Response of Vegetable Crops to Heavy Metal Exposure in Contaminated Irrigation Water and Its Implications for Food Safety

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Abstract

Heavy metal contamination in irrigation water poses serious risks to crop productivity and food safety, particularly for leafy vegetables that readily accumulate toxic elements. This study evaluated the growth response, bioaccumulation behavior, and health risks of three commonly consumed leafy vegetables, velvetleaf (*Limnocharis flava*), kailan (*Brassica oleracea* var. *alboglabra*), and kangkong (*Ipomoea aquatica*), exposed to Pb at 50 mg L^{-1}, Cd at 1 mg L^{-1}, and Cu at 2 mg L^{-1} under controlled irrigation conditions. Plant length, leaf number, and leaf area were monitored over an eighteen-day exposure period. Heavy metal concentrations in roots and leaves were quantified using ICP-OES with high analytical accuracy. Data were analyzed using analysis of variance, and means compared using the Duncan Multiple Range Test (DMRT). The results showed specific growth responses for each cultivar. Kailan exhibited the strongest stress symptoms, including a twenty-four percent reduction in plant length and a thirty-three percent decrease in leaf area under Cu exposure, while velvetleaf and kangkong showed comparatively higher tolerance. Bioconcentration factors exceeded one for all metals, indicating strong accumulation potential, particularly in kailan with Pb at 2873.21, Cd at 99.87, and Cu at 414.13. Translocation factors were consistently below one, indicating restricted metal movement from roots to leaves. Despite low translocation, Pb and Cd concentrations in edible tissues exceeded FAO and WHO safety limits, resulting in very high Hazard Index values, especially for kailan at 3097.6. These findings demonstrate that plant tolerance alone does not guarantee food safety. The integration of accurate analytical measurement, mathematical performance evaluation, and health risk assessment provides a robust framework for identifying safer crop choices and guiding irrigation management in heavy metal-contaminated environments.

Keywords: heavy metal stress, plant tolerance, bioaccumulation, food safety, polluted irrigation water.

1. INTRODUCTION

Heavy metal contamination in agricultural ecosystems is an increasingly significant global concern, particularly in peri-urban areas and wetlands exposed to industrial activities and intensive agricultural practices with inadequate waste management (Kim et al., 2020). The scarcity of clean water resources has resulted in the use of low-quality water as an alternative irrigation source in agriculture (Dotaniya et al., 2023). Heavy metals in irrigation water can accumulate in crops, significantly increasing the risk of human exposure through dietary consumption (Bounar et al., 2020; Khan et al., 2023). These metals are toxic to plants, causing substantial physiological and morphological changes that adversely affect plant growth and the nutritional quality of vegetables (Noor et al., 2022; Rahman et al., 2022). Previous studies have shown that heavy metal contamination in irrigation water leads to reduced biomass, altered growth patterns, and disturbed plant development (Tekle et al., 2023; Waheed et al., 2022). The toxic effects of these metals are further exacerbated by the fact that many vegetables absorb and accumulate heavy metals in their edible parts, such as leaves and fruits, which increases the potential risks to human health.

Exposure to heavy metals such as chromium (Cr), manganese (Mn), iron (Fe), copper (Cu), nickel (Ni), zinc (Zn), cadmium (Cd), lead (Pb), and mercury (Hg) has been found in high concentrations in various vegetables sold in the market (Manwani, Vanisree, et al., 2022). Pb, Cd, and Cu are of particular concern which are commonly found in high concentrations in various vegetables, including Japanese spinach (*Spinacia oleracea*), tomatoes (*Solanum lycopersicum*), mustard and cabbage (*Brassica parachinensis L.*, *Brassica compestris L.*, *Brassica oleracea L.*), lettuce (*Lactuca sativa*) (Abbas et al., 2023; Zhou et al., 2016), and spinach (*Amaranthus viridis, Amaranthus tricolour, Amaranthus paniculatus L.*) (Islam & Hoque, 2014). Pb, Cd, and Cu contamination in irrigation water affects different growth responses among plant species. Previous study indicates that plant species exhibit varying levels of tolerance to heavy metals (Singh et al., 2024a), with some plants demonstrating better adaptation and resilience than others. As the global demand for safe and nutritious vegetables increases, understanding the morphological responses of plants to heavy metals is crucial for developing sustainable agricultural practices.

Various scientific studies have investigated the morphological responses of vegetables to heavy metal contamination. Studies on spinach have shown that this plant is capable of accumulating high levels of Pb and Cd in its roots and leaves when irrigated with contaminated water (Bonanno & Cirelli, 2017; Ogunkunle et al., 2015). A study on cucumbers has shown significant changes in growth patterns, including reduced root and shoot biomass, indicating toxicity due to heavy metal exposure (Othman et al., 2021; Somda et al., 2019). In addition to growth inhibition, heavy metals often cause chlorosis in leaves, further exposing the noticeable morphological impact of contamination on irrigation water (Aksouh et al., 2024). Furthermore, research indicates that certain vegetables, such as spinach, lettuce, and cabbage, show increased heavy metal concentrations when exposed to contaminated water and soil (Abbas et al., 2023). Understanding plant responses to heavy metal stress and their ability to accumulate and tolerate metals can enhance vegetable safety and mitigate risks associated with heavy metal contamination in vegetable cultivation.

Although previous studies have reported heavy-metal accumulation in various vegetables, the current literature still lacks comparative evidence on how different leafy species respond physiologically to specific metal contaminants supplied through irrigation water under controlled conditions. Most studies use soil-based exposure, focus on a single crop, or assess only one metal, which limits the understanding of species-specific tolerance, accumulation, and translocation pathways relevant to real irrigation risks. Moreover, few studies integrate morphological responses with quantitative bioaccumulation metrics and health risk assessment to determine the safe-use potential of vegetables grown under contaminated water.

The novelty of this study lies in its combined experimental and risk-based approach. First, the work provides a controlled, comparative assessment of three widely consumed leafy vegetables exposed to Pb, Cd, and Cu at environmentally relevant concentrations delivered through irrigation water. Second, the study quantifies both bioconcentration and translocation factors to identify species-specific metal movement from roots to edible leaves, which directly determines food safety. Third, the analysis links plant responses to a health risk framework based on Estimated Daily Intake, Hazard Quotient, and Hazard Index, allowing a direct interpretation of the implications for consumers. This integrated approach enables the identification of species with lower translocation potential and supports crop selection strategies for cultivation in areas where irrigation water may contain heavy metals.

2. LITERATURE REVIEW

Heavy metal contamination of irrigation water and soil has emerged as a critical environmental and food safety issue worldwide, particularly in regions where untreated or partially treated wastewater is reused for crop production. Such practices are common in peri-urban and water-scarce agricultural systems due to limited freshwater availability. Heavy metals such as Pb, Cd, and Cu are persistent, non-biodegradable contaminants that accumulate in soils and crops, posing significant risks to ecosystem health and human consumers. Studies have documented that continuous irrigation with contaminated water increases soil metal concentrations over time, subsequently enhancing the uptake and accumulation of metals in crops and increasing the likelihood of entry into the food chain. This process remains a major concern for agricultural sustainability and public health globally (Khaliq et al., 2022).

Leafy vegetables are particularly vulnerable to heavy metal contamination due to their morphological and physiological characteristics, including shallow root systems and high transpiration rates, which facilitate efficient absorption of metals from soil and irrigation water. Research indicates that leafy vegetables such as spinach and okra often accumulate higher metal concentrations compared to other vegetables under similar exposure conditions, making them important indicators of environmental contamination (Khaliq et al., 2022). Metal uptake and accumulation in plants are influenced by several factors, including metal speciation, soil pH, organic matter content, and plant species traits. The extent of metal uptake and internal distribution can vary widely among species, with some metals like Cd and Pb being readily absorbed by roots and subsequently transported to aerial parts, while others may be more strongly retained in root tissue. Copper, although essential at trace levels, becomes toxic at higher concentrations, leading to physiological stress and reduced crop performance (Ali & Ahirwar, 2025).

To quantify metal accumulation in plants, researchers frequently use mathematical indicators such as the bioconcentration factor (BCF) and translocation factor (TF). The BCF represents the ratio of metal concentration in plant tissues to that in the soil or water medium, providing a measure of the plant's capacity to accumulate metals. The TF describes the efficiency of metal movement from roots to shoots or edible parts. High BCF values indicate strong accumulation potential, whereas TF values below one suggest limited internal metal mobility. Studies have shown that many leafy vegetables exhibit high BCF values but low TFs, indicating considerable metal retention in roots despite potential accumulation in edible tissues (Kaur et al., 2025).

Assessment of plant tolerance to heavy metal stress often involves morphological and physiological parameters such as plant height, leaf area, and biomass. Tolerant species are generally those capable of maintaining growth despite stress by activating detoxification mechanisms such as metal sequestration, synthesis of metal-binding proteins, and antioxidant defenses. However, tolerance in terms of growth does not necessarily correlate with safety for human consumption, as tolerant plants may still accumulate metals at concentrations exceeding food safety thresholds. This decoupling underscores the need for integrated evaluation frameworks that consider both plant performance and potential health risks (Ali & Ahirwar, 2025). From a human health perspective, consumption of vegetables contaminated with heavy metals represents a primary exposure route. Health risk assessment models, including Estimated Daily Intake (EDI), Hazard Quotient (HQ), and Hazard Index (HI), are widely used to evaluate potential non-carcinogenic risks associated with dietary exposure. HQ values greater than one indicate potential health concerns, and HI integrates multiple metal risks to reflect combined exposure. Studies consistently report that Pb and Cd contribute disproportionately to overall health risk due to their high toxicity and low tolerable intake limits, while children are found to be especially vulnerable due to higher intake relative to body mass (Ahmed et al., 2022).

Despite extensive research on heavy metal uptake and health risk assessment, the literature reveals a fragmented focus on isolated aspects, such as accumulation metrics or health implications alone, without an integrated performance evaluation framework. Many studies lack a unified methodology linking plant growth response, metal accumulation behavior, translocation patterns, and human health risk, which limits applicability for decision-making in agricultural management. Recent efforts have emphasized the integration of morphological measures with mathematical performance indices to provide a more comprehensive and reproducible evaluation of crop suitability under contaminated irrigation (Mafuyai & Ugbidye, 2021).

3. MATERIALS AND METHODS

3.1 Study site, design, experimental unit, and replication

The experiment was conducted at an open experimental site in Pematang Sulur village, Telanaipura District, Jambi Province, Indonesia (1°36′25.6″ S, 103°33′52.1″ E). The site was selected because it is located in a peri-urban agricultural area with potential exposure to low-quality irrigation water. Daily meteorological data including air temperature and humidity were obtained from the nearest official station of Badan Meteorologi, Klimatologi, dan Geofisika (BMKG), located approximately 5 km from the experimental site. Sampling of plant tissues was conducted at harvest on day 18. Roots and leaves from each treatment pot were collected as composite samples to reduce variability between individual plants. The details of daily temperatures during the experimental can be seen in **Figure 1.**

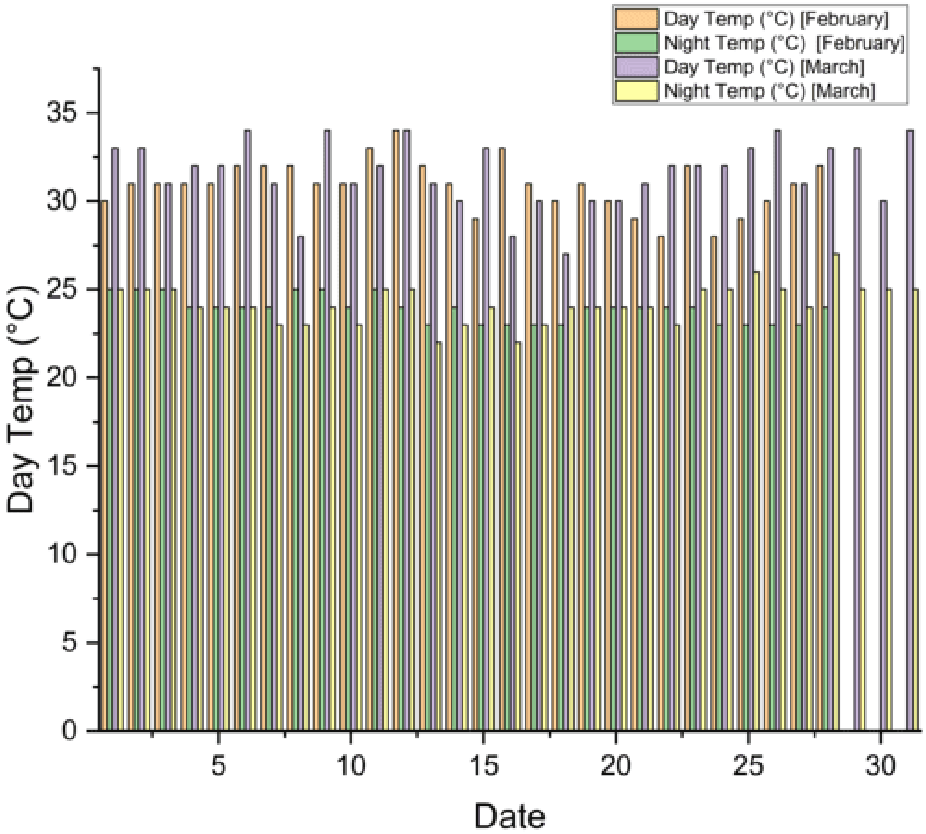

Figure 1. Daily temperature during the experimental period (February–March 2025) recorded by the Badan Meteorologi, Klimatologi, dan Geofisika (BMKG) Station of Sultan Thaha Airport, Jambi

The experimental used a Randomized Block Design (RDB) with three replications, consisting of one factor, namely the combination of plant cultivars and type of heavy metal. The plants used consist of three vegetables: velvetleaf (*Limnocharis flava*), kangkong (*Ipomoea aquatica*), and kailan (*Brassica oleracea* var. *alboglabra*) and the heavy metals: non-metals, Plumbum (Pb), Cadmium (Cd), and Copper (Cu), resulting in 12 treatment combinations. Each treatment combination was repeated in three replications (three blocks). There was a total of 36 treatment combinations, where each treatment unit consist of 4 plants, with 2 plants as samples. The total number of plants in this study was 144. Pots (25 cm × 20 cm) were arranged under a transparent polyethylene shelter to prevent rainfall interference while maintaining natural photoperiod and ventilation. Treatments were randomized within blocks to minimize positional effects.

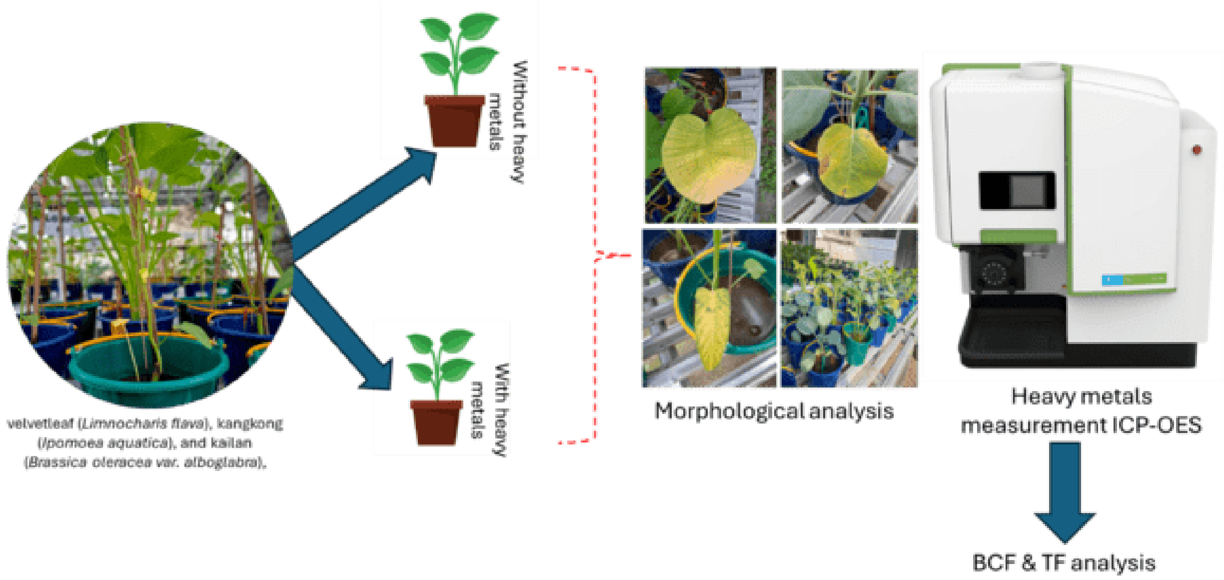

Figure 2. Experimental workflow of the study

3.2 Experimental Setup and Instrumentation

The experiment was conducted inside a transparent polyethylene shelter (6 m × 3 m × 2.5 m) constructed to exclude rainfall while maintaining natural light and ventilation (**Figure 2**). The shelter frame was built from galvanized steel and covered with 0.12 mm UV-stabilized polyethylene film. Ambient temperature and humidity inside the shelter were monitored continuously using a digital thermo-hygrometer (Testo 608-H2, Germany) placed at plant canopy height (≈ 50 cm) and recorded every 30 min.

The experimental bench was arranged in three blocks, each measuring 1.8 m × 0.9 m, separated by 50 cm aisles to minimize edge effects and ensure uniform exposure. Within each block, twelve 25 cm × 20 cm plastic pots were positioned on elevated trays (height = 60 cm). Pots were randomly assigned to treatment combinations of plant species × metal type. Each pot was labeled with an alphanumeric code. Irrigation and metal dosing were performed manually using graduated glass cylinders (500 mL, Pyrex) to ensure a precise daily application of 400 mL solution per pot. Fresh working solutions were prepared every morning from analytical-grade salts ($Pb(NO_3)_2$, $Cd(NO_3)_2$, and $CuSO_4{\cdot}5H_2O$) dissolved in distilled water and stored in amber glass containers to prevent photodegradation.

Concentrations of Pb, Cd, and Cu in the working solutions were verified using Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES; PlasmaQuant 9100, Analytik Jena, Germany). The instrument was calibrated daily using five-point external standards for each metal (0.01, 0.1, 1, 5, and 10 mg L^{-1}) prepared from certified stock solutions (Merck, Germany). Calibration curves were accepted when the coefficient of determination (r^2) was greater than 0.999. Concentration drift was monitored by analyzing continuing calibration verification standards every ten samples. If the measured value deviated by more than ±5 percent of the expected value, the instrument was recalibrated and previous samples were re-run. All laboratory glassware was soaked in 10 percent HNO_3 for 24 h, rinsed three times with deionized water, and dried in a clean cabinet before use. Method blanks and field blanks were included in each analytical batch to detect background contamination. Duplicate samples were analyzed at a frequency of one in every ten samples to evaluate analytical precision.

For morphological observations, a fixed-position digital camera (Canon EOS 250D, 24.1 MP) was mounted on a tripod at 1.0 m distance perpendicular to the plant canopy. This setup ensured identical framing, distance, and illumination for all photographs. Reference scale markers (2 cm × 2 cm grid) were placed beside each pot for image calibration. Plant length was measured with a digital caliper (Mitutoyo 500-196-30, Japan); leaf area at harvest was measured using Easy Leaf Area software (Easlon & Bloom, 2014) based on individual leave scanned on a flat A4 surface. Oven-drying of plant samples was performed in a forced-air drying oven (Memmert UF110, Germany) at 80 °C until constant weight, and dry weight was measured using an analytical balance (Shimadzu ATX224, ±0.0001 g). All laboratory glassware was acid-washed (10 % HNO_3) and rinsed with deionized water before use. Lastly, all analytical procedures followed internal quality-management protocols of the Saraswanti Indo Genetech Laboratory, which are aligned with ISO/IEC 17025 standards.

To quantitatively evaluate plant performance under heavy metal exposure, a mathematical framework integrating growth response, metal accumulation, translocation efficiency, and human health risk was applied. Morphological performance was expressed as relative growth change (%) compared to the control treatment. Metal accumulation performance was quantified using the bioconcentration factor (BCF), while metal mobility within plants was assessed using the translocation factor (TF). Food safety performance was evaluated using Estimated Daily Intake (EDI), Hazard Quotient (HQ), and Hazard Index (HI). These indices collectively represent the ability of a plant to tolerate metal stress while minimizing human exposure risk. Plants exhibiting high growth tolerance, BCF > 1, TF < 1, and HI < critical thresholds are considered to have superior performance under contaminated irrigation conditions.

3.3 Plant material and substrate

Velvetleaf, kangkong, and kailan seeds were treated differently depending on the plant's characteristics. Velvetleaf seeds were sown in seedling trays filled with soil until sprouts appeared in approximately 1 month, then transferred to the nursery. Kangkong was propagated vegetatively using 10 cm stem cuttings from the base of the stem. The kangkong cuttings were planted in soil, and once four leaves had developed, the seedlings moved to the nursery. Meanwhile, kailan seeds were sown in rockwool until sprouts appeared in approximately 3 days and transfer to the nursery. The nursery medium for all three plants is a mixture of soil and manure in a 1:1 ratio. After two weeks in the nursery, the roots were gently washed free of adhering soil and each plant was transferred to a plastic pot (25 cm × 20 cm) containing 2 kg of washed, sieved river sand per pot to provide an inert, low-background substrate. Natural ventilation and sunlight were maintained; rainfall was excluded by the polyethylene roof.

3.4 Heavy-metal solutions: preparation and treatment on plants

The exposure phase involved the application of aqueous heavy metal solutions through the root zone of each plant in 18 days exposure. The metals used were Pb, Cd, and Cu, applied in the form of $Pb(NO_3)_2$, $Cd(NO_3)_2$, and $CuSO_4 \cdot 5H_2O$, respectively. All chemical reagents were obtained from certified analytical-grade suppliers. Stock solutions were prepared using distilled water and subsequently diluted to final concentrations of 50 ppm Pb, 1 ppm Cd, and 2 ppm Cu. These concentrations were determined based on previous phytotoxicity studies that demonstrated observable yet sub-lethal effects on plant growth (Aboelkassem et al., 2022; Doğan et al., 2022). Fresh working solutions were prepared daily. Concentrations at each renewal were verified by ICP-OES PlasmaQuant 9100 Series using five-point external calibration (0.01–10 mg L^{-1}; $r^2 \geq 0.999$). Continuing calibration verification, method blanks, and duplicates were run every 10 samples.

Each pot received 400 mL day^{-1} of the assigned solution for 18 days to ensure continuous root contact. Control plants were irrigated with distilled water only. To prevent nutrient deficiency during exposure, foliar fertilization using Bayfolan D was applied at a concentration of 2 mL L^{-1} every four days throughout the experimental period.

3.5 Morphological observation and harvest

Morphological observation in this study was conducted to measure and visually document the growth dynamics of plants under different heavy metal exposure conditions. The observation included plant length, total leaf number, maximum leaf length, and leaf width, which were measured every two days beginning two days before exposure until harvest (day 18). To complement quantitative measurements, visual documentation was performed throughout the experiment. Each plant was photographed at the same distance, angle, and illumination using a fixed-position digital camera, enabling accurate visual comparison of morphological growth across treatments (**Figure 3**). These photographs provided qualitative evidence of growth inhibition and stress symptoms under Pb, Cd, and Cu exposure. At the end of the exposure period (day 18), all plants were carefully harvested and separated into roots, stems, and leaves. Each tissue was thoroughly rinsed with deionized water, followed by a brief wash in 0.01 M EDTA for 30 seconds to remove any surface-adsorbed metals, then rinsed again, blotted dry, and oven-dried at 80 °C to constant weight. Leaf area at harvest was quantified using scanned-image analysis with the Easy Leaf Area software (Easlon & Bloom, 2014). During the growth phase, daily leaf area was estimated using a validated linear regression model based on non-destructive morphological measurements (Lakitan et al., 2023, 2025). Overall, this combined quantitative and photographic approach allowed real-time tracking of morphological responses, providing both visual and analytical evidence of stress adaptation in velvetleaf, kangkong, and kailan exposed to Pb, Cd, and Cu.

Figure 3. Morphological observation of velvetleaf (*Limnocharis flava*), kangkong (*Ipomoea aquatica*), and kailan (*Brassica oleracea var. alboglabra*) during 18 days of exposure to Pb (50 ppm), Cd (1 ppm), and Cu (2 ppm).

3.6 Heavy metal measurements, Bioaccumulation and Translocation Factors

Heavy metal analysis was performed on dried plant roots and leaves. Plant samples were taken as composites by collecting leaves and roots from each treatment. Measurement of heavy metal concentration using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

Bioconcentration factor (BCF) and translocation factor (TF) were calculated as

$$BCF = \frac{C_p}{C_w} \tag{1}$$

where C_p represents the metal concentration in the plant tissue (mg kg^{-1}) and C_w denotes the metal concentration in irrigation water (mg L^{-1}). The TF, which represents the efficiency of metal transport from roots to leaves, was calculated as:

$$TF = \frac{C_{leaf}}{C_{root}} \tag{2}$$

where C_{leaf} and C_{root} are the metal concentrations in the leaves and roots, respectively. BCF values greater than 1 indicate a high accumulation capacity, while TF values greater than 1 suggest efficient translocation from roots to aerial tissues (Wibowo et al., 2023).

3.7 Health Risk Assessment

Health Risk Assessment (HRA) was conducted to evaluate the potential risk of heavy metal contamination in vegetables grown under polluted irrigation water. The analysis focused on Estimated Daily Intake (EDI), Hazard Quotient (HQ), and Hazard Index (HI), which are commonly used to assess the potential health risks posed by heavy metal accumulation through consumption of contaminated plants. The EDI for each metal (Pb, Cd, Cu) was calculated using the following equation:

$$EDI = \frac{C_{metal} \times F \times W}{BW \times 1000} \tag{3}$$

C_{metal} is the metal concentration in the edible part of the vegetable (mg/kg), F is the average consumption rate of the vegetable (g/day), W is the weight of the edible part (kg), BW is the average body weight (kg) of the population (typically 60 kg for adults).

The Hazard Quotient (HQ) is used to estimate the potential health risk of individual metals by comparing the intake with a reference dose (RfD), which is a level of daily exposure to a contaminant that is considered to be without risk of harmful effects.

$$HQ = \frac{EDI}{RfD} \tag{4}$$

RfD is the reference dose (mg/kg/day), which is a standard value determined by health agencies, the standard for Pb RfD: 0.0035 mg/kg/day; Cd RfD: 0.001 mg/kg/day and Cu RfD: 0.05 mg/kg/day.

The Hazard Index (HI) is the sum of the HQ values for each metal. If the HI is greater than 1, it indicates a potential health risk due to the combined exposure to multiple metals.

$$HI = \sum_i HQ_i \tag{5}$$

Where HQi is the Hazard Quotient for each metal (Pb, Cd, Cu). HQ < 1: No significant health risk; HQ ≥ 1: Potential health risk; HI ≥ 1: Cumulative health risk from exposure to multiple heavy metals.

3.8 Statistical Analysis

All analyses were performed in using IBM SPSS Statistics 25 for Windows (IBM Corporation, Armonk, USA). Pot-level means were used as observations. Normality and homoscedasticity were assessed by Shapiro–Wilk and Levene tests; variables were transformed when needed. Treatment effects within each species were tested by one-way ANOVA followed by Duncan's Multiple Range Test at $\alpha = 0.05$. Results are reported as mean ± SD across pots ($n = 3$). Last, reliability and reproducibility were strengthened by the use of independent experimental replications, standardized measurement protocols, and analytical quality control procedures, ensuring that observed treatment effects were not artifacts of measurement or sampling bias.

4. RESULTS

4.1 Morphological Response of Plants

The results showed that different types of plants exhibited different growth patterns due to exposure to various kinds of heavy metals. Exposure to Pb and Cd in velvetleaf showed a decrease in petiole length compared to velvetleaf without metals. Conversely, Cu exposure did not have a significant effect on velvetleaf, as velvetleaf did not experience any inhibition in petiole length. A decrease in plant length began to appear on days 10 to 18 in velvetleaf samples (**Figure 4**). The leaf number of velvetleaf tends to decrease in both exposure to metals and without metals. **Figure 4A** shows that petiole + midrib length increased gradually in all treatments during the initial exposure phase (0–8 days), indicating that early growth was not immediately inhibited by heavy metal stress. However, plants exposed to Pb and Cd exhibited a slower rate of elongation compared with the control, suggesting mild growth suppression due to metal toxicity. Among the metals, Cd had the most pronounced inhibitory effect after 12 days, consistent with its strong affinity for binding to cellular components and disrupting physiological processes such as photosynthesis and enzyme activity. **Figure 4B** presents the total leaf number per plant, which remained relatively stable throughout the exposure period. A slight reduction in leaf number was observed in the Cd and Cu treatments after day 10, indicating that prolonged exposure may have interfered with leaf initiation or accelerated senescence. Pb exposure produced minimal effect, suggesting that Velvetleaf may exhibit some degree of tolerance to Cd stress compared with Pb and Cu. **Figure 4C** illustrates the trend in leaf area, which followed a similar pattern to petiole and midrib elongation. The control plants showed continuous increases in leaf area, reaching the highest values around day 12–14. In contrast, plants treated with Cd and Cu exhibited reduced leaf expansion toward the later stage of exposure. This decline in leaf area may reflect both inhibited cell enlargement and potential oxidative damage induced by heavy metal accumulation.

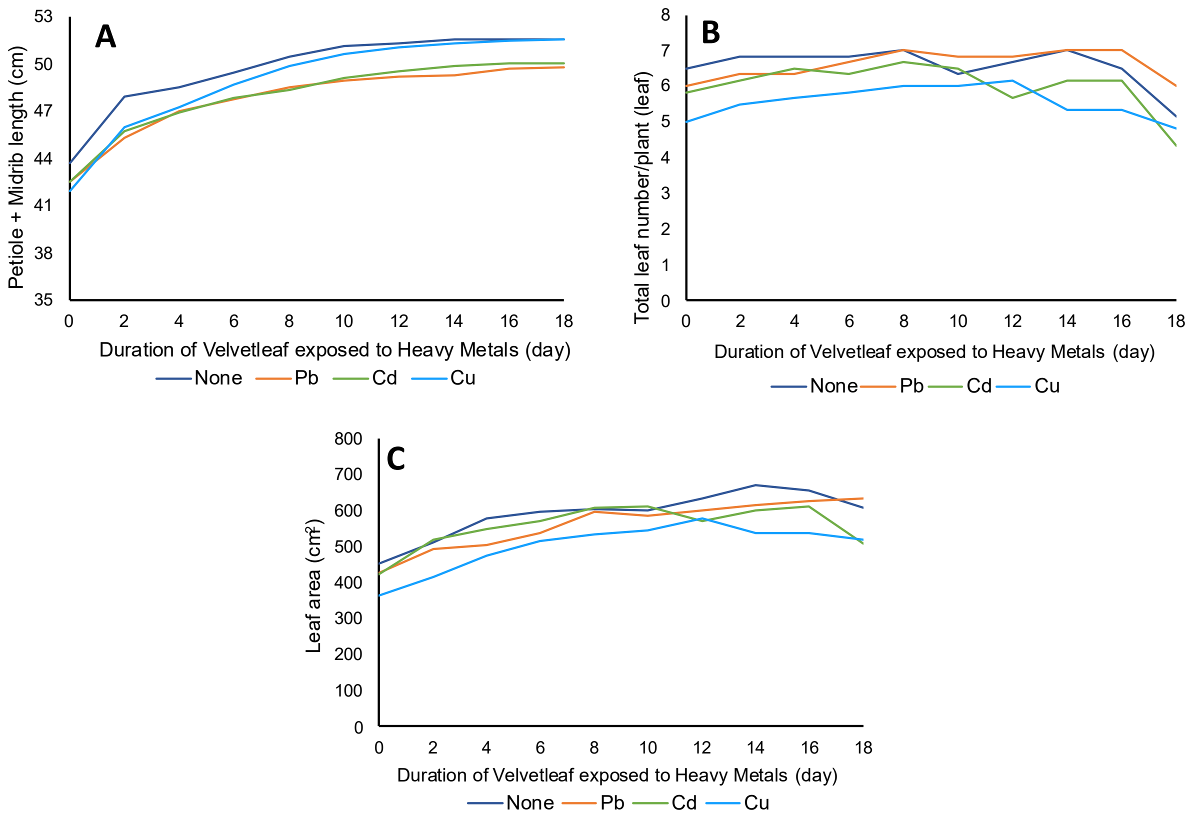

Figure 4. Effects of heavy metal exposure (Pb, Cd, and Cu) on Velvetleaf growth parameters over time. (A) Petiole + midrib length (cm), (B) total leaf number per plant, and (C) leaf area (cm^2) were measured during 18 days of exposure. “None” represents treatment without heavy metal addition.

Cd exposure resulted in the lowest reduction in both leaf number and total leaf area of velvetleaf. Meanwhile, in velvetleaf exposed to Pb, the decrease in leaf number occurs over a longer period, with leaves beginning to wilt and die on the 16th day after exposure. The total leaf area of velvetleaf also decreased along with the decrease in leaf number, which starts on day 10 after exposure. Velvetleaf exposed to Cd and Cu tended to experience a lower decrease in leaf area compared to exposure to Pb. However, there is a similar pattern between velvetleaf exposed to heavy metals and the control group.

Compared to velvetleaf, kangkong tends to be more resistant to heavy metal stress. At the beginning of exposure, kangkong showed normal growth until the 14th day of exposure. A decrease in plant length and leaf number of kangkong was seen after the 14th day. The lowest plant length, leaf number, and leaf area of kangkong occurred in the Cd exposure. Meanwhile, Cu exposure had a positive effect on kangkong growth, as indicated by higher increases in length, leaf number, and leaf area compared to kangkong without metals (**Figure 5**). **Figure 5A** shows that main stem length increased steadily in all treatments during the experimental period, though plants subjected to Pb, Cd, and Cu exhibited reduced elongation rates compared with the control. The control plants reached the greatest stem length by day 18, while Pb and Cd treatments produced the most pronounced inhibition. This suggests that these metals interfere with cell division and elongation processes, likely by disrupting hormonal regulation and nutrient uptake. **Figure 5B** presents total leaf number per plant, which also increased over time in all treatments. However, the growth rate of new leaves was consistently lower under heavy metal exposure, particularly for Pb and Cd. Cu exposure caused only moderate suppression, implying that Kangkong may possess higher tolerance to Cu stress. The reduced leaf proliferation under Pb and Cd treatments might be linked to impaired photosynthetic efficiency and limited carbohydrate allocation to developing leaves. **Figure 5C** illustrates the effect of heavy metals on leaf area expansion. Control plants maintained the largest leaf areas throughout the experiment, while Pb- and Cd-treated plants showed the smallest. Cu exposure caused an intermediate response. The decline in leaf area under metal stress reflects the inhibitory effects of heavy metals on cell enlargement and chlorophyll synthesis, both of which are essential for optimal photosynthetic performance.

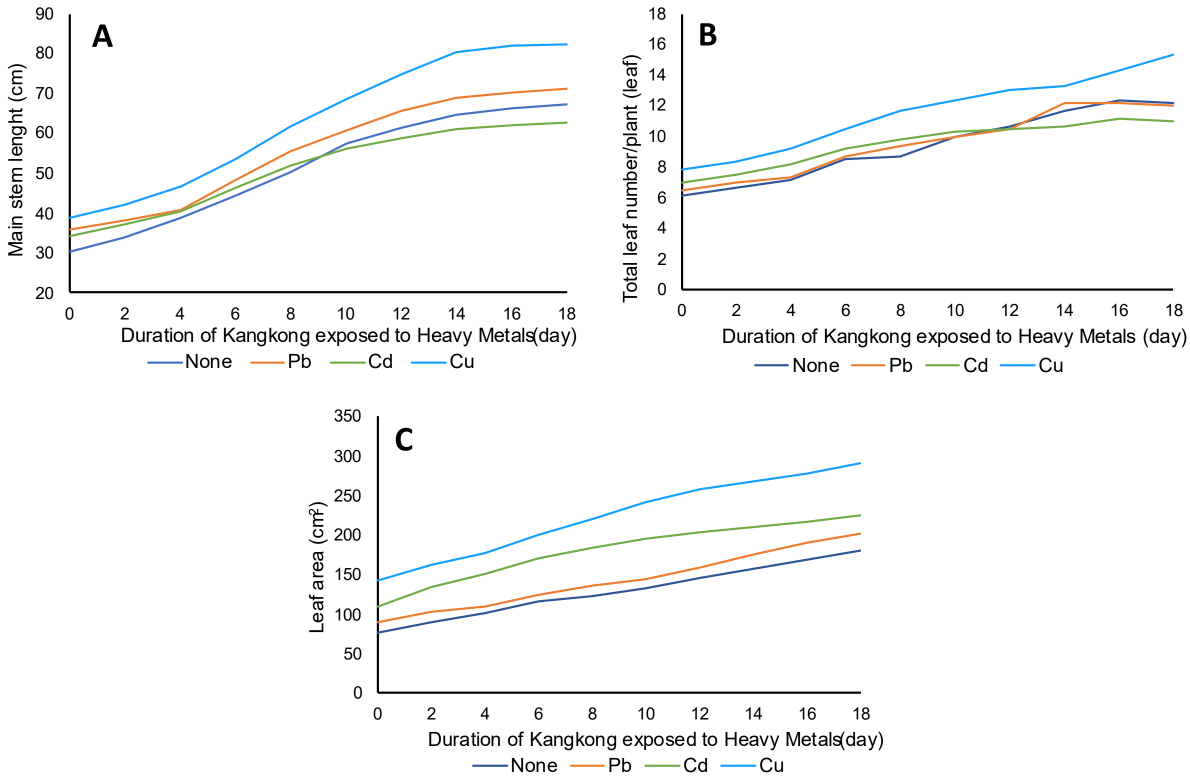

Figure 5. Growth response of Kangkong to heavy metals during exposure time: (A) Plant length, (B) Total leaf number, and (C) Total leaf area

Unlike velvetleaf and kangkong, kailan showed a different growth response. In the initial stages of exposure, there was a suppression of kailan length until day 12. However, after day 12, kailan length growth began to increase again, indicating a recovery mechanism against heavy metal stress. The increase in leaf number and leaf area of kailan leaves also showed a similar trend to plant length, where kailan experienced growth inhibition at the initial stage of exposure. Exposure to Cd and Cu resulted in lower increases in plant length, leaf number, and leaf area compared to Pb. The three types of heavy metals tested (Pb, Cd, and Cu) tended to have a negative impact on kailan growth (**Figure 6**). **Figure 6A** shows that main stem length increased progressively in all treatments over time, indicating continued growth despite the presence of heavy metals. However, plants exposed to Cd and Cu exhibited lower elongation rates relative to the control and Pb treatments. The inhibitory effect was more pronounced under Cu exposure, suggesting that Cu toxicity more strongly affects stem elongation, possibly by impairing cell wall extensibility and interfering with auxin-regulated growth processes. **Figure 6B** illustrates the total leaf number per plant, which gradually increased throughout the experimental period. The differences among treatments were relatively small, indicating that Kailan maintained leaf production under moderate heavy metal stress. However, slight suppression in leaf number was evident under Cd and Cu treatments, particularly after 12 days, which may reflect a delayed impact of metal accumulation on leaf initiation and expansion. **Figure 6C** shows the trend in leaf area, where the control plants maintained the largest leaf area throughout the 18-day exposure period. Pb-treated plants showed a smaller but relatively stable reduction compared with the control, whereas Cd and Cu caused greater declines in leaf expansion. This reduction in leaf area under Cd and Cu stress may result from disturbances in photosynthetic efficiency, chlorophyll degradation, and decreased turgor pressure factors commonly linked with heavy metal-induced oxidative stress.

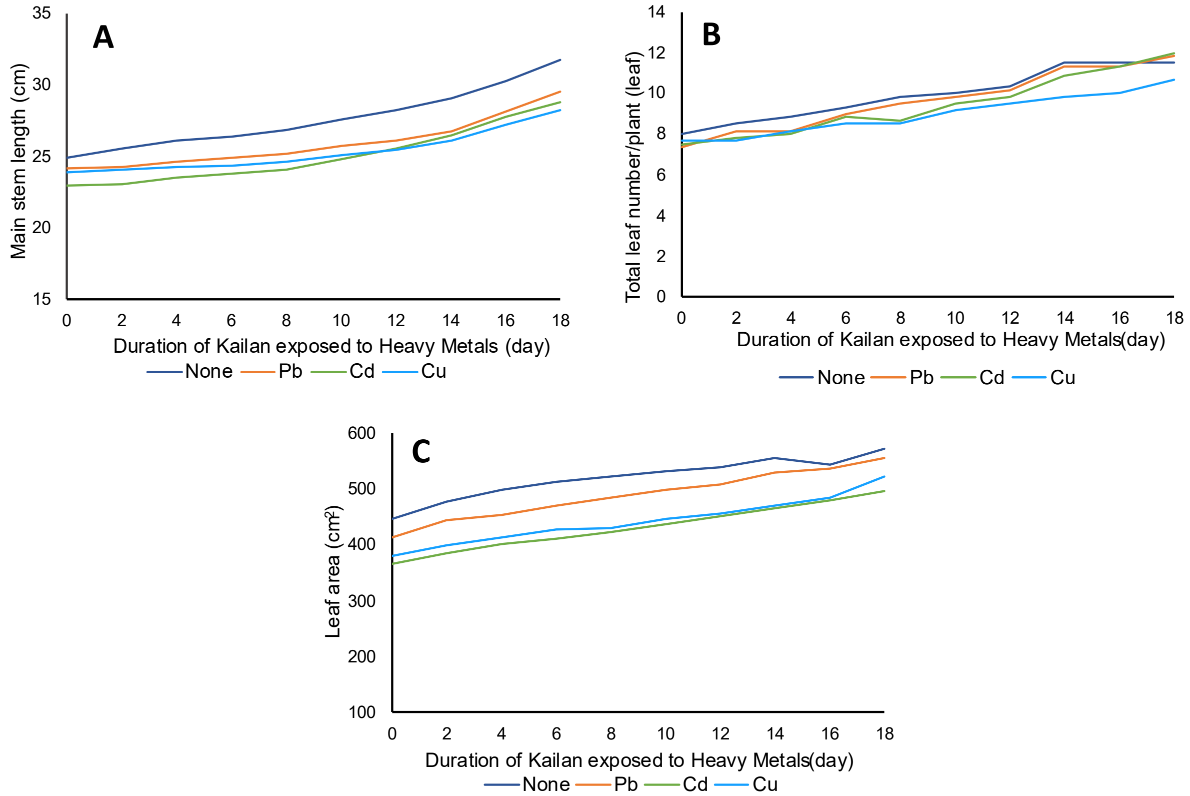

Figure 6. Growth response of Kailan to heavy metals during exposure time: (A) Plant length, (B) Total leaf number, and (C) Total leaf area

The analysis of variance reveals no significant differences in the increase in leaf length and leaf area in velvetleaf and kailan following exposure to Pb, Cd, and Cu metals. It was different for kangkong, where there were significant differences in plant length between kangkong exposed to Cu and kangkong exposed to Pb and Cd. Metal exposure gives significant differences in the increase in leaf number of velvetleaf and kailan. Velvetleaf exposed to Cd has the lowest leaf number and is significantly different from other metals. Meanwhile, exposure to Cu in the leaf number of kailan showed a significant difference compared to Pb and Cd. However, exposure to Pb, Cd, and Cu metals showed substantial differences in the increase in leaf area in all plants. (**Table 1**).

Exposure to heavy metals Pb, Cd, and Cu caused a decrease in the dry weight of kailan. However, it did not have a negative effect on the dry weight of velvetleaf. Meanwhile, in kangkong, only exposure to Pb caused a decrease in dry weight. The analysis of variance showed that the dry weight of kangkong exposed to Pb was significantly different from that exposed to Cd and Cu (**Figure 7)**. Identification and comparison of morphological plants showed in **Table 2**.

Table 1. Increase in plant length, leaf number, and total leaf area in various types of metal

Parameter	**Treatment**	**Velvetleaf**	**Kangkong**	**Kailan**
Increase in plant length (cm)	Without metal	7.90 a	36.93 ab	6.63 a
	Pb	7.22 a	35.40 bc	5.40 a
	Cd	7.48 a	28.50 c	5.80 a
	Cu	9.57 a	43.47 a	4.30 a
Increase in leaf number (leaves/plant)	Without metal	–1.33 bc	6.00 a	3.50 ab
	Pb	0.17 a	5.50 a	4.50 a
	Cd	–1.50 c	4.00 a	4.50 a
	Cu	–0.17 ab	7.50 a	3.00 b
Increase in total leaf area (cm^2)	Without metal	156.23 a	111.08 a	123.91 a
	Pb	204.13 a	112.68 a	142.73 a
	Cd	136.78 a	123.64 a	131.75 a
	Cu	154.84 a	147.85 a	140.49 a

Values followed by different letters in the same column indicate significant differences based on DMRT at $p < 0.05$. Negative values indicate a decrease.

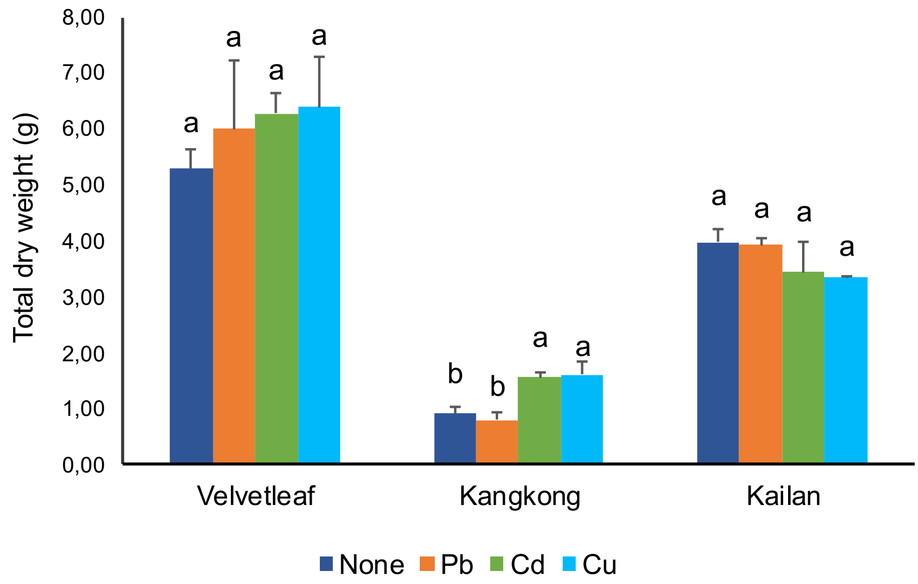

Figure 7. Total dry weight of plants on various types of metal. Different letters on each bar indicate significant differences based on DMRT at $p < 0.05$.

Table 2. Morphological Responses of Plants to Heavy Metal Exposure

Plant species	Pollutants Tested	Morphological effects	Ref.
Velvetleaf, Kangkong, Kailan	Pb, Cd, Cu	Chlorosis, reduced leaf size, stunted growth, leaf curling, necrosis	This study
Spinacia oleracea, Amaranthus grain, Mentha spicata	Cr, Ni, Zn, Cu, Pb	Growth inhibition, yellowing, wilting, leaf deformation	(Singh et al., 2024b)
Malabar spinach, 13 leafy species	Pb, Cd, As, Cu	Growth retardation, biomass reduction, low Cd accumulation in select spinach varieties	(Cui et al., 2022)
Lettuce, radish, carrot	Fe, Cu, Cr, Zn	Lettuce: chlorosis; Radish: reduced root length; Carrot: mild symptoms	(Qureshi et al., 2016)

General vegetables (review)	As, Cd, Hg, Pb	Overall yield reduction, leaf necrosis, root blackening	(Manwani, Devi, et al., 2022)
Pumpkin, spinach, gourd varieties	Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Pb	Leaf curling, reduced chlorophyll, thin leaves, early senescence	(Jolly et al., 2024)

4.2 Heavy metal uptake in plants

Heavy metal content in the three types of plants showed the highest accumulation of metals in plant tissues, namely Pb, followed by Cd and Cu (**Figure 8**). In terms of distribution, metal absorption in the underground organs (roots) was much higher than in above-ground organs (leaves). Pb uptake in roots was found to be highest in kailan at 2873.21 mg/kg, velvetleaf at 1473.88 mg/kg, and kangkong at 363.20 mg/kg.

In contrast to roots, the highest Pb concentration in leaves was found in kangkong, while Cd and Cu were found to be highest in velvetleaf leaves. The DMRT analysis ($p < 0.05$) showed that there were significant differences between the concentrations of Pb, Cd, and Cu in kailan roots and other plants. The results also showed significant differences between the concentrations of Cd and Cu in velvetleaf leaves and the leaves of other plants.

The Bioconcentration Factor (BCF) for the three types of plants exposed to Pb, Cd, and Cu were greater than 1. Kailan had the highest BCF value, followed by kangkong, and velvetleaf. Among the three heavy metals tested (Pb, Cd, and Cu), the highest BCF values were recorded in kailan, namely 2873.21 Pb, 99.87 Cd, and 414.13 Cu. The DMRT test results ($p < 0.05$) showed that the BCF values of kailan showed a significant difference compared to velvetleaf and kangkong. Meanwhile, the BCF values of velvetleaf and kangkong did not differ significantly (**Table 3**).

The Translocation Factor (TF) values for the three plant species for Pb, Cd, and Cu metals were greater than 1. The TF values for kailan for Cd and Cu were the lowest compared to other plants, indicating that heavy metal translocation from the roots to the leaves was very low. The test results showed that there were significant differences in TF for Cd and Cu in kailan and velvetleaf. Meanwhile, TF for Pb did not differ significantly among the three plant species (**Table 3**). In addition, **Table 4** showed the heavy metals concentration in plants comparing with the WHO/FAO standard. This study showed that the concentration of metals are higher than limit concentration in mg/kg plants.

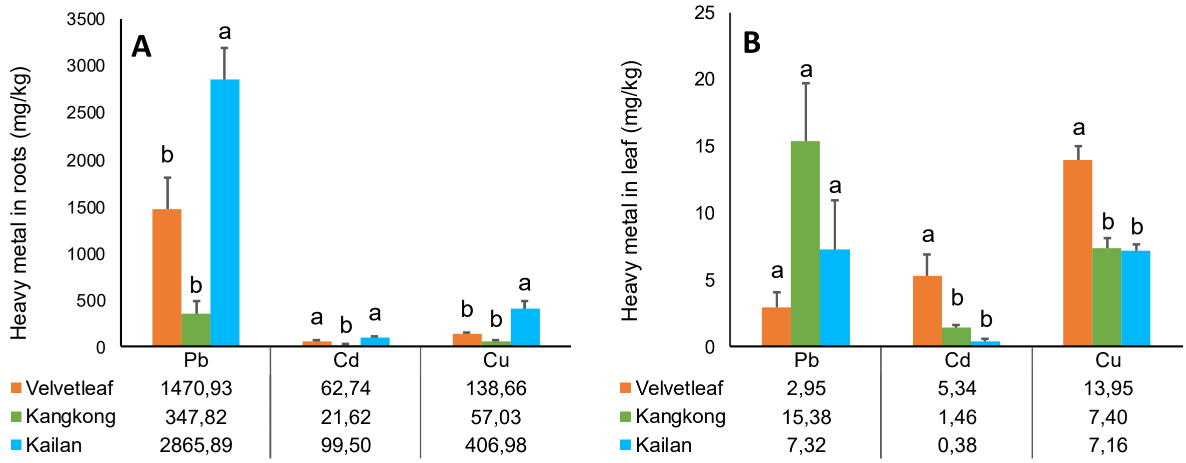

Figure 8. Heavy metal concentrations in plants (A) in roots (B) and leaves. Different letters on each bar indicate significant differences based on DMRT at $p < 0.05$.

Table 3. Bioconcentration factor (BCF) and translocation factor (TF) values for Pb, Cd, and Cu metals in various types of plants

	Pb	Cd	Cu
BCF			
Velvetleaf	29.48 b	1.36 b	3.05 b
Kailan	2873.21 a	99.87 a	414.13 a
Kangkong	181.60 b	11.54 b	32.21 b
TF			
Velvetleaf	0.0028 a	0.0976 a	0.1027 a
Kailan	0.0028 a	0.0041 b	0.0192 b
Kangkong	0.0074 a	0.0677 ab	0.1442 a

Values followed by different letters in the same column indicate significant differences based on DMRT at $p< 0.05$.

Table 4. Comparison the heavy metals concentration in plants vs FAO/WHO standard

Heavy metals	**Plant**	**Concentration in leaf**	**Max concentration (FAO/WHO, 2016) (mg/kg)**

Pb	Velvetleaf Kangkong Kailan	2.95* 15.28* 7.32*	0.3
Cd	Velvetleaf Kangkong Kailan	5.34* 1.46* 0.38*	0.2
Cu	Velvetleaf Kangkong Kailan	13.95 7.4 7.16	40

*Exceeding the maximum level

4.3 Health Risk Assessment

Tables 5–7 show the Health Risk Assessment (HRA) results, which indicate that all three vegetables pose severe health risks due to Pb and Cd contamination in the edible parts. The Hazard Index for kale (3097.6) was the highest, followed by velvetleaf (1421.2) and water spinach (349.1), clearly exceeding the safe threshold of 1. Pb contributed the most to the HQ and HI, suggesting that Pb contamination is the dominant factor affecting human health. Cd also showed a significant contribution, particularly in kale. Cu posed a negligible risk as its HQ values were below 1. The results emphasize the urgent need for monitoring irrigation water and selecting crops with limited translocation of metals to edible parts to ensure food safety.

Table 5. Estimate Daily Intake of heavy metals in each plants

Plant	Metal	Concentration (mg/kg)	EDI (mg/kg/day)
Velvetleaf	Pb	1473.88	4.91
	Cd	5.50	0.018
	Cu	2.50	0.0083
Kangkong	Pb	363.20	1.21
	Cd	1.20	0.004
	Cu	1.50	0.005

Kailan	Pb	2873.21	9.58
	Cd	99.87	0.333
	Cu	414.13	1.38

*Estimated Daily Intake (EDI)

Table 6. Analysis of Hazard Quotient (HQ)

Plant	Metal	EDI (mg/kg/day)	RfD	HQ
Velvetleaf	Pb	4.91	0.0035	1403
	Cd	0.018	0.001	18
	Cu	0.0083	0.05	0.166
Kangkong	Pb	1.21	0.0035	345
	Cd	0.004	0.001	4
	Cu	0.005	0.05	0.1
Kailan	Pb	9.58	0.0035	2737
	Cd	0.333	0.001	333
	Cu	1.38	0.05	27.6

*Estimated Daily Intake (EDI); Reference Dose (RfD); Hazard Quotiens (THQ)

Table 7. Hazzard Index

Plant	HI	Interpretation
Velvetleaf	1421.2	Very high health risk
Kangkong	349.1	Very high health risk
Kailan	3097.6	Extremely high health risk

4.4 Challenges and Limitations

Despite providing valuable insights into the morphological responses, bioaccumulation, and translocation of heavy metals in leafy vegetables, this study has several challenges and limitations that should be acknowledged. First, the experimental setup was conducted under controlled pot-scale conditions, which, although useful for isolating variables, may not fully replicate field-scale dynamics. Factors such as fluctuating temperature, rainfall, microbial activity, and soil heterogeneity can alter metal bioavailability and plant uptake under real agricultural conditions. Future studies should therefore validate these findings across multiple growing seasons and varied soil types to confirm ecological consistency.

Second, the analysis primarily focused on total metal concentrations without differentiating between free ionic and complexed forms. This limits the interpretation of true bioavailability, as metal speciation strongly influences plant uptake and toxicity. Incorporating speciation-aware analysis and rhizosphere measurements such as pH, redox potential, and dissolved organic carbon would provide a more mechanistic understanding of plant-metal interactions.

Third, while this research focused on morphological and accumulation parameters, the molecular and biochemical pathways underlying plant tolerance and detoxification were not investigated. Future research integrating transcriptomic or proteomic profiling could elucidate gene-level defense mechanisms and help identify biomarkers of metal stress.

Fourth, the health risk assessment used standard reference consumption rates and exposure assumptions, which may vary across populations. Region-specific dietary data and chronic exposure modeling should be incorporated to refine risk estimations and enhance applicability to local food systems. Finally, current predictive and statistical approaches, though effective for experimental analysis, could be enhanced through hybrid data-driven models that integrate environmental sensing and optimization algorithms, such as Al-Biruni Earth Radius Optimization and Vision Graph Neural Networks. These advanced computational tools could improve the precision of contamination prediction and enable spatial mapping of risk zones.

5. DISCUSSION

Each type of plant has a different response to heavy metal exposure. The results showed that the growth of plants exposed to heavy metals tends to be lower than that of plants without heavy metals. Among the three types of plants tested, kailan showed initial stress to the toxicity of heavy metals Pb, Cd, and Cu, where plant length and leaf number were significantly inhibited at the beginning of exposure. Heavy metal exposure causes morphophysiological, biochemical, and molecular changes in plants, thereby inhibiting plant growth and productivity (Noor et al., 2022; Raza et al., 2020). This is evident from the decrease in plant height, number of branches, leaf area and biomass, to the decrease in wet weight and dry weight (Hu et al., 2023; Waheed et al., 2022). After 12 days of exposure, kailan growth increased again, indicating a recovery or adaptation phase of plants in a heavy metal-contaminated environment (Ghori et al., 2019).

When exposed to heavy metals, some plants experience initial stress characterised by the production of Reactive Oxygen Species (ROS), which can cause cell damage (Liu et al., 2023; Pande et al., 2022). To overcome this, plants activate various defence mechanisms by enhancing the antioxidant system and producing proteins, hormones, metabolites, and chelating molecules such as phytochelatin and metallothionein to reduce oxidative damage (Ghori et al., 2019; Liu et al., 2023). Phytochelatin and metallothionein chelate toxic metal ions, forming complex compounds that are transported to vacuoles for storage, thereby reducing the concentration of heavy metals in the cytosol (Faizan et al., 2024). This response is crucial for enhancing metal detoxification capacity, repairing damage, and helping plants recover from initial stress and adapt to contaminated environments.

The concentration of heavy metals in kailan tissue was found to be very high (Pb 2873.21 mg/kg, Cd 99.87 mg/kg, and Cu 414.13 mg/kg). It indicates that high heavy metal content in the environment causes plants to be unable to limit the amount of heavy metals absorbed. The ability of plants to limit the absorption of heavy metals in the environment is limited, among other things, by their absorption capacity. The capacity of vacuoles to store heavy metal complexes is limited, causing saturation and a decrease in the efficiency of metal absorption, especially in contaminated environmental conditions (Choppala et al., 2014; Teng et al., 2024). Results in this study are consistent with previous reports, which found that the increase in heavy metal concentrations in plants correlates with higher heavy metal concentrations in the environment and more prolonged exposure durations (Chen et al., 2015; Ghori et al., 2019).

In contrast to kailan, exposure to heavy metals did not have a significant effect on the growth of velvetleaf and kangkong. Neither plant showed initial signs of stress during exposure. This indicates that the concentrations of heavy metals used in the study were still tolerable for the plants. These plants are effectively able to avoid stress by limiting the entry of metals through root exudates and maintaining metal ion homeostasis (Ejaz et al., 2023; Ghori et al., 2019). This exudate plays a role in limiting metal absorption by stimulating peroxidase enzyme activity, which thickens the root cell walls (Noor et al., 2022). The root apoplast acts as a physical barrier that inhibits the movement of metal ions into plant tissues (Ejaz et al., 2023; Singh et al., 2024a).

In velvetleaf, a decrease in plant growth was observed after 10 to 14 days of exposure, including without metal. This indicates that the decrease in velvetleaf growth was not caused by heavy metal exposure, but rather because the plants entered the generative stage (flowering phase). It can be seen from the similarity in the pattern of growth reduction in the control group that was not exposed to metal. Heavy metal exposure also had no significant effect on the dry weight of velvetleaf and kangkong. It indicates that velvetleaf and kangkong have better tolerance and adaptation mechanisms to heavy metal exposure than kailan.

Based on the type of heavy metal, exposure to Pb and Cd resulted in lower plant length and slowed leaf growth in all three plants. Cd exposure had the most adverse effect on plant growth, both in velvetleaf, kailan, and kangkong. Pb and Cd are non-essential metals that are toxic to plants. However, Cd has a higher density than Pb, so even at low concentrations, it causes toxicity that disrupts metabolism and inhibits plant growth. Cd is easily absorbed by plant roots and accumulates with exposure time, then transported to leaves and fruit (Ran et al., 2024). High Cd concentrations in plants caused a decrease in leaf stomatal density, limiting stomata and mesophyll conductance, resulting in slow growth due to disrupted photosynthesis (Guo et al., 2023; Hu et al., 2023). Increased Cd concentrations and exposure time in the three plant accelerate leaf yellowing (chlorosis) due to chlorophyll pigment degradation, leading to plant death (**Figure 9**). Previous studies have indicated a direct correlation between Cd exposure and chlorophyll breakdown, where increased Cd concentration caused a reduction in chlorophyll content in spinach (*Spinacia oleracea L.*) by up to 68% (Rydzyński et al., 2019), and 9–20% in Hybrid pennisetum (Song et al., 2019)

Meanwhile, Cu has a positive effect on the growth of velvetleaf and kangkong. Cu is an essential micronutrient that plays a role in various metabolic processes and physiological reactions in plants, including participating in the electron transport chain of photosynthesis and respiration, oxidative metabolism, cell wall metabolism, and hormone signaling (Nazir et al., 2019; Shabbir et al., 2020). Plants require Cu in low concentrations, but it becomes toxic at higher concentrations. There was a slight different between the essential and harmful concentrations of Cu in organisms. The presence of Cu in the growing medium encourages plants to absorb Cu because of their need as a micronutrient, but plants cannot control the amount of Cu absorbed, causing it to exceed the limit (Kumar et al., 2021; Xu et al., 2024).

Figure 9. Chlorosis on the leaves of Velvetleaf, Kangkong, and Kailan

Table 2 summarizes the morphological alterations observed in various vegetable species exposed to different heavy metals, highlighting both interspecific variability and pollutant-specific responses. In this study, velvetleaf, kangkong, and kailan exhibited visible stress symptoms such as chlorosis, leaf curling, and growth inhibition under Pb, Cd, and Cu contamination. These morphological changes indicate disruptions in photosynthetic efficiency and nutrient translocation caused by metal toxicity. Similar manifestations have been reported in Spinacia oleracea, Amaranthus grain, and Mentha spicata subjected to Cr, Ni, Zn, Cu, and Pb exposure, where leaf deformation and wilting were attributed to oxidative damage and impaired water balance (Singh et al., 2024b).

Consistent with these findings, Basella alba (Malabar spinach) and other leafy vegetables showed reduced biomass and visible growth retardation under Pb, Cd, As, and Cu exposure, suggesting that metal accumulation interferes with cell division and elongation processes (Cui et al., 2022). Root vegetables such as lettuce, radish, and carrot demonstrated organ-specific sensitivity, where chlorosis, root shortening, and mild necrotic symptoms were evident depending on the dominant metal present (Qureshi et al., 2016). A broader review by Manwani et al. (2022) confirmed that exposure to As, Cd, Hg, and Pb generally results in yield reduction, leaf necrosis, and blackening of roots, reinforcing that morphological deterioration is a reliable indicator of physiological stress.

Furthermore, studies on pumpkin, spinach, and gourd varieties exposed to multiple metals (Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, and Pb) demonstrated cumulative toxicity manifested through leaf thinning, early senescence, and reduced chlorophyll content (Jolly et al., 2024). Collectively, these results emphasize that morphological abnormalities serve as rapid, visible markers of heavy metal stress and can be used as preliminary diagnostic criteria before conducting biochemical or molecular analyses. The present study therefore aligns with previous reports, confirming that both leaf deformation and growth suppression are universal adaptive responses among leafy vegetables subjected to heavy metal contamination.

The ability of plants to accumulate pollutants from growing media, both water and soil, is calculated using the Bioconcentration Factor (BCF). Several studies have used BCF values to evaluate the potential for metal bioaccumulation by plants. The BCF for Pb, Cd, and Cu in the three types of plants tested were greater than 1, indicating that all three plants have a high potential for heavy metal accumulation (Dogan et al., 2018; Sipos et al., 2023). Based on the BCF values, the order of plant ability to accumulate Pb, Cd, and Cu is kailan<kangkong<velvetleaf. All plants are capable of absorbing heavy metals at high concentrations, thereby increasing the risk of heavy metal exposure through consumption.

Based on the distribution of heavy metals in plant parts, higher concentrations of heavy metals were detected in underground organs (root tissue) than in above-ground organs (leaf tissue) in all plant tested. Higher heavy metal accumulation in roots is a tolerance strategy of plants to reduce the effects of toxicity on photosynthetic tissues (Bonanno & Cirelli, 2017). The highest accumulation of Pb, Cd, and Cu was found in kailan roots, 2-3 times higher than velvetleaf and kangkong. However, the heavy metals translocated to the kailan leaves were lower than those of velvetleaf and kangkong. The highest accumulation of heavy metals in the leaves was found in kangkong leaves. It indicates that kangkong tends to transfer more heavy metals from underground organs to above-ground organs.

In kangkong, 88–95% of heavy metals accumulate in the roots, and 4–11% are translocated to the leaves. Most heavy metals (90–99%) in velvetleaf also accumulate in the roots, with only 0.2–10% translocated to the leaves. Meanwhile, in kailan, heavy metal accumulation in the roots reaches 98–99%, while the concentration in the leaves is very low (0.25–1.73%). The Translocation Factor (TF) showed that all three plants have TF greater than 1. The order of plants based on TF values was kangkong>velvetleaf >kailan, indicating that kangkong transferred more heavy metals to leaf tissue than velvetleaf and kailan. All three plant species have BCF>1 and TF<1; thus, they can be categorised as phytostabilizers, where the plants are capable of retaining heavy metals in the roots and preventing their mobilisation to leaf tissues (Hidayati & Rini, 2020).

The concentrations of heavy metals Pb, Cd, and Cu in the three plants tested exceeded the maximum levels set by FAO/WHO for leafy vegetables, namely 0.3 mg/kg for Pb, 0.2 mg/kg for Cd, and 40 mg/kg for Cu The comparison between the measured heavy-metal concentrations in plant tissues and the FAO/WHO permissible limits shows a clear pattern of excessive accumulation for Pb and Cd across all three vegetable species. Lead concentrations in velvetleaf (2.95 mg/kg), kangkong (15.28 mg/kg), and kailan (7.32 mg/kg) exceed the recommended maximum limit of 0.3 mg/kg by 9.8- to 51-fold. Kangkong demonstrates the highest Pb uptake, suggesting strong bioaccumulation potential, possibly due to its aquatic growth habit that enhances metal absorption from contaminated irrigation water or sediment.

Cadmium levels follow a similar trend, with velvetleaf (5.34 mg/kg), kangkong (1.46 mg/kg), and kailan (0.38 mg/kg) all exceeding the FAO/WHO limit of 0.2 mg/kg (FAO/WHO, 2016). The concentration in velvetleaf is particularly high, reaching more than 26 times the permissible level. These results indicate a substantial health risk because Cd is known for its high mobility in soil–plant systems and can accumulate in edible tissues even at relatively low environmental concentrations. In contrast, copper concentrations in all plant samples remain below the FAO/WHO limit of 40 mg/kg. Even though Cu is an essential micronutrient, excessive levels can become toxic; however, the measured values (7.16–13.95 mg/kg) suggest that Cu contamination is not a primary concern at the study site. The distinction between toxic (Pb, Cd) and non-toxic (Cu) levels highlights the influence of metal speciation, soil chemistry, and plant physiology on uptake behavior.

Food security under conditions of heavy metal contamination can be achieved through integrated control at multiple stages of the food production chain. At the irrigation level, regular monitoring and regulation of water quality are essential to prevent chronic input of Pb and Cd into agricultural systems. When complete water treatment is not feasible, the selection of vegetable species with low translocation potential becomes critical. The present study demonstrates that although all tested vegetables accumulated metals (BCF > 1), differences in translocation behavior strongly influence food safety outcomes. Species with lower TF values restrict metal movement to edible tissues, thereby reducing direct dietary exposure. However, low TF alone is insufficient; it must be complemented with agronomic mitigation strategies such as soil pH adjustment, organic amendments, and metal-immobilizing materials to reduce bioavailable fractions. From a public health perspective, security is achieved not by plant tolerance alone, but by reducing human exposure below acceptable risk thresholds. Therefore, integrating crop selection, water quality management, and regulatory enforcement is essential to ensure safe vegetable production in contaminated environments.

The HRA revealed alarming implications for human consumption. The Target THQ values for Pb and Cd greatly exceeded the safe threshold (HQ>1) in all three plants, particularly in kale, which had a Hazard Index of 3097.6. Velvetleaf and kangkong also posed severe health risks, with HIs of 1421.2 and 349.1, respectively. Pb was the dominant contributor to risk, followed by Cd, while Cu presented negligible risk (HQ<1). These results highlight that even crops with higher tolerance to heavy metal stress, like velvetleaf and kangkong, can accumulate metals to hazardous levels, emphasizing the necessity of monitoring irrigation water quality and implementing mitigation strategies before cultivation.

Several studies have highlighted the risks of human exposure to heavy metals and their associated health impacts. For instance, in China, rice samples contained Cd concentrations 9.35 times above the Chinese maximum permissible level, emphasizing the risk of dietary exposure (Cai et al., 2019). Similarly, in Iran, Pb in cow meat showed the highest Hazard Quotient (HQ) among the metals analyzed, while Cd in cow kidney posed the greatest carcinogenic risk (CR). Although the overall Hazard Index (HI) was greater than 1, children were found to be more susceptible, indicating that frequent consumption of contaminated meat could pose significant health concerns (Zeinali et al., 2019).

In vitro studies further support the harmful effects of heavy metals, particularly when combined. Human gastric epithelial cells exposed to Cd (5 μM) and Cu (10 μM) simultaneously exhibited a 40% loss in cell viability, a 3.5-fold increase in reactive oxygen species (ROS) indicative of oxidative stress, 36% cell cycle arrest in the S phase, and a 22%

apoptosis rate (Wang et al., 2021). These findings indicate that co-exposure to Cd and Cu is more detrimental than exposure to either metal individually, highlighting the synergistic toxicity of heavy metals (Wang et al., 2021). Biomonitoring data also reflect chronic exposure levels in human populations. Overall, these studies demonstrate that lead, cadmium, and copper are strongly linked to adverse health effects through multiple exposure routes, including food, air, and occupational contact. Even low-level, chronic exposure can lead to oxidative stress, organ damage, and carcinogenic outcomes, particularly when metals act synergistically. These findings underscore the critical importance of monitoring metal contamination in food sources, irrigation water, and the environment to minimize cumulative health risks in vulnerable populations.

The present findings have direct implications for public health because leafy vegetables are widely consumed and can meaningfully contribute to dietary exposure when irrigation water contains bioavailable Pb, Cd, and Cu. Lead is a developmental neurotoxin; even low prenatal and early-life exposures are linked to decrements in intelligence quotient and adverse neurocognitive outcomes, while adult exposures are associated with hypertension and kidney disease (Ramírez Ortega et al., 2021). Cadmium exhibits very long biological half-lives in humans and preferentially accumulates in the kidney, elevating risks of tubular dysfunction and chronic kidney disease; diet is a dominant exposure route globally (Charkiewicz et al., 2023). Although copper is an essential micronutrient, sustained intakes above updated health-based guidance values raise concern for hepatic retention and potential liver injury, underscoring the importance of controlling cumulative exposures from food (More et al., 2023).

Figure 10 showed the pathway of heavy-metal contamination from vegetables to human health impacts. Irrigation-water contaminants such as lead (Pb), cadmium (Cd), and copper (Cu) can be taken up by plants and accumulate in edible tissues; consumption elevates non-carcinogenic risk (HQ and HI). Documented outcomes include neurodevelopmental deficits and hypertension for Pb, renal tubular dysfunction and chronic kidney disease for Cd, and hepatic effects at excessive Cu intake. Arrows indicate the progression from contaminated plants to exposure and health impacts; mitigation can target water quality, soil and cultivar management, and household preparation to reduce bioaccessibility.

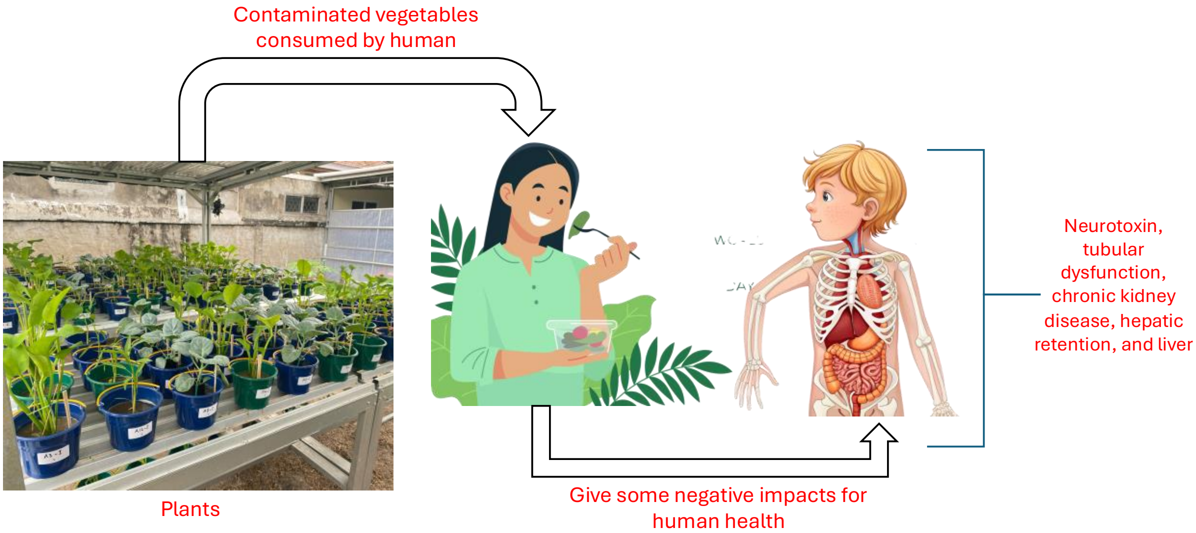

Figure 10. Conceptual pathway from heavy-metal–contaminated vegetables to human health outcomes

Risk characterization for leafy-vegetable consumption should therefore use established non-cancer metrics based on the hazard quotient (HQ) and hazard index (HI). HQ values greater than 1 for individual metals, or HI values greater than 1 for mixtures, indicate potential concern for non-carcinogenic effects and should trigger mitigation. Because consumers experience co-exposure to multiple metals and foods, reliance on single-metal assessments can underestimate total risk. Mixed-metal ingestion may also worsen intestinal barrier integrity and perturb the gut microbiome, amplifying systemic inflammation and metabolic risk, which suggests that risk estimates based solely on total concentrations may understate overall health burdens.

The findings of this study complement recent developments that combine environmental monitoring, artificial intelligence, and sustainable agricultural management. Advanced computational models have demonstrated a strong ability to improve decision-making in food and crop systems. For instance, the reuse of nutrient-rich water using Recurrent Neural Networks (RNN) and Natural Language Processing techniques has shown potential for optimizing nutrient recovery and supporting healthier food practice (K et al., 2025). Similarly, a comparative study using Support Vector Machines (SVM) and Decision Tree algorithms for predicting hydroponic tomato growth revealed that data-driven models can effectively capture non-linear relationships between environmental factors and crop yield (B et al., 2024). In addition, real-time irrigation optimization in coffee plantations using Bi-Directional RNNs and Internet of Things (IoT) sensors demonstrated the value of integrating continuous data streams to enhance water use efficiency and crop productivity (Shahriar et al., 2021). Research on lettuce farming using Random Forest algorithms also indicated that this model outperforms conventional Decision Tree techniques in predicting plant performance and managing cultivation parameters (Jegan et al., 2024) .

Future research should prioritize multi-season field validation across contrasting soils and climatic regimes to determine whether the pot-scale responses observed here persist under on-farm irrigation practices. Studies should incorporate speciation-aware exposure, distinguishing free ions from complexed forms, together with rhizosphere measurements of pH, redox potential, and dissolved organic carbon. This approach will help disentangle bioavailability from total concentration and clarify mechanisms that govern uptake, bioconcentration, and translocation. Longer observation windows that span full growth cycles, including a post-exposure depuration phase in clean water, are also recommended to assess whether edible tissues can reduce accumulated metals and to quantify any agronomic costs. Because real wastewaters contain mixtures, future designs should shift from single-metal tests to factorial or response-surface studies that span realistic multi-metal ranges, for example Pb×Cd×Cu with Zn or Ni as co-contaminants. In parallel, screening of cultivars and landraces within each species is needed to identify low-translocation phenotypes that maintain yield and quality under stress. Mechanistic depth can be added through ionomics and multi-omics, including transcriptomics, proteomics, and metabolomics, to resolve antioxidant responses, chelation pathways, and vacuolar sequestration. Spatial imaging such as micro-XRF or LA-ICP-MS can verify tissue-level sequestration targets suggested by physiological measurements. Translational work should assemble integrative mitigation packages that combine soil amendments, for example liming, silicon, phosphate, organic matter, or biochar, with foliar nutrition and microbe-assisted strategies. Candidate consortia include plant growth-promoting and metal-immobilizing microbes, mycorrhizae, and mineral additives such as iron or manganese oxides and layered double hydroxide-based sorbents. The goal is to suppress bioavailability and leaf translocation without penalizing yield. On the consumer side, experiments that quantify how washing, blanching, pickling or fermentation, and common cooking methods alter both total concentrations and gastrointestinal bioaccessibility will refine health-risk estimates in ways that are directly relevant to household practice. These datasets should feed into probabilistic exposure models that use locally stratified intake distributions by age, sex, and season, and that report both uncertainty and variability. The same models should be used to derive irrigation-water trigger values, defined as concentration–time safe operating envelopes that keep edible-leaf metals below regulatory limits.

In addition, next study also should considerate environmental monitoring and precision agriculture increasingly rely on advanced sensing, data-analysis and modelling techniques to characterise contamination, plant stress and crop performance (Maruthai et al., 2025; Sridharan Sivasubramanian et al., 2025). Finally, methodological enhancements will strengthen reproducibility and policy uptake. Standardized imaging for longitudinal leaf-area tracking, photographic documentation of all experimental stages, preregistered analysis plans, and a priori power calculations will improve inference on treatment effects. Socio-economic and policy integration, including cost–benefit and life-cycle assessments of on-farm versus water-side mitigation, hotspot mapping, and co-designed risk communication with farmers and local regulators, will help convert experimental insights into practical guidance. This should include crop-selection recommendations for settings where water treatment is infeasible and decision frameworks that link routine water monitoring to actionable farm-level responses.

6. CONCLUSIONS

This study revealed that vegetables exposed to Pb, Cd, and Cu-contaminated irrigation water exhibit different morphological responses. Kailan showed initial growth inhibition, while velvetleaf and kangkong tolerated exposure for longer periods. Quantitatively, kailan accumulates the highest concentrations. All plants displaying BCF >1 and TF<1 are classified as phytostabilizers. Despite root retention, Pb and Cd concentrations in leaves consistently exceeded FAO/WHO safety limits, resulting in very high health risk indices for kailan, followed by velvetleaf and kangkong. Therefore, effective mitigation strategies are needed, including monitoring and managing irrigation water quality, and selecting vegetable varieties that are capable of root sequestration without leaf accumulation. This strategy is necessary to reduce the risk of contamination and support the development of safe vegetable cultivation for human health. Future research should prioritize multi-season field trials across diverse soil types and climatic conditions to validate whether the pot-scale responses observed in this study are consistent under real on-farm irrigation practices. Subsequent studies should also incorporate speciation-sensitive analyses that distinguish between free ionic and complexed metal forms, alongside comprehensive rhizosphere assessments of pH, redox potential, and dissolved organic carbon dynamics to better elucidate metal bioavailability and plant uptake mechanisms. In addition, food security in heavy metal–affected irrigation systems can be achieved through a combination of preventive water management, selection of low-translocation crops, and agronomic mitigation to suppress metal bioavailability. Although plant tolerance allows survival under contamination, safety for human consumption requires minimizing metal transfer to edible tissues and maintaining exposure levels below health-based limits. These findings provide a scientific basis for risk-informed crop selection and irrigation management to protect public health in peri-urban agricultural systems.

Future research should extend this work beyond pot-scale conditions through multi-season field trials across different soil types and climatic settings to validate the consistency of plant responses under real irrigation practices. Further studies should incorporate metal speciation and rhizosphere processes, including pH, redox potential, and dissolved organic carbon, to better elucidate bioavailability-driven uptake mechanisms. In addition, experiments involving realistic multi-metal mixtures and longer exposure periods covering complete crop cycles are needed to reflect actual wastewater irrigation scenarios. Screening of cultivars with low translocation potential and the application of mitigation strategies such as soil amendments, biochar, silicon-based materials, or microbial-assisted approaches should also be prioritized. Finally, future work should integrate region-specific dietary data and probabilistic health-risk models to improve the accuracy and relevance of food safety assessments for local populations.

7. Acknowledgements

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8. Data Availability Statements

All raw data, laboratory notebooks, and photographic documentation are archived under the institution's data retention policy and are available upon reasonable request.

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