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Determination of Heavy Metal accumulation in vegetables and their potential health risk analysis in Bargarh, Odisha

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Abstract
Agriculture is integral to global food security and economic stability, while vegetables constitute a significant component of the human diet and provide essential elements necessary for health and well-being. Nevertheless, the growing global emphasis on nutrition-sensitive agriculture necessitates a heightened evaluation of crop nutrient density and the quality of soils utilized for food cultivation. Advancement is unattainable without understanding the significance of vegetables in nutrition and healthcare services. Human exposure to heavy metal contamination primarily occurs through the consumption of green vegetable crops irrigated with wastewater. Objective of the current study was focused on to determine the concentration of heavy metals (cadmium, manganese, chromium, copper, iron, nickel, lead, zinc) in plant and soil from collected from five different samples from Sambalpur, Odisha. The study also determines bioconcentration factor, daily intake of metals from vegetables and health risk index from vegetable intakes. In tomato higher concentration of cadmium, manganese, chromium, copper and Zinc were found whereas, iron concentration was found in maximum concentration parwal (25.07±0.547 mg/kg) in soil. In case of vegetables maximum concentration of heavy metals such as Cd, Mn, Cr, Cu were found in chilli and parwal. Additionally, metals such as Fe, Ni and Zn was found in parwal and brinjal. The concentration of Cd, Mn, Cr and Ni have surpassing the permissible limit given by WHO/FAO. The human DIM has been calculated and found to be below the recommended values set by the FAO/WHO. However, to prevent any chronic health risk and extent of heavy metal contamination, steps must be taken to reduce human activities at the sites. Consistent surveillance of heavy metals in vegetables cultivated in wastewater-irrigated regions is essential.

Keywords: Heavy metals, Bioconcentration factor, Daily intake of metals

Introduction

Vegetables are esteemed for their abundant vitamins, minerals, dietary fibers, and phytochemicals, as well as their role in mitigating chronic ailments such as cardiovascular diseases, diabetes, and specific cancers (Woodside *et al*., 2013) [1]. The growing population and urbanization have significantly elevated vegetable consumption, thereby generating considerable interest in various agricultural strategies aimed at enhancing both crop output and nutritional quality. The quality of vegetables has emerged as a significant concern for researchers, policymakers, and consumers alike. The composition of soil is a primary factor influencing the quality of vegetables. Consequently, the nutritional composition for assessing quality is of paramount importance. Plants intrinsically necessitate nutrients such as macronutrients for growth and development. Microelements, although needed in minute quantities, are as essential for a plant's physiological functions (Nieder *et al*., 2018) [2]. Further, understanding the interaction between soil and plants is essential for advancing agricultural practices. The quantification of soil nutrients is essential for assessing the nutrient content of the crops cultivated in that soil. Human exposure to heavy metal contamination primarily occurs through the consumption of green vegetables irrigated with chemically polluted wastewater (Ismail *et al.,* 2014; Qureshi *et al.,* 2016) [4, 3]. The accumulation of Cu, Ni, Zn, Cr, Fe, Mn, Co, and Pb in red onion (*Allium cepa*), garlic (*Allium sativum*), tomato (*Solanum lycopersicum*), and eggplant (*Solanum melongena*) irrigated with wastewater was examined by Hussain *et al.* (2013) [3]. Zhou *et al.* (2016) examined the accumulation of heavy metals in 22 vegetable species and employed the target risk ratio to assess the health hazards linked to vegetable consumption. Leafy vegetables absorb a greater quantity of heavy metals than melon vegetables, indicating that the cultivation of melon vegetables is appropriate for soil contaminated with heavy metals. Leafy vegetables cultivated in soil contaminated with heavy metals accumulate higher levels of these metals compared to those grown in uncontaminated soil (Jassir *et al.,* 2005) [6].

The world's industrial production is growing at an accelerated rate, increasing the likelihood of heavy metal pollution in the environment. Toxic substances accumulate in soil, water, and air due to accelerated industrialization, unregulated development, and prolonged application of fertilizers and pesticides (Kumar *et al.,* 2015) [8]. Vegetables cultivated near abandoned industrial areas provide a significant danger of heavy metal pollution (Murray *et al.,* 2011; Chen *et al.,* 2018) [10, 17]. The purpose of this study is to evaluate the accumulation of heavy metals in various regularly cultivated vegetables. The investigation aimed to compute the bioconcentration factor of metals and the daily dietary intake of metals from vegetable eating in adults to assess the impact on their health.

Materials and Methods

Geographical location and Soil sampling

The present study was conducted in Bargarh district, Odisha, located in the western part of the state adjacent to Chhattisgarh. It is located between 20° 43' N and 21° 41' N latitude, and between 82° 39' E and 83° 58' E longitude. The district exhibits a tropical climate characterized by arid, sweltering summers, a rainy monsoon season, and frigid winters. Sampling was conducted using the methodology established by Parkinson *et al*., 1971.Soil samples were obtained from agricultural fields cultivated with five distinct crops in the local farmers' land region of Bargarh, Odisha. The crop areas from which samples were gathered include *Solanum melongena* L. (Brinjal, *Solanaceae*), *Solanum lycopersicum* L. (Tomato, *Solanaceae*), *Capsicum frutescens* L (Parwal; *Solanaceae*), *Brassica oleracea var. capitata* L. (Cabbage *Brassicaceae*), and *Momordica charantia* L. (Chilli; Cucurbitaceae).Each site was divided into blocks, and soil samples were randomly collected at a depth of 0-15 cm using 15 × 15 × 15 cm trenches. Soil, following appropriate sieving, was submitted to the laboratory in sterile polyethylene packets for further analysis.

Estimation of heavy metals from vegetables

Methods of Latif *et al.* (2018) was followed for estimation of heavy metals from soil and vegetables. To evaluate the content of heavy metals in plant samples, around 0.3 g of the ground dried materials was placed in a vessel (Model number XP-1500) of the microwave digester, to which 4 ml of concentrated HNO_3 was added. The heating system utilized was that specified in the client's directives. The three-stage temperature protocol comprised an initial connection at -180°C, a ramp length of 10 minutes, a holding period of 15 minutes, and a cooling duration of 10 minutes. Subsequent to digestion, it is important to reduce the rotor's temperature to 60°C. Upon cooling, carefully remove the upper screw of the vessel with a torque wrench to relieve pressure beneath a fume hood. The gathered samples were subsequently separated and diluted to the calibration line with deionized water in a 10 ml volumetric flask. The samples were subsequently analysed using AAS to quantify heavy metals.

Estimation of heavy metals from soil

One gram of soil was placed in a 250 cm³ digestion tube containing 10 cm³ of pure HNO_3.The tubes were subsequently cooked for 30 to 45 minutes. Following oxidation, the samples were cooled, and 5 cm³ of 70% $HClO_4$ was added, after which the mixture was heated until white vapours were observed. The tubes were allowed to cool for an extended period, after which 20 cm³ of distilled water was added and heated until the vapour had dissipated. The solution was thereafter allowed to cool and was filtered using Whatman filter paper No. 42. The filtrate was transferred to a 25 cm³ volumetric flask. The final volume was designated with deionized water. A blank was similarly prepared following the appropriate protocol without incorporating a soil sample. Water samples were prepared by measuring 50 cm³ into a beaker and treating it with 10 cm³ of concentrated HNO_3. The solution in the container was heated until a volume of 40 cm³ remained. The solution was re-filtered using Whatman filter paper No. 42 and diluted to 50 cm³ with deionized water. A blank solution was prepared along the same pathway without the inclusion of a water sample.

A serial dilution method was utilized to create the working standards and determine the metal concentrations in each sample. The concentration was ascertained utilizing AAS (Buck Model 210 VGP) with an integrated digital readout device.

Daily intake of heavy metals from vegetables

Bio-concentration Factor (BCF)- BCF is the ratio of elemental concentration in plant tissues, including leaves, stems, and roots, to the "exchangeable" or potentially accessible fraction of metals in rhizospheric soils. The BCF for the edible portion of the vegetable was computed using the following formula:

$$BCF = Cp/Cs$$

Where, Cp= Concentration of metal in edible part of vegetable (mg/kg), Cs= Metal content in soil (mg/kg).

Daily intake estimate

$$DIM = M \times K \times I/W$$

Where, DIM=Daily intake of metals, M=concentration of heavy metals in plants (mg/kg), K= Conversion factor, I= Daily intake of vegetables, w=Average body weight.

The fresh weight of vegetables was converted to dry weight using a conversion factor of 0.085, as previously outlined. The average body weights for adults and children were 57.1 kg and 30.3 kg, respectively, while the average daily vegetable intakes were 0.330 kg per adult and 0.208 kg per child.

Health Risk Index (HRI): The HRI for Cr, Ni, Cu, Pb, Cd, Mn and Zn by consumption of contaminated vegetables was calculated by following equation (Jan *et al.,* 2010)

$$HRI = DIM/ Rfd$$

Where, DIM-daily intake of metals, Rfd = reference oral dose.

Rfd value for Cr, Ni, Cu, Pb, Cd, Mn and Zn is 1.5, 0.02, 0.04, 0.004, 0.001, 0.033 and 0.30(mg/Kg bw/day) respectively

Result

Estimation of heavy metals in soils

The present study aimed to analyse the accumulation of heavy metals in vegetable soil, quantified in mg/kg (Table 1). Cadmium is a heavy metal naturally present in the Earth's crust; nevertheless, human activities, particularly mining, industrial emissions, and the application of phosphate fertilizers, have augmented its accumulation in agricultural soils. The present study revealed that the aggregation of this metal ranged from 0.09±0.02 to 0.07±0.01 mg/kg. Manganese is a crucial micronutrient vital for the growth and development of vegetable crops. Manganese plays a vital role in essential physiological processes in plants, including photosynthesis, respiration, and nitrogen absorption. Mn concentration in the current study was ranged from 7.45±0.087 to 20.76±0.046 mg/kg. The soil of vegetable tomato (20.76±0.046) was exposed to high Mn concentration as compare to other vegetable soil. Cr concentration was higher in soil of tomato (0.98±0.019) mg/kg and lowest in soil of *Solanaceous* vegetable soil i.e chilli (0.92±0.029) mg/kg. Iron is a crucial micronutrient necessary for the growth and development of plants, as it plays a vital role in numerous physiological processes, including photosynthesis. In the current study the concentration of Iron (Fe) was higher in Parwal (25.07±0.547) mg/kg and less concentration was found in Tomato (9.58±0.216) mg/kg.

Table 1: Metal concentration in five different type of soil (0-15cm)

Sites	Cd	Mn	Cr	Cu	Fe	Ni	Pb	Zn
site 1 (Cabbage)	0.09±0.02	7.45±0.087	0.96±0.015	0.27±0.02	23.07±0.954	0.9±0.054	nd	0.05±0.001
site 2 (Bringal)	0.07±0.01	14.26±0.052	0.95±0.023	0.3±0.001	19.97±0.745	0.88±0.041	nd	0.16±0.014
Site 3 (Tomato)	0.09±0.05	20.76±0.046	0.98±0.019	0.42±0.004	9.58±0.216	0.92±0.032	nd	1.58±0.012
site 4 (Parwal)	0.08±0.04	8.45±0.054	0.97±0.034	0.32±0.001	25.07±0.547	0.89±0.018	nd	0.1±0.014
site 5 (Chilli)	0.09±0.03	15.21±0.074	0.92±0.029	0.28±0.012	17.25±0.554	0.88±0.047	nd	0.2±0.023

*Values are Mean±SD

Copper is a crucial micronutrient vital for sustaining plant life and functioning as a cofactor in significant physiological processes. The present study revealed that the concentration of Cu was highest in tomato (0.42±0.004) mg/kg, whereas the lowest concentration was seen in cabbage (0.27±0.02) mg/kg. Nickel is a vital micronutrient necessary for plant growth and development due to its function as a cofactor in key enzymatic biological processes. The concentration of Ni was greater in tomato (0.92±0.032) mg/kg and lesser in chilli (0.88±0.047) mg/kg. Zinc is an element essential for the synthesis of enzymes, proteins, and the regulation of plant hormones, as well as for other physiological and biochemical processes within the plant. The content was determined to be greater in tomatoes (1.58±0.012) mg/kg and lesser in *Brassicaceae* vegetables (0.05±0.001) mg/kg (Table 1).

Estimation of heavy metal concentration in vegetables

In the current study, the accumulation of heavy in different parts of vegetables was analysed and represented in table 2 with respect to mg/kg. Cadmium is a non-essential element and its concentration is also limit with no role in plant's nutrient uptake. Excessive accumulation will inhibit important processes like photosynthesis and respiration which result in stunted growth, chlorosis and yield reduction. As compare to men, women accumulates high concentration of Cd, because of their high Fe necessities. The level of Cd was shown significant variation in all selected vegetables. The level of Cd was higher in Chilli as compare to other. The accumulation Cd in five vegetable samples was Cabbage (0.125), Brinjal (0.188), Tomato (0.149), Parwal (0.293), Chilli (0.388) mg/kg respectively. The result was also supported by Rapheal and Adebayo (2011). The resulted concentration was slightly higher than the safe limit 0.2 mg/kg given by FAO/ WHO. The Cd concentration in Chilli was 0.388 mg/kg which crossed the safe limit, thus proved to be dangerous for consumption may be due to excessive use of chemical fertilizers. The result shown that the Mn range in different vegetable was from 19.621- 30.252mg/kg. Maximum Mn concentration was found in Parwal (30.252) while low concentration was found in Cabbage (19.621). The safe limit for consumption of Mn in vegetables is 0.2 mg/kg and concentration of Mn in all vegetables crossed the safe limit proposed by FAO/WHO. The concentration of Mn between tomato and brinjal was not shown significant difference. Maximum Mn (26.891) mg/kg was found in *Solanaceous* vegetables. The reason behind high Mn accumulation is soil type and application of pesticides and fertilizer (Chary *et al.*, 2008).The sequence of metal accumulation in vegetables was Cabbage <Tomato< Brinjal < Chilli < Parwal. Cr level was ranged from 1.8 to 2.8 mg/kg in different parts of vegetables. Maximum Cr concentration was seen in vegetables of *Brassicaceae* (2.8) mg/kg followed by *Solanaceous* vegetables i.e brinjal (2.5) and chilli (2.2).The concentration of Cr was exceed from limit given by FAO/WHO of 2.3. This might be because of application of chemical fertilizers or lack of proper management practices during growing period .The sequence of vegetables accumulated with Cr as follows cabbage < parwal < brinjal < chilli < tomato (Table 2). Cu is an essential micronutrient functions as biocatalyst, need for pigmentation of body. The level of Cu content was found between 21.68 to 50.3 mg/kg. Maximum concentration of Cu was maximum in 50.3 mg/kg while minimal accumulation was found in cabbage (21.68) mg/kg. Results are lined with findings of Rapheal and Adebayo (2011). Cu concentration of all vegetables was not crossed safe limit of FAO/WHO of 73mg/kg. Iron (Fe) is an important fundamental element of living life form and essential to maintain cell homeostasis. It is also important for chlorophyll synthesis and its deficiency cause chlorosis in plants. Fe concentration was higher in parwal (190.22) mg/kg and less concentration was seen in cabbage (120.12) mg/kg. The concentration of Fe was found in all vegetables are 120.12 to 190.22 mg/kg. In the current study, the concentration of Fe found in all vegetable has not crossed safe limit of 425 mg/kg. The Fe values in vegetables were in following sequence i.e cabbage < brinjal < tomato < chilli < parwal. The result was also studied by Ismail *et al*. (2014). Ni is a poisonous heavy metal and its concentration was maximum in parwal (5.05) and minimum in brinjal (1.7).These concentration slightly exceeds the standard limit given by FAO/WHO i.e 0.1 mg/kg. Ni concentration in all vegetables was found in sequence as followed brinjal <chilli< tomato< cabbage < parwal. Zn is minimally toxic and an essential element in the human diet, necessary for maintaining immune system function. A deficiency of zinc in the dietary regimen may be significantly more detrimental to human health than an excess of zinc in the diet. In the current study, concentration of Zn was found between range from 22.18 to 42.16mg/kg. Maximum concentration was found in brinjal (42.16) mg/kg while minimum concentration was seen in parwal 22.18 mg/kg. Zn values present in vegetables are brinjal<chilli <tomato<cabbage <parwal (Table 2).

Table 2: Represents accumulation of heavy metals in vegetables

	Cd	Mn	Cr	Cu	Fe	Ni	Pb	Zn
Site 1 (Cabbage)	0.125±0.001	19.621±0.025	2.8±0.089	21.68±0.096	120.12±0.156	2.9±0.056	Nd	24.8±0.265
Site 2 (Bringal)	0.188±0.012	23.112±0.056	2.5±0.024	31.9±0.123	164.22±0.256	1.7±0.096	Nd	42.16±0.236
Site 3 (Tomato)	0.149±0.003	21.322±0.034	1.8±0.056	32.15±0.213	171.21±0.325	2.88±0.024	Nd	31.86±0.562
Site 4 (Parwal)	0.293±0.023	30.252±0.035	2.6±0.028	50.3±0.325	190.22±0.165	5.05±0.264	Nd	22.18±0.564
site 5 (Chilli)	0.388±0.005	26.891±0.065	2.2±0.006	30.9±0.016	181.9±0.125	2.14±0.09	Nd	40.22±0.562
FAO/WHO	0.2	0.2	2.3	73	425	0.1	0.3	99

*Values represents Mean ± Standard Deviation

Estimation of bio-concentration factor (BCF)

From the results of the current study shown that, BCF values in different vegetables were significantly different because for a given metal the rate of transfer is varies with different plant species (Cui *et al*. 2004). BCF values for different heavy metals in targeted vegetables were shown in Table 4. BCF in case of Cd metal was maximum in chilli (4.32) and minimum in tomato (0.544). BCF of all solanceous vegetables are brinjal (1.257), chilli (4.32), tomato (0.544). The highest value of BCF in Mn was found to be 3.182 (parwal) followed by cabbage (2.633). BCF values for vegetables was 2.39 according to Bi *et al*. (2018). BCF values of Cr was found maximum in cabbage while minimum BCF values were tomato (1.836). BCF values of Cu was observed maximum in parwal (157.2) and minimum was found in tomato (76.54). In case of Fe, maximum BCF was found in tomato (17.871) followed by 7.587 in parwal. BCF value was greatest in parwal in case of Ni content and minimum was found in brinjal (1.93). BCF value for Zn was highest in cabbage and lower in tomato. BCF values of metals such as Mn, Cu, Zn, Pb and Cd can vary from 300-500, 20-100, 100-400, 30-300, 5-30 mg/kg respectively on the basis of values provided by Kabata- Pendias and pendias (1985). The variation in transfer factor of metals was related to the absorption capacity, management of nutrients and soil characteristics of different vegetables (Qazi *et al*. 2004). Therefore, by selecting a suitable crop, the risk of human exposer to metal will reduced (Table 3)

Table 3: Bioconcentration factor with respect to various heavy metals

	Cd	Mn	Cr	Cu	Fe	Ni	Pb	Zn
site 1 (Cabbage)	1.388	2.633	2.916	80.3	5.206	3.22	nd	496
site 2 (Bringal)	1.257	1.62	2.631	106.3	8.223	1.93	nd	264
Site 3 (Tomato)	0.544	1.027	1.836	76.54	17.871	3.13	nd	20.2
site 4 (Parwal)	3.662	3.182	2.68	157.2	7.587	5.67	nd	222
site 5 (Chilli)	4.32	1.767	2.391	110.4	10.544	2.43	Nd	201

Daily intake of heavy metals through vegetables and health risk index

Quantification of level of exposure and the way through which contaminants get into the target are of greater significance for watching the underlying health risks. An essential pathway of human to get into these heavy metals is food chain. In children and adults, the DIM was calculated from considering normal measure of vegetables they consumed on daily basis. The DIM values for different vegetables for both adult and children were calculated and shown in table 4. In the current study, DIM values for both child and adults are free of risks, as dietary intake provisional tolerable intake are given by FAO/WHO. Health risk index related to these heavy metals were calculated from ratio of daily intake metals to their reference doses given table. HRI measures how much risk for a human to suffered by consuming these metals in food. HRI < 1 showing probability of not going into health risk. HRI> 1 exactly not represents the health risk rather over dose creates health risk for human. In the current study the value of HRI shows, the consumption of these vegetables are fit for consumption without any risk, but over consumption may cause health risk in human.

Table 4: Estimation of heavy metal intake from consumption of vegetables

Heavy metal	Mean CONC. (mg/kg)	Daily intake in adults (mg/kg)	Daily intake child (mg/kg)	FAO /WHO Limits (mg/µg))	HRI	Rfd (mg/kg/bw/day)
Cd	0.23	0.000296	0.0001002	60µg	0.29696	0.001
Mn	24.23	0.031296	0.01056	20µg	0.94839	0.033
Cr	2.38	0.003072	0.00103		0.00204	1.5
Cu	33.38	0.043106	0.01455	3µg	1.07765	0.04
Fe	165.53	0.213729	0.072181	48µg		
Ni	2.93	0.003788	0.001279		0.18941	0.02
Pb	nd	nd		214µg		0.004
Zn	32.24	0.041631	0.014059	60µg	0.13877	0.3

Conclusion

The current study selected vegetables (Cabbage, brinjal, tomato, parwal, chilli) variate to accumulate metals in their parts. Vegetables such as cabbage have the great tendency to absorb metal due to high leaf surface area. In our study, it was shown that Solanaceous vegetables should not grown in heavy metal contaminated soil. Foods are major dietary source and play a significant part in nutrition commitment to human. Except some vegetables, others are found within safe limit given by FAO/WHO and some others crossed the limits shows human health affected by consuming these vegetables in food. DIM value and HRI in the current study were under safe limit. Thus, suitable management practices make the food enable to consume without any risk by human.

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