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Utilizing earthworm and microbial assays to assess the ecotoxicity of chromium mine wastes

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ABSTRACT

Mining plays an important role in the South African economy which results in environmental impacts. This holds a potential hazard for ecosystems surrounding mining areas and also for public health in the surrounding communities. The aim of this study was to use soil enzymatic analyses and earthworm (*Eisenia andrei*) responses *viz.* growth, reproduction, lysosomal membrane stability and tissue metal concentrations to determine the effect caused by chromium mine waste on the activity of soil microbial community and soil invertebrates. Results indicated that chromium mining did have an ecotoxic effect on enzymatic activity, as the material which exceeded the Cr benchmark for microorganisms showed the least amount of enzymatic activity. Significant differences in enzymatic activity were observed between the different samples. Earthworm biomass increases were low in the mining material exposed worms and might have been correlated with the low enzymatic activities in the materials. Biomass was however not considered a sensitive endpoint. Lysosomal membrane stability, measured as NRRT, proved to be a sensitive endpoint, showing the same pattern from day 7 up to day 28. Hatching success of cocoons was not considered a sensitive endpoint, due to the low cocoon production in the mining material exposed worms. Since mine waste materials often contain complex mixtures of metals that might be toxic on their own or in combination with other factors, it is difficult to attribute any observed effect to any of the specific metals analyzed. The metal concentrations were however compared to benchmarks in order to determine which of the metals could have had a toxic effect on the soil organisms. The only benchmark exceeded, was the PNEC for microorganisms, for Cr in the unrehabilitated silt (TDF1) material. None of the other benchmarks were exceeded, indicating that perhaps granular composition of the materials might have had a greater influence than the metals.

1. Introduction

Soil is vital for the survival of flora and fauna but is threatened by contamination; mining being one of the main contributors (Chester et al., 1989; Godgul and Sahu, 1995).

South Africa is the largest producer of ferrochrome, producing 75% of the global demand (Mbendi Information Services, 2010) which is widely used in ferro-alloys, high temperature furnace linings, chemical products, plating, and as coating on metals or steels to protect against corrosion (Vermaak, 1986). Chromium has been found to be hazardous to fauna and flora at elevated levels (Nriagu et al., 1988; Shanker et al., 2005). Sivakumar and Subbhuraam (2005) investigated toxicity of Cr(III) and Cr(VI) to the earthworm *Eisenia fetida* and found 14-day LC_{50} values of Cr(III) from 1656 mg/kg and Cr(VI) from 222 mg/kg in soil. In organic substrate, LC_{50} values of Cr(III) and Cr(VI) were 1635 mg/kg and 219 mg/kg, respectively.

Opencast mining of minerals has a serious impact on the environment, causing the destruction of natural soils (Vega et al., 2004). The waste material formed after mineral extraction is unstable with an unfavourable texture and structure, increasing its erodability (Michelutti and Wiseman, 1995).

Chromium is commonly found in two oxidation states in the environment, i.e. Cr(III) and Cr(VI). These two forms will react differently in the environment, for example, Cr(III) in the air will not undergo any reaction, while Cr(VI) will eventually react with dust particles or other pollutants and form Cr (III) (Grevatt, 1998). In soil systems Cr is mostly present either as insoluble $Cr(OH)_3$.aq or as Cr(III) adsorbed to soil components, preventing plant-uptake and leaching into ground water systems (Bartlett and Kimble, 1976). Hexavalent chromium in turn is scarce and mostly related with anthropogenic activities. Both species of chromium [Cr(III) and Cr(VI)] have been found to be toxic to *E. fetida* in both water and soil, although the concentrations at which the toxicity of chromium manifested were lower in water and higher in soil (Sivakumar and Subbhuraam, 2005). Studies have furthermore shown that Cr(VI)

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is more toxic than Cr(III) to most organisms (Taylor and Parr, 1978; Langard and Norseth, 1979; Ecological Analysts, 1981). Chromium (VI) exerts several toxic effects on biological systems due to free diffusion across cell membranes and strong oxidative potential (Kotaś and Stasicka, 2000). The highly soluble and bioavailable Cr(VI) acts as an oxidizing agent within the cell. This leads to the formation of free radicals, including reactive oxygen species (ROS), during the reduction from Cr(VI) to Cr(III). These ROS in turn have serious effects on the cellular system, damaging the DNA, and could therefore interrupt cellular activity (Manerikar et al., 2008).

A healthy and active soil microbial community is essential in the recovery of ecologically sensitive ecosystems such as post-mining sites, as it is able to mineralize nitrogen and sulphur, while also decomposing organic material (Renella et al., 2006) and improving root development (Zhou et al., 2009). Enzymatic activity affects soil fertility and is affected by pH (Dick et al., 2000). Dehydrogenase, β-glucosidase, acidic- and alkaline phosphatase and urease reflect the microbiological activity in soil and are indicators of soil change (Dick et al., 2000; Li et al., 2004) caused by contamination, degradation or different soil treatments (Tabatabai, 1994; Aon et al., 2001).

Dehydrogenase activity in soil measures the microbial oxidative capacity of soil, which in turn acts as an indicator of the viable organisms (Dick, 1994; Taylor et al., 2002). β-Glucosidase is involved in the saccharification of cellulose (Tabatabai, 1994) and it is commonly found in animals, plants and microorganisms (Dick et al., 1996). Phosphatases are divided into two groups: alkaline phosphatase which is produced by soil microorganisms, and acidic phosphatase which is mainly attributed to plant roots (Criquet et al., 2004). Phosphatases transforms organic and inorganic phosphorus compounds in soil (Amador et al., 1997), and because of its association with organic matter content, acidic phosphatase is a useful indicator for the recovery of degraded soils (Gil-Sotres et al., 2005). Urease plays an important role in the nitrogen cycle and has been found to be correlated with microbial biomass (Klose and Tabatabai, 1999).

Earthworms play a vital role in soil as they enhance structure, fertility and nutrient availability (Little, 1990). They are closely linked to their soil environment and widely distributed (Cortet et al., 1999). Earthworms are furthermore also able to accumulate metals from their environment into their body tissue (Lapinski and Rosciszewska, 2008) making them ideal for the assessment of bioavailability of metals in soils contaminated with mining waste material. *E. andrei* which is considered a sibling species of *E. fetida* (Bouché, 1992) is used for toxicity testing due to its short generation time, allowing investigation of contamination effects on reproduction and second generation survival. These worms are also maintained with ease in a laboratory, making them ideal for such studies (Na et al., 2011).

Based on the information stated above, the aim of this study was to utilize soil microbial assays and earthworm (*E. andrei*) responses to assess the ecotoxic effect of chromium mining on the environment by determining the effect caused by chromium mine waste on the activity of soil microbial community and soil invertebrates. A further objective was to determine if several earthworm endpoints and the NRRT biomarker could be utilized to assess metal contamination as well as its effects on organisms in the ecosystem with regards to chromium mining.

2. Materials and methods

2.1. Site description

Samples were collected from different sites at an opencast chrome mine in Rustenburg, South Africa including a reference soil (RS) and two tailings disposal facilities (TDF).

At the mine where this study was conducted, the chrome mining process takes place as follows. Chromite occurs in a pyroxenite rock clast, where the chromite rich layers alternate with silicate layers such as pyroxenite (Boyd and Meyer, 1979). Pyroxenite is found in between the chromium bands and during the mining process the two materials are separated from each other and placed on different heaps: silt (TDF1) and pyroxenite (TDF2) heap. The RS is representative of the natural soil in the area surrounding the mining site and is used as topsoil in the mine's rehabilitation process. The materials (pyroxenite, silt and turf) from the three sites were randomly collected in triplicate from each site/heap respectively from an opencast chrome mine. A minimum of 1 kg from the top cover layer (15 cm) was collected from each site and sealed in a Ziploc® bag. The samples were kept at 4 °C to preserve biological properties for the enzymatic assays, which were carried out within 5 days of sampling.

2.2. Material properties

The sand, silt and clay (SSC) distribution of the materials were determined by means of the hydro method. One hundred grams of each sample was weighed off and sifted through a 2 mm sieve. Fifty grams of the sifted material was placed into a 500 ml container and soaked with distilled water. Then 10 ml hydrogen peroxide was added and the suspension left for 10 min. The suspension was stirred well and heated for 4 h where after it was cooled. Next 125 ml Calgon was added and it was again stirred well. A 53 μm sieve was placed into a funnel set up to drain into a 100 ml sedimentation cylinder. The suspensions were transferred into the funnel and washed with running water (no more than 1000 ml) and a small brush until the water ran clear. The fraction of material remaining in the funnel was placed in a glass beaker and dried in an oven where after it was sifted through a 53 μm sieve. The sifted fractions were weighed. The 1000 ml suspension in the cylinder was shaken and the first reading was taken after 40 s while the second reading was taken 7 h later. The fraction dried in the oven is sieved with an electrical sieve for 3 min through a 53 μm sieve and the last fraction caught up in a pan. The sieved fractions are weighed and noted. The International Soil Science Society (ISSS) classification system was used.

The pH (H_2O) was also determined in a 1:2,5 soil/water relationship suspension on a mass basis. Twenty grams of material (<2 mm) was weighed off in a 100 ml plastic beaker and 50 ml distilled water (dH_2O) added. The suspension was stirred with a glass rod at a fast pace for 5 s, where after it stood for 4 h.

The suspension was again stirred and then left to stand for 10 min. The pH was determined by means of a pH meter. The pH meter was recalibrated every hour to compensate for drift. The pH of the top part of the fluid was determined which is why care should be taken not to put the electrode in the soil fraction during pH measurement. The material should also not be brought to suspension since this may influence the reading (Coleman and Thomas, 1967).

2.3. Soil enzymatic activities

Before analyses the samples were passed through a 2 mm sieve. The materials were kept at field moist levels for the determination of dehydrogenase activity and air dried for the determination of β-glucosidase, urease, acidic phosphatase and alkaline phosphatase analyses, as is determined by the protocols for each enzyme assay. The same control as were used for earthworm assays were not used, as these assays focused only on the effects of heavy metals on the enzymatic activity in the material. Each enzyme assay used its own set of controls as is described in the different methods.

Dehydrogenase activity was determined by incubating of soil with iodonitrotetrazolium chloride (INT) at 40 °C for 2 h followed