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Highway increases concentrations of toxic metals in giant panda habitat

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Abstract

The Qinling panda subspecies (*Ailuropoda melanoleuca qinlingensis*) is highly endangered with fewer than 350 individuals inhabiting the Qinling Mountains. Previous studies have indicated that giant pandas are exposed to heavy metals, and a possible source is vehicle emission. The concentrations of Cu, Zn, Mn, Pb, Cr, Ni, Cd, Hg, and As in soil samples collected from sites along a major highway bisecting the panda's habitat were analyzed to investigate whether the highway was an important source of metal contamination. There were 11 sites along a 30-km stretch of the 108th National Highway, and at each site, soil samples were taken at four distances from the highway (0, 50, 100, and 300 m) and at three soil depths (0, 5, 10 cm). Concentrations of all metals except As exceeded background levels, and concentrations of Cu, Zn, Mn, Pb, and Cd decreased significantly with increasing distance from the highway. Geo-accumulation index indicated that topsoil next to the highway was moderately contaminated with Pb and Zn, whereas topsoil up to 300 m away from the highway was extremely contaminated with Cd. The potential ecological risk index demonstrated that this area was in a high degree of ecological hazards, which were also due to serious Cd contamination. And, the hazard quotient indicated that Cd, Pb, and Mn especially Cd could pose the health risk to giant pandas. Multivariate analyses demonstrated that the highway was the main source of Cd, Pb, and Zn and also put some influence on Mn. The study has confirmed that traffic does contaminate roadside soils and poses a potential threat to the health of pandas. This should not be ignored when the conservation and management of pandas is considered.

Keywords

Giant panda Habitat Heavy metal Highway Pollution Risk assessment

Introduction

The giant panda is one of the most endangered animals in the world, especially Qinling subspecies (*Ailuropoda melanoleuca qinlingensis*) which is fewer than 350 individuals in the wild (SFA, State Forestry Administration 2015). Since 1978, 13 conservation zones have been established in the Qinling Mountains to protect the giant panda, and the largest density area of pandas is the Foping Nature Reserve that belongs to Xinglongling population habitat. However, a recent study had found that pandas in the Foping Nature Reserve were exposed to toxic metals via their food, the bamboo species *Fargesia qinlingensis* and *Bashania fargesii*, and soil in their core activity area (Chen et al. 2016). In order to comprehensively explore the ecological risk of metals in Qinling giant panda habitat and the giant pandas' exposure levels to toxic metals and health risk, soil, water, bamboo, and feces samples were collected in independent distribution areas of six Qinling giant panda populations. The result showed that the Qinling giant panda habitat had a certain degree of ecological risk, especially Cd, which could cause serious threat to pandas (Zheng 2016). According to the result of multivariate statistical analysis and actual investigation, Cd and some metals may come from the traffic-related activities (Zheng 2016).

With the development of the economy and improvement of the transportation enterprise, the number of roads and traffic flow are becoming increased in the Qinling Mountains. Therefore, the typical road in typical area was selected in this study to verify whether the road traffic could threaten giant panda and their habitat. The 108th National Highway that bisects Foping Nature Reserve is the potentially important anthropogenic source of metals in this reserve. Elevated concentrations of heavy metals in roadside soil have been reported in previous studies (e.g., Forman and Alexander 1998; Trombulak and Frissell 2000), and the concentrations of heavy metals in roadside soil decrease with increasing distance from the road (Werkenthin et al. 2014). Toxic metals derived from road abrasion, vehicle emission, and the wear of vehicle parts could be transported to roadside soils via spray, surface water runoff, or wind dispersal. Contaminants could be transported up to 10 m from the road via surface runoff, whereas fine particulate matter could be transported and deposited up to 250 m away from the roadside by wind and airflow (Werkenthin et al. 2014). To verify whether the highway is an important source of toxic metals, especially Cd in giant panda habitat, roadside soil samples were taken at different depths and distances.

In general, metals, essential for plant growth, are taken from soils (Kumar et al. 1995; Li et al. 2015; Liu et al. 2015), and many plants can accumulate high concentrations of metals in their tissues (Khan et al. 2009). For instance, bamboo (*Fargesia dura*) growing in a mining area accumulated Cd, Cu, Pb, and Zn with concentrations of Cd and Zn being greater in bamboo tissue than in the soil (Yanqun et al. 2004), and likewise, bamboo (*Fargesia qinlingensis* and *Bashania fargesii*) have higher concentrations of Cd and Pb in the Foping Nature Reserve that is one of the core activity areas of Qinling giant panda (Chen et al. 2016). Pandas can consume an average 30 kg of bamboo shoots and leaves per day (Tuanmu et al. 2013). Consequently, even relatively low metal concentrations in bamboo tissue can result in a higher dietary exposure threatening the pandas' health. Understanding the potential ecological risk and health risk of toxic metals for panda habitat and giant panda is therefore important for the effective conservation of this iconic species.

The geo-accumulation index (I_{geo}) and the potential ecological risk index (RI) were used to evaluate potential ecological risk for giant panda habitat (Müller 1969; Hakanson 1980), and the hazard quotient (HQ) was used to assess potential health risk for giant panda. These

methods have been used previously to evaluate the exposure risk of toxic contaminant using sediments (Yi et al. 2011; Zhang et al. 2014) and soils (USEPA 1997; Loska et al. 2004; Leung and Wong 2008; Chen et al. 2015).

The objectives of this paper are to (1) verify whether the road traffic is an important source of toxic heavy metals, especially Cd in giant panda habitat; (2) parse the rule of spatial variation about the heavy metal concentrations in roadside soil; and (3) identify the classification of heavy metals in the surrounding environment of highway and confirm which heavy metals came from the road traffic.

Materials and methods

Study area

The study was conducted along a 30-km section of the 108th National Highway in the Foping Nature Reserve, China (N 33.61° and E 107.95° to N 33.73° and E 107.97°, elevation range was 1132 to 1771 m). The highway has been built in the 1960s and is one of the main transport corridors in China. According to a recent survey, the daily average traffic flow of this road was about 30,000 vehicles in 2014 (Xi'an Evening Daily 2014).

Sample collection, preparation, and determination

A total of 132 soil samples were collected from 11 sites along the 108th National Highway in March 2015. There were four sampling locations per site, at distances of 0, 50, 100, and 300 m from the carriageway, and at each sampling location, three soil samples were collected at three soil depths (0, 5, and 10 cm). The humus layer was scraped off by a wooden or bamboo shovels, and soil profiles were produced, and then, 0, 5, and 10 cm depth of soil were collected in the vertical plane that was perpendicular to the ground using the sampler. Replicate samples were pooled, and the mass of the combined sample was 0.5 kg.

Soil samples were transported to the laboratory using the clean polyethylene bags, where they were air-dried at room temperature before dried in an oven at 50 °C to a constant weight. Approximately 50 g of each sample was then ground and homogenized by passing through a 0.15-mm nylon sieve before analysis.

Two hundred and fifty milligrams of soil was placed in a Teflon digestion vessel, and each sample was prepared in triplicate. In addition, nine 250 mg samples of standard reference materials (soil GBW 07427) obtained from the Center of National Standard.

Reference Material of China and 12 blank controls were prepared. Guaranteed Reagent (GR)-grade acid digestion mixture (1 mL HNO₃, 3 mL HCl, 5 mL HF, 2 mL HClO₄) was added to each vessel, and the samples were digested using an electric hot plate with the following conditions: ramp to 80 °C hold for 120 min, ramp to 100 °C in 5 min and hold for 120 min, ramp to 115 °C in 5 min and hold for 120 min, ramp to 135 °C in 5 min and hold for 300 min, ramp to 135 °C in 5 min and hold for 300 min, and then ramp to 165 °C in 5 min and hold for 300 min. After digestion, 0.5 mL GR-grade nitric acid was added to each sample and diluted to 25 mL with ultrapure water (18.2 MΩ/cm² Milli-Q water, Millipore).

The concentrations of metals were analyzed using atomic absorption spectroscopy (AAS, ZEEnit 700 P, Analytik Jena, Germany). Concentrations of Cu, Zn, Mn, and Cr were measured by air acetylene flame method of the AAS with electrically modulated deuterium-HCL background correction. Hydride-forming elements of As and Hg were measured by the HS55 Hydride System of the AAS. Concentrations of Pb, Cd, and Ni were measured by graphite furnace AAS coupled with a MPE 60 graphite autosampler and by using two-field mode Zeeman effect background correction. All concentrations of metals in this study are expressed in micrograms per gram on dry weight basis ($\mu\text{g/g dw}$).

Metal recoveries for standard reference materials were within 10 % of the certified values, and all analyses for the standard reference materials were performed in triplicate. Limit of detection (LOD) calculated using triple standard deviation values of blanks and average dry weight of soils were 0.53, 0.21, 0.71, 0.04, 1.50, 0.75, 0.005, 0.01, and 0.03 $\mu\text{g/g}$ for Cu, Zn, Mn, Pb, Cr, Ni, Cd, Hg, and As, respectively.

Evaluation methods of data analysis

The geo-accumulation index (I_{geo}) was calculated to assess heavy metal pollution (Müller 1969), and the I_{geo} is defined by the following equation:

$$I_{\text{geo}} = \log_2 \left(\frac{C_n}{1.5B_n} \right)$$

where C_n is the concentration of metal in the sample and B_n is the soil background value of heavy metal. Factor 1.5 is the background matrix correction factor (Müller 1969). The background value of metals in Shaanxi, obtained from the Chinese Ministry of Environmental Protection (CNEMC 1990), were presented in Table S1, and classification of pollution levels were presented in Table S3.

The monomial potential ecological risk index of each metal (E_r^i) and the potential ecological risk indexes of all metals (RI) are calculated by the following equations:

$$C_f^i = C_i / C_n^i$$

$$E_r^i = T_r^i \times C_f^i$$

$$RI = \sum E_r^i$$

where C_f^i is the single metal pollution index, C_i is the determined concentration of metal, C_n^i is the background value, which is the same as B_n , and T_r^i is the toxic response factor. Based on the standardized toxic factor of metals (Hakanson 1980), the order of toxicity is $\text{Mn} = \text{Zn} = 1 < \text{Cr} = 2 < \text{Cu} = \text{Ni} = \text{Pb} = 5 < \text{As} = 10 < \text{Cd} = 30 < \text{Hg} = 40$. Classification standard of pollution levels based on E_r^i and RI was presented in Table S4.

Health risk assessment for giant panda is calculated based on equations detailed in USEPA's Exposure Factors Handbook (USEPA 1997). Average daily dose (ADD) is determined by the following equation:

$$ADD=(C\times IR_S\times EF\times ED)/(BW\times AT)$$

where C is the mean concentration of heavy metal (mg/kg), IR_S is the soil ingestion rates, the IR_S of giant panda compared with the conservative estimates of adult's $IR_S = 100$ mg/day (USEPA 1997), EF is the exposure frequency, 350 day/year, ED is the exposure duration (according to the data provided by the experts in Shaanxi Wild Animal Research Center (SWARC) that is the only breeding center for the Qinling subspecies conservation, ED is 10.36 years), and BW is the average body weight; the average weight of giant panda is 80–130 kg (Zhang and Wei 2006); we selected the average weight 105 kg in this study. AT is averaging time, $AT = 3781.4$ days.

Noncancer toxic risk is determined by the model hypothesis of hazard quotient (HQ):

$$HQ=ADD/RfD_o$$

RfD_o is the reference dose of a specific metal (USEPA 1997). If $HQ < 1$, it is considered to be relatively safe for risk exposure. If $1 < HQ < 10$, it suggests that there exists considerable threat for health effects, If $HQ > 10$, it suggests a high chronic risk. While the value of HQ increases, the probability of risk expose also increases (Hang et al. 2009).

Statistical analysis

The spatially matched samples from soil samples were analyzed using two-way ANOVA, the fixed factors being location (0, 5, 10 cm) and distances (0, 50, 100, 300 m). Site was included as a random factor.

Correlation analysis (CA), principal component analysis (PCA), and hierarchical cluster analysis (HCA) were used to explore associations between metals in soil samples with a view of identifying possible sources of contamination (Einax and Soldt 1999; Singh et al. 2004; Han et al. 2006; Li and Feng 2012). PCA was widely used to reduce data and to extract a small number of latent factors for analyzing relationships among the observed variables (Martin et al. 2006; Gou et al. 2007). HCA was performed to further classify the different sources of metals on the basis of the similarities of their chemical properties and pathways (Han et al. 2006). Kolmogorov–Smimov test was used to test the normal distribution, and the suitability was tested by Kaiser–Meyer–Olkin and Bartlett to make sure that the original data met the requirements of HCA and PCA. In addition, prior to HCA, the raw data was standardized by z-scores and the Ward's method was performed. All these statistical analyses were performed with the statistical package SPSS 20.0 (IBM SPSS Statistics, IBM Corp., USA).

Results

Spatial variation of heavy metal concentrations

The concentrations of all metals except As exceeded the background values (Fig. 1). Before ANOVA, the data of heavy metal concentration was tested to meet an approximation of normal distribution. Two-way ANOVA indicated a significant effect of distance from highway for all metals except mercury ($F_{3,110} > 4.1$, $p < 0.05$). There was a monotonic reduction in metal concentration with increasing distance for Cu, Zn, Mn, Pb, and Cd, but Hg and As concentrations increased with distance in general; Cr and Ni concentrations decreased and then increased with distance (Fig. 1). Concentrations of Zn, Pb, and Cd decreased with soil depth ($F_{2,110} > 5.9$, $p < 0.01$), whereas concentrations of Cr, Ni, and As increased with soil depth ($F_{2,110} > 3.3$, $p < 0.05$). There was no significant effect of soil depth on concentrations of Cu, Mn, or Hg (Fig. 1).

The I_{geo} was calculated for each sample, and values ranged from -1.50 to 2.73 (Table S1). Values of I_{geo} for Cu, Mn, Cr, Ni, Hg, and As (except for one sample) were less than 0 indicating that soil samples were uncontaminated by these elements (Table S1). All the samples were classified as “uncontaminated to moderately contaminated” for Zn ($I_{geo} = 0.07$ – 0.62). Except topsoil and 5-cm samples taken with 0 m of the road that were classified as “moderately contaminated” ($I_{geo} = 1.13, 1.06$), other samples were classified as uncontaminated to moderately contaminated for Pb ($I_{geo} = 0.01$ – 0.47). The highest levels of contaminations were recorded for cadmium, with all topsoil samples being classified as “moderately to heavily contaminated” ($I_{geo} = 2.18$ – 2.73 , Fig. 2a). Further, the 3D response surface for I_{geo} of Cd showed that the I_{geo} values of Cd were largest and showed a decreasing trend with the increasing distance and sampling depth (Fig. 3a). The pollution of Cd was more serious, especially the topsoil and the area that was near the road closely.

The E_r^i was calculated for each sample, and values ranged from 1.08 to 298.10 (Table S1). Values of E_r^i for Cu, Zn, Mn, Pb, Cr, Ni, and As were less than 15 indicating that E_r^i of these elements was in low pollution degree (Table S4). All the samples were classified as “considerable pollution degree” for Hg ($E_r^i = 44.14$ – 59.28). The highest levels of pollution degree were also recorded for cadmium, with all topsoil samples being classified as very high pollution degree ($E_r^i = 214.69$ – 298.10), and other samples were in high pollution degree ($E_r^i = 75.32$ – 216.58 , Fig. 2b).

The regional comprehensive potential ecological risk (RI) was calculated with nine elements, and the values ranged from 149 to 379 (Table S2). All the sites were determined as in very high degree of potential hazards (>120 , Table S4), and the value of RI presented that potential hazards in closer distance from road and in topsoil were larger than that in the faster distance and deeper soil ($0 > 50 > 100 > 300$ m, $0 > 5 > 10$ cm) (Fig. 3b). The results of I_{geo} and RI showed that the study area had a higher ecological risk, which was mainly due to the pollution of Cd, Pb, Zn, and Hg especially Cd. But whether these higher pollution elements can directly threaten the giant pandas or what elements can pose a direct threat for them, the hazard quotient was used for the further analysis. The mean HQ of nine heavy metals was in the order of Cd (4.57) > Pb (1.80) > Mn (1.10) > Hg (0.97) > Ni (0.82) > Cu (0.44) > As (0.28) > Zn (0.25) > Cr (0.03). The values of Cd, Pb, and Mn were greater than 1, which indicated that these elements can pose health risk to the giant panda, and the HQ tendencies of Pb and Cd were $0 > 50 > 100 > 300$ m and $0 > 5 > 10$ cm (Table 1).

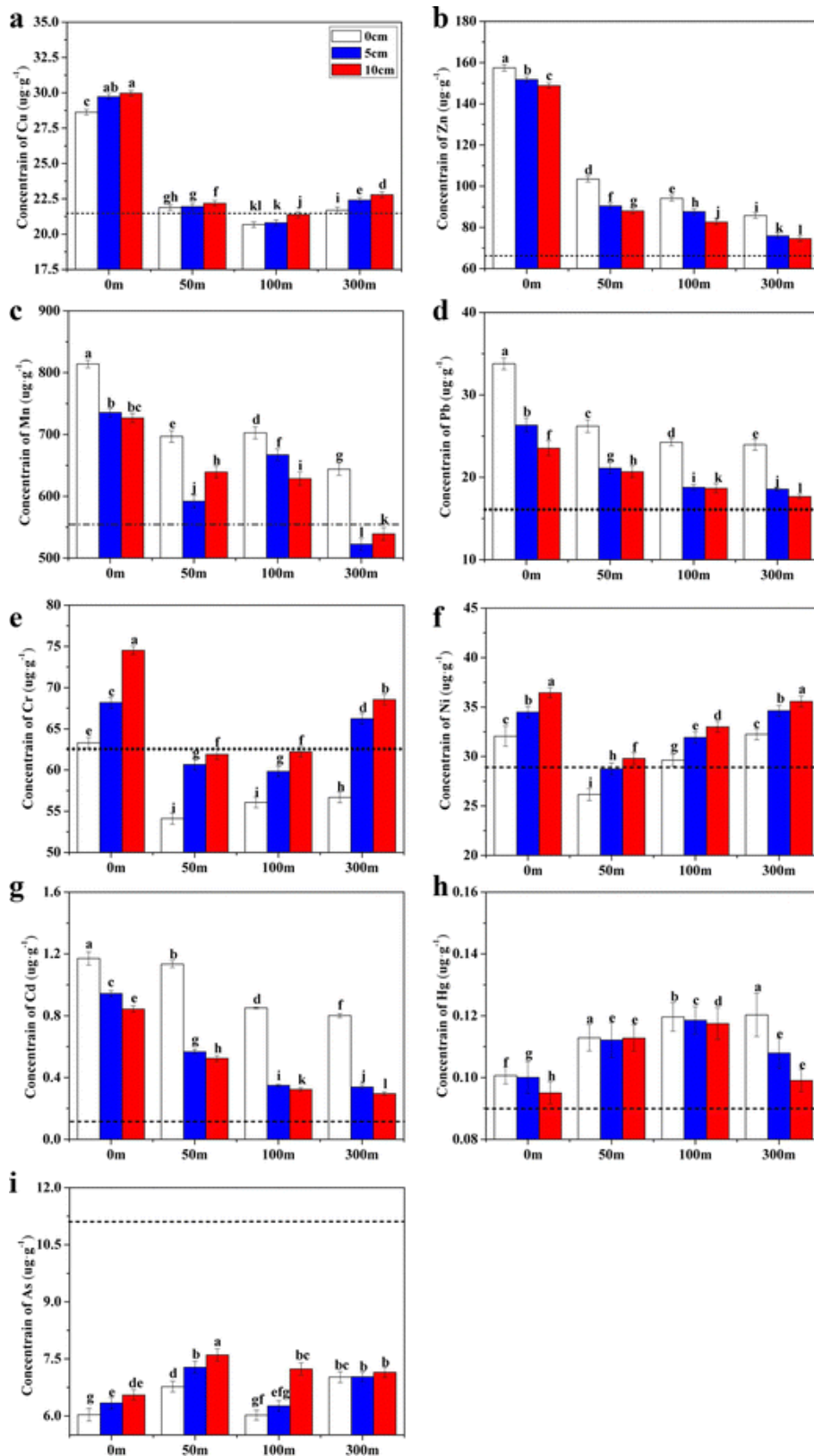


Fig. 1: Spatial variation of concentrations of Cu (a), Zn (b), Mn (c) Pb (d), Cr (e), Ni (f), Cd (g), Hg (h), and As (i) with distance in roadside soil from the 108th National Highway. In the figures, 0, 50, 100, and 300 m express the sampling sites from roadbed at distances of 0, 50, 100, and 300 m. Soil samples collected at depths of 0 cm (white bars), 5 cm (blue bars), and 10 cm (red bars) at each sampling sites. Dotted line denotes background concentration

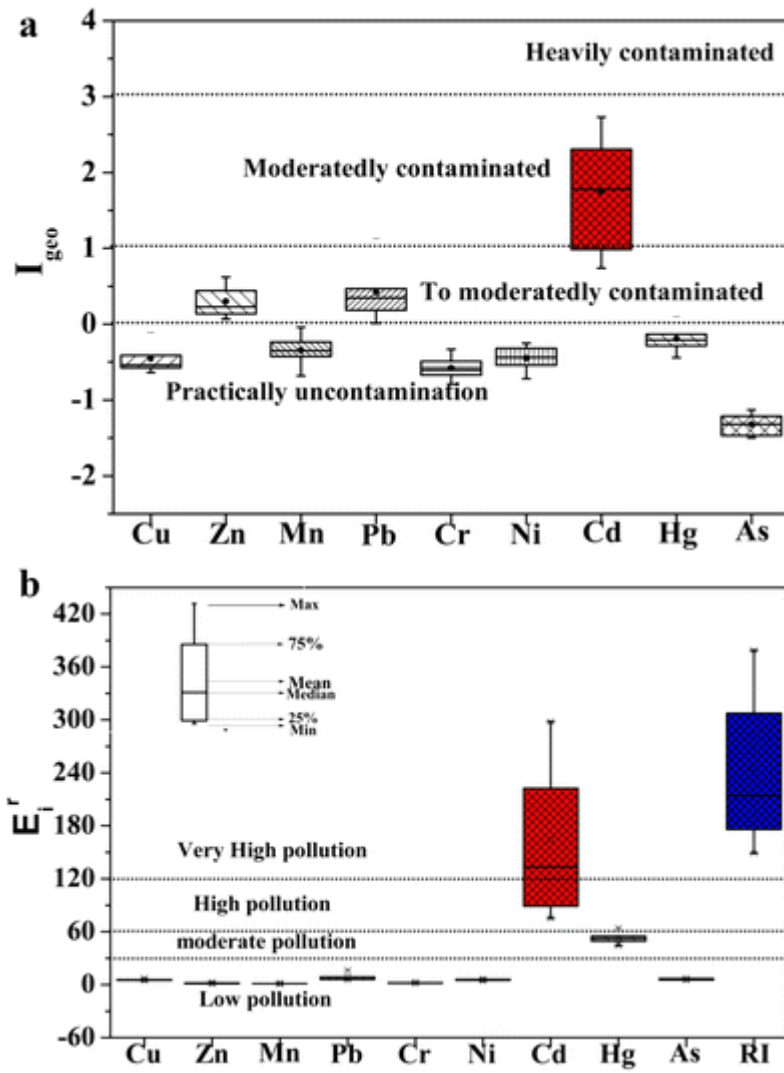


Fig 2: The geo-accumulation index (I_{geo}) (a) and the total potential ecological risk (RI), monomial potential ecological risk index (E_i') (b) for Cu, Zn, Mn, Pb, Cr, Ni, Cd, Hg, and As in soil samples collected along the 108th National Highway

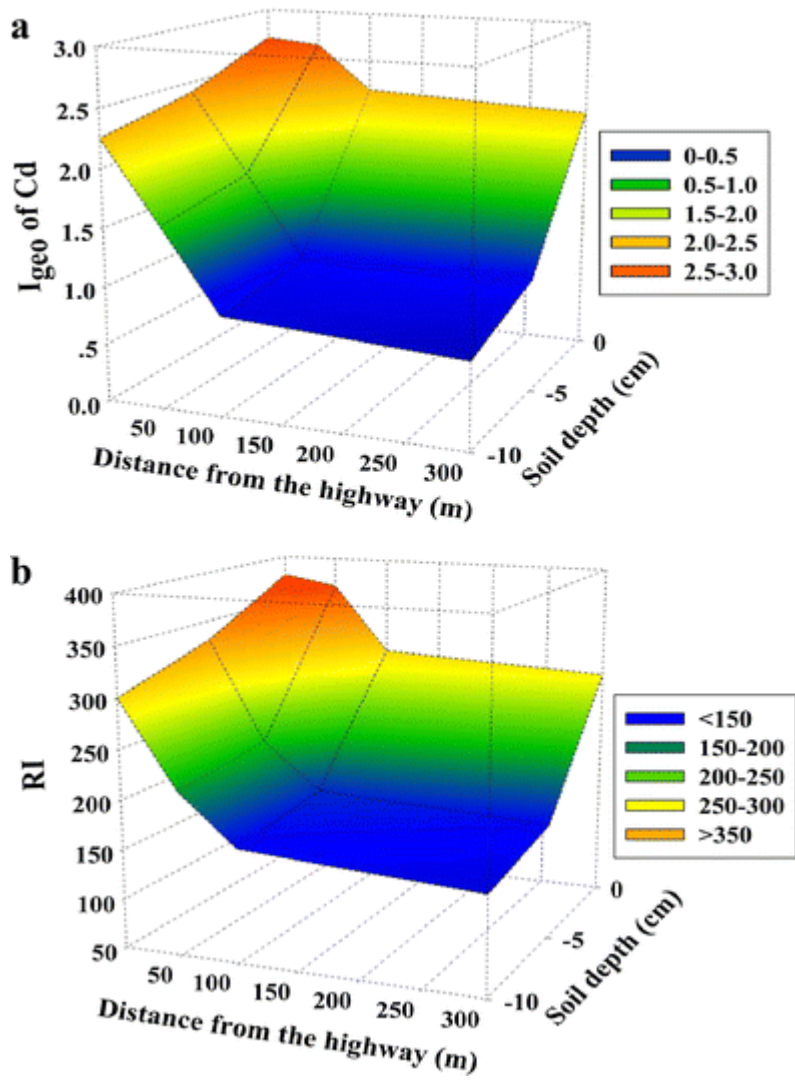


Fig. 3: The 3D response surface of the I_{geo} of Cd (a) and RI of heavy metal (b)

Table 1: The HQs of nine heavy metals in soil collected along the 108th National Highway. Bold values indicate the mean of HQs are greater than 1, which means that these elements can pose health risk to giant panda

Distance	Depth	Cu	Zn	Mn	Pb	Cr	Ni	Cd	Hg	As
	0 cm	0.53	0.37	1.35	3.46	0.03	0.83	6.24	0.87	0.23
0 m	5 cm	0.54	0.37	1.25	1.70	0.03	0.89	4.41	0.86	0.25
	10 cm	0.55	0.36	1.21	1.55	0.04	0.92	3.96	0.85	0.27
50 m	0 cm	0.40	0.25	1.16	3.34	0.03	0.68	5.52	1.10	0.28
	5 cm	0.41	0.22	1.00	1.53	0.03	0.74	4.45	1.05	0.28
	10 cm	0.42	0.22	1.04	1.25	0.03	0.77	4.36	0.98	0.28
100 m	0 cm	0.38	0.23	1.17	2.51	0.03	0.77	5.11	1.05	0.29
	5 cm	0.38	0.22	1.11	1.03	0.03	0.82	3.96	0.91	0.29
	10 cm	0.39	0.20	1.06	0.95	0.03	0.86	4.15	0.87	0.34
300 m	0 cm	0.40	0.21	1.07	2.36	0.03	0.70	5.05	1.01	0.24
	5 cm	0.41	0.19	0.87	1.03	0.03	0.89	3.91	1.03	0.27
	10 cm	0.41	0.19	0.90	0.87	0.03	0.94	3.72	1.03	0.30
Mean		0.44	0.25	1.10	1.80	0.03	0.82	4.57	0.97	0.28

Multivariate statistical analysis

CA, HCA, and PCA were used to explore the association between metals in roadside soil samples in order to gain insight into whether the potential source of toxic contaminants is the road and which elements are derived from traffic. All the raw data was tested and standardized before multivariate statistical analysis, and the data met the certain requirements.

The most significant positive correlations were detected between Cd, Pb, and Zn ($r = 0.768$ – 0.831) and between Cr, Cu, Mn, and Ni ($r = 0.429$ – 0.833). Cu, Mn, Pb, and Zn also had the more significant correlation ($r = 0.158$ – 0.482). Hg and As ($r = 0.466$) were negatively correlated with the other seven elements (Table 2).

HCA (Fig. 4a) and PCA (Fig. 4b) also grouped the metals into three main clusters, Cr–Ni–Cu–Mn, Pb–Zn–Cd, and As–Hg with the Cr–Ni–Cu–Mn cluster being further subdivided into Cr–Ni and Cu–Mn. These analyses suggested that the different clusters of metals were derived from different sources, with Pb, Zn, and Cd being most strongly associated with the same source.

Table 2: The correlation matrix for metals measured in soil samples. Bold values indicate that the correlation is significant at 0.01 level, which means that these metals have the most significant positive correlation

Elements	Cd	Cr	Cu	Hg	Mn	Ni	Pb	Zn	As
Cd		-0.153	0.367*	-0.104	0.277*	-0.016	0.768**	0.827**	-0.061
Cr			0.501**	-0.110	0.599**	0.833**	-0.109	0.071	-0.170
Cu				-0.331	0.619**	0.573**	0.366*	0.476*	-0.278
Hg					-0.230	0.026	-0.118	-0.201	0.466**
Mn						0.429**	0.158*	0.482*	-0.172
Ni							0.053	0.138	-0.323
Pb								0.831**	-0.237
Zn									-0.258
As									

**Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level

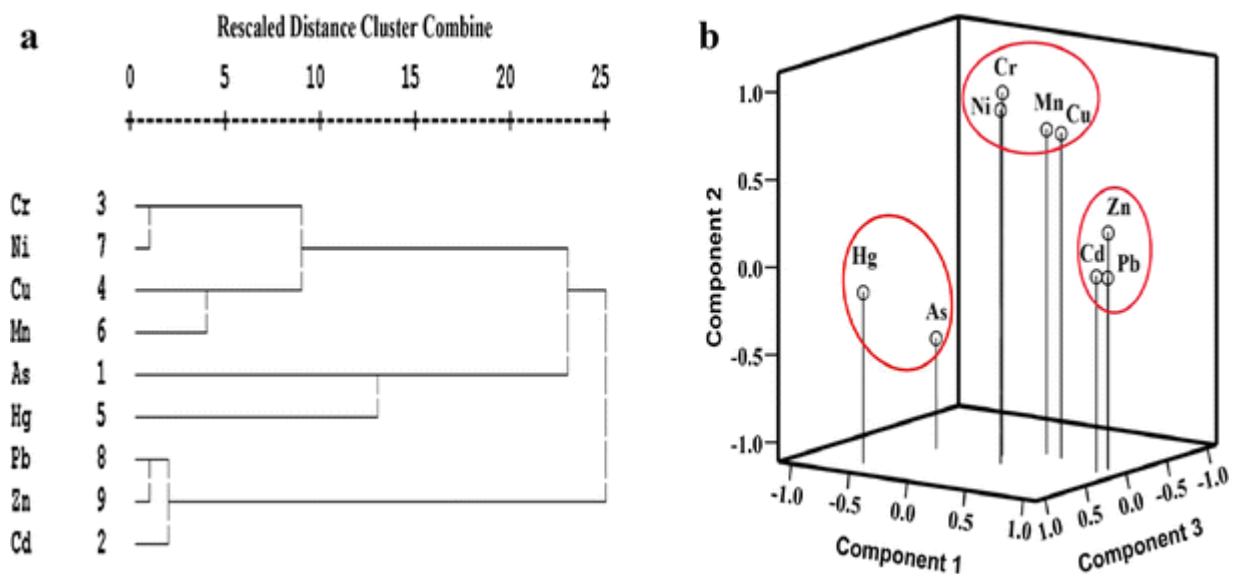


Fig. 4

Fig. 4: Hierarchical clustering analysis of metals (a) and rotated component loading 3D plot for nine heavy metals (b) in soil samples collected from the 108th National Highway that bisects the Xinglongling giant panda habitat (n = 132). The suitability test was tested by Kaiser–Meyer–Olkin (0.694) and Bartlett (sig < 0.05); the original data met the requirements that were analyzed by HCA and PCA

Discussion

The pollution level of heavy metals in roadside soil of 108th National Highway that was a typical road bisecting the giant panda habitat was evaluated to verify whether the road traffic can harm giant panda, which was the first objective of this study. Concentrations of all metals except As exceeded background levels, and the contaminations were particularly high for Cd up to a distance of 300 m away from the highway and for Pb and Zn next to the highway. Some studies have shown that the concentrations of traffic-related metals in soil decreased with the growing distance from the road edge (Liu et al. 2009; Yan et al. 2013; Werkenthin et al. 2014). Whereas, the pollution scope, such as Cd, Pb, and Zn, can be up to a greater distance from the highway (Viard et al. 2004; Chen et al. 2010). The contaminated soil could also cause a wide range of ecological hazard if the contaminants were transferred to other spheres: hydrosphere, atmosphere, and biosphere (Sutherland 2000; Sutherland et al. 2000). Contaminated soil particles could be washed into nearby water bodies that like human blood and form a potential threat for the whole water environment (Sansalone and Buchberger 1997), and fine particulate matter could be transported by wind and deposited up to the further area, completing the secondary atmospheric precipitation (Werkenthin et al. 2014; Schreck et al. 2014). Previous research indicated that diet intake was the direct way that giant panda exposed to toxic metals and the metal in soil directly influences on security and quality of bamboo (Zheng 2016). Bamboos growing in contamination area accumulated high concentrations of metals (Kumar et al. 1995; Khan et al. 2009; Yanqun et al. 2004), and metals were transferred through the food chain, posing a potential health risk to panda (Parker and Hamr 2001; Burger 2008; Brahmia et al. 2013). The giant panda physical activity was found by the camera traps at a small distance from the 108th road in March 2015 (WWF China News Center); long-term exposure to contaminated soil and consumption of contaminated bamboo could threaten pandas' health. Moreover, the contaminated roadside soils transferred to other spheres (Sutherland 2000; Sutherland et al. 2000) leading to the pollution scope extended and threatening the whole habitat and Qinling subspecies (Zheng 2016).

In this study, the higher I_{geo} of Cd, Pb, and Zn and the higher E_r^i of Cd and Hg showed that these elements can pose a higher ecological risk to the environment in research area. Moreover, to further explore whether this pollution situation could directly influence the giant panda health or which elements could pose a direct threat for panda's health, the hazard quotient was calculated, and the higher HQ of Cd, Pb, and Mn, which were greater than 1, indicated that these elements did pose a higher health risk to the giant panda, especially Cd (Zheng 2016). It has been suggested that Cd is involved in carcinogenesis in multiple organs including the kidney, prostate, liver, and pancreas (Waalkes 2003; Goyer et al. 2004; Valko et al. 2005; Thompson and Bannigan 2008) of animals. Cadmium can induce hepatic nuclear DNA damage and testicular injury and increase the sperm aberration rate (Shiva 1982; Masters et al. 1994; Larision et al. 2000; Dalton et al. 1996; Bagchi et al. 1997; Siu et al. 2009). Whereas Pb with low dose can induce nervous system disorders, interfere with hemoglobin synthesis, damage the cardiovascular system, etc. (Michelle et al. 2015), Pb as the carcinogenic substance can cause renal cortical adenocarcinoma, brain tumors, and DNA oxidative damage (Silbergeld et al. 2000) and reproductive system damage (Eibensteiner et al. 2005; Mario et al. 2015). Mn is an essential metal that can pose a low ecological risk to the environment in this study, but the high level exposure to Mn has a risk for health of giant panda. The clinical manifestation of manganese poisoning is a serious mental disorder affecting the transmission ability of nerve synapses, with movement disorders, Parkinsonism, or permanent disability (Cowan et al. 2009; Long et al. 2014; Safa et al. 2016).

This is the first time to verify the harm of road traffic to giant panda in Qinling; therefore, the values could only be compared to those in one of the core areas, Foping Nature Reserve. The concentrations of Cu, Zn, Mn, Pb, Cr, Ni, Cd, Hg, and As are 20, 90, 559, 25, 19, 17, 0.25, 0.028, and 6.4 $\mu\text{g/g}$, respectively, in soil samples collected from core area of reserve (Liu 2015), and the concentrations of Cd ($0.25 > 0.118$), Pb ($25 > 16.3$), and Zn ($90 > 65.8$) exceeded the background values of soil in Shaanxi (CNEMC 1990). The mean concentrations of nine elements in our study are higher than those reported in the core area, which, due to human activities leading to the concentrations of metals in buffer area, were significantly higher than that in the core area (Zheng 2016).

Another objective of this study was to identify classification of heavy metals, parse the possible source of heavy metal contamination in the surrounding environment of highway, and validate which metals came from the road traffic. Multivariate analyses identified three groups of metals: (1) Cr, Ni, Cu, and Mn; (2) Pb, Zn, and Cd; and (3) Hg and As, suggesting three primary sources.

For the first group of metals, the concentrations of Cr and Ni did not present the exponential trend that descended with the increasing distance, but the concentrations were first decreasing and then slightly increasing. Wei and Yang pointed out that the concentrations of Cr, Cu, Pb, Zn, Ni, and Cd $\mu\text{g/g}$ in urban road dusts of cities from China were much higher than background values (Wei and Yang 2010). Studies have demonstrated that vehicle emission and oil pump wear were the important sources of Ni and 80 % of Ni in atmosphere of roadsides came from automobile exhaust (Allen et al. 2001; Faiz et al. 2009; Johansson et al. 2009). But in this study, there was the suddenly increasing phenomenon that appeared in 300 m, and the reason might be because other natural or man-made factors disturbed the pure influence of road. Depending on the specific position of research area, there were very few industrial and agricultural activities, so the man-made factors were eliminated. Some research have demonstrated that Cr and Ni were affected by the heterogeneity of the soil parent material that was controlled primarily by natural lithogenic and/or pedogenic processes (Sutherland and Tolosa 2001), and “natural” heavy metal contents in soils could exhibit a higher variability controlled by the parent material (Facchinelli et al. 2001), so the interference factor was considered as soil parent material ultimately.

The concentrations of Cu and Mn in the first group did not vary significantly with soil depth but roughly decrease with increasing distance from the highway. Fujiwara indicated that the brake pad wear was the main source of Cu, Sb, and Ba (Fujiwara et al. 2011). Methylcyclopentadienyl manganese tricarbonyl (MMT) antiknock additive was the main source of Mn in roadside environment, and Loranger found that manganese concentration in ambient air and emission rate increased about 10 % annually since the use of MMT in 1976 (Loranger and Zayed 1992). In addition, the traffic that led to the concentrations of Cu and Mn decreased with the increasing distance from the road (Turer and Maynard 2003; Curtin et al. 2011; Li et al. 2013). However, the fluctuating decrease and the sudden increase indicated that there was also another disturbance factor to disturb pure impact of traffic. We learned the Qinling Mountains were rich in minerals including Cu and Mn (Zhu et al. 1992), so Cu and Mn should be under the influence of soil parent material like Cr and Ni. It was therefore proposed that metals in this group originate from traffic and soil parent material, but the main source was the traffic with the significant differences in different distance from the highway.

The second group of metals, Pb, Zn, and Cd, exhibited decreasing concentration with increasing soil depth and distance from the highway. This pattern was consistent with

previous studies and indicated that the highway was the main source of these contaminants. The study of traffic-related metals in roadside soils along the Qinghai–Tibet highway indicated that Cu, Zn, Pb, Cr, Cd, and As were related to road transportation (Zhang et al. 2015) and the contents of Zn, Pb, and Cd in roadside soils decreased exponentially with the distance from the road (Yan et al. 2013; Zhang et al. 2015). The heavy metal in soil alongside mountain railway in Sichuan showed that the concentrations of Cu, Zn, Mn, Pb, and Cd decreased with increasing distance from the railroad and indicated that railway transport was the source of such pollutants (Liu et al. 2009). A review of Cu, Zn, Pb, Cr, and Ni in European roadside soils showed that road construction and transportation affected the surrounding roadside soils significantly and the heavy metal contaminations also decreased with increasing distance (Werkenthin et al. 2014). It is commonly believed that vehicle emissions were an important source of Pb pollution (Lee et al. 2006; Zechmeister et al. 2006; Yang et al. 2008, 2011). Zn mainly came from the aging wear of automobile tires and car body, and the loss of gasoline and lubricating oil was also a source of Zn and Cd (Ellis and Revitt 1982; Alloway et al. 1990; Miguel et al. 1997; Hashisho and EL-Fadel 2004; Blok 2005; Nabulo et al. 2006). Besides, the application of fertilizer to soils could be an important source of Cd (Mattigod and Page 1983; Alloway et al. 1990; Li et al. 2001), but few farmlands in this area, the pesticide source of Cd was eliminated, so the elements in this group was mainly affected by traffic.

The third group of metals, Hg and As, indicated that the traffic did not affect both of elements because in this study area, the concentration of Hg and As did not decrease with increasing distance from the highway. It is generally believed that the coal combustion and solid waste incineration and wastewater discharge were generally regarded as the main pollution resource of Hg and As. Research showed the concentrations of Hg and As in fly ash of coal combustion were 3.5 and 63.5 mg/kg, respectively, in China (Li and Feng 2012; Pirrone et al. 2010; Yang et al. 2011; Liu et al. 2015).

Conclusion

To our knowledge, this is the first investigative study to explore whether the highway can pose a potential threat for giant pandas. The results showed that the 108th National Highway bisecting Qinling giant panda habitat was indeed a major source of some toxic metals. All the determined metals except As exceeded background levels, and the concentrations of Cu, Zn, Mn, Pb, and Cd decreased with increasing distance from the highway. I_{geo} indicated that topsoil next to the highway was moderately contaminated with Pb and Zn, whereas topsoil up to 300 m away from the highway was extremely contaminated with Cd. RI indicated that study area was in a high degree of potential hazards, which were also due to the serious Cd contamination. The HQ indicated that Cd ($HQ_{Cd} = 4.57 > 1$), Pb ($HQ_{Pb} = 1.80 > 1$), and Mn ($HQ_{Mn} = 1.10 > 1$) could be threatening the pandas' health especially Cd. In addition, it is worth noting that although Mn was an essential metal that pose a low ecological risk to the environment, the high level exposure to Mn had a risk for giant panda's health. Moreover, the contaminated roadside soils could be transferred to other spheres (Sutherland 2000; Sutherland et al. 2000) leading to the pollution scope extended and threatening the whole habitat and Qinling subspecies, which had already been confirmed in the previous study (Zheng 2016).

Given the potential for metals to lead to adverse ecology and health effects and thereby compromised conservation efforts, it is important to explore the contamination sources of heavy metal. The multivariate analysis of metals in this study was consistent with that in soil,

water, bamboo, and feces collected from different distribution area of six giant panda populations. Cd, Pb, and Zn belonged to the same category, and these toxic pollutants did come from the road traffic. Controlling these metals at source would be desirable, but it was difficult to immediately cut off the contamination. In the short term, therefore, efforts may be focused on controlling traffic flow and transplanting some hyperaccumulators along the road to alleviate the influence of heavy metal contamination to protect the giant panda. In the long run, the giant panda nature reserve should be completely taken into consideration in the road location project, and it is best to choose some location that is far from the nature reserve and human settlement and gradually replace the road that bisects the giant panda habitat.

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