

Use of an Artificial Sweetener to Identify Sources of Groundwater Nitrate Contamination

by W.D. Robertson¹, D.R. Van Stempvoort², J.W. Roy², S.J. Brown², J. Spoelstra^{2,3}, S.L. Schiff³, D.R. Rudolph³, S. Danieleescu^{2,4}, and G. Graham⁵

Abstract

The artificial sweetener acesulfame (ACE) is a potentially useful tracer of waste water contamination in groundwater. In this study, ACE concentrations were measured in waste water and impacted groundwater at 12 septic system sites in Ontario, Canada. All samples of septic tank effluent ($n=37$) had ACE $>6 \mu\text{g/L}$, all samples of groundwater from the proximal plume zones ($n=93$) had ACE $>1 \mu\text{g/L}$ and, almost all samples from the distal plume zones had ACE $>2 \mu\text{g/L}$. Mean mass ratios of total inorganic nitrogen/ACE at the 12 sites ranged from 680 to 3500 for the tank and proximal plume samples. At five sites, decreasing ratio values in the distal zones indicated nitrogen attenuation. These ratios were applied to three aquifers in Canada that are nitrate-stressed and an urban stream where septic systems are present nearby to estimate the amount of waste water nitrate contamination. At the three aquifer locations that are agricultural, low ACE values ($<0.02\text{--}0.15 \mu\text{g/L}$) indicated that waste water contributed $<15\%$ of the nitrate in most samples. In groundwater discharging to the urban stream, much higher ACE values ($0.2\text{--}11 \mu\text{g/L}$) indicated that waste water was the likely source of $>50\%$ of the nitrate in most samples. This study confirms that ACE is a powerful tracer and demonstrates its use as a diagnostic tool for establishing whether waste water is a significant contributor to groundwater contamination or not.

Introduction

Nitrate is considered the most pervasive groundwater contaminant worldwide (Spalding and Exner 1993). In order for management and reduction of nitrate contamination to be successful, the sources of the nitrate contamination must be established. In rural areas, agricultural operations are often the largest loading source of nitrogen, but other sources are also usually present in these areas. In a 1991-1992 screening survey of 1292 farmstead wells in southern Ontario, 14% were found to exceed the drinking water criteria of $10 \text{ mg/L NO}_3^- \text{-N}$ (Goss et al. 1998). Although agriculture was the largest source of nitrogen loading in this study, septic systems were also present at these rural farmstead sites; their minimum regulated setback distance from water supply wells

is only 15 m in Ontario (OSCIA 1999). Thus, onsite waste water disposal likely also contributed to the nitrate contamination observed in this study.

In urban settings, nitrate sources may include the use of lawn fertilizers, spills from industrial sites, leakage of waste water from aging sewers, and legacy loading from past use of septic systems. Consequently, there has been considerable interest in the development of tracer tools to distinguish sources of groundwater nitrate contamination. Nitrate isotopes (N^{15} and O^{18}) have been widely used to distinguish between nitrate derived from chemical fertilizers and sanitary waste water (Kreitler et al. 1979; Heaton 1986; Aravena et al. 1993; Wassenaar 1995). However, there is overlap between the isotopic composition of different nitrate sources, and processes such as NH_4^+ volatilization, soil zone exchange, and denitrification can alter the isotopic signature of the source material. In addition, when manure is used in agricultural operations, the isotopic signature can be similar to that of sanitary waste water. As a result, source identification becomes difficult in many cases (Xue et al. 2009). Assessing the oxygen isotope ratio of nitrate can assist in identifying sources and nitrogen cycling, but it does not distinguish between waste water and manure-derived nitrate.

Boron, which is present as a minor constituent in laundry detergents, has been used successfully as a sanitary waste water tracer in both groundwater (Vengosh

¹Corresponding author: Department Earth and Environmental Sciences, University of Waterloo, Waterloo, ON N2L 3G1, Canada; (519) 885 1211 ext. 36800; fax: (519) 746 7484; wroberts@uwaterloo.ca

²Environment Canada, Burlington, Ontario, Canada.

³Department Earth and Environmental Sciences, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada.

⁴Agriculture and Agri-Food Canada, Fredericton, New Brunswick, Canada.

⁵Environment Canada, Pacific & Yukon Region, Vancouver, British Columbia, Canada.

Received March 2015, accepted December 2015.

© 2016, National Ground Water Association.

doi: 10.1111/gwat.12399

et al. 1994; Bassett et al. 1995; Bussey and Walter 1996) and surface water (Chatelat and Gaillardet 2005). However, B concentrations in waste water (about 0.3–1 mg/L) are usually higher than background values by only a small factor (e.g., about 5, Bassett et al. 1995) and some natural waters such as brackish and irrigation-impacted water can have B values as high as that of waste water. Other tracers such as chloride (Cl^-), commonly used to identify groundwater contamination, are often not definitive for source identification because there are numerous other potential anthropogenic sources (e.g., road salt).

A number of trace organic constituents used in pharmaceutical and personal care products are relatively unique to sanitary waste water, and several of these that persist during sewage treatment have also been utilized as waste water tracers (e.g., carbamazepine, Fenz et al. 2005; Nakada et al. 2008). Recently, the artificial sweetener acesulfame (ACE), which is widely used in the food and beverage industry and particularly in diet soft drinks, has also received considerable attention as a potential tracer. ACE is a substituted ring compound ($\text{C}_4\text{H}_5\text{NO}_4\text{S}$) that is highly soluble and it is not prone to sorption (Lange et al. 2012). It occurs ubiquitously in most sanitary waste waters at the microgram per liter level (which is several orders of magnitude higher than current detection limits [DLs]) and persists during sewage treatment (Lange et al. 2012) and in a variety of surface water (Buerge et al. 2009; Wolf et al. 2012; Spoelstra et al. 2013) and groundwater environments (Buerge et al. 2009; Van Stempvoort et al. 2011a, 2013, Roy et al. 2014). However, laboratory tests have indicated the ACE is prone to degradation under UV irradiation and under natural sunlight conditions (Gan et al. 2014; Sang et al. 2014) and can generate intermediate products that may be persistent.

In a recent study of a groundwater plume from a septic system servicing a large campground in southern Ontario, Canada (Long Point site, Robertson et al. 2013), ACE was found to be elevated along the entire 200 m mapped length of the plume by a factor of more than 1000 compared to the background DL value (0.008 $\mu\text{g/L}$). This plume was about 15 years old, based on tritium/helium age dating, yet ACE concentrations remained relatively uniform (18–75 $\mu\text{g/L}$) throughout the entire length of the plume, indicating that ACE could persist in groundwater flow systems over decadal timescales and, secondly, that ACE in Canadian sanitary waste water has likely been within 50% of current values for at least 15 years (since 1995). ACE was approved for use in Canada in 1988 (Van Stempvoort et al. 2011a). The consistently high ACE concentrations observed in this plume study indicated that this compound had the potential to be a powerful tracer capable of revealing even very small inputs of waste water.

To further unlock this potential, the objectives of the current study were to (1) assess the consistency of ACE concentrations, and its concentration ratios with other waste water constituents, particularly Cl^- and N, for various sanitary waste waters, and (2) determine

its persistence in a range of groundwater environments. To accomplish this, ACE was measured at 12 septic system sites in Ontario, Canada, in both the septic tank effluent and associated groundwater plumes. These sites range from single households (seasonal cottages) to communal facilities (campgrounds, resort, townhouse complex, described below). The sampled plume zones encompassed a broad range of subsurface redox conditions and sediment types. We examined the mass ratios of inorganic nitrogen/ACE (TIN/ACE) in this large dataset to determine whether this ratio could be used as a diagnostic tool for fingerprinting and quantifying nitrate contamination originating from sanitary waste water (septic) sources. Examples are presented, utilizing three nitrate-stressed aquifers in Canada and an urban stream, to demonstrate how this ratio can be used to establish whether waste water is a significant contributor to the observed nitrate contamination. Previous studies have suggested the use of artificial sweetener ratios as a means for revealing the persistence of other waste water contaminants (e.g., Spoelstra et al. 2013; Van Stempvoort et al. 2013, Roy and Bickerton 2014); however, this is the first study of which we are aware that directly applies this ratio method to quantify a contaminant source.

Site Selection

The 12 septic systems examined in this study are all located in southern and central Ontario and include seven large seasonal-use campgrounds, a tourist resort, a communal residence (townhouse complex), two seasonal-use family cottages, and a trailer depot that receives highly concentrated waste water from recreational motor homes. All of the systems are at least 10 years old and most are of conventional design, consisting of a septic tank and associated infiltration bed with perforated infiltration pipes installed into the vadose zone at 0.5–1 m depth. Two of the sites (family cottage, GB, and communal residence, BU) also provide tertiary treatment using aerobic fixed-bed filters prior to effluent discharge to the infiltration beds, and the latter site also includes a denitrification module. Most of these are communal systems that receive relatively high waste water loading rates with the exception of the two family cottage sites (Georgian Bay [GB] and Killarney [JL]), which receive lower loading. The effluent at the seasonal-use sites could be affected by seasonal usage patterns, such as increased consumption of diet soft drinks in the summer, but otherwise the effluent can be considered typical of sanitary waste water generated in Canada. The exception is the trailer depot site, which receives higher strength waste water because of the use of water-limiting fixtures in the mobile homes that use this facility. At two of the sites, the potable water supply undergoes water softening and consequently the effluent has elevated Cl^- concentrations (MA and BU sites). These septic systems are located in a variety of sediment and terrain types. Permeable sand aquifers are present at three sites (Long Point Park [LP], Blind Bay Park [KN], Lake

Joseph Resort [CB]); silt is present at one site (Killarney Cottage [JL]); and infiltration beds exist at seven sites constructed of imported coarse filter sand because either low-permeability clay sediments are present (Killarney Park [KP], Kilcoursie Park [KR], McRae Park Trailer Depot [MC], Mara Park [MA], McRae Park [MB] sites) or shallow bedrock is present (Craigleith Park [CR], Georgian Bay Cottage [GB] sites). Water table depths range from 1 to 4 m. The site characteristics are summarized in Table 1, and additional details are provided in Appendix S1, Supporting Information.

Methods

Sampling and Analyses

In the current study, most sampling of the septic system sites was carried out from June 2011 to October 2013, but earlier results from the LP campground site, which was sampled from September 2008 to October 2010 (Van Stempvoort et al. 2011b; Robertson et al. 2013), are also included. Sampling focused on three discrete zones at these sites: (1) direct effluent from the septic tank and associated tertiary treatment modules; (2) shallow groundwater within 1 m below the water table and lying directly underneath the infiltration beds (proximal plumes); and (3) distal plume zones located beyond the edges of the tile beds and up to 200 m downgradient, where varying amounts of dilution with background groundwater was encountered. Tank samples were obtained only from 7 of the 12 sites.

Groundwater samples were obtained using multilevel bundle piezometers at most sites, consisting of 2-6 sampling tubes set at varying depths. These were installed into a single borehole using direct-push casing or a manual soil auger, without the use of filter sand or sealing material. Five to thirty-five multilevel piezometer bundles were installed at each site (Appendix S1). Several of the sites have considerable monitoring detail aligned along the plume centrelines (e.g., LP site, with 90 monitoring points extending up to 200 m from the tile bed).

Septic tank samples were collected from dedicated sampling tubes installed at the outlets of the tanks. Groundwater samples were collected using a peristaltic pump with silicone tubing and were filtered (0.45 μm) inline prior to atmospheric exposure. Samples for NH_4^+ analyses were acidified to pH 5-6 immediately after collection, whereas samples for anions, NO_3^- , and artificial sweeteners were left untreated. All samples were immediately transported to the laboratory where they were either refrigerated at nearly 4 °C or frozen (sweetener and NH_4^+ samples) until analysis. Nitrate, Cl^- , and SO_4^{2-} were analyzed at the Environmental Geochemistry Laboratory (EGL), Department of Earth and Environmental Sciences, University of Waterloo, Waterloo, ON, by ion chromatography using a Dionex ICS-90 instrument (Dionex, Sunnyvale, California), which provided an analytical reporting limit of <0.5 mg/L for each of these parameters. NH_4^+ was also analyzed at EGL using a colorimetric technique

with a Beckman DU 530 spectrophotometer, which provided a DL of <0.05 mg/L N. ACE was analyzed at the Canada Centre for Inland Waters, Burlington, ON, by ion chromatography (Dionex 2500 system) coupled with tandem mass spectrometry (AB Sciex QTRAP 5500 triple-quadrupole mass spectrometer; AB Sciex LLC, Framingham, Massachusetts), following the methods described by Van Stempvoort et al. (2011a). This technique provided a DL of 0.008 $\mu\text{g/L}$ and a quantification limit (QL) of 0.02 $\mu\text{g/L}$ for ACE. Further details on how the DL and QL values were established are presented in Appendix S2.

Results and Discussion

Variability and Persistence of ACE

Table 2 shows the mean ACE concentrations at the 12 sites, considering both the septic tank effluent and proximal zones of groundwater plumes, which ranged from 9.9 to 265 $\mu\text{g/L}$. Also shown are the mean concentrations of Cl^- , the commonly used tracer parameter for contaminant plumes, and total inorganic nitrogen (TIN, $\text{NH}_4^+ + \text{NO}_3^- - \text{N}$). Organic nitrogen was not considered in this study but is likely only a minor N component in these plumes (with the possible exception of the trailer depot [MC] site) because plume dissolved organic carbon values are low (<10 mg/L). With the exception of one site (JL), at the six sites (LP, KP, KR, CB, JL, GB) where data is available from both the septic tank and the proximal plumes zones, the mean Cl^- values in the proximal zones remained within 65% of the tank values (Table S1), indicating that dilution by mixing in the proximal zones was relatively minor (<35%). Although almost all of the samples from distal plume zones also had highly elevated ACE (>2 $\mu\text{g/L}$), Cl^- values indicated more variable amounts of dilution, and, consequently, the distal zones are not included in the site mean values given in Table 2. The mean Cl^- concentrations in the septic tank/proximal zones at the MA site (740 mg/L) and the communal residence (BU) site (332 mg/L) were much higher because water softeners were used to treat potable water supply. The mean TIN values ranged from 15 to 88 mg/L at these sites (Table 2) and are considered typical of domestic waste water, except the trailer depot site (MC) had a much higher TIN of 256 mg/L because of the high-strength effluent at that site.

All samples of septic tank effluent ($n = 37$) had ACE >6 $\mu\text{g/L}$, and all samples from the proximal plume zones ($n = 93$) had ACE >1 $\mu\text{g/L}$ (Figure 1), demonstrating the ubiquitous presence of this sweetener in the sanitary waste water at these sites, even at one site where the residents were unaware of their consumption of diet food or beverage products (JL cottage site). Overall, ACE values between 15 and 25 $\mu\text{g/L}$ were found most frequently (Figure 2), representing 43% of the septic tank samples and 38% of the proximal zone samples (excluding the high-strength trailer depot [MC] site). Site mean values for 11 of the 12 sites ranged from 9.9 to 62 $\mu\text{g/L}$ (Table 2), reflecting differences in consumption

Table 1
Summary of Site Characteristics at 12 Septic System Sites in Ontario, Canada¹

Site	KN	KR	KP	CR	MC	LP
Waste water source	Campground	Campground	Campground	Campground	Trailer depot	Campground
Persons using	180	120	130	150	40	250
Tile bed size (m ²)	1300	2500	2700	1200	700	225
Age (years)	16	20	8	12	12	20
Sediment type	Sand	Imported sand	Imported sand	Imported sand	Imported sand	Sand
Loading rate (cm/day)	1.5	0.5	0.5	1.4	0.4	6
Number of monitoring points	70	39	30	50	45	210

Site	MA	MB	BU	JL	GB	CB
Waste water source	Campground	Campground	Communal residence	Cottage	Cottage	Resort
Persons using	100	120	70	3	3	85
Tile bed size (m ²)	2400	770	480	40	30	2400
Age (years)	12	26	12	30	7	>15
Sediment type	Imported sand	Imported sand	Imported sand + tertiary	Silt	Imported sand	Sand
Loading rate (cm/day)	1.3	5	4	0.3	0.3	0.4
Number of monitoring points	20	20	6	16	16	60

¹Additional site information is available in Supporting Information.

Table 2
Summary of Site Mean Cl⁻, NO₃⁻-N, TIN, ACE, and TIN/ACE Values at Each of the 12 Septic System Sites

Site	<i>n</i>	Cl ⁻ (mg/L)	NO ₃ ⁻ -N (mg/L)	TIN (mg/L)	ACE (µg/L)	TIN/ACE
LP campground	24	71	78	88	57	1600
KP campground	25	31	17	28	19	1500
KR campground	14	28	3	15	15	940
KN campground	4	39	61	79	27	3500
CR campground	4	33	12	12	16	990
MA campground	2	740	23	44	27	1900
MB campground	4	63	62	62	25	2700
MC trailer depot	17	237	60	256	265	980
CB resort	12	121	23	28	9.9	3300
BU communal res.	2-7	332	39	44	18	2200
JL cottage	4	54	0.5	52	62	840
GB cottage	5	49	25	44	54	680
Mean of all sites					30 ²	1800

¹Values represent samples from both the septic tank effluent and proximal groundwater plumes; NO₃⁻-N values are from the proximal plume only.

²Excludes trailer depot (MC) site.

patterns of diet food and beverage products at individual sites. In contrast, the trailer depot site (MC) had a much higher mean value of 265 µg/L. All proximal plume and tank samples had ACE concentrations that were >50 times higher than the QL for this artificial sweetener (0.02 µg/L); thus this parameter has the potential to reveal even very small waste water impacts, diluted to 2% or less in groundwater that otherwise has not been impacted by waste water. The values measured in the septic tank effluents in this study (6-103 µg/L, seven sites, *n* = 37) are similar to those reported for sanitary waste waters in other areas: for example, 12-43 µg/L in 10 Swiss municipal sewage treatment plant effluents (Buerge et al. 2009); 6-19 µg/L in nine samples of municipal waste water from Jasper, AB and Whitehorse, YK, Canada (Van Stempvoort et al. 2011a); and 9-40 µg/L in effluent

from seven German municipal waste water treatment plants (Lange et al. 2012). Thus, ACE appears to occur ubiquitously in sanitary (household and municipal) waste water at the microgram per liter level in many parts of the world. Our data also include a number of relatively high values (>50 µg/L, Figure 2), which may reflect seasonal effects such as increased consumption of diet soft drinks during the summer. However, not all of the seasonal sites have elevated ACE values (e.g., 15-19 µg/L mean values at the KP, KR, and CR campgrounds, Table 2). At the six sites where samples were obtained from both septic tanks and proximal plumes (LP, KP, KR, CB, JL, GB sites, Figure 1), ACE concentrations in both zones remained similar (considering dilution evident at the JL site, Table S1). This indicated that ACE persisted during migration through the 1-3-m-thick vadose zones present

at these sites and that the proximal zone values were generally representative of the waste water, with some minor dilution. Aerobic conditions were present in the vadose zones at all sites (with the possible exception of the JL site), as indicated by the complete or partial conversion of effluent NH_4^+ to NO_3^- in the proximal zones (Table 2). Effluent residence times in the proximal zones are estimated to vary from several weeks to several months at these sites.

At the eight sites where the distal plumes were also sampled (LP, KP, KR, CR, MC, CB, JL sites, Figure 1), ACE persisted throughout these plume zones (most samples $>2 \mu\text{g/L}$, $n=44$) at concentrations generally as expected based on simple dilution. There were two exceptions. At the LP Park site, distal zone ACE concentrations declined by about 65% to approximately $20 \mu\text{g/L}$ (Figure 1), which is somewhat lower than would be expected based solely on dilution (Robertson et al. 2013). This portion of the plume is about 10-15 years old (since infiltration from the septic bed) based on tritium/helium age dating (Robertson et al. 2013), and this is much older than distal zones at the other sites, which are likely <5 years old. Robertson et al. (2013) suggested that this decrease in the distal zone at LP could be the result of one or more factors such as dilution estimates being perturbed by other sources of Cl^- present at the site, increased usage rates of ACE in recent years, or possibly slow degradation of ACE.

The other site that exhibited lower than expected ACE concentrations in the distal zone was the Lake Joseph resort (CB) site. Values decreased by about 80% to a mean of $0.4 \mu\text{g/L}$ (Figure 1), which is much lower than would be expected from dilution alone (Table S1), and several very low values ($\sim 0.04 \mu\text{g/L}$) were measured. However, this tile bed is located only 50 m from a highway that receives heavy applications of de-icing salt in the winter, and the shallow groundwater here has elevated Cl^- . Consequently, using Cl^- as a plume tracer and to assess dilution is problematic at this site. On the other hand, this was the only site investigated where the groundwater plume was distinctly aerobic throughout (dissolved oxygen $[\text{DO}] > 2 \text{ mg/L}$), which raises the possibility that ACE may have experienced enhanced degradation under aerobic conditions. Although aerobic degradation of ACE has apparently not yet been documented in groundwater, Buerge et al. (2011) observed ACE degradation in aerobic laboratory microcosm tests with half-lives of 3-49 days. The soil slurries used in these tests were relatively carbon rich however (2-18% OC), which may have enhanced degradation. Sang et al. (2014) observed degradation of ACE under UV irradiation and identified 12 chromatograph peaks that were attributed to breakdown products, and Gan et al. (2014) identified a number of breakdown products that were generated under natural sunlight conditions. These breakdown products have apparently yet to be reported in groundwater, however (Lange et al. 2012).

At the other seven sites, distal zones were suboxic ($\text{DO} < 1 \text{ mg/L}$) or had denitrifying (LP, JL sites) or SO_4^{2-} -reducing conditions present (MC site, Figure 1), and at

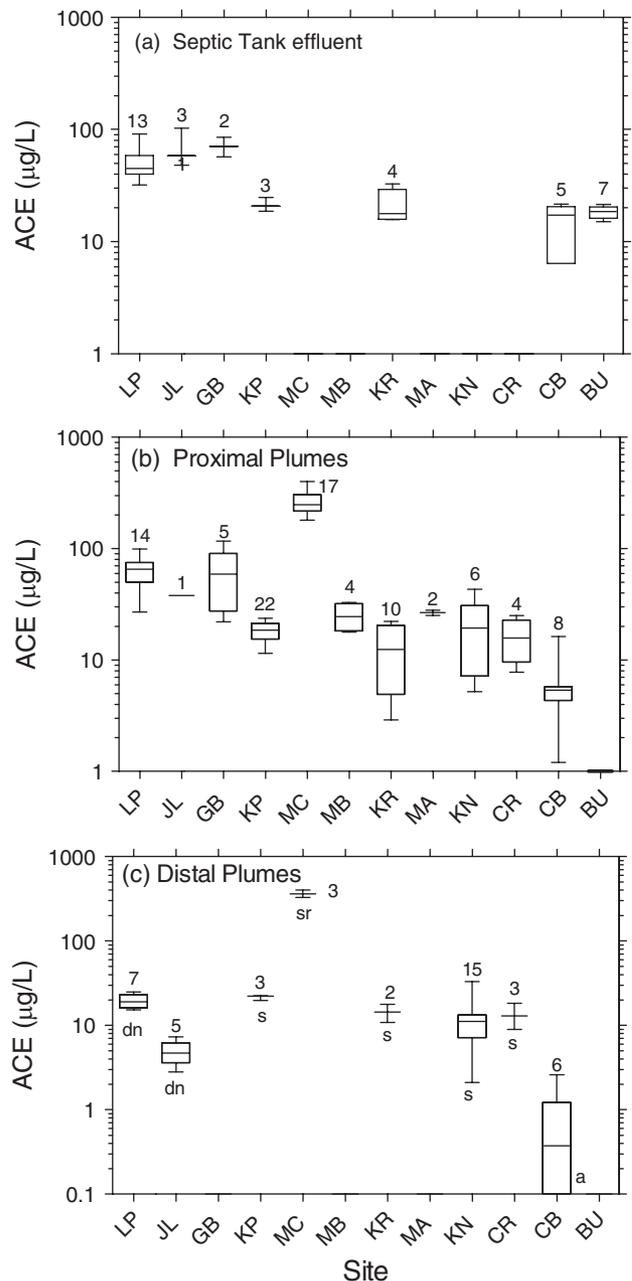


Figure 1. Boxplots showing ranges of acesulfame measured at 12 septic system sites: (a) septic tank effluent, (b) proximal plume zones (below tile bed), and (c) distal plume zones (beyond tile bed). Numbers above boxplots indicate number of samples, horizontal lines indicate median values, boxes indicate upper and lower quartiles, and whiskers indicate full range of measured values at each site. Letters below boxplots indicate the redox status of the distal plumes: a, aerobic; s, suboxic; dn, denitrifying; sr, sulfate reducing.

these sites ACE was generally persistent (Figure 1). Future studies should further evaluate the persistence of ACE particularly in aerobic groundwater environments.

Most of the samples in this study were collected from the filter bed sands and associated sand aquifers. However, at two sites the septic plumes occurred in less permeable fine sand, silt, and clay sediments (JL and GB cottage sites) and at these sites organic compounds could

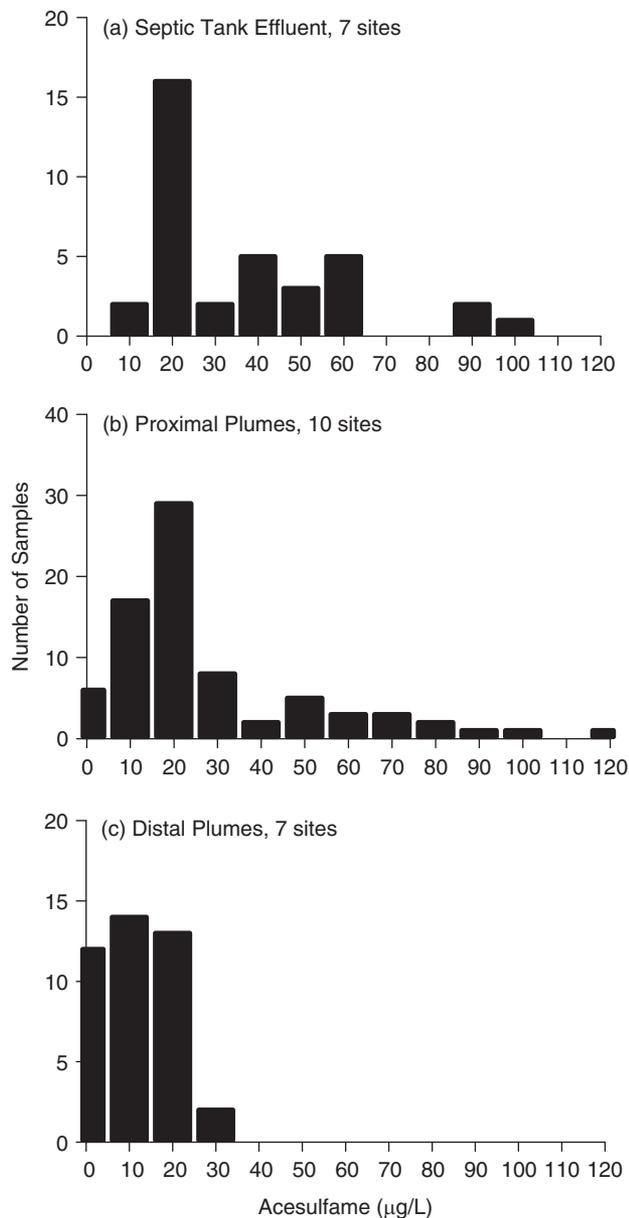


Figure 2. Frequency distribution of ACE concentrations in (a) septic tank effluent, (b) proximal plumes, and (c) distal plumes, considering 11 of the 12 septic system sites. Trailer depot site (MC) is not included.

experience reduced mobility due to increased sorption effects. At both of these sites, the Cl^-/ACE ratios were higher in the plumes by factors of 1.3-2.7 compared to the tank values (Table S1), which suggests that ACE may be less mobile than Cl^- in these finer grained sediments. Although researchers have stated that ACE sorption is not important in coarse-grained soils (Lange et al. 2012), additional sorption studies are likely warranted for finer grained soils.

Using TIN/ACE Ratios to Determine Waste Water N Input and Persistence

These data show the ubiquitous presence of ACE in Canadian sanitary waste water and its persistence in groundwater plumes derived from septic beds. By

examining the ratios of ACE to other waste water contaminants of interest, ACE can potentially provide insight into the persistence of these other contaminants in groundwater flow systems (Van Stempvoort et al. 2011a). At five of the sites (LP, KP, KR, JL, GB), decreasing ratios of TIN/ACE from tank/proximal zone values of nearly 1000-2000 (Table 2) to lower values of 30-800 in the distal plume zones (Table S1) indicated that inorganic N was being consumed (likely transformed to N_2 , N_2O , or organic N). At the LP site, inorganic N consumption from both denitrification and anammox has been documented previously (Robertson et al. 2012), and these reactions could also be occurring at the other sites that are suboxic.

The site mean values of the TIN/ACE ratios, considering both tank and proximal plume samples, vary from 680 to 3500 at the 12 sites (Table 2). These values range by a factor of 5 and are likely representative of the majority of sanitary waste water generated in Canada. As a demonstration, we used this range of TIN/ACE ratios to obtain estimates of the amount of waste water nitrate present in three aquifers in Canada (Woodstock, ON; Abbotsford, BC; and Black Brook, NB), which are nitrate-stressed and have NO_3^- -N levels that approach or exceed the drinking water limit of 10 mg/L. These are unconfined or semiconfined sand and gravel or fractured bedrock aquifers that are used for potable water supply, and therefore these elevated nitrate levels are of concern. All are in agricultural terrain, but the Woodstock and Abbotsford sites are located near urban centers, and thus multiple potential nitrate sources could be present. Also included are samples of shallow riparian zone groundwater discharging to a stream that passes through an urban area where septic systems are in use (Hewitt Creek, ON) (Appendix S1).

In applying the TIN/ACE ratio method to estimate the potential waste water nitrate contribution, several assumptions or simplifications have been made:

- 1 ACE is not degraded or adsorbed appreciably along the groundwater flow system. If this is not true, the calculation would tend to underestimate the potential amount of nitrate derived from waste water.
- 2 The loss of TIN due to processes such as denitrification and anammox in the distal plume is negligible. Actually, TIN attenuation beyond the waste water source would result in an overestimation of the potential contribution of nitrate derived from waste water.
- 3 Sources of TIN, other than waste water, are not accompanied by ACE. This is almost certainly true, except in the case of nitrate derived from municipal landfill leachate (e.g., Roy et al. 2014).
- 4 The groundwater being analyzed does not predate the widespread incorporation of ACE in food and beverage products consumed in the area.
- 5 The TIN/ACE ratio in waste water has not changed significantly over time, or if so, has remained within the range of 680 to 3500 found in this study.

Table 3**Examples of Using ACE to Estimate Wastewater Nitrate Contribution in Three Nitrate-Stressed Aquifers in Canada (Woodstock, Black Brook, and Abbotsford Sites) and an Urban Stream¹**

Well Depth (m)	DO (mg/L)	Cl ⁻ (mg/L)	NO ₃ ⁻ -N (mg/L)	ACE (µg/L)	N/ACE	Waste Water NO ₃ ⁻ -N	
						(mg/L)	%
Woodstock, ON: Agricultural							
WO11-6 m	7	28	8.4	<0.02	>420,000	<0.01-<0.07	<1
WO11—8 m	6	45	13.0	<0.02	>650,000	0.01-0.07	<1
WO11—10 m	6	28	8.2	<0.02	>410,000	0.01-0.07	<1
WO11—13 m	5	46	12.4	<0.02	>620,000	<0.01-<0.07	<1
WO11—18 m	1	13	0.4	<0.02	>20,000	<0.01-<0.07	<3-<18
Woodstock, ON: Agricultural and septic systems							
WO12—8 m		28	5.3	0.093	57,000	0.06-0.33	1-6
WO12—10 m	6	30	5.3	0.060	88,000	0.04-0.21	1-4
WO12—13 m	6	31	5.6	0.11	51,000	0.07-0.39	1-7
WO12—15 m	3	26	4.6	0.10	46,000	0.070-0.35	2-8
WO12—19 m	4	26	3.2	0.14	23,000	0.09-0.49	3-15
Abbotsford, BC: Agricultural							
94SH26—11.5 m	8.3	11	16	<0.02	>800,000	<0.01-<0.07	<1
94SH29—15.6 m	8.2	9.6	13	<0.02	>650,000	<0.01-<0.07	<1
94Q14—6.3 m	9.5	4.6	8.1	0.031	260,000	0.02-0.11	<1-1
94Q20—12.6 m	8.8	5.9	7.9	<0.02	>395,000	<0.01-<0.07	<1
94Q27—18.8 m	4.8	11	12	<0.02	>600,000	<0.01-<0.07	<1
ABB2—5.3 m	7.9	21	16	0.041	390,000	0.03-0.14	<1
Black Brook, NB: Agricultural							
PZ3—2-14 m	8.1	22	8.0	<0.02	>400,000	<0.01-<0.07	<1
PZ7—85 m ²	2.4	29	0.4	0.08	5000	0.05-0.28	13-70
PZ8—18 m	7.7	20	9.5	0.05	190,000	0.03-0.18	<1-2
PZ10—1-69 m	10.8	23	8.1	<0.02	>405,000	0.01-<0.07	<1
PZ10—2-34 m	9.9	20	9.7	0.15	65,000	0.10-0.53	1-5
PZ11—24 m	9.0	9.7	4.7	<0.02	>235,000	<0.01-<0.07	<2
Hewitt Creek, ON: Groundwater discharging to urban stream							
HC-S1—0.30 m	1.3	75	2.5	0.48	5200	0.3-1.7	13-68
HC-S2—0.43 m	0.6	152	7.7	6.4	1200	4.3-22	56100
HC-S3—0.38 m	0.6	248	8.5	8.4	1000	5.6-29	66-100
HC-S4—0.55 m	0.5	291	8.9	10.7	830	7.2-37	81-100
HC-S5—0.42 m	0.6	145	7.4	4.2	1800	2.8-15	38-100
HC-S6—0.30 m	0.5	37	3.6	0.24	15,000	0.2-0.8	4-23

¹Groundwater discharging to an urban stream (Hewitt Creek). Range considers the variations in site mean values for TIN/ACE at the 12 septic system sites (680-3500, Table 2). All of the sites except Hewitt Creek, are unconfined or semiconfined sand and gravel or bedrock aquifers. Dominant land use at each site is indicated. Additional site and sample details are provided in Appendix S1.

²Enriched values of nitrate N¹⁵ and O¹⁸ indicate this well is affected by denitrification.

Table 3 gives DO, Cl⁻, NO₃⁻-N, and ACE concentrations measured in 6-10 wells from each site and then estimates the potential amount of waste-water-sourced nitrate, which is indicated by the TIN/ACE ratios. A potential range of waste water N percentages is given based on the range of ratio values observed at the 12 septic system sites (680-3500). At the three aquifer locations where land use is predominantly agricultural (Woodstock, Abbotsford, and Black Brook), low ACE values (<0.02-0.15 µg/L, Table 3), and extremely high N/ACE ratios (5000 to >800,000, Table 3) suggest that waste water contributes <15% of the nitrate present in most samples. Interestingly, at the second Woodstock site (WO12), a small town with nearly 150 homes on septic systems is located 1-2 km upgradient, and at this location ACE concentrations were generally higher (0.06-0.14 µg/L, Table 3). In groundwater discharging to the urban stream (Hewitt Creek, ON) with

septic systems present nearby, elevated ACE values of 0.2-11 µg/L and N/ACE ratio values (832-5200, Table 3) that were similar to the septic plumes values of 680-3500 indicate that waste water was the dominant source of nitrate loading and potentially represented >50% of the nitrate present in most samples. Many of the samples in Table 3 are from shallow water table zones at <15 m depth and likely represent recharge that originated during the post-1988 period when ACE was used as a sweetener in Canada. The Woodstock wells were age-dated using tritium/helium analysis in an earlier study (Sebol 2004) and estimated to be <25 years old. Although the wells at the Black Brook site are somewhat deeper (14-85 m), these wells are in fractured bedrock with presumably low effective porosity values, which would lead to large vertical migration rates in response to recharge. Thus, it is likely that these wells also sample post-1988 groundwater.

All three of the agricultural sites are dominantly aerobic with $\text{DO} > 3 \text{ mg/L}$ in most wells (Table 3). Thus, with the exceptions of wells WO11-18 and PZ7-85, which have lower DO values of 1 and 2.4 mg/L, respectively, it is unlikely that NO_3^- levels have been substantially affected by denitrification at these sites. This is also supported by NO_3^- isotopic evidence at the Abbotsford (Wassenaar 1995) and Woodstock sites (Heagle 2000), where isotopic enrichment that would be expected from denitrification activity was not found. Many aquifers that have NO_3^- contamination problems also have DO present, and at these sites the use of the ACE ratio method becomes more straightforward because the complicating factor of possible nitrate attenuation (denitrification or anammox) is less likely to occur.

Figure 3 shows the relationship between TIN/ACE and Cl/ACE ratios at the three aquifer sites and the urban stream site (Hewitt Creek), in comparison to the values from the 12 septic system sites. All of the aquifer samples plot above and to the right of the septic system box, indicating additional sources of both Cl^- and N that are not related to sanitary waste water. In addition to N, fertilizers contain potash (KCl), and thus fertilizers likely represent a separate source of both Cl^- and N in these aquifers. At Hewitt Creek, a second source of Cl^- is evident, but most of the TIN/ACE ratios fall within the range expected for sanitary waste water. This evidence supports previous interpretations at the site, indicating that septic systems are likely a major source of N, but additionally there is substantial impact from road salt use (Van Stempvoort et al. 2013).

Although the results of this study allow only an approximate estimate of waste water inputs because site mean TIN/ACE ratio values varied by a factor of 5, the ratio method nonetheless has the potential to reveal whether waste water could be a significant contributor to observed nitrate contamination.

Conclusions

A survey of 12 sites in Ontario indicated that the artificial sweetener ACE occurred ubiquitously in sanitary (septic) waste water which had associated groundwater plumes at concentrations generally in the range 5-100 $\mu\text{g/L}$. This is more than 250 times higher than our analytical QL for this sweetener. Based on these results and previous studies, this compound appears mobile and persistent in a variety of groundwater environments. It is inferred to be a powerful tracer that is useful for revealing the presence of waste water in groundwater and for establishing the relative persistence of other waste water contaminants such as NO_3^- . By applying the range of mean TIN/ACE ratios measured at these sites (680-3500), it was inferred that waste water represented <15% of the nitrate loading in three NO_3^- -stressed aquifers in Canada, whereas in groundwater discharging to an urban stream, waste water was likely the dominant nitrate source. Such evidence can guide management decisions

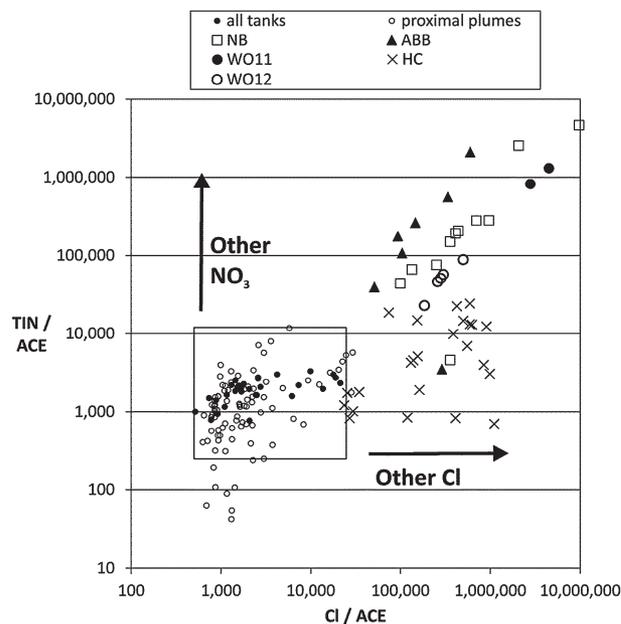


Figure 3. Ratios of TIN/ACE vs. Cl/ACE in the three nitrate-stressed aquifers (Abbotsford, ABB; Woodstock, WO; Black Brook, NB) and the urban stream (Hewitt Creek, HC), compared to ratios observed in the septic tank effluent and proximal plumes at the 12 septic system sites. Additional samples not included in Table 3 but with detectable ACE ($>0.008 \mu\text{g/L}$) are also shown. The vertical and horizontal arrows indicate expected shifts for samples containing additional TIN and Cl^- , respectively, from sources other than waste water.

aimed at reducing nitrate contamination, in this case by shifting attention away from some post-1988 sanitary waste water sources at the aquifer sites and toward other potential sources, particularly agricultural operations. Future studies should also include individual household sites, which were not well represented in the current study.

Acknowledgments

The authors would like to thank Environment Canada for funding support, which contributed to the success of this study, and the site owners who kindly provided access to the study sites. Particularly, the Ontario Ministry of Natural Resources provided access to a number of the sites.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Detailed Site Descriptions for the 12 Septic System and Four Aquifer Study Sites.

Appendix S2. Methodology for Determination of Acesulfame DL and QA/QC Procedures.

Table S1. Mean Waste Water and Groundwater Compositions at the 12 Septic System Sites.

References

- Aravena, R., M.L. Evans, and J.A. Cherry. 1993. Stable isotopes of oxygen and nitrogen in source identification of nitrate from septic systems. *Ground Water* 31, no. 2: 180–186.
- Bassett, R.L., P.M. Buszka, G.B. Davidson, and D. Chong-Diaz. 1995. Identification of groundwater solute sources using boron isotopic composition. *Environmental Science & Technology* 29, no. 12: 2915–2921.
- Burge, I.J., H.R. Buser, M. Kahle, M.D. Müller, and T. Poiger. 2009. Ubiquitous occurrence of the artificial sweetener acesulfame in the aquatic environment: An ideal chemical marker of domestic wastewater in groundwater. *Environmental Science & Technology* 43, no. 12: 4381–4385.
- Burge, I.J., M. Keller, H.R. Buser, M.D. Müller, and T. Poiger. 2011. Saccharin and other artificial sweeteners in soils: Estimated inputs from agriculture and households, degradation, and leaching to groundwater. *Environmental Science & Technology* 45, no. 2: 615–621.
- Bussey, K.W., and D.A. Walter. 1996. Spatial and temporal distribution of specific conductance, boron, and phosphorus in a sewage-contaminated aquifer near Ashumet Pond, Cape Cod, Massachusetts. Open-File Report 96-472. Marlborough, Massachusetts: U.S. Geological Survey.
- Chetelat, B., and J. Gaillardet. 2005. Boron isotopes in the Seine River, France: A probe of anthropogenic contamination. *Environmental Science & Technology* 39, no. 8: 2486–2493.
- Fenz, R., A.P. Blaschke, M. Clara, H. Kroiss, D. Mascher, and M. Zessner. 2005. Monitoring of carbamazepine concentrations in wastewater and groundwater to quantify sewer leakage. *Water Science and Technology* 52, no. 5: 205–213.
- Gan, Z., H. Sun, R. Wang, H. Hu, and P. Zhang. 2014. Transformation of acesulfame in water under natural sunlight: joint effect of photolysis and biodegradation. *Water Research* 64: 113–122.
- Goss, M.J., D.A.J. Barry, and D.L. Rudolph. 1998. Contamination of Ontario farmstead domestic wells and its association with agriculture: 1. Results from drinking water wells. *Journal of Contaminant Hydrology* 32, no. 3–4: 267–293.
- Heagle, D.J. 2000. Nitrate geochemistry of a regional aquifer in an agricultural landscape, Woodstock, ON. MSc thesis, University of Waterloo, Waterloo, ON.
- Heaton, T.H.E. 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: A review. *Chemical Geology (Isotope Section)* 59, no. 1: 87–102.
- Kreitler, C.W., S.E. Rogone, and B.G. Katz. 1979. 15N/14N ratios of ground-water nitrate, Long Island, New York. *Ground Water* 16, no. 6: 404–409.
- Lange, F.T., M. Scheurer, and H.J. Brauch. 2012. Artificial sweeteners—A recently recognized class of emerging environmental contaminants: A review. *Analytical and Bioanalytical Chemistry* 403, no. 9: 2503–2518.
- Nakada, N., K. Kiri, H. Shinohara, A. Harada, K. Kuroda, S. Takizawa, and H. Takada. 2008. Evaluation of pharmaceuticals and personal care products as water-soluble markers of sewage. *Environmental Science & Technology* 42, no. 17: 6347–6353.
- OSCIA (Ontario Soil and Crop Improvement Association). 1999. Septic smart! New ideas for household septic systems on difficult sites. Technical Brochure. Guelph, ON: OSCIA.
- Robertson, W.D., D.L. Van Stempvoort, D.K. Solomon, J. Homewood, S.J. Brown, J. Spoelstra, and S.L. Schiff. 2013. Persistence of artificial sweeteners in a 15-year-old septic system plume. *Journal of Hydrology* 477: 43–54.
- Robertson, W.D., T. Moore, J. Spoelstra, L. Li, R. Elgood, I.D. Clark, S.L. Schiff, R. Aravena, and J.D. Neufeld. 2012. Natural attenuation of septic system nitrogen by anammox. *Ground Water* 50, no. 4: 541–553.
- Roy, J.W., D.R. Van Stempvoort, and G. Bickerton. 2014. Artificial sweeteners as potential tracers of municipal landfill leachate. *Environmental Pollution* 184: 89–93.
- Roy, J.W., and G. Bickerton. 2012. Toxic groundwater contaminants: an overlooked contributor to urban stream syndrome? *Environmental Science & Technology* 46, no. 2: 729–736.
- Sang, Z., Y. Jiang, Y.K. Tsoi, and K. Sze-Yin Leung. 2014. Evaluating the environmental impact of artificial sweeteners: A study of their distributions, photodegradation and toxicities. *Water Research* 52: 260–274.
- Sebol, L. 2004. Use of CFCs and their replacement compounds for age dating groundwater. PhD thesis, Department of Earth and Environmental Sciences, University of Waterloo, Waterloo, ON.
- Spalding, R.F., and M.E. Exner. 1993. Occurrence of nitrate in groundwater—A review. *Journal of Environmental Quality* 22, no. 3: 392–402.
- Spoelstra, J., S.L. Schiff, and S.J. Brown. 2013. Artificial sweeteners in a large Canadian river reflect human consumption in the watershed. *PLoS One* 8, no. 12: e82706. DOI:10.1371/journal.pone.0082706.
- Van Stempvoort, D.R., J.W. Roy, S.J. Brown, G. Bickerton, and E. Sverko. 2013. An artificial sweetener and pharmaceutical compounds as co-tracers of urban wastewater in groundwater. *Science of the Total Environment* 461–462: 348–359.
- Van Stempvoort, D.R., S.J. Brown, J.W. Roy, and G. Bickerton. 2011a. Artificial sweeteners as potential tracers in groundwater in urban environments. *Journal of Hydrology* 401, no. 1–2: 126–133.
- Van Stempvoort, D.R., W.D. Robertson, and S.J. Brown. 2011b. Artificial sweeteners in a large septic plume. *Ground Water Monitoring & Remediation* 31, no. 4: 95–102.
- Vengosh, A., K.G. Heumann, S. Juraske, and R. Kasher. 1994. Boron isotope application for tracing sources of contamination in groundwater. *Environmental Science & Technology* 28, no. 11: 1968–1974.
- Wassenaar, L.I. 1995. Evaluation of the origin and fate of nitrate in the Abbotsford Aquifer using the isotopes of ¹⁵N and ¹⁸O in NO₃⁻. *Applied Geochemistry* 10, no. 4: 391–405.
- Wolf, L., C. Zwiener, and M. Zemmann. 2012. Tracking artificial sweeteners and pharmaceuticals introduced into urban groundwater by leaking sewer networks. *Science of the Total Environment* 403: 8–19.
- Xue, D., J. Botte, B. De Baets, F. Accoe, A. Nestler, P. Taylor, O. Van Cleemput, M. Berglund, and P. Boeckx. 2009. Present limitations and future prospects of stable isotope methods for nitrate source identification in surface- and groundwater. *Water Research* 43, no. 5: 1159–1170.