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Photochemical and Bacterial Transformations of Disinfection By-Product Precursors in Water

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In situ grab sampling from source waters and water extraction from source materials are common methods for determining disinfection by-product (DBP) formation potential (FP) of water samples or reactivity of dissolved organic matter (DOM) in forming DBPs during chlorination. However, DOM, as the main DBP precursor, collected using these techniques may not represent the DOM reacting with disinfectants due to biogeochemical alterations during water conveyance to drinking water treatment facilities. In this study, we exposed leachates from fresh litter and associated decomposed duff to natural sunlight or *Escherichia coli* K-12 for 14 d and evaluated the changes, if any, on the propensity to form trihalomethane (THM), haloacetonitrile (HAN), and chloral hydrate (CHD) during chlorination. Sunlight treatment did not significantly change dissolved organic carbon (DOC) concentration but caused a 24 to 43% decrease in the specific ultraviolet absorbance (SUVA) at 254 nm, indicating that UV-active chromophores were transformed or degraded. There were significant increases ($P < 0.05$) in specific HAN formation potential (HAN-FP) and specific CHD formation potential (CHD-FP) (i.e., HAN and CHD formation potentials per unit carbon), but no change in specific THM formation potential (THM-FP) after sunlight exposure. In contrast, bacterial treatment did not show any significant effect on SUVA, specific chlorine demand, or any specific DBP-FPs, although bacterial colony counts suggested DOM in leachates was utilized for bacterial growth. Results of this study confirmed that the reactivity of DOM in forming DBPs could be different after biogeochemical processes compared with its source materials. For this study, photochemical reactions had a greater effect on DBP-FPs than did microbial degradation.

WATER DISINFECTION such as chlorination is an essential step to minimize pathogen contamination in municipal drinking water supplies. However, dissolved organic matter (DOM), consisting of both dissolved organic carbon (DOC) and dissolved organic nitrogen (DON), in source water can react with chlorine to yield a variety of carcinogenic organic compounds termed disinfection by-products (DBPs) (Xie, 2004). Generally, DBP formation in chlorinated water is proportional to the DOC concentration. Depending on the types of source waters, DOC concentrations can exceed 50 mg L^{-1} , such as in the Sacramento–San Joaquin Delta of California (Chow et al., 2007) and coastal blackwater rivers in South Carolina (Xie et al., 2004; Chow et al., 2013). Chlorination of source waters with high DOC concentration for drinking water purification could result in high DBP formation in the finished waters (Xie, 2004; Chow et al., 2008b, Díaz et al., 2009).

Disinfection by-products are of great human-health concern because they are potential carcinogens, mutagens, or developmental toxicants if ingested over extended periods of time (Muellner et al., 2007). Over 600 different DBPs have been identified in finished waters. For example, trihalomethanes (THMs), haloacetonitriles (HANs), and chloral hydrate (CHD) have been reported in treatment water facilities across the United States with a median of 31, 3, and $1 \mu\text{g L}^{-1}$ and a maximum of 164, 14, and $16 \mu\text{g L}^{-1}$, respectively (Krasner et al., 2006). However, only a few DBPs are currently regulated. The maximum contaminant level (MCL) in the USEPA stage 2 DBP rule for THM is $80 \mu\text{g L}^{-1}$, but current regulations do not include a MCL for HAN or CHD (USEPA, 2006).

Numerous studies have evaluated the reactivity of DOM in forming THMs, HANs, and CHD from a variety of terrestrial DOM sources derived from agricultural drainage and soils, constructed wetlands, forest soils, and foliar litter. Depending on the chlorination procedure, the carbon normalized DBP

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Abbreviations: CHD, chloral hydrate; DBP, disinfection by-product; DOC, dissolved organic carbon; DOM, dissolved organic matter; DON, dissolved organic nitrogen; FP, formation potential; HAN, haloacetonitrile; MCL, maximum contaminant level; SCD, specific chlorine demand; SUVA, specific ultraviolet absorbance; THM, trihalomethane; UVA₂₅₄, ultraviolet absorbance at 254 nm.

formation potential (DBP-FP), termed specific DBP-FP, can range from 0.57 to 12.4 mmol THM mol C⁻¹, 0.12 to 0.37 mmol HAN mol C⁻¹, and 0.05 to 0.28 mmol CHD mol C⁻¹ (Díaz et al., 2008; Engelage et al., 2009; Kraus et al., 2010; Chow et al., 2011).

In most DBP-related studies, DBP-FPs have been determined with source water samples and plant residue leachates under controlled laboratory conditions to isolate physiographic conditions that contribute to highly reactive DOM. However, various biogeochemical processes including microbial decomposition, sunlight irradiation, and microbial utilization could alter the composition and structure of organic matter during transport before entering water treatment facilities for chlorine disinfection. Therefore, the characteristics of DOM collected in situ could be different from DOM in water that reaches treatment facilities. Photochemical processes can increase the amount of hydroxyl, carboxyl and keto groups and decrease the amount of methoxyl groups in organic compounds (Xie et al., 2004; Benner and Kaiser, 2011). Further photooxidation can cause depolymerization into phenolic monomers and aliphatic compounds that arise from terminal methyl and methylene groups in alkyl chains of lipids and proteins (Argyropoulos and Sun, 1996; Moran and Zepp, 1997). Also, microbial processes can rapidly consume lignin phenols and amino acids while refractory dissolved humic substances and hydrophilic acid fractions can persist (Qualls, 2005; Pellerin et al., 2010; Benner and Kaiser, 2011). A few studies have examined the effects of photo- and biodegradation on DBP formation (mainly focused on THMs and haloacetic acids) (Chow et al., 2008b; Engelage et al., 2009; Pellerin et al., 2010). To our knowledge, the effects of photo- and biodegradation on other DBPs of emerging concern, such as HAN and CHD, have not been reported.

The hypothesis of this study was that reactivity of DOM in forming DBPs is different from source materials after exposure to biogeochemical transformation. In this study, we collected leachate from fresh and decomposed litter from the four dominant vegetation types in California oak woodlands. California oak (*Quercus* sp.) woodlands were selected as these watersheds play a major role in the state's water supply system with two-thirds of all drinking water reservoirs located within oak woodland ecosystems. Waters from these landscapes (an estimated 3 million ha; Griffin, 1977) ultimately flow to the Sacramento–San Joaquin Delta, which is a source of drinking water for more than 23 million people. The leachates were exposed to natural sunlight and *Escherichia coli* K-12 (*E. coli* K-12) activity, and all samples were then chlorinated under uniform formation conditions (Summers et al., 1996). *Escherichia coli* was chosen to represent bacterial processes because of (i) the occurrence of *E. coli* in surface waters, (ii) its growth dependence on nutrients and DOM composition, and (iii) ease of quantification of bacterial growth. An increase of both *E. coli* and DOM were observed after rainstorms in the oak woodland watersheds where we collected our DOM samples (Knox et al., 2007; University of California Davis, 2013). Oak woodland watersheds, typically managed for cattle grazing, provide drinking water to many Californians. Cattle manure is washed into the lakes and streams, resulting in an increase of *E. coli* in surface waters (Derlet et al., 2012). In fact,

significant concentrations of *E. coli* were reported in many water bodies in California (Walters et al., 2011). Bioassay showed the growth of *E. coli* in water depends on the nutrient and DOM composition (McCrary et al., 2013; Vital et al., 2010; Arana et al., 2000). In many other studies examining microbial effects on DBP precursors, only indirect methods (e.g., measuring biological oxygen demand) or even no measurements were performed to determine the microbial activity (e.g., Engelage et al., 2009; Krasner et al., 2009; Pellerin et al., 2010). However, growth of *E. coli* can be quantified relatively easily through bacterial plate counts.

Chlorinated samples were evaluated for their reactivity in forming THMs, HANs, and CHD, representing carbonaceous, nitrogenous, and oxygenated DBP classes, respectively. Understanding the effects of photo- and biodegradation processes on the characteristics of DBP precursors can link the existing in situ studies to practical use in water treatment facilities.

Materials and Methods

Sunlight Irradiation and Bacterial Utilization

Leachates of foliage from fresh litterfall and associated decomposed litter (hereafter termed duff) including blue oak (*Quercus douglassii* H. & A.), live oak (*Quercus wislizenii* A. DC.), foothill pine (*Pinus sabiniana* Dougl.), and mixed annual grasses were used in this study. Detailed leachate collection methods were described in previous studies (Chow et al., 2009, 2011). Leachates from 3 out of a total of 11 rainfall events were selected randomly (but corresponding to early, middle, and late season rainfall events) for sunlight and bacterial exposure treatments. General water quality parameters for plant leachates used in the experiments are summarized in Supplemental Table S1. All leachate samples were diluted with organic carbon-free deionized water to ~5 mg L⁻¹ DOC before incubation. To ensure sterility, samples were refiltered through a 0.22- μ m autoclaved polycarbonate membrane filter, and 125-mL aliquots were transferred into sterile 250-mL glass flasks.

For microbial degradation simulation, *E. coli* K-12 was used as described in our previous works (W.J. Wang et al., 2012b). Briefly, an overnight culture of *E. coli* K-12 was prepared by inoculating one colony into 10% nutrition broth and cultured at 30°C and 200 rpm agitation. Cell density was estimated by measuring absorbance at 520 nm and calculated from a standard curve. An appropriate number of cells were diluted with 0.9% NaCl solution and inoculated into samples to reach ~1 \times 10⁴ colony forming units mL⁻¹. Controls without *E. coli* inoculation were included. The samples were incubated at room temperature (25 \pm 2°C) with 200 rpm agitation in the dark. Subsamples were taken daily. After suitable dilution, 0.1 mL was spread onto a nutrient agar plate and incubated at 30°C for 24 h before counting. Another subsample was measured for ultraviolet absorbance at 254 nm (UVA₂₅₄). We measured UVA₂₅₄ on a Helios Gamma UV/VIS spectrophotometer (Thermo Fisher Scientific Inc.) according to standard method 5910B (APHA, 2012). After 14 d of incubation, water samples were analyzed for DOC and DBP-FP.

For sunlight treatment, sealed glass flasks with 125 mL of sterile sample were exposed to ambient sunlight conditions

outside the laboratory. Every 24 h, a subsample was taken aseptically to measure UVA_{254} . Measurement of visible light, UV-A, UV-B, and UV-C were taken three times per day using a digital radiometer placed beneath a glass plate to compensate for glass absorption of radiation by the flask. Average maximum daily values for visible light were $1069 \pm 232 \text{ mW m}^{-2}$; UV-A: $312 \pm 59 \text{ } \mu\text{W cm}^{-2}$; UV-B: $210 \pm 48 \text{ } \mu\text{W cm}^{-2}$; and UV-C: $48 \pm 16 \text{ } \mu\text{W cm}^{-2}$. Air temperature was monitored continuously throughout the experimental period (maximum daily temperatures ranged between 20 and 39°C). After 14 d of exposure to natural sunlight, water samples were analyzed for DOC and DBP-FP. For the experimental controls, filtered samples were placed in sterile 250 mL glass flasks and incubated at room temperature for 14 d, and then analyzed for DOC and DBP-FP. Three replicates were performed for each treatment and control.

Disinfection By-Product Formation

The DOM in water samples untreated and treated with sunlight and *E. coli* exposure was chlorinated following the uniform formation condition that yielded 1 (± 0.4) mg L⁻¹ free chlorine residual at pH 8 (± 0.2) after a 24-h incubation in the dark at 20°C (Summers et al., 1996). Chlorinated samples were analyzed for four bromine- and chlorine-containing THM species (chloroform, bromodichloromethane, bromoform, and dibromochloromethane), three HAN species (dibromoacetonitrile [DBAN], dichloroacetonitrile [DCAN],

and trichloroacetonitrile [TCAN]), and CHD. Detailed analytical methods have been described in associated studies (Chow et al., 2009, 2011). Minimum detection limit for each THM, HAN, and CHD species was $\sim 1 \text{ } \mu\text{g L}^{-1}$.

Results

Specific Ultraviolet Absorbance and Specific Chlorine Demand

For both sunlight and bacterial treatments, all samples had comparable DOC concentrations to those before treatment. Constituents containing unbonded electrons on oxygen, sulfur, and conjugated C=C double bonds show absorption in the ultraviolet and visible regions; therefore, UVA_{254} has been widely used to characterize DOC in soils and waters (Chow et al., 2008a). During the 14-d sunlight exposure, UVA_{254} of all samples decreased in a linear fashion. The reduction in UVA_{254} ranged from 24 to 43% by the end of the 14-d experiments (Fig. 1a and 1b). Leachates of duff samples tended to have a greater reduction (32–43%) compared with associated fresh litter (24–37%); however, the differences were not statistically significant ($P > 0.05$). In contrast, UVA_{254} in bacterial treatments were relatively constant and showed no change compared with the 14-d incubation with no bacteria in the dark (Fig. 1c and 1d), although bacterial counts showed leachates supported growth of bacteria, particularly in fresh litter of blue oak and live oak (Supplemental Fig. S1a and b). Since all treatments had similar

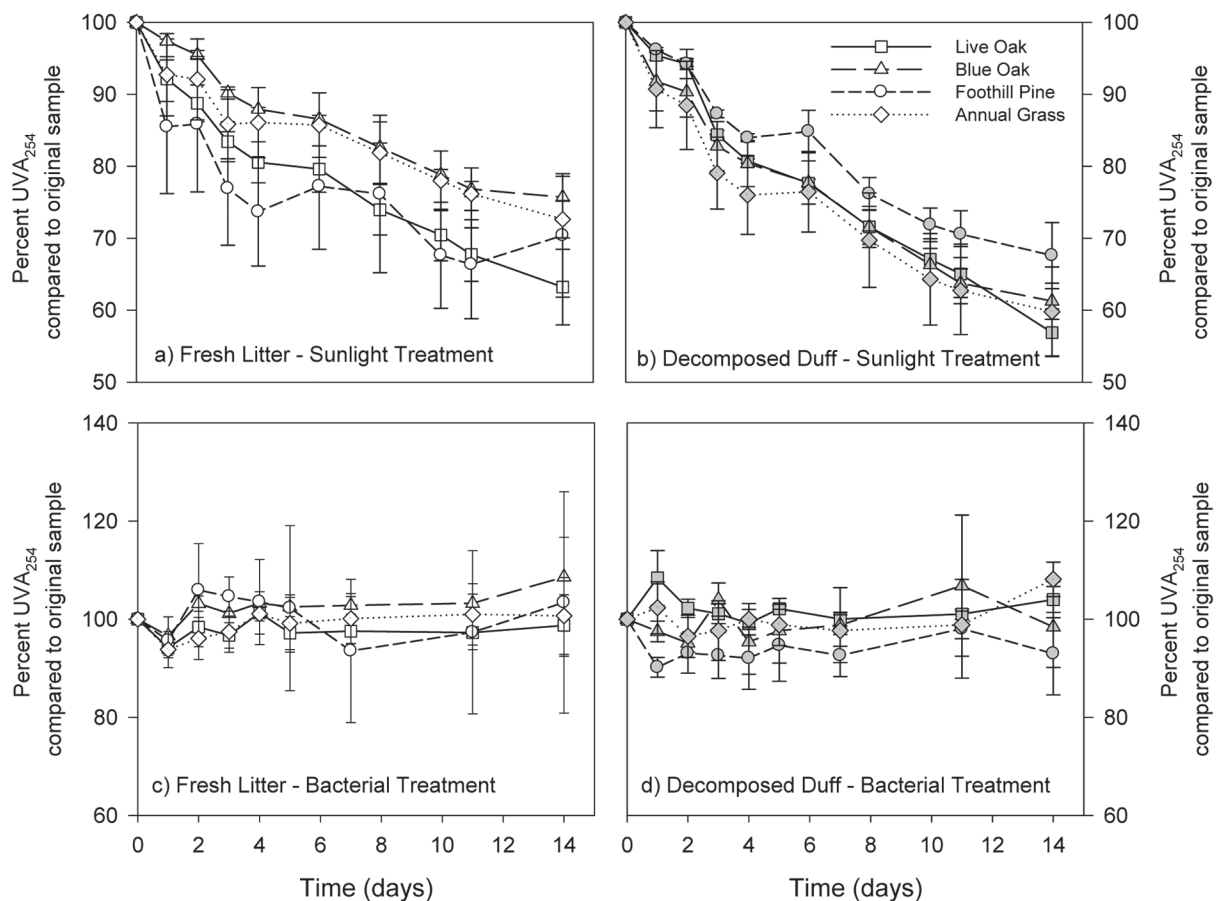


Fig. 1. Sunlight irradiation changed UV absorbance at 254 nm (UVA_{254}) in leachates from (a) litter and (b) duff. Bacterial utilization changed UVA_{254} in leachates from (c) litter and (d) duff. Each treatment was run in triplicate; error bars represent the standard deviation of the measured values.

DOC concentrations before and after treatment, the changes in specific ultraviolet absorbance at 254 nm (SUVA), which is defined as carbon normalized UVA_{254} ($SUVA = UVA_{254}/[DOC]$), were closely matched with the changes in UVA_{254} .

Water-specific UVA_{254} and specific chlorine demand (SCD) are commonly used in water science and industry as surrogates for aromatic carbon content and chlorine consumption of DOM in source waters. The reactivity of DOM with oxidants, such as chlorine and ozone, correlates to certain extent to the aromaticity of the organic matter (Weishaar et al., 2003). In the United States, drinking water needs to be treated by enhanced coagulation or softening if its SUVA is greater than $2.0 \text{ L mg}^{-1} \text{ m}^{-1}$ (USEPA, 1999). Results of SUVA and SCD for four fresh litter and four decomposed duff samples before and after 14 d of sunlight and bacterial exposures are summarized in Fig. 2. No statistical differences were observed in the control samples ($P > 0.05$). There were significant decreases in SUVA ($P < 0.05$) in most samples after sunlight treatment. In contrast, no differences ($P > 0.05$) were observed in bacterial treatments. In terms of SCD, there were no statistical differences ($P > 0.05$) in either sunlight or bacterial treatments (Fig. 2c and 2d).

Reactivity of Dissolved Organic Carbon in forming THMs, HANs, and CHD

The specific trihalomethane formation potential (THM-FP), specific haloacetonitrile formation potential (HAN-FP), and specific chloral hydrate formation potential (CHD-FP) after sunlight and bacterial exposures are shown in Fig. 3. The values were calculated by normalizing the chlorinated-induced THM, HAN, and CHD in a water sample with its DOC concentration. These measures represent the reactivity of carbon in forming THMs, HANs, and CHD during chlorination. Results showed no statistical differences ($P > 0.05$) in specific THM-FP after sunlight and bacterial exposure, although exposed samples apparently had slightly greater reactivity than the original samples. Apart from THM formation, a significant ($P < 0.05$) increase in specific HAN-FP was observed in fresh litter samples after sunlight exposure, but the increase in reactivity of duff samples was not statistically different ($P > 0.05$), except for annual grass duff. An increase of specific CHD-FP was observed in both fresh litter and duff samples after sunlight exposure. No differences were observed in specific THM-FP, specific HAN-FP, or specific CHD-FP in bacterial exposures compared with the original samples.

Discussion

Photochemical Transformation

The controlled experiments confirmed that the reactivity of DOC in forming DBPs is sensitive to photochemical reactions

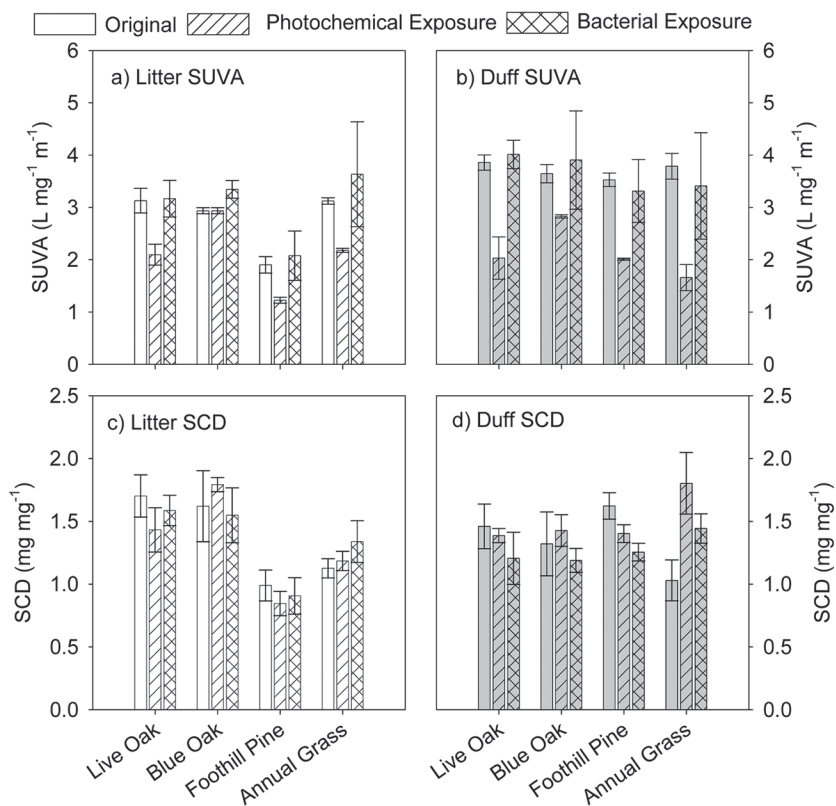


Fig. 2. Specific ultraviolet absorbance at 254 nm (SUVA) and specific chlorine demand (SCD) of litter and duff leachates before and after photo and bacterial exposures. Each treatment was run in triplicate; error bars represent the standard deviation of the measured values.

(Chow et al., 2008b). Although there was no significant increase in THM formation, a significant increase in HAN and CHD formation occurred, representing nitrogenous and oxygenated DBPs. Notably, the different responses to the sunlight treatment suggest that precursors to THM formation could be different from those of HANs and CHD. Phenolic aromatic carbons such as resorcinol have been considered as reactive precursors for THM formation (Luilo and Cabaniss, 2011). Although SUVA indicated that sunlight transformed or partially broke down UV-active chromophores of DOC into low molecular weight organic acids (Brinkmann et al., 2003; Xie et al., 2004; Chow et al., 2008b) and probably depolymerized lignin to phenolic monomers (Argyropoulos and Sun, 1996; Moran and Zepp, 1997; Benner and Kaiser, 2011), such processes apparently did not affect the THM reactivity in our samples. A decreasing SUVA may indicate reduction of the DOC in aromaticity but did not necessarily suggest decreased reactivity of the DOM and reduced DBP formation potential. Research found that THM formation in chlorinated drinking water was influenced by a number of factors including DOC content and composition, temperature, bromide, and pH of the source water (Liang and Singer, 2003). The THM-FP of drinking water showed a poor correlation with the aromaticity or the SUVA of DOC in the source water (Fram et al., 1999; Weishaar et al., 2003; Chow et al., 2008a).

In contrast to THM reactivity, photochemical reactions generated reactive precursors for HAN and CHD. Organic nitrogen such as amino acids and oxidized natural organic matter, such as aldehydes, are considered as the primary

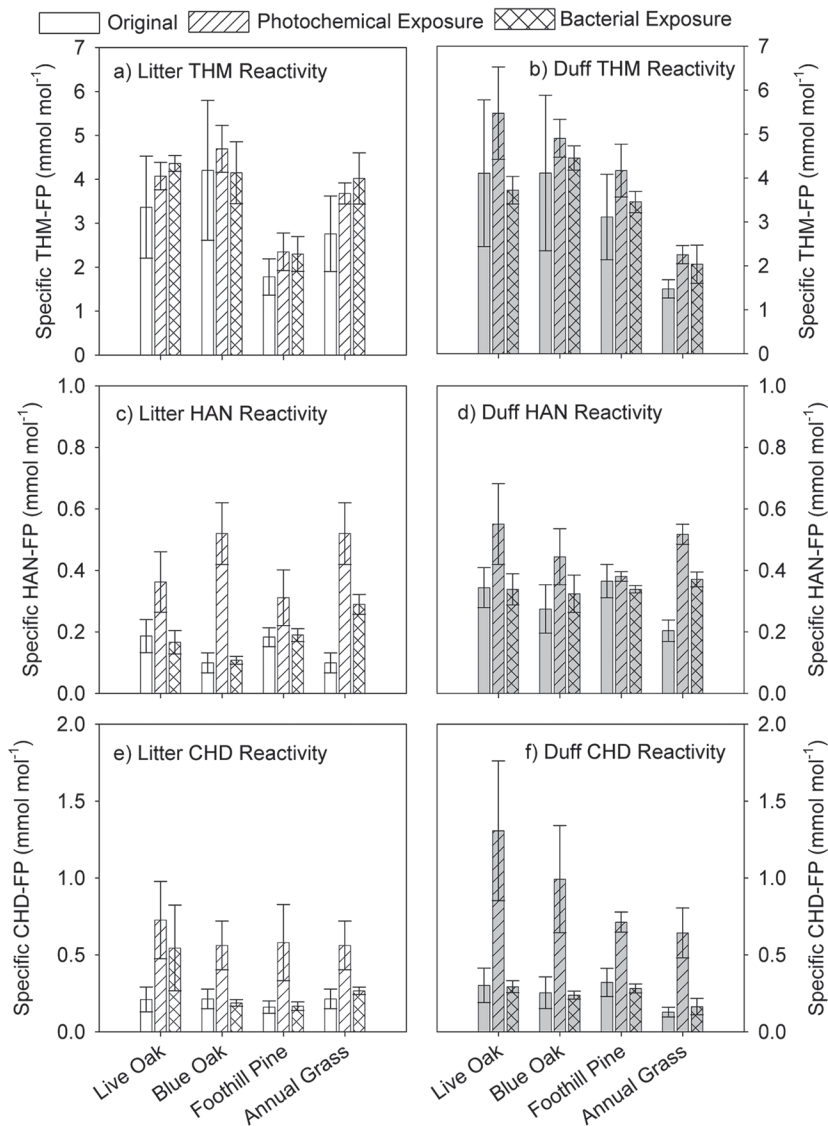


Fig. 3. (a–b) Specific trihalomethane formation potential (THM-FP), (c–d) specific haloacetonitrile formation potential (HAN-FP), and (e–f) specific chloral hydrate formation potential (CHD-FP) of litter and duff leachates before and after photo and bacterial exposures. Each treatment was run in triplicate; error bars represent the standard deviation of the measured values.

precursors of HAN and CHD (Barrott, 2004; Bond et al., 2012; Shah and Mitch, 2012). Formic, acetic, oxalic, and other small organic acids and aldehydes can derive from direct or indirect photolysis of natural DOM (Brinkmann et al., 2003; Chow et al., 2008b; Southwell et al., 2010). Furthermore, photochemical reactions may cause oxidative degradation of the amine side-chain, converting the tertiary amine into secondary or primary nitrogen species, and increase DON and inorganic nitrogen species in sun-irradiated water samples (Mayer et al., 2009; Benner and Kaiser, 2011). Notably, the Cl₂/N ratio could affect the formation of monochloramine or dichloroamine, which could yield different DBP species during the disinfection process (Huang et al., 2012). A decrease in the C/N ratio of DOM can enhance HAN and other nitrogenous DBP formation under the same chlorination procedure (Lee et al., 2007; Chow et al., 2011).

Bacterial Transformation

No significant change was observed in all testing parameters (i.e., SUVA, SCD, specific THM-FP, specific HAN-FP, and specific CHD-FP) in the 14-d bacterial exposures. Biodegradable components from litter leachates, such as carbohydrates and amino acids that have been identified as THM and HAN precursors (Navalon et al., 2008; Bond et al., 2012), should be easily utilized by bacteria (Pellerin et al., 2010; Benner and Kaiser, 2011). The increase of bacterial counts (Supplemental Fig. S1) indicated that bacteria could have utilized DOC, and such microbial processes would transform organic matter pools from a high to low C/N ratio, which would enhance HAN and CHD formation (J.J. Wang et al., 2012a). However, changes in reactivity with chlorination were not observed, suggesting that the contributions of these biodegradable DOM components to the overall DBP formation are less significant when compared to other relatively recalcitrant DOM. In other words, the major DBP precursors are not altered by *E. coli* utilization of DOM in the 14-d incubation.

Implication to Drinking Water Quality

Our previous study showed that litter quality (i.e., plant species and decomposition stage) determined the characteristics of DOM in leachates, resulting in different reactivity in forming DBPs during chlorination (Chow et al., 2011). This follow-up experiment shows that the reactivity of DBP precursors can be further altered by sunlight. Formation of HAN and CHD were particularly sensitive to sunlight irradiation. Importantly, toxicological research has indicated that nitrogenous DBPs are more often genotoxic, cytotoxic, or carcinogenic than many of the carbonaceous DBPs (Shah and Mitch, 2012). Furthermore, previous studies explored the effects of sunlight and biodegradation on DBP precursors, but they mainly focused on THM formation. Chow et al. (2008b) and Engelage et al. (2009) showed no changes in specific THM-FP from natural waters (e.g., riverine and wetland waters) after sunlight irradiation and biodegradation incubations for short incubation periods (<2 wk). Pellerin et al. (2010) showed an increase in specific THM-FP from the leachates of crop plants such as alfalfa and tomato after a 21-d incubation. Differences can be attributed to different sources of DOM, incubation length and conditions, and chlorination procedures (Summers et al., 1996; Xie, 2004). Although inconsistent results were observed from different studies, in any case, no decrease of reactivity in forming DBPs was observed after photochemical or bacterial incubations. In fact, several studies (including this one) showed an increase in the propensity of DOM to form a variety of DBPs, suggesting that

the reactivity of DOM after biogeochemical transformation is greater than DOM extracted from the original source materials. As a result, previous studies determining DBP formation from in situ samples may underestimate the DBP reactivity of DOM entering water treatment facilities.

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