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Mothabi E. Makaleng

University of South Africa

Nompumelelo R. Sibanyoni

University of South Africa

Thabo Nkambule

University of South Africa

Dineo E. Moema

University of South Africa

Illunga Kamika

kamiki@unisa.ac.za

University of South Africa

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Seasonal occurrence and removal of artificial sweeteners in wastewater from five treatment plants in Tshwane, South Africa.

Mothabi E. Makaleng, Nompumelelo R. Sibanyoni¹, Thabo Nkambule¹, Dineo E. Moema¹, Illunga Kamika^{1*}

¹School of Science, College of Science, Engineering and Technology, University of South Africa, Florida, South Africa

*Correspondence: E-mail: kamiki@unisa.ac.za

Abstract

Artificial sweeteners (ASWs) are increasingly detected in aquatic environments. This likely reflects their widespread consumption and incomplete elimination in conventional wastewater treatment. This research examined the presence and removal of four artificial sweeteners (acesulfame-K, saccharin, aspartame, and neotame) at five wastewater treatment facilities in the City of Tshwane, South Africa. Samples were taken in triplicate from mid-March to the end of August 2023, covering both summer and winter conditions. Solid-phase extraction (SPE), followed by high-performance liquid chromatography (HPLC) coupled with ultraviolet (UV) detection, was used for analysis. SPE-HPLC/UV demonstrated good selectivity, linearity, sensitivity, and recovery for each target compound. Each artificial sweetener was detected in at least some influent and effluent samples from all five plants. Neotame concentrations generally exceeded those of aspartame, and saccharin was often comparable to or higher than those of acesulfame-K. Removal rates varied among plants and seasons, suggesting dependence on plant-specific operations and influent characteristics. Complete removal was not achieved, and detectable amounts of each compound remained in some effluent samples, indicating persistence through standard treatment. These results demonstrate that artificial sweeteners have been detected in wastewater treatment systems across the City of Tshwane and indicate the need for enhanced monitoring and treatment technologies to minimize their discharge into the surrounding environmental media.

Keywords: Artificial sweeteners (ASWs), High-performance liquid chromatography coupled with ultraviolet (HPLC-UV), Solid-phase extraction (SPE), Tshwane, Wastewater treatment plants (WWTPs)

Introduction

Water quality decline is a significant problem in Southern Africa, with increased water scarcity, urbanization, industrial activities, and climate variability placing even greater stress on already limited freshwater resources [1; 2]. Consequently, the role of Wastewater Treatment Plants (WWTPs) has become a critical control point for receiving a wide variety of contaminants from domestic, commercial, and industrial sources before releasing treated effluent into surface waters. Conventional WWTP processes do not remove all emerging pollutants (EPs), and the compounds that remain after treatment will likely continue to enter aquatic ecosystems and affect downstream water users [3; 4]. Among the

37 numerous EPs, artificial sweeteners (ASWs) have gained recognition due to their global use,
38 environmental persistence, and frequent detection in wastewater and surface waters [5-7]. ASWs are
39 commonly added to foods, beverages, pharmaceuticals, and personal care products as a low-calorie
40 substitute for sugar; consequently, substantial amounts of ASWs continue to enter municipal
41 wastewater systems. Compared with most organic pollutants, which can degrade during wastewater
42 treatment, many ASWs exhibit high chemical stability, are polar, and are thus poorly degraded by
43 traditional biological treatment processes [8]. These properties make them not only relevant
44 environmental contaminants, but also useful tracers of wastewater influence and treatment performance.

45 ASWs such as acesulfame-K, saccharin, aspartame, and neotame exhibit distinct structural differences,
46 environmental behaviors, and degradability [6, 9]. The presence, distribution, and concentration of
47 ASWs in wastewater can provide insights into human consumption habits, inflow characteristics, and
48 treatment plant hydraulic retention times. Additionally, variations in seasonal water use and the
49 diet/composition of inflows may affect concentrations or removal efficiencies. Although ASWs in
50 wastewater may hold a wealth of information relevant to human and wastewater treatment practices,
51 very little research has examined their presence or fate in South African WWTPs [10], particularly in
52 the City of Tshwane. Various WWTPs within the City of Tshwane have been reported to consistently
53 fail to produce effluent that meets regulatory requirements [11].

54 Limited local data exist on ASWs in South African WWTPs, as WWTP performance can vary
55 significantly with factors such as influent loading, technology, treatment efficacy, hydraulic retention
56 time, and seasonality. Therefore, it is essential to understand how ASW behaves within WWTPs to
57 assess overall performance and identify potential pathways of release into the environment. Research
58 on ASW in WWTPs would also contribute to improved methods for monitoring water quality and
59 wastewater management, as well as more effective treatment technologies. Therefore, the primary
60 objective of this study was to assess the distribution and removal efficiencies of four common ASWs:
61 four common ASWs: acesulfame-K (ACE-K), saccharin (SAC), aspartame (ASP), and neotame (NEO)
62 in influent and effluent samples collected from five WWTPs in the City of Tshwane during summer
63 and winter. Samples were analysed using solid-phase extraction followed by HPLC-UV to quantify
64 each ASW compound and to compare removal efficiencies across WWTPs and seasons. The data
65 generated from this study represent a preliminary assessment of artificial sweetener contamination in
66 South African WWTPs. To our knowledge, this is the first study to report seasonal occurrence and
67 removal of multiple ASWs across several municipal WWTPs within the City of Tshwane, South Africa.
68 By combining influent and effluent monitoring at five plants, we provide a multi-site dataset that
69 extends previous South African work on emerging pollutants beyond single-plant or non-seasonal
70 assessments.

Methods

Study Area

The research was conducted within the boundaries of the City of Tshwane, in Gauteng Province, South Africa. The five WWTPs (Rooiwal, Baviaanspoort, Klipgat, Daspoort, and Sunderland Ridge) were chosen for their locations, operational suitability, and distinct performance characteristics. All of these plants have a common feature: each receives a combination of residential, commercial, industrial, and agricultural effluent from a mixed catchment area. In addition to providing an opportunity to evaluate the presence and effectiveness of ASW removal across different treatment regimes, these WWTPs are spread throughout the city.

Sample collection

Wastewater influent and effluent samples were collected from all treatment plants between mid-March and the end of August 2023, covering both summer and winter conditions. Each sampling period included two consecutive fortnight rounds of sampling, and three replicate samples were collected at each time point using 500 mL pre-washed high-density polyethylene bottles with Teflon-lined lids. The samples were cooled with ice packs immediately after sampling, then transported to the laboratory, where they were chilled and refrigerated at 4°C prior to analysis. A total of 40 composite samples, combining replicates by equal volume, were used in this study.

Preparation of standard solutions

Stock solutions of each artificial sweetener (acesulfame-K, saccharin, aspartame, and neotame; Sigma-Aldrich, Steinheim, Germany) were made at 100 mg/L using ultrapure water. Solutions were sonicated for 20 min to ensure full dissolution. Working analytical standards were prepared by serial dilution of the stock solution with ultrapure water to the required concentration for calibration and validation studies.

Preparation of wastewater sample

Wastewater samples were first filtered through a 1.0 µm pore Whatman filter paper; secondly, through a 0.45 µm syringe filter to remove all particulate matter in suspension. Each sample's pH was lowered to 2.7 before being extracted. A modified solid-phase extraction (SPE) procedure was used with Oasis HLB cartridges [12]. For conditioning the SPE cartridges, 2 mL of methanol was applied, followed by 2 mL of deionized water. Next, 50 mL of the wastewater sample was poured into each SPE cartridge under gravity flow. After pouring the sample into each SPE cartridge, the cartridges were washed with 2 mL of deionized water, dried for 10 minutes under vacuum, and finally eluted with 10 mL of methanol. Methanol was evaporated under nitrogen gas, with nitrogen gently blown over the mixture at 35°C to near dryness. The remaining extracts were mixed with 1 mL of acetonitrile to yield a total volume of 1 mL, which was placed in amber glass vials. Finally, the prepared solutions were filtered through a 0.45 µm syringe filter before being analysed by HPLC-UV.

Chromatographic conditions

The chromatography was performed using an Agilent 1100 Series HPLC system (Agilent Technologies, Santa Clara, CA, USA). This HPLC instrument can be configured to work with a variety of column types. The UV detector uses wavelengths from 190 nm to 400 nm. Since many organic compounds have some degree of absorption in this range of wavelengths, the detector can typically detect almost all these compounds. In order to identify and quantify SAC, ACE-K, NEO, and ASP in the ASW samples, the method was developed to separate the four ASWs based upon a mobile phase that consisted of a mixture of acetonitrile (ACN) and 2.25 mM ammonium acetate with 0.01 % formic acid added to water at a pH of 2.7. Separation of the four ASW components was achieved by gradient elution. Two different solvents were used in the gradient elution method: Solvent A was pure acetonitrile (ACN), and Solvent B was a solution of 2.25 mM ammonium acetate and 0.01 % formic acid in water. During the course of elution, the composition of the mobile phase changed as follows: 0 min., 100% water; 9.00 min., 93% water and 7% ACN; 15.00 min., 59% water and 41% ACN; 28.00 min., 70% water and 30% ACN; and finally, 35.00 min., the composition was again at 95% water and 5% ACN. The wavelengths selected for detection were 210 nm and 220 nm, and real-time analysis data were collected across the 190-380 nm range throughout the analysis period. Standards were made at concentration levels of 0 ppm to 5 ppm (in steps of 1 ppm) for each ASW component. The injection volume was set to 10 μ L/minute, and an X-Terra C18 column (3.5 μ m particle size, 4.6 x 100 mm) was used. The flow rate for the HPLC System was set to 0.5 mL/min, and the temperature was maintained at 30 $^{\circ}$ C.

Method validation and sample analysis

Validation of the analytical technique involved assessing linearity, accuracy, precision, and sensitivity. Linearity was confirmed by linear least-squares regression of calibration graphs for each compound analysed. Each graph showed 4 standards at concentrations of 1, 2, 3, and 4 ppm. Regression values (R^2) were obtained from the calibration plots to assess the fit of the data. An external calibration method was used to quantify the sample to quantify the analytes in samples. The analyses were processed using Agilent ChemStation software. Precision was determined by conducting mixed standard solutions at four varying concentrations. Accuracy was determined utilizing Spiking Recovery Experiments. Known quantities of the compounds were added to Milli-Q Water. The percentage recovery (%) was calculated as the ratio of the measured compound concentration to the spiked quantity. A blank solution containing 5% acetonitrile, devoid of compounds, was also used as a quality control measure. To evaluate instrument stability, a 3-ppm mixed standard was introduced into the HPLC System after every tenth injection. The HPLC System was calibrated once per day. Limits of Detection (LODs) and Limits of Quantitation (LOQs) for all target compounds were determined based upon IUPAC definitions as described below using Signal-to-Noise Ratios of three (3) and ten (10). Additionally, LODs and LOQs were computed from the standard deviations of both the Y-Intercepts (σ) and Slopes (m) of the calibration plots using the following formulas:

$$LOD (mg/L) = \frac{3\sigma}{m}$$

$$LOQ (mg/L) = \frac{10\sigma}{m}$$

To assess method performance under matrix-free conditions, recovery and precision tests were also carried out using Milli-Q water spiked with artificial sweeteners at 1, 2, 3, 4, and 5 ppm [13].

Determination of mass load and removal efficiency

The mass load of selected artificial sweeteners in wastewater treatment plants was estimated using influent and effluent concentrations measured during sampling. Removal efficiency was calculated using the following equation:

$$Removal (\%) = \frac{C_{influent} - C_{effluent}}{C_{influent}} \times 100$$

where $C_{influent}$ is the concentration of the analyte in influent wastewater, and $C_{effluent}$ is the concentration of the analyte in effluent wastewater [13].

Results and discussion

Method validation:

Selectivity and matrix effects

The selectivity of the HPLC-UV procedure was determined by comparing chromatograms of the blank solution, mixed standards, and SPE-treated water. There were no peaks corresponding to endogenous materials at the retention time of ACE, SAC, ASP, and/or NEO, which demonstrated acceptable separation and clear identification of the target analytical compounds (Fig. 4.1). The existence of matrix effects was assessed through an evaluation of possible interfering substances present in the wastewater matrix. A large peak corresponding to a matrix-related material was observed in the chromatogram of both blank and sample solutions. However, this peak eluted before all analytical targets, so there would be no interference with the analytical quantitation. As the matrices were cleaned up using SPE, no noticeable signal suppression or enhancement in the analyte's retention time was observed. Therefore, the use of SPE eliminated any matrix effects under the developed optimum conditions. This data has previously been shown to be consistent with reported data, indicating that SPE eliminates matrix interferences for artificial sweeteners in complex environmental samples [14].

Therefore, these results indicate that the developed HPLC-UV procedure has sufficient selectivity to simultaneously analyse ACE, SAC, ASP, and NEO in wastewater.

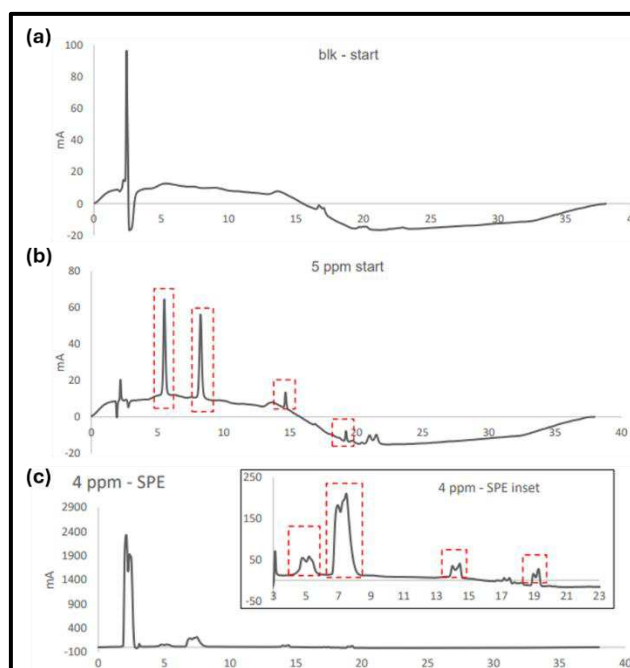


Figure 4.1. Chromatographic analysis of standards for artificial sweetener analysis by HPLC-UV. (a) Blank injection at the start of the analysis showing no interfering peaks at analyte retention times. **(b)** 5 ppm mixed standard solution confirming retention times matched those from previous runs. **(c)** First SPE-extracted wastewater sample analyzed at 4 ppm equivalent concentration.

Linearity

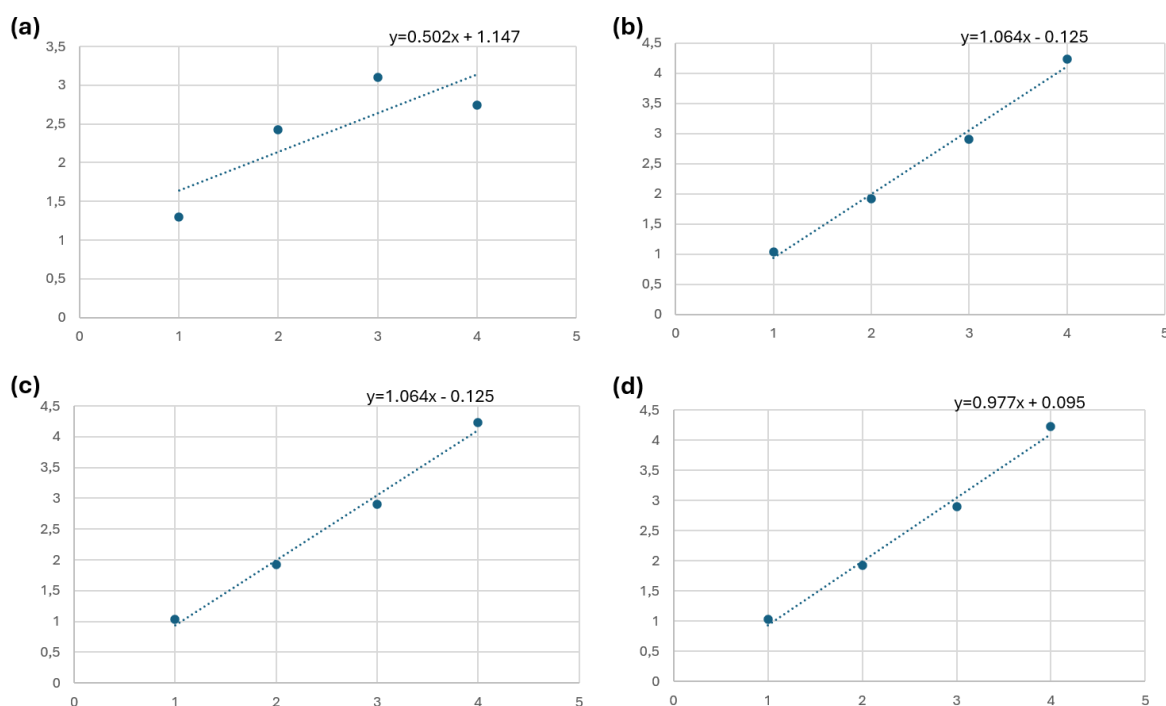
To test if there is a linear relationship between analyte concentrations and detector responses, calibration standards were made with concentrations ranging from 1-4ppm for each of the target analytes. High correlations (greater than $R^2 = .99$) were observed between the detector response and the concentration of acesulfame-K (ACE), saccharin (SAC), aspartame (ASP), and neotame (NEO) (Table 4.1 and Fig. 4.2). These calibration equations provided evidence of similar detector responses at various concentrations in this studied range, indicating the suitability of the analytical method to accurately determine the presence/absence of the above listed compounds in waterways. Other researchers have demonstrated very similar levels of linearity when using HPLC to determine ASWs in waterway and wastewater samples [15; 16]. Overall, based on the correlation values determined in this study, this analytical technique appears to accurately represent analyte concentrations across the tested concentration range.

Table 4.1. Results of validation of the proposed HPLC -UV method for simultaneous analysis of artificial sweeteners.

	<i>Acesulfame K (ACE)</i>	<i>Saccharin (SAC)</i>	<i>Aspartame (ASP)</i>	<i>Neotame (NEO)</i>
<i>Retention time/min</i>	5.65	8.425	14.749	19.409

<i>Slope</i>	63.06	119.15	12.40	6.172
<i>Intercept</i>	-30.13	-14.36	1.56	0.6684
<i>Correlation</i>	0.9978	0.9967	0.9947	0.9965
<i>Standard error</i>	17.48	20.41	3.20	1.33
<i>LOD ug/ml (ppm)</i>	0.915	0.564	0.853	0.712
<i>LOQ ug/ml (ppm)</i>	2.773	1.713	2.586	2.158

194



195

196 **Figure 4.2. Standard linear calibration curves for artificial sweeteners analyzed by HPLC-UV. (a)**
 197 **Acesulfame-K (ACE), (b) Saccharin (SAC), (c) Aspartame (ASP), and (d) Neotame (NEO).** Calibration was
 198 performed over the concentration range of 1–4 ppm (four points). Linear regression yielded correlation
 199 coefficients (R^2) >0.99 for all analytes, confirming a strong linear relationship between analyte concentration and
 200 peak area.

201 Sensitivity

202 The sensitivity of the HPLC-UV method for detecting artificially sweetened compounds was evaluated
 203 using LOD and LOQ values for each analyte of interest. LOD ranged from 0.564 ppm for SAC to 0.915
 204 ppm for ACE; LOQ ranged from 1.713 ppm to 2.773 ppm. The method's ability to accurately quantify
 205 the presence of artificial sweeteners in wastewater samples analysed during this project is demonstrated
 206 by the fact that all measured concentrations exceeded the LOQ. While the LOD and LOQ values
 207 reported here are higher than those commonly reported for other LC–MS/MS-based analytical methods,
 208 they are similar to those reported in literature studies using HPLC–UV to determine artificial sweeteners
 209 in environmental matrices. The range of concentrations determined using these methods can vary

greatly depending on the type of matrix being analysed (environmental vs. biological), the complexity of the matrix, and sample preparation steps (e.g., preconcentration) [17; 18].

The decreased sensitivity compared to LC–MS/MS-based methodologies is due to several inherent characteristics of UV detection [19]. UV detection is less selective than MS detection and is also susceptible to interference from absorbance by non-analyte matrix components [20; 21]. The preconcentration step utilised in the SPE-HPLC-UV methodology provided an increase in method performance; sufficient data were collected regarding ACE, ASP, NEO, and SAC to demonstrate that the concentrations found in the influent and effluent samples could be accurately quantified. In summary, the sensitivity of the SPE-HPLC-UV method provides sufficient information to monitor ASWs in municipal wastewater and assess their removal during treatment. For larger-scale screening or routine monitoring activities, particularly when access to MS instruments is limited, the SPE-HPLC-UV method provides a suitable, analytically reliable alternative.

Precision and Recovery

To assess the precision and accuracy of the HPLC-UV method, triplicate spiked standards were prepared at 4 concentration levels for each of the 4 analytes. Overall recovery ranges were found to be between 68% and 130%. Most of the results for saccharin (SAC), aspartame (ASP), and neotame (NEO) fell within the well-known analytical range of about 80–120%, with many in the narrower range of 95–105% (Table 4.2). Overall, these results are consistent with previously published studies reporting recoveries of 73–112% for artificial sweeteners in water after preconcentration by SPE followed by chromatographic analysis [22]. Higher recoveries were observed for acesulfame-K (ACE) at low spiking levels than at the higher spiking level of 4 ppm, indicating some degree of concentration dependence in either extraction or detection efficiency.

Table 4.2. Measured average concentrations of triplicate samples of standard ASWs.

	Ace (ppm)				Sac (ppm)				Asp (ppm)				Neo (ppm)			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Spiked sample 1	1.0 6	2.0 1	2.6 4	3.7 1	1.0 1	1.3 3	3.6 7	4.6 8	1.03 9	1.9 9	2.0 1	5.5 1	1.01 7	2.1 3	3.1 3	5.5 5
Spiked sample 2	1.9 4	3.1 7	2.8 2	1.2 9	0.9 3	2.3 4	2.0 3	3.7 7	1.32 6	2.0 3	3.1 4	1.9 4	1.35 5	1.6 6	2.4 6	2.1 6
Spiked sample 3	0.9 2	2.0 9	3.8 4	3.2 3	1.1 6	2.1 1	3.0 2	4.2 4	1.2 5	2.3 8	3.1 5	4.1 8	1.03 1	2.0 1	3.1 1	4.6 4
average	1.3 2	2.4 2	3.1 2	2.7 4	1.0 3	1.9 2	2.9 2	4.2 3	1.18 1	2.1 9	2.7 9	3.8 7	1.13 4	1.9 4	2.9 4	4.1 4
% Recoveries	130	121	103	68	103	96	96	105	118	105	93	96	113	97	96	102
Standard deviation	0.4 5	0.5 2	0.5 2	1.0 4	0.0 9	0.4 3	0.6 7	0.3 7	0.11 3	0.1 3	0.4 9	1.4 7	0.15 1	0.2 1	0.3 1	1.4 2

RSD	42, 5	26, 7	20, 8	46, 7	11, 3	27, 5	28, 4	10, 7	7,19	7,7 0	21, 7	46, 6	16,8	13, 7	13, 1	42, 6
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The relative standard deviations (%RSD) of the measurement results ranged from 7.2% to 46.7%, depending on the analyte and the spiking level. Lower %RSD values (approximately $\leq 10\text{--}15\%$) were generally observed in the middle of the calibration curves, whereas higher variability was observed for ACE and ASP at both ends. These larger %RSD values greatly exceeded those normally expected from optimized HPLC-MS/MS methods for artificial sweeteners; i.e., values $< 10\%$ are generally reported. However, considering prior studies examining trace organic contaminants in environmental waters, it can be assumed that the increased %RSD values in this investigation result from additive effects arising from sample preparation, SPE extraction, and instrumental response in a complex wastewater matrix [22; 23]. Despite large variations in %RSD across several measurements, most values showed acceptable recoveries, reasonable linearity, and sufficient selective separation of the analytes.

The relatively high %RSD values observed at certain spiking levels indicate substantial measurement uncertainty under those conditions. Consequently, environmental concentrations near the LOQ should be interpreted with caution, as small analytical fluctuations can lead to disproportionately large apparent changes. As such, the SPE-HPLC-UV method appears to be useful for initial quantitative determinations and qualitative screening of ACE, SAC, ASP, and NEO in wastewater. This method could also serve as a simple tool for evaluating the presence and removal patterns of artificial sweeteners in municipal wastewater treatment plants, where LC-MS/MS instrumentation is limited.

Occurrence of artificial sweeteners in wastewater treatment plants

This is one of the first comprehensive evaluations of how frequently ASW chemicals occur at WWTP intake points and are removed in South African WWTPs. Artificial sweetener chemicals acesulfame-K (ACE), saccharin (SAC), aspartame (ASP), and neotame (NEO) were found to be present in influent and/or effluent water samples obtained from five municipal WWTPs in the City of Tshwane: Rooivaal (ROI), Daspoort (DAS), Sunderland Ridge (SUN), Baviaansport (BAV), and Klipgat (KLI). Detection of these chemical additives for artificial sweeteners across multiple WWTP locations and sampling campaigns suggests that these substances continue to enter municipal wastewater collection systems and may remain present in WWTP-treated effluents; findings consistent with those of numerous other studies conducted internationally, which report that artificial sweeteners commonly appear in surface and wastewater.

Concentration profiles across WWTPs

Four synthetic sweeteners were found in each of the WWTPs examined, though they were present in varying quantities and frequencies. NEOTAME (NEO) was the most consistently observed, with the

highest measured level of 58.995 ppm in the influent at BAV during the summer period. ACE and SAC were usually less than 4 ppm, while ASP was consistently between them. The presence of ASWs is evident in both inflow and outflow samples, indicating ongoing input of ASWs into WWTPs from the residential, commercial, and industrial sectors. This pattern has also been observed in other studies on WWTPs. Studies by [18] and [24] demonstrated that ASWs can persist during standard WWTP treatments. Thus, it is necessary to determine if and how much ASWs will be discharged into a water body after standard WWTP treatment.

Table 4.3. Concentrations of ASWs across different WWTPs and sample types at different seasons.

<i>wwtp id</i>	<i>season</i>	<i>sample type</i>	<i>acesulfame k in ppm</i>	<i>saccharin in ppm</i>	<i>neotame in ppm</i>	<i>aspartame in ppm</i>
<i>ROI</i>	summer	influent	3.89	0.2	32.25	0
<i>ROI</i>	summer	effluent	0.725	0	10.645	3.245
<i>ROI</i>	winter	influent	0.935	0.74	6.4	1.025
<i>ROI</i>	winter	effluent	0	0	5.78	0
<i>DAS</i>	summer	influent	0	0.31	12.92	1.79
<i>DAS</i>	summer	effluent	0	0.405	10.275	0
<i>DAS</i>	winter	influent	2.26	0	53.585	1.06
<i>DAS</i>	winter	effluent	0.19	0	33.45	0.925
<i>SUN</i>	summer	influent	1.54	0.095	32.905	8.37
<i>SUN</i>	summer	effluent	1.86	1.37	0	2.595
<i>SUN</i>	winter	influent	0.495	0.955	3.995	0.5
<i>SUN</i>	winter	effluent	1.62	0.545	50	0
<i>BAV</i>	summer	influent	0	1.325	58.995	0
<i>BAV</i>	summer	effluent	1.69	0.09	1.19	0
<i>BAV</i>	winter	influent	1.97	1.36	49.995	3.84
<i>BAV</i>	winter	effluent	1.075	0	10.14	1.2
<i>KLI</i>	summer	influent	1.6	0.11	54.275	1.79
<i>KLI</i>	summer	effluent	0	0	38.485	0
<i>KLI</i>	winter	influent	0	0.41	0.785	1.46
<i>KLI</i>	winter	effluent	1.22	0	1.735	0

Seasonal Variation

Seasonal variations in ASW concentrations were found for each of the WWTPs studied (Table 4.3; Figure 4.3). For example, Neotame (NEO) concentrations at Rooivaal (ROI) decreased from 32.25 ppm in summer to 6.40 ppm in Winter. Conversely, winter concentrations exceeded summer concentrations at Daspoort (DAS). Similar seasonal variation in concentrations was observed for Acesulfame-K (ACE) at numerous WWTPs, with higher influent concentrations typically measured during the warmer summer months. These variations could be due to one or more variables, including changes in seasonal consumer behavior (e.g., consumption of sweetened drinks and processed foods); wastewater generation patterns; hydraulic loading; dilution effects; and temperature-dependent biodegradation. As previously noted by Lange et al. [25] and Marazuela et al. [26]. There has been considerable seasonal variation in ASW concentrations in both wastewater and receiving water bodies, attributable to seasonal

changes in usage and hydrologic conditions. Lastly, Saccharin (SAC) and Aspartate (ASP) did not consistently exhibit seasonal trends across WWTPs, suggesting that regional watershed characteristics, industrial inputs, and facility-specific operating practices may have a greater effect on them.

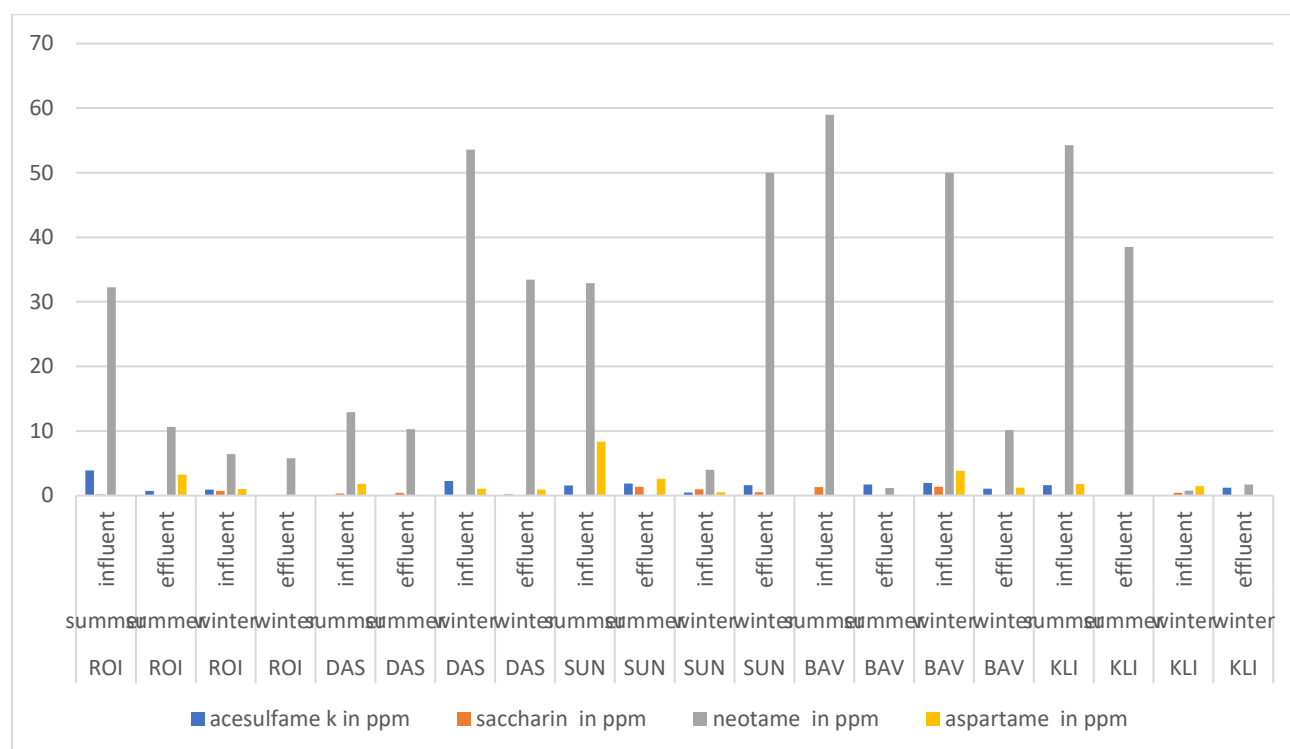


Figure 4.3: Artificial sweetener concentrations in influent and effluent wastewater from 5 different treatment plants within the city of Tshwane for both Summer and Winter seasons. Measured (ppm) acesulfame-K (ACE), saccharin (SAC), aspartame (ASP) and neo-tame (NEO) concentrations are shown in bar format to show the relative presence of each compound across all five sampling locations (ROI, DAS, SUN, BAV & KLI) sampled for both influent and effluent samples, showing some compounds appear in influent and/or effluent samples regardless of the season.

4.2.3 Removal Efficiency

Removal efficiencies varied considerably among WWTPs and among individual ASWs (Table 4.4; Figure 4.4). The highest removal efficiencies were generally observed for SAC and ASP, with complete removal (100%) achieved during at least one sampling period at several facilities. For example, ROI achieved complete removal of SAC in both seasons and of ASP in winter, indicating that these compounds are more amenable to removal under the prevailing treatment conditions. In contrast, NEO exhibited lower and more variable removal efficiencies across the plants. Although BAV achieved high NEO removal (>79%) in both summer and winter, DAS and KLI showed substantially lower reductions; in some instances, only modest decreases in concentration were observed. These findings suggest that NEO may be more resistant to conventional biological treatment processes than SAC and ASP, consistent with previous reports of incomplete removal of certain artificial sweeteners in municipal treatment systems.

Table 4.4. Percentage removal of ASWs from selected WWTPs (ND: not detected influent concentration =0, making removal calculation impossible).

WWTPs	Season	ACE	SAC	NEO	ASP
ROI	summer	81,36247	100	66,99225	ND
ROI	winter	100	100	9,6875	100
DAS	summer	ND	-30,6452	20,47214	100
DAS	winter	91,59292	ND	37,57581	12,73585
SUN	summer	73,3871	30,29197	ND	80,73218
SUN	winter	-227,273	42,93194	-1151,56	100
BAV	summer	ND	93,20755	97,98288	ND
BAV	winter	45,43147	100	79,71797	68,75
KLI	summer	100	100	29,09258	100
KLI	winter	ND	100	-121,019	100

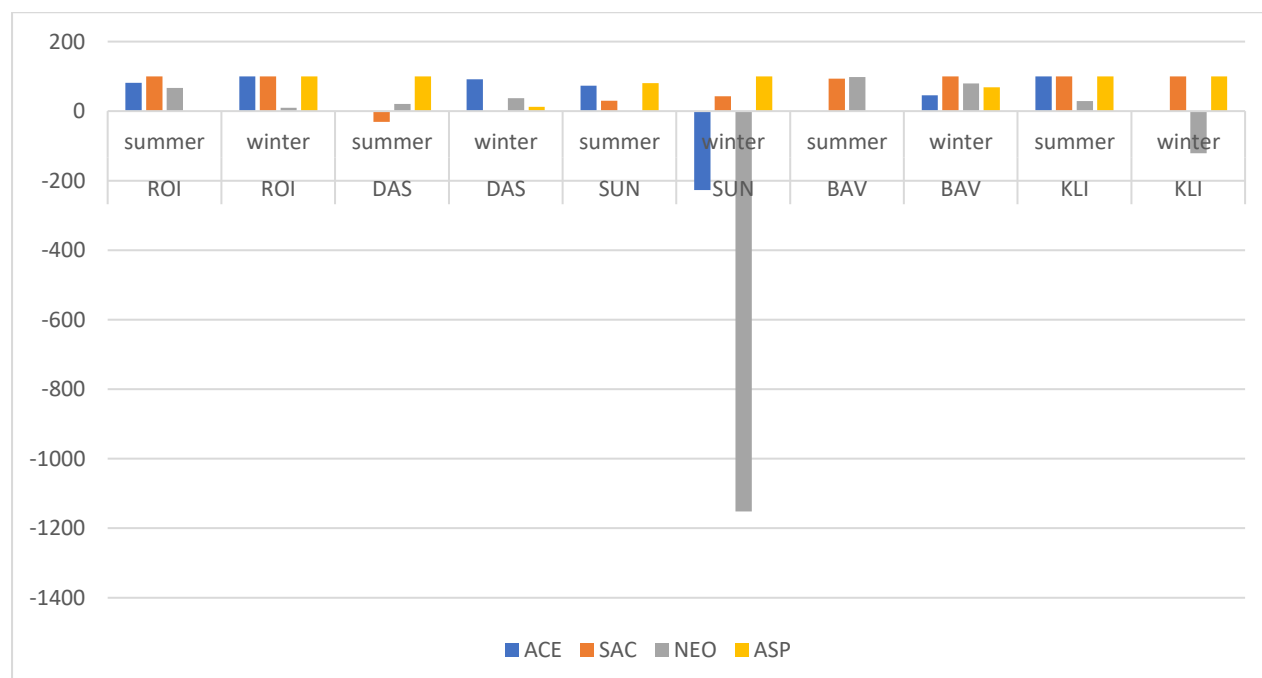


Figure 4.4. Percentage removal of artificial sweeteners in five wastewater treatment plants in the City of Tshwane during summer and winter. Bars show calculated removal efficiencies (%) for acesulfame-K (ACE), saccharin (SAC), aspartame (ASP), and neotame (NEO) at Rooivaal (ROI), Daspoort (DAS), Sunderland Ridge (SUN), Baviaansport (BAV), and Klipgat (KLI). Positive values indicate a net reduction from influent to effluent, whereas negative values indicate higher effluent concentrations than influent, illustrating both spatial and seasonal variability in treatment performance across compounds.

Several facilities exhibited negative removal efficiencies, indicating that measured concentrations in the effluent exceeded those in the influent samples. The most pronounced example was Sunderland Ridge (SUN), where NEO concentrations increased from 3.995 ppm in winter influent to 50 ppm in the corresponding effluent. Negative or near-zero removal values have been reported for a range of emerging contaminants, including artificial sweeteners, and may arise from multiple factors such as

transformation of conjugated or precursor compounds into the measured parent compound, desorption from suspended solids or sludge, deconjugation reactions, temporal mismatches between influent and effluent sampling, or analytical uncertainty [6; 25; 27; 28]. Because influent and effluent were sampled on the same day without accounting for plant-specific hydraulic retention times, temporal mismatches likely contributed to negative removal estimates. Such behavior highlights the complexity of contaminant dynamics within activated sludge systems, and the apparent “production” of a compound within the treatment train does not necessarily imply true formation de novo; it may instead reflect redistribution or transformation processes.

Therefore, the results demonstrate that while conventional WWTPs in the City of Tshwane can substantially reduce certain artificial sweeteners, complete removal is not consistently achieved for all compounds or at all facilities. The persistence of detectable concentrations, particularly of NEO, in treated effluents indicates that artificial sweeteners can be discharged into receiving waters and supports the need for continued monitoring and consideration of advanced treatment options where environmental protection goals require more complete removal.

Environmental implications

The persistence of ASWs in treated effluents raises concerns about their continuous discharge into receiving aquatic environments. In this study, most measured concentrations were below the guideline values reported by Naik et al. [29]; however, NEO exceeded the recommended limit of 33 ppm at several locations, most notably at Daspoort (DAS) during winter. These elevated concentrations indicate that conventional treatment processes may not consistently achieve adequate NEO removal under all operating conditions and may allow episodic high-level releases.

Ongoing discharge of ASWs into surface waters may contribute to their accumulation in aquatic systems and support their proposed use as chemical tracers or indicators of wastewater contamination. Given their high persistence, mobility, and frequent detection in both wastewater effluents and downstream environments reported globally, there is a clear rationale for considering additional or upgraded treatment technologies, such as ozonation, activated carbon adsorption, or membrane-based processes, to enhance removal efficiencies where necessary. Future studies in South Africa should focus on the fate and transport of these compounds in receiving waters, the formation and behaviour of transformation products, and potential ecological impacts on aquatic organisms to better define the environmental significance and risk associated with ASWs.

Conclusion

This study assessed the occurrence and removal of four ASWs: ACE, SAC, ASP, and NEO in influent and effluent from five municipal wastewater treatment plants in the City of Tshwane. All target

compounds were detected in wastewater, and measurable concentrations frequently remained in treated effluents, indicating that conventional treatment processes do not consistently achieve complete removal. Removal efficiencies varied among plants and compounds, with SAC and ASP generally showing higher removal, whereas NEO exhibited lower, more variable removal and occasionally negative apparent removal. Seasonal differences were evident at some facilities, suggesting that consumption patterns, catchment characteristics, and plant operation jointly influence artificial sweetener loads.

The persistence of these compounds in effluents highlights WWTPs as ongoing sources of emerging contaminants to receiving waters and supports the use of artificial sweeteners as indicators of wastewater impact. Routine monitoring, with particular attention to NEO, and consideration of advanced or optimised treatment options are recommended where elevated concentrations persist. Future work should expand temporal coverage, employ more selective analytical techniques, and investigate the fate, transformation products, and ecological implications of artificial sweeteners in South African aquatic environments.

Abbreviations

ACE-K = Acesulfame-k

ASP = Aspartame

ASWs = Artificial sweeteners

DAD = Diode array detection

DNA = Deoxyribonucleic acid

DLLME = Dispersive liquid-liquid micro-extraction

EDCs = Endocrine-disrupting chemicals

EF = Enrichment factor

EMP = Emerging environmental pollutants

HLLME = Homogeneous liquid-liquid microextraction

HPLC = High-pressure liquid chromatography

LLE = Liquid-liquid extraction

Log Kow = Octanol-water partition coefficient

LOD = Limit of detection

LOQ = Limit of quantification

386 ng/L = Nano gram per litre
387 mg/L = Milli gram per litre
388 POPs = Persistent Organic Pollutants
389 pKa = Acid Dissociation Constant
390 PPCPs = Pharmaceuticals and personal care products
391 RSD = Relative Standard Deviation
392 SAC = Saccharin
393 Ksp = Solubility constant
394 SPE = Solid-phase extraction
395 SPME = Solid phase microextraction
396 WHO = World Health Organization
397 WWTPs = Wastewater treatment plants
398 µg/L = Micro gram per litre
399 %R = Relative standard deviation
400 %RSD = Percentage relative standard deviation
401 R2 = Correlations Correlation coefficient
402 ROI = Rooivaal
403 BAV = Baviaanspoort
404 SUN = Sunderland ridge
405 KLI = klipgat
406 BAV = Baviaanspoort

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410 **Author contributions**

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412 Investigation: M.E.M., N.R.S., and I.K., Visualisation: M.E.M., N.R.S., Funding acquisition: I.K.,
413 Project administration: I.K., Supervision: M.E.M., I.K. and T.N., Writing – original draft: N.R.S.,

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Data availability

All data generated or analyzed during this study are included in this published article. Additional data are available from the corresponding author on reasonable request.

Declarations

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Ethics approval and consent to participate

Not applicable. This study did not involve human participants, human data, human tissue, or animals.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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