

Assessment of Heavy Metal Concentrations in Penang, Malaysia's Wastewater Treatment Plants: A Wastewater-Based Epidemiology Approach

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Abstract

Heavy metals and trace metals are among the most prevalent contaminants in sewage. Depending on the concentration and frequency of exposure, certain heavy metals are toxic, carcinogenic, and hazardous to humans. In this study, copper (Cu), nickel (Ni), zinc (Zn), iron (Fe), and cadmium (Cd) levels were measured in the collected wastewater samples from 10 selected domestic sewage treatment plants (STPs) in Penang, Malaysia in December 2021. All metals in the wastewater were determined using the atomic absorption spectrometric (AAS) method followed by the wastewater-based epidemiology (WBE) analysis. The mean concentrations of heavy metals in the wastewater samples were found to be in the following order (unit of mg/L): Fe > Ni > Zn > Cu > Cd (influent), and Fe > Ni > Zn > Cd > Cu (effluent). Among the metals, Fe concentrations were found to be the highest in effluent, with average values at 5.93 ± 5.319 mg/L, slightly over than a maximum permissible Fe concentration. Cadmium level in all STPs was significantly higher when compared to the maximum permissible Cd concentration (0.02 mg/L) allowed by Malaysia's regulation, with an average of 0.13 ± 0.011 mg/L. Other metals concentration such as Cu, Ni and Zn in wastewater at the studied STPs showed low level and were considered safe. Using the WBE approach, the collected data were then calculated to estimate the population's exposure consumption to heavy metals. Mass loading demonstrated high Fe exposure in all STPs but low Cd exposure, with the mean exposure of $1,924.86 \pm 2451.772$ and 46.91 ± 20.936 mg/1000p/d, respectively. Given the World Health Organization's recommendation for tolerable weekly cadmium intake, the estimated human exposure to Cd in this study was particularly concerning, in addition to the lack of efficiency of Cd removal in the studied STPs.

Keywords: Wastewater, Heavy metals, Toxicology, Wastewater based epidemiology

Introduction

Access to clean water is essential for both human well-being and the ecosystem. Nonetheless, during the last decades, water quality has been negatively influenced by a continuously increasing population, rapid industrialisation, urbanisation, and irresponsible utilisation of natural resources [1,2]. Heavy metal contamination in wastewater that originates from anthropogenic activities in numerous sectors, for instance, agricultural activities, industries (mining, battery, nuclear, textile dyes, tannery, etc.), domestic sewage, and others, has always been a threat to human health and environment. The most immediate impact on the environment is the destruction of aquatic habitats, soil degradation that lowers crop yields, and often heavy metals present in contaminated water entering food chains and causing adverse consequences to humans and animals [3].

Heavy metals are not biodegradable, therefore, they tend to bioaccumulate, which is their overtime increase concentration in living organisms [4-6]. Many heavy metals such as arsenic, cadmium, and lead are known for their toxicity or carcinogenic effect on humans. Even at low concentrations, some heavy

metals can induce multiple organ damage affecting the lungs, kidneys, liver, prostate, oesophagus, stomach, skin, and as well as neurodegenerative disorders and diseases, such as Alzheimer's and Parkinson's diseases [7-10]. On the other hand, metals can also affect aquatic organisms (phytoplankton, zooplankton, and fish), accumulating in several organs and causing oxidative damage, endocrine disruption, and depression of the immune system [11].

Heavy metals have been found in water bodies such as rivers [12,13], lakes [14,15], ponds [16-18], and sewage water treatment plants (STP) [19-21]. Among these, heavy metals in STP may indicate the level of metals contributed by human exposure. It could also result in metal accumulation in the river, which returns to humans as the primary water source. Second, the presence of heavy metals in STP could alert whether or not a specific treatment for removing metals is required. Therefore, the failure of the STP to remove heavy metal pollutants from the wastewater will result in the release of effluents into the environment, which can negatively affect human health and causing environmental implications.

In Malaysia, domestic sewage treatment is primarily designed for biological treatment, which may ignore the possibility of heavy metal contamination. Monitoring heavy metals levels at the STP is done on the occasional basis; in other words, it is not adequately regulated. Metals like cadmium (Cd), lead (Pb) and nickel (Ni) were clearly detected primarily in STP sludge, and the presence of metals in STP running water is not uncommon [19-23]. As a result, this study took the initiative to investigate the potential of some heavy metals occurrence in Penang's STP. In addition to monitoring metal levels, this study also had investigated the population level of metal exposure for the first time in Malaysia using the wastewater-based epidemiology (WBE) approach as been proposed by the previous study [24].

Materials and methods

Study site

This study site is located within the Seberang Perai district, a bisection of Penang, a northern state of Malaysia (5.3700° N, 100.4139° E) as shown in **Figure 1**. The selection of sewage treatment plants (STPs) is based on the requirement that each STP serve at least 10,000 residents. Thus, 10 STPs were chosen for sampling points; STP1 (Bandar Baru Bertam Perdana), STP 2 (Tasek Mutiara), STP 3 (Batu Kawan), STP 4 (Kota Permai), STP 5 (Perda), STP 6 (Butterworth North), STP 7 (Butterworth South), STP 8 (Seberang Jaya), STP 9 (Pauh Jaya), and STP10 (Perindustrian Bertam). About 700,000 people from these STPs were included in this study, which equates to 40 % of Penang's population.



Figure 1 Location of studied STPs in Penang, Malaysia. The blue indicator represents 10 STPs from which samples are collected.

Sample collection

Wastewater was collected as influent and effluent samples, with 500 mL of unfiltered water samples were taken and transferred into acid-cleaned polypropylene bottles. All samples were collected between 0900 and 1100 at each STP in the morning, when the study anticipated that morning waste would already be present. Approximately 3 mL of formic acid was added to each polypropylene bottle for pre-treatment purposes. The samples were then filtered through 47 mm glass fibre GF/F filters, nominal cut-off size of 0.7 μm (Whatman, Fontenay Sous Bois, France), and stored in the refrigerator freezer at 4 $^{\circ}\text{C}$ until further analytical use. On the other hand, physicochemical parameters such as Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Ammoniacal Nitrogen (NH_3N), Nitrate Nitrogen (NO_3N), Total Suspended Solids (TSS), pH, Oil and Grease (OG) and temperature were determined in effluent discharge weekly by the STP personnel. The influent and effluent wastewater samples were collected weekly for 3 consecutive weeks in December 2021. All samples were collected at the same sampling point at each STP, noting the weather conditions (dry or rainy) to consider the runoff impact.

Sample preparation and analysis

Cadmium (Cd), copper (Cu), nickel (Ni), iron (Fe), and zinc (Zn) in wastewater were determined by an atomic absorption spectroscopy (AAS) AA800 (Perkin Elmer, Foster City, CA, USA) based on the guideline from the American Public Health Association (APHA) [25]. Briefly, a 100 mL of wastewater mixture with 5 mL of concentrated Nitric acid (HNO_3) was evaporated to 15 to 20 mL on the hot plate. Then, 10 mL of both concentrated HNO_3 and Perchloric acid (HClO_4) were added to the previous mixture (the flask was cooled between additions). The mixture was evaporated gently on a hot plate until dense white fumes appeared. After acid digestion, the mixture was cooled for a few minutes before it was diluted to 50 mL and boiled to expel any chlorine or oxides of nitrogen. A calibration blank and independent calibration and verification standards of Cd, Cu, Ni, Fe, and Zn were analysed together with all samples to confirm the calibration status of the AAS. The calibration curves with $r^2 > 0.999$ were accepted for quantification, and the results were reported as the averages of triple measurements. The limit of quantification (LOQ) for Cd, Cu, Ni, Fe and Zn was 0.01, 0.02, 0.05, 0.1 and 0.5 (mg/L), respectively.

Heavy metal exposure from the population

The daily mass load of each heavy metal per 1000 people at specific STPs was estimated using the WBE approach suggested by previous study [24], and the formula by Du *et al.* [26]. All the calculation related to WBE used Eq. (1). C_i is the influent concentration of the selected heavy metal; F_{In} is the influent flow rate of the specific STPs, and PS is the population served by the STPs. The exposure (mg/1000p/d) of selected heavy metal will be estimated by Eq. (2), where E_{Fi} is the excretion factor of a selected heavy metal excreted as an unchanged parent or metabolite when excreted out from the body, MW_{pi} is the molecular weight of the parent, and MW_{mi} is the molecular weight of the metabolite. In the case of heavy metals, metabolites were unusually rapidly degraded, and our study only measured the total concentration of heavy metals, so MW_{mi} was negligible. E_{Fi} was sought in the literature for each studied metals where the excretion factor of Cu $\sim 72.4\%$ [27], Cd $\sim 84\%$ [28], Ni $\sim 85\%$ [29], Zn $\sim 70\%$ [30] and Fe $\sim 90\%$ [31].

$$Load (mg/1000 p/d) = \frac{C_i (ng/L) \times F_{In} (L/d)}{\frac{PS}{1000}} \times \frac{1}{10^6} \left(\frac{mg}{ng} \right) \quad (1)$$

$$Exposure = Load \times \frac{MW_{pi}}{MW_{mi}} \times \frac{1}{E_{Fi}} \quad (2)$$

Statistical analysis

All tests were carried out in triplicates, and their mean value was considered the final result. Microsoft Excel 2016, Microsoft Corporation, 2016, and GraphPad Prism version 9.1.0 for Windows, GraphPad Software, San Diego, California, USA, were used to tabulate and analyse the data. Data were presented as mean \pm standard deviation (SD), and suitable analysis was done according to the type of data obtained for each parameter.

Results and discussion

Physicochemical properties of the effluent discharge

Table 1 shows the result of the monthly effluent discharge standards parameter testing conducted during the study period. Nine parameters, including BOD, COD, NH_3N , NO_3N , TSS, pH, OG and

temperature, were all found within the permissible range according to Malaysia Sewage and Industrial Effluent Discharge Standards provided by Malaysia's Environmental Law, Environmental Quality Act, 1974, and the Malaysia Environmental Quality (Sewage and Industrial Effluents) Regulations, 1979, 1999, 2000, and 2009. Data on flow rate and population equivalence were also obtained for each STP for further analysis. Monitoring the physicochemical characteristics of wastewater is a fairly common practice in Malaysian STPs, where effluent discharge rather than influent is collected for sample analysis to determine the risk of water discharge from STPs to the environment. Despite the safe physicochemical characteristics, it was reasoned that other contaminants, such as unknown chemicals and heavy metals, could not be ignored because, in Malaysia, they are frequently transferable from STPs to rivers, lakes, and water bodies that serve as water resources for farming, wildlife, and humans.

Table 1 Physicochemical properties of effluent discharge from selected STPs in Penang, Malaysia (mean \pm SD; n = 3).

Parameter	STP1	STP2	STP3	STP4	STP5	STP6	STP7	STP8	STP9	STP10	*Effluent standard
Flow rate (m ³ /day)	11017.7	9778.5	7786.8	26874	8744	14413.9	30139.3	14868	6024.5	6684.6	-
PE	25270	62481	17879	161272	40978	135646	147931	49219	21329	32945	-
BOD (mg/L)	26.2 \pm 5.67	18.0 \pm 3.74	2.4 \pm 0.55	13.8 \pm 4.09	11.6 \pm 4.39	44.4 \pm 9.84	15.2 \pm 2.17	24.4 \pm 4.22	10.4 \pm 4.72	5.0 \pm 1.58	20 - 50
COD (mg/L)	80.0 \pm 19.39	79.2 \pm 20.08	23.2 \pm 3.35	71.2 \pm 21.98	48.8 \pm 6.57	121.6 \pm 18.46	67.2 \pm 3.35	124.8 \pm 35.49	40.0 \pm 9.80	21.6 \pm 3.58	120 - 200
NH ₃ N (mg/L)	14.4 \pm 2.61	14.8 \pm 2.59	8.6 \pm 3.21	17.0 \pm 1.41	16.4 \pm 1.82	17.0 \pm 1.58	15.2 \pm 1.30	11.4 \pm 3.44	17.8 \pm 7.26	6.2 \pm 1.64	20 - 50
NO ₃ N (mg/L)	< 1	< 1	4.4 \pm 1.80	< 1	< 1	-	< 1	-	< 1	< 1	10 - 20
TSS (mg/L)	45.2 \pm 11.80	33.0 \pm 16.14	3.6 \pm 0.55	31.6 \pm 18.08	16.6 \pm 4.04	56.0 \pm 8.72	25.8 \pm 1.79	66.8 \pm 26.21	12.6 \pm 4.62	6.8 \pm 4.21	50 - 100
pH	7.1 \pm 0.29	7.2 \pm 0.19	7.0 \pm 0.19	7.1 \pm 0.15	7.1 \pm 0.13	7.2 \pm 0.122	7.22 \pm 0.22	7.74 \pm 0.21	7.18 \pm 0.16	7.0 \pm .030	5.5 - 9.0
OG	3.2 \pm 2.05	2.6 \pm 0.89	1.8 \pm 0.84	4.4 \pm 3.05	2.6 \pm 1.95	4.0 \pm 2.12	2.4 \pm 1.14	2.0 \pm 1.00	2.2 \pm 1.10	2.4 \pm 1.14	5 - 10
TEMP (°C)	29.6 \pm 2.07	27.2 \pm 1.30	29.8 \pm 0.45	26.0 \pm 0	26.6 \pm 0.89	-	29.0 \pm 1.22	-	28.2 \pm 1.30	29.2 \pm 1.92	40

STP; Sewage Treatment Plant, PE; Population Equivalence, BOD; Biochemical Oxygen Demand, COD; Chemical Oxygen Demand, NH₃N; Ammoniacal Nitrogen, NO₃N; Nitrate Nitrogen, TSS; Total Suspended Solids, OG; Oil and Grease, TEMP; Temperature. *Effluent standard, adopted from Malaysia's Environmental Law for effluent wastewater [32].

Heavy metal concentrations in domestic wastewater

The presence of Cd, Cu, Ni, Fe and Zn in the wastewater samples are shown in **Table 2**. These 5 metals were detected in both influent and effluent from the selected STPs, with varied concentrations. The order of the heavy metal mean concentrations in wastewater samples was Fe > Ni > Zn > Cu > Cd (influent), and Fe > Ni > Zn > Cd > Cu (effluent). Assuming that the studied STPs were from the residential areas, it was believed that these heavy metals found in the wastewater could probably originate from the domestic discharge from the population. On top of that, few studies had opinionated that the presence of heavy metals in the wastewater environment could be caused by a variety of other phenomena such as soil erosion, urban runoff, or aerosol particles [29-31]. The presence of metal residues in effluent is more substantial than in influent because effluent is drained as water resources in rivers or lakes. Among the metals, Fe concentrations were found to be the highest in effluent, with average values at 5.93 \pm 5.319 mg/L, slightly over than a maximum permissible Fe concentration where STP 10 was the only site that recorded Fe effluent concentration above the limit at 9.54 \pm 0.038 mg/L. STP 10 was Perindustrian Bertam which covered not only the residential areas but also housing to universities, boarding schools and industrialization. In comparison to other studies from different countries, the Fe level obtained in this study was the highest, followed by Pakistan, Thailand, and the rest of the countries, as shown in **Table 3**. Many factors might contribute to the situation, and this study would like to propose that there might be some contamination from the other sources such as eroding roofing sheets, the usage of steel and iron cookware, and other associated home products [36]. Given the population at approximately 700,000 people from the studied STPs, this would also contribute to the higher concentrations of Fe than other studies. It is assumed that high Fe concentrations in wastewater may be contributed by high Fe exposure or consumption in the population. Ingestion of high iron concentration can result in serious complications, such as direct caustic injury to the gastrointestinal mucosa, which causes nausea, vomiting, abdominal pain, and diarrhoea [33,34]. Thus, a necessary remediation is suggested to particularly, STP 10 in regard to Fe concentration.

The Cd level in all STPs was significantly higher when compared to the maximum permissible Cd concentration (0.02 mg/L) allowed by Malaysia's Environmental Law for effluent wastewater standards, with an average of 0.13 \pm 0.011 mg/L. Seven STPs had recorded the highest Cd concentration in effluent samples, STP 1 (0.14 \pm 0.003 mg/L), STP 2 (0.14 \pm 0.006 mg/L), STP 3 (0.14 \pm 0.004 mg/L), STP 4 (0.14

± 0.005 mg/L), STP 5 (0.14 ± 0.005 mg/L), STP 9 (0.14 ± 0.004 mg/L), and STP 10 (0.14 ± 0.003 mg/L). Cd level obtained in this study were the second highest after Vietnam when compared to other studies (Table 3). The data obtained from this study is very concerning since the effluent containing Cd will be released into the environment and might disturb the food chain and living ecosystem. For example, long-term use of sludge or the effluent from these STPs on farmland or irrigation with cadmium-contaminated wastewater can result in elevated cadmium levels in crops cultivated on such land, impairing the quality of agricultural output and providing a health risk to the end-users [32,35]. Ingesting very high levels of Cd will severely irritate the stomach, leading to vomiting and diarrhoea, and long-term exposure to some levels of Cd can lead to a build-up in the kidneys and possible kidney disease, lung damage, and fragile bones [36,37]. It is suggested to avoid, using effluent discharge for irrigation watering or animal farmlands [42]. It is learnt that high concentration of Cd in wastewater discharged into the environment is toxic and bioavailable to humans, plants, and animals [7]. Besides, other metals concentration such as Cu, Ni and Zn in wastewater at the studied STPs showed low level and were considered safe.

Table 2 Concentration of heavy metals in influent and effluent discharge from selected STPs in Penang, Malaysia (mean \pm SD; n = 3).

Elements/ STPs	Cu (mg/L)		Cd (mg/L)		Ni (mg/L)		Zn (mg/L)		Fe (mg/L)	
	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
STP1	0.71 \pm 0.014	0.06 \pm 0.002	0.15 \pm 0.002	0.14 \pm 0.003	0.84 \pm 0.060	0.859 \pm 0.0670	0.28 \pm 0.003	0.25 \pm 0.002	4.00 \pm 0.022	3.65 \pm 0.010
STP2	0.16 \pm 0.005	0.03 \pm 0.002	0.15 \pm 0.005	0.14 \pm 0.006	1.00 \pm 0.052	0.795 \pm 0.0965	0.30 \pm 0.003	0.26 \pm 0.002	4.68 \pm 0.037	4.29 \pm 0.030
STP3	0.12 \pm 0.004	0.05 \pm 0.002	0.15 \pm 0.002	0.14 \pm 0.004	0.93 \pm 0.093	0.911 \pm 0.0691	0.25 \pm 0.006	0.22 \pm 0.018	17.93 \pm 0.162	2.69 \pm 0.028
STP4	0.22 \pm 0.012	0.06 \pm 0.001	0.15 \pm 0.004	0.14 \pm 0.005	0.92 \pm 0.048	0.834 \pm 0.0800	0.28 \pm 0.002	0.26 \pm 0.002	4.27 \pm 0.032	3.57 \pm 0.011
STP5	0.18 \pm 0.017	0.07 \pm 0.002	0.15 \pm 0.005	0.14 \pm 0.005	0.76 \pm 0.115	0.759 \pm 0.0709	0.27 \pm 0.003	0.23 \pm 0.005	2.22 \pm 0.043	1.59 \pm 0.021
STP6	0.13 \pm 0.006	0.12 \pm 0.003	0.19 \pm 0.005	0.12 \pm 0.004	1.03 \pm 0.106	0.997 \pm 0.1044	0.28 \pm 0.002	0.17 \pm 0.005	2.03 \pm 0.101	1.53 \pm 0.012
STP7	0.17 \pm 0.008	0.11 \pm 0.002	0.15 \pm 0.003	0.13 \pm 0.002	0.96 \pm 0.105	0.989 \pm 0.0970	0.39 \pm 0.002	0.34 \pm 0.021	3.16 \pm 0.086	1.90 \pm 0.030
STP8	0.14 \pm 0.009	0.11 \pm 0.003	0.20 \pm 0.005	0.11 \pm 0.003	1.05 \pm 0.081	0.671 \pm 0.0351	0.39 \pm 0.006	0.26 \pm 0.003	5.38 \pm 0.062	4.04 \pm 0.010
STP9	0.43 \pm 0.004	0.32 \pm 0.007	0.15 \pm 0.005	0.14 \pm 0.004	0.95 \pm 0.100	0.839 \pm 0.3559	0.33 \pm 0.003	0.20 \pm 0.003	2.42 \pm 0.121	1.48 \pm 0.006
STP10	0.19 \pm 0.006	0.06 \pm 0.002	0.15 \pm 0.003	0.14 \pm 0.003	0.83 \pm 0.081	0.863 \pm 0.0775	0.36 \pm 0.011	0.26 \pm 0.006	13.23 \pm 0.102	9.54 \pm 0.038
Mean \pm SD	0.25 \pm 0.186	0.10 \pm 0.083	0.16 \pm 0.019	0.13 \pm 0.011	0.93 \pm 0.093	0.85 \pm 0.099	0.31 \pm 0.051	0.25 \pm 0.045	5.93 \pm 5.319	3.43 \pm 2.408

STP; Sewage Treatment Plant, Permissible limit - Cu (0.2 - 1 mg/L), Cd (0.01 - 0.02 mg/L), Ni (0.2 - 1 mg/L), Zn (1.0 mg/L) and Fe (1.0 - 5.0 mg/L) (adopted from Malaysia's Environmental Law for effluent wastewater [32])

Table 3 Comparison of heavy metal levels in STP effluent discharge from multiple countries.

Country/Elements	Cu (mg/L)	Cd (mg/L)	Ni (mg/L)	Zn (mg/L)	Fe (mg/L)	References
China ^b	0.010 \pm 0.017	0.0001 \pm 0.0001	0.043 \pm 0.105	0.072 \pm 0.213	-	[19]
Egypt ^t	0.035 - 0.042	0.017 - 0.026	0.021 - 0.027	0.053 - 0.060	-	[22]
Indonesia ^a	0.020 - 0.472	ND - 0.020	0.005 - 0.083	-	-	[21]
Iraq ^a	0.031 - 0.151	ND - 0.119	0.019 - 0.334	0.382 - 1.460	ND - 0.092	[23]
Morocco ^b	0.102 \pm 0.047	0.076 \pm 0.006	-	1.590 \pm 0.195	-	[20]
Pakistan ^a	0.050 - 1.180	0.020 - 0.030	0.030 - 0.080	0.760 - 1.220	0.780 - 4.620	[43]
South Africa ^a	ND - 0.050	ND - 0.130	-	-	ND - 0.636	[44]
Taiwan ^a	ND - 0.721	ND - 0.001	ND - 0.295	ND - 0.255	-	[45]
Thailand ^a	ND - 0.104	ND - 0.003	ND - 0.179	0.050 - 0.140	0.700 - 0.950	[46]
Vietnam ^a	1.963 - 3.859	0.203 - 0.406	-	1.989 - 3.891	-	[47]
Penang, Malaysia ^a	0.031 - 0.322	0.109 - 0.143	0.671 - 0.997	0.168 - 0.341	1.529 - 9.543	This study

STP; Sewage Treatment Plant, ND; Not detectable, ^a, mean \pm sd, ^b, range value

Correlation between heavy metals concentrations and wastewater properties

Figure 2 from this study also noticed a significant negative correlation between Fe and one of the studied water parameters, NH₃N ($r = 0.750, p \leq 0.05$). **Figure 2** also shows that there was a significant negative correlation between the amount of Cd and the pH value in the effluent sample ($r = 0.841, p \leq 0.01$), which was aligned with the previous study by Francisca and Glatstein [48], which states the addition of Cd as a metallic solution will reduce the water’s pH value.

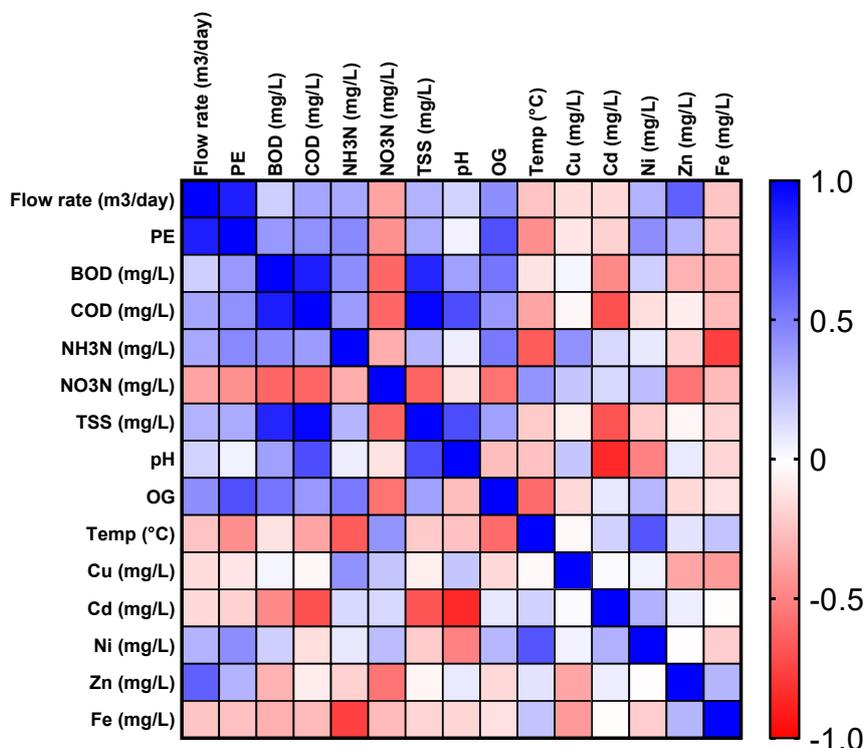


Figure 2 Pearson’s correlation coefficients (r) for the physicochemical parameters and heavy metal levels from selected STPs effluent in Penang, Malaysia. Correlations are defined as weak ($0 < |r| < 0.3$), moderate ($0.3 < |r| < 0.7$) or strong ($|r| > 0.7$). STP; Sewage Treatment Plant, PE; Population Equivalence, BOD; Biochemical Oxygen Demand, COD; Chemical Oxygen Demand, NH₃N; Ammoniacal Nitrogen, NO₃N; Nitrate Nitrogen, TSS; Total Suspended Solids, OG; Oil and Grease, TEMP; Temperature, Cu; Copper, Cd; Cadmium, Ni; Nickel, Zn; Zinc, Fe; Iron

Removal efficiency of heavy metals

Figure 3 shows the efficiency of each STPs in removing Cd, Cu, Ni, Fe and Zn. It is quite interesting that, according to our current understanding, all STPs in Penang, Malaysia did not perform any specific or special wastewater treatment modalities (only biological), yet the heavy metals gained the reduction in the effluent samples. In addition, the percentage of heavy metal removal after the standard treatment differed between all STPs. The removal efficiency of the STPs followed the order: Cu > Fe > Zn > Cd > Ni. Copper and Fe removal were significantly higher, with the maximum removal efficiencies of $81.68 \% \pm 0.050$ and $84.37 \% \pm 0.112$, respectively, indicating that all of the STPs had a high capacity for eliminating Cu and Fe elements from the influent wastewater. According to a recent study, the presence of biological adsorption in aeration tanks and secondary sedimentation tanks in wastewater treatment plants may aid in the removal of Fe and Cu, and it has also shown a significant influence of biosorption and bioaccumulation on the elimination of Cu and Fe [49]. In contrast, elimination of nickel was very poor in most of the STPs, with the amount entering and exiting the plant being unaltered even after treatment. Only 3 STPs recorded a significant low nickel removal, which were STP 8 ($30.8 \% \pm 0.395$), STP 2 ($20.63 \% \pm 0.034$), and STP 9 ($12.23 \% \pm 0.088$). Both Cd and Zn were also had less than 50 % removal in the effluent wastewater, suggesting implementation of a new method such as nano-adsorbents, photocatalysts, electrocatalysts and nano-membrane, which were proven to significantly remove the heavy metals from wastewater [46,47].

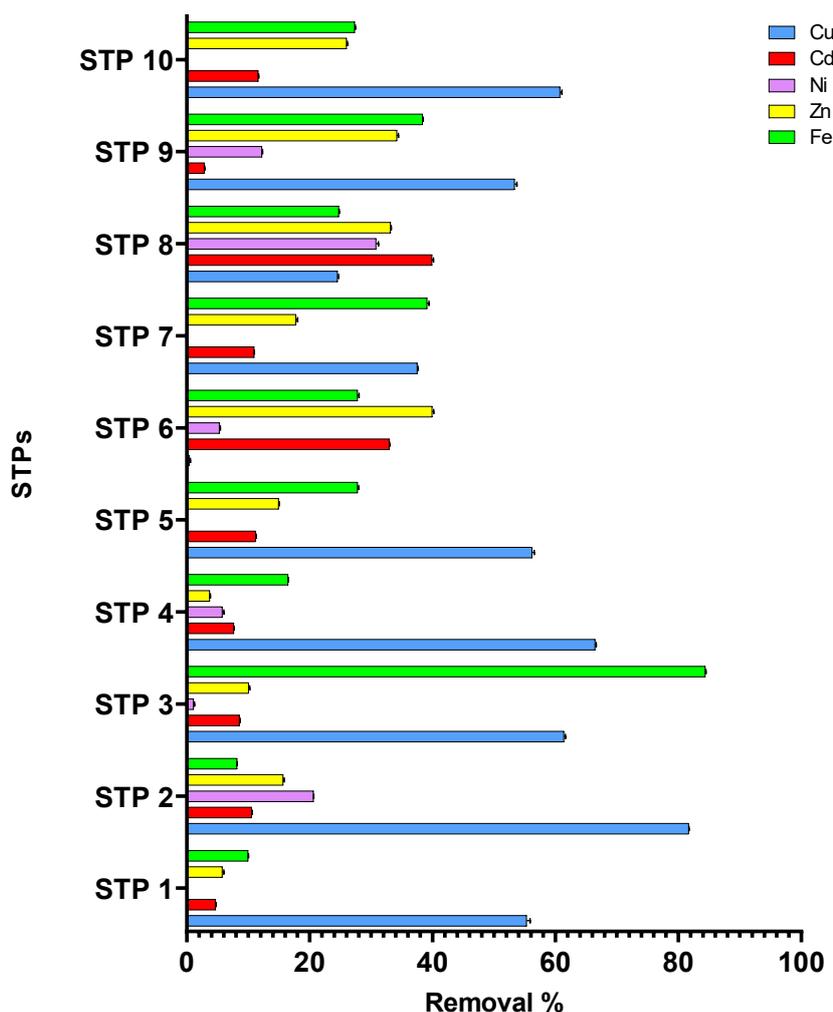


Figure 3 The removal efficiency of heavy metals in the selected STPs in Penang, Malaysia (n = 3).

Estimated exposure rate of heavy metals to Penang's population

Cd, Cu, Ni, Fe and Zn daily exposure rate were expressed as mg/1000p/d, as reported in **Table 4**. The daily exposure rate of Fe was estimated using the mass loads of the total Fe in the influent wastewater. When estimating exposure rates to heavy metals from household waste, it is necessary to use influent samples. The daily exposure rate of Fe was recorded as the highest exposure rate among other studied heavy metals (1924.7 ± 2451.772 mg/1000p/d), with STP 3 recorded the highest daily exposure rate of Fe at ($7960.48 - 10065.53$ mg/1000p/d). This is to be expected given that Fe is the second most abundant metal in the earth's crust [52] and one of the essential metals required by the body. The daily exposure rate of Ni, Zn, and Cu were also estimated using the mass loads of total Ni, total Zn, and total Cu in the wastewater, with Ni having the second highest daily exposure rate at 270.70 ± 122.752 mg/1000p/d during this study period, followed by Zn at 110.17 ± 50.678 mg/1000p/d and Cu at 98.57 ± 192.953 mg/1000p/d. The daily exposure rate of Cd, estimated from the mass loads of total Cd, was found to be the lowest when compared to the other heavy metals (46.91 ± 20.936 mg/1000p/d). Despite the fact that the daily exposure rate to Cd was the lowest, and assuming that all of this exposure is originated from human consumption, the value found in this study was quite concerning because it exceeded the WHO tolerable weekly intake limit for cadmium, which is $7 \mu\text{g/kg/body weight/week}$ for a person [27]. One study also had suggested that the safe daily levels of Cd intake should be kept below $30 \mu\text{g}$ per person because in their view on previous guideline for a maximum recommended Cd intake ($1 \mu\text{g/kg body weight/d}$) was too high to ensure that renal dysfunction does not occur as a result of dietary Cd intake [53]. Overall, this is the first study in Malaysia to use a wastewater-based approach (WBE) to assess population exposure to heavy metals, and there are no comparable data in the literature.

Table 4 Weekly estimated exposure rate for heavy metal in wastewater samples from STPs in Penang, Malaysia.

STPs	Week	Estimated exposure rate (mg/1000p/d)				
		Cu ^a	Cd ^b	Ni ^c	Zn ^d	Fe ^e
STP1	1	1,044.23	75.78	390.86	194.33	1,290.56
	2	40.95	76.82	510.89	148.24	2,480.35
	3	193.91	82.01	393.94	170.66	2,047.26
STP2	1	22.48	27.20	204.74	62.60	816.95
	2	31.13	28.69	172.34	58.13	842.68
	3	52.74	30.18	175.65	83.17	781.47
STP3	1	60.16	80.88	508.29	149.32	10,065.53
	2	87.83	77.77	444.75	179.19	7,999.19
	3	73.39	75.70	470.37	130.66	7,960.48
STP4	1	72.73	28.96	225.45	99.03	744.32
	2	52.02	29.76	145.46	59.04	702.10
	3	28.08	29.76	171.73	41.90	927.62
STP5	1	39.49	35.56	159.16	81.70	527.77
	2	39.49	43.69	173.72	67.06	463.75
	3	78.99	37.60	240.49	93.28	588.94
STP6	1	13.21	22.77	160.02	47.67	291.39
	2	17.91	28.59	103.26	36.43	195.52
	3	23.78	18.98	124.51	40.99	230.70
STP7	1	56.28	39.78	209.97	151.35	760.17
	2	48.96	35.41	251.20	94.30	656.04
	3	38.27	34.44	225.31	96.05	729.84
STP8	1	43.39	64.01	264.41	164.85	1,693.65
	2	77.61	90.62	371.73	218.36	1,905.11
	3	58.41	56.10	482.61	126.01	1,819.18
STP9	1	46.04	48.42	359.55	170.28	708.02
	2	46.04	46.40	265.18	69.40	866.20
	3	410.42	51.11	319.67	162.21	706.77
STP10	1	87.44	38.65	219.13	92.76	2,763.52
	2	30.27	38.65	178.08	107.83	2,937.12
	3	41.48	32.85	198.61	108.41	3,243.72
Min		13.21	18.98	103.26	36.43	195.52
Max		1,044.23	90.62	510.89	218.36	10,065.53
Average		98.57 ± 192.953	46.91 ± 20.936	270.70 ± 122.752	110.17 ± 50.678	1,924.86 ± 2451.772

STP; Sewage Treatment Plant

^aThe excretion factor of Cu was assumed as 72.4 %.^bThe excretion factor of Cd was assumed as 84 %.^cThe excretion factor of Ni was assumed as 85 %.^dThe excretion factor of Zn was assumed as 70 %.^eThe excretion factor of Fe was assumed as 90 %.**Study limitation**

The estimation of heavy metal exposure may provide a more tangible interpretation of WBE data, but the study may also include inaccuracy from all possible errors. The error is often caused by deviations in sample collection and preparation, analyte loss, population variation, and contamination of the analyte from other sources. Several approaches must be taken to address the current limitation of WBE estimation. For instance, the stability and metabolism rate of the biomarker in humans and sewage are the most pivotal key

point in estimating the heavy metal exposure in the population. This study also proposes that the next study to compute the corrective factor for the biomarker to decrease the probable inaccuracy, like the contamination from the external sources or biomarker deposited into the sludge. Our study was restricted to a single Malaysian state for a brief period of time. Consequently, these data cannot represent a long-term exposure, and estimation may be skewed, and the tropical environment may restrict the seasonal impact of human heavy metals exposure. Future research should include other states and prolong the investigation periods for a more comprehensive exposure evaluation of the study area.

Conclusions

In conclusion, this is the most comprehensive assessment of human exposure to heavy metals by the WBE method in Malaysia, which includes about 40 % of Penang's population. This research may aid in enhancing the comprehension and representativeness of future study results and ensuring more precise estimates of population-wide exposure to heavy metals. The mass load and exposure rate of all heavy metals was identified in the influent wastewater from the STPs. The findings of this study also suggested that the heavy metal exposure patterns may differ from the other countries population where this WBE approach may be used. Further research with large samples from many regions over a long period is needed to get more representative heavy metal exposure values for the human population.

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