<td>6.8</td>
<td>1.7</td>
<td>0.7</td>
</tr>
<tr>
<td>ROC mg/g soil</td>
<td>18</td>
<td>3.5</td>
<td>0.5</td>
<td>4.4</td>
<td>6.4</td>
<td>15</td>
</tr>
<tr>
<td>CEC m.e./100 g soil</td>
<td>15.4</td>
<td>4.16</td>
<td>1.56</td>
<td>14.4</td>
<td>10.5</td>
<td>13.5</td>
</tr>
<tr>
<td>Volatile solids %</td>
<td>7.4</td>
<td>4.50</td>
<td>3.9</td>
<td>5.3</td>
<td>5.7</td>
<td>6.5</td>
</tr>
<tr>
<td>Metal content (mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al</td>
<td>32,700</td>
<td>2360</td>
<td>10,600</td>
<td>30,000</td>
<td>26,200</td>
<td>–</td>
</tr>
<tr>
<td>Fe</td>
<td>48,000</td>
<td>4760</td>
<td>48,800</td>
<td>48,300</td>
<td>48,500</td>
<td>–</td>
</tr>
<tr>
<td>Cd</td>
<td>2.04</td>
<td>1.46</td>
<td>1.96</td>
<td>2.1</td>
<td>1.82</td>
<td>0.8</td>
</tr>
<tr>
<td>Cr</td>
<td>16.05</td>
<td>19.6</td>
<td>21.3</td>
<td>15.9</td>
<td>21.3</td>
<td>6.9</td>
</tr>
<tr>
<td>Cu</td>
<td>4060</td>
<td>195.2</td>
<td>536.3</td>
<td>639.2</td>
<td>2503</td>
<td>31.8</td>
</tr>
<tr>
<td>Ni</td>
<td>76.1</td>
<td>3.7</td>
<td>2</td>
<td>1.7</td>
<td>10.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Pb</td>
<td>2140</td>
<td>1100</td>
<td>7480</td>
<td>10,100</td>
<td>29,800</td>
<td>34.9</td>
</tr>
<tr>
<td>Zn</td>
<td>587</td>
<td>57.9</td>
<td>90.2</td>
<td>127</td>
<td>343</td>
<td>44.2</td>
</tr>
</tbody>
</table>

* PSD, particle size distribution; ROC, readily oxidisable carbon; CEC, cation exchange capacity.

Lindsay (1979).
In order to correlate the relationship between the heavy metal concentrations of the soils and the loss in tensile strength, graphs of metal content in the contaminated soils against final CTSL (%) were plotted (Fig. 4a, b, c for Cu, Pb and Zn, respectively). There is no significant relationship between the heavy metal content of the contaminated soil samples and the loss in tensile strength, although weak but positive correlations were noted between CTSL and concentrations of Cu ($R^2=0.387$), and Zn ($R^2=0.429$). There is no relationship between contaminated soil Pb concentration and cellulose decomposition ($R^2=0$). If the metals were adversely affecting cellulose degradation one would expect a negative correlation between metal content and CTSL.

Heavy metal concentration was not, therefore, a major factor in affecting the differences in decomposition rate of the cotton strips within the contaminated soils. The soil samples with the highest metal concentrations did not necessarily take the longest time to reach the CTSL (%) target value, suggesting that heavy metal content is not the key factor in affecting cellulose decomposition in the contaminated soils tested. When different soil properties (i.e. ROC, CEC and volatile solids content) were plotted against the final CTSL (%), it is found that the CTSL (%) values were positively correlated to these soil properties. The linear trend lines plotted have regression coefficient values ranging between 0.6, 0.75 and 0.97 for ROC, volatile solids (loss-on-ignition) and CEC, respectively (refer to Fig. 5).

The CEC of the contaminated soils is directly related to the total Al content of the soils (Fig. 6). A ‘least-squares’ regression analysis of the Al (in mmol/100 g) and CEC gave a line of slope of 0.176 and a $r^2$ value of 0.833. This suggests that one out of every six available Al atoms is involved in the exchange, and that the Al species has a very high surface area (most probably as an Al hydroxide coating on the surface of the sand particles). Assuming that the Al in the soils is present as Al(OH)$_3$ and Fe as Fe(OH)$_3$, the analysed parameters (silicate, Al, Fe, heavy metals) account for between 98.1 and 104% of the total weight. The loss of water from Fe and Al hydroxides to form the metal oxides accounts for almost all the loss of weight of the volatile solids. In short, the soil consists of sand with Fe and Al hydroxides, with very little else present at significant concentrations (except for the metal contaminants). The exchange capacity, therefore, must come from the Al or Fe. Since the exchange capacity is closely correlated with the Al content, but not with Fe, the Al must be the active agent.

### Table 2

<table>
<thead>
<tr>
<th>Harvest time (days)</th>
<th>Cotton tensile strength loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Al</td>
</tr>
<tr>
<td>2</td>
<td>2.9</td>
</tr>
<tr>
<td>5</td>
<td>6.1</td>
</tr>
<tr>
<td>10</td>
<td>7.7</td>
</tr>
<tr>
<td>15</td>
<td>17.1</td>
</tr>
<tr>
<td>20</td>
<td>27.7</td>
</tr>
<tr>
<td>25</td>
<td>41.9</td>
</tr>
</tbody>
</table>

![Fig. 2](image-url) Loss in cotton strip tensile strength (CTSL) over time for background, nursery and averaged delta soils.
4. Discussion

In this study, the presence of heavy metal pollutants appears to inhibit microbial activity in the contaminated soils when compared with the uncontaminated soils. However, within the contaminated soils, the metal content of the soils was not the main factor affecting the microbial decomposition of the cotton strips, and hence cellulose, in the rifle range soils despite the high concentrations present. Other measured chemical properties of the soil samples were found to be the main determinants affecting the degradation of cellulose including CEC, ROC and the volatile carbon content. Possible reasons for the lack of relation between heavy metal content and the loss in tensile strength, include the following:

1. CEC is known to affect the bioavailability of the trace elements (Morel, 1996). CEC for these soils is directly correlated with the Al content, and probably reflects adsorption on Al hydroxide rather than on the clays or organics. Higher CEC values result in metals being more strongly absorbed onto the soil surface, thus reducing bioavailability. It has been previously shown by Doelman and Haanstra (1979b) that inhibitory effects of Pb on microbial respiration and dehydrogenase activity are dependent on soil buffering capacity as expressed by its CEC. Enzyme activity is also positively related to amount of clay material, and thus related to CEC (Ladd, 1985). Hence, soil CEC is likely to affect the mineralisation rate of the cellulose by microorganisms, as well as the bioavailability of heavy metals.

2. The microbial population has adapted to the presence of heavy metal concentrations. Given the long-term contamination of the rifle range site (i.e. in excess of 50 years), the potential for microbial adaption is a distinct possibility. Soil microbial populations are known to be highly adaptive to changing soil conditions and can develop resistance to toxic heavy metals (Tyler, 1981; Olson and Thornton, 1982; Baath, 1989; Silver et al., 1989). Mechanisms of microbial resistance to heavy metals in contaminated soils have previously been noted and include: energy-dependent efflux of intracellular metals; oxidation to less toxic forms; biosynthesis of intracellular polymers to reduce metal bioavailability; binding of metals to the cell surface; precipitation at the cell surface; and biomethylation (Doelman, 1986).

All of the metal-contaminated soils showed slower degradation rates for the cotton strips than did the uncontaminated soils, indicating that the metals are adversely affecting the microbial populations. However, within the contaminated soils the degradation rates are not correlated with metal contents, but rather with other soil properties. Presumably the other soil parameters are influencing the bioavailability of the metals and reducing the impact of the metals on the microbial populations. The results indicate that the total metal content of the soils may not be a good indicator of the impact of the metals on the microbial cellulose decomposition.
Fig. 4. Loss in tensile strength versus (a) copper concentration, (b) lead concentration and (c) zinc concentration in the contaminated soils.
References

Baath, E., 1989. Effects of heavy metals on microbial processes and populations (a review). Water, Air and Soil Pollution 47, 335–379.


