Acidification and Metal Contamination: Implications for the Soil Biota of Sudbury

Christine D. Maxwell

Considerable attention has been focused on the improvements in vascular plant communities in Sudbury that have occurred as a result of the land reclamation programs and reduced industrial emissions during the past two decades. Relatively little is known about the corresponding changes in soil microbial populations even though they are essential for the successful development of plant communities. In this chapter, the effects of industrial emissions on the diverse biological communities within the soil are discussed. The focus is on the effects of acidification and toxic metals on Sudbury soils, but because of the limited number of studies in this area, a more general but very brief review of other literature is included.

Soil Biota

The importance of the soil biota in soil formation, nutrient cycling, and the production of organic matter is a key factor in the functioning of ecosystems. Disruption of these activities by anthropogenic stresses such as smelter emissions can have a profound effect on the development of higher plant communities. Our general lack of knowledge of the effects of anthropogenic stress on soil microbial populations is attributable, in part, to the very nature of the soil environment, which has been described by Stotsky (1974) as undoubtedly the most complex microbial habitat. Soil is composed of (1) mineral particles of various dimensions and chemical characteristics; (2) organic matter in many stages of decay; (3) a liquid phase in the form of soil water that contains soluble materials; (4) a gaseous phase, usually composed of oxygen, carbon dioxide, and nitrogen; and (5) an active living metabolizing soil community.

The components of the soil biota are diverse, including viruses, bacteria, cyanobacteria, fungi, algae, and the soil fauna. This biotic community contributes to the structural integrity of the soil through the process of aggregate formation and to the fertility of the soil through nitrogen fixation. Also, through the production of humus, the soil biota contribute to soil texture, water-holding capacity, and the complex formation of minerals important in plant nutrition (Rhulich 1981; Paul and Clark 1989). Soil microorganisms, in turn, are affected by many factors, including the chemical status of the soil (e.g., nutrient content and ionic composition), the physical conditions (e.g., temperature, moisture, gaseous composition, pH, redox potential, particulates), and the biological interactions between organisms (e.g., competition, parasitism, and predator-prey relationships).

Soil microorganisms are not randomly distributed in the soil profile. A general trend is for soil populations to show a decrease in size...
with increasing depth, and this is matched by a decrease in soil organic matter with depth. The A horizon is the most active zone from a biological point of view, because it harbors the roots of higher plants, the bacterial flora, fungi, algae, and the fauna. Bacteria frequently form microcolonies on the surface of aggregate particles, to which they are attached by means of fibrils and mucigel. There is increased bacterial and fungal activity in the vicinity of plant roots, as a result of an increase in organic matter provided by live, senescent, and dead roots. This region has been termed the rhizosphere. The root surface itself harbors a distinct microflora and is referred to as the rhizoplanae. Most actively growing algae and cyanobacteria are restricted to the upper few millimeters of the soil surface where light permits photosynthesis.

**Toxic Metals, Acid Conditions, and Soil Microorganisms—General Considerations**

In unpolluted soils, the abundance of toxic metals (copper, nickel, etc.) is generally low (Table 17.1), and they are most often in forms unavailable to living organisms. By contrast, metals introduced as a result of anthropogenic activities are usually present as microscopically small particles, which may be sorbed, bound, chelated, or in salt form and are therefore more available to microorganisms (Doelman 1985). The availability, and hence potential toxicity, of metals is also governed by several physicochemical characteristics of the soil system, such as pH, oxidation-reduction potential, aeration, inorganic anions and cations, particulate and soluble organic matter, clay minerals, salinity, and temperature (Babich and Stonsk 1985).

Not all microorganisms are eliminated from industrially damaged areas. In fact, a wide range of relatively resistant or tolerant microorganisms can be found in soils contaminated by toxic metals. They possess physiological mechanisms that allow them to survive and reproduce (Gadd 1990). These mechanisms include the ability to produce polysaccharides, which act as biosorbents for metal cations, the ability to precipitate and crystallize metals on the cell surface, reduced permeability of the cell wall or cell membranes, or the ability to compartmentalize or blind metals to proteins intracellularly. In many bacterial strains, the genetic determinants of resistances to metals are located on plasmids, which are pieces of DNA that are separate from the chromosome (Gadd 1990).

Bacteria can carry out transformation of toxic metals through oxidation, reduction, methylation, and demethylation, and this may have

<table>
<thead>
<tr>
<th>Table 17.1. Metal Concentrations (µg/g) in Sudbury Surface Soils from Surveys Conducted within 20 km of Smelters*</th>
<th>OMSO (1992)</th>
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<tbody>
<tr>
<td><strong>Background level</strong></td>
<td><strong>Mean</strong></td>
</tr>
<tr>
<td>Arsenic</td>
<td>10</td>
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<td>Cobalt</td>
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<td>Copper</td>
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<td>Lead</td>
<td>150</td>
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<tr>
<td>Nickel</td>
<td>60</td>
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</table>

*Surveys by Duda et al. (unpublished) and Ontario Ministry of the Environment (OMSO). Modern background levels for non-agricultural soils is also indicated (computed by Neguseni and McIver, 1990). Geometric means and ranges are presented.

— no data.
some implications for the soil community in terms of oxidizing the effects of toxic metals (Shannon and Usterman 1993). The ability to transform metals as a detoxification mechanism could be potentially very useful in the remediation of metal-contaminated habitats; however, little is known about the importance of these organisms in the natural environment (Lovely 1993).

It is difficult to separate the effects of toxic metals from those of high soil acidity, because soil acidification increases the solubility of metals. Soil acidity in natural (unpolluted) soils is determined by several processes, some of which produce acids (e.g., decomposition of organic material through the production of organic and carbonic acids and the mineralization of plant nutrients) and some of which consume acids (weathering of soil minerals, and the anaerobic reduction of nitrogen and sulfur) (Abrahamsen 1987). Acid deposition has resulted in increased soil acidity in the vicinity of point sources such as smelters (Hutchinson and Whitby 1974); however, it is difficult to determine the relative contributions from atmospheric deposition and naturally occurring processes. There is some evidence to suggest the occurrence of seasonal changes in pH under some vegetation covers as a result of the uptake and return of base cations to the soil (Johnson 1987). Whether small fluctuations in pH can significantly influence the soil biota remains a matter of conjecture.

Sudbury Soils: Implications for Soil Microorganisms

A diverse set of habitats exists for the soil microorganisms in the Sudbury area, ranging from heavily contaminated soils that are undergoing slow changes over time to less contaminated areas undergoing the same changes to sites in which amelioration has been rapid as a result of revegetation treatment.

The natural soils of the Sudbury area are podsols, which are acidic soils with a pH range of approximately 3.8-5.2. On hillsides close to the smelters where acidification and metal contamination are greatest, mineral soils may be found in small pockets, but the loss of vegetation has resulted in severe soil erosion, with the loss of typical soil profiles. In areas farther away, the typical soil profile is retained; however, high concentrations of toxic metals and low pH conditions often occur in the surface layers of the soil, the layers of greatest biological activity (Hutchinson and Whitby 1974).

Among the elements found at elevated concentrations in surface soils near the smelters are copper, nickel, cadmium, cobalt, chromium, iron, manganese, sulfur, and zinc (Duda et al. in press).

There is some evidence of reductions in the copper and nickel concentrations in surface soils over the past 20 years (Gundernann and Hutchinson 1993, Duda et al. in press). The authors of the studies suggested that these changes in concentration of metals in surface soils are due partly to leaching and erosion of the physically unstable barren soils and possibly due to reduced atmospheric inputs. Despite these reductions, bioassay results using lettuce seedlings suggest that the concentrations of metals at sites close to the smelters are still inhibitory to root growth (Gundernann and Hutchinson 1993). These inhibitory effects may also be significant for the soil microbial community.

Diversity and Abundance of Soil Organisms

Soil Algae and Cyanobacteria

The photosynthetic components of the soil community, the soil algae and cyanobacteria, play several important roles. They are responsible for the addition of carbon and organic matter to the soil, and in addition, the growth of filamentous forms can result in the formation of crusts (sometimes with fungal hyphae and moss protonemata) that reduce water and wind erosion (Metting 1981).

The soil flora of three contaminated sites near Sudbury, were characterized by a low

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diversity of chlorophytes, one or two diatom species, and a notable absence of cyanobacteria (Maxwell 1991). Similar results have been reported for soils subject to emissions from various metallurgical plants in Russia (Shina et al., 1985).

The chlorophyte-dominated flora of the Sudbury barrens is characteristic of acid soils, *Chlamydomonas acidophila* (Fig. 17.1), a single-celled motile alga, is ubiquitous in these soils. As the name suggests, this alga is acid-tolerant and is reported to occur in such widespread areas as Smoking Hills in the Canadian Arctic, where it is found in acidic ponds with a pH of 1.8 (Sheath et al., 1982), and strip-mine ponds in Ohio with a pH of 3.3 (Rhodes 1981). Not only is it acid-tolerant, but it also exhibits copper tolerance. Twiss (1990) compared the copper tolerance of isolates from three Sudbury soils, including a roost bed soil, and concluded that in comparison with a laboratory strain of *C. acidophila*, the isolates were indeed tolerant. A second unicellular green alga, *Chlorella saccharophila*, has also been recognized as a metal-tolerant organism in the Sudbury soils (Hutchinson et al., 1981).

The absence of cyanobacteria from soils in the Sudbury barrens area is not surprising. Several authors, including Brock (1973) and Dooley and Houghton (1974), have concluded that cyanobacteria are unable to live at pH levels less than 4.4, conditions that exist over much of the Sudbury area.

The response of the autotrophic soil microflora to liming and fertilizer treatments was surprisingly rapid. Maxwell (1991) followed the changes that occurred at a Wahnapitae site for 46 weeks. The first cyanobacteria were found just 2 weeks after the reclamation treatment, when a pH of 5.3 was recorded. The diversity of the cyanobacteria and chlorophytes increased with time. A nearby untreated control site showed no change over the 46 weeks (Table 17.2). Maxwell (1991) also examined soils that had been subjected to revegetation treatment, 2, 4, and 5 years before her study. A progressive increase both in the diversity of green algae and cyanobacte-
TABLE 17.2. Changes in the Soil Microflora in 46 Weeks after Treatment
with Lime and Fertilizer at a Contaminated Site at Washaapte.

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<thead>
<tr>
<th>Control site</th>
<th>Pre-treatment</th>
<th>2</th>
<th>6</th>
<th>12</th>
<th>36</th>
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<tr>
<td>Cyanobacteria</td>
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<td>Anaerobius sp.</td>
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<td>Gloeocapsa sp.</td>
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<tr>
<td>Lyngbya sp. (2)</td>
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<tr>
<td>Nostoc muscorum</td>
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<td>Oscillatoria sp. (1)</td>
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<td>Chlorophyta</td>
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<td>Chlamydomonas acidophila</td>
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<td>Chlorella sp.</td>
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<td>Chlorococcum sp.</td>
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<td>Gloeocapsa sp.</td>
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<td>Oscillatoria sp.</td>
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<td>Chroococcus nitellus</td>
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<td>Hamatococcus</td>
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<td>Nostoc sp.</td>
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<td>Euglena sp.</td>
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x, isolated in enrichment culture; —, not isolated in enrichment culture.

Soil Bacteria

Little information is available on the diversity and abundance of soil bacteria in Sudbury. That which is available relates to specific soil processes such as nitrogen fixation and nitrification and is covered in the next section. In a summary of the effects of various concentrations of nickel on soil bacteria, Doelman (1985) reported significant decreases in populations both in field and laboratory experiments and noted that the bacteria are more sensitive to metals than fungi are. Metal contamination generally causes an increase in the fungal biomass to bacterial biomass ratio, as a result of the different sensitivities of prokaryotes and eukaryotes.

Hutchinson and Nakatsu (personal communication) found a vigorous acid and metal-tolerant bacterial flora in the Coniston Valley, with pseudomonads predominating. Tolerances to nickel, copper, cobalt, and aluminum were especially high. Nickel tolerance appeared to be controlled by a nickel-tolerant plasmid. Nordgren et al. (1983, 1985) reported decreases in bacterial numbers close to the bass mill at Gusum, where the soil is heavily contaminated with copper (10–15 mg/g) and zinc (15–20 mg/g), and close to a smelter in Northern Sweden, which emits a wide spectrum of metal particulates including lead, copper, zinc, and arsenic.
Fungi
The soil fungi are of particular importance in the decomposition of litter and the mineralization of organic matter, especially under acid conditions. Some basidiomycetes form symbiotic mycorrhizal associations, thereby directly affecting the nutrient status of vascular plants. Freedman and Hutchinson (1980) compared the abundance and diversity of soil fungi on contaminated sites close to the Copper Cliff smelter with uncontaminated sites, and although there were consistently fewer colonies isolated from contaminated sites, the numbers were not significantly different from those at uncontaminated sites.

Carter (1978) compared soil fungi in terms of species diversity and abundance at four sites in Sudbury. The soils at the sites differed in terms of copper and nickel concentrations, although care was taken to select sites that had similar conditions of moisture, pH, and organic matter and also similar leaf litter from red maple (Acer rubrum). A. two highly contaminated sites, he found a lower species diversity index and reduced number of colony-forming units than at the two less-contaminated sites. Also, Trichoderma koningii appeared to be restricted to contaminated sites. In laboratory tests, Carter demonstrated that the fungal flora of soil contaminated sites were more tolerant of high nickel concentrations than those of the less-contaminated sites (approximately 0.4 mg/g of copper, nickel). Similar effects on soil fungi have been reported for metal-contaminated areas in Sweden (Ruhl et al. 1984).

Soil Fauna
The soil fauna is composed of protozoans and metazoans, living both in the soil and in the surface litter. The fauna play an important part in the early breakdown of litter, greatly facilitating the activities of the soil microflora. Those that fragment litter include the millipedes, mites, isopods, and collemabolans.

There is limited information on the soil fauna in Sudbury. Rohan-Pelleter and Winterhalder (unpublished manuscript) recorded 35 species of orbibid mites, representing 22 families from sites around Sudbury. In the barren areas, they found a very limited orbibid fauna with only three species. The semibarren fauna was more diverse with 35 species, although this number is very low in comparison with uncontaminated forest sites in Ontario and Quebec. Revegetated sites had 15 species, indicating a slow recovery.

Reports from other contaminated areas, such as Palmerton, Pennsylvania (Strojan 1978), show a reduced number and diversity of arthropods in forest litter compared with control sites. Springtails (species of collemabolans) that live in the litter or surface layer of the soil decrease in the vicinity of the brass mill at Gusum, Sweden (Bengtson and Rundgren 1984), but species that live deeper in the soil are not affected.

Carbon Dioxide Fixation and Litter Decomposition
The fixation of carbon dioxide in terrestrial ecosystems is mainly carried out by higher plants, soil algae, and cyanobacteria. Several groups of bacteria, the green bacteria, the methanogenic bacteria, and acetogena bacteria, also fix carbon dioxide, but little is known about their contribution of carbon fixation to the ecosystem. Organic carbon from plants enters the soil through the process of litter decomposition or, more directly, through the activity of the soil algae and cyanobacteria. Mycorrhizal fungi can also provide substantial quantities of organic matter, derived from the photosynthetic activities of the host plants, and also, there are recent reports of direct carbon dioxide fixation by both ectomycorrhizal fungi (Lapeyrie 1988) and vesiciarous basidiomycetes (Rechard and Piche 1989).

The amount of organic matter entering the soil depends on the amount of litter input and the rate of decomposition. On the barren slopes close to the smelters where there is little or no vegetation, organic matter in the soil is either in the form of residual humus or derived from the reduced flora of soil algae or perhaps from mosses such as Pohlia. Such organisms are often
the primary colonizers of bare soil. Land reclamation programs should permit a progressive increase in this limited flora, although changes may be slow (see Chapter 13).

Metals from smelter emissions tend to accumulate in litter layers (Hutchinson and Whitham 1974; Dumontet et al. 1992), and their toxicity can result in the decreased activity of the decomposers. Measurements of soil respiration and soil metabolic activity have both shown reduced microbial activity in litter at contaminated sites near the Copper Cliff smelter in Sudbury (Freedman and Hutchinson 1980), near the smelter at Gouin, Sweden (Fredgren et al. 1983), and also downwind from the smelter at Rouyn-Noranda, Quebec (Dumontet et al. 1992). Studies using litter bags have also shown reduced decomposition rates at contaminated sites compared with unamendeded sites (Freedman and Hutchinson 1980; de Canadamo 1983). In a laboratory study, copper was shown to be more inhibitory to the decomposition process than nickel.

The Nitrogen Cycle—Nitrogen Fixation

The biological fixation of nitrogen from the atmosphere is carried out by prokaryotes that possess the enzyme nitrogenase. The organisms fall into two main categories: the asymbiotic forms, which may be autotrophic or heterotrophic, and the symbiotic forms, which form associations with higher plants.

Many of the free-living photosynthetic cyanobacteria are capable of nitrogen fixation. The ability to fix nitrogen is found most often in filamentous forms such as Nostoc and Anabaena; these possess heterocysts, although some non-heterocystous forms may also have the ability (Fig. 17.2).

Maxwell (1991) found that cyanobacteria were absent on contaminated sites in Sudbury and concluded that pH was a limiting factor. Rapid colonization of amended sites occurred after the revegetation treatment, and among the colonists were two heterocystous forms, Nostoc muscorum and N. paludosum. Their presence suggests that nitrogen fixation could be occurring; however, experimental evidence for Nostoc punctiforme (Granhall 1970) shows the fixation process occurring in a range from pH 5.0 to 10.0, with an optimum of pH 7.6. If this is the case with species found on revegetated sites, the pH may still be too low to permit significant nitrogen fixation in Sudbury soils.

A free-living heterotrophic bacterium, similar to Azotobacter was isolated from contaminated
soils in Sudbury by Winterhalder (1987). Although this bacterium was capable of fixing nitrogen in laboratory conditions, no fixation could be detected in the field. De Catanzano (1983) was unable to detect any biological nitrogen fixation occurring in the contaminated soils of remnant jack pine stands in Sudbury. The symbiotic fixation of nitrogen far exceeds the contributions of the symbiotic forms, and the rates of fixation are several orders of magnitude higher. Two associations are of importance, the legume RhiZobium symbiosis, (Fig. 7.3) and the non-legume angiosperms-acinomyces symbiosis, in which the acinomyces is often of the genus Frankia. The efficiency of the two systems is apparently comparable, but the acinomyces association has the advantage of being able to function in more acidic conditions (pH 4.2) than the rhizobia association.

There are no native legumes occurring on the barren and semi-barren soils in the Sudbury area; however, two exotic acid-tolerant legumes, Birdsfoot trefoil (Lotus corniculatus) andAlsike clover (Trifolium hybridum), have been introduced successfully as part of the revegetation program. Nodulation by Rhizobi um and nitrogen fixation have been demonstrated for both species, despite the fact that the pH of the soils was considered to be low for the survival of Rhizobia (Winterhalder 1987). Another leguminous, exotic, the woody black locust (Robinia pseudo-acacia) has also been introduced to the Sudbury soils, where it is hardy and vigorous (see Chapter 8).

Frankia is a non-motile filamentous actinom ycete that forms root nodules with several plants native to the Sudbury area, including sweet iron (Comptonia peregrina) and the alders (Alnus crispa and Alnus rugosa). Whereas Frankia itself is non-motile, there is a recent report that spores of Frankia, which are resistant to desiccation, may be transported by the activities of birds (Paschke and Dawson 1993) and that the nitrogen-fixing ability of this actinomycete allows actinorhizal vascular plants to become very invasive.

Other Nitrogen Cycling Processes

Studies on the effects of acidity and metals on the remaining stages of the nitrogen cycle in Sudbury are limited, and none relates to top soil changes that have occurred over the past decade.

De Catanzano (1983) compared mineralization and nitrification in the contaminated soils of remnant jack pine (Pinus banksiana) stands in Sudbury with uncontaminated sites at Windy Lake (60 km northwest of Sudbury) and Burt Lake (50 km west of Kirkland Lake) and found higher levels of ammonium in the Sudbury soils. Laboratory studies confirmed that nickel was causing a stimulation of the mineralization process. Similar results have been shown for forest soils in Germany, contaminated with various metals (Necker and Kunze 1986). Stimulation of mineralization is not necessarily beneficial, because ammonium, if not taken up and used by higher plants or immobilized by microorganisms, may be lost in leaching.

The study of nitrification in soils is important from the standpoint of soil fertility, because higher plants readily take up nitrate ions into their roots for assimilation into nitrate compounds. Nitrate not used by higher plants may be leached from the soil or used by microorganisms capable of assimilatory nitrogen reduction (denitrification). Nitrification is a two-stage process involving two chemautotrophic bacteria, Nitrosomonas and Nitrobacter. The former oxidizes NH₃ to NO₂, the latter NO₂ to NO. It is also considered to be a process that is highly pH-sensitive. Optimum pH values range from pH 6.6 to 8.0. Below pH 6.0, nitrification rates decrease, and less than 4.5, they cease. Other environmental factors such as soil aeration, moisture, temperature, and organic matter are also important in determining the rate of nitrification.

De Catanzano (1983) found both Nitrosomonas and Nitrobacter in soils at contaminated sites in Sudbury in higher numbers than in uncontaminated forest soils. The contaminated sites had soil pH values of 3.2-3.4, whereas
the uncontaminated soils were also acidic (pH 3.4–3.6). Whereas pronounced seasonal trends were seen, nitrate levels in organic Sudbury soils were generally higher than those of uncontaminated sites, perhaps as a result of the higher numbers of nitrifiers. Incubation studies in which 100 µg g⁻¹ and 500 µg g⁻¹ nickel additions were made to uncontaminated soils showed an increase in nitrification at the lower concentration and a decrease at the higher concentration. This suggests that low concentrations of nickel may stimulate nitrification, whereas high concentrations will inhibit the process.

There are two possible explanations for the occurrence of nitrification in such acidic soils. In the first, there may be microsites in the soil in which the pH is higher than that of the soil solution. These could result from the decomposition of nitrogen-rich material with the production of ammonia in localized areas.
the second case, several heterotrophic bacteria, actinomycetes, and soil fungi are known to produce NO; from N₂ via laboratory conditions; however, the significance of these findings in field conditions is not known (Paul and Clark 1989).

**Myccorrhizae**

Myccorrhizae are symbiotic associations formed between fungi and the roots of most vascular plants (Fig. 17.4). The importance of myccorrhizae in ecosystem dynamics has only recently been recognized. Potentially, they can affect all aspects of a functioning ecosystem, including carbon allocation and nitrogen and phosphorus cycling. The association can confer greater abilities to withstand drought, nutrient stress, and environmental perturbation on the higher plants, allowing them to colonize areas previously unavailable to them (Allen 1991).

The activity and diversity of myccorrhizal fungi may be severely reduced by pollutants in smelter emissions. In profoundly disturbed sites, myccorrhizae may be completely absent. Ruhling et al. (1984) reported a decrease in most myccorrhizal forms, with an increase in copper concentrations in the soil at Gusum; however, some species, such as Laccaria lacarina, were apparently tolerant of fairly high levels of contamination.

Ectomycorrhizae may increase the tolerance of their hosts to metals. Jones and Hutchinson (1986) investigated the role of several species of ectomycorrhizal fungi in conferring copper and nickel tolerance on the paper birch (Betula papyrifera), which is widespread on contaminated sites in Sudbury. They reported that the ectomycorrhizal fungi conferred a degree of metal tolerance on the birch seedlings; however, the four myccorrhizal isolates used in this experiment differed in the degree of protection afforded to the seedlings. There were also differences in tolerance to nickel and copper. At lower nickel levels (34 μM nickel) one of the fungal isolates, Sirodesma flavidum, stimulated growth, whereas at higher levels (85 μM nickel), growth was 86% of control seedlings grown without nickel. Higher concentrations of nickel in seedling roots, compared with stems, suggested that nickel was...
Conclusion

The recovery of vegetation after environmental destruction is dependent on several key factors. First, there must be a reduction or cessation of the conditions that led to the degradation. Second, an active, integrated soil community must be re-established; and third, important biogeochemical cycles such as the carbon and nitrogen cycles must become functional. Over the past 20 years, there have been significant reductions in emissions in South Africa and improvements in some vascular plant communities, which is one step towards the help of soil amelioration. To many people, the "greening" of South Africa provides concrete evidence of this; however, this chapter has shown, we know little of the activities of the soil microbial communities despite the fact that the success of the higher plant communities is dependent on the activities of the below-ground biota. The biotic communities of South Africa soils remain the least investigated of all communities in the area. The main reasons for this may be the inherent complexity of the soil biota, the spatial heterogeneity of soil conditions, and the temporal aspects of colonization and species interactions.

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