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Original Research

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

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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_2MoO_4 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_2MoO_4 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_2MoO_4, 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

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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_2MoO_4 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_2MoO_4 was applied in contaminated ranches [34-37].

The aims of this study were to explore the effects of the applying Nano-K_2MoO_4 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^2) were randomly divided into four groups (10 hm^2/group). The treatments consisting of group C(no fertilizer), group I (8.00 kg of Nano-K_2MoO_4/hm^2), group II (9.00 kg of Nano-K_2MoO_4/hm^2), and group III (10.00 kg of Nano-K_2MoO_4/hm^2). 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 20-25ºC 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 20-25ºC until analysis [38].
Forage samples	The samples of forages were gleaned by using a mower, dried in a forced-air oven at 80ºC, 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_2 [31]. The samples of blood were stored at 4ºC until analysis. The serum samples were separated by centrifuge of 3 000 g for 15 min, and were stored at –20ºC until analysis. Liver samples collections were performed by a trained technician, and stored at –20 ºC for analysis.

Forage Samples

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

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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.

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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_2MoO_4 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_2MoO_4 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_2MoO_4 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_2MoO_4 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_2MoO_4 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_2MoO_4 on the blood indexes from the polluted ranches.

Items	Group C	Group I	Group II	Group III
Hb (g L^{-1})	87.83±11.23**	121.17±13.75	120.14±12.66	122.95±11.37
RBC (10^{12} L^{-1})	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 (10^9 L^{-1})	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 (×10^9/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_2MoO_4 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_2MoO_4 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_2MoO_4 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_2MoO_4 is able to decrease strikingly Pb contents of forage, blood and liver [51]. The mechanism of Nano-K_2MoO_4 reducing Pb content requires still further researching and exploring in heavy metal-polluted ranches.

The Effect of Nano-K_2MoO_4 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_2MoO_4 in the polluted ranches has markedly decreased the contents of Cu an 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_2MoO_4 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_2MoO_4 remarkably were higher than those from the control ranches. Therefore, Our results indicate that the fertilization of Nano-K_2MoO_4 in heavy metal-polluted ranches has strikingly mitigated the toxicity of heavy metal.

Conclusion

The application of Nano-K_2MoO_4 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_2MoO_4 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.

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Compliance with Ethical Standards

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

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