Bioavailability of (Geno)toxic Contaminants in Polycyclic Aromatic Hydrocarbon–Contaminated Soil Before and After Biological Treatment

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Abstract

Contaminated soil from a former manufactured-gas plant site was treated in a laboratory-scale bioreactor. Desorbability and biodegradability of 14 polycyclic aromatic hydrocarbons (PAHs) and 4 oxygenated PAHs (oxy-PAHs) were investigated throughout a treatment cycle. Desorbability was determined using a mixed-function sorbent (Oasis$^{®}$ HLB) or a hydrophobic sorbent (Tenax$^{®}$) in dialysis tubing suspended in the soil slurry. Toxicity and genotoxicity of the whole soil and the desorbable fractions were determined by DNA damage response analysis with the chicken DT40 B-lymphocyte isogenic cell line and its DNA repair-deficient mutant $Rad54^{-/-}$. Biological treatment significantly removed both PAHs and oxy-PAHs, and their desorbability decreased throughout the bioreactor treatment cycle. Collectively, oxy-PAHs were more desorbable and biodegradable than the corresponding PAHs; for example, the oxy-PAH present at the highest concentration, 9,10-anthraquinone, was more desorbable and biodegradable than anthracene. For both PAHs and oxy-PAHs, the percentage removed in the bioreactor significantly exceeded the percentage desorbed from untreated soil, indicating that desorption did not control the extent of biodegradation. Consistent with previous results on the same soil, genotoxicity of the whole soil slightly increased after biological treatment. However, both toxicity and genotoxicity of the desorbable constituents in the soil decreased after treatment, suggesting that any genotoxic constituents that may have formed during treatment were primarily associated with less accessible domains in the soil.

Key words: bioavailability; bioremediation; genotoxicity; MGP; quinones; soil

Introduction

HISTORICALLY, THE POTENTIAL effects of contaminated sites on human or ecological health have been assessed based on the total contaminant concentrations as determined by vigorous chemical extraction techniques (Alexander, 2000). However, because of their association with different soil components, hydrophobic organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) are only partly available for biodegradation by microorganisms or for exerting toxic effects (Alexander, 2000; Reid *et al.*, 2000; Lei *et al.*, 2004; Kreitinger *et al.*, 2007; Vasseur *et al.*, 2008). Poor correlation has been observed between vigorous solvent extraction of a given compound and its bioavailability to

bacteria and earthworms in soil (Kelsey and Alexander, 1997; Kelsey *et al.*, 1997). Recently, the U.S. Interstate Technology and Regulatory Council has advised incorporating bioavailability considerations into the evaluation of contaminated sediment sites to reduce the extent of cleanup required to be protective of the environment (Interstate Technology & Regulatory Council Contaminated Sediments Team, 2011).

A variety of biological and chemical techniques have been developed to estimate and predict bioavailability. Biological techniques involve measuring substrate uptake, mineralization, or toxicity (Kelsey *et al.*, 1997; Stroo *et al.*, 2000; Braida *et al.*, 2004). Chemical techniques involve nonexhaustive extraction with ''mild'' solvents (Hatzinger and Alexander, 1995; Kelsey *et al.*, 1997; Breedveld and Karlsen, 2000; Reid *et al.*, 2000; Liste and Alexander, 2002) and solid-phase extraction (ten Hulscher *et al.*, 2003; Lei *et al.*, 2004; Gomez-Eyles *et al.*, 2011). Solid-phase extraction is common for estimating bioavailability in contaminated soils and sediments, with polymeric adsorbent resins such as Tenax$^{®}$ serving as an infinite sink (Loehr *et al.*, 2003). These sorbents work well with nonpolar compounds such as PAHs.

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In a complex system such as soil, it is likely that some transformations of organic pollutants by microorganisms do not lead to complete metabolism of the parent compounds. In PAH-contaminated soil undergoing bioremediation, hundreds of hazardous compounds have been identified, covering a wide range of physicochemical properties. For example, log K_{ow} values can range from 2 to 8 (Lundstedt *et al.*, 2003), including polar and semi-polar metabolites of PAHs. Compounds other than parent PAHs are responsible at least in part for the toxicity and genotoxicity observed in studies on bioremediation of field-contaminated soil (Hu *et al.*, 2012).

The objective of this study was to investigate the potential bioavailability of PAHs, selected oxygenated PAH (oxy-PAHs) metabolites, and other constituents in contaminated soil that collectively lead to toxicity or genotoxicity during biological treatment in a lab-scale, slurry-phase, sequencing batch bioreactor. Potential bioavailability was evaluated by desorption to both hydrophobic (Tenax) and universal (Oasis® HLB) sorptive resins. The DNA damage response assay using the chicken DT40 B-lymphocyte isogenic cell line and its DNA repair-deficient mutant $Rad54^{-/-}$ was applied to evaluate the toxicity and genotoxicity of whole soil and desorbed constituents throughout a treatment cycle in the bioreactor.

Materials and Methods

Materials

PAH standards (EPA 610 PAH Mixture), 9,10-phenanthrenequinone (PQ), and 9,10-anthraquinone (AQ) were purchased from Sigma-Aldrich (St. Louis, MO). 9-Fluorenone (FLO) and benz[*a*]anthracene-7,12-quinone (BAQ) were purchased from Acros Organics (Morris Plains, NJ). All solvents were high-pressure liquid chromatography (HPLC) grade and were purchased from Fisher Scientific (Pittsburgh, PA).

Tenax TA beads (60/80 mesh) and the universal (mixed-function) Oasis HLB resin (particle size 60 μm) were obtained from Alltech (Deerfield, IL) and Waters (Milford, MA), respectively. They were cleaned by Soxhlet extraction in acetone:hexane (50:50, v/v) and methanol:dichloromethane (50:50, v/v), respectively, overnight and air-dried before use. Snake-Skin® dialysis tubing (10,000 MWCO, 22 mm diameter, 10.5 m) was obtained from Thermo Scientific (Rockford, IL).

Contaminated soil (feed soil) used in this study was collected from a former manufactured-gas plant (MGP) site in Salisbury, North Carolina, processed, and characterized as described elsewhere (Richardson and Aitken, 2011; Hu *et al.*, 2012). The total concentration of target PAHs (14 of the 16 USEPA priority PAHs, excluding acenaphthylene and indeno[1,2,3-*cd*]pyrene) was 362 ± 23 μg/g and the total concentration of target oxy-PAHs (FLO, PQ, AQ, and BAQ) was 22.3 ± 0.5 μg/g (dry mass basis, w/w). Among the oxy-PAHs, AQ was at the highest concentration (18.3 μg/g). Concentrations of individual PAHs and the other oxy-PAHs in the feed soil are shown in Supplementary Table S1. The feed soil was treated in a continuously stirred, semi-continuous (draw-and-fill or sequencing batch), laboratory-scale aerobic bioreactor with a working volume of ∼2 L, a solids concentration of 14% (w/w), and solids retention time of 35 days (Hu *et al.*, 2012); treatment in the reactor relied on indigenous microorganisms present in the contaminated soil (i.e., no

exogenous inoculum was necessary). Every week, 20% of the treated slurry was removed from the bioreactor and replaced with feed soil in a pH 7.5 buffer containing 5 mM phosphate and 5 mM ammonium nitrate.

Treated slurry was sampled at three time intervals during a given 7-days feeding cycle: immediately after feeding (d0), 1 day (d1) and 7 days (d7) after feeding. Concentrations of constituents in the d0 sample represent a weighted average of the concentration remaining at the end of the previous 7-day treatment cycle (80% of the reactor contents immediately after feeding) and the concentration in the added feed soil (20% of the reactor contents immediately after feeding); accordingly, concentrations in the d0 sample are substantially lower than in the feed soil. Samples of the slurry were centrifuged for 20 min to remove excess water prior to use for desorption experiments and toxicity assays. PAH and oxy-PAH concentrations of treated soil samples are shown in Supplementary Table S1.

Desorption experiments

The infinite-sink desorption method (Loehr *et al.*, 2003) was employed to investigate desorption of PAHs and oxy-PAHs from feed soil or treated soil to the sorbents (Tenax beads or HLB resin). Because of the small particle size of HLB resin and difficulty separating it from the soil slurry, the conventional method (adding the sorbent directly to soil slurry) was adapted by placing the sorbent into dialysis tubing suspended in the soil slurry. Briefly, an aliquot of 0.1 g sorbent was weighed and transferred into 5 cm of dialysis tubing closed with knots on both ends. Approximately 4 g (wet wt.) of centrifuged soil sampled from the bioreactor and one dialysis tubing containing the sorbent were suspended in 50 mL phosphate buffer (pH 7.5) amended with 4.15 g/L NaN$_3$ in a 60-mL centrifuge tube with a PTFE-lined septum and screw cap.

To evaluate the effects of incubation time on the extent of desorption, six centrifuge tubes were prepared for each soil sample for each desired time interval (desorption for 1, 2, 3, 7, 10, or 15 days): three tubes with dialysis tubing containing Tenax beads and three tubes with dialysis tubing containing HLB resin. The tubes were placed on a wrist-action shaker in the dark. After each desired time interval, the six centrifuge tubes for each soil sample were sacrificed. The dialysis tubing was removed from the centrifuge tube and rinsed with deionized water to detach soil particles from the exterior of the tubing. The dialysis tubing was then unknotted and the sorbents were rinsed with deionized water into a vacuum filtration apparatus to separate water from the resin. The soil slurry left in the centrifuge tube was centrifuged for 20 min and the supernatant was discarded. The sorbents and the soil after desorption were then extracted. These time-course measurements demonstrated that desorption of total PAHs (sum of 14 compounds) and total oxy-PAHs (sum of 4 compounds) from both feed soil and treated soil increased with time and approached apparent equilibrium in 7 days, as shown for the HLB resin in Supplementary Fig. S1. Therefore, all subsequent desorption experiments were conducted for 7 days. Recoveries of PAHs and oxy-PAHs were calculated by comparing the initial mass in the soil with the mass desorbed to Tenax beads or HLB resin plus the mass remaining in the soil after 7 days of desorption. Individual PAH

and oxy-PAH recoveries are shown in Supplementary Table S2. The dialysis tubing did not sorb PAHs or oxy-PAHs (data not shown).

The conventional Tenax-bead desorption method was carried out as described elsewhere (Hu and Aitken, 2012). Briefly, ~4 g of wet feed soil and 0.1 g Tenax beads were suspended in 50 mL phosphate buffer (pH 7.5) amended with 4.15 g/L NaN$_3$ in each of triplicate 60-mL centrifuge tubes with a PTFE-lined septum and screw cap. The tubes were placed on a wrist-action shaker in the dark. After 7 days, the tubes were centrifuged for 20 min, and Tenax beads were removed from the tubes for subsequent extraction.

Sample extraction, chemical analysis, and DT40 bioassay

Tenax beads and HLB resin were extracted overnight with 10 mL methanol or 10 mL methanol:dichloromethane mixture (50:50, v/v), respectively, in a 20-mL glass vial with a PTFE-lined septum and screw cap. The extracts were filtered through a 0.2-μm pore-size nylon filter and brought to 25 mL with acetone. The soil samples were extracted overnight twice each with a mixture of 10 mL acetone and 10 mL dichloromethane as described elsewhere (Richardson *et al.*, 2011). The extracts were filtered through a 0.2-μm pore-size nylon filter and brought to 50 mL with acetonitrile.

The filtered extracts were analyzed by HPLC for PAHs as described elsewhere (Richardson *et al.*, 2011) and by liquid chromatography-tandem mass spectrometry (LC-MS/MS) for oxy-PAHs as described in Supplementary Data. Toxicity and genotoxicity of the filtered extracts were determined by DNA damage response analysis using a DT40 isogenic cell line and its mutant $Rad54^{-/-}$ knocked out in homologous recombination DNA repair pathway as described elsewhere (Ridpath *et al.*, 2011; Hu *et al.*, 2012). For the bioassays, extracts were first dried and the residue reconstituted in dimethyl sulfoxide as the delivery solvent (Hu *et al.*, 2012).

Data analysis

SPSS® (v16.0; SPSS, Inc., Chicago, IL) was applied for statistical analysis. Student's *t*-test and one-way analysis of variance (ANOVA) followed by Tukey's test were employed to test for differences between two groups and among multiple groups, respectively. The LD$_{50}$ of a sample for the parental DT40 cells and for the $Rad54^{-/-}$ mutant was calculated based on the dose–response relation and converted to an equivalent mass of soil as described elsewhere (Hu *et al.*, 2012).

Results

### Desorption from feed soil

Dialysis tubing was used to contain Tenax beads or HLB resin to investigate desorption of PAHs and oxy-PAHs from soil. Total PAH recovery in the Tenax-bead system was $95.7\% \pm 0.7\%$ and in the HLB-resin system was $93.6\% \pm 1.1\%$; total oxy-PAH recovery in the Tenax-bead system was $101\% \pm 8\%$ and in the HLB-resin system was $110\% \pm 5\%$ (Supplementary Table S2). There was no significant difference in desorption from feed soil to Tenax beads versus HLB resin for any analyte (Fig. 1). To validate the dialysis tubing desorption approach, we compared it to the conventional method of suspending Tenax beads in the soil slurry. There was no difference between desorption to Tenax using the conventional approach versus the dialysis tubing method (Fig. 1).

Biodegradability and desorbability of PAHs and oxy-PAHs

Treatment in the bioreactor significantly removed PAHs and oxy-PAHs from the contaminated soil, and the removal increased over the duration of the 7-day treatment cycle (Fig. 2). Note that removal is reported relative to concentrations in the feed soil; because the d0 sample represents dilution of the feed soil with slurry remaining in the reactor from the
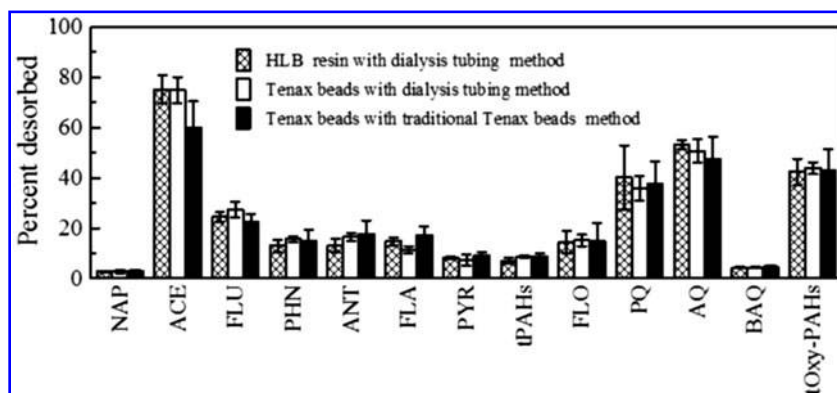
FIG. 1. Comparison of desorption of polycyclic aromatic hydrocarbons (PAHs) and oxygenated PAHs (oxy-PAHs) from feed soil (FS) to HLB resin and Tenax beads in the dialysis-tubing desorption method and in the conventional Tenax-bead desorption method. Desorption was less than 2% for benz[*a*]anthracene (BaA), chrysene (CHR), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), dibenz[*a,h*]anthracene (DBA), and benzo[*g,h,i*]perylene (BgP) under all methods. Desorption time was 7 days. Values represent mean and standard deviation of triplicates. There was no significant difference ($p > 0.05$) between methods for any of the analytes. NAP, naphthalene; ACE, acenaphthene; FLU, fluorene; PHN, phenanthrene; ANT, anthracene; FLA, fluoranthene; PYR, pyrene; tPAHs, total PAHs; FLO, 9-fluorene; PQ, 9,10-phenanthrene quinone; AQ, 9,10-anthraquinone; BAQ, BaA-7,12-quinone; tOxy-PAHs, total oxy-PAHs.
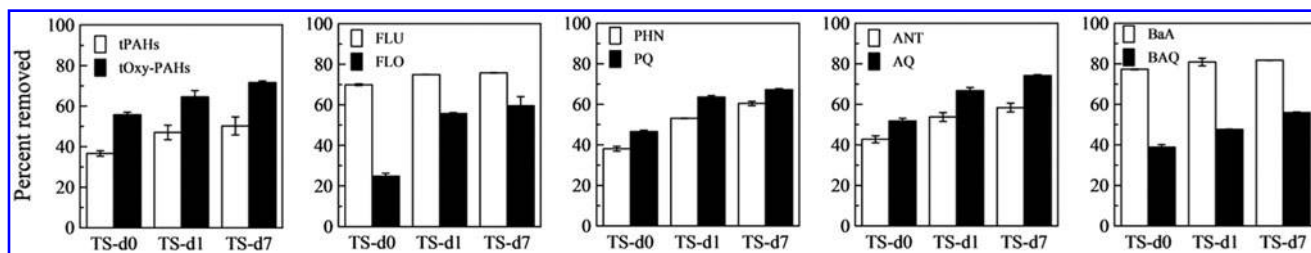
FIG. 2. Removal of PAHs and oxy-PAHs in treated soils (TS) sampled at three time intervals during the 7-day treatment cycle: immediately after feeding (TS-d0), 1 day (TS-d1), and 7 days (TS-d7) after feeding. In all cases, there was a significant difference ($p < 0.05$) between removal of an oxy-PAH and the corresponding parent PAH for a given sample. Removal is relative to the initial concentration of the respective analyte in the FS. Values represent mean and standard deviation of triplicates.

previous 7-day cycle (see **Materials and Methods**), the indicated removal is an apparent removal. The overall removal in the bioreactor is represented by the d7 sample, which is the effluent from the bioreactor at the end of a cycle. After 7 days, removal of total PAHs and total oxy-PAHs was 50% and 72%, respectively. Removal of each individual oxy-PAH and the corresponding parent PAH in the contaminated soil also increased with time. Compared to the respective parent PAH, there was greater removal of AQ and PQ but less removal of BAQ and FLO throughout the 7-day treatment cycle.

Desorption of PAHs and oxy-PAHs to HLB resin and Tenax beads from soils decreased with longer treatment time, except for benz[*a*]anthracene (BaA; Fig. 3); desorption of BaA in feed soil was slightly less than that in treated soil

sampled at the end of the 7-day treatment cycle, although the desorbable fraction of BaA was negligible (2% or less in all samples). In both feed soil and treated soils, the percent desorption of total oxy-PAHs was significantly greater than that of total PAHs. At the end of the 7-day treatment cycle, $7.6\% \pm 1.1\%$ and $6.0\% \pm 0.9\%$ of total oxy-PAHs were still desorbable to HLB resin and Tenax beads, respectively, while only $0.5\% \pm 0.1\%$ and $0.6\% \pm 0.1\%$ of total PAHs were desorbable to HLB resin and Tenax beads, respectively. However, when comparing each individual oxy-PAH with its corresponding parent compound, desorption of only PQ and AQ were significantly greater than that of phenanthrene (PHN) and anthracene (ANT) throughout the bioreactor treatment cycle. Desorption of BAQ was significantly greater
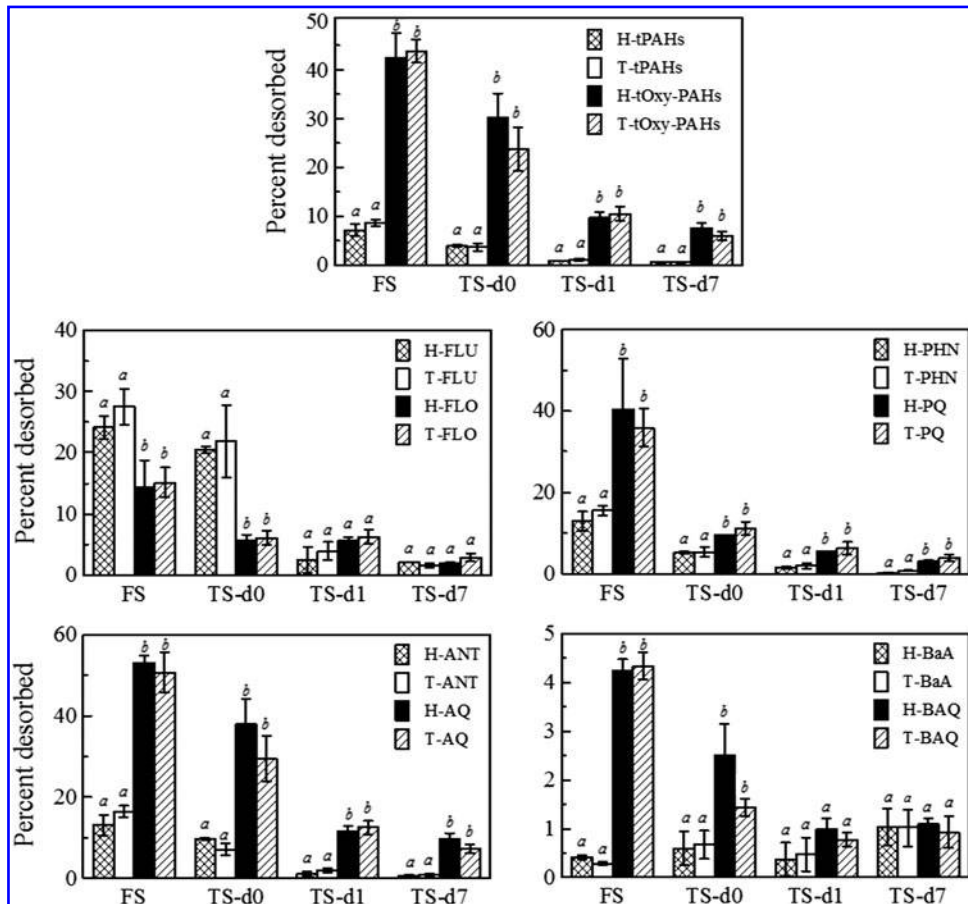
FIG. 3. Desorption of PAHs and oxy-PAHs to HLB resin (H) and Tenax beads (T) from FS and TS sampled at three time intervals during the 7-day treatment cycle (abbreviations as in Fig. 2). [a,b]Different letters are assigned to conditions for which there was a significant difference ($p < 0.05$) among analytes in a given sample. Values represent mean and standard deviation of triplicates.
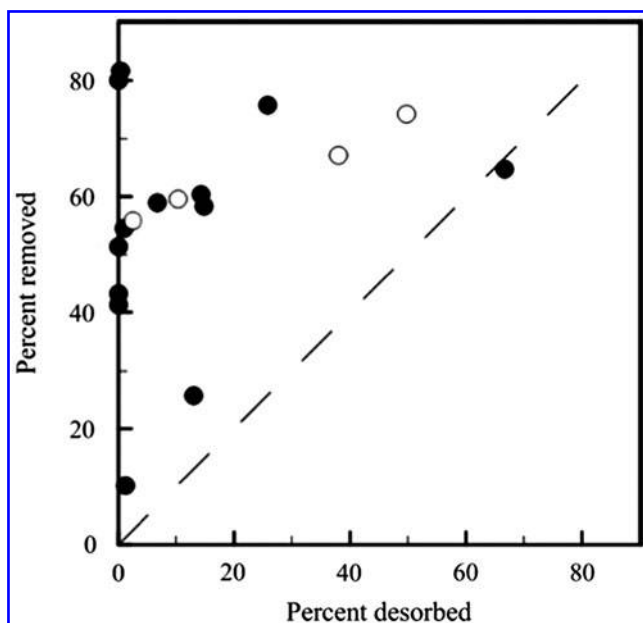
FIG. 4. Percentage of PAH and oxy-PAH removed at the end of the 7-day treatment cycle versus percentage of PAH and oxy-PAH desorbed from the FS. Each point represents the mean value for an individual PAH or oxy-PAH. The dashed line represents a 1:1 correlation. Closed and open circles represent PAHs and oxy-PAHs, respectively. NAP is not included because no removal was observed.

than that of BaA only in feed soil and in treated soil sampled immediately after feeding, while desorption of FLO was significantly less than that of FLU in those two samples. No differences between desorption of FLO or BAQ and that of their corresponding parent compounds were observed in treated soils sampled 1 and 7 days after feeding.

The percentage of PAH and oxy-PAH desorbed from the feed soil was compared to the percentage of PAH and oxy-PAH removed in the bioreactor at the end of the bioreactor treatment cycle (Fig. 4). For most compounds, the removal in the bioreactor was greater than its desorption.

Toxicity and genotoxicity

Toxicity and genotoxicity of whole-soil solvent extracts and of the desorbable constituents of soil samples were examined using the DT40 bioassay. For both the DT40 parental cell line and its $Rad54^{-/-}$ mutant, the LD_{50} of the solvent

extracts increased with time during biological treatment (Fig. 5). The same trend was also observed for the constituents desorbed to HLB or Tenax. The LD_{50} of constituents desorbed to HLB or Tenax for both DT40 and $Rad54^{-/-}$ was significantly higher than that of the solvent extracts for all samples. No significant differences of LD_{50} were observed among the solvent extracts of each sample before or after desorption to HLB or to Tenax (Supplementary Fig. S2). The LD_{50} of constituents desorbed to HLB was significantly lower than that of constituents desorbed to Tenax in feed soil and treated soil sampled immediately after feeding (d0) for both DT40 and $Rad54^{-/-}$. In contrast, the LD_{50} of constituents desorbed to HLB was significantly higher ($p < 0.05$) than that of constituents desorbed to Tenax as treatment progressed during the 7-day treatment cycle (d1 and d7 samples) for DT40, while no significant differences were observed among LD_{50} values for $Rad54^{-/-}$ in these samples.

For a quantitative comparison of genotoxicity, we also calculated the relative LD_{50} (LD_{50} of $Rad54^{-/-}$ divided by the LD_{50} of the parental DT40 cell line; Fig. 6). If the relative LD_{50} of a sample is significantly < 1.0, that sample is defined as genotoxic; the lower the relative LD_{50}, the more genotoxic the sample. The relative LD_{50} of solvent extracts decreased after biological treatment, while the relative LD_{50} of constituents desorbed to either HLB or Tenax increased as a result of biological treatment (Fig. 6a). The relative LD_{50} of constituents desorbed to HLB or Tenax was significantly higher than that of the solvent extracts for all samples of the treated soil (Fig. 6b). No significant differences of relative LD_{50} were observed among the solvent extracts of each sample before or after desorption to HLB or to Tenax (Supplementary Fig. S3). The relative LD_{50} of constituents desorbed to the HLB resin was not significantly different from that of constituents desorbed to Tenax in the feed soil and treated soil sampled immediately after feeding (d0), but was significantly lower in treated soil sampled at d1 and d7 during the 7-day treatment cycle (Fig. 6b). The relative LD_{50} of the constituents desorbed to Tenax in treated soil sampled at d1 and d7 was not significantly different from 1.0 (Fig. 6b).

Discussion

In the previous work (Hu *et al.*, 2012), we reported that treatment of contaminated soil from an MGP site in a lab-scale bioreactor led to an increase in genotoxicity of the soil. In this study, we used solid-phase extraction to evaluate the potential bioavailability of the genotoxic constituents. Extraction with Tenax beads is commonly used to estimate and

FIG. 5. LD_{50} of solvent extracts (SE) and constituents desorbed to HLB (H) and Tenax (T) of soils for the parental DT40 cell line and its $Rad54^{-/-}$ mutant. The inset enlarges the results for LD_{50} of solvent extracts. Values represent mean and standard deviation of three separate experiments to quantify LD_{50} of a given sample.
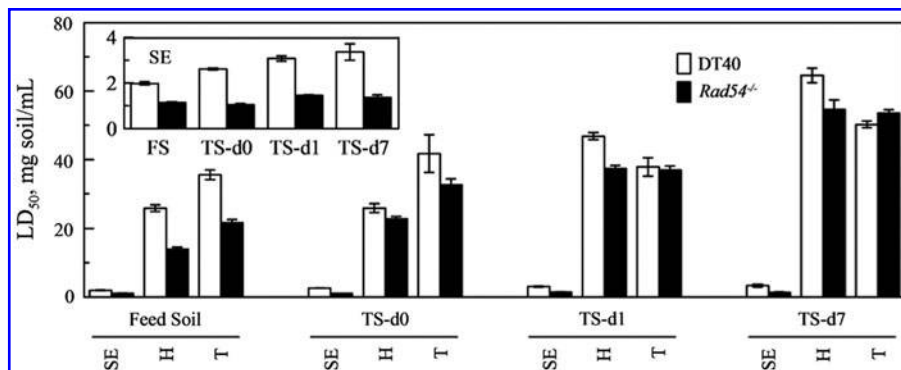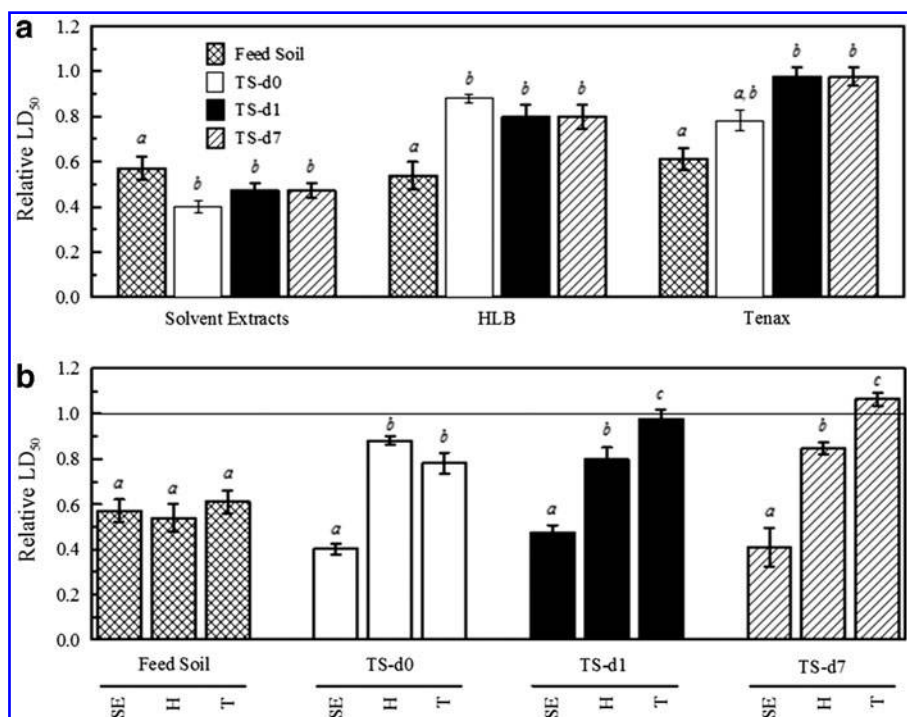
FIG. 6. Relative LD_{50} of solvent extracts (SE) and constituents desorbed to HLB (H) and Tenax (T) of soils. Data are organized by **(a)** extract type and **(b)** sample type. [a,b,c]Different letters are assigned to conditions for which there was a significant difference ($p < 0.05$) among the samples for a given extract type **(a)** or among the extract types for a given sample **(b)**.

predict bioavailability of organic pollutants in contaminated soils and sediments (ten Hulscher *et al.*, 2003; Gomez-Eyles *et al.*, 2011). However, because oxy-PAHs and other possible products of aerobic bacterial metabolism can be more polar than the parent compounds, we decided to evaluate both hydrophobic (Tenax) and mixed-function (Oasis HLB) resins; the HLB resin is a hydrophilic-lipophilic balanced copolymer sorbent (Buchberger, 2007). We suspended the resins in dialysis tubing because the HLB resin particles could not be separated from the soil for quantitative analysis using the conventional method of mixing the sorbent with soil slurry directly. The method was validated by demonstrating that there was no significant difference between extraction with Tenax using the dialysis-tubing method and the conventional slurry-based desorption method (Fig. 1).

Bioreactor treatment significantly removed PAHs and oxy-PAHs from the contaminated soil (Fig. 2). In soils and sediments, the desorbability of PAHs is believed to be one of the major factors influencing the extent of biodegradation (Luthy *et al.*, 1997; Lei *et al.*, 2004). Some studies have suggested that fast-desorbing or total desorbing fractions of a hydrophobic contaminant can be used to predict bioavailability and, correspondingly, the achievable endpoint of bioremediation (Cornelissen *et al.*, 1998; Hawthorne *et al.*, 2001; Braida *et al.*, 2004; Lei *et al.*, 2004). However, we found that the percentage of most PAHs and oxy-PAHs removed at the end of the bioreactor treatment cycle exceeded the percentage desorbed from the feed soil (Fig. 4). This phenomenon was also observed for PAHs in a long-term study on the same soil using laboratory columns to simulate *in situ* bioremediation (Richardson and Aitken, 2011). The oxy-PAHs PQ and AQ were more biodegradable and desorbable than their parent compounds PHN and ANT, but FLO was less biodegradable and desorbable than the parent compound fluorene. The differences in desorbability could at least partially explain the differences in biodegradability between these oxy-PAHs and their respective parent compounds.

However, we also observed that BAQ was more desorbable but less biodegradable than the parent compound BaA, which suggests that factors other than bioavailability limited the biodegradation of BAQ when compared to BaA.

Biological treatment decreased the toxicity of both desorbable and nondesorbable compounds in the soil; comparing LD_{50} values, the toxicity mainly came from the nondesorbable constituents (Fig. 5). For the desorbable compounds, the constituents that desorbed to HLB resin were more toxic than the constituents that desorbed to Tenax beads in the feed soil and in the treated soil immediately after feeding (d0), but less toxic in the treated soil sampled at d1 and d7 (Fig. 5). These results suggest that HLB resin and Tenax beads sorb different categories of compounds, although their ability to sorb 14 PAHs and 4 oxy-PAHs analyzed in this study was similar (Fig. 1). Moreover, the as-yet-unidentified toxic compounds sorbed by HLB resin were removed to a greater extent during the bioreactor treatment cycle than the toxic compounds sorbed by Tenax beads (Fig. 5).

Biological treatment slightly increased the genotoxicity of the whole soil, but it significantly reduced the genotoxicity of the desorbable constituents, especially the constituents desorbed to Tenax (which were not genotoxic at all after 1 day treatment during the 7-day treatment cycle; Fig. 6). Consistent with our previous work (Hu *et al.*, 2012), these results indicate that bioremediation generated genotoxins, but it appears that the genotoxins were not desorbable. It is interesting that the desorbability of putative PAH metabolites such as oxy-PAHs also decreased during the bioreactor treatment cycle (Fig. 3); however, the increased genotoxicity resulting from treatment cannot be attributed to the four oxy-PAHs we analyzed because there was a net removal of these compounds during treatment (Fig. 2).

Findings from this work have potential implications for the influence of bioremediation on overall risk to human health and to ecological receptors. Bioremediation can clearly lead to the

formation of products that are more genotoxic than the original constituents in a contaminated soil, which presumably can lead to increased risk upon exposure to the soil. However, if the genotoxic constituents in treated soil are less desorbable than those in untreated soil, they may be less bioavailable, with a corresponding decrease in overall risk. Further work is necessary to elucidate the nature of genotoxic constituents resulting from bioremediation and their bioavailability to relevant receptors.

Acknowledgments

Author Disclosure Statement

All authors confirm that no competing financial interests exist.

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