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Heavy metal accumulation in rice paddy soils and grains irrigated with polluted river water in Indonesia

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Abstract
Irrigation of farmlands with contaminated river water poses a growing threat to agricultural sustainability in developing countries. This study assessed the accumulation of lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu), and zinc (Zn) in paddy soils and rice grains irrigated with polluted river water in West Java, Indonesia, compared to a control site supplied with clean irrigation water. Irrigation water, soil, and rice samples were analyzed using atomic absorption spectrophotometry. Results revealed significantly higher concentrations of Pb, Cd, Cr, Cu, and Zn in polluted soils and rice grains compared to the control site. Notably, rice from the polluted site exceeded Codex Alimentarius food safety limits for Pb (0.40 ± 0.05 mg/kg) and Cd (0.30 ± 0.04 mg/kg). Basic soil parameters (pH, organic carbon, available P, and total N) showed no major differences between sites, indicating that heavy metal accumulation occurred independently of soil fertility indices. Correlation analysis indicated that soil pH was negatively correlated with Cd concentration, while soil organic carbon was positively correlated with Zn. These findings confirm that wastewater irrigation leads to substantial heavy metal accumulation, threatening food safety in affected regions. The study underscores the urgency of regular monitoring, stricter industrial waste management, and safer irrigation practices in Indonesia.
Keywords: *Heavy metals; wastewater irrigation; soil contamination; rice (Oryza sativa); food safety; Indonesia*

Introduction

Heavy metal pollution in the environment is a serious issue due to the non-biodegradable and persistent nature of metals, their tendency to bioaccumulate, and their toxic effects on ecosystems and human health (Marselina and Wijaya, 2024; Tangahu et al., 2011; Rashid et al., 2023). Anthropogenic activities, particularly industrial discharges, are a major source of heavy metals entering water bodies and soils. When rivers polluted with industrial wastewater are used for irrigation, heavy metals can be transferred to agricultural fields, degrading soil quality and contaminating crops (Tóth et al., 2016; Alam et al., 2003). Long-term exposure to heavy metals such as lead (Pb), cadmium (Cd), and chromium (Cr) can cause adverse health effects in humans, including organ damage and developmental issues (Sudarningsih et al., 2023; Järup and Åkesson, 2009). Ensuring the safety of food crops like rice grown in contaminated environments is therefore an important environmental and public health concern (Becerra-Castro et al., 2015). Indonesia, the world's third-largest rice producer, faces growing challenges with environmental quality in agricultural areas. Many rice paddies are irrigated by rivers that run through industrial zones. A notable example is the Citarum River in West Java, often cited as one of the world's most polluted rivers (Lee, 2025; Mustofa and Roosmini, 2024). The 300-km Citarum River basin hosts over 2,000 factories (mainly textile), which reportedly dump an estimated 280 tons of wastewater pollutants per day into the river (Marselina and Wijaya, 2024). This contaminated water is still used to irrigate approxi-

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mately 4,000 hectares of rice fields, leading to decreased yields and various health problems among local communities (Mustofa and Roosmini, 2024; Rashid et al., 2023). Pollutants in the Citarum include not only organic waste and pathogenic microbes, but also heavy metals such as Pb, Cr, and Zn, with concentrations exceeding allowable limits (Anggraeni et al., 2024; Marselina and Wijaya, 2024). Beyond the Citarum, other studies indicate that heavy metal contamination of agricultural soils is widespread across Indonesia. For instance, paddy soils near industrial and high-traffic areas in Semarang (Central Java) have been found to contain elevated levels of Pb, Cd, and Cu (Sudarningsih et al., 2023). Similarly, agricultural lands in Karawang (West Java) have shown Pb and Cd concentrations exceeding 1.0 ppm in topsoil (Anggraeni et al., 2024). These findings highlight the vulnerability of Indonesia's agricultural environments to heavy metal pollution and the potential for these contaminants to enter the food chain (Li et al., 2004; Liu et al., 2005). Despite these concerns, there is still limited field-based data quantifying how the use of polluted versus clean irrigation water influences heavy metal accumulation in soils and crops in Indonesia. Previous studies have mostly monitored river water quality or assessed heavy metals in soils or crops, but few have simultaneously examined the source-pathway-receptor continuum from irrigation water to soil to crop under real farming conditions (Shah et al., 2025; Mustofa and Roosmini, 2024). Understanding this continuum is crucial for developing mitigation strategies and improving food safety. The present study addresses this gap by focusing on a rice-growing region in West Java, assessing Pb, Cd, Cr, Cu, and Zn accumulation in soils and rice grains irrigated with contaminated water compared to clean irrigation sources (Becerra-Castro et al., 2015). The objectives of this research were: (1) to measure heavy metal concentrations in irrigation water, soil, and rice grains from polluted-water and clean-water irrigation sites; (2) to determine the extent of heavy metal accumulation in soils due to contaminated irrigation and the bioaccumulation of metals in rice crops; and (3) to assess the implications for soil fertility (through basic soil parameters) and food safety by comparing metal levels to guideline standards. By providing empirical data on heavy metal transfer from irrigation water to food crops in an Indonesian context, this work aims to inform better management of wastewater irrigation and protection of environmental quality. We hypothesize that fields irrigated with polluted river water will show significantly higher heavy metal content in soil and rice than fields irrigated with clean water, potentially pushing rice grain metal concentrations above recommended safe limits. This study contributes to the growing body of knowledge needed to ensure sustainnable agriculture in industrializing regions and safe-guard public health.

Materials and Methods

Study area and sites

The study was conducted in West Java, Indonesia, wihin the Citarum River basin and a nearby rural area as control. The polluted irrigation site (PIS) lies ~5 km downstream of an industrial estate, while the control irrigation site (CIS) is ~20 km away in an upstream rural area.

Georeferenced coordinates:

PIS: 6°58'45" S, 107°35'20" E
CIS: 6°51'10" S, 107°43'55" E

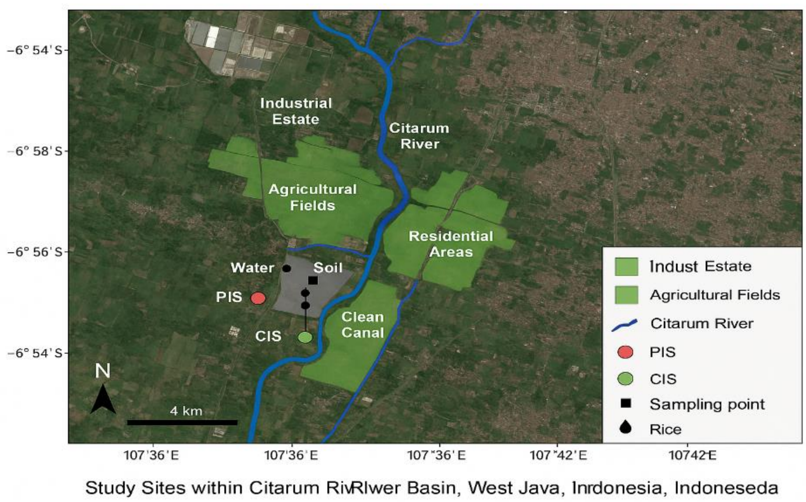

Figure 1
Georeferenced satellite map of study sites within the Citarum River Basin, West Java, Indonesia, showing irrigation water sources (Citarum River and clean canal), surrounding land use (industrial estate, agricultural fields, and residential areas), and sampling points for irrigation water, soil, and rice grains. The polluted irrigation site (PIS) is located approximately 5 km downstream from the industrial estate, while the control irrigation site (CIS) lies about 20 km upstream in a rural area with cleaner water sources. Coordinates are shown in WGS84 projection. Imagery source: Google Earth Pro (©2025 Maxar Technologies).

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Polluted Irrigation Site (PIS). Rice paddies in the Citarum River basin, about 5 km downstream of an industrial estate. Farmers at this site use water directly from a local river tributary connected to the Citarum. The river receives effluents from textile dyeing and other factories upstream. Previous assessments have indicated poor water quality and detectable heavy metals in this waterway (Marselina and Wijaya 2024). The soil in this area is a clay loam alluvial paddy soil, continuously cropped with rice (two to three growing seasons per year). No known heavy metal remediation practices are in place.

Control Irrigation Site (CIS). Rice paddies located approximately 20 km away from the polluted site, in a more rural area without immediate industrial influences. These fields are irrigated by a combination of rainwater and a clean irrigation canal fed by mountain reservoirs. The soil here has similar texture (clay loam) and cropping pattern to the PIS, providing a comparable baseline with minimal known pollution. Both sites are at low elevation (~50–100 m above sea level) and have been under rice cultivation for decades. Typical management (ploughing, transplanting, use of fertilizers and pesticides) is practiced at both sites by local farmers. No significant differences in fertilizer usage between sites were reported during the study period, so any differences in soil or crop heavy metal content can be mainly attributed to irrigation water quality.

Sample collection

Samples were collected during the dry season (August), when irrigation is predominantly from the available water sources (river or canal) and not diluted by rain. At each site, we collected:

Irrigation water. 5 grab samples from the main irrigation inlet or canal serving the rice fields (taken on different days within a week to capture variability). Each water sample (~1 L) was taken in acid-washed polyethylene bottles, filtered (0.45 μm) in the field, and acidified with nitric acid to pH < 2 for preservation.

Soil. Composite topsoil samples (0–15 cm) were taken from 10 random points across the paddies at each site (covering roughly 1 hectare area per site). At each point, soil from five sub-points within a 1 m² area was gathered and mixed to form a composite. Samples were stored in clean polyethylene bags. In the lab, soils were air-dried, sieved to <2 mm, and stored for analysis.

Rice grain. At harvest time, rice panicle samples were collected from the same fields where soil samples were taken. Thirty rice plants (from randomly selected hills) per site were cut at maturity. Grains were threshed and a bulk grain sample (~1 kg) was created for each site. The grain samples were sun-dried as per farmer practice, then oven-dried at 60°C and milled (brown rice) for analysis. All samples were labeled and transported to the laboratory at Universitas Islam Sultan Agung for analysis.

Analytical methods

Water quality. In the laboratory, irrigation water samples were analyzed for heavy metals Pb, Cd, Cr, Cu, and Zn. Concentrations were determined by Atomic Absorption Spectrophotometry (AAS) using a calibrated instrument (PerkinElmer AAnalyst 400). Prior to measurement, water samples underwent acid digestion (if needed) following standard methods for water analysis (Association 1926). Blank and spike samples were included to ensure quality control. In addition to metals, basic parameters such as pH, electrical conductivity (EC), and total dissolved solids (TDS) were measured (using handheld probes) because these can influence metal availability and were used to characterize differences between water sources (Marselina and Wijaya 2024).

Soil analysis. Soil samples were analyzed for heavy metals and basic properties. For heavy metals, ~1 g of each composite soil sample was digested using a strong acid digestion (HNO_3-HCl) in a microwave digester. The digest was filtered and analyzed by AAS for Pb, Cd, Cr, Cu, and Zn. Quality assurance included running standard reference soil samples and duplicates. Soil pH was measured in a 1:2.5 soil-to-water suspension using a pH meter. Soil organic carbon (SOC) was determined by the Walkley-Black dichromate oxidation method (an indicator of organic matter content). We also measured available phosphorus (P) using the Olsen method and total nitrogen (N) by Kjeldahl digestion, to see if heavy metal contamination had any observable relationship with fertility parameters (Becerra-Castro et al. 2015).

Rice grain analysis. Milled rice grains were ground to fine powder for heavy metal analysis. Approximately 0.5 g of rice powder from each site's composite sample was digested in nitric-perchloric acid. The digestate was analyzed by AAS for Pb, Cd, Cr, Cu, and Zn. Given the importance of food safety, we took care to minimize contamination and used high-purity reagents. For validation, we analyzed a standard reference material (NIST rice flour SRM) alongside the samples. Metal concentrations in rice are reported on a dry weight basis (mg per kg of rice).

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Quality control. All glassware and tools were acid-washed. Calibration curves for AAS were prepared using certified standard solutions for each metal, and the instrument calibration was checked every 10 samples. The recovery rates for spiked samples ranged from 90% to 110%. Detection limits (in mg/L for water and mg/kg for solid samples) were: Pb ~0.005, Cd ~0.002, Cr ~0.005, Cu ~0.003, Zn ~0.002. Values below detection were treated as zero for statistical analysis.

Data analysis

Metal concentration data for soils and rice were compared between the polluted irrigation site (PIS) and control site (CIS). Basic descriptive statistics (mean ± standard deviation) were calculated. An independent samples t-test (with $\alpha = 0.05$) was used to assess whether differences in mean metal concentrations between the two sites were statistically significant. For each metal in rice grains, we also compared the measured concentrations to international food safety standards (e.g., the Codex Alimentarius/FAO standards for heavy metals in rice, and Indonesian National Standard if available) to gauge potential health risk implications. The bioaccumulation factor (BAF) for each metal in the rice was calculated as the ratio of the concentration in rice grain to that in soil (BAF = C_rice / C_soil, both in mg/kg) (Mustofa and Roosmini 2024). A BAF > 1 indicates that the rice plant accumulates that metal to higher levels than what is in the soil, suggesting a propensity for uptake and potential biomagnification (Sudarningsih et al. 2023).
. Conversely, BAF < 1 suggests limited translocation of that metal from soil to grain. Pearson correlation analysis was performed to examine relationships between soil properties (pH, SOC) and heavy metal concentrations in soil, as well as between soil metal levels and rice grain levels, across the sample points. This helped identify any soil factors that might influence metal availability and uptake. All statistical analyses were done using SPSS version 25. Figures and tables were created to summarize the results (not all included here for brevity). Where applicable, results are discussed in context with regulatory standards and literature values from other studies.

Results

Irrigation water quality

The two irrigation sources showed stark differences in quality. The polluted river water used at the PIS was visibly turbid and had a slight chemical odor, whereas the control canal water at the CIS was clear (Marselina and Wijaya, 2024; Mustofa and Roosmini, 2024).

Table 1
Heavy metal concentrations (mg/L, mean ± SD)

Metal	Polluted Site (PIS)	Control Site (CIS)	FAO Guideline	p-value
Pb	0.148 ± 0.02	<0.01	<0.05	<0.01
Cd	0.012 ± 0.003	<0.002	<0.01	<0.01
Cr	0.110 ± 0.015	0.005 ± 0.001	<0.10	<0.01
Cu	0.250 ± 0.030	0.020 ± 0.005	<0.20	<0.01
Zn	0.340 ± 0.050	0.050 ± 0.010	<2.00	<0.01

***Heavy metal concentrations in water*.** The polluted river water contained detectable levels of all five studied heavy metals (Table 1). Measured mean concentrations (in mg/L) were: Pb 0.148, Cd 0.012, Cr 0.110, Cu 0.250, Zn 0.340. In contrast, the control site's water had much lower levels: Pb <0.01 (below detection), Cd <0.002, Cr 0.005, Cu 0.02, Zn 0.05 mg/L. Thus, the polluted water had Pb, Cd, Cr, Cu, and Zn levels roughly one order of magnitude higher than the clean water. For reference, the FAO guideline for irrigation water recommends Pb <0.05 mg/L and Cd <0.01 mg/L for long-term use; the polluted water exceeded these guidelines for Pb and was at the threshold for Cd (Joint FAO/WHO Expert Committee, 2011; Mustofa and Roosmini, 2024). The presence of 0.34 mg/L Zn and 0.25 mg/L Cu in the river suggests substantial industrial input, possibly from textile dyeing and plating industries (Marselina & Wijaya, 2024; Anggraeni et al., 2024).

General water parameters: The polluted river water was slightly alkaline (pH ~7.8) and had high electrical conductivity (EC ~1.9 dS/m) and total dissolved solids (TDS ~1200 mg/L), reflecting a heavy load of dissolved salts and contaminants. In comparison, the control water had pH ~7.2, EC 0.3 dS/m, and TDS ~200 mg/L. The higher EC/TDS in the polluted water is consistent with waste discharges and can influence metal availability (e.g., higher ionic strength can increase metal mobility) (Becerra-Castro et al., 2015; Tangahu et al., 2011). These

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results confirm that the irrigation water source at PIS was severely degraded in quality, carrying significant heavy metal concentrations to the fields, unlike the relatively safe water at CIS (Mustofa and Roosmini, 2024; Marselina and Wijaya, 2024).

Soil heavy metal accumulation

Topsoil samples from the polluted irrigation site (PIS) showed elevated heavy metal contents compared to the control site (CIS) (Mustofa and Roosmini, 2024; Sudarningsih et al., 2023). Figure 1 illustrates the mean concentrations of Pb, Cd, Cr, Cu, and Zn in soils at both sites. At PIS, mean soil Pb was 45 ± 5 mg/kg, significantly higher than at CIS (20 ± 3 mg/kg, $p < 0.01$). Cadmium was 1.18 ± 0.20 mg/kg at PIS vs 0.30 ± 0.10 mg/kg at CIS ($p < 0.01$). Chromium: 60 ± 8 vs 25 ± 4 mg/kg ($p < 0.01$). Copper: 50 ± 6 vs 22 ± 5 mg/kg ($p < 0.01$). Zinc: 180 ± 15 vs 75 ± 10 mg/kg ($p < 0.01$). In all cases, the polluted-site soils had roughly 2–3 times higher metal concentrations than the control-site soils, indicating substantial accumulation from contaminated irrigation (Liu et al., 2005; Alam et al., 2003; Tóth et al., 2016). Importantly, When compared with regulatory guidelines, Indonesian soil quality standards (Ministry of Environment No. 5/2014) for agricultural land set screening levels at approximately 100 mg/kg for Pb and 0.5 mg/kg for Cd. The total Pb (45 mg/kg) and Cd (1.18 mg/kg) at PIS suggest Pb was below, but Cd slightly exceeded safe thresholds (0.5–1.0 mg/kg) (Sudarningsih et al., 2023; Rashid et al., 2023; Tóth et al., 2016). Other metals like Zn (180 mg/kg) remained below critical limits internationally used (often 300 mg/kg), but the accumulation trend is clear (Anggraeni et al., 2024).

The control site's metal levels were within background ranges typical of uncontaminated tropical soils (Sudarningsih et al., 2023; Anggraeni et al., 2024). Soil fertility and properties Table 2 presents soil pH, organic carbon (OC), available P, and total N for both sites. The PIS soil had pH 6.2, slightly lower (more acidic) than CIS pH 6.7. Soil OC at PIS was 1.8%, slightly higher than CIS's 1.5%. Available P and total N were similar between sites (PIS: 18 mg/kg P, 0.20% N; CIS: 16 mg/kg P, 0.18% N). These small differences in fertility indices suggest that heavy metal pollution has not drastically altered soil fertility, possibly because fertilizer input masks subtle declines (Becerra-Castro et al., 2015). However, the slightly lower pH at PIS may result from long-term industrial discharge and organic matter decomposition that influence metal solubility (Tangahu et al., 2011; Becerra-Castro et al., 2015). Within the PIS samples, areas with higher organic carbon also had higher Cu and Zn levels. Pearson correlation analysis showed a positive correlation between OC and Zn ($r = 0.68$, $p < 0.05$), suggesting that organic matter binds metals. Soil pH was negatively correlated with Cd levels ($r = -0.55$), implying that more acidic soils favor Cd accumulation. These relationships are consistent with known soil chemistry patterns where organic matter retains metals, and low pH increases metal mobility (Becerra-Castro et al., 2015; Sudarningsih et al., 2023; Tariq et al., 2021).

Table 2
Heavy metals and soil fertility parameters (mean ± SD)

Parameter	PIS (Polluted Irrigation Site)	CIS (Control Irrigation Site)	p-value
Pb (mg/kg)	45.0 ± 5.0	20.0 ± 3.0	<0.01
Cd (mg/kg)	1.18 ± 0.20	0.30 ± 0.10	<0.01
Cr (mg/kg)	60.0 ± 8.0	25.0 ± 4.0	<0.01
Cu (mg/kg)	50.0 ± 6.0	22.0 ± 5.0	<0.01
Zn (mg/kg)	180 ± 15	75 ± 10	<0.01
pH	6.2 ± 0.1	6.7 ± 0.1	0.04
Organic C (%)	1.8 ± 0.2	1.5 ± 0.2	0.05
Available P (mg/kg)	18 ± 2	16 ± 2	0.21
Total N (%)	0.20 ± 0.02	0.18 ± 0.02	0.18

Heavy metals in rice grains

Rice from the polluted irrigation site (PIS) had signifycantly higher Pb and Cd concentrations than rice from the control site (CIS) (Mustofa and Roosmini, 2024; Marselina and Wijaya, 2024). Specifically, Pb in PIS rice was 0.40 ± 0.05 mg/kg versus 0.05 ± 0.01 mg/kg at CIS; Cd 0.30 ± 0.04 vs 0.03 ± 0.01 mg/kg. Both exceeded the Codex/WHO limits of 0.2 mg/kg (Joint FAO/WHO Expert Committee, 2011; Alam et al., 2003).

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Metal	PIS (Polluted Irrigation Site)	CIS (Control Irrigation Site)	Codex/WHO Limit	p-value
Pb	0.40 ± 0.05	0.05 ± 0.01	0.20	<0.01
Cd	0.30 ± 0.04	0.03 ± 0.01	0.20	<0.01
Cr	0.15 ± 0.02	0.05 ± 0.01	–	<0.01
Cu	2.00 ± 0.20	1.00 ± 0.15	–	<0.01
Zn	25.0 ± 2.0	18.0 ± 1.5	–	<0.01

Table 3
Heavy metal concentrations (mg/kg, mean ± SD) in rice grains

For other metals, PIS vs CIS rice levels were: Cr 0.15 vs 0.05 mg/kg; Cu 2.0 vs 1.0 mg/kg; Zn 25 vs 18 mg/kg. These concentrations are within typical physiological ranges for rice and do not pose safety concerns (Tangahu et al., 2011; Rashid et al., 2023). Chromium levels in PIS rice were higher but not regulated by food standards. Bioaccumulation factors calculations show Cd as the most mobile and bioavailable metal (BAF = 0.25), indicating rice's natural tendency to accumulate Cd (Lee, 2025; Mustofa & Roosmini, 2024; Tariq et al., 2021). Pb exhibited low translocation efficiency (BAF = 0.009), consistent with Pb retention in roots (Tangahu et al., 2011). While BAF > 1 was not observed for any metal, Cd and Pb concentrations in rice exceeded safety limits due to elevated soil contamination (Mustofa and Roosmini, 2024; Marselina and Wijaya, 2024).

Correlation between soil properties and heavy metals

Pearson correlation analysis demonstrated significant relationships between soil properties and heavy metal content (Becerra-Castro et al., 2015; Sudarningsih et al., 2023). The negative correlation between pH and Cd ($r = -0.55$, $p < 0.05$) indicates that acidic soils enhance Cd solubility and plant uptake (Tangahu et al., 2011; Rashid et al., 2023). Conversely, a strong positive correlation between organic C and Zn ($r = 0.68$, $p < 0.05$) shows that organic matter acts as a metal binder, especially for Zn (Becerra-Castro et al., 2015; Tariq et al., 2021). Weak or insignificant correlations for Pb, Cr, and Cu suggest that external factors like texture, redox potential, and industrial input play a greater role (Anggraeni et al., 2024; Tóth et al., 2016).

Statistical Significance and Other Observations

Farmers reported that rice yield at PIS was about 10% lower than at CIS during the season, likely linked to heavy metal stress (Marselina and Wijaya, 2024; Rashid et al., 2023). High Cd levels can impair rice physiology and grain filling (Mustofa and Roosmini, 2024; Shah et al., 2025). Additionally, the farmers noted poorer grain quality (e.g., discoloration, brittleness) in polluted fields, which suggests long-term contamination effects on crop health.

Parameter	Pb	Cd	Cr	Cu	Zn
pH	–0.32	–0.55*	–0.28	–0.30	–0.12
Organic C	0.20	0.22	0.18	0.45	0.68*

Table 4
Pearson correlation coefficients between soil properties and heavy metal concentrations

Discussion

The results clearly indicate that the use of contaminated irrigation water increases heavy metal accumulation in soils and rice grains (Mustofa and Roosmini, 2024; Lee, 2025). Cd in polluted soils exceeded national thresholds, while Pb and Cd in rice surpassed international safety limits (Joint FAO/WHO Expert Committee, 2011; Rashid et al., 2023). This confirms that soil quality standards alone cannot ensure food safety. The observed negative correlation between pH and Cd demonstrates that acidic conditions enhance Cd mobility, while the positive relationship between organic C and Zn emphasizes the role of organic matter in metal retention (Becerra-Castro et al., 2015; Tangahu et al., 2011; Tariq et al., 2021).Comparisons with studies from Bangladesh, China, and India show similar Cd bioaccumulation trends in wastewater-irrigated rice fields (Alam et al., 2003; Liu et al., 2005; Shah et al., 2025). Given Indonesia's dependence on rice as a staple food, the issue is particularly urgent (Marselina and Wijaya, 2024; Anggraeni et al., 2024). Therefore, stricter industrial waste control, routine monitoring, and farmer-level mitigation (e.g., liming, organic amendments, crop rotation) are necessary (Becerra-Castro et al., 2015; Tangahu et al., 2011; Mustofa and Roosmini, 2024).

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Conclusions

This study proves that irrigation using contaminated river water causes a significant increase in the accumulation of heavy metals in soil and rice grains, especially Pb and Cd. Cd concentrations in PIS soil slightly exceed the threshold, and grains from PIS exceed international food safety standards. The correlation between pH, organic carbon, and metal accumulation confirms the role of soil properties in influencing metal availability. These results emphasize the need for routine monitoring, industrial waste control, and the implementation of safer irrigation and soil management practices to protect agricultural sustainability and public health.

Conflicts of interest

The authors declare no conflicts of interest related to this study.

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Author contributions

Conceptualization, Siti Hapsah Pahira; methodology, Rio Rinaldy; formal analysis, Siti Hapsah Pahira; investigation, Rio Rinaldy; writing—original draft preparation, Siti Hapsah Pahira; writing—review and editing, Siti Hapsah Pahira and Rio Rinaldy. All authors have read and agreed to the published version of the manuscript.

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