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The stability of textile azo dyes in soil and their impact on microbial phospholipid fatty acid profiles

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ABSTRACT

The aim of this study was to examine the stability of structurally different azo dyes in soil and their impact on the microbial community composition by analyzing phospholipid fatty acid (PLFA) profiles. Sterile and non-sterile soils were amended with three azo dyes, including: Direct Red 81, Reactive Black 5 and Acid Yellow 19 at 160 mg kg^{-1} soil. The results showed that the azo dyes were quite stable and that large amounts of these dyes ranging from 17.3% to 87.5% were recoverable from the sterile and non-sterile soils after 14 days. The maximum amount of dye was recovered in the case of Direct Red 81. PLFA analysis showed that the azo dyes had a significant effect on microbial community structure. PLFA concentrations representing Gram-negative bacteria in dye-amended soil were substantially less as compared to the PLFA concentration of Gram-positive bacteria. Acid Yellow 19 dye had almost similar effects on the PLFA concentrations representing bacteria and fungi. In contrast, Reactive Black 5 had a greater negative effect on fungal PLFA than that on bacterial PLFA, while the opposite was observed in the case of Direct Red 81. To our knowledge, this is the first study reporting the stability of textile azo dyes in soil and their effects on soil microbial community composition.

1. Introduction

The textile industry uses several wet processes that produce large amounts of highly colored wastewater containing azo dyes, which are the largest and the most common class of synthetic dyes used by the industry (Enayatzamir et al., 2010; Singh, 2015). During the dyeing process, approximately 15–50% of the original concentration of the azo dyes that does not bind to the fabric is released into wastewater (McMullan et al., 2001; Pratum et al., 2011). O'Neill et al. (1999) reported that the concentration of the dyes in textile wastewater may vary from 10 to 250 mg L^{-1}, while others have reported concentrations as high as 1500 mg L^{-1} (Pierce, 1994). Besides azo dyes, textile wastewater also presents a heavy pollution load in terms of biological oxygen demand (BOD), chemical oxygen demand (COD) and total dissolved solids.

In developing countries, the use of textile dye-containing wastewater for irrigation is a common practice. A substantial amount of these dyes can thus accumulate in the soil, particularly near the textile processing industries. For example, the average concentration of azo dyes reported in China for surface soil near dyeing and printing units is 456 mg kg^{-1} (Zhou, 2001). This is of concern as azo dyes are generally resistant to aerobic degradation and are commonly reduced to potentially carcinogenic aromatic amines under anaerobic conditions (Franciscon et al., 2009). A number of reports have also revealed phytotoxic effects of azo dyes on plant growth (Dawkar et al., 2008, 2010; Khadhraoui et al., 2009; Saratale et al., 2009; Ayed et al., 2011; Phugare et al., 2011), although the mechanisms by which these dyes affect plants and soil microorganisms are not yet understood.

Soil is a biologically balanced system and any drastic change in the soil can also affect microbial community structure in addition to the biochemical processes that are carried out. Changes in soil biology after addition of a contaminant can be estimated by analyzing phospholipid fatty acids (PLFA), which is a powerful tool for assessing changes in soil microbial community composition (Frostegard et al., 2011; Pratt et al., 2012). To our knowledge, no studies have been conducted to examine the effect of azo dyes on

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soil microbial communities. Similarly, the stability and persistence of the azo dyes in soil have not been explored.

The present study was conducted to establish a method for extracting azo dyes from contaminated soil to estimate their residual concentration and intrinsic degradation in soil over time. The effects of structurally different azo dyes on the soil microbial communities as revealed by PLFA profiles were also examined.

2. Material and methods

2.1. Materials and chemicals

Azo dyes including Reactive Black 5 (dye content 55%), Direct Red 81 (dye content 50%), Acid Red 88 (dye content 75%) and Acid Yellow 19 (dye content 60%), purchased from Sigma-Aldrich (USA), were used. All other chemicals were of analytical grade. The soil used for this research was an Arlington fine sandy loam taken from a vegetable garden in the urban residential area of Riverside, California, USA. General properties of this soil were: pH 7.8, soil organic matter 0.75%, 71% sand, 17% silt, and 12% clay. Homogenizer (FS110H, Fisher Scientific) was used to disperse the soil particles prior to the extraction of azo dyes. A rotary evaporator was used to reduce the volume of the soil extract. PLFA soil analysis was performed using gas chromatograph (Hewlett Packed HP 6890 Series). UV–visible 87 spectrophotometer and NH2-SPE columns were also used in this study.

2.2. Recovery of azo dyes from soil

Direct Red 81 dye was used to establish the method for maximum recovery of the dye from the soil. In this experiment, sterile garden soil was used to measure the efficiency of different extracting agents for removing azo dyes from the soil. For sterilization, 10 g soil was placed into 50 mL Erlenmeyer flasks, covered with a cotton plug and aluminum foil, and autoclaved at 121 °C for 15 min. After cooling, the sterilized soils were amended with 4 mL dye solution at a final dye concentration of 160 mg kg^{-1}. The flasks were incubated under sterile conditions at 28 °C for one week. A control treatment used 4 mL of sterile distilled water, which was added to the soil instead of the azo dye solution.

To evaluate the different extractants, individual flasks of soil containing the dye were extracted with 16 mL of solvent (water, acetone, methanol, chloroform and selected combinations). The flasks were incubated at 28 °C with shaking at 120 rpm for 60 min. Soil particles were dispersed using a Homogenizer for 15 min. Subsequently, the soil suspension was centrifuged at 10,000*g* for 10 min at 4 °C and the supernatant was collected in plastic bottles. The soil was returned back to the respective flasks and the extraction process was repeated three times to recover the maximum amount of the dye. The extracts were then pooled and concentrated using a rotary evaporator. Deionized water was used to bring the final volume to 25 mL. Absorbance was measured at 505 nm (λ_{max} for DR 81) with a spectrophotometer. A calibration curve was constructed using dye standards to determine the dye concentrations in the extracts.

Based on the high recovery efficiency, a mixture of water, methanol, acetone and chloroform (1:1:1:1, v/v) was selected for subsequent experiments to extract Reactive Black 5, Acid Yellow 19 and Acid Red 88 from the soil and the dye concentrations were measured at their respective λ_{max} using the procedures described above.

2.3. Stability and degradation of azo dyes in sterile and non-sterile soil

After optimizing the recovery method, stability of the dyes in the soil was determined by adding 4 mL (400 mg L^{-1} dye concentration) of each dye to 10 g sterile and non-sterile soils. Each treatment was repeated four times. The dyes were extracted from both sterile and non-sterile soils after 7, 14 and 28 days of incubation at 28 °C. It was assumed that the difference in the amount of dye recovered from sterile and non-sterile soil was equivalent to biodegradation.

2.4. Effect of azo dyes on bacterial growth in liquid medium

To assess the toxicity of the selected azo dyes on bacterial growth, 100 mL tryptic soy broth medium (Atlas, 2004) containing 400 mg L^{-1} of the respective dyes was inoculated with the cultures of previously isolated plant growth promoting rhizobacteria (PGPR), namely *Pseudomonas fluorescens* (Gram-negative) and *Bacillus megaterium* (Gram-positive) (Nadeem et al., 2012). For inoculum preparation, the pure cultures of both bacteria were grown in 250 mL Erlenmeyer flasks containing tryptic soy broth medium. These flasks were incubated at 28 °C under shaking conditions for 24 h. Optical densities of the cultures were adjusted to 0.6 at 600 NM for a uniform population of both bacteria. After inoculation, the flasks were incubated under shaking (150 rpm) at 28 °C for 48 h. The control was comprised of broth medium without dyes. After 48 h, bacterial cells were harvested by centrifugation (4000*g* for 5 min) and the cell pellets were dried at 105 °C in an oven to obtain their dry weights (Kim et al., 2012).

2.5. Effect of azo dyes on soil microbial PLFA composition

About 250 g moist garden soil was placed in plastic beakers and 50 mL of dye solution (400 mg L^{-1}) was applied. The soil was kept moist by reapplying the dye solution every 7 days. Soil samples were collected after 28 days and subjected to lyophilization before PLFA analysis.

Phospholipid fatty acids were extracted using the protocol described by Buyer et al. (2010) except that the external standard used in the present study was methyl nonadecanoate fatty acid (19:0). In brief, 4 mL of 50 mM phosphate buffer solution (pH 7.4), 10 mL of methanol and 5 mL of chloroform were added into 5 g of dry soil. Forty microliters of 0.5 M internal standard (19:0) were added to each soil sample after sonication. Thereafter, the samples were suspended using an end-over-end mixer for 2 h. The samples were then centrifuged and the supernatants were transferred to 30 mL test tubes and covered with Teflon-lined screw caps. Thereafter, 5 mL each chloroform and deionized water were added

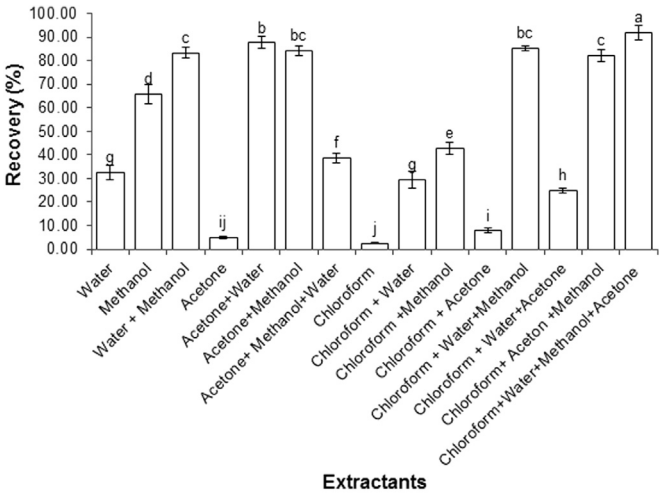

Fig. 1. Recovery (%) of DR 81 azo dye from soil by various extractants after 1 week. Data are shown as mean ± SD ($n=3$). Bar showing different alphabets are statistically ($p \leq 0.05$) different from others.
LSD = 3.99.