Short communication

The impact of sewage irrigation on the uptake of mercury
in corn plants (*Zea mays*) from suburban BeijingS.E. Rothenberg^{a,c,*}, X. Du^a, Y.-G. Zhu^a, J.A. Jay^b^a Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, 18 Shuangqing Road, Beijing, 100085, China^b Department of Civil and Environmental Engineering, Box 951593 Boelter Hall, University of California, Los Angeles, CA 90095-1593, USA^c Environmental Science and Engineering Program, Box 951772 CHS, University of California, Los Angeles, CA 90095-1772, USA

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*Hg content in the roots of corn plants (*Zea mays*) was positively correlated with soil Hg content, but negatively correlated with Hg content in stems.*

Abstract

The impact of sewage irrigation on the uptake and translocation of mercury (Hg) in corn plants (*Zea mays*) was investigated. Corn plants were harvested the same day from two nearby fields in suburban Beijing, one irrigated historically with sewage effluent, and one irrigated solely with groundwater. Hg content was analyzed in the soil, roots and stems, while percent moisture and soil organic content were analyzed in the soil samples. The concentration of Hg in the soil and roots, and the soil organic content were not significantly different between the two fields, despite the historic practice of sewage irrigation. Hg content in roots was positively correlated with soil Hg concentration ($r = 0.95$, $n = 6$). The transfer coefficients between roots and stems were significantly higher in the control site (control: 2.06, sewage-irrigated: 0.44, $p < 0.05$), indicating that the barrier effect of the roots was not consistent between the two fields.

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1. Introduction

Mercury (Hg) is a global contaminant and a potent neurotoxin, affecting human health and wildlife reproductive potential. Between 70 and 80% of current atmospheric Hg is from anthropogenic sources (Mason et al., 1994), mainly combustion of coal. Pacyna and Pacyna (2001) reported between 1990 and 1995 anthropogenic mercury emissions from Asia increased from approximately 30% of total global emissions to 56% (1074 t/1912 t), due to rapid industrialization. The authors also reported China alone increased emissions by 250 t within the 5-year period, while emissions from Europe and North America decreased, due to tighter emission standards, and the development of Hg-removal technology.

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When coal is incinerated, Hg is released into the environment as elemental mercury (Hg^0) or inorganic mercury (Hg^{2+}). Hg^0 will enter the global circulation cycle and will be transported great distances due to its relatively long atmospheric residence time (~ 1 year, Mason et al., 1994), while most Hg^{2+} will be scavenged by particles, and deposited locally (Schroeder and Munthe, 1998). Sediments act as a reservoir for Hg^{2+} , primarily due to organic matter, which provides sorption sites for positively charged metal ions (Kim et al., 1996; Schuster, 1991). Of all metals, the most strongly retained is Hg, due to the affinity between Hg and sulfhydryl groups, a main constituent of humic material (Evans, 1989). In terrestrial environments, a positive correlation between organic content and Hg^{2+} has been reported, although intercepts and slopes varied between sites (Grigal, 2003, and references within).

In China, soil retention of Hg by organic matter may be exacerbated by the use of sewage effluent for agricultural fields

(Chen et al., 1999). Scarce water resources have forced farmers in the upland regions of north and northeastern China to use untreated sewage and reclaimed wastewater for their crops, despite an official government prohibition on this practice (Bai and Imura, 2001). Organic matter from sewage binds heavy metals, elevating their concentration in the soil. This may lead to increased phytoavailability of metals (e.g. Wang et al., 2001; Yu et al., 2004). However, uptake of Hg within plants may be stifled by the barrier effect of the roots (Beauford et al., 1977; Cavallini et al., 1999; Greger et al., 2005; Lindberg et al., 1979), as non-essential and/or toxic elements are not readily translocated (Streit and Stumm, 1993).

Most human exposure to Hg occurs through the consumption of fish, however, Hg exposure also occurs through rice (Bennett et al., 2000; Horvat et al., 2003; Nakagawa and Yumita, 1998), and vegetables (de Temmerman et al., 1986; Mosbaek et al., 1988). Hg concentration may be lower in agricultural crops, but this route of exposure is important because these foods, especially rice, are considered staples throughout Asia.

Sewage irrigation is widespread in developing countries, but little research exists documenting its effect on the uptake of Hg in terrestrial plants. The primary aim of this study was to investigate the impact of historic sewage irrigation on Hg uptake in edible plants, by comparing the concentration of Hg in the soil, roots and stems of corn plants (*Zea mays*) harvested from two fields, one historically irrigated with sewage effluent, and one nearby control site irrigated solely with groundwater. In addition, we hypothesized no differences in stem Hg between the two field sites, due to the barrier effect of the roots. While some plant species may translocate Hg from leaves to the sub-terrestrial portions (Mosbaek et al., 1988; Schwesig and Krebs, 2003), we assumed this represented a negligible portion of Hg levels in roots or stems. This was an observational study, completed 2 years after the cessation of sewage irrigation.

2. Materials and methods

2.1. Study sites and field collection

The sampling area consisted of two field sites across the road from one another in the Bei Ye Chang irrigation district of suburban Beijing (Latitude 39-43-119, Longitude 116-26-752). In 2002, the field historically irrigated with sewage effluent switched to secondarily treated wastewater, which removed about 90% of suspended solids and organic matter. The control site was continuously irrigated with groundwater. Both fields were planted with corn (*Z. mays*) at approximately the same time.

In July 2004, soil samples and corn plants were collected. Plants were harvested before fruit was formed. From each field, four neighboring soil and plant samples (within 1 m²) were collected using Teflon-coated trowels from three sites approximately 10 m apart, for a total of 12 samples from each field. Plant and soil samples were stored in polyethylene bags, and were refrigerated the same day at the lab (4 °C). Laboratory analyses for plants were completed within 4 weeks of sample collection. Hg concentration in soil samples was analyzed twice; once within 4 weeks of sample collection, and then in 2006, 23/24 samples were re-analyzed (see Section 2.2).

2.2. Sediment and plant analyses

Hg content was analyzed in the soil, roots, and stems. For Hg analysis, all labware was pre-soaked overnight in 5% (v/v) HNO₃, and triple-rinsed with

double-distilled water. Plant samples were rinsed thoroughly with double-distilled water to remove sediment particles. Prior to Hg analysis, roots and stems were freeze-dried separately until all plant material was thoroughly dried (approximately 48 h). To release Hg²⁺, freeze-dried plant samples (<0.50 g) were digested in closed 100 mL Teflon[®] vials with either 5 mL of 3:1 HNO₃:HCl or 10 mL HNO₃ in the Mars 5 Microwave Digester (CEM Corporation, USA) (lower acid blanks were recorded using the latter). Microwave digestion included 15 min ramping at 10 °C min⁻¹ to a final temperature of 160 °C, then holding at 160 °C for 15 min. Hg²⁺ was reduced to Hg⁰ with 0.05% KBH₄ mass vol⁻¹ (in 0.1% KOH mass vol⁻¹) and vaporous Hg⁰ was analyzed by cold vapor atomic fluorescence spectrometry (CVAFS-2202E Beijing Haiguang Instruments).

Soil samples were analyzed twice for Hg, once in 2004 and again in 2006, to verify initial findings and to compare Hg analysis between two methods. The same steps described for plants were used to measure mercury in soil samples (by CVAFS, referred to as Method 1). In 2006, 23/24 archived soil samples (refrigerated at 4 °C) were re-measured in triplicate using direct Hg analysis by thermal decomposition, amalgamation and atomic absorption spectrophotometry (Milestone, DMA-80, Method 7473 (USEPA, 1998), referred to as Method 2). All plant material was consumed in 2004, and therefore not re-measured in 2006. Using Method 2, recovery of standard reference material (IAEA-405, estuarine sediment) yielded between 103 and 105% recovery ($n = 4$), and precision, defined as the relative percent difference between triplicate analyses, averaged 19% ($n = 23$). For Method 2, the method detection level (MDL), defined as $3 \times$ standard deviation (SD) of method blanks ($n = 8$), was 0.0039 µg g⁻¹. The MDL for Method 1 was not established through analysis of blanks; however, the aqueous concentration of Hg in all digests was >2 ng liter⁻¹ (i.e. >10 pM) (after subtraction of the acid blank), which was $10 \times$ higher than the typical MDL for CVAFS (see Method 1631; USEPA, 2002).

For both sediment methods, moist samples were analyzed, and the Hg concentration was converted to dry mass using a wet:dry ratio, measured by drying sediments overnight in a 60 °C oven. The data for the wet:dry ratio were also used to calculate the percent soil water content. Soil organic matter was measured as the percentage loss on ignition (LOI) following one additional hour in a 550 °C oven (Method 2540-E, APHA, 1998). Total plant mass (stems and leaves) was measured prior to drying, to verify plants were approximately the same age in the two fields. All results are reported in dry weight (dw).

2.3. Statistical analyses

Each field was sampled at three sites, from which four soil and plant samples were collected. For statistical analyses, each site represented the average of the four replicates, and the grand mean for each field represented the average of the three sites. The *t*-test was used to compare the means between the sites from the sewage-irrigated and control fields ($n = 3$ from each field), assuming unequal variance and unpaired observations. The statistical package, Stata, was used for *t*-tests, while graphs were drawn in Excel. Regression was not used due to the small sample size ($n = 6$).

3. Results and discussion

3.1. Comparison between two Hg methods

Hg concentration in soil samples was compared using two methods: Method 1 (by CVAFS), and Method 2 (by direct Hg analysis). Fig. 1 includes a side-by-side comparison between the results for the control and sewage-irrigated fields. There was more homogeneity among samples re-measured using Method 2, which was likely due to higher precision from triplicate measurements. The overall average for samples measured using Method 2 was lower, especially for the sewage-irrigated sites (see Table 1). Disparities between the two methods may be due to heterogeneity within the soil samples. The average percent difference between results from both

Fig. 1. Comparison between soil Hg concentration ($\mu\text{g g}^{-1}$ dw) using two methods (Method 1, CVAFS; Method 2, direct mercury analysis; see text for details).

methods was 19% ($n = 23$, figure not shown), which was also the average percent difference between triplicate samples (see Methods). All statistical tests for soil Hg were re-calculated, and conclusions did not differ for those reported in Sections 3.2 and 3.3 (e.g. when comparing control and sewage-irrigated fields, p -values were >0.05 for soil Hg and for root/soil bioconcentration factors; soil $>$ roots $>$ stems hierarchy was the same; and the correlation between roots and soil was weaker, although positive).

Since findings for control and sewage-irrigated fields did not differ between the two methods, the remainder of the paper refers to soil and plant material measured using Method 1.

Table 1

Grand mean and SD (in parentheses) for comparison of soil Hg levels ($\mu\text{g g}^{-1}$ dw) using two methods (Method 1, CVAFS; Method 2, direct mercury analysis; see text for details)

	Soil Hg Method 1 ($n = 24$)	Soil Hg Method 2 ($n = 23$)
Control	0.14 (0.0084)	0.12 (0.030)
Sewage-irrigated	0.17 (0.026)	0.11 (0.0098)

3.2. Hg concentration in soil and roots

The main objective of this work was to investigate the impact of sewage irrigation on Hg uptake in corn plants. Tables 2 and 3 include raw data and summary statistics for the two field sites. For both soil and roots, Hg concentration was not significantly higher in the sewage-irrigated field. Soil Hg concentration in the control and sewage-irrigated sites averaged 0.14 and $0.17 \mu\text{g g}^{-1}$, respectively ($p = 0.16$), while Hg concentration in roots averaged $0.017 \mu\text{g g}^{-1}$ (control) and $0.021 \mu\text{g g}^{-1}$ (sewage-irrigated) ($p = 0.13$). Also, organic matter was not significantly different between the two fields (control: 4.2%, sewage: 3.7%, $p = 0.20$). Despite historic irrigation with sewage, there were no significant differences between organic content and Hg in soil and roots between the two fields. This was surprising given the history of the two fields. There were no pre/post data in order to compare exposure scenarios before cessation of sewage irrigation. However, since all three parameters lack disparity between

Table 2

Raw data for the control field (sites 1a–3d) and sewage-irrigated field (sites 4a–6d)

Site id	Hg in soil Method 1 ($\mu\text{g g}^{-1}$ dw)	Hg in soil Method 2 ($\mu\text{g g}^{-1}$ dw)	Hg in roots ($\mu\text{g g}^{-1}$ dw)	Hg in stems ($\mu\text{g g}^{-1}$ dw)	Organic content (%LOI)	Soil moisture content (%)	Stems + leaves (g wet weight)
Control							
1a	0.11	0.093	ND	0.0096	4.23	17.3	2.46
1b	0.12	0.082	ND	0.013	3.55	16.1	3.09
1c	0.15	0.10	0.0081	0.019	3.61	15.6	4.73
1d	0.16	0.078	0.027	0.020	3.45	17.3	2.62
2a	0.15	0.11	0.021	0.0037	4.56	17.4	5.00
2b	0.15	0.15	0.033	0.014	4.00	15.9	3.63
2c	0.15	0.20	0.0021	0.015	3.64	17.3	1.92
2d	0.14	0.13	0.022	0.015	4.74	15.6	3.30
3a	0.056	0.11	ND	0.0018	4.76	16.6	4.57
3b	0.15	0.11	0.0036	0.015	4.60	15.8	2.32
3c	0.16	0.11	0.025	0.020	4.33	15.0	3.61
3d	0.15	NA	ND	0.022	4.54	15.7	2.73
Sewage-irrigated							
4a	0.17	0.11	0.017	0.0028	3.61	10.5	4.35
4b	0.26	0.12	0.024	0.0018	3.20	14.1	4.92
4c	0.22	0.12	0.027	0.0033	3.20	11.3	1.78
4d	0.13	0.15	0.030	0.0055	3.34	15.2	2.70
5a	0.13	0.12	0.011	0.010	4.14	14.2	1.72
5b	0.18	0.096	0.038	0.0070	4.09	14.9	3.64
5c	0.14	0.085	0.0099	0.014	4.02	11.0	2.08
5d	0.24	0.12	0.025	0.0094	3.67	14.0	5.48
6a	0.14	0.13	0.017	0.0068	3.84	11.0	3.93
6b	0.16	0.11	0.018	0.0092	4.07	12.4	6.72
6c	0.15	0.11	0.013	0.0080	3.60	12.8	2.92
6d	0.12	0.10	0.026	0.0085	3.49	12.4	6.64

ND indicates non-detectable values, which were $<$ acid blank. NA indicates a sample not available for measurement.

Table 3

Grand mean and SD (in parentheses) for Hg concentration in the soil, roots and stems, organic matter, percent soil moisture content, and plant mass (stem + leaves)

Variable	Control	Sewage-irrigated	<i>p</i> -Value
	Grand mean (1 SD)	Grand mean (1 SD)	
Hg in soil ($\mu\text{g g}^{-1}$ dw)	0.14 (0.0084)	0.17 (0.026)	0.16
Hg in roots ($\mu\text{g g}^{-1}$ dw)	0.017 ^a (0.0026)	0.021 (0.0029)	0.13
Hg in stems ($\mu\text{g g}^{-1}$ dw)	0.014 (0.0017)	0.0072 (0.0035)	0.062
Organic matter (%LOI)	4.2 (0.40)	3.7 (0.33)	0.20
Soil moisture content (%)	16 (0.47)	13 (0.69)	0.0030
Stems + leaves (g wet weight)	3.3 (0.12)	3.9 (1.0)	0.43

The grand mean represents the average of three sites, of which there were four replicates (unless indicated by ^a). The *p*-values are for two-tailed *t*-tests between control and sewage-irrigated sites ($n = 3$ sites for each field), assuming unequal variance and unpaired observations.

^a Two replicate samples from two sites were non-detectable (ND).

the two fields, this suggests a scenario in which organic content controls soil Hg levels and, consequently, availability and uptake in roots.

Hg levels in soil and roots were compared to other sites in China. In a 1990 monitoring report on trace elements throughout China, soil Hg content ranged between 0.001 and $45.9 \mu\text{g g}^{-1}$, and averaged $0.038 \mu\text{g g}^{-1}$ (CNEMS, 1990). Soil Hg levels found in both sites were above the national average but was still considered uncontaminated ($<0.4 \mu\text{g g}^{-1}$, Grigal, 2003; $<1 \mu\text{g g}^{-1}$, Leonard et al., 1998). In Guizhou Province, located in southwestern China, one of the most highly mercury-contaminated regions, Feng et al. (2006) investigated Hg soil concentration and uptake in corn plants (*Z. mays* L.) from agricultural fields near zinc smelting sites. The authors reported that background soil Hg levels (below 10 cm depth) averaged $0.14 \pm 0.05 \mu\text{g g}^{-1}$, while top soil Hg ranged between 0.14 and $0.86 \mu\text{g g}^{-1}$, and Hg in roots of corn plants averaged $0.072 \mu\text{g g}^{-1}$ (range: 0.021–0.202). Average soil and root Hg levels for the current study were both near the minimum levels measured in Guizhou Province, which may be explained by lower atmospheric Hg emissions in Beijing. Streets et al. (2005) reported the 1999 annual anthropogenic Hg emissions for each province in China. Higher total Hg emissions were recorded for Guizhou Province compared to Beijing (Beijing = 8.83 t, Guizhou = 39.00 t), while the percentage of Hg^{2+} deposited locally was approximately equal (Beijing: 22%, Guizhou: 24%). Higher atmospheric Hg^{2+} deposition in Guizhou likely led to higher soil Hg concentration and uptake in the roots of corn plants, compared to the fields in suburban Beijing.

The transfer coefficient between roots and soil is defined as the ratio between Hg levels in roots and soil (unitless). The transfer coefficients between the two fields were not significantly different (control: 0.12, sewage: 0.13, $p = 0.73$, Table 4), which indicated the uptake of Hg within roots was similar between the two fields. The range was also comparable to other sites. Pereira et al. (2005) measured Hg in soil and seven species of plants, including corn plants (*Z. mays*), from the vicinity of a chlor-alkali plant in Portugal. Soil Hg was higher (0.64 – $1.44 \mu\text{g g}^{-1}$)

Table 4

Transfer coefficients (unitless) between roots and soil, and between stems and roots

Variable	Control	Sewage-irrigated	<i>p</i> -Value
	Grand mean (1 SD)	Grand mean (1 SD)	
Roots/soil	0.11 ^a (0.022)	0.13 (0.0089)	0.25
Stems/roots	2.06 ^a (0.49)	0.44 (0.30)	0.013

The grand mean represents the average of three sites ($n = 3$), of which there were four replicates (unless indicated by ^a). The *p*-values are for two-tailed *t*-tests between control and sewage-irrigated sites ($n = 3$ sites for each field), assuming unequal variance and unpaired observations.

^a Two replicate samples from two sites were non-detectable (ND).

compared to the fields sampled outside Beijing, but the transfer coefficients between soil and roots (calculated from the data tables provided) were comparative, ranging between 0.11 and 0.17. The transfer coefficients for the other six plant species varied between 0.0033 and $0.21 \mu\text{g g}^{-1}$, indicating the percentage of Hg translocated between soil and roots may be species-dependent.

A two-way scatter plot (Fig. 2) between Hg content in soil and roots indicated a positive correlation ($r = 0.95$, $n = 6$). This result was consistent with other studies (Cocking et al., 1995; Du et al., 2005; Feng et al., 2006; Leonard et al., 1998; Maserti and Ferrara, 1991; Pereira et al., 2005), and indicated the Hg concentration in roots was dependent on the in situ soil Hg concentration.

3.3. Hg concentration in stems

The second objective was to investigate whether roots served as a barrier to Hg translocation between roots and stems. If this hypothesis was true, then lower Hg concentration was expected in the stems, compared to the roots. For both the control and sewage-irrigated sites, this hierarchical relationship between soil, roots and stems was observed (data in parentheses represent the grand mean, $\mu\text{g g}^{-1}$):

	Soil	>	Roots	>	Stems
Sewage ($\mu\text{g g}^{-1}$ dw)	(0.17)		(0.021)		(0.0072)
Control ($\mu\text{g g}^{-1}$ dw)	(0.14)		(0.017)		(0.014)

The same hierarchy between soil, roots and stems was also observed by Greger et al. (2005) and Leonard et al. (1998) for other plant species. The decrease between roots and stems indicated a barrier effect; however, the proportion of Hg retained in the roots was not consistent between the control and sewage-irrigated fields. Stem Hg concentration from the control site was nearly twice the concentration from the sewage-irrigated site (control: 0.014, sewage-irrigated: 0.0072, $p = 0.062$, Table 3), and the transfer coefficient, defined as the ratio between stem and root Hg, was significantly higher for the control site (control: 2.06, sewage-irrigated: 0.44, $p = 0.013$, Table 4). Table 5 includes the correlation matrix between Hg concentration in soil, roots, stems, and soil water content. Unlike Hg in soil and roots, there was a negative correlation between Hg concentration in roots and stems ($r = -0.86$, $n = 6$). Feng et al. (2006)

Fig. 2. Hg concentration in roots ($\mu\text{g g}^{-1}\text{ dw}$) vs. Hg concentration in soil ($\mu\text{g g}^{-1}\text{ dw}$), $\circ$ = sewage-irrigated sites; $\bullet$ = control sites ($r = 0.95$, $n = 6$).

also reported a negative correlation between Hg in roots and stems of corn plants.

Differences in stem Hg concentration between control and sewage-irrigated fields may be due to soil water content, which were significantly higher in the control site (control: 16%, sewage-irrigated: 13%, $p = 0.0030$). Even though neither field was considered dry, there was a positive correlation between stem Hg and soil water content ($r = 0.83$, $n = 6$, Table 5). Increased moisture content may have led to a higher transpiration rate within corn plants, and increased Hg uptake from the roots to the stems. Although roots serve as a barrier to the translocation of most Hg, a small fraction may be transported to the leaves via the transpiration stream (Bishop et al., 1998). Lindberg et al. (1998) observed lower Hg flux during drought stress in a mature forest, most likely due to stomatal closure. This implies photosynthesis may drive Hg translocation between roots, stems and possibly leaves, although this hypothesis needs further testing.

4. Conclusion

The concentration of soil and root Hg, and organic content from the control site were not significantly different from the sewage-irrigated site, indicating no disparity between the two fields, despite the historic practice of sewage irrigation. Hg concentration in roots was positively correlated with soil Hg concentration, while Hg concentration in stems was negatively correlated with root Hg concentration. Differences in stem Hg content between the two fields may be due to differences in soil water content, which was significantly higher in the control site, and positively correlated with stem Hg. Lower

moisture content may lead to stomatal closure, decreased transpiration, and lower Hg levels in stems; however, this hypothesis needs further testing.

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Table 5

Correlation matrix for Hg concentration in soil, roots and stems, and soil water content ($n = 6$ for all parameters)

	Soil Hg ($\mu\text{g g}^{-1}\text{ dw}$)	Root Hg ($\mu\text{g g}^{-1}\text{ dw}$)	Stem Hg ($\mu\text{g g}^{-1}\text{ dw}$)	Soil water content (%)
Soil Hg ($\mu\text{g g}^{-1}\text{ dw}$)	1	0.95	−0.86	−0.59
Root Hg ($\mu\text{g g}^{-1}\text{ dw}$)		1	−0.86	−0.56
Stem Hg ($\mu\text{g g}^{-1}\text{ dw}$)			1	0.83
Soil water content (%)				1

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