- **Altered composition of the Indonesian gut microbiome and heavy metal resistance genes abundance in response to heavy metal exposure**

by Fitria Nungky Harjanti, Maulida Aisyah Khairunnisa, Ruri Agung Wahyuono, Arif Luqman, Anjar Tri Wibowo

Abstract: Exposure to heavy metals poses a significant risk to human health due to their accumulation through inhalation, ingestion, and contact with contaminants. Such exposure can profoundly alter the gut microbiome, potentially causing gastrointestinal disorders and heightened infection vulnerability. Indonesia, like other nations, grapples with heavy metal-related health issues. Nonetheless, the extent of heavy metal contamination in the Indonesian population and its impact on their gut microbiome remain unexplored. Our study aimed to evaluate heavy metal prevalence in stools from coastal and highland Indonesian populations and discern its potential effect on gut microbiome. We detected copper (Cu), barium (Ba), manganese (Mn), and zinc (Zn) in fecal samples, with notable concern regarding barium contamination (13 of 20 participants), known for its adverse health impacts. Genes linked to heavy metal resistance were widely distributed in both populations' stool samples, including znuC, nikE, modC, mntH, and arsB. Copper levels correlated negatively with Prevotella abundance, suggesting inhibition of its growth, while barium levels correlated positively with Prevotella, Faecalibacterium, and Ruminococcus abundance, indicating an antagonistic Ba-Cu relationship in shaping the microbiome.
Keywords: *heavy metals; barium; metal resistance genes; gut microbiome.*

- **Peak carbon emission prediction of tourist attractions based on fuzzy support vector machine**

by Xiumei Feng

Abstract: In order to address the issues of low stability, low sensitivity, and low accuracy in traditional peak carbon emission prediction methods, a peak carbon emission prediction of tourist attractions based on fuzzy support vector machine is proposed. Tourist attraction carbon emission

Altered composition of the Indonesian gut microbiome and heavy metal resistance genes abundance in response to heavy metal exposure

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Abstract: Exposure to heavy metals poses significant health risks through inhalation, ingestion, and contact with contaminants, potentially causing gastrointestinal disorders and heightened infection vulnerability by altering the gut microbiome. In Indonesia, heavy metal contamination and its impact on the gut microbiome remain underexplored. This study assessed heavy metal prevalence in stool samples from coastal and highland populations and its potential microbiome effects. Copper (Cu), barium (Ba), manganese (Mn), and zinc (Zn) were detected, with barium contamination particularly concerning (13 of 20 participants). Genes for heavy metal resistance, such as znuC, nikE, modC, mntH, and arsB, were common in both populations. Copper levels negatively correlated with Prevotella abundance, while barium levels positively correlated with Prevotella, Faecalibacterium, and Ruminococcus abundance, indicating an antagonistic Ba-Cu relationship in shaping the microbiome. This study highlights the health risks of heavy metal exposure in Indonesia and its potential impact on gut microbiome composition.

Keywords: heavy metals exposure; copper; barium; health risks; gut microbiome.

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1 Introduction

Heavy metals are elements with high atomic weights that can be toxic to humans, even in small quantities (Hordyjewska et al., 2014). They can accumulate in the human body typically through inhalation, ingestion, or skin contact with contaminated substances or environments (Wu et al., 2019). Certain foods, especially those grown in contaminated soil or exposed to contaminated air or water sources, may contain higher levels of heavy metals (Sandeep et al., 2019; Turkyilmaz et al., 2020). Seafood can also be a source of heavy metal exposure (Ziyuan et al., 2022). When heavy metals are ingested, they can be absorbed into the bloodstream through the digestive tract, leading to potential health risks (Sarpong-Kumankomah et al., 2018).

Some heavy metals can cause direct damage to the gastrointestinal tract, leading to symptoms such as abdominal pain and diarrhoea (Akpor, 2014). Heavy metals absorbed from the digestive system can also have systemic effects on the body beyond the gastrointestinal tract. They can accumulate in the liver and kidneys, which can impact their function over time (Sumino et al., 1975). They can affect the nervous system, causing cognitive impairments and behavioural changes (Sankhla et al., 2017; Sumino et al., 1975). Heavy metals exposure also increases the risk of various cancers (Lim et al., 2019; Sá et al., 2016), affects the cardiovascular system (Sevim et al., 2020), and increases the risk of diabetes (Javaid et al., 2021).

Recent work shows that heavy metals can have significant effects on the human gut microbiome. Heavy metal exposure can lead to alterations in the composition and diversity of the gut microbiota, causing shifts in the abundance and diversity of certain microbial species in the gut (Dong et al., 2017; Zhang et al., 2021, 2023). They also can also alter the expression of genes involved in various metabolic pathways, including those related to nutrient metabolism, energy production, and detoxification (Fatema et al., 2021; Gao et al., 2017). Further altered microbial composition and function due to heavy metal exposure may contribute to gastrointestinal disorders and increased susceptibility to infections (Brabec et al., 2020; Zhang et al., 2023).

Indonesia, the fourth-most populous country, also faces challenges related to heavy metal exposure due to various factors, including industrial activities and environmental pollution (Ahmad et al., 2022; Yap and Al-Mutairi, 2021). The use of certain fertilisers and pesticides containing heavy metals resulted in the accumulation of these metals in

agricultural soils (Budianta et al., 2023). Additionally, the application of sewage sludge or improperly treated wastewater for irrigation purposes introduced heavy metals into the food chain (Sudarningsih et al., 2017). Heavy metals contamination in water sources due to mining activities, industrial discharges, and improper waste disposal practices also reported (Gafur et al., 2018; Juliani, 2021; Palapa and Maramis, 2015). There are considerable reports regarding heavy metals contamination in drinking water (Astuti et al., 2021), agricultural products (Wahyuningsih et al., 2023), fresh water fish (Nasir et al., 2021) and seafood (Puspitasari et al., 2023) in Indonesia.

Despite the high risk of heavy metal exposure through water and food among Indonesians, there is limited data on the contamination rates of heavy metals in the human body within the country. While contamination of heavy metals in urine and blood samples has been reported among high-risk populations such as mining (Ekawanti and Krisnayanti, 2015) and industrial workers (Damastuti et al., 2017), there is a notable absence of data regarding heavy metal contamination in the digestive tract and in the general population. Further, the effect of heavy metal exposure to Indonesian gut microbiome is never evaluated before. This study focuses on reporting heavy metal contamination in faecal samples collected from both the coastal and highland populations of Indonesia, as well as investigating their potential effects on gut microbial composition.

2   Material and method

2.1   Study participants, stool sample collection and DNA extraction

For this study, we utilised stool samples that had been collected in previous studies (Luqman et al., 2021; Wibowo et al., 2021). These samples originated from two distinct study populations: the coastal population of Kenjeran, Surabaya, Indonesia (consisting of 7 males and 2 females), and the highland population of Pacet, Mojokerto, Indonesia (comprising 5 males and 6 females). The selection of participants was based on specific criteria, including being in a healthy condition, aged between 20 and 50 years old. The DNA extraction from the collected stool samples was conducted using the Zymbiomic DNA Miniprep Kit (Zymo Research), following the provided protocol from the company.

2.2   Normalised read counts

The analysis of gene read counts was performed using previously published methods (Luqman et al., 2021; Nugrahapraja et al., 2022). The metagenomic data used in this study was sourced from the DNA Data Bank of Japan (DDJP) with the submission number SSUB023028 (Nugrahapraja et al., 2022). The sequences corresponding to the target or reference genes (provided in Table S1) were downloaded from the NCBI database. To compare the data between samples and genes, all reads from the samples were aligned against these sequences using BWA (Li et al., 2019), and the resulting samfiles were processed with SAM tools to extract the number of mapped reads (Li et al., 2009). The read counts were subsequently normalised by taking into account the number

of sequenced reads and the length of the respective genes, ensuring comparability across samples and genes.

2.3   Detection and measurement of heavy metals from faecal samples

As much as 10 g of faecal samples was analysed using X-Ray Fluorescence NitonTM XL2 GOLDD (Thermo Scientific, Carlsbad, CA, USA) to calculate heavy metals content in the samples. The heavy metals measurements were presented as percentages of the weight of the faeces.

2.4   Data analysis

Comparison of heavy metals concentration in stool samples collected from coastal and highland populations of Indonesia were analysed using t-test. In addition, to determine possible connection between heavy metal concentration and heavy metal related-genes abundance, and also connection between heavy metal concentration and bacterial genus abundance in both population (highland population and coastal population) were analysed using Spearman's correlation.

3   Results

### 3.1   Detection of heavy metals in coastal and highland faecal samples

Stool samples were collected from a total of 20 participants, with 9 individuals (7 males and 2 females) representing a fisherman community residing in the coastal region of Surabaya, Indonesia, and the remaining 11 participants (5 males and 6 females) belonging to a farmer community residing in the highland region of Pacet, Indonesia. Heavy metal analysis revealed the presence of four types of heavy metals, namely copper (Cu), barium (Ba), manganese (Mn), and zinc (Zn) in the study population. Cu, Mn, and Zn were detected in all samples, while Ba was found in 13 participants (Table S2). The average concentrations of Cu, Ba, Mn, and Zn in the coastal samples were 0.534 µg/g, 0.037 µg/g, 0.454 µg/g, and 1.01 µg/g respectively. In the highland samples, the average concentrations of Cu, Ba, Mn, and Zn were 0.282 µg/g, 0.082 µg/g, 0.240 µg/g, and 0.509 µg/g respectively (Figure 1). These findings indicate that heavy metal was observed in the digestive tracts of all study participants, with higher levels of Cu, Mn, and Zn detected in the coastal population, while Ba contamination was more pronounced in the highland population.

3.2   Heavy metals related-genes are widely spread in Indonesians gut microbiome

We conducted an analysis of the relative gene abundance using previously collected stool samples from coastal and highland populations in Indonesia. The samples were mapped against a reference set of heavy metal-related genes (Table S1) known to be involved in the response and metabolism of heavy metals in living organisms. These genes play a crucial role in the uptake, transport, detoxification, and excretion of heavy metals from cells and tissues (Pal et al., 2014). They are responsible for regulating various cellular

processes and pathways that enable organisms to cope with heavy metal exposure (Xavier et al., 2019).

Figure 1   Concentration of heavy metals in stool samples collected from coastal and highland populations of Indonesia
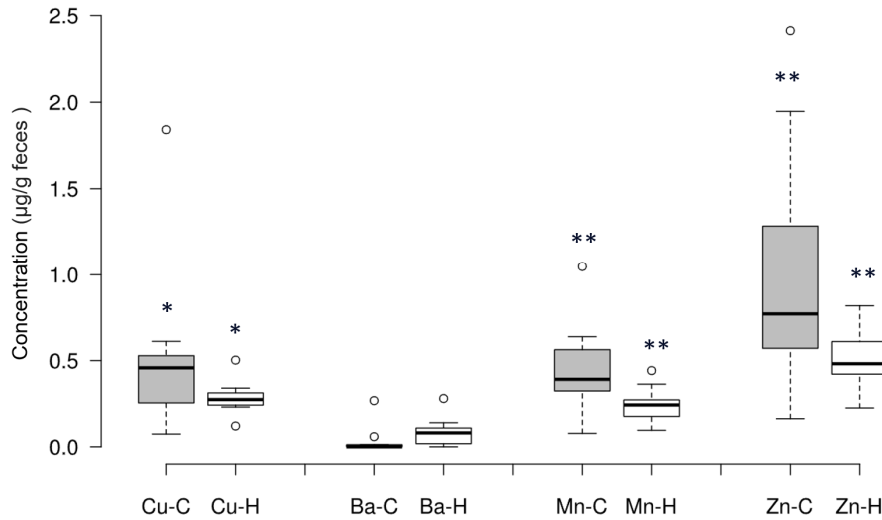
Abbreviations of samples: C – Coastal, H – Highland, *$P \leq 0.1$, **$P \leq 0.05$ on t-test.

Figure 2   The prevalence and abundance of heavy metal related genes in Indonesian gut microbiota (A) the heatmap of the relative reads of the heavy metal related genes with RpoB (housekeeping gene) as a comparison (see online version for colours)
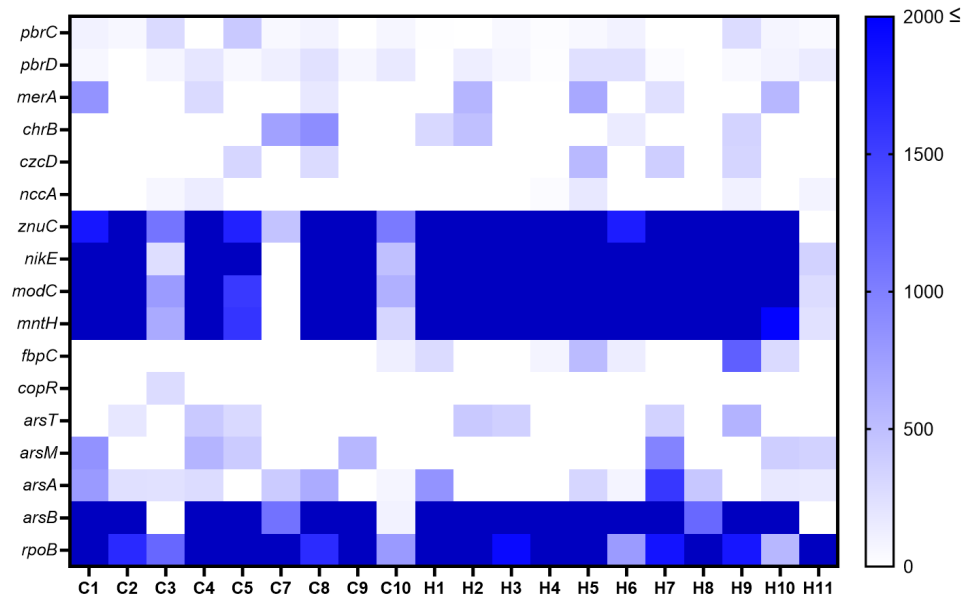
Abbreviations of samples: C – Coastal, H – Highland.

Our analysis revealed that the heavy metal-related genes were widespread in the stool samples from both populations (Figure 2). Certain genes, such as znuC, nikE, modC, and mntH that regulate zinc, nickel, molybdenum, and manganese transport, respectively, were prevalent and detected in 19 out of 20 stool samples, while arsB that regulate arsenic resistance was detected in 18 out of 20 samples (Table S3). These genes also exhibited high relative abundance in the stool samples, indicating their strong and widespread distribution in the human gut. On the other hand, the gene copR, which is involved in the regulation of copper resistance mechanisms, was only detected in one sample and showed relatively low abundance (Figure 2 and Table S3).

3.3 The effect of heavy metal contamination on heavy metal related-genes abundance

The expression and activity of heavy metals related-genes can be influenced by exposure to heavy metals (Das et al., 2016). Understanding their functions and regulation is important for studying the mechanisms of heavy metal toxicity, as well as for developing strategies to mitigate the adverse effects of heavy metal exposure in biological systems. Therefore, we performed spearman correlation analysis to determine correlations between heavy metal contamination rate with heavy metal related-genes abundance.

Table 1   Correlation between heavy metal concentration and heavy metal related-genes abundance

Heavy metal related genes	Significantly correlated metals	Correlation coefficient	P-value
modC	Ba	0.562	0.009913
znuC	Ba	0.552	0.01161
mntH	Cu	–0.4719	0.03926
	Ba	0.5527	0.0115
nikE	Cu	–0.4647	0.04231
	Ba	0.4822	0.03132
merA	Cu	–0.4552	0.04374
	Ba	0.4737	0.03487

Our findings revealed a notable positive correlation between barium (Ba) levels and the abundance of specific heavy metal-related genes (Table 1). Notably, genes such as modC, znuC, mntH, nikE, and merA displayed a significant positive correlation with barium levels observed in the stool samples. These results suggest that elevated concentrations of Ba may impact the abundance of these genes, which are involved in the response and metabolism of heavy metals. Conversely, copper (Cu) levels appeared to have a negative influence on the abundance of mntH, nikE, and merA genes (Table 1). This indicates that higher concentrations of Cu may contribute to reduced abundance of these particular heavy metal-related genes. Overall, our study demonstrates that heavy metal exposure, particularly in relation to Ba and Cu can significantly affect the abundance of heavy metal-related genes within the human gut. These findings shed light on the

potential impact of heavy metal exposure on gene regulation and highlight the complex relationship between heavy metals and gene abundance in the context of gut health.

3.4   The effect of heavy metal contamination on gut microbial composition

Previous research has reported substantial impact of heavy metal contamination on the composition of the human gut microbiome. Heavy metal exposure can lead to a decrease in microbial diversity, causing shifts in the relative abundance of different bacterial taxa. Some studies have shown that heavy metals can selectively promote the growth of certain metal-resistant bacteria while suppressing the growth of other beneficial microbial species (Brabec et al., 2020; Zhang et al., 2023). To investigate the influence of heavy metal exposure on the gut microbiome of the Indonesian population, we conducted Spearman correlation analysis to assess the relationship between heavy metal levels and the relative abundance of microbial species in two distinct study populations.

In the highland population, we observed a robust negative correlation between Cuprum (Cu) levels and the abundance of Prevotella (Table 2), indicating that Cu has a detrimental effect on the growth and propagation of Prevotella. Furthermore, we found a strong negative correlation between Barium (Ba) and Escherichia, suggesting that Ba may limit the growth of Escherichia in the gut. Interestingly, we observed a positive correlation between Ba levels and the abundance of Faecalibacterium and Ruminococcus, implying that these bacterial genera can thrive in an environment with elevated Ba levels. Similarly, we noted a positive correlation between manganese (Mn) levels and the abundance of Ruminococcus and Clostridium. This suggests that these two bacterial genera are susceptible to higher Mn levels and may exhibit increased abundance in the presence of Mn.

Table 2   Correlation between heavy metal concentration and bacterial genus abundance in highland population

Bacterial genus	Significantly correlated metals	Correlation coefficient	P-value
Prevotella	Ba	0.722	0.02806

In the coastal population, we observed a significant positive correlation between Barium (Ba) levels and the abundance of Prevotella (Table 3), suggesting an antagonistic relationship between Ba and Cu in influencing Prevotella abundance. Specifically, Ba appeared to promote the growth of Prevotella, while Cu seemed to have a limiting effect on its abundance. Overall, these findings shed light on the complex interactions between heavy metals and specific bacterial genera in the gut microbiome. The observed correlations provide insights into how heavy metal exposure can influence the growth and distribution of particular microbial species, thereby impacting the overall composition and functioning of the human gut microbiome.

Table 3 Correlation between heavy metal concentration and bacterial genus abundance in coastal population

Bacterial genus	Significantly correlated metals	Correlation coefficient	P value
Prevotella	Cu	–0.7973	0.003292
Faecalibacterium	Mn	0.6821	0.02077
	Ba	0.6929	0.01809
Ruminococcus	Ba	0.8904	0.0002383
	Mn	0.6228	0.04068
Escherichia	Ba	–0.7715	0.005417
Clostridium	Mn	0.6844	0.02018

4 Discussion

Indonesia, like many other countries, faces health problems related to heavy metal pollution (Ahmad et al., 2022; Yap and Al-Mutairi, 2021), which arises from various sources such as industrial activities (Palapa et al., 2015; Juliani et al., 2021), improper waste disposal (Sudarningsih et al., 2017), and agricultural practices (Budianta et al., 2023). These contaminants can enter the environment, contaminate water sources (Astuti et al., 2021), soil (Budianta et al., 2023), and food (Puspitasari et al., 2023; Wahyuningsih et al., 2023), and eventually affect human health. Despite the evident hazards, there is currently a lack of data regarding heavy metal contamination in faecal samples from the general population in Indonesia and. To address this knowledge gap, our study focuses on examining the prevalence of heavy metal exposure among Indonesia's coastal and highland populations, while also evaluating their impact on the composition of the gut microbiome.

In our analysis, we did not detect the presence of highly toxic heavy metals such as lead, mercury, cadmium, arsenic, and chromium in the samples, indicating that contamination with these highly toxic metals is rare among the general population. However, we did identify the presence of other heavy metals including copper (Cu), barium (Ba), manganese (Mn), and zinc (Zn) in the samples. Copper, manganese, and zinc is an essential nutrient for living organisms, playing a vital role in enzyme function and metabolism of proteins and carbohydrates (Pajarillo et al., 2021). While copper, manganese, and zinc are necessary for human health in small amounts, excessive exposure to these metals can lead to health issues (Chasapis et al., 2020; Taylor et al., 2020). The average concentrations of copper (Cu), manganese (Mn), and zinc (Zn) in the coastal samples were measured at 0.534 µg/g, 0.454 µg/g, and 1.01 µg/g, respectively. In the highland samples, the average concentrations of Cu, Mn, and Zn were found to be 0.282 µg/g, 0.240 µg/g, and 0.509 µg/g, respectively. These concentrations fall within the normal range typically observed in healthy adult humans (Bost et al., 2016; Hordyjewska et al., 2014). Therefore, it can be inferred that the study population does not exhibit any significant metal intoxication issues related to these three metals.

Barium (Ba) exposure in humans has garnered attention due to its increasing industrial and medicinal utilisation over the past four decades. Although Ba has always been present as a trace element in food and drinking water, its prevalence has significantly risen (Kravchenko et al., 2014; Peana et al., 2021). Our study revealed a high prevalence of barium contamination among the participants, with 13 out of 20 individuals affected. This finding is worrisome as barium exposure has been associated with various health concerns, including elevated blood pressure (Zeng et al., 2022), gastrointestinal disturbances and muscle weakness (Bhoelan et al., 2014), kidneys and cardiovascular malfunction (Dallas and Williams, 2001), neurological impairments (Wang et al., 2021), and mental disorders. Further investigations are imperative to fully understand the extent of barium exposure in the general Indonesian population and identify potential sources of contamination, particularly amongst vulnerable groups such as the elderly, pregnant women, and children.

To evaluate the impact of heavy metal on the human gut microbiome, we first investigate their effect on the abundance of heavy metal related-genes. We found that some genes such as znuC, nikE, modC, mntH, and ArsB are highly prevalent and abundant in the human gut. These genes regulate the uptake and efflux of zinc (Kandari et al., 2021), nickel (Higgins, 2019), molybdenum (Huang et al., 2022), manganes (Kajfasz et al., 2020), and arsenic (Shen et al., 2013), respectively, in various bacterial species. The abundance of these genes in the human gut suggest the selective pressure of metals concentration in the human gut that favour the growth of metal resistance bacteria. Consistent with these findings, previous studies have reported higher abundance and expression of ArsB in children with high arsenic exposure compared to matched controls (Dong et al., 2017). Further, we found that the abundance of metal resistant genes is significantly correlated with Ba dan Cu concentration in faecal samples, where Ba has a strong positive effect on gene abundance while Cu shows the opposite effect. This result indicates that high Ba contamination in the gut could lead to the selection of bacteria that carry metal resistance genes, thus affecting the composition of gut microbiome. In accordance, several works have reported that various heavy metal pollution could induce the emergence and enrichment of metal resistant bacteria (Mustapha and Halimoon, 2015; Zampieri et al., 2016).

Consistent with these findings, our study revealed a positive correlation between barium (Ba) concentration in faecal samples and the abundance of several bacterial genera, including Faecalibacterium, Ruminococcus, and Prevotella. Conversely, we observed a strong negative correlation between Ba concentration and the abundance of Escherichia. These results align with previous research indicating that exposure to heavy metals such as arsenic and cadmium can have a positive effect on Prevotella (Li and Durbin, 2009) and Faecalibacterium abundance but a negative effect on Escherichia. reported an association between arsenic exposure and the depletion of specific gut commensal bacteria Ruminococcus (Brabec et al., 2020). In contrast, our findings revealed a positive correlation between barium exposure and the abundance of Ruminococcus. These results indicate that different metals can have distinct effects on specific bacteria, which may also be influenced by the physiological state of the host. Taken together, our findings support the notion that heavy metal exposure can selectively promote the growth of certain bacteria, potentially those with metal-resistant properties, while suppressing the growth of other microbial species. This suggests that heavy metals

may exert a significant influence on the composition and dynamics of the gut microbiome, favouring the proliferation of metal-tolerant bacteria. Further research is warranted to elucidate the underlying mechanisms and explore the broader implications of these findings on human health and microbial ecology.

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Author contributions

Conceptualization, A.L. and A.T.W.; methodology, A.L., A.T.W, F.N.H; validation, A.L., A.T.W., and R.A.W.; formal analysis, A.L., F.N.H., and A.T.W; investigation, F.N.H and M.A.K..; resources, A.L., A.T.W., and R.A.W.; data curation, A.L., F.N.H., M.A.K., and A.T.W; writing—original draft preparation, A.L. and A.T.W.; writing– review and editing, A.L and A.T.W.; visualization, A.L. and A.T.W.; supervision, A.L.; project administration, A.L., AT.W., and R.A.W..; funding acquisition, A.L. and A.T.W. All authors have read and agreed to the published version of the manuscript.

Ethical approval

The collection of human stool samples was approved by the Health Research Ethic Committee of Universitas Surabaya (No. 005-OL/KE/III/2021). Stool samples were obtained from 11 adult participants from the highland population of Pacet, Mojokerto, Indonesia and 11 adult participants from the coastal population of Kenjeran, Surabaya, Indonesia. All samples were anonymized and obtained with written consent from the participants.

Declaration of competing interest

The authors declare no conflict of interest.

References

Ahmad, K., Iqhrammullah, M., Rizki, D.R., Aulia, A., Mairizal, A.Q., Purnama, A., Qanita, I., Abdulmadjid, S. and Puspita, K. (2022) 'Heavy metal contamination in aquatic and terrestrial animals resulted from anthropogenic activities in Indonesia: a review', *Asian Journal of Water, Environment and Pollution*, Vol. 19, No. 4, pp.1–8, https://doi.org/10.3233/AJW 220049

Akpor, O.B. (2014) 'Heavy metal pollutants in wastewater effluents: sources, effects and remediation', *Advances in Bioscience and Bioengineering*, Vol. 2, No. 4, p.37, https://doi.org/10.11648/j.abb.20140204.11

Astuti, R.D.P., Mallongi, A., Amiruddin, R., Hatta, M. and Rauf, A.U. (2021) 'Risk identification of heavy metals in well water surrounds watershed area of Pangkajene, Indonesia', *Gaceta Sanitaria*, Vol. 35, pp.S33–S37, https://doi.org/10.1016/j.gaceta.2020.12.010

Bhoelan, B.S., Stevering, C.H., van der Boog, A.T.J. and van der Heyden, M.A.G. (2014) 'Barium toxicity and the role of the potassium inward rectifier current', *Clinical Toxicology*, Vol. 52, No. 6, pp.584–593, https://doi.org/10.3109/15563650.2014.923903

Bost, M., Houdart, S., Oberli, M., Kalonji, E., Huneau, J-F. and Margaritis, I. (2016) 'Dietary copper and human health: current evidence and unresolved issues', *Journal of Trace Elements in Medicine and Biology*, Vol. 35, pp.107–115, https://doi.org/10.1016/j.jtemb.2016.02.006

Brabec, J.L., Wright, J., Ly, T., Wong, H.T., McClimans, C.J., Tokarev, V., Lamendella, R., Sherchand S., Shrestha, D., Uprety, S., Dangol, B., Tandukar, S., Sherchand J.B. and Sherchan, S.P. (2020) 'Arsenic disturbs the gut microbiome of individuals in a disadvantaged community in Nepal', *Heliyon*, Vol. 6, No. 1, p.e03313, https://doi.org/10.1016/j.heliyon.2020.e03313

Budianta, D., Napoleon, A. and Bolan, N. (2023) 'Heavy metals in Indonesian paddy soils', in Almayyahi, P.B.A. (Ed.): *Heavy Metals – Recent Advances*, IntechOpen, 18 October, 2023, doi: 10.5772/intechopen.109027.

Chasapis, C.T., Ntoupa, P-S.A., Spiliopoulou, C.A. and Stefanidou, M.E. (2020) 'Recent aspects of the effects of zinc on human health', *Archives of Toxicology*, Vol. 94, No. 5, pp.1443–1460, https://doi.org/10.1007/s00204-020-02702-9

Damastuti, E., Adventini, N., Syahfitri, W.Y.N., Kurniawati, S., Lestiani, D.D. and Santoso, M. (2017) 'Assessment of heavy metals on occupationally exposed workers from hair analysis', *Atom Indonesia*, Vol. 43, No. 3, p.119, https://doi.org/10.17146/aij.2017.772

Das, S., Dash, H.R. and Chakraborty, J. (2016) 'Genetic basis and importance of metal resistant genes in bacteria for bioremediation of contaminated environments with toxic metal pollutants', *Applied Microbiology and Biotechnology*, Vol. 100, No. 7, pp.2967–2984, https://doi.org/10.1007/s00253-016-7364-4

Dong, X., Shulzhenko, N., Lemaitre, J., Greer, R.L., Peremyslova, K., Quamruzzaman, Q., Rahman, M., Hasan, O.S. I., Joya, S.A., Golam, M., Christiani, D.C., Morgun, A. and Kile, M.L. (2017) 'Arsenic exposure and intestinal microbiota in children from Sirajdikhan, Bangladesh', *PLOS ONE*, Vol. 12, No. 12, p.e0188487, https://doi.org/10.1371/journal.pone.0188487

Dallas, C.E. and Williams, P.L. (2001) 'Barium: rationale for a new oral reference dose', *Journal of Toxicology and Environmental Health, Part B*, Vol. 4, No. 4, pp.395–429, https://doi.org/10.1080/109374001753146216

Ekawanti, A. and Krisnayanti, B.D. (2015) 'Effect of mercury exposure on renal function and hematological parameters among artisanal and small-scale gold miners at Sekotong, West Lombok, Indonesia', *Journal of Health and Pollution*, Vol. 5, No. 9, pp.25–32, https://doi.org/10.5696/2156-9614-5-9.25

Fatema, K., Shoily, S.S., Ahsan, T., Haidar, Z., Sumit, A.F. and Sajib, A.A. (2021) 'Effects of arsenic and heavy metals on metabolic pathways in cells of human origin: similarities and differences', *Toxicology Reports*, Vol. 8, pp.1109–1120, https://doi.org/10.1016/j.toxrep.2021.05.015

Gafur, N., Sakakibara, M., Sano, S. and Sera, K. (2018) 'A case study of heavy metal pollution in water of bone river by artisanal small-scale gold mine activities in eastern part of Gorontalo, Indonesia', *Water*, Vol. 10, No. 11, p.1507, https://doi.org/10.3390/w10111507

Gao, B., Chi, L., Mahbub, R., Bian, X., Tu, P., Ru, H. and Lu, K. (2017) 'Multi-omics reveals that lead exposure disturbs gut microbiome development, key metabolites, and metabolic pathways', *Chemical Research in Toxicology*, Vol. 30, No. 4, pp.996–1005, https://doi.org/10.1021/acs.chemrestox.6b00401

Higgins, K. (2019) 'Nickel metalloregulators and chaperones', *Inorganics*, Vol. 7, No. 8, p.104, https://doi.org/10.3390/inorganics7080104

Hordyjewska, A., Popiołek, Ł. and Kocot, J. (2014) 'The many 'faces' of copper in medicine and treatment', *BioMetals*, Vol. 27, No. 4, pp.611–621, https://doi.org/10.1007/s10534-014-9736-5

Huang, X-Y., Hu, D-W. and Zhao, F-J. (2022) 'Molybdenum: more than an essential element', *Journal of Experimental Botany*, Vol. 73, No. 6, pp.1766–1774, https://doi.org/10.1093/jxb/erab534

Javaid, A., Akbar, I., Javed, H., Khan, U., Iftikhar, H., Zahra, D., Rashid, F. and Ashfaq, U.A. (2021) 'Role of heavy metals in diabetes: mechanisms and treatment strategies', *Critical Reviews in Eukaryotic Gene Expression*, Vol. 31, No. 3, pp.65–80, https://doi.org/10.1615/CritRevEukaryotGeneExpr.2021037971

Juliani, A. (2021) 'Heavy metal characteristics of wastewater from batik industry in Yogyakarta area, Indonesia', *International Journal of GEOMATE*, Vol. 20, No. 80, pp.59–67, https://doi.org/10.21660/2021.80.6271

Kajfasz, J.K., Katrak, C., Ganguly, T., Vargas, J., Wright, L., Peters, Z.T., Spatafora, G.A., Abranches, J. and Lemos, J.A. (2020) 'Manganese uptake, mediated by SloABC and MntH, is essential for the fitness of streptococcus mutans', *MSphere*, Vol. 5, No. 1, pp.1–19, https://doi.org/10.1128/mSphere.00764-19

Kandari, D., Joshi, H. and Bhatnagar, R. (2021) 'Zur: zinc-sensing transcriptional regulator in a diverse set of bacterial species', *Pathogens*, Vol. 10, No. 3, p.344, https://doi.org/10.3390/pathogens10030344

Kravchenko, J., Darrah, T.H., Miller, R.K., Lyerly, H.K. and Vengosh, A. (2014) 'A review of the health impacts of barium from natural and anthropogenic exposure', *Environmental Geochemistry and Health*, Vol. 36, No. 4, pp.797–814, https://doi.org/10.1007/s10653-014-9622-7

Li, H. and Durbin, R. (2009) 'Fast and accurate short read alignment with burrows–wheeler transform', *Bioinformatics*, Vol. 25, No. 14, pp.1754–1760, https://doi.org/10.1093/bioinformatics/btp.324

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G. and Durbin, R. (2009) 'The sequence alignment/Map format and SAMtools', *Bioinformatics*, Vol. 25, No. 16, pp.2078–2079, https://doi.org/10.1093/bioinformatics/btp.352

Li, X., Brejnrod, A.D., Ernst, M., Rykær, M., Herschend, J., Olsen, N.M.C., Dorrestein, P.C., Rensing, C. and Sørensen, S.J. (2019) 'Heavy metal exposure causes changes in the metabolic health-associated gut microbiome and metabolites', *Environment International*, Vol. 126, pp.454–467, https://doi.org/10.1016/j.envint.2019.02.048

Lim, J.T., Tan, Y.Q., Valeri, L., Lee, J., Geok, P.P., Chia, S.E., Ong, C.N. and Seow, W.J. (2019) 'Association between serum heavy metals and prostate cancer risk – A multiple metal analysis', *Environment International*, Vol. 132, p.105109, https://doi.org/10.1016/j.envint.2019.105109

Luqman, A., Nugrahapraja, H., Wahyuono, R.A., Islami, I., Haekal, M.H., Fardiansyah, Y., Putri, B.Q., Amalludin, F.I., Rofiqa, E.A., Götz, F. and Wibowo, A.T. (2021) 'Microplastic contamination in human stools, foods, and drinking water associated with Indonesian coastal population', *Environments*, Vol. 8, No. 12, p.138, https://doi.org/10.3390/environments 8120138

Mustapha, M.U. and Halimoon, N. (2015) 'Screening and isolation of heavy metal tolerant bacteria in industrial effluent', *Procedia Environmental Sciences*, Vol. 30, pp.33–37, https://doi.org/10.1016/j.proenv.2015.10.006

Nasir, M., Muchlisin, Z., Saiful, S., Suhendrayatna, S., Munira, M. and Iqhrammullah, M. (2021) 'Heavy metals in the water, sediment, and fish harvested from the Krueng Sabee River Aceh province, Indonesia', *Journal of Ecological Engineering*, Vol. 22, No. 9, pp.224–231, https://doi.org/10.12911/22998993/141643

Nugrahapraja, H., Sugiyo, P.W.W., Putri, B.Q., Ni'matuzahroh, N., Fatimah, F., Huang, L., Hafza, N., Götz, F., Santoso, H., Wibowo, A.T. and Luqman, A. (2022) 'Effects of microplastic on human gut microbiome: detection of plastic-degrading genes in human gut exposed to microplastics–preliminary study', *Environments*, Vol. 9, No. 11, p.140, https://doi.org/10.3390/environments9110140

Pajarillo, E.A.B., Lee, E. and Kang, D-k. (2021) 'Trace metals and animal health: interplay of the gut microbiota with iron, manganese, zinc, and copper', *Animal Nutrition*, Vol. 7, No. 3, pp.750–761, https://doi.org/10.1016/j.aninu.2021.03.005

Palapa, T.M. and Maramis, A.A. (2015) 'Heavy metals in water of stream near an amalgamation tailing ponds in Talawaan – Tatelu gold mining, North Sulawesi, Indonesia', *Procedia Chemistry*, Vol. 14, pp.428–436, https://doi.org/10.1016/j.proche.2015.03.058

Peana, M., Medici, S., Dadar, M., Zoroddu, M.A., Pelucelli, A., Chasapis, C.T. and Bjørklund, G. (2021) 'Environmental barium: potential exposure and health-hazards', *Archives of Toxicology*, Vol. 95, No. 8, pp.2605–2612, https://doi.org/10.1007/s00204-021-03049-5

Puspitasari, R., Takarina, N.D., Soesilo, T.E.B. and Agustina, H. (2023) 'Potential risks of heavy metals in green mussels (Perna viridis) harvested from Cilincing and Kamal Muara, Jakarta bay, Indonesia to human health', *Marine Pollution Bulletin*, Vol. 189, p.114754, https://doi.org/10.1016/j.marpolbul.2023.114754

Sá, I., Semedo, M. and Cunha, M.E. (2016) 'Kidney cancer. heavy metals as a risk factor', *Porto Biomedical Journal*, Vol. 1, No. 1, pp.25–28, https://doi.org/10.1016/j.pbj.2016.03.006

Sandeep, G., Vijayalatha, K.R. and Anitha, T. (2019) 'Heavy metals and its impact in vegetable crops', *International Journal of Chemical Studies*, Vol. 7, No. 1, pp.1612–1621.

Sankhla, M.S., Sharma, K. and Kumar, R. (2017) 'Heavy metal causing neurotoxicity in human health', *International Journal of Innovative Research in Science Engineering and Technology*, Vol. 6, No. 5, pp.7721–7726.

Sarpong-Kumankomah, S., Gibson, M.A. and Gailer, J. (2018) 'Organ damage by toxic metals is critically determined by the bloodstream', *Coordination Chemistry Reviews*, Vol. 374, pp.376–386, https://doi.org/10.1016/j.ccr.2018.07.007

Sudarningsih, S., Bijaksana, S., Ramdani, R., Hafidz, A., Pratama, A., Widodo, W., Iskandar, I., Dahrin, D., Jannatul Fajar, S. and Agus Santoso, N. (2017) 'Variations in the concentration of magnetic minerals and heavy metals in suspended sediments from citarum river and its tributaries, West Java, Indonesia', *Geosciences*, Vol. 7, No. 3, p.66, https://doi.org/10.3390/geosciences7030066

Sumino, K., Hayakawa, K., Shibata, T. and Kitamura, S. (1975) 'Heavy metals in normal Japanese tissues', *Archives of Environmental Health: An International Journal*, Vol. 30, No. 10, pp.487–494, https://doi.org/10.1080/00039896.1975.10666759

Taylor, A.A., Tsuji, J.S., Garry, M.R., McArdle, M.E., Goodfellow, W.L., Adams, W.J. and Menzie, C.A. (2020) 'Critical review of exposure and effects: implications for setting regulatory health criteria for ingested copper', *Environmental Management*, Vol. 65, No. 1, pp.131–159, https://doi.org/10.1007/s00267-019-01234-y

Turkyilmaz, A., Cetin, M., Sevik, H., Isinkaralar, K. and Saleh, E.A.A. (2020) 'Variation of heavy metal accumulation in certain landscaping plants due to traffic density', *Environment, Development and Sustainability*, Vol. 22, No. 3, pp.2385–2398, https://doi.org/10.1007/s106 68-018-0296-7

Wahyuningsih, N.E., Setiawan, H., Nabiha, P.I., Kartasurya, M.I. and Azam, M. (2023) 'Heavy metals contamination of local and imported rice in Semarang, Central Java, Indonesia', *Journal of Ecological Engineering*, Vol. 24, No. 7, pp.49–60, https://doi.org/10.12911/ 22998993/163308

Wang, C., Pi, X., Chen, Y., Wang, D., Yin, S., Jin, L., Li, Z., Ren, A., Wang, L. and Yin, C. (2021) 'Prenatal exposure to barium and the occurrence of neural tube defects in offspring', *Science of The Total Environment*, Vol. 764, p.144245, https://doi.org/10.1016/j.scitotenv.2020. 144245

Wibowo, A.T., Nugrahapraja, H., Wahyuono, R.A., Islami, I., Haekal, M.H., Fardiansyah, Y., Sugiyo, P.W. W., Putro, Y.K., Fauzia, F.N., Santoso, H., Götz, F., Tangahu, B.V. and Luqman, A. (2021) 'Microplastic contamination in the human gastrointestinal tract and daily consumables associated with an Indonesian farming community', *Sustainability*, Vol. 13, No. 22, p.12840, https://doi.org/10.3390/su132212840

Wu, F., Yang, L., Islam, M.T., Jasmine, F., Kibriya, M.G., Nahar, J., Barmon, B., Parvez, F., Sarwar, G., Ahmed, A., Eunus, M., Islam, T., Slavkovich, V., Hu, J., Li, H., Graziano, J.H., Pei, Z., Ahsan, H. and Chen, Y. (2019) 'The role of gut microbiome and its interaction with arsenic exposure in carotid intima-media thickness in a Bangladesh population', *Environment International*, Vol. 123, pp.104–113, https://doi.org/10.1016/j.envint.2018.11.049

Yap, C.K. and Al-Mutairi, K.A. (2021) 'Ecological-health risk assessments of heavy metals (Cu, Pb, and Zn) in aquatic sediments from the ASEAN-5 emerging developing countries: a review and synthesis', *Biology*, Vol. 11, No. 1, p.7, https://doi.org/10.3390/biology11010007

Zampieri, B.D.B., Pinto, A.B., Schultz, L., de Oliveira, M.A. and de Oliveira, A.J.F.C. (2016) 'Diversity and distribution of heavy metal-resistant bacteria in polluted sediments of the araça bay, são sebastião (SP) and the relationship between heavy metals and organic matter concentrations', *Microbial Ecology*, Vol. 72, No. 3, pp.582–594, https://doi.org/10.1007/ s00248-016-0821-x

Zeng, H., Wang, Q., Wang, H., Guo, L., Fang, B., Zhang, L., Wang, X., Wang, Q., Yang, W. and Wang, M. (2022) 'Exposure to barium and blood pressure in children and adolescents: results from the 2003-2018 national health and nutrition examination survey', *Environmental Science and Pollution Research*, Vol. 29, No. 45, pp.68476–68487, https://doi.org/10.1007/s11356- 022-20507-4

Zhang, J., Wang, Y., Chen, G., Wang, H., Sun, L., Zhang, D., Tao, F., Zhang, Z. and Yang, L. (2023) 'The associations between multiple essential metal(loid)s and gut microbiota in Chinese community-dwelling older adults', *Nutrients*, Vol. 15, No. 5, p.1137, https://doi.org/10.3390/nu15051137

Zhang, Y., Chen, T., Zhang, Y., Hu, Q., Wang, X., Chang, H., Mao, J-H., Snijders, A.M. and Xia, Y. (2021) 'Contribution of trace element exposure to gestational diabetes mellitus through disturbing the gut microbiome', *Environment International*, Vol. 153, p.106520, https://doi.org/10.1016/j.envint.2021.106520

Supplementary material

Table S1 Reference genes

No.	Gene	Reference species	Function	Accession number	Locus tag
1	*rpoB*	*Bacteroides intestinalis*	DNA-directed RNA polymerase subunit beta	NZ_QRKQ01000009.1	DW169_RS10850
2	*merA*	*S. aureus*	Mercury reductase (II)	NC_013352.1	HHF46_RS00110
3	*chrB*	*Ralstonia solanacearum*	Chromate resistance protein	NC_021745.1	F504_3817
4	*czcD*	*Caulobacter vibrioides*	cobalt-zinc-cadmium resistance protein	NC_011916.1	CCNA_00305
5	*nccA*	*Parachlamydia acanthamoebae*	nickel-cobalt-cadmium resistance protein	NC_015702.1	PUV_25170
6	*znuC*	*E. coli*	Zn(2(+)) ABC transporter ATP binding subunit	NC_000913.3	ECK1859
7	*nikE*	*E. coli*	Ni(2(+)) ABC transporter ATP binding subunit	NC_000913.3	ECK3464
8	*modC*	*E. coli*	molybdate ABC transporter ATP binding subunit	NC_000913.3	ECK0754
9	*mntH*	*E. coli*	Mn(2(+))/Fe(2(+)): H(+) symporter	NC_000913.3	ECK2387
10	*fbpC*	*Neisseria meningitidis*	iron(III)-transport system ATP-binding	NC_017512.1	NMAA_0482
11	*copR*	*Salmonella enterica*	Copper resistance transcriptional regulatory protein	NC_003197.2	STM1096
12	*arsT*	*Corynebacterium urealyticum*	putative arsenic-transport protein	NC_020230.1	CU7111_0032
13	*arsM*	*Thermacetogenium phaeum*	S-adenosyl-L-methionine:arsenic(III) methyltransferase	NC_018870.1	Tph_c04450
14	*arsA*	*Enterobacter cloacae*	arsenite efflux transporter ATPase subunit		EC036_RS04535
15	*arsB*	*E. coli*	arsenite/antimonite:H(+) antiporter		b3502, ECK3487, arsF
16	*pbrD*	*Cupriavidus metallidurans CH34*	Pb(II) binding protein	NC_007971.2	Rmet_5949
17	*pbrC*	*Cupriavidus metallidurans CH34*	lead resistance fusion protein	NC_006466.1	pMOL30_090

Table S2   Concentration of heavy metals in stool samples collected from coastal and highland populations of Indonesia

Heavy metals concentration (mg/g)	*Coastal population samples*								
	C1	C2	C3	C4	C5	C7	C8	C9	C10
Cu	0.242556	0.499106	0.611812	0.298659	0.458076	0.528025	1.840979	0.074127	0.25441
Mn	0.225828	0.472962	0.638413	0.323547	0.391447	0.563227	1.047454	0.077834	0.352261
Zn	0.602208	1.283414	2.412668	0.771536	0.978617	1.946653	0.571338	0.16308	0.410971
Ba	0.267648	0	0	0.012444	0	0	0	0	0.05871

	Highland population samples										
	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11
Cu	0.502876	0.330391	0.230499	0.252223	0.241124	0.277939	0.120737	0.242437	0.2947	0.273743	0.339967
Mn	0.158482	0.172905	0.186172	0.278839	0.242585	0.265306	0.095757	0.178942	0.442049	0.263967	0.363146
Zn	0.640023	0.451535	0.301422	0.481631	0.686838	0.581145	0.22482	0.392517	0.574664	0.449721	0.819011
Ba	0	0	0.088654	0.139419	0.029227	0.101069	0.06245	0.080812	0.279965	0.117319	0.007727

Table S3   Abundance of heavy metals related genes in stool samples from coastal and highland population of Indonesia

Heavy metals related genes	Coastal population samples									
	C1	C2	C3	C4	C5	C7	C8	C9	C10	
znuC	1826.65	52678.53	1087.99	14308.77	1734.78	470.15	3428.32	7246.33	1039.08	
nikE	3422.41	39377.25	254.81	5828.04	3656.59	0	5709.61	5656.98	486.71	
modC	2608.02	55922.18	776.7	8882.4	1548.04	0	3535.15	10346.03	618.15	
mntH	2229.13	62453.69	663.86	8351.17	1587.77	0	6043.13	7000.69	317.01	
arsB	3746.75	68622.37	0	15677.43	4320.82	1102.11	7813.37	13447.83	101.49	
copR	0	0	273.08	0	0	0	0	0	0	

Heavy metals related genes	Highland population samples										
	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11
znuC	13667.83	25642.11	7017.03	55841.14	5185.22	1773.5	24307.83	6054.09	3509.37	2833	0
nikE	13901.56	27536.96	4045.27	42538.33	4857.53	4799.66	19864.63	2835.74	6164.22	3412.23	350.34
modC	17562.97	29913.72	4623.99	59330.4	3172.83	2672.81	19568.16	4321.9	6889.49	4044.85	266.98
mntH	12628.68	25186.29	2305.46	51072.67	4519.81	3967.84	20827.77	5541.03	4550.28	1975.55	228.19
arsB	20597.1	39218.02	8857.27	62324.38	3472.89	3810.97	27581.75	1182.66	11825.75	2608.99	0
copR	0	0	0	0	0	0	0	0	0	0	0