



Levels and temporal variations of urinary lead, cadmium, cobalt, and copper exposure in the general population of Taiwan

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Received: 21 June 2018 / Accepted: 4 December 2018 / Published online: 6 January 2019
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Abstract

Toxic metal contamination in food products and the environment is a public health concern. Therefore, understanding human exposure to cadmium (Cd), lead (Pb), cobalt (Co), and copper (Cu) levels in the general population of Taiwan is necessary and urgent. We aimed to establish the human biomonitoring data of urine toxic metals, exposure profile changes, and factors associated with metal levels in the general population of Taiwan. We randomly selected 1601 participants older than 7 years of age (36.9 ± 18.7 years (7–84 years)) from the Nutrition and Health Survey in Taiwan (NAHSIT) conducted during 1993–1996 (93–96) and 2005–2008 (05–08) periods and measured the levels of four metals in the participants' urine samples using inductively coupled plasma-mass spectrometry. The median (range) levels of urinary Cd, Pb, Co, and Cu in participants from the NAHSIT 93–96 ($N = 821$)/05–08 ($N = 780$) were 0.60 (ND–13.90)/0.72 (ND–7.44), 2.28 (ND–63.60)/1.09 (0.04–48.88), 0.91 (0.08–17.30)/1.05 (0.05–22.43), and 16.87 (2.62–158.28)/13.66 (1.67–189.70) $\mu\text{g/L}$, respectively. We found that the urinary median levels of Pb and Cu in our participants were significantly lower in the NAHSIT 05–08 (Pb 1.09 $\mu\text{g/L}$, Cu 13.66 $\mu\text{g/L}$) than in the NAHSIT 93–96 (Pb 2.28 $\mu\text{g/L}$, Cu 16.87 $\mu\text{g/L}$; $P < 0.01$), whereas those of Cd and Co were significantly higher in the NAHSIT 05–08 (Cd 0.72 $\mu\text{g/L}$, Co 1.05 $\mu\text{g/L}$; $P < 0.01$). Youths had higher exposure levels of Pb, Co, and Cu than adults. Participants with alcohol consumption, betel quid chewing, or cigarette smoking had significantly higher median levels of urinary Pb or Cu ($P < 0.01$) than those without. Principal components and cluster analysis revealed that sex had different exposure profiles of metals. We concluded that levels of urinary Cd, Pb, Co, and Cu exposure in the general Taiwanese varied by age, sex, and lifestyles.

Keywords Taiwan · Human biomonitoring · General population · Metals · Urine

Responsible editor: Philippe Garrigues

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11356-018-3911-0>) contains supplementary material, which is available to authorized users.

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Introduction

Taiwan is one of the most important countries producing commercial electronic products, including mobile phone, printed circuit board, and computers, which may release toxic metals (Pb, Cd, etc.) during the process of production and in waste. In Taiwan, some studies revealed high exposure levels of certain petrol-related metals in residents residing near industrial complexes (Chang et al. 2006; Wang et al. 2006; Yuan et al. 2016). Toxic metal contamination may occur because of the increasing use of these metals for different land use, such as industrial, agricultural, and domestic purposes, as well as the improper treatment of e-wastes in Asian countries, especially a restriction or ban of e-waste treatment in China (Doong et al. 2008; Song and Li 2014). In addition, cadmium (Cd) and lead (Pb) in rice (Chen 1991; Watanabe et al. 1989) and mercury (Hg) in fish (Hsu et al. 2007) have raised concerns regarding environmental pollution. Toxic metal contamination concerns in agricultural and seafood products, such as the accumulation of high level of copper (Cu) in oysters due to water contamination, has caused public health concern in Taiwan (Han and Hung 1990; Han et al. 1993). Therefore, exposure to heavy metals in the general population should be assessed and sub-populations with high exposure should be identified.

Cd has been classified as a group 1A human carcinogen by the International Agency for Research on Cancer (IARC) (IARC 2012). It is associated with an increased risk of itai-itai disease in Japanese people (Nogawa and Kido 1993) and cardiovascular diseases (Tellez-Plaza et al. 2013), blood pressure elevation (Tellez-Plaza et al. 2008), and renal dysfunction in general population (Chung et al. 2014). Rice is identified as a major source of Cd exposure in Asian populations (Uraguchi and Fujiwara 2012). Some studies have reported that the high levels of blood Pb, Cd, and Hg in the Asian-American and East Asian immigrated population, which indicated the exposure sources, may link to the characteristic of exposure profile in Asian population (Awata et al. 2017; Dix-Cooper and Kosatsky 2018). However, limited information is available for those toxic metals in a national-wide scale in the Chinese population.

Pb is neurotoxic and has been classified as a group 2A human carcinogen by the IARC (IARC 2006a). It is associated with several human diseases, such as osteopenia or osteoporosis in adult women (Tsai et al. 2015; Tsai et al. 2016). Neurotoxicity that inhibits early cognitive and intellectual development in children may occur after Pb exposure (Bellinger et al. 1987; Huang et al. 2012). Some paints, other materials, and toys may contain Pb; however, no study was systematically conducted to assess the impact of unleaded gasoline use (since 2000) on urinary Pb in the general Taiwanese population (Wu et al. 2012).

Cobalt (Co) is classified as a group 2B human carcinogen by the IARC (IARC 2006b). It is usually mixed with other metals, and its compounds are often used as trace element additives in medicine and agriculture (ATSDR 2004a). Co-exposure episodes result from long-term occupational exposure, which causes a reduction of erythropoietic variables (median 70 $\mu\text{g/g}$ cre.) (Swennen et al. 1993). Indeed, Co is widely used in electroplating industry and a side pollutant or waste of the electronics industry, which may be released into groundwater. Co is also an essential component of vitamin B12 in humans, but exposure to high Co level can result in adverse lung and heart conditions, asthma, dermatitis, and a higher risk of post-operative fibroid diagnoses and potentially deleterious effects to the mother or fetus (ATSDR 2004a; Fort et al. 2015; Johnstone et al. 2014; Rehfisch et al. 2012; Walters et al. 2014). However, the level of urinary Co in the general population of Taiwan is limited.

Cu can enter the environment through release from factories that use Cu or Cu compounds. A previous study reported unusual green oysters in the downstream close to the scientific park of Northern Taiwan, which could possibly be contaminated by wastewater of certain industry, like the printed circuit board, in the early 90s in Southern Taiwan (Han and Hung 1990). Besides, Cu is an essential metal for the human body; however, beyond its tolerable limit, Cu can be toxic. The adverse effects of exposure to high doses of Cu include nausea, vomiting, stomach cramps, and diarrhea (ATSDR 2004b). Several studies address the association between high Cu exposure and health issues, including acute myocardial infarction (Singh et al. 1985), cognitive decline (Zatta et al. 2008), and effects in the immune function (Turnlund et al. 2004). Even within the nutritional range, uncertainties remain regarding Cu reference values for humans, and reliable biomarkers of Cu status are needed.

In this study, we aimed to assess the exposure levels of urinary Cd, Pb, Co, and Cu as well as associated factors and temporal changes in the general population of Taiwan using the Nutrition and Health Survey in Taiwan (NAHSIT) in a two-period survey at 1993–1996 (NAHSIT 93–96) and 2005–2008 (NAHSIT 05–08).

Materials and methods

Ethics statement

This study was approved by the Research Ethics Committee of the National Health Research Institutes (NHRI, No. EC0990103) in Taiwan. Written informed consent from each participant was obtained prior to study

enrollment. This study follows the guidance of the research ethics committee.

Study participants

NAHSIT is a nationally representative cross-sectional survey of the health and nutrition in Taiwan, and all detailed sampling methods have been described in previous studies (Pan et al. 2011; Tu et al. 2011; Yeh et al. 2011). In order to obtain an adequate participation from the general population of Taiwan, the sample number in each age and gender was according to the population density and urbanization of each city in Taiwan. The survey divided whole townships and city districts in Taiwan and was divided into seven and eight strata in the NAHSIT 93–96 and NAHSIT 05–08, respectively. After the strata were defined, three townships or city districts were selected from each stratum with probability proportional to size (PPS) sampling methods. The survey excluded participants who were pregnant or breastfeeding women, had severe diseases, were foreigners, or were citizens in military units, nursing homes, schools, hospitals, or jails. Participants had to be Taiwanese and ≥ 7 years old. We recruited 892 and 870 participants from the NAHSIT 93–96 and NAHSIT 05–08, respectively. We excluded 71 and 90 participants from the NAHSIT 93–96 and NAHSIT 05–08, respectively, for whom sufficient urine sample or questionnaire data were not available. From the NAHSIT 93–96, we included 632 adults and 189 youths, yielding a total of 821 participants. From the NAHSIT 05–08, we included 677 adults and 103 youths, yielding a total of 780 participants. The total of 1601 participants from the NAHSIT studies consists of 821 (response rate 92%) and 780 (response rate 90%) participants from the NAHSIT 93–96 and NAHSIT 05–08 sampling periods, respectively (Fig. S1).

The participants were categorized into groups according to NAHSIT period, sex, age, regions [northern, central, southern, eastern, or remote island (Penghu)], and urbanization (urban, suburban, rural, harbor, or industrial). Participants were classified as youths [children (7 to 11 years), adolescents (12 to 17 years)] or adults [young adults (18 to 39 years), middle-aged adults (40 to 64 years), elderly adults (≥ 65 years)]. The definition of urbanization was categorized by previous studies in Taiwan (Liu et al. 2006; Lin et al. 2013). All townships were classified based on the population density, population ratio of education, population ratio of elderly people over 65 years, population ratio of people of agriculture workers, and the number of physicians per 100,000 people (Liu et al. 2006; Lin et al. 2013). Townships with harbors or industrial areas classified by the Taiwan

Fisheries Agency and the Industrial Development Bureau were categorized as “harbor” and “industrial” areas, respectively.

Data and specimen collection

All participants were interviewed by well-trained interviewers. We assessed the recorded data and specimens of each participant from the NAHSIT 93–96 and NAHSIT 05–08 and obtained basic demographic information (i.e., age, sex, urbanization, and region). The first void spot urine specimens were collected and stored at 4 °C during transport to the laboratory and then frozen at -80 °C until analysis, when they were analyzed for levels of the major ubiquitous heavy metals, namely Cd, Pb, Co, and Cu.

Measurement of urinary metals

We used a published method to analyze certain metals in the urine sample (Chen et al. 2011; Tsai et al. 2017). Briefly, after thawing the samples, 1 mL samples were diluted 10-fold with 9 mL of 1% (v/v) nitric acid (J.T. Baker Chemical Company, Phillipsburg, New Jersey, USA) in 15 mL polypropylene tubes. Inductively coupled plasma-mass spectrometry (ICP-MS, 7700 Series; Agilent Technologies, Inc., Santa Clara, CA, USA) was used to determine the concentrations of urinary Cd, Pb, Co, and Cu. In addition, a guideline of quality control and method detection limit (MDL) was modified from the National Institute of Environmental Analysis (NIEA PA-103, PA-104, PA-107) and the Taiwan Environmental Protection Agency (NIEA PA-103, PA-104, PA-107, 2005), which are based on the standard of the National Environmental Laboratory Accreditation (NELAC 2001). The calibration curves were ranged from 0.01 to 50 $\mu\text{g/L}$ for Cd, Co, Pb, and Cu [correlation coefficient (R^2) > 0.995], and one check sample for the calibration curve was performed. To ensure the quality of the analysis results, one blank, spike, duplicate, and standard reference material (SRM) were checked in every 10 samples (each batch). The blank sample (1% (v/v) nitric acid solution) of all metals was less than 2-fold MDL. The spike recoveries using 5 $\mu\text{g/L}$ urinary matrix for every 10 urine samples were 92.6 ± 5.9 , 98.6 ± 6.0 , 114.1 ± 7.9 , and 93.3 ± 5.3 for Cd, Pb, Co, and Cu, respectively. Additionally, the duplicate sample mean relative error of each metal was within $\pm 15\%$. The SRM (Seronorm™ Trace Elements Urine, Billingstad, Norway) for Cd, Pb, and Co was tested for every 10 samples, with relative errors of 10.0, 1.65, and 5.65%, respectively. The data qualifying our quality control criteria were used for subsequent

analyses. The limit of detection (LOD) of Cd, Pb, Co, and Cu was 0.008, 0.005, 0.003, and 0.003 $\mu\text{g/L}$, respectively. In both periods, the detectable rate of urinary Cd and Pb levels in the general population of Taiwan was higher than 98.8% and the detectable rate of urinary Co and Cu levels was 100%. Notably, when the levels of the metals were below their MDL (Cd 0.02 $\mu\text{g/L}$, Pb 0.012 $\mu\text{g/L}$, Co 0.019 $\mu\text{g/L}$, Cu 0.16 $\mu\text{g/L}$), they were recalculated as $\text{MDL}/\sqrt{2}$ (Paschal et al. 1998).

Statistical analysis

Demographic statistics of the participants in NAHSIT 93–96 and NAHSIT 05–08 are presented. The geometric mean (GM), geometric standard deviation (GSD), 95% confidence interval (95% CI), mean, standard deviation (SD), and selected percentiles (25th, 50th, 75th, and 95th) of the metal levels were also calculated. The correlations between each urinary level of the metals were determined using Pearson's correlation coefficient. Non-parametric statistics (Mann–Whitney *U* test and Kruskal–Wallis test) were used to assess the differences in the levels of each metal according to characteristics, such as the participants' NAHSIT period, sex, age, urbanization, and regions. Finally, a principal component analysis (PCA) was applied to extract the exposure profiles of various metals. PCA was used for eigenvalues > 1 , and we set independent interpretable factors according to a factor loading > 0.5 (or < -0.5) with specific factors considering its major constituents. Hierarchical clustering analysis (HCA) was performed to illustrate the relationships of metals with each group (Chang et al. 2017; Liao et al. 2018). The significance level was set at $P < 0.05$, and all statistical analyses were performed using SPSS statistical software (version 22.0, IBM Corporation).

Results

Characteristics of the study participants

Table 1 illustrates the demographic characteristics of all participants. The distribution of sex was approximately equal for the entire population across both time periods. The majority of the participants were adults in NAHSIT 93–96 (77.0%) and NAHSIT 05–08 (86.8%). There were 72.9 and 48.9% with alcohol consumption in NAHSIT 93–96 and NAHSIT 05–08, respectively. Around 18% of the participants have betel quid chewing in NAHSIT 05–08; however, data were not available in NAHSIT 93–96. In both periods, more than 30% of the participants have cigarette smoking. Additionally, regarding regional and

urbanization distributions, most of the participants were from Northern Taiwan ($n = 542$, 33.8%) and urban ($n = 391$, 24.4%) area (Table 1).

Urinary Cd, Pb, Co, and Cu levels in different periods and sexes

The selected percentiles and GM of urinary Cd, Pb, Co, and Cu levels in all participants were classified by youths and adults in the periods of NAHSIT 93–96 and NAHSIT 05–08 (Table 2). In the two periods, the median (range) levels of urinary Cd [0.72 (ND–7.44) $\mu\text{g/L}$] and Co [1.05 (0.05–22.43) $\mu\text{g/L}$] in participants from the NAHSIT 05–08 period were significantly higher than in those from the NAHSIT 93–96 [Cd: 0.60 (ND–13.90) $\mu\text{g/L}$; Co: 0.91 (0.08–17.30) $\mu\text{g/L}$; $P < 0.001$]. However, the median (range) levels of urinary Pb [1.09 (0.04–48.88) $\mu\text{g/L}$] and Cu [13.66 (1.67–189.70) $\mu\text{g/L}$] in participants from the NAHSIT 05–08 were significantly lower than in those from the NAHSIT 93–96 [Pb: 2.28 (ND–63.60) $\mu\text{g/L}$, Cu: 16.87 (2.62–158.28) $\mu\text{g/L}$; $P < 0.001$].

In urinary Cd, the median (range) level in boys [0.49 (ND–3.07) $\mu\text{g/L}$] was significantly higher than that in girls [0.34 (ND–5.11) $\mu\text{g/L}$; $P = 0.014$] in the NAHSIT 93–96. For females, the median levels of urinary Cd in girls and women in the NAHSIT 05–08 were 1.9- and 1.2-fold higher than those in girls and women in the NAHSIT 93–96, respectively, and these differences were significant (both $P < 0.01$). In urinary Pb, the median levels in adults and youths were lower overall in participants from the NAHSIT 05–08 than in those from the NAHSIT 93–96. For the adult participants, we found that the median urinary Pb level in men was significantly higher than that in women in both periods (both $P < 0.01$). In urinary Co, the median (range) level was significantly higher in girls [1.59 (0.57–10.82) $\mu\text{g/L}$] than that in boys [1.31 (0.55–3.74) $\mu\text{g/L}$] in the NAHSIT 05–08 ($P = 0.028$) and the median level in women was significantly higher than that in men in both periods (both $P < 0.01$). In urinary Cu, we found that the median level in men was significantly higher than that in women in both periods (93–96: 17.40 vs. 15.28 $\mu\text{g/L}$; 05–08: 14.53 vs. 12.45; both $P < 0.01$). For the adult participants, the urinary Cu level in the NAHSIT 05–08 was significantly lower than that in the NAHSIT 93–96 (both men and women; $P < 0.01$) (Table 2).

Urinary Cd, Pb, Co, and Cu levels in different age groups

Table 3 depicts the selected percentiles and GM of urinary metal levels in our samples in the general population of Taiwan, classified by age, from the NAHSIT 93–96 and NAHSIT 05–08. We noted that participants' exposure to Cd, Pb, Co, and Cu varied significantly according to their age. For example, the GM of urinary Cd was high in all adults from both sampling periods, and the median level of urinary

Table 1 Demographic characteristics of all the participants in this study ($N=1601$)

Characteristics	Items	NAHSIT 93–96 ($N=821$)		NAHSIT 05–08 ($N=780$)	
		<i>n</i>	%	<i>n</i>	%
Sex	Male	410	49.9	378	48.5
Age (years)	Youths (7–17)	189	23.0	103	13.2
	Adults (≥ 18)	632	77.0	677	86.8
Alcohol consumption	Yes	515	72.9	310	48.9
Betel quid chewing	Yes	— ^a	— ^a	113	17.9
Cigarette smoking	Yes	271	38.4	201	31.7
Region	Northern Taiwan	310	37.8	232	29.7
	Central Taiwan	106	12.9	181	23.2
	Southern Taiwan	125	15.2	133	17.1
	Eastern Taiwan	161	19.6	136	17.4
	Remote Island	119	14.5	98	12.6
Urbanization	Urban	192	23.4	199	25.5
	Suburban	45	5.5	177	22.7
	Rural	276	33.6	166	21.3
	Harbor	119	14.5	124	15.9
	Industrial	189	23.0	114	14.6

^a Did not survey this question in the NAHSIT 93–96

Co in all participants increased, regardless of age, from the NAHSIT 93–96 to the NAHSIT 05–08. The median level of urinary Pb in youths and adults in the NAHSIT 05–08 was significantly lower than that in the NAHSIT 93–96 (both $P < 0.01$). Furthermore, the median level of urinary Cu in adults in the NAHSIT 05–08 was lower than that in the 93–96, particularly significant in the 18–39 and 40–64 age groups (both $P < 0.01$) (Table 3, Fig. S2).

Urinary Cd, Pb, Co, and Cu levels in different regions

We presented the distribution of urinary metal levels by different regions (Fig. 1a–d, Table S1). Our data revealed that the median level of urinary Cd in participants from the NAHSIT 93–96 varied by region; however, a significantly higher urinary Cd level was observed in central ($P < 0.001$) and eastern ($P = 0.001$) Taiwan between the two sampling periods. The median level of urinary Pb was lower among the participants across all regions from the NAHSIT 93–96 to the NAHSIT 05–08. The median level of urinary Co was significantly higher among the participants from the NAHSIT 05–08 who lived in northern ($P < 0.001$) and eastern ($P = 0.044$) Taiwan, compared with the participants from the NAHSIT 93–96 who lived in those same regions. Our data also revealed that the median level of urinary Cu indicated significant regional differences ($P =$

0.019), but in overall, it was lower in the participants from the NAHSIT 05–08 compared with that in the NAHSIT 93–96, except in central Taiwan (Fig. 1a–d, Table S1).

Urinary Cd, Pb, Co, and Cu levels in different urbanization

We also categorized our participants from both sampling periods into the following urbanization: urban, suburban, rural, harbor, and industrial areas. The median level of urinary Pb was lower, regardless of urbanization, from the NAHSIT 93–96 to the NAHSIT 05–08. Moreover, the participants in the NAHSIT 05–08 who lived in suburban, harbor, and industrial areas had a lower median level of urinary Cu than those in the NAHSIT 93–96 who lived in those areas, whereas higher Cd and Co exposure was observed in urban and rural areas between the two sampling periods (Fig. 1e–h, Table S1).

Urinary Cd, Pb, Co, and Cu levels in different lifestyles

We analyzed the urinary metal levels in the two study periods in relation to the characteristics of alcohol consumption, betel quid chewing, and cigarette smoking. In both periods, the median levels of urinary Cu and Pb in participants with alcohol consumption and cigarette smoking were significantly higher than

Table 2 Selected percentiles and geometric mean of urinary metal levels (µg/L) in the general Taiwanese population (N = 1601) by youths and adults in the periods of the NAHSIT 93–96 and NAHSIT 05–08

Metals	Groups	Detectable rate (%)	n	GM (GSD)	Selected percentiles							95% CI (95th)	P value ^b	P value ^c	
					Min ^a	25th	50th	75th	90th	95th	Max				
Cd	Periods														
	1993–1996	98.8	821	0.54 (2.44)	ND	0.32	0.60	0.97	1.53	1.93	13.90	1.75–2.22			<0.001**
	2005–2008	99.9	780	0.69 (2.18)	ND	0.41	0.72	1.17	1.90	2.40	7.44	2.21–2.66			
	1993–1996														
	Youths	Boys	97.9	96	0.45 (2.34)	ND	0.28	0.49	0.79	1.23	1.69	3.07	1.22–2.36	0.014*	0.267
		Girls	97.8	93	0.35 (2.46)	ND	0.20	0.34	0.62	0.89	1.54	5.11	0.89–1.45		<0.001**
	Adults	Men	99.0	314	0.64 (2.39)	ND	0.38	0.67	1.07	1.88	2.50	13.90	2.10–3.33	0.099 [#]	0.747
		Women	99.1	318	0.55 (2.39)	ND	0.33	0.64	1.07	1.51	1.73	7.56	1.63–1.90		<0.001**
	2005–2008														
	Youths	Boys	100	49	0.54 (1.99)	0.13	0.28	0.56	0.89	1.38	1.61	2.06	0.94–1.86 ^d	0.320	
		Girls	100	54	0.61 (2.20)	0.05	0.44	0.64	0.87	1.21	2.24	3.48	0.96–2.31 ^d		
	Adults	Men	99.7	329	0.67 (1.95)	ND	0.40	0.70	1.11	1.76	2.38	7.44	2.01–2.84	0.050 [#]	
	Women	100	348	0.75 (2.20)	0.07	0.44	0.76	1.27	2.06	2.66	5.60	2.33–3.10			
Pb	Periods														
	1993–1996	99.9	821	2.21 (2.33)	ND	1.41	2.28	3.72	6.20	7.62	63.60	7.03–8.72			<0.001**
	2005–2008	100	780	0.90 (3.14)	0.04	0.51	1.09	1.94	3.15	4.53	48.88	4.05–4.98			
	1993–1996														
	Youths	Boys	100	96	2.52 (2.46)	0.14	1.59	2.51	4.18	6.57	7.84	17.34	5.57–12.57	0.089 [#]	0.003**
		Girls	98.9	93	2.06 (2.27)	ND	1.30	2.06	3.93	6.13	7.47	15.41	6.12–11.72		0.018*
	Adults	Men	100	314	2.65 (2.18)	0.11	1.62	2.78	4.36	7.08	9.64	36.15	7.99–13.03	<0.001**	<0.001**
		Women	100	318	1.81 (2.29)	0.10	1.17	1.99	3.06	4.57	6.34	63.60	5.09–7.20		<0.001**
	2005–2008														
	Youths	Boys	100	49	1.46 (3.03)	0.08	0.89	1.43	3.48	5.87	6.61	7.98	4.02–7.19 ^d	0.634	
		Girls	100	54	1.32 (3.16)	0.08	0.78	1.75	2.66	4.17	5.48	12.60	3.22–6.37 ^d		
	Adults	Men	100	329	0.96 (3.00)	0.04	0.55	1.20	2.09	2.93	4.45	15.55	3.66–5.32	0.001**	
	Women	100	348	0.75 (3.03)	0.05	0.44	0.88	1.55	2.58	3.57	48.88	3.14–4.92			
Co	Periods														
	1993–1996	100	821	0.95 (1.97)	0.08	0.60	0.91	1.41	2.32	3.03	17.30	2.67–3.52			<0.001**
	2005–2008	100	780	1.07 (2.04)	0.05	0.70	1.05	1.65	2.59	3.43	22.43	3.07–3.96			
	1993–1996														
	Youths	Boys	100	96	1.19 (1.99)	0.36	0.77	1.04	1.74	2.92	5.04	8.81	2.85–7.06	0.693	0.021*
		Girls	100	93	1.08 (2.08)	0.08	0.64	1.07	1.67	2.44	3.82	5.63	2.39–5.08		<0.001**
	Adults	Men	100	314	0.77 (1.93)	0.17	0.54	0.78	1.08	1.53	2.06	9.16	1.66–2.47	<0.001**	0.003**
		Women	100	318	1.06 (1.77)	0.16	0.62	1.00	1.68	2.61	3.50	17.30	2.95–4.26		0.088 [#]
	2005–2008														
	Youths	Boys	100	49	1.38 (1.90)	0.55	1.01	1.31	2.00	2.37	3.17	3.74	2.10–3.33 ^d	0.028*	
		Girls	100	54	1.81 (2.08)	0.57	1.25	1.59	2.55	4.73	7.86	10.82	2.97–7.87 ^d		
	Adults	Men	100	329	0.88 (1.55)	0.05	0.60	0.90	1.28	1.98	2.59	22.43	2.26–2.78	<0.001**	
	Women	100	348	1.15 (1.95)	0.09	0.73	1.10	1.85	2.88	3.98	10.59	3.40–5.12			
Cu	Periods														
	1993–1996	100	821	16.67 (1.97)	2.62	11.87	16.87	23.99	32.83	37.86	158.28	35.98–40.40			<0.001**
	2005–2008	100	779	13.81 (1.86)	1.67	8.85	13.66	21.50	29.88	36.36	189.70	34.24–39.92			
	1993–1996														
	Youths	Boys	100	96	19.93 (1.70)	5.14	14.03	20.62	28.33	35.49	40.28	47.05	34.18–43.26	0.096 [#]	0.887
		Girls	100	93	17.29 (1.75)	4.08	13.35	17.53	25.97	34.26	37.59	44.87	35.33–46.45		0.098 [#]
	Adults	Men	100	314	17.67 (1.57)	3.94	12.34	17.40	25.12	35.30	40.21	95.66	37.22–52.13	<0.001**	<0.001**
		Women	100	318	14.74 (1.73)	2.62	10.66	15.28	21.54	28.19	34.05	158.28	30.20–40.04		<0.001**
	2005–2008														
	Youths	Boys	100	49	19.85 (1.82)	4.28	12.89	22.26	29.14	45.20	48.79	61.10	31.56–51.93 ^d	0.746	
		Girls	100	54	20.49 (1.79)	5.78	12.65	22.80	33.01	40.69	52.11	64.04	35.18–57.79 ^d		
	Adults	Men	100	329	14.16 (1.82)	1.67	9.74	14.53	21.19	29.09	36.04	189.70	31.16–40.63	<0.001**	
	Women	100	347	12.04 (1.84)	2.27	7.89	12.45	18.26	25.81	30.64	62.76	28.09–35.29			

[#] P < 0.1, * P < 0.05, ** P < 0.01

^a ND, not detectable; Cd 0.02 µg/L; Pb 0.012 µg/L; Co 0.019 µg/L; Cu 0.16 µg/L

^b Comparison of the two groups by Mann–Whitney U test

^c Comparison of the two time periods by Mann–Whitney U test

^d 95% confidence interval of the 90th percentile

those without (93–96: Cu: 17.51 vs. 15.36 $\mu\text{g/L}$, $P=0.014$, Pb: 2.63 vs. 2.07 $\mu\text{g/L}$, $P<0.001$; 05–08: Cu: 13.55 vs. 12.65 $\mu\text{g/L}$, $P=0.031$, Pb: 1.34 vs. 0.88 $\mu\text{g/L}$, $P<0.001$, respectively). By contrast, the Co levels were significantly higher in the non-smoking participants than in the smoking participants (93–96: 0.93 vs. 0.87 $\mu\text{g/L}$, $P=0.039$; 05–08: 1.01 vs. 0.90 $\mu\text{g/L}$, $P=0.036$) in the two periods. We assessed betel quid chewing in the NAHSIT 05–08 participants only, and we found that participants with betel quid chewing had significantly higher median levels of urinary Pb and Cu than those without (Pb: 1.67 vs. 0.91 $\mu\text{g/L}$, $P<0.001$; Cu: 14.54 vs. 12.74 $\mu\text{g/L}$, $P=0.004$). Considering the co-exposure by different lifestyles, we evaluated the urinary levels in both lifestyles. We found that the median levels of urinary Pb and Cu in participants with alcohol consumption, betel quid chewing, and cigarette smoking were significantly higher than those without (Pb: 1.67 vs. 0.86 $\mu\text{g/L}$, $P<0.001$; Cu: 14.45 vs. 12.64 $\mu\text{g/L}$, $P=0.004$) (Table 4).

The percentage differences of the four metal levels among the five age groups in the NAHSIT 05–08 compared with the NAHSIT 93–96

The percentage differences in the NAHSIT 05–08 were higher at 15–37% and 15–55% for urinary Cd and Co and were lower at 29–58% and 1–3% for urinary Pb and Cu than those in the NAHSIT 93–96, respectively. The adolescent group exhibited the most substantial lowest percentage difference in Pb exposure and the highest percentage difference in Cd exposure, whereas the middle-aged group exhibited the highest percentage difference in Co exposure and the most substantial lowest percentage difference in Cu exposure (Fig. 2).

The correlation between urinary Cd, Pb, Co, and Cu levels

We used the Pearson's correlation to investigate the correlations between urinary metal levels in the NAHSIT 93–96 and NAHSIT 05–08 periods. The correlation coefficients of the four metals were 0.349 to 0.563 in the NAHSIT 93–96 and 0.361 to 0.556 in the NAHSIT 05–08 (Table S2). A weak to moderate correlation between metals in this study represented the route of simultaneous exposure of Cd, Pb, Co, and Cu during the two periods.

Principal component and hierarchical clustering analysis of urinary heavy metal exposure in the general population of Taiwan

We performed a PCA on the individual values of the urinary levels of the four metals in the participants from the NAHSIT 93–96 and NAHSIT 05–08 and analyzed the participants' exposure to metals according to age and sex; the results are summarized in Fig. 3. Notably, two factors were extracted from the

urinary metals which explained the total variance in the participants, regardless of age and sampling period. For the youths, the following two principal components (PCs; PC1 and PC2) explained 62.3% of the total variance in the NAHSIT 93–96: PC1 correlated with all four metals in girls, whereas PC2 correlated with all four metals in boys (Fig. 3a). In the NAHSIT 05–08, PC1 and PC2 explained 62.0% of the total variance in youths: PC1 correlated with all four metals in girls, whereas PC2 correlated with all four metals in boys (Fig. 3b). For adults, two PCs explained 49.1% of the total variance in the NAHSIT 93–96: PC1 correlated with all four metals in men, whereas PC2 correlated with Cd and Cu in women (Fig. 3c). In the NAHSIT 05–08, PC1 and PC2 explained 61.5% of the total variance in adults: PC1 correlated with all four metals in men, whereas PC2 correlated all four metals in women (Fig. 3d). Moreover, in each group, hierarchical clustering analysis identified two major clusters by sex (Fig. 3e–h).

Comparison of metal exposure in different countries

We compared the four metal GM levels in the population of various countries for similar periods. A comparison of the GM levels of the four metals in the general population of Taiwan (based on the NAHSIT 93–96 and NAHSIT 05–08), the United States (based on the National Health and Nutrition Examination Survey (NHANES) periods 1999–2000 and 2007–2008), Germany (based on the German Environmental Survey (GerES) II period 1990–1992 and GerES III period 1998), the Czech Republic (based on the Human Biomonitoring (HBM) project periods 2001–2003 and 2005–2009), Canada (based on the Canadian Health Measures Survey (CHMS) 2007–2013) (Haines et al. 2017), Spain (based on 2009 data) (Castano et al. 2012), China (based on 2008 data) (Wang et al. 2011), Korea (based on the Korea National Survey for Environmental Pollutants in the Human Body (KorSEP-III), 2008) (Lee et al. 2012), and Japan (based on 2000–2005 data) (Ohashi et al. 2006) is shown in Fig. 4. The GM levels of Cd in the Taiwanese population according to the NAHSIT 93–96 (0.54 $\mu\text{g/L}$) and NAHSIT 05–08 (0.69 $\mu\text{g/L}$) were 2.8- and 3.6-fold higher, respectively, than in the U.S. population according to the NHANES 2007–2008 (0.19 $\mu\text{g/L}$), 1.9- and 2.4-fold higher than in the Germany population according to the GerES II (0.29 $\mu\text{g/L}$), and 1.6- and 2.1-fold higher in the Canada population according to the CHMS 2007–2009 (0.34 $\mu\text{g/L}$), respectively. A 2.5-fold decrease in the GM of urinary Pb in the Taiwanese population was observed between the NAHSIT 93–96 and NAHSIT 05–08. Nevertheless, the GM of urinary Pb in the Taiwanese population according to the NAHSIT 05–08 (0.9 $\mu\text{g/L}$) was 1.8-fold higher than that in the U.S. population according to the NHANES 2007–2008 (0.49 $\mu\text{g/L}$), 1.9-fold higher than that in the Canada population according to the CHMS 2007–2009 (0.48 $\mu\text{g/L}$), and 3.1-fold higher

Table 3 Selected percentiles and geometric mean of urinary metal levels (µg/L) in the general Taiwanese population (N= 1601) by age in the periods of 1993–1996 and 2005–2008

Metals/ groups	n	GM (GSD)	Selected percentiles					95% CI (95th)	P value ^b	P value ^c	P value ^d		
			Min ^a	25th	50th	75th	90th					95th	Max
Cd													
1993–1996													
Youths	189	0.39 (2.41)	ND	0.24	0.39	0.71	1.07	1.59	5.11	1.12–1.81	< 0.001**	0.465	< 0.001**
7–11	73	0.38 (2.29)	ND	0.24	0.35	0.69	0.93	1.20	5.11	0.92–5.02		0.006**	
11–17	116	0.40 (2.49)	ND	0.24	0.43	0.73	1.21	1.62	4.93	1.25–2.84		0.001**	
Adults	632	0.59 (2.40)	ND	0.37	0.65	1.07	1.62	2.03	13.90	1.88–2.40	0.038*	0.002**	
18–39	359	0.54 (2.38)	ND	0.34	0.63	0.99	1.37	1.64	3.74	1.43–1.90		0.498	
40–64	200	0.68 (2.30)	0.04	0.41	0.70	1.25	1.88	2.47	13.90	1.97–3.07		0.068 [#]	
≥ 65	73	0.67 (2.62)	0.06	0.36	0.69	1.17	2.37	4.20	7.56	2.35–7.52		0.127	
2005–2008													
Youths	103	0.58 (1.97)	0.05	0.41	0.62	0.87	1.26	1.75	3.48	1.30–2.52	< 0.001**	0.057 [#]	
7–11	65	0.54 (1.93)	0.13	0.36	0.54	0.78	1.22	1.87	3.48	0.90–2.13 ^c			
11–17	38	0.65 (2.02)	0.05	0.49	0.72	0.98	1.42	1.81	2.21	1.01–2.15 ^c			
Adults	677	0.71 (2.20)	ND	0.41	0.72	1.22	1.97	2.44	7.44	2.32–2.78	< 0.001**		
18–39	274	0.60 (2.18)	ND	0.37	0.61	1.02	1.55	2.06	6.42	1.74–2.59			
40–64	310	0.78 (2.16)	0.10	0.48	0.78	1.31	2.09	2.69	5.60	2.36–3.44			
≥ 65	93	0.82 (2.27)	0.14	0.44	0.86	1.56	2.37	2.96	7.44	2.37–5.70			
Pb													
1993–1996													
Youths	189	2.28 (2.33)	ND	1.43	2.35	4.06	6.19	7.59	17.34	6.56–8.64	0.009**	0.543	< 0.001**
7–11	73	2.46 (1.87)	0.53	1.57	2.52	3.52	6.10	6.88	8.67	6.02–8.66		0.064 [#]	
11–17	116	2.18 (2.61)	ND	1.32	2.18	4.17	6.56	7.80	17.34	6.78–14.26		< 0.001**	
Adults	632	2.19 (2.33)	0.10	1.39	2.23	3.60	6.11	7.72	63.60	7.00–9.19	0.002**	< 0.001**	
18–39	359	2.37 (2.32)	0.10	1.53	2.47	3.97	5.60	8.31	63.60	7.13–10.24		< 0.001**	
40–64	200	2.11 (2.27)	0.11	1.25	2.07	3.56	5.62	7.62	22.51	6.35–12.51		< 0.001**	
≥ 65	73	1.64 (2.44)	0.14	1.00	1.71	2.95	3.98	4.96	34.25	3.87–33.53		< 0.001**	
2005–2008													
Youths	103	1.39 (3.00)	0.08	0.83	1.64	2.96	4.61	6.30	12.60	4.77–8.80	< 0.001**	0.004**	
7–11	65	1.73 (2.88)	0.08	1.05	2.06	3.70	5.87	6.76	12.60	4.23–7.23 ^c			
11–17	38	0.95 (2.94)	0.08	0.53	1.17	2.08	3.54	4.48	5.17	2.41–5.06 ^c			
Adults	677	0.85 (3.12)	0.04	0.47	1.03	1.78	2.85	4.23	48.88	3.46–4.84	0.634		
18–39	274	0.90 (2.85)	0.07	0.52	1.04	1.88	2.85	3.78	15.55	2.38–3.24			
40–64	310	0.80 (3.32)	0.04	0.43	0.97	1.68	2.85	4.23	48.88	2.56–3.72			
≥ 65	93	0.84 (3.24)	0.07	0.47	1.06	1.94	2.88	4.67	11.23	2.91–7.95			
Co													
1993–1996													
Youths	189	1.13 (1.96)	0.08	0.73	1.04	1.69	2.56	4.47	8.81	2.89–5.39	< 0.001**	0.509	< 0.001**
7–11	73	1.08 (1.72)	0.36	0.80	0.98	1.54	2.11	3.25	5.01	2.08–5.01		0.002**	
11–17	116	1.17 (2.11)	0.08	0.69	1.08	1.86	2.91	4.97	8.81	3.49–5.94		< 0.001**	
Adults	632	0.91 (1.96)	0.16	0.58	0.87	1.34	2.24	2.73	17.30	2.57–3.15	< 0.001**	0.001**	
18–39	359	0.99 (2.02)	0.17	0.61	0.90	1.45	2.41	3.15	17.30	2.67–3.95		0.022*	
40–64	200	0.85 (1.90)	0.18	0.53	0.83	1.26	2.10	2.59	7.29	1.80–2.46		0.010*	
≥ 65	73	0.70 (1.61)	0.16	0.51	0.75	1.01	1.25	1.44	1.53	1.24–1.53		0.032*	
2005–2008													
Youths	103	1.59 (1.77)	0.55	1.14	1.47	2.17	3.32	4.78	10.82	3.38–8.39	< 0.001**	0.032*	
7–11	65	1.46 (1.81)	0.55	0.95	1.37	2.09	2.72	4.37	10.82	2.20–5.58 ^c			
11–17	38	1.85 (1.66)	0.88	1.26	1.59	2.55	3.55	5.18	7.86	2.67–7.44 ^c			
Adults	677	1.01 (2.04)	0.06	0.66	0.98	1.55	2.50	3.31	22.43	2.82–3.85	0.004**		
18–39	274	1.11 (2.09)	0.05	0.69	1.06	1.81	2.84	3.85	22.43	3.13–4.51			
40–64	310	0.97 (2.03)	0.06	0.67	0.94	1.48	2.50	2.89	7.91	2.67–3.82			
≥ 65	93	0.86 (1.83)	0.18	0.58	0.91	1.20	1.78	2.05	9.61	1.78–7.14			
Cu													
1993–1996													
Youths	189	18.58 (1.63)	4.08	13.52	18.97	27.60	34.58	37.93	47.05	35.18–43.21	0.001**	0.998	0.189
7–11	73	18.79 (1.54)	4.19	14.21	17.82	27.11	34.13	36.69	40.02	33.91–40.00		0.102	
11–17	116	18.46 (1.69)	4.08	13.28	19.53	27.92	35.13	41.78	47.05	35.94–46.06		0.932	
Adults	632	16.13 (1.76)	2.62	11.38	16.43	23.00	31.73	38.09	158.28	34.99–40.81	0.029*	< 0.001**	
18–39	359	16.48 (1.77)	2.86	11.39	17.43	24.30	31.93	37.63	95.66	33.92–40.92		0.002**	
40–64	200	16.21 (1.67)	4.34	12.10	16.23	21.89	31.55	37.23	81.68	33.35–53.48		< 0.001**	
≥ 65	73	14.32 (1.93)	2.62	9.33	14.28	19.57	30.83	47.29	158.28	30.11–156.40		0.299	
2005–2008													
Youths	103	20.18 (1.81)	4.28	12.74	22.53	30.81	41.13	48.79	64.04	41.69–61.61	< 0.001**	0.196	

Table 3 (continued)

Metals/ groups	<i>n</i>	GM (GSD)	Selected percentiles						95% CI (95th)	<i>P</i> value ^b	<i>P</i> value ^c	<i>P</i> value ^d
			Min ^a	25th	50th	75th	90th	95th				
7–11	65	21.26 (1.80)	4.28	13.70	23.06	32.01	40.88	49.72	64.04	36.36–53.32 ^c		
11–17	38	18.47 (1.83)	5.78	11.92	20.65	26.31	43.49	47.92	58.08	30.00–56.48 ^c		
Adults	676	13.03 (1.83)	1.67	8.51	13.17	19.86	27.76	33.21	189.70	30.51–36.24	0.001**	
18–39	274	14.55 (1.77)	1.67	9.48	14.06	21.99	28.89	35.18	189.70	30.47–40.26		
40–64	309	11.87 (1.83)	1.85	8.15	12.44	18.27	24.26	30.83	75.06	27.91–35.56		
≥65	93	12.83 (1.87)	2.91	7.73	12.71	19.85	28.79	34.05	81.09	28.92–68.68		

$P < 0.1$, * $P < 0.05$, ** $P < 0.01$

^aND, not detectable; Cd 0.02 µg/L; Pb 0.012 µg/L; Co 0.019 µg/L; Cu 0.16 µg/L

^bComparison of the five age groups by Kruskal–Wallis test

^cComparison of the age groups by Kruskal–Wallis test (youths)/Mann–Whitney *U* test (adults)

^dComparison of the two time periods by Mann–Whitney *U* test

^e95% confidence interval of the 90th percentile

than that in the Czech Republic population (0.29 µg/L). Conversely, the GM of urinary Pb in the Taiwanese population according to the NAHSIT 05–08 was lower than that in Spain (1.11 µg/L) and China (1.90 µg/L) population. Except for the Czech Republic population, according to the 2005–2009 survey period, the GM of urinary Cd in the Taiwanese population was higher than all of the other countries. In addition, the GM of urinary Co in the Taiwanese population according to the NAHSIT 05–08 (1.07 µg/L) was 1.6-, 2.9-, and 4.7-fold higher than that of Japan population (0.68 µg/L) and the U.S. population according to the NHANES 2007–2008 (0.37 µg/L) and the Canada population according to the CHMS 2009–2011 (0.23 µg/L), respectively. Finally, the GM of urinary Cu decreased in the Taiwanese population from the NAHSIT 93–96 to the NAHSIT 05–08 (16.67 vs. 13.81 µg/L), it but was still 8- and 6-fold higher, respectively, than in the Chinese population (2.07 µg/L). In addition, the GM of urinary Co in the Taiwanese population according to the NAHSIT 05–08 was 1.5-fold higher than that of the Canada population according to the CHMS 2007–2009 (9.00 µg/L) (Fig. 4).

Discussion

This is the first study that systematically evaluated exposure to Cd, Pb, Co, and Cu in the general population of Taiwan over the past decade. We observed significant differences in Cd, Pb, Co, and Cu exposure levels according to sex, age, urbanization, regions, and lifestyles. We found that metal exposure in youths was higher than in adults and exposure in men was higher than in women. In addition, the urinary levels of Pb and Cu in the Taiwanese participants in the NAHSIT 05–08 were lower than the corresponding levels in the NAHSIT 93–96, whereas the levels of Cd and Co were higher than those in the NAHSIT 93–96. However, between those same time periods,

the urinary levels of Cd and Co in the participants were significantly higher in eastern Taiwan and in the urbanization of urban and rural. Differences in lifestyle may have contributed to differences in urinary metal levels.

Different lifestyles may cause different human metal exposure. Our data found that the median level of urinary Cu in the participants with alcohol consumption was significantly higher than that without ($P < 0.05$). Navarro-Alarcon et al. indicated that consuming alcoholic beverages is an important human exposure source (Navarro-Alarcon et al. 2007). We observed that the participants with cigarette smoking have a higher urinary Pb level ($P < 0.01$) and lower urinary Co level ($P < 0.05$) in both periods, which is consistent with the previous report from the NHANES 1999–2004 (Richter et al. 2009). Cigarette smoking is an important source of Pb exposure and is increasingly relevant as leaded gasoline has declined (Bernhard et al. 2005; Richter et al. 2009). Co is a component of vitamin B12, and the previous study indicated that cigarette smoking interferes with absorption of vitamin B12 (Cogswell et al. 2003). This may be the reason why the urinary Co levels were significantly lower in the cigarette-smoking participants. Besides, our data showed that the median levels of urinary Pb and Cu in the participants with betel quid chewing were significantly higher than those without (Cd: $P = 0.067$; Pb and Cu: $P < 0.01$). One study has reported that the betel quid chewing components have higher arsenic, Cd, and Pb (Al-Rmalli et al. 2011). Considering that betel quid may contain higher levels of heavy metals, the possible health impacts associated with exposure to these metals should be a concern. Although some studies have revealed that human activities like alcohol consumption and cigarette smoking can affect urinary metal levels, further researches are needed for other lifestyles. We suggested that the implementation of public health education or intervention strategies of

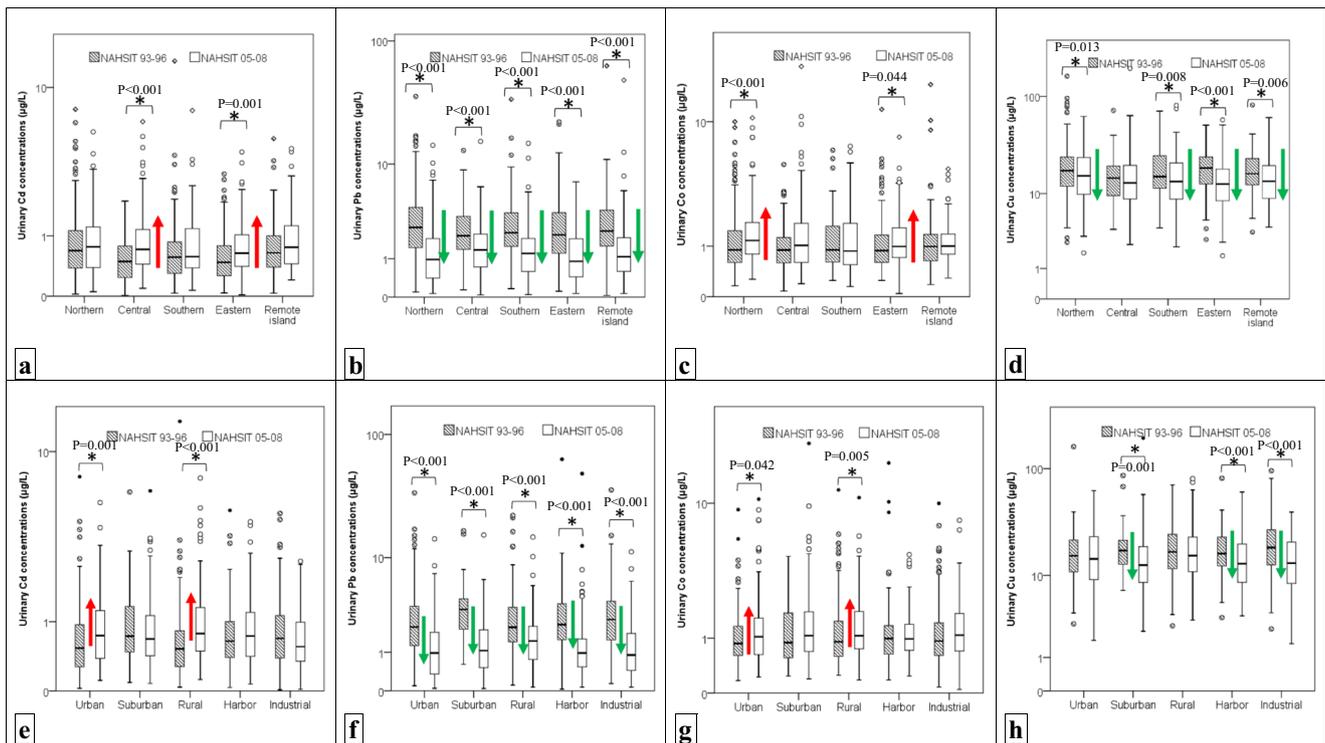


Fig. 1 Distribution (median and range) of urinary Cd (a, e), Pb (b, f), Co (c, g), and Cu (d, h) levels ($\mu\text{g/L}$) in Taiwanese living in different regions (upper) and urbanization (lower) in the NAHSIT 93–96 and 05–08. The Mann–Whitney U test was applied. $*P < 0.05$

alcohol consumption, betel quid chewing, and cigarette smoking could be beneficial for the reduction of certain metal exposure in the general population of Taiwan.

The exposure to Cd in Taiwan is considerably higher than that in other countries. The main exposure routes of cadmium are through foods, especially in rice. In Asian countries like Japan and Taiwan, rice is the main staple food, which is known as the major source of exposure to Cd. In this study, around 23–29% of the participants have over the threshold Cd level ($1 \mu\text{g/L}$), which might cause renal, bone, hematological, etc., problems that are well-documented (Nordberg et al. 2007). One previous meta-analysis indicated that even at low levels of Cd exposure, urinary Cd levels ($\geq 0.48 \mu\text{g/g}$ cre.) are associated with increased cardiovascular disease mortality in the general population (hazard ratio 1.33–1.87) (Larsson and Wolk 2016). As we discussed previously, the prevalence of cigarette smoking in Taiwan from 2001 to 2013 significantly decreased (Chiang and Chang 2016); however, in the present study, the Cd level in the NAHSIT 05–08 was significantly higher than that in the NAHSIT 93–96 period ($P < 0.01$). Although these results contradict the reduced smoking rate in Taiwan, the consumption of foods, such as fish, meat, fruit, and rice, is a Cd exposure pathway for non-smokers; according to a previous NAHSIT study, Taiwan’s intake frequency of sea fish, shellfish, poultry, vegetables, and fruit increased from the NAHSIT 93–96 to NAHSIT 05–08 (Yeh et al. 2011). This may be one reason for the

increased Cd exposure between our studied sampling periods; however, these other sources of Cd exposure must be examined to confirm our results.

Exposure to Pb in the general population of Taiwan in the NAHSIT 05–08 ($0.90 \mu\text{g/L}$) was significantly lower than that in the NAHSIT 93–96 ($2.21 \mu\text{g/L}$, $P < 0.01$), regardless of age, sex, or regions. Martinez et al. (2013) showed that banning leaded gasoline in Argentina resulted in a 66.5% reduction in blood lead levels (Martinez et al. 2013). This phenomenon is possibly attributable to Taiwan’s ban on leaded gasoline and implementation of unleaded gasoline in 2000. In the present study, we found that children (age group 7 to 11 years) exhibited the highest levels of urinary Pb. This observation may be because children of this age tend to ingest Pb via soil and be exposed in lead-contaminated dust (Lanphear et al. 2002) and have higher absorption from the gastrointestinal tract than adults (Ziegler et al. 1978). Smoking habit is also a critical factor leading to Pb exposure (Castano et al. 2012; Richter et al. 2009). A recent study showed that the prevalence of cigarette smoking in Taiwan from 2001 to 2013 significantly decreased from 44.4 to 34.2% (Chiang and Chang 2016); this may be another reason to explain the decrease in Pb levels between the sampling periods. Moreover, although Pb levels decreased, our previous study indicated that exposure to even low levels of Pb may have lagging effects on the intellectual development of children (Huang

Table 4 The median (range) urinary metal levels ($\mu\text{g/L}$) in the general Taiwanese population by alcohol consumption, betel quid chewing, and cigarette smoking in the periods of NAHSIT 1993–1996 and 2005–2008

Lifestyles	NAHSIT 1993–1996 ($N=706$)					NAHSIT 2005–2008 ($N=634$)				
	<i>n</i>	Cd	Pb	Co	Cu	<i>n</i>	Cd	Pb	Co	Cu
Alcohol consumption										
Yes	515	0.64 (ND–13.90)	2.36 (0.11–63.60)	0.90 (0.08–17.30)	17.51 (2.62–95.66)	310	0.70 (0.08–6.42)	1.10 (0.07–15.55)	0.96 (0.06–22.43)	13.55 (2.45–189.7)
No	191	0.51 (0.03–7.56)	2.18 (ND–22.51)	0.93 (0.25–10.20)	15.36 (2.86–158.28)	324	0.74 (ND–4.45)	0.91 (0.04–48.88)	0.99 (0.05–9.61)	12.65 (1.67–62.76)
<i>P</i> value ^a		0.096 [#]	0.084 [#]	0.565	0.014 [*]		0.314	0.069 [#]	0.841	0.031 [*]
Betel quid chewing										
Yes	^b	–	–	–	–	113	0.79 (0.14–4.51)	1.67 (0.07–11.23)	0.99 (0.15–6.28)	14.54 (2.73–75.06)
No	^b	–	–	–	–	518	0.71 (ND–6.42)	0.91 (0.04–48.88)	0.96 (0.05–22.43)	12.74 (1.67–189.7)
<i>P</i> value ^a							0.067 [#]	< 0.001 ^{**}	0.186	0.004 ^{**}
Cigarette smoking										
Yes	271	0.65 (0.04–13.90)	2.63 (0.11–36.15)	0.87 (0.08–6.44)	17.43 (4.04–158.3)	201	0.77 (ND–6.42)	1.34 (0.07–15.55)	0.90 (0.05–22.43)	13.99 (1.67–189.7)
No	435	0.58 (ND–4.93)	2.07 (ND–63.60)	0.93 (0.16–17.30)	16.45 (2.62–95.66)	433	0.68 (0.07–4.45)	0.88 (0.04–48.88)	1.01 (0.06–10.59)	12.69 (1.86–62.76)
<i>P</i> value ^a		0.010 [*]	< 0.001 ^{**}	0.039 [*]	0.068 [#]		0.052 [#]	< 0.001 ^{**}	0.036 [*]	0.021 [*]
Alcohol consumption + cigarette smoking										
Yes	251	0.64 (0.04–13.90)	2.64 (0.11–36.15)	0.87 (0.08–6.44)	17.42 (4.04–81.68)	149	0.75 (0.08–6.42)	0.88 (0.04–48.88)	1.03 (0.20–9.12)	12.65 (1.85–62.76)
No ^c	171	0.51 (ND–3.07)	2.13 (ND–22.51)	0.93 (0.25–10.20)	15.04 (2.86–47.05)	272	0.73 (0.07–4.45)	1.27 (0.07–15.55)	0.93 (0.15–22.43)	14.14 (2.45–189.70)
<i>P</i> value ^a		0.008 ^{**}	< 0.001 ^{**}	0.130	0.006 ^{**}		0.415	< 0.001 ^{**}	0.157	0.003 ^{**}
Alcohol consumption + betel quid chewing										
Yes	–	–	–	–	–	92	0.79 (0.14–4.51)	1.61 (0.07–10.19)	1.05 (0.15–6.28)	14.52 (2.73–75.06)
No ^d	–	–	–	–	–	267	0.72 (0.07–4.45)	0.86 (0.04–48.88)	1.00 (0.20–9.12)	12.64 (1.85–62.76)
<i>P</i> value ^a							0.304	< 0.001 ^{**}	0.776	0.004 ^{**}
Betel quid chewing + cigarette smoking										
Yes	–	–	–	–	–	89	0.85 (0.14–4.51)	1.67 (0.07–11.23)	0.96 (0.15–5.07)	14.14 (2.73–75.06)
No ^d	–	–	–	–	–	267	0.72 (0.07–4.45)	0.86 (0.04–48.88)	1.00 (0.20–9.12)	12.64 (1.85–62.76)
<i>P</i> value ^a							0.097 [#]	< 0.001 ^{**}	0.477	0.029 [*]
Alcohol consumption + betel quid chewing + cigarette smoking										
Yes	–	–	–	–	–	73	0.86 (0.14–4.51)	1.67 (0.07–10.19)	0.99 (0.15–5.07)	14.45 (2.73–75.06)
No ^d	–	–	–	–	–	267	0.72 (0.07–4.45)	0.86 (0.04–48.88)	1.00 (0.20–9.12)	12.64 (1.85–62.76)
<i>P</i> value ^a							0.078 [#]	< 0.001 ^{**}	0.917	0.004 ^{**}

[#] $P < 0.1$, * $P < 0.05$, ** $P < 0.01$

^a Mann–Whitney *U* test

^b Did not survey this question

^c Without alcohol consumption and cigarette-smoking habits

^d Without alcohol consumption, betel quid chewing, and cigarette-smoking habits

et al. 2012) and that women with higher Pb exposure may have increased risk of osteopenia and osteoporosis (Tsai et al. 2016). Thus, strategies to decrease Pb exposure in Taiwan should continue, such as the elimination of Pb pipelines, restrictions on the use of Pb in the paint, and the prohibition of Pb additives in products for children.

Data regarding urinary Co levels in the general population of Taiwan are limited. Co and some of its components may be toxicants (Domingo 1989). One study conducted in Taiwan indicated that urinary Co levels in steel production workers ($n = 63$, mean \pm SD $8.18 \pm 2.73 \mu\text{g/L}$) and quality control workers ($n = 63$, mean \pm SD: $7.39 \pm 1.26 \mu\text{g/L}$) were

significantly higher than those in the general population ($n = 63$, mean \pm SD: $3.18 \pm 1.59 \mu\text{g/L}$) (both $P < 0.01$) (Horng et al. 2003). All participants in the aforementioned study were men, and their mean age was approximately 45 years. In the present study, the mean (\pm SD) urinary Co levels of male participants were $1.06 (\pm 0.95)$ and $1.19 (\pm 1.36) \mu\text{g/L}$ in the NAHSIT 93–96 and NAHSIT 05–08, respectively. Thus, urinary Co levels identified in the present study were lower than those reported in the previous study in Taiwan. However, the exposure levels of Co in the two periods examined in the present study were still 1.6- to 4.7-fold higher than those in the U.S. NHANES, Japan, and Canada for the corresponding periods.

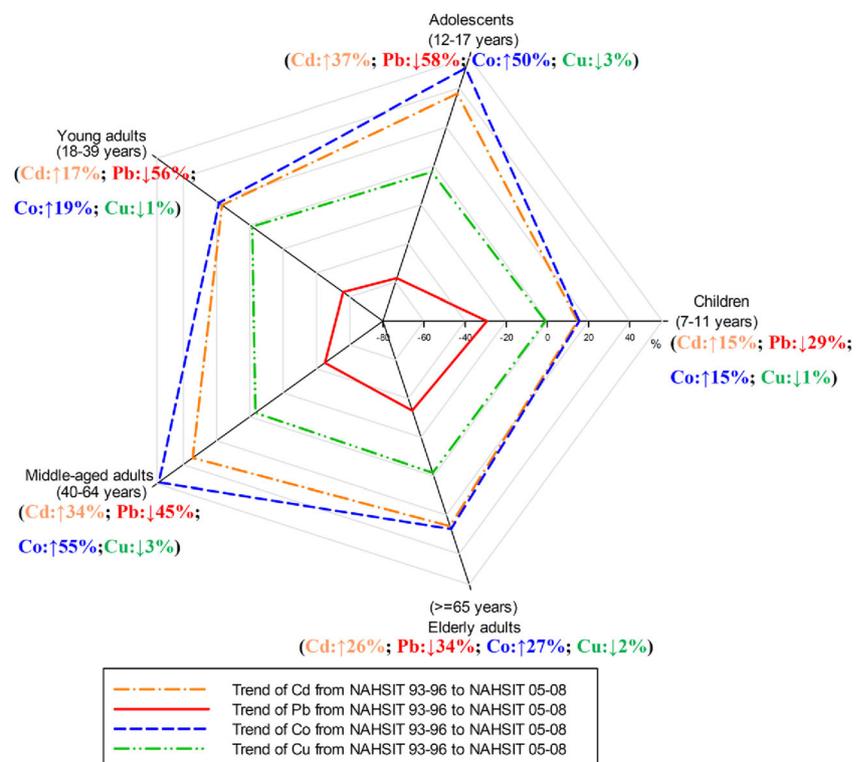
Cu is an essential nutritional element required in small amounts for the well-being of humans; by contrast, high levels of Cu can be harmful, particularly in children (ATSDR 2004a). Maintaining adequate Cu levels in the human body is crucial. A study indicated that a basic relationship exists between the serum Cu level and atherosclerosis (Bagheri et al. 2015). Cu is also a crucial trace element in the brain; when a deficiency occurs, Menkes disease, Wilson’s disease, and other serious neurological disorders may develop (de Bie et al. 2007). In this study, we found that the urinary Cu levels in the NAHSIT 05–08 in young and middle-aged adults were significantly lower than those in the NAHSIT 93–96 period ($P < 0.01$) and that participants from northern Taiwan exhibited the highest urinary Cu levels across both sampling periods (Table 3). Furthermore, the median levels of urinary Cu in youths were approximately 1.2- to 1.5-fold higher than those of adults in both sampling

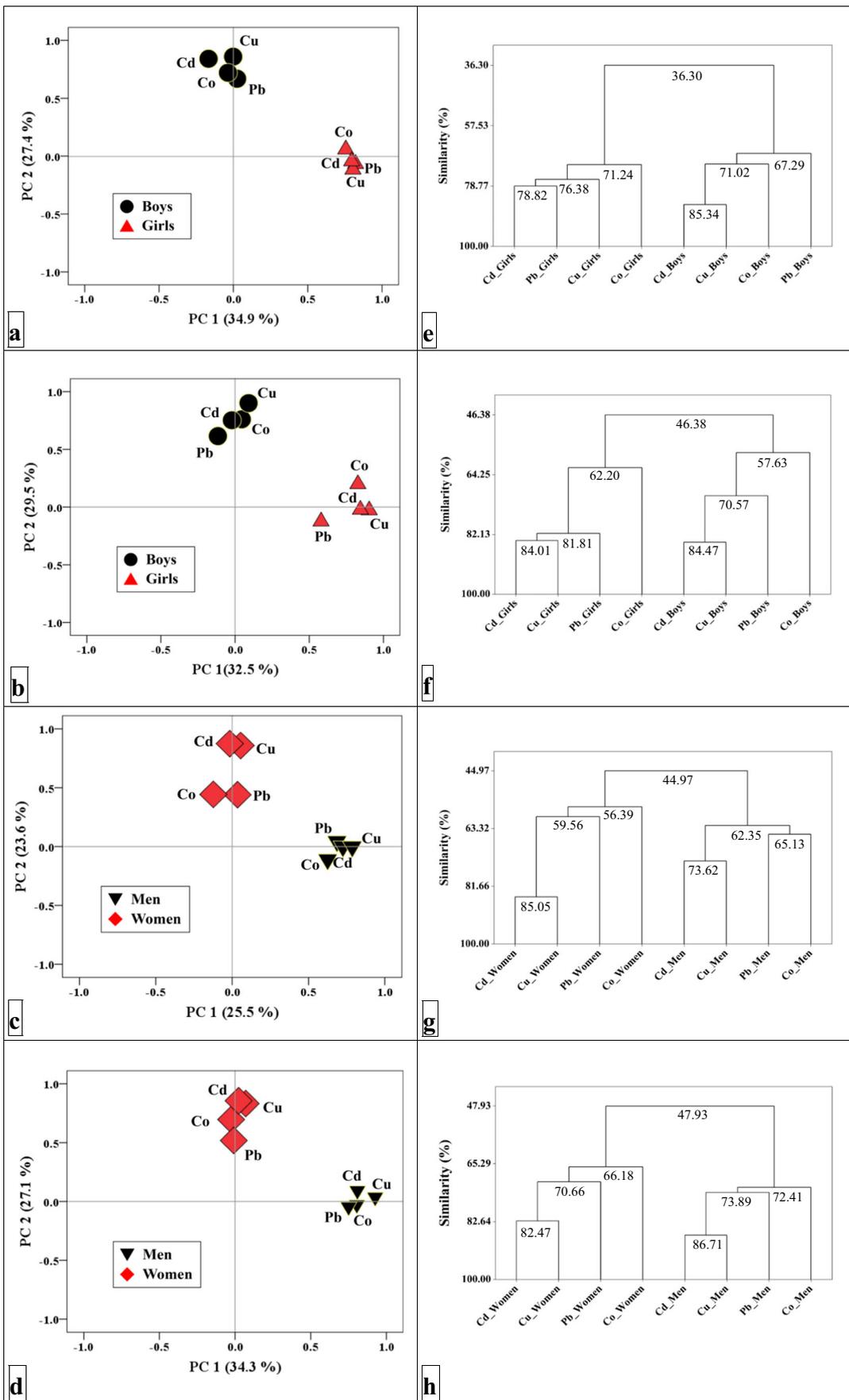
periods. Hence, monitoring and reducing the exposure of children to heavy metal is necessary.

Gender could be a factor dominating the exposure profile of metal exposure. Results of PCA and HCA revealed that two components and clusters of metal exposure were classified by sex regardless of different age or periods. Furthermore, HCA results showed that exposure profile in youth was changed for both sexes between the two periods whereas no similar phenomena were found in adults. It is indicated that youths could be more sensitive to environmental metal exposure. Meanwhile, we observed that 2 PCs for NAHSIT 93–96 adults only accounted for nearly 50%, which is much lower than others. HCA result showed an obviously different similarity for the exposure of the four metals between sexes in NAHSIT 93–96. The change of similarity for metal exposure between the two periods could be possibly influenced by the dramatic environmental change in the population, like the ban of leaded gasoline (Huang et al. 2012; Hwang et al. 2004).

The strengths of this study include its large sample size, evaluation of levels of urinary metal content over time, and survey of different regions in Taiwan. With a large sample size of 1601 participants, we found significant differences in urinary metal content in the Taiwanese population according to sex, age, urbanization, regions, and sampling period. A PCA was also performed to identify the PCs of the different age- and sex-exposure profiles. The PCA provided an overview of the urinary Pb, Cd, Co, and Cu content found in the participants from the NAHSIT 93–96 and NAHSIT 05–08 and

Fig. 2 The percentage differences of urinary Cd, Pb, Co, and Cu levels in the five age groups in the NAHSIT 05–08 compared with the NAHSIT 93–96





◀ **Fig. 3** Principal components (left) and hierarchical clustering (right) analysis of the four urinary metals in youths by sex in the NAHSIT 93–96 (a, e) and NAHSIT 05–08 (b, f) and in adults by sex in the NAHSIT 93–96 (c, g) and NAHSIT 05–08 (d, h)

revealed the participants' exposure to metals according to age and sex. Notably, we analyzed urinary heavy metal levels, rather than blood heavy metal levels. This strategy enabled us to avoid invasive extraction methods for all the age groups (which is a particular concern for children) and mitigate participants' refusal to allow blood collection. Urinary metal levels are suitable for representing recent human exposure (ATSDR 2007; Ince et al. 2008; Ishizaki et al. 2015; Linder 1984). We collected over 90% of the participants' specimens to establish an exposure database in Taiwan. To the best of our knowledge, this is the first study to examine the urinary levels of heavy metals in the general population and discuss sampling period-based differences on a national scale in Taiwan. Our data provide useful exposure reference values for Cd, Pb, Co, and Cu in the general population of Taiwan.

This study also has some limitations in data interpretation. First, this was a cross-sectional, one-time measurement study examining survey data from 1993 to 1996 and from 2005 to

2008. The exposure data only reflected the participants' exposure immediately before the survey and, thus, may not be representative of the long-term exposure. Second, we presented only the basic demographic data (e.g., sex, age, urbanization, and region) and partly lifestyles (alcohol consumption, betel quid chewing, and cigarette smoking) of the participants. The detailed information regarding exposure, lifestyles, and other associated factors, which is required for a comprehensive assessment of potential exposure sources, was not presented. Third, we could not exclude the possibility of residual confounding factors associated with the co-exposure to other metals and metal concentration in soil that is potentially related to personal metal levels. Finally, because of the limited volume of specimens collected, we could not conduct a creatinine-based estimation of metal levels in this study. However, we have compared the correlation between unadjusted and adjusted urine creatinine of the urinary metal levels ($n = 377$) in the NAHSIT 05–08, and we found the significant correlation coefficients of urine creatinine adjusted and unadjusted of Cd, Pb, Co, and Cu levels which were 0.642, 0.790, 0.516, and 0.288 (both $P < 0.01$), respectively (Table S3). Despite the lack in creatinine, the results are suitably comparable to those of other nation-wide studies, namely the NHANES in the U.S., the GerES in Germany, and the CHMS in Canada.

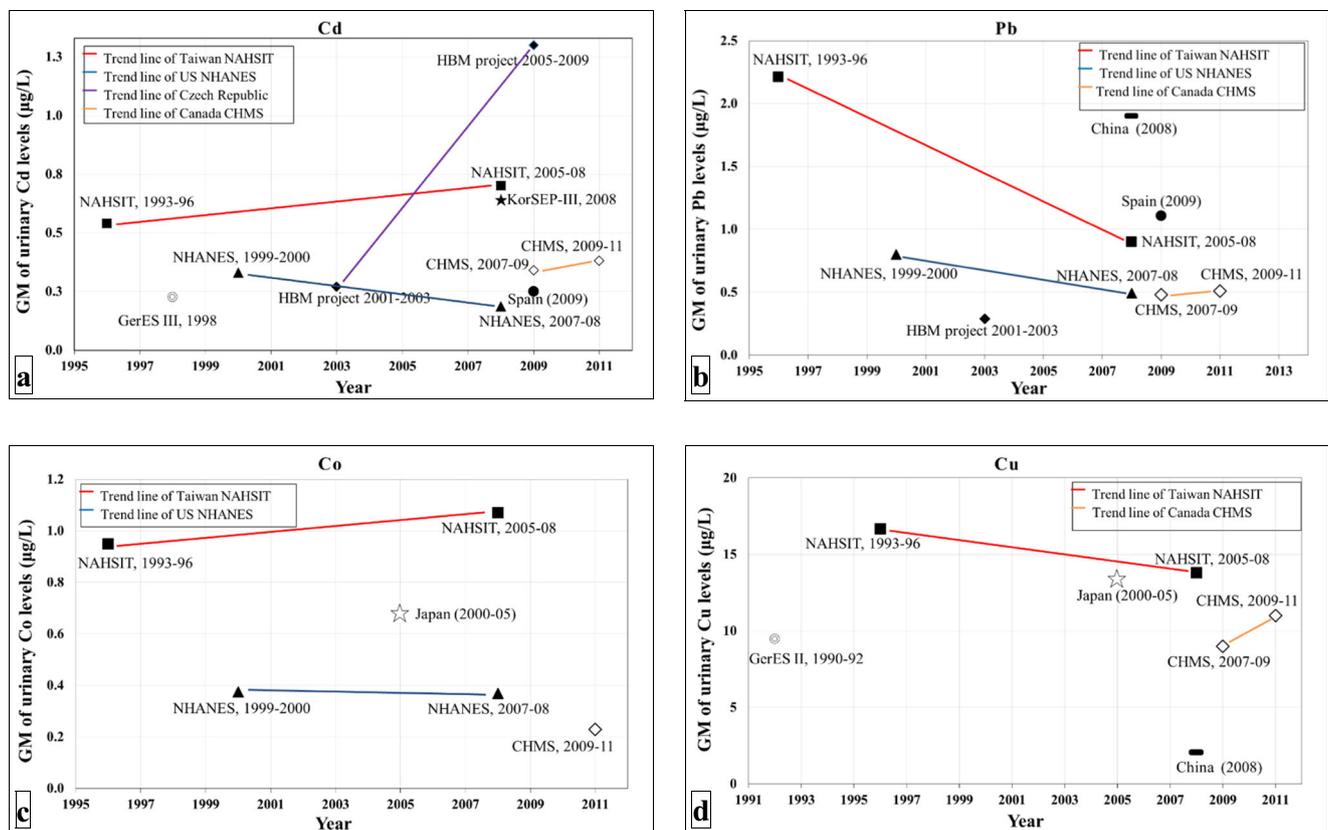


Fig. 4 Comparison of urinary levels of Cd (a), Pb (b), Co (c), and Cu (d) (µg/L) in the following: ■, general population of Taiwan [≥ 7 years, NAHSIT 93–96 ($n = 821$) and 05–08 ($n = 780$); ▲, America [≥ 6 years, NHANES 99–00 ($n = 1007$) and 07–08 ($n = 2627$); ◆, Czech Republic [15–58 years, HBM 2001–03 ($n = 657$) and 2005–09 ($n = 896$); ◎,

Germany [18–69 years, GerES II 1998 ($n = 4740$) and GerES III 2003–06 ($n = 1734$); ●, Spain [23–66 years, 2009 ($n = 165$); ▴, China [25–64 years, 2008 ($n = 117$); □, Korea [□ 20 years, KorSEP-III, 2008 ($n = 4702$); ☆, Japan [20–81 years, 2000–05 ($n = 13,000$); and ◻, Canada [3–79 years, CHMS 2007–09 ($n = 5319$) and 09–11 ($n = 6070$)]

In conclusion, the levels of Cd, Pb, Co, and Cu exposure among the general population of Taiwan varied with age, sex, and lifestyles. Although the overall exposure to Pb and Cu in the NAHSIT 05–08 was lower than that in the NAHSIT 93–96 period, youths are determined to be exposed to higher levels of Pb, Co, and Cu metals. In both periods, the metal exposure profiles in youths were changed for sex. Metal exposure was possibly affected by distinct lifestyles. Related heavy metal exposure sources and the influence of lifestyle in health warrant further investigation. Our findings also indicated that the urinary levels of metals in Taiwan were higher than those in other developed countries. We suggest that the implementation of public health education or intervention strategies of relative risk factors shall be taken for the reduction of metal exposure in the general population of Taiwan.

Acknowledgments We thank Dr. Jein-Wen Chen, Dr. Ya-Tang Liao, Dr. Yu-Te Chung, Dr. Hsiu-Ying Ku, and Mr. Yun-Hsiang Liu from the NHRI in Taiwan for their assistance with data and specimen collection.

Authors' contributions PCH, SLW, SHL, and WHP conceived and designed the experiments. CWS performed the experiments. KWL analyzed the data. PCH and SLW contributed the reagents, materials, and analysis tools. KWL, PCH, and SLW wrote the paper. WHP directed the specimens and questionnaire collection. PCH and SLW contributed to the critical revision of the manuscript.

Funding information This study was funded by the NHRI (EO-99-SP-02, EM-105-PP-05, EH-102-PP-05, EH-103-PP-05, EM-105-SP-16, EM-105-PP-15, EM-106-PP12, EM-107-PP12).

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

Abbreviations *Cd*, Cadmium; *CHMS*, Canadian Health Measures Survey; *Co*, Cobalt; *Cu*, Copper; *GerES*, German Environmental Survey; *GM*, Geometric mean; *GSD*, Geometric standard deviation; *HBM*, Human biomonitoring; *HCA*, Hierarchical clustering analysis; *IACR*, International Agency for Research on Cancer; *ICP-MS*, Inductively coupled plasma-mass spectrometry; *KorSEP*, Korea National Survey for Environmental Pollutants in the Human Body; *LOD*, Limit of detection; *MDL*, Method detection limit; *NAHSIT*, Nutrition and Health Survey in Taiwan; *NHANES*, National Health and Nutrition Examination Survey; *NHRI*, National Health Research Institutes; *NIEA*, National Institute of Environmental Analysis; *Pb*, Lead; *PCA*, Principal component analysis; *SRM*, Standard reference material

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