Captive pandas are at risk from environmental toxins

Captive pandas are at risk from environmental toxins

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Ex situ conservation efforts are the last resort for many critically endangered species and captive breeding centers are thought to provide a safe environment in which to produce individuals for eventual re-introduction to the wild. 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. Here, we report that captive pandas of the Sichuan and Qinling subspecies are exposed to high concentrations of persistent organic pollutants, including polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PDCFs), and biphenyls (PCBs), as well as heavy metals (arsenic, cadmium, chromium, and lead). Further analysis of the *ex situ* environment of the Qinling subspecies demonstrated that contaminated food supplies exposed captive Qinling pandas to high concentrations of PCDD, PCDFs, PCBs, As, Cd, Cr, and Pb). In the short term, these endangered animals should be relocated to breeding centers in less contaminated areas. Their long-term survival, however, depends on reducing emissions of toxic pollutants throughout China.

Key words: Endangered species, *ex situ* conservation, Giant panda, heavy metals, persistent organic pollutants.

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. The panda lineage is at least 11.6 million years old (Abella *et al.* 2012); fossils > 2 million years old and historical records have revealed that pandas once were distributed in at least 18 of China's 23 provinces (Zhu and Long 1983). Until the mid-19th century, giant pandas still inhabited most of eastern and southern China (Hunan, Hubei, Sichuan, Shaanxi and Gansu provinces), but their range has declined in recent years as a result of hunting, habitat destruction, logging, resource exploitation, and tourism (Zhang *et al.* 2013). Giant pandas now survive only in small, fragmented conservation zones in the Qinling, Bashan and Qionglai Mountains (Zhang *et al.* 2013) and in *ex situ* breeding centers including the zoos of Beijing and the breeding centers of Wolong and Chengdu.

It is generally assumed that the conservation areas and the captive breeding centers protect giant pandas from the adverse impacts of human activities. However, their presumed safety may be compromised by the dissemination of widespread pollutants into conservation zones or the proximity of breeding centers to more heavily-polluted urban areas. For example, perfluorinated compounds used in consumer and industrial products as surfactants, surface protectors, and fire-fighting foams have been found in serum samples taken from giant pandas in the Beijing zoo as well as from red pandas (*Ailurus fulgens*) in a number of other zoos and wild animal parks in

China (Dai et al. 2006). However, the extent to which either wild pandas or pandas in breeding centers are exposed to persistent organic pollutants (POPs) and heavy metals that can accumulate in their tissues, compromise their health, and potentially affect the success of ongoing conservation programs remains unknown.

Here, we present data illustrating that giant pandas in *ex situ* breeding centers are exposed to much greater concentrations of POPs and heavy metals than their wild counterparts. Our data suggest that the bamboo fed to the pandas is the proximate source of these compounds. Consequently, urgent action is needed to safeguard these conservation icons, both in captivity and in the wild.

Materials and Methods

Faecal droppings, which can be used as non-invasive indicators of pollutant exposure (Christensen *et al.* 2013), were collected from wild pandas in the Wolong and Foping National Nature Reserves, and from captive pandas housed in China Conservation and Research Center for the Giant panda (CCRCGP) and the Shaanxi Wild Animal Research Center (SWARC) (Fig.1). The CCRCGP is the largest captive panda breeding center for the Sichuan subspecies of giant panda, and SWARC is the only breeding center for the Qinling subspecies. Samples of bamboo, the primary food for giant pandas, were collected in the wild from Foping and from plants grown at SWARC.

Mixed feedstuff, fed to pandas as a nutrient supplement, was also sampled from SWARC. Additional details on sample collection are provided in the Supplemental Online Material.

The faecal droppings, plant tissue, and feedstock samples all were dried to constant mass, digested, and analyzed using standard methods. Determination of concentrations of POPs in the samples was done using (high-resolution mass spectrometry (Liu *et al.*2006; Li *et al.* 2008) at the Research Center for Eco-environmental Sciences of the Chinese Academy of Sciences. Concentrations of heavy metals were determined using atomic absorption or fluorescence spectrometry at the Institute of Earth Environment of the Chinese Academy of Sciences. Complete details on analytical methods, including QA/QC protocols, can be found in the Supplemental Online Material).

Data were analyzed using the SPSS software, version 19.0 (IBM SPSS Inc.). Contaminant concentrations in droppings from wild and captive giant pandas among and between the two subspecies were compared using *t*-tests.

Results and discussion

It is generally thought that pandas in captive breeding centers are better protected from human activities than are wild pandas in nature conservation zones, primarily because *in situ* conservation zones have become more fragmented and less suitable for giant pandas (Liu *et al*. 2001). However, *ex situ* breeding centers usually are close to urban areas and there is an increasing concern that *ex situ* conservation efforts may be being compromised due to environmental pollution associated with urbanization. With China's rapid industrialization and urbanization, environmental pollution is increasing in seriousness and following as it follows a trajectory similar to that previously traversed by developed countries (Seinfeld 2004). This pollution trajectory is having major impacts on public health, as seen in, for example, the > 200 "cancer villages" in China (Yang 2013).

Among the many pollutants, POPs and heavy metals are of significant environmental concern because they may be transported over long distances in air and water (Lohmann *et al*. 2007), are very persistent in the environment, accumulate readily in fatty tissues, and are highly toxic to humans and other mammals (*e.g.,* Qiu 2013; Adriano *et al*. 2014; Fernandez-Rodriguez *et al*. 2015; Syed Ali *et al*. 2015). Three classes of POPs – PCDDs (polychlorinated dibenzo-p-dioxins), PCDFs (polychlorinated dibenzofurans), and PCBs (polychlorinated biphenyls) were found in much higher concentrations in faecal droppings of captive giant pandas than in wild pandas (Fig. 2, WebTables1, 2). POPs were also found at elevated levels in the bamboo fed to captive pandas and their nutrient-supplement feedstock (WebFigures 1, 2). A variety of forms ("congeners") of PCDDs and PCDFs are generated as by-products from various combustion and chemical processes, whereas polychlorinated biphenyls (PCBs) were widely used as dielectric fluids in transformers and capacitors, heat exchange fluids, and as additives in pesticides, adhesives, plastics, and paints because of their insulating and nonflammable properties (Fiedler 2007). Although production of PCBs ceased in 1974, they are still released from old capacitors and transformers and can still be found in various environmental components and in human tissues (Mai *et al.* 2005; Imamura *et al.* 2007).

Because PCDDs, PCDFs, and PCBs occur as congeners that differ in toxicity and toxic equivalency factors, the World Health Organization has defined a single toxic equivalent (WHO-TEQ) that can be calculated to determine total POP exposure (Van den Berg *et al.* 2006). Both total concentrations and the WHO-TEQ for PCDDs, PCDFs, and POPs were higher in droppings collected from captive pandas than they were in wild pandas (Fig. 3). These results are paralleled by total concentrations and WHO-TEQs for the bamboo fed to the pandas and their nutrient-supplement feedstock (WebFigure 2).

Four heavy metals with known toxicity – arsenic (As), cadmium (Cd), chromium (Cr), and lead (Pb) (Brahmia *et al*. 2013; Neal and Guilarte 2013; Uddh-Soderberg *et al*. 2015) – also were found at elevated levels in droppings of captive pandas relative to wild ones (Fig. 3), as well as in their food and their nutrient-supplement feedstock (WebFigure 3). Unlike POPs, these heavy metals occur in the natural environment, but they are readily mobilized by human activities such as mining, automobile use, and overuse of chemical fertilizer.

Our results provide direct evidence that giant pandas are exposed to PCDDs, PCDFs, PCBs, and heavy metals in both *ex situ* captive breeding centers and *in situ* conservation areas, but concentrations of these toxins in pandas are far greater for pandas in captivity. Previous studies have shown that PCDDs and PCDFs are associated with developmental toxicity, immunotoxicity, and reproductive toxicity. PCBs and their breakdown products are known endocrine disrupters, cause the loss of renal cell viability, and are associated with increased risk of chloracne, goiter, anemia, and cancer (Lohmann *et al*. 2007; Qiu 2013; Adriano *et al*. 2014; Fernandez-Rodriguez *et al*. 2015; Gustavson *et al.* 2015; Syed Ali *et al*. 2015). Heavy metal exposure has been associated with increased incidence of cancer (Cr and As), nephrotoxicity and bone damage (Cd), and reduced reproductive function (Pb) (Neal and Guilarte 2013; Brahmia *et al*. 2013; Uddh-Soderberg *et al*. 2015). We conclude that our results belie the notion that captive breeding centers and zoos provide a safe haven from human impacts.

Our results also illustrate that dietary exposure is the dominant, proximal pathway through which giant pandas are exposed to POPs and heavy metals (WebFigures 1-3). Although the food of both captive and wild pandas was enriched in POPs (WebFigures 1, 2) and heavy metals (WebFigure 3), the concentrations of both POPs and metals, and WHO-TEQs of POPs were significantly greater in bamboo eaten by captive pandas (WebFigures 1–3). We note that the nutrient-supplemented feedstock (baked into steamed bread for the pandas) was enriched only in Cd, Cr, and Pb, but not in As, relative to fresh bamboo.

In sum, our data provide clear evidence that giant pandas both in the wild and in captivity are exposed to PCDDs, PCDFs, PCBs, and heavy metals through their diet, and that exposure to these environmental toxins is greater in *ex situ* breeding centers than in *in situ* nature reserves. Because exposure to these environmental toxins is likely to impact negatively the health of these animals, we suggest that urgent action is needed to safeguard these conservation icons. In the short-term, captive breeding centers should be relocated to areas less impacted or contaminated by environmental toxins, and the food provided to captive pandas should be strictly monitored to ensure that it lacks POPs and heavy metals, and is of consistent high quality. In the long term, however, a more sustainable solution will rely on improving air quality through reducing emissions of toxic pollutants.

Acknowledgments

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Figure legends

Figure 1. Sites of sample collection (a); typical dropping of wild pandas (b); and captive pandas (c) at the Shaanxi Wild Animal Research Center (SWARC).

Figure 2. Concentrations of 12 PCB congeners (top) and 17 CDD/F congeners (bottom) in the droppings of wild and captive pandas of the Sichuan and Qinling subspecies of giant pandas. In each of these star plots, the radius is equal to the maximum observed concentration, and concentrations of each individual congeners are scaled to the maximum. The conclusion from these plots is that captive pandas have both more congeners and higher concentrations of them in their faecal samples than wild pandas. Tabular data (actual mean concentrations and the standard errors of the means) are given in WebTables 1 and 2.

Figure 3. Concentrations of (a) all (summed) PCDDs and PCDFs; (b) all (summed) PCBs; (c) WHO-TEQs of PCDDs and PCDFs; and (d) WHO-TEQ of PCBs in faecal samples collected from two subspecies of wild (blue) and captive (red) giant pandas. Bars (means ± 1 SE of the mean from $N = 4$ independent replicates comprising three or four pooled samples) with different letters between the wild and captive pandas for the same subspecies (A or B), or between Sichuan and Qinling subspecies (X or Y) are significantly different ($P < 0.05$, *t*-test).

Figure 4. Concentrations of heavy metals in faecal samples collected from two subspecies of wild (blue) and captive (red) giant pandas. (a) Arsenic (As); (b) Cadmium (Cd); (c) Chromium (Cr); (d) Lead (Pb). Bars (means ± 1 SE of the mean from N = 4 independent replicates comprising three or four pooled samples) with different letters between the wild and captive pandas for the same subspecies (A or B), or between Sichuan and Qinling subspecies (X or Y) are significantly different ($P < 0.05$, *t*-test).

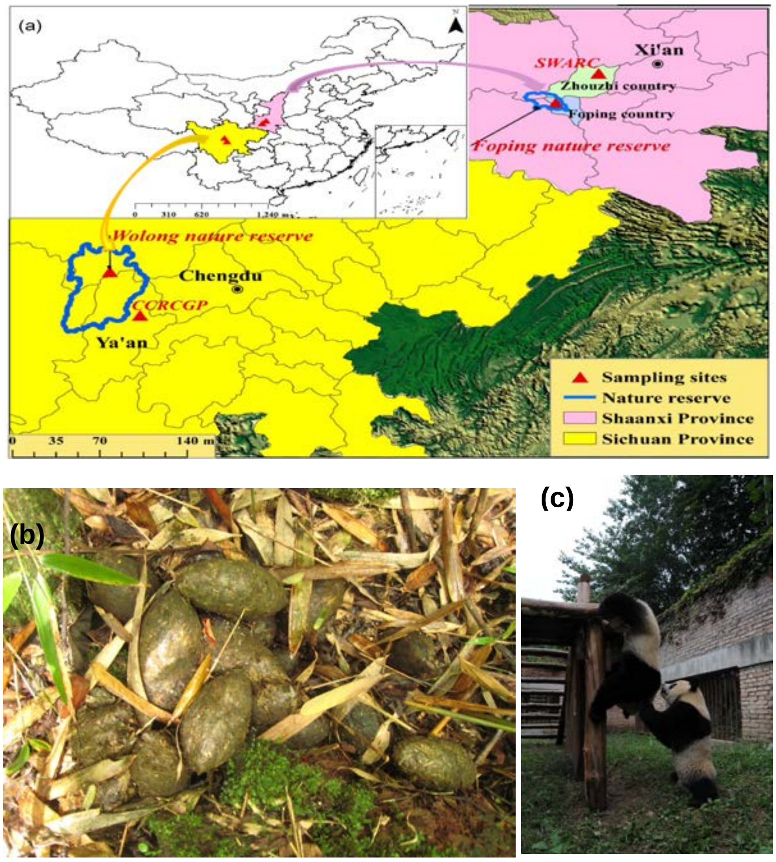
(a)
SWARC
Xi'an
Zhouzhi country
Foping country
Foping nature reserve
Wolong nature reserve
Chengdu
CCRCGP
Ya'an
Sampling sites
Nature reserve
Shaanxi Province
Sichuan Province
(b)
(c)

Figure 1

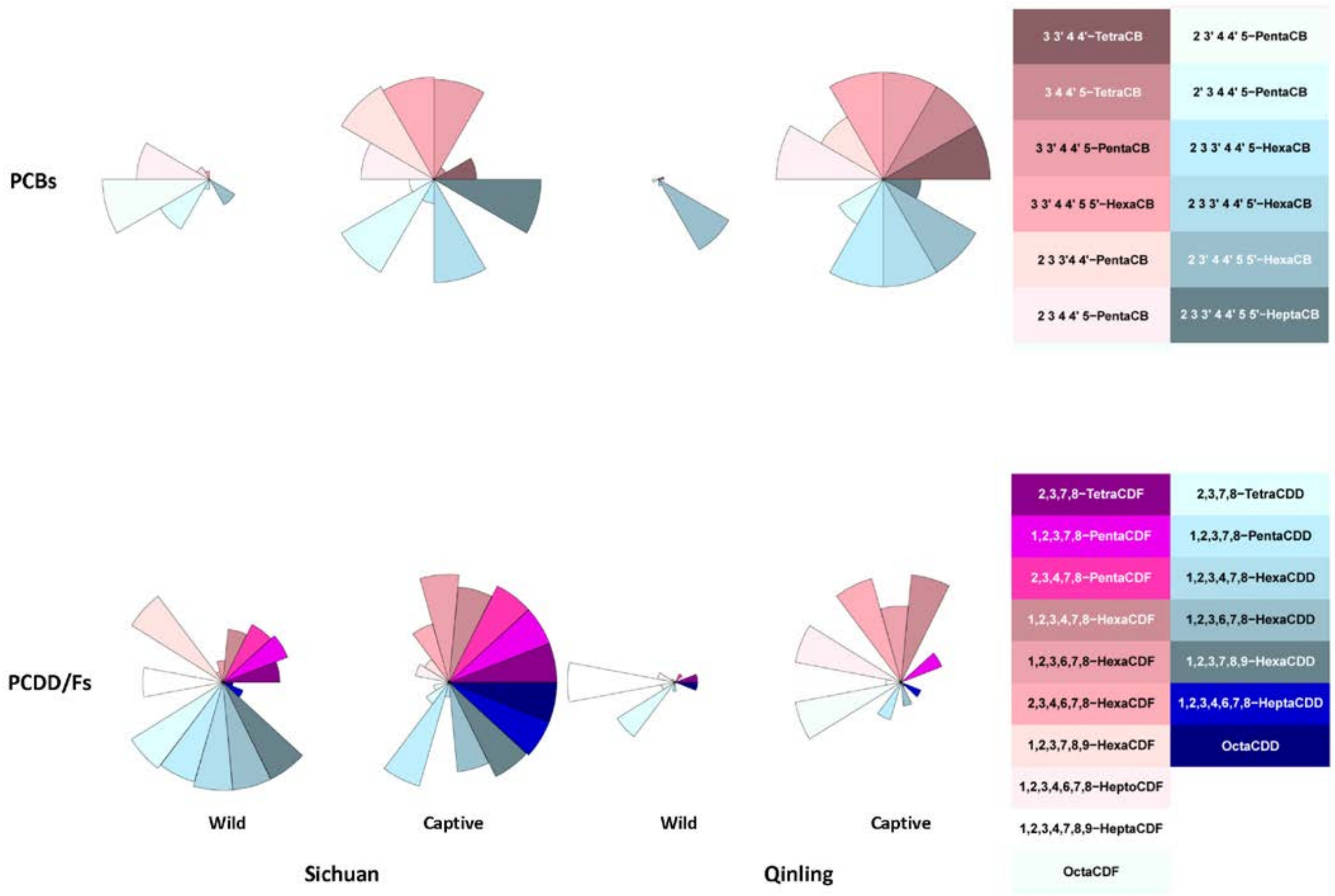
PCBs
PCDD/Fs
Wild
Captive
Wild
Captive
Sichuan
Qinling
3 3' 4 4'-TetraCB
3 4 4' 5-TetraCB
3 3' 4 4' 5-PentaCB
3 3' 4 4' 5 5'-HexaCB
2 3 3'4 4'-PentaCB
2 3 4 4' 5-PentaCB
2 3' 4 4' 5-PentaCB
2' 3 4 4' 5-PentaCB
2 3 3' 4 4' 5-HexaCB
2 3 3' 4 4' 5'-HexaCB
2 3' 4 4' 5 5'-HexaCB
2 3 3' 4 4' 5 5'-HeptaCB
2,3,7,8-TetraCDF
1,2,3,7,8-PentaCDF
2,3,4,7,8-PentaCDF
1,2,3,4,7,8-HexaCDF
1,2,3,6,7,8-HexaCDF
2,3,4,6,7,8-HexaCDF
1,2,3,7,8,9-HexaCDF
1,2,3,4,6,7,8-HeptoCDF
1,2,3,4,7,8,9-HeptaCDF
OctaCDF
2,3,7,8-TetraCDD
1,2,3,7,8-PentaCDD
1,2,3,4,7,8-HexaCDD
1,2,3,6,7,8-HexaCDD
1,2,3,7,8,9-HexaCDD
1,2,3,4,6,7,8-HeptaCDD
OctaCDD

Figure 2

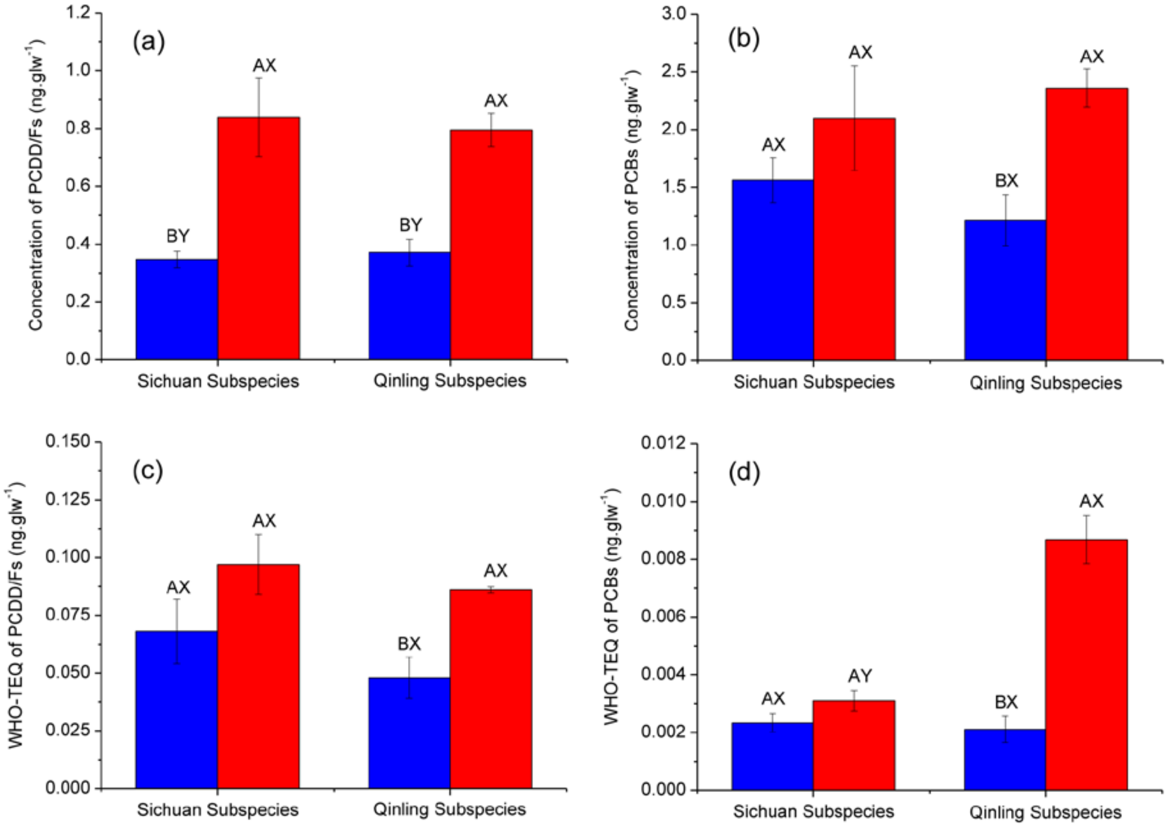
(a)
Concentration of PCDD/Fs (ng.glw-1)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
BY
AX
BY
AX
Sichuan Subspecies
Qinling Subspecies
(b)
Concentration of PCBs (ng.glw-1)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
AX
AX
BX
AX
Sichuan Subspecies
Qinling Subspecies
(c)
WHO-TEQ of PCDD/Fs (ng.glw-1)
0.150
0.125
0.100
0.075
0.050
0.025
0.000
AX
AX
BX
AX
Sichuan Subspecies
Qinling Subspecies
(d)
WHO-TEQ of PCBs (ng.glw-1)
0.012
0.010
0.008
0.006
0.004
0.002
0.000
AX
AY
BX
AX
Sichuan Subspecies
Qinling Subspecies

Figure 3

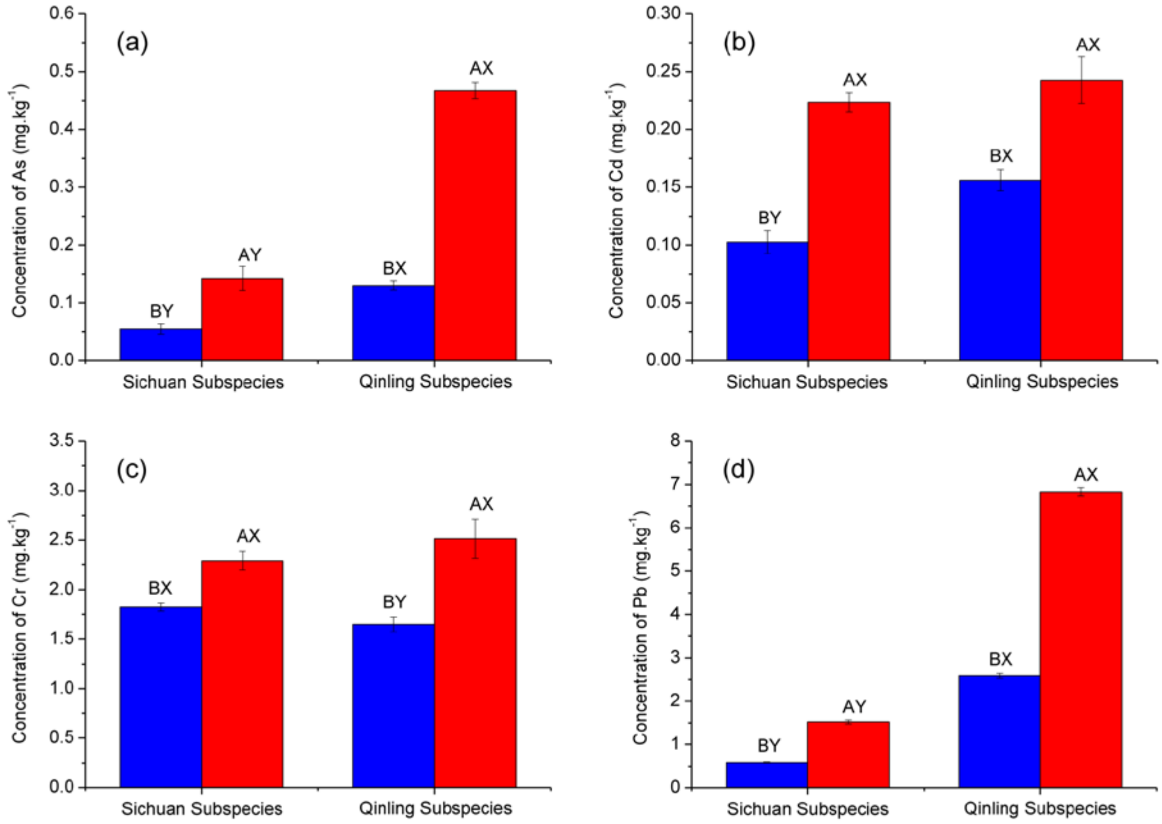
(a)
Concentration of As (mg.kg-1)
BY
AY
BX
AX
Sichuan Subspecies
Qinling Subspecies
(b)
Concentration of Cd (mg.kg-1)
BY
AX
BX
AX
Sichuan Subspecies
Qinling Subspecies
(c)
Concentration of Cr (mg.kg-1)
BX
AX
BY
AX
Sichuan Subspecies
Qinling Subspecies
(d)
Concentration of Pb (mg.kg-1)
BY
AY
BX
AX
Sichuan Subspecies
Qinling Subspecies

Figure 4

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Supplemental Online Material

Additional Materials and Methods

Additional References

WebTables 1, 2

WebFigures 1 – 3

Additional Materials and Methods

Sample collection

All faecal, plant, and feedstock samples were collected from the Wolong National Nature Reserve in the Qionglai Mountains (“Wolong NNR”: 30°45’-31°25’N, 102°52’-103°25’E), the Foping National Nature Reserve in the Qinling Mountains (“Foping NNR”: 33°33’-33°46’N, 107°40’-55’E), the China Conservation and Research Center for Giant Panda (“CCRCGP”: 30°04’N, 102°59’E) and the Shaanxi Wild Animal Research Center (“SWARC”: 34°06’N, 108°32’ E). The CCRCGP is the largest captive breeding center for the Sichuan subspecies of the giant panda. It was relocated to its current location in Bifengxia Ya'an city from Wolong after the 2008 Wenchuan earthquake. SWARC is located in Louguantai, Zhouzhi County, Xi'an city. It was established in 1987 and is the only center for conservation of the Qinling subspecies of the giant panda.

Faecal droppings of wild pandas were collected from 12 sites within the Wolong NNR and 16 sites within the Foping NNR. Sampling locations were 10 km apart and samples were pooled to give three samples/replicate from the Wolong NNR and four samples/replicate from the Foping NNR. Droppings of captive pandas of the Sichuan subspecies were collected at CCRCGP whereas droppings of captive pandas of the Qinling pandas were collected at SWARC. Droppings from either 12 individuals (CCRCGP) or 16 (SWARC) individuals were sampled and pooled into four replicates each comprising of three or four independent samples.

Source of the Environmental Toxins

To investigate the source of the pollutants detected in panda droppings, the Qingling subspecies was studied in more detail. This subspecies was selected because there are about 350 individuals left (State Forestry Administration of the People's Republic of China.2015), so its conservation is much more urgent than that of the Sichuan subspecies. Further, as noted in the Results, the faecal droppings of the Qinling pandas contained significantly higher concentrations of As, Cd and Pb than droppings of the Sichuan subspecies.

Fresh leaves of the primary bamboo fed to these pandas (*Fargesia qinlingensis, Bashania fargesii*) and mixed feedstock used to make nutrient-supplements for the Qingling subspecies were collected from the Foping NNR, from plants cultivated at SWARC, and from feedstock at SWARC. Twelve samples of each food type were collected per location and pooled to produce four replicates each consisting of three samples.

Heavy metal analysis

All samples were dried to constant mass at 60ºC before being homogenized using a ball mill. Dried samples (500 mg) were placed into Teflon bombs to which were added 5 ml of HNO_3 for digestion with a microwave system (CEM, Mars 6, CEM, USA). After digestion, samples were diluted to 50 mL with deionized water. Concentrations of cadmium (Cd), chromium (Cr), and lead (Pb) were measured using a graphite furnace atomic absorption spectrometer (220-FS; Varian Company, USA.) with a hollow cathode lamp (Vigorous Instruments Co., Ltd., Beijing, China) (Yu *et al.* 2001). Concentrations of arsenic (As) were measured using an Atomic Fluorescence Spectrometer (AF-7500; Beijing Dongxi Instruments Co., Ltd., China) with a hollow cathode lamp (Vigorous Instruments Co., Ltd., Beijing, China) (Rahman *et al.* 2000).

Analysis of PCDDs, PCDFs, and PCBs

Samples (dropping, bamboo and feedstuff) were freeze-dried before being spiked with ^{13}C-labeled surrogate standards (Environmental Protection Agency [EPA] method 1613B and 1668A) and underwent accelerated solvent extraction with dichlorinmethene: hexane (1:1). After determining the lipid content of each sample, the 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 of the extracts were concentrated to 2 ml by rotary evaporation.

All solvents were purchased from Fisher (Fairlawn, NJ, USA). Silica gel was obtained from Merck (silica gel 60; Darmstadt, Germany). Basic alumina was obtained from Aldrich (Brockmann I, standard grade; Milwaukee, USA). Florisil was obtained from Riedel-de Haën (60–100 mesh ASTM; Seelze, Germany). Calibration standard solutions, $^{13}C_{12}$-labeled surrogate standards, and $^{13}C_{12}$-labeled injection standards were purchased from Wellington Laboratories (Guelph, Canada).

PCBs, PCDDs, and PCDFs were analyzed at the POP laboratory of the Research Center for Eco-environmental Sciences, Chinese Academy of Sciences; all concentrations were corrected for lipid weight. Sample extraction, cleanup, and chemical analysis followed established methods with some modifications (Liu *et al.*2006; Li *et al.* 2008). Twenty-five PCB congeners, including 12 dioxin-like congeners, were quantified by 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, TX, 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 house.

The quantification of 17 PCDD/PCDF homologues was done using HRGC/HRMS on an Agilent 6890 gas chromatograph coupled with an Autospec Ultima mass spectrometer (Waters Micromass, Manchester, UK) operating in the EI mode at 35 eV with the trap current was 600 lA. The GC was equipped with a CTC PAL autosampler. One or two μL samples 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^{-1} held for 18 min, 230-235°C at 5°C min^{-1} held for 10 min, 235-320°C at 4°C min^{-1} held for 3 min; for PCBs, start 120°C held for 1 min, 120-150°C at 30 °C min^{-1}, 150-300°C at 2.5°C min^{-1} held for 1 min.

Quality control and quality assurance

All data were subject to quality control and quality assurance. All glassware was washed two times with distilled water, and then with dichloromethane after use. After washing, glassware was dried for 6 hours at 400 °C in a muffle furnace.

All performance criteria required for the analysis of PCBs and PCDD/PCDFs followed US EPA methods (1668A and 1613B).^{13}C-labeled surrogated standards

(1668A-LCS and 1613-LCS) were spiked in the sample for qualification and quantification, and ^{13}C-labeled injection standards (EPA 68A-IS and 1613-IS) were added for recovery calculation. The recoveries of the surrogate standards ranged from 76.7±25.2% and 49.2±13.6% for PCB sand PCDD/PCDFs, respectively, which met the requirements of US EPA methods 1668 A and1613 B. Limit of detection (LOD) in the sample was defined as a signal to noise (S/N) ratio = 3. The LOD values were in the range of 0.01–0.82pg g^{-1} for PCBs and 0.04–8.40 pg g^{-1} for PCDD/PCDFs. Laboratory blanks were analyzed with samples quality control at set intervals, and there was no detection of target compounds in the blanks.

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WebTable 1. Concentrations of PCDD and PCDF congeners in faecal droppings from wild and captive pandas of both Sichaun (SS) and Qinling (QS) subspecies. Values are means ± 1 standard error of the mean for *N* = 4 independent replicates each comprising three or four pooled subsamples.

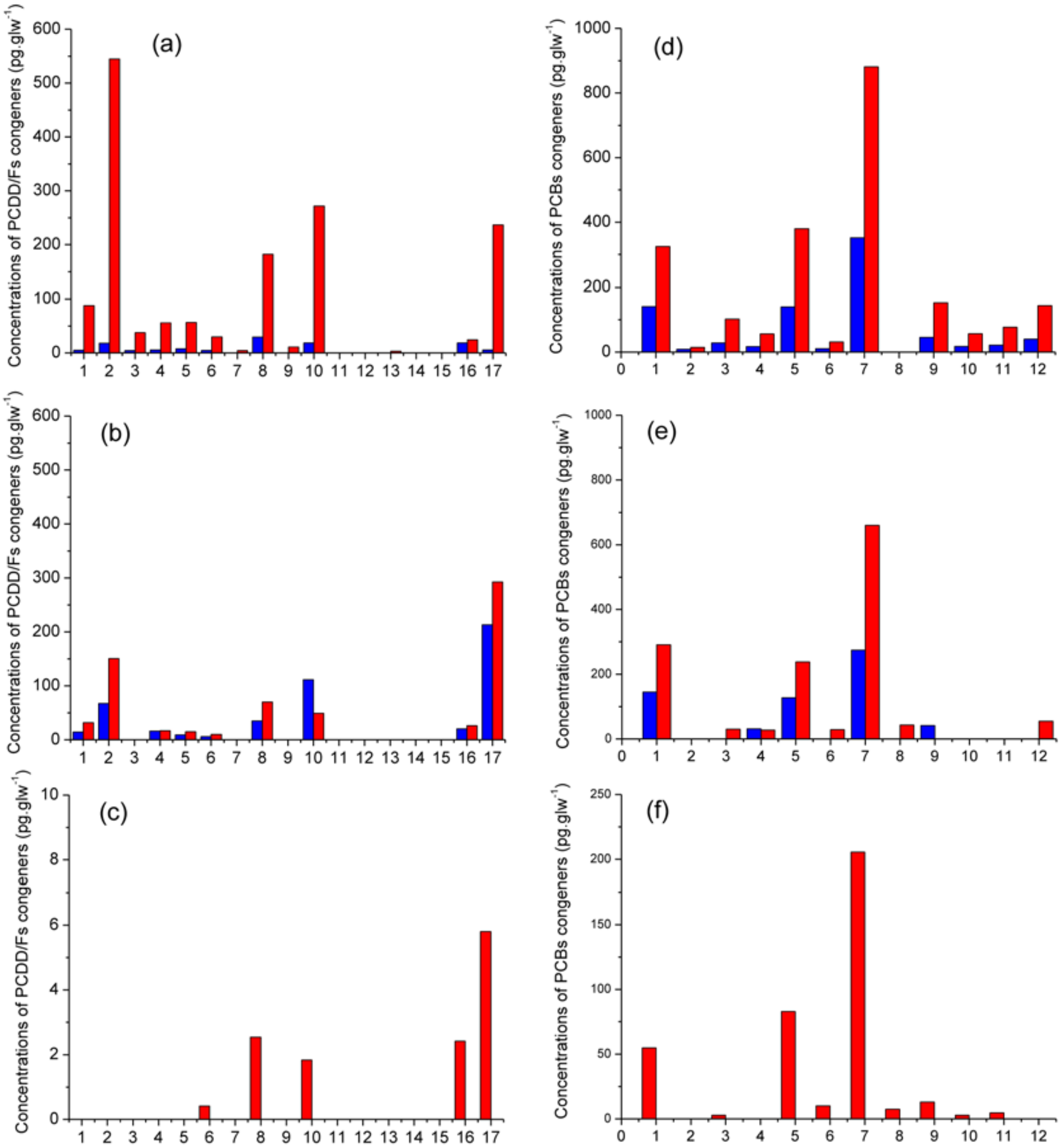
Congeners (pg.glw^{-1})	Wild SS	Captive SS	Wild QS	Captive QS
2,3,7,8-TetraCDF	7.802 ± 2.509	14.278 ± 4.946	3.402± 1.244	0.536 ± 0.473
1,2,3,7,8-PentaCDF;	10.098 ± 2.402	14.536 ± 5.413	2.074 ± 1.278	7.117 ± 7.117
2,3,4,7,8-PentaCDF;	11.585 ± 2.027	18.100 ± 3.694	3.214 ± 1.565	1.816 ±1.816
1,2,3,4,7,8-HexaCDF	11.002 ± 1.477	15.934 ± 6.152	4.868 ± 2.764	17.300 ± 9.700
1,2,3,6,7,8-HexaCDF	8.900 ± 3.097	19.125 ± 5.736	6.352 ± 3.043	15.397 ± 8.576
2,3,4,6,7,8-HexaCDF;	6.568 ± 3.119	15.621 ± 5.895	4.507 ± 2.000	24.320 ± 8.676
1,2,3,7,8,9-HexaCDF	6.582 ± 4.347	2.186 ± 2.001	0.800 ± 0.289	0.579 ± 0.579
1,2,3,4,6,7,8-HeptoCDF	42.796 ± 14.568	65.054 ± 13.461	54.471± 17.196	113.048 ± 37.687
1,2,3,4,7,8,9-HeptaCDF	4.365 ± 2.732	0.566 ± 0.382	5.661 ± 3.843	1.283 ± 1.222
OctaCDF	34.857±5.274	72.098 ± 31.329	67.689 ± 19.035	291.502 ± 165.188
2,3,7,8-TetraCDD	2.521 ± 1.456	0.631 ± 0.494	1.591 ± 1.334	☆
1,2,3,7,8-PentaCDD;	2.846 ± 1.582	2.952 ± 1.450	0.356 ± 0.235	1.289 ± 1.289
1,2,3,4,7,8-HexaCDD	7.514 ± 3.456	1.051 ± 0.698	0.680 ± 0.324	0.053 ± 0.053
1,2,3,6,7,8-HexaCDD	5.676 ± 2.729	4.926 ± 2.046	1.247 ± 0.428	2.216 ± 2.174
1,2,3,7,8,9-HexaCDD	6.654 ± 4.893	6.483 ± 3.772	☆	0.288 ± 0.288
1,2,3,4,6,7,8-HeptaCDD	37.442 ± 5.204	87.879 ± 24.617	24.736 ± 8.696	37.214 ± 22.064
OctaCDD	148.753 ± 8.656	469.836 ± 97.490	189.186 ± 29.664	115.741 ± 92.914

☆ less than the limit of determination.

WebTable 2. Concentrations of PCB congeners in faecal droppings from wild and captive pandas of both Sichaun (SS) and Qinling (QS) subspecies. Values are means ± 1 standard error of the mean for *N* = 4 independent replicates each comprising three or four pooled subsamples.

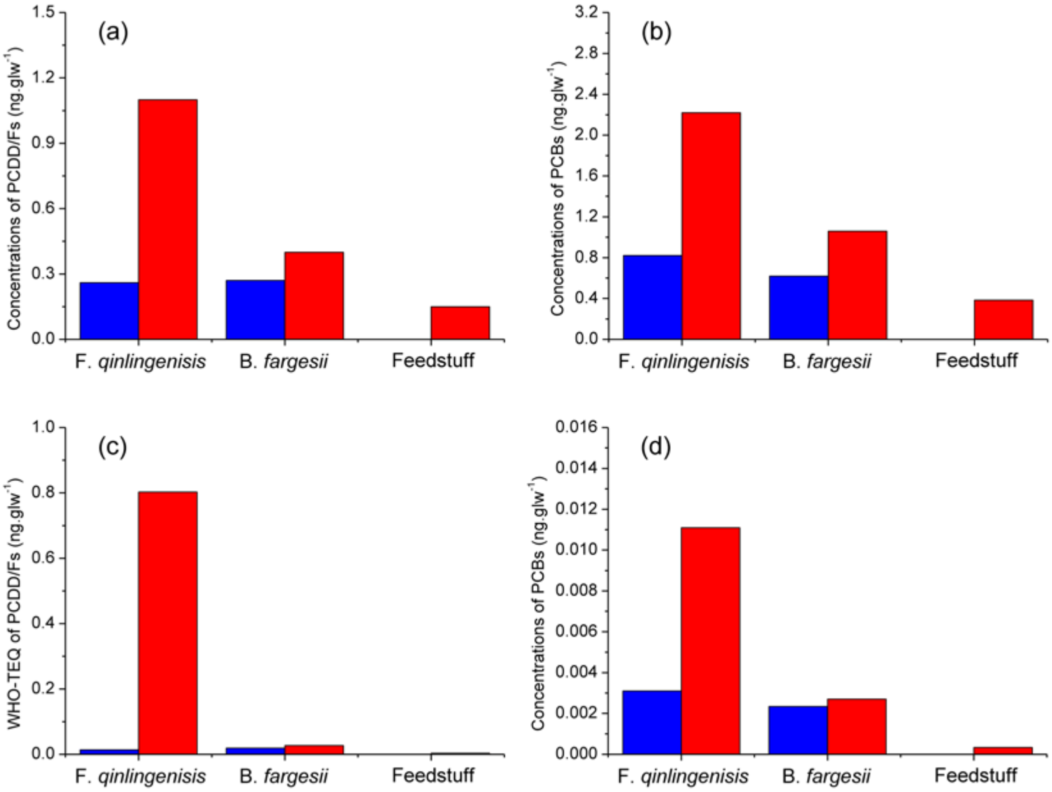
Congeners(pg.glw^{-1})	Wild of SS	Captive of SS	Wild of QS	Captive of QS
3,3',4,4'-TetraCB	72.745 ± 10.721	151.170 ± 22.013	82.564 ± 32.295	270.217 ± 44.520
3,4,4',5-TetraCB	☆	5.768 ± 4.476	☆	45.409 ± 19.320
3,3',4,4',5-PentaCB	7.910± 3.856	155.379 ±101.403	9.495 ± 5.967	165.775 ± 99.209
3,3',4,4',5,5'-HexaCB	1.721 ± 1.629	21.159 ±14.862	☆	22.145 ± 22.145
2,3,3'4,4'-PentaCB	295.723 ± 31.734	716.133 ±246.199	230.065 ± 57.408	556.994 ± 204.311
2,3,4,4',5-PentaCB	54.729 ± 6.431	54.830 ±15.421	21.003 ± 8.061	70.455 ± 9.945
2,3',4,4',5-PentaCB	976.121 ±57.739	788.122 ± 446.010	744.270 ± 135.106	731.055 ± 238.280
2',3,4,4',5-PentaCB	54.104± 5.305	79.313 ±19.382	25.294 ± 3.305	51.228 ±12.658
2,3,3',4,4',5-HexaCB	64.191 ± 4.816	68.530 ±25.965	61.105 ± 4.694	94.781 ± 24.436
2,3,3',4,4',5'-HexaCB	11.179 ± 2.846	35.653 ±13.606	12.693 ± 9.960	36.634 ± 6.900
2,3',4,4',5,5'-HexaCB	24.245 ± 7.676	18.822 ± 10.687	33.975 ± 5.332	38.804 ± 13.105
2,3,3',4,4',5,5'-HeptaCB	☆	4.982 ± 4.860	☆	1.763 ± 1.763

☆ less than the limit of determination.

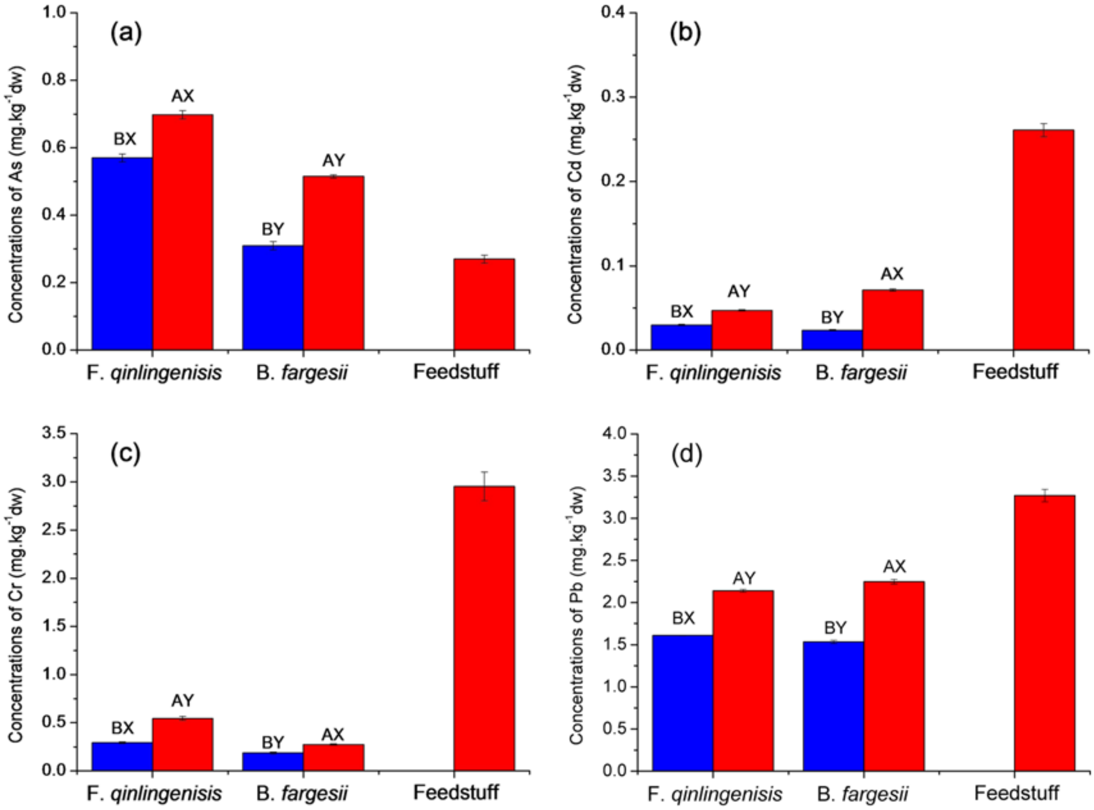


WebFigure 1. The concentrations of congeners of (a, b, c) PCDDs and PCDFs; and (d, e, f) PCBs in the bamboos *Fargesia qinlingensis* and *Bashania fargesii*, and feedstuff of wild (blue) and captive (red) pandas. Numbers on the *x*-axis of panels (a), (b) and (c) denote different congeners of PCDD/F. 1=2,3,7,8-TetraCDF; 2=1,2,3,7,8-PentaCDF; 3=2,3,4,7,8-PentaCDF; 4=1,2,3,4,7,8-HexaCDF; 5=1,2,3,6,7,8-HexaCDF; 6=2,3,4,6,7,8-HexaCDF; 7=1,2,3,7,8,9-HexaCDF; 8=1,2,3,4,6,7,8-HeptoCDF; 9=1,2,3,4,7,8,9-HeptaCDF; 10=OctaCDF;

11=2,3,7,8-TetraCDD; 12=1,2,3,7,8-PentaCDD; 13=1,2,3,4,7,8-HexaCDD; 14=1,2,3,6,7,8-HexaCDD; 15=1,2,3,7,8,9-HexaCDD; 16=1,2,3,4,6,7,8-HeptaCDD; 17=OctaCDD; Numbers on the *x*-axis of panels (d), (e) and (f) denote different congeners of PCBs. 1=3,3',4,4'-TetraCB; 2=3,4,4',5-TetraCB; 3=3,3',4,4',5-PentaCB; 4=3,3',4,4',5,5'-HexaCB; 5=2,3,3'4,4'-PentaCB; 6=2,3,4,4',5-PentaCB; 7=2,3',4,4',5-PentaCB; 8=2',3,4,4',5-PentaCB; 9=2,3,3',4,4',5-HexaCB; 10=2,3,3',4,4',5'-HexaCB; 11=2,3',4,4',5,5'-HexaCB; 12= 2,3,3',4,4',5,5'-HeptaCB.



WebFigure 2. Concentrations of (a) ∑PCDDs and PCDFs, (b) ∑PCBs, (c) WHO-TEQ of PCDDs and PCDFs, and (d) WHO-TEQ of PCBs in the bamboos *Fargesia qinlingensis* and *Bashania fargesii*, and from panda feedstuff. Bars are the value from a single replicate comprising five pooled samples.



WebFigure 3. Concentrations of heavy metals in Qinling subspecies food of wild (blue) and captive (red) from bamboo species (*Fargesia qinlingensis* and *Bashania fargesii*) and feedstuff. (a) Arsenic (As); (b) Cadmium (Cd); (c) Chromium (Cr); (d) Lead (Pb). Bars (means ± 1 SE of the mean from N = 4 independent replicates from three pooled samples) with different letters between the two bamboo species (*Fargesia qinlingensis* and *Bashania fargesii*) (A and B) or between the bamboos fed to wild and captive (X or Y) are significantly different ($P < 0.05$, t-test).