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BIOACCUMULATION OF COPPER AND STRESS RESPONSE IN CHILI PLANTS UNDER ORGANIC MANAGEMENT

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ABSTRACT
Copper (Cu) is an essential micronutrient for plant growth, but at elevated concentrations it can become toxic, particularly in agricultural systems transitioning to organic practices where organic fertilizers are applied intensively. This study aimed to evaluate the bioaccumulation of Cu and physiological stress responses in chili plants (Capsicum annuum L.) cultivated under varying doses of cattle manure in a transitional organic farming system. The experiment was arranged in a completely randomized block design with manure application rates ranging from 15 to 50 tons per hectare. Observed parameters included yield, Cu concentration in the fruit, and food safety indicators such as Bioaccumulation Factor (BAF), Estimated Weekly Intake (EWI), Provisional Tolerable Weekly Intake (PTWI), and Target Hazard Quotient (THQ). The results showed that increasing manure dosage significantly enhanced fruit yield and Cu accumulation. However, no traces of cadmium (Cd) or lead (Pb) were detected, and Cu levels remained within acceptable food safety limits. The highest Cu concentration was observed at the highest manure dose, yet BAF and PTWI values indicated minimal risk to human health. These findings suggest that while cattle manure can improve crop productivity, it may also contribute to Cu accumulation that could trigger oxidative stress in plants. This research contributes to the scientific understanding of micronutrient dynamics, food safety, and plant stress physiology within sustainable organic farming systems.

KEYWORD
Bioaccumulation, Cattle Manure, Copper, Organic Farming, Oxidative Stress

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1. INTRODUCTION

In recent years, the transition from conventional to organic farming has gained momentum as a sustainable alternative to reduce environmental degradation and improve food safety. One of the major shifts in this transition is the replacement of synthetic inputs with organic amendments, particularly animal manure, to enhance soil fertility and crop productivity. However, organic inputs such as cattle manure may contain trace amounts of heavy metals, especially copper (Cu), which can accumulate in the soil and be absorbed by crops over time (Sandeep et al., 2019; Adrees et al., 2015). Accumulation in living organisms can cause biological and physiological complications, while in the environment they turn into pollutants (Novellasari et al., 2023). Heavy metals such as Hg, Cr, Pb, Cd and Cu are contaminants that are widespread in the environment due to anthropogenic (use of agrochemicals in agriculture), industry, and mining (Zhao et al., 2018).

Copper plays an essential role as a micronutrient in plant metabolism, being involved in vital processes such as photosynthesis, respiration, and lignin synthesis (Mir et al., 2021). Nevertheless, at elevated levels, Cu becomes toxic, causing metabolic disorders and inhibiting plant growth. Excess Cu can induce oxidative stress by disrupting cellular redox homeostasis, leading to the overproduction of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide anions (O_2^-), and hydroxyl radicals (OH^-) (Fang et al., 2021; Rehman et al., 2021). To counter oxidative damage, plants activate complex antioxidant defense systems, including enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), as well as non-enzymatic antioxidants such as proline and glutathione. These responses are part of the plant’s adaptive mechanisms to tolerate Cu-induced stress (Mir et al., 2021; Chen et al., 2022). Moreover, recent studies indicate that lipid-based signaling pathways, such as those involving sphingolipids, are crucial for regulating the oxidative stress response under metal toxicity (Marques-da-Silva & Lagoa, 2023).

Despite growing awareness of heavy metal contamination, most studies focus on industrial pollution or mining areas, while limited attention is given to organic farms in transition that repeatedly apply manure-based fertilizers. Emerging evidence suggests that continuous use of livestock manure can lead to a gradual build-up of heavy metals, especially Cu, in the soil (Dhaliwal et al., 2019), potentially impairing plant physiology and raising food safety (Adrees et al., 2015; Mir et al., 2021). Red chili (Capsicum annuum L.) is a high-value horticultural crop widely consumed across Southeast Asia. Due to its sensitivity to environmental changes, it serves as an effective bioindicator for assessing heavy metal stress in agroecosystems (Srivastava et al., 2017). In our study site currently transitioning to organic production using cattle manure the post-harvest soil analysis revealed Cu concentrations exceeding the critical threshold for agricultural soils, similar to the results of research conducted (Swain et al., 2021), while cadmium (Cd) was undetected and lead (Pb) remained within permissible limits according to FAO & WHO and USEPA standards.

Although oxidative stress biomarkers such as SOD, CAT, or proline content were not directly measured in this study, Cu concentrations detected in the soil and plant tissues may serve as indirect indicators of potential oxidative damage. Previous research has demonstrated that even moderate increases in Cu levels can lead to significant physiological and biochemical changes in plants (Hasanuzzaman, 2021; Reckova et al., 2019; Mir et al., 2021).

Given this context, it is crucial to examine the bioaccumulation of Cu in red chili and its potential implications on plant health and food safety, especially under organic fertilization systems. Understanding the plant's physiological responses to Cu stress could provide insights for developing safer and more sustainable fertilization practices during the organic transition period. This study aims to evaluate copper (Cu) bioaccumulation in red chili (Capsicum annuum L.) cultivated under cattle manure fertilization during the transition to organic farming. It further seeks to explore the potential role of Cu in inducing oxidative stress responses in plants and to discuss the broader implications for food safety and sustainable agricultural management.

2. MATERIAL AND METHODS

2.1. Study Site and Experimental Design

The study was conducted in an agricultural field undergoing a transition from conventional to organic cultivation in Central Java, Indonesia, during the dry season of 2017. The experimental layout employed a randomized complete block design (RCBD) with three replications. The main crop studied was red chili (*Capsicum annuum* L.), planted in plots treated with organic fertilizers derived from cattle manure applied at a rate of 20 tons ha^{-1}.

2.2. Soil and Plant Sampling

Soil samples were collected before planting and immediately after harvest (90 days after transplanting) at a depth of 0–20 cm using a soil auger. Simultaneously, chili leaf and fruit tissues were harvested for heavy metal analysis. Each sample consisted of a composite from five sub-samples per plot, air-dried, sieved through a 20 mesh (for soil), and oven dried at 70°C (for plant tissues).

2.3. Sample Preparation, Determination and Treatments

Heavy metal contaminants were analyzed in the Analytical Chemistry Laboratory of the Faculty of Mathematics and Sciences, Gadjah Mada University. Sample preparation was carried out using the following work procedure: 1) Five grams of sample was taken and put into a 250 ml erlenmeyer flask, 2) Forty milliliters of nitric acid and perchloric acid were added in a ratio of 2:1, 3) The Erlenmeyer flask was heated on an electric bath at 100 °C, 4) After the solution in the erlenmeyer flask came to a boil and the red smoke disappeared heating was continued to 170 °C until the solution became clear and white smoke appeared, 5) The erlenmeyer flask was lowered from the bath and let to cool, 6) The sample was transferred into a 25-ml measuring flask and added with distilled water, 7) The sample solution was subjected to atomic absorption spectroscopy (AAS) at 324.8, 228.8, and 283.3 nm for Cu, Cd, and Pb respectively. Fulfillment of nutrient needs in the cultivation of large red chilies in this research, comes entirely from cattle manure obtained from farmers. Application of manure in this research was given together with soil cultivation, with doses as treatments: 15, 20, 25, 30, 35, 40, 45, and 50 (kg ha–1).

2.4. Land Use History

Looking back at the history of land use used in this research is an important aspect because it can identify the input of agricultural production facilities used in previous cultivation systems. The aim is to be able to investigate the activities that have been carried out, so that the origin of heavy metal pollutants found in agricultural land can be traced. The agricultural land used for this research activity is rainfed land, regosol soil type, and based on brief communication with farmers, each year applies a "rice - peanuts - corn" planting pattern. In the annual rotation for the three plant species, each is fertilized with urea at a dose of (100, 0, and 75 kg ha–1); SP-36 (50, 0, and 50 kg ha–1); and NPKS (15:10:12:10) dose for each plant species 50 kg ha–1. Control of plant pests and diseases from season to season using organochlorine pesticides (G), and triflumezopyrim (G), application according to the recommendations on the product label.

2.5. Measurement

In this research, it is also discussed about the potential human health risks that may arise from consuming red chilies contaminated with heavy metals at the trophic level, and their residues in agricultural land related to the ecological quality of the land. In an effort to understand and evaluate the environmental impacts and evaluation of human health due to heavy metal exposure, various parameters include: bioaccumulation factor (BAF), provisional tolerable weekly intake (PTWI), estimated daily intake (EDI), target hazard quotient (THQ), and cancer risk (CR).

Bioaccumulation factor (BAF) is a parameter used to measure the ability of a chemical substance, in this case (Cu, Cd and Pb) to accumulate in the tissue of living organisms (chili fruit). BAF provides an overview of the potential accumulation of Cu, Cd and Pb in the food chain, calculated using equation (1) (Hu et al., 2017).

$$BAF = \frac{Cc}{Cs} \qquad (1)$$

When calculating BAF for different types of crops (fruits, and nuts), Cc indicates the concentration of heavy metals in the crop sample, while Cs indicates the concentration of heavy metals in the corresponding soil sample (Hu et al., 2017).

Provisional tolerable weekly intake (PTWI) is a recommendation from the World Health Organization (WHO), which establishes an acceptable daily intake level of a chemical without causing significant adverse effects on human health. PTWI is often used to assess the health risks associated with chronic exposure to a chemical. Risk assessment was calculated by equation (1) (Bae et al., 2023) .

$$\%PTWI = \frac{Weekly\ Exposure}{PTWI}\ x\ 10^2 \qquad (2)$$

Estimated weekly intake (EWI) and provisional tolerance weekly intake (PTWI) calculations were conducted and compared with the values recommended by the WHO/FAO expert committee. In this case, EWI refers to the estimated weekly intake (mg kg−1 body weight per week), C indicates the heavy metal content in food (mg kg−1), WC is the weekly food consumption per individual (g week−1), and BW is the average body weight, which is 60 kg. Furthermore, this EWI value is compared with the PTWI provided by WHO/FAO. EWI is calculated using equation (3) (Jafari et al., 2018).

$$EWI = C\ x\ \frac{WC}{BW} \qquad (3)$$

Estimated daily intake (EDI) is an estimate of the amount of a chemical substance consumed by humans every day through food, drinking water, or air. EDI is used to evaluate the level of human exposure to certain chemical contaminants and compare it to established safety limits. Potential health risk assessment equation (Yap et al., 2015), (Mol et al., 2017), and (Tay et al., 2019).

$$EDI = \frac{Mc\ x\ consumption\ rate}{body\ weight} \qquad (4)$$

where Mc is the metal concentration in plant tissue.

$$THQ = \frac{EF\ x\ ED\ x\ CR\ x\ Mc}{RfD\ x\ ABW\ x\ AET}\ x\ 10^3 \qquad (5)$$

The target hazard quotient (THQ) value proposed by the USEPA (1989, 2000, 2008, 2012) is an integrated risk index by comparing the amount of consumption of a pollutant with a standard reference dose and has been widely used in assessing the risk of metals in contaminated food. Where EF is the exposure frequency (365 days year−1), ED is the exposure duration (70 years), equivalent to the average lifetime, CR is the consumption rate (93 and 50 g day−1 for adults and children respectively, as cited above), Mc is the metal concentration in wet weight basis, RfD is the oral reference dose as cited above, was used in this study to evaluate the EDIs of metals in chili fruit. The RfD values (mg kg−1 day−1) used in this study were as follows: Cd, Cu, and Pb were 0.001, 0.04, and 0.0014 respectively, (USEPA, 2015). ABW is the average body weight (60 kg for adults), AET is the average exposure time for non-carcinogens (365 days year−1 x ED), and 10−3 is the unit conversion factor. If the THQ value is below 1, then the exposed population is not expected to experience significant side effects. Conversely, a THQ value exceeding 1 indicates the potential for non-carcinogenic effects, where the possibility increases as the THQ value increases. (Yap et al., 2016).

$$HI = \sum_{i=1}^{n} THQ_i \quad (6)$$

where THQi is the targeted hazard quotient of an individual metal and n in the present study is 3 (Cd, Cu, and Pb) (Yap et al., 2015). HI < 1, there is no concern for potential human health risks caused by exposure to non-carcinogenic elements, and where, HI >1, there may be a concern for potential human health risks caused by exposure to non-carcinogenic elements (Tay et al., 2019).

CR is the possibility of developing cancer throughout the life of a person because of the digestion of a potential carcinogen. The cancer risk of Cu, Cd, and Pb was determined by the following equation (Naseri et al., 2021):

$$CR = CSF \ x \ EDI \quad (7)$$

Where CSFingest relates to the carcinogenic slope factor of 0.0085 mg kg−1 day−1 for Pb, 0.38 mg kg−1 day−1 for Cd recommended by USEPA (Naseri et al., 2021), and 1.70 for Cu mg kg−1 day−1 (Pan et al., 2019). Cancer risk is an estimate of the probability of cancer occurring due to chronic exposure to a particular carcinogenic substance in a population (Aendo et al., 2022). The CR value for arsenic is above the safe limit (10^{-6} to 10^{-4}), indicating a significant carcinogenic risk for long-term consumers. (Sultana et al., 2017).

3. RESULTS AND DISCUSSION

Prior to the cultivation of chili plants, an analysis of heavy metal content in the soil and cattle manure intended as the treatment variable revealed that copper (Cu) was the only heavy metal detected at a significant level. The pre-cultivation soil contained 52.25 ± 0.75 mg kg^{-1} of Cu, while the cattle manure contained 35.12 ± 0.98 mg kg^{-1}. Although these individual concentrations fall within the environmentally acceptable range (25–50 mg kg^{-1}, according to environmental standards), the combined Cu load reached 87.37 mg kg^{-1}. This suggests a potential risk of cumulative Cu exposure to the plants. In contrast, cadmium (Cd) and lead (Pb) were not detected (nd), indicating a minimal risk of toxicity from these elements under the current organic farming system (Table 1).

Table 1. Concentration of heavy metals (Cu, Cd, and Pb) in pre-cultivation soil and cattle manure used as organic input.

Diversity	Heavy metal (mg kg−1)		
	Cu	Cd	Pb
Land*	52.25 ± 0.75	nd	33.61 ± 0.15
Cattle manure	35.12 ± 0.98	nd	15.66 ± 0.70
Amount	87.37	nd	49.27
Range value**	25 – 50	-	40 – 60

Notes:
nd : non-detected/below the detection limit of the AAS instrument, for Cd ≤ 0.01 mg kg−1.
* : pre-cultivation land (remains of the previous planting season) as a history of land use.
** : Range value (Duncan et al., 2018).

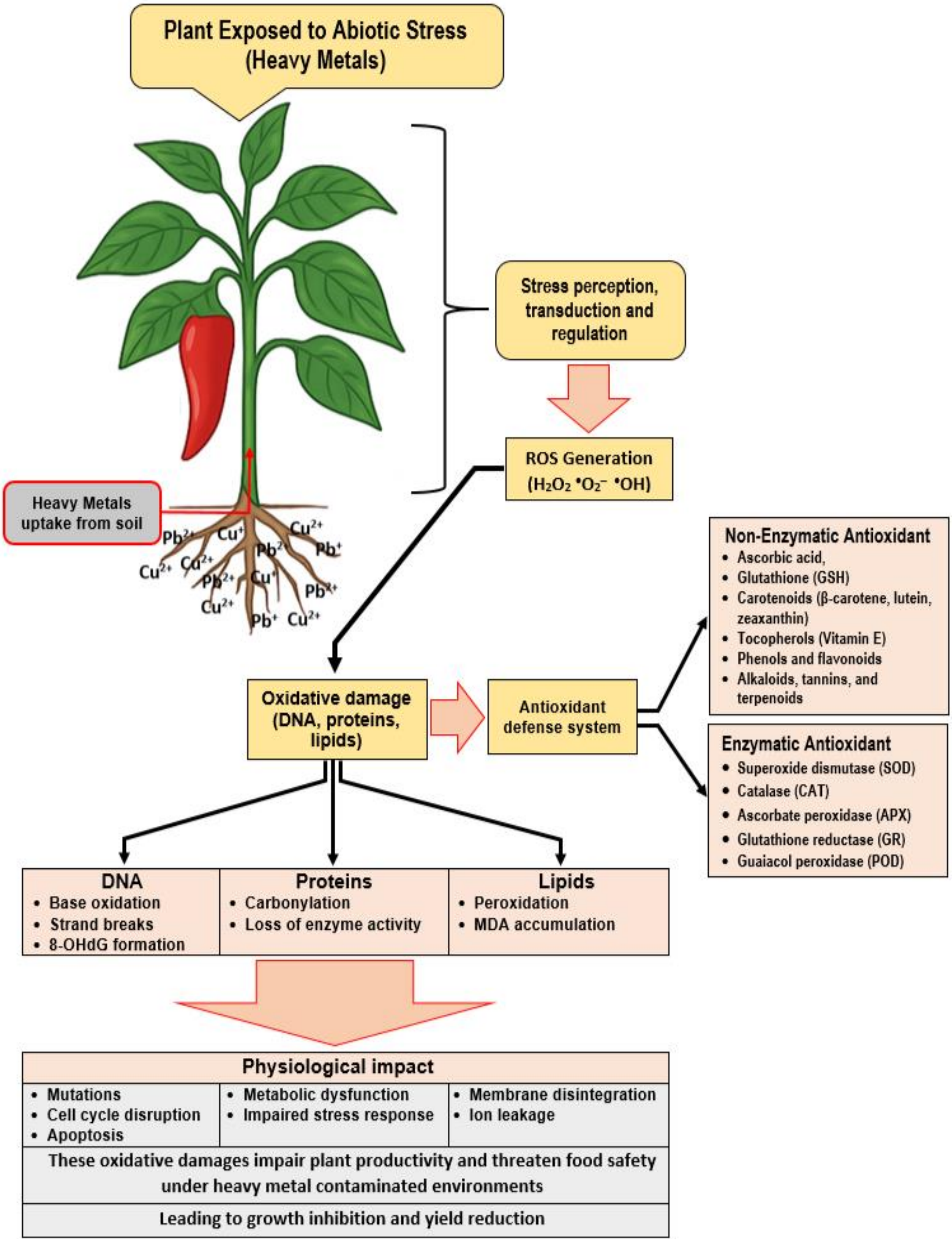
The results showed that the concentration of copper (Cu) in red chili fruits increased progressively with higher doses of cattle manure, ranging from 8.50 ± 0.56 mg kg^{-1} at 15 tons ha^{-1} to 10.18 ± 0.47 mg kg^{-1} at 50 tons ha^{-1}. This trend suggests that cattle manure may serve as a source of Cu accumulation in plant tissues. Correspondingly, fruit yield also rose significantly, peaking at 5,530.29 ± 191.22 kg ha^{-1} under the highest manure dose (Table 2). While this indicates a positive correlation between organic amendment and crop productivity, it also raises concern regarding heavy metal uptake.

Table 2. Yield performance and Cu, Cd, Pb concentration in chili fruits at different cattle manure application rates.

Diversity	Cow Manure Dose (ton^{-ha})							
	15	20	25	30	35	40	45	50
	Red Chili Cultivation							
Yield (kg ha^{-1})	1,936.53±135.01 a	2,238.91±183.37 ab	2,529.76±184.05 ab	3,014.53±102.68 bc	3,220.86±105.61 bc	3,582.86±140.39 cd	4,262.33±157.38 d	5,530.2±191.229 e
Fruits (mg kg^{-1})								
Cu	8.50 ± 0.56	8.89 ± 0.96	8.72 ± 0.96	8.349 ± 0.65	9.760 ± 0.31	9.634 ± 0.96	9.815 ± 0.15	10.18 ± 0.47
Cd	nd	nd	nd	nd	nd	nd	nd	nd
Pb	nd	nd	nd	nd	nd	nd	nd	nd

Notes:
The same letter notation in a row indicates no significant difference at the 5% level.
nd: non-detected/below the detection limit of the AAS instrument, for Cd ≤ 0.01 mg kg−1.

The observed increase in copper concentration in chili fruits alongside the rise in yield with higher manure application suggests a trade-off between productivity and potential heavy metal accumulation. While the use of cattle manure effectively boosts crop yield, it also appears to contribute to copper uptake in plant tissues. This finding raises important considerations regarding the balance between organic fertilization and food safety, particularly with respect to the accumulation of metals in edible plant parts. Excessive Cu accumulation in edible parts of plants is a potential risk factor, as copper is known to trigger oxidative stress in plants when present above physiological thresholds. Cu stress can interfere with photosynthetic processes, particularly by impairing photosystem II (PSII) and substituting iron in Fe–S clusters, thereby disturbing electron transport chains in both chloroplasts and mitochondria (Singh et al., 2016; Schmidt et al., 2020; Lilay et al., 2024). These disruptions enhance the production of reactive oxygen species (ROS), including superoxide radicals and hydrogen peroxide. In response, plants may activate antioxidant defense systems such as Cu/Zn-superoxide dismutase (SOD), catalase (CAT), and peroxidases (POD) to detoxify ROS and restore redox balance (Hasanuzzaman et al., 2018; Sachdev et al., 2021). To better understand the cascade of physiological and molecular events triggered by Cu-induced oxidative stress, a schematic representation is provided (Figur 1). This illustration outlines the uptake of heavy metals, ROS generation, oxidative damage to cellular components, and the activation of antioxidant defense systems in plants



Figur 1. Oxidative Stress Mechanism in Plants Exposed to Heavy Metal Contamination. This schematic illustrates the physiological and biochemical responses of plants, particularly chili (Capsicum annuum L.), under heavy metal induced abiotic stress. Heavy metals such as copper (Cu^{2+}) and lead (Pb^{2+}) are absorbed from contaminated soils through the roots, initiating a cascade of cellular responses. These metals trigger the generation of reactive

oxygen species (ROS), including hydrogen peroxide (H_2O_2), superoxide anion ($\bullet O_2^-$), and hydroxyl radicals ($\bullet OH$), leading to oxidative stress. ROS accumulation causes oxidative damage to key biomolecules DNA (e.g., base oxidation, strand breaks, and 8-hydroxy-2'-deoxyguanosine/8-OHdG formation), proteins (e.g., carbonylation and enzyme inactivation), and lipids (e.g., peroxidation and malondialdehyde/MDA buildup) (Al-Khayri et al., 2021). This damage disrupts vital physiological functions such as metabolism, membrane integrity, ion homeostasis, and stress signaling, ultimately resulting in cell cycle arrest, apoptosis, or impaired growth (Hasan et al., 2017; Ghori et al., 2019; Goncharuk & Zagoskina, 2023; (Mansoor et al., 2023). To counteract these effects, plants activate antioxidant defense systems: non-enzymatic (e.g., ascorbic acid, glutathione, carotenoids, and flavonoids) (Khan & Khan, 2017; Orabi & Abouhussein, 2019; Rudenko et al., 2023), and enzymatic antioxidants (e.g., superoxide dismutase, catalase, and peroxidases) (Ighodaro & Akinloye, 2018; Rajput et al., 2021), which collectively mitigate oxidative damage and enhance plant resilience under environmental stress.

These findings support the relevance of the previously described oxidative stress mechanism, particularly in the context of chili cultivation under organic management practices involving cattle manure application. The findings demonstrate that among the heavy metals analyzed, only copper (Cu) was detected in the fruit of chili plants, whereas cadmium (Cd) and lead (Pb) were not detected (nd) across all treatments. The concentration of Cu in chili fruit increased in response to higher doses of cattle manure, ranging from 8.85 mg kg^{-1} at 15 ton ha^{-1} to 10.18 mg kg^{-1} at 50 ton ha^{-1} (Table 3). This pattern suggests a dose-dependent bioaccumulation of Cu, likely originating from the organic amendments applied. Cattle manure, although beneficial for soil fertility, can contain trace levels of micronutrients and heavy metals, which may accumulate in plant tissues over time.

Table 3. Assessment of food safety and human health consuming red chili in the transitional cultivation system to organic farming.

RED CHILI										
	(ton ha^{-1})	Heavy metal concentration in fruit (mg kg^{-1})			Food safety			Human health assessment		
		Cu	Cd	Pb	BAF	EWI	% PTWI	THQ	HI	CR
		3.5	0.007	0.025	PTWI* (standard)					
Cow manure dosage	15	8.85	nd	nd	0.10	0.553	15.79	0.0020		
	20	8.89	nd	nd	0.10	0.578	16.51	0.0021		
	25	8.72	nd	nd	0.10	0.567	16.19	0.0020		
	30	8.44	nd	nd	0.10	0.549	15.67	0.0020		
	35	9.76	nd	nd	0.11	0.634	18.13	0.0023		
	40	9.63	nd	nd	0.11	0.626	17.88	0.0022		
	45	9.82	nd	nd	0.11	0.638	18.24	0.0023		
	50	10.18	nd	nd	0.12	0.662	18.91	0.0024		
									0.0021	2.43E-06

Notes:
nd : non-detected/below the detection limit of the AAS instrument for Cd and Pb ≤ 0.01 mg kg−1
BAF: bioaccumulation factor
EWI: estimated weekly intake
PTWI*: provisional tolerable weekly intake standard (Cu, Cd, Pb) FAO/WHO, 2004, 2003, 1993 (Pourgheysari et al., 2012), (Bae et al., 2023).
THQ : target hazard quotient
HI : hazard index
CR : cancer risk.

The Bioaccumulation Factor (BAF) for Cu slightly increased from 0.10 to 0.12, confirming a consistent but moderate accumulation trend in chili fruits. While the BAF values remained below 1, indicating low uptake efficiency relative to soil content, the upward trend is noteworthy, especially within organic transition systems that rely heavily on repeated organic inputs. According to the scale, it is stated that there is no accumulation if BAF < 0.01, low bioaccumulation if 0.01 – 0.1, medium bioaccumulation if 0.1 – 1.0, and high bioaccumulation if > 1.0 (Pachura et al., 2016). From a food safety perspective, the Estimated Weekly Intake (EWI) of Cu ranged from 0.549 to 0.662 mg/week, corresponding to 15.67–18.91% of the Provisional Tolerable Weekly Intake (PTWI) standard of 3.5 mg person−1 week−1. These values suggest that under normal consumption rates, the intake of Cu from chili fruits does not pose an acute health risk to humans. The PTWI for Cu is 3.50 mg week−1 kg−1 body weight; thus according to JECFA (2010), PTWI for an adult weighing 60 kg is equivalent to 210 mg week−1 for Cu (Yap et al., 2016), (Bae et al., 2023). Similar research in Iran on the heavy metals Cu due to the consumption of table salt is 1.64% of the established provisions FAO/WHO (Pourgheysari et al., 2012).

Risk assessment parameters, including the Target Hazard Quotient (THQ) and Hazard Index (HI), were consistently below 1 (THQ: 0.0020–0.0024; HI: 0.0021), indicating negligible non-carcinogenic risk. Additionally, the calculated Cancer Risk (CR) value of 2.43E-06 falls within the acceptable range of ≤1.0E-04, further affirming the safety of the chili fruit for consumption under current conditions. Review of the food safety aspect, these heavy metals remain within safe limits according to international guidelines, although there is accumulation from fertilizer use (Y. M. Liu et al., 2020) (Aendo et al., 2022). Nonetheless, the steady increase in Cu concentration, alongside rising values of EWI, %PTWI, THQ, and HI, underscores the potential for long-term accumulation, especially if high-dose organic fertilization is practiced continuously. Similar studies, consumption of red mullet, whiting, and turbot were declared safe for human health, based on EWI values that are far below PTWI, and THQ and HI values that remain below 1. This shows that both individual and combined heavy metal intakes from all these species do not pose a health risk to consumers (Mol et al., 2017), (Ozyurt et al., 2017).

In plant physiological terms, excessive Cu accumulation may induce oxidative stress by triggering the overproduction of reactive oxygen species (ROS), disrupting chloroplast and mitochondrial functions, and impairing photosynthesis and respiration (Sablok, 2019). While the use of cattle manure in organic management appears to support fruit productivity and remains within acceptable safety thresholds, caution is warranted to avoid chronic accumulation effects. Sustainable nutrient management strategies are essential to maintain the delicate balance between organic enrichment, and crop quality (Sarabia et al., 2019); (Panhwar et al., 2018).

Cupprum (Cu) is an essential micronutrient, its excessive accumulation can disrupt physiological processes. The observed Cu levels in leaf tissues (> 50 ppm in several plots) suggest potential oxidative stress conditions. While this study did not directly assess oxidative stress biomarkers (e.g., malondialdehyde or hydrogen peroxide content), the accumulation patterns observed resemble those reported by (Mei et al., 2015; Mosa et al., 2018; Moreira et al., 2015), where Cu excess led to elevated ROS production and reduced photosynthetic performance.

Moreover, Cu content in chili fruits approached the critical threshold for food safety, raising potential risks to consumers if such levels persist over time. Although all fruit samples were within the FAO/WHO recommended limits (< 30 mg/kg fresh weight), a few plots registered values nearing this ceiling. This emphasizes the need for regular monitoring in transitional organic systems, especially considering that animal manures if not quality-controlled can become a source of metal contamination (Yang et al., 2017; W. R. Liu et al., 2020).

Interestingly, the bioaccumulation potential of chili plants under these conditions positions them as effective **bioindicators** for low-level Cu pollution in organically managed soils. This insight supports previous findings by (Outa et al., 2020; Vasile et al., 2024), where Cu uptake correlated with visible morphological stress and antioxidant enzyme activation.

Furthermore, while the transition to organic agriculture is widely promoted for its environmental and health benefits, this study highlights a hidden risk: organic amendments can harbor trace heavy metals that accumulate silently over time. Without appropriate monitoring and regulations, organic systems may inadvertently contribute to subclinical toxicity in plants and humans. Thus, the role of extension services and organic certification bodies becomes crucial in ensuring both agronomic performance and food safety.

From a physiological standpoint, it is likely that the elevated Cu interfered with photosynthetic efficiency, membrane stability, and root enzymatic activity common symptoms of metal-induced oxidative stress in *plant* species (Hasanuzzaman et al., 2017; Hasanuzzaman et al., 2018b; Sachdev et al., 2021; Seneviratne et al., 2019; Chen et al., 2022; Younis et al., 2018; Hoque et al., 2021). Although further biochemical analyses are needed, the patterns seen here offer early evidence of a physiological Cu stress response (Roy et al., 2016; Li et al., 2019).

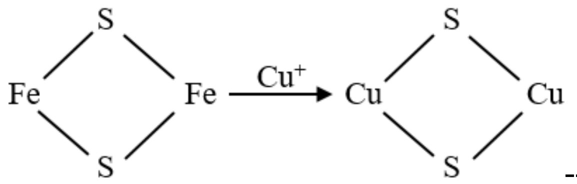
In summary, our findings confirm that red chili cultivated under transitional organic fertilization is prone to Cu bioaccumulation and potential oxidative stress. This poses dual implications: for crop health and human safety. Continuous monitoring, combined with best practices in organic input management, is essential to prevent long-term environmental and nutritional risks.

Moreover, excessive Cu accumulation in plant tissues is frequently associated with oxidative stress, primarily through the overproduction of reactive oxygen species (ROS). ROS such as hydrogen peroxide (H_2O_2), superoxide radicals (O_2^-), and hydroxyl radicals (•OH) can damage cellular structures, disrupt photosynthesis, and impair enzymatic systems (Hasanuzzaman, 2021); Nazir et al., 2019; Ugya et al., 2020; Mansoor et al., 2023). In Capsicum annuum, oxidative stress due to Cu toxicity has been linked to increased malondialdehyde (MDA) levels, reduced chlorophyll content, and a decline in photosystem II efficiency (Z. Chen et al., 2016; Giannakoula et al., 2021; Huihui et al., 2020; Hamed et al., 2017; Da Costa & Sharma, 2016). Although these biochemical markers were not measured in the current study, the observed accumulation patterns and physiological symptoms suggest that similar oxidative stress mechanisms may be at play. Copper (Cu), while essential as a micronutrient, can induce significant oxidative stress when present in excess. It serves as a cofactor for various enzymes involved in electron transport and redox catalysis within mitochondria and chloroplasts, playing a vital role in plant metabolism (Mir et al., 2021; Tripathi et al., 2015; Lilay et al., 2024). However, at toxic concentrations, Cu disrupts cellular homeostasis by catalyzing redox reactions that lead to the formation of reactive oxygen species (ROS), including superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH) (Tsang et al., 2021; Gupta et al., 2016; Schulten & Krämer, 2017; Wang et al., 2022). These ROS are highly reactive and can initiate lipid peroxidation, as well as inflict damage on cellular membranes, proteins, and nucleic acids (Andrés Juan et al., 2021; Sachdev et al., 2021; Choudhary et al., 2020).

The oxidative stress induced by Cu is primarily driven by three biochemical mechanisms: participation in Fenton-type reactions that convert H_2O_2 into hydroxyl radicals (1) (Andrés Juan et al., 2021), reduced glutathione (GSH) acts as a reducing agent, converting Cu^{2+} (cupric) ions into Cu^{+} (cuprous) ions. In this process, two molecules of GSH are oxidized into one molecule of oxidized glutathione (GSSG), and protons (H^{+}) are released. This leads to a depletion of the cellular GSH pool, weakening the plant's antioxidant defenses (2). The accumulation of Cu^{+} is particularly concerning because it can participate in a Fenton reaction (Tiwari et al., 2017), a key intracellular antioxidant, and substitution of iron (Fe) within Fe–S protein clusters (3) (Rydz et al., 2021), impairing essential enzymatic functions. These pathways collectively exacerbate oxidative damage and contribute to the physiological disorders observed in Cu-stressed plants, including chlorosis, necrosis, and growth inhibition (Pandey et al., 2022; Al-Khayri et al., 2021). Therefore, the symptoms identified in this study such as chloroplast membrane disruption and possible enzyme dysfunction are consistent with known Cu-induced oxidative stress responses described in the literature.

$Cu^{+} + H_2O_2 \longrightarrow Cu^{2+} + OH^{-} + \bullet OH$ -------------------------- (1)

$2Cu^{2+} + 2GSH \longrightarrow Cu^{+} + GSSG + 2H$ ---------------------- (2)



---------------- (3)

Furthermore, the formation of reactive oxygen species is amplified through the Haber–Weiss reactions (4 & 5) (Andrés Juan et al., 2021), which mediate electron transfer processes leading to the generation of highly reactive superoxide (O_2^{-}) and hydroxyl (OH) radicals.

$H_2O_2 + OH \longrightarrow H_2O + O_2^{-} + H^{+}$ ------------------------------ (4)

$H_2O_2 + O_2^{-} \longrightarrow O_2 + OH^{-} + \bullet OH$ --------------------------- (5)

Plants exposed to heavy metal stress, such as excess copper (Cu), activate a complex defense system composed of both enzymatic and non-enzymatic antioxidants. Key enzymatic defenses include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR), while the non-enzymatic components involve reduced glutathione (GSH) and ascorbate (Rajneesh et al., 2019; Rajput et al., 2021; Hasanuzzaman et al., 2018b; Ansari et al., 2024). These systems function synergistically to mitigate oxidative damage induced by reactive oxygen species (ROS). When Cu accumulates beyond physiological thresholds (typically 100 – 500 µM), it catalyzes redox reactions particularly those involving the Haber–Weiss cycle thereby increasing the production of ROS such as superoxide anion (O_2^{-}) and hydrogen peroxide (H_2O_2) (Andrés Juan et al., 2021). As a result, the activities of antioxidant enzymes are elevated to maintain redox homeostasis and protect cellular components from oxidative injury.

In addition to enzymatic responses, non-enzymatic antioxidants like GSH play a vital role in stress mitigation. GSH, a tripeptide abundantly present in plant cells, reacts directly with free radicals to shield protein thiol groups and maintain cellular redox balance. However, under conditions of Cu toxicity, GSH levels may decline due to its accelerated consumption during detoxification processes (Bela et al., 2015; Sabetta et al., 2017). Moreover, Cu stress can disrupt iron–sulfur (Fe–S) clusters essential cofactors for electron transport in both mitochondria and chloroplasts by replacing Fe with Cu. This substitution impairs the function of redox-active proteins such as ferredoxin (Fd), a critical component downstream of PSI in the photosynthetic electron transport chain. The destabilization of Fe–S clusters in Fd leads to reduced electron transfer to ferredoxin-$NADP^+$ reductase (FNR), thereby inhibiting the production of NADPH. Consequently, the Calvin cycle receives fewer reducing equivalents, which hampers carbon fixation and leads to excessive accumulation of electrons. This electron backlog increases the formation of reactive oxygen species (ROS), particularly $\bullet O_2^-$ and H_2O_2, triggering oxidative damage within chloroplasts (Schmidt et al., 2020). This substitution compromises the efficiency of electron transfer chains, ultimately impairing energy production and reducing plant growth. Plants often respond to such metal-induced stress by elevating antioxidant enzyme activity, including superoxide dismutase (SOD), catalase (CAT), and peroxidases (POD), as part of their defensive strategy (Rajneesh et al., 2019; Rajput et al., 2021; Hasanuzzaman et al., 2018b; Ansari et al., 2024). Future studies are therefore warranted to quantify these responses and confirm the oxidative stress hypothesis under organic management systems.

4. CONCLUSION AND SUGGESTION

This study demonstrates that the application of cattle manure in organic transition farming systems can enhance chili pepper yields while contributing to the accumulation of copper in the fruit. Although copper concentration increased with higher manure doses, its accumulation remained within safe limits for human consumption and was not accompanied by the presence of other harmful heavy metals such as cadmium and lead. These findings address the research questions regarding the potential for copper bioaccumulation due to organic fertilization and its impact on food safety and plant physiology. The results also confirm that carefully managed organic systems can minimize the risk of heavy metal toxicity. This study contributes to the advancement of sustainable organic farming by emphasizing the need to monitor copper accumulation in plant tissues, as elevated levels although still within food safety thresholds may disrupt key physiological processes in chili plants. Therefore, integrating physiological stress indicators into organic farming assessments is critical to maintaining both crop productivity and long-term plant vitality.

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