



RESEARCH COMMUNICATION

Accumulation of copper in tissues of *Sonchus arvensis* L. grown in copper-polluted soil

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Abstract

Copper (Cu) contamination in agricultural soils poses environmental risks and potential health hazards through food-chain transfer. *Sonchus arvensis* L., a widely consumed medicinal plant, has not been fully assessed for its capacity to accumulate heavy metals. This study investigated whether the species exhibits sufficient tolerance and uptake ability to evaluate its safety implications for human consumption while also assessing its ability to function as a phytoremediation agent. *S. arvensis* was cultivated in soils spiked with graded Cu concentrations (0, 50, 100, 150 and 200 mg/kg). Morphological responses, Cu partitioning between roots and shoots and phytoremediation ability were measured to determine its remediation potential. The plant survived across all treatments, showing dose-dependent stress symptoms but maintaining structural viability. Copper accumulation followed a clear pattern of root greater than shoot across all concentrations. Removal efficiency reached up to 43 % within 14 days, indicating rapid metal sequestration and demonstrating the species' potential for phytostabilisation. However, Cu concentrations in aerial tissues exceeded international food safety limits (WHO) even at the lowest contamination level. These findings highlight a dual implication: *S. arvensis* is a promising, cost-effective candidate for stabilising Cu-contaminated soils, yet its ability to accumulate Cu in edible parts presents significant safety concerns. Regulation of its harvest in polluted environments is therefore essential.

Keywords: accumulator; copper; food safety; heavy metal; *Sonchus arvensis*

Introduction

Heavy metal contamination of soils is a major environmental concern with profound implications for both ecosystems and human health. These metals are toxic, non-biodegradable and can persist for decades, ultimately entering food chains and accumulating in living organisms (1). The World Health Organization (WHO) estimates that over 600 million people are exposed annually to environments contaminated with metals such as copper (Cu), lead (Pb), mercury (Hg) and cadmium (Cd), primarily through agricultural products (2). Key sources include metal plating industries, non-ferrous smelting, mining, intensive pesticide and fertiliser use and poorly managed domestic waste disposal (3). These realities highlight the urgent need for strategies to mitigate heavy metal contamination to protect both environmental quality and food safety.

Among heavy metals, Cu presents a unique challenge. Although Cu is essential in trace amounts (0.05–0.5 mg/L in water), excess accumulation can be toxic (4). Elevated Cu exposure has been linked to gastrointestinal distress including nausea, vomiting and diarrhea as well as cardiovascular disorders such as hypertension, stroke and ischemic heart disease (5–7). Consequently, understanding Cu contamination is critical not only from an ecological perspective but also for public health risk assessments.

In many regions, including Indonesia, wild plants are still widely consumed as vegetables or herbal remedies, particularly species from the Zingiberaceae, Asteraceae and Solanaceae families (7). One of the most used species is *S. arvensis* L. (tempuyung), a cosmopolitan plant that thrives in diverse soils, often colonising roadsides, abandoned fields and agricultural lands due to its wind-dispersed seeds (8). It is characterised by distinctive morphological features, including a rosette growth form with spear- and lanceolate-shaped leaves, as well as the presence of abundant trichomes on the stem which are not observed in other *Sonchus* species (9). Traditionally, *S. arvensis* has been used in folk medicine for its antioxidant, antibacterial, anti-inflammatory and antihypertensive properties (10). Recent studies have analyzed the metabolite profiles of *S. arvensis* leaf and callus extracts, revealing metabolites with potential health-related applications (11). In separate studies, several health-benefiting effects including antiplasmodial, antimicrobial, and antioxidant activities have been confirmed which provide a scientific basis for its traditional use (12).

Recent studies have also highlighted the potential of *Sonchus* species for heavy metal accumulation. For instance, *S. maritimus* and *S. oleraceus* have been reported to absorb up to 12 mg/kg Pb and 56 mg/kg Cu from contaminated soils (13).

Likewise, *S. asper* can accumulate up to 5000 µg/kg Cd in roots with notable translocation to aerial tissues (14). The *S. oleraceus* grown in wastewater-irrigated soils has been found to accumulate multiple metals, including iron (Fe), zinc (Zn), copper (Cu), lead (Pb) and cadmium (Cd), raising concerns about potential health risks. In Peru, *S. oleraceus* accumulated up to 2900 mg/kg Pb and 1910 mg/kg Zn in polluted areas (15), while in Indonesia, *S. arvensis* absorbed up to 4.34 ppm Pb (16). These findings demonstrate that *Sonchus* species can act as significant accumulators of heavy metals in contaminated environments.

However, little is known about Cu accumulation in *S. arvensis* specifically. This is concerning because the WHO sets a maximum safe Cu intake of 0.5 mg/kg body weight, while the Food and Nutrition Board of the United States and Canada recommend 0.9–1.3 mg/kg and the Indonesian Food and Drug Authority (BPOM) limits Cu in fresh vegetables to 5.0 mg/kg (17). Understanding the translocation of Cu in *S. arvensis* is essential for dual purposes: evaluating public health risks and assessing the plant's potential for cost-effective phytoremediation technologies. The present study aims to address this gap, providing evidence for food safety regulation and the sustainable use of wild plants in polluted ecosystems.

Materials and Methods

Experimental conditions and plant material

The experiment was conducted in a controlled greenhouse environment under natural light conditions (12 hr photoperiod), with a temperature range of 28–32 °C and relative humidity of 70–80 %. Seedlings of *S. arvensis* L. (10–15 cm height) were obtained from UPT Materia, Medica, Batu, Malang, Indonesia. Prior to treatment, the seedlings were acclimatised for 30 days in plastic pots filled with uncontaminated commercial potting soil media composed of soil, organic compost and carbonised rice husk at a ratio of 2:1:1, to ensure proper adaptation to greenhouse conditions before the Range Finding Test (RFT) and phytoremediation experiment (18).

Chemicals and soil preparation

Copper sulphate pentahydrate (CuSO₄·5H₂O, analytical grade, Merck KGaA, Darmstadt, Germany) was used as the source of copper. The growth medium consisted of commercial organic potting soil which was air-dried and sieved (2 mm). Contaminated soils were prepared by spiking the soil with CuSO₄ solutions to achieve target concentrations of 0, 50, 100, 150 and 200 mg/kg dry soil weight. The spiked soils were thoroughly mixed and incubated for 1 week to ensure metal equilibrium before transplanting.

Experimental design

Experimental design based on a preliminary range finding test (RFT) conducted to determine sublethal toxicity thresholds (19, 20), 5 CuSO₄ treatment levels were selected: 0 (control), 50, 100, 150 and 200 mg/kg. The study employed a completely randomized design (CRD) with 3 replicates per treatment (n=15). Each experimental unit consisted of one plant per pot containing 500 g of soil. The main exposure period lasted for 14 days. Soil pH was monitored every 48 hr using a digital pH meter (Sojipro soil tester 4 in 1).

Sample preparation and heavy metal analysis

Sample preparation

At harvest (day 14), *S. arvensis* plants were carefully separated into roots and shoots. The samples were thoroughly rinsed with tap water followed by deionised water to remove adhering soil particles. Plant tissues were then oven-dried at 60 °C until constant weight and ground into a fine powder using a stainless-steel mill. Soil samples were similarly oven-dried, ground and sieved to obtain a homogenous sample.

Digestion procedures

Plant samples (0.5 g) were digested following the U.S. EPA Method 3050 B for open acid digestion. Briefly, samples were treated with 10 mL of concentrated HNO₃ (65 %; Merck, Darmstadt, Germany) and heated at 95 °C. To ensure complete oxidation of organic matter, 30 % hydrogen peroxide (H₂O₂) was added sequentially. The digestates were filtered through Whatman No. 42 filter paper and diluted to 50 mL with deionised water.

Soil samples were digested using a microwave digestion system (Mars 6, CEM Corporation, USA). Approximately 1 g of soil was treated with a mixture of HCl and HNO₃ (Merck, Darmstadt, Germany) and digested at 90 °C for 4 hr. The resulting solutions were filtered through 0.45 µm membrane filters and diluted to a final volume of 50 mL.

Heavy metal quantification and quality control

Total Cu concentrations in both plant and soil extracts were quantified using atomic absorption spectrophotometry (AAS; Shimadzu AA-7000, Tokyo, Japan) with a detection limit of 0.004 mg/L. Quality assurance procedures included the analysis of blanks, standard solutions (Certipur, Merck) and duplicates to ensure method accuracy and precision. Instrument detection limits and calibration curves were verified prior to analysis.

Remediation efficiency calculations

Bioconcentration factor (BCF)

The bioconcentration factor (BCF) measures the plant's ability to accumulate heavy metals from the soil into its root system, indicating the potential for phytostabilisation. It is calculated as the ratio between the metal concentration in the roots and the corresponding concentration in the soil, using the following equation (21):

$$\text{BCF} = \text{Cu in roots} / \text{Cu in soil}$$

where C root is the metal concentration in the roots (mg/kg) and C soil is the metal concentration in the soil (mg/kg). BCF values greater than 1 suggest that the plant can efficiently accumulate heavy metals in the root system, whereas values below 1 indicate limited accumulation ability.

Bioaccumulation coefficient (BAC)

The bioaccumulation coefficient (BAC) quantifies the overall accumulation of heavy metals in the entire plant (roots and shoots) relative to soil concentrations (22). The BAC is computed using the following equation:

$$\text{BAC} = \text{Cu in shoots + roots} / \text{Cu in soil}$$

where C root and C shoot are metal concentrations in roots and shoots (mg/kg), respectively and C soil is the metal concentration in the soil (mg/kg). BAC values higher than 1 indicate high metal uptake efficiency by the plant, suggesting potential as

accumulator, while values below 1 denote low accumulation capacity.

Translocation factor (TF)

The translocation factor (TF) describes the ability of plants to transport heavy metals from the roots to the aerial parts (shoots), which is essential for phytoextraction (23). TF is expressed as:

$$TF = \text{Cu in shoots} / \text{Cu in roots}$$

Where C shoot and C root represent metal concentrations in shoots and roots (mg/kg), respectively. $TF > 1$ indicates effective translocation from roots to shoots, whereas $TF < 1$ suggests metal retention in the roots.

Removal efficiency (RE)

The removal efficiency (RE) assesses the capacity of plants to reduce heavy metal concentrations in the soil through uptake and accumulation processes (24). It is calculated as:

$$RE = (\text{Cu reduction in soil} / \text{Initial Cu in soil}) \times 100\%$$

where C initial is the initial metal concentration in the soil (mg/kg) and C final is the final concentration after the treatment (mg/kg). Higher RE values indicate greater remediation efficiency, while lower values reflect limited removal potential.

Data analysis

Data analysis was performed using Microsoft Excel 2019. One-way Analysis of Variance (ANOVA) was performed to determine significant differences ($p < 0.05$), followed by Duncan's Multiple Range Test (DMRT) post hoc analysis. Results are presented as mean \pm standard deviation (SD).

Results

Outcomes of range finding test (RFT) and morphological responses

The preliminary 14-day range finding test (RFT) confirmed that *S. arvensis* exhibits strong tolerance to Cu stress. All plants survived Cu exposures up to 200 mg/kg, although dose-dependent morphological stress symptoms were clearly visible (Fig. 1). Early responses included light green foliage and slight leaf wrinkling, which progressively developed into marginal chlorosis, inward curling and tissue desiccation by day 14, particularly in the 150 and 200 mg/kg treatments.

These stress indicators continued during the main experimental phase. Although all plants remained viable, growth suppression was significant. A marked reduction in leaf number and plant height was observed relative to baseline measurements, with statistically significant differences across treatments ($p < 0.05$). The most severe decline in leaf number occurred at 200 mg/kg, where plants lost an average of 5 leaves over the 14-day period (Fig. 2). Height inhibition was most pronounced in the 100 mg/kg treatment (Fig. 3). Visual comparisons at harvest (Fig. 4) showed that high Cu

concentrations induced visible stress phenotypes such as wilting and chlorosis, yet overall plant structure remained intact, indicating physiological resilience.

Soil physicochemical changes

Copper contamination substantially altered soil chemical characteristics. A consistent acidification trend was recorded, with soil pH decreasing as Cu concentration increased. Control soil remained near-neutral (pH 6.87), whereas soil in the 200 mg/kg treatment became distinctly acidic (pH 4.87) (Fig. 5). The pH values exhibited minor temporal fluctuations but maintained a clear downward trajectory in response to increasing Cu. A strong linear relationship between Cu concentration and pH decline was observed (Fig. 6), suggesting that Cu hydrolysis and associated ion exchange processes contributed to rhizosphere acidification.

Copper depletion in soil and accumulation in plants

Soil analysis demonstrated significant Cu depletion after 14 days of plant growth. All Cu-treated soils exhibited declining Cu concentrations ($p < 0.05$), with the greatest reduction observed in the 200 mg/kg treatment (50.63 mg/kg), followed by the 150 mg/kg treatment (47.88 mg/kg) (Fig. 7). These results confirm the efficient extraction capability of *S. arvensis* over a short cultivation period.

Plant tissue analysis further verified Cu uptake. Copper accumulation followed a clear partitioning pattern, with roots consistently retaining higher concentrations than shoots in all treatments (Fig. 8). The 200 mg/kg treatment showed the highest accumulation, reaching 48.19 mg/kg in roots and 20.07 mg/kg in shoots. This pattern aligns with the mass balance between soil Cu loss and plant Cu gain, confirming active translocation from the soil into plant tissues.

Phytoremediation efficiency indices

The phytoremediation performance of *S. arvensis* varied with Cu concentration (Table 1). BCF values ranged from 0.28 to 0.84, with the highest value at the 0 mg/kg treatment, indicating efficient uptake of background Cu but reduced accumulation capacity under higher contamination levels. The BAC values (0.41–0.86) followed a similar trend, remaining below 1 across all treatments and confirming that the plant acts as a moderate accumulator during short-term exposure.

The TF showed its highest value at 50 mg/kg (0.68), suggesting moderate Cu transfer to shoots at low contamination. TF values < 0.50 in the 100–200 mg/kg treatments indicate that Cu is increasingly retained in the roots as soil concentrations rise.

Despite low BCF, BAC and TF values, *S. arvensis* demonstrated meaningful RE, removing 23–43% of soil Cu within 14 days. The highest RE occurred in the control treatment (43%) and 50 mg/kg treatments (38%), while moderate removal was maintained at higher concentrations (25–32%). Overall, the plant primarily exhibits phytostabilisation, retaining most Cu in the root system while still reducing soil Cu effectively over a short period.

Table 1. Metal BCF, TF, BAC and RE under different copper treatments

Treatment	BCF	TF	BAC	RE
0 mg/kg (control)	0.84	0.04	0.86	43%
50 mg/kg	0.29	0.68	0.49	38%
100 mg/kg	0.28	0.44	0.41	23%
150 mg/kg	0.38	0.46	0.56	32%
200 mg/kg	0.32	0.42	0.46	25%

BCF: Bioconcentration factor; TF: Translocation factor; BAC: Biological absorption coefficient; RE: Removal efficiency.



Fig. 1. Morphological progression of stress symptoms during the range finding test (RFT) under increasing Cu concentrations.

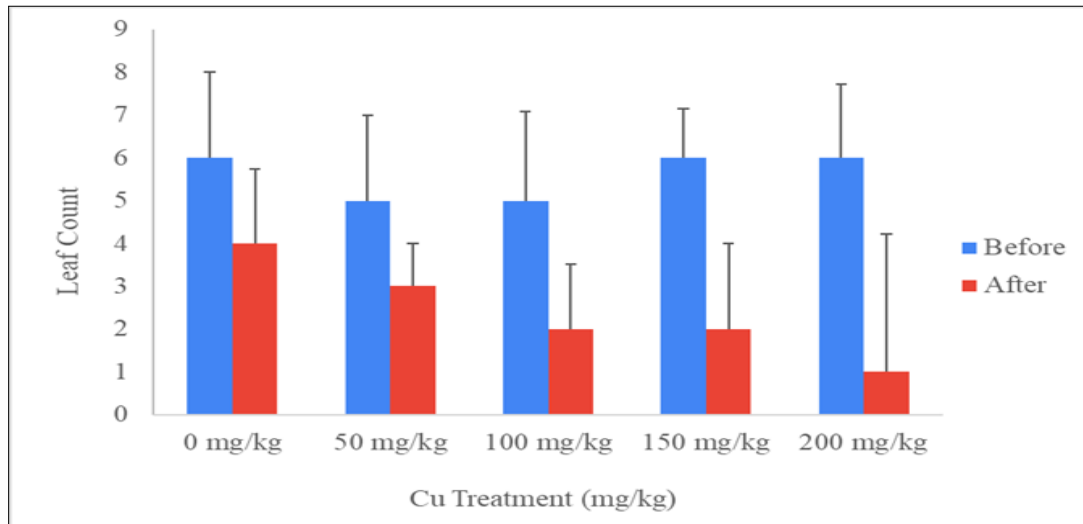


Fig. 2. Changes in leaf number of *Sonchus arvensis* during 14 days of copper exposure.

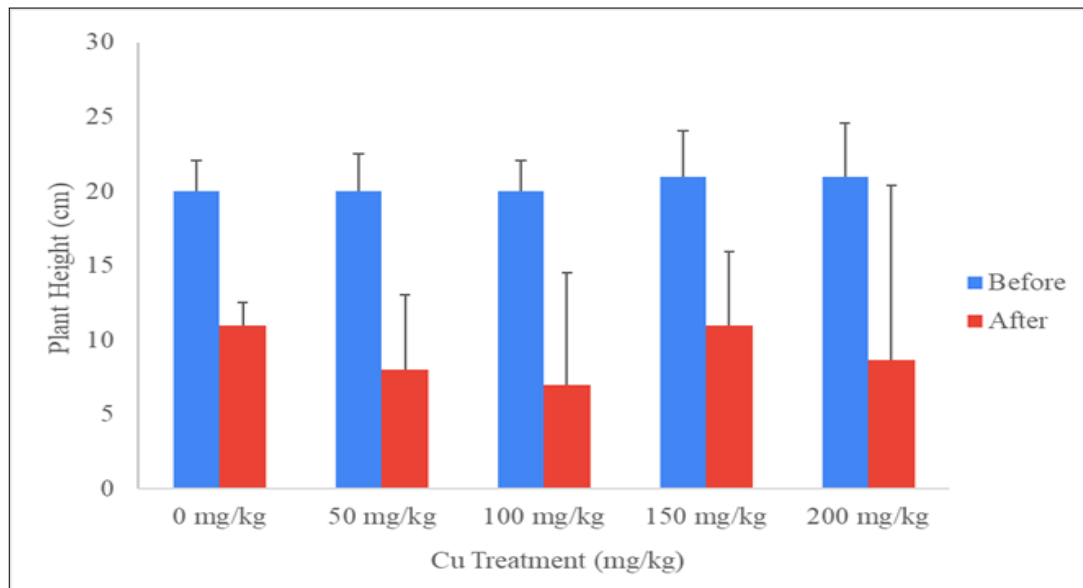


Fig. 3. Plant height variation of *Sonchus arvensis* under different copper treatments over 14 days.



Fig. 4. Morphological appearance of *Sonchus arvensis* at harvest under different copper treatments, showing dose-dependent stress responses. (a) 0 mg/kg; (b) 50 mg/kg; (c) 100 mg/kg; (d) 150 mg/kg; (e) 200 mg/kg.

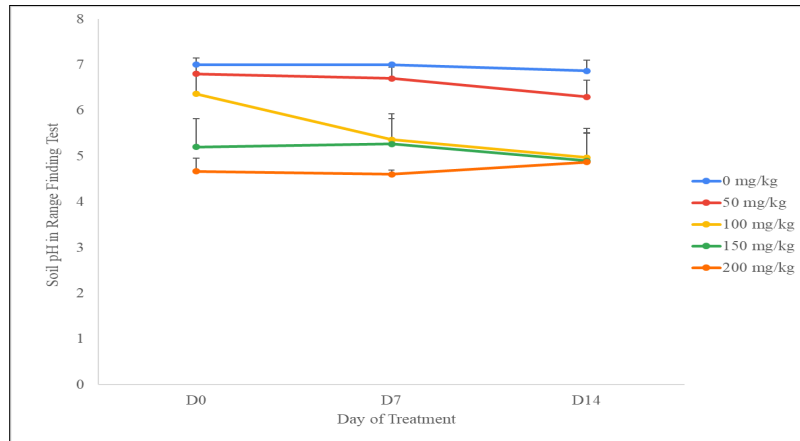


Fig. 5. Soil pH values measured at harvest across all copper treatments, showing dose-dependent acidification.

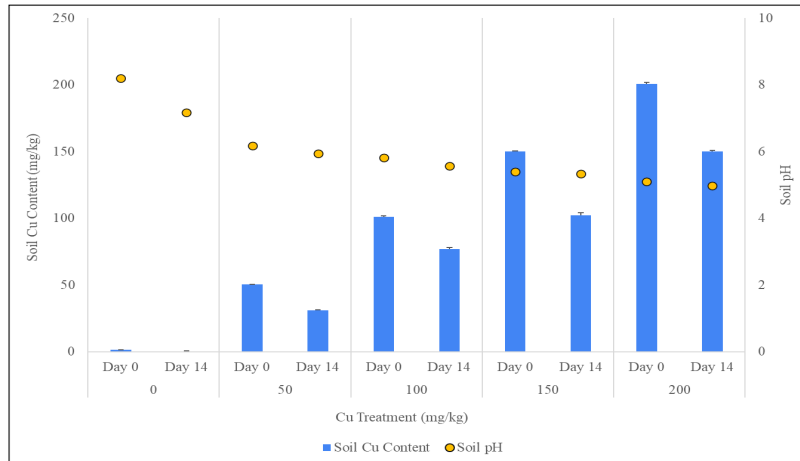


Fig. 6. Linear relationship between initial soil copper concentration and pH reduction.

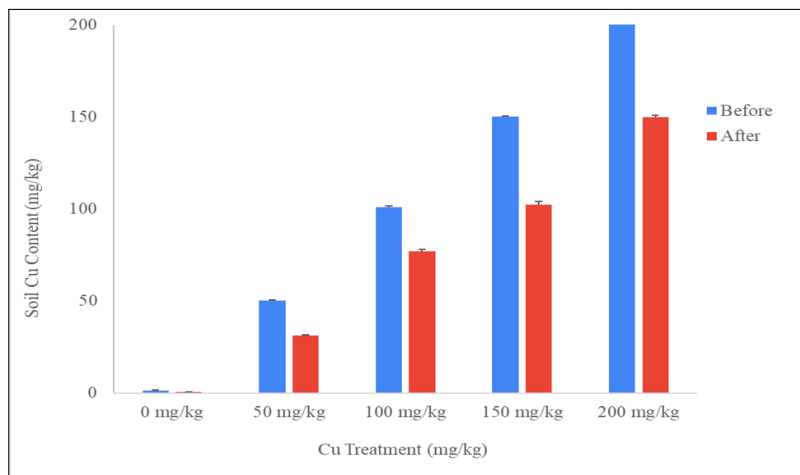


Fig. 7. Reduction of soil copper concentrations after 14 days of cultivation across all treatment levels.

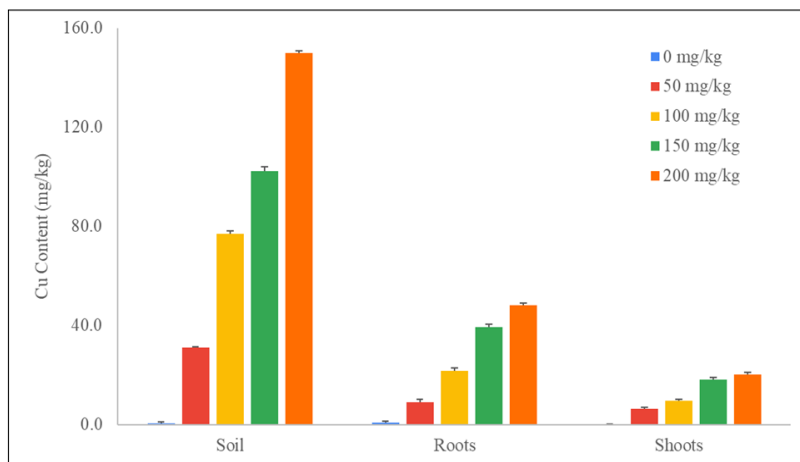


Fig. 8. Copper accumulation in roots and shoots of *Sonchus arvensis* following 14-day exposure to varying copper concentrations.

Discussion

Sonchus arvensis is recognised as a cosmopolitan medicinal plant capable of growing in a wide range of habitats. Its ability to tolerate copper-contaminated soil were shown in this study by surviving 200 mg/kg treatment. Elevated Cu levels in soil may impair plant nutrient uptake through a reduction in soil pH, which interferes with the absorption of essential nutrients leading to growth impairment (25). On the other side, its accumulation in plant tissues was directly correlated with soil Cu levels (26). In this study, the transfer of Cu from soil to plant tissues was evident from the concurrent decline in soil Cu concentration and increase of Cu concentration in plant tissue. Uptake occurs through root plasma membrane transporters, followed by distribution via the xylem and phloem. However, systemic copper redistribution is limited, as Cu is not readily translocated through the phloem (27). This restriction was reflected in the low TF values observed, indicating limited movement of Cu from roots to shoots, except in the control treatment. The presence of Cu in plant tissue caused oxidative stress inside plant cells which disrupt cell membrane, chlorophyll biosynthesis, inhibiting photosynthesis and inhibiting other mineral uptake that leads to growth impairment (28). However, despite reduced growth performance, *S. arvensis* demonstrated survival capacity under Cu-contaminated conditions.

Sonchus arvensis demonstrated higher copper accumulation within a relatively short exposure period compared with the same genus plant. Previous studies using same genus plant showed that *S. oleraceus* able to accumulate 44.5 mg/kg Cu in wastewater-irrigated soil (29). Other studies showed that *S. oleraceus* found in residential areas were able to accumulate 43 mg/kg while *S. oleraceus* planted in artificially contaminated Cu soil accumulated 449 mg/kg Cu (13, 30). In this study, *S. arvensis* able to accumulate higher Cu with the highest treatment accumulate up to 68.26 mg.kg⁻¹ Cu. These differences are attributable to species differences, as studies reporting the Cu accumulation ability of *S. arvensis* remain limited. Accumulated Cu in plant tissue were far exceeded the safety thresholds for human consumption. The WHO recommends a daily Cu intake of 0.5 mg.kg⁻¹ body weight, while the Food and Nutrition Board of the United States and Canada sets the limit at 0.9–1.3 mg.kg⁻¹ and the Indonesian National Agency of Drug and Food Control (BPOM) establishes a threshold of 5 mg.kg⁻¹. Even under relatively low levels and short time exposure to contaminated soil, Cu concentrations inside plant tissue far exceeded the safety limits established by international health organisations.

Although the BCF and BAC were below 1 due to the short exposure period of 14 days, it highlights the potential of *S. arvensis* as a Cu accumulator. *Sonchus arvensis* was able to remove approximately 25–43 % of the total soil Cu within a relatively short time frame, suggesting that prolonged exposure or repeated cultivation cycles could further enhance Cu removal efficiency. The BCF and BAC value suggest that *S. arvensis* has the potential as phytostabiliser where it can retain Cu on its roots and limiting its translocation into the shoots. Copper retention in the roots occurs via binding to root cell walls, phytochelatins binding and intracellular compartmentalisation which collectively prevent Cu translocation to aerial tissue (31). Taken together, these findings indicate that *S. arvensis* is suitable for early-stage or short-term phytoremediation where rapid Cu reduction is required.

Despite the clear evidence of copper accumulation in *S. arvensis*, several limitations of the present study should be acknowledged. The exposure period was limited to 14 days which may not fully represent the cumulative Cu burden in plant tissues over the entire life cycle of *S. arvensis*. Although the sample size was sufficient for inferential analysis, it remains limited in the context of extrapolating these findings to large-scale or commercial applications where broader environmental variability and population heterogeneity must be considered. In addition, while this study assessed safety from a chemical accumulation perspective, no *in vivo* toxicological evaluation was conducted. The absence of animal-based consumption models restricts the ability to directly translate tissue Cu concentrations into physiological safety risks. Finally, environmental and soil variability may influence the transferability of these findings across different regions or continents. Therefore, further studies incorporating extended exposure periods and biological safety assessments are required to comprehensively evaluate the risks associated with the consumption of *S. arvensis* grown in Cu-contaminated environments.

Conclusion

This study confirms that *S. arvensis* possesses high tolerance to Cu stress, surviving soil concentrations up to 200 mg/kg despite observing growth reduction. Notably, this tolerance was accompanied by a strong capacity to accumulate Cu in plant tissues, resulting in concentrations that exceeded established food safety limits and posing a potential risk to consumers. These findings establish *S. arvensis* as a potential candidate for remediation but highlight critical health risks regarding its consumption as an herbal remedy from contaminated environments.

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Authors' contributions

ASPP, FDS and RKA contributed to the research design, experiment execution, data collection, data analysis, data interpretation, sample analysis and manuscript writing. AFMF contributed to the research design, experiment execution, data collection and sample analysis. DKW developed the research design, served as the research supervisor and acted as a research consultant. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT and DeepL to review and refine the English translation of the

manuscript, ensuring that the language is presented in proper academic English. After using this tool/service, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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