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BIOTRANSFORMATION OF CAFFEINE, COTININE, AND NICOTINE IN STREAM SEDIMENTS: IMPLICATIONS FOR USE AS WASTEWATER INDICATORS

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Abstract—Microbially catalyzed cleavage of the imadazole ring of caffeine was observed in stream sediments collected upstream and downstream of municipal wastewater treatment plants (WWTP) in three geographically separate stream systems. Microbial demethylation of the *N*-methyl component of cotinine and its metabolic precursor, nicotine, also was observed in these sediments. These findings indicate that stream sediment microorganisms are able to substantially alter the chemical structure and thus the analytical signatures of these candidate waste indicator compounds. The potential for in situ biotransformation must be considered if these compounds are employed as markers to identify the sources and track the fate of wastewater compounds in surface-water systems.

Keywords—Biodegradation Emerging contaminants Oxic Anoxic Streams

INTRODUCTION

Suitable tracers for the biological and chemical components of wastewater effluent are needed to identify the sources and track the fates of wastewater compounds in surface-water systems [1,2]. Effective surrogate compounds reflect the source and magnitude of contamination and are readily detectable under surface-water conditions [1]. Thus, chemical indicators of human contamination ideally would have the following characteristics: readily detected at environmentally relevant concentrations using currently available methods; incompletely removed during wastewater treatment plant (WWTP) treatment; produced by or passed through humans before entering the waste stream; and behave in the environment in a manner representative of other important effluent-derived contaminants [1–4].

Caffeine and cotinine are perhaps the two most commonly proposed indicators of human-derived waste in surface- and groundwater systems [1,5,6]. Caffeine is arguably the most widely consumed nonprescription, human drug in the world [1]. Even though caffeine occurs in more than 60 plant species, the natural range of these species is primarily tropical. Thus, detection of caffeine in the surface-water systems of the northern hemisphere is largely attributable to the discharge of human waste ([1–3,7,8]; http://pubs.usgs.gov/circ/circ1133/). Nicotine is also a plant-derived drug in common use worldwide, but the presence of nicotine in North American surface-water systems cannot be exclusively attributed to human waste discharge due to ongoing cultivation and processing of nicotine-containing (primarily tobacco) products in this region. Cotinine, on the other hand, is a by-product of nicotine metabolism in the liver. Consequently, detection of cotinine in surface-water environments is a compelling indication of human waste impacts. Caffeine and cotinine are essentially ubiquitous in WWTP effluent in the United States [2] and are detectable in surface waters at nanogram per liter concentrations using existing analytical methods [3,9]. Both compounds have high aqueous solubilities and, consequently, tend to track the movement of water rather than partitioning into the sediment phase.

The utilization of caffeine and cotinine as indicators of human waste impacts in surface-water systems, however, requires a fundamental understanding of their fates in this environment. Little is known about the potential for biotransformation of caffeine and cotinine in wastewater-impacted streams, but the fact that both chemicals derive from natural flora suggests a potential for biotransformation in these systems. This expectation is consistent with the reported elimination of 81 to 99.9% of caffeine in Swiss WWTP and the apparent, nonconservative behavior of caffeine in some Swiss lake systems [1]. However, caffeine has also been reported to persist in soil [10]. This environmental variability emphasizes the need for environment-specific assessment of the degradation character of the proposed indicator compounds.

The purpose of this study was to assess the potential impact of in situ biotransformation on the proposed use of caffeine and cotinine as indicators of human waste impacts in streams. A series of microcosm studies were prepared with ^{14}C-substrates and samples from three, effluent-impacted streams in order to assess the relative efficiency of caffeine biotransformation in sediments under oxic and anoxic conditions; the potential for significant biotransformation of caffeine in the water column; the impact of sediment location (relative to WWTP outfall) on the efficiency of caffeine biotransformation; and the potential for biotransformation of cotinine and its metabolic precursor, nicotine, in sediment.

MATERIALS AND METHODS

Chemicals

The potential for in situ biotransformation of caffeine was investigated using an aqueous solution of [8-^{14}C] caffeine (53

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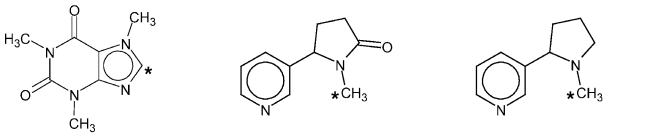

Fig. 1. Molecular structures of [8-^{14}C] caffeine, [*N*-methyl-^{14}C] cotinine, and [*N*-methyl-^{14}C] nicotine. Asterisk indicates the location of the ^{14}C-radiolabel. The enantiomers of cotinine and nicotine are not shown.

μCi/μmol; Moravek Biochemicals, Brea, CA, USA). The radiolabeled carbon of [8-^{14}C] caffeine is located in the imadazole ring of the purine skeleton (Fig. 1). Thus, mineralization of the ^{14}C-radiolabel in [8-^{14}C] caffeine implies substantial ring destruction and indicates significant degradation of the caffeine molecule. The potential for in situ biotransformation of cotinine and its metabolic precursor, nicotine, was investigated using aqueous solutions of L-[*N*-methyl-^{14}C] cotinine and DL-[*N*-methyl-^{14}C] nicotine (55 μCi/μmol; American Radiolabeled Chemicals, St. Louis, MO, USA). These ^{14}C-substrates are *N*-methyl-^{14}C labeled compounds (Fig. 1). Thus, mineralization of the ^{14}C-radiolabel of L-[*N*-methyl-^{14}C] cotinine or DL-[*N*-methyl-^{14}C] nicotine indicates demethylation and, therefore, transformation that may affect detection in the environment but not necessarily significant degradation of the substrate molecule. The radiochemical compositions of all ^{14}C-substrates were evaluated in the laboratory by direct injection, radiometric detection (RD), high-performance liquid chromatography (HPLC) and direct injection, radiometric detection gas chromatography (GC) and found to be greater than 99% pure. $H^{14}CO_3^-$ (Sigma Biochemicals, St. Louis, MO, USA) and $^{14}CH_4$ (New England Nuclear, Boston, MA, USA) were used as radiolabeled standards for calibration and methods development. Both had radiochemical purities >98%.

Study sites

The ability of stream microorganisms to transform [8-^{14}C] caffeine, L-[*N*-methyl-^{14}C] cotinine, and DL-[*N*-methyl-^{14}C] nicotine was examined in sediments collected from two locations at each of three effluent-dominated streams (Table 1). Surface water and sediments, consisting of fine-grain sands, were collected from Fourmile Creek near Ankeny, Iowa, USA, at locations approximately 30 m upstream and 30 m downstream of the Ankeny WWTP outfall. The Fourmile Creek system is described in detail elsewhere [11]. Water and sediments, consisting of coarse sands and gravels, were collected from Boulder Creek near Boulder, Colorado, USA, from locations approximately 200 m upstream and 20 m downstream of the Boulder 75th Street WWTP outfall. The Boulder Creek system has been described in detail earlier [12]. South Platte River water and streambed sediments consisting of coarse sands and gravels were collected from locations approximately 100 m upstream and 100 m downstream of the Metro Wastewater Reclamation District outfall near Denver, Colorado, USA. The South Platte River system has been described elsewhere [13].

Microcosm studies

Experimental bed sediment microcosms were prepared in quadruplicate as described previously [14,15] and were composed of 10-ml serum vials with 5 ml of saturated sediment and an atmosphere of air (oxic treatments) or nitrogen (anoxic treatments). Triplicate control microcosms were prepared as described for each sediment and sample location and autoclaved three times for 1 h. To assess the effects of sediment oxygen condition on caffeine biotransformation, sediment microcosm treatments were prepared using material collected upstream of the WWTP outfall in each system. To assess the effects of WWTP effluent on substrate biotransformation, sediment microcosm treatments were prepared for all three test substrates using material collected from upstream and downstream of the WWTP outfall in each system. To compare differences in caffeine biotransformation between the bed sediments and the overlying water column, sediment and water-only microcosm treatments were prepared for each system. Quadruplicate experimental and triplicate control water microcosms were prepared as for the sediments but with 5 ml of unfiltered surface water collected only at the upstream sediment sample locations. All microcosms were amended with approximately 0.027 μCi of [8-^{14}C] caffeine, L-[*N*-methyl-^{14}C] cotinine, or DL-[*N*-methyl-^{14}C] nicotine to yield initial dissolved concentrations of approximately 0.25 μmol/L. Microcosms were incubated statically, in the dark, at 23°C, for 40 to 74 d.

Analytical methods

Headspace concentrations of CH_4, $^{14}CH_4$, CO_2, and $^{14}CO_2$ were monitored by analyzing 0.5 ml of headspace using GC/RD combined with thermal conductivity detection. Compound separation was achieved by isocratic (80°C), packed-column (300 cm of 13× molecular sieve) gas chromatography using

Table 1. Surface water system characteristics for Fourmile Creek near Ankeny, Iowa; Boulder Creek near Boulder, Colorado; and South Platte River near Denver, Colorado, USA

Characteristic	Units	Fourmile Creek	Boulder Creek	South Platte River
Drainage basin area	km^2	410	1,160	10,000
Stream gradient	m/km	1	4	2
Human population[a]	1000	27	110	1,700
Stream flow upstream of WWTP	m^3/s	0.617	0.161	0.566
WWTP discharge[b]	m^3/s	0.244	0.093	5.61
WWTP contribution to downstream flow[b]	%	28	37	>91
Increase in sediment organic content[c]	%	29	33	200
Caffeine apparent half-life[d]	h	23	5.3	—

[a] Population data are from 2002.
[b] Data are for the period of sample collection in spring 2005. WWTP = wastewater treatment plant.
[c] Percentage increase in sediment organic content (loss on ignition) between upstream and downstream locations.
[d] Apparent half-life for caffeine in water column based on Lagrangian sampling events conducted in the Fourmile Creek and Boulder Creek systems in 2002.

a Hewlett-Packard 5890 series II plus GC (Agilent Technologies, Santa Clara, CA, USA). The headspace sample volumes were replaced with pure oxygen (oxic treatments) or nitrogen (anoxic treatments). Dissolved phase concentrations of $^{14}CH_4$ and $^{14}CO_2$ were estimated based on Henry's partition coefficients that were determined experimentally as described previously [14,15]. The GC/RD output was calibrated by liquid scintillation counting using $H^{14}CO_3^-$. To confirm the presence of oxygen (headspace $[O_2]$ = 2–21% by volume) in oxic treatments or the absence of oxygen (headspace $[O_2]$ minimum detection limit equals 0.2 parts per thousand by volume, 10 μmol/L) in anoxic treatments, headspace concentrations of O_2 were monitored throughout the study using GC with thermal conductivity detection.

RESULTS AND DISCUSSION

This investigation focused on the ability of indigenous microorganisms to alter the chemical structures and, thus, the analytical signatures of caffeine and cotinine, in order to assess the potential impact of biotransformation processes on the use of these compounds as indicators of human waste discharges in surface-water systems. Because current methods for identification and detection of emerging wastewater contaminants at nanogram per liter concentrations are based on mass spectral analyses, the ^{14}C-substrates used in this study were single labeled compounds selected to monitor microbial transformations of the chemical structure that may affect contaminant detection in the environment rather than uniformly ^{14}C-labeled compounds appropriate for assessing complete contaminant degradation. With respect to [8-^{14}C] caffeine, the ^{14}C-radiolabel was located in the imadazole ring of the purine skeleton of the caffeine molecule (Fig. 1). Thus, significant recovery of ^{14}C-radioactivity as $^{14}CO_2$ indicated ring cleavage and significant alteration of the caffeine molecule. In contrast, L-[*N*-methyl-^{14}C] cotinine and DL-[*N*-methyl-^{14}C] nicotine were *N*-methyl-^{14}C labeled compounds (Fig. 1). Mineralization of the ^{14}C-radiolabel of L-[*N*-methyl-^{14}C] cotinine or DL-[*N*-methyl-^{14}C] nicotine indicated demethylation and a potential for transformation that may hamper detection in the environment but did not necessarily indicate significant degradation of the molecule. The products of biotransformation were not assessed in this study. In all cases, the recovery of $^{14}CO_2$ and $^{14}CH_4$ observed in experimental treatments was attributed to biological activity, because no significant recovery of ^{14}C-radiolabel as $^{14}CO_2$ or $^{14}CH_4$ was observed in control microcosms regardless of the ^{14}C-substrate.

Biotransformation of [8-^{14}C] caffeine in oxic and anoxic sediments

The impact of oxic and anoxic sediment conditions on the microbial transformation of [8-^{14}C] caffeine was assessed in sediments collected upstream of the WWTP outfall in each stream (Fig. 2). Upstream sediments were selected in order to evaluate the caffeine degradation potential of stream sediment microbial communities that were not under the immediate influence of wastewater discharge. Because all three stream systems were in or near urban centers, however, human waste impacts existed even at the upstream sample locations.

For all three systems, substantial recovery of ^{14}C-radioactivity as $^{14}CO_2$ was observed within 2 d of incubation under oxic conditions (Fig. 2). The Fourmile Creek sediments demonstrated the lowest initial rate of recovery of $^{14}CO_2$, with 53 ± 16 % recovered within 5 d of incubation. In contrast, South Platte River sediments demonstrated a mean recovery of $^{14}CO_2$ greater than 90% of theoretical within 48 h of incubation under oxic conditions. In all cases, the recovery of ^{14}C-radioactivity as $^{14}CO_2$ was greater than 95% of theoretical within 32 d. These results demonstrate that a substantial potential for oxic biotransformation of caffeine exists in stream sediments.

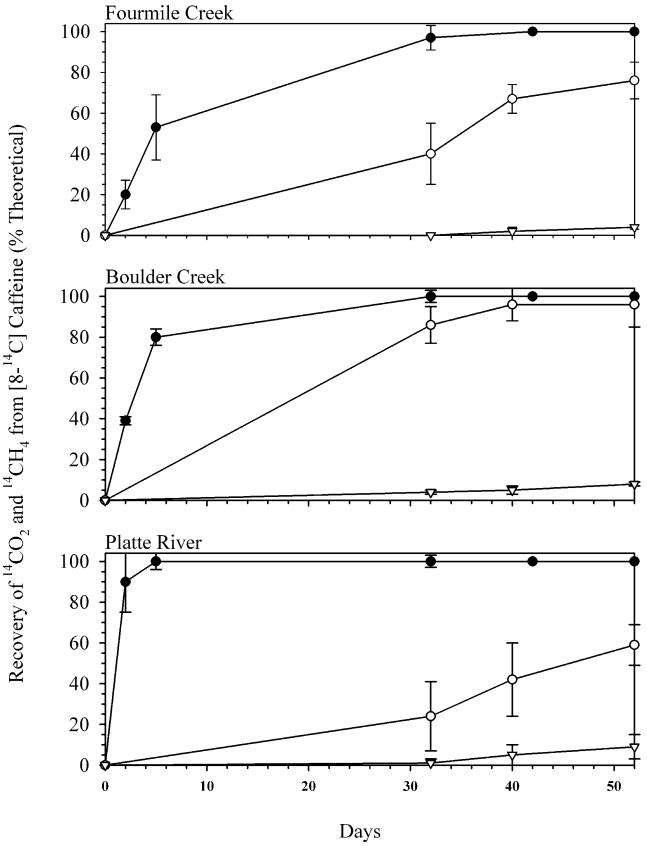

Fig. 2. Recovery of $^{14}CO_2$ (● or ○) and $^{14}CH_4$ (▽) from [8-^{14}C] caffeine in oxic (●) and anoxic (○) microcosms containing sediment from Fourmile Creek, Ankeny, Iowa; Boulder Creek, Boulder, Colorado; and South Platte River, Denver, Colorado, USA. Data are means ± standard deviation for quadruplicate experimental microcosms. No significant recovery of $^{14}CO_2$ or $^{14}CH_4$ was observed in autoclaved or sediment-free control microcosms.

Significant biotransformation of caffeine also was observed in these sediments under anoxic conditions (Fig. 2). The sediments from all three streams were characterized by methanogenic activity, when incubated under anoxic conditions. Even under these reducing conditions, significant recovery of [8-^{14}C] caffeine radioactivity as $^{14}CO_2$ was observed in all sediments within 32 d. Recovery of [8-^{14}C] caffeine activity as $^{14}CH_4$ also was observed in this study in anoxic sediment treatments. The delayed onset and comparatively low magnitude of $^{14}CH_4$ accumulation, however, may indicate methanogenic reduction of $^{14}CO_2$ to $^{14}CH_4$. The cumulative recoveries as $^{14}CO_2$ and $^{14}CH_4$ after 52 d of anoxic incubation ranged from a minimum of 68 ± 10% of theoretical in South Platte River sediment microcosms to 100% of theoretical in Boulder Creek sediment microcosms. These results indicated that a substantial potential for biotransformation of caffeine exists in stream sediments even under methanogenic conditions.

The efficiency of caffeine biotransformation was substantially lower under anoxic conditions than under oxic conditions (Fig. 2). The initial linear rate of $^{14}CO_2$ production was 85 to 98% lower in anoxic sediment microcosms than in the cor-

responding oxic treatments. This observation indicated that the rate of caffeine biotransformation is sensitive to in situ redox conditions. The results of this study suggest that factors affecting the oxygen status of surface-water systems will significantly impact the persistence of caffeine in these environments. The potential for microbial caffeine transformation under anoxic redox conditions other than methanogenesis was not assessed in this study, but merits investigation.

Changes in water-column, caffeine concentrations downstream of the respective WWTP outfalls were assessed previously in the Fourmile Creek and Boulder Creek systems using a Lagrangian sampling approach [12]. Water-column, caffeine concentrations were observed to decrease with increasing distance downstream from the WWTP outfalls and exhibited apparent half-lives of approximately 23 and 5 h in Fourmile Creek and Boulder Creek, respectively (Table 1). The biodegradation of caffeine observed in the current investigation under oxic and anoxic conditions is consistent with those in situ observations and suggests that degradation of caffeine by indigenous microorganisms contributes to the downstream decay in water-column, caffeine concentrations observed in the Fourmile Creek and Boulder Creek systems.

Biotransformation of [8-^{14}C] caffeine in the water column

In general, the experimental approach focused on the biotransformation potential of the streambed sediments, because the purpose of the study was to qualitatively assess the possible impact of biotransformation on the in situ fate of the contaminants and because the per volume biotransformation activity was expected to be greater in the sediments than in the water column. Microbial transformations occurring during infiltration and recirculation of surface water through coarse bed sediments of the type found in all three test streams are a significant determinant of the chemistry of the overlying water column [13]. Infiltration and recirculation of surface water through coarse-grained bed sediments is particularly important immediately downstream of WWTP outfalls in stream systems where WWTP discharge is a substantial contributor to downstream flow (Table 1) and, consequently, a principal determinant of hydraulic heads [13]. In light of the high aqueous solubility of caffeine and its potential use as an indicator of water-borne contamination, however, a limited assessment of the potential for biotransformation of caffeine in the water column was conducted using surface water collected upstream of the respective WWTP outfalls.

Biotransformation of [8-^{14}C] caffeine in the water column was observed in this study, but the efficiency of transformation varied considerably between systems (Fig. 3). In South Platte River water treatments, the recovery of $^{14}CO_2$ was 80 ± 2% of theoretical in 26 d and 100% of theoretical within 46 d of incubation. In contrast, the 3 ± 2% of theoretical final recovery of $^{14}CO_2$ observed in Fourmile Creek water microcosms was just above the radiochemical purity limit of 2% of theoretical for the [8-^{14}C] caffeine stock (Fig. 3). No significant recovery of $^{14}CO_2$ was observed in Boulder Creek water (Fig. 3). The apparent differences in caffeine biodegradation potential between surface-water systems may derive from differences in prior exposure to wastewater compounds. No major WWTP discharges exist in the Fourmile Creek or Boulder Creek systems upstream of the Ankeny WWTP and Boulder 75th Street WWTP outfalls, respectively. Waste effluents are estimated to contribute less than 1% of the flow upstream of the Ankeny and Boulder 75th Street WWTP outfalls. In contrast, the Metro

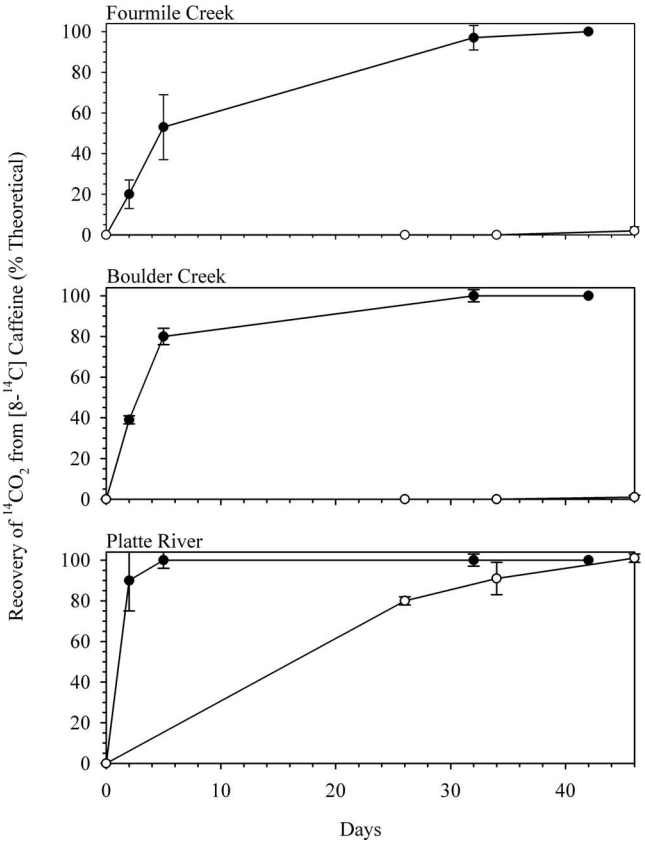

Fig. 3. Recovery of $^{14}CO_2$ from [8-^{14}C] caffeine in oxic microcosms containing sediment (●) or water (○) from Fourmile Creek, Ankeny, Iowa; Boulder Creek, Boulder, Colorado; and South Platte River, Denver, Colorado, USA. Data are means ± standard deviation for quadruplicate experimental microcosms. No significant recovery of $^{14}CO_2$ or $^{14}CH_4$ was observed in autoclaved or sediment-free control microcosms.

Wastewater Reclamation District outfall is located downstream of a number of WWTP outfalls and greater than 50% of the flow in the South Platte River upstream of the Metro Wastewater Reclamation District outfall is effluent derived. Thus, the substantial potential for caffeine biodegradation observed in microcosms containing South Platte River water may be attributable to prior exposure and a resultant acclimation of the indigenous microbial community.

Even in the South Platte River water microcosms, however, the initial linear rate of $^{14}CO_2$ recovery in surface-water microcosms was less than 10% of that observed in corresponding microcosms prepared with an equivalent volume of streambed sediments. Thus, these results indicate that biotransformation of caffeine in the water column can be appreciable in some systems but may not be an important caffeine attenuation mechanism in all surface waters. In light of the considerable variability in the apparent rate of [8-^{14}C] caffeine biotransformation in different surface-water systems, factors affecting caffeine biotransformation in the water column merit further investigation.

Impact of WWTP effluent on biotransformation of [8-^{14}C] caffeine

The discharge of WWTP effluent in surface-water systems has the potential to affect the in situ biotransformation of caffeine in a number of ways. Potential positive effects of waste discharge on in situ biotransformation of [8-^{14}C] caffeine in-

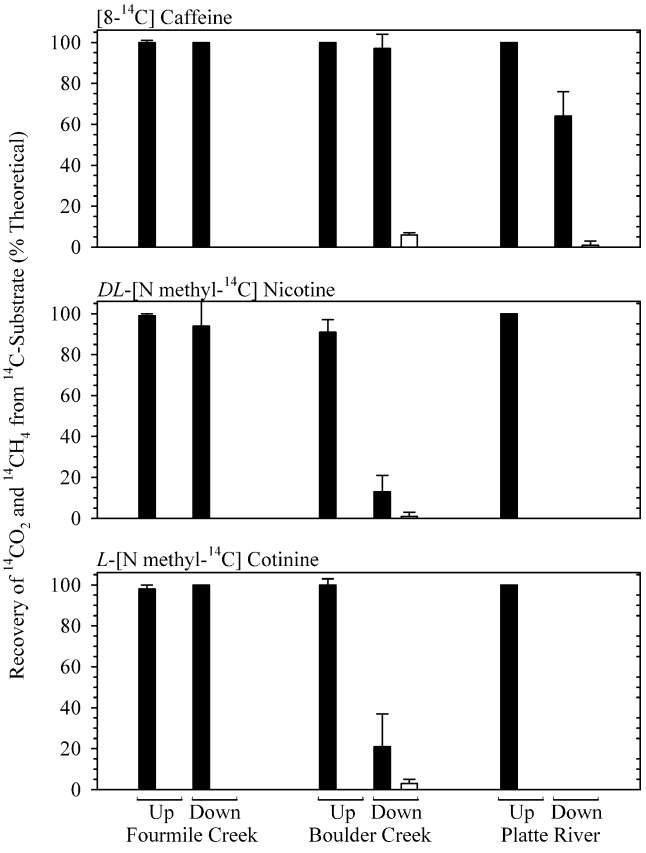

Fig. 4. Recovery of $^{14}CO_2$ (■) and $^{14}CH_4$ (□) from [8-^{14}C] caffeine, L-[*N*-methyl-^{14}C] cotinine, or DL-[*N*-methyl-^{14}C] nicotine in oxic microcosms containing sediment collected upstream or downstream of the wastewater treatment plant at Fourmile Creek, Ankeny, Iowa; Boulder Creek, Boulder, Colorado; and South Platte River, Denver, Colorado, USA. Sediment data are means ± standard deviation for quadruplicate experimental microcosms. No significant recovery of $^{14}CO_2$ or $^{14}CH_4$ was observed in autoclaved or sediment-free control microcosms.

clude increased efficiency of caffeine degradation by indigenous microorganisms induced by ongoing exposure to effluent-associated compounds and introduction of effluent-associated microbial populations capable of efficient degradation of wastewater-specific compounds. Potential negative impacts of waste discharge on caffeine biotransformation include noncompetitive inhibition of microbial transformation due to the introduction of biological inhibitors (biocides, antibiotics, toxic metals, etc.) present in the waste stream; competitive inhibition of contaminant degradation due to the introduction of preferential carbon substrates associated with effluent discharge; and depressed biotranformation due to competitive depletion of efficient terminal electron acceptors such as oxygen and a concomitant shift toward redox conditions less conducive to the biodegradation of complex molecules.

Preliminary insight into the effect of prolonged exposure to WWTP effluent on the potential for caffeine biotransformation was provided by a single time-point comparison of the recovery of [8-^{14}C] caffeine radioactivity as $^{14}CO_2$ and/or $^{14}CH_4$ at 42 d in sediments collected upstream and downstream of the respective WWTP outfalls (Fig. 4) No difference in the recovery of $^{14}CO_2$ from [8-^{14}C] caffeine was apparent in Fourmile Creek sediment treatments after 42 d incubation under oxic conditions. Because the recovery of $^{14}CO_2$ was 100% of theoretical in upstream and downstream Fourmile Creek treatments, intermediate time points would be needed to resolve any differences in [8-^{14}C] caffeine biotransformation rates. Nevertheless, the results indicated that the potential for [8-^{14}C] caffeine biotranformation remained high in both locations. Likewise, comparable recovery of $^{14}CO_2$ was observed in oxic microcosms containing upstream or downstream sediments from Boulder Creek (Fig. 4). Despite the fact that the headspace of microcosms containing downstream sediments from Boulder Creek remained oxic ($[O_2] > 5\%$ v/v), active methanogenesis and significant recovery of $^{14}CH_4$ (6 ± 1% of theoretical) were observed. In contrast to the Fourmile Creek and Boulder Creek sediment treatments, the recovery of $^{14}CO_2$ in downstream South Platte River microcosms was significantly lower than that observed in upstream sediment microcosms after 42 d. Significant methanogenesis also was observed in downstream South Platte River sediment microcosms, even though the headspace concentration of O_2 remained greater than 2% v/v.

These results indicate that the geochemical changes associated with WWTP discharge have the potential to negatively impact caffeine biotransformation in surface-water sediments (Fig. 4). No evidence of a positive effect of WWTP effluent on caffeine biotransformation was observed in this study. On the other hand, the 36% decrease in the mean recovery of $^{14}CO_2$ in downstream South Platte River microcosms (Fig. 4), the methanogenic character of downstream South Platte River microcosms, and the 200% increase in sediment organic content between upstream and downstream sediment collection locations in the South Platte River (Table 1), are consistent with inhibition of [8-^{14}C] caffeine biotransformation activity due to the introduction of preferential carbon substrates associated with effluent discharge and/or the competitive depletion of oxygen and a shift toward anoxic conditions within the sediment column. Likewise, the presence of potential biological inhibitors (including biocides, antibiotics, and heavy metals) in Metro WWTP effluent is consistent with the hypothesized, noncompetitive inhibition of [8-^{14}C] caffeine biotransformation in downstream South Plate River microcosms.

Biotransformation of cotinine and nicotine in oxic sediments

A single time point assessment of the ability of surface-water sediment microorganisms to chemically alter cotinine and its precursor, nicotine, was conducted under oxic conditions (Fig. 4). The fact that complete recovery of L-[*N*-methyl-^{14}C] cotinine radioactivity as $^{14}CO_2$ was observed after 72 d in all oxic microcosms containing sediment collected upstream of the respective WWTP outfalls indicated that the potential for significant cotinine biotransformation existed in all three streams. No statistical difference in the final recovery of $^{14}CO_2$ from L-[*N*-methyl-^{14}C] cotinine was observed between microcosms containing upstream or downstream sediments from Fourmile Creek. However, microcosms containing sediment collected downstream of the Boulder Creek WWTP outfall demonstrated a 76% decrease in the cumulative recovery of L-[*N*-methyl-^{14}C] cotinine activity as $^{14}CO_2$ and $^{14}CH_4$ compared with upstream sediment microcosms. No significant recovery of $^{14}CO_2$ or $^{14}CH_4$ was observed in South Platte River microcosms containing sediments collected downstream of the WWTP outfall. As reported for the [8-^{14}C] caffeine study, downstream sediment microcosms for Boulder Creek and South Platte River were methanogenic despite the presence of greater than 2% v/v of O_2 in the microcosm headspace at the

end of the study. The behavior of DL-[*N*-methyl-^{14}C] nicotine in oxic sediment microcosms was essentially identical to that of L-[*N*-methyl-^{14}C] cotinine (Fig. 4). These results demonstrate that the microbial communities associated with stream sediments have a significant potential for biotransformation of cotinine and its metabolic precursor, nicotine. Moreover, these results confirm the results obtained with [8-^{14}C] caffeine and indicate that prolonged exposure to WWTP effluent can negatively impact the potential for biotransformation of wastewater contaminants in surface-water sediments.

Implications for use as human waste indicators

The results of this investigation demonstrated that the microbial communities indigenous to stream sediments can catalyze substantial transformation of the candidate human waste indicators, caffeine and cotinine, and thereby alter or remove the analytical signatures of these compounds in the environment. Thus, while detection of caffeine and cotinine in surface waters provides compelling evidence of an upstream discharge of human waste, the absence of such indicator compounds is less easily interpreted. Consequently, the potential for in situ biotransformation must be considered if these compounds are employed as tracers to identify the sources and track the fates of wastewater compounds in surface-water systems.

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REFERENCES

1. Buerge IJ, Poiger T, Müller MD, Buser H-R. 2003. Caffeine, an anthropogenic marker for wastewater contamination of surface waters. *Environ Sci Technol* 37:691–700.
2. Glassmeyer ST, Furlong ET, Kolpin DW, Cahill JD, Zaugg SD, Werner SL, Meyer MT, Kryak DD. 2005. Transport of chemical and microbial compounds from known wastewater discharges: Potential for use as indicators of human fecal contamination. *Environ Sci Technol* 39:5157–5169.
3. Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT. 2002. Pharmaceuticals, hormones and other organic wastewater contaminants in U.S. streams, 1999–2000: A national synthesis. *Environ Sci Technol* 36:1202–1211.
4. Standley LJ, Kaplan LA, Smith D. 2000. Molecular tracers of organic matter sources to surface water resources. *Environ Sci Technol* 34:3124–3130.
5. Chen Z, Pavelic P, Dillon P, Naidu R. 2002. Determination of caffeine as a tracer of sewage effluent in natural waters by on-line solid-phase extraction and liquid chromatography with diode-array detection. *Water Res* 36:4830–4838.
6. Seiler RL, Zaugg SD, Thomas JM, Harcroft DL. 1999. Caffeine and pharmaceuticals as indicators of wastewater contamination in wells. *Ground Water* 37:405–410.
7. Barber LB, Leenheer JA, Pereira WE, Noyes TI, Brown GK, Tabor CF, Writer JH. 1995. Organic contamination of the Mississippi River from municipal and industrial wastewater. In Meade RH, ed, *Contaminants in the Mississippi River, 1987–1992*, U.S.G.S. Circular 1133. U.S. Geological Survey, Reston, VA, pp 114–135.
8. Weigel S, Kuhlmann J, Huhnerfuss H. 2002. Drugs and personal care products as ubiquitous pollutants: Occurrence and distribution of clofibric acid, caffeine and DEET in the North Sea. *Sci Total Environ* 295:131–141.
9. Kolpin DW, Skopec M, Meyer MT, Furlong ET, Zaugg SD. 2004. Urban contribution of pharmaceuticals and other organic wastewater contaminants to streams during differing flow conditions. *Sci Total Environ* 328:119–130.
10. Padmanabhan P, Padmanabhan S, DeRito C, Gray A, Gannon D, Snape JR, Tsai CS, Park W, Jeon C, Madsen EL. 2003. Respiration of 13C-labeled substrates added to soil in the field and subsequent 16S rRNA gene analysis of 13C-labeled soil DNA. *Appl Environ Microbiol* 69:1614–1622.
11. Schnoebelen DJ, Kolpin DW, Skopec M. 2007. Chemicals of concern for Iowa's water resources—Iowa's first field research area for emerging contaminants. *J Iowa Acad Sci* (in press).
12. Barber LB, Murphy SF, Verplank PL, Sandstrom MW, Taylor HE, Furlong ET. 2006. Chemical loading into surface water along a hydrological, biogeochemical, and land use gradient: A holistic watershed approach. *Environ Sci Technol* 40:475–486.
13. McMahon PB, Tindall JA, Collins JA, Lull KJ, Nuttle JR. 1995. Hydrologic and geochemical effects on oxygen uptake in bottom sediments of an effluent-dominated river. *Water Resour Res* 31: 2561–2569.
14. Bradley PM, Chapelle FH, Landmeyer JE. 2001. Effect of redox conditions on MTBE biodegradation in surface water sediments. *Environ Sci Technol* 35:4643–4647.
15. Bradley PM, Landmeyer JE, Chapelle FH. 2002. TBA biodegradation in surface-water sediments under aerobic and anaerobic conditions. *Environ Sci Technol* 36:4087–4090.