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Review:

Assessing potential dietary toxicity of heavy metals in selected vegetables and food crops*

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Abstract: Heavy metals, such as cadmium, copper, lead, chromium and mercury, are important environmental pollutants, particularly in areas with high anthropogenic pressure. Their presence in the atmosphere, soil and water, even in traces can cause serious problems to all organisms, and heavy metal bioaccumulation in the food chain especially can be highly dangerous to human health. Heavy metals enter the human body mainly through two routes namely: inhalation and ingestion, ingestion being the main route of exposure to these elements in human population. Heavy metals intake by human populations through food chain has been reported in many countries. Soil threshold for heavy metal toxicity is an important factor affecting soil environmental capacity of heavy metal and determines heavy metal cumulative loading limits. For soil-plant system, heavy metal toxicity threshold is the highest permissible content in the soil (total or bioavailable concentration) that does not pose any phytotoxic effects or heavy metals in the edible parts of the crops does not exceed food hygiene standards. Factors affecting the thresholds of dietary toxicity of heavy metal in soil-crop system include: soil type which includes soil pH, organic matter content, clay mineral and other soil chemical and biochemical properties; and crop species or cultivars regulated by genetic basis for heavy metal transport and accumulation in plants. In addition, the interactions of soil-plant root-microbes play important roles in regulating heavy metal movement from soil to the edible parts of crops. Agronomic practices such as fertilizer and water managements as well as crop rotation system can affect bioavailability and crop accumulation of heavy metals, thus influencing the thresholds for assessing dietary toxicity of heavy metals in the food chain. This paper reviews the phytotoxic effects and bioaccumulation of heavy metals in vegetables and food crops and assesses soil heavy metal thresholds for potential dietary toxicity.

Key words: Heavy metals, Dietary toxicity, Vegetables, Food crops
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INTRODUCTION

Regulation, handling and bioremediation of hazardous materials require an assessment of the risk to some living species other than human being, or assessment of hazard to the entire ecosystem. Assessment endpoints are values of the ecosystem that are to be protected and are identified early in the analysis. Such endpoints may include life cycle stages of a species and reproductive or growth patterns. Ecosystem risk assessment is at its dawn with this area of environment sciences still requiring extensive work in the industrialized nations of the world for sustainability of the global ecosystem.

Heavy metals, such as cadmium, copper, lead, chromium and mercury, are important environmental pollutants, particularly in areas with high anthropogenic pressure. Their presence in the atmosphere, soil

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and water, even in traces, can cause serious problems to all organisms. Heavy metal accumulation in soils is of concern in agricultural production due to the adverse effects on food quality (safety and marketability), crop growth (due to phytotoxicity) (Ma *et al*., 1994; Msaky and Calvert, 1990; Fergusson, 1990) and environmental health (soil flora/fauna and terrestrial animals). The mobilization of heavy metals into the biosphere by human activity has become an important process in the geochemical cycling of these metals. This is acutely evident in urban areas where various stationary and mobile sources release large quantities of heavy metals into the atmosphere and soil, exceeding the natural emission rates (Nriagu, 1989; Bilos *et al*., 2001). Heavy metal bioaccumulation in the food chain can be especially highly dangerous to human health. These metals enter the human body mainly through two routes namely: inhalation and ingestion, and with ingestion being the main route of exposure to these elements in human population. Heavy metals intake by human populations through the food chain has been reported in many countries with this problem receiving increasing attention from the public as well as governmental agencies, particularly in developing countries.

Vegetables constitute essential diet components by contributing protein, vitamins, iron, calcium and other nutrients, which are usually in short supply (Thompson and Kelly, 1990). They also act as buffering agents for acidic substances produced during the digestion process. However, they contain both essential and toxic elements over a wide range of concentrations. Metal accumulation in vegetables may pose a direct threat to human health (Türkdogan *et al*., 2003; Damek-Poprawa and Sawicka-Kapusta, 2003). Chinese cabbage (*Brassica chinensis* L. cv. Zao-Shu 5), winter greens (*B. rosularis* var. Tsen et Lee cv. Shang-Hai-Qing), pakchoi (*Brassica chinensis* L.) and celery (*Apiumg graveolens* L. var. dulce DC) are some crops, which were assessed for heavy metal toxicity. Vegetables take up metals by absorbing them from contaminated soils, as well as from deposits on different parts of the vegetables exposed to the air from polluted environments (Zurera-Cosano *et al*., 1989). It has been reported that nearly half of the mean ingestion of lead, cadmium and mercury through food is due to plant origin (fruit, vegetables and cereals). Moreover, some population groups seem to be more exposed, especially vegetarians, since they absorb more frequently 'tolerable daily doses'.

Food contamination by heavy metals depends both on their mobility in the soil and their bioavailability. Though some of the mobility and bioavailability factors are easy to measure, determination of the food risk contamination is tricky. The aim of the present paper is to review concisely the phytotoxic effects and bioaccumulation of heavy metals in vegetables and food crops and assessment of soil heavy metal thresholds for potential dietary toxicity.

HAZARDOUS EFFECTS OF HEAVY METALS ON HUMAN HEALTH

Chronic low-level intakes of heavy metals have damaging effects on human beings and other animals, since there is no good mechanism for their elimination. Metals such as lead, mercury, cadmium and copper are cumulative poisons. These metals cause environmental hazards and are reported to be exceptionally toxic (Ellen *et al*., 1990). Vegetables take up metals by absorbing them from contaminated soils, as well as from deposits on parts of the vegetables exposed to the air from polluted environments (Zurera-Cosano *et al*., 1989).

Metal contamination of garden soils may be widespread in urban areas due to past industrial activity and the use of fossil fuels (Chronopoulos *et al*., 1997; Sánchez-Camazano *et al*., 1994; Sterrett *et al*., 1996; van Lune, 1987; Wong, 1996). Heavy metals may enter the human body through inhalation of dust, direct ingestion of soil, and consumption of food plants grown in metal-contaminated soil (Cambra *et al*., 1999; Dudka and Miller, 1999; Hawley, 1985). Potentially toxic metals are also present in commercially produced foodstuffs (DEFRA, 1999). Exposure to potentially toxic metals from dust inhalation or soil ingestion is usually modelled simply as the concentration of a contaminant measured in the soil multiplied by the quantity of dust inhaled or soil ingested (Konz *et al*., 1989). This is a conservative approach to estimate dose, because the bioaccessibility of heavy metals adsorbed on ingested soil is not 100% (Ruby *et al*., 1999). However, predicting exposure to potentially toxic metals from consumption of food crops is more complicated because uptake of metals by plants depends on soil properties and plant physiologic factors. This leads to much larger uncertainties associated with estimating potential doses through food chains compared to the uncertainties associated with other exposure pathways such as soil ingestion and dust inhalation (McKone, 1994).

Lead is a toxic element that can be harmful to plants, although plants usually show ability to accumulate large amounts of lead without visible changes in their appearance or yield. In many plants, Pb accumulation can exceed several hundred times the threshold of maximum level permissible for human (Wierzbicka, 1995). The introduction of Pb into the food chain may affect human health, and thus, studies concerning Pb accumulation in vegetables have increasing importance (Coutate, 1992). Although a maximum Pb limit for human health has been established for edible parts of crops (0.2 mg/kg) (Chinese Department of Preventive Medicine, 1994), soil Pb thresholds for producing safe vegetables are not available.

Knowledge of Zn toxicity in humans is minimal. The most important information reported is its interference with Cu metabolism (Barone *et al*., 1998; Gyorffy and Chan, 1992). The symptoms that an acute oral Zn dose may provoke include: tachycardia, vascular shock, dyspeptic nausea, vomiting, diarrhea, pancreatictis and damage of hepatic parenchyma (Salgueiro *et al*., 2000). Although maximum Zn tolerance for human health has been established for edible parts of crops (20 mg/kg) (Chinese Department of Preventive Medicine, 1995), soil Zn threshold for producing safe vegetables is not available.

According to Hough *et al*.(2004) under Part IIA of the Environmental Protection Act 1990, the UK government favors a “suitable for use” approach to redevelopment (DETR, 2000): Land is contaminated only if the current or intended use of a site has the potential to cause an unacceptable health risk to human occupants or to the environment. Under the UK Town and Country Planning Act 1990 (DETR, 2000), this approach requires that land be assessed for redevelopment on a site-specific basis. At present, concentrations of metals in the soil are compared to metal-specific “trigger values” (termed “maximum contaminant levels” or “maximum contaminant concentrations” in North America). In the past these trigger values were based on total contaminant concentration in the soil (ICRCL, 1987). More recently, the introduction of Contaminated Land Exposure Assessment (CLEA) (DEFRA and Environment Agency, 2002a) in April 2002 has replaced these trigger values with generic soil guidance values (SGVs) (DEFRA and Environment Agency, 2002b). The SGVs are considered a significant improvement on the previous ICRCL values and for Cd at least, soil pH categories are employed where food plants are to be grown. Where a soil exceeds the SGV, it is recommended that a risk assessment or remediation measure be conducted for the site in question (DEFRA and Environment Agency, 2002b). Additionally, exceeding of an SGV indicates that some further risk management action should be undertaken. However, the use of single trigger values or SGVs for most scenarios may represent a poor indication of the risk associated with a specific site. There is therefore a requirement for site-specific risk assessment based on commonly measured geochemical and population parameters (Hough *et al*., 2004).

GROWTH RESPONSES OF VEGETABLES TO HEAVY METALS

Crop plants growing on heavy metal contaminated medium can accumulate high concentrations of trace elements to cause serious health risk to consumers. Long *et al*.(2003) studied the effects of excess zinc on plant growth of three selected vegetables i.e. Chinese cabbage, celery and pakchoi. They found that excess Zn in growth media caused toxicity to all three vegetable crops. Toxicity symptoms included chlorosis in young leaves, browning of coralloid roots, and serious inhibition on plant growth. Shoot fresh weight (FW) progressively decreased with increasing Zn concentrations. Large differences in Zn tolerance were also noted among the three vegetable crops (Table 1). Celery was more sensitive to higher Zn levels and reduced shoot growth than Chinese cabbage and pakchoi. Shoot FW decreased to approximately 63%, 73% and 36% of the control for Chinese cabbage, pakchoi and celery, respectively, when plants were grown at Zn level of 50 mg/L.

Although no visible Zn toxicity symptoms were observed in the soil experiment, shoot growth was significantly inhibited at Zn levels above 200 mg/kg for celery and Chinese cabbage, and above 300 mg/kg for pakchoi (Table 1). Shoot DW (dry weight) decreased by 10% for Chinese cabbage and celery, but increased by 13% for pakchoi when grown at the soil diethylenetriamene pentaacetic acid (DTPA) extractable Zn level of 72 mg/kg. However, at soil DTPA-Zn levels up to 172 mg/kg, similar yield reduction was observed with celery and pakchoi (Long *et al*., 2003). Root DW was reduced more than shoot DW for pakchoi grown at high Zn levels. Similar sensitivity of both root and shoot growth to Zn toxicity was noted for celery. The results showed that pakchoi required higher soil Zn concentrations for optimal growth and was more tolerant to Zn at soil available Zn concentrations less than 132 mg/kg than the other two vegetable species (Long *et al*., 2003).

Xiong and Wang (2005) showed that seed germination was significantly adversely influenced by Cu (P<0.001) in *Brassica pekinensis*. The 0.5 mmol/L Cu treatment remarkably reduced the germination rate, and the LC_{50} (median lethal concentration), calculated as the lethal effect on seed germination, was 0.348 mmol/L. Root and shoot lengths of the young seedlings were also inhibited by Cu, but stimulatory elongation of the shoots occurred with the 0.008 mmol/L treatment.

Further investigations (Zhang and Zhou, 2005) showed that the Al-based coagulants at the tested concentrations had a poisonous effect on the germination of vegetable seeds. There were positive curvilinear or linear relationships between the inhibitory rate of seed germination and the concentration of Al in the acidic and neutral conditions except for the toxic effects of PAC (polyaluminum-chloride) on *Brassica chinensis* in the neutral condition (Zhang and Zhou, 2005). Moreover there were obvious differences in root elongation of *Brassica chinensis* exposed to $AlCl_3$ in various pH conditions.

High copper levels in growth media caused toxicity to all the three vegetable crops, resulted in chlorosis in new leaves, brown, stunted, coralloid roots, and plant growth was inhibited (Yang *et al*., 2002). Shoot fresh weight (FW) progressively decreased with increasing Cu levels in the nutrient solution. Great differences in Cu tolerance were also noted among the three vegetable crops. Shoot fresh weight of pakchoi, celery and Chinese cabbage decreased to about 33%, 37% and 50% of the control, respectively, when grown with Cu supply of 10 mg/L. These results indicate that celery is more tolerant to the toxicity of Cu than Chinese cabbage or pakchoi grown in nutrient solution (Yang *et al*., 2002).

Table 1 Effects of soil Zn levels on growth and yield of different vegetable crops grown under soil culture conditions*

Species	Added Zn (mg/kg)	DTPA-Zn (mg/kg)	Plant height (cm)	*FW* (g/pot)		*DW* (g/pot)	
				Shoot	Root	Shoot	Root
Chinese cabbage	CK	18.1	15.4 a	78.35 a A	2.83 a	5.16 a A	0.42 a
	100	71.7	14.8 ab	70.72 b B	2.78 a	4.55 b B	0.41 a
	200	132.2	14.3 ab	66.22 bc BC	2.77 a	4.32 b B	0.40 a
	300	172.3	13.9 a	61.29 c CD	2.76 a	3.82 c C	0.41 a
	400	233.2	14.7 ab	54.84 d D	2.74 a	3.69 c C	0.40 a
Pakchoi	CK	18.1	18.1 a A	93.22 a A	3.66 a A	5.53 ab C	0.65 a A
	100	71.7	19.1 a A	104.96 a A	3.06 b B	6.22 a A	0.57 a AB
	200	132.2	18.5 a A	88.57 a AB	2.49 c C	5.51 ab A	0.46 b BC
	300	172.3	15.3 b B	61.77 b BC	2.11 d C	4.44 bc AB	0.38 b CD
	400	233.2	13.1 c B	40.11 c C	1.47 e D	3.35 c B	0.26 c D

				FW (g/pot)			*DW* (g/pot)		
				Stem	Leaf	Root	Stem	Leaf	Root
Celery	CK	18.1	24.7 a	39.46 a A	32.61 a A	23.86 a A	3.68 a A	5.01 A	4.16 a A
	100	71.7	25.8 a	36.41 a A	28.85 b B	17.59 b B	3.04 b B	4.22 B	3.08 b B
	200	132.2	25.0 a	29.82 b B	22.56 c C	16.31 b BC	2.58 c C	3.33 C	2.88 b BC
	300	172.0	25.4 a	28.72 bc B	20.37 d D	14.83 c CD	2.47 cd C	3.01 D	2.60 c CD
	400	233.2	24.9 a	26.25 c B	19.26 e D	13.34 d C	2.24 dc	2.73 E	2.32 d D

*All data are means of 4 replications; Values followed by the same letter within the same column for each vegetable species are not significantly different by Duncan's multiple range test (lowercase: P>0.05; uppercase: P>0.01) within the same column for each vegetable species; FW: Fresh weight; DW: Dry weight

Other studies conducted on vegetables i.e. celery, Chinese cabbage and winter greens by Ni *et al.*(2002) demonstrated that no nutrient deficiency or toxicity symptoms caused by cadmium were visible on any plant. Biomass productions of root and shoot for Chinese cabbage and winter greens, as well as root, petiole and leaf blade for celery, expressed as FW were not significantly different among the treatments (Table 2).

HEAVY METAL UPTAKE AND ACCUMULATION IN DIFFERENT PLANT PARTS OF VEGETABLES

Plant species and varieties vary in their capacity for heavy metal accumulation. Long *et al.*(2003) showed that zinc uptake and accumulation by shoots and roots varied with Zn levels in growth media and vegetable types (Table 3). Both shoot and root Zn concentrations increased sharply with increasing Zn concentrations for Chinese cabbage, celery and pakchoi. However, shoots contained over 3-fold less Zn than roots when grown under nutrient solution culture conditions. The three vegetable crops differed greatly in their ability to take up Zn from the growth media and to transport it to the shoots. At an external Zn level of 25 mg/L, shoot Zn concentration of Chinese cabbage was almost 2-fold lower than that of pakchoi or celery (Table 3). Zinc concentration in the edible part of celery was nearly 2-fold higher than that of the other two species when grown at higher Zn levels (50 mg/L). Moreover, under soil culture conditions, the zinc accumulation coefficient (AF) in shoots increased for pakchoi, but decreased for celery and Chinese cabbage when soil available Zn was raised from 18 to 172 mg/L (Table 4). However, root Zn AF increased to varied extents, with increasing soil Zn for all the vegetables. Celery showed the highest AF in edible parts at low soil Zn i.e. CK (control), whereas pakchoi had the highest AF of Zn at higher soil available Zn levels. The AF for zinc in edible parts of the three vegetable crops decreased in the order: pakchoi, celery (stem) and Chinese cabbage. Significant positive correlations were noted between shoot Zn and soil available Zn level (Long *et al.*, 2003). Zn threshold for human health has been established to be 20 mg/kg (Chinese Department of Preventive Medicine, 1995).

Ni *et al.*(2002) studied the effect of Cd on the

Table 2 Biomass production of vegetable crops in pot experiment

Extractable Cd con. of soil (mg/kg)	Biomass production expressed as FW (g/pot)						
	Chinese cabbage		Winter greens		Celery		
	Root	Shoot	Root	Shoot	Root	Petiole	Blade
0.15	3.0 a	62.7 a	3.0 a	64.7 a	23.6 a	32.6 a	32.4 a
0.89	3.1 a	69.1 a	2.7 a	60.0 a	23.7 a	32.9 a	33.6 a
1.38	3.0 a	61.3 a	2.5 a	63.3 a	22.5 a	33.5 a	32.0 a
1.84	3.1 a	64.7 a	2.8 a	65.1 a	22.7 a	33.2 a	32.2 a
2.30	3.2 a	69.8 a	2.7 a	62.8 a	22.9 a	32.6 a	32.2 a
F-anova	NS	NS	NS	NS	NS	NS	NS

NS: Non-significant; Values followed by the same letter in the same column are not significantly different by Duncan's multiple range test (P>0.05)

Table 3 Zn concentration in different parts of different vegetable crops grown in nutrient solution*

Zn supply levels (mg/L)	Zn concentrations (mg/kg DW)						
	Chinese cabbage		Pakchoi		Celery		
	Root	Shoot	Root	Shoot	Root	Stem	Leaf
0.03	135.5 e	65.9 e	127.5 e	61.7 d	115.0 e	72.5 e	125.8 e
25	1167 d	494 d	1587 d	925 c	2308 d	870 d	1295 d
50	2313 c	1024 c	2623 c	977 c	4350 c	2820 c	2070 c
100	3595 b	1835 b	4642 b	1883 b	7567 b	4075 b	2342 b
200	12807 a	2975 a	7483 a	2375 a	12823 a	4514 a	2978 a

*All data are means of 3 replications; Values followed by the same letter in the same column are not significantly different by Duncan's multiple range test (P>0.05) growth of three vegetable crops i.e. Chinese cabbage (*Brassica chinensis* L. cv. Zao-Shu 5), winter greens (*B. rosularis* var. Tsen et Lee cv. Shang-Hai-Qing), and celery (*Apiumg graveolens* L. var. dulce DC). Their results indicated that the cadmium concentrations in shoots and roots varied both with different Cd levels and type of vegetable. Generally Cd accumulation in various plant parts in vegetable crops increased with the increasing cadmium concentrations in the growth medium. Root Cd increased more sharply than shoot Cd. Celery contained higher Cd in the edible parts than other vegetable species (Table 5).

Yang *et al.*(2002) studied the response of three vegetables to Cu toxicity and found that Cu levels in both root and shoot increased, but root Cu concentration increased more sharply than shoot with increasing Cu levels in growth media. Cu mainly accumulated in roots while a small fraction (10%~20%) of absorbed Cu was transported to shoot (Table 6). Celery accumulated higher Cu content both in roots (1557 mg/L) and shoot (166.7 mg/L in leaves).

Copper AFs in the shoots of vegetable species were relatively small when grown at soil addition Cu levels of 200~400 mg/kg and dramatically increased at soil addition Cu levels above 600 mg/kg (Table 7).

While investigating copper toxicity and bioaccumulation in Chinese cabbage (*Brassica pekinensis*

Table 4 Zn accumulation coefficients (AFs) of different vegetable crops grown at various soil Zn levels[*]

Zn added to soil (mg/kg)	DTPA-Zn (mg/kg)	Zn AFs						
		Chinese cabbage		Pakchoi		Celery		
		Root	Shoot	Root	Shoot	Root	Stem	Leaf
CK	18.1	0.158 b	0.039 b	0.169 e	0.056 e	0.290 d	0.113 c	0.195 a
200	71.7	0.133 c	0.030 c	0.284 d	0.078 d	0.507 c	0.068 a	0.138 b
400	132.2	0.135 c	0.036 b	0.292 c	0.098 c	0.419 b	0.069 a	0.114 c
600	172.3	0.143 d	0.042 d	0.415 b	0.119 b	0.577 a	0.066 b	0.135 b
800	233.2	0.235 a	0.058 a	0.709 a	0.189 a	0.572 a	0.068 a	0.130 d

[*] *AF*=Zn in plant tissues (mg/kg)/total Zn in soil (mg/kg); All data are means of 4 replications; Values followed by the same letter in the same column are not significantly different by Duncan's multiple range test (P>0.05)

Table 5 Cd concentration in different parts of vegetables in pot experiment

Extractable Cd of soil (mg/kg)	Plant Cd contents (mg/kg FW)						
	Chinese cabbage		Winter greens		Celery		
	Root	Shoot	Root	Shoot	Root	Stem	Leaf
0.15	0.017 e	0.010 e	0.023 e	0.022 e	0.096 e	0.020 e	0.020 e
0.89	0.158 d	0.049 d	0.095 d	0.038 d	0.265 d	0.085 d	0.040 d
1.38	0.332 c	0.076 c	0.151 c	0.103 c	0.385 c	0.113 c	0.072 c
1.84	0.455 b	0.086 b	0.235 b	0.119 b	0.439 b	0.204 b	0.082 b
2.30	1.095 a	0.151 a	0.428 a	0.166 a	0.653 a	0.241 a	0.253 a
F-anova	**	**	**	**	**	**	**

[**] represents significant differences between values at P=0.01 by ANOVA test; Values followed by the same letter in the same column are not significantly different by Duncan's multiple range test (P=0.01)

Table 6 Cu concentration in different parts of different vegetables grown in nutrient solution

Cu levels (mg/L)	Cu concentration (mg/kg DW)						
	Chinese cabbage		Pakchoi		Celery		
	Root	Shoot	Root	Shoot	Root	Stem	Leaf
0.01	13.00	13.34	18.67	9.00	27.00	21.50	31.17
10	238.30	45.00	235.80	17.67	566.70	53.00	65.83
20	361.30	58.75	475.00	32.33	1312.00	81.50	101.00
30	565.60	71.13	528.30	128.30	1423.00	95.00	123.30
40	711.30	97.50	705.60	179.20	1557.00	145.00	166.70
$LSD_{0.05}$	5.36	3.58	8.34	6.67	11.23	9.47	10.32

Table 7 Cu accumulation coefficients (AFs) of different vegetable crops grown at various soil Cu levels

Cu added to soil (mg/kg)	Cu AFs						
	Chinese cabbage		Pakchoi		Celery		
	Root	Shoot	Root	Shoot	Root	Stem	Leaf
CK	0.223	0.060	0.438	0.074	0.117	0.005	0.090
200	0.081	0.016	0.091	0.013	0.062	0.008	0.071
400	0.081	0.014	0.071	0.010	0.134	0.009	0.084
600	0.091	0.019	0.072	0.017	0.318	0.015	0.107
800	0.167	0.030	0.157	0.036	0.324	0.017	0.150
$LSD_{0.05}$	0.007	0.003	0.006	0.002	0.017	0.003	0.009

Rupr.), Xiong and Wang (2005) found that Cu concentration in the shoots was significantly influenced by Cu treatment ($P<0.001$). Cu concentration increased markedly with an increase in the soil Cu concentration. With a background level of Cu (the control, 13.6 mg/kg dry soil), Cu concentration in the shoots was 9.9 mg/kg. With the 0.2 mmol/kg treatment, shoot Cu concentration rose to 42.5 mg/kg. With the 1.0 mmol/kg treatment, shoot Cu concentration was 119.0 mg/kg (1.9 mmol/kg). According to the *LSD* test, shoot Cu concentration in both treatments was significantly higher than that in the control. These facts showed that when Chinese cabbage (cultivar Xiayangbai) plants were exposed to certain levels of Cu pollution, the shoots could accumulate a relatively high amount of Cu.

SOIL THRESHOLD VALUES OF POTENTIAL DIETARY TOXICITY

Soil threshold for heavy metal toxicity is an important factor affecting soil environment capacity of heavy metal and determining heavy metal cumulative loading limit. For the soil-plant system, the heavy metal toxicity threshold is the highest permissible content in the soil (total or bioavailable concentration) that does not produce any phytotoxicity (i.e., inhibition of plant growth and decrease of yield), or the heavy metal in the edible parts of crops does not exceed the critical dietary heavy metal threshold for human health. During the recent years much effort was exerted to calculate the soil thresholds of potential dietary toxicity by different ways.

The critical food Cu threshold for human health has been established to be 10 mg/kg (Chinese Department of Preventive Medicine, 1995). Yang *et al.* (2002) reported that from the regression lines between shoot DM yields and Cu concentration in plant tissue or soil, soil Cu thresholds for phytotoxicity (10% yield reduction) and potential dietary toxicity in edible parts of the vegetables could be calculated. Soil total and available Cu thresholds for potential dietary toxicity in the edible parts of vegetable crops were 5-fold higher than those for phytotoxicity (at 10% yield reduction) (Table 8). Among the three vegetable crops, pakchoi had much lower soil total and available Cu thresholds, as compared with the other two vegetable species.

The critical dietary Zn threshold for human health has been established to be 20 mg/kg (Chinese Department of Preventive Medicine, 1995). Long *et al.*(2003) showed that from the regression equations between shoot yield or Zn concentration and soil total or available Zn, soil Zn thresholds for yield reduction (decreased 10%) and potential dietary toxicity in edible parts of the vegetables could be calculated (Table 9). The total soil Zn thresholds for shoot dry matter yield reduction were higher for pakchoi and only slightly lower for celery (stem), than that for potential dietary toxicity. Soil available Zn thresholds for Zn potential dietary toxicity were 175.6, 74.9 and 101.0 mg/kg for Chinese cabbage, pakchoi, and celery (stem), respectively. For pakchoi, a higher soil available Zn threshold for yield reduction (10%) (103 mg/kg) was again noted relating to that for potential dietary toxicity (74.9 mg/kg). The lower soil available Zn threshold for Zn potential dietary toxicity for pakchoi than for the other vegetable species is mainly associated with its greater ability to absorb Zn from the soil and to translocate and accumulate Zn in the shoots. These results indicate that some vegetable species, like pakchoi, may accumulate Zn in the edible part over dietary toxic threshold before yield reduction occurs.

Based on the regression equation established in our study and the limit of cadmium concentration in vegetable products (0.05 mg/kg fresh weight, GBN 238-84 in China), the threshold of Cd concentration in growth media was evaluated as 0.5 mg/kg of soil extractable Cd for soil. The results indicated that the criteria for extractable Cd content in soil were 0.869, 0.730 and 0.489 mg/kg soil for Chinese cabbage, winter greens and celery, respectively (Table 10) (Ni *et al.*, 2002).

BIOAVAILABILITY OF ADDED HEAVY METAL IN THE VEGETABLE GARDEN SOIL

Total soil metals can be used to estimate the degree of soil exposure to heavy metal pollution, although this is not generally well correlated with metal mobility and bioavailability (Kuo *et al.*, 1983). Metals such as Zn exist in soils in various fractions, chemical species or forms including: exchangeable, carbonate-bound, oxide-bound, organic matter-bound and crystal lattice metals (Shuman, 1991). Availability of soil Zn to plants differs and may be governed by dynamic equilibrium among these fractions (Kiekens, 1990). The biologically active fractions of Zn in soils mainly consist of its soluble, exchangeable and complexed forms. Many studies showed that DTPA-extractable Zn is correlated well with plant uptake Zn (Arnesen and Singh, 1998; Miner *et al.*, 1997). DTPA extractable Zn decreased progressively with incubation time, and leveled off in 8~12 weeks of incubation. However, after 12-week incubation, 60%~70% of added Zn was still extractable by the DTPA method (Long *et al.*, 2003). The results showed that a major portion of the Zn added to the garden soil is phytoavailable, which is in agreement with other studies (Cajuste *et al.*, 2000; Darmawa and Wada, 1999).

Total copper in soil includes six pools classified according to their physicochemical behavior. The pools are soluble ions and inorganic and organic complexes in soil solution; exchangeable Cu; stable organic complexes in humus; Cu adsorbed by hydrous oxides of Mn, Fe and Al; Cu adsorbed on the clay-humus colloidal complex; and crystal lattice-

Table 8 Soil Cu thresholds for yield reduction and potential dietary toxicity in edible parts of the vegetables

Crop species	Total Cu (mg/kg)		Available Cu (mg/kg)	
	PDT≤10 mg/kg	SDMYR by 10%	*PDT*≤10 mg/kg	SDMYR by 10%
Chinese cabbage	835.56	142.84	338.98	50.58
Pakchoi	429.93	167.52	269.14	57.64
Celery (stem)	608.22	174.18	312.72	70.68
Celery (Leaf)	161.07	232.03	57.22	92.09

Note: PDT: Potential dietary toxicity; SDMYR: Shoot dry matter yield reduction

Table 9 Soil Zn thresholds for yield reduction and potential dietary toxicity in edible parts of the vegetables

Crop species	Total Zn (mg/kg)		Available Zn (mg/kg)	
	PDT≤20 mg/kg	SDMYR by 10%	*PDT*≤20 mg/kg	SDMYR by 10%
Chinese cabbage	413	244	175.6	85.6
Pakchoi	224	277	74.9	103.0
Celery (stem)	272	220	101.0	73.0
Celery (Leaf)	122	187	19.4	55.8

Note: PDT: Potential dietary toxicity; SDMYR: Shoot dry matter yield reduction

Table 10 Correlative relationship between Cd concentration in edible parts of vegetable crops (C_p) and extractable Cd content in soil (C_s) and the evaluated criteria of Cd pollution in growth media

Species	Correlative equation	Correlative coefficient	Significance	Criteria of Cd concentrations (mg/kg soil)
Chinese cabbage	C_p=0.0575C_s	0.965 (*n*=5)	**	0.869
Winter greens	C_p=0.0685C_s	0.969 (*n*=5)	**	0.730
Celery	C_p=0.1022C_s	0.983 (*n*=5)	**	0.489

** represents that the correlative relationship is significant at the level of 1%

bound Cu (Baker, 1990). When added to soil, Cu may react with soil constituents, changes its chemical form, then its availability to plants is also altered. The amount of soil Cu removed by a chemical chelating agent like DTPA or EDTA is considered as the plant available portion (Baker, 1990). The DTPA extractable Cu decreased with incubation time, especially in the first 8 weeks. After 12-week incubation, 60% of added Cu was not extractable by DTPA. These may have resulted from transformation of the added soluble Cu fraction to slowly available fractions of Cu in the soil (Yang *et al*., 2002).

Although the amount of soil heavy metal removed by a chelating agent like DTPA or EDTA is considered to be the plant-available portion, many results have shown that the concentration of Pb is not well correlated to the amount of plant uptake (Yang and Zhang, 1993). Some results showed that significant and positive correlations existed between shoot Pb and soil NH_4NO_3-extractable Pb levels (Song, 2002).

SOIL-PLANT-MAN RELATIONS DETERMINING HEAVY METAL TOXICITY

Soil-to-plant transfer is one of the key components of human exposure to metals through the food chain. Lăcătuşu *et al*.(1996) studied soil-plant-man relationships in heavy metal polluted areas in Romania and detected significant overclark levels of Cd and Pb from the geogenic abundance viewpoint. Although the polluted soils were neutral to slightly alkaline and well supplied with organic matter, the soluble forms of heavy metals in EDTA-CH_3COONH_4, pH=7.0 represented on average 37% Cd, 17% Cu, 28% Pb and 14% Zn, respectively of their global concentration, exceeding the maximum allowable limits (MAL), for soluble forms, by on average up to 14.8 (Pb), 4.2 (Cd) and 2.1 (Zn) times. The relationship between their contents in plants and in soil (soluble forms) showed significant correlations for Cd, Cu, Pb and Zn. As a result, the contents of these elements in vegetables often exceed those allowable for normal human and animal consumption. In this case, if an adult consumed 2 kg potatoes, 2 kg tomatoes and 1 kg carrots in a week, his/her food would exceed by 12% the MAL for Cd (0.525 mg). The daily maximum allowable rate of ingested Pb (0.430 mg) could be reached by consuming 880 g of vegetables (equal parts of potatoes, tomatoes, carrots and cucumbers). The higher acidity of soils enhances the transfer of large amounts of heavy metals in soluble forms, exceeding MAL on average up to 23.4 (Pb), 2.1 (Cd), 2.8 (Cu) and 2.7 (Zn) times. As a result the average Pb content in carrots was 10 times higher than the MAL and the Pb accumulation in the lettuce, parsley and garden orach, significantly above the critical contents. At the same time, the Cd content in the analyzed vegetables exceeded by 5 times the MAL, while the Cu and Zn contents were close to critical levels (Lăcătuşu *et al*., 1996). Ingestion of vegetables containing high concentrations of heavy metals is one of the main ways in which these elements enter the human body. Typical diseases recorded were Pb and Cd intoxication, saturnine encephalopathy, radial nerve paralysis and saturnine colic. The most affected group of inhabitants was children (Lăcătuşu *et al*., 1996). Estimates from various countries showed that the dietary intake for lead in adults is between 54 mg per day (Dabeca *et al*., 1987) and 412 mg per day (Dick *et al*., 1978), and that of cadmium is between 10 and 30 mg per day (Reilly, 1991). For zinc and copper, the estimated daily intake is from 1 to 3 mg, and 10 to 20 mg, respectively (Fox, 1982). Lăcătuşu *et al*.(1996) found that their estimations for lead and zinc were above those reported from other countries whereas the estimation for cadmium was within the range. The levels of copper were observed to be below the estimation. Bahemuka and Mubofu (1999) suggested that a large daily intake of these vegetables is likely to cause a detrimental health hazard to the consumer.

Since the dietary intake of food may constitute a major source of long-term low-level body accumulation of heavy metals, the detrimental impact becomes apparent only after several years of exposure. Regular monitoring of these metals from effluents, sewage, in vegetables and in other food materials is essential for preventing excessive buildup of the metals in the food chain (Bahemuka and Mubofu, 1999).

CURRENT TECHNOLOGIES TO DECREASE DIETARY TOXICITY OF HEAVY METALS

Different technologies have been employed for soil decontamination. Two strategies depending on the amount of heavy metal contents in soil can be adopted. First is the application of different agronomical practices in order to minimize the availability of heavy metals in soils. These include pH modification, organic matter management, fertilizer management, and choice of the most suitable vegetables for a particular soil. This strategy can be employed in areas where heavy metal pollution is not extensive. Another way is the use of phytoremediation techniques in which metal accumulating plants are used to transport and concentrate metals from soil into the aboveground shoots, which are then harvested. This technique is ideal in soils where heavy metal pollution is very high.

Phytoremediation i.e. use of higher plants to absorb heavy metals from polluted lands, has gained much interest recently. Phytoremediation may include phytoextraction, phytoviolatilization, rhizofiltration or phytostabilization. A number of plant species from Angiospermic groups have been employed to carry out phytoremediation. Certain plants such as *Thlaspi caerulenscens*, *Haumaniastrum robertii*, *Ipomoea alpina*, *Macadamia neurophylla*, *Psychotria douarret*, *Thlaspi rotundifolium*, etc. which can selectively accumulate heavy metals are employed to remove heavy metals from soils. Such plants can accumulate 10~500 times higher levels than crops.

Uptake of metal ions is an essential part of plant nutrition. Several heavy metals such as Cu, Mn, Zn, Fe and Ni play important roles in enzyme induction and reaction, membrane function, and isozyme activity. The response of plants to high concentrations of metals varies across a broad spectrum from toxic reaction to tolerance; some plants are obligate metallophytes with a physiological requirement for elevated metal contents in soils (Nedelkoska and Doran, 2000). Of those plant species that actively accumulate metals i.e. hyperaccumulators store metals in their tissues at concentrations far exceeding those in the environment. Heavy metal contents in hyperaccumulators are at least 100 times those found in non-hyperaccumulator plants grown in soil under the same conditions (Brooks *et al*., 1998). The number of confirmed hyperaccumulating species is expanding rapidly, and includes about 300 plants that hyperaccumulate nickel, 26 cobalt, 24 copper, 16 zinc and 11 manganese. Hyperaccumulators of selenium, thallium, cadmium, lead and uranium have also been reported (Brooks *et al*., 1998).

Although the existence of hyperaccumulating plants has been known for more than 20 years, their potential environmental and commercial applications have only recently been considered in detail (Robinson *et al*., 1997a; 1997b). Hyperaccumulators that can remove metals from contaminated soils and polluted rivers and lakes are being investigated for phytoremediation of sites affected by sewage sludge; metal-rich mine tailings, and downwash or aerial fallout from smelting and electroplating factories (Brooks and Robinson, 1998; Salt *et al*., 1998). Another application is phytomining, whereby hyperaccumulator plants are grown on low-grade ores, the metal-laden crop harvested, and the biomass treated to recover the metal (Brooks *et al*., 1998). The advantage of this agricultural approach to mining is its low cost relative to conventional methods, allowing economic exploitation of ore or mineralized soil that is too metal-poor for direct mining operations (Nedelkoska and Doran, 2000).

So far, the mechanisms of metal uptake by hyperaccumulating plants and the basis of their metal specificity are poorly understood; the phytochemistry involved varies considerably depending on the metal and plant species (Brooks, 1998). Several reports have suggested that metals are detoxified in hyperaccumulators by binding with low-molecular-weight ligands such as histidine (Krämer *et al*., 1996) or organic acids such as citric, malic and malonic acids (Kersten *et al*., 1980; Sagner *et al*., 1998; Tolrà *et al*., 1996b). However, complexation with such universal plant metabolites is considered by some researchers to be insufficient explanation for the special properties of hyperaccumulator plants, including their metal specificity (Homer *et al*., 1991). Other mechanisms that could be involved in hyperaccumulation include metal translocation within the plant (Krämer *et al*., 1996; Tolrà *et al*., 1996a) and compartmentation within the cells, for example, in vacuoles (Vázquez *et al*., 1992). The role of phytochelatins in hyperaccumulators is unclear at present, although many plants for normal metal homeostasis use these cysteine rich peptides (Nedelkoska and Doran, 2000). Several reports have concluded that phytochelatins are not primarily responsible for hyperaccumulation of Ni or Zn (Krämer *et al*., 1996; Shen *et al*., 1997).

Most studies of hyperaccumulation have been carried out using whole plants grown either in soil or hydroponically. For investigation of the mechanisms and metabolic responses of hyperaccumulators rather than agronomic characteristics, there are advantages associated with alternative plant cultivation systems (Nedelkoska and Doran, 2000). In vitro culture of organs such as roots and shoots allows indefinite propagation and experimentation using tissues derived from the same plant, thus avoiding the effects of variability between individual specimens (Pollard and Baker, 1996). Axenic conditions in tissue culture prevent microbial symbiosis disguising the metal uptake characteristics of plants grown in soil, while better control over environmental conditions at the roots can be obtained compared with pot cultivation. Experiments using separately cultured organs also allow the metal accumulation properties of each organ to be identified without interference from translocation effects. Genetically transformed hairy roots produced by infection of plants with *Agrobacterium rhizogenes* are a convenient form of organ culture (Doran, 1997) and have been used previously in phytoremediation studies (Hughes *et al.*, 1997; Macek *et al.*, 1994; Maitani *et al.*, 1996; Metzger *et al.*, 1992). However, as generation of hairy roots of hyperaccumulating species has not been reported previously, their abilities to hyperaccumulate metals and their utility in hyperaccumulation research have not yet been demonstrated (Nedelkoska and Doran, 2000).

CONCLUSION

Factors affecting the thresholds of dietary toxicity of heavy metal in soil-crop system include: soil type involving soil pH, organic matter content, clay mineral and other soil chemical and biochemical properties; and crop species or cultivars regulated by genetic basis for heavy metal transport and accumulation in plants. In addition, the interactions of soil-plant roots-microbes play important roles in regulating heavy metal movement from soil to the edible parts of crops. Agronomic practices such as fertilizer and water management and crop rotation system can affect bioavailability and crop accumulation of heavy metals, thus influencing the thresholds of assessing dietary toxicity of heavy metals in food chain.

Further research is needed to find out the variations in metal uptake by different vegetable species, and the site-specific risk assessment guidelines to highlight and to minimize the potential health risks of ingesting vegetables containing high levels of heavy metals.

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