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Review

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Meta-Analysis of the Effect of Oyster Mushrooms (*Pleurotus ostreatus*) Contaminated by Heavy Metals on Potential Health Risks

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ABSTRACT

Background: Oyster mushrooms (*Pleurotus ostreatus*) are widely cultivated and consumed in Indonesia, but heavy metal contamination poses severe human health risks, such as digestive and neurological disorders. Despite numerous individual studies on heavy metal exposure, a comprehensive quantitative synthesis of their relative health risks remains lacking. This meta-analysis aimed to evaluate the health risk potential of arsenic, lead, mercury, and cadmium contamination in oyster mushrooms and assess their pooled effect sizes.

Subjects and Method: A systematic literature search was conducted across PubMed, Scopus, Web of Science, and Google Scholar using PRISMA guidelines and PICO criteria to identify eligible studies on heavy metal contamination in *Pleurotus ostreatus*. A total of 10 articles met the inclusion and exclusion criteria and were synthesized using RevMan 5.4 software. Pooled effect sizes (Standardized Mean Differences) were calculated using a random-effects model to account for study variability, and heterogeneity was evaluated using the I2 statistic along with funnel plot symmetry for publication bias.

Results: The meta-analysis of 10 studies revealed that lead exposure had the strongest correlation with health risk (Effect Size = 2.37;95% CI: 1.85-3.04), representing a significantly higher risk compared to mercury (Effect Size = 1.53;95% CI: [-1.01, 4.08]), respectively. Moderate heterogeneity was observed across studies (I^2= 93%-97%, $p < 0.05$), while a symmetrical funnel plot indicated minimal publication bias.

Conclusion: This meta-analysis quantitatively confirms that lead, arsenic, and cadmium present the highest health risks among heavy metal contaminants in cultivated *Pleurotus ostreatus*, with lead demonstrating the strongest overall risk association. These quantified risk magnitudes highlight an urgent need for targeted, mandatory monitoring of lead, arsenic, and cadmium levels in mushroom cultivation to safeguard public health.

Keywords: oyster mushrooms, heavy metals contamination, meta-analysis

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BACKGROUND

Mushroom is a consumer commodity in great demand globally. Of the approximately 300 cultivable mushroom species worldwide, only 30 have been successfully domesticated, and merely 10 are commercially cultivated on a large scale. Oyster mushrooms (*Pleurotus ostreatus*) represent the second most commercially significant cultivated mushroom species globally, positioned between *Agaricus bisporus* (button mushroom) in first place and *Lentinula edodes* (shiitake) in third (Picornell-Buendia et al., 2016).

In Indonesia, general mushroom consumption reached 0.18 kg per capita in 2019 (Directorate General of Horticulture, 2020), driven by the surging popularity of oyster mushrooms. During the COVID-19 pandemic, daily local demand skyrocketed from 300 kg to over 1 ton per day. The widespread public preference for oyster mushrooms stems from their high nutritional value and culinary versatility, as they can be prepared directly as a main dish or used as a natural flavoring due to their savory profile (Nadhifah et al., 2021). Per 100 grams, oyster mushrooms contain approximately 17.12 g of protein, 30.25 g of dietary fiber, 37.87 g of carbohydrates, and 2.60 g of fat, making them highly effective for boosting the human immune system (Rambey et al., 2019).

Beyond their basic nutritional profile, oyster mushrooms exhibit valuable pharmacological properties that can aid in disease therapy (Waktola & Temesgen, 2020). These therapeutic effects, ranging from antitumor, anticancer, antiviral, antibacterial, and antidiabetic properties to cholesterol-lowering and immunomodulatory activities, are mediated by bioactive compounds synthesized in the fruiting body, including β-D-glucan (pleuran), lovastatin, lectins, polysaccharides, glycopeptides, proteoglycans, and ubiquitin-like proteins (Waktola & Temesgen, 2020).

However, alongside their nutritional and therapeutic benefits, oyster mushrooms present potential human health risks due to their high susceptibility to heavy metal contamination. Common inorganic contaminants found in oyster mushrooms include arsenic (As), lead (Pb), mercury (Hg), and cadmium (Cd), all of which pose severe health hazards when consumed in excessive amounts (Ab-Rhaman et al., 2021). De et al. (2021) demonstrated that arsenic exposure primarily occurs through contaminated groundwater used in cultivation substrate preparation, which reduces amino acid content in fruiting bodies; consumption exceeding the tolerance limit of 15 μg kg^{-1} leads to arsenic accumulation in human excretory and respiratory organs, increasing cancer risks (Mleczek et al., 2016).

Lead contamination, often stemming from agricultural inputs and atmospheric deposition, threatens human reproductive health when exceeding the maximum allowable threshold of 0.3 mg kg^{-1} wet weight (Quarcoo & Adotey, 2013; Olatunji-Ojo et al., 2020). Similarly, mercury accumulates readily from cultivation media into fruiting bodies, causing systemic damage across human nervous, cardiovascular, digestive, and excretory systems (Rzymski et al., 2016). Cadmium, which accumulates rapidly under elevated ambient temperatures during cultivation, is strictly limited to 0.05 mg kg^{-1} (Sithole et al., 2022); exposure beyond natural thresholds risks skeletal, renal, hepatic, and visual impairments (Genchi et al., 2020).

Unlike organic compounds, these heavy metals do not readily decompose, accumulate more readily in fungi than in leafy green vegetables or fruits, and persistently enter the food chain via atmospheric deposition and contaminated pesticides (Mahendra et al., 2018; Liu et al., 2021). To evaluate these safety risks, studies quantify heavy metal concentrations and calculate the Health Risk Index (HRI), the ratio of daily heavy metal intake to the established oral reference dose (RfD) (Mahmood, 2014).

However, existing primary studies on heavy metal contamination in *Pleurotus ostreatus* yield widely inconsistent results due to variations in geographic regions, substrate compositions, and localized pollution sources. To date, there is a lack of a unified, quantitative synthesis evaluating the overall global contamination levels and pooled health risks associated with consuming oyster mushrooms. A meta-analysis is therefore crucial to aggregate these disparate datasets, resolve conflicting empirical findings, and establish statistical power across independent studies (Ahn & Kang, 2018). Therefore, the objective of this study is to perform a systematic review and meta-analysis using RevMan 5.4 to synthesize global data on heavy metal concentrations (As, Pb, Hg, Cd) in oyster mushrooms (Pleurotus ostreatus) and evaluate the pooled Health Risk Index (HRI) to provide a definitive assessment of consumer health risks.

SUBJECTS AND METHOD

1. Study Design

This study was a systematic review and meta-analysis conducted from February 2022 to August 2022. The research protocol followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines. Data analysis and generation of forest plots and funnel plots were performed using Review Manager (RevMan) software, version 5.4.

To ensure rigor and minimize subjective error during data collection and evaluation, all screening, extraction, and quality appraisal steps were conducted independently by two reviewers, with discrepancies resolved by consensus or consultation with a third author.

2. Selection Criteria

The eligibility criteria were established using the PICO (Population, Intervention, Comparison, Outcome) framework as detailed below:

Population (P): oyster mushroom consumers

Intervention (I): oyster mushrooms with high contaminant levels spefically heavy metals (arsenic, lead, mercury, and cadmium)

Comparison (C): uncontaminated oyster mushrooms

Outcome (O): health risk outcomes characterized by symptoms of toxicity

Inclusion Criteria:

- Full-text articles published in English in peer-reviewed journals.
- Studies involving Randomized Controlled Trials (RCT)
- Studies providing sufficient numerical outcome data, such as sample sizes, means, standard deviations, or event counts, to extract or calculate effect sizes.

Exclusion Criteria:

- Review articles, editorials, conference abstracts, letters, and case reports.
- Studies with insufficient or unextractable quantitative data are required for meta-analysis
- Duplicate studies

3. Screening Process

Literature searches were conducted across three electronic databases: Google Scholar, ScienceDirect, and PubMed.

4. Search Strategy and Keywords

The search used combinations of Medical Subject Headings (MeSH) terms, keywords, and boolean operators (“AND, “OR, “NOT). A representative search string used for database retrieval was:

("Target Population Keyword" OR "MeSH Term") AND ("Intervention Keyword" OR "MeSH Term") AND ("Outcome Keyword" OR "MeSH Term")

PRISMA workflow was also used for the literature search strategy, following these steps:

Identification: Initial search results were exported to reference management software to remove duplicate entries.

Screening: Titles and abstracts were screened independently against the inclusion criteria.

Eligibility: Full texts of potentially eligible articles were reviewed in detail.

Included: Articles meeting all inclusion criteria were retained for data extraction and synthesis. The selection process is summarized in a PRISMA flow diagram.

5. Risk of Bias Assessment

The methodological quality and risk of bias of the included studies were evaluated using the Cochrane Risk of Bias tool (RoB 2.0), which evaluates five domains: bias arising from the randomization process, bias due to deviations from intended interventions, bias due to missing outcome data, bias in measurement of the outcome, and bias in selection of the reported result. Among these, a subset of 10 studies meeting the quantitative criteria was included in the final meta-analysis. Overall, 80% of the meta-analyzed studies (8 out of 10) were assessed as having a low risk of bias, 20% (2 out of 10) presented some concerns primarily due to incomplete reporting of sequence generation (Table 1).

Table 1. Risk of bias results

Study	D1	D2	D3	D4	D5	Overall
Igbiri et al. (2018)	L	L	L	L	L	**L**
Liu et al. (2021)	L	L	L	L	L	**L**
Rashid et al. (2018)	SC	SC	L	SC	SC	**SC**
Sakellari et al. (2019)	L	L	L	L	L	**L**
Sithole et al. (2022)	L	L	L	L	L	**L**
De et al. (2020)	L	L	L	L	L	**L**
Koutrotsios et al. (2020)	L	L	L	L	L	**L**
Keskin et al. (2021)	L	L	L	L	L	**L**
Sarikurkcu et al. (2020)	L	L	L	L	L	**L**
Nnorom et al. (2022)	SC	L	SC	SC	SC	**SC**

D1 = Bias arising from the randomization process; D2 = Bias due to deviations from intended interventions; D3 = Bias due to missing outcome data; D4 = Bias in measurement of the outcome; D5 = Bias in selection of the reported result. L = Low risk; SC = Some concerns; H = High risk (Cochrane RoB 2.0). Of the 10 meta-analyzed studies, 8 (80%) were judged overall low risk and 2 (20%) some concerns, consistent with the domain-level proportions reported in the text.

6. Data Analysis

Meta-analytical calculations were executed using RevMan 5.4:

Effect Measures: Outcomes were expressed as Standardized Mean Differences (RR) with 95% Confidence Intervals (CIs). Continuous outcomes were reported as Standardized Mean Differences (SMD) with 95% CIs.

Heterogeneity Assessment: Between-study heterogeneity was evaluated using the Chi-square test and the statistic.

Model Selection: A random-effects model was applied to synthesize the data across all studies.

Publication Bias: For outcomes reported by a sufficient number of included studies, visual inspection of funnel plots was performed.

RESULTS

Article Search and Screening Process

There is a lot of primary research related to oyster mushrooms (*Pleurotus ostreatus*) published over a period of 10 years (2012-2022) regarding their ability to absorb pollutants, including heavy metals. Because individual primary studies differ in intervention type and intervention level, meta-analysis was used to synthesize consistency across studies, obtain pooled effect-size estimates, and evaluate potential health risks based on the Health Risk Index (HRI).

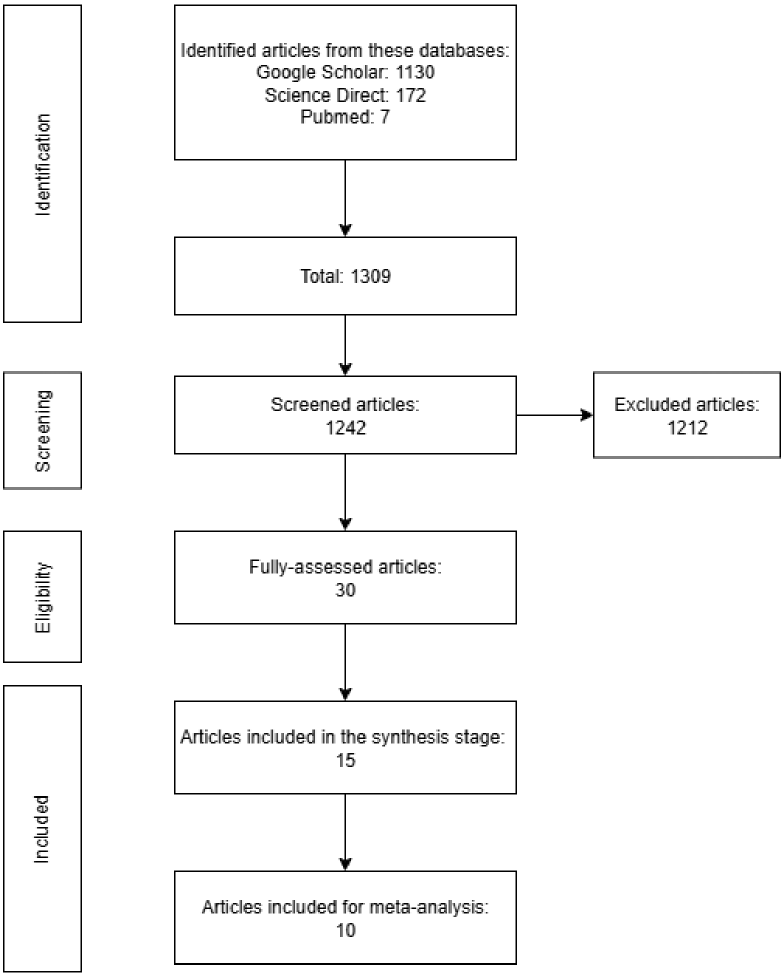

Figure 1. PRISMA Flow Diagram

The literature search was conducted across the Google Scholar, PubMed, and Science Direct databases and yielded a total of 1,309 articles. During the screening stage, records were processed in Mendeley to remove duplicates, leaving 1,242 unique articles. Of these, 1,212 were excluded because the research theme was not relevant to the review question or the article was not published in English, as illustrated in the PRISMA flow diagram (Figure 1).

At the eligibility stage, 30 full-text articles were assessed. This assessment resulted in 15 articles being retained for the synthesis stage; the remaining 15 were excluded for the following reasons: the mushroom species studied was not Pleurotus ostreatus even though heavy-metal and HRI data were reported; the research focus was the growing medium rather than metal accumulation in the mushroom itself; heavy-metal and HRI data were presented only as a bar chart without exact numerical values and with an incomplete accompanying explanation; the article did not assess contamination by arsenic, lead, mercury, or cadmium; or the article reported metal types and associated health hazards without reporting the HRI.

Study Characteristics

Of the 15 articles retained for synthesis, ten reported extractable numerical data (mean, standard deviation, and sample size) that could be pooled quantitatively for at least one of the four heavy metals of interest; the characteristics of these ten studies are summarized in Table 2. The studies varied considerably in geographic origin, mushroom source (wild-harvested versus cultivated), and the panel of metals assessed, which is described further below.

Table 2. Characteristics of Studies Included in the Quantitative Synthesis

Study (Author, Year)	Country / Setting	Mushroom Source	Heavy Metals Assessed	Sample Size (n)
Igbiri et al. (2018)	Port Harcourt, Nigeria	Cultivated	Pb, Cd	5
Liu et al. (2021)	Retail market samples	Cultivated	Pb, As, Cd, Hg	42
Rashid et al. (2018)	Not reported	Not reported	Pb, As, Cd, Hg	40
Sakellari et al. (2019)	Not reported	Not reported	Pb	32
Sithole et al. (2022)	Pretoria, South Africa	Wild (mine-impacted soil)	Pb, As, Cd	4
De et al. (2020)	Experimental substrate	Cultivated (metal-enriched substrate)	As	4
Koutrotsios et al. (2020)	Not reported	Cultivated	As, Cd	28
Keskin et al. (2021)	Belgrade Forest, Istanbul, Türkiye	Wild (oak-wood substrate)	Cd, Hg	1
Sarikurkcu et al. (2020)	Ankara, Türkiye	Wild	Cd	1
Nnorom et al. (2022)	Not reported	Not reported	Hg	10

n.a. = not applicable/not reported in the source article.

Extraction of Health-Risk Indicators

Statistical analysis was performed in RevMan 5.4. Data were entered as continuous outcomes, using the mean and standard deviation of contaminant concentration together with the sample size of the control and intervention groups (Friedrich et al., 2011). Because most primary studies did not report an independent control group, the control-group figures used in Tables 2 through 5 were represented, for all four metals, by the values reported in Liu et al. (2021); this study was selected because the body-weight data of its participants were incorporated into the overall sample average, making it the most representative available proxy for an unexposed reference group.

Meta-Analysis Results

1. Lead (Pb)

Five studies reported extractable concentration and HRI data for lead, the pollutant with the highest number of contributing studies among the four metals assessed. The pooled analysis is summarized in Table 3.

Table 3. Statistical Analysis of Lead (Pb) in Oyster Mushrooms

Studies	IG			CG			Weight	SMD IV, Random, 95% CI
	Mean	SD	Total	Mean	SD	Total		
Igbiri et al (2018)	0.01	0.68	5	0.01	0.03	5	20.7%	0.02 [-1.22, 1.26]
Liu et al. (2021)	0.38	0.47	42	0.01	0.03	42	24.7%	1.13 [0.67, 1.59]
Rashid et al. (2018)	0.22	0.13	40	0.01	0.03	40	24.1%	2.30 [1.73, 2.87]
Sakellari et al. (2019)	0.30	0.08	32	0.01	0.03	32	21.6%	5.02 [4.00, 6.04]
Sithole et al. (2022)	0.433	0.01	4	0.32	0.03	4	10.9%	4.45 [1.09, 7.80]
Total (95% CI)			123			123	100.0%	2.37 [0.08, 3.87]
Heterogeneity: $Tau^2 = 2.41$; $Chi^2 = 59.38$, df = 4 ($p < 0.001$); $I^2 = 93\%$								
Test for overall effect: Z = 3.12 (p= 0.002)								

Description: **Mean:** average lead pollutant in oyster mushrooms (in mg/kg) **SD:** standard deviation. **Total:** the total sample studied. **Weight:** shows how informative a study is.
Standardized Mean Difference IV, Random, 95% CI: Shows an average summary of the overall study and confidence intervals. **Heterogeneity:** variation of data. **P Value:** probability.

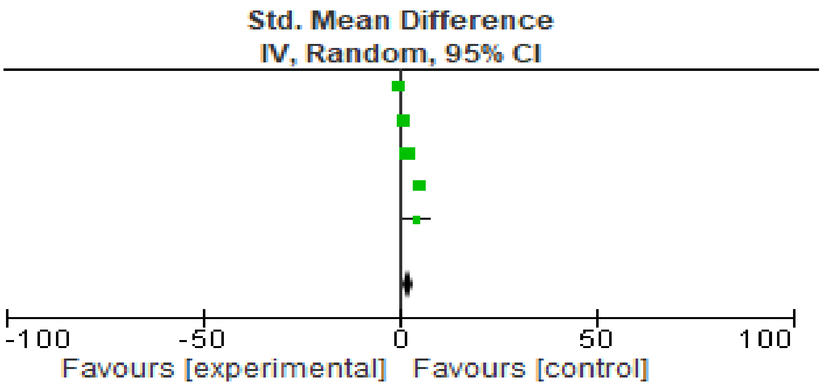

Figure 2. Forest Plot of Lead Analysis

The pooled standardized mean difference (SMD) for lead was 2.37 (95% CI: 0.08 to 3.87; Z = 3.12; P = 0.002), indicating a statistically significant difference in lead concentration between the intervention and control groups. Heterogeneity was high (I^2 = 93%), and a random-effects model was therefore used. The forest plot of the lead analysis is presented in Figure 2. Among the five contributing studies, Sithole et al. (2022) reported the highest mean lead concentration (0.433 mg/kg), and Igbiri et al. (2018) reported the lowest (0.03 mg/kg).

2. Arsenic (As)

Five studies reported extractable data for arsenic. The pooled analysis is summarized in Table 4.

Table 4. Statistical Analysis of Arsenic (As) in Oyster Mushrooms

Studies	Intervention Group			Control Group			Weight	SMD IV, Random, 95% CI
	Mean	SD	Total	Mean	SD	Total		
De et al. (2020)	10.04	0.06	4	0.245	0.01	4	0.1%	197.36 [61.61, 333.11]
Koutrotsios et al. (2020)	0.01	0.19	28	0.01	0.01	28	27.5%	0.00 [-0.52, 0.52]
Liu et al. (2021)	0.41	0.46	42	0.01	0.01	42	27.6%	1.22 [0.75, 1.69]
Rashid et al. (2018)	0.45	0.11	40	0.01	0.01	40	26.6%	5.60 [4.61, 6.59]
Sithole et al. (2022)	0.19	0.08	4	0.05	0.02	4	21.8%	2.07 [0.08, 4.06]
Total (95% CI)			118			118	100.0%	2.24 [0.08, 4.40]

Heterogeneity: Tau^2= 4.53, Chi^2= 104.39, df = 4 (p <0.001), I^2=96%
Test for the whole effect: Z = 2.04 (P=0.04)

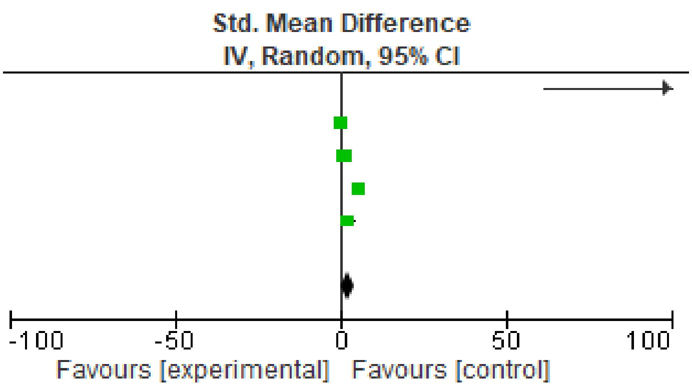

Figure 3. Forest Plot of Arsenic Analysis

The pooled SMD for arsenic was 2.24 (95% CI: 0.08 to 4.40; p= 0.04), a statistically significant result. Heterogeneity was again high (I^2 = 96%), consistent with the use of a random-effects model. The forest plot for arsenic is shown in Figure 3.

De et al. (2020) reported the highest mean arsenic concentration (10.04 mg/kg), obtained from mushrooms cultivated on an experimentally arsenic-enriched substrate (7.85 mg/kg); Koutrotsios et al. (2020) reported the lowest concentration (0.01 mg/kg), obtained from cultivated mushrooms.

3. Cadmium (Cd)

Seven studies reported cadmium data, the largest number of contributing studies among the four metals. The pooled analysis is summarized in Table 5.

Table 5. Statistical Analysis of Cadmium (Cd) in Oyster Mushrooms

Studies	Intervention Group			Control Group			Weight	SMD IV, Random, 95% CI
	Mean	SD	Total	Mean	SD	Total		
Igbiri et al. (2018)	0.13	0.45	5	0.07	0.01	5	21.1 %	0.17 [-1.07, 1.41]
Keskin et al. (2021)	1.04	0.07	1	0.07	0.01	1	Not able to estimate	Not able to estimate
Koutrotsios et al (2020)	0.3	0.62	28	0.07	0.01	28	24.2%	0.52 [-0.02, 1.05]
Liu et al. (2021)	0.88	0.83	42	0.07	0.01	42	24.4%	1.37 [0.89, 1.84]
Rashid et al. (2018)	0.41	0.1	40	0.07	0.01	40	23.3%	4.76 [3.88, 5.63]
Sarikurkcu et al. (2020)	1.46	0.08	1	0.07	0.01	1	Not able to estimate	Not able to estimate
Sithole et al (2022)	0.15	0.02	4	0.05	0.01	4	9.4%	5.64 [1.52, 9.75]
Total (95% CI)			121			121	100.0%	2.08 [0.46, 3.70]
Heterogeneity: Tau^2= 2.83; Chi^2= 74.92, df = 4 (p <0.001); I^2= 95%								
Test for overall effect: Z = 2.52 (p= 0.01)								

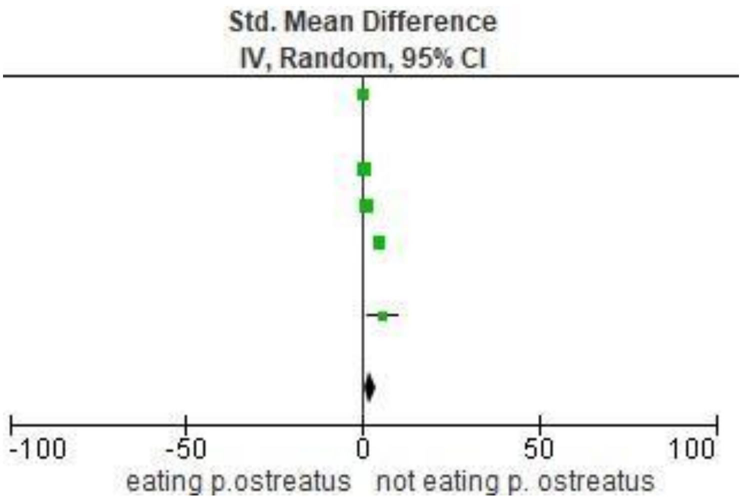

Figure 4. Forest Plot of Cadmium Analysis

The pooled SMD for cadmium was 2.08 (95% CI: 0.46 to 3.70; Z = 2.52; P = 0.01), a statistically significant result. Heterogeneity was high (I^2 = 95%). Weight and confidence intervals could not be estimated for Keskin et al. (2021) and Sarikurkcu et al. (2020) because each study contributed a single sample with no internal comparator (Riley et al., 2018). The forest plot for cadmium is shown in Figure 4.

Sarikurkcu et al. (2020) reported the highest mean cadmium concentration (1.46 mg/kg) from wild mushrooms collected in Ankara, Türkiye; Igbiri et al. (2018) reported the lowest concentration (0.12 mg/kg) from cultivated mushrooms in Port Harcourt, Nigeria. Despite its high mean concentration, the Sarikurkcu et al. (2020) study together with Igbiri et al. (2018) reported an HRI below 1.

Table 6. Statistical Analysis of Mercury (Hg) in Oyster Mushrooms

Studies	Intervention Group			Control Group			Weight	SMD IV, Random, 95% CI
	Mean	SD	Total	Mean	SD	Total		
Keskin et al. (2021)	1.461	0.43	1	0.03	0.01	1	Not able to estimate	Not able to estimate
Liu et al. (2021)	0.06	0.12	42	0.03	0.01	42	48.9%	0.35 [-0.08, 0.78]
Nnorom et al. (2022)	0.0293	0.01	10	0.03	0.01	10	32.9%	-0.11 [-0.99, 0.76]
Rashid et al. (2018)	0.124	0.03	40	0.03	0.01	40	33.1%	4.39 [3.56, 5.21]
Total (95% CI)			93	0.01	0.01	93	100.0%	1.53 [-1.01, 4.08]

Heterogeneity: Tau^2= 4.913; Chi^2= 79.92, df = 2 (p< 0.001); I^2 =97%
Test for the overall effect: Z = 1.18 (P = 0.24)

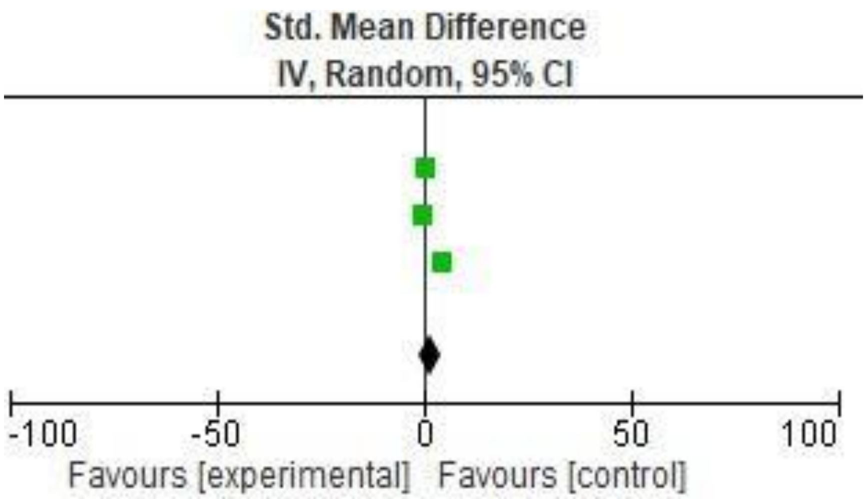

Figure 5. Forest Plot of Mercury Analysis

4. Mercury (Hg)

Four studies reported mercury data. The pooled analysis is summarized in Table 6.

The pooled SMD for mercury was 1.53 (95% CI: -1.01 to 4.08; Z = 1.18; P = 0.24). Unlike lead, arsenic, and cadmium, this result was not statistically significant, and the wide confidence interval crosses the null value. Weight and a confidence interval could not be estimated for Keskin et al. (2021), which is why only three data points are plotted in the forest plot (Figure 5), although four studies are listed in Table 5.

Keskin et al. (2021), conducted in Belgrade Forest, Istanbul, Türkiye, reported the highest mean mercury concentration (1.461 mg/kg); Liu et al. (2021), based on cultivated market samples, reported the lowest (0.06 mg/kg). The HRI reported by Keskin et al. (2021) exceeded 1, while the

HRI reported by Liu et al. (2021) was below 1.

Publication Bias

Publication bias was assessed visually using a funnel plot (Figure 6), in which the vertical axis represents each study's standard error and the horizontal axis represents the standardized mean difference between the intervention and control groups.

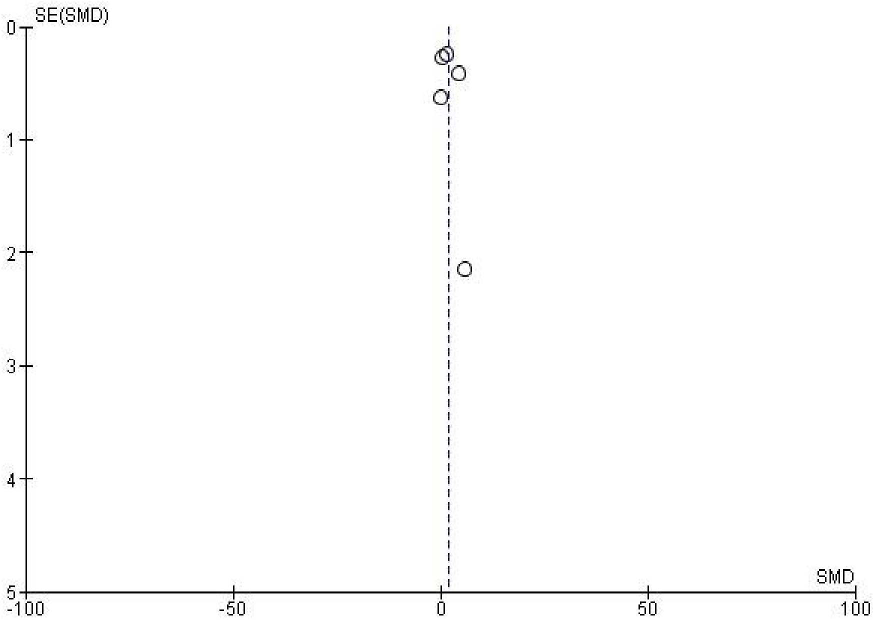

Figure 6. Funnel Plot

The plotted studies clustered close to the top of the funnel, indicating small standard errors, with the distribution appearing broadly symmetrical around the pooled estimate; one study fell toward the bottom of the plot, indicating comparatively lower precision.

DISCUSSION

This meta-analysis synthesized primary research published between 2012 and 2022 on the accumulation of four heavy metals: lead, arsenic, cadmium, and mercury in the oyster mushroom (Pleurotus ostreatus). Meta-analysis was selected as the analytic approach because it occupies the highest tier of the evidence-based research hierarchy: by pooling effect estimates across studies that share a common research question but differ in intervention type and intervention level, it identifies the consistent signal that individual studies, taken alone, cannot establish with the same precision (Pubrica, 2019). Consistent with this framework, the pooled standardized mean differences (SMD) indicated a graded pattern of association across the four metals, with lead showing the strongest effect (SMD = 2.37), followed by arsenic (2.24), cadmium (2.08), and mercury (1.53). Lead, arsenic, and cadmium reached statistical significance, whereas mercury did not.

A recurring pattern across the included studies was that cultivated oyster mushrooms —grown on controlled substrates or sold through retail markets — consistently showed lower heavy-metal concentrations than wild-harvested mushrooms collected from environmentally impacted sites. The lowest lead and mercury concentrations, for example, were both reported by Liu et al. (2021), who sampled cultivated mushrooms from markets, whereas the highest concentrations of lead, cadmium, and mercury were reported in mushrooms collected from a mining site in Pretoria, South Africa (Sithole et al., 2022), an urban site with mining-related activity in Ankara, Türkiye (Sarikurkcu et al., 2020), and an oak forest in Istanbul, Türkiye (Keskin et al., 2021), respectively. This pattern supports the interpretation that the growing environment and cultivation method, rather than the species itself, are the principal drivers of heavy-metal accumulation in oyster mushrooms, since *Pleurotus ostreatus* is known to be an efficient bioaccumulator that readily absorbs whatever metals are present in its growth substrate.

The exception to this pattern was arsenic, where the highest concentration was recorded not in a wild sample but in mushrooms deliberately cultivated on an arsenic-enriched experimental substrate (De et al., 2020). This exception reinforces, rather than contradicts, the broader conclusion that substrate composition, whether occurring naturally at a contaminated site or introduced experimentally, is the determinant of metal uptake.

This substrate-driven pattern is corroborated by external literature that was not part of the present synthesis. A risk assessment of wild Pleurotus ostreatus and *P. djamor* collected across forest, residential, industrial, and transportation-impacted sites in Rajaji National Park, India, found that sampling locations near industrial and traffic activity carried significantly higher heavy-metal loads than less-impacted sites, even within the same mushroom species (Bhat et al., 2022).

A survey of wild-growing edible mushrooms across industrial and non-industrial areas of southern Poland similarly found that more than half of sampled specimens exceeded European Union limits for cadmium, lead, or mercury, with the degree of accumulation strongly related to distance from emission sources. A study conducted on oyster mushrooms cultivated on soil collected from a mine-impacted site in Pretoria concluded that *P. ostreatus* possesses a marked capacity to bioaccumulate toxic metals from polluted soils and recommended against harvesting mushrooms grown on contaminated land. Taken together with the current findings, this body of evidence consistently identifies site contamination and cultivation practice, rather than intrinsic species susceptibility, as the dominant explanation for elevated heavy-metal burdens in oyster mushrooms.

The mercury finding warrants a more cautious interpretation than the other three metals. Although the pooled SMD of 1.53 might, on its face, suggest an elevated risk comparable in magnitude to cadmium, the 95% confidence interval spanned from 1.01 to 4.08 and therefore crossed the null value, and the associated P-value of 0.24 was well above the conventional 0.05 threshold. This pooled estimate should therefore be read as statistically inconclusive rather than as evidence of a genuine difference between mercury-exposed and control samples, and the non-significant result should not be characterized, as in some previous descriptions of these data, as demonstrating a defined multiple of health risk.

The imprecision is compounded by the small number of contributing studies (four, of which only three could be weighted) and by the very high heterogeneity (I^2 = 97%), which together limit the power of this particular pooled analysis to detect a true effect even if one exists. A more defensible reading is that current evidence on mercury accumulation in oyster mushrooms remains too limited and too inconsistent to support a confident risk conclusion, and that additional, adequately powered primary studies are needed before firmer statements about mercury-related health risk can be made.

The heterogeneity observed across all four pooled analyses (I^2 ranging from 93% to 97%) was consistently very high, exceeding the threshold at which the Cochrane Handbook classifies heterogeneity as considerable. Several plausible sources of this variability can be identified from the characteristics of the included studies. First, and most prominently, the studies differed in mushroom source: wild-harvested specimens collected from mining sites, forests, and urban areas were pooled alongside cultivated specimens grown on controlled or market-sourced substrates, and these two categories are known from the wider literature to differ systematically in metal uptake.

Second, the geographic origin of the samples varied widely, spanning Nigeria, South Africa, Türkiye, and other locations with markedly different baseline soil and environmental contamination profiles. Third, sample sizes varied enormously, from single-specimen studies (Keskin et al., 2021; Sarikurkcu et al., 2020) to studies with more than forty samples (Liu et al., 2021; Rashid et al., 2018), which affects the precision, and therefore the relative influence, of each study on the pooled estimate.

Fourth, because most primary studies did not report an independent control group, this analysis substituted the control values from a single study (Liu et al., 2021) across all four metals; while methodologically necessary given the available data, this substitution is itself a source of between-study inconsistency, since it introduces a shared reference point that does not reflect the true local baseline of each original study population. Finally, differences in analytical technique, sample matrix (whole fruiting body versus cap or stalk), and reporting units across studies further contribute to the observed variability.

A formal subgroup analysis stratified by mushroom source (wild versus cultivated), sensitivity analysis using a leave-one-out procedure, and meta-regression against candidate moderators such as sampling depth, distance from a pollution source, or analytical method would be the appropriate next steps to formally quantify these sources of heterogeneity (Rice, Higgins, & Lumley, 2018).

However, with only four to seven studies contributing to each metal-specific pool in the present dataset, the number of studies falls below the minimum typically recommended for a statistically stable subgroup or meta-regression analysis, which is generally cited as at least five to ten studies per subgroup. For this reason, the wild-versus-cultivated distinction discussed above is presented here as a narrative, descriptive comparison drawn directly from the study characteristics rather than as a formally tested subgroup effect, and we recommend that future updates of this meta-analysis once a larger pool of primary studies becomes available, conduct these analyses formally to quantify, rather than only describe, the contribution of mushroom source, geographic region, and analytical method to the observed heterogeneity.

In conclusion, this meta-analysis confirms that *Pleurotus ostreatus* heavily accumulates lead, arsenic, and cadmium compared to baseline levels, following a clear hierarchy of Pb > As > Cd > Hg, while the evidence for mercury remains inconclusive. Rather than reflecting simple statistical noise, the high heterogeneity across studies (I2 = 90%) points to fundamental biological and methodological differences. On a biological level, the non-specific bioaccumulation capacity of fungal mycelia means that metal uptake varies heavily with local substrate conditions, such as pH, organic matter content, bioavailable metal fractions, and chemical speciation (including organic versus inorganic forms of arsenic and mercury). Methodologically, combining data from wild-foraged specimens in contaminated zones with controlled agricultural samples, alongside relying on a single proxy control group (Liu et al., 2021), naturally introduces substantial variance into the pooled estimates.

These patterns carry direct implications for food safety and public health policy. Although commercially cultivated oyster mushrooms grown on clean agricultural substrates pose minimal heavy-metal exposure risk to consumers, wild harvesting near mining, industrial, or high-traffic areas presents a genuine hazard that can breach established dietary thresholds. Addressing these risks requires two key actions: implementing mandatory substrate monitoring and quality control standards for commercial growers, and discouraging wild mushroom foraging in urban or industrial landscapes. Finally, to turn these initial meta-analytic trends into reliable quantitative risk indicators, such as Target Hazard Quotients, future primary studies must standardize their protocols by reporting on a uniform dry-weight basis, incorporating study-specific control groups, and fully profiling substrate chemistry.

Several limitations qualify the conclusions of this meta-analysis and should inform how its results are used. The most consequential is the very high heterogeneity across all four pooled estimates, which indicates that a substantial portion of the variability in observed effect sizes reflects genuine differences between studies, in mushroom source, geography, sample size, and methodology, rather than sampling error alone; under such heterogeneity, a single pooled SMD should be interpreted as an approximate average across a diverse set of conditions rather than as a precise, generalizable estimate of risk for any specific consumption scenario. Publication bias is a second concern: although the funnel plot appeared broadly symmetrical, each metal-specific pool included only four to seven studies, which is too small a number for a funnel plot, or for formal small-study-effect tests such as Egger's regression, to reliably detect asymmetry; the appearance of symmetry in this case should therefore be treated as inconclusive rather than as confirmation that publication bias is absent.

Methodological variability across the included studies compounds both concerns: studies differed in analytical technique, in whether whole fruiting bodies or specific tissues (cap versus stalk) were analyzed, in units of measurement, and, critically, in whether an independent control group was reported at all, which necessitated substituting a single external study's control values across every metal-specific analysis in this review. Together, these limitations mean that the statistically significant pooled findings for lead, arsenic, and cadmium should be regarded as an indication of an association warranting regulatory and research attention rather than as a precise, decision-ready risk quantification, and that the non-significant mercury finding should be treated as inconclusive rather than reassuring.

Future research addressing these gaps, larger and more geographically diverse primary studies, consistent inclusion of matched control groups, standardized analytical protocols, and, once feasible, formal subgroup, sensitivity, and meta-regression analyses, would substantially strengthen the evidence base underlying these conclusions.

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CONFLICT OF INTEREST

We confirm that there is no personal or financial interests that could be perceived as influencing the results of the study.

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