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Research Article

Potentially Toxic Metals in Cucumber *Cucumis sativus* Collected from Peninsular Malaysia: A Human Health Risk Assessment

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Abstract

The purposes of this study were to assess the concentrations of Fe, Cu, Ni, Pb, and Zn in the cucumber *Cucumis sativus* from four farming areas of Peninsular Malaysia, to assess the HHRA of the five heavy metals in the collected samples. The cucumber was collected between May and December 2016 from Kg Ara Kuda (Penang), Kg. Sitiawan (Perak), Kuala Ketil (Kedah) and Jerantut (Pahang) of Peninsular Malaysia. For the edible fruity cucumber, the ranges of metal concentrations (mg/kg dry weight) from the four sites were 9.56-13.6 for Cu, 39.5-109 for Fe, 0.18-2.19 for Ni, 0.74-2.78 for Pb and 17.5-62.0 for Zn. All the target hazard quotient values for Fe, Cu, Ni, Pb, and Zn in adults and children were found below 1.00 for the health risk assessment. The present investigation found no evidence of non-carcinogenic hazards associated with the intake of cucumber in relation to Fe, Cu, Ni, Pb, and Zn. However, it is important to regularly evaluate the levels of heavy metals in vegetables cultivated in these soils and adopt appropriate remediation procedures to reduce harmful effects on human health.

Introduction

Contamination of vegetables due to wastewater irrigation is a significant issue that poses potential risks to human health. Using wastewater for irrigating vegetables introduces contaminants such as toxic metal ions, dyes, and waterborne pathogenic bacteria into the soil [1-4]. The plant roots can absorb these contaminants and gradually accumulate in the edible parts of the vegetables, making them unsafe for consumption. Furthermore, hazardous pollutants in wastewater can adversely affect aquatic life and immobilize plant enzymes [5]. Moreover, the contamination of vegetables with potentially toxic metals is often caused by anthropogenic inputs such as sewage sludge and residues from mining and various industries. Improper application of fertilizers and pesticides from atmospheric sources can also contribute to elevated concentrations of pollutants in the soils [5-9].

Human activities, including manufacturing industries, urbanization practices, and agro-based industries, contribute to the presence of potentially toxic metals in wastewater used for irrigation, further exacerbating the contamination of vegetables [10-15]. This contamination of vegetables not only poses a threat to human health but also has implications for the overall ecosystem.

Vegetable consumption is a significant pathway for potentially toxic metals (PTMs) entering the human body [16]. Improper application of sewage sludge might negatively affect agro-systems' productivity [17]. Excessive levels of trace elements may pose a risk to human health. The presence of heavy metal residues in food crops that are irrigated with wastewater has been extensively documented in China [18]. Eid, et al. [17] found that the sewage sludge they studied might be utilized as a fertilizer in cucumber production systems in Saudi Arabia.

Previous research has documented the presence of metal exposure and toxicities in edible cucumbers. These studies include the works of Alcantara, et al. [19], Romera, et al. [20], Munzuroglu and Geckil [21], Tabaldi, et al. [22], Janicka-Russak, et al. [23], Prakash, et al. [24], Eid, et al. [17], Minich, et al. [25], Stevic, et al. [26], Kabala, et al. [27], Freitag, et al. [28], and Kim, et al. [29]. Romera, et al. [20] conducted a study to investigate the impact of bicarbonate and specific metal ions on the development of increased root Fe(III) reduction capacity in young cucumber plants (*Cucumis sativus* L) cultivated in a nutrient solution. This reaction is a result of Fe shortage in dicotyledons. Their findings indicated that bicarbonate can hinder the growth of root Fe(III), lowering capacity by limiting the accessibility of certain metal ions necessary for this process.

Tabaldi, et al. [22] conducted a study to examine the impact of several metals on the activity of acid phosphatase in cucumber seedlings (*C. sativus* L.) in a laboratory setting. They found that Zn is a stronger acid phosphatase inhibitor from cucumbers than Hg. Zhang, et al. [30] examined the impact of the heavy metal cadmium (Cd) on the root development and the activity of antioxidant enzymes, namely superoxide dismutase (SOD) and peroxidase (POD), in *C. sativus* L. hairy roots. Additionally, they investigated the combined effects of cadmium (Cd) and zinc (Zn) on these parameters. Their findings indicated that concentrations of Cd below 10 mg/L stimulated the development of *C. sativus* hairy roots and specifically increased root diameter within a 5-15 days of root culture. Arata, et al. [31] conducted experiments to examine the effects of elevated levels of lead (Pb), nickel (Ni), and copper (Cu) on the germination and development of *C. sativus*. The analysis revealed that the metals' bioaccumulation data in the seeds indicated the toxicity level of the metals being evaluated.

However, the literature lacks reports on the human health risk assessment (HHRA) of PTMs in the edible cucumber *C. sativus*. Therefore, the objectives of this study were to 1) assess the concentrations of Fe, Cu, Ni, Pb, and Zn in the cucumber *C. sativus* from four farming areas of Peninsular Malaysia and 2) assess the HHRA of the five heavy metals in the collected samples.

Materials and methods

The samples *C. sativus* were collected between May and December 2016, from Kg Ara Kuda (Penang), Kg. Sitiawan (Perak), Kuala Ketil (Kedah) and Jerantut (Pahang) of Peninsular Malaysia (Figure 1). The collected samples were stored in clean polyethylene bags and transferred to the laboratory for further analysis. The morphology and classification of the cucumber from the present study were identified according to Chin and Yap [32] and Prohens and Nuez [33,34].

The collected samples were washed with distilled water to remove soil particles. Then, they were cut into small pieces using a clean knife and dried in an oven at 60 °C for 72 hours until they reached constant dry weights. After drying, the vegetable samples were ground using a commercial blender and stored in polyethylene bags until further analysis.

For the determination of heavy metals, all samples stored in acid-washed pillboxes were analyzed by using a flame atomic absorption spectrophotometer (AAS) model Thermo Scientific iCE 3000 series for Fe, Cu, Ni, Pb, and Zn at the Chemistry Department of the Faculty of Science at Universiti Putra Malaysia (UPM). Standard solutions were prepared from 1000ppm stock solution provided by Sigma-Aldrich for the five metals. All data obtained from the AAS were presented on a mg/kg dry weight basis.

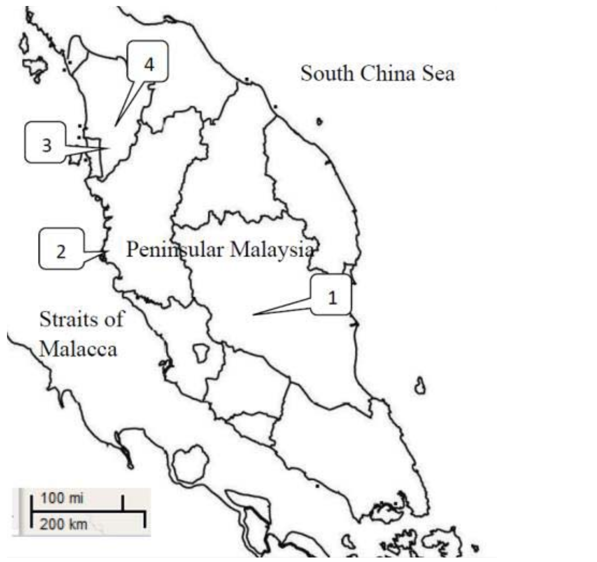

Figure 1: The sampling sites (estimation only) for cucumber *Cucumis sativus* in Peninsular Malaysia (1 = Jerantut; 2 = Sitiawan; 3 = Ara Kuda; 4 = Kuala Ketil).

All the glassware used in this study was acid-washed for quality assurance and quality control to avoid external contamination. Two certified reference materials (CRMs) were used to check for the analytical procedures and accuracy of the method used. The CRMs included were *Lagarosiphon major* N.60 and Peach Leaves (NIST 1547). The recoveries for the CRM *Lagarosiphon major* N.60 were 97.4, 120.2, and 119% for Zn, Cu and Pb, respectively, while CRM Peach Leaves (NIST 1547) were 97.0 and 117% for Ni and Fe, respectively (Table 1).

For the HHR assessment, the present concentrations in dry weight were converted into wet weight because cucumber consumption (or cooking) is assumed to be in wet weight. Therefore, the present concentrations (mg/kg dry weight) of Fe, Cu, Ni, Pb, and Zn were converted to wet weight basis by using a conversion factor of 0.043 [35,36].

The HHR assumes a once-or long-term potential hazardous exposure to metals through consumption of the vegetables. The HHR assessments included estimated daily intake (EDI) and target hazard quotient (THQ) values were calculated by using the following formulas:

EDI = (Mc × CR)/BW

where;

Mc = The metal concentration in cucumber (mg/kg wet weight).

CR = The consumption rate of cucumber (345 g/day for adults and 232 g/day for children) and average body weight (55.90 kg for adults and 32.70 kg for children) [37].

In this study, a non-carcinogenic risk assessment method was based on THQ, a ratio between the estimated contaminant dose and the oral reference dose (RfD), below which there will not be any appreciable risk. The THQ was determined with a formula described by USEPA [38]:

THQ = EDI/ RfD

where;

EDI = Estimated daily intake calculated previously; RfD = The oral reference dose. The RfD (µg/kg wet weight/day) values used in this study were Fe: 700, Ni: 20.0, Cu: 40.0, and Zn: 300, provided by the EPA's Integrated Risk Information System online database [39]. Since RfD for Pb was unavailable according to the EPA's IRIS [39], the present study employed the RfD as 4.00 µg/kg wet weight/day proposed by FAO/ WHO [40]. It is estimated that if the THQ ratio is more than one, vegetable consumption will result in a non-carcinogenic risk of heavy metals to human health.

Table 1: Comparison of metal concentrations (mg/kg dry weight) between certified and measured values. The certified values are based on certified reference materials were *Lagarosiphon major* N.60 and Peach Leaves (NIST 1547).

	Lagarosiphon major N.60		
	Certified value	Measured value	Recovery (%)
Cu	51.20 ± 1.9	61.54 ± 1.4	120.2
Zn	313 ± 8	304.85 ± 3.4	97.4
Pb	64 ± 4.00	76.3 ± 2.40	119
	Peach Leaves (NIST 1547).		
Ni	0.689	0.81	117
Fe	219.8	211	97.0

Results

The heavy metal concentrations (mg/kg dry weight) in the cucumber collected from the four sites in Peninsular Malaysia are presented in Table 2. For the edible fruity cucumber, the ranges of metal concentrations (mg/kg wet weight) from the four sites were 0.41-0.58 for Cu, 1.70-4.69 for Fe, 0.01-0.09 for Ni, 0.03-0.12 for Pb and 0.75-2.67 for Zn (Table 2). The maximum permissible limits for Cu, Pb and Zn set by the Malaysian Food Act 1983 and Regulation 1985 are 30.0, 2.00 and 100 mg/kg wet weight [41]. Therefore, the Cu, Pb and Zn levels found in the present study's cucumbers are well below the MPLs.

The Fe limit has not been found in the literature. Similarly, the Ni maximum permissible limits (MPL), known as the action level (80 mg/kg WW) for molluscan shellfish (FDA Guidance Document), has been set at 80 mg/kg wet weight [42]. However, the Ni limit for fruit and vegetables has not been found in the literature or is lacking, or validation of the Ni limits is needed. Therefore, comparisons of the MPLs for Fe and Ni are not possible.

Overall, the PTM values are comparable to those reported from Tongling [43], Pearl River Estuary [44,45], and Saudi Arabian markets [46]. However, the present data are significantly lower than those reported from Guangdong, especially for Pb and Zn (Table 2) [47].

The values of EDI and THQ of the five heavy metals in the cucumber for adults and children from the present study are presented in Tables 3,4, respectively. All the THQ values for Fe, Cu, Ni, Pb, and Zn in both adults and children were found below 1.00. This indicates there is no non-carcinogenic risk of Fe, Cu, Ni, Pb, and Zn via the consumption of the cucumber from the present study. Overall, the EDI and THQ values are comparable those reported from Tongling [43], Pearl River Estuary [44,45], and Saudi Arabian markets [46] but are significant lower than those reported from Guangdong especially for Pb and Zn in which the THQ values are higher than 1.0 for both Pb and Zn [47] (Tables 3 and 4).

In general, the THQ values of heavy metals in children are higher than in adults. Zhang, et al. [48] collected greenhouse

Table 2: Mean heavy metal concentrations (mg/kg dry weight) in cucumber *Cucumis sativus* collected from four farms in Peninsular Malaysia. Note: Values in brackets are converted into wet weight basis using a conversion factor of 0.043.

Site	Cu	Fe	Ni	Pb	Zn	SD	Source of irrigation	Reference
Ara Kuda	9.56 (0.41)	109 (4.69)	1.40 (0.06)	0.74 (0.03)	26.1 (1.12)	12-Oct-16	Tube well and stream	This study
Kg Sitiawan	11.1 (0.48)	56.6 (2.43)	1.40 (0.06)	1.39 (0.06)	17.5 (0.75)	17-Nov-16	Domestic wastewater, tube well and stream	This study
Kuala Ketil	12.4 (0.53)	39.5 (1.70)	2.19 (0.09)	2.78 (0.12)	20.0 (0.86)	21-Dec-16	Tube well and stream	This study
Jerantut	13.6 (0.58)	82.0 (3.53)	0.18 (0.01)	1.60 (0.07)	62.0 (2.67)	12-May-16	Private farms, a river, distance from the highway, surrounded by forest.	This study
Tongling, China	0.36	NA	NA	0.018	1.539	Unspecified	mining area	Ding, et al. [41]
Pearl River Estuary, China*	0.52	NA	0.074	0.023	1.615	Unspecified	Reclaimed tidal flat soil	Li, et al. [42]
Chongqing, China	32.2	NA	NA	10.2	57.0	2008-2009	27 supermarkets	Yang, et al. [43]
Guangdong Province, China	17.0	NA	NA	164	806	Unspecified	Polluted sites	Wang, et al. [45]
Saudi Arabian markets	3.21	113	NA	3.67	29.8	2011	Dammam City	Ali and Al-Qahtani [44]

Note: SD: Sampling date; *: in wet weight basis; NA: data is not available.

Table 3: Values of estimated daily intake (EDI) of heavy metal concentrations in cucumber *Cucumis sativus* collected from four farms in Peninsular Malaysia.

	Adults					Children				
Consumption rate of vegetables (g/day)	345					323				
Body weight (kg)	55.9					32.7				
	Cu	Fe	Ni	Pb	Zn	Cu	Fe	Ni	Pb	Zn
Ara Kuda	2.54	28.9	0.37	0.20	6.91	4.06	46.3	0.59	0.31	11.1
Kg Sitiawan	2.94	15.0	0.37	0.37	4.63	4.70	24.1	0.59	0.59	7.42
Kuala Ketil	3.29	10.5	0.58	0.74	5.32	5.26	16.8	0.93	1.18	8.51
Jerantut	3.61	21.8	0.05	0.42	16.5	5.78	34.8	0.08	0.68	26.3
Tongling mining area, China*	2.23	NA	NA	0.11	9.50	3.57	NA	NA	0.18	15.2
Pearl River Estuary, China*	3.18	NA	0.46	0.14	9.97	5.10	NA	0.73	0.23	15.9
Chongqing, China	8.55	NA	NA	2.71	15.1	13.7	NA	NA	4.33	24.2
Guangdong Province, China	4.51	NA	NA	43.5	214	7.22	NA	NA	69.7	342
Saudi Arabian markets	0.85	29.9	NA	0.97	7.91	1.36	47.9	NA	1.56	12.7

Note: All metal data were concerted to wet weight basis using a conversion factor of 0.043 for the calculation of EDI. *: The data were not converted since the data is already in wet weight basis. NA: Data is not available.

Table 4: Values of target hazard quotient (THQ) of heavy metal concentrations in cucumber *Cucumis sativus* collected from four farms in Peninsular Malaysia.

	Adults					Children				
RfD (µg/kg wet weight/day)	40.0	700	20.0	4.00	300	40.0	700	20.0	4.00	300
	Cu	Fe	Ni	Pb	Zn	Cu	Fe	Ni	Pb	Zn
Ara Kuda	0.063	0.041	0.019	0.049	0.023	0.102	0.066	0.030	0.079	0.037
Sitiawan	0.073	0.021	0.019	0.092	0.015	0.117	0.034	0.030	0.148	0.025
Kuala Ketil	0.082	0.015	0.029	0.184	0.018	0.132	0.024	0.047	0.295	0.028
Jerantut	0.090	0.031	0.002	0.106	0.055	0.144	0.050	0.004	0.170	0.088
Tongling mining area, China*	0.056	NA	NA	0.028	0.032	0.089	NA	NA	0.044	0.051
Pearl River Estuary, China*	0.080	NA	0.023	0.035	0.033	0.127	NA	0.037	0.057	0.053
Chongqing, China	0.214	NA	NA	0.677	0.050	0.342	NA	NA	1.083	0.081
Guangdong Province, China	0.113	NA	NA	10.881	0.713	0.181	NA	NA	17.414	1.141
Saudi Arabian markets	0.021	0.043	Na	0.243	0.026	0.034	0.068	NA	0.390	0.042

NA = data is not available.

surface soils (0–20 cm) and 30 vegetables from Kunming City, Yunnan Province, southwestern China, and analyzed for total Cd, Pb, Cu, Zn, As, Hg, and Cr. They found that the THQ value was greater than one for adolescents, indicating a non-carcinogenic risk of heavy metals in adolescents. To reduce the health risk effects, it is suggested that industrial wastes be treated properly and phyto-extract the overload of heavy metals and metalloids from polluted sites.

Discussion

The low human health risk of potentially toxic metals in cucumber collected from farming sites can be attributable to controlling and minimizing pre-harvest contamination. By implementing effective measures to control and minimize pre-harvest contamination, the risk of potentially toxic metals in cucumbers collected from farming sites can be reduced to levels that pose low human health risks. Additionally, studies have shown that their concentrations influence the bioaccumulation of metals in vegetables in the soil. For instance, higher levels of metals such as Cd, Fe, and Ni in vegetables were found to be correlated with higher levels of these metals in the soil [49].

Factors such as the geochemical composition of the soil, levels of metals in the organic matter and sulphides of the soil, and solubility and interchangeable geochemical fractions of the surrounding soils can influence the bioaccumulation of metals in vegetables [5]. Furthermore, the bioavailability of metals in the soil can vary depending on the specific type of metal. For example, studies have shown that the bioavailability of Zn in vegetables can be assessed by the combined concentrations of Zn in the surrounding soils. Meanwhile, vegetable metal levels may not always be strongly related to those in the habitat topsoils.

Based on the THQ values, it has been determined that the levels of Cd, Fe, Ni, and Zn in cucumbers collected from farming sites pose low human health risks. This is supported by studies showing that the THQ values for these metals in cucumbers were all below 1. This indicates that consuming cucumbers from these farming sites would not result in non-carcinogenic risks to consumers, including children and adults. Furthermore, studies have reported that the levels of Fe in lettuce are significantly higher than in other vegetables but still below the threshold for adverse effects. Therefore, the potential human health risks from potentially toxic metals in cucumbers collected from farming sites are low when effective pre-harvest contamination control measures are implemented. However, there is always a need to continuously monitor and assess the levels of potentially toxic metals in cucumbers and other vegetables and implement effective measures to minimize contamination risks.

The levels of potentially toxic metals in cucumbers collected from farming sites have been found to pose low human health risks. However, it is important to note that continued monitoring and implementation of effective measures to minimize contamination risks are still necessary to ensure the safety of cucumbers and other vegetables for human consumption. Therefore, based on the available evidence, it can be concluded that the risk of high human health risks from potentially toxic metals in cucumbers collected from farming sites is minimal.

Finally, the biomonitoring of PTMs in the edible cucumber is crucial for several reasons. Firstly, cucumbers are widely consumed as a food source by humans, making it essential to ensure their safety and quality. Secondly, cucumber plants have the ability to absorb and accumulate PTMs from contaminated soil and water, making them potential indicators of environmental pollution. Thirdly, the presence of PTMs in cucumbers can harm human health, as these metals can be toxic and may lead to various health issues, such as heavy metal poisoning and organ damage. Therefore, by monitoring PTMs in cucumber plants, we can identify and mitigate potential risks to human health and the environment. Furthermore, PTM monitoring in cucumber for wastewater and soil analysis is important for environmental management and remediation. It allows us to identify areas that may be contaminated with PTMs, determine the extent of contamination, and develop strategies for remediation and pollution control.

Conclusion

In sum, the HHRA of metals in the cucumbers investigated in the present study indicated no non-carcinogenic risks of Fe, Cu, Ni, Pb, and Zn from consuming the cucumbers. Nevertheless, the findings emphasized the need for routine monitoring and management to avoid cucumber contamination from the wastewater irrigation system. In conclusion, effective pre-harvest control measures can minimise the risk of PTM contamination in cucumbers collected from farming sites.

Author contributions

Conceptualisation, C.K.Y.; methodology and validation, C.K.Y. and A.Y.; formal analysis, A.Y. and Z.S.; investigation, C.K.Y.; resources, M.C.O.; data curation, C.K.Y. and M.C.O.; writing—original draft preparation, C.K. Y.; writing—review and editing, W.M.S., R.N., H.O., Y.H., C.S.L., AD.S., K.K., W.H.C. and K.A.A. All authors have read and agreed to the published version of the manuscript.

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