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ABSTRACT

In this study, the mediating role of 2, 2-Dichlorovinyl Dimethyl Phosphate (diclorvos) in the uptake of metal ions in vegetables is investigated and the health risk assessment conducted toward establishing a health-based approach in vegetable farming for human consumption. The concentrations of Cd and Pb in the vegetables analyzed using Atomic Absorption Spectroscopy (AAS) were observed to be higher in the pesticides treated vegetables compared to the untreated (control). The consumption of the vegetables treated with the pesticides were observed to increase the estimated daily intake (EDI) of the metal ions in humans compared to the EDI level in the untreated groups. The EDI levels were observed to fall in this order Spinach>Sorrel and Pb>Cd. The consumption of the vegetables treated with the pesticides were observed to expose children to a highest EDI of 1.50E-02 mg/kg/day and 2.46E-02 mg/kg/day for Cd and Pb compared to the 1.19E-02 mg/kg/day and 1.55E-02 mg/kg/day in the adults. The susceptibility of the children to non-carcinogenic risk from Cd and Pb exposure through the consumption of the pesticides treated Spinach were observed to be higher. For the metals, a target hazard quotient (THQ) of 1.76E-01 and 8.26E-01 were observed in the treated groups compared to the untreated (1.40E-01 and 5.21E-01). Based on species, the THQ established in the Spinach specie is higher than in Sorrel.

Keywords: pesticides; sorrel; spinach; heavy metals; dichlorvos; trace elements; risk assessment; estimated daily intake; target hazard quotient.

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In this study, the mediating role of 2, 2-Dichlorovinyl Dimethyl Phosphate (diclorvos) in the uptake of metal ions in vegetables is investigated and the health risk assessment conducted toward establishing a health-based approach in vegetable farming for human consumption. The concentrations of cadmium (Cd) and lead (Pb) in the vegetables analyzed using Atomic Absorption Spectroscopy (AAS) were observed to be higher in the pesticides treated vegetables compared to the untreated (control). The consumption of the vegetables treated with the pesticides were observed to increase the estimated daily intake (EDI) of the metal ions in humans compared to the EDI level in the untreated groups. The EDI levels were observed to fall in this order Spinach>Sorrel and Pb>Cd. The consumption of the vegetables treated with the pesticides were observed to expose children to a highest EDI of 1.50E-02 mg/kg/day and 2.46E-02 mg/kg/day for Cd and Pb compared to the 1.19E-02 mg/kg/day and 1.55E-02 mg/kg/day in the adults. The susceptibility of the children to non-carcinogenic risk from Cd and Pb exposure through the consumption of the pesticides treated Spinach were observed to be higher. For the metals, a target hazard quotient (THQ) of 1.76E-01 and 8.26E-01 were observed in the treated groups compared to the untreated (1.40E-01 and 5.21E-01). Based on species, the THQ established in the Spinach specie is higher than in Sorrel. All the THQs values recorded in the study are observed to be <1. The health index (HI) for the non-carcinogenic risk were also observed to follow similar trend with the THQ values. Though, the THQ and HI values were observed to be <1 and safe for human consumption, the continual application of the pesticides will increase the concentrations of Cd and Pb and hence, the non-carcinogenic (THQ and HI) risk to a level of concern. Furthermore, the application of pesticides were observed to increase the carcinogenic risk for Cd and Pb exposure through the consumption of the vegetables. The CRI for Cd were higher than the unacceptable range (>10^{-4}). The combine effect (TCRI) further show high carcinogenic risk for children compared to the adults. From the results it will suffice to say that the application of pesticides in vegetable cultivation for human consumption should be regulated or discouraged where possible.

Keywords: pesticides; sorrel; spinach; heavy metals; dichlorvos; trace elements; risk assessment; estimated daily intake; target hazard quotient.

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I. INTRODUCTION

Food in a more practical sense are source of nutrition that can modulate toxicity, inhibits diseases, reduce risk from environmental stressors, and improves life expectancy [1]. Besides been a reservoir of nutrient and an antidote for quenching hunger, in contaminated form can also increase a person's vulnerability to additional environmental stressors. In food profiling, vegetables are ranked among the widely accessible nutrient-dense foods, providing the essential nutrients, dietary fibres and other bioactive components necessary for human health and vitality [2]. Dietary daily consumption of vegetables

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have significant health effect, such as lowering the risk of cardiovascular diseases, regulating body weight and increasing the immune system to fight carcinogens [3,4]. These growing popularity of vegetables as a source of healthy, affordable and nutrient-rich food has attracted considerable array of research to improve output and make it readily available.

In Nigeria, community garden are fast becoming a household practice, virtually every available space is being cultivated with locally grown vegetables. This is presumably expected to meets the demands for fresh vegetables owed largely to the health-improving awareness as highlighted above. However, gardening on polluted soils were observed to contaminate the whole food chain. Soil as a repository of contaminants can readily accumulate heavy metals and transfer same into the plants. The buildup of contaminants in soils is multidimensional, emanating from human activities such as waste incineration, mining, oil combustion, use of leaded gasoline, paints and from galvanized metal parts [5]. Gardening on soils contaminated from such activities can increase the risk of exposure to both adults and children through either incidental soil ingestion, inhalation, dermal contacts or from the consumptions of plants grown on such a soil [6]. Several research relates the association of heavy metals in contaminated soils to the levels in edible plants [7-9]. Voutsa *et al*, [8], and De Nicola *et al*, [9] reported high accumulation of Pb, Cr and Cd in leafy vegetables. The level of preponderance were observed to be predominant in the leafy or root potion of the plants [10-13]. Other studies shows a direct relationship between population/vehicular density and other anthropogenic activities with heavy metals concentration on leafy vegetables [14-22].

Excessive applications of plant protection products to bust the cultivation of vegetables were observed to influence the chemistry of the soil and the health-status of the plants. Plant protection products were observed to modulate metal bioavailability in soil and streaming rate in plants [23], This were observed to be influenced by the complexation chemistry between the pesticides and the metal ions in the soil [23, 24].This complexation reaction helps in facilitating the mobility of the pesticides-metal complex in the soil and translocation in plants [23-25]. Pesticides residues were reported to facilitate the mopping of heavy metals in soil by chelating the metals ions and stimulates the uptake and translocation in plants [23, 26]. In our previous study, 2, 2-dichlorovinyl dimethyl (diclorvos) were observed to influence heavy metal uptake and bioavailability in the roots, stem and leaves of spinach (Spinacia oleracea) and sorrel (Rumex acetosa), compared with the same vegetable cultivated without the pesticides [23]. Related study were also reported by Chiroma et al, [18]. Metals ions like Cd, Pb, Zn and Fe readily chelates with the phospheryl oxygen groups on dichlorvos and by so doing enhances the uptake by plants [24, 27].

This study is intended as a community project and conducted to assess the level of exposure to heavy metals through the consumption vegetables grown on experimental garden. The study further examine the potential association between pesticides application in the garden and heavy metal concentrations and their potential health risk on consumption. Dietary intake of lead (Pb) and cadmium (CD) through the consumption of spinach and sorrel were used to determine the level of concern for both non-carcinogenic and carcinogenic risks.

II. MATERIALS AND METHODS

2.1 Sample collection and treatment

A representative standard plot of garden were evenly demarcated into four beds (B1, B2, B3, and B4). *Spinacia oleracea* (Spinach) were planted in plot B1 and B2 while sorrel (*Rumex acetosa*) in B3 and B4. Three weeks after planting, B2, B4 beds (treated plants) were sprayed with 2, 2-dichlorovinyl dimethyl phosphate, while B1, B3 beds were used as the control beds (untreated plants) following the same procedure described by Garba *et al* [23]. After harvesting, the powdered samples taken from each beds were digested and analysed for Cd and Pb using atomic absorption spectroscopy (VGP 210, Buck Scientific) [23].

2.2 Health Risk Characterization

The estimated daily intake of Cd and Pb based on the average consumption of the vegetable by adults and children in Nigeria were determined

using the USEPA recommended procedure described in equation 1 [28, 29].

$$EDI = C\frac{F_{IR}}{BW} \quad (1)$$

Where EDI is the average daily intake (mg/kg body weight/day); C is the concentration of the elements in the vegetable plant, and F_{IR} is the average daily consumption of vegetables (0.2 kg). The BW is the body weight (kg); set at 60 for an average adult and 15 for children.

Furthermore, target hazard quotient (THQ) as described in equation 2 was used to determine the human health risk posed by the long-time exposure to the metals following the consumption of the vegetables.

$$THQ = \frac{C \times F_{IR} \times EF \times ED}{BW \times AT \times RfD} \quad (2)$$

Where, EF is the exposure frequency (365 days/year); ED is the exposure duration (70 years); BW is the body weight in kg; and AT is the average time for non-carcinogens (365 days/year × ED). The oral reference dose (RfDs) of 0.001, and 0.00035 mg/kg/day were adopted for Cd and Pb respectively. The parameters, C, and F_{IR} are already described in Eq.1 [30-33]. The description hypothesis a THQ<1 to signify no associated risk, meaning the exposed population is unlikely to experience any adverse health hazard. However, if the THQ ≥1, then there is a potential health risk [33].

The health index (HI), expressed as the sum of THQ as described in equation 3 is used in this study to describe the cumulative effect pose by the combination of the individual metal ions presents in the vegetable plants. Thus, the greater the value of HI, the greater the level of concern. [28, 29].

$$HI = \Sigma THQs \quad (3)$$

The description in equation (4) is use to estimate the carcinogenic risk (CRI), which is the lifetime probability of an individual developing any type of cancer following the consumption of the vegetables contaminated with the metal ions.

$$CRI = EDI \times CSF_{ing} \quad (4)$$

Where, EDI is the estimated daily intake of each heavy metal (mg/kg/day), CSF_{ing} is ingestion cancer slope factor which is used to evaluates the probability of an individual developing cancer from oral exposure to the heavy metals over a period of a lifetime. The CSF for Cd and Pb are 0.38, and 0.0085 mg/kg. A CRI values of 10^{-6} (1 in 1,000,000) to 10^{-4} (1 in 10,000) represent a range of permissible predicted lifetime risks for carcinogens [30].

III. RESULTS

3.1 Concentration of Cd and Pb in the vegetables

The average mean concentrations of Cd and Pb analyzed in the Spinach and Sorrels plants are presented in Fig. 1. The results show a significant ($p<0.05$) increase in the concentrations of Cd (13.20±0.87 µg/g) and Pb (21.67±1.47 µg/g) in the spinach treated with the pesticides when compared to the untreated (control) groups (10.47+1.22 µg/g and 13.67±1.21 µg/g). Furthermore, an insignificant ($p>0.05$) increase in Cd (10.23±0.92 µg/g) and Pb (12.53±2.26 µg/g) were observed in the pesticide treated Sorrels compared to the untreated groups (10.00±0.57 µg/g and 10.17±2.18 µg/g). For both species, the metal accumulation were observed to follow the trend, Pb>Cd. The concentration of both Cd and Pb were observed to be significantly ($p<0.05$) higher in the Spinach than in the sorrel.

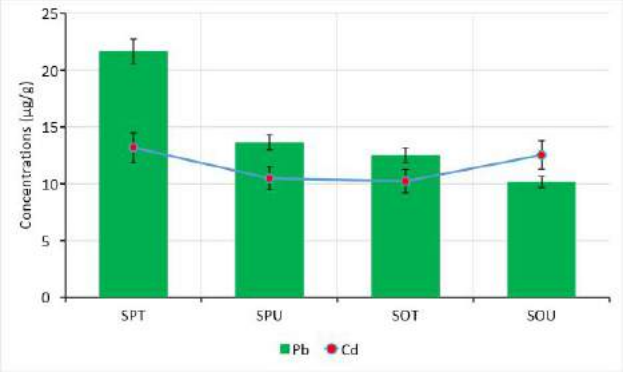

Fig. 1: Concentration of heavy metals (Cd and Pb) in spinach cultivated with (SPT) and without (SPU) pesticides treatment. The SOT and SOU stands for Sorrel cultivated with and without the pesticides treatment. Results are presented as Mean ± SD of triplicate analysis..

3.3 Estimated daily intake of metals for adults and children

As shown in table 1, the consumption of the vegetables treated with the pesticides were observed to increase the EDI for Cd and Pb in the adults and children respectively. The EDI of Cd and Pb in the Spinach treated with the pesticides are 3.74E-03 mg/kg/day and 6.14E-03 mg/kg/day compared to the control groups (2.97E-03 mg/kg/day and 3.87E-03 mg/kg/day). The EDI for Cd and Pb in the children were 1.50E-02 mg/kg/day and 2.46E-02 mg/kg/day following the consumption of Spinach treated pesticides compared to the EDI of 1.19E-02 mg/kg/day and 1.55E-02 mg/kg/day in the control groups. Similar trend were also observed for Sorrel. An EDI of 2.90E-03 mg/kg/day and 3.55E-03 mg/kg/day were observed in the treatment groups while, 2.83E-03 mg/kg/day and 2.88E-03 mg/kg/day were observed in the control groups for the adults. In children, an EDI of 1.16E-02 and 1.42E-02 were observed in the treatment groups compared to the 1.13E-02 and 1.15E-02 in groups cultivated without pesticides treatment. Overall, the EDI were observed to be higher for children than the adults and the activity were equally higher in Spinach compared to Sorrel. The EDI for both Spinach and Sorrel and for each weight category were observed to be lower than the RfD values of 1.00E-03 and 3.50E-04 for Cd and Pb respectively

Table 1: Estimated Daily Intake (EDI) in mg/kg/d for Cd and Pb in the Vegetables

	Spinach				Sorrel			
	Adults		Children		Adults		Children	
Elements	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
Cd	3.74E-03	2.97E-03	1.50E-02	1.19E-02	2.90E-03	2.83E-03	1.16E-02	1.13E-02
Pb	6.14E-03	3.87E-03	2.46E-02	1.55E-02	3.55E-03	2.88E-03	1.42E-02	1.15E-02

3.3 Target hazard quotient (THQ) and Health index (HI) in adults and children for Cd and Pb in the vegetables:

The non-carcinogenic risk of the two heavy metals in the Spinach and Sorrel are presented in table 2. The THQ value of the heavy metals in the Spinach treated with the pesticides were observed to be higher (4.40E-02 and 2.06E-1) than the untreated (3.49E-03 and 1.30E-01) for Cd and Pb in the adults. The values for the adults were observed to be much lower to the THQ values in children. Showing a THQ of 1.76E-01 and 8.26E-01 for the treated and 1.40E-01 and 5.21E-01 for the control groups for Cd and Pb respectively. Target hazard quotient of 3.41E-02 and 1.19E-01 were observed in Sorrel treated with the pesticides compared with the 3.33E-02 and 9.68E-02 in the untreated for the adults. For the same species in children, a THQ of 1.36E-01 and 4.77E-01 were observed in the treated groups, while THQ of 1.33E-01 and 3.87E-01 were observed in the untreated. In all, the non-carcinogenic risk were observed to be higher in children than in adults and higher for Pb than Cd. Based on species, THQ of Spinach is higher than in Sorrel. All the THQs values recorded in the study are observed to be <1. The health index (HI) for the non-carcinogenic risk were also observed to follow similar trend with the THQ values except in children whose HI were observed to be 1.00E+00 through the consumption of pesticides treated Spinach

Table 2: Target hazard quotient (THQ) and Health index (HI) for Cd and Pb in the Vegetables

	Spinach				Sorrel			
	Adults		Children		Adults		Children	
Elements	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
Cd	4.40E-02	3.49E-02	1.76E-01	1.40E-01	3.41E-02	3.33E-02	1.36E-01	1.33E-01
Pb	2.06E-01	1.30E-01	8.26E-01	5.21E-01	1.19E-01	9.68E-02	4.77E-01	3.87E-01
HI	2.50E-01	1.65E-01	1.00E+00	6.60E-01	1.53E-01	1.30E-01	6.14E-01	5.21E-01

3.4 Cancer risk index (CRI) in adults and hildren for Cd and Pb in the vegetables

The CRI for Cd and Pb following the consumption of the vegetables treated with and without pesticides are presented in table 3. Highest CRI of 5.68E-03 were observed in children exposed to Cd through the consumption of treated spinach when compared to the untreated (4.57E-03). A CRI of 1.42E-03 and 1.13E-03 were observed in the adults exposed to the metals following the consumption of the treated and untreated spinach respectively. From the result, CRI $\leq 10^{-4}$ were observed in both age categories exposed to Pb through the consumption of spinach treated with and without pesticides. For the treated groups, a CRI of 5.22E-05 and 2.09E-04 were observed in adults and children exposed to the metal ion compared to the CRI of 3.25E-05 and 1.32E-04 for the untreated. The cumulative cancer risk of the metal ions (TCRI) following the consumption of Spinach were also observed to be above the unacceptable limits ($> 10^{-4}$). For Cd levels in the pesticides treated Sorrel, a CRI of 1.10E-03 and 4.41E-03 were observed in the adults and children, compared to the 1.08E-03 and 4.31E-03 observed in the control groups. The CRI for Pb following the consumption of the vegetable by children were 3.02E-05 and 1.24E-04 for Sorrel treated with the pesticides while, 2.45E-05 and 9.79E-05 were observed for the untreated groups respectively. The TCRI following the consumption of the vegetables containing both Cd and Pb are 1.13E-03 and 4.53E-03 in the pesticides treated sorrel for both the adults and children. The untreated groups show a TCRI of 1.10E-03 and 4.40E-03 for the same age categories. The highest TCRI were observed in children. Overall, the results show a higher CRI and TCRI in children exposed to the heavy metals through the consumption of the vegetables than adults.

Table 3: Cancer risk index (CRI) in adults and children for Cd and Pb in the vegetables

	Spinach				Sorrel			
	Adults		Children		Adults		Children	
Elements	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
Cd	1.42E-03	1.13E-03	5.68E-03	4.51E-03	1.10E-03	1.08E-03	4.41E-03	4.31E-03
Pb	5.22E-05	3.29E-05	2.09E-04	1.32E-04	3.02E-05	2.45E-05	1.21E-04	9.79E-05
TCRI	1.47E-03	1.16E-03	5.89E-03	4.64E-03	1.13E-03	1.10E-03	4.53E-03	4.40E-03

V. DISCUSSIONS

Pesticides application were reported to mediate metal ions bioavailability in soils and plants [23, 34-40]. As observed in Fig.1, the concentrations of Cd and Pb in the vegetables were higher in the pesticides treated groups compared to the untreated. Study shows that the phospheryl oxygen groups in organophosphate pesticides has strong affinity to chelates metal ions such as Cd, Pb, Zn and Fe in soil/water medium [24, 26, 27]. The application of the dichlorvos might have initiated a change in the soil pH, increasing the solubility of

the sparingly soluble metals ions and chelating potential with the biological components of the plants [24]. Based on this chemistry increases the absorption capacity and translocation of the metal-ions by the plants [41, 42]. The concentrations of the metals ions in the vegetables were observed to be higher than the maximum permissible (PL) limits of 0.2 mg/kg and 0.3 mg/kg set by the FAO/WHO for Cd and Pb in edible plants [43]. The concentration was observed to fall in this order Cd<Pb. Lead ions compared to other heavy metals were reported to have the ability to preferential chelate with the uronic acids expressed on the root surface of plant [44, 45] mostly in the form of extracellular precipitates [46]. Besides this pathway, atmospheric deposition of the Pb-pesticides complexes on the leaves is another possible explanations for the observed higher concentration of the ions in the vegetables [47, 48]. The high concentration of the metal ions in the Spinach compared to Sorrel could be due to the difference in their cell biology response to physiological stress. Schaider *et al*, [49] observed that Pb or Cd complexes in the soil can physiologically increase stress in plant, initiating both transient and pronounced changes in the cells biology, facilitating the absorption of metal ions in dissociation with the diffusive boundary layer [50]. On the contrary, the poor absorption of the metal ions by Sorrel could be connected to the barrier limiting properties of the roots applying exclusion mechanism on sensing toxic-induce stress to inhibit absorption [51-53].

As discussed earlier, the metal ion absorption potentials were enhanced following the application of pesticides in the cultivation of the vegetables [23]. The increase in the EDI values influence by the pesticides were observed to be more pronounce in the Spinach species than in the Sorrel. Even though, the EDI for both the Spinach and Sorrel were observed to be lower than their RfD values of 1.00E-03 mg/kg/day and 3.50E-04 mg/kg/day for Cd and Pb, the results however draw attention on the need to apply caution on the indiscriminate applications of pesticides in food cultivation. The RfD often used for the assessment of non-carcinogenic health risk is a reference oral dose values set as an estimate for the tolerable daily intake of metals that will pose no health risk during a lifetime [54]. The cultivation of the vegetables using pesticides poses potential health risk to the consumers and more so to the populace that eat more of spinach. On the same note, body weight and age variations were also observed to influence the vulnerability to Cd or Pb toxicity. The EDI were observed to be higher for children than the adults and the activity were equally higher in Spinach compared to Sorrel. Which further suggest that children could be more susceptible to possible non-carcinogenic or carcinogenic risk from Cd and Pb exposure than the adults through the consumption of the vegetables especially the consumption of Spinach.

The target hazard quotient for non-carcinogenic risk were conducted to further share light into the level of concerns to Cd or Pb exposure following the consumption of these vegetables cultivated with or without pesticides. The risk characterization processes based on THQ analysis is a health-based statistical probability expressed as a function of the quantified level of concern; a process developed to estimate the potential health risk associated with long-term exposure to environmental pollutants [30, 32, 55]. The THQ values of <1 observed in the study suggest no health risk associated with the level of Cd or Pb in the vegetables, thus the population consuming these vegetable are in no immediate danger for non-carcinogenic risk. The HI for Cd and Pb in the vegetables were observed to be <1. The result therefore means that the consumption of the vegetables by the population posed no immediate health risk. However, The HI of 1.00E+00 observed for Spinach suggest possible health concerns for children eating the vegetable if the exposure to the metals persisted. Polluted vegetable increases the likelihood of heavy metal induced toxicity. An HI values >1 were observed in some vegetables in Kpanshia and Swali markets in Bayelsa state, Nigeria [13]. Similar finding are reported in leafy vegetables collected in Lagos state, Nigeria by Adedokun et al, [56]. Higher THQ for Cd, Pb, and Ni were also reported by Singh et al., [57] in vegetables from wastewater irrigated area. Higher THQ for Cd and Pb in an area near a lead (Pb) and antimony (Sb) were also reported by Cui et al. [58] and Zhou et al. [59] in vegetable species planted in contaminated soils.

Though the risk characterization conducted in this study might have suggest no immediate danger for non-carcinogenic risk, the continual application of pesticides in the cultivation of vegetables will increase the likelihood of heavy metal buildup in the plants and consequently transmit potential non-carcinogenic risk to a level of concern.

The lifetime cancer risk (CRI) for the adults and children as presented in table 3 were analyzed for the ingestion exposure pathways only. For regulatory purposes, a cancer risk in the range of 10^{-6} to 10^{-4} are considered acceptable [55, 59]. From the results presented in the table, the CRI were found to be in this order Cd>Pb and Children>Adults. The CRI for Cd were higher than the unacceptable range ($>10^{-4}$). The level were found to be higher in the pesticides treated groups compared to the untreated suggesting that the consumption of vegetables cultivated with pesticides will significantly increase the likelihood of heavy metal exposure. From the results it will suffice to say that children are more susceptible to carcinogenic risk than the adults. The combine effect (TCRI) further show high carcinogenic risk for children. The order for exposure was observed to be in the decreasing order, Sorrel<Spinach. According to the results, the carcinogenic risk is higher in the pesticides treated vegetables and largely due to the presence of Cd, which was observed to exceed the limit for acceptable risk of developing cancer indicating some concern about the application of pesticides in vegetable garden.

IV. CONCLUSION

Cadmium and Pb ions concentrations were observed to be influence by the pesticides, with significantly ($p < 0.05$) higher activities recorded in leads levels in both vegetables. The 2, 2-dichlorovinyl dimethyl phosphate was observed to facilitate significant ($p < 0.05$) metal uptake in the spinach compared to the sorrel plants. The EDI of the metal ions were observed to be higher in children and significantly higher through the consumption of pesticides treated Spinach compared to the adults and Sorrel species. Though, the THQ and HI values were observed to be <1 and safe for human consumption, the consumptions of the vegetables treated with the pesticides were observed to increase THQ and HI values closer to a point where continual application of the pesticides will increase the likelihood for non-carcinogenic risk to a level of concern. The application of pesticides were observed to increase the carcinogenic risk from Cd and Pb exposure through the consumption of the vegetables. The CRI for Cd were higher than the unacceptable range ($>10^{-4}$). The combine effect (TCRI) further show high carcinogenic risk for children compared to the adults.

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