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Atmospheric deposition exposes Qinling pandas to toxic pollutants

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Abstract. The giant panda (*Ailuropoda melanoleuca*) is one of the most endangered animals in the world, and it is recognized worldwide as a symbol for conservation. A previous study showed that wild and captive pandas, especially those of the Qinling subspecies, were exposed to toxicants in their diet of bamboo; the ultimate origin of these toxicants is unknown. Here we show that atmospheric deposition is the most likely origin of heavy metals and persistent organic pollutants (POPs) in the diets of captive and wild Qinling pandas. Average atmospheric deposition was 199, 115, and 49 $g \cdot m^{-2} \cdot yr^{-1}$ in the center of Xi'an City, at China's Shaanxi Wild Animal Research Center (SWARC), and at Foping National Nature Reserve (FNNR), respectively. Atmospheric deposition of heavy metals (As, Cd, Cr, Pb, Hg, Co, Cu, Zn, Mn, and Ni) and POPs was highest at Xi'an City, intermediate at SWARC, and lowest at FNNR. Soil concentrations of the aforementioned heavy metals other than As and Zn also were significantly higher at SWARC than at FNNR. Efforts to conserve Qinling pandas may be compromised by air pollution attendant to China's economic development. Improvement of air quality and reductions of toxic emissions are urgently required to protect China's iconic species.

Key words: air pollution; atmospheric deposition; Conservation biology; heavy metals; POPs pollution; Qingling panda.

Introduction

The giant panda (*Ailuropoda melanoleuca* David, 1869) is one of the most endangered animals in the world and a worldwide symbol for conservation. Two strategies, developed in the last several decades, are now used to protect this flagship endangered species. One strategy uses ex situ breeding in, for example, the zoos of Beijing and the seven breeding centers, established since the 1950s, of Wolong and Chengdu. The other strategy has been to establish natural conservation zones that preserve panda habitat: 50 conservation zones, with a total area >20000 km^2, have been delimited (Zhang and Wei 2006). In these conservation zones, efforts are ongoing to reduce habitat destruction, logging, resource exploitation, and tourism, all of which threaten wild panda populations.

As China's economy has developed rapidly, environmental problems have emerged. This trade-off of environmental quality for economic development has been common in developed nations (Seinfeld 2004), and in China it has had predictable effects of particulate pollution influencing air quality, regional and global climates, and human health (Cao et al. 2011, Wang et al. 2014, Wang et al. 2015). For example, in 2013, China experienced extremely severe and persistent haze pollution: measurements of average daily concentrations of $PM_{2.5}$ (particulate matter with an aerodynamic particles <2.5-μm diameter) in 74 major cities exceeded the Chinese pollution standard of 75 $\mu g/m^3$. During the same year, a maximum daily concentration of 772 $\mu g/m^3$ was observed over 1.3 million km^2, affecting the health of at least 800 million people (China National Environmental Monitoring Centre 2013).

Xi'an, one of the largest cities in China, is situated on the Guanzhong Plain at the northern edge of the Qinling Mountains. This city has a resident population of approximately eight million people and receives at least two million visitors annually. Between 2005 and 2010, the 24-h $PM_{2.5}$ in Xi'an ranged from 130 to 351 $\mu g/m^3$ (Han et al. 2010, Shen et al. 2011), exceeding Chinese government standards two- to fivefold. Intense "haze-fog" events occur regularly, making air pollution one of the most important environmental issues in Xi'an (Cao et al. 2012),

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and of far more concern than in the much-less industrialized and nearby smaller cities of Xiangyang and Baoji.

Xi'an also is home to the Shaanxi Wild Animal Research Center (SWARC; 34°06′ N, 108°32′ E). Established in 1987, SWARC is on the north slope of the Qinling Mountains and is dedicated to the conservation of the golden monkey (*Rhinopithecus roxellana* Milne-Edwards, 1870), golden takin (*Budorcas taxicolor* Hodgson, 1850), Crested Ibis (*Nipponia nippon* Temminck, 1835), and the Qinling subspecies of giant panda, of which only 345 individuals remain (Sun et al. 2005, SFA 2015). Captive pandas at SWARC and wild pandas elsewhere in the region are exposed to heavy metals and persistent organic pollutants (POPs), including PCBs (polychlorinated biphenyls), PCDDs (polychlorinated dibenzo-p-dioxins), and PCDFs (polychlorinated dibenzofurans) through their diet of bamboo (Chen et al. 2016). However, the ultimate origin of these pollutants is not known. Here we test the hypothesis that these pollutants are derived from atmospheric deposition.

Methods and Materials

Sample collection

Atmospheric deposition samples were collected from November 2013 to November 2014 at the Foping National Nature Reserve (FNNR; Qinling Mountain, 33°33′–33°46′ N, 107°40′–107°55′ E), Shaanxi Wild Animal Research Center (SWARC; Louguantai, Zhouzhi County, Xi'an city, 34°06′ N, 108°32′ E), and Xi'an City (34°23′ N, 108°89′ E) using standard collection methods (full details in Qian and Dong 2004). Samples of dry deposition and precipitation were collected continuously for one year into 66 × 40 × 12 cm plastic containers located at four sites at FNNR, three at SWARC, and four in Xi'an City. During the sampling period, purified water was added to the containers to avoid the collected deposition being blown out of the containers. After collection, the containers were rinsed with purified water to release particles deposited or sorbed onto the container walls. At the same time, soils samples also were collected from FNNR and at SWARC where bamboos are planted to feed captive pandas. Both the suspensions and the soil samples were dried to a constant mass at 60°C before being homogenized with a ball mill.

Heavy metal analysis

A 500-mg portion of each sample was placed into a Teflon digestion vessel to which was added 11 mL GR-grade acid digestion mixture (1 mL HNO3, 3 mL HCl, 5 mL HF, 2 mL $HClO_4$) for digestion with an electric hot plate. After digestion, samples were diluted to 50 mL with ultrapure water (18.2 MΩ/cm^2 Milli-Q water, Millipore, France). Heavy metal concentrations were analyzed using atomic absorption spectroscopy (AAS; ZEEnit 700P, Analytik, Jena, Germany). Concentrations of Cu, Co, Zn, Mn, and Cr were measured using the air–acetylene flame method with electrically modulated deuterium–HCl background correction. The hydride-forming elements As and Hg were measured using the HS55 Hydride System (AAS; ZEEnit 700P, Analytik, Jena, Germany). Concentrations of Cd, Ni, and Pb were measured using a graphite furnace AAS coupled to a MPE 60 (ZEEnit 700P, Analytik, Jena, Germany) graphite autosampler with two-field mode Zeeman effect background correction. Heavy metal concentrations are expressed as μg/g dry mass.

Analysis of persistent organic pollutants

Sample extraction, cleanup, and chemical analysis of POPs followed established methods with some modifications (Liu et al. 2006, Li et al. 2008, Chen et al. 2016). Samples from atmospheric deposition were freeze dried before being spiked with ^{13}C-labeled surrogate standards (Environmental Protection Agency [EPA] methods 1613B and 1668A) and undergoing accelerated solvent extraction with dichloromethane: hexane (1:1). Each sample extract was adjusted to 50 mL with hexane; 15 g of acid silica (30% w/w) was added to remove lipids. The acid silica was stirred for 2 h and the extract was poured through ≈5 g of anhydrous sodium sulfate. All extracts were concentrated to 2 mL by rotary evaporation before cleanup. All solvents were purchased from Fisher (Fair Lawn, New Jersey, USA). Silica gel was obtained from Merck (silica gel 60; Merck, Darmstadt, Germany). Basic alumina was obtained from Aldrich (Brockmann I, standard grade; Aldrich, Milwaukee, Wisconsin, USA). Florisil was obtained from Riedel-de Haën (60–100 mesh ASTM; Seelze, Germany).

PCBs, PCDDs, and PCDFs were analyzed by the POP laboratory at the Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences; all concentrations were corrected for lipid mass. Twenty-five PCB congeners, including 12 dioxin-like congeners, were quantified with an isotope dilution method using high-resolution gas chromatography coupled with high-resolution mass spectrometry (HRGC/HRMS). Total organic carbon (TOC) concentration was analyzed on a TOC Analyzer (O.I. Analyzer, College Station, Texas, USA). A 0.1-g sample was weighed and loaded into the combustion cup, which was packed with quartz wool. Prior to combustion, the samples were wetted with 5% phosphoric acid and heated to 250°C for 1 min to purge inorganic carbon. The signal was detected by non-dispersed infrared (NDIR) detection when flashed at 900°C for 6 min in the combustion chamber. Calibration standard solutions, $^{13}C_{12}$-labeled surrogate standards, and $^{13}C_{12}$-labeled injection standards were purchased from Wellington Laboratories (Guelph, Ontario, Canada).

Quantification of 17 PCDD and PCDF homologues was done by HRGC/HRMS on an Agilent 6890 gas chromatograph coupled with an Autospec Ultima mass spectrometer (Waters Micromass, Manchester, UK) operating in EI mode at 35 eV; the trap current was 600

IA. The GC was equipped with a CTC PAL autosampler. Samples of 1 or 2 mL were injected in splitless mode (splitless time, 2 min for PCDD/Fs) in a DB-5MS fused silica capillary column (60 m for PCDD/Fs and PCBs) with helium as carrier gas at a constant flow rate of 1.2 mL/min. The oven temperature programs were as follows: for PCDD/Fs, start 150°C held for 3 min, 150–230°C at 20°C/min held for 18 min, 230–235°C at 5°C/min held for 10 min, 235–320°C at 4°C/min held for 3 min; for PCBs, start 120°C held for 1 min, 120–150°C at 30°C/min, 150–300°C at 2.5°C/min held for 1 min.

Statistical analysis

All statistical analyses were done using the SPSS 20.0 software (IBM SPSS Statistics, IBM Corp., USA); the significance level was set at $P < \alpha = 0.05$. Amounts of atmospheric heavy metals deposition from FNNR, SWARC, and Xi'an City were compared using one-way ANOVA followed by Tukey post-hoc tests. Comparisons of heavy metals concentrations in soils were done using *t* tests. Because PCCD, PCDF, and PCB congeners differ in toxicity, toxic equivalency factors (set by the World Health Organization), were used to calculate a single toxic equivalent (WHO-TEQ) for each sample (Van den Berg et al. 2006).

Results

The annual average rate (2013–2014) of atmospheric deposition of dust was 199 ± 6.50 $g \cdot m^{-2} \cdot yr^{-1}$ in the center of Xi'an City, 115 ± 9.84 $g \cdot m^{-2} \cdot yr^{-1}$ at SWARC, but only 49 ± 6.79 $g \cdot m^{-2} \cdot yr^{-1}$ at FNNR. Deposition rates of all assayed heavy metals were significantly lower at FNNR than at SWARC (Fig. 1), and all but As were significant lower at SWARC than at Xi'an. In parallel, concentrations of all assayed heavy metals except for As and Zn in soils around SWARC were significantly higher than in soils around FNNR (Fig. 2). Concentrations of Cd, Pb, Zn, and Mn at both SWARC and FNNR exceeded soil background criteria, whereas concentrations of Hg exceeded the soil background criterion only at SWARC (Fig. 2). There were significant positive correlations in the concentrations of these metals in deposited dust between Xi'an and SWARC ($r = 0.98$), and between Xi'an and FNNR ($r = 0.87$).

Deposition rates of dioxin and dioxin-like compounds (PCDDs and PCDFs) and PCBs were highest in Xi'an, intermediate at SWARC, and lowest at FNNR (Fig. 3). Seventeen congeners of PCDD/Fs and 12 of PCBs were detected in atmospheric deposition of PM (Table 1). The most prevalent PCDD/Fs were 1,2,3,4,6,7,8-HeptoCDF, OctaCDF, and 1,2,3,4,6,7,8-HeptaCDD, whereas the most prevalent PCBs were 3,3′,4,4′-TetraCB, 2,3,3′4,4′-PentaCB, 2,3′,4,4′,5-PentaCB. The WHO-TEQ for PCDD/Fs and PCBs (Fig. 3c, d) paralleled trends in atmospheric deposition rates of total PCDD/Fs and PCBs (Fig. 3a, b).

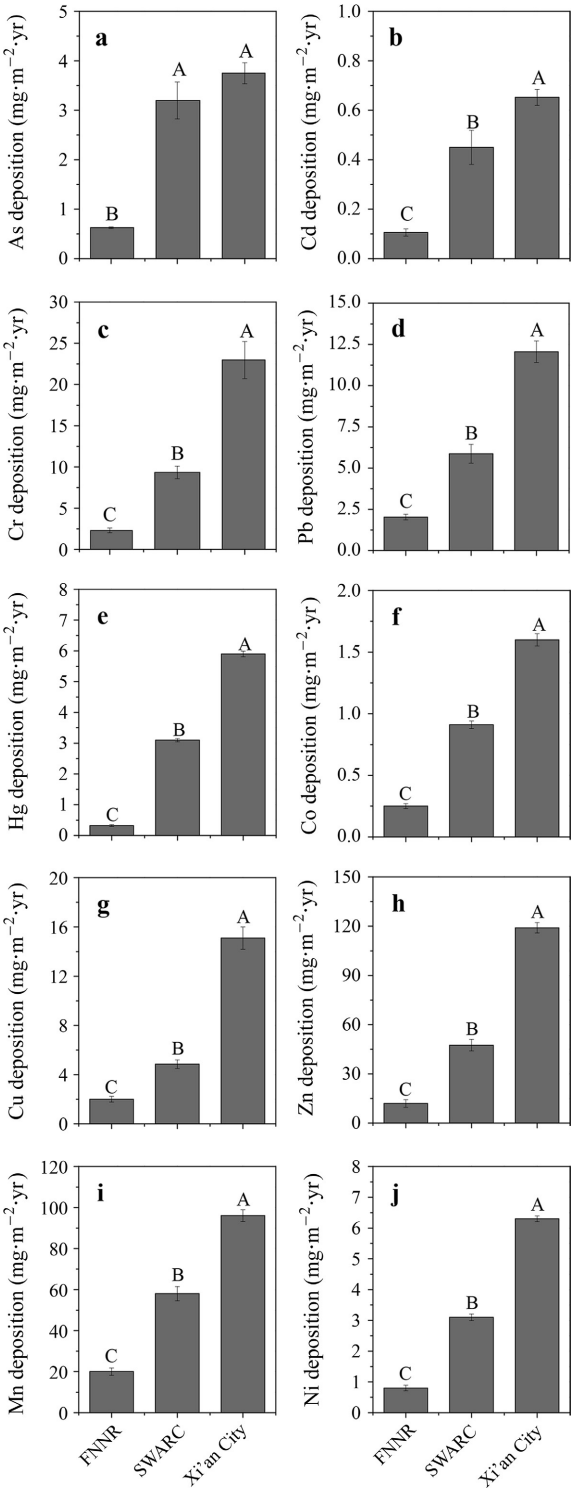

Fig. 1. Atmospheric deposition (mean ± SE) of heavy metals: (a) As, (b) Cd, (c) Cr, (d) Pb, (e) Hg, (f) Co, (g) Cu, (h) Zn, (i) Mn, and (j) Ni at the three studied sites (FNNR [$n = 4$], SWARC [$n = 3$], and Xi'an City [$n = 4$]) over a 1-yr period. Differences among means at the three sites were compared using one-way ANOVA; different letters denote significant differences ($P < 0.05$; Tukey post-hoc test).

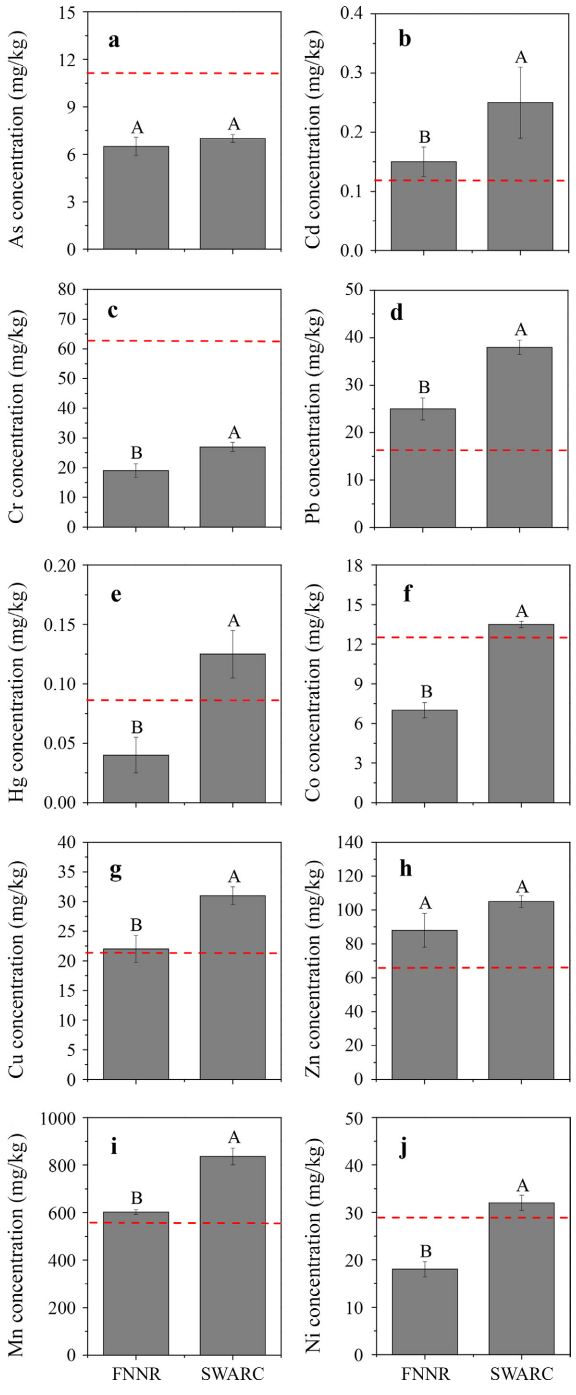

FIG. 2. Comparison of toxic metals concentrations in soils at the southern (FNNR) and northern (SWARC) slopes of the Qinling Mountains. Bars illustrate amounts (mean ± SE) of soil (a) As, (b) Cd, (c) Cr, (d) Pb, (e) Hg, (f) Co, (g) Cu, (h) Zn, (i) Mn, and (j) Ni at FNNR ($n = 4$) and SWARC ($n = 3$). A dashed red line in each panel indicates the soil background criteria value for polluted soils (CNEMC 1990). Different letters denote significant differences ($P < 0.05$; t test). [Color figure can be viewed at wileyonlinelibrary.com]

DISCUSSION

The Qinling Mountain region is home to a number of threatened and endangered species, including the golden monkey, golden takin, Crested Ibis, and the Qinling subspecies of the giant panda. The Shaanxi Wild Animal Research Center, which focuses on ex situ conservation of these species, is on the North Slope of the Qinling Mountains and near Xi'an city. Intense "haze-fog" events have taken place in this region many times in recent years, and air pollution has become one of the important environmental issues in Xi'an. We explored possible relationships between atmospheric deposition of heavy metals and POPs in light of the previously documented exposure to all of these toxicants in the diet of pandas (Chen et al. 2016) and to some heavy metals in captive monkeys at SWARC (Liu et al. 2015).

The high annual deposition rate of metal- and pollutant-laden dust at Xi'an and SWARC originates from coal combustion, from bare soil surfaces in the surrounding areas, heavy traffic and a large number of on-going construction sites in the cities (Wang et al. 2014, Wang et al. 2015). The elevated levels of metals in deposition parallels that found in bamboo fed to captive pandas at SWARC (Chen et al. 2016) and far exceeds that found in both deposition (Figs. 1 and 2) and in bamboo eaten by pandas in the wild (at FNNR; Chen et al. 2016). Correlations in the concentrations of metals between Xi'an City and either SWARC or FNNR suggest a source for these metals in the industrial activity and traffic in Xi'an (Dun and Tan 2013, Ha et al. 2014), upwind from both SWARC and FNNR. Bamboo fed to pandas at SWARC is grown without fertilizers or supplemental irrigation, and so there appear to be no other local or regional sources other than atmospheric deposition for the toxicants measured in these plants. Although direct effects of these heavy metals have not been measured in pandas, these metals all have serious health effects in humans and other mammals (e.g., Rodier 1955, Friberg et al. 1985, Buchet and Lauwerys 1989, Winge and Mehra 1990, Rowbotham et al. 2000, Falcón et al. 2003, Doreswamy et al. 2004).

In parallel, deposition rates of dioxin and dioxin-like compounds (PCDDs and PCDFs) and polychlorinated biphenyls (PCBs) were highest in Xi'an, intermediate at SWARC, and lowest at FNNR (Fig. 3). Like heavy metals, PCDDs and PCDFs are by-products of combustion and industrial processes (Fiedler 2007); these persistent organic pollutants are known human carcinogens and endocrine disruptors (Mai et al. 2005, Imamura et al. 2007). In contrast, PCBs were once used widely as nonflammable insulators and heat-exchange fluids (De Voogt et al. 1990), but their production ceased in 1974. Nonetheless, their long-term and continuing persistence in the environment and in tissues of living organisms has been associated with reduction in reproductive success,

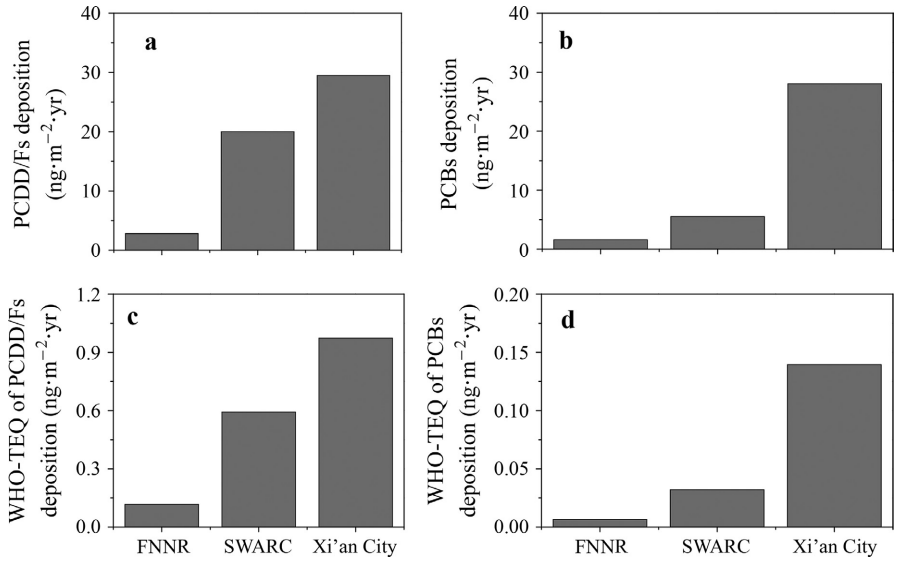

FIG. 3. Total atmospheric deposition of (a) PCDD/Fs and (b) PCBs (b), and the WHO-TEQs of (c) PCDD/Fs and (d) PCBs at the three different sites (FNNR, SWARC, and Xi'an City) over a 1-yr period. Values are from four pooled parallel samples (collection at same time) for FNNR and Xi'an City and three pooled parallel samples for SWARC. Data for individual congeners of PCBs, PCDDs, and PCDFs are given in Table 1.

TABLE 1. Concentrations (ng·m^{-2}·yr^{-1}) of congeners of persistent organic pollutants (POPs) in atmospheric deposition at the three sampled sites (FNNR, SWARC, and Xi'an City).

Type of POP and congener	FNNR	SWARC	Xi'an City
PCDD/F			
2,3,7,8-TetraCDF	0.04	0.23	0.71
1,2,3,7,8-PentaCDF	0.07	0.37	0.66
2,3,4,7,8-PentaCDF	0.05	0.40	0.64
1,2,3,4,7,8-HexaCDF	0.11	0.70	1.21
1,2,3,6,7,8-HexaCDF	0.10	0.71	1.06
2,3,4,6,7,8-HexaCDF	0.13	0.73	1.21
1,2,3,7,8,9-HexaCDF	0.03	0.13	0.26
1,2,3,4,6,7,8-HeptoCDF	0.46	3.32	3.95
1,2,3,4,7,8,9-HeptaCDF	0.10	0.37	0.45
OctaCDF	0.39	3.48	4.32
2,3,7,8-TetraCDD	0.01	0.03	0.05
1,2,3,7,8-PentaCDD	0.03	0.05	0.08
1,2,3,4,7,8-HexaCDD	0.02	0.14	0.26
1,2,3,6,7,8-HexaCDD	0.05	0.18	0.22
1,2,3,7,8,9-HexaCDD	0.05	0.15	0.27
1,2,3,4,6,7,8-HeptaCDD	0.80	1.38	2.51
OctaCDD	0.48	5.68	10.30
PCB			
3,3′,4,4′-TetraCB	0.24	1.13	6.38
3,4,4′,5-TetraCB	0.04	0.15	0.72
3,3′,4,4′,5-PentaCB	0.06	0.27	1.26
3,3′,4,4′,5,5′-HexaCB	0.03	0.08	0.27
2,3,3′4,4′-PentaCB	0.21	0.75	5.32
2,3,4,4′,5-PentaCB	0.08	0.17	0.73
2,3′,4,4′,5-PentaCB	0.05	1.79	9.31
2′,3,4,4′,5-PentaCB	0.16	0.11	0.29
2,3,3′,4,4′,5-HexaCB	0.17	0.32	1.30
2,3,3′,4,4′,5′-HexaCB	0.03	0.15	0.39
2,3′,4,4′,5,5′-HexaCB	0.06	0.07	0.66
2,3,3′,4,4′,5,5′-HeptaCB	0.07	0.14	0.50

Note: Data are from four pooled samples from FNNR and Xi'an City and from three pooled samples from SWARC.

birth defects, and behavioral changes (Mai et al. 2005, Imamura et al. 2007).

The most prevalent congeners of PCDD/Fs recovered in samples of atmospheric deposition were 1,2,3,4,6,7,8-HeptoCDF, OctaCDF and 1,2,3,4,6,7,8-HeptaCDD, whereas the most prevalent PCBs were 3,3′,4,4′-TetraCB, 2,3,3′4,4′-PentaCB, 2,3′,4,4′,5-PentaCB (Table 1). Total Concentrations of PCDD/Fs were higher at Xi'an than SWARC. Together, these data indicated that the main toxic organic chemicals deposition at SWARC originated from Xi'an.

Atmospheric deposition of pollutants in China, including in and around Xi'an, has been increasing rapidly (Cao et al. 2011). Deposition of heavy metals and POPs has resulted in high concentrations of these toxicants in soils, well in excess of established soil background criteria. POPs and heavy metals can be inhaled directly by pandas or taken up by bamboo and subsequently bioaccumulate in pandas. Our results suggest that urban and industrial areas are the main sources of these environmental toxicants, and pandas in captive breeding centers near cities are at greater risk than pandas in natural reserves further from urban areas and industrial centers. Rapid action to improve atmospheric conditions, including efforts to decrease automobile emissions, reduce coal usage, and improve urban efficiency should parallel efforts to relocate pandas from urban-based captive breeding centers to environmentally cleaner areas.

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Data Availability

Data associated with this paper have been deposited in a Dryad digital repository https://doi.org/10.5061/dryad.g0p8b