A Comprehensive Evaluation of Heavy Metal Contaminations in Soils of Ohio and West Virginia and Cucurbitaceous Vegetables Grown in Respective Farms and Implications on Human Health

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Posted Date: 12 July 2023

doi: 10.20944/preprints202307.0811.v1

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Article

A Comprehensive Evaluation of Heavy Metal Contaminations in Soils of Ohio and West Virginia and Cucurbitaceous Vegetables Grown in Respective Farms and Implications on Human Health

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Abstract: Contamination of soil and crops with heavy metals (HMs) poses a significant environmental challenge in the United States. Hence, this study aimed to assess HM contamination levels from sampled Ohio and West Virginia soils using various pollution indicators analyses, including Enrichment Factor (EF), Geo-accumulation index (I_{geo}), Contamination Factor (CF), and Pollution Load Index (PLI) and identify the Translocation Factor (TF) of HMs in the edible part of the vegetables and further evaluate health risks associated with dietary exposure through estimations of Estimated Daily Intake (EDI), Hazard Index (HI), Target Hazard Quotient (THQ), Cancer Risk (CR) and Target Cancer Risk (TCR) on adults and children. In this study, Fe was the most predominant contaminant, ranging from 28.41 to 67.36 g/kg in the soil. Cancer risk assessment revealed that Ni poses significant risks. Therefore, regular monitoring of metal concentrations in soil and vegetables grown in these regions might mitigate potential health hazards in the future.

Keywords: Heavy metals; Vegetables; Contamination; Pollution indicators; Cancer risk; Health risk

1. Introduction

Heavy metal (HM) pollution is one of the most troublesome environmental issues that has significantly accelerated since the beginning of the industrial revolution. A wide variety of HMs are released into the environment through anthropogenic sources such as mining, smelting, metallurgy, combustion of fossil fuels, electroplating, agriculture, sewage sludge amendments, etc., [1]. HMs can be uptake by the plants from the contaminated soil, and into the organisms through the biomagnification process [2]. HMs such as Iron (Fe), Zinc (Zn), Manganese (Mn), Copper Cu, Chromium (Cr), and Selenium (Se) are classified as essential HMs for human metabolisms and involved in various biological processes at trace level. However, when these metals exceed the threshold levels, they become toxic to living forms [3,4]. In addition, HMs such as Arsenic (As), Mercury (Hg), Cadmium (Cd), and Lead (Pb) have no beneficial biological roles and are hazardous to the organisms even at lower concentrations [5,6]. Even in lower doses and prolonged exposure, these metals can induce serious health risks causing cancer, lung inflammation, liver and kidney damage, fibrosis, emphysema, and dysfunction of the enzymatic, endocrine, and immune systems [7–9]. The US Environmental Protection Agency (EPA) recently estimated that HM contamination posed a health threat to more than 10 million people worldwide [10].

Plants from the *Cucurbitaceae* family consist of several fruits and vegetables consumed worldwide. Cucurbit fruits and vegetables are pivotal in dietary and nutritional aspects, especially as sources of minerals, dietary fibers, vitamin C, thiamine, niacin, folic acid, and pyridoxine [11,12]. Moreover, these fruits and vegetables have played an important role in the ethnopharmacological and traditional medicinal systems globally, and their evidence is well established in several classic literatures [13]. Due to these nutritional and pharmaceutical benefits, consumption has increased in recent years. According to the statistics of the Food and Agriculture Organization of the United

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Nations [14], the consumption rate of fruits and vegetables increased to 22.4% compared to the last ten years. However, fruits and vegetables of *Cucurbitaceae* are the most exposed food to HM contaminations, which accumulate metals in their edible parts at quantities high enough to cause health hazards [15]. It is important to identify safer limits of the essential and non-essential HMs in the major consumable cucurbit fruits and vegetables to ensure food safety and sustainability.

Ohio and West Virginia have a long history of mining coal, oil, natural gas, etc. They are also a major hub of various industrial and production sectors supporting the economy [16]. On the other hand, these anthropogenic activities release different HMs into the environment [17,18]. The last comprehensive survey of HMs in farmlands in the United States was published in 1993 [19]. Hence, its highly essential to understand metal contamination in high environmental risk states like Ohio and West Virginia. Based on the facts stated above, the present research was to i) assess HMs contamination in farmlands from Ohio and West Virginia states through the Enrichment Factor (EF), Geo-accumulation index (I_{geo}), Contamination Factor (CF), and Pollution Load Index (PLI) analysis, ii) identify the bioaccumulation and Translocation Factor (TF) of HMs in the edible part of the vegetables, iii) validate the HM dynamics through correlation factor, principal component and hierarchical cluster analysis, and iv) predict health risks associated with dietary exposure through modeling using Estimated Daily Intake (EDI), total EDI (TEDI), Hazard Index (HI), Target Hazard Quotient (THQ), Cancer risk (CR) and Target Cancer Risk (TCR) on adults and children.

2. Materials and Methods

2.1. Site Description and Samples Collection

Soil samples were collected from different counties of Ohio and West Virginia (Athens, Highland, Meigs, and Mason), which are home to many mining activities and hence are highly vulnerable to HMs contamination. The geographical map of the sampled locations is presented in Figure 1. Eighty-two vegetable samples (cucrbits) were collected from the 13 sampled sites from September to November 2022 (Table S1). Simultaneously, thirteen soil samples were collected using a zig-zag pattern from the close vicinity of the roots of the same vegetable plants at a depth ranging from 5 to 15 cm. Collected soil samples were bulked to form a composite sample.

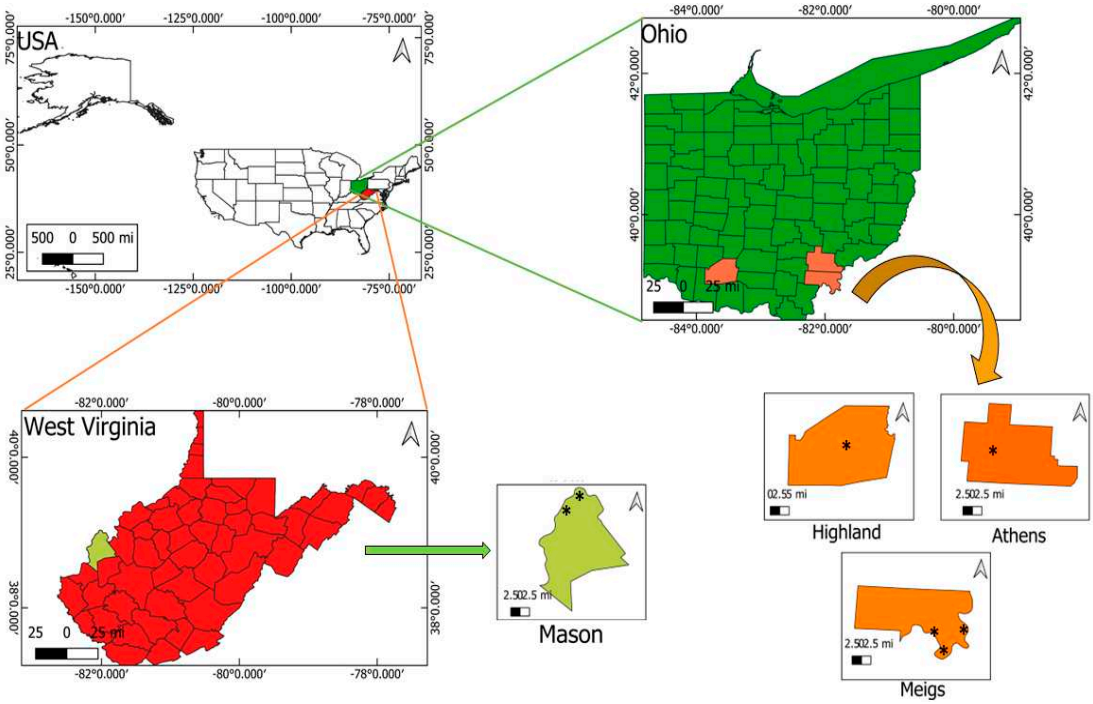

Figure 1. Map of the sampling sites.

2.2. Sample Preparation and ICP – MS Analysis

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Collected soil samples were air-dried at laboratory, sieved through a nylon mesh (2 mm) to remove the non-soil particles, and stored at 4 °C for further analysis. Vegetable samples were rinsed and washed with deionized water. Further, vegetables were cut into small pieces, completely oven dried at 65 – 75 °C for 24 h, powdered, sieved, and stored at 4 °C in an airtight plastic container for analysis [1]. Processed soil and vegetable samples were digested with 3:1 ratio of HCl and HNO_3 through a microwave digester (MARS 6, CEM Corporation, Matthews, NC, USA). The resulting mixture was then filtered and diluted with metal-free HPLC grade water. The concentrations of HMs in the test samples were estimated through Inductively Coupled Plasma – Mass Spectrometry (ICP – MS, X – serous II, Thermo, US) [8].

2.3. Pollution Indicator Assessment

2.3.1. Enrichment Factor (EF)

The EF is a primary geochemical index used to assess the influence of natural and anthropogenic sources on HM contamination in soil. The soil samples' EF was calculated using the following formula (Eq. 1) [20].

$$EF = \left(\frac{Cn}{Cref}\right) sample / \left(\frac{Bn}{Bref}\right) background \quad (1)$$

where C_n referred to the concentration of metal in the soil, C_{ref} referred to concentrations of reference elements in the soil. Similarly, B_n is referred to as the background reference value of metals and B_{ref} as the background value of the reference element. This study used Aluminium (Al) as a reference element to calculate the EF. Reference values used for EF calculations are listed in Table S2. The EF levels are categorized as follows: (i) no or minimal enrichment (EF < 2), (ii) moderate enrichment (2 EF < 5), significant enrichment (5 EF < 20), very high enrichment (20 EF < 40) and extremely high enrichment (EF40) [21].

2.3.2. Geo-Accumulation Index (I_{geo})

The I_{geo} comprehensively assesses HMs in the soil by comparing contemporary and pre-industrial values. This study determined the I_{geo} values soil samples with the following formula (Eq. 2) [22].

$$Igeo = log2 \frac{Ci}{1.5GBV} \quad (2)$$

Ci represents the measured concertation of HM in the soil, and GBV represents the HM's geochemical background value, compiled in Table S2. Based on the rate of l_{geo}, contamination ratios were classified into seven different classes, which include: $l_{geo} \leq 0$ – uncontaminated, $0 < l_{geo} \leq 1$ – uncontaminated to moderately contaminated, $1 < l_{geo} \leq 2$ – moderately contaminated, $2 < l_{geo} \leq 3$ – moderately to heavily contaminated, $3 < l_{geo} \leq 4$ – heavily contaminated, $4 < l_{geo} \leq 5$ – heavily to extremely contaminated and $I_{geo} \geq 5$ – extremely contaminated [22].

2.3.3. Contamination Factor (CF)

The CF of the soil samples was assessed to quantify the impact of individual HMs on the soil. The CF ratio was calculated by dividing the metal concentrations in the test soil (C_t) by the background (C_{Ref}) value (Eq. 3) [23].

$$Cf = \frac{Ct}{CRef} \quad (3)$$

Metal-specific reference concentrations used for CF calculation are given in Table S2. Further, CF values are classified based on the degree of metal concentrations, as follows; if CF <1 – low contamination; if $1 \leq CF < 3$ – moderate contamination; if $3 \leq CF < 6$ – considerable contamination and if $CF \geq 6$ - very high contamination [24].

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2.3.4. Pollution Load Index (PLI)

The PLI is also used to determine the overall concentration of HMs in the target soil samples. The PLI was calculated based on the CF values of each metal in the soil as follows (Eq. 4) [25].

$$PLI = \sqrt[n]{Cf1 \times Cf2 \dots \dots \dots \dots Cfn} \quad (4)$$

where the C_f^1 contamination factor of element 1, C_f^2 contamination factor of element 2, and C_n^f contamination factor of the nth element. PLI is classified as PLI < 0 – no pollution, < 0 PLI <1 – low degree of pollution, < 1 PLI <2 moderate degree of pollution, <2 PLI <4 – high degree of pollution, <4 PLI <8 – very high degree of pollution and <8 PLI <16 – extremely high degree of pollution.

2.4. Translocation Factor (TF)

The HM uptaking ability of the plants was determined by calculating the concentration of HM in test soil and plant tissues (Eq. 5) [23].

$$TF = \frac{Cveg}{Csoil} \quad (5)$$

C_{veg} represents the total HM concentration in the vegetable, and C_{soil} represents the total HM concentration in the corresponding soil where the vegetable was grown. A TF value greater than 1 indicates that the plant is potentially accumulating HM [26].

2.5. Calculation of Human Health Risk Assessment

2.5.1. EDI Assessment

The EDI of the HMs was determined by their concentrations in each sample and the daily intake of vegetables in grams. The EDI value of each HM of interest was determined with the following formula (Eq. 6) [27].

$$\text{EDI} = \frac{EF \times ED \times EIR \times CM \times CF}{BW \times TA} \times 0.001 \quad (6)$$

where EF represents the exposure frequency, ED represents the exposure duration, EIR represents the average vegetable consumption by adults and children, CM represents the concentration of HM present in the vegetables, CF represents the concentration of conversion factor for fresh weight to dry weight, BW represents average body weight of adult and children, TA represents the average exposure duration and 0.001 represent the unit conversion factor. The comprehensive reference data employed for EDI calculation is compiled in Table S2.

2.5.2. THQ and HI Assessment

The noncarcinogenic health hazards for people who consume HM-contaminated vegetables were calculated through THQ using the following equation (Eq. 7) [27].

$$\text{THQ} = \frac{EDI}{RfD} \quad (7)$$

EDI represents the estimated daily intake of HMs in mg/day/kg body weight, and RfD means the oral reference dosage values of HMs as listed in Table S2. If THQ value is < 1, it is considered safe for the risk of noncarcinogenic effects. If THQ value >1, it is supposed that there is a chance of noncarcinogenic effects with an increasing probability as the value increases.

The HI is a vital index that assesses the likely consequences of exposure to more than one metal. The HI was calculated following equation (Eq. 8) [26].

$$HI = \sum_{n=1}^{i} THQn;\ i = 1, 2, 3, \dots\dots n \quad (8)$$

If the HI value is <1, there is no apparent health impact; however, if HI value is >1 indicates a greater possibility of health impact.

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2.5.3. CR and TCR Assessment

The CR induced by HM-contaminated vegetable ingestion was estimated following the equation (Eq. 9) [28]. Further, the target cancer risk (TCR) was calculated with the following equation (Eq. 10) [29].

$$CR = EDI \times CPSo \quad (9)$$

$$TCR = \sum_{n=1}^{i} CR; i = 1, 2, 3, \ldots\ldots n \quad (10) \quad (10)$$

CR represents the cancer risk over a lifetime due to specific HM intake, and CPSo represents the oral slop factor (mg/kg/day). The cancer risk is categorized as no significant health risk (CS/TCR < 1.00E-06), admissible risk (1.00E-06 < CR/TCR < 1.00E-04), and inadmissible risk (CR/TCR > 1.00E-04). The CPSo value used for CR calculation was described in Table S2, while we couldn't find the corresponding value of Cu, Fe, Mn, and Zn; hence, their cancer risk was not calculated.

2.6. Statistical Analysis

Soil and vegetable samples were collected in triplicates. The mean difference between the samples was compared using one-way ANOVA. The data analysis and statistical interpretations were conducted using SPSS 20.0 (IBM Corporation, New York, NY, USA). Further, correlation coefficient factor, Principal Component Analysis (PCA), and hierarchical cluster analysis were performed by Origin 2022 (OriginLab Corporation, Corporation, Northampton, MA, USA).

3. Results and Discussion

3.1. HM Analysis in Agricultural Soil

Soil contamination with HMs directly impacts plant growth and yield. Moreover, these contaminants are transported to the edible parts, significantly increasing food safety concerns. Hence, in this study, the concentrations of HMs in the soil samples collected from different agricultural sites in Ohio and West Virginia were analyzed through ICP – MS analysis, and results are presented in Table 1. Across the 13 sampling sites, Cd, Cu, Fe, Mn, Ni, and Zn were identified. Contents ranged from 0.13 to 2.91 µg/g of Cd, 0.016 to 0.043 g/kg of Cu, 28.41 to 67.36 g/kg of Fe, 0.42 to 1.86 g/kg of Mn, 0.018 to 0.044 g/kg of Ni, and 0.064 to 0.234 g/kg of Zn. These metals were significantly varied in HM contaminations in the study areas. Fe (67.36 g/kg) was identified as the predominant contaminant in the study area. Mn, Ni, and Zn were also identified, with concentrations of 1.8, 0.044, and 0.234 g/kg, respectively. In contrast, Cd, and Cu, were detected at less than the background levels and can be classified as the least contaminants in the study area. The descending order of HM concentrations was Fe > Mn > Zn > Ni > Cu > Cd. Higher Fe, Mn, and Ni concentrations were observed among the tested soil samples in the MS2, HI, and RS6 sampling sites, respectively. In contrast, increased Cd, Cu, and Zn concentrations in the RS3 sampling site. Our results showed that the farmlands in Meigs County might have been exposed to greater HM concentrations. These increased concentrations of HMs may affect the soil structure, plant growth, nutrient, and water uptake by impairing the primary root growth and root hair formation [30,31].

Table 1. HM concentrations from the sampled soils.

Sampling location	HM concentrations (g/kg)					
	Cd (µg/g)	Cu	Fe	Mn	Ni	Zn
RS1	0.397^{c}	0.027^{b}	39.96^{c}	1.39^{c}	0.036^{ab}	0.114^{c}
RS2	0.250^{d}	0.02^{b}	28.41^{d}	0.737^{d}	0.019^{c}	0.097^{cd}
RS3	2.91^{a}	0.043^{a}	37.29^{c}	1.02^{d}	0.03^{b}	0.234^{a}
RS4	0.257^{d}	0.024^{b}	28.41^{d}	0.7^{d}	0.018^{b}	0.097^{cd}
RS5	0.295^{d}	0.021^{b}	30.13^{d}	1.01^{d}	0.022^{bc}	0.102^{c}
RS6	0.992^{b}	0.04^{a}	52.89^{b}	1.54^{b}	0.044^{a}	0.17^{b}

 doi:10.20944/preprints202307.0811.v1

RS7	0.134^{f}	0.016^{bc}	29.34^{d}	0.57^{f}	0.019^{c}	0.064^{e}
NM1	0.17^{e}	0.022^{b}	38.74^{c}	0.5^{f}	0.029^{b}	0.09^{cd}
NM2	0.17^{e}	0.022^{b}	39.57^{c}	0.57^{f}	0.028^{b}	0.079^{d}
MS1	0.205^{de}	0.023^{b}	34.88^{cd}	0.83^{de}	0.028^{b}	0.095^{cd}
MS2	0.189^{d}	0.043^{a}	67.36^{a}	0.42^{f}	0.03^{b}	0.119^{c}
MS3	0.203^{de}	0.021^{b}	30.66^{d}	1.28^{cd}	0.026^{b}	0.087^{d}
HI	0.166^{e}	0.017^{bc}	40.31^{c}	1.86^{a}	0.023^{bc}	0.081^{d}
Mean	0.487	0.026	38.32	0.959	0.027	0.11
Median	0.205	0.022	37.29	0.83	0.028	0.097
Mode	0.17	0.022	28.41	0.57	0.028	0.097
SD	0.761	0.009	11.08	0.44	0.007	0.045

Note: Superscripts show differences $P < 0.05$ level.

3.2. Pollution Indicators Analysis

The EF analysis is a widely employed tool for characterizing various HM derived from multiple anthropogenic activities. The EF analysis of various HM, showed an increased level of Fe (Table 2). Of 13 soil samples, EF of Fe ranged from 2.041 to 4.125, with the maximum EF of 4.125 observed at the RS1 sampling site. Conversely, the EF for Cu, Cd, Mn, Ni, and Zn ranged from 0.051 to 0.481, 0.183 to 0.533, 0.249 to 1.64, 0.137 to 0.431, and 0.247 to 0.814, respectively, indicating either no enrichment or minimal enrichment in the study areas. Understanding the sources and mechanisms of Fe accumulation would be critical for assessing the possible impact of Fe enrichment on soil health. Like EF, the I_{geo} values exhibited high variability, ranging from uncontaminated to moderately contaminated, depending on the specific HMs analyzed (Table 2). Across all study areas, Fe showed a higher prevalence than other analyzed HMs. Sampling sites RS2, RS4, and RS7 were uncontaminated to moderately contaminated ($0 < I_{geo} \leq 1$) with Fe; while RS1, RS3, RS5, RS6, NM1, NM2, MS1, MS3, and HI sites were moderately contaminated with Fe ($1 < I_{geo} \leq 2$). Noteably, site MS2 was identified as moderate to heavily contaminated ($2 < I_{geo} \leq 3$) in terms of Fe level. On the other hand, five sampling sites (RS1, RS3, RS5, MS1, and MS3) were polluted with Mn, ranging from uncontaminated to moderately contaminated, and two sampling sites (RS6 and HI) were moderately contaminated. Further, sampling sites RS3, RS6, and MS2 were uncontaminated to moderately contaminated with Cu, while RS3 and RS6 were uncontaminated to moderately contaminated with Zn.

In addition to the EF and I_{geo} indices, the CF is also a useful indicator for comparing the HM contamination in the soil. The distribution pattern of CF values revealed significant variations among the analyzed metals, ranging from 0.017 to 0.08, 0.87 to 2.34, 1.40 to 2.80, 0.68 to 2.80, 1.08 to 2.61, and 1.15 to 4.23 for Cd, Cu, Fe, Mn, Ni, and Zn, respectively (Figure 2A). The sampling sites, namely RS1, RS2, RS3, RS4, RS5, RS6, MS1, and MS3, were moderately contaminated (CF>1) with Cu, Fe, Mn, Ni, and Zn. These values indicated that the soil's Cu, Fe, Mn, Ni, and Zn levels exceeded background levels, signifying a certain degree of contamination in the study area. Moreover, the CF values for Fe in the MS2 site and Zn in the RS3 and RS6 sites were higher ($3 \leq CF < 6$), indicating the considerable contamination of respective HMs in the study areas. Collectively, all the above pollution indicator analyses revealed contamination of Fe, Mn and Zn, which may source from various anthropogenic activities such as agrochemical, mining, geological weathering, mineralization, and parent material characteristics [32,33]. Additionally, substantial quantities of Ni and Cu metals were also found, indicating their contribution to overall pollution levels. Understanding the sources and mechanisms of these HMs' enrichment are critical for assessing the possible impact on soil health [31]. Moreover, these parameters can aid in planning risk mitigation strategies for HM contamination to minimize health hazards. Further, the overall contamination levels in the study areas were assessed through PLI analysis, which provided an important metric for evaluating the pollution load and highlighting areas of concern. In this study, most sampling sites exhibited PLI ranging from 0 to 1, except for the RS3 and RS6 sites which exceeded the limit (Figure 2B). The RS3 and RS6 sites were classified as polluted, with PLI values of 1.017 and 1.024, respectively. The elevated PLI values indicate a significant amount of HMs contributed to the overall pollution load in the areas [5].

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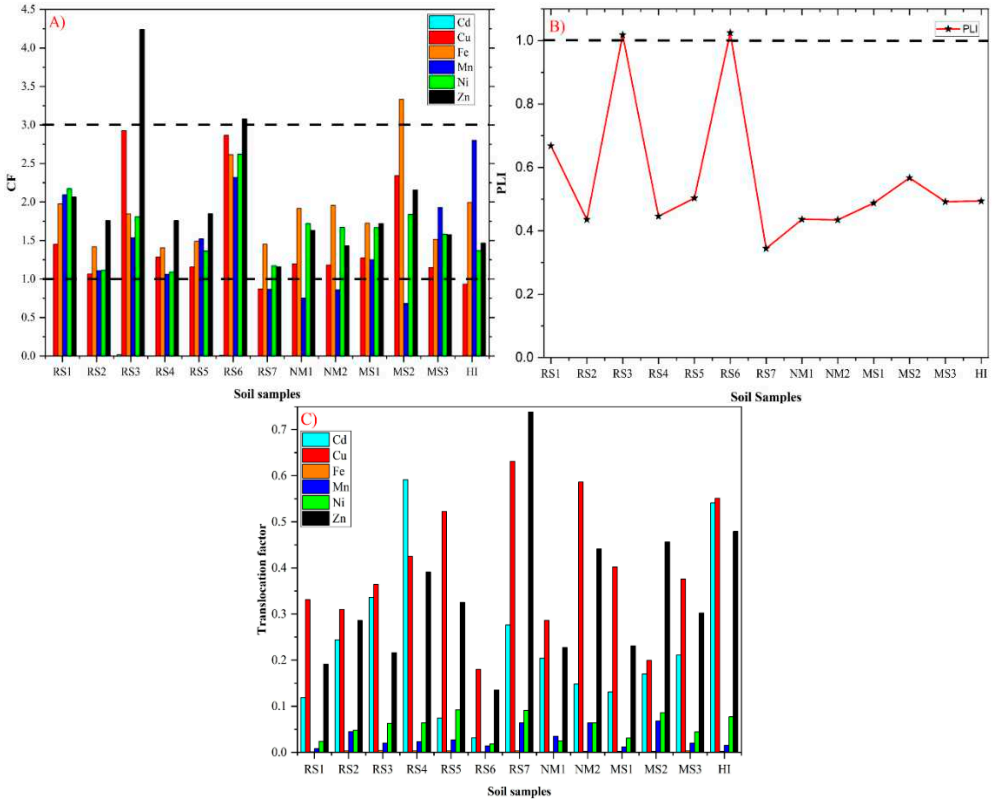

Figure 2. Pollution indicator analysis: A) CF of HMs, B) PLI of the study area, and C) TF of HMs in vegetables. The black dash indicates the HM contamination range of the soil.

Table 2. EF and I_{geo} analysis in various agricultural fields.

Sampling	EF						I_{geo}					
location	Cd	Cu	Fe	Mn	Ni	Zn	Cd	Cu	Fe	Mn	Ni	Zn
RS1	0.257	0.533	**4.125**	1.641	0.431	0.748	-10.88	-0.146	**1.413**	**0.890**	-1.038	-0.395
RS2	0.151	0.365	**2.766**	0.812	0.206	0.594	-11.55	-0.592	**0.936**	-0.0252	-2.003	-0.628
RS3	0.481	0.453	**2.041**	0.638	0.190	0.814	-8.480	**0.536**	**1.313**	**0.443**	-1.302	**0.641**
RS4	0.141	0.400	**2.489**	0.706	0.183	0.539	-11.51	-0.321	**0.922**	-0.089	-2.035	-0.628
RS5	0.127	0.283	**2.076**	0.796	0.156	0.446	-11.31	-0.473	**1.006**	**0.429**	-1.711	-0.556
RS6	0.372	0.461	**3.160**	1.052	0.300	0.645	-9.562	**0.432**	**1.818**	**1.037**	-0.769	**0.180**
RS7	0.051	0.189	1.785	0.400	0.137	0.247	-12.45	-0.880	**0.967**	-0.380	-1.928	-1.228
NM1	0.053	0.210	1.910	0.282	0.163	0.282	-12.10	-0.421	**1.368**	-0.584	-1.375	-0.736
NM2	0.056	0.224	**2.106**	0.347	0.171	0.267	-12.10	-0.440	**1.399**	-0.395	-1.416	-0.925
MS1	0.090	0.317	**2.439**	0.664	0.224	0.422	-11.83	-0.334	**1.217**	**0.146**	-1.421	-0.658
MS2	0.057	0.403	**3.253**	0.249	0.170	0.365	-11.95	**0.545**	**2.166**	-0.730	-1.279	-0.334
MS3	0.080	0.258	1.930	0.922	0.191	0.348	-11.85	-0.480	**1.031**	**0.771**	-1.495	-0.785
HI	0.057	0.183	**2.216**	1.170	0.144	0.283	-12.14	-0.777	**1.426**	**1.310**	-1.705	-0.888

3.3. Traces of HMs in Vegetables

Monitoring the accumulation of HM residues in agricultural products is essential for ensuring food safety. The rate of HM accumulation in plant species widely differs, even among cultivars and varieties within the same species. HM contents in cucurbit fruits and vegetables grown in different sampling sites were analyzed (Table 3). Our results showed zucchini grown in MS2 had higher concentrations of Cd (0.152 mg/kg), Fe (176.42 mg/kg), Ni (2.67 mg/kg), and Zn (54.32 mg/kg). Pumpkins cultivated in the RS6 and RS7 sites showed the highest contents of Cu (11.29 mg/kg) and Mn (37.29 mg/kg), respectively. On the other hand, among all the tested vegetable samples, squash exhibited lower contents of Cu (6.18 mg/kg), Fe (60.91 mg/kg), Ni (0.73 mg/kg), and Zn (20.46 mg/kg). Similarly, watermelon displayed lower contents of Cd (0.02 mg/kg) and Mn (10.71 mg/kg), respectively. These results indicated significant accumulation of HMs in the tested vegetable samples,

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which can be attributed to species variation, genetic mechanisms related to metal-specific afficinty with the root, metal bioavailability, soil pH, and nutrient availability in a specific site [34,35]. Furthermore, certain species possess efficient metal transporter systems that selectively uptake, transport, and sequester certain HMs based on their requirements, thereby regulating the entry [36]. Further, TF was estimated in this study to identify the rate of HM uptake from soil to aerial plant parts. In the present experiment, TF was less than 1, ranging from 0.074 to 0.591 for Cd, 0.18 to 0.631 for Cu, 0.002 to 0.004 for Fe, 0.08 to 0.064 for Mn, 0.018 to 0.092 for Ni, and 0.135 to 0.738 for Zn, respectively (Figure 6). Among the analyzed vegetable samples, the maximum TF values were observed in pumpkin, followed by cucumber, zucchini, watermelon, and squash. Published studies have also reported a similar trend of TF ranges for Cd, Cu, Pb, and Zn [37].

Table 3. Concentrations of HMs in fruit and vegetable samples.

Sampling location	HM concentrations (g/kg)					
	Cd (µg/g)	Cu	Fe	Mn	Ni	Zn
RS1-W	0.047^{d}	8.5^{d}	80.19^{de}	11.39^{d}	1.05^{d}	22.46^{cd}
RS2-S	0.061^{c}	6^{e}	101.26^{c}	34.31^{a}	1.12^{d}	28.92^{c}
RS3-Z	0.098^{b}	16.04^{a}	160.07^{b}	20.4^{c}	1.4^{cd}	50.21^{a}
RS4-C	0.152^{a}	10.14^{c}	104.02^{c}	16.28^{cd}	1.65^{c}	38.11^{b}
RS5-P	0.031^{e}	7.1^{e}	73.07^{e}	22.4^{c}	0.8^{e}	22.97^{cd}
RS6-P	0.022^{ef}	11.12^{bc}	90.19^{d}	28.0^{b}	2.1^{b}	33.2^{bc}
RS7-P	0.037^{de}	10.18^{c}	90.02^{d}	37.3^{a}	1.8^{bc}	47.3^{ab}
NM1-S	0.035^{de}	6.14^{e}	61.11^{ef}	17.62^{c}	1.12^{d}	20.49^{d}
NM2-P	0.043^{d}	8.33^{d}	100.19^{c}	26.45^{b}	1.4^{cd}	25.82^{c}
MS1-W	0.027^{e}	10.21^{c}	77.8^{e}	11.05^{d}	1.06^{d}	21.88^{cd}
MS2-Z	0.152^{a}	9.07^{cd}	176^{a}	31.09^{ab}	3^{a}	53.16^{a}
MS3-P	0.025^{e}	13.05^{b}	88.14^{d}	20.01^{c}	1.8^{bc}	35.7^{b}
HI-C	0.09^{b}	10^{c}	108.09^{c}	28.31^{b}	1.4^{cd}	38.3^{b}
Mean	0.063	9.684	100.7	23.43	1.515	33.73
Median	0.043	10	90.6	22.4	1	33.2
Mode	0.152	10	-	11	1	22
SD	0.045	2.75	32.78	8.207	0.673	11.59

Note: Superscripts show differences $P < 0.05$ level. W – watermelon, S – squash, Z – zucchini, C – cucumber, and P – pumpkin.

3.4. Identification of HM Contamination Source

Correlation coefficients were estimated to examine the dynamics of HMs in soil-vegetable systems and gain insights into the sources of HMs in the sampling sites (Figure 7 A), which revealed a significant positive correlation between Cd and Cu (r = 0.94), and Cd and Cu with Fe (r = 0.73 and r = 0.65, respectively). Cu also positively correlated with Ni (r = 0.67) and Zn (r = 0.87). Similarly, Fe and Ni showed positive correlations with Ni (r = 0.65) and Zn (r = 0.54), respectively. Such significant positive correlations among the tested HMs suggest common mode of accumulation [5]. However, Mn did not significantly correlate with the other metals analyzed, indicating that the pattern of Mn intake and accumulation could be different from those of the other metals. Notably, Mn accumulation in the vegetable samples was negatively correlated with Mn concentrations within the soil samples. Such negative correlation coefficients could be attributed to differential sources of Mn when compared with other HMs [38]. This weak correlation and lack of a linear relationship between the concentration of HMs in soil and vegetables might affected by various environmental factors which could influence the plant uptake mechanisms [39].

In this study, a PCA was constructed using standardized forms of the dataset and analyzed through varimax rotation with Kaiser Normalization to understand HMs' sampling distribution. The multivariate PCA of HMs in the study area explained approximately 94.6% of the cumulative variance and identified three major factors for HM distribution (Figure 3B and Table S3). Cd, Cu, and Zn primarily influenced the first factor, contributing 56.6% of the total variance. The second factor was Ni and Fe, contributing 20.4% of the variance. In contrast to the first two factors, the third factor

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was predominantly influenced by Mn, which accounted for 17.6% of the variance and highly varied from the other tested HMs.

Further, the source of the HMs was validated through hierarchical cluster analysis (Figure 3C). This study provided insights into the grouping and associations among the HMs analyzed, shedding light on potential pollution sources and their interconnections. Similar to the PCA, Cd, and Zn were closely grouped, indicating a strong association. Cu is moderately associated with Cd and Zn, forming a subcluster within the first major cluster. The PCA and cluster analysis suggested that Cd, Zn, and Cu may share familiar sources or interact with each other through shared environmental pathways. Moreover, these HMs may originate from the same sources by applying agrochemicals during intensive agricultural activities in the study area [40]. Furthermore, Fe and Ni were grouped in the second cluster and moderately associated with the first cluster. This moderate association implies that shared pollution sources or environmental factors influence the dynamics of these metals in the soil. Sources of Fe and Ni could be attributed to various natural and anthropogenic activities, including geogenic processes, mining, atmospheric deposition, and irrigation with contaminated wastewater [41]. In contrast, Mn formed a separate third cluster and showed limited association with the other two clusters. This disassociation of Mn with other HMs concludes that the source of Mn in agriculture sights completely different from other HMs. Rollin et al. [42] suggested some possible sources of Mn entry in agricultural soil, such as mining, mineral processing, sewage sludge, and other anthropogenic emissions.

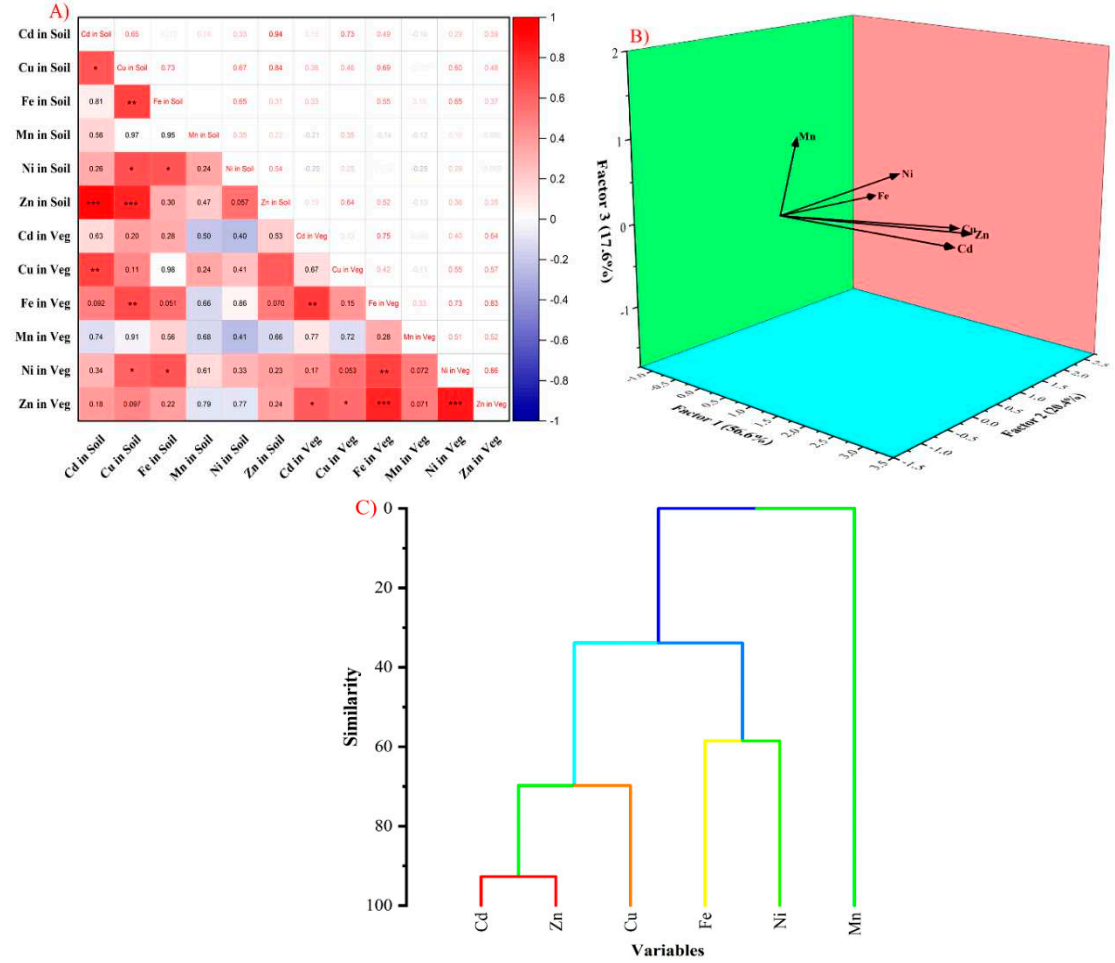

Figure 3. Identification of HM dynamics and sources: A) Correlation plot analysis *P< 0.05, **P< 0.01, and ***P< 0.001, B) PCA and C) hierarchical cluster analysis.

3.5. Noncarcinogenic Health Risk Assessment

Cucurbit have been an important vegetables, health risks from consuming contaminated with HMs were determined by calculating the EDI, THQ, and HI for different age groups (Figure S1). The

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EDI, TEDI, THQ and HI rates of consuming HMs through vegetables were lower than the maximum tolerable daily intake of each metal. In terms of the overall HM load in HI percentage, Cu contributed the most with 27.6%, followed by Mn (19.2%), Fe (17.6%), Cd (14.5%), Zn (12.9%), and Ni (8.2%) (Figure S2). While comparing the adults and children, the EDI, TEDI, THQ and HI rates were significantly higher in children, suggesting that consuming HM-contaminated vegetables poses relatively significant health risks to the younger population. Furthermore, children's nervous system is highly vulnerable to HM stress; therefore, even a relatively lower concentration of HMs in children's blood can have an irreversible impact on their mental growth and functions [21]. Within the sample set, the highest EDI, TEDI, and THQ rates were observed in zucchini, whereas the lowest rate was observed in squash.

3.6. Carcinogenic Risk

Furthermore, CR and TCR analysis validated the carcinogenic effects of consuming HM-contaminated vegetables. In this study, The CR value of Cd were within the acceptable range from 9.4962E-06 to 1.47288E-05 and from 8.241E-06 to 1.216E-05, for adults and children, respectively. On the other hand, the CR and TCR values of Ni in all the tested vegetables were higher than the permissible limit, ranging from 3.487E-4 and 1.172E-3 for adults and from 5.362E-4 and 1.286E-3 for children, respectively (Figure 4A and B). In the analyzed vegetables, the maximum CR and TCR values were observed in zucchini, followed by pumpkin, cucumber, watermelon, and squash. Prolonged consumption of vegetables contaminated with HMs poses a carcinogenic risk to human health, potentially leading to cancer [43]. Previous studies have documented a similar trend of enhanced CR and TCR rates through consuming HM-contaminated vegetables [15,26]. These carcinogenic risk results should be considered in planning and agriculture policy-making; for instance, selecting plants with low metal accumulation ability, such as squash and watermelon instead of zucchini, may help restrict the entry of metals into the food chain. Moreover, contaminated agriculture sites such as RS3 and RS6 could be used to cultivate wood and ornamental crops as an alternative to edible crops. However, it is crucial to identify the pollutant source and regulate the entry of metals into agricultural soil, which is an essential step to prevent such contamination. This would provide a significant balance between food safety and environmental protection, cost-effectively minimizing health risks.

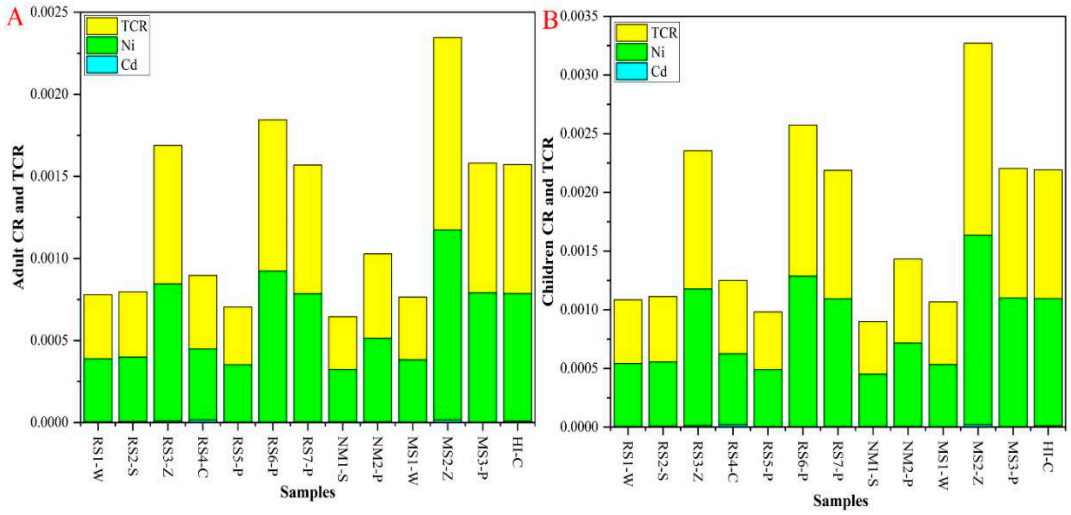

Figure 4. Carcinogenic health risks assessment of HMs from the consumption of cucurbit fruit and vegetables. CR and TCR of A) adults and B) children from different vegetables.

4. Conclusion

The range of HM contamination in the major agriculture sites of Ohio and West Virginia was analyzed, revealing high levels of Fe contamination in the test soil samples. The calculated values of

the HMs, obtained through various pollution indicator analyses, indicate that Cu, Fe, Mn, and Zn contaminated the study areas. Through PCA and hierarchical cluster analysis, it can be concluded that Cd, Cu, and Zn mainly accumulated from agricultural activities. In contrast, Fe, Mn, and Ni are primarily accumulated from mining and/or atmospheric deposition. The carcinogenic risk assessment indicated that the CR and TCR of Ni were higher than the allowable limit. Hence, it is strongly recommended to implement management practices and monitor HM levels in the soil and vegetables to prevent carcinogenic health risks to consumers.

Author contributions: UR and PN: conceptualization, project administration, funding acquisition, manuscript editing, supervision, KC: experimentation, data analysis, writing-original manuscript, PS and HY: sample collection and processing. All authors have read and approved the final manuscript.

Acknowledgments and Funding: Authors thankful to NASA WV EPSCoR (NASA-EPSCoR-R3-21-0051) for their financial support. The authors would like to thank Elemental Analysis Laboratory, Dept. of Chemistry Texas A&M University, for providing ICP – MS facilities.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflict of interest: The authors have declared no conflict of interest.

Compliance with Ethics requirements: This article does not contain any studies with human or animal subjects.

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