Urinary Level of Dimethoate, Bisphenol a and Benzo[a]pyrene in the First-year Students of Hohai University From Different Geographical Regions

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Research Article

Keywords: Dimethoate, Benzo(a)pyrene, Bisphenol A, Exposure, First-year students

DOI: https://doi.org/10.21203/rs.3.rs-400314/v1

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Abstract

**Background:** The objective of this study was to detect the urinary level of dimethoate, benzo(a)pyrene (BaP), and bisphenol A (BPA) in the first-year Hohai University students of different geographic origins.

**Methods:** First-morning urine samples were collected from 540 healthy freshmen aged 17 to 19. Toxin levels were measured using β-glucuronidase hydrolysis followed by a high-performance liquid chromatography-tandem mass spectrometry-based method. Geometric means (GM) are presented by BMI (Body Mass Index) and different locations for these three toxins in a volume-based and creatinine-standardized way.

**Results:** GM concentration of dimethoate, BPA and BaP were 9.47 µg/L (10.80 µg/g creatinine), 3.54 µg/L (4.04 µg/g creatinine) and 0.34 ng/L (0.39 ng/g creatinine), respectively. The GM concentration of dimethoate in males was significantly higher than that in females. The BMI higher than 23.9 had a higher GM concentration of dimethoate, BPA, and BaP. The inhabitant in the Southwest of China had a significantly lower GM concentration of dimethoate, BPA, and BaP than those who live in other locations of China.

**Conclusion:** The average level of environmental toxins accumulation in freshmen is relatively high and differs in the youth who live in different regions. Besides, obesity is correlated to higher toxins levels in youth.

Introduction

Pollution problems of air, water, and food have been increasing as a consequence of global climate change, pesticide misuse, and industry development[1], thus the negative effects of environmental pollution on human health have lately become a great concern. Inevitably, humans are exposed to pollutants such as heavy metals, pesticide residues, polycyclic aromatic hydrocarbons (PAHs), bisphenol A (BPA), and so on through drinking water, food, dust, and ambient air[2] which tightly link to the initiation and progression of multiple diseases[3].

China is one of the largest agricultural countries in the world, with >300,000 tons of agricultural pesticides used annually[4]. Dimethoate, one of the most commonly used organophosphorus pesticides, is wildly used for broad-spectrum control of a wide range of insects including mites, flies, aphids, and plant hoppers[5]. Overuse of dimethoate can lead to massive residues on fruits, vegetables, and grains[6]. Existing research confirmed that even very low levels of dimethoate may have adverse health effects[7, 8] which is potentially carcinogenic[9, 10]. At the same time, the extensive industrial development model has made China fall into an “environmental pollution–economic development” cycle[11]. PAHs, which are originated from diverse sources including petrochemical products and the combustion of fossil fuels, are pervasive pollutants characterized by their hazardous carcinogenic and mutagenic potential which exist not only in the air but also in food and drinking water[12, 13]. Benzo(a)pyrene (BaP) is one of the most studied PAHs classified by IARC as a Group 1 carcinogen[14]. Multiple studies have shown that BaP requires metabolic activation to exert its carcinogenic effects[15, 16]. It was reported that a higher incidence of forestomach tumors was observed when applied with BaP in food to B6C3F1 mice over a lifetime[17]. BPA is a synthetic plasticizer with more than 8 million pounds produced worldwide each year which can be found everywhere from plastic bottles, the medical devices to the undercoat of the food packages[18]. Likewise, there is growing evidence that BPA may adversely affect human health. Several studies have proved that BPA has negative effects on human reproduction including female fertility[19], male sexual function[20], sperm quality[21], etc. Moreover, BPA has an impact on gene expression processes, such as the enzymatic proteins which have important roles during fetal development[22]. Besides, metabolic syndromes such as type 2 diabetes, non-alcoholic fatty liver disease, and obesity are also associated with BPA[23].

Different subjects originating from distinct parts of the country may carry the pattern of environmental exposure in their regions. It was reported that the blood Pb levels of the populations who live in Wuhan of central China were lower than those in Beijing[24]. It was also reported that the healthy Chinese who live in the mining area of manganese mines or nonferrous metal mines have a significantly higher urinary manganese level than those who live in other regions. Another study showed that higher urinary levels for As and Cd in the Wuhan population are observed when compared with other countries[25]. Besides the geographic distributions, a preliminary study has reported that the hair and urinary Al levels in the obese subjects are significantly higher by 31% and 46% compared to that in the healthy group, respectively[26].

Hohai University (former Hohai Civil Engineering School of China, established in 1915, HHU) is a national key university under the direct administration of the Ministry of Education. As a comprehensive university with at least 20 colleges, she enrolls more than tens of thousands of students each year from every province in China. Before entering university, the lifestyle of high school students is relatively unitary during nearly 10 years study period at the place of birth. Considering that environmental background may have a significant effect on long-term health effects. Meanwhile, these environmental toxin markers can be easily detected in blood and urine. It's meaningful to detect the level of environmental toxin which could reflect the local environment and individual health.
Therefore, the objective of the present study was to provide the baseline information of the levels of dimethoate, BaP, and BPA in the urine samples from the first-year Hohai University students of different geographic origins and to assess the correlation between the level of pollutants exposure with geographic origins and BMI through the baseline. More importantly, it will facilitate the improvement of the overall health level of Chinese people by advocating a healthier lifestyle and provide suggestions for environmental protection policy design.

**Materials And Methods**

**Study Design and Participants**

All procedures including sampling and examination were performed in agreement with the principles set in the Declaration of Helsinki and its later amendments (2013). All examinees were invited to participate and took part in the present study voluntarily. All subjects were informed about the objectives of the study and experimental procedures and signed the informed consent form. The study protocol has been reviewed and approved by the Ethical Review Committee of Sir Run Run Hospital, Nanjing Medical University (2019-SR-018).

A total of 540 freshmen of the HHU originating from East (n = 319), Northeast (n = 10), North (n = 85), Northwest (n = 41), Southwest (n = 43), and South (n = 42) of China were enrolled in the present study (Table 1), in which there were 253 males and 287 females aged from 17 to 19. And the precise geographical location of China was shown in figure 1.

**Table 1. Demographic characteristics and particular residence of origin of the examined subjects**

| Region     | East | Northeast | North | Northwest | Southwest | South |
|------------|------|-----------|-------|-----------|-----------|-------|
| n          | 319  | 10        | 85    | 41        | 43        | 42    |
| Age        | 17.9±0.6 | 18.3±0.7 | 17.8±0.7 | 17.9±0.7 | 17.9±0.6 | 17.8±0.5 |
| Gender     | 175/144 | 6/4       | 49/36 | 17/24     | 22/21     | 18/24 |
| BMI        | 20.93±3.1 | 21.2±3.1 | 21.2±2.7 | 20.8±3.4 | 20.2±3.2 | 21.9±2.5 |
| Waist      | 72.9±8.2 | 73.3±10.3 | 73.2±8.4 | 73.4±8.3 | 71.0±9.0 | 75.2±7.2 |
| Province   | Zhejiang-16 | Liaoning-3 | Tianjin-7 | Shaanxi-10 | Yunnan-14 | Hunan-17 |
|            | Shanghai-2 | Jilin-4 | Shandong-14 | Shanxi-10 | Sichuan-17 | Guangxi-16 |
|            | Jiangxi-18 | Heilongjiang-3 | Hubei-10 | Qinghai-5 | Guizhou-12 | Guangdong-9 |
|            | Jiangsu-248 | Henan-31 | Ningxia-1 |
|            | Fujian-13 | Hebei-23 | Gansu-15 |
|            | Anhui-22 |

**Sample Collection**

Examination and sample collection were performed during the first medical screening on admission to the university directly after arrival to HHU using noninvasively collected substrates (urine) in September 2019. Only healthy subjects without chronic diseases were involved in the current investigation to avoid side effects and interactions of diseases on the studied parameters.

**Sample Processing**

Collection of urine samples (second portion) was performed in the morning using plastic Vacuette\textsuperscript{®} Urine Collection Cups (Greiner Bio-One International AG, Austria).

Evaluation of dimethoate, BPA, and BaP levels in the urine of examinees was performed using Liquid chromatography-mass spectrometry (LC-MS). The levels of omethoate and 3-hydroxypyrene, metabolites of dimethoate and BaP respectively, and BPA, were examined. The 1 µg/mL standard working solutions of dimethoate, BPA, and BaP were prepared with methanol as solvent. After continuous dilution of 10\textsuperscript{4} times, it became the standard working solution of 100 pg/mL. Take BaP as an example, different concentrations of benzo (a) pyrene standard working solution were prepared. 50 µL of each BaP standard working solution was prepared and injected into the system. The collected urine samples (2 mL) were filtered with 0.22 µM filter membrane, pH was adjusted to 5.4 by adding acetic acid-sodium acetate buffer (0.5 M), then β-glucuronidase/arylsulfatase (10 µL) and vitamin C (5 mg) were added and incubated overnight at room temperature to complete the enzymatic hydrolysis. The samples were extracted, after enzymatic hydrolysis, by solid-phase extraction with SPE column (C18 ENVI 0.25 g).
The extract was eluted with methanol (2 mL) and dried with nitrogen. Finally, methanol (100 µL) was used to re-dissolved the analyte to be determined. 50 µL of the analyte to be tested was transferred to a liquid chromatography bottle with a microsyringe, which was especially used for the injection analysis of BaP levels. The detection methods of dimethoate and BPA were consistent with those of BaP[27].

Statistical Analyses

Statistical treatment of raw data was performed using SPSS 26.0 (IBM Corp., Armonk, NY, USA) software. Geometric median (GM) values were used as descriptive statistics for pollutants levels. T-tests were used to compare GMs between categories. Multiple regression analysis was performed to specify the association among the pollutant levels, BMI, and region of origin. All models were adjusted for age and gender variability. The results of the tests were considered significant at $p < 0.05$.

Results

Urine dimethoate, BPA, BaP were detected in 100% of the recruited people. And the results were presented by volume-based and also by creatinine standardized which was used to eliminate the effect of the time of urine collection, urine concentration, and urine flow rate[28].

Hohai University is a multi-disciplinary comprehensive university located in Jiangsu Province of East China. The freshmen came from all over the country including East, North, South, Northeast, Northwest, and Southwest. We choose East China as the reference category because subjects originating from a distinct part of China carry the patterns of environmental exposure in their domestic region. The obtained data demonstrated that the origin of the students had an important impact on the urine toxins (Table 2). Particularly, the volume-based GM of urine dimethoate, BPA, and BaP concentrations in students from Southwest China was significantly lower than East China by 9.49%, 10.14%, and 8.82%. Likewise, the standardized GM of urine dimethoate, BPA, and BaP concentrations in students from Southwest China was also significantly lower than East China by 10.81%, 11.49%, and 10.26%. Data on other regions were more homogenous.

Table 2. Weighted geometric means of urinary dimethoate, bisphenol A and benzo(a)pyrene, by sex and location group, freshmen aged 17-19 years, China
| Location          | Total     | Males       | Females     |
|-------------------|-----------|-------------|-------------|
|                   | Geometric | 95%         | Geometric   | 95%         | Geometric | 95%         |
|                   | mean      | confidence  | mean        | confidence  | mean      | confidence  |
|                   | from      | to          | from        | to          | from      | to          |
| Urinary dimethoate |           |             |             |             |           |             |
| Total µg/L        | 9.47      | 9.28        | 9.67        | 10.64       | 10.34     | 10.95       | 8.55        | 8.35        | 8.74        |
| µg/g creatinine   | 10.80     | 10.58       | 11.02       | 11.11       | 10.76     | 11.46       | 10.53       | 10.26       | 10.80       |
| Northeast China µg/L | 9.76      | 8.00        | 11.91       | 11.65       | 7.50      | 18.11       | 8.38        | 7.32        | 9.58        |
| µg/g creatinine   | 11.17     | 9.49        | 13.15       | 11.61       | 7.91      | 17.03       | 10.84       | 8.36        | 14.04       |
| East China† µg/L  | 9.38      | 9.13        | 9.65        | 10.84       | 10.38     | 11.31       | 8.49        | 8.24        | 8.75        |
| µg/g creatinine   | 10.82     | 10.52       | 11.13       | 11.29       | 10.78     | 11.83       | 10.48       | 10.12       | 10.85       |
| North China µg/L  | 9.77      | 9.34        | 10.22       | 10.59       | 9.83      | 11.40       | 9.02        | 8.57        | 9.50        |
| µg/g creatinine   | 10.98     | 10.50       | 11.49       | 10.79       | 10.00     | 11.63       | 11.16       | 10.57       | 11.79       |
| South China µg/L  | 9.96      | 9.47        | 10.48       | 10.48       | 9.95      | 11.03       | 9.14        | 8.41        | 9.93        |
| µg/g creatinine   | 11.15     | 10.67       | 11.66       | 11.04       | 10.48     | 11.64       | 11.31       | 10.43       | 12.27       |
| Northeast China µg/L | 9.81      | 8.92        | 10.79       | 10.95       | 9.71      | 12.34       | 8.43        | 7.44        | 9.55        |
| µg/g creatinine   | 10.81     | 9.77        | 11.96       | 11.54       | 10.09     | 13.21       | 9.87        | 8.40        | 11.59       |
| Southwest China µg/L | 8.49*     | 7.82        | 9.23        | 9.71        | 8.45      | 11.17       | 7.47        | 7.01        | 7.96        |
| µg/g creatinine   | 9.65*     | 8.91        | 10.45       | 10.26       | 8.81      | 11.95       | 9.10        | 8.55        | 9.67        |
| Urinary bisphenol A |           |             |             |             |           |             |             |             |             |
| Total µg/L        | 3.54      | 3.48        | 3.61        | 3.52        | 3.43      | 3.62        | 3.56        | 3.49        | 3.65        |
| µg/g creatinine   | 4.04      | 3.96        | 4.12        | 3.68        | 3.57      | 3.79        | 4.39        | 4.28        | 4.50        |
| Northeast China µg/L | 3.68      | 3.17        | 4.26        | 3.68        | 2.66      | 5.09        | 3.65        | 3.01        | 4.43        |
| µg/g creatinine   | 4.21      | 3.47        | 4.11        | 3.67        | 2.77      | 4.86        | 4.70        | 3.38        | 6.53        |
| East China† µg/L  | 3.55      | 3.47        | 3.63        | 3.56        | 3.43      | 3.70        | 3.54        | 3.43        | 3.64        |
| µg/g creatinine   | 4.09      | 3.98        | 4.21        | 3.75        | 3.59      | 3.91        | 4.36        | 4.22        | 4.52        |
| North China µg/L  | 3.65      | 3.51        | 3.80        | 3.54        | 3.32      | 3.78        | 3.75        | 3.57        | 3.95        |
| µg/g creatinine   | 4.11      | 3.90        | 4.32        | 3.57        | 3.33      | 3.84        | 4.64        | 4.40        | 4.90        |
| South China µg/L  | 3.65      | 3.47        | 3.83        | 3.50        | 3.29      | 3.74        | 3.86        | 3.59        | 4.16        |
| µg/g creatinine   | 4.08      | 3.87        | 4.31        | 3.66        | 3.44      | 3.88        | 4.78        | 4.45        | 5.13        |
| Northwest China µg/L | 3.50      | 3.23        | 3.78        | 3.58        | 3.21      | 3.98        | 3.39        | 2.99        | 3.85        |
| µg/g creatinine   | 3.85      | 3.51        | 4.23        | 3.77        | 3.33      | 4.26        | 3.97        | 3.36        | 4.69        |
| Southwest China µg/L | 3.19*     | 2.98        | 3.40        | 3.22        | 2.85      | 3.63        | 3.16        | 2.94        | 3.39        |
| µg/g creatinine   | 3.62*     | 3.36        | 3.89        | 3.40        | 2.96      | 3.90        | 3.84        | 3.61        | 4.08        |
| Urinary benzo(a)pyrene |           |             |             |             |           |             |             |             |             |
|                | ng/L 0.34 | 0.33 | 0.35 | 0.38 | 0.37 | 0.39 | 0.31 | 0.30 | 0.32 |
|----------------|-----------|------|------|------|------|------|------|------|------|
| ng/g creatinine| 0.39      | 0.38 | 0.40 | 0.40 | 0.39 | 0.41 | 0.38 | 0.37 | 0.39 |
| Northeast China| ng/L 0.35 | 0.30 | 0.41 | 0.39 | 0.30 | 0.52 | 0.32 | 0.27 | 0.39 |
| ng/g creatinine| 0.40      | 0.34 | 0.47 | 0.39 | 0.31 | 0.50 | 0.41 | 0.30 | 0.57 |
| East China†    | ng/L 0.34 | 0.33 | 0.35 | 0.38 | 0.37 | 0.39 | 0.31 | 0.30 | 0.32 |
| ng/g creatinine| 0.39      | 0.38 | 0.40 | 0.41 | 0.40 | 0.42 | 0.38 | 0.37 | 0.39 |
| North China    | ng/L 0.35 | 0.34 | 0.37 | 0.38 | 0.36 | 0.41 | 0.33 | 0.31 | 0.34 |
| ng/g creatinine| 0.40      | 0.38 | 0.41 | 0.39 | 0.36 | 0.42 | 0.40 | 0.38 | 0.43 |
| South China    | ng/L 0.36 | 0.34 | 0.38 | 0.38 | 0.35 | 0.41 | 0.33 | 0.31 | 0.36 |
| ng/g creatinine| 0.40      | 0.38 | 0.42 | 0.40 | 0.37 | 0.42 | 0.41 | 0.39 | 0.44 |
| Northwest China| ng/L 0.34 | 0.31 | 0.38 | 0.39 | 0.35 | 0.43 | 0.29 | 0.25 | 0.33 |
| ng/g creatinine| 0.38      | 0.34 | 0.42 | 0.41 | 0.36 | 0.46 | 0.34 | 0.29 | 0.40 |
| Southwest China| ng/L 0.31* | 0.29 | 0.33 | 0.35 | 0.31 | 0.39 | 0.27 | 0.25 | 0.29 |
| ng/g creatinine| 0.35*     | 0.33 | 0.37 | 0.37 | 0.32 | 0.42 | 0.33 | 0.31 | 0.35 |

**Note.** † reference category

* significantly different from estimate for reference category \( p < 0.05 \)

The volume-based geometric mean (GM) concentration was 9.47 µg/L (Table 3). The GM of urine dimethoate in the female group (8.55 µg/L) was significantly lower than that in the male group (10.64 µg/L). The GM dimethoate concentration rose significantly from 9.12 (µg/L) at the normal BMI \( (18.5 \leq \text{BMI} \leq 23.9) \) to 14.68 (µg/L) at the overweight BMI \( (\text{BMI}>23.9) \). However, only a moderate change of GM dimethoate concentration was observed between the normal BMI (9.12 µg/L) and the less-weight BMI \( (\text{BMI}<18.5) \) (8.74 µg/L).

**Table 3.** Weighted geometric means of urinary dimethoate, bisphenol A and benzo(a)pyrene, by sex and BMI group, freshmen aged 17-19 years, China
| BMI group | Total | Males | Females |
|-----------|-------|-------|---------|
|           | Geometric mean | 95% confidence interval from to | Geometric mean | 95% confidence interval from to | Geometric mean | 95% confidence interval from to |
| Urinary dimethoate | | | | | | |
| Total | µg/L | 9.47 | 9.28 | 9.67 | 10.64 | 10.34 | 10.95 | 8.55‡ | 8.35 | 8.74 |
| µg/g creatinine | 10.80 | 10.58 | 11.02 | 11.11 | 10.76 | 11.46 | 10.53‡ | 10.26 | 10.80 |
| <18.5 | µg/L | 8.74 | 8.28 | 9.23 | 9.66 | 9.02 | 10.36 | 7.88 | 7.39 | 8.40 |
| µg/g creatinine | 10.01 | 9.48 | 10.57 | 10.33 | 9.66 | 11.06 | 9.68 | 8.86 | 10.58 |
| 18.5 to 23.9† | µg/L | 9.12 | 8.96 | 9.29 | 10.19 | 9.95 | 10.42 | 8.30 | 8.13 | 8.47 |
| µg/g creatinine | 10.43 | 10.23 | 10.62 | 10.60 | 10.32 | 10.89 | 10.28 | 10.01 | 10.55 |
| >23.9 | µg/L | 14.68* | 13.68 | 15.75 | 17.49* | 16.10 | 19.00 | 12.41* | 11.68 | 13.18 |
| µg/g creatinine | 16.21* | 15.13 | 17.37 | 18.16* | 16.27 | 20.27 | 14.54* | 13.63 | 15.51 |
| Urinary bisphenol A | | | | | | |
| Total | µg/L | 3.54 | 3.48 | 3.61 | 3.52 | 3.43 | 3.62 | 3.56 | 3.49 | 3.65 |
| µg/g creatinine | 4.04 | 3.96 | 4.12 | 3.68 | 3.57 | 3.79 | 4.39 | 4.28 | 4.50 |
| <18.5 | µg/L | 2.93* | 2.79 | 3.07 | 2.83 | 2.64 | 3.04 | 3.03 | 2.84 | 3.23 |
| µg/g creatinine | 3.35 | 3.15 | 3.56 | 3.03 | 2.83 | 3.24 | 3.72 | 3.41 | 4.07 |
| 18.5 to 23.9† | µg/L | 3.50 | 3.44 | 3.55 | 3.47 | 3.39 | 3.55 | 3.52 | 3.44 | 3.60 |
| µg/g creatinine | 4.00 | 3.91 | 4.08 | 3.61 | 3.51 | 3.71 | 4.36 | 4.24 | 4.47 |
| >23.9 | µg/L | 4.94* | 4.70 | 5.20 | 5.13* | 4.72 | 5.57 | 4.77* | 4.49 | 5.07 |
| µg/g creatinine | 5.46* | 5.14 | 5.80 | 5.32* | 4.77 | 5.94 | 5.59* | 5.24 | 6.00 |
| Urinary benzo(a)pyrene | | | | | | |
| Total | ng/L | 0.34 | 0.33 | 0.35 | 0.38 | 0.37 | 0.39 | 0.31‡ | 0.30 | 0.32 |
| ng/g creatinine | 0.39 | 0.38 | 0.40 | 0.40 | 0.39 | 0.41 | 0.38‡ | 0.37 | 0.39 |
| <18.5 | ng/L | 0.27* | 0.25 | 0.28 | 0.29 | 0.27 | 0.31 | 0.25 | 0.23 | 0.26 |
| ng/g creatinine | 0.31 | 0.29 | 0.32 | 0.31 | 0.29 | 0.33 | 0.30 | 0.28 | 0.33 |
| 18.5 to 23.9† | ng/L | 0.34 | 0.33 | 0.35 | 0.38 | 0.37 | 0.39 | 0.31 | 0.30 | 0.32 |
| ng/g creatinine | 0.39 | 0.38 | 0.40 | 0.40 | 0.39 | 0.41 | 0.38 | 0.37 | 0.39 |
| >23.9 | ng/L | 0.45* | 0.42 | 0.48 | 0.52* | 0.48 | 0.57 | 0.39 | 0.37 | 0.41 |
| ng/g creatinine | 0.50* | 0.46 | 0.53 | 0.54* | 0.49 | 0.61 | 0.45 | 0.43 | 0.48 |

**Note.** † reference category

* significantly different from estimate for reference category (p < 0.05)

‡ significantly different from estimate for males (p < 0.05)
Males with a BMI of more than 23.9 had significantly higher GM dimethoate concentration (17.49 µg/L) compared with the normal BMI (10.19 µg/L). Likewise, females with a BMI of more than 23.9 had significantly higher GM dimethoate concentration (12.41 µg/L) compared with the normal BMI (8.3 µg/L).

Standardizing dimethoate with urinary creatinine concentrations resulted in a GM dimethoate concentration of 10.80 µg/g for all recruited people. The standardized GM of dimethoate concentrations in the female group (10.53 µg/g) was significantly lower than that in the male group (11.11 µg/g). The standardized GM of dimethoate concentrations in people with an overweight BMI (16.21 µg/g) was significantly higher than other BMI groups. The only moderate change was observed between the normal BMI (10.43 µg/g) and the BMI less than 18.5 (10.01 µg/g).

The standardized GM of dimethoate concentrations in the overweight male (18.16 µg/g) was significantly higher than that in normal (10.60 µg/g) or less weight male (10.33 µg/g). Likewise, overweight females had significantly higher standardized GM of dimethoate concentration (14.54 µg/g) than normal (10.28 µg/g) or fewer weight females (9.68 µg/g).

The volume-based GM of urine BPA concentration was 3.54 µg/L (Table 3). Interestingly, the GM of urine BPA in the female group (3.56 µg/L) had no significant difference from that in the male group (3.52 µg/L). People with overweight BMI had higher GM of urine BPA concentration (4.94 µg/L) compared with the one with normal (3.50 µg/L) or less weight BMI (2.93 µg/L). Notably, a significant change of GM BPA concentration was also observed between the normal BMI and the less-weight BMI.

Males with overweight BMI had significantly higher GM BPA concentration (5.13 µg/L) compared with the normal BMI (3.47 µg/L) and less weight BMI (2.83 µg/L). Likewise, females with overweight BMI also had significantly higher GM BPA concentration (4.77 µg/L) compared with the normal BMI (3.52 µg/L) and less weight BMI (3.03 µg/L).

Standardizing BPA with urinary creatinine concentrations resulted in a GM BPA concentration of 4.04 µg/g for all recruited people. There was no significant difference found between the male group (3.68 µg/g) and the female group (4.39 µg/g). The standardized GM of BPA concentrations in people with an overweight BMI (5.46 µg/g) was significantly higher than other BMI groups. The only moderate change was observed between the normal BMI (4.00 µg/g) and the less-weight BMI (3.35 µg/g).

The standardized GM of BPA concentrations in the overweight male (5.32 µg/g) was significantly higher than that in normal (3.61 µg/g) or less weight male (3.03 µg/g). Likewise, overweight females had significantly higher standardized GM of BPA concentration (5.59 µg/g) than normal (4.36 µg/g) or fewer weight females (3.72 µg/g).

The volume-based GM of urine BaP concentration was 0.34 ng/L (Table 3). The GM of urine BaP in the female group (0.31 ng/L) was significantly lower than that in the male group (0.38 ng/L). People with overweight BMI had a higher GM of urine BaP concentration (0.45 ng/L) compared with the ones with normal (0.34 ng/L) or less weight BMI (0.27 ng/L). Notably, a significant change of GM BaP concentration was also observed between the normal BMI and the less-weight BMI.

Males with overweight BMI had significantly higher GM BaP concentration (0.52 ng/L) compared with the normal BMI (0.38 ng/L) and less weight BMI (0.29 ng/L). Likewise, females with overweight BMI also had significantly higher GM BaP concentration (0.39 ng/L) compared with the normal BMI (0.31 ng/L) and less weight BMI (0.25 ng/L).

Standardizing BaP with urinary creatinine concentrations resulted in a GM BaP concentration of 0.39 ng/g for all recruited people. A significant difference was found between the male group (0.40 ng/g) and the female group (0.38 ng/g). The standardized GM of BaP concentrations in people with an overweight BMI (0.50 ng/g) was significantly higher than other BMI groups. A significant change was also observed between the normal BMI (0.39 ng/g) and the less-weight BMI (0.31 ng/g).

The standardized GM of BaP concentrations in the overweight male (0.54 ng/g) was significantly higher than that in normal (0.40 ng/g) or less weight male (0.31 ng/g). Likewise, overweight females had significantly higher standardized GM of dimethoate concentration (0.45 ng/g) than normal (0.38 ng/g) or fewer weight females (0.30 ng/g).

Association between urinary toxins levels and BMI, as well as the potential confounding effects of age, gender, and waist, was additionally studied in the regression model (Table 4). Particularly, in this regression model, urinary dimethoate, BPA, and BaP were not associated with age and waist. In turn, gender and BMI were considered as significant predictors of the volume-based concentration of urinary dimethoate, BPA, and BaP. Notably, the male gender was positively associated with the volume-based concentration of urinary dimethoate and BaP while inversely associated with urinary BPA. However, after being standardized by urinary creatinine, the male gender was only inversely associated with urinary BPA. It was also notable that BMI was positively associated with both volume-based and standardized concentrations of urinary dimethoate, BaP, and BPA.
Table 4. Multiple linear regression analysis of the impact of BMI, waist, and gender on the urine toxins in the freshmen

| Predictor         | Unstandardized |             | Standardized by creatinine |             |
|-------------------|----------------|-------------|----------------------------|-------------|
|                   | Dimethoate     | Bisphenol A | Benzo(a)pyrene              | Dimethoate  | Bisphenol A | Benzo(a)pyrene |
| Gender            | β              | p           | β                           | p           | β            | p              |
|                   | 1.739          | <0.001*     | -0.183                     | 0.001*      | 0.063        | <0.001*       |
| Age               | 0.011          | 0.923       | -0.013                     | 0.734       | -0.001       | 0.797          |
| BMI               | 0.572          | <0.001*     | 0.170                      | <0.001*     | 0.016        | <0.001*       |
| Waist             | 0.007          | 0.648       | 0.004                      | 0.476       | 7.743E-5     | 0.877          |
|                   | 0.010          | 0.647       | 0.005                      | 0.477       | 0.000224     | 0.756          |

* significantly different from estimate for the reference category

**Discussion**

In this study, we chose 3 kinds of very common pollutants in the Chinese environment to represent the exposure level of pollutants in Chinese young people aged from 17~19. Besides, the detection does not rely on a blood sample, but urinary samples obtained from non-invasive sources which were easily obtained and low cost. Urine could also better reflecting the changes in human metabolism because the metabolite concentration is higher in urine than in human plasma or serum[29].

The obtained data demonstrate the freshmen of Hohai University originating from distinct geographic regions of China are characterized by increased pollutants exposure levels. Bushnik, T. reported urinary level of BPA in Canada (1.16 µg/L) is almost 1/3 of that in our data (3.54 µg/L)[30]. This may reflect the differences in the situation of BPA pollution between the Chinese and other countries’ environment. Yu., et al reported urinary 1-OHP concentrations increased with increasing concentrations of B[a]P in the industrial area in Lanzhou city[31]. Notably, there is no data report for the urinary level of dimethoate in other countries. So it is meaningful to detect the baseline of these three pollutants to reflect the effects on the human body of the local environment. Furthermore, the male gender shows a significantly higher urinary level of BPA than the female gender in the data from Canada, while no significant sexual difference of BPA level was observed in our data. This may reflect the differences in pharmacokinetic factors between genders and races, the relevance of which is not currently known[32].

Despite no differences in urinary BPA between genders in our data, the male gender exhibited a significantly higher level of urinary dimethoate and BaP than the female gender (Table 3). Moreover, gender was considered as a significant predictor of the urinary level of dimethoate and BaP in the regression model. This may reflect the differences in the lifestyle between males and females.

Association of BMI and the urinary level of dimethoate, BaP, and BPA was additionally studied in regression models. In particular, BMI could be considered as a significant predictor of the urinary level of these three pollutants no matter whether be standardized by urinary creatinine or not (Table 4). Since most persistent organic pollutants (POPs) are lipophilicity[33], it has been widely studied that POPs could be stored in the adipose tissue[33-35]. Moreover, the accumulated POPs could also increase the risks of obesity and diabetes by inducing adipogenesis[36, 37] and inhibiting glucose uptake[38]. This reflects that adipose tissue can act as a storage for most pollutants in our bodies. The stored pollutants in the adipose tissue can further enhance adipogenesis and insulin resistance. Together with our data, the increasing BMI and obesity can be a risk factor for higher pollutants accumulation in our body which could further act as the obesogens.

Besides the index of BMI, we found that the students from Southwest China had a significantly lower level of all three pollutants compared with East China which was considered as the control group (Table 2). Interestingly, there is no significant difference in BMI between students from Southwest China and East China. This reflects that the living environment and lifestyle may determine this difference.[39]

Although there are very few reports to study the difference among various provinces in China, it has been reported that air pollution in North China is much worse than in South China[40]. This may partially explain our data and indicates that the living environment can determine the level of accumulated pollutants in our bodies. To investigate the relationship of the accumulated pollutants in our body and our health, further researches are needed to study the detailed differences in the living environment and lifestyle between people from Southwest China and other geographic regions in China. The baseline information of dimethoate, BaP, and BPA level will improve public health awareness and have important implications for health policy formulation.

**Abbreviations**
Declarations

Ethics approval and consent to participate

All procedures including sampling and examination were performed in agreement with the principles set in the Declaration of Helsinki and its later amendments (2013). All examinees were invited to participate and took part in the present study voluntarily. All subjects were informed about the objectives of the study and experimental procedures and signed the informed consent form. The study protocol has been reviewed and approved by the Ethical Review Committee of Sir Run Run Hospital, Nanjing Medical University (2019-SR-018).

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the Key R&D Program of Jiangsu Province (BE2017708), the Chinese National Science Foundation (32071145, 31771572), the Nature Science Foundation of Jiangsu Province (BK20191356), the Six talent peaks project in Jiangsu Province (yy-014), Qin Lan Project of Jiangsu Province (KY520R202025), the Science and Technology Development Fund of Nanjing Medical University - General Project (NMUB2019079), the Natural Science Foundation of the Jiangsu Higher Education Institutions of China (20KJB180004), the Chinese National Science Foundation (81700710), the joint project of Sir Run Run Hospital, Nanjing Medical University and China Exposomics Institute (CEI) Precision Medicine Co. Ltd (YFHX2020-001) and the joint project of Hohai University and China Exposomics Institute (CEI) Precision Medicine Co. Ltd (1094/CZ819129916).

Authors’ contributions

YJX, CJL, JW, NS, and BX contributed equally to the design of the study and the direction of its implementation, including supervision of the field activities, quality assurance, and control. FZ and XHX supervised the field activities. YJX, NWZ, and JY were responsible for sample collection. YJX, HLG, HL, NS, and BX helped conduct the literature review and prepare the Materials and methods and the Discussion sections of the text. QC, FRZ, AQW, BM, YJD, and NS designed the study’s analytic strategy and conducted the data analysis. All authors read and approved the final manuscript.

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Figures
Map of study area showing sampling sites Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.