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Phosphatase Activity and Populations of Microorganisms from Cadmium- and Lead-Contaminated Soils

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Phytoextraction is a long-term phytoremediation technology which involves harvesting of shoot biomass containing the accumulated metals (Blaylock and Huang 2000). The harvested plant materials may be used for non-food purposes, or they can be ashed followed by recycling of the metals if economically feasible, or disposal in a landfill (Chaney et al. 2000). One of the current strategies to enhance the efficacy of phytoextraction is the use of soil-applied synthetic chelates. However, many synthetic chelates and their complexes with heavy metals are toxic (Sillanpaa and Oikari 1996) and poorly photo-, chemo-, and biodegradable in soil environments (Nortemann 1999). To ensure the success of phytoextraction, it is imperative to sustain the quality of the soil and to enable vigorous growth of the phytoextracting plants. Soil microorganisms are critically important for normal functioning of soils and are common indicators of soil quality (Grcman et al. 2003).

Enzyme activity is a soil property that is chemical in nature but has a direct biological origin. This activity arises from the presence of many types of enzymes that are present in the soil, and within soil microorganisms. From an assortment of enzymes present and active in soil, phosphatases are interesting groups of enzymes that catalyze the hydrolysis of phosphate from organic mono-ester linkages (Tabatabai and Bremner 1969). Phosphates released from such phosphatase action are very important to the plants and microorganisms that depend on soil for their phosphorus requirements. Studies have shown that long-term heavy metal contamination of soils, has harmful effects on soil microbial activity, especially microbial respiration (Doelman and Haanstra 1984; Rajapaksha et al. 2004). Aside from metal-mediated changes in soil enzyme activities, many reports have shown large reductions in microbial activity due to short-term exposure to toxic metals (Doelman and Haanstra 1979; Hemida et al. 1997). Bacterial activity, measured by thymidine incorporation technique, had been shown to be very sensitive to metal pollution both under laboratory (Diaz-Ravina and Baath 1996a,b) and field (Pennanen 1996) conditions. Moreover, habitats that have high levels of metal contamination for years still have microbial populations that are smaller than the microbial populations and activities in uncontaminated habitats (Kandeler et al. 2000; Roane and Kellog 1996).

This study was therefore conducted to investigate the effects of metals (e.g., lead, cadmium) and synthetic chelates (e.g.,ethylenediamine tetraacetic acid, EDTA; ethylenebis (oxyethylenenitrilo) tetraacetic acid, EGTA) on phosphatase activity and populations of bacteria and fungi from metal- and chelate-amended soils.

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MATERIALS AND METHODS

Plants were maintained inside a greenhouse with day/night temperatures set at 30°C/25°C and 50% relative humidity. Supplemental light for 12 hrs were provided by high intensity super halide lamps (1000W H.Y. Lites Horizontal System, High Yield, Inc., Camas, WA). The photosynthetically active radiation (PAR; 400-700 nm) measured at the canopy level was no less than 900 μmol photons m^{-2} sec^{-1} as measured with a LI-COR 6200 portable photosynthesis system (LI-COR, Inc., Lincoln, NE). Wheat (*Triticum aestivum* L. cv. TAM-109) seeds were obtained from Arrowhead Mills, Hereford, TX through a local store. Unless otherwise specified, four seeds were sown in each 1.9L (half gallon) plastic pot containing a 2:1 (v/v) mixture of sieved silty clay loam soil (pH 8.2; 1.5% organic matter) and peat. Emerged seedlings were thinned out to 2 plants per pot at 5 d after planting.

Three wks before planting, four concentrations (0, 500, 1000, 2000 mg Pb/kg dry soil) of Pb (supplied as lead nitrate) or Cd (supplied as cadmium nitrate) were thoroughly mixed with the soil using a hand throwel. Also, two concentrations (0, 5 mMol/kg dry soil) of either ethylenediamine tetraacetic acid (EDTA) or ethylenebis (oxyethylenenitrilo) tetraacetic acid (EGTA) were mixed as powder with the metal-spiked soil before planting. Based from the evaporative demand, 100 mL of nutrient solution were periodically added to each pot to ensure that soil moisture content was maintained at field capacity and that no excess soil moisture drained from perforations at the bottom of each pot.

Any symptoms of metal toxicity (e.g., discoloration, pigmentation, yellowing, necrosis, stunting) exhibited by plants were visually noted during the experimental period. All plants were harvested at six wks after planting. During harvest, soil samples devoid of any root fragments were put in polyethylene bags and immediately stored at 4°C until further analyses.

Microbial assessment from a 10-g sieved (< 2mm) soil sample consisted of viable microbial counts (after at least 48 hrs incubation), using spread plates for each treatment. Spread plates were made from tryptic soy agar (TSA) for bacteria and rose bengal Martin's agar for fungi. Soil phosphatase activity of a 1-g sieved (< 2 mm) soil sample from each treatment was assayed using the procedures developed by Tabatabai and Bremner (1969).

In both experiments (Pb/EDTA or Cd/EGTA), each treatment replicate consisted of a pot containing 2 plants. Treatments were arranged in a Completely Randomized Design (CRD) with four replications. Data were analyzed using Statistical Analysis System (SAS, software version 8). Treatment comparisons were done using Fisher's Protected Least Significant Difference (LSD) test. In this

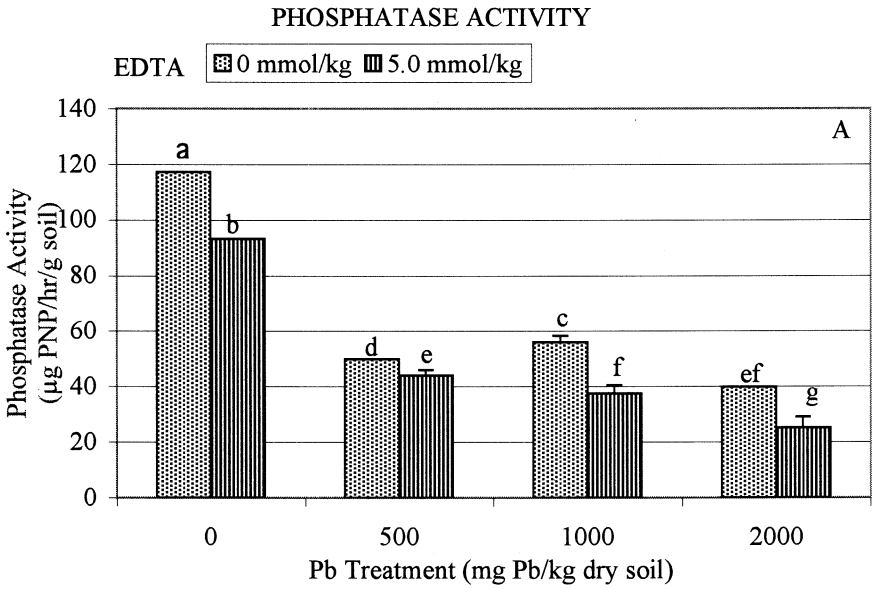
study, a probability $P \leq 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Phosphatase activity exhibited similar patterns of sensitivity to the metals (Pb, Cd) and synthetic chelates (EDTA, EGTA). Generally, phosphatase activity decreased with increasing levels of soil Pb and Cd treatments compared to the control (Figs. 1A and 1B). This reduction in phosphatase activity was more pronounced in the presence of the chelates, EDTA and EGTA. Stuczynski et al. (2003) recently showed that Pb treatment strongly impeded acid phosphatase, but the inhibition was usually lower than 10%. Based from our data, we could not ascertain whether the decrease of phosphatase activity was caused by a heavy metal-induced reduction of enzyme synthesis by soil microorganisms or by a heavy metal-induced inhibition of enzyme activities. This line of research is being pursued in our laboratory because of our cognizance of the relative importance of phosphatase as a suitable indicator for soil pollution by metals such as Pb.

Pb and Cd differentially affected both soil fungal and bacterial populations. Soil fungal population decreased with increasing level of soil-applied Pb (Fig. 2A). It was noted however, that EDTA diminished the toxic effect of Pb especially at the lowest (500 mg Pb/kg) treatment. It is well established that the free, hydrated metal ion is the toxic form of metals present in the environment. For instance, Vassil et al. (1998) demonstrated that a chelated heavy metal such as a Pb-EDTA complex was less toxic to plants than a free protonated Pb. Soil fungi were tolerant to both Cd and EGTA as evidenced by their similar or higher numbers compared to those present in the control treatments (Fig. 2B). Previous studies showed that ethylenediaminedisuccinate (EDDS), a synthetic chelate like EGTA, was less toxic to fungi as determined by phospholipid fatty acid (PLFA) analysis (Grcman et al. 2003) and caused less stress to microorganisms as indicated by the trans-to-cis PLFA ratio (Guckert et al. 1986). Moreover, chelate addition did not prevent the development of arbuscular mycorrhiza on red clover (Grcman et al. 2003).

Generally, soil bacteria were tolerant to all levels of Pb except at 1000 mg Pb/kg treatment, where a significant reduction in population was observed (Fig. 3A). Stimulatory effects of Pb are not necessarily indicative of a lack of negative effects of this metal on microbial processes. The Pb-induced increases in biological activities may be related to their lethal effect on sensitive microbial populations, promoting growth of resistant species, which may feed on cell debris leading to restructuring of soil microbial populations as previously observed by Stuczynski et al. (2003). Also, Doelman and Haanstra (1979) found that in Pb-polluted soils, a higher proportion of tolerant bacteria occurred than in normal unpolluted soils. These previous findings are compatible with our present observations depicting



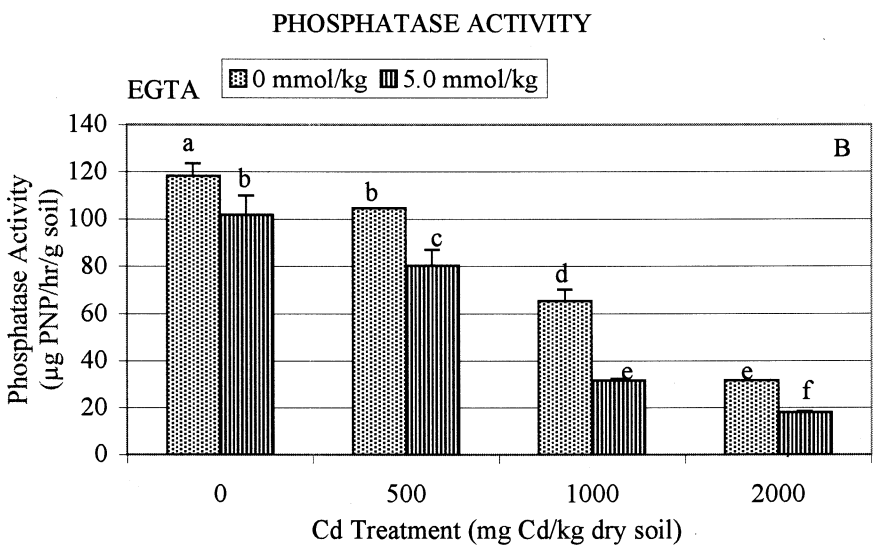


Figure 1. Phosphatase activity of microorganisms from previously cropped soils contaminated with Pb/EDTA (A) or Cd/EGTA (B). A vertical bar in each column represents the standard error of the mean of four replications. Means with a common letter are not significantly different from each other according to Fisher's protected Least Significant Difference Test (P = 0.05).

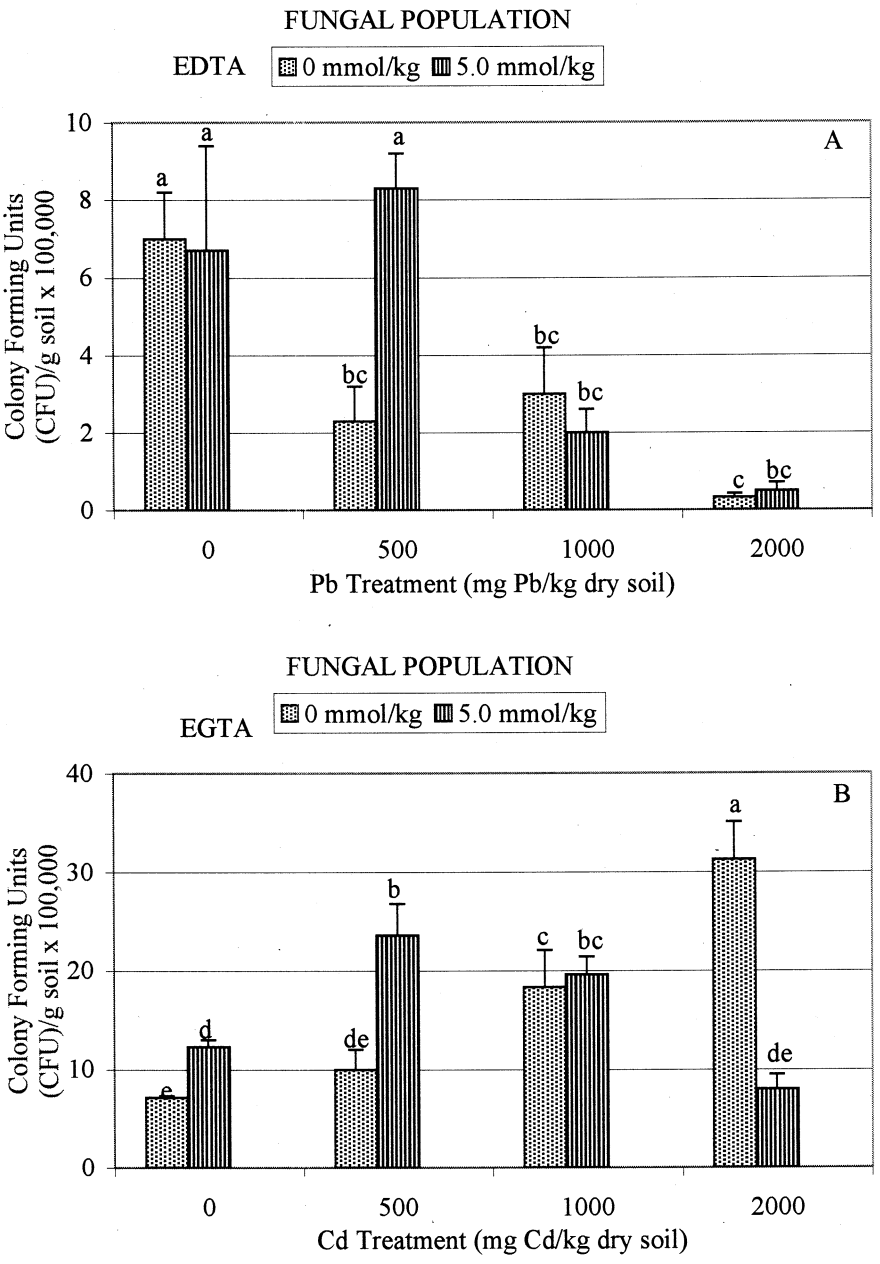
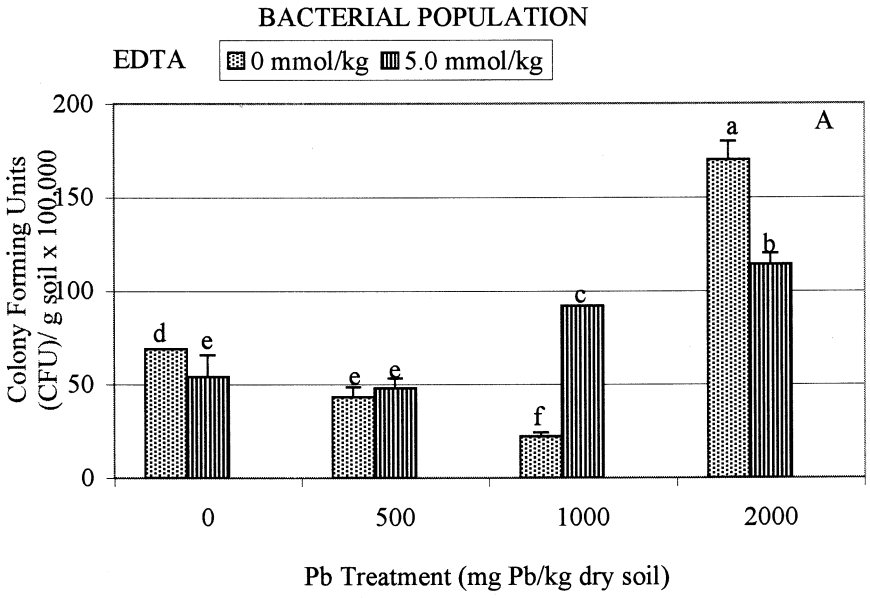


Figure 2. Fungal populations (CFU/g soil) from previously cropped soils contaminated with either Pb/EDTA (A) or Cd/EGTA (B). A vertical bar in each column represents the standard error of the mean of four replications. Means with a common letter are not significantly different from each other according to Fisher's protected Least Significant Difference Test (P= 0.05).



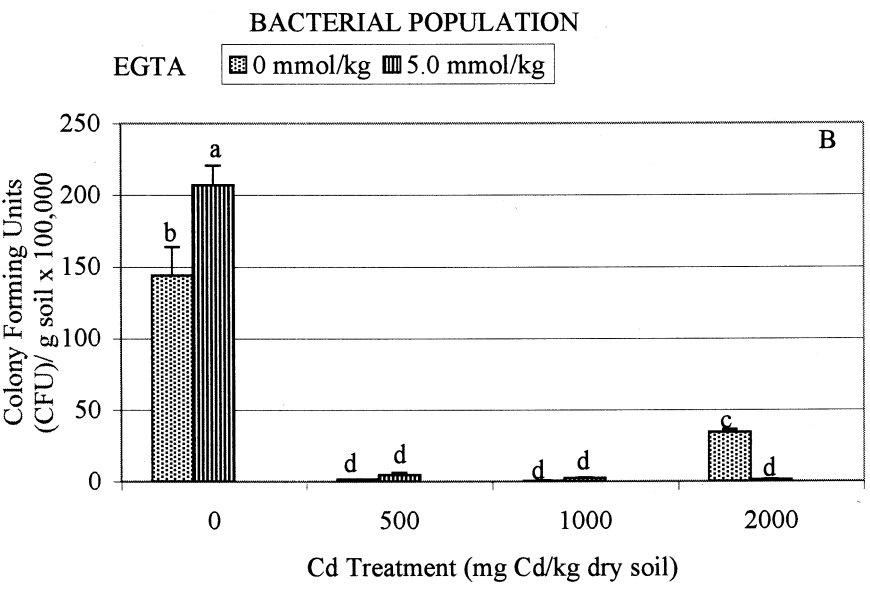


Figure 3. Bacterial populations (CFU/g soil) of previously cropped soils contaminated with either Pb/EDTA (A) or Cd/EGTA (B). A vertical bar in each column represents the standard error of the mean of four replications. Means with a common letter are not significantly different from each other according to Fisher's protected Least Significant Difference Test (P=0.05).

significant increases in bacterial numbers at 2000 mg Pb/kg. EDTA alleviated the toxic effect of Pb especially at 1000 mg Pb/kg. In a related study, Saeki et al. (2002) found that complexation of a metal such as copper and soil organic matter may alleviate the toxicity of copper on the soil bacterial community.

Soil bacterial populations were greatly reduced by Cd, especially at the two highest concentrations in combination with 5 mMol EGTA/kg (Fig.3B). In a study (Khan and Chang-yong 1999) using Cd levels comparable to those used in our study, acetate addition to a red soil spiked with 900 and 2700 μg Cd/g caused two- to six-fold more reductions in biomass C compared to similar Cd levels with no acetate. The increased Cd toxicity was attributed to the adsorption of free acetate in the soil that in turn reduced the Cd adsorption, increasing its bioavailability and hence toxicity to the microbial biomass.

The resistance of the soil fungi and bacteria to soil-applied Pb (Fig. 2B) and Cd (Fig. 3A) indicated that these microorganisms have resistance mechanisms to deal with metal toxicity. These resistance mechanisms are currently being determined in our laboratory using our microbial isolates from this study.

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