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Health Risk Assessment of Toxic Metal Accumulation in *Pleurotus ostreatus* Cultivated on Agricultural Residues Amended with Calcium Carbonate

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Abstract

Agricultural residues provide sustainable substrates for mushroom cultivation, but their potential to accumulate toxic metals may compromise food safety. This study evaluated the concentrations of Cd, Cr, Ni, and Pb in *Pleurotus ostreatus* cultivated on cassava peel, maize stalk, paddy straw, cotton stalk, and their combinations supplemented with 0, 5, 10, and 15 g calcium carbonate. Heavy metal concentrations were determined, and non-carcinogenic and carcinogenic health risks associated with mushroom consumption were assessed using hazard quotient (HQ), hazard index (HI), and cancer risk (CR) models. Significant differences ($p < 0.05$) were observed among the substrate treatments, demonstrating that substrate composition and calcium carbonate supplementation strongly influenced heavy metal accumulation. Paddy straw-based substrates accumulated the highest concentrations of Cr, Ni, and Pb, whereas cassava peel-based substrates generally contained higher Cd levels. Increasing calcium carbonate supplementation consistently reduced metal accumulation across most substrate combinations, indicating reduced metal

bioavailability under alkaline conditions. The HQ values for individual metals and the cumulative HI remained below the USEPA safety threshold of 1.0 for all treatments, suggesting negligible non-carcinogenic health risks. Although Cd and Ni contributed most to the HI, their estimated dietary exposures remained within acceptable limits. Carcinogenic risk assessment identified Cd and Ni as the primary contributors to lifetime cancer risk, while Cr and Pb made comparatively smaller contributions. Calcium carbonate amendment substantially lowered cumulative cancer risk across the substrates, with the greatest reductions observed at the 15 g supplementation level. The findings demonstrate that agricultural wastes can be safely utilized for *P. ostreatus* cultivation when appropriate substrates are selected and supplemented with calcium carbonate.

Keywords: *Pleurotus ostreatus*; agricultural residues; heavy metals; health risk assessment; food safety; mushroom cultivation

1. Introduction

The generation of agricultural residues has increased substantially due to the expansion of agricultural production and population growth. Although these lignocellulosic by-products are rich in organic matter and nutrients, their disposal remains a major environmental challenge. In many developing countries, including Nigeria, agricultural residues are frequently managed through open-field burning, a practice that releases particulate matter, greenhouse gases, and other air pollutants, thereby contributing to environmental degradation, climate change, and adverse human health effects (FAO, 2021; Jain et al., 2022). Consequently, sustainable approaches for converting agricultural wastes into value-added products have become increasingly important as part of circular bioeconomy and waste valorisation strategies (IEA, 2020; Jain et al., 2022).

Mushroom cultivation represents one of the most effective biological methods for agricultural waste recycling. Species of *Pleurotus*, particularly *Pleurotus ostreatus*, possess efficient lignocellulolytic enzyme systems capable of degrading cellulose, hemicellulose, and lignin, enabling them to grow on a wide range of agricultural residues (Ahmed et al., 2013; Adebayo & Martinez-Carrera, 2015; Fufa et al., 2021). Besides reducing environmental pollution, mushroom cultivation transforms low-value agricultural wastes into nutritious food rich in proteins, dietary fibre, vitamins, minerals, and bioactive compounds.

The nutritional importance of mushrooms has become increasingly significant in regions experiencing food insecurity. Rising food prices, declining purchasing power, and increasing demand for affordable protein sources have stimulated greater interest in edible mushrooms as an alternative source of high-quality dietary protein (Pashaei et al., 2024). Unlike animal-derived proteins, mushrooms can be cultivated using inexpensive agricultural by-products with relatively low environmental impacts, making them an economically viable component of sustainable food systems.

Despite these advantages, food safety remains an important consideration because mushrooms possess an exceptional capacity to absorb and accumulate trace elements from their cultivation substrates (Adebiyi & Adeyemi, 2020). This characteristic, while beneficial for environmental remediation, may also result in the accumulation of toxic heavy metals in edible fruiting bodies. Essential elements such as Fe, Zn, Mn, and Cu play important physiological roles at appropriate concentrations but may become toxic when present at elevated levels (Taiwo et al., 2018). Conversely, non-essential metals including Cd, Pb, Hg, and As exhibit no known biological function and may pose serious health risks even at relatively low concentrations (Ndimele et al., 2017).

Heavy metal contamination originates from both natural geological processes and anthropogenic activities, including industrial emissions, agricultural inputs, wastewater irrigation, mining, and atmospheric deposition. Because mushrooms readily accumulate metals from their growth substrates, substrate selection has a direct influence on mushroom quality and consumer safety (Obodai et al., 2014). While numerous studies have investigated heavy metal contamination in wild mushrooms, comparatively fewer studies have evaluated cultivated mushrooms produced from agricultural residues under controlled conditions, particularly in Nigeria. Moreover, information regarding the human health risks associated with consuming mushrooms cultivated on different agricultural waste substrates remains limited.

Assessing dietary exposure to toxic metals through cultivated mushrooms is therefore essential for ensuring food safety and promoting consumer confidence. Human health risk assessment models, including estimated daily intake (EDI), target hazard quotient (THQ), hazard index (HI), and lifetime cancer risk (CR), provide internationally accepted approaches for evaluating potential health risks associated with food consumption. Accordingly, this study investigated the accumulation of selected toxic metals (Cd, Cr, Ni and Pb) in *Pleurotus ostreatus* cultivated on different agricultural waste substrates amended with calcium carbonate and evaluated the associated non-carcinogenic and carcinogenic health risks to consumers. The findings provide important evidence supporting the safe utilization of agricultural residues for sustainable mushroom production and agricultural waste management.

2. Materials and Methods

2.1. Study Area

The experiment was conducted at the Mushroom Research Station of the Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria, under controlled environmental

conditions (Figure 1). Established in 1988, FUNAAB occupies approximately 10,200 ha and is dedicated to teaching, research, and extension services in agriculture. The controlled mushroom production facility provided suitable environmental conditions for the cultivation of *Pleurotus ostreatus*, minimizing the influence of external climatic variations on mushroom growth.

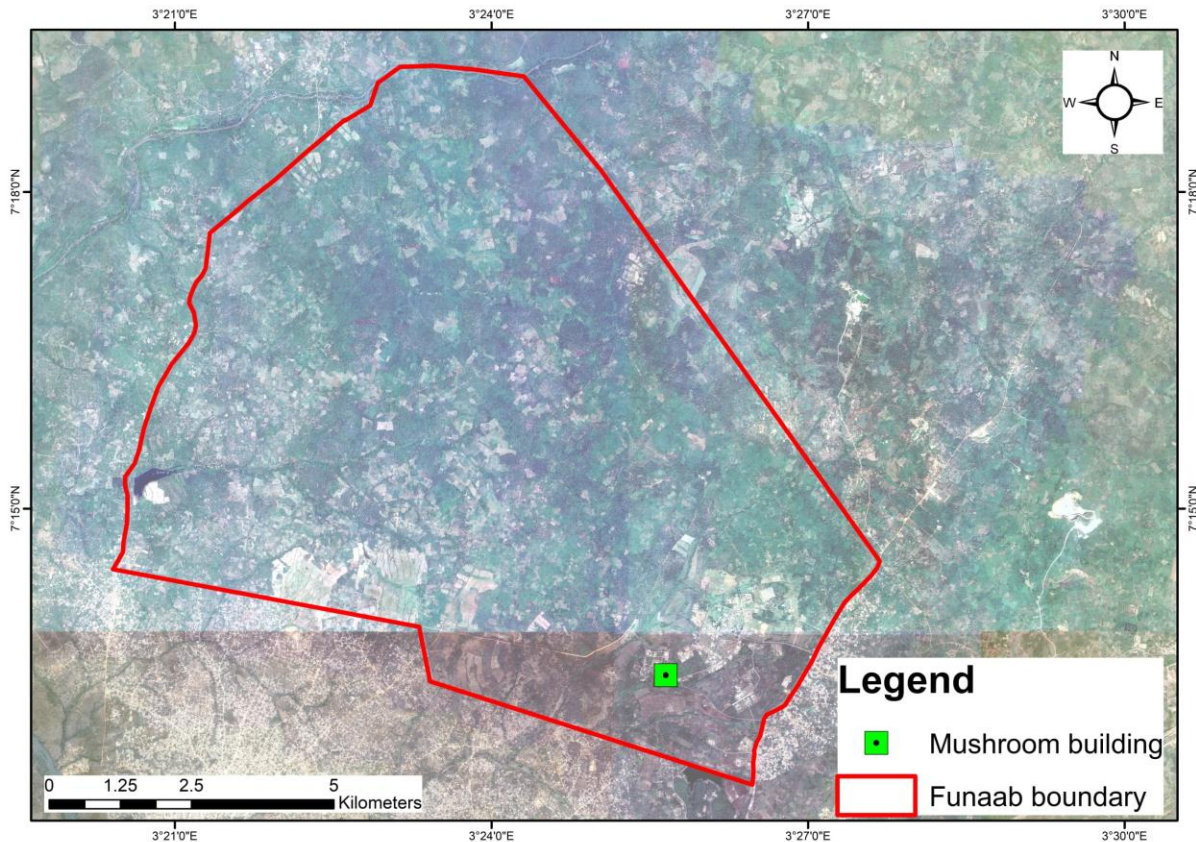


Figure 1: Map showing mushroom station in FUNAAB

2.2. Collection and Preparation of Substrates

Four lignocellulosic agricultural residues [paddy straw (PS), cotton stalk (CS), maize stalk (MS), and cassava peel (CP)] were used as cultivation substrates. Paddy straw, cotton stalk, and maize stalk were collected from the National Centre for Agricultural Mechanization (NCAM), Ilorin,

Nigeria, while cassava peels were obtained from a commercial cassava processing facility at Ganmo along the Abuja–Lokoja Highway.

Before substrate preparation, the physicochemical characteristics, including pH, moisture content, and heavy metal concentrations, were determined to establish baseline properties. The substrates were mechanically shredded into pieces approximately 0.5–1.0 cm in length using a purpose-built shredding machine, ensuring uniform particle size and improved substrate compaction.

The substrates were evaluated both individually and as binary mixtures prepared at a 1:1 (w/w) ratio. Each substrate was soaked separately in clean water for 24 h to achieve adequate hydration following the procedure described by Jasińska and Siwulski (2021). Excess water was removed manually, and the substrates were spread on a clean tarpaulin to attain the desired moisture content while minimizing contamination.

A 600 g (wet weight) of each prepared substrate was packed into sterile polyethylene bags (20 × 33 cm; 8 × 13 inches). The bags were sealed with plastic neck rings fitted with absorbent cotton plugs and secured with rubber bands to facilitate gas exchange while preventing microbial contamination.

2.3. Experimental Design and Substrate Formulation

Calcium carbonate (CaCO_3) was incorporated as a pH-adjusting additive at four application rates (0, 5, 10, and 15 g per 600 g substrate). Ten substrate treatments were prepared using either single agricultural residues or binary substrate combinations. Binary mixtures consisted of equal proportions (300 g each) of the two constituent substrates.

The experiment therefore consisted of ten substrate formulations, each evaluated under four CaCO_3 supplementation levels (Table 1), resulting in forty treatment combinations. All treatments were prepared in triplicate using a completely randomized design (CRD).

Table 1. Experimental treatments used for the cultivation of *Pleurotus ostreatus* on agricultural residues supplemented with calcium carbonate.

Treatment	Substrate Composition	Substrate Weight (g/bag)	CaCO_3 Level (g)	Treatment Code
T ₁	Paddy straw (PS)	600	0, 5, 10, 15	PS, PS+5, PS+10, PS+15
T ₂	Cotton stalk (CS)	600	0, 5, 10, 15	CS, CS+5, CS+10, CS+15
T ₃	Maize stalk (MS)	600	0, 5, 10, 15	MS, MS+5, MS+10, MS+15
T ₄	Cassava peel (CP)	600	0, 5, 10, 15	CP, CP+5, CP+10, CP+15
T ₅	PS + CS (1:1)	300 + 300	0, 5, 10, 15	PS+CS, PS+CS+5, PS+CS+10, PS+CS+15
T ₆	PS + MS (1:1)	300 + 300	0, 5, 10, 15	PS+MS, PS+MS+5, PS+MS+10, PS+MS+15
T ₇	PS + CP (1:1)	300 + 300	0, 5, 10, 15	PS+CP, PS+CP+5, PS+CP+10, PS+CP+15
T ₈	MS + CP (1:1)	300 + 300	0, 5, 10, 15	MS+CP, MS+CP+5, MS+CP+10, MS+CP+15
T ₉	MS + CS (1:1)	300 + 300	0, 5, 10, 15	MS+CS, MS+CS+5, MS+CS+10, MS+CS+15
T ₁₀	CP + CS (1:1)	300 + 300	0, 5, 10, 15	CP+CS, CP+CS+5, CP+CS+10, CP+CS+15

T-treatment; PS = Paddy straw; CS = Cotton stalk; MS = Maize stalk; CP = Cassava peel.

2.4. Substrate Pasteurization

Prepared substrate bags were pasteurized using a steam-heating system powered by liquefied petroleum gas (LPG) (Figure 3). The bags were placed inside cotton sacks, submerged in water contained in a metal drum, and heated at approximately 100°C for 90 min. Following pasteurization, the hot water was completely drained before processing subsequent batches to prevent cross-contamination and changes in substrate physicochemical properties. The pasteurized

substrates were allowed to cool naturally to room temperature (approximately $23 \pm 2^{\circ}\text{C}$) before inoculation.

2.5. Inoculation and Mushroom Cultivation

Spawn of *Pleurotus ostreatus* was prepared on sterilized sorghum grains following the protocol described by Jiskani et al. (2007). Each substrate bag was inoculated aseptically with approximately one tablespoon (≈ 20 g) of spawn placed near the upper surface of the substrate. The inoculum was gently pressed into the substrate to promote rapid mycelial colonization. To improve aeration, a 1.5-inch PVC pipe (approximately 0.5 cm in length) was inserted into the upper portion of each bag to create an air channel. The opening was loosely covered with sterile cloth to permit air exchange while preventing insect infestation and microbial contamination.

The inoculated bags were incubated in a dark cropping room until complete mycelial colonization. Upon full colonization, small openings were made in the bags to facilitate fruiting body emergence. Throughout the fruiting period, the bags were irrigated twice daily to maintain adequate relative humidity. Mature fruiting bodies were harvested by gently twisting the base of the stipe to avoid damage to the substrate. Harvesting was carried out continuously for seven consecutive weeks, and the fresh weight of mushrooms harvested from each treatment was recorded weekly.

2.6. Toxic Metal Analysis

Harvested mushroom samples were air-dried to constant weight and pulverized into a fine powder. Approximately 1.0 g of each sample was transferred into a digestion flask and digested using an aqua regia mixture ($\text{HNO}_3\text{:HCl}$, 3:1, v/v), consisting of 9 mL concentrated nitric acid (HNO_3) and 3 mL concentrated hydrochloric acid (HCl). The digestion mixture was heated on a hot plate until

dense brown nitrogen oxide fumes disappeared, indicating complete digestion. After cooling to room temperature, the digest was diluted with distilled water, filtered through Whatman No. 42 filter paper, and made up to a final volume of 50 mL in a volumetric flask. The filtrate was transferred into acid-washed polyethylene bottles for analysis at the University of Ilorin Central Laboratory, Ilorin, Kwara State.

Toxic metal concentrations (Cd, Cr, Ni, and Pb) were determined using a Buck Scientific Atomic Absorption Spectrophotometer (BUCK Scientific ACCUSYS 230, Buck Scientific, East Norwalk, CT, USA). The instrument specifications are provided in Table S1 (Supplementary Material). Calibration curves were established using standard solutions prepared over a concentration range of 0–30 mg L⁻¹. All chemicals and reagents employed in the analysis were of analytical grade (Sigma-Aldrich Chemie GmbH, Darmstadt, Germany). Reagent blanks were analyzed alongside the samples to account for potential matrix effects introduced during extraction and to determine the instrument's limit of detection (LOD), calculated as three times the standard deviation of the blank measurements (Taiwo et al., 2014). Concentrations below the LOD were assigned a value equal to one-half of the detection limit, following the approach recommended by Croghan and Egeghy (2003).

2.7. Statistical Analysis

All experiments were conducted in triplicate, and results are presented as mean ± standard deviation (SD). Statistical analyses were performed using IBM SPSS Statistics version 23.0 (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) was used to evaluate differences among treatment means. Duncan's Multiple Range Test (DMRT) was applied for post hoc comparisons at a significance level of $p < 0.05$. Principal Component Analysis (PCA) was further employed to identify possible relationships among heavy metals and to determine potential

sources of metal accumulation in the mushroom samples. PCA is a multivariate statistical technique that reduces the dimensionality of complex datasets by transforming correlated variables into a smaller number of uncorrelated principal components, thereby facilitating the identification of contamination patterns and potential pollution sources (Iannone et al, 2025).

2.8 Health Risk Assessment of Toxic Metals

Potential health risks associated with the consumption of *Pleurotus ostreatus* were assessed using the United States Environmental Protection Agency (USEPA) risk assessment model. Both non-carcinogenic and carcinogenic risks were evaluated by estimating the Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), Hazard Index (HI), and Lifetime Cancer Risk (CR) using Equations (3)–(6) (USEPA, 2007).

Estimated Daily Intake (EDI)

The estimated daily intake of each heavy metal was calculated as follows:

$$EDI = \frac{C \times IR \times ED \times EF}{BW \times AT} \quad (3)$$

Where, C = Concentration of metals in mushroom; IR = Ingestion rate of metals in mushroom (17 g/day; EF = Exposure frequency = 156 days/year (3 times per week); ED = Exposure duration (30 years for carcinogenic effects); AT = Averaging time. For carcinogenic risk, AT = 61.33 years, whereas for non-carcinogenic risk, AT = ED.; BW = Body Weight (60 kg).

$$\text{Hazard quotient (HQ)} = \frac{EDI}{RfD} \quad (4)$$

$$\text{Hazard index (HI)} = \sum_{i=1}^n HQ \quad i=1 \dots n \quad (5)$$

Where, EDI = estimated daily intake of metals in mushroom; RfD = Reference dose (USEPA, 2002); n = numbers of elements observed.

HQ/HI > 1.0 establishes non-carcinogenic adverse health effects, HQ/HI <1.0 shows no adverse effects.

$$\text{Cancer Risk} = \text{EDI} \times \text{CSF} \quad (6)$$

Where, EDI = estimated daily intake of metals in mushroom; CSF = Cancer Slope Factor.

CR > 1.0×10^{-4} indicates possible development of cancer, while CR < 1.0×10^{-4} establishes no possible cancer development.

3. Results and Discussion

3.1 Heavy Metal Concentrations in *Pleurotus ostreatus*

Table 2 presents the concentrations of Cd, Cr, Ni, and Pb in *Pleurotus ostreatus* cultivated on different agricultural waste substrates amended with varying levels of calcium carbonate. Significant differences ($p < 0.05$) were observed among the treatments for all four metals, indicating that both substrate composition and calcium carbonate supplementation played important roles in determining the extent of metal accumulation in the mushroom fruiting bodies. These differences are likely associated with variations in the chemical composition, pH, cation exchange capacity, and metal-binding properties of the agricultural residues, all of which influence the mobility and availability of heavy metals for uptake by the growing mushrooms (Kulshreshtha, 2019).

Cadmium concentrations ranged from $<0.001 \pm 0.000$ to $0.90 \pm 0.05 \text{ mg kg}^{-1}$. The highest concentration was found in mushrooms cultivated on the paddy straw–cassava peel (PS+CP) substrate without calcium carbonate, while the lowest values were recorded in paddy straw (PS) and paddy straw–cotton stalk (PS+CS) amended with 15 g of calcium carbonate.

223 Table 2: Toxic metal concentrations in mushroom (N=120)

	Cd		Cr		Ni		Pb	
Substrate	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CP	0.80 ^m	0.05	0.12 ^{ef}	0.01	8.93 ⁷⁸	0.16	0.18 ⁸⁹	0.00
CP+5g	0.70 ^{kl}	0.05	0.09 ^{cde}	0.01	8.13 ⁵⁶⁷	0.05	0.16 ⁷⁸	0.00
CP+10g	0.57 ^j	0.03	0.06 ^{bc}	0.01	6.60 ⁵⁶	0.37	0.13 ⁶	0.01
CP+15g	0.35 ^{hi}	0.05	<0.01 ^a		5.98 ⁴⁵	0.16	0.12 ⁵	0.00
MS	0.72 ^{klm}	0.01	0.13 ^{fg}	0.01	4.00 ³	0.25	0.08 ⁴⁵	0.01
MS+5g	0.69 ^{kl}	0.005	0.08 ^{cde}	0.01	3.25 ²	0.25	0.07 ³⁴	0.01
MS+10g	0.63 ^j	0.01	0.06 ^{bc}	0.01	1.58 ^{ab}	0.38	0.03 ^{abc}	0.01
MS+15g	0.56 ^j	0.005	0.04 ^{ab}	0.01	0.92 ^a	0.14	0.02 ^a	0.00
PS	0.003 ^a	0.000	1.09 ^p	0.03	21.42 ¹²	0.88	0.43 ^p	0.02
PS+5g	0.002 ^a	0.000	1.02 ^o	0.01	18.75 ¹¹¹²	0.25	0.38 ^{op}	0.01
PS+10g	0.002 ^a	0.000	0.52 ⁿ	0.01	16.83 ¹⁰¹¹	0.52	0.34 ⁿ	0.01
PS+15g	<0.001 ^a		0.35 ^{lm}	0.01	10.83 ⁹	0.80	0.22 ^{lm}	0.02
PS+CP	0.90 ⁿ	0.05	0.13 ^g	0.01	9.45 ⁸	0.09	0.19 ⁹	0.00
PS+CP+5g	0.80 ^m	0.05	0.09 ^{cde}	0.01	9.18 ⁸	0.05	0.18 ⁸⁹	0.00
PS+CP+10g	0.70 ^{kl}	0.05	0.08 ^{cd}	0.00	8.72 ⁶⁷⁸	0.05	0.17 ⁸⁹	0.00
PS+CP+15g	0.40 ^{ij}	0.05	0.07 ^{bcd}	0.00	8.43 ⁶⁷⁸	0.04	0.17 ⁸	0.00
PS+CS	0.003 ^a	0.000	1.10 ^p	0.03	19.33 ¹¹¹²	0.38	0.39 ^{op}	0.01
PS+CS+5g	0.002 ^a	0.000	1.03 ^o	0.01	14.25 ¹⁰	0.50	0.29 ^m	0.01
PS+CS+10g	0.002 ^a	0.000	0.53 ⁿ	0.02	10.92 ⁹	0.52	0.22 ^l	0.01
PS+CS+15g	<0.001 ^a		0.35 ^{lm}	0.01	6.17 ⁴⁵	5.33	0.19 ^k	0.01
PS+MS	0.03 ^{bc}	0.000	0.27 ^k	0.00	0.03 ^a	0.00	0.17 ⁸⁹	0.00
PS+MS+5g	0.03 ^{bc}	0.001	0.26 ^{jk}	0.00	0.03 ^a	0.00	0.16 ⁷⁸	0.00
PS+MS+10g	0.02 ^{bc}	0.001	0.21 ^{ij}	0.01	0.03 ^a	0.00	0.16 ⁷⁸	0.00
PS+MS+15g	0.02 ^b	0.001	0.19 ^{hi}	0.01	0.02 ^a	0.00	0.12 ⁵	0.00
CS	0.17 ^{efg}	0.004	0.17 ^h	0.01	0.13 ^a	0.01	0.17 ⁸⁹	0.00
CS+5g	0.16 ^{efg}	0.002	0.13 ^{fg}	0.01	0.10 ^a	0.01	0.16 ⁷⁸	0.00
CS+10g	0.12 ^{def}	0.004	0.08 ^c	0.02	0.08 ^a	0.00	0.12 ⁵	0.00
CS+15g	0.09 ^c	0.01	<0.01 ^a		0.07 ^a	0.01	0.11 ⁴⁵	0.00
MS+CP	0.45 ^j	0.02	0.17 ^h	0.01	0.10 ^a	0.01	0.17 ⁸⁹	0.00
MS+CP+5g	0.29 ^g	0.02	0.13 ^g	0.01	0.08 ^a	0.00	0.16 ⁷⁸	0.00
MS+CP+10g	0.18 ^f	0.02	0.10 ^{def}	0.02	0.07 ^a	0.01	0.12 ⁵	0.00
MS+CP+15g	0.09 ^{bc}	0.02	0.04 ^{ab}	0.01	0.06 ^a	0.01	0.11 ⁴⁵	0.00
MS+CS	0.02 ^{bc}	0.000	0.37 ⁿ	0.01	0.25 ^a	0.05	0.09 ⁵	0.01
MS+CS+5g	0.02 ^{bc}	0.001	0.33 ^m	0.01	0.03 ^a	0.01	0.08 ⁴	0.00
MS+CS+10g	0.02 ^{bc}	0.001	0.26 ^l	0.00	0.02 ^a	0.00	0.04 ^{2a}	0.01
MS+CS+15g	0.01 ^{ab}	0.001	0.23 ^k	0.01	<0.01 ^a		0.03 ^a	0.00
CP	0.40 ^{ij}	0.03	0.23 ^j	0.01	0.10 ^a	0.01	0.12 ⁵	0.00
CP+5g	0.34 ^{gh}	0.02	0.18 ^{hi}	0.01	0.08 ^a	0.01	0.11 ⁴⁵	0.00
CP+10g	0.22 ^f	0.02	0.13 ^g	0.01	0.05 ^a	0.01	0.07 ³	0.00
CP+15g	0.12 ^{de}	0.02	0.09 ^{cd}	0.01	0.04 ^a	0.01	0.06 ²	0.00

224 CP: cassava peel; MS: maize stalk; PS: paddy stalk; CS: cotton stalk: 5, 10 and 15 g represents the varying
225 concentrations of lime introduced as additives. ; Values are presented as mean ± standard deviation. Means with different
226 superscripts within the same column differ significantly (p < 0.05) according to Tukey B Multiple Analysis. SD-standard deviation.

227 Across most substrates, cadmium concentrations decreased progressively as the level of calcium
228 carbonate increased, suggesting that lime reduced cadmium availability by increasing substrate pH
229 and promoting the immobilization of Cd ions. Similar observations have been reported by Pan et
230 al. (2022) and Zhang et al. (2021), who showed that alkaline substrate amendments can
231 significantly reduce cadmium uptake by cultivated mushrooms. Although relatively high cadmium
232 concentrations were observed in untreated PS+CP and cassava peel substrates, calcium carbonate
233 supplementation markedly reduced these levels, with many treatments producing mushrooms that
234 were close to or below the European Commission maximum limit of 0.2 mg kg^{-1} for cultivated
235 mushrooms (European Commission, 2023). These findings highlight the potential of calcium
236 carbonate as a simple and effective amendment for improving mushroom safety by limiting
237 cadmium accumulation.

238 Chromium concentrations ranged from $<0.01 \pm 0.00$ to $1.10 \pm 0.03 \text{ mg kg}^{-1}$. The highest values
239 were observed in mushrooms grown on PS+CS ($1.10 \pm 0.03 \text{ mg kg}^{-1}$) and untreated paddy straw
240 ($1.09 \pm 0.03 \text{ mg kg}^{-1}$), whereas the lowest concentrations occurred in cassava peel and cotton stalk
241 amended with 15 g calcium carbonate. Similar to cadmium, chromium concentrations generally
242 declined as calcium carbonate application increased, indicating that liming reduced chromium
243 bioavailability within the substrates. Although this study measured total chromium rather than
244 distinguishing between Cr(III) and Cr(VI), reducing total chromium in edible mushrooms remains
245 desirable because it lowers overall dietary exposure. The chromium concentrations observed in the
246 present study are within the range reported for cultivated edible mushrooms by Dong et al. (2024),
247 who found total Cr concentrations of $0.09\text{--}4.71 \text{ mg kg}^{-1}$ dry weight in commercially cultivated
248 mushrooms from China. Furthermore, previous studies have consistently demonstrated that the
249 accumulation of chromium and other trace metals in cultivated mushrooms is strongly influenced

by the contamination status and chemical composition of cultivation substrates, with mushrooms grown on contaminated agricultural or industrial residues exhibiting substantially higher metal concentrations than those cultivated on cleaner substrates (Ab Rhaman et al., 2021; Mohamadhasani & Rahimi, 2022). These findings support the observed differences among substrates in the present study.

Nickel exhibited the widest variation among all the metals analysed, with concentrations ranging from $<0.01 \pm 0.00$ to $21.42 \pm 0.88 \text{ mg kg}^{-1}$. The highest nickel concentrations occurred in mushrooms cultivated on untreated paddy straw and paddy straw-based substrate mixtures, whereas the lowest values were recorded in maize stalk–cotton stalk (MS+CS) substrates amended with calcium carbonate. Increasing calcium carbonate supplementation consistently reduced nickel accumulation across nearly all substrate combinations, with the greatest reductions observed at the 15 g application rate. Nevertheless, several paddy straw-based treatments still exceeded the dietary limit of $0.2 - 0.3 \text{ mg kg}^{-1}$, suggesting that paddy straw either contained higher nickel concentrations or promoted greater nickel availability during cultivation (Levická and Orliková, 2024). This observation is consistent with reports that rice straw can accumulate trace metals from agricultural soils and irrigation water, which may subsequently be transferred to cultivated mushrooms. Similar trends were reported by Zhang et al. (2022), who found that the choice of agricultural residue significantly influences nickel accumulation in edible mushrooms. In contrast, mushrooms cultivated on maize stalk, cotton stalk, maize stalk–cotton stalk, and calcium carbonate-amended cassava peel generally contained nickel concentrations well below the recommended limit, indicating that these substrates are more suitable for producing mushrooms with lower nickel burdens.

Lead concentrations ranged from 0.02 ± 0.00 to 0.43 ± 0.02 mg kg⁻¹. The highest concentration was recorded in mushrooms grown on untreated paddy straw, while the lowest values occurred in maize stalk and maize stalk–cotton stalk supplemented with 15 g calcium carbonate. As observed for the other metals, calcium carbonate generally reduced lead accumulation, most likely by decreasing lead solubility and mobility under more alkaline conditions. Importantly, all lead concentrations measured in this study remained below the European Commission maximum level of 0.50 mg kg⁻¹ fresh weight for cultivated mushrooms, suggesting that the mushrooms produced under these conditions are unlikely to pose a significant health concern with respect to lead exposure (European Commission, 2023). The Pb concentrations obtained in the present study are comparable to those reported for cultivated edible mushrooms by Liu et al. (2024), who identified cultivation substrates as important sources of potentially toxic elements, and by Huang et al. (2015), who demonstrated that heavy metal concentrations in cultivated mushrooms closely reflect the contamination status of their growth substrates. Nevertheless, because lead is a cumulative toxic metal with no known biological function, continued monitoring of cultivation substrates remains essential, particularly when agricultural residues are sourced from areas affected by anthropogenic pollution.

The findings demonstrate that substrate type is a major determinant of heavy metal accumulation in *Pleurotus ostreatus*, while calcium carbonate supplementation provides an effective means of reducing metal uptake. Paddy straw consistently resulted in the highest chromium, nickel, and lead concentrations, whereas maize stalk and cotton stalk, particularly when supplemented with 10–15 g calcium carbonate, produced mushrooms with substantially lower concentrations of these metals. The consistent reduction in metal accumulation observed following calcium carbonate application suggests that modifying substrate chemistry through liming can improve the safety of cultivated

oyster mushrooms. Consequently, selecting appropriate agricultural residues and incorporating calcium carbonate during substrate preparation could serve as practical and cost-effective strategies for minimizing heavy metal contamination and enhancing the quality and safety of commercial mushroom production.

3.2 Health Risk Assessment of Toxic Metals in Mushroom

3.2.1 Non-carcinogenic adverse effects

Table 3 presents the hazard quotient (HQ) and hazard index (HI) values for Cd, Cr, Ni, and Pb associated with the consumption of *Pleurotus ostreatus* cultivated on different agricultural waste substrates amended with varying levels of calcium carbonate. Significant differences were observed among the treatments, showing that both substrate type and calcium carbonate supplementation influenced heavy metal uptake and the resulting non-carcinogenic health risks. Across all treatments, the HQ values for individual metals and the cumulative HI were below the critical threshold of 1.0, indicating that consumption of the cultivated mushrooms is unlikely to pose adverse non-carcinogenic health effects according to the United States Environmental Protection Agency (USEPA) risk assessment criteria.

Among the metals evaluated, Cd contributed the largest proportion to the total hazard index (HI) in most substrate combinations. The HQ values for Cd ranged from 0.000 to 0.213, with the highest value recorded in mushrooms cultivated on the untreated paddy straw–cassava peel (PS+CP) substrate, while the lowest values occurred in paddy straw (PS) and paddy straw–cotton stalk (PS+CS) amended with 10–15 g calcium carbonate. A steady decline in Cd HQ was observed as the level of calcium carbonate increased across most substrates, reflecting the reduction in Cd concentrations reported in Table 2.

317 Table 3: Hazard quotient and hazard index of toxic metals in mushroom

	Cd		Cr		Ni		Pb		HI
Substrate	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
CP	0.189	0.012	0.005	0.000	0.053	0.001	0.006	0.000	0.253
CP+5g	0.166	0.012	0.003	0.000	0.048	0.000	0.005	0.000	0.222
CP+10g	0.134	0.007	0.002	0.000	0.039	0.002	0.004	0.000	0.180
CP+15g	0.083	0.012	0.000	0.000	0.035	0.001	0.004	0.000	0.123
MS	0.169	0.002	0.005	0.001	0.024	0.001	0.003	0.000	0.200
MS+5g	0.163	0.001	0.003	0.000	0.019	0.001	0.002	0.000	0.188
MS+10g	0.149	0.002	0.002	0.000	0.009	0.002	0.001	0.000	0.161
MS+15g	0.132	0.001	0.001	0.000	0.005	0.001	0.001	0.000	0.140
PS	0.001	0.000	0.043	0.001	0.127	0.005	0.014	0.001	0.185
PS+5g	0.000	0.000	0.040	0.001	0.111	0.001	0.013	0.000	0.164
PS+10g	0.000	0.000	0.020	0.000	0.100	0.003	0.011	0.000	0.132
PS+15g	0.000	0.000	0.014	0.000	0.064	0.005	0.007	0.001	0.085
PS+CP	0.213	0.012	0.005	0.000	0.056	0.001	0.006	0.000	0.280
PS+CP+5g	0.189	0.012	0.004	0.000	0.054	0.000	0.006	0.000	0.253
PS+CP+10g	0.166	0.012	0.003	0.000	0.052	0.000	0.006	0.000	0.226
PS+CP+15g	0.095	0.012	0.003	0.000	0.050	0.000	0.006	0.000	0.153
PS+CS	0.001	0.000	0.043	0.001	0.114	0.002	0.013	0.000	0.171
PS+CS+5g	0.001	0.000	0.040	0.001	0.084	0.003	0.010	0.000	0.135
PS+CS+10g	0.000	0.000	0.021	0.001	0.065	0.003	0.007	0.000	0.093
PS+CS+15g	0.000	0.000	0.014	0.000	0.036	0.032	0.006	0.000	0.057
PS+MS	0.006	0.000	0.011	0.000	0.000	0.000	0.006	0.000	0.023
PS+MS+5g	0.006	0.000	0.010	0.000	0.000	0.000	0.005	0.000	0.022
PS+MS+10g	0.005	0.000	0.008	0.000	0.000	0.000	0.005	0.000	0.019
PS+MS+15g	0.004	0.000	0.007	0.000	0.000	0.000	0.004	0.000	0.015
CS	0.041	0.001	0.007	0.000	0.001	0.000	0.006	0.000	0.054
CS+5g	0.038	0.000	0.005	0.000	0.001	0.000	0.006	0.000	0.049
CS+10g	0.029	0.001	0.003	0.001	0.000	0.000	0.004	0.000	0.036
CS+15g	0.022	0.002	0.000	0.000	0.000	0.000	0.004	0.000	0.026
MS+CP	0.106	0.005	0.007	0.000	0.001	0.000	0.006	0.000	0.120
MS+CP+5g	0.069	0.005	0.005	0.000	0.000	0.000	0.006	0.000	0.080
MS+CP+10g	0.043	0.005	0.004	0.001	0.000	0.000	0.004	0.000	0.051
MS+CP+15g	0.020	0.005	0.001	0.000	0.000	0.000	0.004	0.000	0.026
MS+CS	0.006	0.000	0.015	0.000	0.001	0.000	0.003	0.000	0.025
MS+CS+5g	0.005	0.000	0.013	0.000	0.000	0.000	0.003	0.000	0.021
MS+CS+10g	0.005	0.000	0.010	0.000	0.000	0.000	0.001	0.000	0.016
MS+CS+15g	0.003	0.000	0.009	0.000	0.000	0.000	0.001	0.000	0.013
CP	0.095	0.007	0.009	0.000	0.001	0.000	0.004	0.000	0.109
CP+5g	0.081	0.005	0.007	0.000	0.000	0.000	0.004	0.000	0.093
CP+10g	0.051	0.005	0.005	0.000	0.000	0.000	0.002	0.000	0.059

318 CP: cassava peel; MS: maize stalk; PS: paddy stalk; CS: cotton stalk: 5, 10 and 15 g represents the varying
319 concentrations of lime introduced as additives; SD-standard deviation.

321 This trend suggests that liming reduced Cd bioavailability by increasing substrate pH and
322 promoting the adsorption and immobilization of Cd, thereby limiting its uptake by the mushroom
323 mycelium. This interpretation is consistent with studies demonstrating that increasing pH is one
324 of the most effective mechanisms for reducing Cd mobility and bioavailability in environmental
325 matrices (Bolan et al., 2003; Pan et al., 2022). Furthermore, studies on cultivated mushrooms have
326 shown that substrate composition and contamination status are major determinants of heavy metal
327 accumulation, including cadmium (Huang et al., 2015; Liu et al., 2024). Although untreated
328 PS+CP produced the highest Cd HQ, all values remained well below the safety threshold ($HQ <$
329 1), indicating an acceptable level of non-carcinogenic dietary exposure.

330 The HQ values for Cr ranged from 0.000 to 0.043, with the highest values observed in mushrooms
331 cultivated on untreated PS and PS+CS substrates. As with Cd, calcium carbonate supplementation
332 consistently reduced Cr HQ across nearly all substrate combinations, suggesting that liming
333 lowered Cr mobility and bioavailability within the cultivation substrates. Despite relatively higher
334 Cr concentrations in some treatments, the corresponding HQ values remained far below 1.0,
335 indicating minimal non-carcinogenic risk from Cr exposure through mushroom consumption. The
336 HQ values for Ni ranged from 0.000 to 0.127, making Ni the second-largest contributor to the
337 cumulative HI after Cd. The highest HQ values occurred in mushrooms grown on untreated PS,
338 whereas the lowest values were recorded in maize stalk–cotton stalk (MS+CS) substrates
339 supplemented with calcium carbonate. Although several paddy straw-based substrates produced
340 comparatively higher Ni concentrations, the estimated HQ values remained well below the
341 acceptable limit of 1.0, indicating that dietary exposure through mushroom consumption is
342 unlikely to cause adverse health effects. The progressive decline in Ni HQ with increasing calcium
343 carbonate supplementation further demonstrates the effectiveness of lime in reducing Ni uptake.

Similar observations have been reported by Liu et al. (2024) and Jasinska et al. (2024), who identified substrate characteristics as a major factor influencing Ni accumulation and the resulting dietary exposure in edible mushrooms.

Among the four metals, Pb produced the lowest HQ values, ranging from 0.001 to 0.014. The highest HQ was recorded in mushrooms cultivated on untreated PS, while the lowest values occurred in maize stalk and MS+CS substrates amended with higher levels of calcium carbonate. Although Pb is recognised as a cumulative toxic metal with no known biological function, its contribution to the overall HI in this study was very small because of the low concentrations detected in the mushroom samples. This finding agrees with reports that edible mushrooms containing low Pb concentrations generally produce HQ values below 1, indicating negligible non-carcinogenic health risks to consumers (Soceanu et al., 2024).

The cumulative HI ranged from 0.013 to 0.280, with the highest value recorded for mushrooms cultivated on the untreated PS+CP substrate and the lowest value observed in MS+CS amended with 15 g calcium carbonate. Importantly, every HI value was substantially lower than the acceptable limit of 1.0, confirming that the combined exposure to Cd, Cr, Ni, and Pb through consumption of the cultivated mushrooms is unlikely to result in adverse non-carcinogenic health effects. The consistent reduction in HI following calcium carbonate supplementation further demonstrates that liming is an effective approach for reducing heavy metal bioavailability and improving the safety of cultivated mushrooms.

The health risk assessment indicates that mushrooms produced on all the agricultural waste substrates evaluated are safe for consumption with respect to non-carcinogenic risks. However, untreated paddy straw and paddy straw-based substrate mixtures consistently produced higher HQ and HI values than maize stalk, cotton stalk, and their combinations. This finding highlights the

importance of substrate selection in mushroom cultivation. The study also demonstrates that calcium carbonate supplementation, particularly at 10–15 g, effectively reduced the health risks associated with heavy metal accumulation, making it a practical and low-cost strategy for producing safer *Pleurotus ostreatus* for human consumption.

Chukwuka et al. (2023) reported substantially higher hazard quotient (HQ) values for toxic metals, particularly Cd, Cr, and Pb, in wild mushrooms collected from different regions of Nigeria. The elevated HQ values were attributed to the ability of wild mushrooms to bioaccumulate heavy metals from contaminated soils and substrates, reflecting the influence of environmental pollution. The authors further observed that the HI frequently exceeded the safe threshold ($HI > 1$), indicating potential non-carcinogenic health risks, especially among children, who exhibited greater susceptibility due to their lower body weight and higher exposure relative to body mass. These findings contrast with the low Pb HQ values observed in cultivated oyster mushrooms produced on relatively uncontaminated agricultural substrates, underscoring the importance of cultivation substrate and growing environment in determining the safety of edible mushrooms.

Figure 2 illustrates the percentage contribution of Cd, Cr, Ni, and Pb to the overall non-carcinogenic health risk (HI) associated with the consumption of *Pleurotus ostreatus* cultivated on the different agricultural waste substrates. The relative contribution of each metal varied considerably among substrate combinations, reflecting differences in metal accumulation patterns and demonstrating that substrate composition strongly influences dietary health risks.

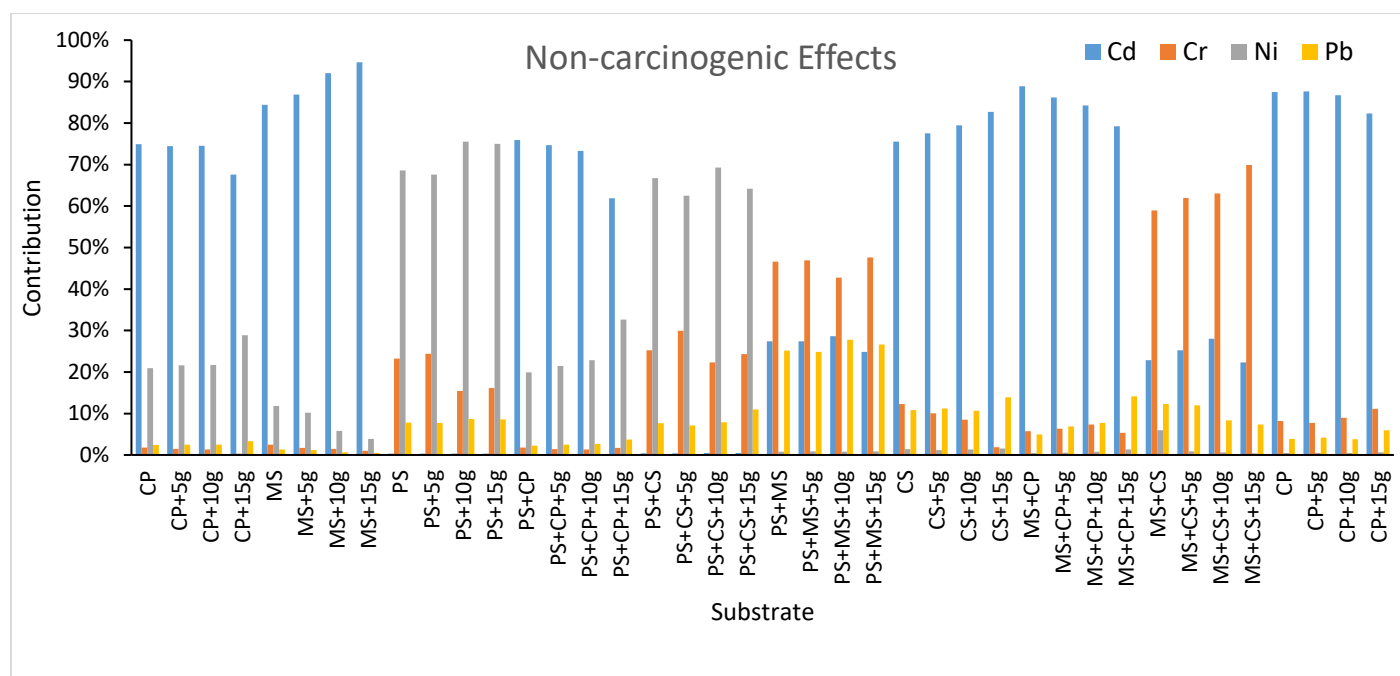


Figure 2: Contributions by toxic metals to noncarcinogenic adverse effects; CP: cassava peel; MS: maize stalk; PS: paddy stalk; CS: cotton stalk: 5, 10 and 15 g represents the varying concentrations of lime introduced as additives

Across most substrate treatments, Cd was the dominant contributor to the overall non-carcinogenic risk. In mushrooms cultivated on cassava peel (CP), maize stalk (MS), cotton stalk (CS), and their mixtures, Cd accounted for approximately 60–95% of the total HI. Its contribution became even more pronounced with increasing calcium carbonate supplementation, particularly in the MS- and CP-based substrates, where Cd contributed more than 85% of the cumulative risk. Although all calculated HQ values remained below the acceptable threshold of 1.0, the predominance of Cd indicates that it remains the principal driver of potential non-carcinogenic effects. This observation agrees with the findings of Mleczek et al. (2021), who reported that Cd often represents the greatest contributor to dietary health risks from cultivated mushrooms because of its relatively low oral reference dose compared with other trace metals.

In contrast, mushrooms cultivated on paddy straw (PS) and paddy straw-based mixtures exhibited a different risk profile. In these substrates, Ni became the major contributor to the total HI, accounting for approximately 60–75% of the cumulative risk in untreated PS, PS+CP, and PS+CS substrates. The higher contribution of Ni reflects the elevated nickel concentrations observed in these treatments (Table 2), suggesting that paddy straw either contained greater background levels of Ni or enhanced its bioavailability during mushroom growth. Previous studies have demonstrated that the elemental composition of cultivation substrates strongly influences the accumulation of trace metals in edible mushrooms. For example, Liu et al. (2024) showed that straw-based cultivation materials constitute major sources of potentially toxic elements in cultivated mushrooms, while Zhang et al. (2022) reported that the type and composition of mushroom substrates significantly affect the distribution and accumulation of trace metals. These findings support the present observation that paddy straw-based substrates contributed more substantially to Ni-associated non-carcinogenic risk than corn stalk-based substrates.

The contribution of Cr was generally low in CP-, MS-, and CS-based substrates, where it accounted for less than 10% of the total hazard index (HI). However, its contribution increased noticeably in paddy straw mixtures, particularly PS+MS and MS+CS, where Cr represented approximately 40–70% of the cumulative risk. Even under these conditions, the corresponding hazard quotient (HQ) values remained well below the safety threshold ($HQ < 1$), indicating that Cr made only a modest contribution to the overall non-carcinogenic risk. The increased proportional contribution of Cr in these treatments is primarily attributable to the relatively lower contributions of Cd and Ni rather than to excessive chromium accumulation. Similar trends have been reported in multi-element health risk assessments of edible mushrooms, where the contribution of individual metals to the overall hazard index varies according to their relative concentrations and toxicological

characteristics rather than their absolute concentrations alone (Huang et al., 2015; Dong et al., 2024). These findings underscore the importance of evaluating cumulative risk using the hazard index rather than interpreting individual metal concentrations in isolation.

Among the four metals, Pb consistently made the smallest contribution to the overall HI, generally accounting for less than 15% of the total risk across all substrate combinations. Although slight increases were observed in some paddy straw-based treatments, Pb remained a minor contributor because of its comparatively low concentrations in the fruiting bodies. These findings are consistent with the study of Boonyingsathit et al. (2025), who similarly reported that Pb contributes only a small fraction of the overall non-carcinogenic risk associated with cultivated oyster mushrooms when grown on relatively uncontaminated agricultural residues.

A notable finding from Figure 2 is the influence of calcium carbonate supplementation on the relative contribution of individual metals. As the amount of calcium carbonate increased from 5 g to 15 g, the contributions of Ni, Cr, and Pb generally declined, particularly in paddy straw-based substrates. This trend indicates that liming reduced the bioavailability of these metals by increasing substrate pH and promoting their adsorption, complexation, or precipitation into less soluble forms. Consequently, the overall hazard index (HI) decreased substantially with increasing calcium carbonate application, demonstrating that substrate amendment can effectively minimise dietary exposure to potentially toxic metals. These findings are consistent with previous studies showing that increasing substrate or soil pH reduces the bioavailability of potentially toxic elements and limits their transfer into biological systems (Pan et al., 2022). Furthermore, studies on cultivated mushrooms have demonstrated that the chemical composition and contamination status of cultivation substrates are major determinants of trace metal accumulation, highlighting

the importance of substrate management in improving mushroom safety (Huang et al., 2015; Liu et al., 2024).

Figure 2 demonstrates that the contribution of individual metals to non-carcinogenic risk depends largely on substrate composition. Cd was the predominant contributor in most substrate combinations, whereas Ni became the principal contributor in paddy straw-based substrates. Despite these differences, the cumulative hazard indices for all treatments remained below the USEPA safety threshold of 1.0, confirming that the mushrooms produced under the different substrate formulations are unlikely to pose significant non-carcinogenic health risks to consumers (USEPA, 2007). The consistent reduction in the contribution of toxic metals following calcium carbonate supplementation further supports the potential use of liming as a practical, inexpensive, and environmentally friendly strategy for reducing the bioavailability of trace metals in mushroom cultivation substrates and improving the safety of *Pleurotus ostreatus* produced on agricultural residues. This interpretation is consistent with previous studies demonstrating that liming increases substrate pH, thereby decreasing the mobility and bioavailability of potentially toxic elements (Bolan et al., 2003; Pan et al., 2022), as well as studies highlighting the critical role of substrate composition in determining trace metal accumulation in cultivated mushrooms (Huang et al., 2015; Liu et al., 2024).

3.2.2 Cancer risk (CR) of toxic metals in mushroom

Table 4 presents the estimated lifetime cancer risk (CR) associated with dietary exposure to Cd, Cr, Ni, and Pb through the consumption of *Pleurotus ostreatus* cultivated on the different agricultural waste substrates. The results show significant variation in the CR values among the substrate treatments, reflecting differences in heavy metal accumulation and the influence of calcium carbonate supplementation on metal bioavailability.

470 Table 4: cancer risk values of toxic metals in mushroom

	Cd		Cr		Ni		Pb		
Substrate	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Σ CR
CP	2.82E-04	1.76E-05	2.73E-06	2.37E-07	4.70E-04	8.22E-06	8.78E-08	1.54E-09	7.55E-04
CP+5g	2.47E-04	1.76E-05	2.02E-06	2.37E-07	4.28E-04	2.63E-06	7.99E-08	4.92E-10	6.77E-04
CP+10g	2.00E-04	1.02E-05	1.42E-06	1.19E-07	3.47E-04	1.97E-05	6.49E-08	3.67E-09	5.49E-04
CP+15g	1.23E-04	1.76E-05	2.21E-07	6.85E-09	3.14E-04	8.22E-06	5.88E-08	1.54E-09	4.38E-04
MS	2.52E-04	3.53E-06	2.96E-06	3.14E-07	2.11E-04	1.32E-05	3.93E-08	2.46E-09	4.66E-04
MS+5g	2.43E-04	1.76E-06	1.98E-06	1.81E-07	1.71E-04	1.32E-05	3.20E-08	2.46E-09	4.17E-04
MS+10g	2.22E-04	2.70E-06	1.42E-06	1.19E-07	8.33E-05	2.01E-05	1.56E-08	3.76E-09	3.06E-04
MS+15g	1.98E-04	1.76E-06	8.30E-07	2.37E-07	4.82E-05	7.60E-06	9.01E-09	1.42E-09	2.47E-04
PS	1.00E-06	2.69E-08	2.58E-05	7.90E-07	1.13E-03	4.62E-05	2.11E-07	8.63E-09	1.15E-03
PS+5g	7.41E-07	1.76E-08	2.41E-05	3.14E-07	9.87E-04	1.32E-05	1.84E-07	2.46E-09	1.01E-03
PS+10g	6.47E-07	2.69E-08	1.23E-05	2.98E-07	8.86E-04	2.74E-05	1.66E-07	5.12E-09	8.99E-04
PS+15g	4.00E-07	2.69E-08	8.30E-06	1.54E-07	5.70E-04	4.23E-05	1.07E-07	7.90E-09	5.79E-04
PS+CP	3.18E-04	1.76E-05	3.08E-06	1.19E-07	4.97E-04	4.74E-06	9.29E-08	8.86E-10	8.18E-04
PS+CP+5g	2.82E-04	1.76E-05	2.13E-06	1.19E-07	4.83E-04	2.74E-06	9.03E-08	5.12E-10	7.68E-04
PS+CP+10g	2.47E-04	1.76E-05	1.78E-06	1.19E-07	4.59E-04	2.74E-06	8.57E-08	5.12E-10	7.08E-04
PS+CP+15g	1.41E-04	1.76E-05	1.58E-06	6.85E-08	4.44E-04	2.01E-06	8.29E-08	3.75E-10	5.87E-04
PS+CS	1.03E-06	2.69E-08	2.60E-05	7.53E-07	1.02E-03	2.01E-05	1.90E-07	3.76E-09	1.04E-03
PS+CS+5g	7.76E-07	1.76E-08	2.43E-05	3.14E-07	7.50E-04	2.63E-05	1.40E-07	4.92E-09	7.75E-04
PS+CS+10g	6.88E-07	3.53E-08	1.25E-05	3.62E-07	5.75E-04	2.74E-05	1.07E-07	5.12E-09	5.88E-04
PS+CS+15g	4.35E-07	2.69E-08	8.33E-06	1.54E-07	3.25E-04	2.81E-04	9.10E-08	2.46E-09	3.34E-04
PS+MS	9.27E-06	7.13E-08	6.37E-06	4.74E-08	1.65E-06	8.04E-09	8.31E-08	8.86E-10	1.74E-05
PS+MS+5g	9.01E-06	7.96E-08	6.22E-06	4.79E-08	1.62E-06	1.47E-08	7.96E-08	7.37E-10	1.69E-05
PS+MS+10g	8.19E-06	1.25E-07	4.92E-06	1.78E-07	1.41E-06	3.07E-08	7.74E-08	8.63E-10	1.46E-05
PS+MS+15g	5.75E-06	1.70E-07	4.43E-06	2.91E-07	1.24E-06	3.50E-08	5.99E-08	1.44E-09	1.15E-05
CS	6.09E-05	1.17E-06	3.99E-06	2.98E-07	6.75E-06	4.02E-07	8.51E-08	1.35E-09	7.18E-05
CS+5g	5.68E-05	6.36E-07	2.96E-06	2.37E-07	5.18E-06	3.04E-07	8.03E-08	6.19E-10	6.50E-05
CS+10g	4.32E-05	1.32E-06	1.86E-06	3.62E-07	4.39E-06	1.52E-07	5.66E-08	9.93E-10	4.95E-05
CS+15g	3.22E-05	2.52E-06	2.92E-07	2.47E-08	3.68E-06	2.63E-07	5.28E-08	8.63E-10	3.63E-05
MS+CP	1.59E-04	8.08E-06	4.11E-06	1.81E-07	5.00E-06	5.26E-07	8.56E-08	1.35E-09	1.68E-04
MS+CP+5g	1.03E-04	7.34E-06	3.04E-06	1.81E-07	3.95E-06	2.63E-07	8.05E-08	3.76E-10	1.10E-04
MS+CP+10g	6.41E-05	7.95E-06	2.25E-06	5.43E-07	3.42E-06	2.63E-07	5.71E-08	9.93E-10	6.98E-05
MS+CP+15g	3.06E-05	7.96E-06	8.30E-07	1.19E-07	2.98E-06	4.02E-07	5.33E-08	6.19E-10	3.44E-05
MS+CS	8.46E-06	1.17E-07	8.81E-06	1.22E-07	1.32E-05	2.63E-06	4.42E-08	2.46E-09	3.05E-05
MS+CS+5g	7.99E-06	6.20E-08	7.89E-06	2.56E-07	1.58E-06	2.63E-07	3.69E-08	2.46E-09	1.75E-05
MS+CS+10g	6.78E-06	2.06E-07	6.15E-06	7.71E-08	8.77E-07	1.52E-07	1.97E-08	2.46E-09	1.38E-05
MS+CS+15g	4.33E-06	2.28E-07	5.47E-06	1.28E-07	4.39E-07	1.52E-07	1.39E-08	1.42E-09	1.02E-05
CP	1.42E-04	1.08E-05	5.38E-06	1.30E-07	5.00E-06	2.63E-07	6.10E-08	1.37E-09	1.53E-04
CP+5g	1.21E-04	7.13E-06	4.29E-06	1.60E-07	4.21E-06	2.63E-07	5.60E-08	2.46E-10	1.30E-04
CP+10g	7.59E-05	8.08E-06	3.17E-06	1.93E-07	2.46E-06	4.02E-07	3.26E-08	1.26E-09	8.15E-05
CP+15g	4.06E-05	6.36E-06	2.21E-06	1.25E-07	1.84E-06	2.63E-07	2.87E-08	5.12E-10	4.47E-05

471 CP: cassava peel; MS: maize stalk; PS: paddy stalk; CS: cotton stalk; 5, 10 and 15 g represents the varying
472 concentrations of lime introduced as additives; SD-standard deviation.

In general, the cumulative cancer risk (Σ CR) decreased progressively with increasing calcium carbonate application, demonstrating that liming can effectively reduce the long-term health risks associated with heavy metal exposure.

Among the investigated metals, Ni was the principal contributor to the cumulative cancer risk in mushrooms cultivated on paddy straw (PS) and paddy straw-based substrates. Individual Ni CR values ranged from 4.39×10^{-7} to 1.13×10^{-3} , with the highest value recorded in untreated PS. Similar trends were observed in PS+CP and PS+CS substrates, where Ni remained the dominant contributor to Σ CR. The elevated Ni risk is consistent with the higher nickel concentrations measured in these substrates (Table 2), suggesting that paddy straw either contained greater background levels of Ni or promoted its bioavailability during mushroom cultivation. Previous studies have similarly reported that rice straw-derived substrates accumulate higher levels of Ni, leading to increased lifetime cancer risks compared with other agricultural residues (Hseu et al., 2017).

In contrast, Cd was the dominant carcinogenic metal in mushrooms cultivated on cassava peel (CP), maize stalk (MS), maize stalk–cassava peel (MS+CP), and cotton stalk (CS) substrates. The CR values for Cd ranged from 4.00×10^{-7} to 3.18×10^{-4} , with the highest values recorded in the untreated PS+CP and CP substrates.

Although the concentrations of Cd were generally lower than those of Ni, its comparatively high carcinogenic potency resulted in a substantial contribution to the cumulative cancer risk. Similar observations have been reported by Senila et al. (2024), who demonstrated that Cd frequently contributes disproportionately to lifetime cancer risk because of its relatively high cancer slope factor. Murtić et al. (2024) also identified Cd as one of the principal determinants of carcinogenic risk in cultivated *Pleurotus ostreatus* despite relatively low tissue concentrations.

The contribution of Cr to the cumulative cancer risk was comparatively small across all substrate combinations. Chromium CR values ranged from 2.21×10^{-7} to 2.60×10^{-5} , with the highest values occurring in untreated paddy straw and paddy straw–cotton stalk substrates. The progressive decline in Cr CR following calcium carbonate supplementation suggests that liming effectively reduced chromium availability in the cultivation substrates. Likewise, Pb consistently produced the lowest CR values, varying from 9.01×10^{-9} to 2.11×10^{-7} . Despite the well-established carcinogenic and cumulative nature of Pb, its contribution to the overall cancer risk remained negligible because of its low concentrations in the mushroom fruiting bodies. Similar findings have been reported by Senila et al. (2024) and Abdullah et al. (2023), who found that Pb generally contributes only a minor fraction of the total carcinogenic risk associated with cultivated edible mushrooms.

The cumulative cancer risk (Σ CR) varied considerably among the substrate treatments, ranging from 1.02×10^{-5} in MS+CS+15g to 1.15×10^{-3} in untreated PS. Relatively high Σ CR values were also recorded for untreated CP (7.55×10^{-4}), PS+CP (8.18×10^{-4}) and PS+CS (1.04×10^{-3}), indicating that these substrates accumulated greater amounts of carcinogenic metals, particularly Ni and Cd. However, supplementation with calcium carbonate consistently reduced Σ CR across nearly all substrate combinations. For example, Σ CR decreased from 1.15×10^{-3} in untreated PS to 5.79×10^{-4} following supplementation with 15 g calcium carbonate, representing almost a 50% reduction in estimated lifetime cancer risk. Similar reductions were observed for CP, MS, PS+CP and PS+CS substrates, highlighting the effectiveness of calcium carbonate in limiting heavy metal uptake.

According to the USEPA, an acceptable lifetime cancer risk generally falls within the range of 1×10^{-6} to 1×10^{-4} , while values exceeding 1×10^{-4} may indicate an increased probability of

carcinogenic effects following long-term exposure (USEPA, 2007). Based on this criterion, mushrooms cultivated on MS+CS, PS+MS, CS, and MS+CP supplemented with 10–15 g calcium carbonate generally remained within the acceptable risk range. In contrast, untreated PS, CP, PS+CP, and PS+CS produced Σ CR values above the recommended limit, suggesting that long-term consumption of mushrooms cultivated on these substrates could present an elevated lifetime cancer risk if consumed regularly over many years. Nevertheless, the substantial reductions in Σ CR achieved through calcium carbonate supplementation demonstrate that simple substrate modification can significantly improve the safety of cultivated mushrooms.

The carcinogenic risk assessment confirms that substrate composition is a major determinant of lifetime cancer risk in *Pleurotus ostreatus*. Paddy straw-based substrates consistently produced the highest cumulative cancer risks, largely because of elevated Ni accumulation, whereas maize stalk, cotton stalk, and their combinations generally produced much lower risks. The consistent decline in individual CR values and Σ CR following calcium carbonate supplementation demonstrates that liming is an effective, inexpensive, and environmentally sustainable strategy for reducing heavy metal bioavailability during mushroom cultivation. These findings further support the use of carefully selected agricultural wastes for mushroom production while emphasizing the importance of substrate amendment in minimizing long-term dietary exposure to carcinogenic metals.

Figure 3 illustrates the relative contribution of Cd, Cr, Ni, and Pb to the cumulative lifetime cancer risk associated with the consumption of *Pleurotus ostreatus* cultivated on the different agricultural waste substrates. The figure complements the findings presented in Table 4 by showing how each metal contributed to the overall carcinogenic risk in the various substrate formulations. The contribution of individual metals differed markedly among the substrates, demonstrating that the

carcinogenic risk profile of the mushrooms was largely governed by substrate composition and, to a lesser extent, calcium carbonate supplementation.

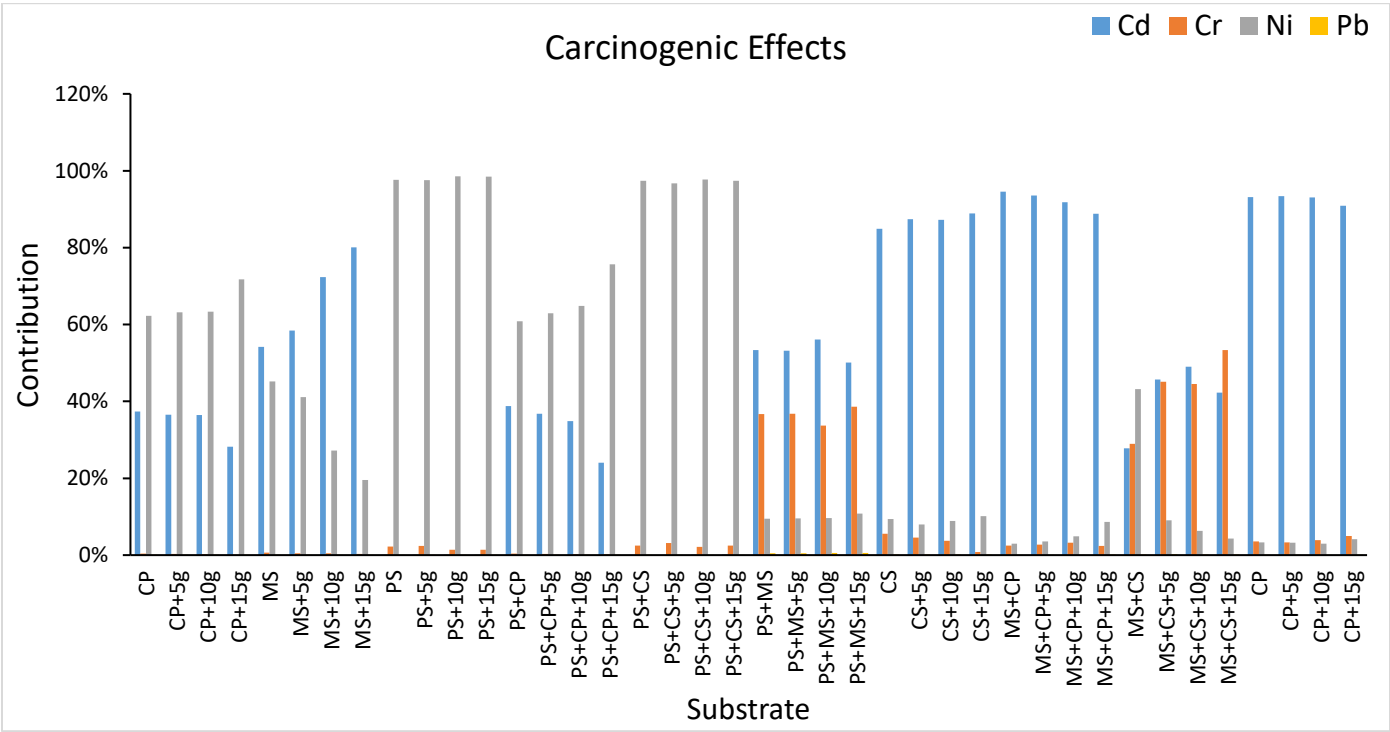


Figure 3: Contributions by toxic metals to carcinogenic adverse effects; CP: cassava peel; MS: maize stalk; PS: paddy stalk; CS: cotton stalk; 5, 10 and 15 g represents the varying concentrations of lime introduced as additives

A clear pattern emerged across the substrate treatments. In mushrooms cultivated on cassava peel (CP), maize stalk (MS), cotton stalk (CS) and their combinations, Cd was the dominant contributor to the total cancer risk, accounting for approximately 80–95% of the cumulative risk in many treatments. This predominance reflects the relatively high carcinogenic potency of Cd, which resulted in a disproportionately greater contribution to lifetime cancer risk despite its moderate concentrations in the mushroom fruiting bodies. Similar observations have been reported by Murtić et al. (2024) and Senila et al. (2024), who identified Cd as one of the principal drivers of carcinogenic risk in cultivated oyster mushrooms because of its relatively high cancer slope factor.

In contrast, Ni was the predominant contributor in mushrooms cultivated on paddy straw (PS) and paddy straw-based substrates, including PS, PS+CP and PS+CS, where it contributed approximately 60–98% of the total cancer risk. This observation is consistent with the elevated Ni concentrations measured in these substrates (Table 2) and the higher individual Ni cancer risk values presented in Table 4. Paddy straw is known to accumulate trace metals from agricultural soils and irrigation water, which may explain the greater transfer of Ni into the mushroom fruiting bodies.

The contribution of Cr remained relatively small across most substrate treatments, generally accounting for less than 10% of the cumulative cancer risk. However, its contribution became more noticeable in PS+MS and MS+CS substrates, where Cr accounted for approximately 30–50% of the total risk. This increase was not due to unusually high chromium concentrations but rather to the substantially lower contributions of Cd and Ni in these particular substrate combinations. Consequently, Cr represented a greater proportion of the total cancer risk even though its absolute cancer risk remained low. Similar shifts in the proportional contribution of individual metals have been reported by Senila et al. (2024), who observed that the dominant carcinogenic metal varies according to the relative abundance of other metals present in the mushroom.

Among all the metals investigated, Pb consistently contributed the least to the cumulative cancer risk, accounting for only a very small proportion across all substrate combinations. Although Pb is a recognised toxic metal, its concentrations in the mushroom samples were sufficiently low that the estimated lifetime cancer risk remained negligible. Similar findings have been reported in studies of cultivated edible mushrooms, where lead concentrations below regulatory limits resulted in low or negligible health risks to consumers (Huang et al., 2015; Liu et al., 2024). These

observations further support the conclusion that, when cultivation substrates are not heavily contaminated, Pb contributes only a minor proportion of the overall health risk associated with mushroom consumption.

Figure 3 also clearly demonstrates the beneficial effect of calcium carbonate supplementation on the carcinogenic risk profile of the mushrooms. Across almost all substrate combinations, increasing calcium carbonate application from 5 g to 15 g reduced the relative contributions of Ni, Cr, and Pb, while simultaneously lowering the cumulative cancer risk reported in Table 4. For example, the Σ CR of untreated PS decreased from 1.15×10^{-3} to 5.79×10^{-4} following supplementation with 15 g calcium carbonate. Similar reductions were observed for CP, MS, PS+CP, and PS+CS substrates. These findings indicate that calcium carbonate effectively reduced heavy metal bioavailability by increasing substrate pH and limiting metal mobility, thereby decreasing their transfer into the mushroom fruiting bodies.

The contribution patterns shown in Figure 3 are in good agreement with the cumulative cancer risk values presented in Table 4. Substrates in which Ni or Cd dominated the carcinogenic profile, particularly untreated PS, PS+CP, PS+CS, and CP, also produced the highest Σ CR values. Conversely, substrates such as MS+CS, PS+MS, CS, and MS+CP, especially after calcium carbonate supplementation, exhibited much lower contributions from the carcinogenic metals and consequently recorded the lowest cumulative cancer risks. This close agreement between the contribution analysis and the quantitative risk assessment confirms that substrate composition is the principal factor controlling lifetime cancer risk in cultivated *Pleurotus ostreatus*.

4. Conclusion

This study demonstrated that both agricultural waste substrates and calcium carbonate (CaCO_3) supplementation significantly influence toxic metal accumulation in *Pleurotus ostreatus*. Metal concentrations varied among substrate types, confirming that substrate characteristics determine metal bioavailability and uptake. Paddy straw generally accumulated higher Cr, Ni, and Pb concentrations, while cassava peel-based substrates contained relatively higher Cd. In contrast, maize stalk, cotton stalk, and their mixtures consistently produced mushrooms with lower toxic metal levels, particularly when supplemented with CaCO_3 .

Calcium carbonate effectively reduced Cd, Cr, Ni, and Pb concentrations across most substrates, with the greatest reductions observed at the 15 g application rate. These decreases lowered estimated dietary exposure, demonstrating that liming is a practical strategy for improving mushroom safety.

Non-carcinogenic risk assessment showed that hazard quotients (HQ) and hazard indices (HI) for all treatments remained below the USEPA threshold of 1.0, indicating negligible health risks. Although Cd and Ni contributed most to the overall hazard, their exposures remained within acceptable limits. Carcinogenic risk varied more among substrates, with Cd and Ni being the primary contributors. Untreated paddy straw and some of its mixtures exhibited the highest cumulative cancer risk due to elevated Ni levels, whereas maize stalk, cotton stalk, and their combinations presented the lowest risks. Calcium carbonate supplementation consistently reduced both individual and cumulative cancer risks.

This present study demonstrates that agricultural wastes can be safely utilized for oyster mushroom cultivation when appropriate substrates are selected and amended with calcium carbonate. This approach enhances food safety while supporting sustainable agricultural waste valorization. Future

625 studies should investigate the long-term effectiveness of CaCO_3 , mechanisms of metal
626 immobilization, and metal bioaccessibility after cooking and digestion.

627 **Author Contribution**

628 AMT conceived and designed the study; MAA carried out the laboratory analysis; AMT, AOA,
629 and OZO supervised the study. AMT carried out the data analysis. AMT and MAA drafted the
630 manuscript. All the authors read and approved the manuscript.

631 **Data and Materials Availability**

632 All data generated or analysed during this study are included in this published article [and its
633 supplementary information files].

634 **Code Availability**

635 Not applicable.

636 **Declarations**

637 **Ethics Approval and Consent to Participate**

638 Not applicable.

639 **Consent for Publication**

640 Not applicable.

641 **Conflict of Interest**

642 The authors declare no competing interests.

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