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Article

Artificial sweeteners in surface waters from Asian, African and Middle Eastern countries: Utility as molecular markers and water pollution status in 2010–2019

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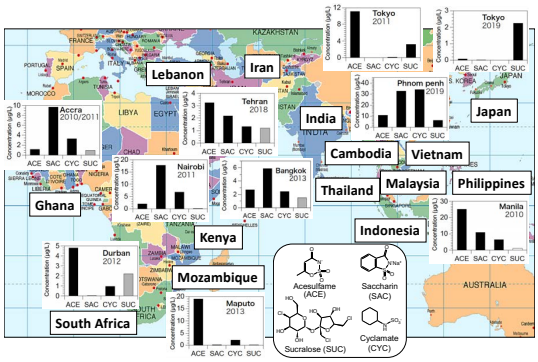
ABSTRACT

To explore the utility of artificial sweeteners (Acesulfame: ACE; sucralose: SUC; saccharin: SAC; cyclamate: CYC) as molecular markers and to establish a historical benchmark of the water pollution status in the 2010 decade, 272 surface water samples, including river water, sewage, and livestock wastewater, were collected from African (Ghana,



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Kenya, Mozambique, South Africa), Asian (Vietnam, Indonesia, Thailand, Philippines, Cambodia, Malaysia, India, and Japan) and Middle Eastern (Iran and Lebanon) countries during 2010–2019 and were analyzed for the artificial sweeteners by high performance liquid chromatograph with tandem mass spectrometer coupled with online solid-phase extractor. ACE was most frequently detected (87% of the samples) due to its widespread use and lower detection limit (2 ng/L). SUC was less frequently detected (51%) because of its lower sensitivity and detection limit of 100 ng/L, and less widespread use in some low-income countries, probably due to its higher price. SAC and/or CYC were abundant in surface waters of most countries, despite their biodegradable nature. This was ascribed to intensive usage of SAC and CYC and extensive inputs of untreated sewage to rivers due to limited installation of sewage treatment systems. Concentrations of the sweeteners were higher in urbanized rivers (~1 μg/L to ~100 μg/L) than those in corresponding suburban and rural sites, reflecting spatial patterns of sewage inputs. Furthermore, concentrations of the sweeteners were positively correlated with those of linear alkylbenzenes (LABs) for all countries except Malaysia. These results confirm the utility of artificial sweeteners as indicators of sewage inputs. SAC and CYC could be indicators of untreated sewage inputs. ACE has proved to be the most sensitive and reliable marker to assess inputs of sewage, i.e., both treated and untreated, to surface waters. However, ACE concentrations in secondary effluents and receiving waters in Tokyo decreased two orders of magnitude from 2011 to 2019, while sucralose did not show such a marked decrease. This is probably due to the upgrading of activated sludge treatment, i.e., introducing anaerobic-anoxic-oxic (A2O) treatment and resultant higher removal of ACE. Therefore, the measurement of multiple markers, including SUC, is recommended for long-term monitoring.

Key words: molecular markers; artificial sweeteners; river water; Africa; Asia; Middle East; linear alkylbenzenes; monitoring; A2O; sewage treatment, antibiotics

INTRODUCTION

Anthropogenic molecular markers are organic compounds specific to certain pollution sources and are useful for identifying sources of the pollutants, to assess the magnitude of anthropogenic impacts, and to trace transport pathways of contaminants (Takada and Eganhouse, 1998; Harwood, 2014). Sewage is a large source of pollutants and pathogens (Kennish, 1997), and molecular markers of sewage, i.e., sewage markers, are important. Coprostanol, derived from human feces (Leeming et al., 1996), and linear alkylbenzenes (LABs) contained in synthetic detergents (Takada and Ishiwatari, 1987) have been used as sewage markers since the 1960s (e.g., Murtaugh and Bunch, 1967; Goodfellow et al., 1977; Readman et al., 1986; Leeming et al., 1996; Peng et al., 2005; Martins et al., 2014) and 1980s (e.g., Eganhouse et al., 1983; Takada and Ishiwatari, 1987; Hong et al., 1995; Gustafsson et al., 2001; Ni et al, 2009; Wang et al., 2012; Thomes et al., 2019; Alkhadher et al., 2023), respectively. Although coprostanol and LABs are persistent, they can be removed through sewage treatment and adsorption by soil because of their hydrophobic and particle-reactive nature. This may limit their applicability as molecular markers in a wide range of environments, including treated-sewage-impacted water bodies and groundwater. Some pharmaceuticals and personal care products (PPCPs) are persistent and hydrophilic and have been used as hydrophilic sewage markers since the 2000s (Clara et al., 2004; Glassmeyer et al., 2005; Buerge et al., 2006; Nakada et al., 2008). Artificial sweeteners (ASs) were first proposed as hydrophilic sewage markers in 2009 (Buerge et al., 2009).

The present study evaluated the utility of 4 ASs (acesulfame: ACE, sucralose: SUC, saccharin: SAC, cyclamate: CYC; Fig. 1) as sewage markers. They are added to beverages and foods to give sweetness instead of sugar, and are widely used around the world. Since they are not absorbed in the human body and are not metabolized, they are found in human urine and feces. ACE and SUC are persistent in the environment, whereas SAC and CYC are biodegradable. Therefore, ACE and SUC have been used as water-soluble sewage markers. ACE was first proposed as an ideal sewage marker because its removal was not observed during sewage treatment (Buerge et al., 2009). However, its removal by sewage treatment was observed in several countries (Cardenas et al., 2016; Castronovo et al., 2017; Yang et al., 2017; Shreve and Brennan, 2019) and Van Stempvoort et al. (2020) concluded that ACE can be degraded by advanced biological sewage treatment. On the other hand, SUC was proposed as an ideal sewage marker because of its abundance in waters in the USA and its more resistant nature in sewage treatment (Oppenheimer et al., 2011). However, SUC is an expensive sweetener (ALIC, 2014) and may not be widely used in low-

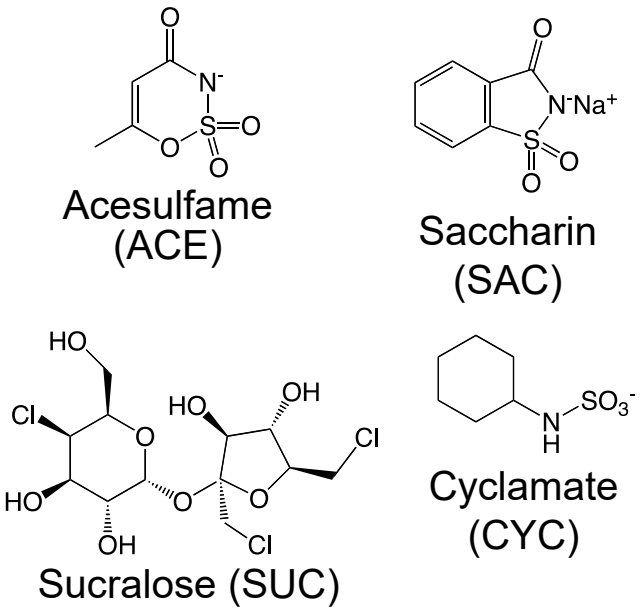

Fig. 1. Chemical structures of the target artificial sweeteners

er-income countries.

Studies on ASs in surface water have been conducted mostly in European countries (e.g., Scheurer et al., 2009; Scheurer et al., 2011; Richards et al., 2017), North America (e.g., Oppenheimer et al., 2011; James et al., 2016; Cantwell et al., 2019; Van Stempvoort et al. 2020) and Australia (Sidhu et al., 2013). Limited information is available on the presence and distribution of ASs in surface water of tropical Asian, African and Middle Eastern countries. ASs were measured as molecular markers for surface waters in China (Gan et al., 2013; Yang et al., 2017; Yang et al., 2018) and Singapore (Tran et al., 2014). The study areas in China and Singapore were partially or fully covered by sewage collection systems. The study areas in the present study, except for Japan, were rarely or limitedly covered by sewage collection systems (Table S1). As a result, the direct discharge of untreated sewage to surface waters is of concern. Thus, we studied the distribution of the 4 ASs in 228 surface water samples collected from 13 countries in tropical Asia, Africa and the Middle East in 2011–2019. Measurement of ASs in the areas covered by this study could reveal a unique pattern of occurrence, e.g., dominance of biodegradable ASs. The analytical results could contribute towards establishing a relationship between sewage inputs and ASs occurrence pattern.

As a reference, ASs were measured in surface water and sewage effluents collected in two major rivers (i.e., the Tamagawa River and Sumidagawa River) in Tokyo, Japan. The entire human population (100%) in the Sumidagawa River catchment (Bureau of Sewerage Tokyo, 2020a) and virtually entire population (99%) in the Tamagawa River catchment (Bureau of Sewerage Tokyo, 2020b) has been serviced by modern (primary + secondary) sewage treatment since 2008. Thus, comparison of concentrations and composition of ASs in Japan and those in tropical Asian, African, and Middle Eastern waters would highlight the utility of ASs as sewage markers in terms of sewage treatment and would contribute to generalizing the relationship between sewage inputs and ASs occurrence.

To evaluate the utility of ASs as molecular markers of sewage, LABs, a traditional sewage marker, were measured in the same water samples except for the samples in Tokyo. LABs are a feedstock of linear alkylbenzenesulfonates (LAS) which are widely used anionic surfactants for synthetic detergents. Unreacted feedstock LABs are carried to the final products and a small but significant amount of LABs are contained in synthetic detergent products (Eganhouse et al., 1983; Takada and Ishiwatari, 1987). LABs are highly hydrophobic and less biodegradable than LAS and have been used as molecular marker of sewage inputs to aquatic systems (Eganhouse et al., 1983; Takada and Ishiwatari, 1987; Isobe et al., 2004; Magam et al., 2016; Mizukawa et al., 2017). LABs are efficiently removed during sewage treatment (Takada and Ishiwatari, 1987; Isobe et al., 2004) and, therefore, LABs can be utilized as markers of untreated sewage inputs. Comparing the occurrence of ASs with LABs could highlight the unique feature of ASs as molecular markers. To our knowledge, such comparison has not been conducted. Furthermore, the occurrence patterns of the ASs were compared with those of antibiotics (sulfonamide, macrolide, and tetracyclines), as reported in our previous papers (Segura et al., 2015; Suzuki et al., 2021) to establish a source-identification relationship between ASs and antibiotics. These comparisons would strengthen the utility of ASs as molecular markers.

Water pollution is closely related to social factors, such as population size, economic status, availability of wastewater infrastructure, mitigation measures, as well as natural factors such as rainfall and geographical configuration. Because of temporal changes in the social factors, environmental pollution is temporarily varied and, therefore, periodic monitoring of environmental pollution is important. The present study archives water pollution in tropical Asian, African, and Middle Eastern countries in the decade of 2010–2019, to provide benchmark data for future monitoring, especially, sewage treatment systems in these countries are yet to be expanded or installed. Thus, this study, could provide a solid benchmark to evaluate the effectiveness of future sewage treatment installations.

The objectives of our study were 1) to explore the utility of the ASs as molecular markers of sewage pollution in countries with no or limited installation of sewage treatment systems; and 2) to provide benchmark data on sewage pollution in Asian, African, and Middle Eastern countries in 2010–2019.

MATERIALS AND METHODS

CHEMICALS

Native ASs standards, i.e., acesulfame K, sucralose, saccharin Na, and cyclamate Na were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Isotopically-labeled ASs i.e., acesulfame-d_4, sucralose-d_6, and saccharin-$^{13}C_6$ were purchased from Toronto Research Chemicals Inc. (Toronto, Canada). Cyclamate-d_{11} was purchased from C/D/N Isotope Inc. (Quebec, Canada). Ammonium acetate was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). A LAB mixture consisting of m-C_n AB (m: 2–7; n = 10–14) was kindly provided by Mitsubishi Petrochemical Co., Ltd, where "m" in "m-C_n AB" means phenyl attachment position on the alkylchain and "n" means number of alkylcarbons. 1-phenyloctane (1-C_8 AB), 1-phenylnonane (1-C_9 AB), 1-phenyldecan (1-C_{10} AB), and 1-phenyldodecane (1-C_{12} AB) were purchased from Aldrich Chemical Company, WI, USA. 1-phenylundecane (1-C_{11} AB) and 1-phenyltridecane (1-C_{13} AB) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 1-phenyltetradecan (1-C_{14} AB) were purchased from Tokyo Chemical Industry, Co Ltd. (Tokyo, Japan).

SAMPLE COLLECTION AND ONSITE TREATMENT

In total, 272 water samples were collected from African (Ghana, Kenya, Mozambique, South Africa), Asian (Vietnam, Indonesia, Thailand, Philippines, Cambodia, Malaysia, India, and Japan), and Middle Eastern (Iran and Lebanon) countries. Samples were collected from 2010–2019, once or in two years for each country except Japan, where samples were collected basically every year from 2011 to 2019, and in Vietnam, where samples were collected in 2014 and 2017. Details of sampling dates and locations are provided in Table S2. The status of sewage collection and treatment systems in

individual cities was obtained through the Joint Monitoring Program (JMP: https://washdata.org), related references, and database and the other references, which are summarized in Table S1. The samples were grab water samples, including sewage treatment plant (STP) influents and effluents, river water, and agricultural wastewater. The surface water samples were collected using a stainless steel bucket and were stored in 1 L glass amber bottles and transported cool to laboratories in the corresponding countries.

The water samples were filtered through pre-baked glass fiber filter (Whatman GF/F), using an all-glass equipment. Approximately 70 mL of the filtrate aliquot was stored in amber polyethylene (PE) bottles and frozen in freezers in the laboratories. Filters containing suspended solids from ~50 to ~500 mL of water samples were wrapped in aluminum foil and frozen. The filtrates and filters were shipped frozen to Japan. In some sampling campaigns, ~100 mL aliquots of water samples were filtered on site using a Hydrophilic PTFE filter (0.45 μm × 25 mm; Millex LH) from Millipore (MA, USA) and plastic syringe. The filtrates were stored in amber PE bottles and shipped frozen to Japan.

ARTIFICIAL SWEETENERS ANALYSIS

Filtrates were thawed at room temperature just before the analysis of the ASs in the laboratory in Japan. ASs were analyzed using a high performance liquid chromatograph equipped with tandem quadrupole mass spectrometers and an online solid phase extraction (SPE) system (online SPE-LC-MS/MS) according to Buerge et al. (2008), with slight modification. The instrument was a Quantum Access MS/MS with binary pump of Accela and equipped with an autosampler (Thermo Scientific). Water samples (9.9 mL) were spiked with an isotopically-labeled AS surrogate standard (0.1 mL of 100 ng/mL each) as surrogates and mixed well. About 1 mL aliquots were taken in a 1 mL PE vial and placed into the autosampler. When the HPLC run started, 0.5 mL of the water sample was taken and transferred to a stainless-steel sample loop with 1 mL volume. The sample water was then delivered to an SPE cartridge consisting of 2 pieces of precolumn (Gemini C_{18}, 5 μm particle size, 4 mm × 3 mm) by using a pump at a rate of 0.4 mL of 1 mM ammonium acetate aqueous solution per minute to trap ASs in the SPE cartridge. After 1.5 min online extraction, the eluent (mixture of 1 mM ammonium acetate aqueous solution and methanol with the gradient described below at 0.2 mL/min) was backflushed, and trapped ASs were injected into the analytical column (Gemini C_{18}, 5 μm particle size, 2 mm × 150 mm). HPLC analysis was performed with linear gradient from 1 mM ammonium acetate aqueous solution (A) at 1.6 min to methanol (B) at 21.6 min with an initial isocratic of 100% A, final flushing for 3.4 min with 100% B, and 11 minutes of reestablishment to the initial condition with 100% A at 0.2 mL/min.

Native ASs and corresponding surrogates were ionized by electrospray in the negative mode and detected and quantified on selected reaction monitoring (SRM) with parent mass > product mass (collision energy), as follows, ACE: $m/z = 162 > 82$ (15 eV) and 78 (35 eV); d_4-ACE: $m/z = 166 > 86$ (15 eV) and 78 (35 eV); SAC: $m/z = 182 > 42$ (24 eV) and 106 (16 eV); $^{13}C_6$-SAC: $m/z = 188 > 42$ (15 eV) and 106 (16 eV); CYC: $m/z = 178 > 80$ (35 eV); d_{11}-CYC: $m/z = 189 > 80$ (40 eV) and 146 (13 eV); SUC: $m/z = 394 > 359$ (12 eV); d_6-SUC: $m/z = 401 > 365$ (12 eV). The summed area of signals for two transitions were used for quantification when two transitions were available. Calibration curves (50, 100, 500, 1,000 ng/L) were used to quantify the analytes. Measured concentrations were corrected for recovery of the surrogate standards. Limit of detection (LOD) and limit of quantification (LOQ) were determined by replicate (n = 5) analyses of a successively diluted standard solution. LOD was defined as a signal to noise ratio of 3. LOD was 2 ng/L for ACE, 10 ngL for SAC, 5 ng/L for CYC, and 100 ng/L for SUC for most of samples. However, for the samples from Iran and Cambodia, which were analyzed just after the intensive maintenance of the LC-MS/MS by the supplier's technician, better LOD for SAC and SUC were obtained at 5 ng/L and 8 ng/L, respectively. LOQ was determined as concentration where the relative standard deviation of the peak areas of replicate analyses was 25% or larger. LOQ was 5 ng/L for ACE, 50 ngL for SAC, 40 ng/L for CYC, and 500 ng/L for SUC for the analyses of most samples, except those from Iran and Cambodia, where the LOQ for SAC and SUC were obtained at 40 ng/L and 200 ng/L, respectively. Travel blanks prepared using distilled water were run with all sample runs, but no ASs more than LOD were detected. The reproducibility of ASs analyses was confirmed by using replicate (n = 4) analyses of secondary effluents from STP-K in Tokyo, Japan. Relative standard deviations of ACE and SUC, which were both frequently detected, were 4% and 6%, respectively. The effluent samples were diluted 20 times and spiked with native and surrogate ASs standards (0.1 mL of 100 ng/mL each), respectively, and analyzed to calculate the recoveries of ASs. The recoveries of ACE, SAC, CYC, and SUC were 94 ± 6%, 89 ± 7%, 91 ± 2%, and 91 ± 10%, respectively.

LINEAR ALKYLBENZENES ANALYSIS

The suspended particles were analyzed using the method described by Sugiura et al. (2021). The filters containing suspended solids were freeze-dried. As surrogate standards, 1-C_n ABs (n: 8–14) were spiked onto the filter. The filters were successively extracted with 15 mL each of methanol, dichloromethane (DCM) / methanol (1 : 1, v/v), and DCM by ultra-sonication for 15 minutes each. The combined extracts were evaporated to dryness using a rotary evaporator. The extracts were re-dissolved into 1 mL of hexane and subjected onto a 10% H_2O-deactivated silica gel column (1 cm × 5 cm) with activated copper (1 cm × 1 cm) on top. LABs were eluted with 15 mL of hexane/DCM (3 : 1, v/v) and the fraction was concentrated to ~0.5 mL and transferred to a 1 mL glass ampoule. The solvent was evaporated under a gentle stream of nitrogen to dryness and the residue was then dissolved into small volume (50 μL to 200 μL) of isooctane containing biphenyl-d_{10} as an injection internal standard. A 1 μL aliquot was injected into a gas chromatograph equipped with a mass spectrometer (GC-MS; Agilent 6890 with 5973). The detailed conditions of the GC-MS are described in Hartmann et al. (2000). Concentrations of LABs were recovery-corrected using surrogates (1-C_n ABs) with the same carbon number.

The sum of 26 LAB congeners with C_{10}–C_{14} alkylcarbons is expressed as total LABs.

To examine contamination through all the processes, including sample collection, filtration, and analytical procedure, distilled water in a glass bottle was brought to the field and subjected to all the analytical processes as a travel blank. Three times the amounts found in the travel blanks divided by the water volume (500 mL for rural samples) were considered the LOQ. LOQ of total LABs was ~80 ng/L. Reproducibility was examined by replicate analyses (n = 4) of sediment from Tokyo Bay. The relative standard deviation of individual LAB congeners was 5–20%. Recovery was examined by spiking the sediment with native standards of LABs and by subjecting the sediment to all the analytical processes. The recovery of individual LAB congeners ranged from 70% to 98%.

RESULTS AND DISCUSSION

DETECTION AND CONCENTRATIONS OF ARTIFICIAL SWEETENERS

All data generated in this study are provided in Table S3. Among the 4 ASs, ACE was detected in 238 of the 272 samples, a detection frequency of 88% (Table 1). This frequent detection is probably due to the widespread use of ACE and its relatively persistent nature together with its low detection limit (2 ng/L). On the other hand, SUC was less frequently detected, i.e., 138 samples with detection frequency of 51%. SUC is similarly or more persistent compared to ACE (Oppenheimer et al., 2011; Van Stempvoort et al., 2020; Takada et al., 2022). However, its detection limit (~100 ng/L in most cases) was two orders of magnitude higher than that for ACE. The low sensitivity could be a reason for the lower frequency of detection in our study. In addition, the lower detection frequency can be ascribed to lower concentrations of SUC in sewage. In sewage influents, i.e. untreated sewage, SUC showed the lowest concentrations amongst the 4 ASs (Fig. 2), suggesting less usage of SUC in the countries in the present study. This may be due to higher price of SUC than ACE. For example, in 2014 the industrial market price of SUC was 2 to 11 times higher than of ACE (ALIC, 2014). SUC was proposed as an ideal marker of sewage in the USA because of its consistent detection in water samples with known wastewater inputs (Oppenheimer et al., 2011). However, because most of the countries in our study are low- or middle-income countries, the use of this expensive sweetener may have been depressed. SAC and CYC were abundant in sewage (Fig. 2). However, due to their relatively higher detection limit (5–10 ng/L) and their degradable nature, their detection frequency (~60%) was less than that of ACE (Table 1).

For individual countries in the present study, concentrations of ASs were higher in urban rivers (sum of 4 ASs: ~1 μg/L to ~100 μg/L) than in suburban (~0.01 μg/L to ~10 μg/L) and rural rivers (~0.01 μg/L ~1 μg/L) (Fig. 3). The concentrations in urban rivers in the Asian, African and Middle Eastern countries were one or more orders of magnitude higher than those reported in other areas of the world. Buerge et al. (2009) reported ASs concentrations in Swiss

Table 1 Detection frequency of artificial sweeteners

		number of observations	ACE	SAC	CYC	SUS
Manila	2010	12	12	8	7	2
Jakarta	2010	13	13	13	12	1
Ghana	2010	12	9	8	7	3
Ghana	2011	14	9	9	9	4
Kenya	2011	10	7	5	5	1
Durban	2012	21	20	8	14	11
Mozambique	2013	16	15	6	9	4
Thailand	2013	12	12	11	11	2
Thailand	2014	14	8	12	8	9
Vietnam-2014	2014	20	17	5	11	9
Malaysia	2016	9	9	9	9	6
Vietnam-2017	2017	17	15	4	7	1
India	2017	11	11	10	5	4
Tokyo-2011	2011	6	5	0	3	5
Tokyo-2013/2014	2013	4	4	2	0	4
Tokyo-2019	2019	10	10	5	0	10
STP-Effluent T	2011–2019	9	9	3	2	9
STP-Effluent K	2011–2019	15	15	9	12	15
Iran	2018	23	19	16	13	19
Cambodia	2019	20	15	12	15	16
Lebanon	2019	4	4	4	4	3
Total		272	238	159	163	138
% detection			88%	58%	60%	51%

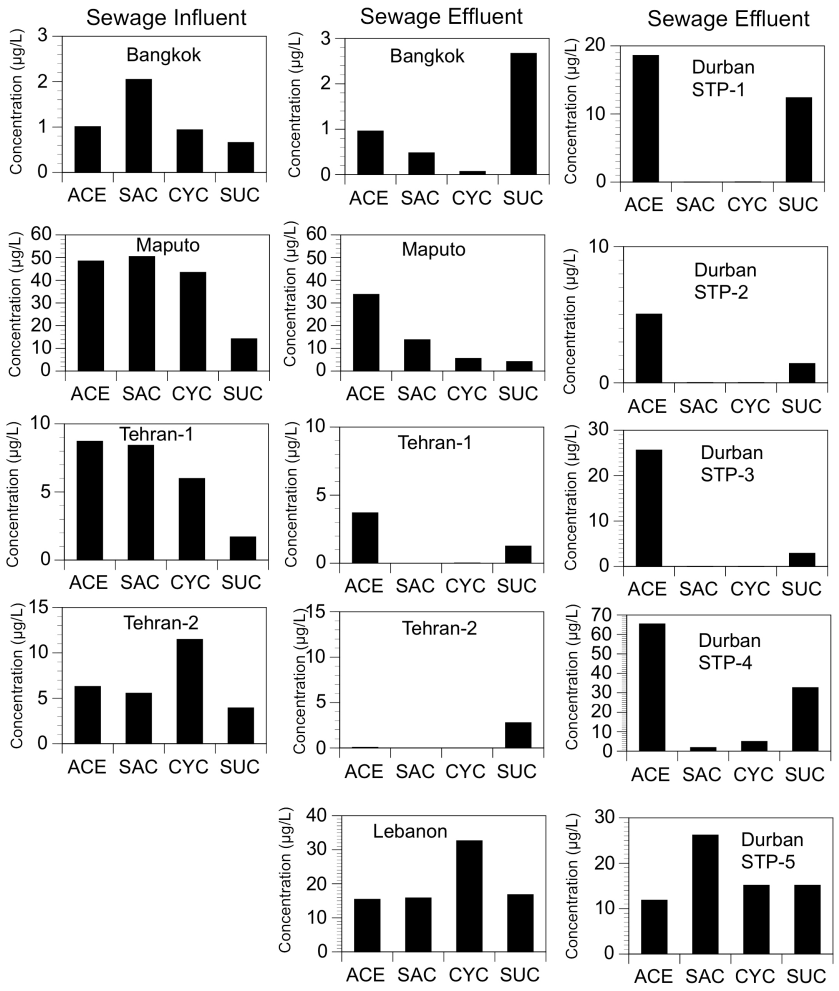

Fig. 2. Concentrations of artificial sweeteners in sewage influent and effluent

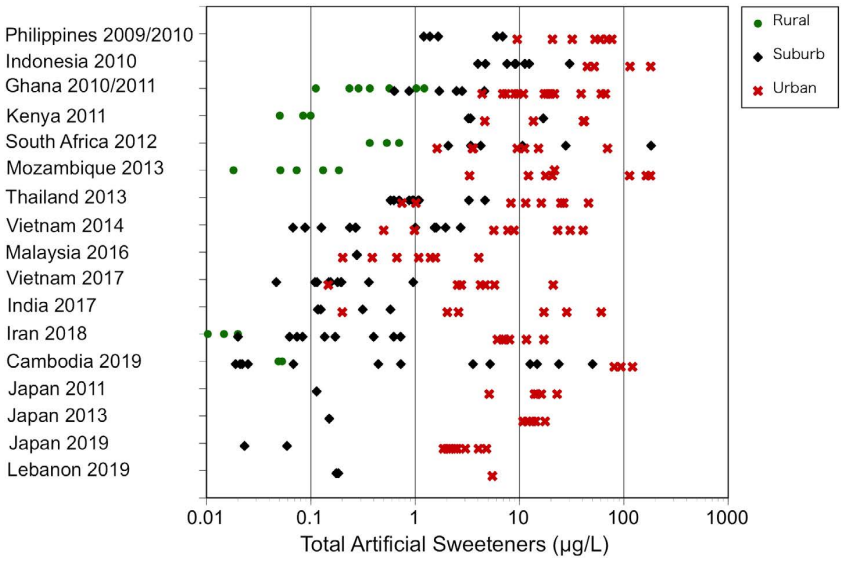

Fig. 3. Concentrations of artificial sweeteners in river water
Total Artificial Sweeteners: sum of concentrations of ACE + SAC + CYC + SUC.

lakes of up to 2.8 μg/L for ACE, 0.18 μg/L for SAC, and 0.13 μg/L for CYC, and 6.9 μg/L for ACE in rivers. Similar concentrations of ASs were reported for German rivers (ACE: several μg/L, SAC: 0.05–0.15 μg/L, CYC: 0.05–0.15 μg/L, and SUC: 0.06–0.08 μg/L). In USA rivers, SUC was observed predominantly at concentrations of 0.12 μg/L to 10 μg/L

(Oppenheimer et al., 2011). In the Pearl River, China, median (n = 28) concentrations of ACE and SUS were reported as 1.43 μg/L and 1.14 μg/L, respectively (Yang et al., 2017). In another Chinese river, the Dongjing River, a similar range of AS concentrations were reported (0.504 μg/L for ACE, 0.145 μg/L for SAC, 0.102 μg/L for CYC, and 0.265 μg/L for SUC; (Yang et al., 2017)). In the surface water from Singapore much lower concentrations of ASs (median concentration of 414 samples) were reported i.e., 0.047 μg/L for ACE, 0.094 μg/L for SAC, 0.0875 μg/L for CYC, and < method quantification limit (0.05 μg/L) for SUC (Tran et al., 2014). The higher AS concentrations in the present study can be ascribed to greater inputs of untreated and treated wastewater to the urban rivers. In some countries, such as Ghana, Mozambique, Indonesia, and Cambodia, sewage collection systems were rarely installed (Table S1) and large volumes of wastewater were discharged directly into rivers and streams in populated cities. In cases of highly populated cities where sewage collection systems were installed, such as Tokyo, treated sewage was discharged to the rivers and persistent ASs such as SUS were abundantly detected in the river water. Types of sewage discharged to the rivers and spatial patterns of AS pollution for individual cities are discussed in the following sections.

COMPOSITIONS OF ARTIFICIAL SWEETENERS

The compositions of ASs are shown in Fig. 4 as averaged relative compositions of ASs for individual cities. The composition of ASs was variable amongst the locations. However, a relatively small deviation of individual components for individual cities, as shown by shorter error bars in Fig. 4, implies that AS compositions were relatively uniform within individual cities. This is probably due to similar compositions of ASs sold and consumed in food and beverages and similar sewage collection and treatment systems within the individual cities. As shown in Fig. 4, SAC and/or CYC were abundant (more than 10%) in surface waters of all target countries except Tokyo, where only trace levels (less than 1%) of SAC and no CYC was detected whereas ACE and/or SUC were

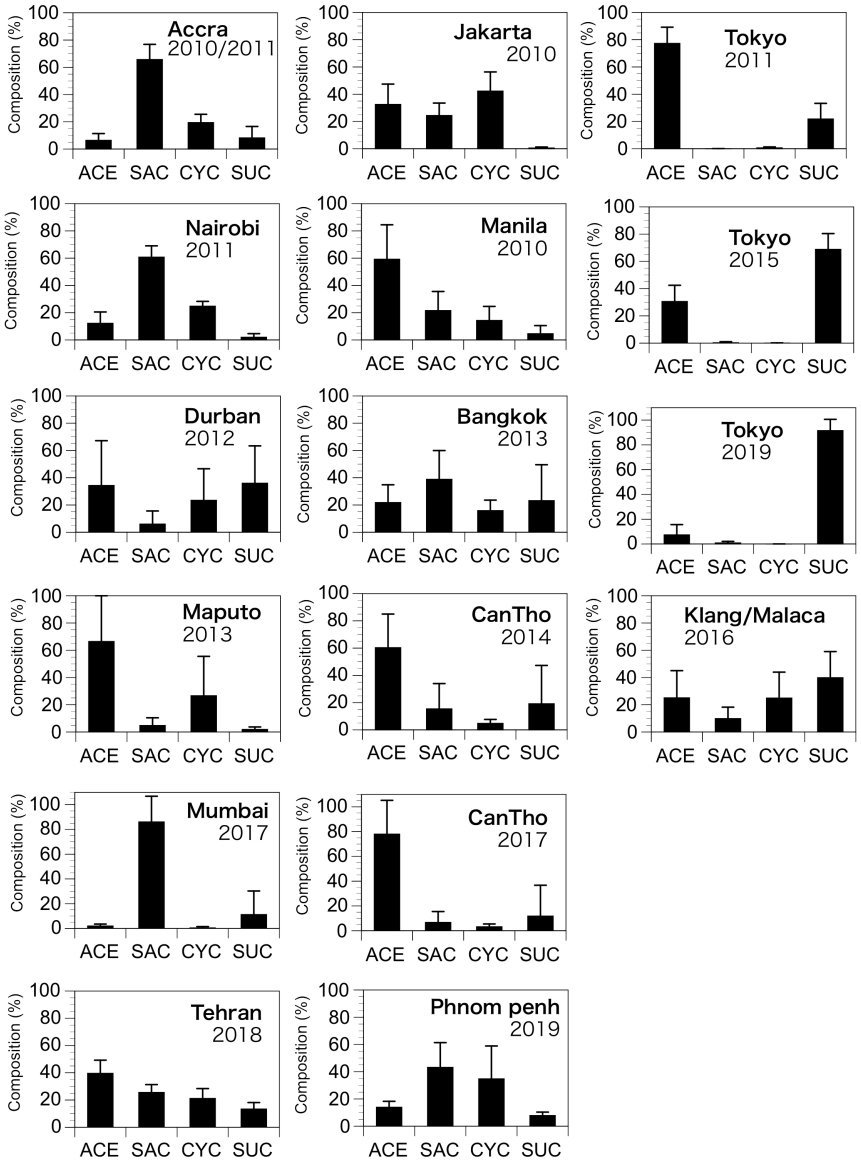

Fig. 4. Average compositions of artificial sweeteners in urban rivers (error bar: standard deviation)

predominantly detected in rivers. The absence of CYC in Japanese rivers reflects regulations on CYC use since 1969 due to concerns regarding its toxicity. Although an acceptable daily intake (ADI) of 11 mg/kg-body weight was established for CYC by WHO in 1982 and the ADI far exceeds the potential uptake of the sweetener, CYC has been regulated in Japan. Similar regulation is not evident in the other countries included in the present study, and CYC was detected in sewage influents in Thailand, Mozambique and Iran and sewage effluent in South Africa and Lebanon, as shown in Fig. 2.

The reason for the trace concentrations of SAC in the rivers in Tokyo is due to the installation of secondary sewage treatment systems and the biodegradable nature of SAC. In influents of STPs in Tokyo, a substantial proportion of SAC (~15% of the 4 ASs) was observed, whereas SAC was not detected in STP effluents (less than 1%) (Takada et al., 2022). The efficient removal of SAC and CYC during sewage treatment was also reported in other studies (Scheurer et al., 2009; Yue et al., 2023) and confirmed for STPs in Iran (Fig. 2) in the present study. Thus, the abundance of SAC and CYC in the river water is ascribed to inputs of untreated sewage due to inadequate sewage collection and treatment systems. In the rivers in Accra, Nairobi, Mumbai, Bangkok, and Phnom Penh, SAC comprised more than 40% of the 4 ASs summed concentration. Due to the insufficient coverage of the sewage collection systems (< 20% of population) in these populated cities (Table S1), a large proportion of untreated sewage was discharged to the rivers. The relatively high abundance of SAC may be an indicator of untreated sewage inputs. In the sewage from India (both STP influents and effluents), Subedi et al. (2015) reported the predominance of SAC (> 87% among the 4 ASs). This may suggest the prevalent use of SAC in the Indian market. The pattern of usage of each AS needs to be considered when evaluating the composition of the ASs as sewage inputs. In addition, SAC was detected at extremely high concentrations up to 79 μg/L in wastewater influent (TRP1-IN and TRP2-IN) and 16 μg/L in effluent (TRP1-OUT and TRP2-OUT) at a pig farm in Thailand (Table S3–7(B)). This is probably due to inclusion of SAC in the feed stock. This is consistent with the report by Buerge et al., (2011). The contribution of wastewater from pig farms should also be considered, depending on the geographic conditions, when SAC is used as a marker of untreated sewage inputs.

CORRELATION OF ARTIFICIAL SWEETENERS WITH THE OTHER MOLECULAR MARKERS AND POLLUTANTS

Correlations between concentrations of individual ASs and the traditional sewage marker (LABs) were examined for individual cities except Tokyo, Japan. Concentrations of one or more of the ASs were positively correlated with those of LABs in all the countries except Malaysia (Table 2). Typical correlations are shown in Fig. 5. Since LABs are introduced into rivers in untreated wastewater, the correlations again indicate the utility of ASs as markers of untreated sewage inputs to rivers. Positive correlations were most frequently observed for CYC, SAC, and ACE, while positive correlations were observed for SUC in a limited number of cities. The less common correlation for SUC can be ascribed to its less frequent detection due to lower concentrations of SUC and the lower sensitivity of analyses. LABs are hydrophobic and associated with particles, while ASs are water-soluble and present in the dissolved phase. Differential transport amongst the markers in rivers and streams may weaken their correlation. Nevertheless, positive correlations were evident between the hydrophobic and hydrophilic markers, suggesting that source-related factors control the occurrence of markers rather than differential transport. This is probably because most of the samples were collected in shallow rivers, which facilitate the resuspension of particles so that the differential transport between LABs and ASs is not evident. Furthermore, in the target cities sewage inputs from houses along the rivers and streams occurred, resulting in non-point and continual inputs of sewage. This also would have depressed the differential transport of both classes of markers.

The reason for the lack of positive correlations for the

Table 2 Correlation (r) of Artificial Sweeteners with Linear Alkylbenzenes (LABs)

		number of observations	ACE	SAC	CYC	SUC
Manila	2010	12	0.89	0.83	0.96	0.89
Jakarta	2010	13	0.82	0.89	0.75	NC
Ghana	2010/2011	24	0.90	0.95	0.94	NC
Kenya	2011	10	NC*	0.83	0.73	NC
Durban	2012	16	NC	NC	0.70	NC
Mozambique	2013	13	0.77	0.94	0.89	0.80
Thailand	2013	18	0.96	0.90	0.78	0.52
Vietnam-2014	2014	20	0.87	0.93	0.81	NC
Malaysia	2016	9	NC	NC	NC	NC
Vietnam-2017	2017	17	0.86	0.83	0.89	0.54
India	2017	9	0.92	0.91	0.58	0.79
Iran	2018	18	0.86	0.97	0.95	0.93
Cambodia	2019	17	0.81	0.84	0.86	0.78

*NC: No significant correlation ($p > 0.05$)

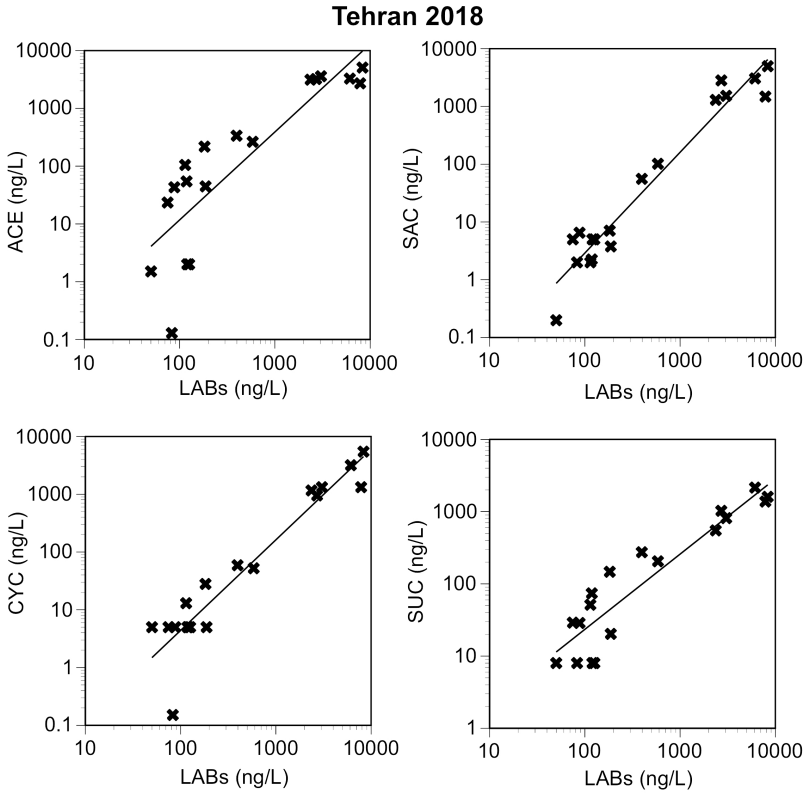

Fig. 5. Correlation between LAB concentrations and concentrations of individual artificial sweeteners in river water samples from Tehran, Iran

Malaysian samples could be lower inputs of untreated sewage into the Klang estuary and Malacca River. In fact, 78% of the population in Malaysia was covered by sewage collection system in 2016 (National Water Services Commission (SPAN), 2016) and ~75% of the population in the catchments of Klang and Malacca rivers was serviced by the collection (National Water Services Commission (SPAN), 2022), and as a result smaller amounts of raw sewage containing LABs could be supplied to the rivers, whereas persistent and water-soluble ASs (ACE and SUC) could be supplied via STP effluents. A similar lower correlation between ASs and LABs was evident in Durban, where coverage of sewage collection systems (63%, Department of Statistics South Africa, 2011) was higher than in other cities. Another reason for the weaker correlation between the markers could be stagnant conditions in the aquatic systems (Klang estuary), which could facilitate differential transport such as sedimentation between water soluble marker (ASs) and hydrophobic particle-reactive markers (LABs).

LABs consist of isomers with different positions of phenyl attachment on the alkylchain. Isomers with external phenyl substitution (external isomers) are more biodegradable than internal isomers. The relative abundance of external isomers to internal isomers can be an index of the degree of biodegradation for LABs (Takada and Ishiwatari, 1987, 1990). For the most abundant homolog, i.e., LABs with C_{12} alkyl carbons, the ratio of isomers is formulated to be I/E as follows (Takada and Ishiwatari, 1990):

I/E ratio = ratio of sum 6-C_{12} AB + 5-C_{12} AB to sum 4-C_{12} AB + 3-C_{12} AB + 2-C_{12} AB. The I/E ratio in synthetic detergent and untreated sewage is ~0.8 and a higher I/E ratio means more degradation (Takada and Ishiwatari, 1990). Regarding ASs, SAC is more biodegradable than ACE and a ratio of SAC to ACE was calculated to examine the index of biodegradation of ASs. The ratio is plotted against the I/E ratio in Fig. 6. The higher abundance of SAC, with a SAC/ACE ratio >1, was observed in samples with an I/E ratio for LABs of <1.5, while a smaller SAC/ACE ratio <1 was observed for samples with an I/E ratio >1.5. This indicates that SAC is derived from untreated sewage (Fig. 6) and suggests that the SAC/ACE ratio can be used as a proxy to identify inputs of untreated sewage. However, extremely high SAC/ACE ratios (i.e., up to 300) were observed for India's river-water samples; these can be explained by the high usage of SAC in the Indian market as discussed above. We, therefore, caution against using the SAC/ACE ratio as an indicator of untreated sewage. Concurrent measurement of the other markers such as LABs can help to identify untreated sewage inputs.

Antibiotics; that is, sulfonamides, macrolides, and tetracyclines were also measured in the same water samples for Thailand, Ghana, Kenya, Mozambique, and South Africa as previously reported by Suzuki et al. (2021) and Segura et al. (2015). The correlation between ACE and antibiotics concentrations was examined for each country. Sulfamethoxazole (SMX), clarithromycin (CLA), and tetracycline (TC) were selected as a representative sulfonamide, macrolide, and tetracyclines, respectively, due to their abundance. As shown in Fig. 7, there is a positive correlation between ACE and SMX concentrations for samples from Ghana. Similarly, ACE concentrations also showed significant positive correlations

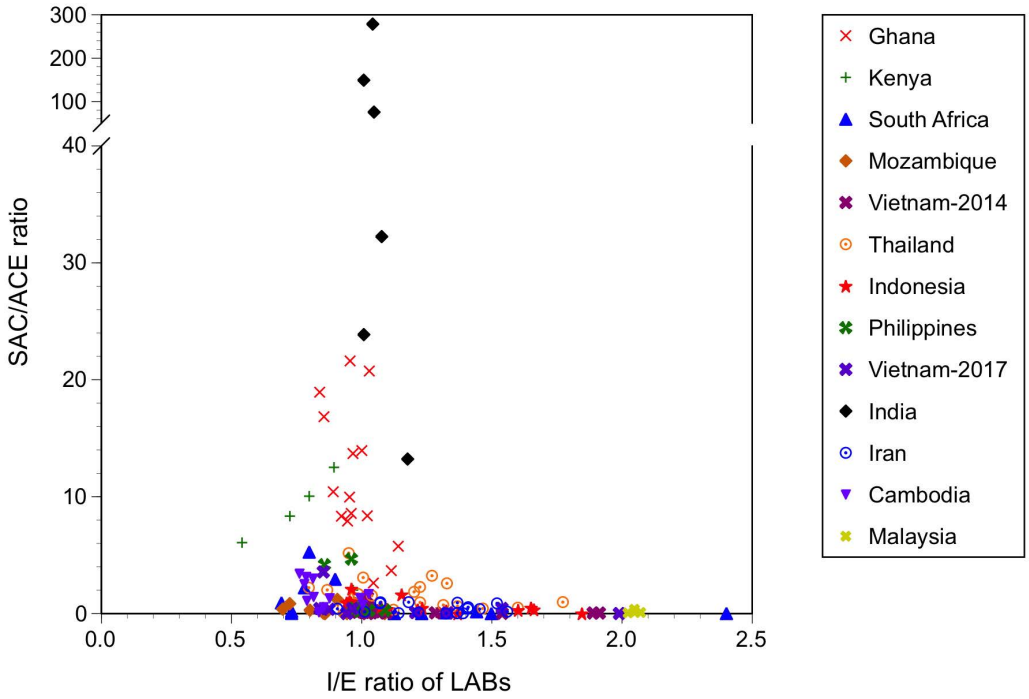

Fig. 6. I/E ratio of Linear alkylbenzenes (LABs) and SAC/ACE ratio of artificial sweeteners in river water

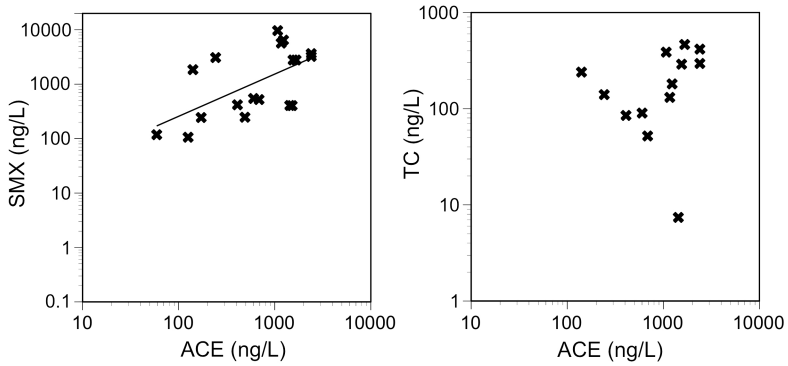

Fig. 7. Correlation between ACE concentrations and concentrations of sulfamethoxazole (SMX, left) and tetracycline (TC, right) in river water samples from Ghana
Data on SMX and TC were cited from Segura et al. (2015).

with SMX for all countries, except Kenya (Table 3). These findings are consistent with that SMX is widely used in both human and veterinary medicine in developing countries (Suzuki and Hoa, 2012; Shimizu et al., 2013; Suzuki et al., 2013), the major route by which these antibiotics reach aquatic environments is the sewage (Shimizu et al., 2013; Segura et al., 2015), and they are stable in the environment (Thiele-Bruhn et al., 2004). Thus, correlation between SMX and ACE indicates that SMX was derived mainly through sewage in these countries. ACE concentrations also exhibited significant positive correlations with CLA in Thailand, Kenya, and Mozambique (Table 3). CLA is also a major antibiotic class administered to humans (Murata et al., 2011) and has been observed in numerous aquatic environments (Murata et al., 2011; Shimizu et al., 2013; Segura et al., 2015; Ilie et al., 2023; Baranauskaite-Fedorova and Dvarioniene, 2023). Unlike the CLA and ACE correlation in Thailand, Kenya, and

Table 3 Correlation (r) between ACE and some antibiotics in the water samples from some Asian and African countries

	n	SMX	CLA	TC
Thailand	18	0.76	0.96	LD*
Ghana	20	0.73	NC****	NC****
Kenya	7	NC****	0.76	LD**
South Africa	16	0.65	NC****	LD*
Mozambique	10	0.92	0.69	LD***

* Limited detection, only one location.
** Limited detection, only two locations.
*** Limited detection, only three locations.
**** No significant correlation ($p > 0.05$).

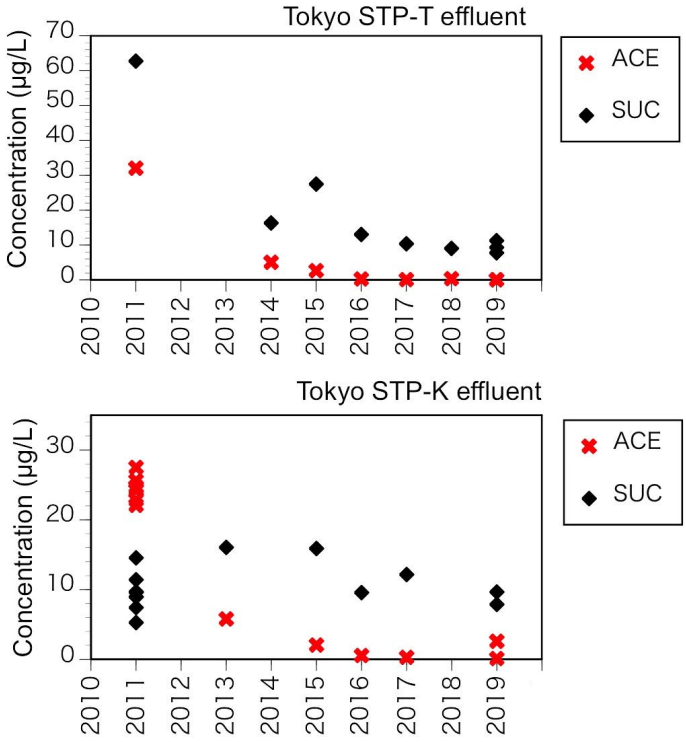

Fig. 8. Temporal trend in concentrations of artificial sweeteners in sewage effluents, Tokyo, Japan

Mozambique, significant correlation was not observed in Ghana and South Africa. This may be due to the infrequent use of this expensive antibiotic (Shimizu et al., 2013) and subsequent lower concentrations of CLA in rivers in these African countries (Segura et al., 2015). Unlike SMX and CLA, positive correlation was not observed between ACE and TC in any of the samples from the five countries (Table 3 and Fig. 7). In Thailand, Kenya, South Africa, and Mozambique, TC was detected in a limited number of locations; that is, 1–3 while ACE was detected in most locations. The fact that correlation between TC and the sewage marker ACE was not observed is consistent with the main use of TC as a veterinary medicine for livestock. High concentrations of TC have been detected in livestock wastewater (Suzuki et al., 2021); however, ACE was not detected in these samples (Table S3–7(B)). The present study demonstrated the utility of ASs for providing insight into the sources of other pollutants.

SEWAGE POLLUTION STATUS IN INDIVIDUAL COUNTRIES CHARACTERIZED BY MOLECULAR MARKERS

In this section, spatial patterns of sewage pollution are visualized in areal maps in Fig. S1 based on the data in Table S3, and discussed by using ASs as markers. A clear gradient in the concentrations of ASs from urban to suburban to rural sites was evident in Ghana (Fig. S1–3). No ASs were detected in reservoirs (GVL01 and GVL02) and rural lagoons (e.g., GL1103), while extremely high concentrations of ASs >50 $\mu g/L$ were evident in rivers running through the capital city Accra (AR09 and AR10) with 4 million capita (Table S4). Moderate concentrations were evident in suburban rivers and lagoons, such as the Densu River (2.8 $\mu g/L$ at GL1106), Sakumono Lagoon (2.5 $\mu g/L$ at SL01), and Chemu Lagoon (39 $\mu g/L$ at 2010CL), as listed in Table S3–3. This spatial pattern in AS concentrations is consistent with the demographic distribution and very low coverage (i.e., ~7%) of sewage collection systems (WHO and UNICEF, 2021a). In and near Jakarta (Fig. S1–2 and Table S3–2) extremely high AS concentrations were evident in coastal canals in downtown Jakarta, such as JKCS1 and JKCS2 at >50 $\mu g/L$, while lower concentrations (ranging from 1 $\mu g/L$–10 $\mu g/L$) were evident in inland suburban rivers, such as JKCS11 and JKCS12. In Jakarta <4% of population was serviced by sewage collection systems (Wirawan et al., 2018; Cahyadi et al., 2021). As a result, large amounts of untreated sewage from ~9 million capita (Table S4) are emitted into the rivers and canals in populated areas of the city. Similar spatial patterns were evident in Mozambique (Fig. S1–6) with extremely high concentrations of ASs (22 $\mu g/L$ to 179 $\mu g/L$ at MZr7 to17) in streams in the capital city Maputo with ~1.6 million capita but no detection in rural river locations (MZr-1 to 6). This is consistent with the situation that only a small percentage (~4%) of the population in the city was covered by sewage collection systems (WHO and UNICEF, 2021b) in 2013. Collected sewage in Maputo is discharged into the ocean via a pipeline or is subjected to lagoon treatment (van Esch and van Ramshorst, 2014) together with black water collected by vacuum truck. The removal efficiency of the lagoon treatment was not high. Based on the ASs concentrations in black water (MZr14) and effluent from the lagoon (MZr16) (Table S3–6) the removal efficiency of SAC was 73%, which is considerably lower than for activated sludge treatment (99%: Takada et al., 2022; >94%: Scheurer et al., 2009). All in all, a large volume of untreated, or poorly-treated sewage was discharged into

rivers in Maputo, Mozambique, in 2013, and caused the extremely high concentrations of ASs in streams in the city.

Phnom Penh (Cambodia), Can Tho (Vietnam), Bangkok (Thailand), and Manila (Philippines) are cities with millions of populations and have large rivers (Mekong, Chao Phraya, and Pasig Rivers) that flow through the cities. Sewage treatment system coverage in Phnom Penh, Can Tho, Bangkok, and Manila are also limited, at up to 17% (JICA, 2016, Trinh et al., 2013; General Statistics Office of Viet Nam and UNICEF, 2021, National Static Office et al., 2012, JICA et al., 2016; Negishi, 2010, respectively). As a result, large volumes of untreated sewage were discharged into unban canals in the cities explaining the extremely high concentrations of ASs of around 100 $\mu g/L$ that were detected in ponds, streams, and canals in the cities and surroundings (Fig. S1–7, Fig. S1–8, Fig. S1–9, Fig. S1–13), such as 120 $\mu g/L$ at PPC-1 in Phom Penh (Table S3–13), 41 $\mu g/L$ at SW-4 in Can Tho (2014) (Table S3–8), 46 $\mu g/L$ at TBC4 in Bangkok (Table S3–7(B)), and 77 $\mu g/L$ at MC6 in Manila (Table S3–1)). However, only trace levels of ASs (<1 $\mu g/L$) were evident in the main stream of the Chao Phraya River and Mekong River. This is due to dilution by the large volume of water in these rivers which have very high flows (~1,000 m^3/sec for Chao Phraya River: Komori et al., 2013; ~10,000 m^3/sec for Mekong River: Mekong River Commission, 2010). On the other hand, higher concentrations (>1 $\mu g/L$) of ASs were detected in the main stream of the Pasig River (e.g., 9.5 $\mu g/L$ at MNR1) and Laguna de Bay (e.g., 6.9 $\mu g/L$ at MNL1). This is probably due to less dilution due to the lower water volume in the Pasig River (~10 to ~300 m^3/sec: Azuma et al., 2017), and the higher population of Metro Manila (~12 million; Table S4). In Can Tho, monitoring was conducted at the same locations in 2014 and 2017, meanwhile a sewage treatment system was developed and proportion of population serviced by sewage collection increased from almost zero in 2014 to ~7% in 2017 (Trinh et al., 2013; General Statistics Office of Viet Nam and UNICEF, 2021). The effectiveness of installing the sewage treatment system can be seen in the sewage markers. Median concentrations of total ASs and SAC for urban locations was 8.29 $\mu g/L$ and 0.28 $\mu g/L$, respectively in 2014 (Table S3–8), compared to 3.50 $\mu g/L$ and 0.02 $\mu g/L$, respectively, in 2017 (Table S3–9). The decrease might have been caused by a temporal change in dilution brought about by changes in river flow, rainfall and freshwater inputs. However, the compositional index of markers, which is not affected by dilution, indicates a temporal decrease in inputs of untreated sewage. That is, the average proportion that SAC contributed to the total AS concentration at urban locations decreased from 16% (±19%) in 2014 to 7% (±9%) in 2017. The I/E ratio for LABs also increased from 1.30 (±0.41) in 2014 (Table S3–8) to 1.72 (±0.67) in 2017 (Table S3–9). These compositional indexes of the sewage markers indicate a relative decrease in the input of untreated sewage.

In Tehran (Iran), a clear pattern was evident in the spatial distribution of AS concentrations in river water (Fig. S1–12). In the headwater of the Jajroud River (IRTR1 to 3) no ASs were detected, whereas stations in mountain villages in the river catchment (IRTR4 to 7) showed significant concentrations (~0.08 $\mu g/L$) of ASs. At suburban sites (IRTR 8, 9, 11, 12) in the Golab dareh River in Tehran, ASs were at more significant concentrations (~0.1 $\mu g/L$ to ~0.7 $\mu g/L$) and much higher concentrations of ASs were evident for urban locations (7 $\mu g/L$–17 $\mu g/L$ at IRTR13 to 18) (Table S3–12). Sixty-seven percent of the population in Tehran was covered by sewage treatment (Tehran Province Water and Wastewater Company, 2017). Higher concentrations of ASs at urban locations can be explained by a portion of the untreated sewage input from ~9 million capita to the river (Table S4). In Kenya (Fig. S1–4 and Table S3–4), high concentrations of ASs were evident in the Nairobi River (~40 $\mu g/L$ at NR07 and NR08), while no (<0.1 $\mu g/L$ at KK01 and KK02) or low concentrations (~3 $\mu g/L$ at KK03 and KK04) were evident in coastal areas. Approximately 25% of population in Nairobi (~3 million capita, Table S4) was covered by sewage collection systems (WHO and UNICEF, 2021c) but mainly by primary treatment only (stabilization pond and wetland treatment) (Kilingo et al., 2021). Thus, the discharge of poorly treated and untreated sewage from 75% of the population must have caused severe sewage pollution and resulted in the high concentrations of SAC and CYC in the Nairobi River, as shown in Fig. S1–4. On the other hand, sites KK01–KK04 were located in coastal small towns with less population density (~90 capita/km^2, Table S4) than Nairobi (~4,500 capita/km^2, Table S4). Even though these areas are poorly covered by sewage collection systems (WHO and UNICEF, 2021c) the smaller volumes of sewage discharged into rivers at these sites appeared to result in much lower levels of sewage pollution. In Durban, clear contrast of AS concentrations was evident between rural locations (<1 $\mu g/L$ for St.01 to 03) and suburban/urban locations (Fig. S1–5). Interestingly, concentrations of ASs at site in lower catchments in the city of Durban were an order of magnitude lower (e.g., ~3 $\mu g/L$ for St.06, 07, 12 and 13) than those in surrounding (upstream) locations (e.g., 15 $\mu g/L$–70 $\mu g/L$ for St.09, 10, and 11) (Table S3–5). In Durban, most suburban and urban areas were covered by sewage collection systems (Department of Statistics South Africa, 2011). However, there were many informal settlements in the city with no connection to the sewer system. The downtown area is covered by modern sewage treatment while informal settlements in surrounding areas are not. A similar situation might have occurred in Mumbai, India (Fig. S1–11). The coverage rate of sewage collection systems was 65%–80% in Mumbai (JICA 2012; Sharma 2021) and sewage was mostly discharged into the ocean. However, extremely and moderately high concentrations of ASs, with a predominance of SAC, were observed at some locations in Mumbai (16 $\mu g/L$ -59 $\mu g/L$ at INMB-4 to 6) (Table S3–11). Exclusive predominance of SAC among the 4 ASs, i.e., 95.3%–97.4% of total ASs (Table S3–11) is more similar to the AS compositions in sewage influent (96.9%–99.8%; Subedi et al., 2015) than those in sewage effluents (86.8%–96.7%; Subedi et al., 2015). This may suggest discharge of untreated sewage from slum areas (Slum Rehabilitation Authority, 2023) near these locations (Vijay et al., 2010 & 2016).

In Beirut, Lebanon, 99.9% of the population was serviced by sewerage collection systems (Lebanese Republic Central Administration of Statistics (CAS) et al., 2020). However, only

8% of collected sewage was treated by primary and/or secondary treatment (Lebanese Republic Central Administration of Statistics et al., 2020). As a result, large volumes of untreated sewage were discharged to rivers and coastal waters. Wastewater effluent from an outfall at Ramlet in Beirut had an extremely high concentration of ASs (80 μg/L) (Fig. S1–14). This resulted in considerable level of ASs (5.5 μg/L) in surface water at Ramlet Beach near (~0.3 km) the outfall (Table S3–14). Even at coastal locations (Costa Brava, Saida) more than 10 km from Beirut, trace but detectable levels of ASs >0.1 μg/L were evident (Table S3–14). This suggests the discharge of untreated or poorly-treated sewage may have occurred not only in the capital city with population density of 5,100 capita/km^2 (Table S4) but also at other suburban location, i.e., Saida with 631 capita/km^2 (Table S4). In Malaysia, on the other hand, relatively lower concentrations (0.1 μg/L to several μg/L) of ASs were evident compared to other countries (Table S3–10 and Fig. S1–10). This is probably due to minimal inputs of untreated sewage into the Klang and Malacca Rivers due to smaller populations (~1 million and ~0.6 million, respectively, Table S4), and relatively higher coverage of sewage collection systems in the river catchments (74% for Klang River and 75% for Malacca River, National Water Services Commission (SPAN), 2016 and 2022), as discussed in previous section.

The population in the catchments of rivers in Tokyo (i.e., Sumidagawa River and Tamagawa River) have been fully covered by secondary (activated sludge) sewage treatment since 2008 (Bureau of Sewerage of Tokyo, 2020a, 2020b). As a result, no SAC was detected in samples collected in 2011 or later. Since a large volume of secondary treated effluent is discharged into the small volume rivers, secondary treated effluent represents more than half the flow volume for both rivers (Murata et al., 2011; Sugiura et al., 2021). Secondary treated effluents in Tokyo contained ~10 μg/L to ~100 μg/L of ASs (Table S3–15). As a result, moderately high concentrations of ASs (~2 μg/L to ~20 μg/L) were observed in the rivers in Tokyo. Interestingly, ACE concentrations in secondary treated effluents and receiving waters in Tokyo decreased by two orders of magnitude from 2011 to 2019 (Fig. 8 and Fig. S1–15), while SUC did not show such a marked decrease. This is probably due to the upgrading of the sewage treatment works from conventional activated sludge treatment to anoxic-anaerobic-oxic (A2O) treatment and resultant higher removal of ACE by the secondary treatment. Van Stempvoort et al. (2020) reported that A2O treatment efficiently removes ACE. This is consistent with observations in our study on wastewater treatment. As shown in Fig. 4, ACE was reduced to trace levels in the Tehran STP-2 where A2O was applied, while no similarly marked reduction was evident for STP-1 where A2O was not introduced. The introduction of A2O in Tokyo has been progressing since 2010. The proportion of A2O in secondary treatment works was 30% in 2011, compared to 70% in 2019 (Bureau of Sewerage Tokyo, 2020c). The introduction of A2O treatment in Tokyo has thus led to a marked decrease in ACE in the treated effluents, and correspondingly in the rivers of Tokyo. On the other hand, the concentration of SUC did not decrease from 2011 to 2019, probably due to its persistent nature against A2O treatment. Thus, SUC could be a useful sewage marker in countries and areas where A2O sewage treatment covers the whole population in the catchment.

As discussed in the case study in Vietnam and Japan, concentrations of ASs could provide a historical benchmark of sewage inputs and water pollution. However, the composition and concentrations of the individual ASs in the sewage were highly variable among countries, as discussed in the case study in India and may vary even within each country. Thus, the rigid usage of ASs as historical benchmark of sewage inputs and water pollution requires measurement of ASs in sewage, especially in untreated wastewater. In addition, simultaneous measurements of other sewage markers such as LABs. as discussed in the Vietnamese case study, and coprostanol could validate the usage of ASs as historical benchmark.

CONCLUSIONS

The spatial patterns of AS concentrations are consistent with sewage inputs and their correlation with traditional sewage markers, i.e., LABs, and their comparison with antibiotics demonstrate the utility of the suite of ASs as markers of sewage inputs into rivers and streams. Among the ASs, SAC and CYC are useful indicators of untreated sewage inputs in countries with sewage system coverage of <70%. ACE is a useful marker of untreated sewage and secondary treated effluents where A2O is not fully installed. SUC could be a useful marker in areas or countries where upgraded secondary treatment systems, i.e., A2O, cover most of the population in the catchment areas. Enhancing the sensitivity of SUC analyses is required in future studies. Overall, the measurement of multiple markers, including SUC, is recommended for long-term and future-oriented monitoring.

The present study archived the water pollution status in Asian, African and Middle Eastern countries in the 2010s, providing a potential benchmark against which water pollution in the 2020s and beyond can be evaluated. The results of the present study demonstrated that population demography, coverage and types of sewage treatment systems, and dilution by natural water are keys to control the sewage pollution status in the individual cities. The lack of efficient sewage treatment systems especially caused serious pollution by untreated sewage in ponds, streams, and rivers in the cities and surroundings in most of tropical Asian, African, and Middle Eastern countries. Sewage treatment systems are urgently required in these countries.

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SUPPLEMENTARY MATERIAL

Table S1, Coverage of sewage collection system (population-based) and the types of the sewage treatment; Table S2–1, Sample information for Phlippines, 2009 and 2010; Table S2–2, Sample information for Jakarta 2010; Table S2–3(A), Sample information for Ghana, 2010; Table S2–3(B), Sample information for Ghana, 2011; Table S2–4, Sample information for Kenya; Table S2–5, Sample information for South Africa; Table S2–6, Sample information for Mozambique; Table S2–7(A), Sample information for Thailand, 2013; Table S2–7(B), Sample information for Thailand, 2014; Table S2–8, Sample information for Vietnam, 2014; Table S2–9, Sample information for Vietnam, 2017; Table S2–10, Sample information for Malaysia; Table S2–11, Sample information for India; Table S2–12, Sample information for Iran; Table S2–13, Sample information for Cambodia; Table S2–14, Sample information for Lebanon; Table S2–15, Sample information for Tokyo 2011–2019; Table S3–1. Molecular markers in surface water in Phlippines, 2009 and 2010; Table S3–2. Molecular markers in surface water in Jakarta 2010; Table S3–3 (A). Molecular markers in surface water in Ghana, 2010; Table S3–3 (B). Molecular markers in surface water in Ghana, 2011; Table S3–4. Molecular markers in surface water in Kenya; Table S3–5. Molecular markers in surface water in Durban; Table S3–6. Molecular markers in surface water in Mozambique; Table S3–7 (A). Molecular markers in surface water in Thailand, 2013; Table S3–7 (B). Molecular markers in surface water in Thailand, 2014; Table S3–8. Molecular markers in surface water in Vietnam, 2014; Table S3–9. Sample information for Vietnam, 2017; Table S3–10. Molecular markers in surface water in Malaysia; Table S3–11. Molecular markers in surface water in India; Table S3–12. Molecular markers in surface water in Iran; Table S3–13. Molecular markers in surface water in Phom Penh; Table S3–14. Molecular markers in surface water in Lebanon; Table S3–15. Sample information for Tokyo 2011–2019; Fig. S1–1, Sum of concentrations of the 4 artificial sweeteners in surface water from Manila; Fig. S1–2, Sum of concentrations of the 4 artificial sweeteners in surface water from Jakarta; Fig. S1–3, Sum of concentrations of the 4 artificial sweeteners in surface water from Ghana; Fig. S1–4, Sum of concentrations of the 4 artificial sweeteners in surface water from Nairobi and Kilifi; Fig. S1–5, Sum of concentrations of the 4 artificial sweeteners in surface water from Durban; Fig. S1–6, Sum of concentrations of the 4 artificial sweeteners in surface water from Mozambique; Fig. S1–7, Sum of concentrations of the 4 artificial sweeteners in surface water from Bangkok; Fig. S1–8, Sum of concentrations of the 4 artificial sweeteners in surface water from CanTho in 2014; Fig. S1–9, Sum of concentrations of the 4 artificial sweeteners in surface water from CanTho in 2017; Fig. S1–10, Sum of concentrations of the 4 artificial sweeteners in surface water from Klang and Malacca rivers; Fig. S1–11, Sum of concentrations of the 4 artificial sweeteners in surface water from Mumbai and Goa; Fig. S1–12, Sum of concentrations of the 4 artificial sweeteners in surface water from Tehran; Fig. S1–13, Sum of concentrations of the 4 artificial sweeteners in surface water from Phnom Penh; Fig. S1–14, Sum of concentrations of the 4 artificial sweeteners in surface water from Beirut and vicinities; Fig. S1–15, Sum of concentrations of the 4 artificial sweeteners in surface water from Tokyo; Table S4, Population and population density of studied cities/regions and the data sources.

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