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SPATIAL-TEMPORAL VARIATIONS OF ARTIFICIAL SWEETENERS IN GROUNDWATER IN XIZANG AND SICHUAN PROVINCES, CHINA

Artificial sweeteners (ASs) are emerging contaminants widely used in food, beverages, and personal care products. Due to their resistance to biodegradation, ASs are frequently detected in aquatic environments, including groundwater. The spatial-temporal distribution, urbanization influences, and human health risks of four ASs (including acesulfame (ACE), cyclamate (CYC), saccharin (SAC), and sucralose (SUC)) in groundwater from the Sichuan and Xizang Provinces, China, were investigated. A total of 153 groundwater samples were collected during the flood (July) and dry (November) seasons in 2022. All targets ASs were detected, with ACE showing the highest detection frequency (100%) and SUC the highest concentrations. The levels of AS were generally elevated in flood seasons and urban areas, showing a decreasing trend from central to eastern Sichuan. Xizang exhibited higher average concentrations than Sichuan, likely due to lower degradation rates under cooler temperatures. Significant positive correlations were found between AS concentrations and both gross domestic product (GDP) and population density ($p < 0.05$), indicating strong urbanization influence. Although estimated daily intakes via groundwater ingestion posed negligible non-carcinogenic risks for adults and children, cumulative exposure remains a concern. This study provides valuable insight into the occurrence and distribution of ASs in groundwater across contrasting geographic and socio-economic regions in China.

1. INTRODUCTION

Artificial sweeteners (ASs) are a group of synthetic sugar substitutes characterized by high sweetness and low caloric content. Since the 1950s, they have been widely used in a variety of products, including food, beverages, personal care items, and animal feed [1, 2]. According to *the Chemical Economics Handbook*, global AS consumption exceeded 156,000 Mg in 2017, with China accounting for approximately 32% of the global total, making it the largest consumer worldwide [3]. ASs have emerged as a class of environmental contaminants of growing concern, particularly after the detection of sucralose (SUC) in Sweden in 2007 [4, 5].

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Due to their resistance to metabolic degradation and high-water solubility, most ASs are excreted from the human body unchanged via urine and feces [5, 6]. Consequently, they enter wastewater treatment plants (WWTPs) and are released into the environment through effluents, making WWTPs a major point source of ASs in aquatic systems [7]. Additionally, direct discharges from domestic, industrial, agricultural, and livestock sources further contribute to ASs contamination in water bodies [7]. As a result of their widespread usage and incomplete removal, ASs have been frequently detected in various environmental media, including wastewater [8], surface water [4, 9], seawater [9], groundwater [9], and even drinking water [10].

The presence of ASs in the environment raises concerns about their potential ecological and human health impacts [11, 12]. Groundwater, a vital source of drinking water, is particularly vulnerable to contamination through leaching from septic systems, leaking sewers, storage tanks, wastewater infiltration systems, and polluted surface water [13]. Such contamination can affect urban streams, wetlands, lakes, and private wells [14]. Numerous studies have reported the presence of ASs in groundwater globally, with concentrations ranging from nanograms per liter (ng/dm^3) to micrograms per liter (μg/dm^3). Detection has been documented in China [9], Singapore [15], Canada [16], South Korea [17], India [18], Sweden [4], and Barbados [19]. Lee et al. [17] observed significant differences in the occurrence and distribution of ASs (especially acesulfame (ACE)) between agricultural and non-agricultural rural areas in South Korea. Moreover, AS concentrations in groundwater are influenced by geographic location and seasonal variation. However, comprehensive comparative studies examining AS levels across different regions in China remain scarce.

This study aims to address these gaps by investigating the spatial and temporal distribution of four commonly used ASs (ACE, CYC, SAC, and SUC) in groundwater across two distinct regions in China: Sichuan Province, a densely populated area with substantial industrial and agricultural activity, and Xizang, a plateau region characterized by animal husbandry and tourism. Groundwater samples were collected during both the flood season (July) and the dry season (November). The objectives of this study are threefold: (1) to provide a detailed assessment of AS concentrations in groundwater from plateau and basin regions, (2) to analyze the spatial-temporal variations and their potential driving factors, and (3) to evaluate the influence of urbanization on AS distribution and assess the associated human health risks via groundwater ingestion.

2. MATERIALS AND METHODS

Study areas. Sichuan Province, located in the upper reaches of the Yangtze River, features a diverse topography comprising plateaus, basins, and hills, and supports a large population and extensive industrial activities. Groundwater samples were collected primarily in the central-eastern basin region of Sichuan during the flood season (July 2022, $n = 66$) and dry season (November 2022, $n = 58$). Xizang, situated on the southwestern

Tibetan Plateau, is characterized by robust tourism and animal husbandry sectors. Groundwater samples from Xizang were collected during the same periods: July 2022 (n = 18) and November 2022 (n = 11). Sampling locations are shown in Fig. 1, and the functional area classifications for Sichuan are provided in Table 1. All samples were stored at 4 °C without any preservatives until analysis.

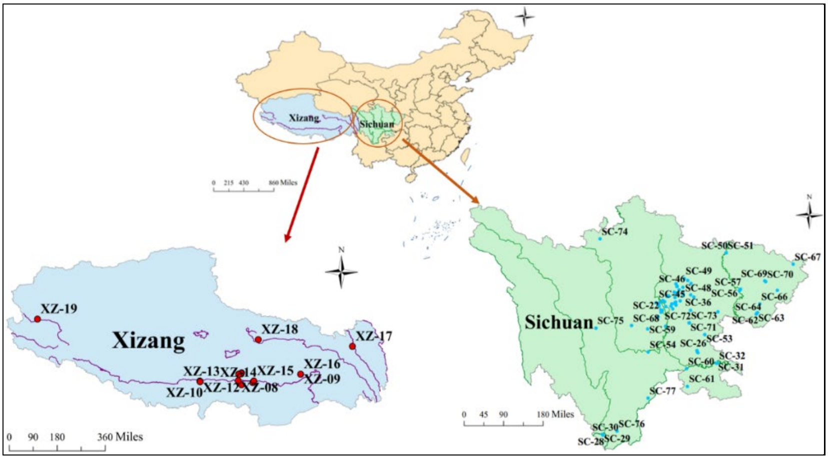

Fig. 1. Map of the sampling sites

Table 1

Functional area division of the sample sites in Sichuan Province, China

Functional area	Sample sites
Agricultural area	SC06, SC12, SC13, SC15, SC16, SC17, SC18, SC19, SC20, SC21, SC22, SC24, SC25, SC26, SC27, SC28, SC30, SC36, SC37, SC38, SC43, SC46, SC53, SC54, SC55, SC56, SC59, SC61, SC66, SC68, SC69, SC70, SC71, SC74, SC75, SC77
Business district	SC02, SC14, SC34, SC44, SC45, SC49, SC50, SC51, SC52, SC58, SC62, SC63, SC65, SC72, SC76
Living quarter	SC07, SC09, SC10, SC11, SC29, SC31, SC32, SC33, SC35, SC41, SC47, SC48, SC60, SC67, SC73

Sample analysis method. Quantification of ASs was performed using an SCIEX Exion LC system coupled with a SCIEX Triple Quad 4500 QTRAP mass spectrometer (Framingham, MA, USA). Electrospray ionization (ESI) was conducted in negative mode with multiple-reaction monitoring (MRM). Data were processed using MultiQuant 3.0.3 and Analyst 1.7 software.

Chromatographic separation was performed isong an Athena C18-WP column (2.1×50 mm, 3 μm, ANPEL Laboratory Technologies, Shanghai). The column temperature was set to 40 °C, with a flow rate of 0.4 cm^3/min. The mobile phase consisted of 5 mM ammonium acetate in water (A) and methanol (B). The gradient profile was as follows: 0–1.0 min, B from 0 to 75%, held for 0.5 min, decreased to 10% in 0.3 min, increased to 75% in 0.2 min, returned to 0% in 0.3 min, equilibrated for 3.7 min. The injection volume was 2 µL. The desolvation gas temperature was 500 °C, curtain gas pressure 0.241 MPa, capillary voltage –4500 V, and dwell time 30 ms.

Groundwater samples (50 cm^3 each) were extracted using Oasis WAX solid-phase extraction (SPE) cartridges (3 cm^3, 60 mg, Macherey Nagel, Düren, Germany), following a modified method from Gan et al. [20]. Cartridges were preconditioned with 6 cm^3 methanol followed by 6 cm^3 of 25 mM sodium acetate buffer (pH 4). Spiked samples containing ISs were passed through the cartridges at a flow rate of 1–2 cm^3/min. After sample loading, cartridges were rinsed with 6 cm^3 of the same buffer and eluted with 6 cm^3 methanol containing 2% ammonia. The eluates were evaporated under nitrogen at 40 °C and reconstituted in 0.5 cm^3 methanol. The final concentration of each IS was 40 ng/cm^3.

The detection limits for ACE, CYC, SAC, and SUC using standard sample analysis methods were 0.3, 0.3, 0.6, and 6.6 ng/dm^3, respectively. Due to a 100-fold concentration during the pretreatment process, the effective detection limits for these compounds were reduced by a factor of 100 compared to the standard methods.

Human risk assessment. Non-carcinogenic risk associated with AS exposure via groundwater ingestion was assessed using the U.S. Environmental Protection Agency's human health risk (HHR) model [21]. Parameters used in the calculations are provided in Table 2.

Table 2

The parameters of exposure assessment

Age group	Intake rate [dm^3/d]	Exposure frequency [d/a]	Exposure cycle [a]	Average body weight [kg]	Average time [d]
Children	1.5	350	6	15	2190
Adults	3.14	350	24	70	8760

The chronic daily intake (*CDI*) and hazard quotient (*HQ*) were as follows [22]:

$$CDI = \frac{C_i IR\, EF\, ED}{BW\, AT} \tag{1}$$

$$HQ = \frac{CDI}{RfD} \tag{2}$$

where *CDI* denotes exposure to the ASs in drinking water by the oral route of intake, mg/kg/d, C_i the concentrations of the ASs in the groundwater, mg/dm^3, *IR* the intake rate, dm^3/d, *EF* exposure frequency, d/year, *ED* denotes exposure cycle, year, *BW* average body weight, kg, *AT* average time. d, days corresponding to *ED* in this study, *HQ* denotes hazard entropy and *RfD* denotes acceptable daily intake, mg/kg/d. When the *HQ* is < 1, the ASs risk to health is acceptable, if *HQ* > 1, indicating unacceptable health risk.

Statistical analysis. All statistical analyses were performed using SPSS 21.0. Since the AS concentration data did not follow a normal distribution, non-parametric methods were applied. Spearman's correlation was used to assess relationships among ASs and between AS concentrations and urbanization indicators. Differences in AS concentrations across seasons and regions were evaluated using the Mann–Whitney *U* test. A significance level of $p < 0.05$ was adopted for all analyses.

3. RESULTS

3.1. OCCURRENCE OF ARTIFICIAL SWEETENERS IN GROUNDWATER

As presented in Table 3, all four targeted artificial sweeteners ASs were detected in groundwater. ACE exhibited the highest detection frequency (*DF*) at 100%, with concentrations ranging from 1.9 to 3180 ng/dm^3. In contrast, sucralose (SUC) showed the lowest *DF* at 71%, with concentrations varying from below the detection limit (*ND*) to 1510 ng/dm^3.

Table 3

Occurrence of the four artificial sweeteners in the study area

Province	Compound	Flood season ($n = 66$)					Dry season ($n = 58$)				
		DF [%]	Max [ng/dm^3]	Min [ng/dm^3]	Med [ng/dm^3]	Ave [ng/dm^3]	*DF* [%]	Max [ng/dm^3]	Min [ng/dm^3]	Med [ng/dm^3]	Ave [ng/dm^3]
Sichuan	ACE	100	3180	1.9	22.1	100.1	100	3,070	1.6	23.5	117.1
	CYC	92	719	0	3.5	31.6	93	508	0	3.5	31.3
	SAC	85	373	0	5.5	16.3	91	158	0	7	15.3
	SUC	71	964	0	47.1	132.1	84	1,170	0	87.7	161.4
Xizang	ACE	100	1,380	2.2	31	100	302	0.9	11.6	ACE	100
	CYC	89	1,370	0	6.5	91	115	0	2.4	CYC	89
	SAC	89	309	0	8.3	82	22.3	0	3.8	SAC	89
	SUC	83	1,510	0	128.5	73	1110	0	102	SUC	83

Max – maximum, Min – minimum, Med – median, Ave – average, *DF* – detection frequency.

The observed concentrations of ACE and SUC in this study were notably higher than those reported in previous investigations in China, Singapore, Canada, South Korea [17], and Barbados [19]. The maximum concentrations of cyclamate (CYC) and saccharin (SAC) were 1370 and 373 ng/dm^3, respectively. CYC levels exceeded those in similar studies conducted in the aforementioned countries, except for those in the Yellow River

Basin, China. SAC concentrations were the lowest, consistent with previous findings. These variations suggest potential geographic differences in AS usage and environmental behavior.

3.2. DISTRIBUTION OF ARTIFICIAL SWEETENERS IN FLOOD AND DRY SEASONS IN THE GROUNDWATER

The concentration ranges of the four ASs in the Sichuan Province are shown in Fig. 2a. ACE was present in all samples across both seasons, with peak concentrations of 3180 ng/dm^3 in the flood season and 3070 ng/dm^3 in the dry season. Total concentrations of ACE, CYC, SAC, and SUC were 6978, 2024, 1056, and 8932 ng/dm^3 in the flood season and 6794, 1813, 886, and 9362 ng/dm^3 in the dry season, respectively. As shown in Fig. 2b, the concentration trend was consistent across both seasons: SUC > ACE > CYC > SAC. SUC dominated the profiles in both periods. The persistence of ACE and SUC in the environment may explain their elevated levels, as they are more resistant to biodegradation than CYC and SAC. ACE is also widely consumed in food and beverages due to its low cost and intense sweetness.

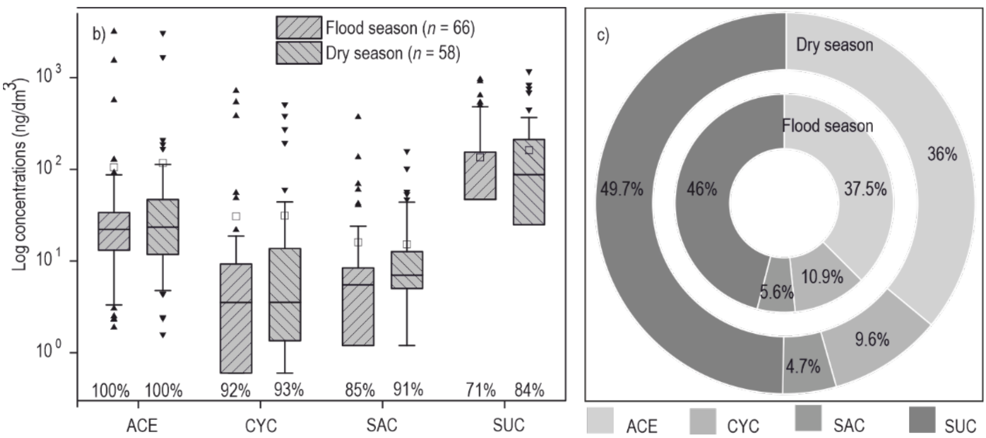

Fig. 2. Box-and-whisker plots showing the concentrations of the investigated ASs in the groundwater in Sichuan (a), and fan charts showing the distribution of the four ASs in the flood and dry seasons

Detection frequencies for all ASs were generally higher in the dry season. While total AS concentrations were higher in the flood season (except for SUC), SAC showed a statistically significant difference between the two seasons ($p < 0.05$, Mann–Whitney U test). This may be attributed to increased consumption of sweetened products during warmer months, tourism-related activities during summer vacation, and the mobilization of pollutants due to stormwater runoff or sewer overflows.

Table 4

Correlation analysis among the ASs in the Sichuan Province

Season	Variable	ACE	CYC	SAC	SUC
Flood season	ACE	1	0.282*	0.086	0.469**
	CYC	0.282*	1.000	0.291*	0.435**
	SAC	0.086	0.291*	1.000	0.216
	SUC	0.469**	0.435**	0.216	1.000
Dry season	ACE	1.000	0.575**	0.442**	0.635**
	CYC	0.575**	1.000	0.454**	0.507**
	SAC	0.442**	0.454**	1.000	0.406**
	SUC	0.635**	0.507**	0.406**	1.000

Asterisks indicate significant correlations: * $p < 0.05$, ** $p < 0.01$.

Spearman correlation analysis (Table 4) indicated significant correlations among most ASs ($p < 0.05$), suggesting common sources or similar transport pathways. SAC exhibited weak correlation with other ASs in the flood season, implying distinct sources such as livestock feed or herbicide degradation products that enter groundwater through soil leaching [23].

3.3. SPATIAL DISTRIBUTION OF THE ARTIFICIAL SWEETENERS IN THE GROUNDWATER IN SICHUAN PROVINCE

Figure 3a shows that SC11 and SC58 recorded the highest AS concentrations in both seasons, with values exceeding those reported for WWTP influents in Shenzhen, Dalian, and Hanoi [24, 25]. SC11, located in a residential area near a major tourist site (Anren Ancient Town), and SC58, in a commercial area, were likely influenced by intense human activity. ACE concentrations were particularly elevated, likely due to its high environmental persistence and mobility in groundwater.

As shown in Fig. 3b, AS concentrations in residential and commercial areas were generally higher than those in agricultural zones, except for SAC. SAC's higher levels in agricultural areas may result from its use in animal feed and from herbicide transformation in soils. Additionally, sites near rivers may exhibit elevated concentrations due to river infiltration. The elevated concentrations in urban areas align with known consumption patterns of sweetened products.

Figure 3c reveals a decreasing trend in AS concentrations with increasing longitude, from central to eastern Sichuan. This likely reflects regional disparities in population density, industrial activity, and tourism. Principal component analysis (PCA) identified a single principal component (PC1, eigenvalue = 2.5) explaining 62.5% of the variance. ACE, CYC, and SUC had high loadings, indicating similar sources, consistent with correlation analysis results.

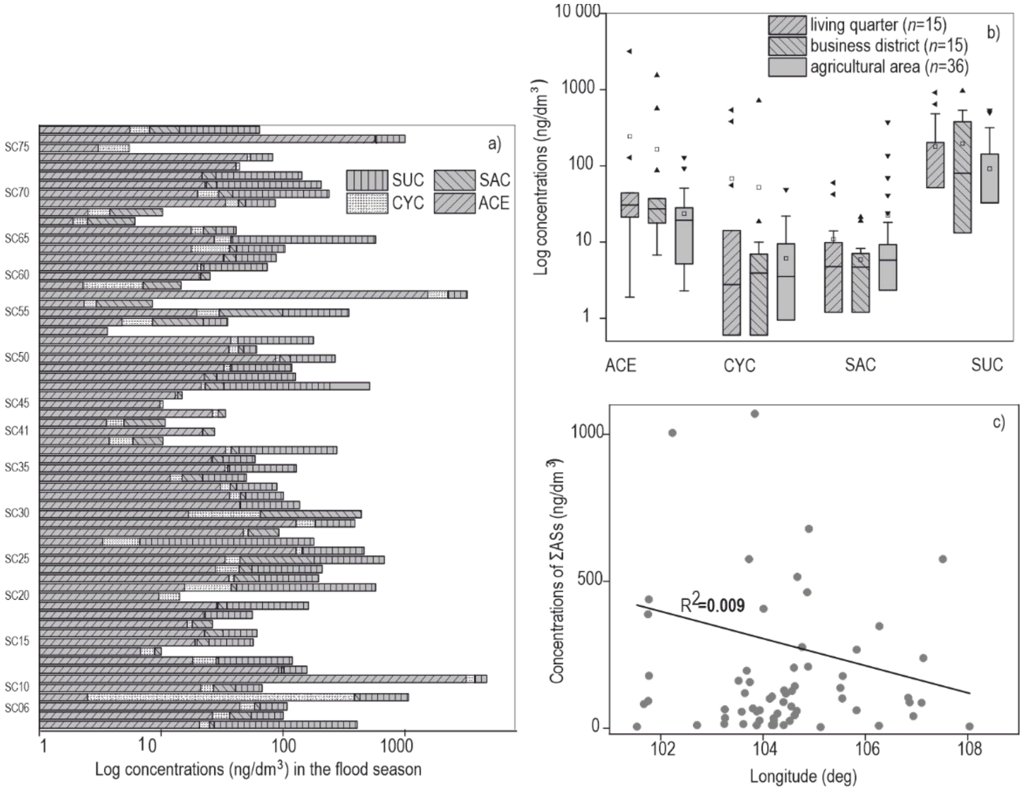

Fig. 3. Concentrations of the ASs for Sichuan Province (a) at sampling sites (b), and box-and-whisker plots showing the concentrations of the investigated ASs in different functional area at longitude (c)

3.4. ARTIFICIAL SWEETENERS IN THE PLATEAU AND BASIN AREAS

AS concentrations in groundwater from Sichuan and Xizang were compared to explore regional differences as shown in Fig. 4. In Sichuan, the ranges were: ACE (1.9–3180 ng/dm^3), CYC (*ND* – 719 ng/dm^3), SAC (*ND* – 373 ng/dm^3), and SUC (*ND* – 964 ng/dm^3). In Xizang, average concentrations were higher: ACE (150.7 ng/dm^3), CYC (93.8 ng/dm^3), SAC (34.9 ng/dm^3), SUC (281.3 ng/dm^3), compared to Sichuan: ACE (108.3 ng/dm^3), CYC (31.4 ng/dm^3), SAC (16.2 ng/dm^3), SUC (132.9 ng/dm^3). SAC levels differed significantly between regions ($p < 0.05$, Mann–Whitney *U* test). These differences may stem from anthropogenic activity and climatic conditions. Warmer temperatures in Sichuan may have enhanced microbial degradation of ASs, especially ACE.

Figure 4b shows SUC as the most dominant AS in both regions, followed by ACE. SAC and CYC contributed the least. The persistence and longer half-lives of ACE and SUC (8.4–12.3 and 7.3–10.8 days, respectively) may facilitate their migration from soil to groundwater more effectively than SAC and CYC. These findings suggest that AS occurrence is influenced by regional consumption patterns, environmental degradation, and population density.

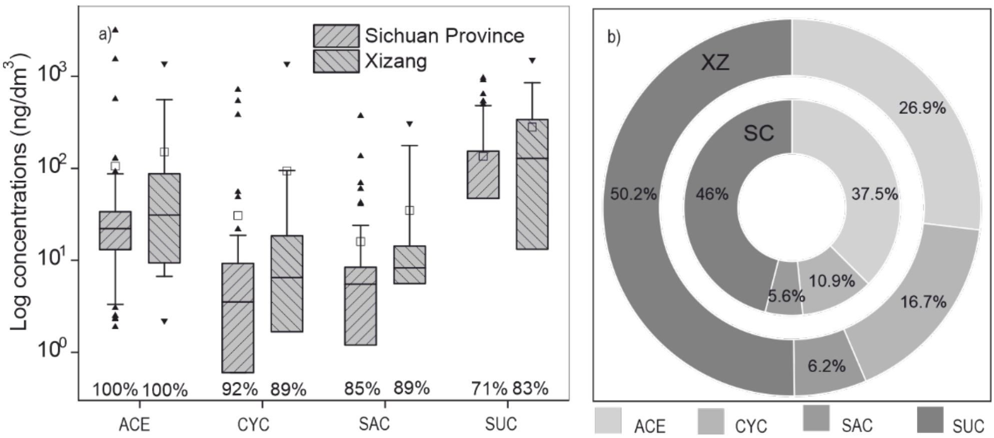

Fig. 4. Box-and-whisker plots show the concentrations of the ASs in groundwater in Sichuan and Xizang (a), and fan charts show the distribution of the four ASs in Sichuan and Xizang (b)

4. DISCUSSION

4.1. POTENTIAL IMPACT OF GDP AND POPULATION DENSITY ON THE ASs CONCENTRATIONS

To assess the impact of urbanization on the distribution of ASs, the potential correlations between AS concentrations and Gross Domestic Product (GDP) as well as population density were analyzed (Fig. 5). Since ASs are commonly used in various food and beverage products, their consumption can reflect regional dietary behavior and, by extension, local living standards associated with economic development.

Significant positive correlations ($p < 0.05$) were observed between city-level GDP and the average concentrations of ASs, indicating that economically developed regions tend to have higher AS consumption. This finding aligns with previous research suggesting that increased income levels are associated with greater consumption of processed foods and beverages containing ASs [26].

Similarly, AS concentrations also showed a significant positive correlation with population density ($p < 0.05$), confirming that densely populated areas are more likely to exhibit higher AS contamination in groundwater. This observation is consistent with the findings of Gao et al. [26], who reported a direct association between population size and AS levels in municipal wastewater.

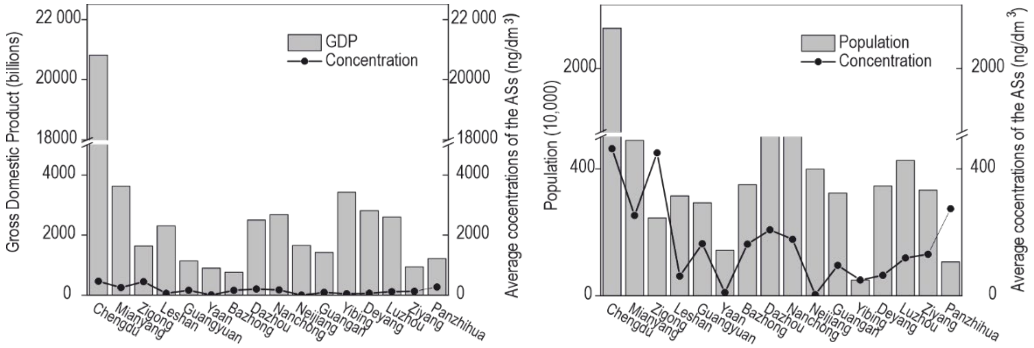

Fig. 5. GDP (2022) (a) and population density versus average AS concentrations in cities of Sichuan Province (b)

Collectively, these results underscore the role of urbanization characterized by both economic prosperity and population aggregation – as a key driver in the spatial distribution of ASs. However, regional discrepancies still exist. For instance, Yang et al. [24] reported no significant correlation between GDP per capita and AS consumption in Dalian, China. This divergence highlights the influence of localized dietary preferences, food industry development, and social habits, suggesting that AS usage patterns may vary significantly across different geographic and cultural contexts.

4.2. HUMAN RISK ASSESSMENT

The estimated daily intakes (EDIs) of ACE and SAC via groundwater ingestion were notably higher in Sichuan than in Xizang, particularly for ACE (Fig. 6). In Sichuan, children's daily intake of ACE reached 305 ng/(kg·day), compared to 137 ng/(kg·day) for adults – more than double the values observed in Xizang. Additionally, across all regions, children consistently exhibited higher AS exposure than adults, likely due to their lower body weight and relatively higher water intake rates. Despite these differences, all EDIs for the four target ASs were substantially below their respective acceptable daily intake (ADI) values (for ACE 15 mg/(kg·d), for CYC 11 mg/(kg·d), for SAC 2.5 mg/(kg·d) and for SUC 15 mg/(kg·d), suggesting that the health risks posed by ASs through groundwater ingestion are negligible in both Sichuan and Xizang.

Nevertheless, groundwater is only one of several potential exposure pathways. ASs are also widely ingested through direct consumption of sweetened beverages, preserved fruits, ice cream, and baked goods. Emerging evidence indicates that certain ASs – particularly SAC – may have adverse health impacts, including impaired glucose tolerance, increased body mass index (BMI), and disruptions to gut microbiota [27]. Furthermore, studies have linked ASs to cardiovascular dysfunction [28] and the development of hepatic steatosis and non-alcoholic fatty liver disease [29].

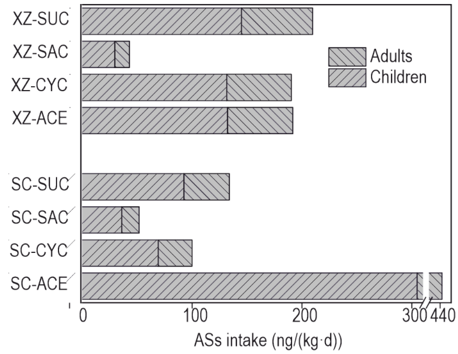

Fig. 6. The ASs intake for adults and children in Sichuan and Xizang in China

Therefore, although the risk from groundwater ingestion is currently minimal, the cumulative effects of multi-source AS exposure merit closer scrutiny. Further research is warranted to evaluate total dietary exposure, long-term health outcomes, and the combined effects of ASs in diverse environmental matrices.

5. CONCLUSIONS

This study provides a comprehensive assessment of the occurrence, spatial-temporal variations, and potential health risks of four commonly used artificial sweeteners (ACE, CYC, SAC, and SUC) in groundwater across the Sichuan and Xizang Provinces in China. All target ASs were detected in groundwater samples, with ACE exhibiting the highest detection frequency and SUC presenting the highest overall concentrations. SUC and ACE dominated the AS profiles, reflecting their high usage and strong environmental persistence.

Seasonal variation analyses revealed higher AS concentrations during the flood season, likely due to increased consumption, tourism, and stormwater mobilization. Spatially, urbanized areas, including residential and commercial zones, showed significantly higher AS levels than agricultural regions, while AS concentrations decreased from central to eastern SICHUAN. In comparison to SICHUAN, Xizang exhibited generally higher AS concentrations, which may be attributed to regional differences in temperature, microbial degradation, and human activities.

Statistical analyses confirmed significant positive correlations between AS concentrations and both GDP and population density, highlighting the influence of urbanization on AS distribution. Although the estimated daily intakes of ASs via groundwater ingestion were below acceptable thresholds and thus pose negligible health risks, children were found to be more vulnerable than adults. Nevertheless, considering additional exposure routes such as food and beverages, the cumulative health risks associated with ASs warrant continued monitoring and research.

Overall, this study advances the understanding of AS contamination in groundwater and provides new insights into their environmental behavior and public health relevance, particularly under the dual influences of urbanization and seasonal dynamics in plateau and basin regions.

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