HUMAN HEALTH RISK, POTENTIALS OF BIOMONITORING AND PHYTOREMEDIATION OF COPPER USING *AMARANTHUS VIRIDIS*

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Abstract. This study aimed to determine copper (Cu) levels in *Amaranthus viridis* collected from 11 Peninsular Malaysia sampling sites and to estimate its human health risk. In addition, it also aims to assess the potential of *A. viridis* as a biomonitor and phytoremediator of Cu pollution. From a biomonitoring point of view, the Cu concentrations in the leaves, stems, and roots of *A. viridis* ranged from 10.8 to 21.9 µg/g dw (1.30-2.63 µg/g ww), 5.96 to 14.60 µg/g dw (0.36-0.88 µg/g ww), and 9.17 to 30.68 µg/g dw (1.01-3.37 µg/g ww), respectively. From the health risk aspect, it was found that the target hazard quotient (THQ) for Cu in the edible leaves of *A. viridis* were all below 1.00, indicating there were no non-carcinogenic risks of Cu to consumers, regarding both children and adults. Still, routine monitoring and managing the vegetable farms are recommended and necessary. From a phytoremediation perspective, with most bioconcentration factor values> 1.0 and the transfer factor> 1.0, *A. viridis* is a very potential phytoextraction agent of Cu. The experimental greenhouse study indicated that *A. viridis* could be a Cu biomonitor.
Keywords: *public concern, toxic metal, phytoextraction, ecotoxicology, vegetable*

Introduction

Public health concerns are raised due to contaminated wastewater irrigating edible vegetables (Atayese et al., 2010; Aguilar et al., 2011; Adekiya et al., 2019). Published papers with scopes of study that covered aspects of biomonitoring (An et al., 2004; Nik et al., 2012; Mwesigye et al., 2016), health risk (Chang et al., 2014; Chary et al., 2014; Eliku and Leta, 2017), and phytoremediation (Ali et al., 2013; Arco-Lázaro et al., 2017; Alaboudi et al., 2018) of heavy metals are currently getting increased attention.

From the biomonitoring perspective, checking the current status of heavy metal contamination in vegetables is important. Some publications (Chunilall et al., 2005; Khurana et al., 2008) on *Amaranthus* species only reported the accumulation and concentrations of heavy metals in the different parts of the plants. According to studies, the Copper (Cu) and other heavy metal levels in vegetables grown near mining sites or smelters can exceed the maximum permissible limits. This poses a significant risk to human health as these contaminated crops enter the food chain. The accumulation of Cu in vegetables is influenced by factors such as growth stage, fertilizer treatment, and plant species. Certain vegetables, such as water spinach, have shown higher Cu accumulation levels than others. This issue calls for monitoring Cu's bioavailability, speciation, exposure levels, and routes in living organisms. Furthermore, it is crucial to assess the

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quantity and speciation of Cu in the soil-plant system to understand its behaviour and potential risks (Kumar et al., 2021).

It is necessary to develop prediction models for Cu concentration in leafy vegetables to address the issue, particularly in tropical regions where no specific prediction model currently exists. This is because the model established elsewhere from the subtropical plants could not be used in the tropical area. The plant *Amaranthus* is a tropical terrestrial species; its abundance and distribution are absent in the subtropical region. Therefore, the findings from subtropical areas cannot be extrapolated to the tropical region. Therefore, studies using different plant species found ecologically in the tropical region are needed to understand similar ecotoxicological impacts.

These models can help farmers and agricultural professionals make informed decisions regarding soil management, irrigation practices, and crop selection to reduce vegetable Cu toxicity. Furthermore, research should also focus on developing safe cultivation methods that minimize plants' Cu and other heavy metals uptake. The potential health implications of Cu toxicity in vegetables necessitate thorough monitoring and assessment of its bioavailability, accumulation, and behaviour in the soil-plant system (Parvin et al., 2014; Chandel et al., 2020; Gungshik et al, 2021; Rezapour and Jalil, 2022).

Improper fertilizer and pesticide applications or atmospheric sources elevated the concentrations of heavy metals in the soil (Zarcinas et al., 2004). As a developing country, Malaysia has numerous industrial and mining activities, especially on the west coast of Peninsular Malaysia. Thus, the main sources of heavy metals are manufacturing industries, urbanization practices, and agro-based industries (Parisa et al., 2010).

The significance of biomonitoring of heavy metals in vegetables is highly connected to the need to know the status of human health risks of heavy metals from a consumer point of view. Therefore, biomonitoring studies have been extended to human health risk assessments of heavy metals in the edible *Amarnthus*. Khalid et al. (2017) examined the impacts of groundwater and wastewater irrigation on Pb elevation in soil and crops and their potential health risks. They, therefore, proposed that Vehari city wastewater/groundwater be cleaned before being used for irrigation to avoid heavy metal contamination of vegetables and reduce the risk of Pb-induced human health effects.

Also, biomonitoring studies have been extended to understand its potential as a phytoremediation agent of heavy metals. Chunilall et al. (2005) investigated the responsiveness and ability of *Amaranthus hybridus* (green herbs) and *Amaranthus dubius* (red herbs) to accumulate and tolerate varying amounts of Cd(II), Ni(II), Pb(II), and Hg(II) combinations in their roots and shoots. Lukatkin et al. (2021) investigated how the accumulation of heavy metals (including Cu) influenced the biochemical and physiological parameters of *Amaranthus retroflexus* seedlings in laboratory settings. The seedlings showed considerably high resistance to all investigated metals and no significant oxidative stress in the leaves.

Phytoremediation is a green technology for degrading, extracting, and remediating pollutants from soil and water (Purakayastha and Chhonkar, 2010). It is ecologically beneficial and a cost-effective alternative to the soil remediation procedures that are currently used. Phytoremediation has been well received in the recent decade because it does not harm the soil structure while protecting microbial communities. The rate and depth of contaminant uptake from the soil, accumulation in the plant cells and the degree of contaminant transformation to ordinary cell metabolites can all be used to measure the plant's detoxifying potential. To achieve the most desired results in the detoxification of

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the soil, the selection of a plant for phytoremediation purposes has to be based on multiple plant characteristics.

According to Baker and Brooks (1989), the content limits of metal elements in the dry biomass of plants to be termed hyperaccumulators are 1,000 mg/kg for Cu. A plant's potential as a phytoremediator can be determined by calculating the plant's Bioconcentration factor (BCF; metal concentration ratio of plant roots to the soil) and transfer factor (TF; metal concentration ratio of plant shoots to roots) values (Oguntade et al., 2015, 2018). If the BCF and TF values are above 1, the species can potentially be a phytoextractor of metals. With a BCF value of above one but a TF value below 1, the plant has the potential as a phytostabilizer of metals (Yoon et al., 2006).

In particular, *Amaranthus* is a human food source (Oguntade et al., 2015, 2018; Adekiya et al., 2019). In this study, *Amaranthus viridis* was focused upon because a) it was among the top ten most popular vegetables grown in Malaysia by various ethnic groups that covered 85% of the Malaysian population's demand; 2) it was used in previous research studies reported in the literature Kabata-Pendias and Pendias (1991), and 3) District Agriculture Department of Hulu Perak recommended it as a research vegetable. Amaranth is a leafy vegetable of the *Amaranthacea* family with many beneficial contents, such as vitamins A, B, and C and other substances such as Cl, Cu, Fe, Fe Mn and Na (Mnkeni, 2005). Furthermore, due to its high biomass and root proliferation, this leafy crop is known to accumulate considerable levels of heavy metal in its shoots and roots (Khurana et al., 2008).

Numerous studies have investigated the levels of Cu in various vegetables to understand their essentiality and potential toxicity to human health. The findings have provided valuable insights into monitoring Cu intake from vegetable consumption to balance its essential role and potential adverse effects. This research has led to a better understanding of how different vegetables contribute to the overall Cu intake and has implications for dietary recommendations and health policies (Quinn et al., 1981; Ginocchio et al., 2002; Wu et al., 2011; Liu et al., 2021; Li et al., 2022). Cu toxicity in vegetables is a growing concern due to its potential health implications for humans. Cu is a heavy metal that can accumulate in vegetables, especially lettuce, spinach, and endive. The ingestion of these Cu-laced vegetables is a key source of heavy metal toxicity in humans (Kumar et al., 2021).

The objectives of this study were to a) assess the human health risk of the Cu concentrations of the edible leafy parts of *A. viridis* based on target hazard quotient (THQ) values from Peninsular Malaysia, b) assess the potentials of *A. viridis* as a biomonitor, by comparing to habitat topsoils and a greenhouse experimental accumulation study; and c) assess the potentials of *A. viridis* as a phytoremediator of Cu by the BCF and TF values.

Materials and Methods

Study area

A series of sampling trips was conducted within six months on 11 sites from July 2017 to November 2018. The sampling locations were chosen after getting initial information from the district agriculture department. A total of 11 sampling sites were covered in this study (*Fig. 1*, *Table S1*).

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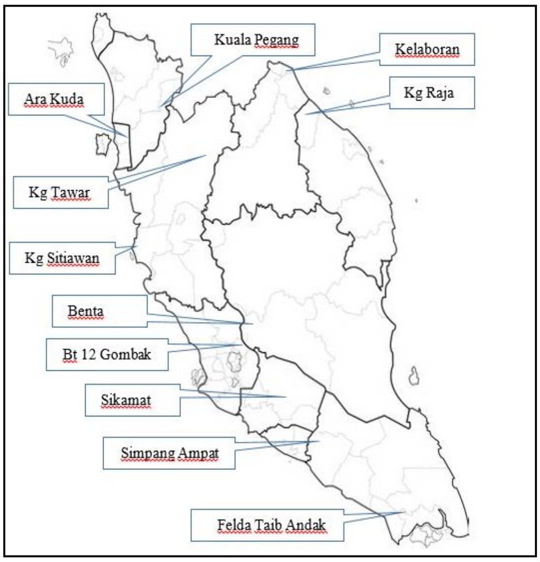

Figure 1. *Sampling map for Amaranthus viridis and their habitat topsoils in Peninsular Malaysia*

Sampling and sample preparation

At each sampling site, *A. viridis* and their habitat topsoils were collected simultaneously. The 11 different sampling sites included farm areas in Perak and Penang that potentially received wastewater for irrigation (*Table S1*). These sites also included sites near mining, industrial, landfill, agricultural and residential areas. At least three subsamples were collected from the sampling sites. At the same time, three replicates of habitat topsoils (0-10 cm) where *A. viridis* were collected and sampled. All collected samples of *A. viridis* and topsoils were stored in clean polythene bags. All the collected *Amaranthus* from the fields in all sampling sites are estimated to be aged 1-2 months. Later, they were transported to the laboratory of Universiti Putra Malaysia in Serdang for further analysis.

The collected samples were washed with distilled water to remove the dust particles in the laboratory. The *A. viridis* were separated into stems, roots, and leaves by cutting them into small pieces using a clean knife. The different parts (roots, stems, and leaves) of *A. viridis* were dried in an oven at 60°C for at least three days. After drying, the separated parts of the vegetable samples were ground into a fine powder using a commercial blender and stored in polyethylene bags until used for acid digestion (Yap et al., 2022).

The collected topsoil samples were dried in an oven at 100°C. After drying, the dried soils were ground into a fine powder using a mortar and pestle, and later, they were sieved using a 63µm mesh size sieve.

Digestion of plant samples

The aqua-regia method was applied to extract Cu from the *A. viridis* samples. About 0.50 g of dried tissues were weighed and placed in a washed digestion tube. Five ml of concentrated nitric acid (HNO_3, AnalaR grade, BDH 69%) was added to the digestion tube to digest the dried tissues. The digestion tubes were then placed in a digestion block at 40°C for 1 hour, and the contents were then fully digested at 140°C for 3 hours, as

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described by Yap et al. (2003). The digested samples were left to be cooled and then topped up (diluted) to 40 ml with double de-ionized water. The digested samples were filtered into acid-washed pill boxes through Whatman No. 1 (filter speed: medium) filter papers in funnels. Later they were stored in the refrigerator (4°C) until metal determination (Yap et al., 2022).

Digestion of soil samples

The direct aqua-regia method was applied to extract the total concentrations of Cu in the topsoils. About one g of each dried sample was weighed and placed into an acid-washed digestion tube. A combination of concentrated nitric acid (HNO_3, AnalaR grade, BDH 69%) and perchloric acid ($HClO_4$, AnalaR grade, BDH 60%) in a ratio of 4:1 (about 10 ml) was added to each digestion tube (Yap et al., 2002). Digestion tubes with samples were placed in a digestion block at 40°C for 1 hour, and the contents were fully digested at 140°C for 3 hours (Yap et al., 2002). They were then cooled before being topped up (diluted) to 40 ml with double de-ionized water. Later, the diluted samples were filtered into acid-washed pill boxes through Whatman No. 1 (filter speed: medium) filter papers. The samples were then stored in the refrigerator (4°C) until metal determination.

For the geochemical fractionations, the topsoils were fractionated into four fractions based on Badri and Aston (1983), namely, 'Easily, freely, leachable, or exchangeable' (EFLE), 'Acid-reducible' (AR), 'Oxidisable-organic' (OO), and 'Resistant' (RES).

Cu analysis

All the samples stored in acid-washed pill boxes and standard solutions of Cu were prepared from 10000ppm stock solution provided by MERCK Titrisol for Cu. The Cu was determined using an air-acetylene Atomic Absorption Spectrophotometer (AAS), and the metal concentrations were presented in μg/g.

All glassware and non-metal apparatuses used in this study were soaked in an acid bath (5% HNO_3) for 72 h after being washed with laboratory-grade detergent (Decon 90), to avoid contamination. The metal-made apparatuses were washed and soaked in laboratory-grade detergent (Decon 90) for at least 3 hours before use. Procedural blanks were employed, and quality control samples were diluted by the standard solutions of the metals to be tested. These standard solutions were analysed after every 5 - 10 samples to check for the accuracy of the analysed samples.

Four types of Certified Reference Materials (CRMs) were checked with the samples to ensure the accuracy of the FAAS measurements. *Table S2* shows a comparison of the measured values (μg/g dry weight) and the Certified Reference Material) values (μg/g dry weight) for *Lagarosiphon major* (NR.60), Dogfish Liver-DOLT-3 (National Research Council Canada), marine sediments-(MESS-3, National Research Council Canada, Beaufort Sea), and NSC DC 73319 (soil) with their certified concentrations for Cu. Their recoveries were mostly acceptable.

Experimental greenhouse study

An experimental greenhouse study was conducted to study the accumulations of Cu in the different parts (leaves, stems and roots) of *A. viridis*. For the experimental design, stock solutions of Cu (1000ppm) were prepared by dissolving 3.9289g Cu (II) sulphate in 1 L of distilled water. Two treatments were investigated, namely Treatment 1 (1.00 ppm of Cu) and Treatment 2 (wastewater water collected from the polluted Tasik

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Sri Serdang (Yap et al., 2008), and a control (rainwater as control). Information on the irrigation types in the control and the two treatments during the 35 days of experimental study are presented in *Table S3*.

The experimental design was completely randomized with 15 replicate polybags (10 x12) for each treatment with 3kg of soil weight for each polybag. All polybags were also fertilized with the same ratio of nitrogen, phosphorus and potassium (NPK green 15:15:15) kg/ha once, obtained from the District Agriculture Department of Hulu Perak, until the plants were harvested to prevent the *A. viridis* plant from nutrient deficiencies during the preparation of the medium. The plants in the replicates were thinned to one plant per poly bag before being given the treatment (N=15). The vegetables were grown in polybags with a mixture of commercial topsoil and sand bought from a hardware supplier in the district in a 3:1 ratio, respectively. Each polybag was approximately ¾ filled. Germination occurred within three days, and the treatment was only started after 14 days of germination (~50 mL/polybag) and followed by three days interval treatments until reaching harvest day. Each polybag was watered with rain water using drip irrigation every other day. Morphological features such as the number of leaves and height were collected every three days after the treatments were started.

The pH readings of water were in the pH 5.6-6.8 range, and the greenhouse temperature was between 32 and 41°C. These readings were taken every 3 days after planting. All the plants from the control and two treatments were harvested at day 35. The edible leaves of the plant were carefully removed, collected in sterile bags, and transported in an ice chest to the laboratory. All plant samples were washed with distilled water to remove any soil particles from the plant surfaces, and they were oven-dried to get constant weights at 60°C.

Human health risk assessments

For the human health risk assessment (HHRA) of Cu, all the metal concentrations formerly presented on a dry weight (dw) basis were converted into a wet weight (ww) basis using a conversion factor of 0.12. To evaluate a once-or long-term potential hazardous exposure to metals through the consumption of edible vegetables (USEPA, 1989) by the population of Peninsular Malaysia, the estimated daily intake (EDI) (IRIS, 2000) of metals was calculated using the following *equation (1)*:

$$\text{EDI} = \frac{\text{Mc} \times \text{consumption rate}}{\text{body weight}} \quad \text{(Eq.1)}$$

where Mc is the vegetables' metal concentration (mg/kg wet weight). The body weights for children and adults were 17 and 69.2 kg, respectively (Nurul Izzah et al., 2012), and the consumption rates were 17.0 and 34.0 g/day, for children and adults, respectively (Yap et al., 2022). Target hazard quotient (THQ) was calculated based on *equation (2)*:

$$\text{THQ= EDI/RfD} \quad \text{(Eq.2)}$$

Cu's RfD (g/kg wet weight/day) in vegetables was compared to the EDIs (g/kg wet weight/day). Cu: 40.0 was utilised as the oral reference dose (RfD) (g/kg wet weight/day) in this investigation, according to the EPA's Integrated Risk Information System online database (IRIS, 2000). The oral reference dosage is the threshold metal absorption concentration below which there is no significant risk (USEPA, 1989).

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Calculation of translocation factor and bioconcentration factor

The translocation factor (TF) and the bioconcentration factor (BCF) were utilised to calculate the plant's ability to uptake and withstand Cu uptake and toxicity (Yoon et al., 2006). These two indices are commonly used to determine the suitability of plants as good phytoremediators. BCF determines the plant's ability to bioaccumulate metals from soils. It is defined as in *equation (3)*:

$$BCF_{root}=\frac{Root_{metal}}{Soil_{metal}}$$
$$BCF_{stem}=\frac{Stem_{metal}}{Soil_{metal}} \qquad (Eq.3)$$
$$BCF_{Leaf}=\frac{Leaf_{metal}}{Soil_{metal}}$$

TF is used to determine the ability of the plant to translocate metals from the roots to the shoots (stem or leaf). It is defined as in *equation (4)*:

$$TF_{stem}=\frac{Stem_{metal}}{Root_{metal}}$$
$$TF_{leaf}=\frac{Leaf_{metal}}{Root_{metal}} \qquad (Eq.4)$$

The principle of phytoextraction is to remove heavy metals from the soil by uptaking and translocating them from the plant's roots to the leaves and stems (the easily harvested components of the plants).

Statistical analysis

Differences in metal concentrations in the different parts of the plants were analyzed using the post-hoc test (Student-Newman-Keuls) in the One-Way ANOVA analysis to see if any significant difference was at $P < 0.05$. This post-hoc test was performed using SPSS Statistics for Windows, version 18.0 (SPSS Inc., Chicago, Ill., USA).

To reduce the variance (Zar, 1996), Pearson's correlation analysis and stepwise multiple linear regression analysis (SMLRA) were based on $\log_{10}$ transformed data of the metals using the STATISTICA (Version 10; StatSoft. Inc., Tulsa, OK, USA, 1984–2011). After the $\log_{10}$ transformation on the data of Cu, the plants and the topsoils showed that all the data were within the normality ranges for skewness (−2 to +2) (Hair, 2010; Garson, 2016).

The SMLRA is used to see the Cu concentration relationships between the plants' different parts and geochemical factions in the habitat topsoils. The general purpose of the SMLRA is to find the most influential independent variables (represented by the Cu concentrations in the geochemical fractions in the habitat topsoils) that could influence the dependent variables (represented by Cu concentrations in the different parts of plants).

Results

Cu concentrations in Amaranthus viridis

The Cu concentrations in the leaves of *A. viridis* from the present study ranged from 10.8 to 21.9 mg/kg dw (1.30-2.63 mg/kg ww) (*Fig. 2*). It was found that several sites had

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the highest concentrations of Cu. Kuala Pegang was found to have the highest levels of Cu (21.91).

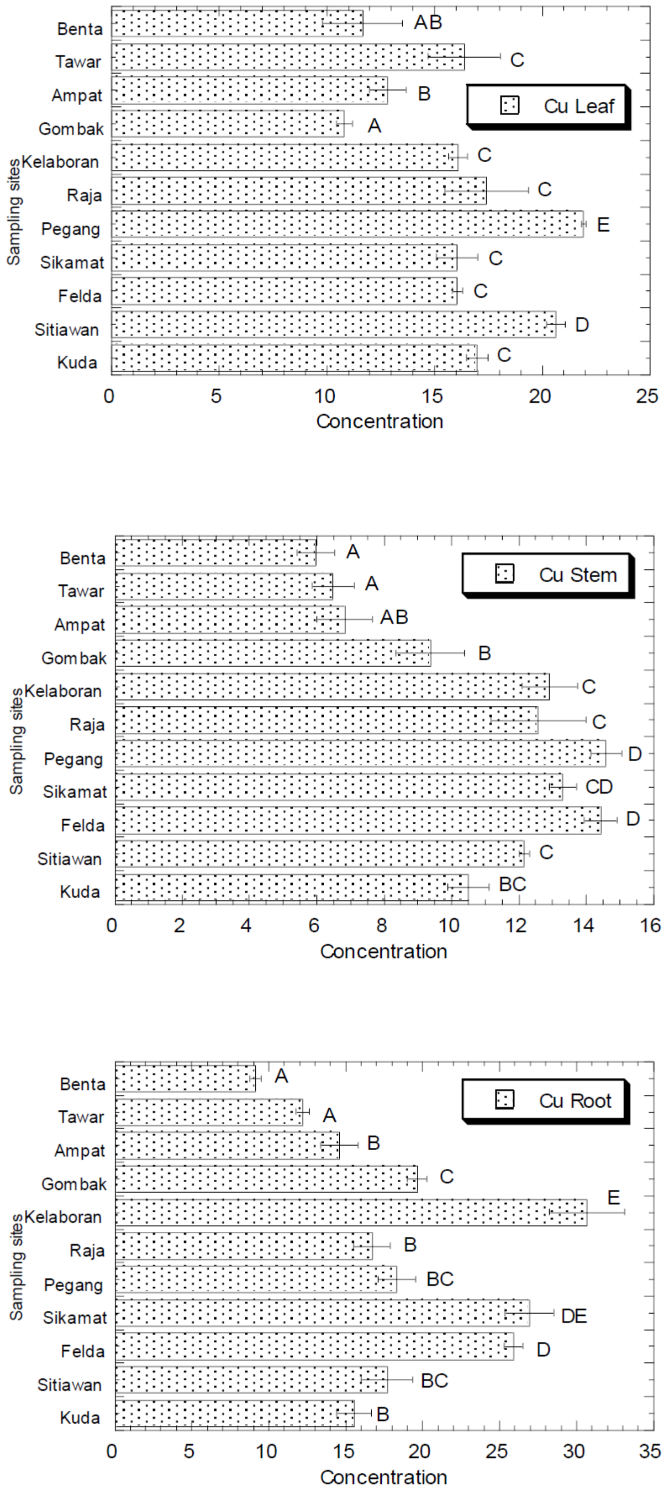

Figure 2. *Concentrations (mean ± SE, mg/kg dry weight) of Cu in the leaves, stems and roots of A. viridis collected from all the sampling sites in Peninsular Malaysia. Note: Metal concentrations sharing a common letter are not significantly different (P > 0.05) based on Post-hoc test (Student-Newman-Keuls) in the One-Way ANOVA analysis*

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The Cu concentrations in the stems of *A. viridis* from the present study ranged from 5.96 to 14.60 mg/kg dw (0.36-0.88 mg/kg ww) (*Fig. 2*). It was found that samples from Kuala Pegang also showed the highest Cu concentration (14.6), followed by Felda Taib Andak (14.4) and Sikamat (13.3).

The Cu concentrations in the roots of *A. viridis* in the present study ranged from 9.17 to 30.68 mg/kg dw (1.01-3.37 mg/kg ww) (*Table 1, Fig. 2*). When compared with the other sites, Cu had the highest concentration in roots at Kelaboran, Sikamat, and Felda Taib Andak (30.7, 26.95, and 25.9, respectively).

Table 1. *Overall concentrations (mean ± SE, mg/kg dry weight) of Cu in the leaves, stems and roots of A. viridis collected from Peninsular Malaysia. WW= converted to wet weight basis*

Cu	Leaves	WW	Stems	WW	Roots	WW
Min	10.82	1.30	5.96	0.36	9.17	1.01
Max	21.91	2.63	14.60	0.88	30.68	3.37
Mean	16.07	1.93	10.83	0.65	18.86	2.07
SE	1.02	-	0.97	-	1.98	-
Skewness	0.09	-	-0.42	-	0.45	-
Kurtosis	-0.64	-	-1.33	-	-0.76	-

Note: The dry weights of leaves, roots and stems were converted into wet weight (WW) basis by using conversion factors of 0.12, 0.11, and 0.06, respectively. Note: Min= minimum; max= maximum; SE= Standard error

Cu concentrations in Amaranthus viridis's habitat topsoils

The concentrations of Cu in geochemical fractions of the habitat topsoils of *A. viridis* collected from all sampling sites in Peninsular Malaysia are presented in *Fig. 3*. The overall concentrations of Cu in the geochemical fractions of the habitat topsoils of *A. viridis* collected from Peninsular Malaysia are presented in *Table 2*.

Table 2. *Overall concentration (mean ± SE, mg/kg dry weight) of Cu in the geochemical fractions of the habitat topsoils of A. viridis collected from Peninsular Malaysia*

Cu	EFLE	AR	OO	RES	SUM
Min	0.88	0.22	2.57	26.42	30.09
Max	3.23	1.76	11.14	224.38	237.68
Mean	1.96	0.87	6.12	111.56	120.52
SE	0.26	0.15	0.90	22.42	23.66
Skewness	0.53	0.70	0.41	0.68	0.67
Kurtosis	-1.17	-0.58	-1.22	-1.09	-1.10

Note: EFLE= easily, freely, leachable or exchangeable; AR= acid-reducible; OO= oxidisable- organic; RES= resistant; SUM= summation of the four geochemical fractions. Min= minimum; max= maximum; SE= Standard error

The Cu concentrations (mg/kg dw) in the EFLE, AR, OO, RES and SUM in the habitat topsoils of *A. viridis* ranged from 0.88 to 3.23, 0.22 to 1.76, 2.57 to 11.1, 26.4 to 224, and 30.1 to 238, respectively.

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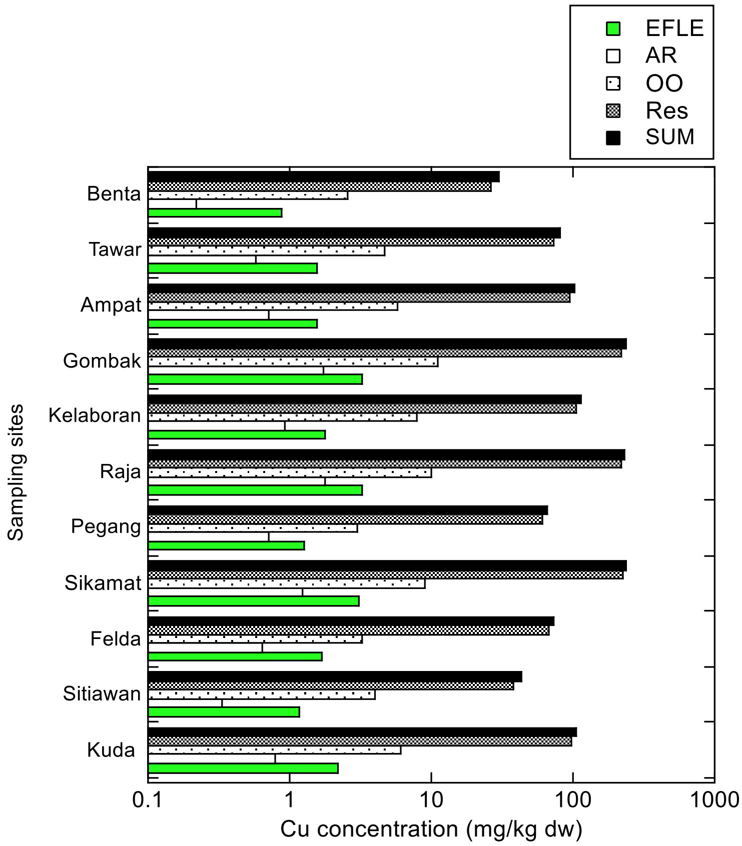

Figure 3. *Concentration (mean, mg/kg dry weight) of Cu in the geochemical fractions of the habitat topsoils of A. viridis collected from Peninsular Malaysia. Note: EFLE= easily, freely, leachable or exchangeable; AR= acid-reducible; OO= oxidisable- organic; RES= resistant; SUM= summation of the four geochemical fractions*

Correlations of metals between Amaranthus viridis and geochemical fractions of the habitat topsoils

The correlation coefficients of Cu concentrations between the *A. viridis* (leaves, stems, and roots) and their habitat topsoils (geochemical fractions) are presented in *Table 3* However, no significant (P> 0.05) correlations existed for all Cu pairwises. Practically, there were no clear correlations for Cu pairwises (*Table 3*). The SMLRA results for Cu, especially in Cu-leaf did not show significant (P> 0.05) selection of Cu geochemical fractions of the habitat topsoils (*Table 4*). However, Cu-stem was significantly (P < 0.05) influenced by AR and OO, while Cu-root was significantly (P < 0.05) influenced by RES (*Table 4*).

Human health risk of Cu in Amaranthus virdis

The values of THQ on the edible leaves of *A. viridis* from the current studies of Cu for children and adults from all the sampling sites in Peninsular Malaysia are presented in *Fig. 4* (*Table S4*). *Table 5* shows the overall values of EDI and THQ on the edible leaves of *A. viridis* from the current study. The EDI values of Cu for adults and children ranged from 1.41 to 2.86, and 0.64 to 1.29, respectively. The THQ values of Cu for adults and children ranged from 0.035 to 0.072, and 0.016 to 0.032, respectively.

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Table 3. *Correlation coefficients of Cu concentrations (log_{10}(mean +1) between the A. viridis (leaves, stems and roots) and their habitat topsoils (geochemical fractions). N= 11*

Cu	EFLE	AR	OO	RES	SUM
Leaf	-0.17	-0.15	-0.25	-0.16	-0.16
Stem	0.32	0.35	0.17	0.30	0.30
Root	0.44	0.45	0.42	0.49	0.49

Note: Values in bold are significantly correlated at P< 0.05. EFLE= easily, free, leachable or exchangeable; AR- acid-reducible; OO= oxidisable-organic; RES= resistant; SUM= summation of the all four geochemical fractions

Table 4. *Stepwise multiple linear regression analysis of Cu concentrations (based on log_{10}[mean+1]) between the different parts of Amaranthus viridis (as dependent variables), and the geochemical fractions of the habitat topsoils (as independent variables) (N= 11)*

Dependent variables	Independent variables selected	R	R^2
Leaf	No significant variable was selected.	-	-
Stem	1.17 + 1.14 (AR) - 0.49 (OO)	0.48	0.23
Root	0.81 + 0.23 (RES)	0.49	0.24

Note: EFLE–easily, freely, leachable or exchangeable; AR–acid-reducible; OO–oxidisable-organic; RES–resistant; SUM–summations of four geochemical fractions; Those dependent variables were significantly (P < 0.05) influenced by the selected independent variables

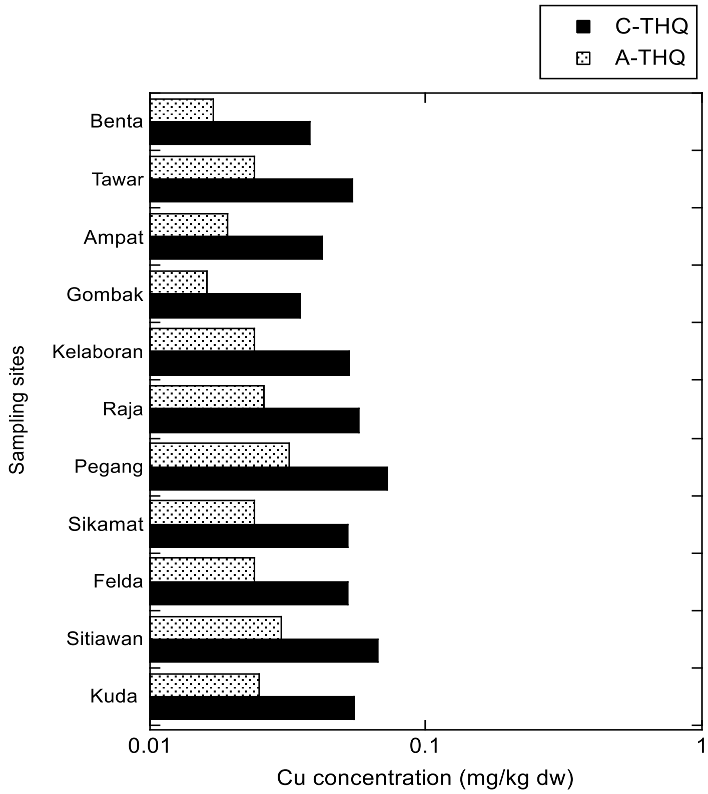

Figure 4. *Values of target hazard quotient (THQ, unitless) values of Cu on the edible leaves of A. viridis by children (C) and adults (A) from the current study. Note: The THQ=1.0 is the threshold value*

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***Table 5.** Overall values of estimated daily intake (EDI, µg/kg wet weight/day) and target hazard quotient (THQ, unitless) values of Cu on the edible leaves of A. viridis from the current study*

	EDI Child	THQ Child	EDI Adult	THQ Adult
Min	1.41	0.035	0.64	0.016
Max	2.86	0.072	1.29	0.032
Mean	2.10	0.052	0.95	0.024
SE	0.13	0.003	0.06	0.001
Skewness	0.08	0.089	0.09	-0.039
Kurtosis	-0.64	-0.597	-0.65	-0.679

Note: Min= minimum; max= maximum; SE= Standard error

Therefore, the THQ for Cu in *A. viridis* was all below 1.00, indicating that Cu did not pose any non-carcinogenic risks to consumers, both children and adults.

The translocation factor (TF) and bioconcentration factor (BCF) of Amaranthus viridis

The values of BCF of Cu on the leaves, stems, and roots of *A. viridis* from all the sampling sites in Peninsular Malaysia are presented in *Fig. 5* and *Table S5*. The overall values of BCF of Cu on the leaves, stems, and roots of *A. viridis* from Peninsular Malaysia are presented in *Table 6*. The values of $BCF_{leaf/EFLE}$ for Cu ranged from 3.34-17.80. The values of $BCF_{leaf/SUM}$ for Cu ranged from 0.05-0.48. The values of $BCF_{stem/EFLE}$ for Cu ranged from 2.90-11.60. The values of $BCF_{stem/SUM}$ for Cu ranged from 0.04-0.28. The values of $BCF_{root/EFLE}$ for Cu ranged from 5.19-17.25. The values of $BCF_{root/SUM}$ for Cu ranged from 0.07-0.41.

***Table 6.** Overall values of bioconcentration factors (BCF) of Cu on the leaves, stems and roots of A. viridis in Peninsular Malaysia*

	$BCF_{leaf/EFLE}$	$BCF_{leaf/SUM}$	$BCF_{stem/EFLE}$	$BCF_{stem/SUM}$	$BCF_{root/EFLE}$	$BCF_{root/SUM}$
Min	3.34	0.05	2.90	0.04	5.19	0.07
Max	17.80	0.48	11.60	0.28	17.25	0.41
Mean	9.76	0.20	6.28	0.13	10.65	0.21
SE	1.42	0.04	0.87	0.02	1.28	0.04
Skewness	0.56	0.77	0.69	0.61	0.29	0.36
Kurtosis	-0.73	-0.62	-0.86	-1.07	-1.41	-1.24

Note: Min= minimum; max= maximum; SE= Standard error

The values of TF of Cu on the leaves, stems, and roots of *A. viridis* from all the sampling sites in Peninsular Malaysia are presented in *Fig. 6* and *Table S6*. The overall values of TF of Cu on the leaves, stems, and roots of *A. viridis* in Peninsular Malaysia are presented in *Table 7*. The values of $TF_{leaf/root}$ for Cu ranged from 0.52 to 1.34.

The values of $TF_{stem/root}$ for Cu ranged from 0.42 to 0.80. Subha and Srinivas (2017) reported the BCF values as Cu (1.48) in the common marsh buckwheat *Polygonum glabrum* collected from Hussain Sagar Lake, India.

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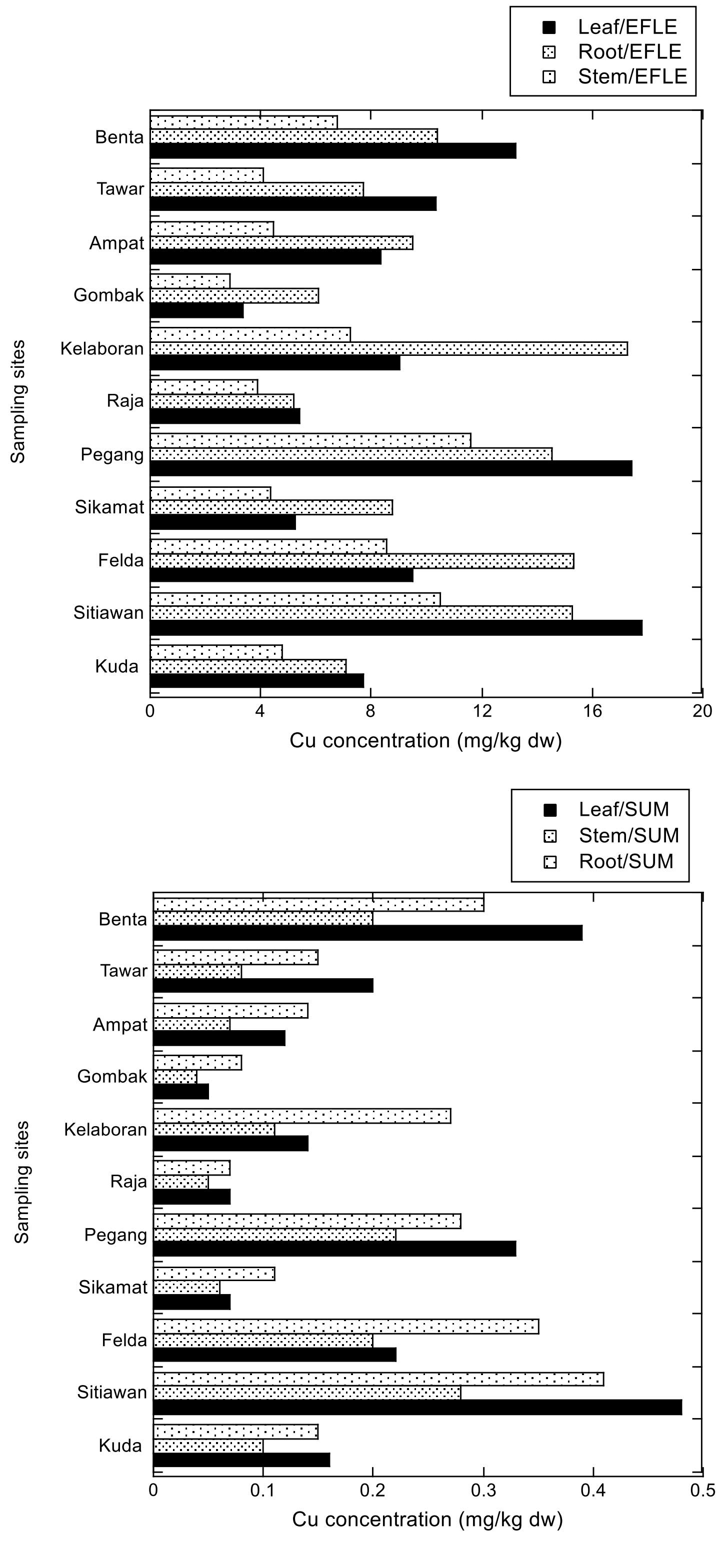

Figure 5. *Values of bioconcentration factors (BCF) of Cu on the leaves, stems and roots of A. viridis divided by EFLE of the topsoils in all sampling sites. Note: EFLE= easily, freely, leachable or exchangeable; AR= acid-reducible; OO= oxidisable- organic; RES= resistant; SUM= summation of the four geochemical fractions*

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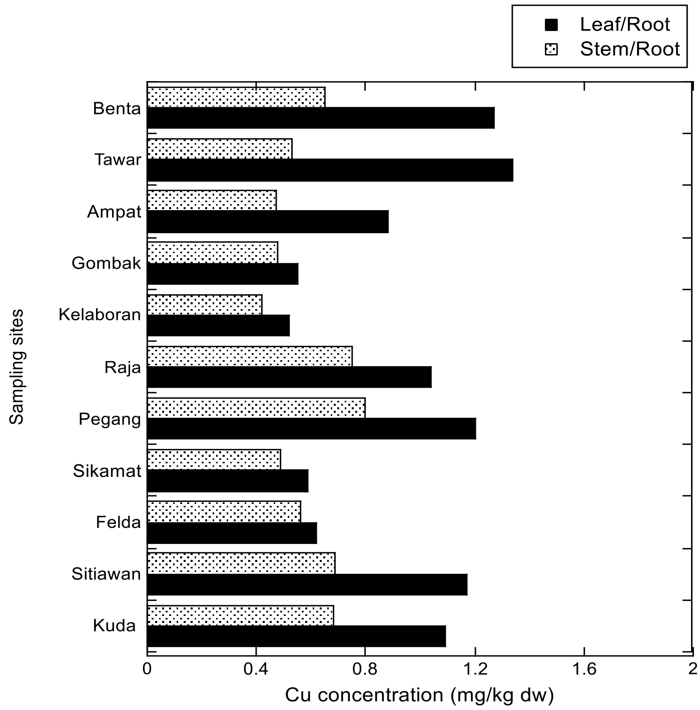

Figure 6. *Values of translocation factors (TF) of Cu on the leaves, stems and roots of A. viridis in all sampling sites*

Table 7. *Overall values of translocation factors (TF) of Cu on the leaves, stems and roots of A. viridis in Peninsular Malaysia*

	$TF_{leaf/root}$	$TF_{stem/root}$
Min	0.52	0.42
Max	1.34	0.80
Mean	0.93	0.59
SE	0.09	0.04
Skewness	-0.19	0.24
Kurtosis	-1.58	-1.30

Note: Min= minimum; max= maximum; SE= Standard error

Based on the values of Cu BCF in the leaves (*Table 7*), all 11 sampling sites were found with $BCF_{leaf/EFLE}$ > 1.0, but all sites were found with $BCF_{leaf/SUM}$ < 1.0. Based on the values of Cu BCF in the stems (*Table 7*), all 11 sampling sites were found with $BCF_{stem/EFLE}$ > 1.0, but all sites were found with $BCF_{stem/SUM}$ < 1.0. Based on values of Cu BCF in the roots (*Table 7*), all 11 sampling sites were found with $BCF_{root/EFLE}$ > 1.0, but all sites were found with $BCF_{root/SUM}$ < 1.0. This showed that the Cu in the topsoil EFLE fraction could be more easily transferred to the roots, leaves, and stems than those in the total concentrations of Cu in the topsoil. Based on the values of Cu TF (*Table 7)*, six sites (54%) were found with $TF_{leaf/root}$> 1.0, while all sites (100%) with $TF_{stem/root}$< 1.0 were found. This showed that Cu transfer to the stems from the roots was inefficient in all sampling sites, while Cu transfer to the leaves from the roots was efficient in some sampling sites. With most BCF> 1.0 and TF> 1.0 values, *A. viridis* is a potential phytoextraction agent of Cu (Yoon et al., 2006).

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Accumulation of Cu under experimental greenhouse study

The morphological measurements of the number of leaves and the maximum heights of *A. viridis* after 35 days of experimental greenhouse study are presented in *Table S7*. Generally, the number of leaves and height of plants of *A. viridis* showed no significant (P> 0.05) difference between the two treatments and the control. Application of Cu showed no significant (P> 0.05) difference in morphological characteristics compared to the control. No damage to the leaves, stems, and roots was observed until 34 days before harvesting.

Table 8 presents the concentrations of Cu in the leaves, stems, and roots of *A. viridis* between the control and the treatment with wastewater, as well as their increment percentages after 35 days under the experimental greenhouse study.

Table 8. *Concentrations (mean ± SE, mg/kg dry weight) of Cu in the leaves, stems and roots of A. viridis between control and treatment with wastewater, and their percentages of increment, after 35 days under experimental greenhouse study*

	Control		Wastewater		
Parts	**Mean**	**SE**	**Mean**	**SE**	**% of increment**
Leaves	11.78	0.17	14.56	0.38	23.60
Stems	8.68	0.17	9.57	0.46	10.25
Roots	9.66	0.21	10.70	0.30	10.77
EFLE	0.32	0.00	0.35	0.00	9.37
SUM	25.36	-	26.22	-	3.39

Note: NA= data not available. EFLE= geochemical fraction of 'easily, freely, leachable or exchangeable'; SUM= summation of all four geochemical fractions. % of increment= (wastewater- control)/control x 100%

After 35 days of experimental study with wastewater irrigation, the concentrations (mg/kg dw) of Cu in the leaves, stems, and roots reached 14.6, 9.57 and 10.70, respectively. These Cu levels were within those found in the 11 sampling sites (leaves: 10.8 to 21.9 mg/kg dw; stems: 5.96 to 14.6 mg/kg dw; roots: 9.17 to 30.7 mg/kg dw).

Cu concentrations in the leaves, stems, and roots of *A. viridis* of the two treatments and control after 35 days of experimental greenhouse study are presented in *Table 9*. After 35 days of experimental study with Treatment Cu (1.00ppm) addition, the concentrations (mg/kg dw) of Cu in the leaves, stems, and roots reached 13.6, 10.0 and 13.8, respectively.

Table 9. *Cu concentrations (mean ± SD, mg/kg dry weight) leaves, stems and roots of A. viridis in control (Con), and Treatment Cu (1 ppm), after 35 days of experimental greenhouse study*

	Control		Cu (1 ppm)		
Parts	**Mean**	**SE**	**Mean**	**SE**	**% of Cu increment**
Leaves	11.78	0.17	13.60	0.23	15.48
Stems	8.68	0.17	10.01	0.06	15.28
Roots	9.66	0.21	13.78	0.02	42.63

Note: % of increment= (Treatment- control)/control x 100%

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The Cu levels of the above treatment were within the range of those found in the 11 sampling sites (leaves: 10.8 to 21.9 mg/kg dw; stems: 5.96 to 14.6 mg/kg dw; roots: 9.17 to 30.7 mg/kg dw). The Cu concentrations (mg/kg dry weight) in the edible leaves were 11.8 and 13.6 for control and Cu treatment, respectively. The Cu concentrations (mg/kg dry weight) in the stems were 8.68 and 10.0 for control and Cu treatment, respectively. The roots' Cu concentrations (mg/kg dry weight) were 9.66 and 13.8 for control and Cu, respectively. The pattern of Cu accumulations followed: leaves> roots> stems for control, while it was rooted> leaves> stems for Cu treatment.

Discussion

Cu concentrations in Amaranthus viridis is comparatively lower and within those reported in the literature

The present study thoroughly analysed Cu concentrations in *A. viridis* and found they were within acceptable limits. Thus, the present study's findings support that *A. viridis* is a safe and viable option for including Cu in one's diet without exceeding the recommended intake.

Similarly, Chary et al. (2008) found that green vegetables had a greater enrichment factor for heavy metals. The root cell wall and ion transmembrane transport in the endoderm cytoplasm were within acceptable limits. Thus, the membrane and water transport in the xylem vessel, according to Yang et al. (2005), governed metal ion translocation from soil to plants. Transpiration influenced the latter far more (Tani and Barrington, 2005).

Based on the cited data from Li et al. (2012), the Cu concentrations (mg/kg ww) in leafy vegetables ranged from 0.35 to 1.38. Mwesigye et al. (2016) reported that based on an analysis of local food vegetables grown in mine tailing soil, 19% of *Amaranthus* had Cu levels which exceeded the EC threshold. Islam et al. (2016), based on vegetables grown in Bogra District (Bangladesh), reported that the range of the mean concentrations was 20.50–75.0 mg/kg for Cu contents in edible sections of different types of plants (leaves and stems) were found to vary considerably. Cu concentrations were greater in the leaves of *A. viridis* than in the stem. This meant that the edible sections of the leaves accumulated more HMs. This outcome was consistent with prior research findings (Yang et al., 2014; Valun et al., 2015; Hu et al., 2017).

Compared to reported studies, the lower and higher levels of Cu in the *A. viridis* can provide three major significances from a biomonitoring point of view. Firstly, from the aspect of health safety assurance, the consistently lower Cu levels in *A. viridis*, as highlighted in this study and supported by existing literature, assure the safety of consuming this vegetable. This is particularly significant in ensuring individuals can obtain dietary Cu without exceeding recommended intake levels, thereby minimizing potential health risks associated with excessive Cu intake. Secondly, from the dietary guidance aspect, incorporating *A. viridis* into one's diet can be guided by the understanding that the vegetable offers a safe and viable source of Cu. The comparative lower Cu concentrations align with food safety guidelines, supporting its inclusion as part of a balanced and healthy diet without the need for excessive concern regarding Cu intake. Thirdly, from the perspective of environmental and agricultural implications, the comparative lower Cu concentrations in *A. viridis* also raise important considerations related to environmental and agricultural factors. These findings underscore the need for ongoing monitoring to account for potential fluctuations in Cu levels influenced by

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varying growing conditions and locations. By doing so, the stability and safety of incorporating *A. viridis* into the diet can be upheld, contributing to a comprehensive understanding of potential health risks associated with Cu intake from this vegetable.

In conclusion, the comparative analysis of Cu concentrations in *A. viridis* reinforces its safety for consumption. It emphasizes the importance of continued biomonitoring to ensure consistency in maintaining safe Cu levels. This proactive approach will ultimately support informed dietary choices and promote the safe utilization of *A. viridis* as a valuable source of dietary Cu.

Consumption of Amaranthus viridis poses no human health risk of Cu toxicity

Cu concentrations in *A. viridis*, a commonly consumed vegetable, have been the subject of numerous studies due to the potential health risks associated with excessive Cu intake. In comparison to the established safety guidelines of Cu for vegetables, the present Cu range (1.30-2.63 mg/kg ww) in the edible leaves of *A. viridis* was below the maximum permissible concentration for Cu (10.0 mg/kg ww) for edible parts of vegetables established by China (AQSIQ, 2001, 2012). The mean concentrations of Cu in the leaves of *A. viridis* were below the maximum permissible levels suggested by FAO/WHO (2011) (Cu: 40 mg/kg ww) for leafy and fruit vegetables.

When compared to the food safety guidelines, the Cu levels in A. viridis were found to be well within the acceptable limits set by regulatory authorities. This further supports the safety of including *A. viridis* in one's diet without the risk of exceeding the recommended Cu intake levels.

Overall, the present study's findings reinforce the notion that *A. viridis* is suitable for individuals seeking to incorporate Cu into their diet while adhering to food safety guidelines. However, despite the promising findings of this study, it is important to note that regular monitoring is still needed in the future. As environmental and agricultural factors can influence the levels of Cu in *A. viridis*, continuous assessment will be crucial to ensure that the Cu concentrations remain within acceptable limits for safe consumption. Additionally, ongoing research and monitoring will contribute to a comprehensive understanding of the potential health risks associated with Cu intake from this vegetable, especially considering variations that may arise due to different growing conditions and locations.

The THQ for Cu in *A. viridis* from Peninsular Malaysia was below 1.00, indicating no non-carcinogenic risks of Cu to consumers, both children and adults. Zhang et al. (2019) examined seven HMs, including Cu and Pb, in greenhouse surface soils (0–20 cm) and 30 vegetables from Kunming City, Yunnan Province, southern China. They found that while there was no possible risk for children and adults, the hazard health risk index for adolescents was higher than one. Eliku and Leta (2017) reported that the metal concentration in cabbage was 2.84 Cu. All of the vegetables had a THQ value of less than one. As a result, the target group was unlikely to be harmed by eating these vegetables. THQs for Cu were reported to be below 1.00 by Islam et al. (2016), based on vegetables from the Bogra District (Bangladesh), indicating that people would not face significant health risks if they ingested a single metal from a single vegetable species. According to Islam et al. (2015), the THQ values of the examined heavy metals from all vegetables from Bangladesh were greater than 1.00.

From the point of target hazard quotient, which assesses the potential health risks associated with a specific exposure, the Cu content in *A. viridis* was found to be lower than the food safety guidelines. Furthermore, the present study's findings are consistent

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with other research showing that leafy and tuberous vegetables accumulate higher levels of Cu than fruity vegetables (Sharma et al., 2021). Cu concentrations in *A. viridis* are comparatively lower than those reported in the literature. The results of the present study on Cu concentrations in *A. viridis* align with previous literature, indicating that the levels of Cu in this vegetable are within acceptable limits and do not pose a significant health risk to consumers. Cu concentrations in *A. viridis* are comparatively lower and within those reported in the literature, suggesting that consuming this vegetable is unlikely to result in excessive Cu intake or associated health risks. The comparative analysis with the literature review indicated that the levels of Cu in *A. viridis* are consistently lower than those reported in the literature. This aligns with the findings of previous studies and affirms that *A. viridis* is a safe and viable option for obtaining dietary Cu without surpassing the established safety thresholds. Additionally, the present study's results agree with other research demonstrating that leafy and tuberous vegetables accumulate higher levels of Cu than fruity vegetables. The comparatively lower Cu concentrations in *A. viridis* further emphasize its safety for consumption.

According to Jolly et al. (2013), Cu consumption from vegetables was low. The THQ for Zn was higher than that of Cu from all plant sections, according to Lion and Olowoyo (2013), and the THQ values showed that humans might be at risk if they consumed spinach grown in waste disposal locations in Tshwane (South Africa). According to Wang et al. (2012), the THQ values of Cu and Zn were less than 1.00, implying that the health effects of HM exposure from vegetable consumption were generally considered to be low in wastewater-irrigated areas, Beijing-Tianjin city. According to Hu et al. (2014), the THQ values of Cu obtained from vegetable consumption in China were larger than 1.00 for leaf vegetables and higher for greenhouse plants than for open-field vegetables.

Incorporating long-term monitoring and further research will provide valuable insights into the stability of Cu concentrations in *A. viridis* and help to uphold its status as a safe and viable option for dietary Cu intake. This ongoing attention to monitoring will ultimately contribute to the consistency and reliability of the safety assessments for incorporating *A. viridis* into the diet.

Amaranthus viridis is a potential biomonitor of Cu pollution

The present study showed no clear correlations for Cu pairwises. This could be because, in addition to metal uptake by the roots, air deposition could affect Cu bioavailability and contamination in local plants (Islam et al., 2014). However, Liu et al. (2013) found a poor correlation between metal concentrations in crops and soils. This was most likely due to other factors, such as cation exchange capacity, influencing Pb availability in habitat topsoils (Khan et al., 2015).

Fan et al. (2017) investigated the relationship between HM levels and soil parameters in greenhouse crops (including the geochemical fractions). They found that the concentrations of Pb in greenhouse vegetables were highly linked with the concentrations of the different forms of HMs in the greenhouse soil, implying that these concentrations in the soil might be used to predict Pb levels in vegetables. Aside from the root uptakes of metals to the leaves of vegetables, air deposition of Pb on local plants could also affect its bioavailability and contamination (Islam et al., 2014).

Based on the experimental Cu exposure study, after 35 days of experimental study with wastewater irrigation, the concentrations of Cu in the leaves, stems, and roots were within the range of those found in the 11 sampling sites; the concentrations of Cu in the leaves, stems and roots were within the range of those found in the 11 sampling sites in

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the leaves but were higher than those in the stems and roots; the concentrations of Cu in the leaves, stems and roots were lower than those found in the 11 sampling sites in the leaves and roots but within the range of those in the stems. The above findings indicated that the wastewater used in this study was not highly contaminated by the six metals investigated. Besides, only seven times (each with 50 ml) of wastewater irrigations were conducted within 34 days of the greenhouse experimental study. However, this study revealed two points. Firstly, the low levels of metals in the irrigation water could be attributable to sedimentation. Even though the wastewater was sourced from the known polluted lake at the Seri Serdang Lake (Yap et al., 2008), polluted suspended particulate matter sedimentation occurred. This could be due to the wastewater being collected in a plastic container and stored for five days before use for irrigation. This showed that the suspended particulate matter absorbed with HMs had been sedimented. This greatly reduced the bioavailable metals that the roots of *A. viridis* could take up. This also indicated that wastewater from polluted lakes could be used for plant irrigation after five days of storage in a container before irrigation. However, this claim needs further studies in the future.

The results based on Treatment Cu, under experimental greenhouse study, were generally within the ranges of Cu in the leaves, stems and roots of *A. viridis* collected from the 11 field sampling sites. The roots of the plants had the highest concentration of Cu because Cu was commonly immobilized in the roots (Pulford and Watson, 2003). Cu was found to accumulate primarily in plant roots, with just a small quantity being transferred from the roots to the other sections of the plant.

The high level of Cu concentration in the roots of the Cu plant treatment was similar to the study result reported by Kabata-Pendias and Pendias (1991), in which Cu was found to accumulate primarily in plant roots, with just a minor quantity being transferred from the roots to the leaves. Besides, the high accumulation of Cu was always related to the disruption of the plasma membrane by high concentrations of Cu, which prevented it from being transferred to the other parts. This showed that Cu was not easily transported from roots to aerial parts such as leaves and stems. The present experimental greenhouse findings showed that wastewater irrigation could lead to the accumulation of Cu in the different parts of *A. viridis*. However, Cu did not accumulate as much by the plant exposed to the wastewater.

A. viridis is a potential biomonitor of Cu pollution. This means that the presence and accumulation of Cu in *A. viridis* can indicate environmental pollution from this metal. When monitoring Cu pollution, researchers can study the levels of Cu in *A. viridis* to assess the extent and impact of contamination in the surrounding environment. Using *A. viridis* as a biomonitor for Cu pollution allows researchers to effectively gauge the presence and accumulation of Cu in the environment, providing valuable insights into the potential risks and impacts on ecosystems and human health. Furthermore, *A. viridis* is a promising biomonitor due to its wide distribution, fast growth rate, and ability to accumulate Cu in its tissues (Tripathi et al., 2021; Chikane et al., 2021).

In sum, using *A. viridis* as a biomonitor for Cu pollution offers a valuable tool for assessing the environmental impact of Cu contamination. Its widespread distribution, rapid growth, and ability to accumulate Cu make it well-suited for monitoring and understanding the presence of this metal in the environment. Researchers can continue to utilize *A. viridis* to gain crucial insights into the potential risks and impacts on ecosystems and human health posed by Cu pollution. However, further studies and routine monitoring of *Amaranthus* farms for trace metal concentrations, including Cu, are still needed to fully

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understand and assess the extent of Cu pollution and its potential impacts on the environment.

Amaranthus viridis as a potential phytoextractor of Cu pollution

The use of other plants as phytoextractors of Cu, as reported in the literature (*Table 10*), supported the present finding for using *A. viridis*.

Table 10. *Plants employed for phytoremediation through the process of phytoextraction of Cu*

No.	Plants	Contaminant(s)	Medium	Country	References
4	Spartina alterniflora	Cr, Cu, Pb, Fe and Zn	Soil	USA	Salla et al., 2011
5	Ipomoea carnea	Cd, Pb, Cu, Cr, Mn and Ni	Fly ash deposits	India	Pandey (2012)
6	Amaranthus spinosus	Cu, Zn, Cr, Pb and Cd	Soil	India	Chinmayee et al. (2012)
7	Amaranthus spinosus (weed)	Heavy metals	Soil	Experimental pot culture	Chinmayee et al. (2012)
9	Typha latifolia	Zn, Mn, Cu, Pb, Cd, Cr and Ni	Soil	India	Pandey et al. (2014)
10	Typha latifolia	Al, As, Cd, Cr, Cu, Hg, Mn, Ni, Pb and Zn	Soil	Italy	Bonanno and Cirelli (2017)
13	Brassica campestris	Cd, Cu, Ni, Pb, and Zn	Soil	Botanical Garden of Komarov Botanical Institute, Russia	Drozdova et al. (2019)
14	Hibiscus cannabinus	Cu	NA	NA	Saleem et al. (2020b)
15	Corchorus capsularis	Cu	NA	NA	Saleem et al. (2020a)
17	Hibiscus mutabilis, H. hamabo and Senna corymbosa	Cu, Pb, and Zn.	Soil	experimental site in Shanghai, China	Shang et al. (2020)
18	Vachellia campechiana	Pb, Fe, Cr, Cu, and Zn	Soil	Mexico	Santoyo-Martínez et al. (2020)
20	Saccharum spontaneum and Saccharum munja	Zn, Pb, Cu, Ni, Cd and As	Soil	Pot experiments	Banerjee et al. (2020)
21	Sun spurge and common nettle	As, Cd, Pb, Cu, and Zn.	Soil	Bor (Serbia)	Petrović et al. (2021)

Note: NA= not available

In this study, the results showed that the phytoextraction process helped to concentrate Cu in the roots and stems. Phytoextraction, the absorption and accumulation of HMs in the plant shoots and their removal from the treatment site through harvesting these plant parts is one of the many strategies for the phytoremediation of the soil (Yoon et al., 2006; Ashraf et al., 2019). This method requires the uptake of pollutants by the plant roots and the translocation of the metals to the other parts (stems and leaves) of the plants (He et al., 2005; Ali et al., 2013). This is followed by the biomass harvest of these above-ground plant parts to safely dispose of the accumulated metals. Islam et al. (2016), based on vegetables grown in Bogra District (Bangladesh), reported the BCF of Cu (0.56).

However, it should be noted that many abiotic factors could influence the efficiency of the phytoextraction processes, such as the physico-chemical properties of the soil, metal bioavailability to the plants, metal speciation, climatic conditions and the weed's characteristics ((Barcelo and Poschenrieder, 2003; Patra et al., 2018). Theoretically,

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plants that act as phytoextractors should accumulate massive amounts of pollutants (Barcelo and Poschenrieder, 2003). However, the suitability of a plant as a phytoextractor species for HMs also depends on the metal concentrations in the shoot (stems and leaves) and the shoot biomass. Being a non-hyperaccumulator, the phytoextraction approach that fits *A. viridis* well is the relatively higher above-ground biomass production due to its fast growth rate despite its lesser metal accumulation. This has also been reported in plants such as *Brassica* sp. (Drozdova et al., 2019; Benavides et al., 2021).

The metal BCF, according to Hu et al. (2017), is a crucial component of human exposure through the food chain. Understanding BCF status is crucial to restricting soil HMs' transmission into edible crop sections. Wang et al. (2012) reported the BCF values of Cu and indicated that their results were dependent on the vegetable species. The fact that metal uptake by vegetables depended on water, soil composition, metal bioavailability, and nutrient balance accounted for the differences in HM concentrations in distinct vegetables (Ahmad and Goni, 2010). Although Cu, Fe, and Zn are necessary for vegetable growth, excessive levels can harm plant growth (Wang et al., 2009). Due to a lack of macronutrients such as phosphorus, excessive metal accumulation could impact vegetable biomass (An et al., 2004). Chang et al. (2014) found that pakchoi had the lowest capacity for HM enrichment of the six leafy vegetables studied and concluded that sewage irrigation and fertilization were likely the main sources of HMs accumulated in leaf vegetables produced in agricultural settings. Metal concentrations in vegetables and matching soils were shown to be poorly linked by Liu et al. (2013).

Cu pollution poses a significant threat to the environment and human health due to its widespread use in mining, agriculture, and manufacturing industries. One potential solution to mitigate Cu pollution is phytoremediation, which utilizes plants to remove, degrade, or immobilize contaminants from the soil or water. *A. viridis*, commonly known as green amaranth, has shown potential as a phytoextractor of Cu pollution. *A. viridis* has been found to have the ability to uptake and accumulate high concentrations of Cu in its root and shoot tissues, making it a promising candidate for phytoremediation of Cu-contaminated sites. Furthermore, studies have shown that *A. viridis* can effectively reduce Cu levels in the soil, improving soil quality and reducing environmental risks. By harnessing the natural capabilities of *A. viridis*, we can potentially restore Cu-contaminated sites and reduce the impact of Cu pollution on the surrounding environment and human health. Many studies have shown the potential of *Amaranthus* as a phytoremediator. Its ability to uptake and accumulate high concentrations of Cu in its tissues makes it a promising candidate for phytoremediation of Cu-contaminated sites. Moreover, using *Amaranthus* for phytoremediation helps reduce Cu levels in the soil, improve soil quality, and reduce environmental risks. Using *Amaranthus* as a natural solution to mitigate Cu pollution can significantly contribute to the restoration of Cu-contaminated sites and minimize the environmental and health impacts of Cu pollution. Further research and application of *Amaranthus* in phytoremediation strategies could offer sustainable and effective solutions for addressing Cu pollution (Chua et al., 2019; Jin et al., 2021; Khan et al., 2021; Debabeche et al., 2022).

In conclusion, the use of *A. viridis* for phytoremediation of Cu-contaminated sites holds great promise for addressing Cu pollution. However, there are potential challenges that need to be considered. One such challenge is the need for further research to optimize the growth conditions and specific mechanisms for maximizing the phytoextraction efficiency of *A. viridis*. Additionally, the long-term effects of using *A. viridis* for

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phytoremediation and its impact on the surrounding ecosystem should be thoroughly investigated to ensure the sustainability of this approach.

Moving forward, future research directions could focus on exploring the potential of genetic engineering to enhance the phytoextraction capabilities of *A. viridis* and its application in combination with other phytoremediation techniques for comprehensive remediation of Cu-contaminated sites. By addressing these challenges and pursuing these future directions, using *A. viridis* in phytoremediation strategies could offer sustainable and effective solutions for mitigating Cu pollution and promoting environmental health.

Relevance to UN Sustainable Development Goals

Monitoring Cu in vegetables for possible metal toxicity through vegetable consumption is directly related to several United Nations' Sustainable Development Goals (UN-SDGs). Specifically, this issue aligns with UN-SDG 2: Zero Hunger, as it pertains to ensuring food security and promoting sustainable agriculture. Additionally, it is linked to UN-SDG 3: Good Health and Well-being, as it addresses the potential health implications of heavy metal toxicity in vegetables and its impact on human well-being.

Furthermore, the focus on developing prediction models and safe cultivation methods corresponds to UN-SDG 9: Industry, Innovation, and Infrastructure, emphasising the need for innovative agricultural practices and infrastructure to mitigate Cu toxicity in vegetables. Additionally, assessing the bioavailability and accumulation of Cu in the soil-plant system is aligned with UN-SDG 15: Life on Land regarding the conservation and sustainable use of terrestrial ecosystems (Dumbrava et al., 2014; Chiou and Hsu, 2019; Rezapour and Jalil, 2022; Adepoju et al., 2023).

These efforts contribute to the broader sustainable development agenda outlined in the UN-SDGs by addressing the Cu toxicity in vegetables. Integrating sustainable agricultural practices and mitigating metal toxicity in food aligns with the global aspirations for a healthier, more secure, and environmentally conscious future. The potential health implications of Cu toxicity in vegetables necessitate thorough monitoring and assessment of its bioavailability, accumulation, and behaviour in the soil-plant system to ensure the safety and quality of our food supply. Research and monitoring of Cu toxicity in vegetables is essential to ensure the safety and quality of our food supply.

Conclusions

Based on 11 sampling sites, the present study found that the Cu concentrations in the leaves of *A. viridis* ranged from 10.8 to 21.9 mg/kg dw (1.30-2.63 mg/kg ww). In general, the THQ for Cu in the *A. viridis* was below 1.00, indicating no non-carcinogenic risks of Cu to the consumers, both children and adults. Still, the routine monitoring and management of vegetable farms are recommended and necessary. With most BCF> 1.0 and TF> 1.0 values, *A. viridis* is a potential phytoextraction agent of Cu. All the experimental greenhouse findings indicated three points. Firstly, wastewater irrigation could cause accumulations of Cu in the different parts of *A. viridis,* although Cu was not highly accumulated by the plant exposed to the wastewater. Secondly, after 35 days of experimental greenhouse study, the accumulated Cu levels were mostly within the Cu ranges in the field-collected samples. Thirdly, the findings also indicated that *A. viridis* could potentially be a Cu biomonitor.

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APPENDIX

Table S1. *Sampling information of all the sampling sites of Amaranthus viridis and their habitat topsoils from Peninsular Malaysia*

No	Sampling sites	Sampling date	GPS	Site descriptions
1	Ara Kuda (Penang)	12-Sept-17	N 05º 27’09’’	Agriculture area and palm oil plantation.
			E 100º 31’12’’	
2	Kg Sitiawan (Perak)	14-Sept-17	N 04º 15’18’’	Near coastal region and residential area
			E 100º 42’27’’	
3	Felda Taib Andak (Johor)	4-Nov-17	N 01º 43’55’’	Palm oil plantation
			E 103º 38’23’’	
4	Sikamat(N.Sembilan)	15-Oct-17	N 02º 46’ 24”	Residential area and road side.
			E 101º 59’ 28”	
5	Kuala Pegang (Kedah)	16-Sept-17	N 05º 39’13’’	Agriculture area.
			E 100º 54’35’’	
6	Kg Raja (Terengganu)	17-Jan-18	N 05º 48’ 19”	Residential area and road side.
			E 102º 35’09”	
7	Kelaboran (Kelantan)	18-Jan-18	N 06º 11’ 14”	Residential area and roadside.
			E 102º 10’ 25”	
8	Batu 12 Gombak (Selangor)	9-Oct-17	N 03º 15’ 46”	Residential area and road side.
			E 101º 43’22”	
9	Simpang Ampat (Melaka)	16-Oct-17	N 02° 26'44"	Residential area and highway
			E 102° 11'12”	
10	Kampung Tawar (Perak)	21-Dec-17	N 05° 26’ 39”	Agriculture area
			E 101° 07' 13"	
11	Benta (Pahang)	14-Nov-17	N 03° 47’ 15"	Rubber plantation.
			E 101° 51’ 31"	

Table S2. *A comparison of the Cu measured values (µg/g dry weight) and Cu Certified Reference Material) values (µg/g dry weight) for A) Lagarosiphon major (NR.60), B) Dogfish Liver-DOLT-3 (National Research Council Canada), C) marine sediments-(MESS-3, National Research Council Canada, Beaufort Sea), D) NSC DC 73319 (soil) with their certified concentrations for Cu. (NA= Not available)*

CRM	Certified value (C)	Measured value (M)	Percentage of recovery (M/C)
A	51.2	40.7	79.5
B	31.2	29.2	93.6
C	33.9	27.9	82.2
D	21.0	17.7	84.2

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Table S3. *Information on the irrigation types in the control and the two treatments during the 35 days of experimental study*

Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Control	Rainwater (50ml)	Rainwater (50ml)*	Rainwater (50ml)	Rainwater (50ml)	Rainwater (50ml)	Rainwater (50ml)*	Rainwater (50ml)	Rainwater (50ml)	Rain water (50ml)	Rain water (50ml)
T1 (Cu)	Rainwater (50ml)	Rainwater (50ml)*	Rainwater (50ml)	Rainwater (50ml)	Rain water (50ml)	Rain water (50ml)	Rain water (50ml)	Rain water (50ml)	Rain water (50ml)	Rain water (50ml)
T2 (Wastewater)	Rain water (50ml)	Rain water (50ml)	Rain water (50ml). Germinating.	Rain water (50ml)	Rain water (50ml)	Rain water (50ml)	Rain water (50ml)	Rain water (50ml)	Rain water (50ml)	Rain water (50ml)
Day	11	12	13	14	15	16	17	18	19	20
Control	Rainwater (50ml)	Rainwater (50ml)*	Rainwater (50ml)	Rainwater (50ml)	Rainwater (50ml)	Rainwater (50ml)*	Rainwater (50ml)	Rainwater (50ml)	Rain water (50ml)	Rain water (50ml)*
T1 (Cu)	Rain water (50ml)	Rain water (50ml)	Rain water (50ml)	Cu (1 ppm) (50ml)*	Rain water (50ml)	Rain water (50ml)	Cu (1 ppm) (50ml)*	Rain water (50ml)	Rain water (50ml)	Cu (1 ppm) (50ml)*
T2 (Wastewater)	Rainwater (50ml)	Rainwater (50ml)	Rainwater (50ml)	Wastewater (50 ml)*	Rain water (50ml)	Rain water (50ml)	Wastewater (50 ml)*	Rain water (50ml)	Rain water (50ml)	Wastewater (50 ml)*
Day	21	22	23	24	25	26	27	28	29	30
Control	Rainwater (50ml)	Rainwater (50ml)*	Rainwater (50ml)	Rainwater (50ml)	Rainwater (50ml)	Rainwater (50ml)*	Rainwater (50ml)	Rainwater (50ml)	Rainwater (50ml)*	Rainwater (50ml)
T1 (Cu)	Rain water (50ml)	Rain water (50ml)	Cu (1 ppm) (50ml)*	Rainwater (50ml)	Rainwater (50ml)	Cu (1 ppm) (50ml)*	Rainwater (50ml)	Rainwater (50ml)	Cu (1 ppm) (50ml)*	Rainwater (50ml)
T2 (Wastewater)	Rain water (50ml)	Rain water (50ml)	Wastewater (50 ml)*	Rainwater (50ml)	Rainwater (50ml)	Wastewater (50 ml)*	Rainwater (50ml)	Rain water (50ml)	Wastewater (50 ml)*	Rainwater (50ml)
Day	31	32	33	34	35					
Control	Rainwater (50ml)	Rainwater (50ml)*	Rainwater (50ml)	Rainwater (50ml)	Harvesting day					
T1 (Cu)	Rainwater (50ml)	Cu (1 ppm) (50ml)*	Rainwater (50ml)	Rainwater (50ml)	Harvesting day					
T2 (Wastewater)	Rainwater (50ml)	Wastewater (50 ml)*	Rainwater (50ml)	Rainwater (50ml)	Harvesting day					

Note: *= Numbers of leaves and heights of plants recorded

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Table S4. *Values of estimated daily intake (EDI, µg/kg wet weight/day) and target hazard quotient (THQ, unitless) values of Cu on the edible leaves of Amaranthus viridis from all the sampling sites in Peninsular Malaysia*

Cu	**Children**		**Adults**	
	EDI	**THQ**	**EDI**	**THQ**
1	2.22	0.055	1.00	0.025
2	2.70	0.067	1.22	0.030
3	2.10	0.052	0.95	0.024
4	2.10	0.052	0.94	0.024
5	2.86	0.072	1.29	0.032
6	2.28	0.057	1.03	0.026
7	2.10	0.053	0.95	0.024
8	1.41	0.035	0.64	0.016
9	1.68	0.042	0.76	0.019
10	2.14	0.054	0.97	0.024
11	1.53	0.038	0.69	0.017

Note: The dry weight basis of metal concentrations in the edible leaves of Amaranthus viridis was converted to wet weight basis using a conversion factor of 0.12

Table S5. *Values of bioconcentration factors (BCF) of Cu on the leaves, stems and roots of Amaranthus viridis from all the sampling sites in Peninsular Malaysia*

Sampling sites	$BCF_{leaf/EFLE}$	$BCF_{leaf/SUM}$	$BCF_{stem/EFLE}$	$BCF_{stem/SUM}$	$BCF_{root/EFLE}$	$BCF_{root/SUM}$
1	7.73	0.16	4.79	0.10	7.08	0.15
2	17.80	0.48	10.49	0.28	15.26	0.41
3	9.49	0.22	8.54	0.20	15.34	0.35
4	5.23	0.07	4.34	0.06	8.79	0.11
5	17.41	0.33	11.60	0.22	14.54	0.28
6	5.41	0.07	3.90	0.05	5.19	0.07
7	9.04	0.14	7.26	0.11	17.25	0.27
8	3.34	0.05	2.90	0.04	6.08	0.08
9	8.34	0.12	4.44	0.07	9.50	0.14
10	10.35	0.20	4.10	0.08	7.71	0.15
11	13.22	0.39	6.75	0.20	10.39	0.30

Table S6. *Values of translocation factors (TF) of Cu on the leaves, stems and roots of Amaranthus viridis from all the sampling sites in Peninsular Malaysia*

	$TF_{leaf/root}$	$TF_{stem/root}$
Site	**Cu**	
1	1.09	0.68
2	1.17	0.69
3	0.62	0.56
4	0.59	0.49
5	1.20	0.80
6	1.04	0.75
7	0.52	0.42
8	0.55	0.48
9	0.88	0.47
10	1.34	0.53
11	1.27	0.65

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Table S7. *Morphological measurements of number of leaves and maximum heights of Amaranthus viridis after 35 days of experimental greenhouse study*

Treatment	Number of leaves (blade)	Height (cm)
Control	12.33[a]	41.94[a]
Cu	12.00[a]	41.86[a]
Wastewater	12.80[a]	42.78[a]

Note: The same alphabets show no significant difference at P> 0.05

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