

*Original Research*

# Decreasing Toxicity of Heavy Metal to the Altai Sheep by Fertilized Nano-Potassium Molybdate

Yunzhuo Zhang<sup>1</sup>, Ping Zhou<sup>2</sup>, Xiaoyun Shen<sup>1,2\*</sup>

<sup>1</sup>School of Life Science and Engineering, Southwest University of Science and Technology, Mianyang, China

<sup>2</sup>State Key Laboratory of Sheep Genetic Improvement and Healthy Production, Xinjiang Academy of Agricultural and Reclamation Sciences, Shihezi, Xinjiang, China

*Received: 31 March 2022*

*Accepted: 17 August 2022*

## Abstract

The concentrations of heavy metals (Cu, Zn, Cd, Hg and Pb) in water, soil and forages have strikingly increased in the Balikun xijiao ranches, Xinjiang, China for the latest 10 years. The concentrations of Cu, Zn, Cd, Hg and Pb in soil in polluted ranches were 6.76, 3.37, 6.77, 5.76 and 11.67 times higher than those in healthy ranches. The impact of fertilized Nano-K<sub>2</sub>MoO<sub>4</sub> on toxicity of heavy metal have been studied in polluted the ranches. Our findings showed that the concentrations of Mo, Se and N in forage from fertilized ranches were strikingly higher than those in the control ranches ( $P < 0.01$ ). The harvest and digestibility of forage in fertilized ranches were strikingly higher than those in the control ranches ( $P < 0.01$ ). The CP and EE in forage in fertilized the ranch were also increased strikingly ( $P < 0.01$ ). The concentrations of Mo and Se in blood and liver in the Altai sheep from fertilized ranches were strikingly higher than those in control ranches ( $P < 0.01$ ). The concentrations of Cu and Pb in blood and liver in the Altai sheep from fertilized ranches were strikingly lower than those in control ranches ( $P < 0.01$ ). The levels of Hb, PLT and RBC in animals from fertilized ranches were strikingly higher than those in control ranches ( $P < 0.01$ ). In conclusion, The fertilization of Nano-K<sub>2</sub>MoO<sub>4</sub> not only strikingly increased the contents of Mo and Se in blood, but also markedly reduced the contents of Cu and Pb in blood and liver, and remarkably relieved damage from heavy metal pollution in the Altai sheep.

**Keywords:** Altai sheep, heavy metal pollution, natural pasture, Nano-K<sub>2</sub>MoO<sub>4</sub>, toxicity of heavy metal

## Introduction

Sheep farming is vital to the production system in the Xinjiang, China. The Balikun prairie is one of the most key sheep bases in Xinjiang of China, and is also a native habitat for the Altai sheep. The Altai sheep is one of the major livestock species in the Balikun

prairie [1-3]. In the latest 10 years, the exploiting mines and the sewage irrigating, resulting in the most of soil and forage are polluted by heavy metal in the Balikun Prairie [4-6]. The contents of copper (Cu), Zinc (Zn), Cadmium (Cd), Mercury (Hg) and Lead (Pb) in soil from the polluted ranches were strikingly higher than those in healthy ranches [7-10]. The planting capacity of soil markedly reduced, and the quality of the forage strikingly declined [11, 12]. The contamination of heavy metal not only delays the development of the sheep industry, but also has put the health of humans

\*e-mail: xyshen@swust.edu.cn

and animals in seriously danger [13-15]. The heavy metals have the characteristics of concealment, non-degradation and irreversibility, resulting in the heavy metal-contaminated soil is very hard to repair [16]. The finding measures of controlling the pollution of heavy metal have been very urgent in Xinjiang, China. The majority of heavy metals are essential trace nutrition for growth and development of animals and humans [17-20]. However, the excessive content can cause very large harm to the organisms. The heavy metal accumulate in the soil will remarkably decrease the quality and production of forage [21, 22]. It has been reported that heavy metal reduced gastrointestinal peristalsis, while lead to a strong stimulation of the gastrointestinal mucosa and caused gastroenteritis anorexia, which bring the decrease of daily gain, slaughter rate, and daily feed intake [23, 24]. At the same time, heavy metal accumulate *in vivo* will also cause the disorder of toxicity. Toxicity of heavy metal has been observed in most the Altai sheep in the polluted ranches [25]. The heavy metal may lead to oxidative stress, a state of the imbalance between oxidation and antioxidant *in vivo*, which not only causes damage to animal health but also decrease animal economic performance [26-28]. The previous studies have shown that the Mo of soils markedly reducing the absorption of Cu and sulfur (S), and increasing absorption of Se in plants. Meanwhile, the Mo of forage also strikingly decreasing absorption of Cu and S, and increasing absorption of Se in animals [29, 30]. Many scholars have reported that the Se in food is able to obviously reduce toxicity of Cd, Hg and Pb for animals and humans [31-33]. Our previous studies have shown that foliar fertilization of Nano-K<sub>2</sub>MoO<sub>4</sub> markedly increases the contents of Mo and N, and markedly decreased Cu content in healthy ranches. Therefore, It is very necessary that foliage dressing of Nano-K<sub>2</sub>MoO<sub>4</sub> was applied in contaminated ranches [34-37].

The aims of this study were to explore the effects of the applying Nano-K<sub>2</sub>MoO<sub>4</sub> on toxicity of heavy metal in grazing the Altai sheep in the heavy metal-contaminated ranches.

## Materials and Methods

### Study Ranches

The tested areas are located in the Balikun xijiao ranches, Xinjiang, China (42°17'-43°31'N, 82°26'-86°178'E). The concentrations of Cu, Zn, Cd, Hg and Pb in soils of polluted the areas were 6.76, 3.37, 6.77, 5.76 and 11.67 times higher than those in healthy ranches. The Other elements are within the normal range. The pH value in soil in the polluted ranches were higher than 7.0 (pH>7.0). We analyzed the heavy metals (Table 1), physical and chemical features (Table 2) of the soil in polluted and healthy ranches.

Table 1. The contents of heavy metal in soil in tested ranches.

Elements	Polluted ranches	Healthy ranches
Zn (mg/kg)	200.95±11.57*	59.63±5.37
Mo (mg/kg)	1.27±0.12	1.35±0.11
Se (mg/kg)	0.12±0.00	0.13±0.00
Cu (mg/kg)	116.47±11.89*	17.23±1.63
Cd (mg/kg)	3.59±0.23*	0.53±0.04
Cr (mg/kg)	6.83±0.69	6.69±0.61
Hg(mg/kg)	0.29±0.03*	0.05±0.17
Pb(mg/kg)	97.68±9.67*	8.37±0.83

\* indicated the soil environmental quality risk control standard of soil contamination of agricultural land (GB 15618-2018, China).

Table 2. The physical and chemical properties in soil in tested ranches.

Items	Polluted ranches	Healthy ranches
OM (g/kg)	36.93±2.62	37.96±3.31
TS (mg/kg)	5577.77±43.67	5317.00±48.83

OM, organic matter; TS, total salt.

### Fertilizer Treatments

The heavy metal-polluted ranches (40 hm<sup>2</sup>) were randomly divided into four groups (10 hm<sup>2</sup>/group). The treatments consisting of group C(no fertilizer), group I (8.00 kg of Nano-K<sub>2</sub>MoO<sub>4</sub>/hm<sup>2</sup>), group II (9.00 kg of Nano-K<sub>2</sub>MoO<sub>4</sub>/hm<sup>2</sup>), and group III (10.00 kg of Nano-K<sub>2</sub>MoO<sub>4</sub>/hm<sup>2</sup>). The fertilized groups accepted foliar fertilization method.

### Laboratory Animals

The forty Altai sheep, weight of (34.43±3.17) kg, were distributed to heavy metal-polluted ranches for 180 days, 10 sheep/group. The contents of heavy metal and the hematological indexes in blood have been analyzed at the beginning of the experiment. The indicators of animal are within healthy ranges.

### Sample Collections

The samples of soil, forage and sheep were gleaned in June 2020 in heavy metal-polluted ranches. No animals were injured in the sampling process.

### Soil Samples

The samples of soil were gleaned from surface layer in randomly distributed locations in each ranch. The soils were dried at 20-25°C until analysis [38].

Table 3. Samples of collection method.

Samples	Collection method
Soil samples	The samples of soil were gleaned from surface layer in randomly distributed locations in each ranches. The soils were dried at 20-25°C until analysis [38].
Forage samples	The samples of forages were gleaned by using a mower, dried in a forced-air oven at 80°C, and ground to pass a 0.5-mm screen [4].
Tissue samples	The blood samples were collected from the jugular vein by vacuum blood collection tubes with EDTA-K <sub>2</sub> [31]. The samples of blood were stored at 4°C until analysis. The serum samples were separated by centrifuge of 3 000 g for 15 min, and were stored at -20°C until analysis. Liver samples collections were performed by a trained technician, and stored at -20 °C for analysis.

### Forage Samples

The samples of forages were gleaned by using a mower, dried in a forced-air oven at 80°C, and ground to pass a 0.5-mm screen [4].

### Tissue Samples

The blood samples were collected from the jugular vein by vacuum blood collection tubes with EDTA-K<sub>2</sub> [31]. The samples of blood were stored at 4°C until analysis. The serum samples were separated by centrifuge of 3 000 g for 15 min, and were stored at -20°C until analysis. Liver samples collections were performed by a trained technician, and stored at -20°C for analysis.

### Sample Analysis

Mineral contents in samples were examined on June 25, 2020. Hematological and biochemical analysis were executed on June 29, 2020.

### Heavy Metal

The analysis of heavy metals, including Cu, Zn, Cd, Pb, Hg, selenium (Se) and chromium (Cr), using

an AA-7000 absorption spectrophotometer (Shimadzu Corporation, Japan) [3]. The Mo was analyzed by using atomic absorption spectrophotometer (Perkin-Elmer 3030 graphite furnace with a Zeeman background correction).

### Soil Properties

The OM in soil was analyzed by potassium dichromate sulfuric acid oxidation titration, and the TS in soil was analyzed by drying residue mass method, and the water-soil ratio was 5:1 [5]. The pH in soil solution (water-soil ratio, 5:1) was analyzed with potentiometric method (PHS-3C, Shanghai Precision Scientific Instrument Co., Ltd) [19].

### Physiology Index

The blood indexes, including hemoglobin (Hb), red blood cell count (RBC), packed cell volume (PCV), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC) and platelet count (PLT), were analyzed by automatic blood cell analyzer (SF-3000, Sysmex-Toa Medical Electronics, Kobe, Japan) [27].

Table 4. The analysis of methods in the samples.

Indicators	Determination methods
Heavy metal	The analysis of heavy metals, including Cu, Zn, Cd, Pb, Hg, selenium (Se) and chromium (Cr), using an AA-7000 absorption spectrophotometer (Shimadzu Corporation, Japan) [3]. The Mo was analyzed by using atomic absorption spectrophotometer (Perkin-Elmer 3030 graphite furnace with a Zeeman background correction).
Soil properties	The OM in soil was analyzed by potassium dichromate sulfuric acid oxidation titration, and the TS in soil was analyzed by drying residue mass method, and the water-soil ratio was 5:1 [5]. The pH in soil solution (water-soil ratio, 5:1) was analyzed with potentiometric method (PHS-3C, Shanghai Precision Scientific Instrument Co., Ltd) [19].
Physiology index	The blood indexes, including hemoglobin (Hb), red blood cell count (RBC), packed cell volume (PCV), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC) and platelet count (PLT), were analyzed by automatic blood cell analyzer (SF-3000, Sysmex-Toa Medical Electronics, Kobe, Japan) [27].
Nutrition values	Crude protein (CP) and crude fat (EE) of forage were analyzed by kjeldahl method and Soxhlet extractor method, respectively. Crude fiber (CF) in forage was analyzed by crude fiber analyzed apparatus (CXC-06, Wuhan Glemo Testing Equipment Co., Ltd).
Digestibility	The digestibility of forage was analyzed with in vitro gas production technique [3]. Organic matter digestibility (OMD) and metabolizable energy (ME) in the forage were calculated by gas production.

### Nutrition Values

Crude protein (CP) and crude fat (EE) of forage were analyzed by kjeldahl method and Soxhlet extractor method, respectively. Crude fiber (CF) in forage was analyzed by crude fiber analyzed apparatus (CXC-06, Wuhan Glemo Testing Equipment Co., Ltd).

#### Digestibility

The digestibility of forage was analyzed with in vitro gas production technique [3]. Organic matter digestibility (OMD) and metabolizable energy (ME) in the forage were calculated by gas production.

#### Statistical Analyses

These datum were shown as mean±standard deviation. The datum were analyzed by using the Statistical Package for the Social Sciences (SPSS, version 23.0, Inc., IL, USA). The differences between two groups were analyzed by using the T- test. The very extreme difference was indicated by \*\* $P<0.01$ .

### Results

#### Effects of Nano- $K_2MoO_4$ on Nutrition Values of Forage from the Polluted Ranches

As shown in Table 5, the values of CP and EE in forage from fertilized the ranches were strikingly higher than those from control ranches ( $P<0.01$ ). Compared to control ranches, the contents of N in forage were strikingly higher ( $P<0.01$ ). The S contents in forage in fertilized ranches were strikingly lower than those from control ranches ( $P<0.01$ ). There were not significantly different in the other indicators.

#### Effect of Nano- $K_2MoO_4$ on the Contents of Heavy Metal in the Forage from the Polluted Ranches

As shown in Table 6. Compared to control ranches, the contents of Mo and Se in forage were strikingly higher in fertilized the ranches ( $P<0.01$ ). The contents of Cu and Pb in fertilized forages were strikingly lower than those in control forages. There are no striking differences in other elements.

Table 5. Effect of Nano- $K_2MoO_4$  on nutrition of forage in the polluted ranches.

Items	Group C	Group I	Group II	Group III
CP (%)	11.37±1.23**	19.35±1.72	21.12±2.17	21.37±2.35
EE (%)	1.97±0.17**	3.97±0.29	4.37±0.43	4.51±0.47
CF (%)	23.61±2.31	24.32±3.22	24.57±2.31	24.75±2.27
S (%)	0.31±0.01**	0.17±0.02	0.16±0.02	0.15±0.01
N (mg/kg)	2.23±0.21**	3.17±0.31	3.23±0.36	3.37±0.41
OMD (%)	47.17±4.33	46.37±4.26	47.99±4.35	47.66±4.21
ME (MJ/kg)	6.64±0.62	6.59±0.63	6.73±0.61	6.71±0.53

\*\*Very remarkable difference ( $P<0.01$ ).

Table 6. Effect of Nano- $K_2MoO_4$  on contents of heavy metals in forage form the polluted ranches.

Elements	Group C	Group I	Group II	Group III
Zn (mg/kg)	286.69±22.89	288.00±34.81	286.83±22.63	287.20±23.84
Cu (mg/kg)	211.32±21.57**	157.35±15.47	159.29±15.49	151.79±15.27
Mo (mg/kg)	1.35±0.12**	2.93±0.24	2.94±0.23	2.97±0.25
Se (mg/kg)	1.14±0.11**	2.32±0.21	2.34±0.19	2.33±0.22
Cd (mg/kg)	6.27±0.71	6.31±0.53	6.29±0.59	6.33±0.57
Cr (mg/kg)	2.19±0.27	2.22±0.23	2.27±0.25	2.19±0.24
Hg (mg/kg)	0.73±0.07	0.69±0.06	0.71±0.05	0.68±0.04
Pb (mg/kg)	97.63±8.33**	77.77±7.13	77.71±7.21	77.59±7.24

\*\*Very remarkable difference ( $P<0.01$ ).

### Effect of Nano-K<sub>2</sub>MoO<sub>4</sub> on Contents of Heavy Metals in Blood from the Polluted Ranches

As shown in Table 7. Compared to group C, the contents of Mo and Se in blood from fertilized ranches were significantly increased ( $P<0.01$ ). The contents of Cu and Pb in blood from fertilized ranches were greatly decreased ( $P<0.01$ ). No significant differences were found in other heavy metals in the blood of the Altai sheep.

### Effect of Nano-K<sub>2</sub>MoO<sub>4</sub> on Contents of Heavy Metal of Liver from the Polluted Pasture

As shown in Table 8, the contents of Mo and Se in liver from fertilized ranches were significantly increased ( $P<0.01$ ). The contents of Cu and Pb in liver from fertilized ranches were remarkably decreased ( $P<0.01$ ). No significant differences were found in other heavy metals in liver.

### The Effect of Nano-K<sub>2</sub>MoO<sub>4</sub> on Blood Indexes from the Polluted Ranches

As shown in Table 9, in the polluted ranches, the levels of Hb, RBC and PLT in blood of the Altai sheep from fertilized pasture were remarkably higher than those in group C ( $P<0.01$ ). There was a significant improvement in anemia in the Altai sheep from the polluted ranches.

## Discussion

### Effects of Nano-K<sub>2</sub>MoO<sub>4</sub> on Nutrition Values of Forage from the Polluted Ranches

The Mo in soil is a key trace nutrition for the plants and animals in the natural ecosystem. The Mo is very closely related to nitrogen (N) metabolism in plant. It not only plays a pivotal role in biological nitrogen fixation, but also is involved in the reduction process

Table 7. The heavy metals in blood in the Altai sheep from the polluted ranches.

Items	Group C	Group I	Group II	Group III
Zn (mg/kg)	51.83±3.31	57.75±5.43	55.32±2.57	57.23±7.63
Cu (mg/kg)	8.50±0.77**	5.14±0.53	5.13±0.59	5.11±0.52
Cd (mg/kg)	0.35±0.03	0.37±0.02	0.36±0.01	0.36±0.02
Mo (mg/kg)	0.17±0.01**	0.37±0.02	0.36±0.02	0.39±0.03
Se (mg/kg)	0.18±0.01**	0.31±0.03	0.32±0.02	0.31±0.02
Cr (mg/kg)	0.23±0.01	0.22±0.02	0.22±0.02	0.21±0.01
Hg (mg/kg)	0.33±0.02	0.35±0.03	0.34±0.03	0.31±0.02
Pb (mg/kg)	0.57±0.04**	0.39±0.03	0.37±0.05	0.38±0.04

\*\*Very remarkable difference ( $P<0.01$ ).

Table 8. Effect of Nano-K<sub>2</sub>MoO<sub>4</sub> on contents of heavy metals of liver from the polluted ranches.

Items	Group C	Group I	Group II	Group III
Zn (mg/kg)	365.95±33.53	369.32±37.82	371.57±41.87	374.97±45.26
Cu (mg/kg)	935.56±76.93**	471.57±42.37	463.31±44.73	476.37±45.73
Cd (mg/kg)	6.67±0.67**	4.71±0.73	4.59±0.68	4.13±0.71
Mo (mg/kg)	5.18±0.57**	7.85±1.39	7.68±1.41	7.57±1.37
Se (mg/kg)	0.79±0.00**	2.11±0.23	2.13±0.25	2.21±0.19
Cr (mg/kg)	1.13±0.12	1.21±0.13	1.26±0.11	1.19±0.15
Hg (mg/kg)	4.17±0.37	4.21±0.39	4.22±0.41	4.19±0.32
Pb (mg/kg)	17.35±2.11**	12.11±1.83	12.65±1.79	12.53±1.63

\*\*Very remarkable difference ( $P<0.01$ ).



Table 9. Effect of Nano-K<sub>2</sub>MoO<sub>4</sub> on the blood indexes from the polluted ranches.

Items	Group C	Group I	Group II	Group III
Hb (g L <sup>-1</sup> )	87.83±11.23**	121.17±13.75	120.14±12.66	122.95±11.37
RBC (10 <sup>12</sup> L <sup>-1</sup> )	7.36±0.51**	10.34±1.12	11.56±2.11	11.12±1.21
PCV (%)	35.33±3.53**	42.21±3.17	41.32±3.23	42.21±3.25
WBC (10 <sup>9</sup> L <sup>-1</sup> )	8.11±0.82	8.21±0.73	8.23±0.78	8.34±0.77
MCV (fl)	47.43±4.21	46.41±4.11	46.36±4.22	46.35±4.15
MCH (pg)	17.61±1.35	17.53±1.47	17.43±1.67	17.35±1.58
MCHC (%)	24.67±2.11	25.15±2.56	25.26±2.37	25.37±2.61
PLT (×10 <sup>9</sup> /L)	413.53±32.67**	474.57±33.27	479.37±23.45	477.35±33.24

\*\*Very remarkable difference (P<0.01)

of the nitric acid [39, 40]. The previous scholar have reported that fertilization of the Mo have remarkably increased the values of N, CP and DMD of the forage in healthy the natural ranches [41, 42]. Our study showed that applying Nano-K<sub>2</sub>MoO<sub>4</sub> increasing strikingly the values of N, CP and EE in the pollute ranches, but decreasing strikingly contents of S in forages. It may be a very important connection with the interaction of S, Mo and Cu in soil solution in heavy metal-polluted ranches [43, 44].

#### Effect of Nano-K<sub>2</sub>MoO<sub>4</sub> on the Contents of Heavy Metal from Forage, Blood and Liver

In plant, the Mo of the soil solution combines with soluble S elements to form the thiomolybdate, then binds with the soluble Cu elements to form the Cu-thiomolybdate, a very insoluble the complex [45], decreasing the contents of soluble Cu and S, and strikingly decreasing absorption of S and Cu in forage, resulting in low contents of Cu and S in plant [46,47]. Meanwhile, molecular structure of S element and Se element are similar, and competing the same absorbing sites of the plant roots in soil solution [48]. The decreasing content of the soluble S, resulting in an increasing absorption of Se nutrition, and increasing contents of Se in the forage [49, 50].

The heavy metals of animals are mainly derived from the feed, but, rate of absorption is also affected by other elements [16]. The S element combines with the Mo elements to form the thiomolybdate in the sheep rumen [34]. The thiomolybdate closes the absorption site of the Cu in the gut. The Cu of liver was stripped by thiomolybdate from metallothionein (MTs), the stripping Cu is excreted by the blood and bile, resulting in occurring the decreasing Cu content of tissues in animals [45]. The current study has shown that applying Nano-K<sub>2</sub>MoO<sub>4</sub> has strikingly decreased the contents of Cu and Pb, and increasing contents of Se and Mo in forage, blood and liver in the polluted ranches. No previous researchers have found that the fertilization of Nano-K<sub>2</sub>MoO<sub>4</sub> is able to decrease strikingly Pb contents of forage, blood and

liver [51]. The mechanism of Nano-K<sub>2</sub>MoO<sub>4</sub> reducing Pb content requires still further researching and exploring in heavy metal-polluted ranches.

#### The Effect of Nano-K<sub>2</sub>MoO<sub>4</sub> on Toxicity of Heavy Metal in the Polluted Ranches

The current study showed that the contents of Cu, Cd, Pb and Hg in the soil have remarkably higher than those in healthy values, the applying Nano-K<sub>2</sub>MoO<sub>4</sub> in the polluted ranches has markedly decreased the contents of Cu and Pb, and remarkably increased the contents of Mo and Se in forage, blood and liver. The Mo is one of a key components of molybdoflavoprotein in nitrogen-fixing bacteria of plants, is also one of a main ingredient of the plant nitrate reductase [24]. The Mo is a key element of enzymes involving in Fe of utilization in the organism, decreasing symptom of anemia, and increasing the growth in the animals [52, 53]. The low Mo contents in forage will cause ruminants suffer from chronic Cu poisoning. The different species of ruminants have different sensitivity to Cu, and tolerance in sheep to Cu is 25 mg/kg [54]. The excessive Cu of forage can cause corrosion and ulcers of the gastrointestinal mucosa, resulting in anorexia, and decreasing the feed intake. The Se element is also a key mineral for animals and performs the main biological functions in animals [45]. The previous researcher showed that the Se is an essential element of GSH-Px, a main enzyme that catalyzes the reducing hydrogen peroxide [55]. The main cause of oxidative stress is the excessive accumulation of free radicals in the animal, which may cause impairing cell structure and organization. The free radicals can be scavenged by antioxidant enzyme [6]. The heavy metal pollution mainly harms the antioxidant system function [56-58]. The antioxidant system is the defense system for scavenging free radicals, comprising vitamin, Cu, Fe, Zn, Se, SOD, GSH-Px, CAT, and so on [17, 26]. The hematological values are able to assess the degree of anemia in animals [59-61]. In the present study, the Hb, PCV and RBC

in Altai sheep were remarkably decreased in heavy metal-polluted ranches, which indicated that the sheep in the polluted ranches had serious anemia. However, applying Nano-K<sub>2</sub>MoO<sub>4</sub> in the polluted ranches has strikingly alleviating the symptoms of anemia. The value of Hb, PCV and RBC in Altai sheep from ranches of Nano-K<sub>2</sub>MoO<sub>4</sub> remarkably were higher than those from the control ranches. Therefore, Our results indicate that the fertilization of Nano-K<sub>2</sub>MoO<sub>4</sub> in heavy metal-polluted ranches has strikingly mitigated the toxicity of heavy metal.

### Conclusion

The application of Nano-K<sub>2</sub>MoO<sub>4</sub> improving strikingly the quality of forage, increasing markedly the contents of Mo and Se in plants, and decreasing markedly contents of Pb and Cu in forages in heavy metal-contaminated ranches. Meanwhile, The Nano-K<sub>2</sub>MoO<sub>4</sub> of the polluted ranches not only strikingly increasing contents of Mo and Se of blood and liver, decreasing strikingly Pb and Cu of blood and liver in the Altai sheep in the fertilized ranches, but also strikingly alleviating the symptoms of anemia, and mitigating the toxicity of heavy metal.

### Author Contribution Statement

Zhang Yunzhuo: Conceptualization, Methodology, Preparation, Investigation, Software, Data curation, Formal analysis, Resources, Writing-original draft. Xiaoyun Shen: Supervision, Methodology, Software, Project administration, Funding acquisition, Writing-reviewing & editing. Zhou Ping: Methodology, Software, Formal analysis, Resources.

### Declaration of Competing Interest

All authors have declared that they have no known competing financial interests or personal relationships which may influence the work reported in the paper.

### Funding

This work was supported by the Innovation and Development Supporting Plan Project of Key Industries in Southern Xinjiang, China (2021DB014).

### Compliance with Ethical Standards

The experiment was approved by the Institutional Animal Care and Use Committee of Southwest University of Science and Technology (SWUST20210356).

### References

- LV X., CHEN L., HE S., LIU C. Effect of nutritional restriction on the hair pollicles development and skin transcriptome of Chinese Merino Sheep. *Animals*, **10** (6), 1058, **2020**.
- JOHN R.P., AHMAD P., GADGIL K., SHARMA S. Heavy metal toxicity: effect on plant growth, biochemical parameters and metal accumulation by brassica juncea l. *Int J Plant Prod*, **3** (3), 27, **2009**.
- SHEN X., MIN X., ZHANG S., SONG C., XIONG K. Effect of Heavy metal contamination in the environment on antioxidant function in Wumeng semi-fine wool sheep in Southwest China. *Biol Trace Elem Res*, **198** (2), 505, **2020**.
- ARTHINGTON J.D., RECHCIGL J.E., YOST G.P., MCDOWELL L.R., FANNING M.D. Effect of ammonium sulfate fertilization on bahiagrass quality and copper metabolism in grazing beef cattle. *J Anim Sci*, **80** (10), 2507, **2002**.
- CHI Y.K., HUO B., SHEN X.Y. Distribution characteristics of selenium nutrition on the natural habitat of Przewalski's Gazelle. *Pol J Environ Stud*, **29** (1), 67, **2020**.
- SHEN X.Y., HUO B., GAN S.Q. Effects of nano-selenium on antioxidant capacity in Se-deprived Tibetan gazelle (*Procapra picticaudata*) in the Qinghai-Tibet Plateau. *Biol Trace Elem Res*, **199** (4), 981, **2021**.
- HUO B., WU T., XIAO H., SHEN X.Y. Effect of copper contaminated pasture on mineral metabolism in the Wumeng semi-fine wool sheep. *Asian J Ecotoxicol*, **14** (06), 224, **2019**.
- NISHITO Y., KAMBE T. Absorption mechanisms of iron, copper, and zinc: An overview. *J Nutr Sci Vitam*, **6** (1), 1, **2018**.
- SHEN, X.Y., SONG, C.J. Responses of Chinese merino sheep (Junkun Type) on copper-deprived natural pasture. *Biol Trace Elem Res*, **199** (4), 989, **2021**.
- ZHANG L., JIAO T., ZHENG Z.C., LIU C.Q., ZHOU X.H., FENG R.L. Analysis of Se concentrations in study farm of Sanjiaocheng in Qinghai at different seasons. *J Tradit Chin Vet Med*, **4** (05), 17, **2005**.
- WILLSCHER S., JABLONSKI L., FONZ Z., RAHMI R., WITTIG J. Phytoremediation experiments with helianthus tuberosus under different pH and heavy metal soil concentrations. *Hydrometallurgy*, **168** (3), 153, **2017**.
- KHAN Z.I., AHMAD K., ASHRAF I., KHAN A., FARDOUS A., SHER M., AKRAM N.A., ASHRAF M., HAYAT Z., LAUDADIO V., TUFARELLI V., HUSSAIN A., ARSHAD F., CAZZATO E. Appraisal of trace metal elements in soil, forage and animal continuum: a case study on pasture irrigated with sewage water. *Philipp Agric Sci*, **99** (1), 80, **2016**.
- JACOB J.M., KARTHIK C., SARATALE R.G., KUMAR S.S., PRABAKAR D., KADIRVELU K., PUGAZHENDHI A. Biological approaches to tackle heavy metal pollution: a survey of literature. *J. Environ. Manage*, **217**, 56, **2018**.
- GALL J.E., BOYD R.S., RAJAKARUNA N. Transfer of heavy metals through terrestrial food webs: A review. *Environ Monit Assess*, **187** (4), 1, **2015**.
- HUANG Y., CHEN Q., DENG M., JAPENGA J., LI T., YANG X. Heavy metal pollution and health risk assessment of agricultural soils in a typical peri-urban area in southeast china. *J. Environ. Manage*, **207**, 159, **2018**.
- SHEN X.Y., CHI Y. K., XIONG K.N. The effect of heavy metal contamination on humans and animals in the vicinity of a zinc smelting facility. *PLoS One*, **14** (10), **2020**.

17. LI Y.F., WANG Y.C., SHEN X.Y. Effects of sulfur fertilization on antioxidant capacity of Wumeng semi-fine wool sheep in the Wumeng Prairie. *Pol J Environ Stud*, **30** (5), 3919, **2021**.
18. G B. 15618-2018. Soil environmental quality risk control standard of soil contamination of agricultural land (trial). Beijing, Ministry of Ecology and Environment of the People's Republic of China.
19. ZHAO K., MIN X.Y., SHEN X.Y. Response of the Wumeng sheep to phosphorus deprived environment in the Southwest China. *Pol J Environ Stud*, **30** (3), 2927, **2021**.
20. PERRY L.G., BLUMENTHAL D.M., MONACO T.A., PASCHKE M.W., REDENTE E.F. Immobilizing nitrogen to control plant invasion. *Oecologia*, **163** (1), 13, **2010**.
21. HUO B., WU T., SONG C.J., SHEN X.Y. Effects of selenium deficiency in the environment on antioxidant systems of Wumen semi-fine wool sheep. *Pol J Environ Stud*, **29** (2), 1649, **2020**.
22. LI Y.F., HE J., SHEN X.Y. Effects of foliar application of nano-molybdenum fertilizer on copper metabolism of grazing Chinese merino sheep (Junkun Type) on natural grasslands under copper and cadmium stress. *Biol Trace Elem Res*, **2021**.
23. WU T., SHEN X.Y. Response of Wumeng semi-fine wool sheep to copper-contaminated environment. *Pol J Environ Stud Environ. Stud*, **29** (4), 2917, **2020**.
24. LI Y.F., HE J., LUO L., WANG Y.C. The combinations of sulfur and molybdenum fertilization improved antioxidant capacity in grazing Nanjiang brown goat. *Biol Trace Elem Res*, **2021**.
25. PELL A.N., SCHOFIELD P. Computerized monitoring of gas production to measure forage digestion in vitro. *J Dairy Sci*, **76** (4), 1063, **1993**.
26. ZHAO K., HUO B., SHEN X.Y. Studies on antioxidant capacity in selenium-deprived the Choko yak in the Shouqu prairie. *Biol Trace Elem Res*, **199** (1-4), 3297, **2021**.
27. CHI Y.K., ZHANG Z.Z., SONG C.J., XIONG K.N., SHEN X.Y. Effects of fertilization on physiological and biochemical parameters of Wumeng sheep in Chinas Wumeng prairie. *Pol J Environ Stud*, **29** (1), 79, **2020**.
28. SHEN X.Y., CHI Y.K., HUO B., XIONG K.N. Studies on phosphorus deficiency in the Qianbei-pockmarked goat. *Asian-Australas J Anim Sci*, **32** (6), 896, **2019**.
29. SONG C.J., QING J., SHEN X.Y. Responses of Przewalski's gazelle (*Procapra przewalskii*) to zinc nutrition in physical habitat. *Biol Trace Elem Res*, **199** (2), 142, **2021**.
30. ZHAI B.W., ZHAO K., SHEN X.Y. Effects of Sulphur Fertilizer on Copper Metabolism in Grazing Tibetan Sheep in Fertilized Pasture. *Pol J Environ Stud*, **30** (6), 5351, **2021**.
31. LI Y.F., HE J., SHEN X.Y. Effects of nano-selenium poisoning on immune function in the Wumeng Semi-fine wool sheep. *Biol Trace Elem Res*, **199** (11), 2919, **2021**.
32. SHEN X.Y., HUO B., GAN S.Q. Effects of nano-selenium on antioxidant capacity in Se-deprived Tibetan gazelle (*Procapra picticaudata*) in the Qinghai-Tibet Plateau. *Biol Trace Elem Res*, **199**, 981, **2021**.
33. QIU J., ZHOU P., SHEN X.Y. Effects of Se-yeast on immune and antioxidant in the Se-deprived Pishan red sheep. *Biol Trace Elem Res*, **2021**.
34. LI Y.F., SHEN X.Y., LIU F.Y., LUO L., WANG Y.C. Molybdenum fertilization improved antioxidant capacity of grazing Nanjiang brown goat on copper-contaminated pasture. *Biol Trace Elem Res*, **1**, 8, **2021**.
35. ZHAO K., CHI Y.K., SHEN X.Y. Studies on edema pathema in Hequ horse in the Qinghai-Tibet Plateau. *Biol Trace Elem Res*, **198**, 142, **2020**.
36. SHEN X.Y., HUO B., WU T., SONG C.J., CHI Y.K. iTRAQ-based proteomic analysis to identify molecular mechanisms of the selenium deficiency response in the Przewalski's gazelle. *J Proteomics*, **203** (1), 103389, **2019**.
37. SHEN X.Y., SONG C.J., WU T. Effects of Nano-copper on antioxidant function in copper-deprived Guizhou black goats. *Biol Trace Elem Res*, **199** (1), 2201, **2021**.
38. BAO S.D. Soil agrochemical analysis [M]. China Agricultural Publishing House, Beijing, 22. **2000**.
39. POTT E.B., HENRY P.R., ZANETTI M.A., RAO P.V., HINDERBERGER Jr. E.J., AMMERMAN C.B. Effects of high dietary molybdenum concentration and duration of feeding time on molybdenum and copper metabolism in sheep. *Anim Feed Sci Technol*, **79** (1-2), 93, **1999**.
40. SOETAN K.O., OLAIYA C.O., OYEWOLE O.A. The importance of mineral elements for humans, domestic animals and plants-a review. *Afr. J. Food Sci*, **4** (5), 200, **2020**.
41. LI Y.F., LIU H.W., He J., SHEN X.Y., ZHAO K., WANG Y.C. The Effects of oral administration of molybdenum fertilizers on immune function of Nanjiang brown goat grazing on natural pastures contaminated by mixed heavy metal. *Biol Trace Elem Res*, **2021**.
42. HUO B., WU T., SONG C.J., SHEN X.Y. Studies of selenium deficiency in the Wumeng semi-fine wool sheep. *Biol Trace Elem Res*, **194** (1), 152, **2020**.
43. LI Y.F., WANG Y.C., SHEN X.Y., LIU F.Y. The combinations of sulfur and molybdenum fertilizations improved antioxidant capacity of grazing Guizhou semi-fine wool sheep under copper and cadmium stress. *Ecotox Environ Safe*, **2021**.
44. LATIF R., MALEK M., MIRMONSEF H. Cadmium and lead accumulation in three endogeic earthworm species. *Bull. Environ. Contam. Toxicol*, **90** (4), 456, **2013**.
45. SHEN X.Y., SONG C.J., WU T. Effects of Nano-copper on antioxidant function in copper-deprived Guizhou black goats. *Biol Trace Elem Res*, **199** (1), 2201, **2020**.
46. SONG C.J., GAN S.Q., SHEN X.Y. Effects of Nano-copper poisoning on immune and antioxidant function in the Wumeng semi-fine wool sheep. *Biol Trace Elem Res*, **198**, 515, **2020**.
47. OBIDA C.B., ALAN G.B., DUNCAN J.W., SEMPLE K.T. Quantifying the exposure of humans and the environment to oil pollution in the niger delta using advanced geostatistical techniques. *Environ. Int*, **111**, 32, **2018**.
48. SHEN X.Y., HUO B., LI Y.F., SONG C.J., WU T., HE J. (2021) Response of the critically endangered Przewalski's gazelle (*Procapra przewalskii*) to selenium deprived environment. *J Proteomics*, **241**, 104218, **2021**.
49. HUO B., WU T., SONG C.J., SHEN X.Y. Effects of selenium deficiency in alpine meadow on antioxidant systems of yaks. *China Anim Husb Vet Med*, **46** (04), 1053, **2019**.
50. HUO B., HE J., SHEN X.Y. Effects of selenium-deprived habitat on the immune index and antioxidant capacity of Przewalski's gazelle. *Biol Trace Elem Res*, **198**, 149, **2020**.
51. CASALINO E., CALZARETTI G., SBLANO C., LANDRISCINA C. Molecular inhibitory mechanisms of antioxidant enzymes in rat liver and kidney by cadmium. *Toxicology*, **179**, 37, **2002**.



52. HUO B., WU T., CHI Y.K., MIN X.Y., SHEN X.Y. Effect of molybdenum fertilizer treatment to copper pollution meadow on copper metabolism in Wumeng semi-fine wool sheep. *J Dom Anim Ecol*, **40** (07), 44, **2019**.
53. SONG C.J., SHEN X.Y. Effects of environmental zinc deficiency on antioxidant system function in Wumeng semi-fine wool sheep. *Biol Trace Elem Res*, **195** (1), 110, **2020**.
54. WU T., SONG M., SHEN X.Y. Seasonal dynamics of copper deficiency in Wumeng semi-fine wool sheep. *Biol Trace Elem Res*, 197, 487, **2020**.
55. WU T., HE J., SHEN X.Y. Study of metabolomics in selenium deprived Przewalski's gazelles (*P. przewalskii*). *Brit J Nutr*, **2021**.
56. SAZUKA Y., TANIZAWA H., TAKINO Y.. Effect of adriamycin on the activities of superoxide dismutase, glutathione peroxidase and catalase in tissues of mice. *Jpn. J. Cancer Res*, **80** (1), 89, **1989**.
57. DING F., WANG C., XU N., WANG M.L., ZHANG S.X. Jasmonic acid-regulated putrescine biosynthesis attenuates cold-induced oxidative stress in tomato plants. *Sci Hortic*, **288**, 110373, **2021**.
58. DING F., REN L.M., XIE F., WANG M.L., ZHANG S.X. Jasmonate and melatonin act synergistically to potentiate cold tolerance in tomato plants. *Front Plant Sci* **V12**, 1, **2022**.
59. SONG C.J., GAN S.Q., HE J., SHEN X.Y. Effects of nano-zinc on immune function in Qianbei-Pockmarked goats. *Biol Trace Elem Res*, **199** (6), 578, **2020**.
60. DING F., WANG C., XU N., ZHANG S., WANG M.L. SIMYC2 mediates jasmonate-induced tomato leaf senescence by promoting chlorophyll degradation and repressing carbon fixation. *Plant Physiology and Biochemistry* **180** (2022) 27-34. EDWARDS J.R., PROZIALECK W.C. Cadmium, diabetes and chronic kidney disease. *Toxicol. Appl. Pharmacol*, **238** (3), 289, **2022**.

