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Effect of Heavy Metal Contamination in the Environment on Antioxidant Function in Wumeng Semi-fine Wool Sheep in Southwest China

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Abstract
Many environmental accidents have led to worldwide heavy metal pollution, raising concern about heavy metal toxicity in Southwest China. To study the effects of Cd and Pb in the environment on antioxidant function in Wumeng semi-fine wool sheep, contents of Cu, Zn, Mn, Mo, Fe, Se, Cd, and Pb were measured in irrigation water, soil, herbage, and animal tissues. Hematological and biochemical parameters were also determined. The concentrations of Cu, Zn, Cd, and Pb in affected samples of irrigation water, soil, herbage, and tissues were significantly higher than those in the control ($P < 0.01$). There was no significant difference in other element contents between affected pastures and control areas. The occurrence of anemia affected Wumeng semi-fine wool sheep. The activities of SOD, CAT, and GSH-Px in affected animals were significantly decreased than those in the control ($P < 0.01$). Content of MDA in serum in affected animals was significantly increased than that in control ($P < 0.01$). Serum T-AOC in affected animal was significantly lower than that in control ($P < 0.01$). Consequently, it is suggested that heavy metal contamination in natural habitat caused serious harm to antioxidant function in Wumeng semi-fine wool sheep.

Keywords Antioxidant function · Heavy metals · Reactive oxygen species · Wumeng semi-fine wool sheep · Natural habitat

Introduction

A lot of environmental accidents have led to worldwide heavy metal pollution, raising concern about heavy metal toxicity in the Yunnan-Guizhou Plateau of China [1]. We have studied the effect of environmental heavy metal on antioxidant function in animals. The antioxidant system of animals is the defense system against free radical damage [2]. Its function is to eliminate excessive free radicals and block the chain reaction of lipid peroxides, thereby preventing the accumulation of malondialdehyde (MDA) in the body [3, 4]. The main manifestations are cell membrane degeneration, protein damage, DNA mutation, and so on, which eventually lead to various diseases, even aging and death of organisms [5, 6]. The activity of antioxidant enzymes is closely related to heavy metal content in blood. Too low or too high contents of heavy metal in the blood will cause the activity of antioxidant enzymes to decrease [7, 8]. Therefore, processes underlying antioxidant responses to heavy metal contaminants must be clearly understood.

The Yunnan-Guizhou Plateau is located in Southwest China, and it is an important pastureland for native sheep

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breeds [9]. The semi-fine wool sheep are vital for the productivity system in the Southwest China [10]. Over the last 10 years, contents of cadmium (Cd) and lead (Pb) in air, water, soil, and herbage have increased greatly in Weining county, Southwest China [9, 11]. Cd and Pb can be transferred freely through food chains, which has potential adverse effects on animal and human health [12–14]. Pb is often combined with Cd as an environmental contaminant and Cd has similar effects to those of Pb; thus, the effects are additive [15–18]. The Yunnan-Guizhou Plateau is important for the production of non-ferrous metals in China and contains extensive heavy metal resources characterized by large quantities of ores containing zinc (Zn), copper (Cu), and Pb [19, 20]. There are many industrial enterprises involved in the extraction of Pb, Zn, Cu, and polymetallic compounds [21, 22]. Over the past few decades, much pasture and farmland have been used for intensive development of metallurgical industries [23]. A number of Wumeng semi-fine wool sheep grazing in polluted pastures have died [10]. Oxidative damage is considered to be one of the main mechanisms of Pd and Cd toxicity. The results show that Pb and Cd can change the redox state of cells, promote free radical metabolism of cells and tissues, and lead to oxidative damage caused by lipid peroxidation. All affected Wumeng semi-fine wool sheep were characterized with anemia, emaciation, anorexia, depression, and weakness. In severe areas, 49.56% of Wumeng semi-fine wool sheep were affected, and the mortality reached 75.66% (911/1204). However, little research has been undertaken on the movement of heavy metal contamination in the natural environment and antioxidant function effects on Wumeng semi-fine wool sheep.

The aims of the present study were to determine the effects of heavy metal contamination on antioxidant functions of Wumeng semi-fine wool sheep and to find new ways for solving problem of heavy metal contamination in natural habitat in Weining county of Southwest China.

Materials and Methods

Experimental Area

The experimental pastures were located in Southwest China (25° 35′–28° 21′ N, 102° 37′–105° 16′ E), with an average elevation of 2250 m above sea level, an annual precipitation of 965 mm, and an average atmospheric temperature of 9–10 °C. The polluted area with heavy metal in the study is located in Weining county of Guizhou province (26° 19′–27° 17′ N, 103° 35′–104° 46′ E). The control area is located in Mianning county of Sichuan province (26° 13′–27° 12′ N, 103° 34′–104° 23′ E).

Experimental Animals

Twenty affected Wumeng semi-fine wool sheep, aged 1 year, were collected from polluted pasture in Weining county, where they grazed for 60 days. All animals showed signs of anemia, emaciation, anorexia, depression, and weakness. Twenty healthy Wumeng semi-fine wool sheep, aged 1 year, were collected from non-contaminated pasture in Mianning county, where they grazed for 60 days. A clinical examination revealed that all control animals were in good health. All sheep are free to eat and drink.

Sample Collection

The collected wool samples from the neck of the Wumeng semi-fine wool sheep were washed and degreased, and stored in a silica gel dryer for analysis. Taking blood samples from the jugular vein collect 10 mL with 1% heparin sodium vacuum blood collection tube, and collect 5 mL with vacuum blood collection tube without additives. The samples were stored at −4 °C until analysis for heavy metals.

All the animals were killed by exsanguination and samples of at least 30 g were taken from the lobus caudatus of the heart, lung, liver, kidney, muscle, and spleen. These samples were packed in labeled plastic bags and immediately transported to the laboratory.

Soil and herbage samples were taken at 30 sampling sites situated at 50-, 500-, 5000-, 10,000-, and 15,000-m distances from the smelters in the Weining county of Southwest China. Multiple small portions of herbage that were herbaceous vegetation were cut from pasture and mixed in the affected area. To reduce soil contamination, the forage samples were cut at 1 cm above ground level and placed into labeled polythene bags. The herbage samples were dried at 80 °C for 48 h and ground by a mortar to facilitate chemical analysis. Soil samples were taken from the surface layer (0–30 cm) of the pastures, using a 30-mm-diameter cylindrical corer and placed into labeled polythene bags. The soil samples were dried out at 80 °C for 48 h and passed through a 5-mm sieve. Ten-milliliter of irrigation water of the area was collected. Samples of irrigation water, soil, and herbage were also sampled from Mianning county in Southwest China as controls.

Sample Treatment and Analysis

Heavy metal contents in irrigation water, soil, forage, and animal tissue were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES). The elements analyzed were molybdenum (Mo), Pb, Cd, Cu, Zn, manganese (Mn), iron (Fe), and selenium (Se).

Hemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), and white blood cells (WBC) were determined using an automated hematology analyzer (SF-3000, Sysmex-

Table 1 Results of clinical examination in affected Wumeng semi-fine wool sheep

Characteristic	Lambs (10)[a]	Adult rams (10)[a]	Adult ewes (10)[a]
Incidence of weakness (%)	100	100	100
Incidence of emaciation (%)	100	100	100
Incidence of anorexia (%)	100	100	100
Incidence of anemia (%)	100	100	100
Incidence of depression (%)	100	100	100
Temperature (°C)	36.76 ± 1.22	36.32 ± 1.21	36.33 ± 1.23
Heart rate (beats/min)	54.47 ± 2.87	53.62 ± 2.85	54.11 ± 2.77
Respiratory rate (breaths/min)	18.77 ± 1.37	18.21 ± 1.51	18.39 ± 1.33

[a] Number of samples

Toa Medical Electronic, Kobe, Japan). Ceruloplasmin (Cp), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AKP), creatinine (CRT), catalase (CAT), cholesterol (Chol), blood urea nitrogen (BUN), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), malondialdehyde (MDA), and total antioxidant capacity (T-AOC) were determined on an automated biochemical analyzer (Olympus AU 640, Olympus Optical Co., Tokyo, Japan). The electrophoretic studies of serum protein (total protein, albumin, and globulin) were performed on cellulose acetate using an EA-4 electrophoresis apparatus (Shanghai Medical Apparatus and Instruments Factory, Shanghai, China).

Data Analysis

The differences between groups were evaluated by one-way ANOVA, and the significance was verified by a two-tailed test, least great differences of 1% ($P < 0.01$) or 5% ($P < 0.05$) [24]. The data in the table were expressed in the form of "mean ± standard deviation." And the correlation was analyzed by Pearson's analysis. All data processing adopted the Statistical Package for Social Sciences (version 20.0, SPSS Inc., Chicago, IL, USA).

Results

All affected animals showed symptoms of anemia, emaciation, anorexia, depression, and weakness (Table 1). Examination results of weight and liver parameters showed that heavy metal pollution could cause significant weight loss in Wumeng semi-fine wool sheep accompanied with liver swelling and increase in coefficients of viscera organs comparing with the control group. Histopathologic observation, granular degeneration of hepatocyte, and serious congestion of the central vein in the liver of affected Wumeng semi-fine wool sheep were found, and it was found that the liver cells of the affected sheep showed granular degeneration and severe congestion of the central vein (Fig. 1a, b).

These results obtained showed that heavy metal levels in soil diminished with increasing distance from the smelter (Table 2). The contents of heavy metals (Pb, Cd, Cu, and Zn) in irrigation water, soil, and herbage in affected pastures were markedly

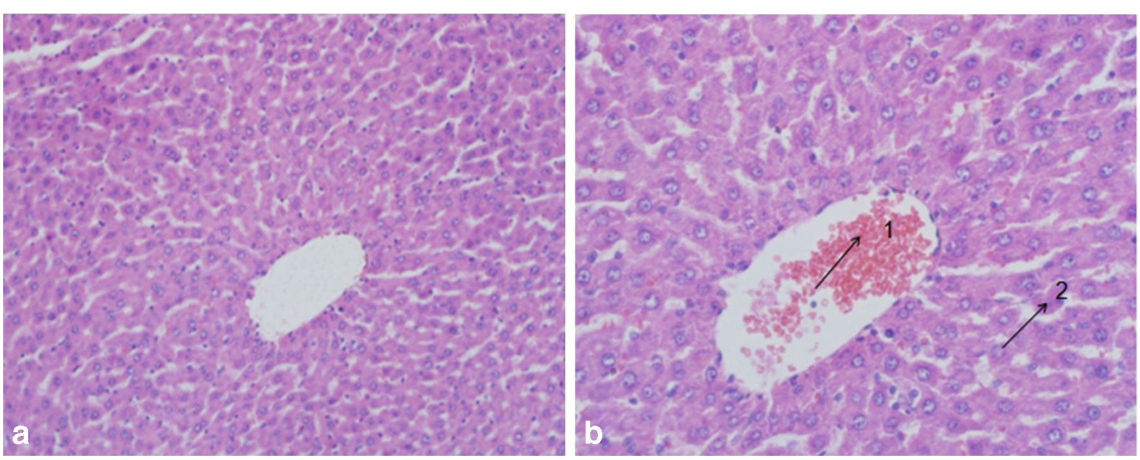

Fig. 1 Pathology slices of liver in control (**a**) and affected animals (**b**). Note: Central venous congestion is indicated by arrow 1 in **b**, and hepatocyte granule degeneration is indicated by arrow 2

Table 2 The concentrations of heavy metals in soils (mg/kg)

Metals	50 m	500 m	5000 m	10,000 m	15,000 m	Mean contents	Controls
Cd	75.21 ± 5.31	58.79 ± 4.53	44.12 ± 3.47	29.69 ± 2.34	23.63 ± 1.97	46.89 ± 3.84	0.67 ± 0.22
Pb	251.25 ± 17.61	202.62 ± 15.79	123.14 ± 12.23	76.86 ± 6.04	41.57 ± 3.79	135.63 ± 13.47	8.64 ± 0.75
Zn	301.71 ± 27.03	272.75 ± 23.53	219.12 ± 17.06	185.94 ± 15.61	156.31 ± 13.42	225.53 ± 21.31	33.63 ± 1.47
Cu	175.30 ± 15.37	137.43 ± 12.21	104.29 ± 9.46	82.01 ± 7.69	67.56 ± 5.32	116.34 ± 9.72	11.67 ± 1.32
Mn	68.53 ± 3.52	69.64 ± 2.81	70.76 ± 3.32	68.46 ± 3.26	69.43 ± 4.65	65.12 ± 3.62	64.53 ± 4.57
Mo	1.55 ± 0.36	1.58 ± 0.41	1.54 ± 0.32	1.56 ± 0.32	1.56 ± 0.42	1.62 ± 0.38	1.63 ± 0.60
Fe	4222.67 ± 72.46	4223.52 ± 73.74	4226.62 ± 73.25	4224.54 ± 73.76	4223.64 ± 72.42	4223.38 ± 71.68	4225.79 ± 72.37
Se	0.23 ± 0.05	0.23 ± 0.07	0.25 ± 0.06	0.24 ± 0.03	0.23 ± 0.05	0.24 ± 0.04	0.25 ± 0.03

Cd cadmium, *Pb* lead, *Zn* zinc, *Cu* copper, *Mn* manganese, *Mo* molybdenum, *Fe* iron, *Se* selenium

Table 3 Heavy metal contents in irrigation water, soil, and forage

Metals	Irrigation water (mg/L)		Soil (mg/kg)		Herbage (mg/kg)	
	Affected	Control	Affected	Control	Affected	Control
Cd	5.91 ± 0.35[a]	0.007 ± 0.001	46.29 ± 3.62[a]	0.63 ± 0.03	7.63 ± 0.35[a]	0.013 ± 0.002
Pb	3.79 ± 0.35[a]	0.027 ± 0.012	139.36 ± 7.17[a]	8.45 ± 0.37	114.78 ± 5.12[a]	6.31 ± 0.65
Zn	12.77 ± 1.93[a]	0.017 ± 0.003	227.17 ± 5.23[a]	33.37 ± 1.51	188.01 ± 6.36[a]	21.48 ± 1.67
Cu	4.53 ± 0.33[a]	0.012 ± 0.004	113.32 ± 5.98[a]	11.77 ± 1.27	25.61 ± 1.37[a]	6.67 ± 1.37
Mn	0.12 ± 0.02	0.13 ± 0.03	67.85 ± 5.37	63.53 ± 4.19	18.95 ± 3.39	18.89 ± 3.76
Mo	0.10 ± 0.02	0.07 ± 0.01	1.67 ± 0.21	1.76 ± 0.31	1.32 ± 0.11	1.35 ± 0.17
Fe	0.29 ± 0.01	0.21 ± 0.02	4327.65 ± 27.15	4224.67 ± 73.55	384.61 ± 12.53	324.67 ± 13.59
Se	0.009 ± 0.002	0.010 ± 0.003	0.26 ± 0.06	0.25 ± 0.07	0.071 ± 0.007	0.063 ± 0.003

Cd cadmium, *Pb* lead, *Zn* zinc, *Cu* copper, *Mn* manganese, *Mo* molybdenum, *Fe* iron, *Se* selenium

[a] $P < 0.01$

higher than those in the control area ($P < 0.01$) (Table 3). The mean contents of Pb and Cd in affected pastures exceeded control levels by 790.00-fold and 140.37-fold in irrigation water, respectively; by 73.48-fold and 16.49-fold in soil, respectively; and by 586.92-fold and 18.19-fold in herbage, respectively. Taking into account that the sheep were exclusively grazing in affected pastures, the ingestion rates of Cd and Pb were estimated. These estimations were based on an average

Table 4 Heavy metal contents in wool and blood in Wumeng semi-fine wool sheep (mg/kg)

Metals	Wool		Blood	
	Affected	Control	Affected	Control
Cd	2.27 ± 0.14[a]	0.37 ± 0.03	0.39 ± 0.0[a]	0.01 ± 0.00
Pb	3.66 ± 0.23[a]	1.17 ± 0.11	0.47 ± 0.0[a]	0.04 ± 0.00
Zn	119.77 ± 6.23[a]	86.61 ± 5.77	17.83 ± 0.92[a]	9.87 ± 2.21
Cu	9.67 ± 0.23[a]	3.53 ± 0.36	1.75 ± 0.06[a]	0.78 ± 0.12
Mn	4.73 ± 0.47	4.74 ± 0.31	0.38 ± 0.04	0.37 ± 0.02
Mo	2.18 ± 0.13	2.17 ± 0.12	0.18 ± 0.03	0.17 ± 0.03
Fe	332.18 ± 37.13	322.77 ± 35.26	532.76 ± 47.88	542.36 ± 46.57
Se	0.168 ± 0.017	0.171 ± 0.016	0.121 ± 0.015	0.123 ± 0.013

Cd cadmium, *Pb* lead, *Zn* zinc, *Cu* copper, *Mn* manganese, *Mo* molybdenum, *Fe* iron, *Se* selenium

[a] $P < 0.01$

Table 5 Heavy metal contents in tissues in Wumeng semi-fine wool sheep (mg/kg)

Metals	Heart		Lung		Liver		Kidney
	Affected	Control	Affected	Affected	Control	Affected	Control
Cd	0.38 ± 0.03^a	0.12 ± 0.01^a	3.32 ± 0.25^a	0.69 ± 0.12^a	7.72 ± 0.36^a	0.49 ± 0.03^a	26.65 ± 0.73^a
Pb	4.86 ± 0.57^a	1.24 ± 0.33^a	4.55 ± 0.31^a	1.75 ± 0.21^a	17.31 ± 1.37^a	0.83 ± 0.05^a	37.57 ± 1.87^a
Zn	157.63 ± 5.26^a	112.97 ± 3.23^a	$147.87 \pm \pm 5.67^a$	117.82 ± 5.63^a	253.96 ± 5.72^a	127.71 ± 6.57^a	267.17 ± 5.43^a
Cu	39.89 ± 2.46^a	13.52 ± 0.73^a	37.23 ± 2.72^a	12.61 ± 1.93^a	378.17 ± 5.93^a	107.67 ± 6.77^a	29.21 ± 2.21^a
Mn	3.77 ± 0.43	3.72 ± 0.33	3.72 ± 0.21	3.73 ± 0.22	4.83 ± 0.42	4.86 ± 0.51	4.88 ± 0.57
Mo	1.52 ± 0.13	1.53 ± 0.14	1.66 ± 0.78	1.67 ± 0.77	1.53 ± 0.16	1.59 ± 0.17	1.97 ± 0.32
Fe	246.56 ± 33.31	341.23 ± 31.36	616.34 ± 67.31	547.55 ± 65.77	446.93 ± 55.36	426.65 ± 57.37	421.59 ± 36.17
Se	1.31 ± 0.13	1.29 ± 0.11	0.49 ± 0.05	0.51 ± 0.06	1.49 ± 0.13	1.51 ± 0.17	2.89 ± 0.23

Metals	Kidney	Muscle		Spleen	
	Affected	Control	Affected	Control	Affected
Cd	1.23 ± 0.08^a	0.72 ± 0.03^a	0.13 ± 0.02^a	0.67 ± 0.11^a	0.17 ± 0.02^a
Pb	0.87 ± 0.11^a	3.85 ± 0.31^a	0.97 ± 0.03^a	4.81 ± 0.53^a	0.93 ± 0.12^a
Zn	112.65 ± 3.69^a	245.13 ± 6.62^a	123.56 ± 5.87^a	247.93 ± 5.17^a	115.62 ± 4.86^a
Cu	11.35 ± 1.53^a	9.91 ± 0.57^a	4.83 ± 0.36^a	9.62 ± 0.27^a	3.47 ± 0.36^a
Mn	4.83 ± 0.35	3.93 ± 0.57	3.96 ± 0.37	2.76 ± 0.27	2.71 ± 0.23
Mo	1.99 ± 0.42	$1.58 \pm 0. 34$	1.56 ± 0.38	0.39 ± 0.04	0.37 ± 0.03
Fe	433.53 ± 35.17	282.89 ± 22.23	293.76 ± 33.33	1972.89 ± 173.23	1863.77 ± 181.33
Se	2.93 ± 0.32	1.19 ± 0.12	1.13 ± 0.11	1.79 ± 0.17	1.83 ± 0.19

Cd cadmium, *Pb* lead, *Zn* zinc, *Cu* copper, *Mn* manganese, *Mo* molybdenum, *Fe* iron, *Se* selenium

[a] $P < 0.01$

Table 6 Heavy metal contents in the rib, radius, and teeth in Wumeng semi-fine wool sheep (mg/kg)

Metals	Rib		Radius		Teeth	
	Affected	Control	Affected	Control	Affected	Control
Cd	4.89 ± 0.23[a]	1.13 ± 0.27	5.72 ± 0.53[a]	1.68 ± 0.31	5.67 ± 0.24[a]	1.53 ± 0.33
Pb	15.53 ± 2.73[a]	5.37 ± 0.13	23.57 ± 3.51[a]	5.37 ± 0.63	28.82 ± 2.93[a]	4.11 ± 0.95
Zn	99.83 ± 3.43[a]	57.55 ± 3.77	157.13 ± 7.63[a]	81.56 ± 7.81	247.37 ± 5.59[a]	103.97 ± 8.61
Cu	9.21 ± 0.51[a]	4.52 ± 0.53	12.17 ± 0.57[a]	4.33 ± 0.76	8.73 ± 1.27[a]	4.73 ± 0.67
Mn	2.37 ± 0.37	2.29 ± 0.36	1.47 ± 0.38	1.46 ± 0.38	1.73 ± 0.37	1.79 ± 0.23
Mo	3.88 ± 0.35	3.79 ± 0.37	4.23 ± 0.53	4.22 ± 0.63	4.23 ± 0.53	4.19 ± 0.36
Fe	195.83 ± 25.77	189.97 ± 22.66	205.55 ± 25.13	198.97 ± 32.53	165.86 ± 15.71	159.97 ± 12.62
Se	0.48 ± 0.07	0.45 ± 0.06	0.36 ± 0.03	0.37 ± 0.04	0.33 ± 0.02	0.35 ± 0.03

Cd cadmium, *Pb* lead, *Zn* zinc, *Cu* copper, *Mn* manganese, *Mo* molybdenum, *Fe* iron, *Se* selenium

[a] $P < 0.01$

herbage ingestion of 73.6 g (dw)/kg body wt/day. Ingested heavy metals ranged from 3.28 to 37.47 mg/kg body wt/day and 0.18 to 3.58 mg/kg body wt/day for Pb and Cd, respectively. The minimum cumulative fatal dosages were 1.17 mg/kg body wt/day and 4.43 mg/kg body wt/day for Cd and Pb, respectively. The contents of Pb, Cd, Zn, and Cu in wool, blood, heart, lung, liver, muscle, spleen, tooth, and bone of affected Wumeng semi-fine wool sheep were markedly increased than those of control animals ($P < 0.01$) (Tables 4, 5, and 6). Pb and Cd mainly accumulated in the kidneys, livers, and skeletons of affected Wumeng semi-fine wool sheep.

The hematological parameters in affected Wumeng semi-fine wool sheep are given in Table 7. Compared with control sheep, Hb and PCV were markedly decreased ($P < 0.01$). Abnormal blood indices indicated hypochromic microcytic anemia in affected Wumeng semi-fine wool sheep. Serum biochemical parameters in affected Wumeng semi-fine wool sheep are given in Table 8. Compared with control animals, the activities of CRT, LDH, SOD, and GSH-Px were significantly diminished ($P < 0.01$). Serum T-AOC in affected animals was also significantly decreased than that in the controls ($P < 0.01$). Serum MDA in affected animals was significantly increased than that in controls ($P < 0.01$). There was no difference in other parameters between affected Wumeng semi-fine wool sheep and control animals [2].

A significant positive correlation was found between heavy metal content in soil and herbage ($r = 0.976$, $R^2 = 0.953$) for Cd and ($r = 0.969$, $R^2 = 0.938$) for Pb, respectively (Fig. 2a, b). There was a significant positive correlation between heavy metal content in herbage and blood ($r = 0.992$, $R^2 = 0.984$) for Cd ($r = 0.987$, $R^2 = 0.974$) and Pb, respectively (Fig. 3a, b). Therefore, it was shown that heavy metals in these sheep were mainly from contaminated soil, and this soil contamination was due to the large number of industrial enterprises involved in the extraction of polymetallic compounds in the area.

Table 7 Hematological parameters in Wumeng semi-fine wool sheep

Parameters	Affected	Control
Hb (g/L)	95.83 ± 3.56[a]	125.56 ± 6.65
RBC (10^{12}/L)	12.76 ± 2.67	12.35 ± 3.61
PCV (%)	32.21 ± 2.21[a]	38.93 ± 2.25
WBC (10^9/L)	6.47 ± 1.93	6.46 ± 2.23
BUN (U/L)	6.63 ± 1.52	6.67 ± 1.37

Hb hemoglobin, *PCV* packed cell volume, *RBC* red blood cell, *WBC* white blood cell, *BUN* blood urea nitrogen

[a] $P < 0.01$

Table 8 Biochemical parameters in serum in Wumeng semi-fine wool sheep

Parameters	Affected	Control
Cp (mg/L)	53.37 ± 7.67	54.76 ± 8.26
LDH (U/L)	2.71 ± 0.33[a]	3.77 ± 0.41
AKP (U/L)	77.71 ± 8.98	77.79 ± 7.56
AST (U/L)	37.71 ± 2.36	38.23 ± 2.57
ALT (U/L)	13.76 ± 1.27	13.98 ± 1.76
SOD (U/L)	121.35 ± 6.73[a]	167.67 ± 7.69
GSH-Px (U/L)	257.67 ± 9.42[a]	379.56 ± 9.35
CAT (U/L)	16.73 ± 1.35	16.82 ± 1.43
T-AOC (U/L)	3.11 ± 0.11[a]	4.52 ± 0.13
CRT (U/L)	267.83 ± 6.57[a]	338.62 ± 7.73
Chol (U/L)	2.87 ± 0.33	2.83 ± 0.32
MDA (nmol/L)	12.37 ± 0.38[a]	6.56 ± 0.25

Cp ceruloplasmin, *LDH* lactate dehydrogenase, *AKP* alkaline phosphatase, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *SOD* superoxide dismutase, *GSH-Px* glutathione peroxidase, *CAT* catalase, *T-AOC* total antioxidant capacity, *CRT* creatinine, *MDA* malondialdehyde

[a] $P < 0.01$

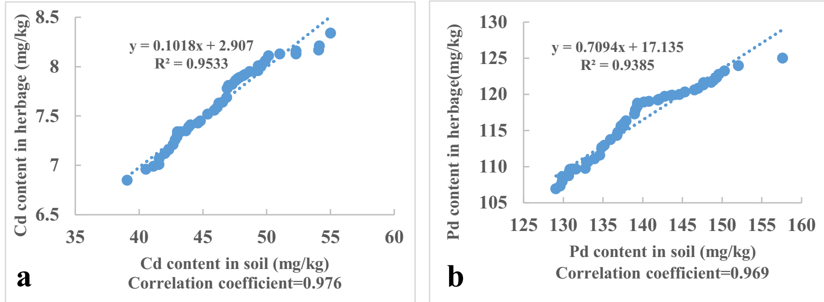

Fig. 2 **a**, **b** Correlation and regression analysis of Cd and Pb in herbage and soil

Discussion

Based on the analysis of heavy metal contents in soil, forage, and animal tissues, combined with the clinical observation results, we preliminarily considered that Wumeng semi-fine wool sheep have been severely affected by heavy metal in polluted pasture.

The polluted pasture with Cd and Pb has occurred in the vicinity of smelters. The maximum tolerable dietary levels of Cd and Pb have been set at 0.5 mg/kg and 30 mg/kg for livestock, respectively [25, 26]. The contents of Cd and Pb in the soil, herbage, and irrigation water in the affected pasture greatly exceeded the abovementioned maximum levels ($P < 0.01$). In addition, wastewater discharged from the smelter was used to irrigate pastures in the vicinity and it was a source of heavy metal contamination in these soils. Pb element, an environmental contaminant, is often combined with Cd element [27–29]. In this study, the contents of heavy metals (Cu, Zn, Pb, and Cd) in tissues of affected Wumeng semi-fine wool sheep with heavy metal pollution were markedly higher than those in controls ($P < 0.01$). Hypochromic microcytic anemia was observed in affected Wumeng semi-fine wool sheep. The present results are consistent with previous studies in livestock and indicate that Pb and Cd accumulate in tissues [30, 31]. As a result of the activities carried out in the smelters in the Weining county of Southwest China, large increases in Cd and Pb were observed in the surrounding soil and herbage. Taking into account that these Wumeng semi-fine wool sheep were exclusively fed herbage from these affected pastures, the ingested heavy metals were estimated to range from 3.28 to 37.47 mg/kg body wt/day and from 0.18 to 3.56 mg/kg body wt/day for Pb and Cd, respectively. Registered values for the minimum cumulative fatal doses for sheep have been estimated to be 4.43 mg/kg body wt/day and 1.17 mg/kg body wt/day for Pb and Cd, respectively [28, 32, 33]. Therefore, the ingestion of herbage growing in these pastures, especially at the sites closest to the smelters, constitutes a clear toxicity hazard for livestock [34, 35].

Oxidative stress is an important mechanism of hepatotoxicity and nephrotoxicity caused by Pb and Cd [36–38]. The organ damage caused by oxidative stress is closely related to lipid peroxidation [39–41]. Patra [42] and Nigam [43] showed that Pb and Cd can induce the organism to produce excessive oxygen free radicals and cause oxidative damage to the organism. In addition, Pb and Cd can be combined with reductive sulfhydryl (–SH) in vivo to antagonize reductive SOD and GSH-Px in sulfhydryl, so as to weaken the ability of the organism to oxidize and metabolize lipid products, and enhance lipid peroxidation [44–47]. Serum T-AOC is an integrative index used to indicate the antioxidant capacity of the body in animals [48–51]. Little is known about the effect of Pb and Cd on the T-AOC of sheep. Our studies indicate that the T-AOC in affected animals was significantly reduced ($P < 0.01$), and enhanced lipid peroxidation in intracellular and extracellular membranes resulted in damage to cells, tissues, and organs [52, 53]. SOD and GSH-Px are important antioxidant enzymes that protect against this process [54, 55]. SOD catalyzes the destruction of superoxide radicals with potential toxicity due to dismutation and hydrogen peroxide formation, and GSH-Px catalyzes the conversion of hydrogen peroxide to water and reduces tissue injury due to lipoperoxidation [56–60]. MDA is a product of excessive oxidation of free radicals against rich unsaturated fatty acids. It is a

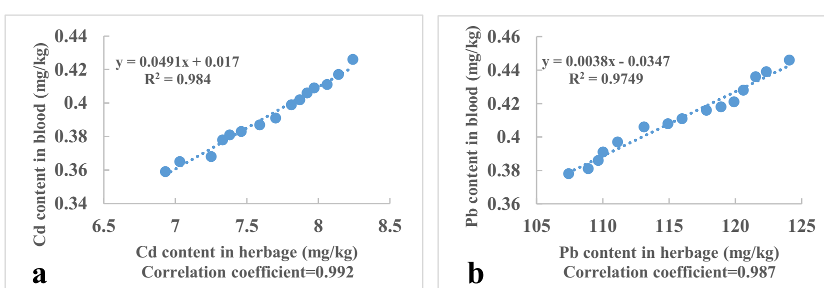

Fig. 3 **a**, **b** Correlation and regression analysis of Cd and Pb in herbage and blood

sensitive and reliable marker reflecting the oxidative damage of tissues and cells caused by reactive oxygen species [61–63]. High concentrations of Pb and Cd will produce a lot of MDA, which will aggravate the accumulation of free radicals [64–66]. The present results show that the contents of Pb and Cd increased, serum SOD and GSH-Px activity in affected animals was markedly decreased, and serum MDA activity in affected animals was markedly increased ($P < 0.01$). It has seriously affected the antioxidant system function and causes various diseases in Wumeng semi-fine wool sheep. Heavy metal contents of forage from affected pastures were 25.61 mg/kg and 188.01 mg/kg, for Cu and Zn, respectively. In general, the maximum tolerable contents in Wumeng semi-fine wool sheep are 28 mg/kg and 300 mg/kg, for Cu and Zn, respectively [11, 67]. Thus, it appears the heavy metal poisoning of the Wumeng semi-fine wool sheep in the pastures was not related to heavy metals Cu and Zn. Whether the contents of heavy metals Cu and Zn in these soils and herbages affect the absorption of heavy metals Pb and Cd in the Wumeng semi-fine wool sheep will be further investigated.

Conclusion

A significant positive correlation was found between heavy metal content in soil and herbage. A significant positive correlation was also found between heavy metal content in herbage and blood. It is presumed that Pb and Cd poisoning in soil and forage is the cause of disease of Wumeng semi-fine wool sheep. Cd and Pb poisoning resulted in significantly increased in serum MDA content and significantly decreased in T-AOC, CAT, GSH-Px, and SOD activities in Wumeng semi-fine wool sheep. It shows that it seriously affects the antioxidant function in the Wumeng semi-fine wool sheep.

Authors' Contributions Xiaoyun Shen conceived the study and carried out its design. Xiaoyun Shen, Xiaoying Min, Shihao Zhang, and Chunjie Song performed the experiments. Xiaoyun Shen conducted the statistical analyses. Xiaoyun Shen and Kangning Xiong commented on the study. Xiaoyun Shen wrote the paper. Xiaoyun Shen and Xiaoying Min revised the paper. Xiaoyun Shen, Xiaoying Min, Shihao Zhang Chunjie Song, and Kangning Xiong supervised the research. All authors read and approved the final manuscript.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethics Statement Experimental Wumeng semi-fine wool sheep were cared for as outlined in the Guide for the Care and Use of Experimental Animals in Agricultural Research and Teaching Consortium. Sample collections from Wumeng semi-fine wool sheep were approved by Southwest University of Science and Technology, Institutional Animal Care and Use Committee (Project B-0006). The study was approved by the relevant ranchers, with the written consent of all participants.

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