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Impact of zinc-copper mixtures on the development of phenanthrene catabolism in soil

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ABSTRACT

Anthropogenic pollutants rarely occur in the environment as single constituents, thus combination effects rather than separate effects of pollutants are expected to occur in the environment. PAH contamination of soil is often associated with the presence of high levels of potentially toxic metals which may impact on PAH biodegradation. In this study, the impact of zinc and copper mixtures (0, 50, 100, 500 and 1000 mg kg^{-1}) on the development of phenanthrene catabolism and impact on bacterial numbers in soil were investigated over 168 day incubation. The presence of low concentrations of Zn and Cu (50 and 100 mg kg^{-1}) had no significant effect ($p > 0.05$) on the development of phenanthrene catabolism, but phenanthrene biodegradation was significantly reduced at higher concentrations of Zn + Cu mixture ($p < 0.05$). Bacterial cell numbers decreased significantly in the higher concentrations of the mixtures relative to the control. Thus, in soils impacted with organic and metallic contaminants, biodegradation may be inhibited.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are chemical compounds consisting of atoms of C and H, arranged in the form of two or more aromatic rings (Thavamani et al., 2011). They are ubiquitous environmental pollutants and the concern about their presence, persistence and loss is increasing since this important class of chemicals have been found to have toxic, mutagenic and carcinogenic properties (Malakul et al., 1998; Thavamani et al., 2011).

Potentially toxic elements (also known as heavy metals) include a range of metals and metalloids which are commonly associated with pollution and toxicity, but also include elements which are essential in the metabolism of living organisms (Zn and Cu), albeit at low concentrations (Wong et al., 2005). Repeated applications of Zn and Cu as fungicides, in sewage sludge and, in animal manure have led to increasing concentrations in many soils (Kim et al., 2008). Though essential metabolic requirements in most organisms, at higher concentrations Zn and Cu can become toxic, impacting on microbial growth, morphology and biochemical activities as a result of adverse interactions with cellular components (Sokhn et al., 2001; Wong et al., 2005; Wang et al., 2007).

Microbial degradation has been proposed as an inexpensive and efficient method to remove PAHs from the environment. Prior exposure of the indigenous soil microflora to PAHs has been shown to be important in the metabolism of PAHs (Lee et al., 2003; Macleod and Semple, 2006). Adaptation of microorganisms to PAHs is thought to occur through (i) the induction of enzymes involved in the biodegradation of the contaminant and/or (ii) an increase in the number of degrading organisms (Macleod and Semple, 2002; Lee et al., 2003).

Soils are complex, multi-component systems with a range of different types of contaminants coexisting in different physical and chemical forms (Ibarrolaza et al., 2009). Anthropogenic pollutants hardly occur in the environment as single constituents thus, combination effects rather than separate effects of pollutants are expected to occur in the environment (Gongolev and Wilke, 1997).

PAH contamination of soil is often associated with the presence of high levels of potentially toxic metals (Malakul et al., 1998; Amezcua-Allieri et al., 2005; Thavamani et al., 2011). For example, 40% of hazardous wastes sites on the U.S. Environmental Protection Agency's National Priority list are co-contaminated with organic and metal pollutants (Sandrin et al., 2000; Sandrin and Maier, 2003; Thavamani et al., 2011). Although PAHs are one of the most studied organic compounds for the past two decades, the impact of toxic metals on their biodegradation has not received much attention so far (Maliszewka-Kordybach and Smreczak, 2003; Thavamani et al., 2011). Maliszewka-Kordybach and Smreczak

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(2003) reported the impact of Zn, Pd and Cd and PAHs compounds (fluorene, anthracene, pyrene and chrysene) on the PAHs dissipation, microbial activity and plant growth in agricultural soil. Thavamani et al. (2011) characterised heavy metals and PAHs contaminated manufacturing gas plant soils with emphasis on heavy metal availability and implications on bioremediation of mixed contaminated soils. Sources of these co-contaminants in soil include metallurgical industries, old gasification and wood preserving plants, as well as from atmospheric deposition, sewage sludge and fungicidal application (Maliszewka-Kordybach and Smreczak, 2003; Thavamani et al., 2011).

Metal-contaminated soils represent one of the most difficult challenges facing bioremediation (Roane and Pepper, 2000). Biodegradation of organic contaminants in metal contaminated soils is complicated because metals are toxic and cannot be degraded by biological processes (Thavamani et al., 2011). These metals often exist as metal mixtures in soil and their elevated concentrations have wide-ranging impacts on animal, plant, and microbial species (Roane et al., 1996). High metal concentrations in soil can inhibit the microbial degradation of organics that are normally easily degraded within soils (Pepper et al., 2002). Due to their toxic nature, the presence of metals in organic-contaminated sites often complicates and limits bioremediation process. These effects include extended acclimation periods, reduced biodegradation rates, and failure of target compound biodegradation (Malakul et al., 1998).

The aims of this study were to assess effects of mixtures of Zn and Cu at increasing concentrations on the development of phenanthrene catabolism in a pristine soil by considering the effects of soil contact time in the presence of Zn and Cu, either freshly added or aged over time.

2. Materials and methods

2.1. Chemicals

Phenanthrene and [9-^{14}C] phenanthrene (55 mCi mmol^{-1}) were obtained from Sigma Aldrich Co., Ltd, UK. Goldstar multipurpose liquid scintillation fluid was obtained from Meridian, UK. Plate count agar (Tryptone, dextrose Yeast agar) was obtained from Sigma Aldrich Co., Ltd, UK. Agar–agar, zinc chloride ($ZnCl_2$) and copper (II) chloride dihydrate ($CuCl_2.2H_2O$) were obtained from Fisher Scientific, UK.

2.2. Soil

A dystric cambisol soil collected from Myerscough Agricultural College (Lancashire, UK) from the upper 10 cm layer, free of any pesticides, nitrogen or phosphate treatment for more than 20 years was used in this study. It had a sandy loam texture (pH 6.5; organic matter (OM) content 2.7%; clay content 19.5%; total sand 60.4%; silt 20.0%). The soil was air dried, sieved with 2 mm sieve to remove stones and was kept in the cold room at 4 ± 1 °C until use.

2.3. Amendment of soil with ^{12}C-phenanthrene, zinc and copper

The soil was amended with 50 mg kg^{-1} of phenanthrene using the spiking procedure described by Doick et al. (2003). Soluble metal salts $CuCl_22H_2O$ and $ZnCl_2$ were used to introduce Cu and Zn at equal amounts to give 50, 100, 500 or 1000 mg kg^{-1} weight of soil, respectively. Soils were each weighed into amber jars and incubated in the dark for 168 d at 20 ± 1 °C (termed metal amendment).

In a second incubation, soil was spiked with ^{12}C-phenanthrene only and incubated in amber jars and stored in the dark for 168 d at 20 ± 1 °C. At each time point (1, 21, 42, 105 and 168 d), 10 ± 0.2 g soils in their various respirometers were amended with 50, 100, 500 or 1000 mg kg^{-1} Zn and Cu (termed fresh metal amendment). These soils were sampled at 1, 21, 42, 105 and 168 d to measure the mineralisation of ^{14}C-phenanthrene and bacterial cell numbers (heterotrophs and phenanthrene degraders) were enumerated. The control had 50 mg kg^{-1} ^{12}C-phenanthrene (without soluble metal amendment) and analytical blanks were prepared to which no amendment was added to give background values, as well as a no phenanthrene control. Zn and Cu were also analysed individually as above (Obuekwe and Semple, 2013).

2.4. Respirometry

At each time point, soil samples were assessed for indigenous microbial phenanthrene catabolism using ^{14}C-phenanthrene. Sterile minimal basal salts containing (0.3 g $NaCl_2$, 0.6 g $(NH_4)_2SO_4$, 0.6 g KNO_3, 0.25 g KH_2PO_4, 0.75 g K_2HPO_4, 0.15 g $MgSO_4.7H_2O$ and 1 ml of trace element solution in 1 l DI H_2O) (30 ml) was added to 10 ± 0.2 g of the soil in respirometers ($n = 3$ for each condition). Within each respirometer, a mixture of ^{12}C- and ^{14}C-phenanthrene (50 mg kg^{-1} and 833 Bq) was added to the soil. Production of $^{14}CO_2$ was assessed in modified Erlenmeyer flasks (250 ml) (Reid et al., 2001). These respirometers incorporated aTeflon lined screw-caped CO_2 trap containing 1 M NaOH (1 ml) within a suspended glass scintillation vial (7 ml). The soils were incubated on a rotary shaker at 100 rpm for 14 d at a constant temperature of 21 °C and 45% relative humidity. The production of $^{14}CO_2$ was assessed daily by the addition of Ultima Gold liquid scintillation fluid to the CO_2 traps and subsequent liquid scintillation counting (LSC, Canberra Packard Tri Carb 2300 TR, UK).

2.5. Bacterial enumeration

Enumeration of viable bacteria (heterotrophs and phenanthrene degraders) was achieved by counting the colony forming unit counts g^{-1} of soil (CFU g^{-1} soil) at each time point before the start of each assay. Bacterial enumeration was carried out using 1 g of soil from the different treatments. Soils were mixed with 9 ml of Ringer's solution by whirl-mixing for 30 s, sonicated for 1 min and allowed to stand for 2 min. Soil solution (0.1 ml) was serially diluted in 0.9 ml Ringer's solution: aliquots (0.01 ml) of these dilutions were spread on both plate count agar plates and agar–agar plates with phenanthrene for isolation of heterotrophs and phenanthrene degraders. CFUs were counted after 24 h, 48 h and 7 d.

2.6. Statistical analysis

Following blank-correction, Sigma Stat for Windows (version 3.5) was used to statistically analyse the data. Significant effects of Zn and Cu mixture concentrations on lag phase (prior to > 5% mineralisation), ^{14}C-Phenanthrene mineralisation and bacterial enumeration (heterotrophs and phenanthrene degraders) were compared statistically with the control. Impact of ageing on lag phase, maximum rates (fastest rate of $^{14}CO_2$ evolved per day during microbial growth) and extents (cumulative $^{14}CO_2$ evolved from microorganisms for a defined period of mineralisation i.e 14 d) of phenanthrene mineralisation in the different treatments were also compared statistically with the control using one way analysis of variance and student t-test, at $P < 0.05$ significant level, using three replicates ($n = 3$).