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Mobility of Heavy Metals from Soil into Hot Pepper Fruits: A Field Study

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Abstract Capsaicin and dihydrocapsaicin contribute to pungency as well as having health-promoting properties, in peppers. Twenty-three genotypes (four spp.) of hot pepper from the USDA germplasm collection were grown in the field to identify accessions having increased concentrations of these two compounds and determine the concentrations of heavy metals, in mature fruits. Concentrations and relative proportions of capsaicin, dihydrocapsaicin, and seven heavy metals varied between and within pepper species. Plant Introduction 547069 (*C. annuum*) contained the greatest concentrations of the two pungent compounds. Fruits of PI-439381 and PI-267729 (*C. baccatum*) accumulated the greatest concentrations of Pb, while PI-246331 (*C. annuum*) accumulated the greatest concentration of Cd among accessions tested.

Keywords *Capsicum* spp · Capsaicin · Dihydrocapsaicin · Lead · Cadmium

Members of the genus *Capsicum* (Family: Solanaceae) exhibit varying degrees of pungency that reflect the relative concentrations of capsaicin and dihydrocapsaicin, the two major capsaicinoids in hot pepper. Capsaicin (*N*-vanillyl-8-methyl-6-nonenamide) and dihydrocapsaicin accounted for an estimated 80%–95% of the naturally occurring capsaicinoids in peppers (Cavett et al. 2004). These compounds exhibit antioxidant activity and potent antimutagenic and anticarcinogenic properties (Surh and Seoul 2002). Pungent chili varieties are grown for their food value, health-promoting properties (Padilla and Yahia 1998) and also as a source of capsaicinoids that have a variety of medicinal uses (Sicuteri et al. 1990). There is a growing interest in the enhancement of compounds in foods having health-promoting attributes, such as antioxidant properties (Van der Sluis et al. 2002; Antonious et al. 2006).

In consideration of the enormous worldwide consumption of fruit of various *Capsicum* spp., the utilization of capsaicin as a food additive and its current medicinal application in humans, the evaluation and assessment of any harmful effects of hot peppers, such as the accumulation of heavy metals in the fruits is warranted in efforts to protect public health. Presently, one of the pollutants of most concern around the world is heavy metals (Thuy et al. 2007). Elevated concentrations of heavy metals in edible plants could expose consumers to excessive levels of potentially hazardous chemicals. Plant uptake is one of the main pathways through which metals enter the food chain. Accumulation of heavy metals varied between plant species (Antonious and Snyder 2007; Melo et al. 2007). Variability in the concentrations of capsaicinoids (capsaicin and dihydrocapsaicin) and heavy metals can be a factor affecting the selection of pepper as parents in hybridizations in the USDA breeding programs aimed to produce fruits with value-added traits and as a source of phytochemicals having antioxidant activity.

The USDA hot pepper (*Capsicum* spp.) germplasm collection contains several thousand accessions that include commercial cultivars, breeding lines and landraces. Accessions of the cultivated species (*C. annuum*, *C. baccatum*,

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C. chinense, *C. frutescens*, and *C. pubescens*) in this collection have not been analyzed for heavy metal content. Data on the heavy metal content in hot pepper fruits is limited. Identifying *Capsicum* species and accessions within species with high levels of capsaicin and dihydrocapsaicin and those that minimally uptake heavy metals is the focus of this study. The main objectives of this investigation were: (1) to select candidate accessions of hot pepper having high concentrations of capsaicin and dihydrocapsaicin for use as parents in breeding for these two compounds, (2) to determine the concentrations of seven heavy metals (Cd, Cr, Ni, Pb, Zn, Cu, and Mo) in soil and their accumulation in hot pepper fruits, and (3) to determine if the heavy metal content of hot pepper fruit that have elevated levels of capsaicin and dihydrocapsaicin are lower than the permitted heavy metal limits.

Materials and Methods

A field study was conducted on a Lowell silty loam soil (2.8% organic matter, pH 6.9) located at Kentucky State University Research Farm, Franklin County, KY. The soil has an average of 12% clay, 75% silt, and 13% sand. Seeds of 23 *Capsicum* accessions were obtained from the USDA *Capsicum* germplasm collection (Plant Genetic Resources Conservation Unit, Griffin, GA) and planted in the greenhouse in the spring and transplanted to the field in June of 2006. Five *C. chinense* Jacq. (PI-594139, PI-438643, PI-438614, PI-435916, and PI-224448); six *C. frutescens* L. (PI-241675, PI-239703, PI-586675, PI-439506, PI-257069, and PI-257051); seven *C. baccatum* L. (PI-260434, PI-281340, PI-238061, PI-439381, PI-370004, PI-267729, and Grif-9354); and five *C. annuum* L. (PI-438649, PI-310488, PI-593566, PI-547069, and PI-246331) were selected to represent four cultivated pepper species, and a cross-section of the geographic range of origin of these species. Seedlings were fertilized with Peters (NPK fertilizer) at 200 ppm in the greenhouse. Two weeks after transplanting to the field, Nature Safe Fertilizer (Advance Turf Solutions, Louisville, KY) containing the elements NPK (10:2:8) was side-dressed at the rate of 5 lbs 1,000 ft^{-2}. Hot pepper seedlings were planted in 10 rows $plot^{-1}$ at 10 plants row^{-1}. Plots were watered twice a week using drip tape (Rainbird Corporation, Glendora, CA) and no pesticide was applied.

At harvest, 120-day-old mature fruits of comparable size and color were collected at random from each accession (six replicates for each accession) and washed with deionized water. Fruits were oven dried at 65°C for 48 h to a constant weight, ground using a mortar and pestle, sieved through a No. 18 (1 mm) mesh, and re-dried to constant weight. To 1 g of each dried sample, 10 mL of concentrated nitric acid were added, the mixture was allowed to stand overnight, and it was then heated for 4 h at 125°C on a hot plate. The mixture was diluted to 50 mL with double distilled water and filtered through Whatman No. 1 filter paper. The Cadmium (Cd), chromium (Cr), nickel (Ni), lead (Pb), zinc (Zn), cupper (Cu), and molybdenum (Mo) contents of 23 *Capsicum* accessions were determined by Inductively Coupled Plasma (ICP Varian Vista-Pro) spectrometer.

Capsaicin and dihydrocapsaicin were extracted by blending 50 g of fresh fruit ($n = 3$) with 100 mL of methanol for one min as described by Antonious and Jarret (2006). Each extract was subsequently passed through a 0.45 µm disposable syringe filter (Fisher Scientific, Pittsburgh, PA). One microliter of this filtrate was injected into a gas chromatograph (GC) equipped with a nitrogen-phosphorus detector (NPD) using a 25 m × 0.20 mm ID capillary column with 0.33 µm film thickness (HP-1). Operating conditions were 230, 250, and 280°C for injector, oven, and detector, respectively with a carrier gas (He) flow rate of 5.2 mL min^{-1}. Peak areas were determined using a Hewlett-Packard (HP) model 3396 series II integrator. Under these conditions, retention times (R_t) were 11.50 and 11.75 min, for capsaicin and dihydrocapsaicin, respectively. Purified standards of capsaicin (*N*-vanillyl-8-methyl-6-noneamide) and dihydrocapsaicin were obtained from Sigma-Aldrich Inc. (Saint Louis, MO 63103, USA) and used to prepare calibration curves. To determine the recovery of the extraction process, clean-up, and quantification procedures, concentrations of capsaicin and dihydrocapsaicin in the range of 20–200 µg g^{-1} fresh fruit were added to 20 g of bell pepper (*C. annuum)* fruits. Recoveries of the added capsaicin and dihydrocapsaicin were 98% and 95%, respectively. Minimum detectable levels of capsaicin and dihydrocapsaicin detected in the fruit extracts averaged 0.02–0.005 µg g^{-1} fruit.

Soil samples were collected to a depth of 15 cm from field plots using a 2.5-cm interior diameter soil core sampler equipped with a plastic liner (Clements Associates, Newton, IA, USA). Soil samples were oven-dried at 105°C and sieved to a size of 2 mm. Cadmium, Cr, Ni, Pb, Zn, Cu, and Mo were determined by ICP (ICP Varian Vista-Pro) spectrometer. Detection limits (µg g^{-1}) were Cd 0.02, Cr 0.04, Cu 0.04, Mo 0.1, Ni 0.2, Pb 0.3, Zn 0.04 at wavelengths (nm) 226.502, 267.716, 324.754, 202.032, 231.604, 220.353, and 213.857, respectively. Data for all fruits analyzed in this investigation are expressed on dry weight basis. Heavy metal concentrations in soil and hot pepper fruits, and capsaicin and dihydrocapsaicin concentrations in fruits were statistically analyzed using a randomized complete block design (ANOVA Procedure) and the means were compared using Duncan's multiple range test.

Results and Discussion

Concentrations of capsaicin and dihydrocapsaicin varied among accessions from undetectable in PI-310488 to 1343 mg g^{-1} in PI-547069 (*C. annuum*). Accession PI-547069 (C. *annuum*) contained the greatest concentrations of each of the two pungent compounds of hot pepper among all the accessions tested (Table 1).

Heavy metal concentrations in soil and fruits are presented in Fig. 1 and Table 2, respectively. Six of the seven heavy metals analyzed in this study were detected in the soil samples. Generally, the average concentration of all metals in soil samples was lower than the permissible limits. Zinc and Cu concentrations in the soil samples were extremely high, compared to other heavy metals (Fig. 1). The Cu concentration was greatest in PI-439506 (*C. frutescens*) when compared to other accessions (Table 2). These data are consistent with those of Morrison et al. (2004) who found that plants rapidly accumulate Cu. In contrast, the Zn concentration was greatest in accession PI-438643 (*C. chinense*). Although Zn has relatively low toxicity to humans, studies have shown that allergies to zinc and zinc poisoning could occur within the food chain, which may also interfere with copper metabolism (Ohnessorge and Wilhelm 1991). The order of elements in various hot pepper genotypes and their concentration ranges (μg g^{-1} dry fruit) were; Mo (0–0.65), Pb (0–0.86), Cd (0.14–0.63), Cr (0.18–1.05), Ni (1.04–3.47), Cu (8.8–111.5), and Zn (19.73–62.64) (Table 2).

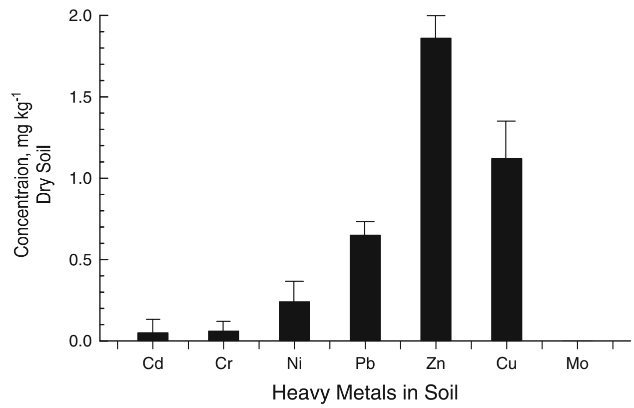

Fig. 1 Concentrations (means ± SE) of seven heavy metals in a silty-loam soil used for growing hot pepper at Kentucky State University Research Farm, Franklin County, Kentucky, USA

Table 1 Concentrations of capsaicin and dihydrocapsaicin in hot pepper fruits of 23 accessions grown at Kentucky State University Research Farm, Franklin county, Kentucky, USA

		Capsaicin		Dihydrocapsaicin	
Taxon	Accession	μg g^{-1} Fresh fruit		μg^{-1} Fresh fruit	
C. annuum	PI-246331	566.60	cde	600.48	b
C. annuum	PI-310488	0.00	l	0.00	m
C. annuum	PI-438649	29.30	kl	16.20	m
C. annuum	PI-547069	1342.90	a	791.13	a
C. annuum	PI-593566	205.80	ijkl	192.78	ghij
C. baccatum	Grif-9354	348.40	efghi	490.75	bc
C. baccatum	PI-238061	284.00	fghij	376.42	de
C. baccatum	PI-260434	255.10	ghijk	145.73	ijkl
C. baccatum	PI-267729	71.70	jkl	65.52	klm
C. baccatum	PI-281340	106.80	jkl	41.23	lm
C. baccatum	PI-370004	394.70	defghi	467.42	cd
C. baccatum	PI-439381	240.50	hijk	73.85	klm
C. chinense	PI-224448	209.10	ijkl	68.34	klm
C. chinense	PI-435916	694.60	c	314.54	ef
C. chinense	PI-438614	501.90	cdef	216.47	fghi
C. chinense	PI-438643	487.20	cdefg	231.43	fghi
C. chinense	PI-594139	288.20	fghij	88.99	jklm
C. frutescens	PI-239703	934.10	b	577.77	bc
C. frutescens	PI-241675	460.30	cdefgh	264.97	efgh
C. frutescens	PI-257051	481.30	cdefgh	170.81	hijk
C. frutescens	PI-257069	405.40	defghi	303.29	efg
C. frutescens	PI-439506	681.60	c	556.02	bc
C. frutescens	PI-586675	626.10	cd	359.56	de

Values within a column for each compound having different letter(s) are significantly different ($p < 0.05$) from each other, using Duncan's multiple range test (ANOVA procedure)

Hot pepper and other vegetables absorb heavy metals from polluted soil, air and water. In general, heavy metals are not biodegradable and can accumulate in vital organs (Demirezen and Aksoy 2006). This accumulation leads to progressive toxic effects. When the plants die and decay, heavy metals taken into the plants are redistributed and the soil becomes enriched with these pollutants. Cadmium and Pb are the heavy metals of greatest concern to human health. Results of the present investigation revealed that concentrations of Cd in hot pepper fruits averaged 0.14–0.63 μg g^{-1} dry fruit.

Accession PI-246331 (*C. annuum*) accumulated significant concentrations of Cd in its' fruits (Table 2). Considering that pepper fruits are 91% water (by weight), the Cd concentrations (0.057 μg g^{-1}) are slightly higher than the Codex-established maximum limit (Codex Alimentarius Commission Levels 2006) of 0.05 μg g^{-1} fresh weight of fruit. Cadmium may accumulate in the human body and induce kidney dysfunction, skeletal damage and reproductive deficiency (Commission of the European Communities 2002).

The Codex Standard (Codex Alimentarius Commission Levels 2006) indicates that the maximum level for lead in most vegetables is 0.1 μg g^{-1}. Pepper accessions PI-267729 and PI-439381 (*C. baccatum*) contained the highest Pb concentrations. Lead is defined by USEPA as potentially toxic to most forms of life. It is possible that root exudates

Table 2 Mean concentrations (μg g^{-1}dry weight) of heavy metals in hot pepper species grown at KSU Research Farm (Franklin County, Kentucky, USA)

Accession	Taxon	Cd	Cr	Ni	Pb	Zn	Cu	Mo
PI-246331	*C. annuum*	0.63 a	0.62 bcde	2.97 ab	0 b	47.2 abc	65.94 cd	0 c
PI-310488	*C. annuum*	0.14 g	0.52 bcdef	2.05 abcde	0 b	49.55 abc	88.07 b	0 c
PI-438649	*C. annuum*	0.37 bcd	0.59 bcde	1.8 bcde	0 b	39.56 abc	90.49 b	0 c
PI-547069	*C. annuum*	0.22 efg	0.34 cdef	1.26 de	0 b	39.35 abc	61.61 de	0 c
PI-238061	*C. baccatum*	0.22 efg	0.43 bcdef	1.47 bcde	0 b	41.69 abc	41.13 fgh	0 c
PI-260434	*C. baccatum*	0.32 cde	0.7 bc	2.83 abc	0 b	42.85 abc	49.7 def	0 c
PI-267729	*C. baccatum*	0.47 b	0.62 bcde	2.26 abcde	0.86 a	20.38 c	26.7 hij	0 c
PI-281340	*C. baccatum*	0.24 efg	0.42 bcdef	2.16 abcde	0 b	35.69 abc	42.76 fgh	0 c
PI-370004	*C. baccatum*	0.42 bc	1.05 a	2.05 abcde	0 b	49.28 abc	79.88 bc	0.65 a
PI-439381	*C. baccatum*	0.37 bcd	0.64 bcde	2.15 abcde	0.79 a	34.01 abc	52.9 def	0 c
Grif-9354	*C. baccatum*	0.42 bc	0.79 ab	3.47 a	0 b	42.4 abc	64.99 cd	0 c
PI-224448	C. chinense	0.36 bcd	0.64 bcde	1.04 e	0 b	38.56 abc	20.66 ijk	0.45 b
PI-435916	*C. chinense*	0.33 cde	0.3 def	1.33 cde	0 b	41.52 abc	8.82 k	0 c
PI-438614	*C. chinense*	0.33 cde	0.64 bcde	1.98 abcde	0 b	22.27 bc	11.28 jk	0.46 b
PI-438643	*C. chinense*	0.29 def	0.67 bcd	1.97 abcde	0 b	62.64 a	31.33 ghi	0 c
PI-594139	*C. chinense*	0.18 fg	0.18 f	1.27 de	0 b	19.73 c	27.28 hij	0.14 c
PI-239703	*C. frutescens*	0.25 efg	0.58 bcde	1.75 bcde	0 b	46.92 abc	9.96 jk	0 c
PI-241675	*C. frutescens*	0.23 efg	0.28 ef	1.09 e	0 b	41.13 abc	52.99 def	0 c
PI-257051	*C. frutescens*	0.23 efg	0.37 cdef	2.71 abcd	0 b	38 abc	91.23 b	0 c
PI-257069	*C. frutescens*	0.22 efg	0.69 bc	1.81 bcde	0 b	33.11 abc	22.45 ijk	0.14 c
PI-439506	*C. frutescens*	0.17 g	0.38 cdef	1.43 bcde	0 b	44.64 abc	111.46 a	0.14 c
PI-586675	*C. frutescens*	0.23 efg	0.35 cdef	2.01 abcde	0 b	53.14 ab	45.62 efg	0.15 c

Values within a column accompanied by the same letter(s) are not significantly different ($p > 0.05$) (ANOVA procedure)

of these hot pepper genotypes contained hydrolyzing enzymes that may have enhanced the solubility of some complex forms of lead the in soil and its mobility from the soil into the plants, thus increasing its accumulation in the fruits. Although higher than the levels present in other accessions examined, Pb levels in PI-267729 and PI-439381 did not exceed acceptable levels on a fresh weight basis. Fruit of accession Grif-9354 (*C. baccatum*) contained the highest levels of Ni (3.47 μg g^{-1}) on dry weight basis. According to the Institute of Medicine (2003) and the Agency for Toxic Substances and Disease Registry (2004), Ni can cause respiratory problems and is carcinogenic.

Heavy metals are among the major contaminants in the food chain. An increasing awareness of the value of vegetables and fruits in the human diet suggests that monitoring of heavy metals in food crops might be warranted. The different absorption patterns of heavy metals among the accessions investigated in this study could be attributed to individual accession (genotypic) characteristics. Plant species have a variety of mechanisms that effect the removal and accumulation of heavy metals. Several reports have indicated that some crop plants accumulate specific heavy metals to a greater extent than others (Antonious and Snyder 2007). This suggests that plants that accumulate heavy metals in their edible portions should be regarded as a potential source of heavy metal contamination in the food supply.

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