Estimating alcohol and tobacco consumption of university students and urban population in Ho Chi Minh city by wastewater-based epidemiology

Thi Thuy Quyen Do (✉ dttquyen@hcmus.edu.vn)
University of Science, Viet Nam National University Ho Chi Minh City

Thi Yen Nhi Tran
University of Science, Viet Nam National University Ho Chi Minh City

Thi Thanh Nhon Nguyen
University of Science, Viet Nam National University Ho Chi Minh City

Thi Hien To
University of Science, Viet Nam National University Ho Chi Minh City

Research Article

Keywords:

Posted Date: October 23rd, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3435598/v1

License: ☒ ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: No competing interests reported.
Abstract

This study employed wastewater-based epidemiology (WBE) to assess alcohol and tobacco consumption of university students and urban residents in Ho Chi Minh City, Vietnam. Ethyl sulfate and cotinine were chosen to be the markers of alcohol and tobacco use, respectively. The estimation showed that on average a student consumed between $0.69 \pm 0.13$ mL of pure alcohol and $1.2 \pm 0.2$ mg nicotine per day. These values were significantly lower when compared to the consumption rates among urban residents, which were $2.41 \pm 0.34$ mL of pure alcohol and $4.34 \pm 0.60$ mg nicotine per day per person aged 15 and above. The estimated alcohol consumption in this study was substantially lower than previous survey-based reports, however, the tobacco consumption rate closely aligned with the findings of surveys conducted among current smokers. The degradation of biomarker within household on-site septic tanks was thought to take account for the low alcohol consumption estimation. Further research is necessary to consider the correction factors of WBE method in the context of sewage system.

1. Introduction

Alcoholic drinking and smoking are the two lifestyle factors linked to many significant public health concern. Frequent heavy drinking can lead to liver disease, cardiovascular problems, addiction and also contribute to millions of deaths each year globally. Smoking tobacco is also a major risk factor for numerous health problems, including respiratory diseases, heart disease and lung cancers. In Vietnam, drinking is favorable in all kind of events and binge drinking is common in young adult. According to the latest report published on World Bank database, it is estimated that an Vietnamese adult drinks 8.7 L of pure alcohol every year (WHO, 2018b) The amount is significant higher in male of 14.1 L compared to female of 3.5 L. Also, the smoking prevalence in Vietnam is one of the highest rates in the world. According to the Global Adult Tobacco Survey (GATS) the overall prevalence of current smokers was 22.5% and male adults smoker is dominant (45.3% male smoker vs. 1.1% female (WHO and Vietnam Ministry of Health, 2015b). To cope with such alarming rate, smoking is ban in certain public places in the country from August, 2023 (Vietnam Ministry of Health, 2023). It is necessary to provide accurate estimation of consumption patterns and inform public health policies and interventions.

Wastewater-based epidemiology (WBE) has been applied as an alternative approach to monitor drinking and smoking behaviours in many countries. The concept is to measure the urinary metabolites of alcohol and tobacco in centralized wastewater treatment plants (WWTPs) and back calculate the consumption of pure compounds consumed in community living in the wastewater catchment (Chen et al., 2019; Ryu et al., 2016). Furthermore, WBE can assess the consumption behaviour of specific population such as school population (Driver et al., 2020; Zheng et al., 2022; Zuccato et al., 2017) specific time event, festival (Andres-Costa et al., 2016; Benaglia et al., 2020). Some research also related the impact of COVID-19 restrictions to alcohol consumption (Boogaerts et al., 2022; Chen et al., 2023). The method was recognized as a relatively low cost approach compared to conventional self-report survey (Driver et al., 2020). Also, WBE ensure personal privacy by focusing on collective consumption patterns community-level data rather than individual behaviors.

To the best of the authors knowledge, only few WBE studies have been conducted in Vietnam. One study in 2022 estimated daily nicotine consumption of $1.28 \pm 0.25$ mg/day/person and concluded that the estimates of
smoking prevalence were slightly lower than the survey data. (Thanh et al., 2022). Another study in Ho Chi Minh city generally investigated drugs and personal care products biomarkers use using one-week grab samples also concluded that the estimates of tobacco and alcohol use were lower than those of European countries (Nguyen et al., 2018). Due to the the lack of autosampler, sampling method in these studies were only grab samples. Besides, both studies reported the consumption pattern of general population. Studies about the alcohol and tobacco use of students demographics is not available.

This study therefore aims to apply the WBE approach to estimate the alcohol and tobacco use of university students and urban residence in Ho Chi Minh city. We also compared the wastewater-derived result with existing sales data and survey reports to understand the consumption trend in different demographics. Further discussion was given upon the uncertainties when applied WBE method in Vietnam and how to improve the precision of the alcohol and tobacco consumption estimation.

2. Materials and Methods

2.1. Chemicals and reagents

Native standards ethyl sulfate (EtS), cotinine (COT), nicotine (NIC) and isotope labelled standards ethyl sulfate-d5 (EtS-d5), cotinine-d3 (COT-d3) were purchased from Cerilliant (Supelco). Analytical grade methanol was purchased from Merck (Germany); formic acid was purchased from Fisher Scientific (Thermo Fisher Scientific UK). MilliQ water from Millipore, 0.22 µm filter, 18.2 MΩ cm⁻¹). Regenerated cellulose 0.2 µm hydrophilic regenerated cellulose (RC) syringe filters were purchased from Minisart (Satorius, Germany).

2.2. Sampling collection and preparation

A total of 52 composite samples of domestic wastewater were collected using an automated sampling system. Sampling period is within April to May 2023, the end of dry season in Ho Chi Minh city. The sampling sites included the WWTP of an undisclosed university town (capacity 1,920 m³/day) and the largest WWTP in the urban area of HCMC (capacity 210,685 m³/day). Details of the sampling sites and sampling periods are shown in Table S1.

Wastewater samples were collected at the pump station after the screening bar. Composite samples were collected using an in-house designed autosampler. 40 mL of wastewater was collected every hour. Day time sample (from 8AM – 6PM) and night time sample (6PM-8AM) were collected separately. Samples were acidified to pH 2 with HCl 1M to reduce biological degradation. After transported to laboratory, samples were filtered and 1 mL filtrate was transfered into a 1.5 mL glass amber vial, into which 10 µL of labeled internal standards standard EtS-d5 and cotinine-d3 (1 µg/mL) were spiked. Samples were frozen until analysis which is within one week after the end of each sampling.

2.3. Instrumental analysis and data analysis

Samples were measure by direct injection method using Exion LC – AC series liquid chromatography coupled with 3500 TripleQuad mass spectrometry (Sciex, Ontario, Canada). LC separation was achieved by Kinetex Biphenyl column (2.6 µm, 100Å, 50 x 2.1mm; Phenomenex). MS/MS determination was done in negative ionization mode for EtS and positive mode for COT and NIC. Instrumental blanks were included in each run.
Procedural blanks were added after every 10 samples to monitor potential carry-over. No contamination was detected in blank samples. Calibration curve included 10 points (0.1–50 ppb) and the linearity of larger than 0.99 was achieved. Detailed information of LC method, MS setting, and QA/QC information were provided in SI.

Data were acquired and processed using Analyst® software (AB Sciex). Statistical analysis and figures were performed using GraphPad Prism version 9.5.1. Non-parametric Mann-Whitney test was used to compare group of data.

### 2.4. Back estimation of stimulant consumption

*Alcohol consumption* were normalized to mL of pure alcohol using Eq. 1, referred from previous study (Rodriguez-Alvarez et al., 2015)

\[
mL\text{ EtOH/day/inhabitant} = C_{EtS}\left[\mu g_L\right] \times 10^{-6}\left[g_{EtS}\right] \times Q\left[L/day\right] \times \frac{CF_{Alc}}{P \times R_{15} \times \rho_{alcohol}} \quad (Eq. 1)
\]

*Tobacco consumption* was estimated following the approach from previous study Gao et al. (2020)

\[
m_{NIC_{\text{mg/person/day}}} = \frac{C_{COT} \times CF_{NIC} \times Q}{P \times R_{15}} \quad (Eq. 2)
\]

Where \( C_{EtS} \) and \( C_{COT} \) are concentrations biomarkers in wastewater influent (\( \mu g/L \)).

- \( Q \): is the daily influent flow (L/d);
- \( P \): number of inhabitants served by WWTP;
- \( \rho_{alcohol} \): density of ethanol 0.789 g/mL;
- \( R_{15} \): prevalence of population aged 15 and above

\( CF_{Alc} \) and \( CF_{NIC} \) are correction factor calculated by Eq. 3

\[
CF = \frac{MW_{\text{parent compound}}}{MW_{\text{metabolite}}} \times \frac{1}{\text{Excretion rate}} \quad (Eq. 3)
\]

Excretion rate of ethyl sulfate is 0.012% and cotinine is 32.3% (Gao et al., 2020; Rodríguez-Álvarez et al., 2015; Zheng et al., 2017). Number of cigarettes smoked per day of population age 15+ is calculated in Eq. 4, where D is the content of NIC per cigarette. A tobacco rod contains 10–14 mg of nicotine and during smoking, about 1-1.5 mg nicotine is absorbed to blood (Benowitz et al., 2009). This study used \( D = 1.25 \) mg for estimation.

\[
n_{15+} = \frac{m_{NIC}}{D \times R_{15}} \quad (Eq. 4)
\]

The calculation was strictly aged-standardized to assist the comparision in the latter part. \( R_{15} \) of 100% was used for the estimation in Utown case and the latest demographic data of 78% of Vietnam population aged 15 and older was used for urban area (United Nations Population Division, 2022).

### 3. Result and Discussion
3.1. The consumption of alcohol and tobacco of students and urban population

The estimation revealed the significant differences in the daily mean consumption of alcohol and tobacco of the two populations. The concentration of alcohol metabolite EtS in wastewater varied from 1.0 to 2.2 µg/L while tobacco metabolites cotinine ranged from 2.2 to 4.8 µg/L. While the average concentration of all sample at two sampling sites was in same range (Table S2) the estimated consumption of alcohol and tobacco of urban residence was 3–4 times higher than students in university town. The difference was statistically significant (p-value < 0.0001) (Fig. 1).

The fact that both drinking and smoking are prohibited in the university town could explain for the difference. On average a student consumed 0.69 ± 0.13 mL pure alcohol while an urban residence (15+) consumed 2.41 ± 0.34 mL. Back calculation from wastewater concentration showed that a student in Utown consumed 1.20 ± 0.2 mg nicotine/day while an adult age 15 and older in urban area consumed 4.34 ± 0.6 mg nicotine/day. It was estimated that each student in Utown smokes 0.96 cigarette per day and the number is 3.47 cigarettes per adult (15+) in urban areas.

We observed the higher concentration of ethyl sulfate in urban wastewater during weekend when compared to weekdays (P value < 0.05). Previous study also reported the same pattern in Belgium urban WWTP (van Wel et al., 2016). However, there is no significant difference of the EtS concentration in Utown WWTP. The fact that students living in university dormitory often return home in neighbor provinces in weekend could explain for this observe. On the other hand, significant difference of COT concentration was observed in daytime and nighttime wastewater samples in U-Town WWTP (P value < 0.05) (Fig. 2). The lower concentration in daytime is likely because students attend classes and do not have chance to smoke. Since there is no restriction for the residence in urban WWTP catchment, the COT concentration showed no difference within a day.

3.2. Compared to other countries

Table 2 summarized the results from previous WBE studies other countries. The estimated alcohol consumption of urban residence in this study (2.4 ± 0.34 mL/day/inhabitant) is substantially lower when compared to previous studies covering large population in other countries.
Table 1
Estimated alcohol and nicotine consumption in previous WBE studies in other countries (converted to same unit mL pure ethanol /day/1000 inhabitants and mg nicotine/day/inhabitant)

<table>
<thead>
<tr>
<th>Research site</th>
<th>Population (1000 people)</th>
<th>Alcohol mL/day/inh</th>
<th>Nicotine mg/day/inh</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban resident</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Italia (8 cities)</td>
<td>80–1,201*</td>
<td>n.a</td>
<td>2.2–4.5</td>
<td>(Castiglioni et al., 2015)</td>
</tr>
<tr>
<td>11 countries</td>
<td>n.a *</td>
<td>6.4–44.3</td>
<td>n.a</td>
<td>(Ryu et al., 2016)</td>
</tr>
<tr>
<td>Australia</td>
<td>10,800</td>
<td>7–24</td>
<td>0.75–3.02</td>
<td>(Lai et al., 2018)</td>
</tr>
<tr>
<td>China (21 cities)</td>
<td>107.6–3,338*</td>
<td>4.7–8.1</td>
<td>1.5–1.8</td>
<td>(Gao et al., 2020)</td>
</tr>
<tr>
<td>Spain (13 cities)</td>
<td>48–1,163*</td>
<td>4.5–46</td>
<td>n.a</td>
<td>(Lopez-Garcia et al., 2020)</td>
</tr>
<tr>
<td>Turkey (14 STPs)</td>
<td>18,000</td>
<td>29.6</td>
<td>5.5</td>
<td>(Asicioglu et al., 2021)</td>
</tr>
<tr>
<td>Vietnam</td>
<td>637.1*</td>
<td>2.4 ± 0.34</td>
<td>4.3 ± 0.6</td>
<td>This study</td>
</tr>
<tr>
<td>U-town student</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>US</td>
<td>n.a</td>
<td>14.3 ± 9.5</td>
<td>0.63 ± 0.22</td>
<td>(Driver et al., 2020)</td>
</tr>
<tr>
<td>China</td>
<td>200</td>
<td>1.3 ± 0.6</td>
<td>n.a</td>
<td>(Zheng et al., 2022)</td>
</tr>
<tr>
<td>Vietnam</td>
<td>16.3</td>
<td>0.7 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>This study</td>
</tr>
</tbody>
</table>

* Prevalance of population age 15 + was considered for estimation

n.a: not available; mL/day/inh: mL/day/inhabitant

However, the nicotine consumption in this study (4.3 ± 0.6 mg/day/inhabitant) was higher than 21 cities in China (1.5–1.8 mg/day/inhabitant) and Australia (0.75–3.02 mg/day/inhabitant). The level was similar to 8 Italian cities (2.2–4.5 mg/day/inhabitant) while it is lower than the estimated amount for 90% population of Istanbul of 5.5 mg/day/inhabitants (Asicioglu et al., 2021).
<table>
<thead>
<tr>
<th>Research site</th>
<th>Population (1000 people)</th>
<th>Alcohol mL/day/inh</th>
<th>Nicotine mg/day/inh</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban resident</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Italia (8 cities)</td>
<td>80–1,201*</td>
<td>n.a</td>
<td>2.2–4.5</td>
<td>(Castiglioni et al., 2015)</td>
</tr>
<tr>
<td>11 countries</td>
<td>n.a *</td>
<td>6.4–44.3</td>
<td>n.a</td>
<td>(Ryu et al., 2016)</td>
</tr>
<tr>
<td>Australia</td>
<td>10,800</td>
<td>7–24</td>
<td>0.75–3.02</td>
<td>(Lai et al., 2018)</td>
</tr>
<tr>
<td>China (21 cities)</td>
<td>107.6–3,338*</td>
<td>4.7–8.1</td>
<td>1.5–1.8</td>
<td>(Gao et al., 2020)</td>
</tr>
<tr>
<td>Spain (13 cities)</td>
<td>48–1,163*</td>
<td>4.5–46</td>
<td>n.a</td>
<td>(Lopez-Garcia et al., 2020)</td>
</tr>
<tr>
<td>Turkey (14 STPs)</td>
<td>18,000</td>
<td>29.6</td>
<td>5.5</td>
<td>(Asicioglu et al., 2021)</td>
</tr>
<tr>
<td>Vietnam</td>
<td>637.1*</td>
<td>2.4 ± 0.34</td>
<td>4.3 ± 0.6</td>
<td>This study</td>
</tr>
<tr>
<td>U-town student</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>US</td>
<td>n.a</td>
<td>14.3 ± 9.5</td>
<td>0.63 ± 0.22</td>
<td>(Driver et al., 2020)</td>
</tr>
<tr>
<td>China</td>
<td>200</td>
<td>1.3 ± 0.6</td>
<td>n.a</td>
<td>(Zheng et al., 2022)</td>
</tr>
<tr>
<td>Vietnam</td>
<td>16.3</td>
<td>0.7 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>This study</td>
</tr>
</tbody>
</table>

* Prevalance of population age 15+ was considered for estimation

n.a: not available; mL/day/inh: mL/day/inhabitant

There were less WBE studies about alcohol and tobacco consumption in university students demographics. The author found one study in a public US university reported the consumption of 14.3 mL pure alcohol/day/student (Driver et al., 2020), which is about 20 times higher than the level observed in this study of 0.7 mL/day/student. Another study in a university town in China estimated the consumption at 1.3 mL/day/student (Zheng et al., 2022), as high as the urban residence demographic in the same research (1.4 mL/day/inhabitant) and nearly two times higher than this study. As mentioned above, the possible reason for the low consumption of student in this study is that drinking is prohibited in the university town of Ho Chi Minh city. However, the estimated nicotine consumption in this study is 2 times higher than in the US university even though smoking is also strictly prohibited in campus and dormitory in Vietnam.

### 3.3. Compared to previous survey-based data on alcohol and tobacco consumption of Vietnam population

We also collected data of alcohol and tobacco consumption in Vietnam from previous survey-based studies in Table 3. The other two relevant WBE studies in Vietnam was also included for reference (Nguyen et al., 2018; Thanh et al., 2022)
Table 3
Estimation of alcohol and tobacco consumption in Vietnam in previous survey studies. The rates was age-standardized (15+) and normalized to L pure alcohol/year/inhabitant and cigarettes/day/smoker

<table>
<thead>
<tr>
<th>Year</th>
<th>Alcohol L/year/inhabitant</th>
<th>Tobacco Cigarettes/day/smoker</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td>2018</td>
<td>8.7</td>
<td></td>
</tr>
<tr>
<td>2018</td>
<td>2.2 (1)</td>
<td></td>
</tr>
<tr>
<td>2015</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>2023</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>2002</td>
<td>4.1 (2)</td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td>13.9</td>
<td></td>
</tr>
<tr>
<td>2015</td>
<td>&gt;10</td>
<td></td>
</tr>
<tr>
<td>2018</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>2018–2020</td>
<td>9.8</td>
<td></td>
</tr>
<tr>
<td>2023</td>
<td>13.9</td>
<td></td>
</tr>
</tbody>
</table>

(1) Estimated from approximately 44.1 liters beer consumed per capita per year, assuming the average ABV is 5%
(2) Calculated from 682,800 VND spending per smoker per year, cost per pack of 20 cigarette was 9,000 VND in 2002 (Viet NguyenC, 2021)

It is estimated that each urban residence in this study consumed 0.9 L pure alcohol per year. This is considerably lower than the estimation from a survey in 2012 (Santosh Kumar, 2012) and the latest report from Global Health Observatory Data Repository (WHO, 2018a), which is 6.7 L and 8.7 L, respectively. Our estimation is even 2 times lower than the estimation of only beer consumption per Vietnamese adult in 2018 which is at 2.2 L (Minh Ngoc Nguyen, 2018). The only one previous WBE study in Ho Chi Minh city reported 1.7 L pure alcohol used per year, which is also higher than our estimation (Nguyen et al., 2018).

Considered 25% Vietnamese population is smoker (World Bank, 2020), we also estimated that a smoker in the wastewater catchment consumed 13.9 cigarettes per day. Smoker is defined as people who currently smoke any tobacco products on a daily basis or non-daily basis. The result is quite close to previous report in 2015 by Global Tobacco Adult Survey, stating approximately 75.9% of daily cigarette smokers smoked more than 10 cigarettes per day and 37.6% smoked more than 20 cigarettes per day (WHO and Vietnam Ministry of Health, 2015b). Another database of Institute for Health Metrics and Evaluation also reported daily use of 13.9 cigarettes per smoker (Ng et al., 2014).
3.4. Uncertainties of the WBE estimation in this study

The current sewage system in Ho Chi Minh city has two drawbacks that might affect the estimation by WBE approach. Firstly, the drainage system combines domestic wastewater and rain water. Hence, the dilution factor will lower the biomarker concentration if sample is collected during heavy rainfall. In this study, the contribution of from rainfall factor could be eliminated as no rain was recorded during sampling periods. The second issue is the existing of individual septic tank in every house in Vietnam.

It was mentioned in previous report that in-sewer degradation rate of EtS was estimated of approximately 8% per hour (Banks APW, 2018). It was thought to be more important during the interpretation of alcohol consumption estimation than for tobacco, as cotinine was shown to be more stable than EtS in the sewer (Banks APW, 2018; Gao et al., 2018). However, no study has estimated the degradation of alcohol and tobacco biomarkers in septic tank condition. The degradation these biomarkers in septic tank unit, could be estimated by comparing the estimation from centralized city WWTPs with small scale WWTPs of apartment buildings where wastewater is collected directly without retention in septic tank units.

4. Conclusion

This study investigated alcohol and tobacco consumption of university students and the urban population of Ho Chi Minh City using the WBE (Wastewater-based epidemiology) approach. Overall, alcohol and tobacco consumption among students are lower than in the urban population. The estimated alcohol use is lower than the result from survey-based data. The existing of septic tank unit in sewage system of Ho Chi Minh city was thought to degrade the ethyl sulfate biomarker and hence, lower the estimation of alcohol use. On the other hand, the estimation of tobacco consumption using nicotine biomarkers showed the comparable result with previous survey-based data. The application of WBE method would be promising to monitor the alcohol and tobacco consumption at university dormitory, residential apartment where the in-sewer degradation of the biomarker is negligible.

Declarations

Ethics approval Not applicable

Consent to participate Not applicable

Consent for publication Not applicable

Availability of data and materials The datasets generated in this study are available from the corresponding author on reasonable request.

Competing interests The authors declare no competing interests.

Funding This study was supported by University of Science, Viet Nam National University Ho Chi Minh City under grant number T2022-71.

Authors’ contributions D.T.T.Quyen wrote the main manuscript text. T.T.Y.Nhi prepared analysis methodology and data curation. N.T.T.Nhon collected samples. T.T.Hien edited manuscript. All authors contributed to
interpreting results and editing the manuscript.

**Acknowledgement** The authors would like to thank Tran Huynh Thuan and Nguyen Thi Hong Tien for their great assistance in wastewater sample collection.

**References**


   https://doi.org/10.1016/j.scitotenv.2022.157310


   https://doi.org/10.1016/j.drugalcdep.2016.03.002

28. Regulation on enforcement of smoke-free places and nomination for Tobacco-Free Award, (2023).


   end=2018&locations=VN&start=2000&view=chart


   https://doi.org/10.1016/j.drugalcdep.2021.109178

   https://doi.org/https://doi.org/10.1016/j.scitotenv.2017.02.214


**Figures**
Figure 1

Daily mean consumption of (left) alcohol and (right) nicotine per day per population aged 15+ served by 2 WWTPs (Note: **** indicated p-value <0.0001)

Figure 2

COT conc. in wastewater (µg/L)

EIS conc. in wastewater (µg/L)

UTown WWTP

Urban WWTP
(left) Cotinine concentration of daytime and nighttime sample of University town WWTP (Utown) and (right) concentration of ethyl sulfate of weekends and weekdays samples in Urban WWTP (Note: * means p-value <0.05)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- DoThiThuyQuyenSupplementaryInformation.docx