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Original Research

Effects of Nano-Potassium Molybdate Preventing the Heavy Metal Poisoning in the Przewalski's Gazelle

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Abstract

In the past 10 years, the level of heavy metals has sharply increased in the Jiangxigou farm in the Qinghai Lake basin, which has seriously affected the health of Przewalski's gazelle. Nano-potassium molybdate (nano-K_2MoO_4), as a new nano-fertilizer, has been applied in agriculture. To study the effect of applying different levels of nano-K_2MoO_4 fertilization (8 kg/hm^2, group I; 9 kg/hm^2, group II; 10 kg/hm^2, group III) on preventing heavy metal toxicity in Przewalski's gazelle. The samples of soil, forage, and animal tissues were collected for testing heavy metals and mineral contents, oxidative stress, and blood indexes. The empirical findings showed that the contents of selenium (Se) and copper (Cu) in the tested forage were significantly higher than those in the control group ($p<0.01$). The levels of Cu and lead (Pb) in the blood and liver of the tested Przewalski's gazelles were remarkably higher than those in the control animals ($p < 0.01$), but the levels of molybdenum (Mo) and Pb were remarkably lower than those in the control animals ($p < 0.01$). The levels of hemoglobin (Hb), platelets (PLT), and red blood cells (RBC) in the tested Przewalski's gazelles were remarkably higher than those in the control animals ($p<0.01$). The enzymatic activities of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT), and total antioxidant capacity (T-AOC) in the serum of the tested Przewalski's gazelles exhibited a significant increase in comparison to the control animals ($p<0.01$), but the content of malondialdehyde (MDA) was lower than that in the control animals ($p<0.01$). In summary, the application of nano-K_2MoO_4 not only significantly improved the antioxidant capacity and alleviated effectively the anemia symptoms of Przewalski's gazelle, but also reduced the heavy-metal toxicity of Przewalski's gazelle in the fertilization areas.

Keywords: Przewalski's gazelle, nano-potassium molybdate, antioxidant capacity, toxicity of heavy metal, anaemia

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Introduction

The Przewalski's gazelle (Procapra przewalskii) belongs to the order Artiodactyla, Bovis, Antelopelae, and Procapra genus. It is a first-class national protected animal on the Qinghai-Tibet Plateau in China and is listed as an "endangered species" on the Red List of the International Union for Conservation of Nature and Natural Resources (IUCN) [1]. Historically, Przewalski's gazelle was widely distributed in Tibet, Inner Mongolia, Gansu, Ningxia, Qinghai, Shanxi, and Xinjiang [2]. In recent years, with the increase in human activities and the change in the environment, the distribution areas and the number of Przewalski's gazelles have been shrinking. Now, it can only be found in the desert around Qinghai Lake on the Tibetan Plateau [3].

During the previous ten years, the application of sewage irrigation and increased exhaust emissions have resulted in widespread soil and forage contamination by heavy metals on the Jiangxigou farm [4-6]. In the polluted areas, notably higher concentrations of Cu, zinc (Zn), cadmium (Cd), hydrargyrum (Hg), and Pb were detected in the soil samples as compared to the uncontaminated areas [7-9]. The planting potential of the soil was significantly reduced, and the quality of the forage was also significantly reduced [10, 11]. Heavy metal pollution not only poses a serious risk to the health of plants and animals, but also causes serious environmental pollution [11, 12].

Heavy metals are characterized by their coverability, non-degradability, and irreversibility, making it difficult to repair soil contaminated with heavy metals [13]. Many heavy metals serve as crucial micronutrients for the growth and development of animals and humans [14]. Nevertheless, excessive levels can result in substantial and severe harm to organisms. The accumulation of heavy metals in the soil can significantly reduce the quality and yield of forage [14, 15].

According to previous reports, heavy metal accumulation reduced the peristalsis of the digestive tract and, at the same time, gave a strong stimulation to the mucous membrane of the digestive tract, causing gastroenteritis and loss of appetite, leading to a reduction in daily intake [16, 17]. Heavy metal accumulation can cause oxidative stress, a state in which the balance of oxidation and antioxidants in the body is broken, which not only harms the health of animals, but also reduces their reproductive efficiency [18]. Toxicity of heavy metals has been observed in most gazelles in the contaminated areas [19].

Therefore, the aim of this research was to investigate the effect of fertilizer application of nano-K_2MoO_4 on heavy metal poisoning of Przewalski's gazelle and to find a new way to protect endangered species in heavy metal polluted habitats. This may provide a basis for the research and prevention of heavy metal poisoning in Przewalski's gazelle.

Materials and Methods

Study Farm

The study sites are located in Gonghe County (35°46′-37°10′N, 98°54′-101°22′E) in the northeast of Qinghai Province, China. The tested areas are located in the Jiangxigou farm, Gonghe (36°33'N, 100°32'E). The concentrations of Cu, Zn, Cd, Hg, and Pb in the soil in the contaminated areas (Jiangxigou farm) were 6.76, 3.37, 6.77, 5.76, and 11.67 times higher than those concentrations in the healthy areas, respectively (Table 1). All the other elements were within the normal range. The pH value in the soil in the contaminated areas was higher than 7.0 (pH>7.0). The analysis did not show any significant difference in soil physicochemical characteristics between polluted and healthy areas (Table 2).

Experimental Design

Forty healthy Przewalski's gazelles (1.5 years) were selected from the Jiangxigou farm and were randomly divided into four groups (10 animals/group). The test duration lasted 180 days.

The treatments consisted of group C (no fertilizer group), 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 the foliar fertilization method.

Sample Collections

In July 2022, samples of soil, forage, and animal tissues were taken from the areas contaminated by heavy metals. No animals were injured during the sampling (Table 3).

Table 1. The contents of heavy metals in the soil from tested and healthy districts.

Elements (mg/kg)	Polluted district	Healthy district
Zinc (Zn)	200.95±11.57*	59.63±5.37
Molybdenum (Mo)	1.27±0.12	1.35±0.11
Selenium (Se)	0.12±0.00	0.13±0.00
Copper (Cu)	116.47±11.89*	17.23±1.63
Cadmium (Cd)	3.59±0.23*	0.53±0.04
Chromium (Cr)	6.83±0.69	6.69±0.61
Hydrargyrum (Hg)	0.29±0.03*	0.05±0.17
Lead (Pb)	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 the soil from tested and healthy districts.

Items	Polluted district	Healthy district
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.

Sample Analysis

The samples were tested for mineral contents on 25 June 2020. Hematological and biochemical analyses were performed on 29 June 2020 (Table 4).

Statistical Analyses

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

Results and Discussion

Detection of Nutritional Value in Forages

The values of CP and EE in the forage from fertilized areas were remarkably higher than those from the control group ($p<0.01$, Table 5). In contrast, the value of N in the forage from fertilized areas was remarkably higher ($p<0.01$) than that from the control areas. The value of S in the forage from fertilized areas was remarkably lower ($p<0.01$) than that from the control group. No significant difference in the other indicators was found.

Detection of Heavy Metal Content in Forages

Compared to the control group, the levels of Se and Cu in the forage from the fertilized areas were remarkably higher ($p<0.01$, Table 6). On the other hand, the levels of Mo and Pb in the fertilized forages were significantly lower than those in the control forages. There were no major differences observed in the levels of other elements.

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 farm. The soils were dried at 20-25ºC until analysis [20].
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 [21]. 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 –20ºC for analysis.

Table 4. The analysis of methods in the samples.

Indicators	Determination methods
Heavy metals	The analysis of heavy metals, including Cu, Zn, Cd, Pb, Hg, Se, and chromium, using an AA–7000 absorption spectrophotometer (Shimadzu Corporation, Japan) [3]. Mo was analyzed by using atomic absorption spectrophotometer (Perkin-Elmer 3030 graphite furnace with a Zeeman background correction).
Soil properties	The organic matter (OM) in the soil was analyzed by potassium dichromate sulfuric acid oxidation titration, and the TS in the soil was analyzed by drying residue mass method, and the water-soil ratio was 5:1 [5]. The pH value in the soil solution (water-soil ratio, 5:1) was analyzed with potentiometric method (PHS-3C, Shanghai Precision Scientific Instrument Co., Ltd) [22].
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) [23].
Nutrition values	Crude protein (CP) and crude fat (EE) of the forage were analyzed by kjeldahl method and Soxhlet extractor method, respectively. Crude fiber (CF) in the forage was analyzed by crude fiber analyzed apparatus (CXC-06, Wuhan Glemo Testing Equipment Co., Ltd).
Digestibility	The digestibility of the forage was analyzed with in vitro gas production technique [3]. Organic matter digestibility (OMD) and metabolic energy (ME) in the forage were calculated by gas production.

Detection of Heavy Metal Content in the Blood

Compared to group C, the levels of Cu and Cd in the blood of Przewalski's gazelle from the fertilized areas were significantly elevated (p<0.01, Table 7). The levels of Mo and Pb in the blood of Przewalski's gazelle from fertilized areas were greatly decreased (p<0.01, Table 7). There were no significant differences observed in the levels of other heavy metals in the blood of Przewalski's gazelle.

Table 5. Effect of nano-K_2MoO_4 on the nutrition values of polluted forages.

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

CP: Crude protein; EE: ether extract; CF: crude fiber; S: sulfur; N: nitrogen; OMD: organic matter digestibility; ME: metabolic energy. **indicated significant difference (p<0.01).

Table 6. Effect of nano-K_2MoO_4 on the contents of heavy metals in polluted forages.

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

** indicated significant differences (p<0.01).

Table 7. Effect of nano-K_2MoO_4 on the contents of heavy metals in the blood of the Przewalski's gazelle from the polluted animals.

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

** indicated significant differences (p<0.01).

Detection of Heavy Metal Content in Livers

The levels of Cu and Cd in the liver of Przewalski's gazelle from the fertilized areas were significantly higher than those from the control group ($p<0.01$, Table 8). Additionally, the contents of Mo, Se, and Pb in the liver of Przewalski's gazelle from the fertilized areas were notably reduced ($p<0.01$, Table 8). However, no significant differences were observed in the levels of other heavy metals in the liver.

Detection of Blood Indexes

The levels of Hb, RBC, PCV, and PLT in the blood of Przewalski's gazelle from fertilization areas were significantly higher than those in group C ($p<0.01$, Table 9). This indicated a substantial improvement in anemia among Przewalski's gazelles from the polluted areas.

Detection of Antioxidant Capacity

In this research, it can be concluded that the levels of serum GSH-Px, T-AOC, CAT, and SOD of Przewalski's gazelle from the fertilized areas were significantly higher than those from group C ($p<0.01$, Table 10). Additionally, the content of MDA in Przewalski's gazelle from the fertilized areas was notably reduced ($p<0.01$, Table 10). This indicated a substantial improvement in antioxidant function among Przewalski's gazelles from the polluted areas.

Discussion

Mo is a crucial micronutrient for plants and animals within the natural ecosystem, as it plays a pivotal role in various biological processes. Specifically, Mo is intimately related to N metabolism in plants, whereby it facilitates biological nitrogen fixation and is involved in the reduction process of nitric acid. Given its integral

Table 8. Effect of nano-K_2MoO_4 on the contents of heavy metals in the liver of the Przewalski's gazelle from the polluted animals.

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

** indicated significant differences ($p<0.01$).

Table 9. Effect of nano-K_2MoO_4 on the gazelle's blood indexes from the polluted animals.

Items	Group C	Group I	Group II	Group III
Hb (g /L)	87.83±11.23**	121.17±13.75	120.14±12.66	122.95±11.37
RBC (10^{12} /L)	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)	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

Hb: hemoglobin; RBC: red blood cell; PCV: packed cell volume; WBC: white blood cell count; WBC: white blood cell count; MCV: mean corpusular volume; MCH: mean corpusular hemoglobin; MCHC: mean corpusular hemoglobin concerntration; PLT: platelet.
** indicated significant differences ($p<0.01$).

Table 10. Effect of nano-K_2MoO_4 on the gazelle's antioxidant parameters from the polluted animals.

Antioxidant capacities	Group C	Group I	Group II	Group III
GSH-Px (IU/L)	45.63 ± 3.53**	152.67±15.21	147.61±14.27	153.78±15.37
MDA (μmol/L)	126.83±11.63**	53.66 ± 5.73	33.66 ± 3.35	33.66 ±3.23
CAT (IU/L)	4.52 ± 0.63**	8.77 ± 0.69	9.33 ± 0.78	9.22 ± 0.83
SOD (IU/L)	55.77 ± 4.43**	75.46 ± 6.55	83.46 ±8.33	84.46 ±8.77
T-AOC (U/mL)	3.32 ± 0.27**	7.76± 0.83	17.66 ± 1.66	17.77 ±1.76

GSH-Px: glutathione peroxidase; MDA: malondialdehyde; SOD: superoxide dismutase; CAT: catalase; T-AOC: total antioxidant capacity. ** indicated significant differences (p<0.01).

function in maintaining the overall ecological balance, Mo is a crucial component of the soil nutrient profile [24, 25]. It has been reported that in healthy natural areas, the application of Mo-containing fertilizer can significantly increase the N, CP, and organic matter digestibility (OMD) values of the forage [26, 27]. In our previous research surveys, the utilization of nano-K_2MoO_4 exhibited a notable impact on the levels of N, CP, and EE, while also demonstrating a significant reduction in S content within the grass samples in polluted regions. This observed outcome might potentially arise from the intricate interplay between S, Mo, and Cu within the soil solution of areas afflicted by heavy metal pollution [28, 29].

Within a plant's biological system, the available Mo in the soil solution undergoes a chemical reaction with the soluble S element, ultimately forming Cu-thiomolybdate, a highly insoluble complex [30, 31], resulting in a notable decrease in the levels of soluble Cu and S, consequently resulting in a significant reduction in the absorption of these elements in forage plants. This, in turn, contributed to lower concentrations of Cu and S in the plant tissues [32, 33]. Simultaneously, it is important to note that the molecular structures of S and Se elements exhibit similarities, thereby competing for the same absorption sites in plant roots within the soil solution. The reduced availability of soluble S leads to an augmented uptake of the Se nutrient, consequently resulting in elevated Se levels in forage plant compositions [34-36]. In our study, the contents of Se and Cu in the forage from fertilized areas were remarkably higher than those from the control group. The Mo and Pb contents were significantly lower than those from the control diet. It might be that nano-k_2MoO_4 applied in the diets competitively inhibited the absorption, thus reducing the content in the forage.

The accumulation of heavy metals in animal tissues is predominantly influenced by the composition of their feed. However, it is pertinent to note that the rate at which these heavy metal elements are absorbed is also contingent upon the presence of other chemical elements within the feed composition [37, 38]. In the digestive system of gazelles, the S element interacts with the Mo element to generate thiomolybdate compounds within the rumen [39]. The results demonstrate that thiomolybdate effectively inhibits the absorption of Cu in the gut through the closure of absorption sites. Furthermore, thiomolybdate effectively removes Cu from metallothionein (MTs) in the liver, subsequently inducing the excretion of stripped Cu via the blood and bile. This process ultimately leads to a reduction of Cu content within animal tissues [40, 41]. In this study, the utilization of nano-K_2MoO_4 has demonstrated a significant reduction in Mo and Pb contents while concurrently increasing Cu and Cd contents in the forage, blood, and liver. Remarkably, no prior research has reported such a distinct reduction in Pb levels in the forage, blood, and liver with the application of nano-K_2MoO_4 fertilization [42, 43].

Mo serves as a vital constituent of molybdoflavoprotein in nitrogen-fixing bacteria found in plants. It is also a principal component of plant nitrate reductase, playing a crucial role in various enzymatic processes. Additionally, Mo is involved in the utilization of Fe within organisms and contributes to the alleviation of anemia symptoms while simultaneously promoting animal growth [44, 45]. Insufficient Mo levels in the forage can lead to chronic Cu poisoning in ruminants. It is important to note that different ruminant species exhibit varying degrees of sensitivity to Cu, with gazelles demonstrating a tolerance threshold of 25 mg/kg [46, 47]. Excessive Cu in the forage can lead to corrosion and ulceration of the gastrointestinal mucosa, causing the onset of anorexia and decreasing feed intake in animals. Furthermore, Se is also an integral mineral essential for various biological functions in animals [48, 49]. This study indicated a significant disparity in the levels of Cu, Cd, Pb, and Hg in the soil between the polluted areas and the healthy areas. Specifically, the polluted areas exhibited substantially higher concentrations of these elements compared to the healthy areas. However, upon the application of nano-K_2MoO_4 in the polluted areas, there was a marked reduction in Mo and Pb levels, accompanied by a notable increase in the Cu content observed in the forage, blood, and liver samples.

Mo can participate in the composition and metabolism of vitamin B12, promote RBC development and maturity, and prevent anemia. Hematological parameters serve as reliable indicators for assessing the severity of anemia in animals [50, 51]. The present research demonstrated that heavy metal pollution in the natural habitat of the gazelles had caused a significant reduction in the levels of Hb, PLT, PCV, and RBC, resulting in severe anemia among the animals. Nevertheless, as evidenced by the markedly elevated levels of Hb, PLT, PCV, and RBC in the gazelle from the nano-K_2MoO_4 fertilized areas as compared to the control group. These findings suggested that the application of nano-K_2MoO_4 to the polluted farm could effectively reduce the toxicity of heavy metals in the animal's environment and significantly mitigate the symptoms of anemia.

The antioxidant system of the animal body is a defense system for the rapid removal of free radicals, which mainly includes the non-enzyme system and enzyme system [52, 53]. On the one hand, non-enzymatic systems play a crucial role in antioxidant defense mechanisms and consist of various components such as vitamin E, vitamin C, GSH, Cu, Mn, Mo, Zn, Fe, and Se. On the other hand, the enzymatic system is composed of specific antioxidant enzymes, including SOD, GSH-Px, and CAT. The assessment of GSH-Px, MDA, CAT, SOD, and T-AOC provides insights into the overall antioxidant function of the organism [54-56]. The experimental investigations demonstrated that heavy metal contamination had a substantially negative impact on the functionality of antioxidant systems. It was observed that this contamination rapidly disrupted the delicate balance between oxidants and antioxidants. Conversely, this result indicated that the fertilization of nano-K_2MoO_4 in heavy metal-polluted areas had remarkably mitigated the decline of antioxidant capacity in Przewalski's gazelle.

Conclusions

The application of nano-K_2MoO_4 significantly increased the concentrations of Se and Cu in the forage, as well as the concentrations of Cu and Cd in both blood and liver samples. While significant decreases in Mo and Pb levels were observed in the forage, blood, and liver samples, there were also significant increases in CP and EE levels in the forage. Additionally, the intervention with nano-K_2MoO_4 effectively mitigated anemia symptoms, enhanced antioxidant capacity, and reduced the toxicity of heavy metals within the fertilized areas.

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Conflict of 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. The authors have no relevant financial or non-financial interests to disclose, and the authors declare no conflicts of interest.

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